# Metabolic and Body Composition Responses to a Moderate Energy Restricted, Abundant Protein Diet in Adults with Type 2 Diabetes

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# Abstract

Obesity and particularly visceral adiposity are important factors in insulin resistance, and contribute to blunted protein anabolism that is observed with diabetes. This insulin sensitivity of protein metabolism in obese adults with type 2 diabetes, does not improve when normalizing glycemia with medication, although rates of protein turnover decrease. On the other hand, insufficient protein intake in weight-loss diets that keep protein at a fixed percentage of energy could exacerbate insulin sensitivity of protein metabolism. Thus the objective of this thesis was to perform a pilot study in 6 obese diabetic adults (3 men, 3 women), testing whether 5 weeks of energy restriction with maintained usual abundant protein intake would improve insulin sensitivity of glucose and protein metabolism. The energy-restricted diet provided 60% of energy requirements with 45% as carbohydrate and 26% as protein (1.8-1.9 g/kg lean body mass/day). Isotopic tracers were used to quantify whole-body glucose (3-$^{3}$H-glucose) and protein ($^{13}$C-leucine) metabolism Pre- and Post-intervention, postabsorptive and during a hyperinsulinemic (~ 500 pM), isoglycemic (8.3 ± 0.5 Pre vs. 5.8 ± 0.3 mmol/L Post), isoaminoacidemic clamp. At 5 weeks of energy restriction, weight loss was mainly attributed to total body and visceral fat losses, measured by dual-energy x-ray absorptiometry, and lean mass was preserved. Fasting plasma glucose was near normal, and serum insulin and C-peptide and HOMA-IR decreased significantly as did other cardiovascular risk factors. Postabsorptive protein turnover and rates of oxidation decreased, resulting in less negative net balance and a sparing of body protein; rates of glucose turnover decreased and the metabolic clearance rate of glucose improved. During clamp, protein turnover rates were lower, but there was no improvement in net anabolism. Thus, maintaining the usual protein intake (~ 110 g/d) preserves lean body mass when confronted with energy deficit and insulin resistance, without improving or worsening protein metabolism.

**Effet d'une restriction énergétique modérée avec apport en protéines généreux sur l'effet métabolique et la composition corporelle, chez des adultes atteints de diabète de type 2**

# Résumé

L'obésité et plus précisément l'adiposité viscérale sont des facteurs importants dans la résistance à l'insuline, et peuvent contribuer à un anabolisme protéique moindre en réponse à l'hyperinsulinémie, tel que observé dans le diabète de type 2. Cette insulino-résistance du métabolisme des protéines démontrée chez des adultes obèses atteints de diabète de type 2 ne s'améliore pas lors d'un contrôle intensif de la glycémie par la médication. Ce dernier pourrait être exacerbé par l'apport protéique insuffisant des régimes amaigrissants qui maintiennent les protéines à un pourcentage fixe de l'énergie. Par conséquent, l'objectif de cette thèse était de vérifier par une étude pilote chez 6 adultes obèses diabétiques (3 hommes, 3 femmes) si une restriction énergétique avec apport en protéines généreux, d'une durée de cinq semaines améliorerait la sensibilité à l'insuline du métabolisme du glucose et des protéines. Le régime hypocalorique fournissait 60% des besoins en énergie, dont 45% provenait de glucides et 26% de protéines (1,8-1,9 g/kg de masse maigre/jour). Les cinétiques globales ont été mesurées pour le métabolisme du glucose (3-$^{3}$H-glucose) et des protéines ($^{13}$C-leucine) Pré- et Post-intervention, à jeûn et durant un clamp hyperinsulinémique (~ 500 pM), isoglycémique (8,3 ± 0,5 Pré vs. 5,8 ± 0,3 mmol/L Post), isoaminoacidémique. A 5 semaines de restriction énergétique, la perte de poids était principalement due à une perte de gras corporel total et viscéral mesuré par absorptiométrie à rayons X en double énergie, alors que la masse maigre a été préservée. On a observé une normalisation de la glycémie à jeun et une réduction significative de l'insuline et du peptide-C sérique et de l'indice HOMA-IR, ainsi qu'une amélioration significative d'autres facteurs cardiovasculaires. A jeun, le turnover des protéines et le taux d'oxydation ont diminué, entraînant une synthèse nette moins négative et une épargne de protéines corporelles; le taux du turnover du glucose a également diminué et celui de la clairance métabolique s'est amélioré. Durant le clamp, le turnover des protéines était plus bas mais l'anabolisme net n'avait pas changé. Donc, maintenir un apport protéique généreux (~ 110 g/jour) face à un déficit énergétique et une insulino-résistance protège la masse maigre sans améliorer ni aggraver le métabolisme des protéines.

# Contribution of authors

I contributed to the completion of the research study in the following ways. After conducting the telephone questionnaires of potential candidates, I was in charge of the physical and nutritional examination during screening and of the anthropometric and bodycircumference measurements during weekly visits.

I was in charge of the nutritional intervention, including ordering meal replacements, selecting key food items and providing the dietary plan with the instructions to follow for the impending week. I completed a thorough analysis of the market for cereals, as well as for ready-to-eat meals. My supervisor and I customized subjects' dietary plan and decided upon the frozen vacuum-packed meals. At each weekly visit, I was involved in counseling participants and making the necessary dietary changes to their original meal plan.

Upon subject admission to the study unit, I was responsible for preparing the room, admitting subjects, measuring vital signs every morning, monitoring capillary blood glucoses, preparing all meals and writing progress notes on their charts. One of my responsibilities was to select questionnaires to evaluate candidates' knowledge, level of physical activity and changes if any to their lifestyle after completion of the study as well as appropriate educational tools and translated them into French.

On the study day, I did the glucose analysis, blood sample withdrawal and breath sampling, depending on the assigned task. I also entered and analyzed the data and became familiar with the required laboratory methodologies and equipment. I was responsible for data compilation, statistical analysis, presentation, interpretation, and composition of the thesis.

# Supervisors and committee members

**Dr. Rejeanne Gougeon**, the candidate's supervisor is the principal investigator on the operating grant secured by the Canadian Institutes of Health Research (CIHR) to fund this study. Dr. Gougeon designed the study and supervised the candidate in all areas of subject recruitment, screening, enrolling, collecting and interpreting the data. Dr. Gougeon also edited all scientific presentations and posters associated with the study. She finally assisted the candidate with achieving the objectives required for the Master degree.

**Dr. Errol Marliss**, the candidate's committee member and co-investigator on the operating grant secured from CIHR, cooperated in training and supervising the candidate's clinical work. Dr. Marliss assisted with study design, subject screening, outpatient and inpatient medical management, clamp medical supervision, data interpretation and editing this thesis.

**Dr. Hope Weiler**, the candidate's committee member, assisted the candidate toward achieving her Master's objectives and in editing this thesis.

**Dr. Jose Morais**, co-investigator in the study, assisted with muscle biopsy collection during the clamp and in screening subjects.

# McGill Nutrition and Food Science Centre -Technicians

Marie Lamarche performed the radioimmunoassays of–insulin, glucagon and C-peptide and analysis of free fatty acids, glucose turnover, and assisted with data management.

Daniel White was in charge of the plasma branched chain amino acid analyses by fluorometric assay, leucine kinetics' preparation and analyses by GC-MS.

Connie Nardolillo assisted with the recruitment of participants, hospital admissions and the clamp experiments.

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# Contents

# List of Tables

# List of Figures

# List of Appendices

# List of abbreviations

| | |
|---|---|
| Δ | difference between clamp and baseline values for a given variable |
| A1C | glycosylated hemoglobin |
| ADA | American Diabetes Association |
| AMDR | Acceptable Macronutrient Distribution Ranges |
| ANOVA | analysis of variance |
| APE | atom percent excess |
| AUC | area under the curve |
| B | rate of protein breakdown |
| β | beta-cell functioning |
| BCAA | branched chain amino acid |
| BIA | bioelectric impedance analysis |
| BMI | body mass index |
| BW | body weight |
| C | protein catabolism |
| CDA | Canadian Diabetes Association |
| CHO | carbohydrates |
| CIHR | Canadian Institutes of Health Research |
| CRP | C-reactive protein |
| DBP | diastolic blood pressure |
| DRI | Dietary Reference Intakes |
| DXA | dual-energy x-ray absorptiometry |
| ECG | electrocardiogram |
| EER | estimated energy requirements |
| eGFR | estimated glomular filtration rate |
| EGP | endogenous glucose production |
| FFA | free fatty acid |
| FFM | fat free mass |
| FPG | fasting plasma glucose |
| FPI | fasting plasma insulin |

| | |
|---|---|
| GCMS | gaz chromatography-mass spectrometry |
| G/I | glucagon-to-insulin ratio |
| GI | glycemic index |
| GOD | glucose oxidase enzyme |
| HDL | high-density lipoprotein |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| Hot ginf | hot glucose infusion; indicates the presence of a glucose tracer in a glucose infusate |
| HPD | high-protein diet |
| I | rate of infusion |
| IAA | indispensable amino acids |
| I: CP | ratio of insulin to C-peptide |
| IFG | impaired fasting glucose |
| IGF-1 | insulin like growth factor-1 |
| IGT | impaired Glucose tolerance |
| IR | insulin resistance |
| IRMS | isotopic ratio mass spectometry |
| LBM | lean body mass |
| LDL | low-density lipoprotein |
| MCR | metabolic clearance rate |
| mg | milligram |
| MGH | Montreal General Hospital |
| Ml | milliliter |
| mmol | millimole |
| mRNA | messenger ribonucleic acid |
| mTOR | mammalian target of rapamycin |
| mTORC1 | mammalian target of rapamycin complex 1 |
| mU | milliunit |
| MUFA | monounsaturated fat |
| MUHC | McGill University Health Centre |
| N | nitrogen |

| | |
|---|---|
| NEFA | nonesterified fatty acids |
| O | rate at which an amino acid is oxidized |
| OGTT | oral glucose tolerance test |
| 2hPG | 2-hour plasma glucose |
| pmol | picomole |
| PRO | protein |
| Q | turnover or flux of whole body proteins |
| R | resistance |
| Ra | rate of appearance; in the context of glucose, endogenous Ra equals EGP |
| RAS | rennin-angiotensin system |
| Rd | rate of disappearance; in the context of glucose, it is the rate of glucose disposal |
| RDA | Recommended Dietary Allowance |
| REE | resting energy expenditure |
| RIA | radioimmunoassay |
| RQ | respiratory quotient |
| RVH | Royal Victoria Hospital |
| S | protein synthesis |
| SA | specific activity |
| SEM | standard error of the mean |
| SA | specific activity |
| S-B | net balance |
| SBP | systolic blood pressure |
| TBW | total body weight |
| TC | total cholesterol |
| T2DM | type 2 diabetes mellitus |
| TG | triglycerides |
| µg | microgram |
| µmol | micromoles |
| $VC0_2$ | rate of $C0_2$ production |
| VLED | very-low-energy-diet |

| | |
|---|---|
| $VO_2$ | rate of $O_2$ is consumption |
| WHO | World Health Organization |
| WHR | waist to hip ratio |
| Wt | weight |
| Xc | reactance |
| α -KIC | α –Ketoisocaproate |

# I. Introduction

The first known mention of diabetes goes back to the age of papyrus (1552 B.C.E.), when Hesy-Ra, an Egyptian physician, listed some remedies to frequent urination as a symptom of a mysterious disease. It is not until 150 B.C.E., that the Greek physician Arateus described the condition as "the melting down of flesh and limbs into urine" (Reed 1954). From then on, more understanding has been brought to the diabetic condition. Centuries later, the word “mellitus”, meaning honey, was added to the elicited name of the condition to acknowledge the sugar in the urine (Warram & Krolewski, 2005).

Diabetes mellitus, a currently incurable metabolic disorder of multiple etiologies, is characterized by chronic hyperglycemia (Alberti et al., 1998). There are three main types of diabetes, namely, type 1 formerly called juvenile diabetes (T1DM), type 2 (T2DM) and gestational diabetes. Previously named maturity-onset diabetes, T2DM now shares with other omnipresent conditions, such as cancer, the hotspots of public health concerns. Early accounts of T2DM are characterized by a diminished sensitivity to the action of insulin in target organs, referred to as insulin resistance. The latter feature of T2DM, is associated first with hypertrophy of, followed later by, decrease in mass and function of the β-cells of the pancreas, leading to insufficient insulin secretion to overcome the resistance to its action (American Diabetes Association, 2006a).

In 2012, 371 million people worldwide were diagnosed with diabetes (International Diabetes Federation, 2012). With a further 7 million people developing diabetes each year, this number is expected to reach 438 million by 2030 (Canadian Diabetes Association, 2012; World Health Organization, 2013). The surge of diabetes, reaching pandemic proportions, is further affecting the pediatric population (Sicree et al., 2010; Demmer et al., 2013). Concurrent with the worldwide obesity epidemic, 45% of new-onset of T2DM in adolescents is attributed to childhood obesity (D’Adamo et al., 2012). In view of that, the global burden of T2DM urges the need for early preventive and diagnostic approaches as well as protective measures from the myriad complications associated with the disease (Ali et al., 2010). In recent times, the Western lifestyle has been blamed for the increasing prevalence of T2DM, owing to large portion sizes, high consumption of refined carbohydrates and trans-fat rich foods, and to a sedentary lifestyle.

Adopting a healthy lifestyle, explicitly by sustained dietary modifications and physical activity is a key component in the management of T2DM (Canadian Diabetes Association Clinical Practice Guidelines, CDA CPGs, 2013). The literature is replete with studies of different dietary interventions for managing T2DM, but controversies still exist as to what the optimal treatment is; however, all researchers agree that achieving a healthy body weight is crucial for the management of the disease. Given the heterogeneity of this condition, the "one size fits all" concept is no longer advocated for diabetes care and needs to be replaced by customized treatments tailored to maximize adherence, for the optimal dietary intervention and glycemic target.

Diabetes is no longer the disease of glucose and lipids, but also that of protein (Bassil & Gougeon, 2013). Recognized by some as "diabetes proteinus" (Gougeon & Marliss, 2002), insulin resistance of protein anabolism is affected by obesity and incrementally aggravated by poor metabolic control in diabetes. Evidence suggests that uncontrolled diabetes is subject to further lean tissue loss beyond that seen with aging, in conjunction with gain in fat mass (Workeneh & Bajaj, 2013), a condition described as sarcopenic obesity (Park et al., 2007). This condition raised concerns as to whether protein requirements in T2DM may differ (Gougeon & al., 1997), compared to the Recommended Dietary Allowance (RDA) of 0.8 g/kg/d for the general population.

Preliminary data suggest that normalizing glycemia with medications does not improve protein anabolic response to insulin; however, the response to a simulated meal by infusing amino acids to postprandial levels indicate that it can compensate for this resistance (Bassil et al., 2011). On the other hand, efficiency of protein utilization is at risk of further compromise by hypoenergetic diets, when the prescribed percentage of energy derived from protein remains constant at ~ 15%. Based on a thorough literature search, this pilot study was designed and appears to be the first to assess the impact of weight loss from energy restriction with protein intake held at the absolute level consumed during an isoenergetic diet (~15-17% of energy), on the insulin resistance of protein metabolism. The latter is determined using a hyperinsulinemic isoglycemic isoaminoacidemic clamp in conjunction with $^{13}$C-leucine tracer methodology. The findings of this thesis research will further assist in defining the optimal protein intake during energy restriction to spare muscle mass and improve insulin sensistivity of protein metabolism in T2DM. Insulin resistance of glucose was studied concurrently.

# II. Literature review

## 2.1. Diabetes Mellitus (DM): An Overview

### 2.1.1. Type 2 Diabetes Mellitus, Definition and Risk factors

#### 2.1.1.1. Definition & Prevalence

Diabetes mellitus is an autoimmune chronic disorder of the pancreas. There are three main types of diabetes, namely type 1 previously called juvenile diabetes, type 2 and gestational diabetes. Biochemically, all types are detected by abnormally elevated plasma glucose levels, i.e., hyperglycemia (Chakraverty, 2013), but are associated with different plasma insulin concentrations. Type 1, most often diagnosed at younger age, results from the failure to produce insulin by the β-cells of the pancreas. Type 2, more common at adulthood, occurs due to a diminished responsiveness of body tissues to the action of insulin, referred to insulin resistance, and a defective secretion of this hormone, resulting in persistent hyperglycemia. In the early stage of T2DM, insulin secretion is permanently increased to overcome insulin resistance, leading to elevated plasma levels in the fasting and the post-prandial states (DeFronzo, 2004; Chakraverty, 2013). This will eventually lead to decline in secretion, and eventually to loss of β-cell mass via apoptosis, and affected persons may then require exogenous insulin (Butler et al., 2003; Ferrannini et al., 2003; Gastaldelli et al., 2004; Stumvoll et al., 2005). The third type, gestational diabetes, can develop at any time during pregnancy in a woman who does not have preexisting diabetes, but is a risk factor for later development of diabetes.

