DISORDERED SKELETAL MUSCLE OXIDATIVE METABOLISM IN HUMAN OBESITY AND TYPE 2 DIABETES

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1 ABSTRACT

Obesity and type 2 diabetes mellitus (T2DM) are both complex diseases with multifactorial etiologies. Together they affect over 640 million people worldwide and have a significant impact on the global healthcare system incurring costs of over 800 billion dollars. The overall goal of my doctoral research has been to elucidate metabolic predictors and underlying mechanisms in obesity and T2DM. Specifically, I have examined mechanisms contributing to disordered oxidative metabolism in skeletal muscle. My research included participants who were recruited from the Ottawa Hospital Weight Management Clinic in which they completed a clinically supervised meal-replacement and lifestyle intervention program. More so, my doctoral studies evaluated characteristics of muscle mitochondrial function in obesity and T2DM and revealed impaired mitochondrial respiration and electron transport chain supercomplex assembly in muscle from patients with T2DM. The first aim was to study the impact of T2DM on weight loss ability in a large population of obese patients participating in a standardized meal replacement and lifestyle modification program. As there is considerable variability in weight loss propensity, it was found that T2DM significantly deters weight loss although the effect is not large. Since skeletal muscle energetics are central in the development and progression of obesity and T2DM, the second and third aims were to study mitochondrial function in this tissue with the idea of uncovering molecular etiologies. The second aim found deficiencies in mitochondrial respiration in individuals with obesity and T2DM compared to individuals with obesity alone. Reductions in mitochondrial respiration were correlated with increasing levels of HbA1C and attributed to paucity in supercomplex formation in the mitochondrial inner membrane (MIM) of the electron transport chain (ETC). The third aim was to delineate differential fuel oxidation

mechanisms and circulating protein biomarkers in obese diet-sensitive (ODS) and obese diet-resistant (ODR) participants following a high fat meal (HFM) challenge. Whole-body analyses were conducted in addition to measures in blood, adipose tissue, skeletal muscle and primary cells. Remarkable increases in oxidative capacity were measured post-HFM. In addition, impaired mitochondrial function was found in the ODR group despite lack of differences in mitochondrial content or the assembly of supercomplexes. Differences were also found in circulating acylcarnitines as well as expression of several proteins including Heat shock 70 kDa protein 1A/1B, Tyrosine-protein kinase Fgr, and Peptidyl-prolyl cis-trans isomerase D. Ultimately, a better understanding of mechanisms involved could lead to significant improvements in personalized medical approaches in obesity and T2DM.

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4 LIST OF ABBREVIATIONS

2hPG	2-hour Plasma Glucose
AA	Antimycin A
ADP	Adenosine Diphosphate
AHR	Aryl Hydrocarbon Receptor Protein Complex
AIP	Aryl Hydrocarbon Receptor-Interacting Protein
ANOVA	Analysis of Variance
ASP	Acid Soluble Products
ATP	Adenosine Triphosphate
BAT	Brown Adipose Tissue
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BMRU	Behavioral Metabolic Research Unit
BN-PAGE	Blue Native Polyacrylamide Gel Electrophoresis
BPA	Bisphenol A
COX	Cytochrome C oxidase
CPT1	Carnitine Palmitoylotransferase 1
Cyt C	Cytochrome C
DAG	Diacylglycerol
DCFDA	Dichloro-Dihydro-Flourescein Diacetate
DTT	Dichlorodiphenyltrichloroethane
ETC	Electron Transport Chain
ETF	Electron Transfer Flavoprotein
ETS	Electron Transfer System
FA	Fatty Acid
FCCP	Carbonyl Cyanide-P-Trifluoro-Methoxyphenyl-Hydrazone
FGR	Tyrosine Protein-Kinase FGR
FPG	Fasting Plasma Glucose
G	Glutamate
Glut 4	Glucose Transporter 4

GWAS	Genome-Wide Association Studies
HbAIC	Glycated Hemoglobin
HFM	High Fat Meal
HSP72	Heat Shock Protein 72
IFG	Impaired Fasting Glucose
LCD	Low Calorie Diet
LPL	Lipoprotein Lipase
M	Malate
Mfn2	Mitofusin-2
MIM	Mitochondrial Inner Membrane
MnSOD	Manganese Superoxide Dismutase
MOM	Mitochondrial Outer Membrane
MPTP	Mitochondrial Permeability Transition Pore
mtDNA	Mitochondrial DNA
OC	Octanoyl Carnitine
ODR	Obese Diet Resistant
ODS	Obese Diet Sensitive
OGTT	Oral Glucose Tolerance Test
OHWMP	Ottawa Hospital Weight Management Program
OXPHOS	Oxidative Phosphorylation
P	Pyruvate
PG	Plasma Glucose
PPID	Pepditylpropyl iIomerase D
PWL	Percentage Weight Loss
RMR	Resting Metabolic Rate
ROS	Reactive Oxygen Species
ROWL	Rate of Weight Loss
RYGB	Roux-en-Y Gastric Bypass
S	Succinate
SR	Sarcoplasmic Reticulum
T2DM	Type 2 Diabetes Mellitus
TCA	Tricarboxylic Acid

TCAG	The Centre for Applied Genomics
TDE	Total Daily Energy Requirements
TG	Triglycerides
TMPD	Tetramethylphosphodinitrate
UCP	Uncoupling Protein
UK	United Kingdom
US	United States
WHO	World Health Organization
XO	Xanthine Oxidase

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1 CHAPTER 1 – GENERAL INTRODUCTION

1.1 THE DISEASES: OBESITY AND DIABETES

1.1.1 OBESITY

Throughout the history of humanity, many diseases have surfaced and many have been treated by modern medicine. Some diseases have had a bigger impact on our society than others. One of the biggest health challenges facing our modern society is obesity. The World Health Organization (WHO) defines obesity as "abnormal or excessive fat accumulation that may impair health" (World Health Organization, 2015). More practically, obesity can be defined by calculating a person's body mass index (BMI). This is done by taking a person's weight (kg) and dividing it by their height (m) squared. The upper limit of a lean BMI is 24.9 kg/m^2; whereas an individual is considered overweight when their BMI is between 25 kg/m^2 and 29.9 kg/m^2, or obese when their BMI is 30 kg/m^2 or higher (Mendis *et al.*, 2014). Since BMI measurements do not account for bodily distribution of adipose tissue, and abdominal obesity is correlated increased cardiovascular risks (Despres, 2012), other means were developed to qualify and quantify obesity in adults. Waist-to-hip ratio is an appropriate measure of *relative* abdominal obesity. However, waist circumference is the generally accepted anthropometric tool used for measuring *absolute* abdominal obesity, and is preferred to waist-to-hip ratio in clinical practice. Due to the cultural and geographic variability of waist circumference, different ethnic-specific values have been established to assess obesity in distinct populations (Obesity Canada Clinical Practice Guidelines Expert Panel, 2007). Moreover, waist circumference is generally not considered useful in extreme/morbid obesity.

1.1.1.1 PREVALENCE

Studies of the distribution of obesity over different ages reveal that in most areas around the world, the proportion of obese individuals increases with age and reaches a peak in mid to late adulthood (Haslam and James, 2005). The prevalence of obesity worldwide has been shown to be greater in women compared to men (Ng *et al.*, 2014). Over time and all around the world, the prevalence of obesity has been increasing and has nearly doubled between the years of 1980 and 2014. In fact, in 2014, more than half a billion adults were considered to be obese, representing 11% of men and 15% of women. The Americas are most affected by this epidemic where over 27% of the population was shown to be obese. On the other hand, South-East Asia represents the lowest prevalence area where obesity affects only 5% of its population (Mendis *et al.*, 2014).

In Canada, there are more men who are obese than women; with prevalence rates of 26.1% and 23.4% respectively. Overall, about a quarter (24.8%) of the national Canadian population, or 6.3 million individuals, was considered obese in 2012 (Navaneelan and Janz, 2014; Ng *et al.*, 2014). The prevalence has more than doubled since 1985, when fewer than 10% of people in all provinces were obese. (Katzmarzyk, 2002).

1.1.1.2 ETIOLOGY

Obesity is a disease of multifaceted development involving a complex interplay between social, environmental, physiological (metabolic), behavioral, genetic, and epigenetic factors (Bray *et al.*, 2016). Ultimately, obesity results from a chronic imbalance between dietary energy intake and energy expenditure. Specifically, when the amount of dietary energy is greater than the amount of energy expended, the body stores this excess energy in the form of fat that accumulates in adipose tissue. In a chronic setting, this leads to increased adiposity and weight gain, and eventually reaches the severity to be considered obesity.

The specific reasons that lead to excessive adiposity are different depending on the age at which obesity develops. Previous studies from our laboratory and others have shown that *in utero* factors, such as growth retardation resulting from *in utero* undernutrition, predispose offspring to the development of obesity later on in life (Beauchamp *et al.*, 2014; Gluckman *et al.*, 2008). In the child and adolescent periods, just as during adulthood, many other factors contribute to the development of obesity. These include genetic factors (Bell *et al.*, 2005; Loos, 2012), endocrine disorders (Bougneres *et al.*, 2008; Reinehr *et al.*, 2007), diet, physical activity, iatrogenic causes (including medications that cause weight gain and diseases that limit physical activity such as orthopedic injuries, nerve injuries, strokes, etc.), limitations in sleep (Jiang *et al.*, 2009), socioeconomic factors (Mendis *et al.*, 2014). Other environmental factors such as access to sugar-sweetened beverages (Gilbert-Diamond *et al.*, 2014; Taber *et al.*, 2013), television (Gilbert-Diamond *et al.*, 2014; Hancox *et al.*, 2004), and video games (Stettler *et al.*, 2004) have also been linked to obesity. Additional emerging theories related to the causes of obesity include factors such as the gut microbiome (Angelakis *et al.*, 2012; Burcelin, 2012; DiBaise *et al.*, 2008; Jess, 2014), and exposure to endocrine-altering toxins (such as dichlorodiphenyltrichloroethane (DDT), and bisphenol A (BPA)) (Carwile and Michels, 2011; Trasande *et al.*, 2012; Warner *et al.*, 2014) and certain strains of adenoviruses (such as adenovirus 36) (Atkinson *et al.*, 2005; Atkinson *et al.*, 2010; Gabbert *et al.*, 2010; Pasarica *et al.*, 2006).

1.1.1.3 COMORBIDITIES

It has been known for over 2000 years that obesity has multiple impacts on the human body (Bray, 2007). Obesity can generally impact most systems of the human body and can ultimately lead to death. In fact, obesity has been associated with an increase of

mortality by two- to three- fold, with a 30% increase in mortality rate for every increase of BMI of 5 kg/m^2 (Adams *et al.*, 2006). Moreover, a study of 1.46 million adults demonstrated that the hazard ratio for mortality is positively correlated with increases of BMI (Berrington de Gonzalez *et al.*, 2010). Other more recent work further established the association of BMI with mortality in over 4 million individuals from 4 different continents (Global BMI Mortality Collaboration, 2016).

With regard to the impact of obesity on other body systems, it has been demonstrated that the most strongly associated comorbidity is T2DM (Guh *et al.*, 2009). Other diseases associated with obesity are: cardiovascular diseases (including hypertension, coronary artery disease, stroke, and congestive heart failure), cancers (including colorectal, kidney, breast, ovarian, endometrial, and pancreatic), asthma, arthritis, gallbladder disease (including non-alcoholic steatohepatitis), and chronic back pain (Guh *et al.*, 2009; Haslam and James, 2005; Whitlock *et al.*, 2009).

Additional diseases with suggestive associations with obesity include: dyslipidemia (Poirier *et al.*, 2006), venous thromboembolism (Tsai *et al.*, 2002), and obstructive sleep apnea (Foster *et al.*, 2009).

1.1.1.4 MANAGEMENT

The treatment goal in obesity generally revolves around weight loss in order to prevent, treat, or reverse the various comorbidities associated with the disease. Weight loss is the major outcome measure that has a significant effect on mortality and the prevalence of comorbidities associated with obesity (Jensen *et al.*, 2014). In addition, just as obesity is a multifactorial and multifaceted disease, its treatment modalities should be varied and address its different contributing factors (Eckel *et al.*, 2014; Garvey *et al.*, 2014; Ryan and Heaner,

2014). The three major categories of treatment options for weight loss in obese patients are: 1) lifestyle modification, which include diet and exercise; 2) medications; and 3) weight loss surgery. With this in mind, weight loss is ultimately only achieved when energy output (expenditure) is greater than energy input (dietary intake).

Lifestyle Modifications

Lifestyle modifications include dietary changes; *i.e*. reducing energy intake, exercise; *i.e.* increasing energy expenditure, and behavioral modification; *i.e.* developing healthy habits over a prolonged period of time. Typically, the behavioral component of most lifestyle modification programs includes routine self-monitoring of food intake, body weight, and physical activity in order to facilitate ongoing changes in those parameters (Ryan and Heaner, 2014). Most lifestyle modification programs also incorporate an educational component whereby participants attend regularly scheduled sessions in order to ensure adherence and reinforce healthy habits.

As the obesity epidemic gained momentum and media attention, many different commercial weight loss diets have emerged. The most popular commercial diets in North America include Atkins, South Beach, Ornish, Zone/Omega Rx, and Weight Watchers (McClendon *et al.*, 2010). Their similarities include the recommendation of certain food groups such as fruits, vegetables, whole grains, legumes, fish, and poultry; whereas some of their differences relate to the recommended proportions of different macronutrients. General trends include balanced low-calorie diets, portion-controlled diets, low-fat diets, and low-carbohydrate diets (Freedman *et al.*, 2001). Although these diets all generally result in some level of weight loss (about 3% weight loss per year (Bray *et al.*, 2016)), scientifically sound data on long-term efficacy and weight maintenance still need to be gathered (Gudzune *et al.*,

2015). In addition, studies that compare the efficacy of different weight loss programs have concluded that adherence to a diet, and therefore sustained caloric restriction, was a greater predictor of weight loss and cardiovascular risk improvement compared to the qualitative compositions of different diets (Dansinger *et al.*, 2005).

Exercise is another important component of lifestyle therapy. Guidelines for recommended physical activity differ in various regions around the world. There is however some agreement regarding the set goal of 150 minutes of aerobic exercise per week (Jakicic *et al.*, 2013; National Clinical Guideline Centre (UK), 2014; Ryan and Heaner, 2014). For an obese and sedentary individual, goals should be set minimally and gradually increased as tolerated. In addition, Ross *et al.* demonstrated that the amount of physical activity has a higher impact on weight loss than the intensity by comparing three groups undergoing low-amount, low-intensity exercise, high-amount, low-intensity exercise, and high-amount, high-intensity exercise (Ross *et al.*, 2015).

Medication

With regard to pharmacotherapy, the only approved drugs in Canada to treat obesity are Orlistat (Xenial®) and Liraglutide (Saxenda®) (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2013; Hollander *et al.*, 1998; Obesity Canada Clinical Practice Guidelines Expert Panel, 2007). Orlistat is a non-systemic pancreas-specific lipase inhibitor that inhibits the digestion and absorption of dietary fats by about 30% (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2013). It is not absorbed systemically and efficacy data have demonstrated that treatment results in weight loss of roughly 6.1% of total body weight over 1 year. (Bray *et al.*, 2016). Liraglutide, on the other hand, is a human glucagon like peptide-1 (GLP-1) receptor agonist

that results in a mean percentage of weight loss of 7.4% over 1 year (Bray *et al.*, 2016). Other weight loss medications currently approved in the United States (US) and the United Kingdom (UK) include Phentermine, Lorcaserin, a combination of Pherntermine and Topiramate ER, and a combination of Naltrexone SR and Buproprion SR (Bray *et al.*, 2016). It is also important to note that all pharmacotherapies should be combined with effective lifestyle modifications that include a regimented diet and exercise program.

Weight Loss Surgery

Weight loss surgery, which is also used to treat T2DM, will be discussed below in section 1.1.3.

1.1.1.5 DIET RESISTANCE

Weight loss, as mentioned above, is the cornerstone of obesity treatment. As such, several meal-replacement programs have been created and data on outcomes are increasingly plentiful. While most programs are successful in inducing weight loss in patients with adequate adherence to diets (Bray *et al.*, 2016), there is still great inter-individual variability in weight loss success with some individuals exhibiting an impaired capacity for weight loss. This phenomenon is known as diet resistance.

At the base, variability in weight loss capacity harbors a complex etiology that is determined by many factors similar to those contributing to the etiology of obesity, as mentioned above. Additionally, other clinical and demographic factors have been demonstrated to impact patients' abilities to lose weight. These include initial body weight, sex, age (Roberts and Rosenberg, 2006), smoking (Chiolero *et al.*, 2008) thyroid hormone status, medications, movement disorders, disabilities, and certain genetic factors as revealed by monozygotic twin studies (Bouchard and Tremblay, 1997; Bouchard *et al.*, 1990).

Patients involved in the different studies described in this thesis were all recruited from the Core Program of the Ottawa Hospital Weight Management Clinic. This consists of a 26-week long program that includes six or twelve weeks of meal replacement with a 900kCal/day product from Nestlé called Optifast900®. The program also involves weekly clinic visits, regular meetings with a physician, and a yearlong course on different subjects relating to nutritional advice and lifestyle modification (Dent *et al.*, 2002; Harper *et al.*, 2002).

Patients in this program, as with others, exhibit variability in their weight loss. In order to study factors affecting this variability, rate of weight loss (ROWL) was calculated in diet adherent patients over the first six weeks of meal replacement when weight loss is typically linear. Data were corrected for factors known to affect weight loss such as sex, age, initial weight, and thyroid hormone status and patients were divided into quintiles based on the adjusted ROWL. The patients in the top quintile exhibiting the highest ROWL were called obese diet sensitive (ODS) whereas those in the bottom quintile exhibiting the lowest ROWL were called obese diet resistant (ODR). A two-fold difference in weight loss was observed between these two populations, even when factors known to affect weight loss were accounted for (Dent *et al.*, 1999).

Additional studies in ODS and ODR populations revealed that gene expression of specific pathways were upregulated in the blood of ODS compared to ODR individuals. These included the "oxidative phosphorylation" (OXPHOS) pathway, and to a lesser extent the "proteasome" pathway (Ghosh *et al.*, 2011). As detailed below in sections 1.2.2. and 1.3.2., studies of differences in skeletal muscle and skeletal muscle mitochondria between these two unique populations further reveal the etiology of this variability in ROWL and diet resistance.

1.1.2 TYPE 2 DIABETES MELLITUS (T2DM)

T2DM is a systemic metabolic disorder in which glycemia is dysregulated due to impaired sensitivity to insulin. It is characterized by insulin resistance in a variety of tissues including liver and skeletal muscle (DeFronzo, 1988). The development of T2DM is correlated with weight gain and decrease in physical activity; each increasing the risk for diabetes independently of the other (Sullivan *et al.*, 2005). The diagnosis of diabetes is established by measuring specific blood markers as follows (Goldenberg and Punthakee, 2013):

- Fasting Plasma Glucose (FPG) ≥ 7.0 mmol/L
  OR
- Glycated Hemoglobin (HbA1C) ≥ 6.5% (in adults)
  OR
- 2-hour Plasma Glucose (2hPG) in a 75 g Oral Glucose Tolerance Test (OGTT) ≥ 11.1 mmol/L
  OR
- Random Plasma Glucose (PG) ≥ 11.1 mmol/L
-

Glycated hemoglobin, or HbA1C, as mentioned above, is an important measure used in the diagnosis of T2DM. As red blood cells uptake glucose, hemoglobin irreversibly binds to it in a fashion correlated to the concentration of glucose in the blood. Since the average lifespan of a red blood cell is 120 days, glycated hemoglobin can be used as an indicator of general glycemia over a period of 8 to 12 weeks to predict the development of T2DM (Goldstein, 1984; Morris *et al.*, 2013; Nathan *et al.*, 1984).

1.1.2.1 PATHOGENESIS

T2DM is a chronic, progressive disease that begins, in early stages, with insulin resistance and abnormal or impaired glucose metabolism. As insulin mediates the uptake of glucose from blood to cells, insulin resistance is characterized by impaired glucose uptake and utilization in the presence of insulin (Moller and Flier, 1991). Major organs responsible

for insulin-mediated glucose uptake include skeletal muscle and adipose tissue. Insulin also targets the liver where gluconeogenesis and glycogenolysis are normally inhibited by this hormone (Pessin and Saltiel, 2000). Insulin resistance affects all tissues, but it is thought that skeletal muscle may be the first site of pathogenesis (Petersen and Shulman, 2006). In reaction to this initial decreased glucose uptake, pancreatic ß-cells in the islets of Langerhans increase production of insulin in order to overcome the insulin resistance and maintain normal glycemia. This state of hyperinsulinemia progressively increases over a prolonged period of time with maintenance of normoglycemia. Hyperglycemia, on the other hand, occurs when the pancreatic ß-cells are no longer able to produce sufficient amounts of insulin to overcome insulin resistance in peripheral tissues. Additionally, in response to the decreased glucose uptake by peripheral tissues, the liver increases the rate of gluconeogenesis (Magnusson *et al.*, 1992), which exacerbates pre-existing hyperglycemia. In severe cases, chronic high blood sugars lead to a reduction in β-cell mass; which, if left untreated, causes pancreatic failure (Weir and Bonner-Weir, 2004).

Furthermore, patients who suffer from impaired glucose tolerance or impaired fasting glucose are at a high risk of developing diabetes. This state of deregulation, in which patients demonstrate higher than normal blood glucose but lower than the positive diagnosis threshold, is considered to be prediabetes and constitutes a major risk factor for the eventual development of full-blown T2DM (Perreault *et al.*, 2012).

1.1.2.2 PREVALENCE

The occurrence of diabetes is on the rise at the global level. The term ‘diabetes’ in this section refers to type 1 diabetes mellitus (T1DM), gestational diabetes, as well as T2DM, the last of which represents over 90% of all diabetes cases. The latest international estimate

states that in 2014, there were 422 million adults living with diabetes. There were also 3.7 million deaths that were due to hyperglycemia and diabetes, of which 1.7 million were directly caused by diabetes (World Health Organization, 2016). This is in sharp contrast to estimates from 2004, which state the prevalence to be 171 million in 2000, and projected this to increase to 366 million by 2030 globally (Wild *et al.*, 2004). This increase in the prevalence of diabetes is thought to be associated with a predicted aging society and a doubling of the urban population in developing countries in the same time period. Wild *et al.* even suggest that the rise in diabetes is not necessarily dependent on obesity levels; and that if the rise in obesity was compounded, the true level of diabetes may be even higher (Wild *et al.*, 2004).

In Canada, the prevalence of diabetes was estimated at 3.4 million people in 2015, which represented 9.3% of the population. In practical terms, this means that 1 in 10 individuals in the country is living with this disease. The same report, published by the Canadian Diabetes Association, estimated that the prevalence would increase 44% in 10 years and reach 5 million individuals with diabetes in 2025 (Canadian Diabetes Association, 2015).

1.1.2.3 Risk Factors

Several risk factors have been associated with the onset and progression of T2DM, the most common of which is obesity. Studies have demonstrated that having a BMI ≥ 25 kg/m^2 makes an individual three times more likely to develop T2DM than an individual with a BMI < 25 kg/m^2 (Brancati *et al.*, 1999). Obesity, along with glucose intolerance, insulin resistance, central obesity, dyslipidemia, and hypertension constitute a constellation of symptoms known as "metabolic syndrome" (Eckel *et al.*, 2005). These

associated conditions have been grouped together given their increased likelihood of affecting an individual simultaneously.

Genetic risk factors have also been identified in patients with T2DM. The genetic influence on T2DM has been demonstrated through several studies that show differential expression of the disease based on race or ethnic background and family history (Carter *et al.*, 1996; Harris *et al.*, 1998; Klein *et al.*, 1996). Moreover, many genes have been associated with T2DM, and those with a larger effect size include: *PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX-IDE, CDKAL1, CDNK2A/B, IGF2BP2, FTO, THADA, NOTCH2, HFN1A, CAPN10* (Florez, 2008). Nonetheless, it is clear that the disease develops through a combination of genetic and environmental factors.

Lifestyle also plays an important role. Reis *et al*. demonstrated that dietary intake, physical activity, smoking, and alcohol consumption impact the onset of diabetes; and that controlling these factors can be linked to a "substantial" reduction in the risk for diabetes. These results were not impacted by adiposity or family history, further reinforcing the independent impact of lifestyle (Reis *et al.*, 2011).

1.1.2.4 COMORBIDITIES

As discussed above, the development of T2DM has been strongly associated with the incidence of obesity and several other comorbidities. Chronic hyperglycemia irreversibly damages small blood vessels and capillary beds. In turn, this causes injury to several nerves and organs and can ultimately lead to multiple complications of the disease such as hypertension, dyslipidemia, chronic kidney disease, retinopathy, peripheral neuropathy, erectile dysfunction, and delayed wound healing leading to potential ulcerations (mostly of the feet) needing amputation (Canadian Diabetes Association Clinical Practice Guideline

Expert *et al.*, 2013; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013a; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013b; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013c; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013e; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013f).

The delayed diagnosis of T2DM is unfortunately quite common. Although Harris and colleagues suggested it in 1992, it is still true today that the clinical diagnosis of diabetes is often delayed from its original onset by up to 12 years. During this pre-diagnosis period, treatment is not being offered, which can lead to clinical complications (Harris *et al.*, 1992).

1.1.2.5 MANAGEMENT

The fundamental principles of T2DM treatment aim to achieve optimum glycemic control. In order to optimally manage patients, healthcare providers generally begin with lifestyle recommendations and supplement with drugs according to the treatment response, progressing from single to combination therapy. Insulin supplement therapy remains a key component of treatment, particularly as pancreatic failure develops. Another important principle in management includes referral to different healthcare specialists in order to manage different comorbidities as they arise.

Lifestyle Therapy

Nutritional and exercise therapy often form the base upon which T2DM treatment is built. A regimented diet program with reduced energy intake of low glycemic index carbohydrates has been shown to improve glycemic control and cardiovascular risk factors for individuals with T2DM who are overweight and obese (Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013d). Exercise therapy has also been shown to

improve glycemic control and reduce the need for pharmacological therapies such as antihyperglycemic drugs and insulin (Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013g).

Antihyperglycemic Drugs

Oral antihyperglycemic drugs are commonly used in conjunction with lifestyle therapies. However, many antihyperglycemic agents in clinical practice tend to cause weight gain. While some of the newer drug alternatives available in Canada are weight neutral, others help cause significant weight loss.

Weight gain inducing agents include insulin, sulfonylureas, and meglitinides. While insulin helps achieve the most significant reductions in HbA1C levels, they also cause the largest weight gain (Harper *et al.*, 2013). Due to the progressive nature of T2DM, most patients eventually end up needing insulin therapy in order to adequately control their condition.

Weight neutral medications include metformin, a frequently used first-line agent, acarbose and DPP-4 inhibitors (Harper *et al.*, 2013).

Weight loss inducing drugs include GLP-1 receptor agonists, which are a new class of medications used in the treatment of T2DM. They are the first to offer substantial weight loss benefits (Harper *et al.*, 2013). These medications can work very well alone or in combination with others in order to minimize weight gain associated with the use of other drugs.

Lipase Inhibitors

Unlike all the above options, lipase inhibitors, like Orlistat, are used to induce weight loss in diabetic patients without typically offering the significant benefit of additional

glycemic control (Harper *et al.*, 2013). It is expected that the role of weight neutral and weight loss inducing agents continue to grow in the treatment of T2DM during the years to come.

1.1.3 BARIATRIC SURGERY

Bariatric surgery is a medical operation whereby the gastrointestinal system is modified to reduce food intake or hinder absorption of consumed foods. Its primary purpose is for weight loss and for secondary treatment of T2DM. It is one of the most effective interventions available to induce weight loss, and has been shown to reduce mortality rates among obese subjects (Sjostrom, 2013; Sjostrom *et al.*, 2007; Sjostrom *et al.*, 2012). Research samples for the study described in Chapter 3 were obtained during routine bariatric surgery.

There are many different types of bariatric surgeries, which can be classified by their mechanisms of weight loss induction. Multiple biological changes result in weight loss and a single surgery can act through multiple mechanisms. Mechanisms include volume restriction of nutrient intake, malabsorption of ingested nutrients, hormonal changes associated with hunger and satiety, as well as whole-body metabolic changes. All of these, in combination with sustained behavioral modification, lead to reductions in weight over time. In addition to weight loss, the most common bariatric surgeries also have been associated with improvement or resolution of T2DM, independently of weight loss (Dixon *et al.*, 2012). In fact, studies have even shown that bariatric surgery, specifically the Roux-en-Y gastric bypass (RYGB) and duodenal switch procedures, produce better outcomes than conventional medical therapy (Mingrone *et al.*, 2012), or intensive medical treatment alone (Schauer *et al.*,

2014; Schauer *et al.*, 2012). As detailed below, this area is still debated given the life-changing repercussions of these surgeries.

In 2013, there were about half a million bariatric surgeries performed worldwide; the majority being completed in North America. The most commonly performed surgery was the Roux-en-Y gastric bypass (Angrisani *et al.*, 2015)

In Canada, eligibility criteria for bariatric surgery include a BMI > 40 kg/m^2 or a BMI > 35 kg/m^2 if severe comorbidities are present (NIH Consensus, 1996). Patients eligible for bariatric surgery should also undergo rigorous assessment by a multidisciplinary team which includes internists, surgeons, psychiatrists, and nutritional experts (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2013).

The most common procedures include (in increasing order of invasiveness and complexity): 1) Laparoscopic adjustable gastric band; 2) Sleeve gastrectomy; 3) Roux-en-Y gastric bypass; and 4) Biliopancreatic diversion with duodenal switch.

Roux-en-Y gastric bypass surgery is the most popular bariatric surgery performed with around 200,000 cases reported worldwide in 2013 (Angrisani *et al.*, 2015). It is considered to be restrictive as well as malabsorptive. It consists of the creation of a small stomach pouch that is attached directly to a distal part of the small intestine. The remaining stomach is left in place and the proximal part of the intestine is anastomosed to the distal intestine creating a Y shape with two limbs converging into one in the distal part of the intestine. One limb is called the alimentary limb (or Roux limb) and contains the bolus. The other limb is called the biliopancreatic limb and it contains the pancreatic and digestive enzymes (Elder and Wolfe, 2007).

Emerging alternatives to contemporary surgeries include: intragastric balloons (Mathus-Vliegen and Tytgat, 2005), vagal blockage (Kral *et al.*, 2009), mini-gastric bypass

(Wang *et al.*, 2005), endoluminal vertical gastroplasty (Majumder and Birk, 2013), and endoscopic gastrointestinal bypass devices (Sandler *et al.*, 2011).

1.2 THE ORGAN: SKELETAL MUSCLE

Skeletal muscle is one of the three main types of muscle, along with cardiac and smooth muscle. It represents one of the major organ groups of the human body and its dysfunction is involved in many different diseases. Skeletal muscle is composed of individual muscle fibrils that are grouped together into muscle fibers. Muscle fibers are the main contractile unit of muscles and are further grouped together to form fascicles. Fascicles are organized into whole muscles that are contained within a fascia and attached to the bones by fibrous connective tissue called tendons. The contractions of skeletal muscles cause the bones to move, allowing us to control our movements through space (Jones and Round, 1990).

Muscle fibers consist of multinucleated cells, whose formation results from the fusion and polarized organization of muscle satellite cells during cellular differentiation. Muscle fibers are rich in mitochondria, which produce the ATP necessary for muscle contraction and other processes. Mitochondria form a reticular network in muscle but some of them are also present as independent structures (Kirkwood *et al.*, 1986). Mitochondrial content in skeletal muscle can be highly variable and can be modified over time, for example as a result of exercise or sedentariness. In addition, mitochondria in skeletal muscle can be further divided in two types based on their location. Subsarcolemmal mitochondria are found just beneath the surface of the sarcolemma and produce ATP mainly for the purposes of cellular and organelle-related functions such as protein synthesis, ion exchange, substrate transport, cell signaling. On the other hand, intermyofibrillar mitochondria are located between the

myofibrils near the Z lines and produce ATP mainly for muscle contraction (Cogswell *et al.*, 1993). Ultimately, muscle fibers adapt to meet the physical and metabolic needs of the individual (Coyle, 2000; Fry, 2004). Muscle cells are also known to secrete cytokines termed myokines which can have endocrine, paracrine and autocrine effects affecting the body systematically (Pedersen and Febbraio, 2012).

Muscle fibers are generally divided into subtypes based on the molecular characteristics of markers present on the surface and within the myofibers. Traditionally, muscle fibers can be classified into type I or type II, based on the nature of the myosin heavy chain. Type I fibers are generally considered slow twitch, to be more aerobic, and to be more fatigue resistant due to their elevated mitochondrial content. These fibers also have higher myoglobin content. Type II fibers on the other hand, exhibit opposite properties, being fast twitch, with decreased mitochondrial and myoglobin contents. In humans, type II fibers are further subdivided into type IIa – more oxidative – and type IIx – more glycolytic (Pette and Staron, 2000). There is considerable variability in the proportion of different muscle fiber types between different individuals (Lexell *et al.*, 1988; Saltin *et al.*, 1977). Muscle fiber types are determined by motor neuron properties and have been shown to exhibit plasticity over time (Burke, 2011; Enad *et al.*, 1989; Greising *et al.*, 2012; Mantilla and Sieck, 2003).

Skeletal muscle contractions are initiated by somatic neuronal activation when action potentials reach the neuromuscular junction, which is also termed “motor unit”. A single somatic neuron can activate between 3 and 1000 muscle fibers. At these motor units, the neuronal axon releases acetylcholine that binds to receptors on the sarcolemma portion of the motor end plate. This stimulates release of sodium ions that potentiate the T-tubules. This, in turn, stimulates release of calcium ions from the sarcoplasmic reticulum (SR), which acts to reduce the total length of the sarcomeres. Specifically, calcium ions bind to troponin on the

thin filaments, causing them to shift and expose myosin-binding sites. Myosin from the thick filaments subsequently binds to actin at which point myosin pivots pulling the actin filaments. The next step in muscle contraction is the release of myosin from actin; which now requires adenosine triphosphate (ATP). Specifically, it is the breakdown of ATP into adenosine diphosphate (ADP) and inorganic phosphate that causes the release of the myosin from the actin filament. Upon release, the myosin returns to its initial position, ready to bind to the adjacent portion of the actin filament. This cycle continues consecutively as long as calcium ions are still present and the quantity of ATP is sufficient to cause the release of myosin heads from the actin filament. This process happens simultaneously across the multiple myofibers of a muscle, which causes gross contraction (Burke, 2011).

1.2.1 SKELETAL MUSCLE AND OBESITY

Obesity is known as a disease with excess accumulation of adipose tissue. One of adipose tissue's main functions is storing excess energy that the body does not use. Skeletal muscle, on the other hand, is one of the major users of that energy and therefore plays a pivotal role in the development and progression of obesity. In a healthy lean individual, it is the largest organ representing approximately 42% of total body mass and accounting for 20% of whole body resting metabolic rate (Rolfe and Brown, 1997). During exercise, metabolic rate can increase by up to 20 times in trained athletes (Rolfe and Brown, 1997), with a disproportionately increasing amount being used by skeletal muscle. In fact, energy expenditure in muscle can account for up to 87% of total energy expenditure during maximal exercise (McGilvery, 1970).

In obesity, many changes happen to skeletal muscle in order for it to adapt to the increased load of weight that it needs to bear over a prolonged period of time. Most evident

of these is increases in muscle size and strength which results in greater absolute maximal muscle strength (Lafortuna *et al.*, 2014). Nevertheless, when comparing muscle strength between lean and obese individuals (in terms of strength per body mass), obese individuals seem to have less strength per unit body mass (Blimkie *et al.*, 1990; Hilton *et al.*, 2008; Hulens *et al.*, 2001; Lafortuna *et al.*, 2014; Maffiuletti *et al.*, 2007). We suggest that this decrease in relative strength may be due, in part, to the functional limitations associated with obesity. These could include reductions in mobility, strength, postural integrity, and dynamic balance.

1.2.2 SKELETAL MUSCLE AND DIET RESISTANCE

As previously mentioned, diet resistance is a phenomenon observed when patients undergoing a hypocaloric diet are relatively unresponsive with little or no documented weight loss. Several factors impact a patient's ability to lose weight, but given the important role played by skeletal muscle, it has been shown to be involved in the etiology of diet resistance (Gerrits *et al.*, 2010; Harper *et al.*, 2002).

Previous studies emerging from our group and others have identified important characteristics of skeletal muscle that affect ROWL and diet resistance. Specifically, it was found that ODS individuals have a greater proportion of oxidative (type I) muscle fibers as well as muscle fiber hypertrophy. On the other hand, ODR individuals were found to have more type IIa fibers. This suggests that intrinsic energetic efficiency may be lower in ODS individuals, which contributes to their increased propensity for weight loss (*i.e.,* the energy is released as heat, rather than being stored. In addition, an upregulation of gene sets involved in oxidative phosphorylation was also identified in the ODS compared to the ODR individuals (Gerrits *et al.*, 2010). This was an interesting finding given that both obesity and

T2DM are generally associated with increased proportion of type IIx fibers (Mogensen *et al.*, 2007) and that fiber type proportions were found to be important in determining metabolic responses to overfeeding (Sun *et al.*, 2002). Taken together, these data suggest a strong role for skeletal muscle fiber type composition in ROWL and diet resistance (Gerrits *et al.*, 2010).