The prevalence of type 2 diabetes mellitus (T2DM) is increasing and has become a growing public health problem (Ali et al., 2010). Presently, diabetes is approaching pandemic proportions, with about 371 million people worldwide diagnosed in 2012 (International Diabetes Federation, 2012). Over 2 million Canadians have diabetes and over 50% of them are between the ages of 25 to 65 years (Sicree et al., 2010; Public Health Agency of Canada, 2011; International Diabetes Federation, 2012). Chronic exposure to hyperglycemia and other metabolic derangements lead to diabetes-related microvascular and macrovascular complications. These affect the eyes, kidneys and nerves, resulting in retinopathy, neuropathy and nephropathy. Macrovascular complications are related to damage of blood vessels and are associated with increased risk of cardiovascular morbidity and mortality, from conditions

like atherosclerosis, ischemic heart disease and peripheral vascular disease (Goldenberg & Punthakee, 2013).

#### 2.1.1.2. Risk factors

The multifactorial etiology of diabetes includes both non-modifiable and modifiable risk factors. Non-modifiable risk factors include age over 40 y, family history of diabetes and certain predisposed ethnicities or high-risk populations, including Aboriginal Peoples, African Americans, Hispanics, Asians and Pacific Islanders (Ripsin et al., 2009; Steinberger & Daniels, 2003). The presence of associated diseases and genetic defects (such as, polycystic ovary syndrome, obstructive sleep apnea, psychiatric disorders and various mutations in relation to insulin secretion) and the usage of diverse drugs (e.g. corticosteroids and atypical antipsychotics) are also linked to diabetes (Ekoé et al., 2013). Although there is a strong genetic predisposition to T2DM, obesity, mainly with abdominal adiposity, is ranked first among the modifiable risk factors and is considered as the main trigger in developing T2DM (Carey et al., 1997; Haffner, 1998; Alberti et al., 2006; Del Prato et al., 2009; Ekoé et al., 2013). Environmental, behavioural and dietary factors are other examples of modifiable factors. These consist of a stressful and sedentary lifestyle, and a high fat, low fibre diet (Zimmet et al., 2001).

Strongly linked with the increased prevalence of diabetes, obesity is the other growing pandemic commonly referred to as "diabesity". According to recent statistics, more than 80 - 90% of people with T2DM are either overweight (BMI 25-29.9 kg/m²) or obese (BMI 30 kg/m² or higher) (Zimmet et al., 2001; Brinkworth et al., 2004; U.S. Preventive Services Task Force, 2003; World Health Organization, 2005; National Diabetes Information Clearinghouse, 2011; Sales & Patti, 2013). It is believed that obesity complicates the management of T2DM by increasing insulin resistance and glucose intolerance (Brinkworth et al., 2004). In addition to BMI, elevated waist circumference is another risk factor for diabetes, where thresholds to denote abdominal obesity in Canadian men and women are correspondingly ≥ 102 cm and ≥ 88 cm, respectively (Douketis et al., 2005). Hence, in addition to aggressive glycemic control, achieving long-term weight management is crucial to minimize associated morbidities (Sicree et al., 2010; Moyer, 2012).

### 2.1.2. Diagnostic criteria

Diabetes is diagnosed by any of the following criteria: fasting plasma glucose (FPG) ≥ 7.0 mmol/L, a 2-hour plasma glucose (2hPG) value of ≥ 11.1 mmol/L in a 75 g oral glucose tolerance test (OGTT), A1C[1] ≥ 6.5% (in adults) and/or random plasma glucose ≥11.1 mmol/L. Prediabetes, however, is a state where individuals are at high risk of developing diabetes and is defined by any of the following criteria: impaired fasting glucose (IFG) with FPG of 6.1 – 6.9 mmol/L, impaired glucose tolerance (IGT) with 2hPG of 7.8 – 11.0 mmol/L and A1C of 6.0 - 6.4% (Goldenberg & Punthakee, 2013). The classic symptoms of untreated diabetes are loss of weight, polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Cooke & Plotnick, 2008).

## 2.2. Insulin action & insulin resistance in T2DM

### 2.2.1. Overview

In T2DM, insulin resistance is no longer known for being solely a disorder of glucose and lipid metabolism, but also of protein (Pereira et al., 2008; Bassil & Gougeon, 2013). Under normal physiological conditions, upon food ingestion insulin is secreted and triggers the cellular uptake of the different nutrients, explicitly, glucose, amino acids, and free fatty acids (FFA) (Pisters et al., 1991). Consequently, impairment in insulin action leads to distorted regulation of the aforementioned substrates including uptake and metabolism.

Gene expression analysis has shed light on different pathways that contribute to insulin resistance and T2DM, such as enhanced gene expression implicated in inflammation, mitochondrial oxidative metabolism and lipogenesis (Sales & Patti, 2013). Physiological defects highlighted in T2DM are associated with insulin resistance at the muscle, adipose tissue and liver sites, and which include impairment in the signaling process of synthesis and action of insulin and regulatory related hormones (such as glucagon). However, genome-wide analyses have identified genes that account for only about 5% of "effect size" (Park et al., 2010).

[1] Glycated hemoglobin (A1C) reflects the average blood glucose concentration over three months.

### 2.2.2. Insulin resistance of glucose metabolism

Recognized as a glucose-regulating hormone, insulin affects glucose transport, uptake and metabolism in adipocytes, skeletal muscle and liver (Kahn & Flier, 2000). Studies in T2DM have shown that in the basal state, the liver overproduces glucose despite normal or elevated plasma insulin and glucose levels. This is regarded as hepatic resistance to the action of insulin (Groop et al., 1987). The association of obesity with T2DM is mainly due to the presence of insulin resistance, which denotes reduced sensitivity to the action of insulin of glucose metabolism. As a consequence of defective insulin signalling, impaired glucose metabolism in T2DM is explained from different facets. One mechanism is related to reduced insulin-stimulated glucose disposal in tissues as a consequence of the downregulation of the major insulin-dependent glucose transporter GLUT4 (Kahn & Flier, 2000). Other mechanisms include dysfunctional muscle mitochondrial ATP and protein production as well as suppression of hepatic gluconeogenesis (Asmann et al., 2006; Møller & Nair, 2008). Reduced mitochondrial ATP production may curtail ATP-dependent processes such as protein synthesis and hence contribute to the impairment of protein metabolism in diabetes (Asmann et al., 2006; Møller & Nair, 2008).

Concurent with insulin resistance and hyperinsulinemia in T2DM, lipolysis suppression is impaired, resulting in increased circulating levels of FFA. A positive correlation was found between fasting plasma FFA concentration and the rate of hepatic endogenous glucose production (EGP), and another between FFA oxidation and EGP in obese T2DM subjects, confirming that fat and glucose metabolism are interrelated. Hence, elevated levels of FFA and abnormal insulin-stimulated suppression of lipid oxidation are associated with impaired EGP suppression and diminished glucose oxidation (Golay et al., 1987; Groop et al., 1989; Lau, 2013). Studies of energy restriction have shown significant improvement in EGP, insulin sensitivity and secretion in obese individuals with T2DM, independently of weight loss (Kelley et al., 1993).

### 2.2.3. Insulin resistance of protein metabolism

#### 2.2.3.1. Insulin resistance of protein metabolism and obesity

According to Adegoke et al. (2009), amino acids stimulate body protein anabolism in an independent and synergistic manner with insulin. Amino acids serve not only as substrates

but also as nutritional signals to mRNA translation initiation of protein synthesis (Patti et al., 1998; Anthony et al., 2001). The greatest protein anabolism occurs during the fed state concurrent with the responses of insulin secretion (Bassil & Gougeon, 2013). Obesity contributes to insulin resistance of protein metabolism via defects in the stimulation of protein synthesis and of its restraint of proteolysis (Jensen & Haymond, 1991; Chevalier et al., 2005). Body fat distribution is believed to be an important predictor of metabolic abnormalities accompanying obesity, and hence has an important impact on protein metabolism (Jensen et al., 1991; Newgard, 2012).

A study conducted by Jensen et al. (1991) examining protein metabolism in response to insulin in upper-body (i.e. abdominal obesity) or lower-body obese (BMI 30 - 36 $kg/m^2$) and in nonobese women, found that impaired antiproteolytic response to insulin was more pronounced in abdominal obesity, which was reflected by greater peripheral hyperinsulinemia at this site. In line with these findings, Toth et al. (2000), using $^{13}C$-leucine kinetics, reported a positive relationship between the magnitude of the intra-abdominal adiposisty and leucine oxidation rate, and a negative relationship between intra-abdominal adipose tissue and glucose disposal rate, in obese premenopausal women. Increased abdominal adiposity was also negatively associated with other regulatory hormones, such as adiponectin. The latter modulates glucose regulation, fatty acid oxidation and the anabolic response to insulin-like growth factor (IGF - 1) (Tai et al., 2010).

An association between amino acid levels and blood lipid profile was found, whereby serine concentration was inversely correlated with serum level of cholesterol and triglycerides in obese T2DM subjects and lower compared to the control obese group, while proline was positively related with A1C, fasting blood glucose, total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) (Zhou et al., 2013). More evidence is required to identify the effect of supplementing some amino acids, on blood glucose and lipid profiles in diabetes.

#### 2.2.3.2. Impaired protein metabolism in T2DM

The novel association of BCAA level with the incidence, progression and efficacy of therapy of diabetes, has emerged from a wide range of epidemiological studies. Strong evidence supports the hypothesis that altered plasma BCAA level starts at the onset of an

insulin-resistant state through to the diagnosis of diabetes (Zhou et al., 2013). Elevated levels of BCAA were observed in prediabetic as well as in insulin-resistant subjects having either normal or excess body weight (Felig et al., 1970; Newgard, 2012).

Numerous studies suggest that whole-body protein turnover is altered and protein synthesis is diminished with both aging and health conditions such as diabetes (Paddon-Jones et al., 2008; Stenholm et al., 2008; Westerterp-Plantega et al., 2009). Gougeon et al. (1997) assessed whole body protein metabolism (using $^{15}$N-glycine), during the integrated 24-hour fed-fasted state in T2DM subjects with poor glycemic control. They found lower net protein synthesis and accelerated turnover rates (defined as higher rates of protein synthesis and breakdown). They also demonstrated that under such conditions, endogenous and dietary proteins are oxidized and serve as substrate for gluconeogenesis (Marliss & Gougeon, 2002). It was then questioned whether the lower net synthesis resulted from a resistance to insulin of protein metabolism or to greater dietary protein needs in T2DM than the 0.8g/kg BW/d recommended in the DRI. Using leucine tracer methodology with the "gold standard" hyperinsulinemic euglycemic isoaminoacidemic clamp (glycemia normalized to 5.5 mmol/L and amino acids maintained at post-absorptive levels), leucine flux and protein synthesis increased less in T2DM men compared to obese non-diabetic and even more so to lean subjects, while the rate of breakdown was not different among the three groups (Pereira et al., 2008). Normalizing glycemia with medication decreased protein turnover rates but did not improve net protein anabolism in response to insulin in obese men with T2DM (unpublished data). A potential explanation for a reduction in protein synthesis responses could be a reduced availability of ATP necessary to fulfill ATP-dependent processes, perhaps induced by a reduced glucose uptake and fuel flux because of insulin resistance (Møller & Nair, 2008).

Few studies have examined whether maintaining moderate hyperglycemia during a clamp would aggravate insulin resistance of glucose and the alteration in protein metabolism (Bassil, 2011). During a hyperinsulinemic, hyperglycemic, hyperaminoacidemic clamp protocol, obese men with T2DM and lean men had similar anabolic protein responses. Hence, hyperaminoacidemia overcomes the insulin resistance of protein metabolism in T2DM (Bassil, 2011). Yet to be determined is the dose-response of hyperaminoacidemia at which protein anabolism becomes normalized, however.

Obese T2DM and nondiabetic obese subjects following a very-low-energy-diet (VLED) for 6 weeks (410 kcal all protein), demonstrated decreasing negative nitrogen balance over time, but that did not reach equilibrium as in nondiabetic controls (Gougeon et al., 1994). Thus, even though the VLED improved glycemia to near normal, and some protein sparing occurred, nitrogen balance was still negative at 4 weeks. This highlighted the attenuated protein-sparing adaptations during weight loss and the capacity for using alternate energy substrates sufficiently to generate energy did not occur by week 4. A less energy restrictive diet (50% of energy requirements with 1.5 g/kg BW/d of protein) was associated with nitrogen balance at equilibrium but protein metabolism was still compromised (Gougeon et al., 2000). Further studies are thus warranted, to find strategies to improve insulin sensitivity of protein anabolism.

Another factor that is prominent in T2DM is the greater glucagon response to dietary protein (Gannon et al., 1998; Franz, 2002). When glucagon, insulin's main antagonist, is secreted it will stimulate proteolysis, gluconeogenesis (requiring more amino acids as substrates) and glycogenolysis. Both plasma glucagon and insulin levels have been shown to decrease with weight loss (Savage et al., 1979; Laferrere et al., 2008).

#### 2.2.3.3. Effect of dietary protein on glucose metabolism

Protein ingestion is just as powerful as glucose in stimulating insulin secretion (Franz, 2002). Protein contributes to glucose metabolism both by providing gluconeogenic substrate and by recycling glucose carbon via alanine and glutamine (Katz & Tayek, 1998; Layman et al., 2003). Recent studies have shown that ingested protein increases insulin secretion without affecting plasma glucose concentrations. These findings might have brought about the idea that a high protein diet may enhance insulin secretion and lead to pancreatic fatigue, an effect shown in animals but not in humans (Tremblay et al., 2007).

To examine the effect of dietary protein on glucose and insulin homeostasis, Layman et al. (2003) randomly assigned overweight and obese adult women (>26 kg/m$^2$) to weight-loss diets 1800 kcal/d) with either a high carbohydrate/protein (CHO/PRO) ratio (> 3.2, 55% of energy from CHO and 0.8 g/kg BW/d for protein) or a low ratio (< 1.5, 40% of energy from CHO and 1.5g/ kg BW/d for protein). The high CHO/PRO group had higher insulin response to meals that was maintained 2 hour post-meal, interpreted as a reduction in insulin

sensitivity. The authors suggested that consuming an energy-restricted diet with a low CHO/PRO ratio stabilizes blood glucose and reduces postprandial insulin response during weight loss. A study conducted by Ma et al. (2009) in T2DM subjects, showed that protein preload administered before a high-CHO meal leads to a significant reduction in postprandial glycemic level and an increase in insulin response compared to providing it within the meal or not providing protein at all. This was explained by an increase in insulin response and a slowing of gastric emptying time induced by the ingested amino acids (Fieseler et al., 1995; Morens et al., 2001; Layman et al., 2004). Gastric emptying time was slowest with the protein preload followed by the provision of protein within a meal (Ma et al., 2009). Delayed gastric emptying time defined as a slower passage of food from the stomach to the small intestine is associated with a rise in satiety and a reduction in food intake (Wilmshurst et al., 1980; Tossetti et al., 1996; Cuomo et al., 2001). Incretin hormones, such as glucagon-like-peptide 1, were greater with protein preload than when provided within the meal (Ma et al., 2009; Kahleova et al., 2012). The latter are released from the gastrointestinal tract in response to food ingestion and assist in glucose homeostasis by reducing gastric transient time, suppressing glucagon and stimulating insulin secretion (Wajchenberg, 2010; Kahleova et al., 2012).

## 2.3. Dietary protein requirements and diabetes

### 2.3.1. Diabetes and sarcopenic obesity

Beyond the devastating sequelae of diabetes, muscle wasting has been recently earmarked for interventional strategies, as it contributes to a reduced quality of life along with diminished capabilities to easily perform daily living activities (Guillet eta l., 2012; Workeneh & Bajaj, 2013). Few studies have examined the effect of T2DM on skeletal muscle reporting changes of body composition after the onset of diabetes. According to Park et al. (2009), there is a rapid decline in appendicular lean mass in older adults with T2DM independently of weight changes over time. The decline in lean mass was more pronounced in undiagnosed diabetes cases and in women. Recent evidence indicates that people with T2DM are at higher risk of sarcopenia as they age, compared to those without diabetes (Park et al., 2007; Kim et al., 2012). This effect was still significant after adjusting for BMI, baseline muscle quality and lean mass, and for physical activity (Park et al., 2007).

Sarcopenia is defined as a condition in which older adults expresses a loss of muscle mass and sarcopenic obesity represents a gain of fat mass in conjunction with the loss of muscle mass (Fielding et al., 2011).

Therapeutic interventions targeting muscle wasting have been attempted to treat diabetic adults, with protein supplements. However, enhancing dietary protein alone did not reverse the loss of lean tissue (Kotler et al., 2000). Among the 20 amino acids, and more precisely the branched chain amino acids (BCAA) group, leucine supplementation has been suggested as a nutritional strategy to promote protein synthesis in skeletal muscle in T2DM (Leenders et al., 2011). Leucine is believed to be the most effective amino acid activating the mammalian target of Rapamycin (mTOR) in complex with raptor mTORC1 (mammalian target of Rapamycin complex 1), whereby this mechanism is insulin-dependent (Öst et al., 2010). mTORC1, a Serine/Threonine kinase signaling complex, is in charge of the transduction cascade of protein synthesis, governed by amino acid supply (Dodd & Tee, 2012). Thus, increasing dietary protein enhances protein anabolism by stimulating both protein synthesis and insulin secretion. Recent findings have postulated that the mTORC1 pathway is deregulated in specific conditions such as cancer, T2DM and with aging, and contributes to muscle wasting commonly found in these conditions (Laplante & Sabatini, 2009; Dodd & Tee, 2012). As a consequence of insulin resistance in T2DM, Öst et al. (2010) noted diminished insulin signaling mediated effect, leading to a decreased downstream stimulation of mTORC1 cascade.

Among other potential interventions to improve protein anabolism in diabetes, insulin and the sulfonylurea agents (acting on the β-cells of the pancreas to secrete insulin) (Gougeon et al., 1999; Gougeon et al., 2000), weight loss and physical activity (Boulé et al., 2001; Fujita et al., 2007; Praet et al., 2008; Bassil, M. S., & Gougeon, R., 2013; Karstoft et al., 2013; Miller et al., 2013; Sénéchal et al., 2013; Solomon et al., 2013) have been suggested. Ideally, an optimal intervention slow or reverse muscle wasting associated with diabetes.

### 2.3.2. Dietary protein recommendations

#### 2.3.2.1. General recommendations

Under normal conditions, the type and quantity of protein ingested are the main determinants of whole-body protein turnover (Westerterp-Plantega et al., 2009). Contrary to

carbohydrates and fats, protein cannot be stored and hence replenishing protein sources within 4-6 hours is needed. An often neglected feature of daily protein intake that may impact net protein balance is its distribution across daily meals (Paddon-Jones et al., 2010). According to them, 25 - 30 g of high quality protein (~ 10 g essential amino acids) at each meal is necessary to maximize daily skeletal muscle protein anabolism in both young and older individuals. Most adults are believed to eat at least 50% more protein than required to replace body proteins lost on a daily basis, whereby, men consume approximately 100 to 125 g/d and women 80 to 100 g/d, which is equivalent to 1.2 - 1.4 g/kg BW/d (Franz, 2002), which is still considered within the Acceptable Macronutrient Distribution Range (AMDR) of 10 to 35% for adults.

#### 2.3.2.2. Specific requirements in diabetes

According to the American Diabetes Association guidelines (ADA, 2004) and the Canadian Diabetes Association (CDA, 2013), there is no evidence to suggest that the usual recommended protein intake of 15 to 20% of total daily energy intake or estimated from the 0.8 - 1.0 g/kg BW/day equation (Trumbo & al., 2002), needs to be modified for people with diabetes. People with T2DM who are in poor metabolic control (FBG: > 9 - 10 mmol/L) may have increased protein requirements due to the increased turnover of protein (Gougeon et al., 1997). Adequate protein intake is also necessary for maintaining nitrogen balance during weight loss interventions (Franz, 2002), and more importantly may need to be calculated based on lean body mass (LBM) in order to spare muscle mass. The general agreement for an even distribution of protein intake throughout a day for greater total protein anabolic response (Paddon-Jones et al., 2010), has not been studied yet in T2DM.

#### 2.3.2.3. Dietary protein restriction in diabetes

It has long been questioned whether protein restriction is essential for the management of diabetes, to prevent complications, especially diabetes nephropathy. However, insufficient evidence exists to suggest that usual protein intake (15 - 20% of energy intake) should be modified for individuals with diabetes and normal renal function (Brinkworth et al., 2004; Bantle et al., 2006). The National Health and Nutrition Examination Survey (NHANES III) reported that protein intake was not associated with microalbuminuria

levels (defined as urinary albumin/creatinine ratio ≥ 30 mg/g) in subjects without renal dysfunction, or those with diabetes (Wrone et al., 2003). Furthermore, consuming up to 30% of dietary energy from protein is believed to cause no detrimental effects on the advancing process of albuminuria and/ or microalbuminuria (i.e. urinary albumin/creatinine ratio of > 2.5 mg/g), nor preventing or else delaying renal damage (Jameel et al., 1992; Skov et al., 1999a; Brinkworth et al., 2004).

At present, epidemiological studies also provide insufficient evidence with regard to the association between protein intake and the predisposition to kidney stone formation (Martin et al., 2005). Changes in dietary protein cause an adaptive alteration in renal size and function without adverse effects, namely increased glomerular filtration rate and renal hypertrophy, for people with normal renal function (Martin et al., 2005). A recent study by Jesudason et al. (2013) comparing two weight-loss diets with either 30% or 20% energy from dietary protein showed similar effects on renal function in both groups, based on estimated glomular filtration rate (eGFR) measurements.

Further research is required to establish evidence-based protein recommendations in T2DM (ADA, 2004), and more precisely to maintain lean muscle mass during weight loss (Marliss & Gougeon, 2002; Arathuzik & Goebel-Fabbri, 2011). Based on existing data to date, protein intakes within the AMDR do not exacerbate renal complications in adults with T2DM.

## 2.4. T2DM management

### 2.4.1. Holistic approach

Applying the terms “remission” or “cure” to describe diabetes management have been deemed inappropriate, since the treatment aims for optimal glycemic control and minimization of micro- and macrovascular complications by improving insulin sensitivity, which will make the diabetic condition “silent” (Buse et al., 2009). β-cell function was proposed to be not quite reversible but improved, particularly at early stages of diagnosis, through lifestyle intervention, namely diet and exercise (Kahleova et al., 2012). Advocating an active lifestyle to manage diabetes includes regular and structured exercise, of > 150 minutes/ week of moderately to vigorously intense physical activity and ≥ 2 sessions/ week of resistance training (Sigal et al., 2013).

The health benefits associated with weight loss has long been recognized for overweight people and those with diabetes and assigned as a primary goal by healthcare professionals (Wharton et al., 2013). Evidence-based strategies for improving diabetes care should concentrate on enhancing self-management, and personalizing the treatment (Berard et al., 2013; Ransom et al., 2013).

#### 2.4.1.1. Improvement of glycemia

Optimal glycemic control is fundamental in the management of diabetes, since uncontrolled hyperglycemia is associated with a myriad of devastating complications. Achievement and maintenance of an A1C level of < 7% is recommended by CDA's CPGs (CDA, 2013) for most people with T2DM to reduce their risk of diabetes-related complications. An A1C of < 6.5% may be considered in some people with T2DM to further lower their risk of diabetes-related comorbidities. Accordingly, a fasting FPG of 4.0 - 7.0 mmol/L and 2-hour postprandial plasma glucose of 5.0 - 10.0 mmol/L should be aimed for in T2DM (Imran et al., 2013).

Based solely on lifestyle changes (i.e. diet and physical activity), the CDA CPGs (CDA, 2013) advocate for 5-10% reduction of initial body weight as a method to improve many of the metabolic markers, such as improved glycemic control. The impact of energy restriction on improving glycemia is apparent shortly after initiating dietary changes and is thought to be independent of weight loss. This was reported by Hensrud (2001) in T2DM subjects assigned to VLED. Clinical studies have shown that at least 3% of initial body weight reduction is needed to begin observing some improvement in glycemic control (Goldstein et al. 1992; Henrud, 2001; Harayama et al., 2013), and more than 15% (Harayama et al., 2008) weight loss was associated with normalized glucose tolerance, measured with the 75g-OGTT test. Diabetes duration has also an important impact on the magnitude of diabetes-related amelioration, whereby, duration of 3.2 ± 2.5 years was needed to normalize glucose tolerance (Harayama et al., 2013).

#### 2.4.1.2. Insulin sensitivity

Being the primary and major concern upon diagnosis of T2DM, insulin resistance affects most cells in the body and impairs their normal function. As for glycemia, weight loss

improves insulin action (Lee et Morley, 1998) and the effect is reported to be observed in less than 2 weeks after starting a hypocaloric diet (Wing et al., 1994).

To examine the effect of different caloric restriction regimens on glycemic control and insulin sensitivity, Wing et al. (1994) assigned obese adults with T2DM to either a 400 or 1000 kcal/d diet. Despite an equal 11% weight loss in the two groups, the VLED mediated a greater amelioration in both glycemic control and insulin sensitivity, with the same change in fasting insulin levels. Hence, the impact of reduced ingested calories on insulin sensitivity and glycemic levels is independent of weight loss (Wing et al., 1994). Hypocaloric diets with low dietary CHO were shown to induce greater decreases in fasting glucose than isocaloric diets with higher CHO content (Grey & Kipnis, 1971). As discussed previously for protein, eating small frequent meals may induce greater glycemic control compared to fewer equicaloric meals in a day (Jenkins et al., 1992).

### 2.4.2. Weight loss and nutritional strategies

Weight-loss programs for obese T2DM subjects consider multifaceted interventional counseling that addresses behaviour modification, diet and exercise (Wing, 1987; Clark, 2004). Lifestyle interventions form the cornerstone of therapy for obese adults with T2DM as reported by the European Association of Diabetes and the ADA treatment algorithm (Del Prato et al., 2009), who are not necessarily required to undergo bariatric surgery (Buchwald et al., 2009). Whereas, achieving better glycemic levels to target goals and managing hyperglycemia are of primary concern, improvements in other cardiovascular risk factors associated with T2DM are also expected with a modest weight-loss (i.e. 5 to 10% of initial body weight). This would include improvements in hypertension, dyslipidemia and inflammatory markers (e.g. CRP levels) (Pi-Sunyer et al., 2007; Wharton et al., 2013).

Weight loss appears to be more difficult to achieve in overweight and obese people with T2DM compared to those without diabetes (Peters et al., 2002). A reduction in calorie intake and/or enhancement in energy expenditure through exercise are two means to weight reduction (Wing, 1989; Franz et al., 2003). A weight loss at a rate of 1-2 lb per week (i.e. 0.5 - 1 kg per week) is advocated for optimal glycemic control and reduced cardiovascular risk factors, which is the equivalent to a caloric deficit of 500 to 1000 kcal/ day (National Heart, Lung and Blood Institute, 1998, Wharton et al., 2013). Several factors affect the magnitude of

weight loss, including the duration of the disease, the pharmacological treatment regimens and the severity of the insulin resistance (Hensurd, 2001). When present in the course of T2DM, hyperinsulinemia may trigger lipogenesis and triglyceride storage and suppress lipolysis in adipocytes (Cahill et al., 1959; Kahn & Flier, 2000), leading to adipose tissue expansion (Lau, 2013) and changes in weight and fat mass (Wycherley et al., 2010).

#### 2.4.2.1. Personalized nutritional interventions

Obesity has recently been evoked as a disease by the American Association of Clinical Endocrinology (AACE) (Dickey et al., 1998) and categorized as a behavioural disorder. Weight loss program should include customized nutritional plan, that are tailored to individuals' lifestyle and eating habits (ADA, 1994; Tate et al., 2001; Inzucchi et al., 2012).

Behavioural treatment has been a common intervention strategy for managing mild to moderate obesity in both diabetic and non-diabetic persons due to its long-term results demonstrated in some studies (Wing, 1987). It consists of promoting self-monitoring and cognitive restructuring by positive thinking (Clark, 2004). Dietary and lifestyle modifications include substituting specific food items with their healthier alternatives and managing stress from daily-living (Wing et al., 1994; Toobert et al., 2003; Pi-Sunyer, et al., 1997). Also, it has been proposed that overeating and preoccupation with food are unintended consequences of dietary restriction. As a consequence, the modified behaviour is acheived by providing specific dietary and portion sizes guidelines, meal replacements and ready-to-eat meals (Ruderman et al., 1986; Ditschuneit et al., 1999; Yip et al., 2001; Heymsfield et al., 2003; Wadden et al., 2003; Cheskin, et al., 2008; Katan et al., 2009).

Findings from the Look Ahead study (Action for Health in Diabetes), demonstrated that continuous participants' monitoring and personalized dietary plan (i.e. intensive lifestyle intervention) resulted in greater dietary adherence as well as improvement in glycemic control and lipid profile compared to the group receiving education group sessions (Wing et al., 2010). Assigning T2DM subjects to the intensive lifestyle intervention also produced a greater percent of weight loss. The higher success rate observed with the intensive lifestyle intervention group may be associated with the higher dietary adherence from administering portion-controlled dietary plans (Wing et al., 2010). Hence, customized dietary plan and behavioural therapy should be included in the nutritional therapy to aquire and maintain

sustained healthy eating habits (Wing, 1987; Wing, 2010). A summay of the different dietary intervention studies is presented in Table 1.

#### 2.4.2.2. Dietary approach

##### 2.4.2.2.1. Macronutrient distribution

Studies of energy-restricted diets to identify the ideal proportions of macronutrients (carbohydrate, fat and protein) remain inconclusive. A minimum of 130 g of CHO per day should be advocated to prevent ketogenesis according to Dworatzek et al. (2013). However, a study by Alford et al. (1990) compared the effect of three diets of 1200 kcal with 25, 45 or 75% of carbohydrate and different proportions of fat and protein and found no significant difference in weight loss or lipid profile.

A low-fat diet defined as < 30 % of energy from dietary fat and < 7% from saturated fat has been recommended to people with T2DM for optimal lipid control (Guldbrand et al., 2002; Dworatzek et al., 2013). Advising participants to follow either a low fat diet (55-60% energy from CHO and < 30% from fat) or a low- CHO diet (20% energy from CHO and 50% from fat) led to similar weight loss in both groups (Westman et al., 2008; Davis et al. 2009; Sasakabe et al., 2011), as well as, reduction in A1C and insulin concentration (Gulbrand et al., 2012). Prescribing a low fat diet (< 30%) with either high protein (30% energy from protein, corresponding to ≥ 1.2g/kg BW/d) or high-CHO diet (55% from CHO) to obese T2DM subjects (BMI ≥ 27 $kg/m^2$) resulted in similar modest weight loss and waist circumference reduction over a two year period (Krebs et al., 2012).

Few studies have reported differences in fat loss and LBM sparing depending on macronutrient distribution within equal provision of energy-restrcited diet. Energy-restricted low fat diet (~ 1450 -1650 kcal/d) contributing either > 25 % of energy from protein or 15–20 % showed similar improvements in glycemic control, cardiovascular risk factors and lipid profile (Seshadri wt al., 2004; Bantle et al., 2008; Barnard et al., 2009). However, the group receiving > 25% protein experienced a larger weight and fat mass reduction as well as a significant reduction in fasting insulin (Parker et al., 2002; Wycherley et al., 2010). Jene & Haymond (1991) documented that the provision of adequate CHO (> 45% of total energy from CHO, or 130 g/d) in combination with high protein intake (HPD > 20%) contribute to protein sparing. Binkworth et al. (2004) noted equal weight loss when assigning T2DM

subjects to LPD (< 15%) or higher-protein diets (HPD, > 25%) with energy-restriction (~ 1500-1800 kcal/d) in ad libitum fat-reduced diets (LFD) (< 30%) and enhanced proteolysis independently of protein intake.