1.2.3 SKELETAL MUSCLE AND T2DM

Early signs of T2DM include insulin resistance that eventually leads to pancreatic insulin hyper-secretion followed by pancreatic failure. One of the main sites of insulin resistance is skeletal muscle. In fact, skeletal muscle accounts for 80-90% of postprandial insulin-stimulated glucose uptake and is an important player in the development of insulin resistance (Petersen and Shulman, 2006). Indeed, insulin resistance is also typically more severe in this tissue compared to others (DeFronzo, 1988), placing it in a central role in the development and progression of T2DM.

Many studies have characterized insulin resistance and energy imbalances in skeletal muscle. It had been demonstrated that insulin resistance in muscle and adipose tissues is linked to decreased insulin-stimulated glucose uptake, mostly mediated by glucose transporter 4 (Glut 4) (Rothman *et al.*, 1992), decreases in insulin-stimulated glycogen synthesis (Ducluzeau *et al.*, 2001; Eriksson *et al.*, 1989; Felber *et al.*, 1987; Vaag *et al.*, 1992), disruption of insulin signal transduction (Pratipanawatr *et al.*, 2001), and reductions in TCA cycle enzymes (Bass *et al.*, 1975; Lithell *et al.*, 1981; Vondra *et al.*, 1977). This could be caused by the accumulation of intramyocellular lipids (Jacob *et al.*, 1999; Koves *et al.*, 2008; Savage *et al.*, 2007), which has been described in muscles of different fiber types (Malenfant *et al.*, 2001). The quantity of intramyocellular lipids has also been correlated with

the severity of insulin resistance, even when corrected for the degree of obesity (Jacob *et al.*, 1999).

In addition, intramyocellular lipids inhibit insulin-stimulated insulin-receptor substrate (IRS)–1 tyrosine phosphorylation and leads to decreases in the activity of IRS-1-associated phosphatidyl inositol 3 kinase (Dresner *et al.*, 1999). Ultimately, muscle cells exhibit decreased glucose metabolism and increased fatty-acid metabolism under insulin-stimulated conditions, which is inappropriate during post-prandial periods where the amount of glucose in the blood is elevated (Phielix and Mensink, 2008). This has been termed "metabolic inflexibility" (Kelley and Mandarino, 2000), or the inability to optimally use different fuel sources, which, in skeletal muscle, has been shown to be linked with intramyocellular accumulation of lipids (Simoneau *et al.*, 1995). The underlying causes of these phenomena remain to be elucidated, but mitochondrial dysfunction, as discussed below, is thought to be a major player (Rieusset, 2015).

1.3 THE ORGANELLE: MITOCHONDRIA

Cells are composed of many different organelles, each having specific roles that contribute to cellular function. Mitochondria are known to have many roles but historically are mostly associated with energy transduction and ATP generation. They are double-membraned with a mitochondrial outer member (MOM) and a mitochondrial inner membrane (MIM). The MOM and the MIM have relatively high levels of the phospholipid, cardiolipin; they also have relatively low cholesterol content, which is unique to mitochondria and essential for adequate mitochondrial respiration (Hatch, 2004; Mileykovskaya *et al.*, 2005; Schlame *et al.*, 2000). Compared to the MOM, the MIM has a much larger surface area that forms invaginations called cristae. The formation and dynamics

of cristae structure are controlled by proteins such as optic atrophy 1 (OPA1) and have a significant effect of mitochondrial respiration (Patten *et al.*, 2014).

Mitochondria are dynamic organelles that are in a constant flux of fusion and fission. This allows the distribution of synthesis products and metabolites across the mitochondrial network, and has been shown to have an impact on other mitochondrial functions including calcium homeostasis, regulation of ROS and respiration (Chen and Chan, 2005; Parone et al., 2008; Soubannier and McBride, 2009). The mechanisms controlling mitochondrial fusion and fission are as yet poorly understood, however it is known that the mitofusins (Mfn1 and Mfn2) play key roles in MOM fusion whereas OPA1 is essential for MIM fusion (Shaw and Nunnari, 2002). These events usually happen in a coordinated manner. On the other hand, dynamin-related protein 1 (Drp1) is necessary for mitochondrial fission (van der Bliek *et al.*, 2013). Mfn2 is of particular interest given its involvement in obesity and T2DM as detailed below.

Mitochondrial spaces can be divided into two compartments. The intermembrane space, between the MOM and the MIM, is where protons accumulate as a result of the proton pumping activity of the ETC. The ETC, found in the MIM, is composed of four protein complexes (Complex I-IV), in addition to electron carriers, which shuttle electrons and pump protons from the matrix to the intermembrane space (Nicholls and Ferguson, 2003b). Electron movement through Complexes I, III and IV provides sufficient energy for these complexes to pump protons from the matrix to the intermembrane space, thereby forming a protonmotive force (PMF). The PMF, which is usually between 140 and 200 mV, drives ATP synthesis as protons return to the matrix through Complex V, also known as ATP synthase. ATP production results in the formation of a high-energy bond between adenosine diphosphate molecules (ADP) and inorganic phosphate (Nicholls and Ferguson, 2003c).

The compartment inside of the MIM is known as the mitochondrial matrix. It contains many metabolic enzymes as well as mitochondrial DNA (mtDNA) and the machinery necessary for protein transcription and translation. Mitochondrial DNA is circular and contains 37 genes, of which 13 are subunits of the ETC. Proteins involved in mitochondrial function are known to be encoded by both nuclear, and mitochondrial genes (Mootha *et al.*, 2003a; Pagliarini *et al.*, 2008).

As mentioned above, mitochondria are involved in a variety of cellular functions. Although they are most widely known for their role in oxidative phosphorylation and ATP generation, mitochondria have other essential functions in metabolism and cell signaling, which include breakdown of amino acids, ketogenesis, urea cycle, control of cytoplasmic calcium, production of Fe/S clusters, and steroidogenesis (Patti and Corvera, 2010).

The oxidative phosphorylation system is powered by the reducing equivalents that are generated through different oxidative pathways in cells, including glycolysis and the tricarboxylic acid (TCA) cycle (majority). The reducing equivalents/electrons converge at the ETC where oxidation/reductions reactions shuttle electrons to the final accepter, molecular oxygen (Bayir and Kagan, 2008; Scialo *et al.*, 2013).

Various factors can affect the efficiency of oxidative phosphorylation. Protons can re-enter the matrix from the intermembrane space without going through Complex V and generating ATP. This process, called proton leak, can occur in multiple different ways and decreases the efficiency of oxidative phosphorylation. In fact, proton leak is responsible for 30% and 50% or resting respiration in hepatocytes and skeletal muscle respectively (Rolfe and Brown, 1997). One of the main sources of proton leak is the family of uncoupling proteins (Nicholls and Ferguson, 2003a). Five different members, numbered one to five, have been identified in mammals. Their expression is variable and depends on the tissue and

the organism in question. Very little as yet is known about the function and control of uncoupling protein (UCP) 4 and UCP5. In humans, UCP3 is present in skeletal muscle, heart and brown adipose tissue (BAT) and plays an important role in mitochondrial respiration (Costford *et al.*, 2006). It is the only uncoupling protein expressed at the protein level in skeletal muscle (Bezaire *et al.*, 2007).

As electrons are shuttled through the complexes of the ETC, reactive oxygen species (ROS) can be produced by the reduction of molecular oxygen to form O_2^-. This free radical can be dismutated into H_2O_2, which is further broken down to generate hydroxyl radicals OH^-. These are the main types of ROS generated through mitochondrial respiration. The main sites of ROS production in the ETC are Complex I and III (Murphy, 2009). ROS have been shown to cause oxidative damage and their negative effects can be prevented through the action of different antioxidants. Recent research demonstrates that ROS have signaling properties and levels of ROS are important in a variety of cellular processes including signaling in hypoxia, insulin release, insulin sensitivity, and adipocyte differentiation (Anderson *et al.*, 2009; Guzy and Schumacker, 2006; Houstis *et al.*, 2006; Li *et al.*, 2012; Tormos *et al.*, 2011). Uncoupling proteins are considered important regulators of ROS levels, acting to regulate ROS production and responding to slight increases in ROS (Mailloux *et al.*, 2012).

In addition, UCP2 and UCP3 are controlled through a post-translational modification called, glutathionylation. The reversible binding of glutathione to UCP2 and UCP3 has been shown to control these proteins and modulate levels of ROS, mitigating oxidative stress (Mailloux *et al.*, 2011). In other words, slight increases in levels of ROS cause the deglutathionylation and activation of UCP2 and UCP3, which in turn decreases membrane potential and ROS emissions, thereby acting in a negative feedback loop. Through the action

of the UCPs, this process additionally impacts mitochondrial respiration and various cell signaling processes (reviewed in Mailloux and Harper, 2011).

Even prior to discoveries showing that mitochondrial oxidative phosphorylation efficiency is variable, classical approaches for the study of mitochondrial bioenergetics defined multiple different respiratory states (Chance and Williams, 1955b). State 1 respiration is defined by the presence of mitochondria in a medium containing inorganic phosphate and minimal energy substrates. Mitochondrial respiration is low in this state. State 2 respiration is induced by the addition of substrate, but respiration is limited due to a lack of ADP. State 3 respiration is observed with the subsequent addition of ADP which induces electron flow through the ETC, coupled to the production of ATP. Mitochondrial respiration is maximal in this state. State 4 respiration is observed when all of the ADP is converted to ATP, causing a marked reduction in oxygen consumption; this is also referred to as non-phosphorylating respiration. Alternatively, when ATP synthase is chemically inhibited by oligomycin, a similar reduction in oxygen consumption is described and termed State 4o; this is also a type of non-phosphorylating respiration. Lastly, State 5 respiration is that which occurs with the depletion of all oxygen (anoxia) in the experimental chambers.

The idea that components of the mitochondrial ETC can be bound together to form supercomplexes was first proposed by Chance and Williams in 1955 (Chance and Williams, 1955a). However, the idea that mitochondrial supercomplexes play a key role in energy transduction and ATP synthesis has been highly controversial and only recently received increased attention with the discovery that mitochondrial supercomplexes involving Complexes I, III and IV exist in both yeast and mammalian cells (Acin-Perez *et al.*, 2008; Schagger and Pfeiffer, 2000). These supercomplexes have further been shown to impact mitochondrial electron flux and respiration (Lapuente-Brun *et al.*, 2013). In fact, the

assembly of supercomplexes enhances ETC activity through spatial restriction of electron carrier diffusion (Acin-Perez *et al.*, 2008). This is thought to increase the efficiency of electron shuttling through the ETC and results in increased rates of oxygen consumption. Despite these important findings, the precise role and functioning of these supercomplexes is still poorly understood and much work is yet to be done in this growing field.

Many factors have been shown to affect the formation and stability of supercomplexes, and many more are presently being discovered as interest in this phenomenon is growing. Among others, cristae structure (Cogliati *et al.*, 2013), mitochondrial membrane integrity (cardiolipin content) (Bazan *et al.*, 2013; McKenzie *et al.*, 2006; Zhang *et al.*, 2002, 2005), as well as certain proteins including UQQCC3 (Desmurs *et al.*, 2015; Wanschers *et al.*, 2014) and supercomplex assembly factor 1 (SCAF1 – also known as Cox7a2l and Cox7RP) (Ikeda *et al.*, 2013) have been shown to impact supercomplex assembly and stability. HIG2A and HIG2B, the mammalian orthologues of yeast rcf1 and rcf2, are additionally thought to be supercomplex assembly factors (Chen *et al.*, 2012; Strogolova *et al.*, 2012; Vukotic *et al.*, 2012) although this is debated (Enriquez, 2016; Hayashi *et al.*, 2012).

1.3.1 MITOCHONDRIA IN OBESITY

As mentioned above, obesity is the result of imbalances between dietary energy intake, and energy expenditure. As mitochondria are the organelles responsible for energy transduction into ATP, they are thought to be important in the development and progression of obesity.

One of the first studies linking mitochondrial dysfunction with obesity was published by Galton and Bray in 1967 and identified a deficiency in the metabolism of α-glycerol

phosphate, which was known to be carried out by mitochondria. This study was conducted in adipose tissue (Galton and Bray, 1967). In skeletal muscle, some of the early studies linking dysfunctional mitochondria with obesity and T2DM came from Simoneau and Kelley (Colberg *et al.*, 1995; Simoneau and Bouchard, 1995; Simoneau *et al.*, 1995) who identified reductions in several key mitochondrial enzymes in the fasting state. These included cytochrome C oxidase (COX), carnitine palmitoyltransferase 1 (CPT1), malate dehydrogenase, and citrate synthase (Simoneau and Bouchard, 1995; Simoneau *et al.*, 1995). They also identified associated increases in other mitochondrial enzymes involved in glycolysis (Simoneau and Kelley, 1997).

Around the same time, other studies revealed that reductions in skeletal muscle oxidative metabolism enzymes were a risk factor (Sun *et al.*, 2002; Zurlo *et al.*, 1994) as well as a causative factor (Kim *et al.*, 2000; Simoneau *et al.*, 1999) for the development of obesity. Kelley *et al.* also reported decreases in general ETC activity as well as reduced mitochondrial size in intermyofibrillar mitochondria in skeletal muscle of subjects with obesity and T2DM (Kelley *et al.*, 2002). This particular study did not assess differences in mitochondrial distribution but the authors further pursued this question and reported a decrease in subsarcolemmal as well as intermyofibrillar ETC activity in subjects with obesity. In the same study, they also found a decrease in mitochondrial content in skeletal muscle of affected subjects and reported that the decreases in mitochondrial ETC activity were present even when activity data was corrected to mitochondrial content. This implies that there is a reduced functional capacity in mitochondria of subjects with obesity and T2DM (Ritov *et al.*, 2005).

In parallel, many genes associated with skeletal muscle function have been identified to be associated with obesity (Loos, 2012). Of relevance to mitochondrial function,

reductions in the expression of OXPHOS genes have been linked with obesity, increases of intramyocellular lipids (Crunkhorn *et al.*, 2007), as well as high-fat feeding (Sparks *et al.*, 2005) and lipid infusion (Richardson *et al.*, 2005).

In fact, deficiencies in fatty acid metabolism have been reported in obesity and have also been thought to be a causative factor many years ago (Randle *et al.*, 1965). Among other deregulated phenomena identified with time, increased fatty acid uptake, lipid accumulation, reduced capacity for lipid oxidations, as well as oxidative stress have all been identified in skeletal muscle of individuals with obesity (Bonen *et al.*, 2004; Colberg *et al.*, 1995; Furukawa *et al.*, 2004; Keaney *et al.*, 2003; Kelley *et al.*, 1999; Kim *et al.*, 2000; Simoneau *et al.*, 1999). These conclusions are, however, still debated as other groups have found little differences in fatty acid metabolism linked with obesity (Holloway *et al.*, 2009; Holloway *et al.*, 2007; Hulver *et al.*, 2003). In some cases, discrepancies were explained by mitochondrial content, which is reduced in obesity, as mentioned above (Boushel *et al.*, 2007; Holloway *et al.*, 2007; Ritov *et al.*, 2005).

Aside from the phenomena mentioned above, incomplete fatty acid oxidation has been observed in obesity and leads to the release of acylcarnitines, which cause disturbances of mitochondrial function and insulin resistance (Bell *et al.*, 2010; Koves *et al.*, 2008; Thyfault *et al.*, 2007). Our research group has further demonstrated that the accumulation of acylcarnitine species and associated oxidative stress are implicated in the development of obesity and T2DM (Aguer *et al.*, 2015).

Finally, irregularities in mitochondrial morphology and dynamics have been linked with obesity in skeletal muscle. These are thought to be important as they have direct impact on mitochondrial function. Specifically, reductions in mitochondrial length and disrupted mitochondrial fission were found in muscle of obese mice and cultured C2C12 cells treated

with palmitate (Jheng *et al*., 2012). In addition, decreases in the expression of Mfn2, which was shown to result in a fragmented mitochondrial network and decreases in mitochondrial metabolism, have been observed in skeletal muscle and implicated in obesity and T2DM as discussed below (Bach *et al*., 2003).

1.3.2 MITOCHONDRIA IN DIET RESISTANCE

Just as in obesity, several different aspects of mitochondrial function have been associated with diet resistance. As mitochondrial proton leak, specifically through UCPs, has been shown to have a significant effect on metabolism, it is thought to play a critical role in determining ROWL and diet resistance.

Initial studies from our research group revealed that ODS individuals, as opposed to ODR individuals, have 51% increased mitochondrial proton leak-dependent (state 4) respiration, as well as 25% greater mRNA expression of UCP3 (Harper *et al.*, 2002). Follow up studies demonstrated that *rectus femoris* and *vastis lateralis* skeletal muscles from ODS individuals also exhibited greater expression of genes and gene sets implicated in oxidative phosphorylation and glucose and fatty acid metabolism (Gerrits *et al.*, 2010). Additional studies in primary cell cultures established from skeletal muscle of these patients identified increased mitochondrial leak, as well as a less oxidized glutathione redox state in myotubes of ODS patients (Thrush *et al.*, 2014). Taken together, these results suggest that mitochondrial function plays a key role in variability of ROWL and diet resistance (Thrush *et al*., 2013)

1.3.3 MITOCHONDRIA IN T2DM

Mitochondrial dysfunction has been associated with insulin resistance and development of T2DM. These mitochondrial dysfunctions are thought to lead to

intramyocellular lipid accumulation that, as described above, can lead to insulin resistance in skeletal muscle. Although some studies have found no differences in skeletal muscle mitochondrial function in T2DM (Boushel *et al.*, 2007; Han *et al.*, 2011), or causal relationships between mitochondrial dysfunction and T2DM (Han *et al.*, 2011; Pospisilik *et al.*, 2007; Wredenberg *et al.*, 2006; Zechner *et al.*, 2010), many studies to date have associated this disease state with reductions in the enzyme activity of various mitochondrial ETC components and reductions in mitochondrial function (Kelley *et al.*, 2002; Mogensen *et al.*, 2007; Ritov *et al.*, 2010; Ritov *et al.*, 2005). DeLany *et al*. have further demonstrated that reductions in mitochondrial respiration are present before the appearance of T2DM in African-American women, who are more than twice as likely to develop T2DM than Caucasian women (DeLany *et al.*, 2014). Although there is still debate about reductions in mitochondrial activity in skeletal muscle in patients with T2DM, no studies to date have delineated the underlying mechanisms.

Dysfunctional fatty acid metabolism has been proposed as a mechanism through which mitochondrial dysfunction leads to insulin resistance and T2DM. Specifically, accumulations of different species, such as ceramides, diacylglycerols, fatty acyl coenzyme A (CoA), and products of incomplete oxidation have all been linked to the development of insulin resistance (Chibalin *et al.*, 2008; Holland *et al.*, 2007; Koves *et al.*, 2008; Savage *et al.*, 2007)

Additionally, many studies have characterized altered mitochondrial morphology, which has been shown to be directly linked to mitochondrial function (Kelley *et al.*, 2002). Impaired mitochondrial dynamics have also been liked to T2DM. Specifically, reduced expression levels of Mfn2, have been found in skeletal muscle cells of individuals with T2DM (Bach *et al.*, 2005). It has been additionally demonstrated that levels of Mfn2 increase

after bariatric surgery, which is known to ameliorate insulin sensitivity (Bach *et al.*, 2005). Other studies have revealed downregulation of genes involved in oxidative phosphorylation (PGC1α-responsive genes) (Mootha *et al.*, 2003b; Patti *et al.*, 2003).

Calcium-related abnormalities have also been linked to reduced insulin sensitivity and signaling. Since calcium homeostasis is required for glucose uptake and fuel oxidation processes following exercise, disruptions of calcium signaling have been shown to decrease glucose uptake and cause insulin resistance (Lanner *et al.*, 2008; Lebeche *et al.*, 2008; Park *et al.*, 2009)

Mitochondrial ROS have also been implicated in the etiology of T2DM (Houstis *et al.*, 2006). Among other players, xanthine oxidase (XO), a key source of ROS production, has been shown to contribute to oxidative stress in skeletal muscle of T2DM individuals, although its inhibition has not been shown to ameliorate the T2DM phenotype in mice (Bravard *et al.*, 2011). On the other hand, the mitochondrial-targeted expression of key enzymes involved in neutralizing ROS, such as catalase and manganese superoxide dismutase (MnSOD), has been shown to reduce oxidative damage in skeletal muscle cells and even ameliorate insulin sensitivity (Anderson *et al.*, 2009; Lee *et al.*, 2010). This suggests that targeting general mitochondrial ROS could yield better outcomes for improving insulin resistance and glucose homeostasis (Rieusset, 2015).

1.4 PROJECT OBJECTIVES AND HYPOTHESES

The overall goal of my doctoral research is to elucidate metabolic predictors and underlying mechanisms in obesity and T2DM. To do this, mechanisms contributing to disordered oxidative metabolism in skeletal muscle were examined. The studies detailed below were conducted with the overarching hypothesis that disordered skeletal muscle

function contributes to diet resistance and development of obesity and T2DM. Specific goals and hypotheses are as follows:

Objective 1: To study the effects of T2DM on weight loss in a population of morbidly obese patients.

Hypothesis: Individuals with T2DM have an impaired propensity for weight loss when participating in a clinically supervised meal-replacement and lifestyle intervention program.

Objective 2: To compare characteristics of skeletal muscle mitochondrial respiration from individuals with obesity and individuals with obesity and T2DM.

Hypothesis: Skeletal muscle from individuals with T2DM have impaired mitochondrial respiration and this is associated with reductions in ETC supercomplex assembly.

Objective 3: To delineate differential skeletal muscle and whole body metabolic responses in diet-sensitive and diet-resistant individuals following a HFM challenge.

Hypothesis: Skeletal muscle from diet-resistant individuals have impaired mitochondrial respiration. Differences in plasma proteomics will further delineate markers of diet resistance.

1.5 REFERENCES

Acin-Perez, R., Fernandez-Silva, P., Peleato, M.L., Perez-Martos, A., and Enriquez, J.A. (2008). Respiratory active mitochondrial supercomplexes. Molecular cell *32*, 529-539.

Adams, K.F., Schatzkin, A., Harris, T.B., Kipnis, V., Mouw, T., Ballard-Barbash, R., Hollenbeck, A., and Leitzmann, M.F. (2006). Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. N Engl J Med *355*, 763-778.

Aguer, C., McCoin, C.S., Knotts, T.A., Thrush, A.B., Ono-Moore, K., McPherson, R., Dent, R., Hwang, D.H., Adams, S.H., and Harper, M.E. (2015). Acylcarnitines: potential implications for skeletal muscle insulin resistance. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *29*, 336-345.

Anderson, E.J., Lustig, M.E., Boyle, K.E., Woodlief, T.L., Kane, D.A., Lin, C.T., Price, J.W., 3rd, Kang, L., Rabinovitch, P.S., Szeto, H.H*., et al.* (2009). Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest *119*, 573-581.

Angelakis, E., Armougom, F., Million, M., and Raoult, D. (2012). The relationship between gut microbiota and weight gain in humans. Future microbiology *7*, 91-109.

Angrisani, L., Santonicola, A., Iovino, P., Formisano, G., Buchwald, H., and Scopinaro, N. (2015). Bariatric Surgery Worldwide 2013. Obesity surgery *25*, 1822-1832.

Atkinson, R.L., Dhurandhar, N.V., Allison, D.B., Bowen, R.L., Israel, B.A., Albu, J.B., and Augustus, A.S. (2005). Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. International journal of obesity *29*, 281-286.

Atkinson, R.L., Lee, I., Shin, H.J., and He, J. (2010). Human adenovirus-36 antibody status is associated with obesity in children. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity *5*, 157-160.

Bach, D., Naon, D., Pich, S., Soriano, F.X., Vega, N., Rieusset, J., Laville, M., Guillet, C., Boirie, Y., Wallberg-Henriksson, H*., et al.* (2005). Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. Diabetes *54*, 2685-2693.

Bach, D., Pich, S., Soriano, F.X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J.R., Lloberas, J., Camps, M., Zierath, J.R*., et al.* (2003). Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. The Journal of biological chemistry *278*, 17190-17197.

Bass, A., Vondra, K., Rath, R., Vitek, V., and Havranek, T. (1975). Metabolic changes in the quadriceps femoris muscle of obese people. Enzyme activity patterns of energy-supplying metabolism. Pflugers Arch *359*, 325-334.

Bayir, H., and Kagan, V.E. (2008). Bench-to-bedside review: Mitochondrial injury, oxidative stress and apoptosis--there is nothing more practical than a good theory. Crit Care *12*, 206.

Bazan, S., Mileykovskaya, E., Mallampalli, V.K., Heacock, P., Sparagna, G.C., and Dowhan, W. (2013). Cardiolipin-dependent reconstitution of respiratory supercomplexes from purified Saccharomyces cerevisiae complexes III and IV. The Journal of biological chemistry *288*, 401-411.

Beauchamp, B., Ghosh, S., Dysart, M.W., Kanaan, G.N., Chu, A., Blais, A., Rajamanickam, K., Tsai, E.C., Patti, M.E., and Harper, M.E. (2014). Low birth weight is associated with adiposity, impaired skeletal muscle energetics and weight loss resistance in mice. International journal of obesity.

Bell, C.G., Walley, A.J., and Froguel, P. (2005). The genetics of human obesity. Nature reviews Genetics *6*, 221-234.

Bell, J.A., Reed, M.A., Consitt, L.A., Martin, O.J., Haynie, K.R., Hulver, M.W., Muoio, D.M., and Dohm, G.L. (2010). Lipid partitioning, incomplete fatty acid oxidation, and insulin signal transduction in primary human muscle cells: effects of severe obesity, fatty acid incubation, and fatty acid translocase/CD36 overexpression. J Clin Endocrinol Metab *95*, 3400-3410.

Berrington de Gonzalez, A., Hartge, P., Cerhan, J.R., Flint, A.J., Hannan, L., MacInnis, R.J., Moore, S.C., Tobias, G.S., Anton-Culver, H., Freeman, L.B.*, et al.* (2010). Body-mass index and mortality among 1.46 million white adults. N Engl J Med *363*, 2211-2219.

Bezaire, V., Seifert, E.L., and Harper, M.E. (2007). Uncoupling protein-3: clues in an ongoing mitochondrial mystery. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *21*, 312-324.

Blimkie, C.J., Sale, D.G., and Bar-Or, O. (1990). Voluntary strength, evoked twitch contractile properties and motor unit activation of knee extensors in obese and non-obese adolescent males. Eur J Appl Physiol Occup Physiol *61*, 313-318.

Bonen, A., Parolin, M.L., Steinberg, G.R., Calles-Escandon, J., Tandon, N.N., Glatz, J.F., Luiken, J.J., Heigenhauser, G.J., and Dyck, D.J. (2004). Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *18*, 1144-1146.

Bouchard, C., and Tremblay, A. (1997). Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. The Journal of nutrition *127*, 943S-947S.

Bouchard, C., Tremblay, A., Despres, J.P., Nadeau, A., Lupien, P.J., Theriault, G., Dussault, J., Moorjani, S., Pinault, S., and Fournier, G. (1990). The response to long-term overfeeding in identical twins. N Engl J Med *322*, 1477-1482.

Bougneres, P., Pantalone, L., Linglart, A., Rothenbuhler, A., and Le Stunff, C. (2008). Endocrine manifestations of the rapid-onset obesity with hypoventilation, hypothalamic, autonomic dysregulation, and neural tumor syndrome in childhood. J Clin Endocrinol Metab *93*, 3971-3980.

Boushel, R., Gnaiger, E., Schjerling, P., Skovbro, M., Kraunsoe, R., and Dela, F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia *50*, 790-796.

Brancati, F.L., Wang, N.Y., Mead, L.A., Liang, K.Y., and Klag, M.J. (1999). Body weight patterns from 20 to 49 years of age and subsequent risk for diabetes mellitus: the Johns Hopkins Precursors Study. Arch Intern Med *159*, 957-963.

Bravard, A., Bonnard, C., Durand, A., Chauvin, M.A., Favier, R., Vidal, H., and Rieusset, J. (2011). Inhibition of xanthine oxidase reduces hyperglycemia-induced oxidative stress and improves mitochondrial alterations in skeletal muscle of diabetic mice. Am J Physiol Endocrinol Metab *300*, E581-591.

Bray, G.A. (2007). The Battle of the Bulge: A History of Obesity Research (Dorrance Publishing Company).

Bray, G.A., Fruhbeck, G., Ryan, D.H., and Wilding, J.P. (2016). Management of obesity. Lancet.

Burcelin, R. (2012). Regulation of metabolism: a cross talk between gut microbiota and its human host. Physiology (Bethesda) *27*, 300-307.

Burke, R.E. (2011). Motor Units: Anatomy, Physiology, and Functional Organization. In Comprehensive Physiology (John Wiley & Sons, Inc.).

Canadian Diabetes Association (2015). Diabetes in Canada.

Canadian Diabetes Association Clinical Practice Guideline Expert, C., Boyd, S.R., Advani, A., Altomare, F., and Stockl, F. (2013). Retinopathy. Can J Diabetes *37 Suppl 1*, S137-141.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Bowering, K., and Embil, J.M. (2013a). Foot care. Can J Diabetes *37 Suppl 1*, S145-149.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Bril, V., Perkins, B., and Toth, C. (2013b). Neuropathy. Can J Diabetes *37 Suppl 1*, S142-144.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Brock, G., and Harper, W. (2013c). Erectile dysfunction. Can J Diabetes *37 Suppl 1*, S150-152.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Dworatzek, P.D., Arcudi, K., Gougeon, R., Husein, N., Sievenpiper, J.L., and Williams, S.L. (2013d). Nutrition therapy. Can J Diabetes *37 Suppl 1*, S45-55.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Goldenberg, R., and Punthakee, Z. (2013e). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Can J Diabetes *37 Suppl 1*, S8-11.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., McFarlane, P., Gilbert, R.E., MacCallum, L., and Senior, P. (2013f). Chronic kidney disease in diabetes. Can J Diabetes *37 Suppl 1*, S129-136.

Canadian Diabetes Association Clinical Practice Guidelines Expert, C., Sigal, R.J., Armstrong, M.J., Colby, P., Kenny, G.P., Plotnikoff, R.C., Reichert, S.M., and Riddell, M.C. (2013g). Physical activity and diabetes. Can J Diabetes *37 Suppl 1*, S40-44.

Canadian Diabetes Association Clinical Practice Guidelines Expert Committee (2013). Canadian Diabetes Association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes *37 Suppl 1*, S1-3.

Carter, J.S., Pugh, J.A., and Monterrosa, A. (1996). Non-insulin-dependent diabetes mellitus in minorities in the United States. Annals of internal medicine *125*, 221-232.

Carwile, J.L., and Michels, K.B. (2011). Urinary bisphenol A and obesity: NHANES 2003-2006. Environmental research *111*, 825-830.

Chance, B., and Williams, G.R. (1955a). A method for the localization of sites for oxidative phosphorylation. Nature *176*, 250-254.

Chance, B., and Williams, G.R. (1955b). Respiratory enzymes in oxidative phosphorylation. III. The steady state. The Journal of biological chemistry *217*, 409-427.

Chen, H., and Chan, D.C. (2005). Emerging functions of mammalian mitochondrial fusion and fission. Hum Mol Genet *14 Spec No. 2*, R283-289.

Chen, Y.C., Taylor, E.B., Dephoure, N., Heo, J.M., Tonhato, A., Papandreou, I., Nath, N., Denko, N.C., Gygi, S.P., and Rutter, J. (2012). Identification of a protein mediating respiratory supercomplex stability. Cell Metab *15*, 348-360.

Chibalin, A.V., Leng, Y., Vieira, E., Krook, A., Bjornholm, M., Long, Y.C., Kotova, O., Zhong, Z., Sakane, F., Steiler, T.*, et al.* (2008). Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. Cell *132*, 375-386.

Chiolero, A., Faeh, D., Paccaud, F., and Cornuz, J. (2008). Consequences of smoking for body weight, body fat distribution, and insulin resistance. Am J Clin Nutr *87*, 801-809.

Cogliati, S., Frezza, C., Soriano, M.E., Varanita, T., Quintana-Cabrera, R., Corrado, M., Cipolat, S., Costa, V., Casarin, A., Gomes, L.C.*, et al.* (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell *155*, 160-171.

Cogswell, A.M., Stevens, R.J., and Hood, D.A. (1993). Properties of skeletal muscle mitochondria isolated from subsarcolemmal and intermyofibrillar regions. The American journal of physiology *264*, C383-389.

Colberg, S.R., Simoneau, J.A., Thaete, F.L., and Kelley, D.E. (1995). Skeletal muscle utilization of free fatty acids in women with visceral obesity. J Clin Invest *95*, 1846-1853.

Costford, S.R., Chaudhry, S.N., Salkhordeh, M., and Harper, M.E. (2006). Effects of the presence, absence, and overexpression of uncoupling protein-3 on adiposity and fuel metabolism in congenic mice. Am J Physiol Endocrinol Metab *290*, E1304-1312.

Coyle, E.F. (2000). Physical activity as a metabolic stressor. Am J Clin Nutr *72*, 512S-520S.

Crunkhorn, S., Dearie, F., Mantzoros, C., Gami, H., da Silva, W.S., Espinoza, D., Faucette, R., Barry, K., Bianco, A.C., and Patti, M.E. (2007). Peroxisome proliferator activator receptor gamma coactivator-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 mitogen-activated protein kinase activation. The Journal of biological chemistry *282*, 15439-15450.

Dansinger, M.L., Gleason, J.A., Griffith, J.L., Selker, H.P., and Schaefer, E.J. (2005). Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. Jama *293*, 43-53.

DeFronzo, R.A. (1988). Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes *37*, 667-687.

DeLany, J.P., Dube, J.J., Standley, R.A., Distefano, G., Goodpaster, B.H., Stefanovic-Racic, M., Coen, P.M., and Toledo, F.G. (2014). Racial differences in peripheral insulin sensitivity and mitochondrial capacity in the absence of obesity. J Clin Endocrinol Metab *99*, 4307-4314.

Dent, R., McPherson, R., and Harper, M.E. (1999). Variability in weight loss in highly compliant women on a controlled dietary regimen. Obes Res *7*.

Dent, R.M., Penwarden, R.M., Harris, N., and Hotz, S.B. (2002). Development and evaluation of patient-centered software for a weight-management clinic. Obes Res *10*, 651-656.

Desmurs, M., Foti, M., Raemy, E., Vaz, F.M., Martinou, J.C., Bairoch, A., and Lane, L. (2015). C11orf83, a mitochondrial cardiolipin-binding protein involved in bc1 complex assembly and supercomplex stabilization. Mol Cell Biol *35*, 1139-1156.

Despres, J.P. (2012). Body fat distribution and risk of cardiovascular disease: an update. Circulation *126*, 1301-1313.

DiBaise, J.K., Zhang, H., Crowell, M.D., Krajmalnik-Brown, R., Decker, G.A., and Rittmann, B.E. (2008). Gut microbiota and its possible relationship with obesity. Mayo Clinic proceedings *83*, 460-469.

Dixon, J.B., le Roux, C.W., Rubino, F., and Zimmet, P. (2012). Bariatric surgery for type 2 diabetes. Lancet *379*, 2300-2311.

Dresner, A., Laurent, D., Marcucci, M., Griffin, M.E., Dufour, S., Cline, G.W., Slezak, L.A., Andersen, D.K., Hundal, R.S., Rothman, D.L.*, et al.* (1999). Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest *103*, 253-259.

Ducluzeau, P.H., Perretti, N., Laville, M., Andreelli, F., Vega, N., Riou, J.P., and Vidal, H. (2001). Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. Diabetes *50*, 1134-1142.

Eckel, R.H., Grundy, S.M., and Zimmet, P.Z. (2005). The metabolic syndrome. Lancet *365*, 1415-1428.

Eckel, R.H., Jakicic, J.M., Ard, J.D., de Jesus, J.M., Houston Miller, N., Hubbard, V.S., Lee, I.M., Lichtenstein, A.H., Loria, C.M., Millen, B.E.*, et al.* (2014). 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation *129*, S76-99.

Elder, K.A., and Wolfe, B.M. (2007). Bariatric surgery: a review of procedures and outcomes. Gastroenterology *132*, 2253-2271.

Enad, J.G., Fournier, M., and Sieck, G.C. (1989). Oxidative capacity and capillary density of diaphragm motor units. J Appl Physiol (1985) *67*, 620-627.

Enriquez, J.A. (2016). Supramolecular Organization of Respiratory Complexes. Annu Rev Physiol *78*, 533-561.

Eriksson, J., Franssila-Kallunki, A., Ekstrand, A., Saloranta, C., Widen, E., Schalin, C., and Groop, L. (1989). Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med *321*, 337-343.

Felber, J.P., Ferrannini, E., Golay, A., Meyer, H.U., Theibaud, D., Curchod, B., Maeder, E., Jequier, E., and DeFronzo, R.A. (1987). Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. Diabetes *36*, 1341-1350.

Florez, J.C. (2008). Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. J Clin Endocrinol Metab *93*, 4633-4642.

Foster, G.D., Sanders, M.H., Millman, R., Zammit, G., Borradaile, K.E., Newman, A.B., Wadden, T.A., Kelley, D., Wing, R.R., Sunyer, F.X.*, et al.* (2009). Obstructive sleep apnea among obese patients with type 2 diabetes. Diabetes Care *32*, 1017-1019.

Freedman, M.R., King, J., and Kennedy, E. (2001). Popular diets: a scientific review. Obes Res *9 Suppl 1*, 1S-40S.

Fry, A.C. (2004). The role of resistance exercise intensity on muscle fibre adaptations. Sports Med *34*, 663-679.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., and Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest *114*, 1752-1761.

Gabbert, C., Donohue, M., Arnold, J., and Schwimmer, J.B. (2010). Adenovirus 36 and obesity in children and adolescents. Pediatrics *126*, 721-726.

Galton, D.J., and Bray, G.A. (1967). Metabolism of alpha-glycerol phosphate in human adipose tissue in obesity. J Clin Endocrinol Metab *27*, 1573-1580.

Garvey, W., Garber, A., and Mechanick, J. (2014). AACE Advanced framework for a new diagnosis of obesity as a chronic disease.

Gerrits, M.F., Ghosh, S., Kavaslar, N., Hill, B., Tour, A., Seifert, E.L., Beauchamp, B., Gorman, S., Stuart, J., Dent, R.*, et al.* (2010). Distinct skeletal muscle fiber characteristics and gene expression in diet-sensitive versus diet-resistant obesity. Journal of lipid research *51*, 2394-2404.

Ghosh, S., Dent, R., Harper, M.E., Stuart, J., and McPherson, R. (2011). Blood gene expression reveal pathway differences between diet-sensitive and resistant obese subjects prior to caloric restriction. Obesity (Silver Spring, Md) *19*, 457-463.

Gilbert-Diamond, D., Li, Z., Adachi-Mejia, A.M., McClure, A.C., and Sargent, J.D. (2014). Association of a television in the bedroom with increased adiposity gain in a nationally representative sample of children and adolescents. JAMA pediatrics *168*, 427-434.

Global BMI Mortality Collaboration (2016). Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet.

Gluckman, P.D., Hanson, M.A., Cooper, C., and Thornburg, K.L. (2008). Effect of in utero and early-life conditions on adult health and disease. N Engl J Med *359*, 61-73.

Goldenberg, R., and Punthakee, Z. (2013). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Canadian Journal of Diabetes *37, Supplement 1*, S8-S11.

Goldstein, D.E. (1984). Is glycosylated hemoglobin clinically useful? N Engl J Med *310*, 384-385.

Greising, S.M., Gransee, H.M., Mantilla, C.B., and Sieck, G.C. (2012). Systems biology of skeletal muscle: fiber type as an organizing principle. Wiley interdisciplinary reviews Systems biology and medicine *4*, 457-473.

Gudzune, K.A., Doshi, R.S., Mehta, A.K., Chaudhry, Z.W., Jacobs, D.K., Vakil, R.M., Lee, C.J., Bleich, S.N., and Clark, J.M. (2015). Efficacy of commercial weight-loss programs: an updated systematic review. Annals of internal medicine *162*, 501-512.

Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L., and Anis, A.H. (2009). The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC public health *9*, 88.

Guzy, R.D., and Schumacker, P.T. (2006). Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Exp Physiol *91*, 807-819.

Han, D.H., Hancock, C.R., Jung, S.R., Higashida, K., Kim, S.H., and Holloszy, J.O. (2011). Deficiency of the mitochondrial electron transport chain in muscle does not cause insulin resistance. PLoS One *6*, e19739.

Hancox, R.J., Milne, B.J., and Poulton, R. (2004). Association between child and adolescent television viewing and adult health: a longitudinal birth cohort study. Lancet *364*, 257-262.

Harper, M.E., Dent, R., Monemdjou, S., Bezaire, V., Van Wyck, L., Wells, G., Kavaslar, G.N., Gauthier, A., Tesson, F., and McPherson, R. (2002). Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. Diabetes *51*, 2459-2466.

Harper, W., Clement, M., Goldenberg, R., Hanna, A., Main, A., Retnakaran, R., Sherifali, D., Woo, V., and Yale, J.F. (2013). Pharmacologic management of type 2 diabetes. Can J Diabetes *37 Suppl 1*, S61-68.

Harris, M.I., Flegal, K.M., Cowie, C.C., Eberhardt, M.S., Goldstein, D.E., Little, R.R., Wiedmeyer, H.M., and Byrd-Holt, D.D. (1998). Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. Diabetes Care *21*, 518-524.

Harris, M.I., Klein, R., Welborn, T.A., and Knuiman, M.W. (1992). Onset of NIDDM occurs at least 4-7 yr before clinical diagnosis. Diabetes Care *15*, 815-819.

Haslam, D.W., and James, W.P. (2005). Obesity. Lancet *366*, 1197-1209.

Hatch, G.M. (2004). Cell biology of cardiac mitochondrial phospholipids. Biochem Cell Biol *82*, 99-112.

Hayashi, H., Nakagami, H., Takeichi, M., Shimamura, M., Koibuchi, N., Oiki, E., Sato, N., Koriyama, H., Mori, M., Gerardo Araujo, R.*, et al.* (2012). HIG1, a novel regulator of mitochondrial gamma-secretase, maintains normal mitochondrial function. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *26*, 2306-2317.

Hilton, T.N., Tuttle, L.J., Bohnert, K.L., Mueller, M.J., and Sinacore, D.R. (2008). Excessive Adipose Tissue Infiltration in Skeletal Muscle in Individuals With Obesity, Diabetes Mellitus, and Peripheral Neuropathy: Association With Performance and Function. Physical Therapy *88*, 1336-1344.

Holland, W.L., Brozinick, J.T., Wang, L.P., Hawkins, E.D., Sargent, K.M., Liu, Y., Narra, K., Hoehn, K.L., Knotts, T.A., Siesky, A.*, et al.* (2007). Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab *5*, 167-179.

Hollander, P.A., Elbein, S.C., Hirsch, I.B., Kelley, D., McGill, J., Taylor, T., Weiss, S.R., Crockett, S.E., Kaplan, R.A., Comstock, J.*, et al.* (1998). Role of orlistat in the treatment of obese patients with type 2 diabetes. A 1-year randomized double-blind study. Diabetes Care *21*, 1288-1294.

Holloway, G.P., Benton, C.R., Mullen, K.L., Yoshida, Y., Snook, L.A., Han, X.X., Glatz, J.F., Luiken, J.J., Lally, J., Dyck, D.J.*, et al.* (2009). In obese rat muscle transport of palmitate is increased and is channeled to triacylglycerol storage despite an increase in mitochondrial palmitate oxidation. Am J Physiol Endocrinol Metab *296*, E738-747.

Holloway, G.P., Thrush, A.B., Heigenhauser, G.J., Tandon, N.N., Dyck, D.J., Bonen, A., and Spriet, L.L. (2007). Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. Am J Physiol Endocrinol Metab *292*, E1782-1789.

Houstis, N., Rosen, E.D., and Lander, E.S. (2006). Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature *440*, 944-948.

Hulens, M., Vansant, G., Lysens, R., Claessens, A.L., Muls, E., and Brumagne, S. (2001). Study of differences in peripheral muscle strength of lean versus obese women: an allometric approach. Int J Obes Relat Metab Disord *25*, 676-681.

Hulver, M.W., Berggren, J.R., Cortright, R.N., Dudek, R.W., Thompson, R.P., Pories, W.J., MacDonald, K.G., Cline, G.W., Shulman, G.I., Dohm, G.L.*, et al.* (2003). Skeletal muscle lipid metabolism with obesity. Am J Physiol Endocrinol Metab *284*, E741-747.

Ikeda, K., Shiba, S., Horie-Inoue, K., Shimokata, K., and Inoue, S. (2013). A stabilizing factor for mitochondrial respiratory supercomplex assembly regulates energy metabolism in muscle. Nat Commun *4*, 2147.

Jacob, S., Machann, J., Rett, K., Brechtel, K., Volk, A., Renn, W., Maerker, E., Matthaei, S., Schick, F., Claussen, C.D.*, et al.* (1999). Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes *48*, 1113-1119.

Jakicic, J.M., Egan, C.M., Fabricatore, A.N., Gaussoin, S.A., Glasser, S.P., Hesson, L.A., Knowler, W.C., Lang, W., Regensteiner, J.G., Ribisl, P.M.*, et al.* (2013). Four-year change in cardiorespiratory fitness and influence on glycemic control in adults with type 2 diabetes in a randomized trial: the Look AHEAD Trial. Diabetes Care *36*, 1297-1303.

Jensen, M.D., Ryan, D.H., Apovian, C.M., Ard, J.D., Comuzzie, A.G., Donato, K.A., Hu, F.B., Hubbard, V.S., Jakicic, J.M., Kushner, R.F.*, et al.* (2014). 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. Circulation *129*, S102-138.

Jess, T. (2014). Microbiota, antibiotics, and obesity. N Engl J Med *371*, 2526-2528.

Jheng, H.F., Tsai, P.J., Guo, S.M., Kuo, L.H., Chang, C.S., Su, I.J., Chang, C.R., and Tsai, Y.S. (2012). Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. Mol Cell Biol *32*, 309-319.

Jiang, F., Zhu, S., Yan, C., Jin, X., Bandla, H., and Shen, X. (2009). Sleep and obesity in preschool children. The Journal of pediatrics *154*, 814-818.

Jones, D.A., and Round, J.M. (1990). Skeletal muscle in health and disease: a textbook of muscle physiology (Manchester University Press).

Katzmarzyk, P.T. (2002). The Canadian obesity epidemic, 1985-1998. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne *166*, 1039-1040.

Keaney, J.F., Jr., Larson, M.G., Vasan, R.S., Wilson, P.W., Lipinska, I., Corey, D., Massaro, J.M., Sutherland, P., Vita, J.A., Benjamin, E.J.*, et al.* (2003). Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arteriosclerosis, thrombosis, and vascular biology *23*, 434-439.

Kelley, D.E., Goodpaster, B., Wing, R.R., and Simoneau, J.A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. The American journal of physiology *277*, E1130-1141.

Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944-2950.

Kelley, D.E., and Mandarino, L.J. (2000). Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes *49*, 677-683.

Kim, J.Y., Hickner, R.C., Cortright, R.L., Dohm, G.L., and Houmard, J.A. (2000). Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab *279*, E1039-1044.

Kirkwood, S.P., Munn, E.A., and Brooks, G.A. (1986). Mitochondrial reticulum in limb skeletal muscle. The American journal of physiology *251*, C395-402.

Klein, B.E., Klein, R., Moss, S.E., and Cruickshanks, K.J. (1996). Parental history of diabetes in a population-based study. Diabetes Care *19*, 827-830.

Koves, T.R., Ussher, J.R., Noland, R.C., Slentz, D., Mosedale, M., Ilkayeva, O., Bain, J., Stevens, R., Dyck, J.R., Newgard, C.B.*, et al.* (2008). Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab *7*, 45-56.

Kral, J.G., Paez, W., and Wolfe, B.M. (2009). Vagal nerve function in obesity: therapeutic implications. World journal of surgery *33*, 1995-2006.

Lafortuna, C.L., Tresoldi, D., and Rizzo, G. (2014). Influence of body adiposity on structural characteristics of skeletal muscle in men and women. Clinical Physiology and Functional Imaging *34*, 47-55.

Lanner, J.T., Bruton, J.D., Katz, A., and Westerblad, H. (2008). Ca(2+) and insulin-mediated glucose uptake. Curr Opin Pharmacol *8*, 339-345.

Lapuente-Brun, E., Moreno-Loshuertos, R., Acin-Perez, R., Latorre-Pellicer, A., Colas, C., Balsa, E., Perales-Clemente, E., Quiros, P.M., Calvo, E., Rodriguez-Hernandez, M.A., *et al.* (2013). Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. Science *340*, 1567-1570.

Lebeche, D., Davidoff, A.J., and Hajjar, R.J. (2008). Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. Nature clinical practice Cardiovascular medicine *5*, 715-724.

Lee, H.Y., Choi, C.S., Birkenfeld, A.L., Alves, T.C., Jornayvaz, F.R., Jurczak, M.J., Zhang, D., Woo, D.K., Shadel, G.S., Ladiges, W., *et al.* (2010). Targeted expression of catalase to mitochondria prevents age-associated reductions in mitochondrial function and insulin resistance. Cell Metab *12*, 668-674.

Lexell, J., Taylor, C.C., and Sjostrom, M. (1988). What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci *84*, 275-294.

Li, N., Li, B., Brun, T., Deffert-Delbouille, C., Mahiout, Z., Daali, Y., Ma, X.J., Krause, K.H., and Maechler, P. (2012). NADPH oxidase NOX2 defines a new antagonistic role for reactive oxygen species and cAMP/PKA in the regulation of insulin secretion. Diabetes *61*, 2842-2850.

Lithell, H., Lindgarde, F., Hellsing, K., Lundqvist, G., Nygaard, E., Vessby, B., and Saltin, B. (1981). Body weight, skeletal muscle morphology, and enzyme activities in relation to fasting serum insulin concentration and glucose tolerance in 48-year-old men. Diabetes *30*, 19-25.

Loos, R.J. (2012). Genetic determinants of common obesity and their value in prediction. Best Pract Res Clin Endocrinol Metab *26*, 211-226.

Maffiuletti, N.A., Jubeau, M., Munzinger, U., Bizzini, M., Agosti, F., De Col, A., Lafortuna, C.L., and Sartorio, A. (2007). Differences in quadriceps muscle strength and fatigue between lean and obese subjects. European journal of applied physiology *101*, 51-59.

Magnusson, I., Rothman, D.L., Katz, L.D., Shulman, R.G., and Shulman, G.I. (1992). Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Invest *90*, 1323-1327.

Mailloux, R.J., Fu, A., Robson-Doucette, C., Allister, E.M., Wheeler, M.B., Screaton, R., and Harper, M.E. (2012). Glutathionylation state of uncoupling protein-2 and the control of glucose-stimulated insulin secretion. The Journal of biological chemistry *287*, 39673-39685.

Mailloux, R.J., and Harper, M.E. (2011). Uncoupling proteins and the control of mitochondrial reactive oxygen species production. Free Radic Biol Med *51*, 1106-1115.

Mailloux, R.J., Seifert, E.L., Bouillaud, F., Aguer, C., Collins, S., and Harper, M.E. (2011). Glutathionylation acts as a control switch for uncoupling proteins UCP2 and UCP3. The Journal of biological chemistry *286*, 21865-21875.

Majumder, S., and Birk, J. (2013). A review of the current status of endoluminal therapy as a primary approach to obesity management. Surgical endoscopy *27*, 2305-2311.

Malenfant, P., Joanisse, D.R., Theriault, R., Goodpaster, B.H., Kelley, D.E., and Simoneau, J.A. (2001). Fat content in individual muscle fibers of lean and obese subjects. Int J Obes Relat Metab Disord *25*, 1316-1321.

Mantilla, C.B., and Sieck, G.C. (2003). Invited review: Mechanisms underlying motor unit plasticity in the respiratory system. J Appl Physiol (1985) *94*, 1230-1241.

Mathus-Vliegen, E.M., and Tytgat, G.N. (2005). Intragastric balloon for treatment-resistant obesity: safety, tolerance, and efficacy of 1-year balloon treatment followed by a 1-year balloon-free follow-up. Gastrointestinal endoscopy *61*, 19-27.

McClendon, D.A., Dunbar, S.B., Clark, P.C., and Coverson, D.L. (2010). An analysis of popular weight loss diet types in relation to metabolic syndrome therapeutic guidelines. Medsurg nursing : official journal of the Academy of Medical-Surgical Nurses *19*, 17-24.

McGilvery, R.W. (1970). Biochemistry: A Functional Approach (Saunders).

McKenzie, M., Lazarou, M., Thorburn, D.R., and Ryan, M.T. (2006). Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients. J Mol Biol *361*, 462-469.

Mendis, S., Armstrong, T., Bettcher, D., Branca, F., Lauer, J., Mace, C., Poznyak, V., Riley, L., Silva, V.D.C.E., and G., S. (2014). Global Status Report on noncommunicable diseases 2014 (World Health Organization).

Mileykovskaya, E., Zhang, M., and Dowhan, W. (2005). Cardiolipin in energy transducing membranes. Biochemistry (Mosc) *70*, 154-158.

Mingrone, G., Panunzi, S., De Gaetano, A., Guidone, C., Iaconelli, A., Leccesi, L., Nanni, G., Pomp, A., Castagneto, M., Ghirlanda, G*., et al.* (2012). Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med *366*, 1577-1585.

Mogensen, M., Sahlin, K., Fernstrom, M., Glintborg, D., Vind, B.F., Beck-Nielsen, H., and Hojlund, K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes *56*, 1592-1599.

Moller, D.E., and Flier, J.S. (1991). Insulin resistance--mechanisms, syndromes, and implications. N Engl J Med *325*, 938-948.

Mootha, V.K., Bunkenborg, J., Olsen, J.V., Hjerrild, M., Wisniewski, J.R., Stahl, E., Bolouri, M.S., Ray, H.N., Sihag, S., Kamal, M.*, et al.* (2003a). Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell *115*, 629-640.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E.*, et al.* (2003b). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet *34*, 267-273.

Morris, D.H., Khunti, K., Achana, F., Srinivasan, B., Gray, L.J., Davies, M.J., and Webb, D. (2013). Progression rates from HbA1c 6.0-6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. Diabetologia *56*, 1489-1493.

Murphy, M.P. (2009). How mitochondria produce reactive oxygen species. Biochem J *417*, 1-13.

Nathan, D.M., Singer, D.E., Hurxthal, K., and Goodson, J.D. (1984). The clinical information value of the glycosylated hemoglobin assay. N Engl J Med *310*, 341-346.

National Clinical Guideline Centre (UK) (2014). Obesity: Identification, Assessment and Management of Overweight and Obesity in Children, Young People and Adults: Partial Update of CG43 (London: National Clinical Guideline Centre, 2014.).

Navaneelan, T., and Janz, T. (2014). Adjusting the scales: Obesity in the Canadian population after correcting for respondent bias. Health at Glance.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F.*, et al.* (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet *384*, 766-781.

Nicholls, D.G., and Ferguson, S.J. (2003a). 4 - The chemiosmotic proton circuit. In Bioenergetics (Third Edition) (London: Academic Press), pp. 57-88.

Nicholls, D.G., and Ferguson, S.J. (2003b). 5 - Respiratory chains. In Bioenergetics (Third Edition) (London: Academic Press), pp. 89-XIII.

Nicholls, D.G., and Ferguson, S.J. (2003c). 7 - The ATP synthase. In Bioenergetics (Third Edition) (London: Academic Press), pp. 195-218.

NIH Consensus (1996). Gastrointestinal surgery for severe obesity. NIH consensus development conference, March 25-7,1991. Nutrition (Burbank, Los Angeles County, Calif) *12*, 397-404.

Obesity Canada Clinical Practice Guidelines Expert Panel (2007). 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne *176*, Online - 1-117.

Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.E., Walford, G.A., Sugiana, C., Boneh, A., Chen, W.K.*, et al.* (2008). A mitochondrial protein compendium elucidates complex I disease biology. Cell *134*, 112-123.

Park, S., Scheffler, T.L., Gunawan, A.M., Shi, H., Zeng, C., Hannon, K.M., Grant, A.L., and Gerrard, D.E. (2009). Chronic elevated calcium blocks AMPK-induced GLUT-4 expression in skeletal muscle. Am J Physiol Cell Physiol *296*, C106-115.

Parone, P.A., Da Cruz, S., Tondera, D., Mattenberger, Y., James, D.I., Maechler, P., Barja, F., and Martinou, J.C. (2008). Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. PLoS One *3*, e3257.

Pasarica, M., Shin, A.C., Yu, M., Ou Yang, H.M., Rathod, M., Jen, K.L., MohanKumar, S., MohanKumar, P.S., Markward, N., and Dhurandhar, N.V. (2006). Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. Obesity (Silver Spring, Md) *14*, 1905-1913.

Patten, D.A., Wong, J., Khacho, M., Soubannier, V., Mailloux, R.J., Pilon-Larose, K., MacLaurin, J.G., Park, D.S., McBride, H.M., Trinkle-Mulcahy, L.*, et al.* (2014). OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. The EMBO journal *33*, 2676-2691.

Patti, M.E., Butte, A.J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R.*, et al.* (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A *100*, 8466-8471.

Patti, M.E., and Corvera, S. (2010). The role of mitochondria in the pathogenesis of type 2 diabetes. Endocrine reviews *31*, 364-395.

Pedersen, B.K., and Febbraio, M.A. (2012). Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nature reviews Endocrinology *8*, 457-465.

Perreault, L., Pan, Q., Mather, K.J., Watson, K.E., Hamman, R.F., Kahn, S.E., and Diabetes Prevention Program Research, G. (2012). Effect of regression from prediabetes to normal glucose regulation on long-term reduction in diabetes risk: results from the Diabetes Prevention Program Outcomes Study. Lancet *379*, 2243-2251.

Pessin, J.E., and Saltiel, A.R. (2000). Signaling pathways in insulin action: molecular targets of insulin resistance. J Clin Invest *106*, 165-169.

Petersen, K.F., and Shulman, G.I. (2006). Etiology of insulin resistance. Am J Med *119*, S10-16.

Pette, D., and Staron, R.S. (2000). Myosin isoforms, muscle fiber types, and transitions. Microsc Res Tech *50*, 500-509.

Phielix, E., and Mensink, M. (2008). Type 2 diabetes mellitus and skeletal muscle metabolic function. Physiology & behavior *94*, 252-258.

Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X., and Eckel, R.H. (2006). Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arteriosclerosis, thrombosis, and vascular biology *26*, 968-976.

Pospisilik, J.A., Knauf, C., Joza, N., Benit, P., Orthofer, M., Cani, P.D., Ebersberger, I., Nakashima, T., Sarao, R., Neely, G.*, et al.* (2007). Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. Cell *131*, 476-491.

Pratipanawatr, W., Pratipanawatr, T., Cusi, K., Berria, R., Adams, J.M., Jenkinson, C.P., Maezono, K., DeFronzo, R.A., and Mandarino, L.J. (2001). Skeletal muscle insulin resistance in normoglycemic subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated insulin receptor substrate-1 tyrosine phosphorylation. Diabetes *50*, 2572-2578.

Randle, P.J., Garland, P.B., Newsholme, E.A., and Hales, C.N. (1965). The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. Ann N Y Acad Sci *131*, 324-333.

Reinehr, T., Hinney, A., de Sousa, G., Austrup, F., Hebebrand, J., and Andler, W. (2007). Definable somatic disorders in overweight children and adolescents. The Journal of pediatrics *150*, 618-622, 622 e611-615.

Reis, J.P., Loria, C.M., Sorlie, P.D., Park, Y., Hollenbeck, A., and Schatzkin, A. (2011). Lifestyle factors and risk for new-onset diabetes: a population-based cohort study. Annals of internal medicine *155*, 292-299.

Richardson, D.K., Kashyap, S., Bajaj, M., Cusi, K., Mandarino, S.J., Finlayson, J., DeFronzo, R.A., Jenkinson, C.P., and Mandarino, L.J. (2005). Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increases the expression of extracellular matrix genes in human skeletal muscle. The Journal of biological chemistry *280*, 10290-10297.

Rieusset, J. (2015). Contribution of mitochondria and endoplasmic reticulum dysfunction in insulin resistance: Distinct or interrelated roles? Diabetes & metabolism *41*, 358-368.

Ritov, V.B., Menshikova, E.V., Azuma, K., Wood, R., Toledo, F.G., Goodpaster, B.H., Ruderman, N.B., and Kelley, D.E. (2010). Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab *298*, E49-58.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H., and Kelley, D.E. (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes *54*, 8-14.

Roberts, S.B., and Rosenberg, I. (2006). Nutrition and aging: changes in the regulation of energy metabolism with aging. Physiol Rev *86*, 651-667.

Rolfe, D.F., and Brown, G.C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev *77*, 731-758.

Ross, R., Hudson, R., Stotz, P.J., and Lam, M. (2015). Effects of exercise amount and intensity on abdominal obesity and glucose tolerance in obese adults: a randomized trial. Annals of internal medicine *162*, 325-334.

Rothman, D.L., Shulman, R.G., and Shulman, G.I. (1992). 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest *89*, 1069-1075.

Ryan, D., and Heaner, M. (2014). Guidelines (2013) for managing overweight and obesity in adults. Preface to the full report. Obesity (Silver Spring, Md) *22 Suppl 2*, S1-3.

Saltin, B., Henriksson, J., Nygaard, E., Andersen, P., and Jansson, E. (1977). Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. Ann N Y Acad Sci *301*, 3-29.

Sandler, B.J., Rumbaut, R., Swain, C.P., Torres, G., Morales, L., Gonzales, L., Schultz, S., Talamini, M., and Horgan, S. (2011). Human experience with an endoluminal, endoscopic, gastrojejunal bypass sleeve. Surgical endoscopy *25*, 3028-3033.

Savage, D.B., Petersen, K.F., and Shulman, G.I. (2007). Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev *87*, 507-520.

Schagger, H., and Pfeiffer, K. (2000). Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. The EMBO journal *19*, 1777-1783.

Schauer, P.R., Bhatt, D.L., Kirwan, J.P., Wolski, K., Brethauer, S.A., Navaneethan, S.D., Aminian, A., Pothier, C.E., Kim, E.S., Nissen, S.E.*, et al.* (2014). Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. N Engl J Med *370*, 2002-2013.

Schauer, P.R., Kashyap, S.R., Wolski, K., Brethauer, S.A., Kirwan, J.P., Pothier, C.E., Thomas, S., Abood, B., Nissen, S.E., and Bhatt, D.L. (2012). Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med *366*, 1567-1576.

Schlame, M., Rua, D., and Greenberg, M.L. (2000). The biosynthesis and functional role of cardiolipin. Prog Lipid Res *39*, 257-288.

Scialo, F., Mallikarjun, V., Stefanatos, R., and Sanz, A. (2013). Regulation of lifespan by the mitochondrial electron transport chain: reactive oxygen species-dependent and reactive oxygen species-independent mechanisms. Antioxid Redox Signal *19*, 1953-1969.

Shaw, J.M., and Nunnari, J. (2002). Mitochondrial dynamics and division in budding yeast. Trends Cell Biol *12*, 178-184.

Simoneau, J.A., and Bouchard, C. (1995). Skeletal muscle metabolism and body fat content in men and women. Obes Res *3*, 23-29.

Simoneau, J.A., Colberg, S.R., Thaete, F.L., and Kelley, D.E. (1995). Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *9*, 273-278.

Simoneau, J.A., and Kelley, D.E. (1997). Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. J Appl Physiol (1985) *83*, 166-171.

Simoneau, J.A., Veerkamp, J.H., Turcotte, L.P., and Kelley, D.E. (1999). Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *13*, 2051-2060.

Sjostrom, L. (2013). Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. Journal of internal medicine *273*, 219-234.

Sjostrom, L., Narbro, K., Sjostrom, C.D., Karason, K., Larsson, B., Wedel, H., Lystig, T., Sullivan, M., Bouchard, C., Carlsson, B.*, et al.* (2007). Effects of bariatric surgery on mortality in Swedish obese subjects. N Engl J Med *357*, 741-752.

Sjostrom, L., Peltonen, M., Jacobson, P., Sjostrom, C.D., Karason, K., Wedel, H., Ahlin, S., Anveden, A., Bengtsson, C., Bergmark, G.*, et al.* (2012). Bariatric surgery and long-term cardiovascular events. Jama *307*, 56-65.

Soubannier, V., and McBride, H.M. (2009). Positioning mitochondrial plasticity within cellular signaling cascades. Biochimica et biophysica acta *1793*, 154-170.

Sparks, L.M., Xie, H., Koza, R.A., Mynatt, R., Hulver, M.W., Bray, G.A., and Smith, S.R. (2005). A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes *54*, 1926-1933.

Stettler, N., Signer, T.M., and Suter, P.M. (2004). Electronic games and environmental factors associated with childhood obesity in Switzerland. Obes Res *12*, 896-903.

Strogolova, V., Furness, A., Robb-McGrath, M., Garlich, J., and Stuart, R.A. (2012). Rcf1 and Rcf2, members of the hypoxia-induced gene 1 protein family, are critical components of the mitochondrial cytochrome bc1-cytochrome c oxidase supercomplex. Mol Cell Biol *32*, 1363-1373.

Sullivan, P.W., Morrato, E.H., Ghushchyan, V., Wyatt, H.R., and Hill, J.O. (2005). Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. Diabetes Care *28*, 1599-1603.

Sun, G., Ukkola, O., Rankinen, T., Joanisse, D.R., and Bouchard, C. (2002). Skeletal muscle characteristics predict body fat gain in response to overfeeding in never-obese young men. Metabolism *51*, 451-456.

Taber, D.R., Chriqui, J.F., Powell, L., and Chaloupka, F.J. (2013). Association between state laws governing school meal nutrition content and student weight status: implications for new USDA school meal standards. JAMA pediatrics *167*, 513-519.

Thrush, A.B., Dent, R., McPherson, R., and Harper, M.E. (2013). Implications of mitochondrial uncoupling in skeletal muscle in the development and treatment of obesity. The FEBS journal *280*, 5015-5029.

Thrush, A.B., Zhang, R., Chen, W., Seifert, E.L., Quizi, J.K., McPherson, R., Dent, R., and Harper, M.E. (2014). Lower mitochondrial proton leak and decreased glutathione redox in primary muscle cells of obese diet-resistant versus diet-sensitive humans. J Clin Endocrinol Metab *99*, 4223-4230.

Thyfault, J.P., Cree, M.G., Zheng, D., Zwetsloot, J.J., Tapscott, E.B., Koves, T.R., Ilkayeva, O., Wolfe, R.R., Muoio, D.M., and Dohm, G.L. (2007). Contraction of insulin-resistant muscle normalizes insulin action in association with increased mitochondrial activity and fatty acid catabolism. Am J Physiol Cell Physiol *292*, C729-739.

Tormos, K.V., Anso, E., Hamanaka, R.B., Eisenbart, J., Joseph, J., Kalyanaraman, B., and Chandel, N.S. (2011). Mitochondrial complex III ROS regulate adipocyte differentiation. Cell Metab *14*, 537-544.

Trasande, L., Attina, T.M., and Blustein, J. (2012). Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. Jama *308*, 1113-1121.

Tsai, A.W., Cushman, M., Rosamond, W.D., Heckbert, S.R., Polak, J.F., and Folsom, A.R. (2002). Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Arch Intern Med *162*, 1182-1189.

Vaag, A., Henriksen, J.E., and Beck-Nielsen, H. (1992). Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Invest *89*, 782-788.

van der Bliek, A.M., Shen, Q., and Kawajiri, S. (2013). Mechanisms of mitochondrial fission and fusion. Cold Spring Harbor perspectives in biology *5*.

Vondra, K., Rath, R., Bass, A., Slabochova, Z., Teisinger, J., and Vitek, V. (1977). Enzyme activities in quadriceps femoris muscle of obese diabetic male patients. Diabetologia *13*, 527-529.

Vukotic, M., Oeljeklaus, S., Wiese, S., Vogtle, F.N., Meisinger, C., Meyer, H.E., Zieseniss, A., Katschinski, D.M., Jans, D.C., Jakobs, S.*, et al.* (2012). Rcf1 mediates cytochrome oxidase assembly and respirasome formation, revealing heterogeneity of the enzyme complex. Cell Metab *15*, 336-347.

Wang, W., Wei, P.L., Lee, Y.C., Huang, M.T., Chiu, C.C., and Lee, W.J. (2005). Short-term results of laparoscopic mini-gastric bypass. Obesity surgery *15*, 648-654.

Wanschers, B.F., Szklarczyk, R., van den Brand, M.A., Jonckheere, A., Suijskens, J., Smeets, R., Rodenburg, R.J., Stephan, K., Helland, I.B., Elkamil, A.*, et al.* (2014). A mutation in the human CBP4 ortholog UQCC3 impairs complex III assembly, activity and cytochrome b stability. Hum Mol Genet *23*, 6356-6365.

Warner, M., Wesselink, A., Harley, K.G., Bradman, A., Kogut, K., and Eskenazi, B. (2014). Prenatal exposure to dichlorodiphenyltrichloroethane and obesity at 9 years of age in the CHAMACOS study cohort. American journal of epidemiology *179*, 1312-1322.

Weir, G.C., and Bonner-Weir, S. (2004). Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes *53 Suppl 3*, S16-21.

Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., Qizilbash, N., Collins, R., and Peto, R. (2009). Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet *373*, 1083-1096.

Wild, S., Roglic, G., Green, A., Sicree, R., and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care *27*, 1047-1053.

World Health Organization (2015). Obesity and overweight. In Fact Sheets (World Health Organization).

World Health Organization (2016). Global Report on Diabetes.

Wredenberg, A., Freyer, C., Sandstrom, M.E., Katz, A., Wibom, R., Westerblad, H., and Larsson, N.G. (2006). Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. Biochem Biophys Res Commun *350*, 202-207.

Zechner, C., Lai, L., Zechner, J.F., Geng, T., Yan, Z., Rumsey, J.W., Collia, D., Chen, Z., Wozniak, D.F., Leone, T.C.*, et al.* (2010). Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. Cell Metab *12*, 633-642.

Zhang, M., Mileykovskaya, E., and Dowhan, W. (2002). Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. The Journal of biological chemistry *277*, 43553-43556.

Zhang, M., Mileykovskaya, E., and Dowhan, W. (2005). Cardiolipin is essential for organization of complexes III and IV into a supercomplex in intact yeast mitochondria. The Journal of biological chemistry *280*, 29403-29408.

Zurlo, F., Nemeth, P.M., Choksi, R.M., Sesodia, S., and Ravussin, E. (1994). Whole-body energy metabolism and skeletal muscle biochemical characteristics. Metabolism *43*, 481-486.

2 CHAPTER 2 - IS TYPE 2 DIABETES ASSOCIATED WITH IMPAIRED CAPACITY FOR WEIGHT LOSS?

Is type 2 diabetes associated with impaired capacity for weight loss?

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2.1 STATEMENT OF MANUSCRIPT STATUS AND CONTRIBUTIONS

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2.1.4 CONTRIBUTION STATEMENT

All authors contributed to the conception and design of the study. RD acquired the clinical data; all authors analyzed and interpreted the data. The manuscript was drafted by GA, MN, and RD and critical revisions were performed by all authors. All authors have approved the final version.

2.1.5 DUALITY OF INTEREST STATEMENT

The authors declare that there is no duality of interest associated with this manuscript.

2.2 ABSTRACT

2.2.1 AIMS/HYPOTHESIS

Type 2 diabetes mellitus (T2DM) and obesity are intricately linked epidemics. In both, weight loss is considered part of the first line of treatment. The goal of this study was to evaluate the effect of T2DM status on weight loss in response to dietary intervention and lifestyle modification.

2.2.2 METHODS

Patients were enrolled in the Core Program of the Ottawa Hospital Weight Management Clinic. This consisted of 12 weeks of energy restriction to 900 calories (total meal replacement with Optifast® 900), within a 26-week lifestyle modification course with nutritional counseling and physician supervision. Data were gathered with software previously described and individuals were excluded for adherence issues and diseases affecting weight loss. Patients with T2DM were compared to those with impaired fasting glucose (IFG) and to those with normoglycemia. The group on antidiabetic medications was further divided to determine the effect of agents associated with weight gain. Rate of weight loss (ROWL) was analyzed at 6 weeks and percentage of weight loss (PWL) was analyzed at 26 weeks. Data are presented unadjusted and adjusted for initial weight, gender and age.

2.2.3 RESULTS

Between 1992 and 2012, 4173 patients entered the program and 2231 remained after all exclusions. There were significant differences in the age, weight, BMI, and HbA1C levels among the groups of individuals without T2DM, those with IFG and those with T2DM ($p < 2.2e\text{-}16$, $p = 3.959e\text{-}11$, $p = 1.232e\text{-}11$, and $p < 2.2e\text{-}16$ respectively). There were no differences in ROWL adjusted for age, sex and initial weight at 6 weeks. At 26 weeks,

adjusted PWL was lower in the group of individuals with T2DM (p=0.013). Data adjusted for age and sex, and data adjusted for age, sex, and initial weight yielded similar results (p= 0.001 and p=0.0002 respectively). Medication used by patients with T2DM did not affect the adjusted ROWL at 6 or PWL at 26 weeks.

2.2.4 CONCLUSIONS/INTERPRETATION

Patients with T2DM have impaired weight loss when undergoing a defined meal replacement and lifestyle modification program. Absence of an effect at 6 weeks is likely related to cessation of diabetes medications commonly occurring upon initial weight loss. While the magnitude of this effect is small in comparison to the contribution of sex, and initial weight, this is an important consideration in the treatment of obesity and T2DM.

2.3 INTRODUCTION

The impact of type 2 diabetes mellitus (T2DM) on weight loss in obese individuals is debated. Meal replacement and lifestyle modification programs are commonly used in the prevention and first-line treatment of obesity and T2DM. It is important to know if individuals with T2DM can have the same expectations when joining a weight loss program, or if more powerful weight management interventions are required. Previous studies have attempted to determine the impact of T2DM and associated pharmacotherapies on weight loss interventions. Some of these studies have found that patients with T2DM lose less weight than patients without T2DM (Henry *et al.*, 1986; Rolland *et al.*, 2013; Wing *et al.*, 1987), while others found that T2DM status had no impact (Baker *et al.*, 2012; Guare *et al.*, 1995; Li *et al.*, 2014; Paisey *et al.*, 1998). In addition, our recent studies of obese patients with T2DM reveal profound deficits in skeletal muscle oxidative activity and disordered

mitochondrial structure ('supercomplexes'), compared to obese control individuals (Antoun *et al.*, 2015).

Here we examine the impact of T2DM and associated pharmacotherapy on weight loss; we have studied the largest cohort to date, using prospectively collected data from the Core Program of the Ottawa Hospital Weight Management Clinic. Data were adjusted for factors known to impact weight loss and the effect of different pharmacotherapy regimens was analyzed. Enrolled patients completed 12 weeks of low calorie diet (LCD) total meal replacement within a 26-week program to accomplish weight loss in a safe and timely manner. There have been 4173 patients enrolled since the program's inception in 1992 until the year 2012 (Dent *et al.*, 2002). Previous studies from this center have evaluated genetic and metabolic predictors of weight loss variability (Antoun *et al.*, 2015; Harper *et al.*, 2002). Here we report that T2DM has a small but significant effect on weight loss, revealing impaired weight loss in individuals with T2DM.