Hence, independently of macronutrient distribution in a structured energy-deficit diet, improvements in CVD risk factors and glycemic control are achieved in obese subjects with T2DM, as well as fat mobilization and lean mass conservation (Heilbronn et al., 1999; Iqbal et al., 2009; Krebs et al., 2012).

#### 2.4.2.2.2. High protein diets

High protein diets (i.e. ≥ 25% of energy from protein) have become omnipresent as weight loss strategies. They are believed to favour a faster weight loss (Skov et al., 1999b) in comparison to lower protein diets while conserving LBM (Westerterp-Plantenga, 2005; Leidy et al.,2007). A study by Gougeon et al. (2000) reported that a protein intake of ≥ 1g/kg BW/d (~ 15% of energy from protein) during 50% restriction of estimated energy requirements, maintained LBM and mobilized fat in obese participants with and without T2DM. A study by Parker et al. (2002), where subjects were assigned to an energy deficit with either 30% energy from protein or 16%, for 8 weeks, was associated with a similar weight loss of 5.2 ±1.8 kg in both groups, but a greater reduction in total (- 5.3 vs. - 2.8 kg, $p$ = 0.009) and abdominal fat (- 1.3 vs. - 0.7 kg, $p$ = 0.006) with the higher protein diet. The latter was also associated with less reduction of fat free mass (FFM) and resting energy expenditure (REE) compared to the isocaloric conventional diet (Wycherley et al., 2012).

Higher protein intake (i.e. ~ 30% energy from protein) has satiety-enhancing and hunger-suppressing properties within and between meals, which increase adherence to weight loss strategies (Paddon-Jones et al., 2007; Katan, 2009; Leidy, et al., 2010), as it is shown to be associated with reduced food consumption and sensation of hunger (Johnstone et al., 2008; Westerterp-Plantenga et al., 2004; Westerterp-Plantenga et al., 2009; Soenen et al., 2013). Studies have shown that higher protein intake can improve glycemic control associated with improved insulin sensitivity (Hamdy, et al., 2008). In particular, the amino acid leucine triggers insulin secretion, in presence of a blunted glucose insulinotropic response that characterizes T2DM (Evan, 2004).

Dietary protein is highly thermogenic, meaning 20 - 30% of its energy content is lost, compared to 5 - 10% for carbohydrate and up to 3% for fat, which is explained in part by the high energy cost of postprandial protein synthesis (Skov et al., 1999b; Franz, 2002; Westerterp-Plantenga, 2008). Higher protein intake possibly also increases thermogenesis, due to the energy cost of gluconeogenesis (Halton et al., 2004; Westerterp-Plantenga et al., 2009). Within a weight loss setting, 1.2 vs 0.8 g/kg BW/d of dietary protein maintained REE due to a sparing of FFM (Westerterp-Plantenga et al., 2006a; Westerterp-Plantenga et al., 2009; Soenen et al., 2013). Assigning obese adults to 35 or 67% of energy-deficit diet with either 30% energy from protein or the Dietary Reference Intake (DRI) protein intake of 10-15% yielded similar loss in body weight and fat mass as well as a similar reduction in insulin concentration, HOMA-IR and improvement in lipid profile. The higher protein intake was associated with a greater FFM-sparing and lowered diastolic blood pressure (Skov et al., 1999b; Trumbo et al., 2002; Leidy et al., 2006; Soenen et al., 2013).

It is thus essential to consider the amount of ingested protein and not the percentage that it contributes to total calories. In fact, the protein contribution of an isoenergetic diet of 10-15% of total energy represents the same amount of protein in grams per day than an energy-restricted diet with 20-30% energy from protein, although the latter can be interpreted as a high protein diet. However, in absolute terms it may consist of the same amount consumed (grams of protein) as for the energy-balanced diet, but less energy in total (Soenen et al., 2013; Westerterp-Plantenga et al., 2006a).

#### 2.4.2.2.3. Dietary patterns

Several dietary patterns have been suggested by CDA CPGs to induce weight loss and improve diabetes-related outcomes. Clinical studies have been intensively interested in the Mediterranean diet for its cardio-protective properties, now commonly recommended by health practitioners (Toobert et al., 2003; Psaltopoulou et al., 2004). This dietary pattern consists of 50-55% low-glycemic index-CHO, 15-20% protein, < 35% fat rich in monounsaturated fat (MUFA) and < 8% of saturated fat. It also advocates for an abundant consumption of legumes, fruits and vegetables, unrefined cereals, > 2 servings/week fish as well as a moderate consumption of dairy and low in meat products, particularly red meat (Psaltopoulou et al., 2004).

The Dietary Approaches to Stop Hypertension (DASH diet), also recommended for people with T2DM, shares common characteristics with Mediterranean diet and emphasize a reduction in dietary salt depending on the class of hypertension. Reducing dietary salt minimizes water retention (Luft et al., 1997), and this approach is based on the fact that sodium exerts a crucial role in fluid and acid-base balance. People with diabetes are believed to be more sensitive to the blood pressure-raising effect of dietary sodium (Luft et al., 1997; Carlson et al., 2000; Otten et al., 2006). A possible explanation may arise from the fact that under normal physiological conditions insulin enhances renal sodium retention, and therefore with hyperinsulinemia, sodium retention may be accentuated (Ferrannini et al., 1993). Weight loss lowers blood pressure and may reverse the sodium excretion impairment in relation to insulin resistance, perhaps in part by lesser hyperinsulinemia and its direct antinatruiretic effect on renal tubular sodium retention (Reisin et al., 1978; Ferrannini et al., 1987; Otten et al., 2006).

Several studies have favoured low-glycemic index (GI) foods (e.g. whole grain food items, non-refined starchy foods, green vegetables and legumes) in the management of T2DM and in the treatment of insulin resistance. GI ranks food based on their glycemic effect (Jenkins et al., 1989; Brouns et al., 2005). Low-GI foods (index of $\leq 55$ on the GI reference scale) improve glucose homeostasis by lowering glycemic and insulin response to meals and reducing A1C levels compared to high-GI foods (index of $\geq 70$ or greater) and offer prolonged satiety (Ludwig, 2000 and 2002; Roberts et al., 2000; Foster-Powell et al., 2002; Ball et al., 2003; Atkinson et al., 2008). Furthermore, low-GI diets have been shown to improve lipid profile by reducing LDL (Jarvie et al., 1999; Jarvandi et al., 2011) and triglycerides levels (Barnard et al., 2009). The low-GI diet was also associated with a higher adherence rate to a weight loss program (Larsen et al., 2010).

Energy density, defined as the total number of calories in a given weight or volume of food (Hensrud et al., 2001) is also implicated in weight management. Low-energy dense foods promote satiety and reduce caloric intake without decreasing food volume (Duncan et al., 1983; Bell et al., 1998; Ello-Martin et al., 2005; Rolls et al., 2005; Drewnowski, 2009). Among food components, water decreases energy density by adding weight and volume to a food item, while fat enhances energy density to even a greater extent than carbohydrate and protein (Ello-Martin et al., 2005). Because of their high fibre and water content, fruits and

vegetables contain a small amount of calories in a large volume; hence, they have a low energy density (Ello-Martin et al., 2005; Rolls, et al., 2005). Ello-Martin et al. (2007) documented a 40% greater weight loss at 6 months following a low-energy dense foods diet compared to reduced portion sizes of all foods.

A meta-analysis of different dietary approaches to T2DM management reported that low-glycemic index (GI) (index < 55), Mediterranean and high protein diet, all led to superior improvement in A1C, similar to the one achieved by medications (Turner et al., 1998).
Thus, these dietary patterns should be considered when planning a dietary intervention to promote sustained weight loss and healthy eating habits.

#### 2.4.2.2.4. Prepared meals and meal replacements

Prepared meals and liquid formulas used as meal replacements have been able to achieve as good or even superior weight loss and glycemic control than conventional dietary instructions and meal plans. After 1 year, T2DM subjects provided with prepared meals experienced greater weight loss and improvement in fasting blood glucose levels and A1C, compared to the group who was assigned a dietary plan (Hensrud, 2001). Similarly, provision of meal replacement was associated with greater compliance and weight loss in the short and long-term compared with the conventional energy-reducing diets with the same caloric targets (Wing et al., 1994; Heymsfield et al., 2003; Cheskin et al., 2008; Wing, 2010). Because they mimic a meal or snack, they engender reduced contact with calorie-dense foods and decision-making in relation to foods (Heymsfield et al., 2010; Craig, 2013). The greater weight loss seen with the packaged meals and liquid formulas may be due to controlled portion sizes (Noakes er al., 2004). More accurate food records can be acheived in a study setting when formula and prepared meals are administered to participants, as obese individuals tend to underreport their actual food intake by as much as 47 % (Lichtman et al., 1992). A systematic evaluation of randomized controlled trials of energy-restricted diets (1200-1500 kcal/d) reported a weight loss of 7-9% in obese subjects receiving meal replacements compared to 1.5 - 3% with a conventional diet (Ditschuneit et al., 1999; Heymsfield et al., 2003; Craig, 2013).

In diabetes, there is a general concern about the effects of type of carbohydrates consumed on postprandial glucose level. In contrast with a fast absorption of sucrose

available in most formulas, those with higher sugar alcohol content instead (e.g. Glucerna®) induce a slower and a smaller peak in postprandial glucose (Fonda et al., 2010; Craig, 2013). Yip et al. (2001) assigned T2DM subjects to either a liquid formula containing lactose, fructose and sucrose, another containing lactose and oligosaccharides, or a customized reduced-calorie dietary plan for 12 weeks. It was found that fasting glucose, LDL and TC levels were significantly lower and weight loss was higher in the meal replacements groups, with a reduction of 6.4, 6.7 and 4.9%, respectively; however, no difference was noted in the effect of the different formulas on glycemic levels.

Hence, meal replacements and ready-to-eat meals are considered good dietary strategies to enhance adherence and convenienece in a weight loss program. The type of meal replacement should also be considered in T2DM.

### 2.4.3. Pharmacological therapy in T2DM

#### 2.4.3.1. Overview

When modifications in dietary composition and activity level are insufficient or not followed for diabetes control, medications are required, and often are even with adherence to lifestyle changes. Antihyperglycemic medications, particularly metformin, enhance insulin receptor binding within the brain (Lee et Morley, 1998). Choice of pharmacological treatment agents should be individualized and take into account the degree of hyperglycemia, presence of comorbidities and side effects. Insulin is the oldest medication used among the different anti-diabetic medications available on the market. It is also the most effective at lowering glycemia (Nathan et al., 2009). Under normal physiological conditions, insulin reduces food intake by acting on the hypothalamus and assisting in weight control (Schwartz et al., 1990). T2DM manifests as insulin insensitivity at the central nervous system level, due to defects in central insulin signal transduction, and fewer insulin receptors on the brain capillaries (Schwartz et al., 1990) and within the hypothalamus (Ikeda et al., 1986). This results in a blunted insulin effect on reducing food consumption.

It should be noted that medications are as important as the nutritional intervention for optimal management of T2DM, and when combined with a balanced dietary regimen, medications can greatly enhance glycemic control.

#### 2.4.3.2. Effect of medications on body weight

Metformin (Glucophage®) widely accepted as the first line monotherapy among the antihyperglycemic medications for T2DM, enhances peripheral glucose uptake and reduces gluconeogenesis (Wollen et Bailey, 1988; Argaud et al., 1993; Lee, et Morley, 1998). Weight loss ranging of 1.2-1.3 kg over the first 12 weeks with no specific diet prescription or lack of weight gain were consistently reported in people with T2DM treated with metformin as monotherapy (Clarke et Duncan, 1968; McAlpine et al., 1988; Johansen, 1999; Lachin et al., 2007; Nathan et al., 2009; Ripsin et al., 2009; Harper et al., 2013). The mechanism underlying its weight-reducing effect is believed to be partially explained by its impact on inducing satiety and decreasing food intake, with no impact reported on energy expenditure (Stumvoll et al., 1995; Lee et Morley, 1998). Among the anti-diabetic drugs, metformin, belonging to the biguanides class, was reported to have the most significant weight-reducing effect, followed by the peptide analogs class that include glucagon-like peptide-1 agonists (GLP-1 agonist) of the incretin mimetic groups (e.g. exenatide category, Byetta®) and amylin agonists (e.g, pamlintide category, Symlin®) (Clarke, B. F., & Duncan, 1968; Herman et al., 1991; DeFronzo et al., 1995; Nathan et al., 1995; Bailey, 1999; Hoffmann et al., 1999). GLP-1 receptor agonists exert an inhibitory effect on appetite and food intake by acting on gut hormones and may also slow gastric emptying time (Lovshin et al., 2009). An emerging antihyperglycemic agent that is currently being tested is the sodium-glucose co-transporter 2 (SGLT2), which has been shown to induce some degree of weight loss in T2DM. SGLT2 inhibitors lower blood glucose levels by reducing glucose reabsoprtion at the kidneys, thereby causing renal loss of calories. Epidemiological studies have reported a modest weight loss with SGLT2 of about 1.8 kg with dapagliflozin and 2.3 kg with canagliflozin and a reduction in A1C level ranging by 0.7 to 0.9 % (Clar et al., 2012; Sheridan, 2012).

Alternatively, dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitor) and the saxagliptin class from the incretin enhancers group were shown to be weight neutral. Further studies are required to determine whether combining metformin with anti-obesity agents, including Orlistat (Xenical®) and fenfluramine (Termium Plus®), is more effective than metformin alone in promoting weight loss.

In contrast, some anti-diabetic medications cause weight gain up to 2 - 4 kg. Examples of such medications include thiazolidinedione, insulin, and the insulin secretagogues (e.g. sulfonylurea and meglitinide) (Nathan et al., 1995; Del Prato et al., 2009; Ripsin et al., 2009; Harper et al., 2013). Insulin therapy is expected to induce a weight gain ranging between 1.5 to 4 kg, and approximately 1.8 to 5.0 kg with sulfonylureas (Turner et al., 1998; Lau, 2013). Other medications prescribed to people with T2DM, which may induce weight gain, include the antihypertensive medications such as the diuretics, which may be presented as water retention (e.g. edema). Therefore, these medications require close monitoring and adjustement with weight loss.

# III. Rationale

Elevated whole-body protein turnover and reduced net protein synthesis in obese adults with T2DM has been attributed to a blunted protein anabolic response to hyperinsulinemia (Gougeon et al., 1997) compared with nondiabetic obese men (Periera et al., 2008). This insulin resistance of protein metabolism did not improve when glycemia was normalized with medication (unpublished data). However, protein anabolism in response to a hyperinsulinemic hyperaminoacidemic clamp, with amino acids raised to postprandial levels, did not differ from that of healthy lean controls. This implies that hyperaminoacidemia may compensate for the insulin resistance of protein metabolism in T2DM (Bassil et al., 2011). High protein diets have been recommended to conserve muscle mass, which decreases further in T2DM with aging (Park et al., 2007). Furthermore, hypoenergetic diets may aggravate protein loss, if percentage of energy from protein is not increased from what it was during an isoenergetic diet. The optimal protein intake to protect from muscle loss during energy restriction remains to be determined. Therefore, it becomes clinically relevant to assess whether weight and fat losses, which improve glycemia, will improve the protein anabolic response to insulin when energy restriction is moderate and abundant typical protein content is maintained and evenly distributed throughout the day.

# IV. Hypothesis & objectives

An energy restricted diet with maintained typical abundant protein intake, evenly distributed at all meals will increase insulin sensitivity of glucose metabolism, protect muscle mass and improve or prevent further deterioration of whole body protein metabolism.

The main objective of this pilot study is to assess the impact of decreasing caloric intake from carbohydrates and lipids but not from protein on whole-body protein and glucose metabolism as well as on protein anabolic response to insulin in T2DM.

1/ Develop and apply a dietary strategy to ensure optimal adherence to an intake of 60% of energy requirements and associated weight, total fat and visceral fat losses while preserving LBM in T2DM adults.

2/ Measure changes in markers of glycemic control, hormones and lipid profile.