2.4 METHODS

Study participants were patients from the Ottawa Hospital Weight Management Clinic enrolled in the Core Weight Management Program between 1992 and 2012. This study was approved by the Ottawa Hospital Research Ethics Board. Details of the meal replacement program are provided in supplementary material. All consenting patients (n=4173) were initially considered for the study. Individuals who were non-adherent and those with conditions that affect weight loss were excluded from the study. Adherence criteria were based on attendance, reported Optifast® use, and physician notes. Medical conditions for which individuals were excluded included surgery or pregnancy onset during

study period, severe edematous states, and medications known to be associated with weight gain during the study period. Patients with gestational diabetes and incomplete information on diabetes were also removed. For the detailed list, see Supplementary Table 1. Patients without T2DM were compared to patients with impaired fasting glucose (IFG) and patients with T2DM. Patients with T2DM were further subdivided in order to study the effect of various pharmacologic regimens. Those who are not taking medications were compared to those taking medications that are known to cause weight gain and those that are taking weight neutral medications. Medications known to cause weight gain include insulin, sulfonylureas and meglitinides while weight neutral drugs include metformin, acarbose and DPP-4.

The rate of weight loss (ROWL) was calculated for the first 6 weeks of meal replacement, since weight loss over this time period is linear. The percentage of weight loss (PWL) was calculated at the 26 weeks time point since weight loss is not linear over 0-26 weeks. Data are presented unadjusted and adjusted for age, sex and initial weight, as these factors are known to impact weight loss. In these cases, ROWL and PWL data presented herein are expressed in arbitrary units defined in relation to aforementioned variables. Statistical analyses were carried out as detailed in supplementary material.

2.5 RESULTS

A total of 2231 patients remained after the filters were applied. Of these, 1667 (74.7%) were female, and 339 (15.2%) had T2DM. Table 2-1 outlines the baseline characteristics of all groups of study participants. Sex, age and initial weight are well known to affect weight loss; therefore, data were adjusted for 1) sex and age; and 2) sex, age, and

initial weight of individual patients. Without these adjustments, there were significant differences in the age, weight, BMI, and HbA1C levels between the groups of individuals without T2DM, ones with IFG and ones with T2DM ($p < 2.2e-16$, $p = 3.959e-11$, $p = 1.232e-11$, and $p < 2.2e-16$ respectively).

After 6 weeks on the meal replacement program, the adjusted ROWL was similar among the three groups of participants (Table 2-1). However, at 26 weeks, the PWL was clearly lower in individuals with T2DM. This is evident in the unadjusted data ($p=0.013$) as well as the data adjusted for sex and age ($p=0.001$) and the data adjusted for sex, age, and initial weight ($p=0.0002$) (Table 2-1).

Joint contribution of baseline characteristics on the ROWL and PWL were further queried by fitting a linear model. Sex, initial weight and age were all highly associated with ROWL at 6 weeks (Table 2-2). At 26 weeks after the initiation of the treatment program, sex, initial weight and T2DM were associated with PWL (Table 2-2). Specifically, PWL was higher in males compared to females ($p<2\times10-16$ at 6 and 26 weeks). Initial body weight was positively correlated with ROWL at 6 weeks and PWL at 26 weeks ($p<2\times10-16$ at 6 weeks, $p=0.0028$ at 26 weeks). Age was negatively correlated with ROWL at 6 weeks only ($p=1.5\times10-10$). Patients without T2DM lost more weight at 26 weeks than those with T2DM ($p=0.0008$).

Since some T2DM medications are known to cause weight gain, we further analyzed the effects of medications. For this purpose, patients with T2DM were segregated into three groups based on their medication status: 1-no medications, 2-medications known to cause

weight gain, and 3-medications not known to cause weight gain. As in the above-described analyses, unadjusted and adjusted ROWL at 6 weeks and PWL at 26 weeks were used. As reported in Table 6-2, there were no differences observed in the adjusted ROWL at 6 weeks and the PWL at 26 weeks in all three groups.

2.6 DISCUSSION

Here we demonstrate that individuals with T2DM, compared to individuals with IFG or without T2DM, lose less weight in this standardized clinical meal-replacement and lifestyle modification program. No differences were observed at the 6-week point. It is likely that this is related to the reduced need for medications, such as insulin and sulfonylureas, which are associated with weight gain. Typically, dosages are decreased or stopped altogether as patients begin the hypocaloric meal replacement phase of the program and acutely lose weight.

Strengths of this study include the analysis of weight loss in patients with IFG and T2DM in the largest cohort of patients studied to-date. It is the first study to examine ROWL and PWL, adjusted for factors known to impact weight loss, and to examine differences among patients on various pharmacotherapy regimens.

Previous to this current work, Li *et al*. had published the largest study comparing weight loss in diabetic and non-diabetic groups (Li *et al.*, 2014). By analyzing data retrospectively, they concluded that patients with T2DM lose weight as effectively as non-diabetic and pre-diabetic patients at 1-, 3-, 6-, and 12-months after the initiation of a meal-replacement program. Their model for calculating the rate of weight loss (kg/week) did not

however account for various medication regimens that groups of patients were taking. Furthermore, the same rigid criteria for removing patients on the basis of adherence and concomitant morbidities was not applied. In the current work, ROWL and PWL values were collected in a prospective manner, patients were excluded for adherence issues and weight-affecting comorbidities, and medications known to affect weight were examined.

Limitations of the current study include the fact that study participants were not randomly selected, but were seeking treatment for obesity. Furthermore, patients pay between CAD2000 - 3000 to complete the program. This may have inherently selected patients who were more serious about adherence and making lifestyle changes. In addition, this study focuses on the active weight loss period, the first 6–12 weeks and the early maintenance phase (26 weeks) and as such gives no information about weight stability over a longer period of time.

In conclusion, our results demonstrate that individuals with T2DM, compared to individuals with IFG or without T2DM, have impaired weight loss when participating in a medically supervised hypocaloric diet and lifestyle modification program. While effect sizes are statistically significant, they rank after sex and initial weight in clinical significance. Individuals with T2DM on medications that cause weight gain were additionally shown to exhibit similar weight loss responses to those with T2DM who are not taking any medication. These findings are relevant to the design of personalized treatment programs for patients with obesity and T2DM and in counseling patients in goal setting for weight treatment.

2.7 REFERENCES

Antoun, G., McMurray, F., Thrush, A.B., Patten, D.A., Peixoto, A.C., Slack, R.S., McPherson, R., Dent, R., and Harper, M.E. (2015). Impaired mitochondrial oxidative phosphorylation and supercomplex assembly in rectus abdominis muscle of diabetic obese individuals. Diabetologia *58*, 2861-2866.

Baker, S.T., Jerums, G., Prendergast, L.A., Panagiotopoulos, S., Strauss, B.J., and Proietto, J. (2012). Less fat reduction per unit weight loss in type 2 diabetic compared with nondiabetic obese individuals completing a very-low-calorie diet program. Metabolism *61*, 873-882.

Dent, R.M., Penwarden, R.M., Harris, N., and Hotz, S.B. (2002). Development and evaluation of patient-centered software for a weight-management clinic. Obes Res *10*, 651-656.

Guare, J.C., Wing, R.R., and Grant, A. (1995). Comparison of obese NIDDM and nondiabetic women: short- and long-term weight loss. Obes Res *3*, 329-335.

Harper, M.E., Dent, R., Monemdjou, S., Bezaire, V., Van Wyck, L., Wells, G., Kavaslar, G.N., Gauthier, A., Tesson, F., and McPherson, R. (2002). Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. Diabetes *51*, 2459-2466.

Henry, R.R., Wiest-Kent, T.A., Scheaffer, L., Kolterman, O.G., and Olefsky, J.M. (1986). Metabolic consequences of very-low-calorie diet therapy in obese non-insulin-dependent diabetic and nondiabetic subjects. Diabetes *35*, 155-164.

Li, Z., Tseng, C.H., Li, Q., Deng, M.L., Wang, M., and Heber, D. (2014). Clinical efficacy of a medically supervised outpatient high-protein, low-calorie diet program is equivalent in prediabetic, diabetic and normoglycemic obese patients. Nutr Diabetes *4*, e105.

Paisey, R.B., Harvey, P., Rice, S., Belka, I., Bower, L., Dunn, M., Taylor, P., Paisey, R.M., Frost, J., and Ash, I. (1998). An intensive weight loss programme in established type 2 diabetes and controls: effects on weight and atherosclerosis risk factors at 1 year. Diabet Med *15*, 73-79.

Rolland, C., Lula, S., Jenner, C., Dyson, L., Macdonald, I., Johnston, K.L., and Broom, I. (2013). Weight loss for individuals with type 2 diabetes following a very-low-calorie diet in a community-based setting with trained facilitators for 12 weeks. Clin Obes *3*, 150-157.

Wing, R.R., Marcus, M.D., Epstein, L.H., and Salata, R. (1987). Type II diabetic subjects lose less weight than their overweight nondiabetic spouses. Diabetes Care *10*, 563-566.

2.8 TABLES

Table 2-1 - Weight loss for patients without T2DM, patients with impaired fasting glucose, and patients with T2DM at 6 and 26 weeks following the initiation of a meal replacement and lifestyle modification program.

Total (n=2231)	Patients without T2DM (n=1667)	Impaired Fasting Glucose (n=225)	Patients with T2DM (n=339)	
Males (n)	369	67	128	3.267×10^{-09}
Female (n)	1298	158	211	
				P value (comparing all three groups)
Age (years)	43.4 +/- 10.8	50.3 +/- 9.2	51.2 +/- 9.6	$< 2.2\times10^{-16}$
Height (m)	1.67 +/- 0.086	1.67 +/- 0.093	1.68 +/- 0.100	0.120
Weight (kg)	115.9 +/- 24.9	122.9 +/- 28.0	126.0 +/- 25.8	3.959×10^{-11}
Body Mass Index (kg/m2)	41.4 +/- 7.6	43.8 +/- 8.7	44.4 +/- 7.7	1.232×10^{-11}
HbA1C (%)	5.32 +/- 0.29	5.93 +/- 0.37	7.32 +/- 1.30	$< 2.2\times10^{-16}$
ROWL 6 weeks	4.100 +/- 1.28	4.329 +/- 1.51	4.434 +/- 1.50	0.0002
PWL 26 weeks	0.191 +/- 0.06	0.192 +/- 0.06	0.180 +/- 0.06	0.013
Data adjusted for age and sex				
ROWL 6 weeks	4.160 +/- 0.24	4.203 +/- 0.26	4.200 +/- 0.27	0.005746
PWL 26 weeks	0.203 +/- 0.31	0.200 +/- 0.31	0.125 +/- 0.33	0.001
Data adjusted for age, sex, and initial weight				
ROWL 6 weeks	4.104 +/- 0.19	4.096 +/- 0.21	4.083 +/- 0.23	0.2581
PWL 26 weeks	0.205 +/- 0.31	0.195 +/- 0.31	0.118 +/- 0.33	0.0002
Data are means +/- standard deviation.				

Table 2-2 - Joint effect of baseline characteristics on the rate of weight loss. Linear regression analysis was conducted to find the significant predictors of weight loss; the null hypothesis tested was that the regression coefficient was equal to zero and $P < 0.05$ was used to reject the null-hypothesis.

	ROWL 6 weeks			PWL 26 weeks		
Trait	Beta	SE	P value	Beta	SE	P value
Sex	-1.202	0.0435	$< 2\times10^{-16}$	-3.062×10^{-02}	3.281×10^{-03}	$< 2\times10^{-16}$
Initial Weight	0.0129	0.0003	$< 2\times10^{-16}$	7.566×10^{-05}	2.527×10^{-05}	0.0028
Age	-0.010	0.0016	1.5×10^{-10}	-1.230×10^{-04}	1.216×10^{-04}	0.3121
T2DM	-0.028	0.0385	0.473	-9.638×10^{-03}	2.865×10^{-03}	0.0008

3 CHAPTER 3 - IMPAIRED MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION AND SUPERCOMPLEX ASSEMBLY IN *RECTUS ABDOMINIS* MUSCLE OF DIABETIC OBESE INDIVIDUALS

Impaired Mitochondrial Oxidative Phosphorylation and Supercomplex Assembly in *Rectus Abdominis* Muscle of Diabetic Obese Individuals

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3.1.4 CONTRIBUTION STATEMENT

All authors contributed to the conception and design of the study. GA, FM, ABT and RD acquired the clinical data; GA, FM, ABT, DAP and ACP generated respirometry data and performed all laboratory work; data were analyzed and interpreted by GA, FM, ABT, DAP, ACP, RSS, RM and M-EH. The manuscript was drafted by GA, FM, ABT, DAP,

ACP, RSS and M-EH and critical revisions were performed by all authors. All authors have approved the final version. GA and M-EH are the guarantors of this work.

3.1.5 DUALITY OF INTEREST STATEMENT

The authors declare that there is no duality of interest associated with this manuscript.

3.2 ABSTRACT

3.2.1 AIMS/HYPOTHESIS

Skeletal muscle mitochondrial dysfunction has been documented in patients with type 2 diabetes mellitus; however, specific respiratory defects and their mechanisms are poorly understood. The aim of the current study was to examine oxidative phosphorylation and electron transport chain (ETC) supercomplex assembly in *rectus abdominis* muscles of 10 obese diabetic and 10 obese non-diabetic individuals.

3.2.2 METHODS

Twenty obese women undergoing Roux-en-Y gastric bypass surgery were recruited for this study. Muscle samples were obtained intraoperatively and subdivided for multiple analyses, including high-resolution respirometry and assessment of supercomplex assembly. Clinical data obtained from referring physicians were correlated with laboratory findings.

3.2.3 RESULTS

Participants in both groups were of a similar age, weight and BMI. Mitochondrial respiration rates were markedly reduced in diabetic vs non-diabetic patients. This defect was observed during maximal ADP-stimulated respiration in the presence of complex I-linked substrates and complex I- and II-linked substrates, and during maximal uncoupled respiration. There were no differences in fatty acid (octanoyl carnitine) supported respiration, leak respiration or isolated activity of cytochrome c oxidase. Intriguingly, significant correlations were found between glycated haemoglobin (HbA1c) levels and maximal respiration or respiration supported by complex I, complex I and II or fatty acid. In the muscle of diabetic patients, blue native gel electrophoresis revealed a striking decrease in complex I, III and IV containing ETC supercomplexes.

3.2.4 CONCLUSIONS/INTERPRETATION

These findings support the hypothesis that ETC supercomplex assembly may be an important underlying mechanism of muscle mitochondrial dysfunction in type 2 diabetes mellitus.

3.3 INTRODUCTION

Type 2 diabetes mellitus is characterised by insulin resistance in a variety of tissues. Skeletal muscle is normally accountable for 80–90% of postprandial glucose uptake, but it becomes a major site of insulin resistance during the development of type 2 diabetes mellitus. Mitochondrial dysfunction in muscle has been the focus of many studies, some of which have identified altered mitochondrial morphology (Kelley *et al.*, 2002) and downregulation of genes involved in oxidative phosphorylation (e.g. peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PGC1α]-responsive genes) (Mootha *et al.*, 2003). Metabolic inflexibility, or the inability to optimally switch between carbohydrate and fatty acid fuel sources (Kelley, 2005), and impairments in mitochondrial electron transport chain (ETC) components (Kelley *et al.*, 2002; Ritov *et al.*, 2010) have also been described in the muscle of patients with type 2 diabetes mellitus. Recently, DeLany et al demonstrated dysfunctional mitochondrial respiration before the appearance of any clinical characteristics of type 2 diabetes mellitus in a population of African-American women (DeLany *et al.*, 2014). Despite these clear associations between mitochondrial dysfunction and the risk of developing type 2 diabetes mellitus, the underlying mechanisms in human skeletal muscle are poorly understood.

Here we report impaired skeletal muscle mitochondrial energetics in individuals with type 2 diabetes mellitus and show that this is significantly correlated with circulating levels of glycated haemoglobin (HbA1c). Moreover, we demonstrate that there are corresponding decreases in the formation of mitochondrial ETC supercomplexes. Since ETC supercomplexes can regulate mitochondrial respiration (Lapuente-Brun *et al.*, 2013), our findings suggest these may represent important mechanistic players of disordered skeletal muscle metabolism in the aetiology of type 2 diabetes mellitus.

3.4 METHODS

Twenty female patients from the Ottawa Hospital Weight Management Clinic were recruited after being confirmed as candidates for bariatric surgery. The study was approved by the Ottawa Hospital Ethics Board and written informed consent was obtained from each patient prior to study participation. Patients were included in this study based on their availability, willingness to participate and eligibility for gastric bypass surgery. They were recruited randomly as part of an ongoing sample collection of consenting individuals referred for bariatric surgery. No a priori selection was made based on medications taken by patients in each group. There were no differences between groups in the use of other commonly prescribed agents for BP, depression, gastroesophageal reflux and dyslipidemia. Diabetes medications included metformin, sulfonylureas (e.g. gliclazide), dipeptidyl peptidase 4 (DPP-4) inhibitors (e.g. sitagliptin), glucagon-like peptide-1 (GLP-1) agonists (e.g. liraglutide), sodium/glucose cotransporter 2 (SGLT2) inhibitors (e.g. canagliflozin) and various insulin derivatives (e.g. detemir [B29Lys(ε-tetradecanoyl),desB30 human insulin], glargine [A21Gly,B31Arg,B32Arg human insulin] and aspart [B28Asp human insulin] insulins). All medications were discontinued on the day of surgery and blood glucose levels were measured. Diabetic patients received intravenous dextrose and/or insulin as necessary.

Exclusion criteria included symptomatic coronary artery disease, inflammatory bowel disease (Crohn's) and oesophageal varices. Patients were also excluded if they were aged over 65 years or if their HbA1c levels were >8.0% (64 mmol/mol). Prior to surgery, all patients underwent routine preparations for Roux-en-Y gastric bypass including OPTIFAST® 900 meal replacement for 3 weeks. Patients fasted for at least 8 h prior to the surgery and sample collection. No muscle-conditioning programme was prescribed to patients and exercise was limited, given their very high BMIs (~50 kg/m^2).

Rectus abdominis samples were obtained during Roux-en-Y gastric bypass surgery. Samples were divided for multiple analyses including high-resolution respirometry, assessment of ATP synthase and ETC supercomplex assembly by blue native polyacrylamide gel electrophoresis and measurement of individual components by traditional Western blotting. Analysis of mitochondrial function by high-resolution respirometry was performed using the Oxygraph-2k modular system for high-resolution respirometry (OROBOROS Instruments, Innsbruck, Austria), as previously described (Pesta and Gnaiger, 2012). For additional details on methods, please refer to Section 6.2.1 Supplementary Methods.

Statistical analysis: GraphPad Prism 6 (La Jolla, CA, USA) was used to perform Unpaired Student's *t* tests and regression analyses, and data are presented as means±SEM.

3.5 RESULTS

Baseline characteristics of all participants are outlined in Table 6-3. There were no differences in mean age, weight, BMI and percentage body fat of participants in the obese non-diabetic and obese diabetic groups. All patients had a BMI >40 kg/m^2 and HbA1c values were significantly higher in diabetic patients (5.5±0.1% [36.8±1.6 mmol/mol] vs 7.3± 0.2% [55.7±2.3 mmol/mol], $p<0.001$).

In order to assess differences in mitochondrial energetics, high-resolution respirometry was performed on permeabilised *rectus abdominis* muscle fibers. Respiration was decreased in muscle samples from diabetic patients (Figure 3-1). This was observed across a variety of phosphorylating respiratory states (state 3), including in the presence of complex I linked substrates (P_{CI}; 20.7±2.5 vs 11.8±2.2 pmol/[s×mg wet weight], p=0.02; Figure 3-1a) and complex I and II linked substrates (P_{CI+CII}; 50.4±4.1 vs 35.0±2.2 pmol/[s×mg wet weight], p=0.004; Figure 3-1b). Maximal respiration in the presence of the

uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) was also significantly lower in diabetic patients (U_C; 57.1±5.3 vs 38.4±2.7 pmol/[s×mg wet weight], p=0.006; Figure 3-1c). There was a trend toward lower fatty acid supported respiration in the presence of electron transfer flavoprotein (ETF) linked substrates, such as octanoyl carnitine (P_{ETF}; Figure 3-1d). No differences were observed in non-phosphorylating leak respiration in the absence of ADP (L_N; Figure 6-1a) or in the presence of oligomycin, an ATP synthase (complex V) inhibitor (L_{Omy}; Figure 6-1b). Further normalisation of data to the activity of cytochrome c oxidase, as assessed by N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD; Tm; Figure 6-1c), did not alter the results (Figure 6-2).

A significant inverse correlation was found between circulating levels of HbA1c and respiration through complex I (P_{CI}; Figure 3-1e), complex I and II (P_{CI+CII}; Figure 3-1f), maximal uncoupled respiration (U_C; Figure 3-1g) and fatty acid oxidative capacity (P_{ETF}; Figure 3-1h). HbA1c was also negatively associated with adenylate-free leak respiration (L_N; Figure 6-1d; p=0.051). In contrast, no correlation was observed between HbA1c and oligomycin-induced leak respiration (L_{Omy}; Figure 6-1e) and mitochondrial content (Tm; Figure 6-1f). When data were analysed in the same manner, but using data from the diabetic women only, maximal uncoupled respiration was the only respiration type to reach near significance (p=0.0674).

In order to further investigate the causes of reductions in mitochondrial respiration, higher order ETC supercomplex assembly was assessed (Figure 3-2). There was less supercomplex formation in the muscle of diabetic patients (Figure 3-2a). This was consistently observed when the subunits of complex I (Figure 3-2b), complex III (Figure 3-2d) and complex IV (Figure 3-2e) were analysed separately. ATP synthase and complex II do not participate in mammalian ETC supercomplex formation, and were similar in both

patient groups (Figure 3-2c, f). For this reason, all protein levels were normalised to the complex II monomer level. Additionally, no differences were observed in the absolute amount of any complex as assessed by reducing SDSPAGE blots (Figure 3-2g, h). This suggests that reductions in supercomplex assembly do not correlate with changes in the total levels of ETC complexes.

3.6 DISCUSSION

Previous reports have examined mitochondrial dysfunction in obese type 2 diabetes mellitus patients (Kelley *et al.*, 2002; Mogensen *et al.*, 2007; Ritov *et al.*, 2010; Ritov *et al.*, 2005). Our results confirm the presence of mitochondrial dysfunction and significantly extend previous findings by characterising the various states of mitochondrial energetics in intact permeabilised human muscle fibers and assembly of ETC supercomplexes. This constitutes the first report of altered mitochondrial supercomplex assembly in type 2 diabetes mellitus and of mitochondrial energetics in permeabilised *rectus abdominis* muscle fibers. We also identify novel correlations between human muscle mitochondrial energetics (including fatty acid metabolism) and circulating HbA1c levels.

Previous reports linking dysfunctional mitochondrial energetics with type 2 diabetes mellitus are limited, and many have been based upon analyses of the maximum velocity (V_{max}) enzyme activities of individual ETC proteins, as measured in frozen muscle samples (Kelley *et al.*, 2002; Ritov *et al.*, 2010; Ritov *et al.*, 2005) or permeabilised mitochondria (Mogensen *et al.*, 2007). Our ex vivo methods in which mitochondrial energetics are analysed in permeabilised muscle fibers are arguably the least disruptive to the in vivo mitochondrial network. Overall, the results presented herein agree with previous findings and demonstrate substantial reductions in the different respiratory states, including fatty acid

supported and maximal respiration. Previous studies have been conducted using *vastus lateralis* muscle samples from obese individuals with considerably lower BMIs (<35 kg/m^2), most often in men (Boushel *et al.*, 2007; Mogensen *et al.*, 2007; Ritov *et al.*, 2005), whereas the current study focuses on differences observed in the *rectus abdominis* muscle of women with a much higher BMI (Table 6-3). These differences in studied muscle groups and patient populations may partly explain why the findings presented herein contradict the findings of Boushel et al (Boushel *et al.*, 2007). A further explanation for these discrepancies could be that the data presented here are normalised against COX activity within the same sample, whereas Boushel et al (Boushel *et al.*, 2007) normalised data to citrate synthase activity measured in a separate sample of whole muscle lysate. Taken together, these results support the idea that deficiencies in muscle mitochondrial respiration are global, surpassing the boundaries of the specific muscle group tested and the sex of the affected individual.

As mitochondrial content can substantially impact respiratory capacity, it was assessed by quantification of protein components of individual respiratory complexes (Figure 3-2g). Consistent with previous work (Boushel *et al.*, 2007; Mogensen *et al.*, 2007), we observed no differences in mitochondrial content of muscle between diabetic and nondiabetic patients. Additionally, functional analysis of complex IV (cytochrome c oxidase) maximal activity (Figure 6-1c), which is sometimes used as a proxy measure of mitochondrial content, revealed no differences between the diabetic and nondiabetic groups.

While investigating the mechanism of this mitochondrial dysfunction, we discovered pronounced reductions in mitochondrial supercomplex assembly. Others have demonstrated that reductions in mitochondrial function precede the development of obesity and type 2 diabetes mellitus in a high-risk population (DeLany *et al.*, 2014). These findings were significant across a variety of respiratory states, leading us to hypothesise that supercomplex

formation may be inherently altered in high-risk populations. The association of ETC proteins into supercomplexes has been proposed to promote electron flow through the ETC, which in turn supports augmented respiration rates (Lapuente-Brun *et al.*, 2013).

Supercomplex formation is an as yet poorly understood process that is affected by factors such as cristae structure, membrane integrity and levels of specific proteins including ubiquinol-cytochrome-c reductase complex assembly factor 3 (C11ORF83) and cytochrome c oxidase subunit 7A-related protein, mitochondrial (COX7RP). An additional protein affecting cristae structure and ETC supercomplex assembly is mitofilin, a component of the mitochondrial contact site and cristae organising system (MICOS). Interestingly, overexpression of mitofilin was shown to rescue deficiencies in respiration in a mouse model of type 1 diabetes mellitus, although rescue of ETC supercomplex assembly was not investigated (Thapa *et al.*, 2015). Furthermore, the cardiolipin content of the mitochondrial inner membrane has been shown to affect mitochondrial function and supercomplex formation, and may be involved in the pathophysiology of diabetes (Ritov *et al.*, 2010). Future studies are required to determine the causes of the supercomplex dysfunction observed in this study.

In conclusion, we report extensive mitochondrial dysfunction in *rectus abdominis* muscle of obese diabetic women. Mitochondrial respiration negatively correlated with circulating levels of HbA1c. Furthermore, dysfunctional mitochondrial energetics in muscle of diabetic patients were linked to reductions in the assembly of mitochondrial supercomplexes, suggesting that this is an important mechanism for development of the disease. Future studies will be required to delineate approaches that could reverse this dysfunction.

3.7 REFERENCES

Boushel, R., Gnaiger, E., Schjerling, P., Skovbro, M., Kraunsoe, R., and Dela, F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia *50*, 790-796.

DeLany, J.P., Dube, J.J., Standley, R.A., Distefano, G., Goodpaster, B.H., Stefanovic-Racic, M., Coen, P.M., and Toledo, F.G. (2014). Racial differences in peripheral insulin sensitivity and mitochondrial capacity in the absence of obesity. J Clin Endocrinol Metab *99*, 4307-4314.

Kelley, D.E. (2005). Skeletal muscle fat oxidation: timing and flexibility are everything. J Clin Invest *115*, 1699-1702.

Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944-2950.

Lapuente-Brun, E., Moreno-Loshuertos, R., Acin-Perez, R., Latorre-Pellicer, A., Colas, C., Balsa, E., Perales-Clemente, E., Quiros, P.M., Calvo, E., Rodriguez-Hernandez, M.A.*, et al.* (2013). Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. Science *340*, 1567-1570.

Mogensen, M., Sahlin, K., Fernstrom, M., Glintborg, D., Vind, B.F., Beck-Nielsen, H., and Hojlund, K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes *56*, 1592-1599.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E.*, et al.* (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet *34*, 267-273.

Pesta, D., and Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods in molecular biology (Clifton, NJ) *810*, 25-58.

Ritov, V.B., Menshikova, E.V., Azuma, K., Wood, R., Toledo, F.G., Goodpaster, B.H., Ruderman, N.B., and Kelley, D.E. (2010). Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab *298*, E49-58.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H., and Kelley, D.E. (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes *54*, 8-14.

Thapa, D., Nichols, C.E., Lewis, S.E., Shepherd, D.L., Jagannathan, R., Croston, T.L., Tveter, K.J., Holden, A.A., Baseler, W.A., and Hollander, J.M. (2015). Transgenic overexpression of mitofilin attenuates diabetes mellitus-associated cardiac and mitochondria dysfunction. J Mol Cell Cardiol *79*, 212-223.

3.8 FIGURES

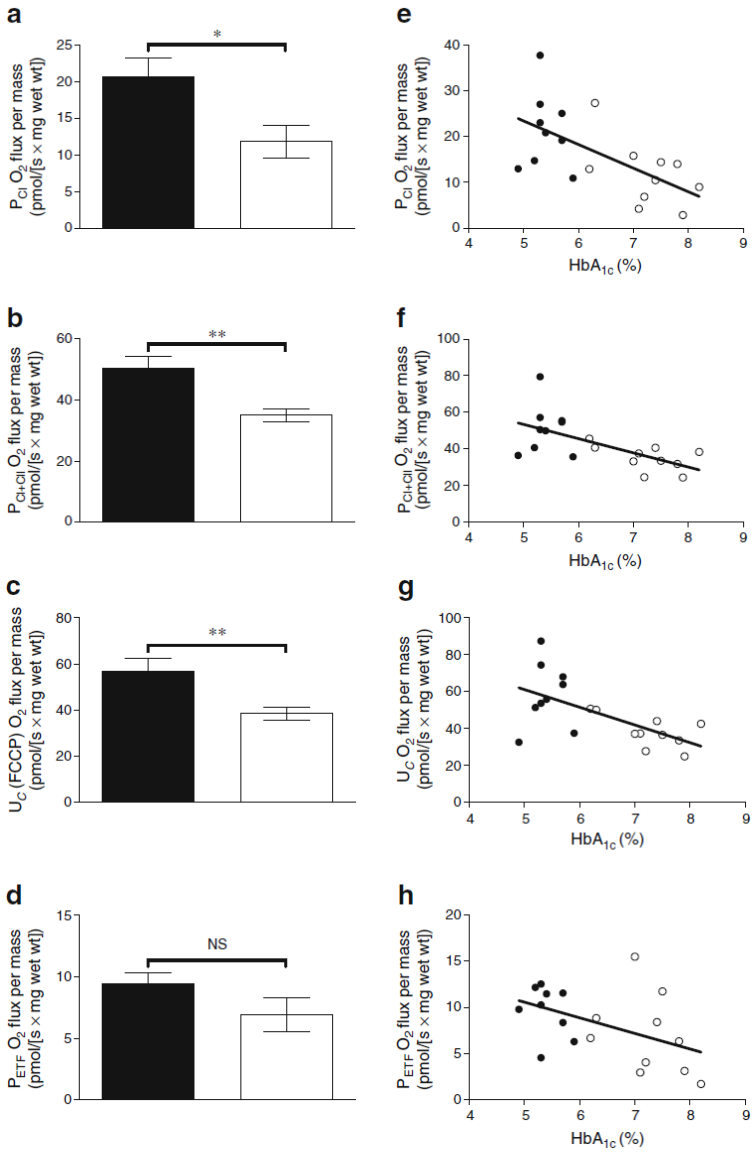

Figure 3-1 - Mitochondrial energetics are altered in permeabilised rectus abdominis *muscle fibers from obese diabetic women compared with obese non-diabetic women, and correlate significantly with HbA1c levels.*

Data are shown for state 3 respiratory capacity through (a, e) complex I (P_{CI}), (b, f) maximal oxidative phosphorylation capacity (P_{CI+CII}), (c, g) maximal uncoupled respiration in the presence of the chemical protonophore carbonylcyanide-ptrifluoromethoxyphenylhydrazone (U_C), and (d, h) maximal electron flow through electron-transferring flavoprotein and fatty acid oxidative capacity (P_{ETF}). (a–d) All values are mean ± SEM, n=10 non-diabetic patients (black bars) and n=10 diabetic patients (white bars). *p < 0.05, **p< 0.01 for the unpaired Student's t test; (e) p = 0.0042, R^2 = 0.3910; (f) p = 0.0034, R^2 = 0.4045; (g) p = 0.0044, R^2 = 0.3886; (h) p = 0.0405, R^2 = 0.2243. (e–h) All data were obtained by linear regression analyses, n=9 non-diabetic patients (black points) and n=10 diabetic patients (white points). To convert values for HbA1c in % into mmol/mol, subtract 2.15 and multiply by 10.929

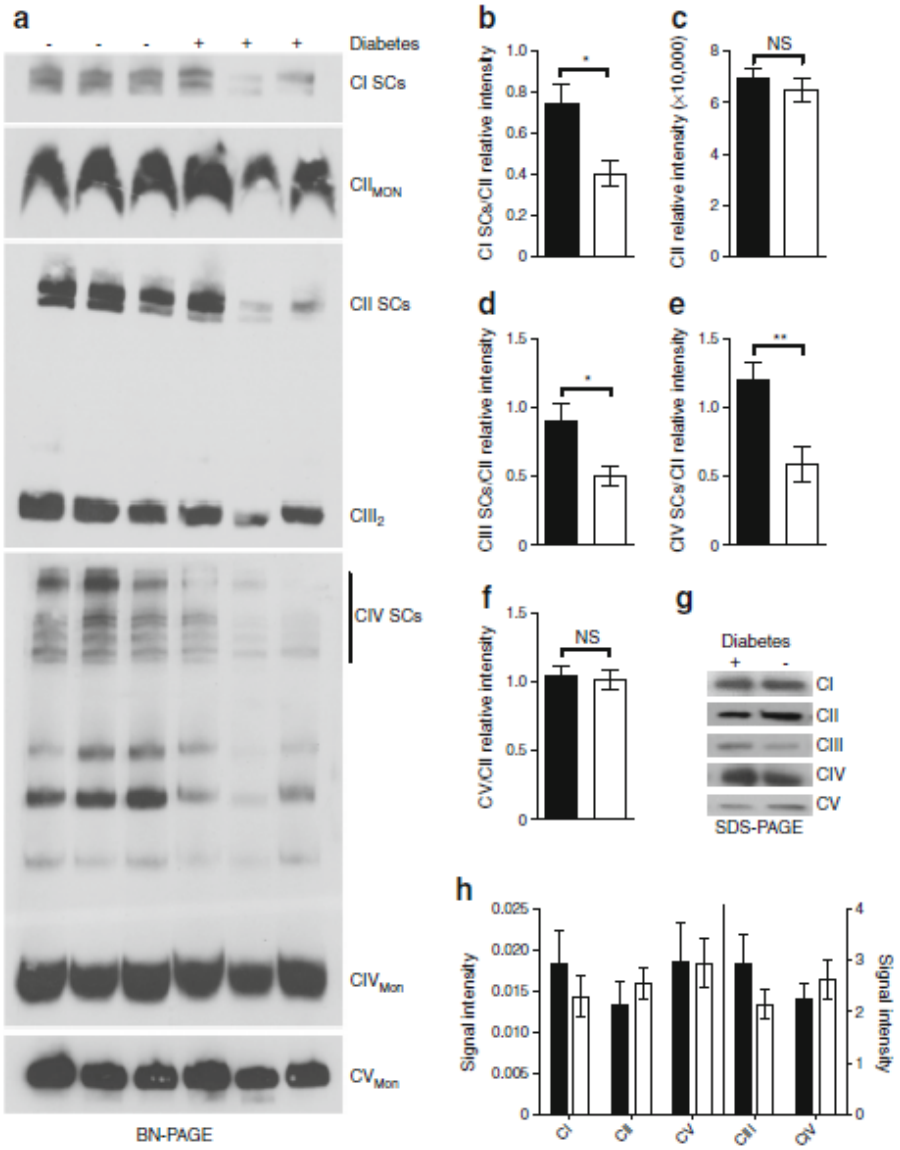

Figure 3-2 - Supercomplex assembly is altered in rectus abdominis *muscle samples from obese diabetic women compared with obese non-diabetic women.*

(a) Representative blue native polyacrylamide gel (BN-PAGE) blot of the indicated respiratory complexes (CI–V), supercomplexes (SCs), and monomers (Mon) using anti-NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial (NDUFA9; complex I), anti-flavoprotein (complex II), anti-ubiquinol-cytochrome c reductase core protein II (UQCRC2; complex III), anti-complex IV subunit I (complex IV) and anti-ATP synthase subunit alpha, mitochondrial (ATP5a; complex V) antibodies. (b–f) Quantification of expression of the indicated respiratory supercomplex normalised to complex II monomer levels. Complex II monomer levels in (c) were normalised to Ponceau S staining. n=6 non-diabetic patients (black bars) and n=7 diabetic patients (white bars). (g) Representative western blot of the indicated respiratory complexes using anti-NDUFA9 (complex I), anti-flavoprotein (complex II), anti-complex III subunit core 1 (complex III), anti-complex IV subunit I (complex IV) and anti-ATP5a (complex V) antibodies. (h) Quantification of the indicated respiratory complexes (total levels). n=5 nondiabetic patients (black bars) and n=7 diabetic patients (white bars). All values are the mean ± SEM. *p<0.05, **p<0.01 for the unpaired Student's t test

4 CHAPTER 4 - DISTINCT PLASMA PROTEOMIC SIGNATURES AND MULTIPLE IMPAIRMENTS IN MUSCLE FIBER ENERGETICS IN DIET RESISTANT OBESITY

Distinct plasma proteomic signatures and multiple impairments in muscle fiber energetics in diet resistant obesity

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4.1 STATEMENT OF MANUSCRIPT STATUS AND CONTRIBUTIONS

4.1.1 STATEMENT OF MANUSCRIPT STATUS

The manuscript "Distinct plasma proteomic signatures and multiple impairments in muscle fiber energetics in diet resistant obesity" has been submitted and is under review in the journal Cell Metabolism. Manuscript ID CELL-METABOLISM-D-16-00197.

4.1.2 AUTHOR CONTRIBUTIONS

Experimental Work: ABT, GA, JFM, DP, BLB, PL; Data Analysis: ABT, GA, MN, JFM, DP, PI, AV, OYA; Interpretation of Results: ABT, DP, ED, PI, RB, RD, RM, MEH; Conception of Ideas: ABT, ED, PI, RB, JH, RD, RM, MEH; Manuscript Writing: All authors.

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4.2 ABSTRACT

Inter-individual variability in weight loss during obesity treatment is a complex and poorly understood phenomenon. A better understanding of factors involved will lead to personalized therapeutic approaches. Here we focus on diet resistance and identify deficiencies in fuel oxidation characteristics in skeletal muscle and distinct circulating protein biomarkers in fasting and following a high fat meal challenge in obese diet-resistant (ODR), compared to matched obese diet-sensitive (ODS) women. Subjects previously completed a standardized clinical weight management program and were weight stable at the time of the analyses. *Vastus lateralis* biopsies, subcutaneous adipose tissue and plasma samples were obtained before and 6 h following a high fat meal (HFM). To comprehensively analyze bioenergetics in the intact mitochondrial reticulum in situ, mitochondrial metabolism was assessed in permeabilized muscle fibers by high-resolution respirometry. Fatty acid (FA) supported respiration and maximal oxidative phosphorylation were significantly greater in fibers of ODS vs. ODR women under baseline fasting conditions and following the HFM. Unbiased plasma proteomic analyses demonstrated higher tyrosine protein-kinase Fgr (FGR), aryl-hydrocarbon receptor-interacting protein (AIP), heat shock protein 72 (HSP72) and pepditylpropyl isomerase D (PPID) in ODS vs. ODR subjects. Furthermore, HSP72 was positively correlated with mitochondrial oxidation, citrate synthase activity and HOMA-IR, thus making it a potential biomarker of diet sensitivity. Altogether, our findings demonstrate that muscle mitochondrial oxidative metabolism is greater in ODS than ODR individuals during fasting and in response to a HFM and reveal a clear link to circulating levels of HSP72 protein.

4.3 INTRODUCTION

Weight loss response in clinical obesity treatment programs is highly variable. There is a growing body of evidence supporting a biological basis to diet resistance that relates to inherent differences in oxidative metabolism, and/or adaptation to energy restriction (Bouchard *et al.*, 1990; Doucet *et al.*, 2001; Gerrits *et al.*, 2010; Leibel *et al.*, 1995; Rosenbaum and Leibel, 2010; Thrush *et al.*, 2014; Tremblay *et al.*, 2015). The phenomenon of diet-resistance in obesity has also been characterized in animal models (Levin and Dunn-Meynell, 2000) and recent work has shown that diet resistance in mice can be due in part to metabolic programming in utero (Beauchamp *et al.*, 2014).

Our previous analyses of 3450 patient records in the Ottawa Hospital Weight Management Program have documented a 2-3 fold difference in weight loss response in diet adherent patients even after controlling for factors known to affect rate of weight loss (e.g., age, sex, initial weight, thyroid status, medication, movement disorders or disabilities) (Dent *et al.*, 1999; Gerrits *et al.*, 2010; Harper *et al.*, 2002; Thrush *et al.*, 2013). We have thus been interrogating the unknown biological factors differentiating patients who lose weight very quickly from age-, sex- and initial body weight- matched patients who lose weight very slowly. We define those in the upper quintile for weight loss in the first 6 weeks of the clinical meal-replacement program as obese diet-sensitive (ODS), those in the lower quintile for weight loss as obese diet-resistant (ODR) groups. We previously reported that ODS patients have a higher proportion of type I oxidative muscle fibers; muscle fiber hypertrophy; increased expression of genes involved in oxidative metabolism; higher proton leak uncoupling in mitochondria isolated from *rectus femoris* muscle, and higher proton leak uncoupling and lower glutathione redox in primary myotubes from distinct groups of patients

(Gerrits *et al.*, 2010; Harper *et al.*, 2002; Thrush *et al.*, 2014). We also observed that the expression of OXPHOS genes in samples of whole blood that were taken prior to any diet intervention was predictive of weight loss success in the clinical weight loss program (Ghosh *et al.*, 2011). Altogether these findings are consistent with the hypothesis that ODS have a higher capacity for oxidative phosphorylation compared to ODR; however, this has not been thoroughly investigated in intact muscle preparations in which the mitochondrial reticulum is preserved, or from the perspective of circulating protein biomarkers. Clearly, the identification of circulating biomarkers of diet-resistance would allow rapid initiation of personalized treatment strategies (e.g., exercise and diet, or bariatric surgery).

Many studies have associated impaired FA metabolism and oxidative stress with obesity (Aguer *et al.*, 2015; Anderson *et al.*, 2009; Bonen *et al.*, 2004; Thrush *et al.*, 2014). The maximal activity of FA metabolizing enzymes have also been shown to be predictive of 24 hr energy expenditure (Doucet *et al.*, 2003). Nonetheless, a key unanswered question is whether the response to weight loss interventions is associated with a greater capacity for FA metabolism in the fasting or postprandial states. Here we aimed to interrogate aspects of whole body and skeletal muscle oxidative metabolism in ODS and ODR individuals under resting fasting conditions and in response to a defined high fat meal (HFM). Given the previously published phenotypes in these clinical cohorts, we hypothesized that oxidative characteristics and capacities would be higher in ODS than those in ODR, consistent with the possibility that ODS individuals are protected against dietary fat induced oxidative stress. Given the previous identification of distinct circulating mRNA transcript profiles between the groups prior to any weight loss, we also hypothesized that distinct circulating proteins distinguish the groups and be relevant for diagnostic test development.

Our findings here show that many measures of oxidative metabolism in skeletal muscle are indeed higher in ODS than ODR individuals when biopsies were obtained under resting fasting conditions, and in response to a HFM. Intriguingly, these differences are not associated with differences in mitochondrial content or mitochondrial supercomplex assembly. Plasma proteomics analyses revealed clear group differences in circulating proteins in the fasted state, and in response to the HFM. These proteins include heat shock protein 70 1A/1B (HSP72), which is associated with protection from obesity and insulin resistance (Chung *et al.*, 2008). Not only do we identify possible mechanistic origins of diet resistance but we also identify potential biomarkers that may be useful in predicting weight loss success.

4.4 RESULTS

In this work we aimed to investigate mechanisms associated with the phenomenon of weight loss variability in obesity. We examined whole body, plasma, tissue and mitochondrial bioenergetics differences in rigorously matched ODS and ODR women. We also used a high fat meal challenge to further probe phenotypic and mechanistic differences.

4.4.1 PATIENT ANTHROPOMETRY AND WEIGHT LOSS CHARACTERISTICS

Prior to enrollment in the clinical weight loss program, and at the time of biopsy, there were no differences in age, body mass or BMI between ODS and ODR women, indicating that subjects in the two groups were well matched (Table 4-1). Following 6 weeks of dietary restriction, ODS women lost approximately 44% more weight compared to ODR (ODR vs. ODS; 6.9 ± 0.5kg, vs. 11.5 ± 0.6 kg, $P<0.001$). BMI was significantly decreased in ODS but not ODR following 6 weeks of weight loss ($p<0.05$; Table 4-1).

Waist circumference, body fat mass and lean mass were also similar between ODS and ODR at the time of biopsy (Table 4-1). Interestingly, body fat distribution was different between ODS and ODR, with ODS having more upper body fat and less lower body fat compared to ODR ($p<0.05$). Resting VCO2, VO2, respiratory exchange ratio, resting metabolic rate, physical activity and total daily energy expenditure were not significantly different between ODS and ODR (Table 4-1).

To investigate the whole body response to the high fat meal, we assessed plasma glucose, lipids, insulin and whole body energy metabolism in ODS and ODR. Following the HFM, there was an initial drop in plasma FA (0-2h) followed by a plateau and subsequent increase, with no difference between groups (Figure 4-1A, $P<0.0001$ significant effect of time). Plasma TG, glucose and insulin significantly increased following the HFM with no difference between groups. Plasma TG and glucose began to decline at approximately 4h, which coincided with the increase in FA and insulin (Figure 4-1A-C; $P<0.0001$ effect of time). The area under the curve for insulin was larger in ODS from 3-6h although this did not reach significance (ODR vs. ODS, 51.1 ± 7.4 vs. 96.1 ± 27.3, $P=0.12$).

Following the HFM, whole body O2 consumption and CO2 production increased, with no difference between groups (Figure 4-1D,E; $P<0.0001$, effect of time). As expected, FA utilization increased over time, whereas reliance on glucose decreased, with no difference between groups (Figure 4-1F,G; $P<0.01$ effect of time). Total energy expenditure also increased over time, compared to time 0, but this was not different between groups (Figure 4-1H; $P<0.0001$, $^{*}P<0.05$ effect of time vs. time 0). When whole body energetics was expressed per lean body mass, there was no difference between groups (data not shown). In summary, indirect calorimetry results do not reveal differences between ODS and ODR, despite clear effects of the HFM.

4.4.2 MITOCHONDRIAL ENERGETICS IN SKELETAL MUSCLE

We have previously reported differences in mitochondrial function in distinct ODS and ODR groups. Here, to further investigate skeletal muscle energetics we used ex vivo techniques to investigate mitochondrial oxidative phosphorylation processes under various metabolic conditions. We used permeablized muscle fibers in order to assess bioenergetics in intact mitochondria in situ. FA supported respiration in permeabilized *vastus lateralis* fibers was measured with octanoyl carnitine as a substrate. Intriguingly, following the HFM, octanoyl carnitine supported respiration was significantly increased in both groups (Figure 4-2A; $P<0.001$) and tended to be higher in ODS compared to ODR ($P=0.09$ effect of group). There was also a post-meal increase in NADH-driven respiration through Complex I respiration (P_{MPG}), although this occurred in both groups (Figure 4-2B; $P<0.01$, effect of meal). Importantly, the maximal oxidative phosphorylation rate with electron supply to the Q-junction through Complex I, II and ETF (P_{MOPGS}) was significantly increased in ODS compared to ODR (Figure 4-2C; $P<0.05$, effect of group) with a significant difference following the meal between ODS and ODR. Similarly, when oxidative phosphorylation was measured in the absence of octanoyl carnitine (P_{MPGS}) respiration was higher in ODS compared to ODR (Figure 4-2D; $P=0.06$, effect of group). Moreover, when we assessed the maximal capacity of the electron transfer system (ETS) using the chemical uncoupler, FCCP, we found that it was significantly higher in ODS compared to ODR (Figure 4-2E; $P<0.05$, effect of group). Non-phosphorylating respiration was measured in the presence of malate and pyruvate (P_{MP4}), but there was no difference between groups (Figure 6-4A) nor following ATP synthase inhibition with oligomycin (State 4o; Figure 6-4B).

We then sought to determine if the observed differences in mitochondrial energetics in the muscle fibers were related to any differences in mitochondrial content. However, we

found that the expression of oxidative phosphorylation proteins was not different between ODS and ODR (Figure 4-2F). Similarly, we observed no difference in citrate synthase activity (Figure 4-2G) in skeletal muscle or COX activity as assessed in muscle fibers (Figure 4-2I). We also measured β-HAD activity, as a marker of FA oxidative capacity in skeletal muscle, and we observed no difference between ODS and ODR (Figure 4-2H). When we expressed FA supported maximal oxidative phosphorylation and maximal uncoupled respiration per unit COX activity, mitochondrial respiration remained higher in ODS compared to ODR (Figure 4-2J-L, $P<0.05$, effect of group). This suggests that the observed differences in myofiber mitochondrial respiration between ODS and ODR are not due to mitochondrial content but rather to mitochondrial function or regulation.

As the formation of oxidative phosphorylation supercomplexes is thought to promote electron flow through the ETS (Lapuente-Brun *et al.*, 2013), we measured their supercomplex assembly in skeletal muscle of ODS and ODR. There were no differences in supercomplex formation between groups or in response to the HFM (Figure 4-3), consistent with the conclusion that the differences in muscle energetics between groups and following the HFM are not due to changes in ETS supercomplex assembly.

4.4.3 FATTY ACID OXIDATION

To thoroughly investigate FA oxidation in ODR and ODS, we measured plasma acylcarnitine content at baseline and in response to the high fat meal. Short, medium and long chain acylcarnitines changed over time (Figure 4-4A-C, $P<0.01$, effect of time), with an initial increase at 15 minutes. Medium chain acylcarnitine peaks were significantly affected by the high fat meal (Figure 4-4B, ODR 15 min vs. 2,3,4,5 h, $p<0.05$; ODS 15 min vs. 3hr, 5hr, $p<0.05$). Medium chain acylcarnitines peaked again at 6hr in ODR (6hr vs. 3hr, $p<0.05$)

but not in ODS. The area under the curve for long chain acylcarnitines was lower in ODS (P=0.06) suggesting that FA oxidation is more complete in ODS. To investigate this further, we measured FA oxidation in primary myotubes of ODS and ODR patients, and studied the myotubes with and without glucose pretreatment (Figure 4-4D-H). As expected, glucose pre-incubation decreased FA oxidation (Figure 4-4E-H, effect of glucose treatment). Complete, incomplete (ASP in the media + ASP in the cells) and total FA oxidation were not different between groups. Interestingly, in ODR cells, cellular ASP and incomplete FA oxidation did not differ in the presence of glucose as compared to the control condition (Figure 4-4E,F), indicating that ODR myotubes have reduced metabolic flexibility compared to ODS myotubes.

4.4.4 OXIDATIVE STRESS

Previous studies in primary myotubes of ODS and ODR revealed higher glutathione redox in ODS cells (Thrush *et al.*, 2014). Here we therefore measured ROS production and markers of oxidative stress pre and post HFM feeding. Interestingly, ROS emissions were higher in ODS compared to ODR, with no effect of the HFM (Figure 4-5A; $P<0.05$, effect of group). We also assessed ROS emission in myotubes following 24h of 500μM palmitate exposure. Palmitate significantly increased ROS emission (Figure 4-5B; $P<0.01$, significant effect of treatment). Post hoc analysis revealed that ROS emission was significantly increased by palmitate in ODS but not ODR (Figure 4-5B, $P<0.05$), consistent with our observations of increased ROS emission in tissue preparations. Oxidative damage was then assessed by protein carbonylation. There was no evidence of oxidative damage in skeletal muscle between groups or in response to the HFM (Figure 4-5C). Protein carbonylation was

however significantly increased following the HFM in adipose tissue in both groups (Figure 4-5D, $P<0.05$, effect of treatment).

4.4.5 ADIPOSE TISSUE FUNCTION

We also sought to investigate adipose tissue function in ODS and ODR as it is an important regulator of whole body substrate utilization, and as adipocyte dysfunction can lead to overall differences in characteristics of energy metabolism. Adipose tissue dysfunction has not previously been investigated in ODS and ODR individuals. Adipocyte size and mitochondrial content did not differ between ODS and ODR. Basal lipolysis measured in isolated subcutaneous abdominal adipocyte did not differ between groups before and after the HFM. Maximal lipolytic response to isoproterenol (β-adrenoceptor agonist) and maximal antilipolytic response to UK-14304 (α2-adrenoceptor agonist) did not differ between groups before and after the meal. Finally, the activity of adipose tissue LPL activity, a rate limiting enzyme for plasma triglyceride clearance and adipose tissue uptake of fatty acids, was similar between ODS and ODR before and after the HFM. Together, these results suggest that the fatty acid mobilization and storage capacities of abdominal subcutaneous adipose tissue were not different between ODR and ODS (Figure 6-5).

4.4.6 PROTEOMIC MARKERS OF DIET RESPONSE

To identify novel mechanisms governing diet sensitivity and to possibly identify circulating biomarkers of diet responsiveness, we conducted unbiased proteomic analyses in plasma samples collected at baseline (fasting), and at 1, 2, 5 and 6 h after the HFM. Findings revealed significant differences between ODS and ODR individuals at baseline and in response to the HFM, notably for tyrosine protein-kinase Fgr (FGR), aryl-hydrocarbon receptor-interacting protein (AIP; also known as ARA9, part of the acryl hydrocarbon

receptor protein complex), heat shock protein 72 (HSP72) and pepditylpropyl isomerase D (PPID; also known as cyclophilin D). As shown in Figure 4-6, plasma FGR, PPID and AIP all increased in ODR following the high fat meal (group x time effect, Bonferroni corrected; 6A FGR, $P<0.05$; 6B, PPID $P<0.001$; 6C, AIP $P<0.05$). Plasma HSP72 levels were higher in ODS (group effect, Bonferroni corrected, $P<0.05$; Figure 4-6D). Further, baseline HSP72 was positively correlated with baseline FA supported respiration (PMO3, $P<0.05$ Figure 4-6H), OXPHOS (PMOPGS3, $P<0.05$, Figure 4-6I), complex I respiration (PMP3, $P=0.05$, Figure 4-6J), citrate synthase activity ($P=0.05$, Figure 6K) and HOMA-IR ($p=0.056$, Figure 6L). HSP72 protein expression was measured in skeletal muscle of ODS and ODR, but was not different between groups (Figure 6-6).

Additional proteins of interest that were nominally significant include glutathione-S transferase P (Figure 4-6E; higher in ODS, group effect, $P<0.01$), tyrosine-protein phosphatase non-receptor 11 (Figure 4-6F; SHP-2; higher in ODS, group effect; $P<0.01$) and brain derived neurotrophic factor (BDNF; Figure 4-6G, group x time effect, higher in ODS).

4.4.7 SKELETAL MUSCLE DNA METHYLATION

DNA isolated from fasting muscle samples from 6 of the matched pairs of ODS and ODR subjects was analyzed for DNA methylation using an Illumina HumanMethylation450K array. Results demonstrated increased methylation of DGKZ, encoding diacylglycerol kinase ζ ($p=1.3X10^{-9}$) and of NDUF5A, encoding NADH:ubiquinone oxidoreductase subunit A5 ($p=7.0X10^{-9}$) in the ODS vs. ODR samples (Table 6-4).

4.5 DISCUSSION

In the current study we aimed to examine whole body, plasma, tissue and mitochondrial bioenergetic characteristics related to variability in weight loss, and examined mechanisms related to fuel oxidation and metabolic flexibility at in vivo and in vitro levels. We focused largely on skeletal muscle energetics given the importance of skeletal muscle to whole body metabolism. Given that mitochondria exist in reticular structures in cells, we assessed mitochondrial function in permeabilized muscle fiber preparations using high resolution respirometry. We demonstrate that both FA supported respiration and maximal oxidative phosphorylation are greater in ODS vs. ODR women under fasting, resting conditions, and following a high fat meal. These differences are independent of muscle mitochondrial content and oxidative phosphorylation supercomplex assembly. Plasma proteomic analyses revealed differences in the levels of tyrosine protein-kinase Fgr (FGR), aryl-hydrocarbon receptor-interacting protein (AIP), pepditylpropyl isomerase D (PPID) and heat shock protein 72 (HSP72). Of the latter circulating biomarkers, HSP72 was positively correlated with muscle mitochondrial respiration, citrate synthase activity and with HOMA-IR. These findings demonstrate overall that muscle mitochondrial oxidative metabolism is greater in ODS than ODR individuals both in a resting fasting state, and in response to a HFM, consistent with earlier reports of differences in expression of OXPHOS genes and muscle fiber type (Gerrits *et al.*, 2010; Harper *et al.*, 2002).

This is the first demonstration of greater oxidative phosphorylation capacity in skeletal muscle of ODS compared to ODR individuals. These and other bioenergetic characteristics were assessed in permeabilized muscle fibers in which mitochondrial structures remain intact and highly functional, and better represent the in vivo condition than do isolated mitochondria. Specifically we demonstrate that FA supported respiration,

oxidative phosphorylation with NADH and FADH2 mediated electron supply through complex I and II and the maximal capacity of the ETS were higher in ODS muscle. We also find that NADH-driven respiration through Complex I respiration is acutely increased following a high fat meal, consistent with the control of mitochondrial oxidative capacity through acute mechanisms, such as post-translational modifications.

Differences in mitochondrial respiration are often related to differences in mitochondrial content (Boushel *et al.*, 2007; DeLany *et al.*, 2014; Kristensen *et al.*, 2014; Ritov *et al.*, 2005). Despite the use of various techniques to measure mitochondrial content, including citrate synthase activity in whole tissue homogenate, COX activity in muscle fibers and ETS protein expression, we observed no difference mitochondrial content, in accord with our prior studies in primary muscle cells (Thrush *et al.*, 2014). When we expressed respiration data per unit COX activity as assessed in the same muscle fibers, oxidative phosphorylation remained higher in ODS. Thus the observed differences in mitochondrial respiration are not due to altered mitochondrial content.

Mitochondrial ETS proteins interact to form supercomplexes and their assembly is critical to oxidative phosphorylation and mitochondrial function. Supercomplex disassembly has been implicated in mitochondrial dysfunction associated with aging (Frenzel *et al.*, 2010; Gomez *et al.*, 2009), cardiovascular disease (Rosca *et al.*, 2008) and type 2 diabetes (Antoun *et al.*, 2015), which may be related to oxidative stress (Maranzana *et al.*, 2013). In the current study, ETS supercomplex assembly was not different between ODS and ODR muscle. The observed differences in mitochondrial respiration may be a result of posttranslational modification or regulation of ETS proteins rather than supercomplex formation. Surprisingly, we observed no difference in supercomplex assembly in response to the high fat meal, despite an increase in various states of respiration, including complex I,

FA supported respiration and OXPHOS (PMOPGS3) in ODS. Due to the large volume of tissue required, supercomplex assembly experiments were conducted on a subset of samples. It is possible that with a larger sample size differences may have been observed between groups or in response to the meal.

Consistent with our finding of a lower capacity for oxidation of fatty acids in muscle of ODR than ODS, we found that plasma long chain acylcarnitines were higher in ODR individuals and that peak plasma medium chain acylcarnitines (15 min, 6 h) were more pronounced in ODR compared to ODS. Acylcarnitines are byproducts of fatty acid oxidation and circulating acylcarnitine accumulations can be indicative of fatty acid oxidation dysfunction (Adams *et al.*, 2009; Koves *et al.*, 2008; Thyfault *et al.*, 2007), and plasma and skeletal muscle acylcarnitine profiles have been shown to differ in obesity (Aguer *et al.*, 2015; Baker *et al.*, 2015; Koves *et al.*, 2008). Due to tissue constraints, we were unable to measure acylcarnitine levels in skeletal muscle. We did however observe evidence of metabolic inflexibility in myotubes of ODR individuals. Indeed, FA supported respiration in permeabilized muscle fibers was also lower in these subjects. Taken together, these findings indicate that FA metabolism is impaired in skeletal muscle of ODR individuals.

Plasma proteomic analyses revealed differences in levels of proteins that are known to interact with mitochondria, consistent with the possibility that the observed differences in oxidative phosphorylation may be due to post-translational modifications or protein-protein interactions in mitochondria. Here we show that FGR, a Src kinase that interacts with succinate dehydrogenase and aconitase (Salvi *et al.*, 2007), is increased in ODR following the HFM. Reversible phosphorylation has been identified as an important regulator of mitochondrial function and oxidative phosphorylation (Hebert-Chatelain, 2013). Src Kinases

are tyrosine kinases found in mitochondria that are redox sensitive and have been shown to be activated by H2O2 or oxidative stress (Hebert-Chatelain, 2013; Salvi *et al.*, 2002). SHP-2 is a tyrosine phosphatase that has also been found in mitochondria (Salvi *et al.*, 2004) and dephosphorylates the inhibitory residue of Src kinases, thus resulting in its activation (Hebert-Chatelain, 2013; Roskoski, 2005). Plasma FGR and SHP-2 levels were significantly higher and lower, respectively, in ODR compared to ODS following the high fat meal. The physiological relevance of the changes of these proteins in plasma is not known but may reflect tissue levels of the protein and phosphorylation activity. Src kinase and SHP-2 have been shown to be decreased and increased, respectively in cardiac tissue in sepsis and associated with decreased tyrosine phosphorylation and activity of OXPHOS proteins (Zang *et al.*, 2012). The observed differences in the levels of these proteins may contribute to the observed differences in oxidative phosphorylation between ODS and ODR individuals and in response to the HFM.

Additional mechanisms of post-translational modification known to alter mitochondrial metabolism include glutathionylation, acetylation, nitrosylation and carbonylation (Mailloux *et al.*, 2011). We have previously shown that myotubes of ODR individuals have a more oxidized glutathione redox state, which is indicative of oxidative stress (Thrush *et al.*, 2014). Due to tissue and experimental constraints, we were unable to measure protein glutathionylation in the current study. Interestingly, proteomics analysis revealed that glutathione-S transferase protein expression was higher in ODS individuals, consistent with the possibility of a greater capacity to deal with oxidative stress.

AIP (ARA9), part of the aryl hydrocarbon receptor protein complex (AHR) was increased in ODR subjects following the high fat meal. The AHR complex has been reported to influence a number of cellular functions (e.g., cell cycle, cell death, cell

homeostasis, xenobiotic metabolism) including mitochondrial function, potentially through its interaction with ATP5α1.

Similarly, PPID (also known as cyclophilin D) was increased in ODR plasma following the HFM. PPID is an important regulator of mitochondrial physiology and is known to interact with a number of mitochondrial proteins including glycogen synthase kinase 3, adenine nucleotide translocator, F1FO ATP synthase and most well studied, the mitochondrial permeability transition pore (MPTP) (Giorgio *et al.*, 2010). The MPTP plays a key role in apoptosis as well as calcium handling and metabolism (Kwong and Molkentin, 2015). The binding of PPID to MPTP modulates its function by increasing the probability of pore opening whereas PPID binding to the lateral stalk of F1FO ATP synthase, decreases its activity (Giorgio *et al.*, 2009). Thus the observed increase in PPID in ODR is not known but may also be a reflection of mitochondrial activity. Of note, the PPID locus has been previously linked to serum metabolite levels (Illig *et al.*, 2010) and PPID null mice are protected against HFD induced insulin resistance (Taddeo *et al.*, 2014).

Plasma BDNF was found to be higher in ODS. BDNF plays a key role in energy balance (Xu *et al.*, 2003). BDNF is an anorexigenic protein thought to play an important role in adaptive responses to energy balance; it is decreased in obese rodents and humans and the infusion of BDNF in rats causes weight loss and decreases in food intake (Pelleymounter *et al.*, 1995). Mutations in the BDNF gene are associated with human obesity (Speliotes *et al.*, 2010). BDNF also stimulates mitochondrial biogenesis in neurons through the activation of peroxisome proliferator activated receptor alpha (PGC1-alpha) (Cheng *et al.*, 2012). The lack of an increase in BDNF in the ODR individuals following the HFM is consistent with an impaired adaptive response to changes in energy intake.

The HSP72 observations were particularly intriguing. Here we found that plasma HSP72 was higher in ODS and positively correlated with FA supported respiration, OXPHOS activity, citrate synthase activity, complex I respiration and HOMA-IR. Heat shock proteins protect against oxidative stress and inflammation by acting as chaperone proteins for naïve, aberrantly folded or mutated proteins (Benjamin and McMillan, 1998). It has previously been reported that skeletal muscle HSP72 expression is decreased in obesity (Chung *et al.*, 2008) and type 2 diabetes (Bruce *et al.*, 2003) and correlates with skeletal muscle oxidative capacity in humans (Bruce *et al.*, 2003). The overexpression of HSP72 in skeletal muscle protects against high fat diet-induced inflammation, weight gain, and increases whole body energy metabolism and mitochondrial content (Chung *et al.*, 2008; Henstridge *et al.*, 2014).

Skeletal muscle accounts for about 20% of total resting energy expenditure (Elia, 1991), which may explain in part why no difference was observed in resting and postprandial whole body energetics between ODR and ODS individuals despite the fact that marked differences in skeletal muscle metabolism have been observed here and in previous studies. Whole body energy expenditure and fat oxidation did increase in response to the high fat meal, but this was similar between groups. In this study whole body energy metabolism was measured hourly for 30 minutes over a 6 h period using a metabolic hood. It is likely that differences in whole body energy expenditure between ODS and ODR are small and documentation of such would require a more sensitive measurement over a longer time frame, such as with whole room calorimetry or direct calorimetry. Participants in this study and in our previous work were in energy balance. It is also possible that in negative energy balance, such as during the energy restriction protocol, whole body energy expenditure may differ between ODS and ODR individuals due to adaptive responses to weight loss (Leibel *et*

al., 1995). Indeed, Reinhardt *et al.* (2015) recently demonstrated that individuals who have a smaller reduction in energy expenditure in response to 24 h fasting lost more weight following 6 weeks of energy restriction (Reinhardt *et al.*, 2015). Change in RMR in response to caloric deficit is also associated with weight loss success (Tremblay *et al.*, 2015). We found no difference in RMR between ODS and ODR but in future studies plan to assess acute changes in RMR in response to weight loss. Finally, differences in whole body energy metabolism may be observed in situations where skeletal muscle has a greater contribution to energy utilization, such as during exercise, and this will be pursued in future studies.

In previously published work, we reported that mitochondrial proton leak was higher in ODS subjects and we proposed that this may protect these individuals against oxidative stress (Harper *et al.*, 2002; Thrush *et al.*, 2014). In the current study we sought to investigate this using a high fat meal in an effort to elicit oxidative stress. We found little evidence of oxidative stress following the high fat meal in either group. We did however find that ROS emission and glutathione-S transferase were higher in ODS and this was corroborated by an increased ROS emission response to 24 h palmitate treatment in primary myotubes from ODS. Taken together this suggests that ODS may have a greater capacity to respond to oxidative stress compared to ODR individuals, where low levels of ROS can function in cell signaling. The lack of oxidative stress response to the HFM may be due to the timing of the post meal biopsy. Anderson *et al.* (Anderson *et al.*, 2009), found increased ROS emission 4 h following a high fat meal; thus we may have missed the maximal effect of the high fat meal. Indeed, plasma TG and insulin began to decline at 4 hours. We also observed no difference in mitochondrial proton leak between ODS and ODR, which is in contrast to our previous work. This discrepancy may be related to differences in tissue preparations and

incubation conditions; previous studies were conducted in isolated mitochondria and primary myotubes (Harper *et al.*, 2002; Thrush *et al.*, 2014).

We observed significant methylation of skeletal muscle DNA of DGKZ encoding diacylglycerol kinase ζ. This signaling molecule phosphorylates diacylglycerol (DAG) to yield phosphatidic acid (PA) and thus attenuates the activity of DAG-activated proteins such as protein kinase C. In skeletal muscle DGKZ forms a complex with syntrophins to maintain a stable association with the sarcolemma (Abramovici *et al.*, 2003). Relevant to differences in the capacity for skeletal muscle OXPHOS, we also noted differential methylation of NDUFA5, a nuclear gene that encodes the B13 subunit of complex I of the respiratory chain. The encoded protein localizes to the mitochondrial inner membrane, where it functions in the transfer of electrons from NADH to ubiquinone.

Altogether we provide novel insights into the etiology of weight loss variability and the diet resistant state in obesity. We show that mitochondrial oxidative characteristics are lower in skeletal muscle fibers of ODR compared to ODS individuals, and that this is independent of mitochondrial content or supercomplex assembly. We also document metabolic inflexibility (cellular FA oxidation, plasma acylcarnitines) in ODR compared to ODS individuals. Multiple differences in the plasma proteome also provide exciting clues relevant to this phenotype and may provide useful biomarkers to predict weight loss success. In particular, levels of HSP72, were higher in ODS and were positively correlated with mitochondrial respiration and HOMA-IR and the lower levels in ODR individuals may contribute to the reduced capacity for oxidative metabolism and impaired metabolic response to a FA stimulus.

4.6 EXPERIMENTAL PROCEDURES

This study was approved by the Ottawa Health Science Network Research Ethics Board and all procedures were conducted in accordance with the Declaration of Helsinki. All participants gave informed written consent prior to any testing.

4.6.1 RESEARCH PARTICIPANTS

Participants were weight stable, sedentary, non-smoking, non-diabetic females, with a BMI >30kg/m2. All had previously completed the Ottawa Hospital Weight Management Program (OHWMP) and were found to be adherent to the program and were in the upper (ODS; n=10) or lower (ODR; n=10) quintiles for rate of weight loss, which is documented in the first six weeks of meal replacement. Program adherence included: attendance to 75% of clinical sessions, strict adherence to the 900kcal/d meal replacement (Optifast 900®, Nestlé Health Sciences, Nestlé Canada Inc., North York, ON) and abstaining from exercise during the initial 12 weeks of the program. Study participants were matched for age (± 10 years), weight (± 10kg) and BMI (± 2kg/m2) at the time of enrollment in the weight management program and for age (± 10 years) at the time of the biopsy.

4.6.2 WEIGHT LOSS INTERVENTION

During the first 12 weeks of the 26 week program, participants consume a 900kcal/d Optifast® meal replacement in the form of four equal liquid meals per day and refrain from exercise. Rate of weight loss, corrected for age, initial weight and BMI is calculated in the first 6 weeks of the program. Following 12 weeks, participants gradually transition to a 1200 to 1800 kcal/d diet with re-introduction of solid food. Throughout the program, participants attend weekly 90 min interactive skill building sessions and bi-weekly physician visits. Body mass, waist circumference, blood pressure and dietary adherence are monitored weekly.

Exclusion criteria were: non-adherence (outlined above), prior bariatric surgery, use of any medication known to affect rate of weight loss or glucose homeostasis, abnormal thyroid indices, congestive heart failure, malignancy and obstructive sleep apnea (during the program or at the time of biopsy).

4.6.3 STUDY DESIGN

Meeting 1: Participants arrived at the Behavioral Metabolic Research Unit (BMRU) in the morning after an overnight fast. After bladder voiding, height was measured with a stadiometer and body mass was determined to the nearest 0.1 kg. Waist circumference was measured at the level of the umbilicus, in triplicate. Body composition was measured by dual energy X-ray absorptiometry (DXA; GE-LUNAR Prodigy module; GE Medical Systems, Madison, WI). During the procedure, participants lay in the supine position and wore a standard hospital gown.

Physical activity was determined using an Actical® accelerometer (Actical; Mini Mitter Co, Inc, Bend, OR), which was worn by each participant during all waking hours for 7 days prior to the testing day. Participants were instructed to maintain their normal daily activities and record their activities and dates and times wearing the accelerometer.

Meeting 2: Trial Day. Details of the protocol are outlined in Figure 6-3. The night before the study, participants consumed a standardized meal and were instructed to have a glass of water as soon as they woke up on the morning of the study. They arrived by taxi at the BMRU at 7am after a 12 h fast and upon arrival, were met at the taxi with a wheelchair, to minimize physical exertion prior to the RMR measurement. Participants voided their bladders, and body mass was measured.

Indirect Calorimetry: Study subjects rested quietly in the dark in a supine position for 20 min. Expired gasses were collected for the next 30 minutes using a ventilated hood (Vmax Encore 29N, SensorMedics Corporation, Yorba Linda, CA, USA). Following the high fat meal, respiratory gases were collected at 1, 2, 3, 4, and 5 h time points for 30 min. Urine was collected over the duration of the day to assess protein oxidation, as determined from urea content using a urea assay kit (Bioassay Systems, Hayward, CA, USA). Protein oxidation was not different between groups (data not shown).

Blood Sampling: To allow for continuous blood sampling, an intravenous catheter was inserted into a forearm vein and kept patent with a saline drip. Blood was sampled at baseline (0h) 0.5, 1, 1.5, 2, 2.5, 3, 3.5 4, 4.5, 5, and 6h. Plasma was separated by centrifugation and stored at -80 °C for future analysis.

Muscle Biopsies: At baseline (fasting) and 6 h following the HFM, the *vastus lateralis* was biopsied under local anesthetic using a Bergstrom needle. Muscle samples were placed immediately in ice-cold saline and any fat or connective tissue was removed. 20-30mg was placed in ice-cold BIOPS (2.77mM CaK2EGTA, 7.23mM K2EGTA, 5.77mM Na2ATP, 6.56mM MgCl2.6H2O, 20mM taurine, 15mM Na2-phosphocreatine, 20mM imidazole, 0.5mM DTT, 50mM MES, pH 7.1) for mitochondrial respiration and ROS measurements. The remaining muscle sample was aliquoted, and snap frozen in liquid nitrogen and stored at -80 °C for later analyses.

Adipose Biopsies: Under local anesthetic (1% lidocaine, without epinephrine), a small incision was made adjacent to the umbilicus and subcutaneous adipose tissue biopsies (~400mg) were obtained at baseline (fasting) and 6 h following the HFM. Samples were placed immediately in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin and 5mmol/L glucose. A portion of the sample was used for the measurement

adipocyte lipolysis and any remaining tissue was snap frozen in liquid nitrogen, and stored at -80°C.