Table 1. Different interventional strategies in relation to weight loss

| Study, first author, year of publication | Subjects enrolled, n | Subject characteristics (sex, age and BMI) | Total study duration (wks) | Intervention | Body composition changes | Significant outcome measures |
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| **Behaviour therapy** | | | | | | |
| Wing et al., 1994 | 93 | Obese adults with T2DM | 12 weeks | Group A: 400 kcal<br>Group B: 1000 kcal (30% fat, 15% protein, 55% CHO)<br>Weekly meetings | Weight loss of 11.0 ± 0.3 kg in body weight at ~ 9 weeks for Group A and at 12 weeks for Group B | Lower FPG in Group B (10.13 ± 1.12 vs 1.7.61 ± 0.56 mM)* and for fasting insulin (188 ± 42 1. 94 ± 13 pM)**. No diet effect was found on fasting insulin reduction ($p < 0.001$). |
| Toobert, et al., 2003 | 279 | Postmenopausal women with T2DM | 24 weeks | Group A: Mediterranean lifestyle program -self-management program: low - saturated fat diet, stress management, exercise, group support, etc.<br>Group B: Usual care | Lower BMI with Group A (-0.37% vs +0.02%)*. Body fat distribution, using waist-to-hip ratio was not statistical significant | Lower A1C with Group A (-0.36 vs + 0.02%). No difference in blood pressure |
| Pi-Sunyer, et al., 2007; Wing et al., 2010<br>Look AHEAD Study | 5145 | Obese adults with T2DM (BMI > 25 kg/$m^2$) | 4 years | Group A: Intensive lifestyle intervention (ILI): caloric restriction, > 15% from protein, < 30 % from fat, > 175 min/week of physical activity, meal replacements, structured meal plans | Greater weight loss with ILI (6.15 vs 0.88%)** | Greater improvements in ILI in A1C (- 0.36 vs -0.09%)**, SBP (-5.33 vs -2.97 mmHg)**, DBP (-2.92 vs -2.48 mmHg), HDL (+3.67 vs 1.97 mg/dl)** and TG (- 25.56 vs -19.75 md/dl)**. Reduction in LDL was greater with DSE (-11.27 vs -12.48 mg/dl)** |

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| | | | | and weekly educational and counseling sessions.<br>Group B: Diabetes support and education (control) | | |
| **<u>Nutritional therapy</u>**<br>**High protein diets**<br>Brinkworth et al., 2004 | 66 | Obese adults with T2DM (BMI: 27 - 40 kg/m$^2$) | 12 months | Group A -Low-protein diet:15% protein, 55% carbohydrate<br>Group B - High-protein diet: 30% protein, 40% carbohydrate | Significant weight loss of 2.2±1.1 kg with the low-protein and 3.7±1.0 kg with the high-protein group, with no diet effect. No change in fat mass. | Both diets reduced SBP and DBP by 6 and 3 mm Hg respectively. CRP decreased by 14 %* with no diet effect. |
| Krebs et al., 2012<br>Diabetes Excess Weight Loss (DEWL) | 419 | Obese adults with T2DM (BMI > 27 kg/m$^2$) | 12 months | Group A – Low fat & high protein diet: 30% protein, 40% CHO, 30% fat<br>Group B - Low fat & high CHO diet: 15% protein, 55% CHO, 30% fat | No differences between groups in weight loss (2 - 3 kg)*, waist circumference ** changes and in body fatness | No significant differences between groups in A1C, lipids and blood pressure |
| **Low fat diets**<br>Heilbronn, et al., 1999 | 35 | Obese with T2DM | 12 weeks | Energy restriction (1,600 kcal/day):<br>Group A - Higher-CHO diet: 10% fat, 4% saturated fat, 3% MUFA<br>Group B – Higher-saturated fat (SFA) diet: 31% fat, 17% saturated fat, 10% | No diet effect on weight loss (- 6.6 ± 0.9 kg). | Reductions in FPG (-14%), A1C (-14%), insulin (-27%), SBP (-7%), DBP (-10%) levels and the glucose response area (-17%), independent of diet composition.<br>Reduction in LDL by 10% and 17%, with higher-CHO and higher-MUFA diets, respectively, with no change with higher-SFA diet |

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| | | | | MUFA<br>Group C- Higher monounsaturated fat (MUFA) diet: 32% fat, 7% saturated fat, 15% MUFA | | |
| Iqbal et al., 2010 | 144 | Overweight adults with T2DM | 12 months | Group A - Low-CHO diet: 35% CHO, 20% protein, 40% fat<br>Group B - Low-fat diet: 40% CHO, 23% protein, 30% fat | No significant difference in weight loss ~ - 4 kg | No significant difference in A1C with - 0.5% in low-CHO group compared to - 0.1% in low-fat group. |
| Gulbrand et al., 2002 | 61 | Obese adults with T2DM (BMI > 27 kg/m$^2$) | 24 months | Group A – Low-fat diet: 55 -60% CHO<br>Group B – Low-CHO diet: 20 % CHO | Weight loss did not differ between groups, i.e. −2.97 ± 4.9 kg* with low-fat diet vs. −2.34 ± 5.1 kg* low-CHO | No diet effect in HDL increase, and reduction in reduction in blood pressure, TG and LDL. |
| **Glycemic index (GI)**<br>Järvi et al., 1999 | 20 | Obese adults with T2DM (BMI 25.3 ± 2.7 kg/m$^2$) | 3 weeks | Energy from protein 16%, fat 28%, and carbohydrate 55%:<br>Group A: Low-GI diet<br>Group B: High- GI diet. | Similar reduction in body weight | Fall in FBG in both groups by 14%*. Incremental area under the curve for plasma insulin was 30%* lower in Group A. C-peptide higher in Group B (120 vs 300)**.<br>LDL lowered on both diets, more pronounced reduction in Group A (-5%)*. Reduction of TG, TC and HDL were not different for both diets. |
| Ma, 2008 | 40 | Obese adults with T2DM | 12 months | Group A - Low - GI diet: 37% CHO, 76GI, 42% fat, 20% protein | Similar reduction in body weight. | Equivalent decrease of A1C (~ 8%)** with less diabetic medication Improvements in HDL and TG were |

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| | | | | Group B - ADA diet: 38% CHO, 80 GI, 43% fat, 20% protein | | similar between groups, however lower LDL in ADA group (-0.42 mmol/L)*. |
| **Meal replacements and prepared meals**<br>Ditschuneit et al., 1999 | 100 | Obese adults (BMI 25-40 kg/m$^2$) | 12 weeks | Energy-restricted diet (1200-1500 kcal/d):<br>Group A: Self-prepared from conventional foods<br>Group B: 2 meals replaced daily with meal replacements | Reduction in weight by ~ 6% in Group A (1.3 ± 2.2 kg) vs ~11% in Group B (7.1± 3.5 kg)** | No significant changes in biomarkers, beside TC in Group A (-0.2 mmol/L)*. In Group B, TG (-22% vs -1.5%)**, FPG (-8% vs +0.5%)**, insulin concentrations (-39% vs -0.6%)** and SBP (-6 % vs -0.7%)* decreased significantly. |
| Yip et al., 2001 | 75 | Obese adults with T2DM (27 to 40 kg/m$^2$) | 12 weeks | Group A: meal replacement containing lactose, fructose, and sucrose<br>Group B: meal replacement containing lactose and oligosaccharides<br>Group C: exchange diet plan based on ADA | Weight losses in groups A & B were comparable (6.4% and 6.7%) and greater than Group C (4.9%) | No significant difference between groups A & B-Pooled for the rest of data analysis.<br>FPG (-22% vs -5%)* significantly reduced in meal replacement group.<br>No differences between Groups A+B and C were observed in lipid concentrations, fasting insulin and A1C. |
| Heymsfield et al., 2003 | 487 | Obese adults (>25 kg/m$^2$) | 12 weeks | Energy-restricted diet (800 -1600 kcal/d):<br>Group A: 1 meal replacement combined with regular foods<br>Group B: 2 meal | Greater weight loss in pooled Group A & B, (7–8% vs 3–7%)* | Not reported |

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| | | | | replacements combined with regular foods<br>Group C: typical reduced calorie diet | | |
| Cheskin, et al., 2008 | 48 | Obese adults (25- 40 kg/m$^2$) | 34 weeks | 75% of predicted energy needs:<br>Group A: meal replacement<br>Group B: standard diet using ADA recommendations | Greater weight loss in Group A (6.84% vs 3.70%)*.<br>WC decreased significantly in both groups, 6.5 cm (5.5%) * and 4.9 cm* (4.2% )*<br>Hip circumference decreased by 4.1 cm (3.3%)** in Group A and by 1.6 cm (1.3%)* in Group B. | Lower FBG (11.3%** vs 7.2%) fasting insulin (-15.7% vs + 7.3%)* and TG (19.4%)** in Group A.<br>No significant differences between groups in reduction of blood pressure. Reduction of glucose lowering medications occurred in users from Group A (13% vs 0%)*. |

ADA, American Diabetes Association; CHO, carbohydrate; CRP, C-reactive protein; DBP, diastolic blood pressure; FPG, fasting plasma glucose; A1C, glycated hemoglobin; TC, total cholesterol; TG, Triglycerides; SBP: systolic blood pressure; *$p < 0.05$ and **$p < 0.001$.

# V. Methods & Analysis

## 5.1. Ethics and consent approval

Ethics approval for the study was obtained from the McGill University Health Centre (MUHC) Royal Victoria Hospital (RVH) Research Ethics Board and renewed annually (Appendix 1). Candidates were informed of the nature, purpose and possible risks of the study before signing the consent form which describes risks and benefits to the participant (Appendix 2) and informs of the possibility to withdraw from the study at any time without any impact on their habitual medical care. During the study, participants were confined to ambulation on hospital grounds and the clinical investigation unit.

## 5.2. Study advertisement

Internal as well as external communication tools were used to recruit participants with type 2 diabetes. Visually appealing posters in English and French were placed in strategic areas designated by the MUHC Communications Office in the RVH and the Montreal General Hospital (MGH) and had to be replaced every two months (Appendix 3). The locations ensured good visibility in order to capture people's attention. Additionally, the study was introduced to health professionals (physicians, dietitians, nurses and administrative assistants) through written notices and verbally and permission was obtained to look through their patients' charts to identify potential candidates and approach them.

External communication included advertisement of the study in Diabète Québec's journal "Plein Soleil" (Appendix 5), as well as posters placed in different medical clinics in Montreal downtown area and on residency posters boards (Appendix 4). External non-profit organizations such as, Notre-Dame-de-Grâce-Diabetic Support Group (Local Community Service Centre of Cavendish, CLSC of Notre-Dame-de-Grâce, Montreal, Qc) were also contacted to promote the study. In addition, others were recruited by contacting previous participants who had expressed an interest in doing more studies in our laboratory and their family members, friends and co-workers. The declines were mostly related to work constraints or personal reasons preventing hospital admission.

## 5.3. Subject recruitment

### 5.3.1. Eligibility: inclusion and exclusion criteria

All participants were required to be overweight and/or obese (BMI: 27 - 40 $kg/m^2$) adults (< 65 years of age), diagnosed with type 2 diabetes > 6 months, having a stable weight for the previous 3 months, not on short-acting insulin and non-smokers. Smoking has been shown to affect glucose metabolism; fasting plasma glucose and insulin concentrations and homeostatic assessment model of insulin resistance (HOMA-IR) were reported to be higher in smokers (Facchini et al., 1992; Jensen et al., 1995; Attvall et al., 2009; Morimoto et al., 2012; Seet et al., 2012). Serious medical concerns, such as cancer, hepatic, hematological, renal (such as, stones, reduced kidney function) pulmonary, thyroid, and cardiovascular (e.g., angina, stroke, myocardial infarction) dysfunctions, as well as the presence of diabetes complications, namely nephropathy, neuropathy and retinopathy were probed if present. Those taking medications that may affect protein and carbohydrate metabolism, including: oral steroids, anti-inflammatory, immunosuppressant, bronchodilators, non-steroidal anti-inflammatory (NSAID) and antiarrhythmics drugs, were excluded from the study (Harper et al., 1995; Kaplan, 1992; Löfberg et al., 2002; Short et al., 2004a). Serum creatinine above 120 µM and hemoglobin below 120 g/L also prevented inclusion of participants in the study, because they are not compatible with donating substantial volume of blood (300 mL) on the study day and also if screened positive for some serology tests, e.g. HIV+, MRSA+, etc. Additionally, abnormal dietary habits assessed by a 24h-recall or following special diets for personal reasons (e.g. kosher, vegan, organic, etc) and/ or therapeutic purposes (e.g. low oxalate diet, gluten free diet, and so on) and having inappropriate eating behaviours (i.e. binging, purging, fasting, etc) were ineligibility criteria. Women were studied during the follicular phase of their menstrual cycle, since hormonal variation can alter protein metabolism (Tipton, 2001). If enrolled in another study or not covered by the Quebec medical insurance program, Medicare, one was not eligible to participate.

Since age and level of obesity can confound insulin resistance along with protein and glucose metabolism, inclusion criteria took these two variables into account. Experimental evidence demonstrates metabolic and functional changes at the muscle level with aging. A lower synthesis rate of "muscle contractile protein myosin heavy

chain occurs in age related muscle wasting and weakness", more intensely over the age of 65 (Balagopal et al., 2001). With T2DM, there is a tendency of having a reduction in LBM concurrently with a gain of fat mass, a condition defined as sarcopenic obesity (Fielding et al., 2011). Also, it has been shown that there is an age related decline in the action of insulin on muscle glucose uptake, even after adjustments for adiposity and fat distribution (Ferrannini et al., 1996; Muller et al., 1996; Narimiya et al., 2013). Consequently, to eliminate the confounding impact of aging on protein metabolism, age limit was set at 65 years for participants' recruitment. The upper limit of participants' BMI was set at 40, due to a weight limit imposed by certain equipments such as dual-energy x-ray absorptiometry machine (DXA) and to ensure ease of veins access.

### 5.3.2. Screening steps

In order for an interested subject to be admitted to the study, several screening procedures were done. Weight, height, age and duration of diabetes, medications and the MUHC identification number (card ID number) were collected by telephone screening (Appendix 6); a revision of the candidate's case using the internal software "OASIS" (if available) with the physician was made before inviting the candidate for the screening visit, which took place at the RVH at 9 am after an overnight fast.

Screening day: After reading and signing the consent forms with the potential candidate and explaining in detail the study, anthropometrics were measured (i.e. weight and height), and fasting blood and urine samples were collected for a standard analysis. To assess participants' nutritional needs and dietary habits, the resting energy expenditure measurement (REE), a 24-hour dietary recall and a food-frequency questionnaire were conducted in that order, and body composition was determined by bioelectrical impedance analysis (BIA). Nutrient analysis software (Food Processor, ESHA Research, Salem, OR) was used to estimate energy and protein intake from the participant's dietary recall. Chest X-ray and ECG were performed to measure pulmonary and cardiac functions respectively. A medical history and physical examination were then carried out by the study physician on a second visit. Based on the screening visit results and the physical exam, the physician determined whether or not the potential subject met all the criteria to do the study.

## 5.4. Study design

### 5.4.1. Overview & Admission to the hospital

The study lasted 39 days, and consisted of two phases: PRE- and POST-5-weeks-weight loss with a high protein energy restricted diet. Participants were admitted twice to the Royal Victoria Hospital's Clinical Research Unit, first for 3 days (PRE-energy-restricted diet) and then for the last week on the energy restricted diet (POST). Participants were instructed to maintain their usual eating and lifestyle habits (i.e. physical activity) in the period between the screening visit and the admission day. No specific guidelines for physical activity were provided; however, participants were encouraged to be active throughout the day and aim for at least 150 min of moderate to vigorous physical activity every week as recommended by CDA's CPGs (CDA, 2013). Questionnaires and forms were given (Appendix 10) for recording sedentary periods during the day in order to enhance awareness. Habitual medications for diabetes and other health concerns (i.e. blood pressure, lipids, prostate enlargement, etc) were maintained except for acetylsalicilic acid which was stopped 10 days before the admission to prevent excess bleeding with muscle biopsies, and other medications that impact water balance (e.g. hydrochlorothiazide) because of the reduction in sodium intake and possibly insulin concentrations. Monitoring of diuretic and antihyperglycemic medications were done during the 5-week intervention.