High Fat Meal: Immediately following the baseline biopsies of muscle and adipose tissues, participants consumed a liquid high fat meal, modified from (Anderson *et al.*, 2009) that was equivalent to 35% of total daily energy requirements (TDE). TDE was determined from the sum of resting metabolic rate (RMR; as determined from indirect calorimetry measurements), average daily energy expended from physical activity and the thermic effect of food (assumed to be 10% of TDE). The HFM consisted of 60% fat, 30% carbohydrate, 10% protein with a fatty acid (FA) distribution of 55% saturated FA, 15% polyunsaturated FA, and 35% monounsaturated FA. Participants were asked to consume the liquid meal within a 5 min period.

4.6.4 EX VIVO ANALYSES OF TISSUE, PLASMA AND PRIMARY CELLS

High Resolution Respirometry: Permeabilized muscle fibers were prepared as described with minor modifications (Boushel *et al.*, 2007). Briefly, muscle fiber bundles were mechanically teased apart with forceps on ice, to achieve a high degree of fiber separation using a dissecting microscope. Fibers were chemically permeabilized in 3mL BIOPS containing 50µg/mL saponin by gentle agitation on ice for 30 min and subsequently washed in respiration media (MIRO5; 0.5mM EGTA, 3mM MgCL2.6H20, 60mM K-lactobionate, 20 mM taurine, 10mM KH2PO4, 20mM HEPES, 110mM sucrose and BSA, pH 7.1) for 10 min. Muscle fibers were carefully weighed (1-4mg to the nearest 0.01mg) using an analytical balance and transferred immediately to the Oxygraph O2K (Oroboros Instruments, Innsbruck, Austria) respiration chamber containing MIRO5. Samples were run

in duplicate, at 37°C, and chamber oxygen levels were maintained between 200-400nmol O_2/L.

Protocol 1: Routine leak in the absence of adenylates was measured in the presence of 1.5mM malate (M) and 200μM octanoyl carnitine (OC), followed by the addition of 5mM ADP for state 3 FA supported respiration. Maximal oxidative phosphorylation (OXPHOS) was measured with the addition of NADH-generating substrates for Complex I (5mM pyruvate (P) and 10mM glutamate (G)) followed by succinate as substrate (10mM succinate (S)) generating FADH2 to the Q-junction from Complex II, and the reinjection of ADP. Mitochondrial proton leak was then assessed with the ATP synthase inhibitor oligomycin (Oligo; 2.5μM; state 4o). Non-mitochondrial respiration was assessed with the complex III inhibitor antimycin A (AA; 2.5μM). The excess capacity of cytochrome c oxidase (COX) activity was measured with the injection of the electron donor tetramethylphosphodinitrate (TMPD; 5mM), maintained in a reduced state by ascorbate (5mM) and reducing cytochrome c as the substrate for COX. The autoxidation of TMPD was inhibited with sodium azide (100mM).

Protocol 2: Concentrations for previously listed substrates are the same as described above. Routine respiration was measured with M and P. NADH-driven state 3 respiration through Complex I and downstream ETS Complexes was achieved with the addition of ADP and G. Maximal OXPHOS was achieved with further electron supply by the addition of S to complex II, generating FADH2, and the reinjection of ADP. Cytochrome C (Cyt C; 10μM) was injected to confirm the integrity of the mitochondrial outer membrane. Respiration rates did not increase > 5% of OXPHOS (data not shown) with Cyt C injection. Maximal uncoupled respiration was measured with the titration of carbonyl cyanide -p- trifluoro-methoxyphenyl-hydrazone (FCCP; 0.25-1μM). Non-mitochondrial respiration was

measured as that remaining in the presence of AA. The maximal capacity of COX was measured with TMPD, as above.

H2O2 Emission: H2O2 emission was measured in ~10mg of mechanically permeabilized muscle prepared using the PBI-shredder using the Oxygraph O2K flourometer (Oroboros Instruments, Innsbruck, Austria) as previously described (Beauchamp *et al.*, 2015) with minor modifications. Briefly, tissue was shredded in 500µL ice-cold ROS buffer containing (120mM KCl, 20mM HEPES, 10mM KH2PO4, monobasic, 2.86mM MgCl2, 0.2mM EGTA, 0.025% BSA, pH 7). Experiments were run in the presence of [1.25 U/mL] horseradish peroxidase and 6µM Amplex UltraRed (ThermoFisher Scientific, MA, USA). Chamber O2 levels were at normoxic levels (<200 nmol O2/L), and all experiments were conducted at 37 °C. H2O2 emission was assessed with the following substrates, added sequentially as previously described concentrations unless specified: M, OC, ADP, P, G, S, Oligo, FCCP (0.5µM) and AA.

Western Blots: Muscle tissue was homogenized in 50mM Tris, 1mM EDTA, 1mM EGTA, 50mM NaF, 5mM Na pyrophosphate, 10% (v/v) glycerol, 1% (v/v) Triton-X, 1mM DTT, 1mM PMSF, (pH 7.5) on ice. Samples were then sonicated, rocked for 30 min at 4oC and spun at 1500g for 15 min at 4°C. Protein content was determined with a bicinchoninic acid assay. Following polyacrylamide gel electrophoresis, proteins were transferred to nitrocellulose membranes, which were then blocked with 5% skim milk in TBST and incubated overnight at 4°C with the following primary antibodies in 2-5% BSA in TBS: Total OXPHOS (1:1000, ab-22604, Abcam Cambridge, MA), heat shock protein72 (HSP72; 1:5000; C92F3A-S, Enzo Life Sciences, Farmingdale, NY). Proteins were visualized using enhanced chemiluminescence, and Image J software was applied for densitrometry. Protein

loading was confirmed with vinculin (1:1000, ab129002, Abcam, Cambridge, MA) or Ponceau S staining (Total OXPHOS).

Oxidative damage: Protein carbonyls were determined in aliquots of muscle using the Oxyblot detection kit (Millipore, Darmstadt, Germany). Samples were prepared according to kit instructions, and spotted onto a nitrocellulose membrane. Ponceau S staining was used as a loading control.

Blue Native Gel Electrophoresis (BN-PAGE): BN-PAGE was used to investigate mitochondrial electron transport chain supercomplex assembly in aliquots of muscle, as previously described (Antoun *et al.*, 2015; Patten *et al.*, 2014; Wittig *et al.*, 2006). Briefly, samples were homogenized in sucrose buffer (250mM sucrose, 20mM imidazole/HCl, pH 7). Mitochondria were pelleted at 10,000xg for 10 minutes and resuspended in 50mM imidiazole/HCl pH 7.0, 50mM NaCl, 5mM 6-aminohexanoic acid, 1mM EDTA with 1.7% digitonin (final digitonin to tissue ratio of 1:18 w/w- experimentally determined) for 30 minutes. Samples were cleared by centrifugation for 30 minutes at 14,000xg. Protein was loaded with 5% glycerol and a 1:10 dye:digitonin ratio of Coomassie Blue G-250 onto 3-13% large gradient gels. Gels were run in high Coomassie Blue cathode buffer for 2 hours at 150V and switched to low Coomassie cathode buffer overnight at 200V. Gels were transferred to nitrocellulose membrane and the following proteins were probed for: Complex I [NDUFA9] (459100, Invitrogen), Complex II [Fp] (459200, Invitrogen), Complex III [UQCRC2] (Ab14745 MitoSciences), Complex IV [subunit I] (459600, Invitrogen), Complex V [ATP5A] (Ab14748, MitoSciences). Bands were visualized using enhanced chemiluminescence and quantified using Image J.

Enzyme Activities: Activities were measured spectrophotometrically at 37°C in muscle homogenate, following repeat freeze thaws. Citrate synthase activity was determined

based on (Srere, 1969). β(3)-hydroxyacyl-CoA dehydrogenase (β HAD) activity was measured based on the disappearance of NADH (Bruce *et al.*, 2006).

DNA methylation: Genomic DNA was isolated from skeletal muscle samples taken in the fasting state using the DNeasy Blood & Tissue kit (Qiagen). 800ng of genomic DNA was subjected to bisulfate transformation using an Epi Mark Bisulfite Conversion kit (New England Biolabs) and analyzed on the Illumina HumanMethylation450K array at The Centre for Applied Genomics (TCAG) in Toronto. The GenomeStudio Methylation Module (version 2011.1) was used to analyze the methylation data from scanned microarray images and calculate methylation levels. To estimate the methylation status at the interrogated CpG site, the Illumina assay uses a pair of fluorescent probes (a methylated probe and an unmethylated probe) and the methylation level (β) is calculated based on the fluorescent signals from the M (methylated) and U (unmethylated) probe as:

$$\beta = \frac{\max(M, 0)}{\max(U, 0) + \max(M, 0) + 100}$$

Beta-value (β) ranges from 0 to 1, a value of zero indicates no methylation and a value of one indicates the CpG site is completely methylated. Since beta-values show severe heteroscedasticity for highly methylated or unmethylated CpG sites, the log ratios of the beta-value (M-value) are used for statistical analysis, as similarly done in most of the microarray literature (Irizarry *et al.*, 2008). We computed the M-values (M) from Beta-values by:

$$M = \log_2\left(\frac{\beta}{1-\beta}\right)$$

and used these M-values to identify the differentially methylated loci. Unpaired two-sample t-test in R (version 3.0.1) was used to determine if the two group means are equal

(our null hypothesis, H0: μ1 = μ2) and the P-value threshold < 2 × 10-8 was used to reject the null hypothesis and conclude that the two group means are different (our alternative hypothesis, Ha: $\mu 1 \neq \mu 2$) at the 0.01 significance level and after correction for the number of probes (N= 485000) in the Illumina HumanMethylation450K array.

mRNA expression: All muscle samples were homogenized in the presence of Tri Pure Isolation Reagent (Roche). 1μg of RNA was reverse-transcribed using the Transcriptor First Strand cDNA Synthesis Kit (Roche) using a combination of oligo(dT) and random hexamer primers. Quantitative PCR of cDNA samples was conducted using the LightCycler 480 II (Roche). Human PPIA was used as the reference gene for qPCR experiments. The following primer sequences were used for qPCR experiments: DGKZ fwd 5'-GCCAACCCAGAGAAATTCAA-3', rev 5'-GTCCTGGATCTTGGGAGTCA; NDUFA5 fwd 5'-AGAGAGGCCATGGGAAGATT-3', rev 5'-TTATCACCAGCCACTTGTGC-3'; PPIA fwd 5'-ACCGTGTTCTTCGACATTGC-3', rev 5'-TTCTGTGAAAGCAGGAACCC-3'.

Plasma Analytes: Glucose was determined using a glucose oxidase kit (Sigma Aldrich, St. Louis, MO). Plasma fatty acids (FA) and triglycerides were determined using a WAKO free fatty acid diagnostic kit and a WAKO triglyceride diagnostic kit (WAKO Diagnostics, Richmond, VA). Plasma insulin was determined using an enzyme linked immunosorbent assay (ELISA) kit (Sigma Aldrich, St. Louis).

Primary Myotubes: Muscle satellite cells were isolated from *vastus lateralis* of ODR (age: 42 ± 5 yr; body mass: 96.7± 4.8 kg; BMI: 34.0 ± 1.5 kg/m2, n=5) and ODS (age: 42 ± 3 yr; body mass: 97.7± 4.8 kg; BMI: 35.8 ± 0.5 kg/m2, n=5) (Costford *et al.*, 2009; Thrush *et al.*, 2014). CD-56 positive cells were induced to differentiate in low glucose (5.5mM)

DMEM with 2% horse serum, 25pM insulin, 1% antibiotic-antimycotic, 2.5µg/mL gentamycin for 7 days. On day 6 of differentiation, cells were treated with differentiation media supplemented with 2% BSA and 1mM carnitine with or without 500µM palmitate for 24 hrs. Media was collected for acylcarnitine analysis.

Reactive Oxygen Species (ROS): Cellular ROS emission was measured in a 96 well plate using dichloro-dihydro-flourescein diacetate (DCFDA) as previously described (Thrush *et al.*, 2014). Data were normalized to protein content.

Fatty Acid Oxidation: Myotubes were grown to confluence in a 12 well plate and induced to differentiate for 7 days. FA oxidation was measured as described elsewhere (Aguer *et al.*, 2013).

Acylcarnitine Levels: Plasma acylcarnitines were measured in samples that were collected at times 0, 15min, 30 min, 1h, 2h, 3h, 4h, 5h and 6h. Acylcarnitine release from primary myotubes was also measured: aliquots of the medium above myotubes were collected following 24 hr incubation with or without 500µM palmitate, based on methods previously described (Turgeon *et al.*, 2008) with minor modifications (Beauchamp *et al.*, 2015).

Isolation of adipocytes and lipolysis: Immediately following the biopsy, roughly 100mg of fresh adipose tissue, free of capillaries, was digested with collagenase (1mg/ml) in 4% BSA Krebs-Ringer buffer at 37°C and filtered through a nylon mesh. Mature adipocyte suspension pictures were acquired using a contrast phase microscope. Mean adipocyte diameter for each sample was calculated from 250 individual measurements. Adipocyte density was adjusted to 500 adipocytes/50µl and distributed in microcentrifuge tubes and incubated at 37°C for 2 hours in the presence of isoproterenol (non selective b-adrenoceptor agonist), and UK 14304 (selective a2-adrenoceptor agonist). To more thoroughly investigate

a2-adrenoceptor-mediated antilipolysis, 5mg/mL adenosine deaminase was added to the incubation medium to remove adenosine released into the incubation medium by the isolated fat cells. Lipolysis was expressed per cell number. Lipolytic rate was determined by glycerol quantification using bioluminescence, as previously described (Imbeault *et al.*, 2000).

Adipose tissue lipoprotein lipase activity: Heparin-releasable LPL activity from subcutaneous abdominal fat was performed as previously described (Imbeault *et al.*, 1999) with minor modifications. The radiolabeled triglyceride previously used was replaced by the commercially available and validated sensitive fluorogenic substrate, the EnzChek lipase substrate (Invitrogen).

Plasma Proteomics: Proteomics analysis was performed on plasma samples collected before (0) and at 1, 2, 5, 6 hr following the high fat meal using SOMAscan technology (Somalogics, Boulder, CO). Each of the 1129 plasma proteins measured in the SOMAscan assay has a targeted SOMAmer® (Slow Off-rate Modified Aptamer) reagent, which is used as an affinity-binding reagent and quantified on a custom Agilent hybridization chip. This multiplex assay measures proteins in their native forms. ODS and ODR samples were randomly assigned to plates within the each assay run along with a set of calibration and normalization samples. Intra-run normalization and inter-run calibration were performed according to SOMAscan v3 assay data quality-control procedures as defined in the SomaLogic good laboratory practice quality system (Gold *et al.*, 2012).

4.6.5 STATISTICAL ANALYSIS

A two-way ANOVA with Bonferonni post hoc analysis was used to assess the effects of the meal and differences between groups. A one-way ANOVA was used to assess differences in body mass, BMI and age at time of enrollment in the weight management

program, following 6 weeks of weight loss and at time of the study. A two-tailed T-test was used to determine differences between groups in which only resting conditions were tested. Significance was accepted as $P<0.05$.

To determine difference in plasma proteomics, a one-way repeated measures Analysis of Variance (ANOVA) in R (version 3.0.1) was used to investigate group effects (ODS/ODR), within subject effects (change over time), and the interaction between them (Group $\times$ Time). Two-way repeated measures Analysis of Variance (ANOVA) corrected with a Bonferroni post hoc in R (version 3.0.1) was used to determine whether any change in protein level is the result of the interaction between response to diet (sensitive/resistant) and time.

4.7 REFERENCES

Abramovici, H., Hogan, A.B., Obagi, C., Topham, M.K., and Gee, S.H. (2003). Diacylglycerol kinase-zeta localization in skeletal muscle is regulated by phosphorylation and interaction with syntrophins. Mol Biol Cell *14*, 4499-4511.

Adams, S.H., Hoppel, C.L., Lok, K.H., Zhao, L., Wong, S.W., Minkler, P.E., Hwang, D.H., Newman, J.W., and Garvey, W.T. (2009). Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. The Journal of nutrition *139*, 1073-1081.

Aguer, C., Fiehn, O., Seifert, E.L., Bezaire, V., Meissen, J.K., Daniels, A., Scott, K., Renaud, J.M., Padilla, M., Bickel, D.R*., et al.* (2013). Muscle uncoupling protein 3 overexpression mimics endurance training and reduces circulating biomarkers of incomplete beta-oxidation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *27*, 4213-4225.

Aguer, C., McCoin, C.S., Knotts, T.A., Thrush, A.B., Ono-Moore, K., McPherson, R., Dent, R., Hwang, D.H., Adams, S.H., and Harper, M.E. (2015). Acylcarnitines: potential implications for skeletal muscle insulin resistance. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *29*, 336-345.

Anderson, E.J., Lustig, M.E., Boyle, K.E., Woodlief, T.L., Kane, D.A., Lin, C.T., Price, J.W., 3rd, Kang, L., Rabinovitch, P.S., Szeto, H.H*., et al.* (2009). Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest *119*, 573-581.

Antoun, G., McMurray, F., Thrush, A.B., Patten, D.A., Peixoto, A.C., Slack, R.S., McPherson, R., Dent, R., and Harper, M.E. (2015). Impaired mitochondrial oxidative phosphorylation and supercomplex assembly in rectus abdominis muscle of diabetic obese individuals. Diabetologia *58*, 2861-2866.

Baker, P.R., 2nd, Boyle, K.E., Koves, T.R., Ilkayeva, O.R., Muoio, D.M., Houmard, J.A., and Friedman, J.E. (2015). Metabolomic analysis reveals altered skeletal muscle amino acid and fatty acid handling in obese humans. Obesity (Silver Spring, Md) *23*, 981-988.

Beauchamp, B., Ghosh, S., Dysart, M.W., Kanaan, G.N., Chu, A., Blais, A., Rajamanickam, K., Tsai, E.C., Patti, M.E., and Harper, M.E. (2014). Low birth weight is associated with adiposity, impaired skeletal muscle energetics and weight loss resistance in mice. International journal of obesity.

Beauchamp, B., Thrush, A.B., Quizi, J., Antoun, G., McIntosh, N., Al-Dirbashi, O.Y., Patti, M.E., and Harper, M.E. (2015). Undernutrition during pregnancy in mice leads to dysfunctional cardiac muscle respiration in adult offspring. Biosci Rep.

Benjamin, I.J., and McMillan, D.R. (1998). Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res *83*, 117-132.

Bonen, A., Parolin, M.L., Steinberg, G.R., Calles-Escandon, J., Tandon, N.N., Glatz, J.F., Luiken, J.J., Heigenhauser, G.J., and Dyck, D.J. (2004). Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *18*, 1144-1146.

Bouchard, C., Tremblay, A., Despres, J.P., Nadeau, A., Lupien, P.J., Theriault, G., Dussault, J., Moorjani, S., Pinault, S., and Fournier, G. (1990). The response to long-term overfeeding in identical twins. N Engl J Med *322*, 1477-1482.

Boushel, R., Gnaiger, E., Schjerling, P., Skovbro, M., Kraunsoe, R., and Dela, F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia *50*, 790-796.

Bruce, C.R., Carey, A.L., Hawley, J.A., and Febbraio, M.A. (2003). Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. Diabetes *52*, 2338-2345.

Bruce, C.R., Thrush, A.B., Mertz, V.A., Bezaire, V., Chabowski, A., Heigenhauser, G.J., and Dyck, D.J. (2006). Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. Am J Physiol Endocrinol Metab *291*, E99-E107.

Cheng, A., Wan, R., Yang, J.L., Kamimura, N., Son, T.G., Ouyang, X., Luo, Y., Okun, E., and Mattson, M.P. (2012). Involvement of PGC-1alpha in the formation and maintenance of neuronal dendritic spines. Nat Commun *3*, 1250.

Chung, J., Nguyen, A.K., Henstridge, D.C., Holmes, A.G., Chan, M.H., Mesa, J.L., Lancaster, G.I., Southgate, R.J., Bruce, C.R., Duffy, S.J., *et al.* (2008). HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A *105*, 1739-1744.

Costford, S.R., Crawford, S.A., Dent, R., McPherson, R., and Harper, M.E. (2009). Increased susceptibility to oxidative damage in post-diabetic human myotubes. Diabetologia *52*, 2405-2415.

DeLany, J.P., Dube, J.J., Standley, R.A., Distefano, G., Goodpaster, B.H., Stefanovic-Racic, M., Coen, P.M., and Toledo, F.G. (2014). Racial differences in peripheral insulin sensitivity and mitochondrial capacity in the absence of obesity. J Clin Endocrinol Metab *99*, 4307-4314.

Dent, R., McPherson, R., and Harper, M.E. (1999). Variability in weight loss in highly compliant women on a controlled dietary regimen. Obes Res *7*.

Doucet, E., St-Pierre, S., Almeras, N., Despres, J.P., Bouchard, C., and Tremblay, A. (2001). Evidence for the existence of adaptive thermogenesis during weight loss. Br J Nutr *85*, 715-723.

Doucet, E., Tremblay, A., Simoneau, J.A., and Joanisse, D.R. (2003). Skeletal muscle enzymes as predictors of 24-h energy metabolism in reduced-obese persons. Am J Clin Nutr *78*, 430-435.

Elia, M. (1991). The inter-organ flux of substrates in fed and fasted man, as indicated by arterio-venous balance studies. Nutr Res Rev *4*, 3-31.

Frenzel, M., Rommelspacher, H., Sugawa, M.D., and Dencher, N.A. (2010). Ageing alters the supramolecular architecture of OxPhos complexes in rat brain cortex. Exp Gerontol *45*, 563-572.

Gerrits, M.F., Ghosh, S., Kavaslar, N., Hill, B., Tour, A., Seifert, E.L., Beauchamp, B., Gorman, S., Stuart, J., Dent, R., *et al.* (2010). Distinct skeletal muscle fiber characteristics and gene expression in diet-sensitive versus diet-resistant obesity. Journal of lipid research *51*, 2394-2404.

Ghosh, S., Dent, R., Harper, M.E., Stuart, J., and McPherson, R. (2011). Blood gene expression reveal pathway differences between diet-sensitive and resistant obese subjects prior to caloric restriction. Obesity (Silver Spring, Md) *19*, 457-463.

Giorgio, V., Bisetto, E., Soriano, M.E., Dabbeni-Sala, F., Basso, E., Petronilli, V., Forte, M.A., Bernardi, P., and Lippe, G. (2009). Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex. The Journal of biological chemistry *284*, 33982-33988.

Giorgio, V., Soriano, M.E., Basso, E., Bisetto, E., Lippe, G., Forte, M.A., and Bernardi, P. (2010). Cyclophilin D in mitochondrial pathophysiology. Biochimica et biophysica acta *1797*, 1113-1118.

Gold, L., Walker, J.J., Wilcox, S.K., and Williams, S. (2012). Advances in human proteomics at high scale with the SOMAscan proteomics platform. New biotechnology *29*, 543-549.

Gomez, L.A., Monette, J.S., Chavez, J.D., Maier, C.S., and Hagen, T.M. (2009). Supercomplexes of the mitochondrial electron transport chain decline in the aging rat heart. Arch Biochem Biophys *490*, 30-35.

Harper, M.E., Dent, R., Monemdjou, S., Bezaire, V., Van Wyck, L., Wells, G., Kavaslar, G.N., Gauthier, A., Tesson, F., and McPherson, R. (2002). Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. Diabetes *51*, 2459-2466.

Hebert-Chatelain, E. (2013). Src kinases are important regulators of mitochondrial functions. The international journal of biochemistry & cell biology *45*, 90-98.

Henstridge, D.C., Bruce, C.R., Drew, B.G., Tory, K., Kolonics, A., Estevez, E., Chung, J., Watson, N., Gardner, T., Lee-Young, R.S.*, et al.* (2014). Activating HSP72 in rodent skeletal muscle increases mitochondrial number and oxidative capacity and decreases insulin resistance. Diabetes *63*, 1881-1894.

Illig, T., Gieger, C., Zhai, G., Romisch-Margl, W., Wang-Sattler, R., Prehn, C., Altmaier, E., Kastenmuller, G., Kato, B.S., Mewes, H.W.*, et al.* (2010). A genome-wide perspective of genetic variation in human metabolism. Nat Genet *42*, 137-141.

Imbeault, P., Almeras, N., Richard, D., Despres, J.P., Tremblay, A., and Mauriege, P. (1999). Effect of a moderate weight loss on adipose tissue lipoprotein lipase activity and expression: existence of sexual variation and regional differences. Int J Obes Relat Metab Disord *23*, 957-965.

Imbeault, P., Prud'Homme, D., Tremblay, A., Despres, J.P., and Mauriege, P. (2000). Adipose tissue metabolism in young and middle-aged men after control for total body fatness. J Clin Endocrinol Metab *85*, 2455-2462.

Irizarry, R.A., Ladd-Acosta, C., Carvalho, B., Wu, H., Brandenburg, S.A., Jeddeloh, J.A., Wen, B., and Feinberg, A.P. (2008). Comprehensive high-throughput arrays for relative methylation (CHARM). Genome Res *18*, 780-790.

Koves, T.R., Ussher, J.R., Noland, R.C., Slentz, D., Mosedale, M., Ilkayeva, O., Bain, J., Stevens, R., Dyck, J.R., Newgard, C.B.*, et al.* (2008). Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab *7*, 45-56.

Kristensen, J.M., Skov, V., Petersson, S.J., Ortenblad, N., Wojtaszewski, J.F., Beck-Nielsen, H., and Hojlund, K. (2014). A PGC-1alpha- and muscle fibre type-related decrease in markers of mitochondrial oxidative metabolism in skeletal muscle of humans with inherited insulin resistance. Diabetologia *57*, 1006-1015.

Kwong, J.Q., and Molkentin, J.D. (2015). Physiological and pathological roles of the mitochondrial permeability transition pore in the heart. Cell Metab *21*, 206-214.

Lapuente-Brun, E., Moreno-Loshuertos, R., Acin-Perez, R., Latorre-Pellicer, A., Colas, C., Balsa, E., Perales-Clemente, E., Quiros, P.M., Calvo, E., Rodriguez-Hernandez, M.A.*, et al.* (2013). Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. Science *340*, 1567-1570.

Leibel, R.L., Rosenbaum, M., and Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. N Engl J Med *332*, 621-628.

Levin, B.E., and Dunn-Meynell, A.A. (2000). Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. Am J Physiol Regul Integr Comp Physiol *278*, R231-237.

Mailloux, R.J., Seifert, E.L., Bouillaud, F., Aguer, C., Collins, S., and Harper, M.E. (2011). Glutathionylation acts as a control switch for uncoupling proteins UCP2 and UCP3. The Journal of biological chemistry *286*, 21865-21875.

Maranzana, E., Barbero, G., Falasca, A.I., Lenaz, G., and Genova, M.L. (2013). Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I. Antioxid Redox Signal *19*, 1469-1480.

Patten, D.A., Wong, J., Khacho, M., Soubannier, V., Mailloux, R.J., Pilon-Larose, K., MacLaurin, J.G., Park, D.S., McBride, H.M., Trinkle-Mulcahy, L.*, et al.* (2014). OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. The EMBO journal *33*, 2676-2691.

Pelleymounter, M.A., Cullen, M.J., and Wellman, C.L. (1995). Characteristics of BDNF-induced weight loss. Exp Neurol *131*, 229-238.

Reinhardt, M., Thearle, M.S., Ibrahim, M., Hohenadel, M.G., Bogardus, C., Krakoff, J., and Votruba, S.B. (2015). A Human Thrifty Phenotype Associated With Less Weight Loss During Caloric Restriction. Diabetes *64*, 2859-2867.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H., and Kelley, D.E. (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes *54*, 8-14.

Rosca, M.G., Vazquez, E.J., Kerner, J., Parland, W., Chandler, M.P., Stanley, W., Sabbah, H.N., and Hoppel, C.L. (2008). Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation. Cardiovasc Res *80*, 30-39.

Rosenbaum, M., and Leibel, R.L. (2010). Adaptive thermogenesis in humans. International journal of obesity *34 Suppl 1*, S47-55.

Roskoski, R., Jr. (2005). Src kinase regulation by phosphorylation and dephosphorylation. Biochem Biophys Res Commun *331*, 1-14.

Salvi, M., Brunati, A.M., Bordin, L., La Rocca, N., Clari, G., and Toninello, A. (2002). Characterization and location of Src-dependent tyrosine phosphorylation in rat brain mitochondria. Biochimica et biophysica acta *1589*, 181-195.

Salvi, M., Morrice, N.A., Brunati, A.M., and Toninello, A. (2007). Identification of the flavoprotein of succinate dehydrogenase and aconitase as in vitro mitochondrial substrates of Fgr tyrosine kinase. FEBS Lett *581*, 5579-5585.

Salvi, M., Stringaro, A., Brunati, A.M., Agostinelli, E., Arancia, G., Clari, G., and Toninello, A. (2004). Tyrosine phosphatase activity in mitochondria: presence of Shp-2 phosphatase in mitochondria. Cell Mol Life Sci *61*, 2393-2404.

Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U., Lango Allen, H., Lindgren, C.M., Luan, J., Magi, R.*, et al.* (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet *42*, 937-948.

Srere, P. (1969). [1] Citrate synthase:[EC 4.1. 3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods in enzymology *13*, 3-11.

Taddeo, E.P., Laker, R.C., Breen, D.S., Akhtar, Y.N., Kenwood, B.M., Liao, J.A., Zhang, M., Fazakerley, D.J., Tomsig, J.L., Harris, T.E.*, et al.* (2014). Opening of the mitochondrial permeability transition pore links mitochondrial dysfunction to insulin resistance in skeletal muscle. Mol Metab *3*, 124-134.

Thrush, A.B., Dent, R., McPherson, R., and Harper, M.E. (2013). Implications of mitochondrial uncoupling in skeletal muscle in the development and treatment of obesity. The FEBS journal *280*, 5015-5029.

Thrush, A.B., Zhang, R., Chen, W., Seifert, E.L., Quizi, J.K., McPherson, R., Dent, R., and Harper, M.E. (2014). Lower mitochondrial proton leak and decreased glutathione redox in primary muscle cells of obese diet-resistant versus diet-sensitive humans. J Clin Endocrinol Metab *99*, 4223-4230.

Thyfault, J.P., Cree, M.G., Zheng, D., Zwetsloot, J.J., Tapscott, E.B., Koves, T.R., Ilkayeva, O., Wolfe, R.R., Muoio, D.M., and Dohm, G.L. (2007). Contraction of insulin-resistant muscle normalizes insulin action in association with increased mitochondrial activity and fatty acid catabolism. Am J Physiol Cell Physiol *292*, C729-739.

Tremblay, A., Lepage, C., Panahi, S., Couture, C., and Drapeau, V. (2015). Adaptations to a diet-based weight-reducing programme in obese women resistant to weight loss. Clin Obes *5*, 145-153.

Turgeon, C., Magera, M.J., Allard, P., Tortorelli, S., Gavrilov, D., Oglesbee, D., Raymond, K., Rinaldo, P., and Matern, D. (2008). Combined newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem *54*, 657-664.

Wittig, I., Braun, H.P., and Schagger, H. (2006). Blue native PAGE. Nat Protoc *1*, 418-428.

Xu, B., Goulding, E.H., Zang, K., Cepoi, D., Cone, R.D., Jones, K.R., Tecott, L.H., and Reichardt, L.F. (2003). Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci *6*, 736-742.

Zang, Q.S., Martinez, B., Yao, X., Maass, D.L., Ma, L., Wolf, S.E., and Minei, J.P. (2012). Sepsis-induced cardiac mitochondrial dysfunction involves altered mitochondrial-localization of tyrosine kinase Src and tyrosine phosphatase SHP2. PLoS One *7*, e43424.

4.8 TABLES

Table 4-1 - Participants characteristics

	Before meal replacement		Following 6 weeks of meal replacement		At time of biopsy	
	ODR	ODS	ODR	ODS	ODR	ODS
Age	44 ± 2	43 ± 2	-	-	48 ± 2	52 ± 2
Weight	104.8 ± 6.9	100.3 ± 3.9	98.0 ± 6.5	88.6 ± 3.4*	100.9 ± 7.3	93.2 ± 5.9
BMI	37.7 ± 2.0	37.8 ± 1.1	35.2 ± 1.5	33.2 ± 1.0*	37.1 ± 2.0	35.6 ± 1.7
% weight loss	-	-	6.5 ± 0.2	11.7 ± 0.2****	-	-
Waist circumference (cm)	-	-	-	-	113.9 ± 4.7	112.1 ± 4.2
Body fat (%)	-	-	-	-	49.8 ± 1.8	47.4 ± 1.2
Body fat distribution	-	-	-	-		
% Upper body	-	-	-	-	56.4 ± 2.1	62.8 ± 2.0*
% Lower body	-	-	-	-	43.6 ± 2.1	37.2 ± 2.0*
Fat mass (kg)	-	-	-	-	46.7 ± 4.1	42.5 ± 3.1
Lean Mass (kg)	-	-	-	-	45.4 ± 2.3	46.9 ± 3.1
Respiratory Exchange Ratio	-	-	-	-	0.79 ± 0.02	0.80 ± 0.02
VO_2 (L/min)	-	-	-	-	0.23 ± 0.01	0.23 ± 0.01
VCO_2 (L/min)	-	-	-	-	0.18 ± 0.01	0.19 ± 0.01
Resting metabolic rate (kcal)	-	-	-	-	1584 ± 85	1604 ± 86
Physical activity (kcal)	-	-	-	-	1222 ± 216	1121 ± 181
Total Daily Energy Expenditure (kcal)	-	-	-	-	3087 ± 213	3004 ± 216
HOMA-IR	-	-	-	-	1.5 ± 0.3	2.2 ± 0.5

Data are mean ± SEM, N=9-10. Weight loss data; 2 way ANOVA, Significantly different compared to before meal replacement within group, P<0.01. ****P<0.0001. Between group data; two tailed unpaired t test, *P<0.05.

4.9 FIGURES

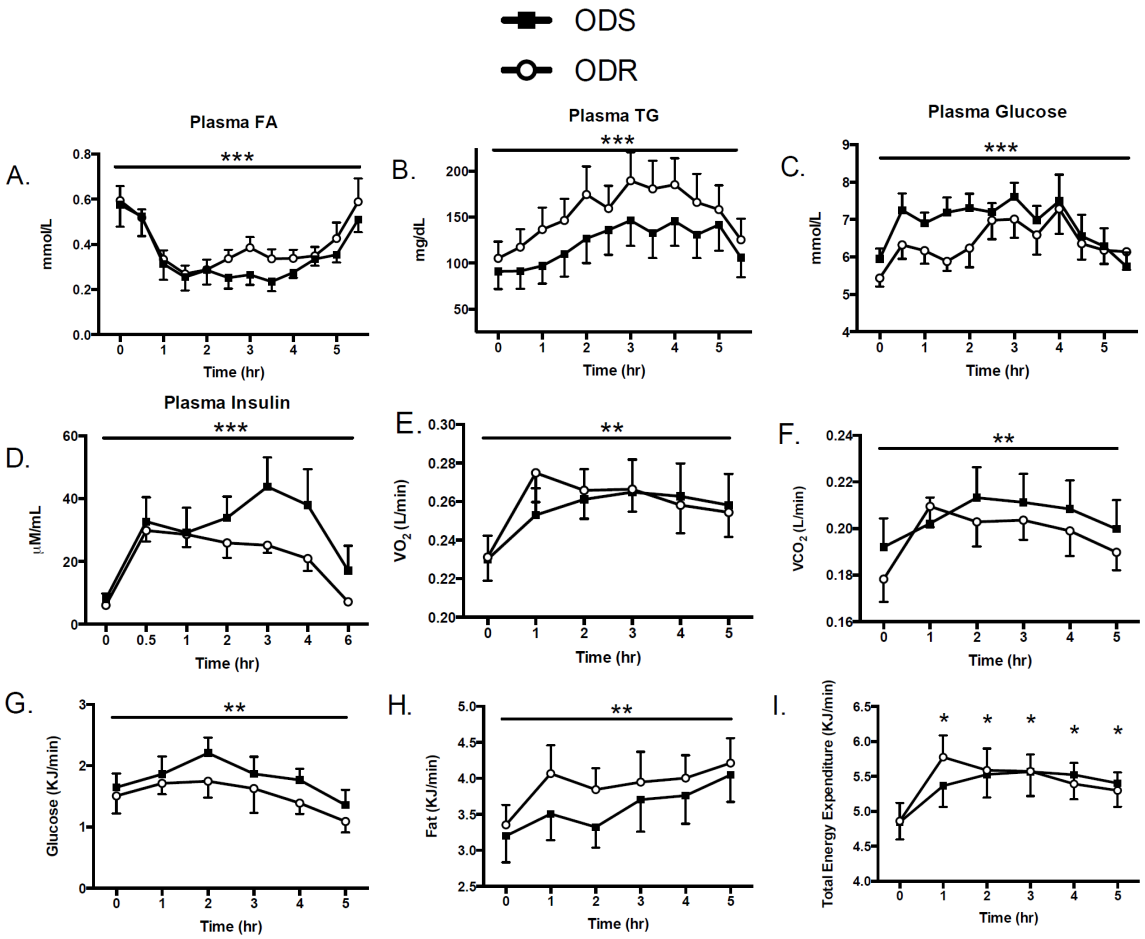

Figure 4-1 - Whole body response to the high fat meal in ODS and ODR individuals.

Plasma fatty acids (FA; A), triglyceride (TG; B), (C) glucose and (D) insulin at baseline (0 min) and following a high fat meal (HFM) in ODR and ODS individuals. Whole body VO2 (E), VCO2 (F), glucose (G), fat metabolism (H) and total energy expenditure (I) at baseline and in response to the HFM. Two way ANOVA, significant effect of time, ***P<0.0001, **P<0.01. Total energy expenditure, *P<0.05 Significant effect of time, post hoc significantly different from baseline within group. Data are mean ± SEM, n=10.