Every morning of their stay at the hospital, weight, blood pressure, heart rate and temperature were measured after voiding. A 24-hour urine collection was done only during the two admission periods, starting at 8 am and ending at 8 am the following day (Figure 3). A log book was filled out by all subjects to report their daily water consumption, bowel movements, adverse symptoms, physical activity if any, blood glucose measurements prior to each meal and treatment of hypoglycemia if present. Subjects were allowed to leave the hospital for few hours, as long as the diet and urine collections were as per protocol.

### 5.4.2. Diet protocol

Candidates were given a high protein meal replacement (Boost®) qid for 5 days pre-admission, to ensure that Pre- study protein intake is at ~ 15% of total energy intake (CDA, 2013) and would not differ from the intervention. Because of caffeine-induced thermogenesis (Acheson, et al., 1980; Bracco et al., 1995; Westerterp-Plantenga et al., 2006b), participants were also instructed to gradually stop their coffee consumption three days before their two admissions to avoid withdrawal symptoms during the hospital stay.

Energy requirements were determined based on REE measured by indirect calorimetry (TrueOne® 2400 Canopy System, Parvo Medics**,** Sandy, UT) with a factor of ~ 1.5 to account for thermic effect of food and physical activity (Gougeon et al., 2000; Harris et al., 1919). The test was conducted on the screening day, unless the admission was delayed of more than three weeks. Protein intake was determined based on each person's FFM determined by BIA (RJL-101A Systems, Detroit, MI) using the Kushner equation for overweight populations (Kushner et al., 1990). The intake amounting to ~ 1.95 - 1.98 g/kg LBM/day determined by DXA was maintained during the entire study; it is equivalent to ~ 1.7 - 1.8 g/kg FFM/day. The two diets met vitamin and mineral requirements. The diet composition is presented in Table 2.

#### 5.4.2.1. Isoenergetic diet

For the first three days of the study, participants consumed a formula-based isonergetic (meeting requirements to ensure weight maintenance), protein controlled diet (Ensure® and Glucerna®, Ross Laboratories, Montreal, Qc, Canada and Boost Diabetes®, Nestle Nutrition) divided into 5 equally distributed meals from 8:00 to 20:00 hours (Appendix 7). Besides the liquid-formula, 30 g of All-Bran® cereal (Kellogg's Canada Inc, Mississauga, ON) and 150 mL of 2% milk were added at breakfast. Nutritional information of the meal replacement formula is presented in Appendix 8.The main goal of this first admission was to maintain body weight and nitrogen balance at equilibrium for baseline data accretion before starting the energy deficit intervention. Glucerna® intake did not exceed three bottles per day because of reported associated diarrhea and gastrointestinal discomfort in some persons mainly attributed to its sugar alcohol content (i.e. glycerine). Orange juice was used to meet energy requirements when needed and/ or

treat hypoglycemia if presented. Subjects were encouraged to drink enough water to quench their thirst, with a maximum of 2 L per day. No other food or beverage was allowed.

#### 5.4.2.2. Energy-restricted diet

The dietary regimen was inspired from the Mediterranean and DASH dietary patterns, whereby, high-volume/low-density foods were advocated (such as plenty of vegetables), two servings of fish per week, low glycemic index foods and low saturated and trans fat. In addition to inducing weight-loss, the dietary intervention was also intended to educate participants on lifelong healthy eating pattern and dietary choices.

The energy-restricted dietary regimen was started after the clamp study (admission.1) and lasted five weeks. The diet provided 60% of calculated energy requirements, 45% of which came from carbohydrate, 26% from protein, which is equivalent to 1.95 g/kg LBM/day (~ 1.1 g/kg BW/d), and 30% from fat. The macronutrient distribution is within the Acceptable Macronutrient Distribution Ranges (AMDR) (Institute of Medicine of the National Academies et al., 2005). The diet was moderately restrictive in sodium and contributed 1500 - 2000 mg/d.

The diet was divided into 3 meals and one snack, and protein and carbohydrates were evenly distributed throughout the day (Appendix 9). Timing of the meals as well as choices for the breakfast meal were customized based on individuals' dietary habits. Accordingly, participants were given specific instructions for their breakfast meals, which mainly included high protein-fibre cereals (Kashi© Go lean and All-Bran®) and other food items, such as skimmed or low fat milk, yogurt, fruit, whole grain toast or an egg. To optimize adherence to diet at home, participants were given meal replacements for lunch and bedtime snack (Glucerna®, Abbott Laboratories Inc and/or Boost Diabetes®, Nestle Nutrition) as well as key food items (i.e. kashi® cereal) and ready-to-heat vacuum packed meals for supper. The meals consisted of 120 - 130 g of lean meat (e.g. porc, veal, beef, and chicken, fish and shrimps), two portions of vegetables and one of starch. Choices of carbohydrate were taken from the low glycemic index list (e.g. wild rice, pearl barley, couscous, sweet potatoes) and the meal adhered to the slow cooking approach to minimize dietary advanced glycation end products (AGEs). To increase food

volume, variety and satiety, as well as ensure regularity and meet CDA CPGs' fibre recommendations of 25 to 50 grams per day for people with diabetes (CDA, 2013), participants were instructed to add one bowl of green salad with 1 tablespoon of salad dressing using olive or canola oil at lunch and supper time. Participants came to hospital weekly to pick-up their meals and meal replacement formulas except during the last week which was spent in the hospital. Admission to hospital was used to better control participants' diet and monitor their glycemia pattern before repeating the same tests as in PRE-energy-restricted diet.

### 5.4.3. Metabolic experiment

#### 5.4.3.1. Design

The experiment presented two states, the postabsorptive state (baseline, fasting for 12hr) and the hyperinsulinemic (~ 600 pmol/L), isoglycemic (as per individuals' own baseline fasting glucose concentration) and isoaminoacidemic (as per individuals' own baseline BCAA concentration, as a marker of total amino acid concentration) clamp state. The same study protocol was conducted twice: PRE-energy-restricted diet during the weight-maintenance phase (Day 4) and at 5 weeks of energy restriction (Day 39). The clamp protocol was based upon the hyperinsulinemic euglycemic clamp of DeFronzo et al. (1979) as described by Banerji & Lebovitz, (1989), Saad el al. (1994) and Chevalier et al. (2004). The time course of the experiment, the infusions and the sampling frequency are illustrated in Figure 1.

#### 5.4.3.2. Preparation

The evening before the experiment, the following syringes were prepared: L-1-$^{13}$C-Leucine, D-3-$^{3}$H- glucose infusate, 10% TrophAmine and the 20% dextrose solution; except for the insulin syringe which was prepared the day of the study. Syringes were placed in separate digital pumps (Harvard Apparatus Inc., Holliston, MA) by which infusion rates were continuously adjusted throughout the study. After an overnight fast, subjects arrived at the Crabtree Nutrition Center at 7:30 am. A catheter was placed into the antecubital vein on one arm for glucose, substrates, insulin and tracers infusion and another catheter was inserted retrogradely into the dorsal hand vein of the other arm for

blood sampling (Chevalier et al., 2004; Saad et al., 1994). This hand was kept in a warming box at 65 - 70ºC to arterialize the venous blood and provide a better representation of intracellular metabolism (Zello et al., 1990; El-Khoury et al., 1994). At first an oral bolus of 0.1 mg/kg of $NaH^{13}CO_2$ (MassTrace Inc., Woburn, MA) mixed in water was ingested, to speed up the reaction. 1.0 mL blood sample was centrifuged (MicroCentrifuge, Model 235B; Fisher Scientific) for 30 seconds and the extracted plasma was for BCAA determination by a fluorometric assay and glucose level by a GM9 glucose analyzer. A specimen of each of the plasma samples was stored at -70°C for consequent analysis of amino acids by high Pressure Liquid Chromatography (HPLC). Measurements at each time point were done in duplicate; i.e., insulin, glucagon, NEFA concentrations as well as plasma sample of D-3-$^3$H-glucose radioactivity. Any urine produced was collected for assessment of urea and glucose.

#### 5.4.3.2.1. Postabsorptive state

The postabsorptive state lasted for 150 minutes, and two tracers were infused: a glucose tracer [D-3-$^3$H-glucose] marked by radioactivity and used to measure hepatic glucose production and disposal and an amino acid tracer [L-1-$^{13}$C-Leucine] marked by a stable isotope. As described in previous similar studies, tracers were administered to assess glucose and leucine kinetics; which were determined at the isotopic steady-state between 2 and 2.5 hour for this first phase of the experiment (Matthews et al., 1980; Banerji & Lebovitz, 1989; Saad et al., 1994; Chevalier et al., 2004). At 1.5 hour a muscle biopsy was taken and indirect calorimetry test conducted. Blood and breath samples were taken at baseline prior to the start of the tracers' infusion, and at 60min, then every 10 minutes from up until completion of the postabsorptive phase at 150 minutes.

#### 5.4.3.2.2. Hyperinsulinemic Isoglycemic Isoaminoacidemic Clamp

After the postabsorptive state, the clamp study was started, and lasted for 180 minutes. The two tracers from the postabsorptive phase were continued, amino acid and glucose solutions were infused. Insulin infusion (Humulin-R; Eli Lilly Canada Inc., Toronto, Canada) was done at a flow rate of 0.096 mL/min, based on a 1.25 mU/kgFFM/min (Banerji & Lebovitz, 1989; Chevalier et al., 2004). A 2 mL aliquot of

heparinized blood from the subject was added to the insulin syringe to prevent insulin from being adsorbed to the syringe walls or the infusion line (DeFronzo et al., 1979). Four minutes after the initiation of the insulin infusion, a potato starch-derived glucose solution of 20% dextrose (Avebe b.a., Foxhol, The Netherlands) was infused at a variable rate to maintain fasting blood glucose at baseline concentrations. Tritiated glucose (D-3-$^{3}$H-glucose) was added to the dextrose solution to minimize changes in circulating glucose specific activity and to avoid underestimating hepatic glucose production (Finegood et al., 1987). Due to the presence of tracer, this mixture is called a "hot glucose infusion" or "hot GINF" (Finegood et al., 1987). This source of glucose was chosen because of its low natural $^{13}$C content (Scrimgeour et al., 1988). Concurrently with glucose infusion, a 10% Amino Acid solution (10% TrophAmine without electrolytes; B Braun Medical Inc, Irvine, CA) was infused at varying rates based on the subject's FFM and BCAA concentrations (Chevalier et al., 2006; Chevalier et al., 2005a; Chevalier et al., 2005b). At 100 minutes post start of insulin infusion, the second biopsy was taken and indirect calorimetry was conducted fom 150 to 170 minutes. Blood and breath samples were taken at 60, 95 minutes and then every 10 minutes from 120 to 180 minutes. All but the 20% glucose pump were stopped at the end of the study; a mean of preventing hypoglycemia. Subjects consumed 12 fl oz of orange juice and glucose infusion was maintained at a lower rate for 20 minutes. A diabetic meal from the hospital was consumed post study and given the fact that an increase in insulin can stimulate potassium cellular uptake and result in a lower serum level (Cohn, et al., 2000), a potassium supplement (Schering-Plough Canada, Inc, Kirkland QC) was also administered.

#### 5.4.3.2.3. Overview & steady state

During the entire experiment, two steady states also called plateau were reached: one during the postabsorptive phase and the other during the clamp phase. Steady states represent an isotopic equilibrium, in which the $^{13}$C-Leucine enrichment and the specific activity (SA) of tritiated glucose in plasma are constant. Additionally, a physiological equilibrium is also attained at the plateau state, in which plasma hormones and substrates concentrations do not vary (Radziuk et al., 1985; El-Khoury, 1999).

## 5.5. Measurements

Different measurements were conducted before and after the low energy diet intervention: body composition was measured by BIA (RJL-101A Systems, Detroit, MI) and DXA (Lunar iDXA, GE healthcare, Madison, WI), indirect calorimetry and Nitrogen balance by 24 hour urine collection. An electronic digital scale (Scale-Tronix, Mettler Toledo Inc., Mississauga, ON) was used to weigh the subjects wearing light clothes to the nearest 100 g, a wall-mounted stadiometer for measuring the height to the nearest 0.1 cm and body mass index (BMI; in $kg/m^2$) was calculated.

### 5.5.1. Assays

#### 5.5.1.1. Urine collection and nitrogen balance (N)

During the hospital stay, a sample of the total daily urine collection was sent to the clinical biochemistry laboratory of the RVH to assess 24-hour creatinine, urea and electrolytes (i.e. sodium, potassium and chloride) excretion (Caggiula et al., 1985; Tasevska et al., 2006). Also, during the first admission, urine samples were used to monitor glycosuria and protein loss. In T2DM, a prolonged hyperglycemia is manifested by the presence of glucose in the urine, named glycosuria. The level of blood glucose at which it spills into the urine is called the renal threshold and is around 10.0 mmol/L in blood drawn from a vein or 11.1 mmol/L in blood drawn from an artery (Ganong, 2001). Replacement of calorie losses was necessary in order to prevent weight loss. Glycosuria was analyzed using GM9 (Analox Instrument USA, Lunenburg, MA). The value was then multiplied by the total urine volume collected during the preceding 24-hour period and multiplied by 4 kcal/g. The energy lost was then replaced by orange juice in order to maintain weight.

Nitrogen (N) balance was determined for each day of the hospital stay as follows:

$$\mathbf{N_{balance} = N_{intake} - N_{losses}}$$

Where, $\mathbf{N_{intake}}$ = **protein intake (g/d) /6.25** Expressed alternately as, $\mathbf{N_{intake}}$ **= 0.16 x protein intake (g/d).** Protein, more precisely from animal sources contains approximately 16% of nitrogen (Morais et al., 1997; Alberta, 2007; Gropper et al., 2009)

And $\mathbf{N_{losses}}$ = **N excretion (urea + creatinine + ammonia + uric acid) + N losses (feces + miscellaneous)**

(1) **Urea N excretion: mmol urea/d x 0.28g N/mmol urea**

(2) **Creatinine N excretion: mmol creatinine/d x 0.042g N/mmol creatinine**

(3) **Ammonia N excretion: 0.28(g)** $N.d^{-1}$

(4) **Uric acid N excretion: 0.30(g)** $N.d^{-1}$

(5) **Fecal N losses: protein intake (g)/d /6.25 x 0.07**

(6) **Miscellaneous N losses: body weight, BW (kg) x 0.005(g)** $N.d^{-1}$

The factor of 0.07g fecal N/g $N_{intake}$ as well as 0.28(g) N/d and 0.30(g) N/d accounting for urinary ammonia and uric acid N excretion respectively were determined from previous studies conducted in our laboratory, in subjects consuming similar formula-based diets (Morais et al., 1997; Gougeon, Styhler et al., 2000). Miscellaneous nitrogen losses were set at 0.005g $N.kg^{-1}.d^{-1}$ (Calloway et al., 1971; Millward & Roberts, 1996; Rand et al., 2003) and represents losses from hair, nails, skin and breath.

5.5.1.2. Glucose Oxidase Technique

In order to maintain participants' baseline glycemia during the clamp, 10 µl sample of plasma glucose was measured every 5 minutes using the GM9 glucose-analyzer (Analox Inst. USA, Lunenberg, MA). β-D-glucose reacts with oxygen forming gluconic acid and hydrogen peroxide, catalyzed by the glucose oxidase enzyme (GOD) at a pH of 6.0.

The following chemical reaction takes place:

Glucose + oxygen $\xrightarrow[GOD]{}$ gluconic acid + H2O2 (hydrogen peroxide)

Under appropriate conditions, the rate of oxygen consumption is directly related to glucose concentration in the plasma sample; thus, the higher the glucose concentration, the more oxygen will be needed for the oxidation reaction to take place. When necessary, the instrument was calibrated with a standard of 144.1 mg/dL (Analox Inc.). Plasma glucose readings were entered into the Andres Glucose Clamp Program version 2.00-8/96, which calculates the glucose infusion rate necessary to maintain isoglycemic target.