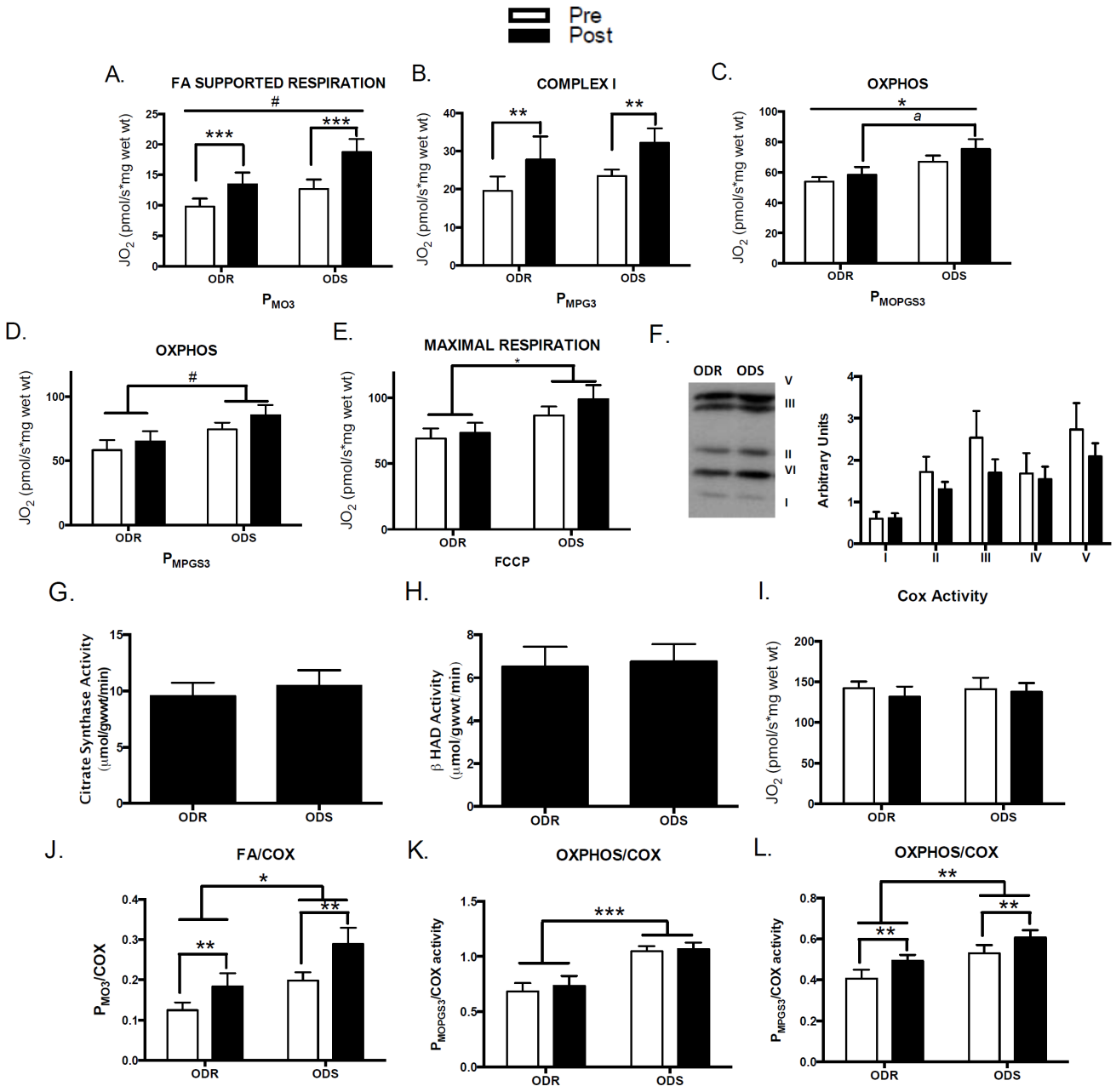

Figure 4-2 - Skeletal muscle mitochondrial respiration but not content is increased in ODS compared to ODR prior to and in response to the high fat meal.

Mitochondrial respiration was measured in permeabilized muscle fibers of ODS and ODR individuals pre and post the high fat meal (HFM). (A) State 3 fatty acid supported respiration with 2mM malate (M), 200μM octanoyl carnitine (OC) and 5mM ADP (PMO3. 2 way ANOVA, #P=0.09, effect of group, *P<0.05 significant effect of meal. (B) State 3 complex I respiration with electron supply from 2mM malate, 5mM pyruvate (P), 10mM glutamate (G) (P_{MPG3}). * P<0.05, significant effect of meal. (C) Maximal oxidative phosphorylation with electron supply to complex I and II with substrate M, OC, P, G and 10mM succinate (S) + ADP (P_{MOPGS3}). *P<0.05 two way ANOVA, significant effect of group, a posthoc significantly different between groups. (D) Maximal oxidative phosphorylation with electron supply to complex I and II with substrate M, P,G, S (P_{MPGS3}), #P=0.06, two way ANOVA, effect of group. (E) Maximal capacity of the electron transfer system, as measured with the chemical uncoupler FCCP. *P<0.05 significant effect of group. (F) Expression of electron transfer system proteins (complex I, II, III, IV, V), (G) citrate synthase activity and (H) beta HAD activity in skeletal muscle of ODR and ODS at baseline. (I) COX activity in permeabilized muscle fibers of ODS and ODR was measured with 5mM TMPD maintained in a reduced state by 5mM ascorbate and reducing cytochrome C as the substrate. (J) FA supported respiration expressed per COX activity. *Two way ANOVA, significant effect of group. **P<0.01, significant effect of meal, posthoc significantly different post between ODS and ODR. (K) Maximal oxidative phosphorylation (PMOPGS3) expressed per COX activity. *P<0.05 two way ANOVA significant effect of group. (L) Maximal oxidative phosphorylation (PMPGS3) expressed per COX activity. *P<0.05 two way ANOVA significant effect of group and meal. Data are mean ± SEM, n=8-10.

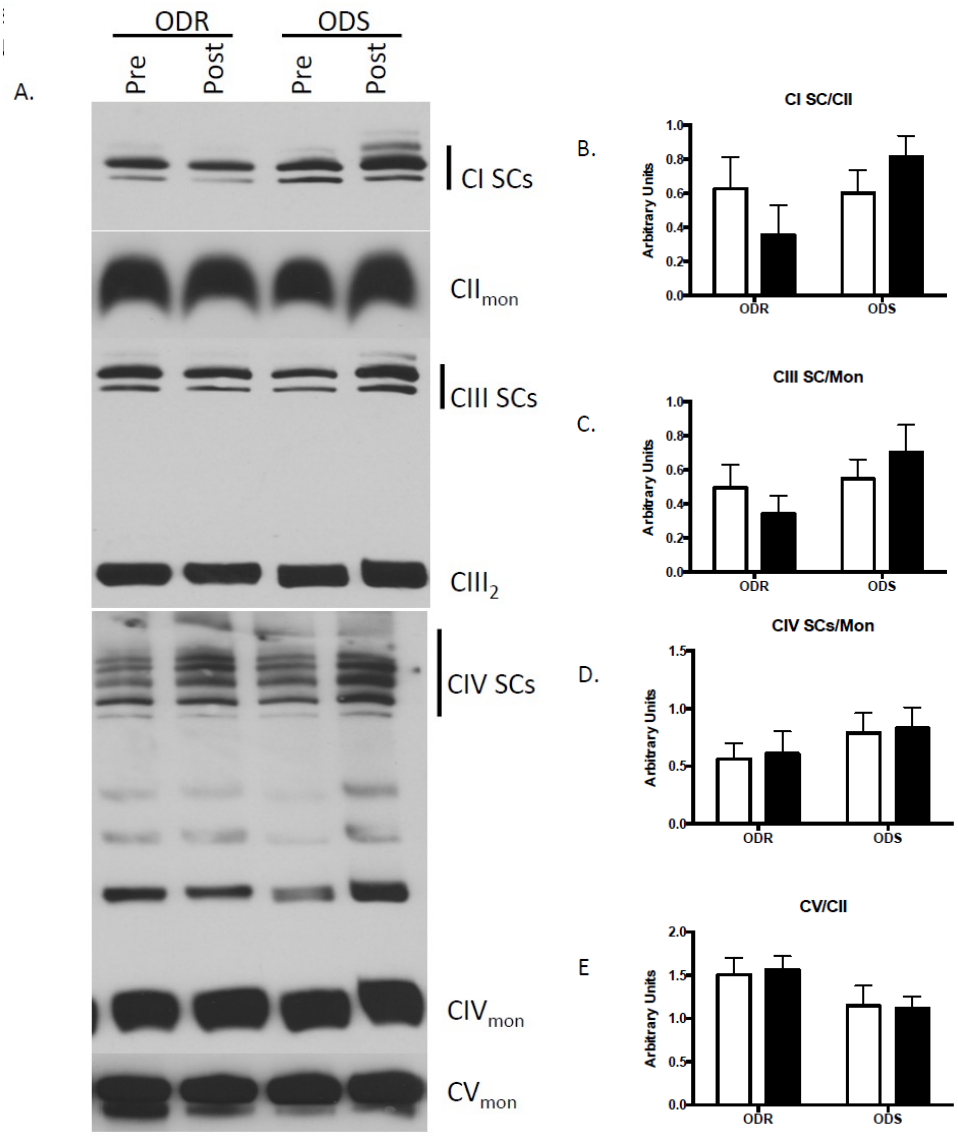

Figure 4-3 - Mitochondrial supercomplex formation is not different in skeletal muscle of ODS and ODR.

(A) Representative BN PAGE of mitochondrial supercomplexes in ODS and ODR pre and post the HFM. Quantification of (B) CI SCs/CII, (C) CIII SCs/CIII monomer, (D) CIV SCs/CIV monomer, and (E) CVSCs/CII. Data are mean ± SEM, n=6-7.

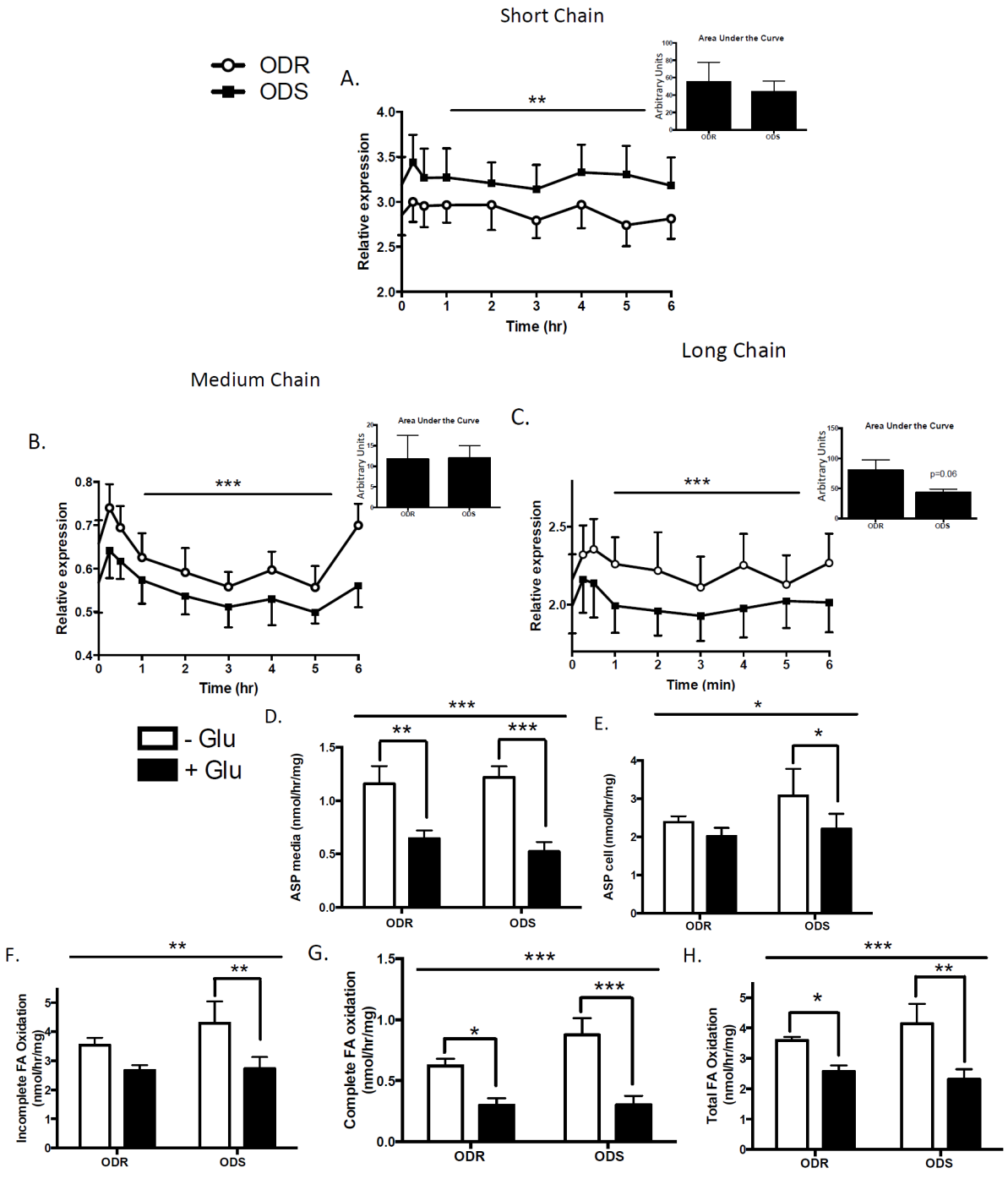

Figure 4-4 - Acylcarnitine and FA oxidation in ODS and ODR.

Plasma (A) short, (B) medium and (C) long chain acylcarnitine content and AUC in ODS and ODR at baseline and in response to the HFM. Two way ANOVA, Bonferroni posthoc, significant effect of time **P<0.01, ***P<0.001. Data are mean ± SEM (n=9). (D-H) FA oxidation in myotubes derived from ODR and ODS ± glucose pretreatment to test metabolic flexibility. (N=5) Data are mean± SEM. Two way ANOVA, line above graph indicates significant effect of glucose *P<0.05, **P<0.01, ***P<0.001. (D) Acid soluble products (ASP) in the media, posthoc effect of glucose ODR **P<0.01, ODS ***P<0.001 (E) ASP in the cell, posthoc effect of glucose, ODS *P<0.05; (F) Incomplete FA oxidation, posthoc effect of glucose, ODS P<0.01; (G) Complete FA oxidation to CO2 production, posthoc effect of glucose ODR, *P<0.05, ODS ***P<0.001; and (E) total FA oxidation, posthoc effect of glucose ODR*P<0.05, ODS **P<0.01.

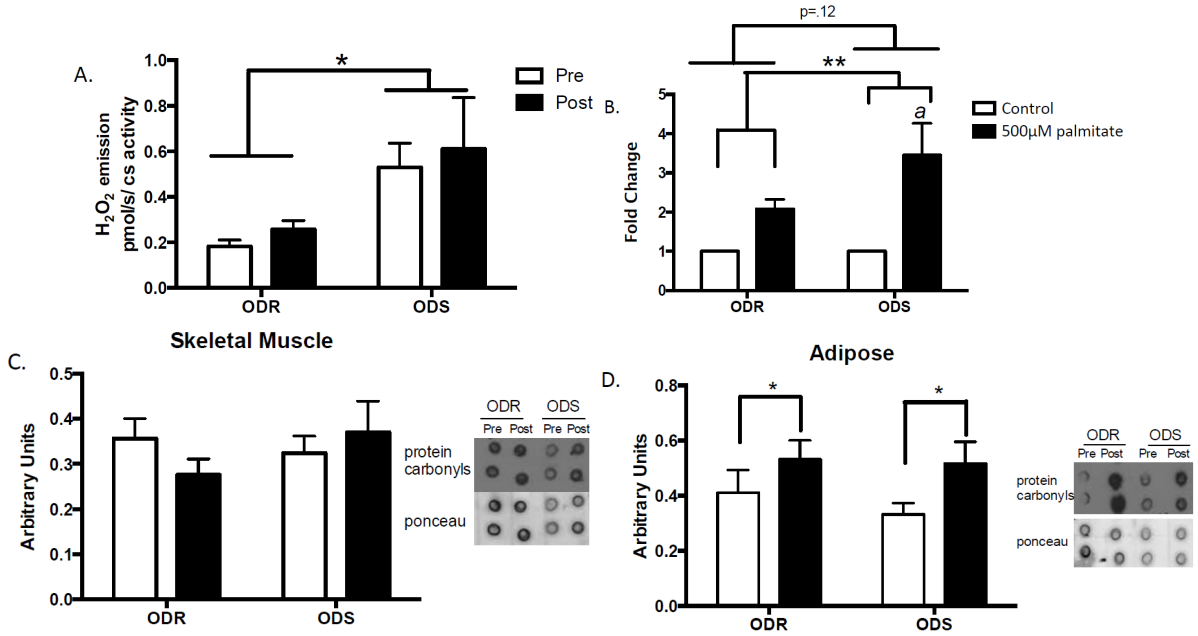

Figure 4-5 - ROS emission and markers of oxidative stress in skeletal muscle and adipose of ODS and ODR.

(A) Oligomycin-induced H2O2 emission in skeletal muscle of ODS and ODR pre and post HFM. *P<0.05 Two way ANOVA, significant effect of group (N=7-8). (B) ROS emission in myotubes derived from ODS and ODR with and without 24 hr 500µM palmitate treatment. Two way ANOVA **P<0.01 significant effect of treatment. P= 0.12 effect of group. a P<0.05 post hoc significant difference from control condition within group n=5. (C) Protein carbonylation in skeletal muscle of ODS and ODR pre and post HFM (E) (n=7-10). (D) Protein carbonylation in adipose of ODS and ODR pre and post the HFM (N=10). *P<0.05 significant effect of meal. Data are mean ± SEM.

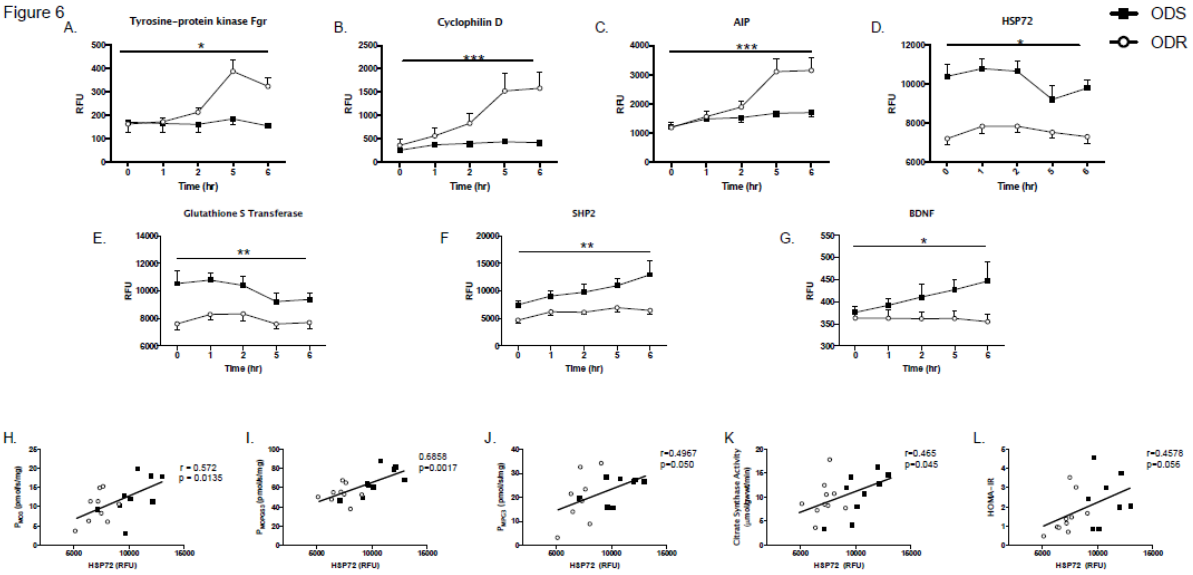

Figure 4-6 - Plasma proteins at baseline and in response to a high fat meal in ODR and ODS individuals.

(A) Tyrosine protein kinase FGR, (B) Cyclophilin D, (C) Aryl hydrocarbon interacting protein (AIP) significant effect of group x time. (D) Heat shock protein 70 1A/1B (HSP72) significant effect of group. Two way repeated measures ANOVA, Bonferonni post hoc. *P<0.05, ***P<0.001, **P<0.01. Nominally significant proteins included (E) Glutathione-S transferase; group effect, (F) tyrosine-protein phosphatase non-receptor 11 (SHP-2), group effect and (G) brain derived neurotrophic factor (BDNF) group x time effect. Two way repeated measures ANOVA *P<0.05, **P<0.01. N=9. Data are mean± SEM. HSP72 is positively correlated to (H) FA supported respiration (PMO3) (I) OXPHOS (P_{MOPGS3}), (J), Complex I respiration (P_{MP3}) (K) citrate synthase activity and (L) HOMA-IR.

5 CHAPTER 5 – GENERAL DISCUSSION

Obesity, T2DM, and their related complications have a significant impact on the healthcare systems internationally, incurring costs of over 800 billion dollars (NCD Risk Factor Collaboration, 2016a, b). With available medical intervention, reductions in these complications can be achieved but not totally eliminated (Gregg *et al.*, 2014). This suggests that further research is needed to uncover underlying pathologies in order to develop more effective treatment options. Obesity and T2DM are both complex diseases with multifactorial etiologies. Metabolic predictors and underlying mechanisms of their pathophysiology are still elusive although significant strides have been made.

The overarching goal of this doctoral thesis was to investigate the interrelation between these two diseases, in addition to elucidating underlying molecular etiologies. The goal of Chapter 2 was to study the impact of T2DM on weight loss ability in a large population of obese patients participating in a standardized meal replacement and lifestyle modification program. It was found that T2DM significantly deters weight loss although the effect is not large. It was further concluded that different pharmacotherapies targeted at T2DM have no impact on weight loss ability in obese patients. Since skeletal muscle energetics are central in the development and progression of obesity and T2DM, Chapter 3 and Chapter 4 aimed to study mitochondrial function in this tissue with the idea of uncovering molecular etiologies. Research in Chapter 3 demonstrated that there are deficiencies in mitochondrial respiration in individuals with obesity and T2DM compared to individuals with obesity alone. Reductions in mitochondrial respiration were correlated with increasing levels of HbA1C and associated with decreases in supercomplex formation in the MIM. Chapter 4 delineated differential fuel oxidation mechanisms and circulating protein

biomarkers in ODS and ODR participants following a high fat meal challenge. Whole-body analyses were conducted in addition to measures in blood, adipose tissue, skeletal muscle and primary cells. Increases in respiratory capacity were measured in skeletal muscle following the consumption of a HFM in all studied groups. Impaired mitochondrial function was found in the ODR group despite lack of differences in mitochondrial content or the assembly of supercomplexes. Differences were also found in circulating acylcarnitines as well as expression of several proteins including Heat shock 70 kDa protein 1A/1B, Tyrosine-protein kinase Fgr, and Peptidyl-prolyl cis-trans isomerase D (more commonly known as cyclophilin D).

Overall findings demonstrate that deficiencies in supercomplex formation in skeletal muscle of patients with obesity and T2DM could not only predispose them to developing these diseases initially, but could also prevent them from losing weight, creating a doubly challenging situation. The proposed sequence of events would start with impaired supercomplex formation, which would lead to decreased flow of electrons through the ETC. Altogether this would result in a compromised capacity to oxidize fuels. An additional effect would hypothetically include sustained reduction of the electrons carriers in the ETC, leading to an increase in ROS emission from the ETC, and increased oxidative damage. All together, these phenomena could be part of a vicious cycle that results in the increased oxidative damage found in tissues of individuals with T2DM (Rani *et al.*, 2016).

There have been few studies linking deficiencies in skeletal muscle mitochondrial energetics with T2DM in subjects with obesity (Kelley *et al.*, 2002; Mogensen *et al.*, 2007; Ritov *et al.*, 2010; Ritov *et al.*, 2005). Our results confirm that there are decreased functional capacities, and significantly extend findings by examining various characteristics of respiration, as well as the assembly of ETC supercomplexes. This constitutes the first report

of decreased supercomplex assembly in any human disease state, and by extension the first study of this phenomenon in T2DM. Furthermore, we identify novel correlations between HbA1C and maximal respiration of the ETC. Our study is also the first to examine mitochondrial respiration in permeabilized muscle fibers of *rectus abdominis*. There have been four previous studies involving mitochondria in this muscle group although none have addressed mitochondrial energetics as measured outcomes. In the first report, mitochondrial function was studied in lean subjects during cholecystectomies (Awad *et al.*, 2010) whereas the second report examined fusion/fission and biogenesis proteins in critically ill intensive care unit patients post-mortem, and compared results with those from *vastus lateralis* (Vanhorebeek *et al.*, 2012).

The question of mitochondrial dysfunction in obesity and T2DM has historically been debated. In obesity alone, several studies have reported reductions in mitochondrial functions (Colberg *et al.*, 1995; Kelley *et al.*, 2002; Kim *et al.*, 2000; Ritov *et al.*, 2010; Ritov *et al.*, 2005; Simoneau and Bouchard, 1995; Simoneau *et al.*, 1995; Simoneau *et al.*, 1999), whereas others have attributed this decline to reductions in mitochondrial content (Ara *et al.*, 2011; Boushel *et al.*, 2007; Chomentowski *et al.*, 2011). Comparisons between subjects who are lean and those who are obese are relatively straightforward as participants can be matched for age, height, medications, and other general medical conditions.

In T2DM, the question of mitochondrial dysfunction is less straightforward as obesity and overweight are associated with this disease in over 90% of cases (Wing, 2001). Therefore, it can be challenging to compare groups of well-matched lean individuals with T2DM to lean individuals who do not have T2DM. In addition, because T2DM is so intricately associated with obesity, results from studies of lean individuals may not be applicable to patients who have obesity and T2DM (the majority) as the etiologies are both

multifactorial and interrelated. In the studies presented herein, groups of individuals with T2DM and obesity were always compared to groups of individuals with obesity alone, allowing specific conclusions to be drawn regarding T2DM. Ongoing studies in our laboratory are examining some of the possible mechanistic origins of T2DM in a mouse model of metabolic programming (offspring of high fat – high sucrose diet fed dams; McMurray *et al.*, In Preparation). The latter research in mice will allow analyses of mitochondrial energetics and ROS at various time-points in the development of the disease.

Boushel *et al*. have previously published the most comparable study to Chapter 3; they reported no difference in mitochondrial function between non-diabetic and diabetic study participants (Boushel *et al.*, 2007). Their initially observed differences were rendered non-significant when they corrected the data to mitochondrial content as assessed by citrate synthase activity and mtDNA copy number. Both of these corrective factors were measured in separate tissue pieces under distinct conditions, *i.e.,* different than the tissue used for the mitochondrial functional studies. In the present study, mitochondrial content was assessed using the specific activity of cytochrome c oxidase (COX) in the tissue that was actually used in the high-resolution respirometry analyses, and all the data were corrected to mitochondrial content. In addition, the two groups of subjects had significantly different BMIs, preventing their conclusions from being specific to T2DM.

One potential weakness in our study (Chapter 3) is the absence of analyses of physical activity levels. Physical activity is well known to affect mitochondrial content and function (Pilegaard *et al.*, 2003; Short *et al.*, 2003). It is theoretically possible that differences observed between both groups are the result of varying levels of physical activity. This is however unlikely since all patients had similar and very high BMIs ($\approx 50 kg/m^2$), and BMI is inversely correlated with levels of physical activity (Brock *et al.*,

2009). Nevertheless, further studies should address the impact of physical exercise on mitochondrial supercomplex assembly. The working hypothesis would be that increasing levels of physical activity, which are known to increase the demand and production of ATP, would lead to greater supercomplex assembly to facilitate increased electron transfer through the ETC.

In addition, given the fact that non-superassembled complexes are fully functional entities (reviewed in Enriquez, 2016), it is possible that the impact of supercomplex assembly is minimal and works simply to enhance electron flow through the ETC. When considering this in the context of disease development, it is possible that the impact of deficiencies in supercomplex formation on whole-body bioenergetics could be negligible in early disease stages but significant later when compensatory mechanisms are no longer adequate. An analogy to this would be a very slow tap dripping in a sink where the main drain is only slightly slower than the incoming water. Early in the disease, compensatory mechanisms would allow water to empty through accessory drains, maintaining a balance of water inflow and output. With time, these accessory drains would progressively fail to work properly, causing accumulation of water in the sink. States of impaired fasting glucose would be the phase during which these energetic changes accumulate enough and fill up the sink, whereas T2DM would occur when the sink is full and additional drops cause overflow.

Nevertheless, the prevalences of obesity and T2DM are rising at alarming rates (Ng *et al.*, 2014), suggesting that mitochondrial dysfunction may compound disease development. It is possible that supercomplex assembly has a minimal impact on whole body energetics; this may explain the fact that no differences in supercomplex assembly were measured in muscle of ODS and ODR patients. Another possibility is that the existing technique used to measure the presences of supercomplexes (BN-PAGE) is not sensitive

enough to detect subtle differences. As mitochondrial supercomplexes become better understood, novel techniques will hopefully be developed in order to better study this essential phenomenon. Additional approaches to analyze supercomplexes could include direct visualization of the MIM ETC, native immunoprecipitation assays or the use of proteins involved in supercomplex assembly, such as SCAF1, as proxy measures. It would be expected that healthy lean adults would have higher levels of assembled supercomplexes than individuals with obesity or T2DM.

Future studies should explore factors underlying supercomplex formation both in T2DM and in different obesity phenotypes. This would include cardiolipin content as well as cristae formation and *bona fide* supercomplex assembly factors, such as SCAF1. Given differences in the levels of assembled supercomplexes found in obese patients with T2DM (Antoun *et al.*, 2015), it would be conceivable to uncover associated decreases in the level of protein factors affecting supercomplex assembly. This could become a novel avenue for research that could help delineate disease etiology or risk factors.

In addition, underlying etiologies of T2DM are thought to be heritable, and research has shown that genetic and epigenetic factors are important (Schwenk *et al.*, 2013). Previous studies have demonstrated that offspring of insulin-resistant parents have decreases in mitochondrial function (with ATP synthesis being reduced by up to 30%), skeletal muscle insulin resistance, as well as accumulation of intramyocellular lipids (Petersen *et al.*, 2004). *In vivo* ^{31}P nuclear magnetic resonance spectroscopy has also revealed that offspring of individuals with T2DM have a 30% reduction in mitochondrial oxidative phosphorylation (Petersen *et al.*, 2004). There is a 38% lower mitochondrial density as measured by electron microscopy (Morino *et al.*, 2005). Additional support for the heritability of muscle mitochondrial function in T2DM stems from our research group where it was found that

myotubes from post-T2DM individuals retain mitochondrial dysfunctions after weight loss and reversal of clinical illness (Costford *et al.*, 2009) With the new understanding that supercomplex assembly is associated with the mitochondrial dysfunction that is central to T2DM development and progression (Antoun *et al.*, 2015), we propose that the predisposition to supercomplex formation is also a heritable trait. As such, the heritability of factors known to affect supercomplex formation, such as SCAF1, could also be studied. This would allow further elucidation of disordered mitochondrial function in offspring of individuals with T2DM.

Findings of this nature would be consistent with results presented by DeLany *et al*. (2014), which demonstrated that reductions in maximal mitochondrial respiration in skeletal muscle are observed in African-American women who are known to be at greater risk of developing T2DM. Reductions seen in these populations, similar to the ones presented in Chapter 3, were measured under a variety of mitochondrial respiratory states including State 3, State 4 and maximal uncoupled respiration (DeLany *et al.*, 2014). Taken together, these data strongly suggest a role for mitochondrial dysfunction, and possibly supercomplex assembly, in the heritability of T2DM.

Additionally, skeletal muscle samples included in the study presented in Chapter 3 were obtained during RYGB surgery at the Ottawa Hospital. Routine preparations for this surgery include 3 weeks of the OPTIFAST® 900 hypocaloric meal replacement diet, as well as fasting for at least 8h prior to the surgery and sample collection. As such, patients were in negative energy balance for a prolonged period of time. This whole-body metabolic stress is important given that dietary changes are known to affect mitochondrial morphology and function (reviewed in Putti *et al.*, 2015). Therefore, conclusions drawn in this study are limited to the specific metabolic states of the patients undergoing the bariatric surgery, in

which the specific phenotype may be revealed. In other words, it is important to note that non-fasting adults with obesity and T2DM may not display dysfunctions of supercomplex formation. In order to answer this question, muscle biopsies should be obtained from individuals with obesity and T2DM and compared to biopsies obtained from individuals with obesity alone under similar states of energy balance, as described in Chapter 4.

It is also possible that medications affect the assembly of supercomplexes. During routine preparations for RYGB, patients are asked to discontinue most medications the evening before the surgery, including medications taken for T2DM. In the context of mitochondrial function and supercomplex assembly, it is therefore important to note that findings of decreased supercomplex assembly in T2DM could be due to T2DM medications taken or discontinued by patients before the muscle sampling. Further studies would be necessary in order to elucidate the effects of medications and medication discontinuation on supercomplex assembly and mitochondrial function.

The findings from our research described in Chapter 4, however, demonstrate no differences in supercomplex assembly in *vastus lateralis* of ODS and ODR patients. Possible reasons to explain these lack of differences include the fact the individuals studied were fasted overnight, but had not undergone prolonged negative energy balance. Participants in this study were indeed asked not to consume anything after their dinner the evening before the study, but they were not under prolonged metabolic stress of the order of days or weeks. This prolonged nutritional deficiency may have been necessary in order to uncover any differences in supercomplex assembly between the ODS and ODR group. It is also possible that metabolic differences in the two groups are unrelated to ETC efficiency and supercomplex formation but further studies are needed in order to shed some light on the question.

It has additionally been established that supercomplex assembly decreases ROS levels. Specifically, inhibition of Complex I association to Complex III increases superoxide generation from Complex I (Maranzana *et al.*, 2013). Similarly, the activity of UCPs has also been demonstrated to reduce ROS levels (Echtay *et al.*, 2002; Mailloux *et al.*, 2011). In a reciprocal manner, ROS and/or their products have been shown to activate the UCPs (Mailloux *et al.*, 2011). ROS are known to be essential signaling molecules (D'Autreaux and Toledano, 2007) and have been shown to cause oxidative damage and apoptosis at high levels (reviewed in Circu and Aw, 2010). The interaction between these two ROS modulators could be related to reductions in mitochondrial respiration although this has not specifically explored. As future studies continue to reinforce the link between supercomplexes, UCPs, and ROS levels, it may become apparent that the formation of supercomplexes could be a novel target for drug development. For example, if mitochondrial metabolism was impaired by preventing supercomplex formation, a more reduced ETC could cause increases in ROS, which would lead to activation of UCPs, increased whole-body energy expenditure, and weight loss.

In Chapter 4, muscle mitochondrial respiration and fatty acid oxidation were found to be lower in ODR compared to ODS individuals. Given the known impact of T2DM on whole-body metabolism and mitochondrial energetics in skeletal muscle (Antoun *et al.*, 2015), glycemia levels were measured and participants were excluded if they were found to have impaired fasting glucose or T2DM. In retrospect, given the fact that T2DM develops gradually, and the fact that underlying mitochondrial dysfunction could be present before overt disease presentation, other markers may have been better suited to identify and remove early-stage insulin resistant patients from the study. For example, given that hyperinsulinemia precedes hyperglycemia, levels of insulin could have been used to identify

participants with early insulin resistance in skeletal muscle. Furthermore, given that insulin resistance, skeletal muscle mitochondrial dysfunction, and diet resistance are all related phenomena, it is conceivable that patients in the ODR group may have been in subclinical early stages of insulin resistance. Future longitudinal studies will be needed in order to track development of T2DM in ODS versus ODR individuals. It would be expected that patients in the ODR group are more likely to develop T2DM than patients in the ODS group. First, however, studies should simply explore the current proportion of individuals with T2DM in the ODS versus the ODR groups. Although it is expected that the proportion of patients with T2DM would be higher in the ODR group, preliminary unpublished data from our group suggest that there are no differences in the proportions of individuals with T2DM in both groups.

Another question needing elucidation in future studies is that of the gut microbiome and its effect on weight loss in obese patients. Previous studies have elegantly demonstrated that the gut microbiome has a significant impact on obesity in both mice and humans (Turnbaugh *et al.*, 2006). The specific link between gut flora and weight loss or T2DM, however, still remains to be established. Given that the gut microbiome specifically impacts energy harvest from consumed foods – and therefore *in vivo* metabolite availability – it would be expected to impact weight loss sensitivity and development of T2DM. In other words, there may microbiome populations that produce higher amounts of energy substrates (e.g., short chain fatty acids) than other microbiome populations, and this could impact the risk for obesity and related metabolic diseases in the host.

In this era of modern medicine, there is a growing need for personalized treatment strategies that increases favorable outcomes for diverse patient populations. Given the findings presented in Chapter 4, it is proposed that circulating plasma biomarkers could be

used to detect weight loss ability in patients, promoting early referral to bariatric surgery or meal replacement and lifestyle modification programs.

In conclusion, mitochondrial dysfunction is an important component of both T2DM and diet resistant obesity. Underlying metabolic imbalances make it exceptionally difficult for patients to curb disease progression. Although medications, diet, and exercise have a role in helping individuals, a prevention strategy based on personalized medicine, which would take into account an individual's genetic and metabolic risk factors, would be the ultimate goal. Further research is needed in order to provide better care to patients and decrease the societal burden of these common chronic diseases.

5.1 REFERENCES

Antoun, G., McMurray, F., Thrush, A.B., Patten, D.A., Peixoto, A.C., Slack, R.S., McPherson, R., Dent, R., and Harper, M.E. (2015). Impaired mitochondrial oxidative phosphorylation and supercomplex assembly in rectus abdominis muscle of diabetic obese individuals. Diabetologia *58*, 2861-2866.

Ara, I., Larsen, S., Stallknecht, B., Guerra, B., Morales-Alamo, D., Andersen, J.L., Ponce-Gonzalez, J.G., Guadalupe-Grau, A., Galbo, H., Calbet, J.A.*, et al.* (2011). Normal mitochondrial function and increased fat oxidation capacity in leg and arm muscles in obese humans. International journal of obesity *35*, 99-108.

Awad, S., Constantin-Teodosiu, D., Constantin, D., Rowlands, B.J., Fearon, K.C., Macdonald, I.A., and Lobo, D.N. (2010). Cellular mechanisms underlying the protective effects of preoperative feeding: a randomized study investigating muscle and liver glycogen content, mitochondrial function, gene and protein expression. Ann Surg *252*, 247-253.

Boushel, R., Gnaiger, E., Schjerling, P., Skovbro, M., Kraunsoe, R., and Dela, F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia *50*, 790-796.

Brock, D.W., Thomas, O., Cowan, C.D., Allison, D.B., Gaesser, G.A., and Hunter, G.R. (2009). Association Between Insufficiently Physically Active and the Prevalence of Obesity in the United States. Journal of physical activity & health *6*, 1-5.

Chomentowski, P., Coen, P.M., Radikova, Z., Goodpaster, B.H., and Toledo, F.G. (2011). Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. J Clin Endocrinol Metab *96*, 494-503.

Circu, M.L., and Aw, T.Y. (2010). Reactive oxygen species, cellular redox systems, and apoptosis. Free Radical Biology and Medicine *48*, 749-762.

Colberg, S.R., Simoneau, J.A., Thaete, F.L., and Kelley, D.E. (1995). Skeletal muscle utilization of free fatty acids in women with visceral obesity. J Clin Invest *95*, 1846-1853.

Costford, S.R., Crawford, S.A., Dent, R., McPherson, R., and Harper, M.E. (2009). Increased susceptibility to oxidative damage in post-diabetic human myotubes. Diabetologia *52*, 2405-2415.

D'Autreaux, B., and Toledano, M.B. (2007). ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol *8*, 813-824.

DeLany, J.P., Dube, J.J., Standley, R.A., Distefano, G., Goodpaster, B.H., Stefanovic-Racic, M., Coen, P.M., and Toledo, F.G. (2014). Racial differences in peripheral insulin sensitivity and mitochondrial capacity in the absence of obesity. J Clin Endocrinol Metab *99*, 4307-4314.

Echtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B., Cadenas, S., Stuart, J.A., Harper, J.A., Roebuck, S.J., Morrison, A., Pickering, S.*, et al.* (2002). Superoxide activates mitochondrial uncoupling proteins. Nature *415*, 96-99.

Enriquez, J.A. (2016). Supramolecular Organization of Respiratory Complexes. Annu Rev Physiol *78*, 533-561.

Gregg, E.W., Li, Y., Wang, J., Burrows, N.R., Ali, M.K., Rolka, D., Williams, D.E., and Geiss, L. (2014). Changes in diabetes-related complications in the United States, 1990-2010. N Engl J Med *370*, 1514-1523.

Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944-2950.

Kim, J.Y., Hickner, R.C., Cortright, R.L., Dohm, G.L., and Houmard, J.A. (2000). Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab *279*, E1039-1044.

Mailloux, R.J., Seifert, E.L., Bouillaud, F., Aguer, C., Collins, S., and Harper, M.E. (2011). Glutathionylation acts as a control switch for uncoupling proteins UCP2 and UCP3. The Journal of biological chemistry *286*, 21865-21875.

Maranzana, E., Barbero, G., Falasca, A.I., Lenaz, G., and Genova, M.L. (2013). Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I. Antioxid Redox Signal *19*, 1469-1480.

Mogensen, M., Sahlin, K., Fernstrom, M., Glintborg, D., Vind, B.F., Beck-Nielsen, H., and Hojlund, K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes *56*, 1592-1599.

Morino, K., Petersen, K.F., Dufour, S., Befroy, D., Frattini, J., Shatzkes, N., Neschen, S., White, M.F., Bilz, S., Sono, S.*, et al.* (2005). Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest *115*, 3587-3593.

NCD Risk Factor Collaboration (2016a). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. The Lancet *387*, 1377-1396.

NCD Risk Factor Collaboration (2016b). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F.*, et al.* (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet *384*, 766-781.

Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med *350*, 664-671.

Pilegaard, H., Saltin, B., and Neufer, P.D. (2003). Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol *546*, 851-858.

Putti, R., Sica, R., Migliaccio, V., and Lionetti, L. (2015). Diet impact on mitochondrial bioenergetics and dynamics. Frontiers in physiology *6*, 109.

Rani, V., Deep, G., Singh, R.K., Palle, K., and Yadav, U.C. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life sciences *148*, 183-193.

Ritov, V.B., Menshikova, E.V., Azuma, K., Wood, R., Toledo, F.G., Goodpaster, B.H., Ruderman, N.B., and Kelley, D.E. (2010). Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab *298*, E49-58.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H., and Kelley, D.E. (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes *54*, 8-14.

Schwenk, R.W., Vogel, H., and Schurmann, A. (2013). Genetic and epigenetic control of metabolic health. Mol Metab *2*, 337-347.

Short, K.R., Vittone, J.L., Bigelow, M.L., Proctor, D.N., Rizza, R.A., Coenen-Schimke, J.M., and Nair, K.S. (2003). Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes *52*, 1888-1896.

Simoneau, J.A., and Bouchard, C. (1995). Skeletal muscle metabolism and body fat content in men and women. Obes Res *3*, 23-29.

Simoneau, J.A., Colberg, S.R., Thaete, F.L., and Kelley, D.E. (1995). Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *9*, 273-278.

Simoneau, J.A., Veerkamp, J.H., Turcotte, L.P., and Kelley, D.E. (1999). Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *13*, 2051-2060.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027-1031.

Vanhorebeek, I., Gunst, J., Derde, S., Derese, I., Boussemaere, M., D'Hoore, A., Wouters, P.J., and Van den Berghe, G. (2012). Mitochondrial fusion, fission, and biogenesis in prolonged critically ill patients. J Clin Endocrinol Metab *97*, E59-64.

Wing, R.R. (2001). Weight loss in the management of type 2 diabetes. Evidence-based Diabetes Care Hamilton, ON: BC Decker Inc, 252-276.

6 APPENDICES

6.1 APPENDIX A: SUPPLEMENTARY MATERIALS FOR CHAPTER 2

6.1.1 SUPPLEMENTARY METHODS

Patients were enrolled in the Core Program of the Ottawa Hospital Weight Management Clinic. This consisted of 12 weeks of calorie restriction of 900 calories (total meal replacement with Optifast® 900 – Nestlé Canada Inc., North York Ontario M2N 6S8), a 26 week lifestyle modification course with nutritional counseling and physician supervision (Dent *et al.*, 2002; Harper *et al.*, 2002). Patients were weighed weekly wearing street clothes and without shoes. Rate of weight loss (ROWL) was calculated using the slope of the weight during the first 6 weeks on meal replacement. Percentage of weight loss (PWL) was calculated at 26 weeks using the averages of weight from weeks 22 to 26. This was done in order to avoid missing data points due to a lack of patient follow up and because most patients were weight-stable around this period of time.

Linear regression analysis was conducted to find the significant predictors of weight loss; the null hypothesis tested was that the regression coefficient was equal to zero and $P < 0.05$ was used to reject the null-hypothesis. Chi-square test of independence with Yates' continuity correction was used to compare categorical variables and to investigate whether the proportions of one variable were different for different values of the other variable; the null hypothesis is that the relative proportions of one variable are independent of the second variable. An independent-samples t-test was used to compare, the means of a quantitative traits under 2-levels (patients with T2DM/patients without T2DM or female/male), our null hypothesis was that the difference in means is equal to zero.

Diagnosis of T2DM and IFG was based on the guidelines published by the Canadian Diabetes Association (Goldenberg and Punthakee, 2013; Meltzer *et al.*, 1998) and the status of each patient was confirmed by the treating physician at the Weight Management Clinic. T2DM was diagnosed using the following criteria: fasting plasma glucose (FPG) ≥ 7.0 mmol/L; or glycated hemoglobin (HbA1C) ≥ 6.5%; or 2-hour plasma glucose (2hPG) in a 75 g oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L; or random plasma glucose (PG) ≥ 11.1 mmol/L. Impaired fasting glucose (IFG) included patients who had FPG ≥ 6.1 mmol/L or ≤ 6.9 mmol/L at week 1 of the program, and those who had a previously documented history of IFG or T2DM. Medications used in the treatment of T2DM may also play a pivotal role in weight management. Among others, insulin, sulfonylureas and meglitinides have been associated with weight gain (Harper *et al.*, 2013) while weight neutral drugs include metformin, acarbose and DPP-4. Patients on weight loss-inducing medications, such as GLP-1 receptor agonists and lipase inhibitors (Orlistat) (Harper *et al.*, 2013), were too few given the dates of data collection and were therefore excluded from this study. Although the effects of all drugs on weight management are typically considered as monotherapy, it is important to note that they are often used in combination with each other to produce the desired outcomes in individual patients.

6.1.2 SUPPLEMENTARY TABLES

Table 6-1 - Exclusion factors for study participants

Patients who completed the program n=4173		
	Factor	Number removed
Removed for adherence issues (n=1070)	Attended <3 sessions in 6 weeks of meal replacement	90
	Unable to calculate rate of weight loss	193
	<50% overall attendance	240
	Product use <80% or >100%	307
	MD notes indicating poor adherence	191
	Late for week 1 labs >6 days on product	49
Removed for other putative factors affecting rate of weight loss (n=728)	TSH out of range (0.3 – 5.5)	160
	Pregnancy onset before week 10	5
	Surgery during study period	20
	Previous bariatric surgery	24
	Tricyclic antidepressant	82
	Paroxetine	70
	Mirtazepine	4
	Typical antipsychotics	8
	Atypical antipsychotics	23
	Mood stabilizers associated with weight gain	34
	Topiramate	9
	Fenfluramine	14
	Sibutramine	9
	Orlistat	3
	Phentermine	0
	Patients with severe oedema including those on dialysis	39
	Patients on β-Blockers	224
	Patients on oral corticosteroids	10
Removed for T2DM-related factors (n=134)	Persons with type 1 diabetes	4
	Information about T2DM not adequate	51
	Gestational T2DM	64
	T2DM on medications with weight loss (Byetta, Januvia, Victoza, Invokana)	6
	T2DM on mixed weight loss and weight gain medications	9
Patients included in the study n=2231		

Table 6-2 - Percentage of weight loss of patients with T2DM who are not on medications, patients with T2DM on medications that do not cause weight gain, and patients with T2DM on medications that cause weight gain at 6 and 26 weeks following the initiation of a meal replacement and lifestyle modification program.

	T2DM not on medications (n=86)	T2DM on medications with no weight gain (n=82)	T2DM on medications with weight gain (n=171)	
Males (n)	31	21	76	0.014
Female (n)	55	61	95	
				P value (comparing all three groups)
Age (years)	49.4 +/- 10.5	51.8 +/- 9.8	51.9 +/- 9.0	0.151
Height (m)	1.68 +/- 0.095	1.66 +/- 0.097	1.69 +/- 0.103	0.039
Weight (kg)	126.9+/- 27.2	121.3 +/- 22.5	127.7 +/- 26.4	0.126
Body Mass Index (kg/m2)	44.7 +/- 8.5	44.0 +/- 7.3	44.4 +/- 7.5	0.836
HbA1C (%)	7.19 +/- 1.05	6.85 +/- 1.12	7.63 +/- 1.40	8.3×10^{-05}
ROWL 6 weeks	4.473 +/- 1.48	3.977 +/- 1.38	4.633 +/- 1.52	0.0032
PWL 26 weeks	0.179 +/- 0.052	0.168 +/- 0.055	0.185 +/- 0.062	0.125
Data adjusted for age and sex				
ROWL 6 weeks	4.140 +/- 0.22	4.083 +/- 0.29	4.149 +/- 0.28	0.2216
PWL 26 weeks	0.134 +/- 0.297	0.088 +/- 0.341	0.137 +/- 0.345	0.595
Data adjusted for age, sex, and initial weight				
ROWL 6 weeks	4.090 +/- 0.21	4.043 +/- 0.26	4.099 +/- 0.23	0.2429
PWL 26 weeks	0.125 +/- 0.299	0.081 +/- 0.337	0.130 +/- 0.341	0.604
Data are means +/- standard deviation.				

6.2 APPENDIX B: SUPPLEMENTARY MATERIALS FOR CHAPTER 3

6.2.1 SUPPLEMENTARY METHODS

6.2.1.1 PATIENT RECRUITMENT AND MUSCLE BIOPSY COLLECTION

Two groups of patients, with and without type 2 diabetes, and meeting all study criteria were selected for study. The length of time between the diagnosis of type 2 diabetes and the muscle sampling was variable and ranged from 2178 days to 241 days. The average duration of this period of time was 1072 ± 211 days. It is however important to note that, given the challenges in dating the exact onset of type 2 diabetes, it is possible and likely that the actual onset of diabetes predated the clinical diagnosis.

Rectus abdominis samples were obtained in the operating room from the incision sites of laparoscopic equipment while patients were under general anesthesia. Skeletal muscle sample collection was limited to regular working hours and varied based on operating room schedules. The *rectus abdominis* was chosen because of ease of access to the site during the surgery, which permitted functional and biochemical characterizations of this stabilizer muscle previously under-appreciated in the literature.

6.2.1.2 HIGH-RESOLUTION RESPIROMETRY

Briefly, *rectus abdominis* samples were placed in ice-cold relaxation medium (BIOPS) (Pesta and Gnaiger, 2012) immediately after harvesting. Individual muscle fibers were mechanically separated and saponin permeabilized (50 µg/ml) on ice for 30 minutes. Fibers were rinsed in mitochondrial respiration medium (MiR05) (Pesta and Gnaiger, 2012), and weighed amounts were placed in the respirometer chambers. Two separate protocols were used and runs were performed at 37°C. The concentration of oxygen was kept between 200 and 400 nmol/mL for the duration of the measurements. The first protocol used involved the progressive additions of 2 mM malate, 5 mM pyruvate (L_N - adenylate free leak

respiration), 10 mM glutamate, 5 mM ADP (P_{CI} – Complex I-supported respiration), 10 mM succinate (P_{CI+CII} – Complex I- and II-supported respiration), 0.25 µM titrations of carbonyl cyanide p-trifluoro-methoxyphenyl hydrazine (FCCP) (U_C – Maximal uncoupled respiration), 2.5 µM antimycin A (AA) and 2 mM N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride (TMPD) with 2 mM ascorbate (Tm - Cytochrome C Oxidase (COX) activity). This last measurement has been used as an indicator of mitochondrial content in various works and is arguably better used to normalized high-resolution respirometry data as the measurement is performed on the same sample as the rest of the protocol (Pesta and Gnaiger, 2012). The second protocol included consecutive additions of 2 mM malate, 200 µM octanoyl carnitine, 5 mM ADP (P_{ETF} – fatty acid-supported respiration), 5 mM pyruvate, 10 mM glutamate, 10 mM succinate, 2.5 µM oligomycin (L_{Omy} – Leak respiration). All runs were performed in duplicate and values are corrected to non-mitochondrial oxygen consumption (AA).

6.2.1.3 Mitochondrial Supercomplex and Total Protein Determinations

ATP synthase assembly and ETC supercomplex assembly were analyzed by blue native polyacrylamide gel electrophoresis (BN-PAGE) (Patten *et al.*, 2014; Wittig *et al.*, 2006). *Rectus abdominis* tissue was flash frozen and subsequent steps were performed on ice. Samples were homogenized in sucrose buffer (250 mM sucrose, 20 mM imidiazole/HCl, pH 7.0). Mitochondria were pelleted at 10,000 x g for 10 minutes and resuspended in 50 mM imidiazole/HCl pH 7.0, 50 mM NaCl, 5 mM 6-aminohexanoic acid, 1 mM EDTA with 1% digitonin (final digitonin to tissue ratio of 1:12 w/w) for 30 minutes. Samples were cleared by centrifugation for 30 minutes at 14,000 x g. Protein was loaded with 5% glycerol and a 1:10 dye:digitonin ratio of Coomassie Blue G-250 onto 3-13% large gradient gels. Gels were

run in high Coomassie Blue cathode buffer for 2 hours at 150 V and switched to low Coomassie cathode buffer overnight at 200 V. Gels were transferred to nitrocellulose membrane at 500 mA for 2.5 hours and membranes were blotted as described below.

For determinations of total mitochondrial protein amounts, samples were subjected to standard Western blotting procedures (SDS-PAGE).

6.2.1.4 IMMUNOBLOTTING

The following primary antibodies were used for BN-PAGE blots: Complex I [NDUFA9] (459100, Invitrogen – 1:2,000), Complex II [Fp] (459200, Invitrogen – 1:10,000), Complex III [UQCRC2] (Ab14745 MitoSciences – 1:2,000), Complex IV [subunit I] (459600, Invitrogen – 1:1,000), Complex V [ATP5A] (Ab14748, MitoSciences – 1:2,000). For SDS-PAGE, antibodies for Complex III and IV were substituted with: Complex III [subunit core I] (459140/D2035, Invitrogen – 1:3,000), Complex IV [subunit I] (MS404/E0594 MitoSciences – 1:2,000). Membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence. Bands were quantified using ImageJ and data was normalized to protein content as assessed by Ponceau S staining of membranes.

6.2.2 SUPPLEMENTARY TABLES

Table 6-3 - Baseline characteristics of non-diabetic and diabetic patients

Variable	Non-diabetic	Diabetic
Female (n)	10	10
Age (years)	49 ± 3	48 ± 2
Weight (kg)	126.9 ± 4.3	129.3 ± 7.0
Body Mass Index (kg/m2)	49.3 ± 1.5	50.2 ± 2.6
Body Fat (%)	52.6 ± 0.8	52.2 ± 1.0
Fasting plasma glucose (mmol/L)	5.8 ± 0.3	6.9 ± 0.3*
HbA1c (%)	5.5 ± 0.1	7.3 ± 0.2***
HbA1c (mmol/mol)	36.8 ± 1.6	55.7 ± 2.3***

Data are means ± SEM. *p<0.05 ***p<0.001

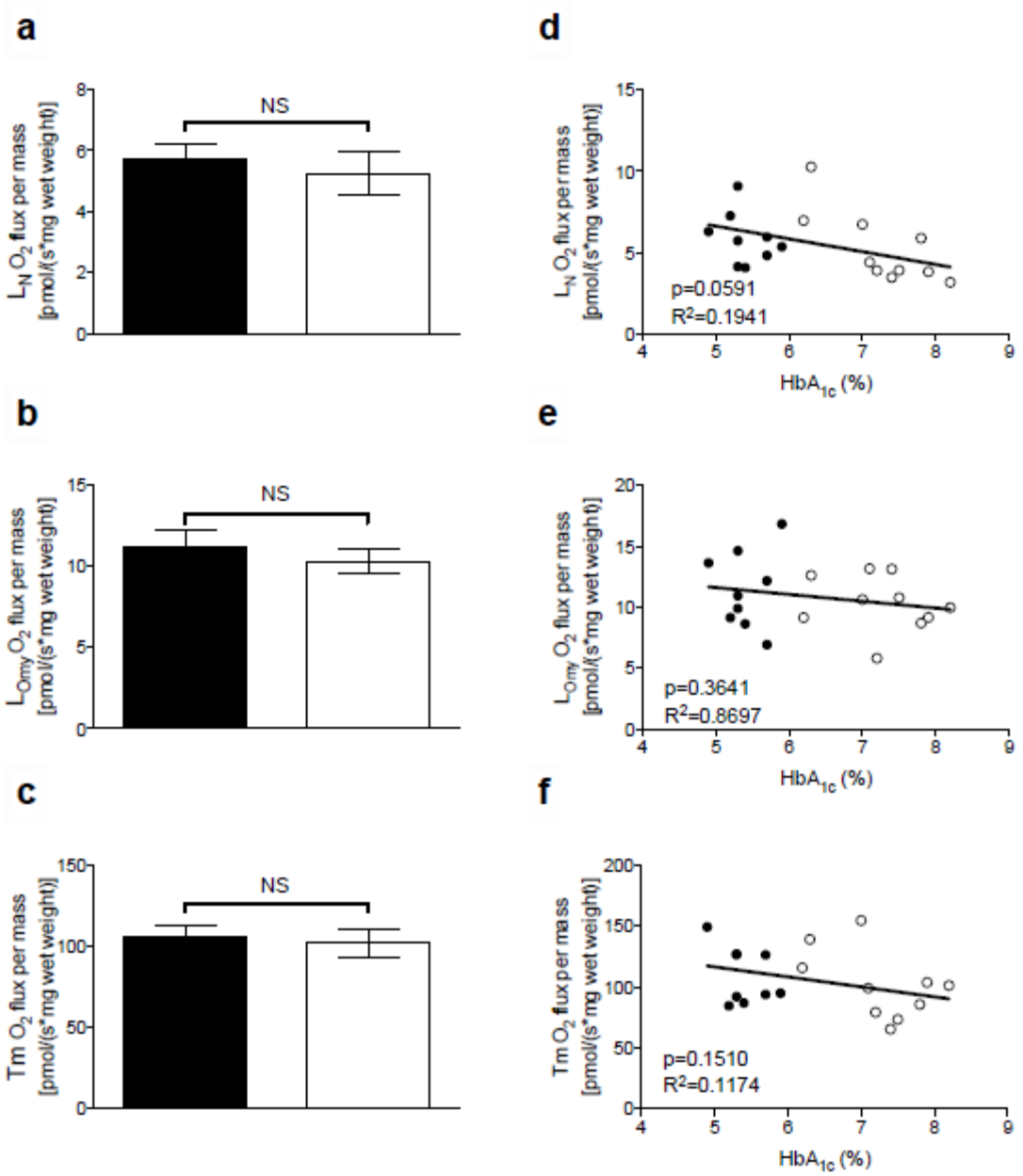

Figure 6-1 - Leak-dependent respiration as well as mitochondrial content are unchanged in permeabilized rectus abdominis *muscle fibers from obese diabetic females compared to obese non-diabetic females.*

There is no correlation between these measures and levels of HbA1c. Data are shown for adenylate-free leak respiration (L_N; A, D), oligomycin-induced leak respiration (L_{Omy}; B, E), and maximal CIV activity in the presence of TMPD and ascorbate (Tm; C, F). A-C: All represented values are mean ± SEM, N = 10 non-diabetic (black bars) and N = 10 diabetic patients (white bars). *P<0.05, **P<0.01 (Unpaired Student's t-test). D-F: All represented data are linear regressions, N = 9 non-diabetic (black points) and N = 10 diabetic patients (white points). To convert values for HbA1c in % into mmol/mol, subtract 2.15 and multiply by 10.929.

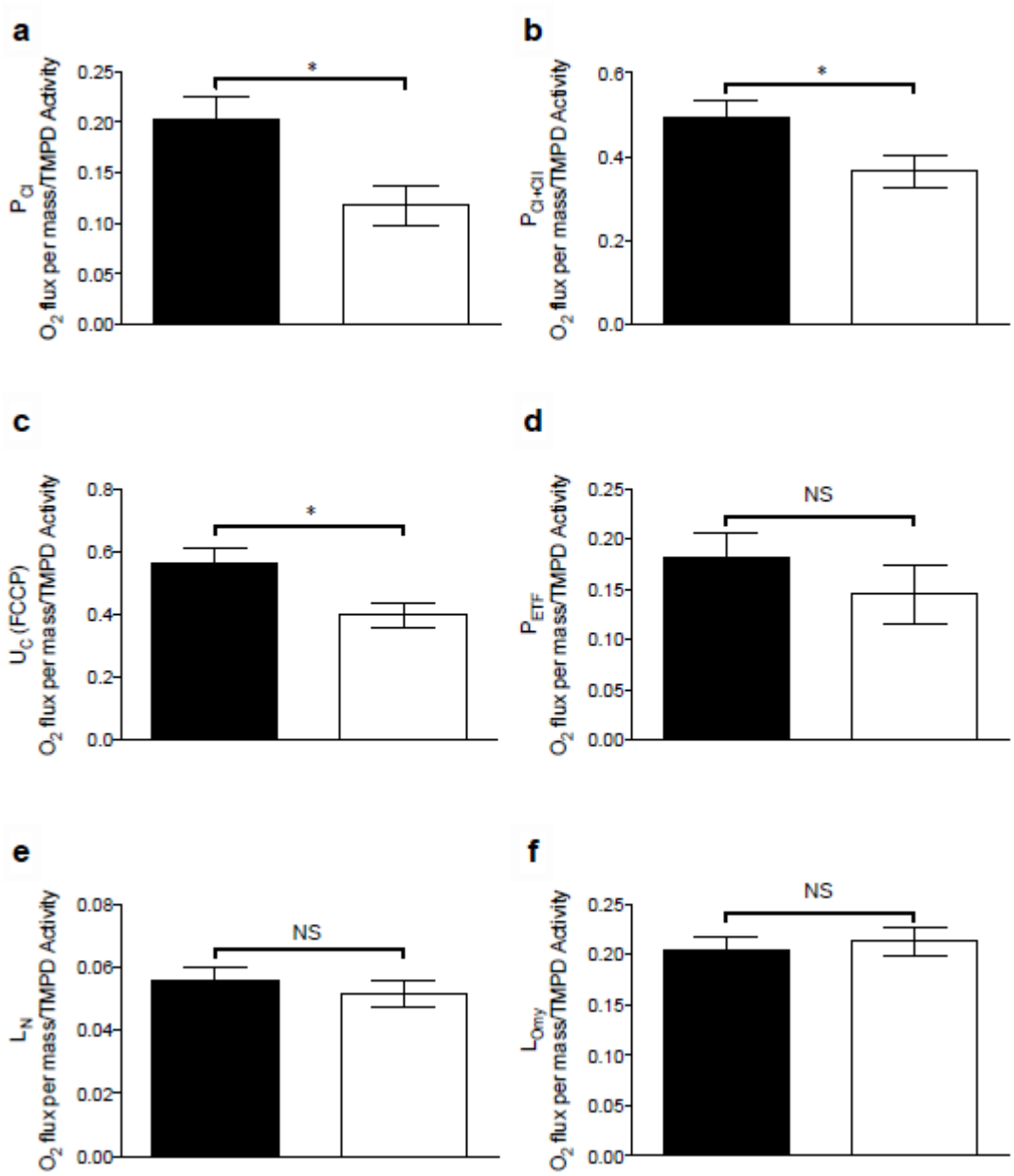

Figure 6-2 - Mitochondrial energetics are altered in permeabilized rectus abdominis *muscle fibers from obese diabetic females compared to obese non-diabetic females when data is normalized to the isolated activity of cytochrome C oxidase (COX).*

Data are reflective of **Error! Reference source not found.** and **Error! Reference source not found.**. Data are shown for state 3 respiratory capacity through complex I (P_{CI}; a), maximal oxidative phosphorylation capacity (P_{CI+CII}; b), maximal uncoupled respiration in the presence of the chemical protonophore FCCP (U_C; c), and maximal electron flow through electron-transferring flavoprotein and fatty acid oxidative capacity (P_{ETF}; d), adenylate-free leak respiration (L_N; e), and oligomycin-induced leak respiration (L_{Omy}; f). All represented values are mean ± SEM, N = 10 non-diabetic (black bars) and N = 10 diabetic patients (white bars). *P<0.05 (Unpaired Student's t-test).

6.3 APPENDIX C: SUPPLEMENTARY MATERIALS FOR CHAPTER 4

6.3 APPENDIX C: SUPPLEMENTARY MATERIALS FOR CHAPTER 4

6.3.1 SUPPLEMENTARY TABLE

Table 6-4 - Increased methylation of DGKZ and NDUFA5 in skeletal muscle DNA from ODS vs ODR Individuals

Probe ID	Tvalue	P	Chr	BP	Gene	Type
cg12856521	22.67	1.26E-09	11	46389249	*DGKZ*	exonic
cg20956366	17.77	7.01E-09	7	123197866	*NDUFA5*	UTR5

6.3.2 SUPPLEMENTARY FIGURES

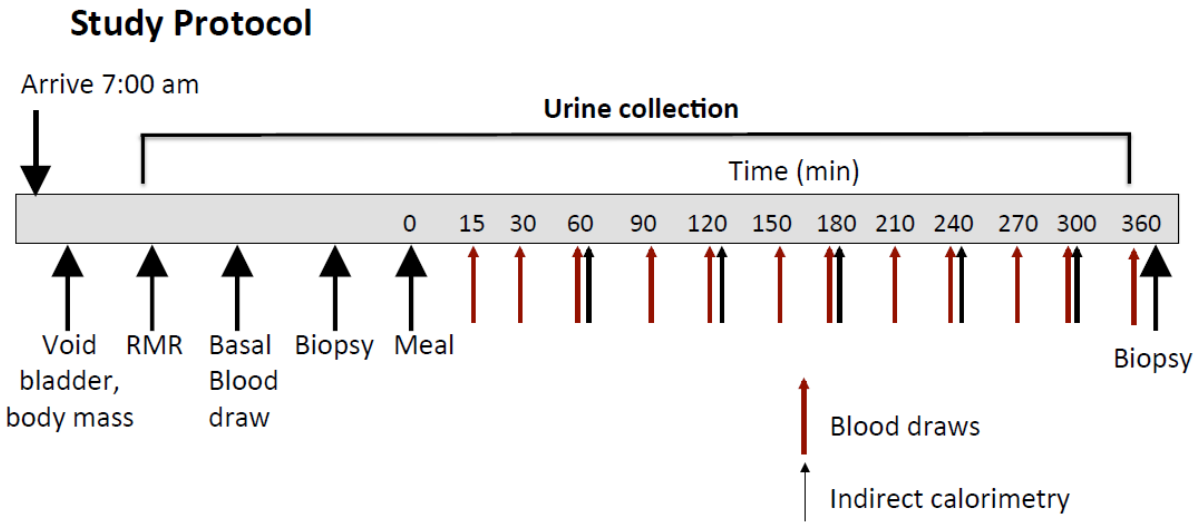

Figure 6-3 - Study Day Protocol

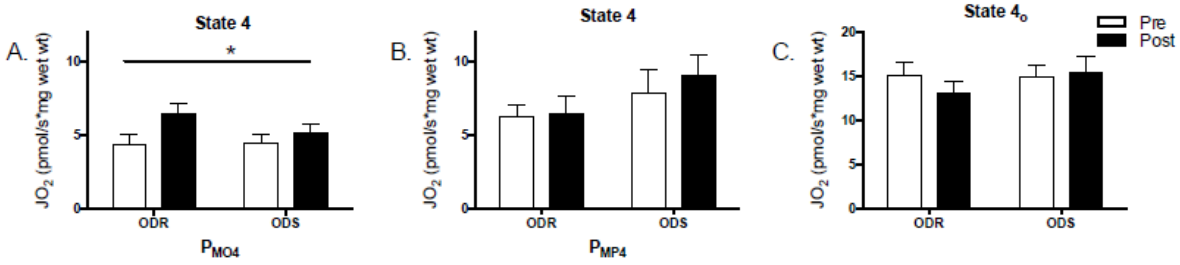

Figure 6-4 - State 4 respiration in permeabilized muscle fibers of ODS and ODR prior to and in response to a high fat meal

Respiration with (A) 2mM malate (M) + 200µM octanoyl carnitine (PMO4), (B) M+ 5mM pyruvate (PMP4) and (C) with oligomycin (2.5µM State 4o). *P<0.05 significantly different between groups. Data are mean ± SEM, n=7-10.

Supplemental Figure 3

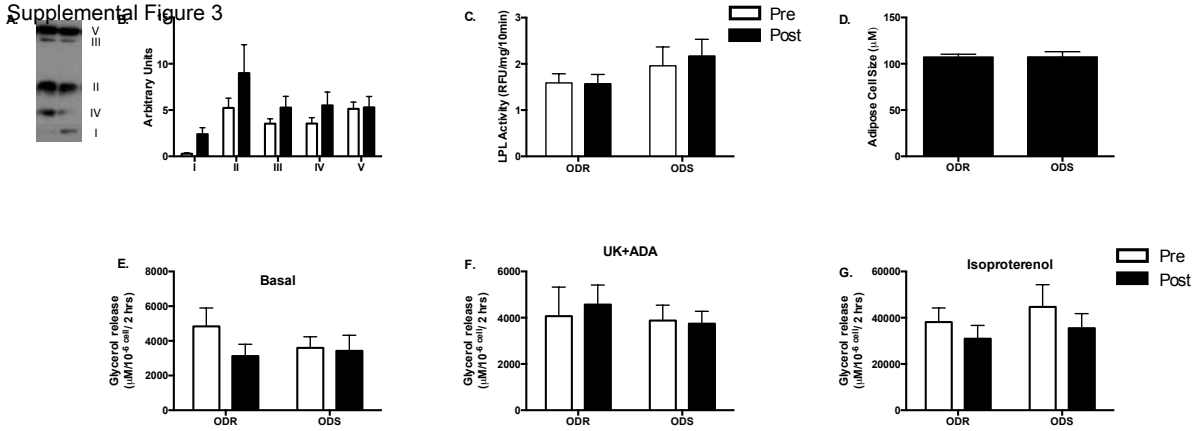

Figure 6-5 - Adipose tissue metabolism and mitochondrial content is not different in ODS and ODR

(A, B) Expression of electron transfer system proteins (complex I, II, III, IV, V) in adipose of ODS and ODR n=6-10. (C) Lipoprotein lipase activity in adipose of ODR and ODS pre and post the high fat meal (HFM). (D) Adipose cell size. Adipose tissue lipolysis in ODS and ODR pre and post the HFM in the under (E) basal conditions and in the presence of (F) UK-14304 + adenosine deaminase (UK+ADA) and (G) Isoproterenol. N=8-10. Data are mean ± SEM.

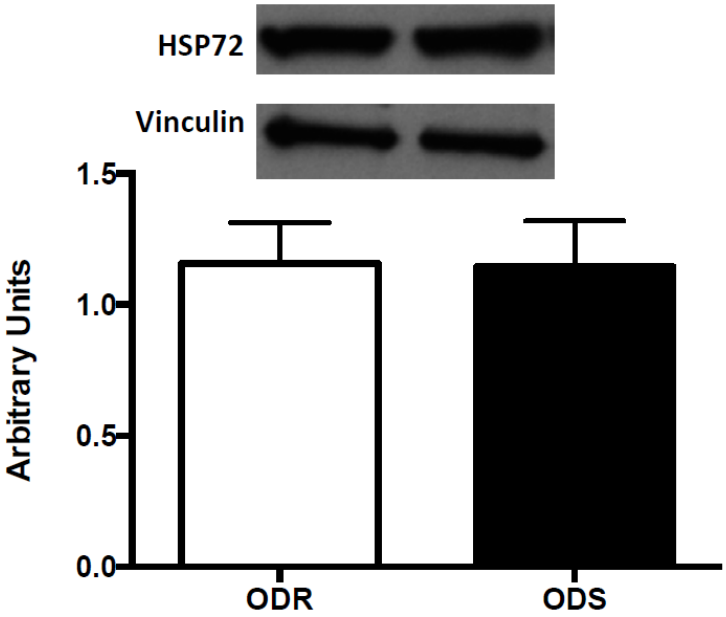

Figure 6-6 - HSP72 protein expression in skeletal muscle of ODS and ODR

(ODR N=9, ODS N=10. Data are mean ± SEM.

6.4 APPENDIX REFERENCES

Dent, R.M., Penwarden, R.M., Harris, N., and Hotz, S.B. (2002). Development and evaluation of patient-centered software for a weight-management clinic. Obes Res *10*, 651-656.

Goldenberg, R., and Punthakee, Z. (2013). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Can J Diabetes *37 Suppl 1*, S8-11.

Harper, M.E., Dent, R., Monemdjou, S., Bezaire, V., Van Wyck, L., Wells, G., Kavaslar, G.N., Gauthier, A., Tesson, F., and McPherson, R. (2002). Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. Diabetes *51*, 2459-2466.

Harper, W., Clement, M., Goldenberg, R., Hanna, A., Main, A., Retnakaran, R., Sherifali, D., Woo, V., and Yale, J.F. (2013). Pharmacologic management of type 2 diabetes. Can J Diabetes *37 Suppl 1*, S61-68.

Meltzer, S., Leiter, L., Daneman, D., Gerstein, H.C., Lau, D., Ludwig, S., Yale, J.F., Zinman, B., and Lillie, D. (1998). 1998 clinical practice guidelines for the management of diabetes in Canada. Canadian Diabetes Association. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne *159 Suppl 8*, S1-29.

Patten, D.A., Wong, J., Khacho, M., Soubannier, V., Mailloux, R.J., Pilon-Larose, K., MacLaurin, J.G., Park, D.S., McBride, H.M., Trinkle-Mulcahy, L.*, et al.* (2014). OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. The EMBO journal *33*, 2676-2691.

Pesta, D., and Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods in molecular biology (Clifton, NJ) *810*, 25-58.

Wittig, I., Braun, H.P., and Schagger, H. (2006). Blue native PAGE. Nat Protoc *1*, 418-428.