5.5.1.3. Enzymatic Fluorometric Assay

To assess insulin sensitivity of proteins metabolism without the confoundig effect of sub-basal plasma amino acid levels as an effect of the increased insulin concentration in the clamp, an isoaminoacidemic state (sustaining postabsorptive amino acid levels) was acheived during the entire clamp protocol. Plasma BCAA concentrations were measured every 5 minutes using an enzymatic fluorometric assay. In the presence of 0.52 U *Bacillus cereus* leucine dehydrogenase (E.C.1.4.9., CAT#431525; specific activity of 47.0 U/mg; Calbiochem-EDM Biosciences, La Jolla, CA) the oxidative deamination of BCAA takes place (Beckett et al., Chevalier et al., 2004), i.e. L-leucine, L-isoleucine and L-valine from a 25 µL plasma sample are converted into their respective keto-analogues, namely α-ketoisocaproate, α-ketomethylvalerate and α-ketovalerate. This chemical reaction uses 4 mmol/L β-NAD as a cofactor (Roche, Roche Diagnostics, Laval, QC) and produces NADH in equimolar amounts to the amount of the substrates (i.e. BCAA) converted (Ohshima et al., 1978; Chevalier et al., 2004). This method has been preferred against the high-performance liquid chromatography (HPLC) because it gives the value within 10 minutes (Chevalier et al., 2004).

The following chemical reaction:

$$\text{Amino Acid} + NAD^{+} \xrightarrow[\text{Leucine dehydrogenase}]{} \text{Keto-acids} + \underset{\downarrow \textbf{Fluorescence}}{\text{NADH}} + H^{+}$$

Using a spectrofluorometer (Jasco model FP-6200 equipped with a xenon lamp, Jasco Corporation, Tokyo, Japan), NADH formation was measured by fluorescence after 4 minutes of enzyme reaction and quantified (µmol/L) using a standard curve with known concentrations of BCAA (i.e. 0, 50, 100, 150, 200, 250 µM) (Sigma, Sigma Chemical, St Louis, MO) (Chevalier et al., 2004). Consequently, NADH appearance was determined with an excitation wavelength of 355 nm and emission wavelength of 485 nm. A potassium phosphate buffer (Fisher Chemicals, Fisher Scientific, St. Laurent, QC), containing 2 mmol/L EDTA and 0.02% mercaptoethanol for a total volume of 2 mL was utilized to keep the pH at 8.4, at which leucine dehydrogenase is most efficient. β -NAD was dissolved in a buffer of 0.1 mol/L sodium carbonate (Fisher Chemicals, Fisher Scientific, St. Laurent, QC) (Chevalier et al. 2004). The cell chamber of the machine and

plasma samples were kept at 37°C, in order to mimic the human body environment.

#### 5.5.1.4. Insulin sensitivity

The homeostatic model assessment (HOMA) was used to quantify insulin resistance (IR) PRE and at 5 weeks, and calculated as follows (Matthews et al., 1985; Wallace et al., 2004):

**HOMA-IR = (FPI x FPG) / 22.5**

Both include fasting plasma insulin (FPI) concentration (mU/L) and fasting plasma glucose (FPG) (mmol/L). Using the same data, the index of tissue sensitivity to insulin can be calculated: G/I ratio (DeFronzo et al., 1979).

Along with HOMA, a modified version of the euglycemic hyperinsulinemic clamp (DeFronzo et al., 1979) was performed, which is more complex and known as the "gold standard test" for identifying changes in insulin sensitivity in response to dietary intervention and weight loss (Wallace et al., 2004; Lotz et al., 2006). Using this method, two aspects of the sensitivity to insulin were determined: 1) the ability of insulin to enhance glucose disposal (Rd, rate of disposal or disappearance) and 2) the ability of insulin to suppress glucose production (Ra, rate of appearance). The metabolic clearance rate of glucose (MCR) was then calculated by dividing Rd (i.e. peripheral glucose uptake) by the glucose concentration (Radziuk et al., 1985; Saad et al., 1994; Tajiri et al., 2011).

Serum insulin, plasma glucagon and C-peptide concentrations were determined by radioimmunoassay (RIA) as described by Finegood et al. (1987) and Sigal et al. (1994), respectively using [$^{125}$I]-labeled human insulin, [$^{125}$I]-labeled glucagon and [$^{125}$I]-labeled human C-Peptide. C-peptide, a measure of insulin secretion, was used in HOMA modeling of IR (Wallace et al., 2004; Cobelli et al., 2007). Additionally, muscle biopsy samples were obtained from the vastus lateralis muscle of the leg to measure cellular proteins of the insulin signaling pathway by Western Blot at a later date. With weight loss, and improved insulin sensitivity, an improvement in insulin signaling (sensitivity) is expected in protein synthesis.

#### 5.5.1.5. Other assays

The following plasma substrates and hormonal concentrations were also measured PRE and Post dietary intevention to appraise the impact of energy restriction and weight loss: glycated hemoglobin (A1C or HbA1c), fructosamine, cytokines (CRP) and lipid profile by hospital laboratories and nonesterified fatty acids (NEFA) concentrations by the enzymatic colorimetric assay (NEFA-C test kit; Wako Chemicals USA Inc, Richmond, VA), using oleic acid as a standard. During hospitalization, overnight-fasted serum electrolytes, calcium, phosphorus, liver and kidney function tests, and complete blood counts were analyzed once. Participants were provided with a glucometer (Accu-Chek III, Boehringer Mannheim) and were asked to measure their blood glucose before each meal and whenever needed during the day during the entire study.

### 5.5.2. Body Composition Assessment

Participants underwent body composition assessment during both admissions and at mid-point of the energy restriction phase, to monitor changes.

#### 5.5.2.1. Body circumferences

Body circumferences taken on the last day of admission and at weekly visits, were measured according to World Health Organization (WHO, 1995) guidelines and the National Health and Nutrition Examination Survey (NHANES, 2009), using a precise tape measure. At the upper body part, chest circumference was measured under the arms and around chest (i.e. at the armpit level), the smallest waist measurement was identified visually and measured, and umbilical waist circumference represented the midpoint between the lower border of the rib cage and the iliac crest, with the tape parallel to the floor. Hip circumference was measured where the greatest circumference at the buttocks level, with the measuring tape being parallel to the floor. Other measurements were taken at the right side of the body: mid-arm circumference at the midpoint between the end of the spine of the right scapula and the tip of the olecranon process, i.e. the bony part of the mid-elbow, calf circumference at the greatest circumference and mid-thigh circumference at the horizontal midpoint level between the inguinal crease of the hip. All measures were taken by the same technician.

#### 5.5.2.2. Bioelectrical Impedance Analysis (BIA)

Using the BIA technique (RJL-101A Systems, Detroit, MI) the total body water (TBW) was estimated by quantifying the impedance of a 50 KHz electrical current on participant's body (Abu Khaled et al., 1988; Dehghan et al., 2008; Canadian Society for exercise physiology, 2010). Impedance refers to the resistance (R) from the extracellular and intracellular fluid and cell membranes (reactance, Xc) (Kyle et al., 2004). The higher the water content of a subject's body, the less resistance there is to the electrical current. On the other hand, adipose tissues are considered less conductive and consequently, the higher the % fat the greater the resistance is (Scharfetter et al., 2001; Dehghan et al., 2008).

The test was conducted with the subject lying supine position on a flat bed for no longer than 5 minutes, with arms on the sides away from the body and legs slightly spread apart. On the subject's dominant side of the body, four electrodes were placed: one behind the middle finger, another on the ulnae bone, one behind the middle toe and last on bisecting the medial malleolus. Clips attached to the four electrodes were repositioned three times to ensure consistency and the average of the three readings of R and Xc was used to estimate FFM, which corresponds to total body mass excluding fat. Using the Kushner equation (1990), FFM was determined and fat mass and percentage of body fat (% BF) were obtained by subtracting FFM from the subject's body weight (kg). The Kushner equation (1990) is derived from BIA measurements conducted on a large group of subjects including lean, normal weight and obese adults (BMI ranging from 17.9 to 54.3 $kg/m^2$), and which is thought to be an accurate FFM measurement tool in healthy obese adults (Kushner et al., 1986; Segal et al., 1988; Newton et al., 2005):

$$\text{TBW (men): } 1.726 + 0.5561\ (Ht^2 / R) + 0.0955\ Wt$$

$$\text{TBW (women): } 8{:}315 + 0{:}382\ (Ht^2 / R) + 0.105\ Wt,$$

$$\text{Then FFM is, FFM: TBW/ } 0.7592$$

Where Ht stands for height (m), Wt for body weight (kg), R for resistance and Rc for reactance.

#### 5.5.2.3. Dual-Energy X-ray Absorptiometry (DXA)

Besides being the mostly widely used bone mineral density (BMD) measurement technology (CDC-NHANES, 2007), DXA provides a great precision of total and regional body measurements (i.e. trunks, arms, legs, pelvis, android and gynoid) and fat distribution (Hind et al., 2001). Consequently, android (i.e. abdominal subcutaneous and visceral fat) and gynoid fat (Hind et al., 2011; Kaul et al., 2012) were quantified and known to be used for investigating cardiovascular risks (Berends et al., 2009). However, the measurement of the android region is believed to be more variant comparing to the total and gynoid region (Rezzi et al., 2009; Rothney et al., 2012). LBM was also determined and represents the weight of muscles, ligaments, tendons, and internal organs.

The DXA machine is recognized as being a great tool to measure visceral fat in larger patients with a weight limit of 204 kg (Rothney et al., 2013). Total body fat, percentage fat content, LBM as well as estimated visceral adipose tissue mass (g) are determined by the test and the computerized imaging provides a representation of the soft tissue fat distribution. Additionally, its ability to perform longitudinal measurements makes it an attractive choice for monitoring changes in body composition with weight loss (Rothney et al., 2012). Accordingly, this tool permitted the detection of where most of the weight loss occurred in terms of body region (e.g. android fat and gynoid fat), as well as the type of tissue lost (fat mass preferably and/ or lean muscle mass). The test which lasted for 15 minutes, was conducted with participants wearing a hospital gown and all metal objects removed. One operator was supposed to conduct the scanning and data acquisition, but this was not always the case, because it depended on the availability of the staff on site. Although the system uses X-ray radiation, the intensity is considered low compared to a standard X-ray (Rothney et al., 2012).

## 5.6. Experimental analysis

### 5.6.1. Glucose kinetics

#### 5.6.1.1. D-3-3H-Gucose tracer

Initially, a 30 μCi bolus of the tritiated D-3-$^{3}$H-glucose (PerkinElmer Inc., Life and Analytical Sciences, Boston, MA) was given intravenously to speed up the experiment and reaches steady state more quickly followed by a constant 0.22 μCi/min

infusion rate that lasted until the end of the experiment (Chevalier et al., 2004). Due to improvement in subjects' fasting glucose POST weight-loss, a 22 μCi bolus injection of the tritiated glucose was administered instead of 30 μCi.

#### 5.6.1.2. Glucose kinetics analyses

Glucose turnover methodology was performed with modifications to the original reported method by DeFronzo et al. (1979) in order to estimate hepatic glucose suppression induced by insulin (Finegood et al., 1987; Banerji and Lebovitz, 1989; Chevalier et al., 2004). The turnover was determined using tritiated glucose and a single pool model, in which glucose Ra represents the endogenous glucose produced mostly by the liver and glucose Rd comprises both the oxidative glucose disposal and nonoxidative glucose disposal. The rate of hepatic glucose production was calculated given that Ra is the sum of hepatic glucose production and exogenously glucose infused (Banerji & Lebovitz, 1989). During steady state of the hyperinsulinemic clamp, Ra is equal to Rd, such that, glucose infused equals glucose uptake, provided that endogenous glucose production is suppressed (Wolfe & Chinkes, 2005) (Figure 2A). D-3-$^{3}$H-glucose specific activity (SA), a measurement of isotopic enrichment (abundance of the radioactive tracer) in μCi, is computed as the amount of radioactivity divided by glucose concentration in a sample (Wolfe & Chinkes, 2005), and is used to calculate glucose Ra and Rd with a modification of Steele's equations (Finegood et al., 1987):

$$\mathbf{Ra}(t) = I/\mathrm{SAp}(t) - [\mathrm{pVG}(t)\,[d\mathrm{SAp}(t)/dt]/\mathrm{SAp}(t)] + [\mathrm{SAg}/\mathrm{SAp}(t) \times \mathrm{GINF}(t)] - \mathrm{GING}(t)$$

$$\mathbf{Rd}(t) = I/\mathrm{SAp}(t) - [\mathrm{pVG}(t)\,[d\mathrm{SAp}(t)/dt]/\mathrm{SAp}(t)] + [\mathrm{SAg}/\mathrm{SAp}(t) \times \mathrm{GINF}(t)] - \mathrm{pV} \times dG(t)/dt$$

*I is* the constant tracer infusion rate (μCi /min /kg), SAp($t$) is the SA of glucose in plasma at a given time (μCi / mg), p is the pool fraction (p=0.65), V is the distribution volume of glucose in the body (dl / kg), G($t$) is the plasma glucose concentration (mg / dl), dSAp($t$)/d$t$ is the rate of change of SA in the plasma (μCi/mg/min), GINF($t$) is the exogenous glucose infusion rate at a given time (mg/min/kg), SAg is the specific activity of the infused glucose solution (μCi/mg), and dG($t$)/d$t$ is the rate of change of the plasma

glucose concentration (mg/dl/min). Variables expressed as derivatives represent changes of these variables with respect to time.

In order to prepare the GINF solution, series of SAg estimations were done and assumed Ra of 2 mg/kg/min at the postabsorptive steady state while completely suppressed at the clamp steady state. The goal was to maintain clamp plasma glucose SA as close as possible to the postabsorptive glucose SA. To determine dSAp (t) and G(t), a smoothing program called Optimal Segments program (OOPSEG) was used and which provided the "error-free curve" of the two variables in time after finding the most probable magnitude of measurement error (Bradley & al., 1993). The amount of radioactive D-3-$^{3}$H-glucose was determined on plasma samples deproteinized with equal volumes of 0.3 N barium hydroxide (Fisher Chemicals, Fisher Scientific, St. Laurent, QC) and of 5.0% (W/V) zinc sulfate solution (Fisher Chemicals, Fisher Scientific, St. Laurent, QC) (Somogyi et al., 1945; Finegood et al., 1987). Subsequently, once the supernatant is dried out to remove tritiated water, 1 ml of distilled water and 10 ml of liquid scintillation cocktail (ICN Biomedical, Irvin, CA) were added and finally counted in a Bekman scintillation counter.

### 5.6.2. Leucine kinetics

#### 5.6.2.1. L-1-13C-Leucine tracer

Similarly as per glucose tracer infusion, a 0.5 mg/kg bolus of the L-1-$^{13}$C-Leucine tracer (Isotech, Sigma-Aldrich, St. Louis, MO) was infused first, and then a constant infusion rate was set at 0.008 mg/kg/min for the remainder of the study (Chevalier et al., 2004; Wolfe & Chinkes, 2005).

#### 5.6.2.2. Leucine kinetics analyses

Leucine turnover, as measurement of whole body protein turnover was performed using $^{13}$C-leucine stable isotope methodology, as detailed by Matthews et al. (1980), Lariviere et al. (1992). The assumption of this model considers that L-1-$^{13}$C-Leucine tracer is metabolized analogously as the natural L-leucine (Cynober, 1995). Consequently, protein turnover is determined using amino acid tracer (marked by a stable isotope) (Wolfe, 1992) and a two pools model (Figure 2B): a whole body protein pool

and a smaller free amino acid pool (Waterlow et al., 1977; El-Khoury, 1999). The exchange between the two models is either by protein catabolism (C) or via protein synthesis (S) and the turnover rate between these two pools is called flux (Q). Accordingly, amino acids enter the free pool by C or amino acid (Trophamine solution) infusion (I) and exit the free pool by protein S or amino acid oxidation (O). At isotopic steady state, Ra and Rd of the amino acids are equal (Abrams and Wong, 2003):

$$\mathbf{Q = I + C = S + O}$$

5.6.2.2.1. Leucine enrichment measurement

In order to assess $^{13}CO_2$ enrichment, leucine kinetics were calculated as per the stochastic model of Mathews et al., (1980), using keto isocarproic acid ($^{13}$C-α-KIC) as an index of the precursor pool enrichment instead of $^{13}$C-leucine (Matthews et al., 1982; Thompson et al., 1988). The higher the level of carbon dioxide ($^{13}$C), the higher the enrichment is considered to be. $^{13}CO_2$ enrichment is initially obtained as delta (Δ) and is converted to Atom Percent Excess (APE); where APE for each sample represents the difference in enrichment at a given time point and the APE value at baseline (Wolfe and Chinkes, 2005). APE is calculated as the following:

$$\mathbf{APE = 100 \times [\Delta r / (\Delta r + 1)] = 100 \times [(r_s - r_b) / ((r_s - r_b) + 1)]}$$

Where, r is the enrichment ratio of $^{13}$α-KIC/$^{12}$α-KIC, subscript s refers to enrichment level taken at specific time either the postabsorptive or clamp steady state and subscript b refers to baseline level (Wolfe et al., 2005).

Amino acid oxidation (O) as per our case, leucine, expires $CO_2$ and which was determined by quantifying $^{13}$C enrichment of expired $CO_2$ in the breath ($E_{CO2}$), measured by isotope ratio mass spectrometry (IRMS) (Micromass 903D; Vacuum Generators, Winsforce, U.K.) as well as $VCO_2$ obtained from indirect calorimetry (Matthews et al., 1980) and $^{13}$C enrichment of plasma α-KIC measured by gas chromatography-mass spectrometry (GCMS) (Agilent Technologies, GCMS MODEL 5973,CA) after derivatization with 50 μL N-methyl-N-(tert-butyldimethylsilyl) trifuoroacetamide (ThermoFisher, Scientific- P/N 60108-101 TD1) (El-Khoury et al., 1994; Chevalier et al., 2005):

**$O = F\,^{13}CO_2 / E_{KIC}$**

**$O = F_{13CO2}\,[1/E_p - 1/E_i] \times 100$,** where **$F_{13CO2} = [((V_{CO2} \times E_{CO2}) \times 60 \times 44.6)/kg \times 100 \times f]$**

F **$^{13}CO2$** represents the rate of $^{13}CO_2$ production in µmol/kg/h, $VCO_2$ is the rate of $CO_2$ production in mL/min and $E_{CO2}$ obtained by breath measurements is the $^{13}C$ enrichment of $CO_2$ exhaled.

Recovery factors (f) of 0.67 and 0.80 were used during the fasting and clamp state correspondingly, to account for the $^{13}C$ bicarbonate of the $CO_2$ produced by $^{13}C$-leucine oxidation and which was retained in the bicarbonate pool instead of being expired in the air (El-Khoury et al, 1994; Chevalier et al., 2004). Furthermore, another correction factor were considered in the calculations of the recovery factors, to account for possible dilution effect of background isotopic $^{13}C$ enrichment of plasma [$^{13}C$] α-KIC from the amino acid and glucose infusate, which this last is considered to have low $^{13}C$ content (Chevalier et al., 2004). Consequently, adjustments to $^{13}CO_2$ enrichment were made during the study (Chevalier et al. 2004; Chevalier et al. 2005) and which mainly depended on the infusion rates of glucose and amino acids (Fukagawa et al. 1989). The exact magnitude of the adjustment was determined from previous studies conducted in our laboratory, which reported a correction factor of 7.0% from the study on obese women and subsequently used for T2DM subjects.

As for leucine flux (Q), which represents the isotopic state, and takes into account $^{13}C$ -leucine in the infusate ($E_i$) and $^{13}C$-α-KIC enrichment in the plasma ($E_p$), the following equation was applied:

**$Q = I\,[E_i/E_p - 1]$,** where I is the rate of L-1-$^{13}C$-leucine infusion in µmol/kg BW/hr

The values of Q and O for each steady state were calculated from the averages of $^{13}CO_2$ and $^{13}C$-α-KIC enrichments for each plateau. Referring to the two pool models of leucine kinetics, B and I are identified as $R_a$ while O and S as rate of disappearance ($R_d$) and S is also known as non-oxidative $R_d$. Since Q and O are determined and I is already known, S and B were deducted by simple subtraction, in which during steady state:

$$\mathbf{B = Q - I} \text{ and } \mathbf{S = Q - O}$$

The following conversion factors were used in the above equations: multiplying by 100 to change APE into a fraction, 44.6 in µmol/mL to exchange units of air from mL to µmol at standard temperature and pressure according to Avogadro's law, and 60 to convert minutes to hours.

#### 5.6.2.2.2. Indirect calorimetry

Indirect calorimetry (TrueOne® 2400 Canopy System, Parvo Medics**,** Sandy, UT) was used to quantify type and rate of substrate utilization and energy metabolism called resting energy expenditure (REE) from gas exchange, i.e. oxygen consumption ($O_2$) and carbon dioxide production ($CO_2$) (Ferrannini, 1988). Accordingly, the respiratory quotient (RQ) was determined, which represents the ratio of metabolic gas exchange of total volume of $VCO_2$ to $VO_2$. Due to variation in the chemical composition of macronutrients (i.e. carbohydrates, fat, protein and a mixture of those), different amounts of $O_2$ are required to completely oxidize them into $CO_2$ and water; correspondingly, RQ is 1.00 for glucose, 0.70 for fat, 0.80 for protein and 0.84 for a mixed diet (McArdle et al., 1986). Once the rates of total substrate oxidation is computed, total energy production can be estimated, by taking into account the caloric equivalent per gram of either three substrates (i.e.~ 9 kcal/g for fats and 4 kcal/g for carbohydrates and proteins) (Ferrannini, 1988). The data generated from indirect calorimetry were most importantly used to determine leucine oxidation by quantifying $^{13}CO_2$ production from $VCO_2$ measurement. REE estimated by this experimental tool was also verified using the Harris-Benedict equation (1919) and an activity factor of 1.5:

Men: REE (kcal/24hrs) = [66.5 + (13.75 x Wt) + (5.003 x Ht)] - (6.755 x A)

Women: REE (kcal/24hr) = [655.1 + (9.563 x Wt) + (1.85 x Ht)] - (4.676 x A)

Where Wt stands for body weight (kg), Ht for height (cm) and A for age

As described by Bogardus et al. (1984), the test was performed twice on the experiment day at the steady states, 20 minutes before insulin infusion (i.e. at the postabsorptive state) and during the last 30 minutes of the clamp. After resting on a bed for a minimum of 30 minutes in a supine position, the test began and lasted for 20 minutes which required the subject to breath under the ventilated hood and the average of

the last 15 minutes was used for calculation of 24-hour REE. The test was conducted in a thermally neutral room temperature with a minimum of environmental disturbance (i.e. noise, lighting, people in the room, etc) and participants were asked not to talk, move, fall asleep and not to alter their breathing pattern.

## 5.7. Monitoring

Continuous monitoring of participants' health and condition state during the study period was done over the phone, by emails and through weekly visits. At home, participants reported their blood glucose values by emails and pertinent information in relation to their health, on a daily basis for the first weeks or upon request. At the weekly visit, participants picked up their meals for the following week, and weight, circumferences and BIA were measured. If judged necessary, diet and medication (i.e. antihyperglycemic, lipid lowering and antihypertensive agents) were adjusted.

The following questionnaires were supplied to assess participants' overall knowledge about diabetes: "Medical Assistant's Diabetes Survey" (St. Peter Family Medicine Residency Program, Olympia, 2003), "Patient's Diabetes Knowledge Questionnaire" (Star County, Gateway Community Health Center, 2003) (Appendix 13), and "Health Belief Questionnaire" (Diabetes Initiative National Program, 2009). Additionally, other questionnaires were given to evaluate the self-efficacy of their condition and the complications issued by diabetes: "Self-Care Activities and Diabetes treatment Questionnaire" (Physical Activity & Nutrition for Diabetes in Alberta, PANDA, 2008), "Self-Efficacy" (Advancing Diabetes Self-Management Project, La Clinica de La Raza, Inc., Oakland, CA, 2005) and "Self-Care Activities and Diabetes Treatment Questionnaire" (PANDA, 2008). Questionnaire on physical activity were also provided to appraise activity level of participants at baseline and to assess the motivational impact of the study (if any) on behavioural and lifestyle changes: "Physical Activity Adherence" (PANDA, 2008), "Baecke Questionnaire of Habitual Physical Activity" (Baecke, et al., 1982) and MOSPA (Iqbal, Rafique et al., 2006).

## 5.8. Post-study follow up

The follow-up was not included in the pilot study, but was offered to participants in recognition for their commitment to the study. Contact was maintained over the phone and by emails. A follow-up visit was scheduled at 3 months post-study, during which blood sample was taken and weight, body circumferences, REE and BIA were measured. Furthermore, participants' 2 - 3 day food diary records were reviewed to provide weight (i.e. weight loss and/or maintenance strategies) and glycemic control counselling.

## 5.9. Statistical Analyses and Power Calculation

Results are presented as means ± SEM. Even though, SD and SEM are related, SEM gives an idea of the accuracy of the mean or the precision for an estimated mean of a group, while the SD illustrates the variability of the data. Given the fact that the sample size is small and variability between subjects is acknowldeged (e.g. gender, diabetes duration and physical activity levels), the main objective of this study remains to assess significant changes in kinetics of glucose and protein metabolism after the nutritional intervention and the weight loss; hence, SEM seems more representative of the statistical significance of the differences Pre vs. Post intervention. Furthermore, a visual contrast is better reflected when using SEM rather than the SD. SD could be used afterwards to compare the findings with those of similar protocols.

Paired t-test was used to assess changes Pre and at 5 weeks of intervention. Repeated-measures analysis of variance (ANOVA) was used to measure changes in kinetics, substrates and hormones within subjects, postabsorptive and during the hyperinsulinemic isoglycemic and isoaminoacidemic clamp. Pearson's coefficient was used to assess if parameters were normally distributed, and Wilcoxon signed-rank test for two related sample was used otherwise (including, visceral adiposity, triglycerides and blood pressure parameters).

Using Hall's formula (1983), a sample size of 10 subjects was needed to detect a 15% difference in protein synthesis rates during hyperinsulinemia and with different glycemic levels, with a power of 90% based on previous results (one-tailed $\alpha = 0.05$, $\beta = 0.10$). Based on 6 subjects, an 8% difference in protein synthesis rates during clamp could

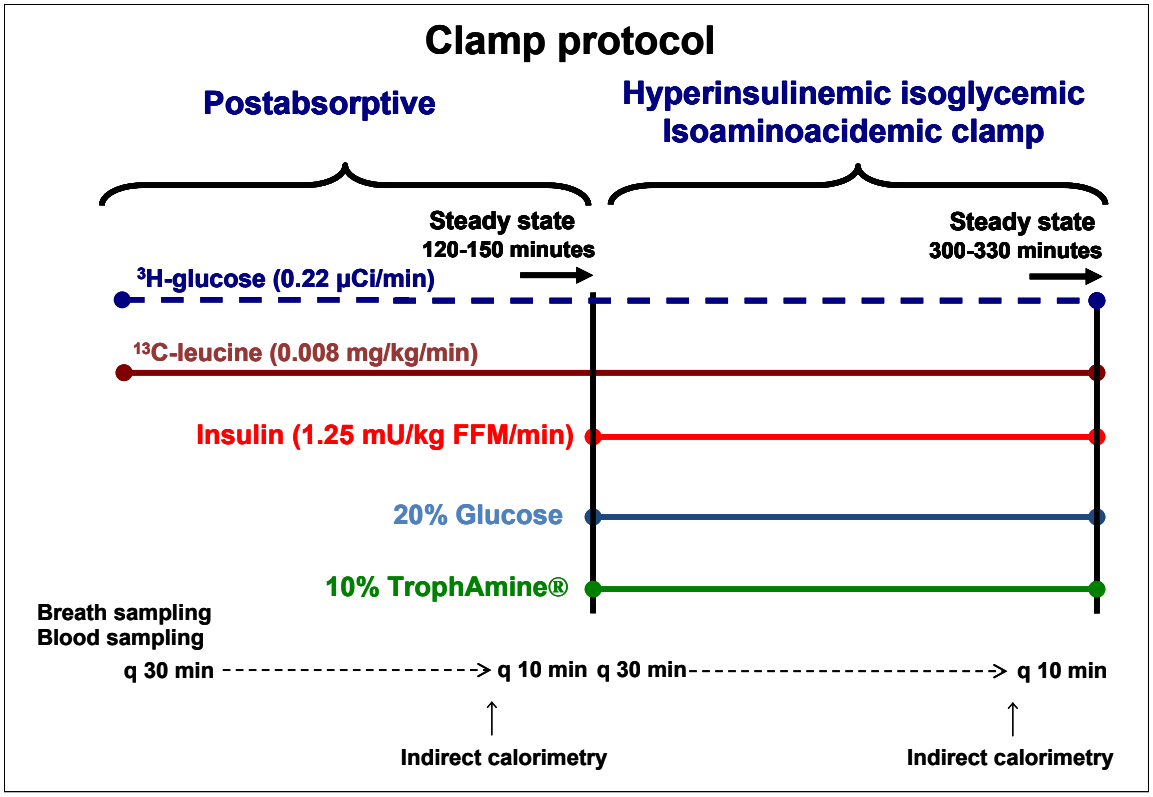
have been detected between Pre and at 5 weeks of energy restriction, instead of our primer goal of 15% with 10 subjects, calculated with the Hall's formula (1983). Analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL).

Table 2. Energy and macronutrient content of the isonergetic weight-maintenance (Pre) and energy-restricted diet

| | **Pre- Energy Restriction** | **During Energy Restriction** | ***p*** |
|---|---|---|---|
| Energy Intake (kcal/d) | 2419 ± 134 | 1477 ± 79 | <0.001 |
| Carbohydrate Intake (g/d) | 361 ± 15 (60%) | 161 ± 8 (45%) | <0.001 |
| Fat Intake (g/d) | 62 ± 3 (23%) | 48 ± 3 (29%) | 0.001 |
| Protein Intake (g/d) | 102 ± 6 (17%)<br>1.98 g/ kg LBM/d | 98 ± 6 (26%)<br>1.95 g/ kg LBM/d | 0.080 |

Data are mean ± SEM. *p* value from paired t-test

Figure 1. Postabsorptive and hyperinsulinemic isoglycemic isoaminoacidemic clamp protocol



## Kinetics Model of Glucose & Protein

Figure 2A. Glucose Kinetics Model

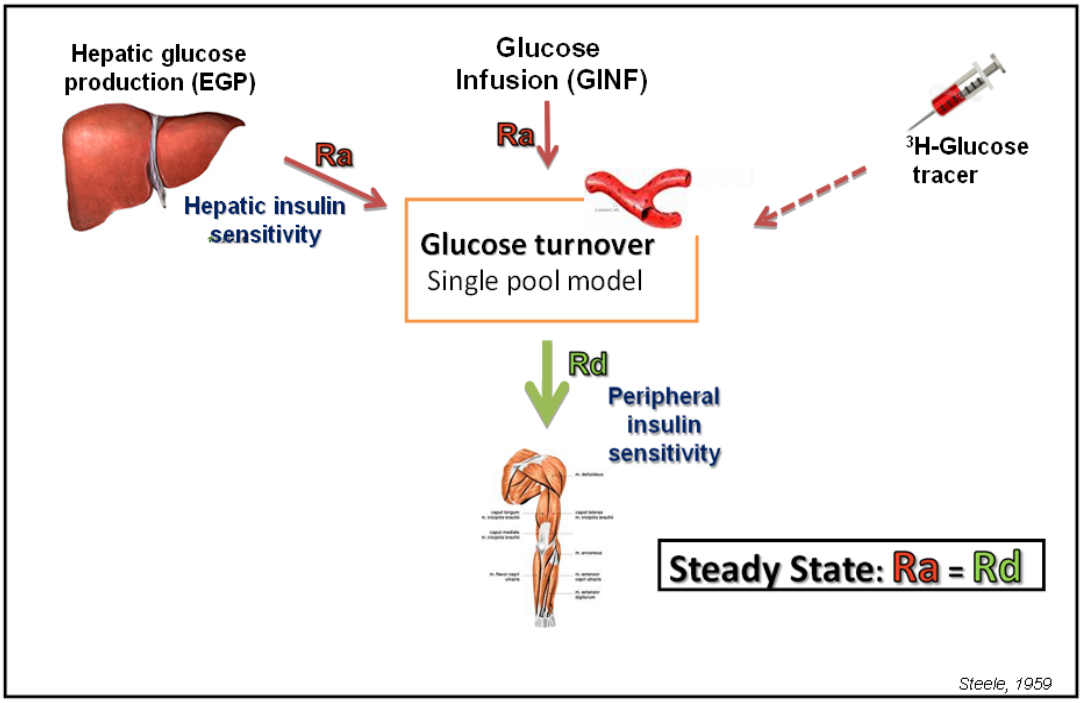



Figure 2B. Leucine Kinetics Model

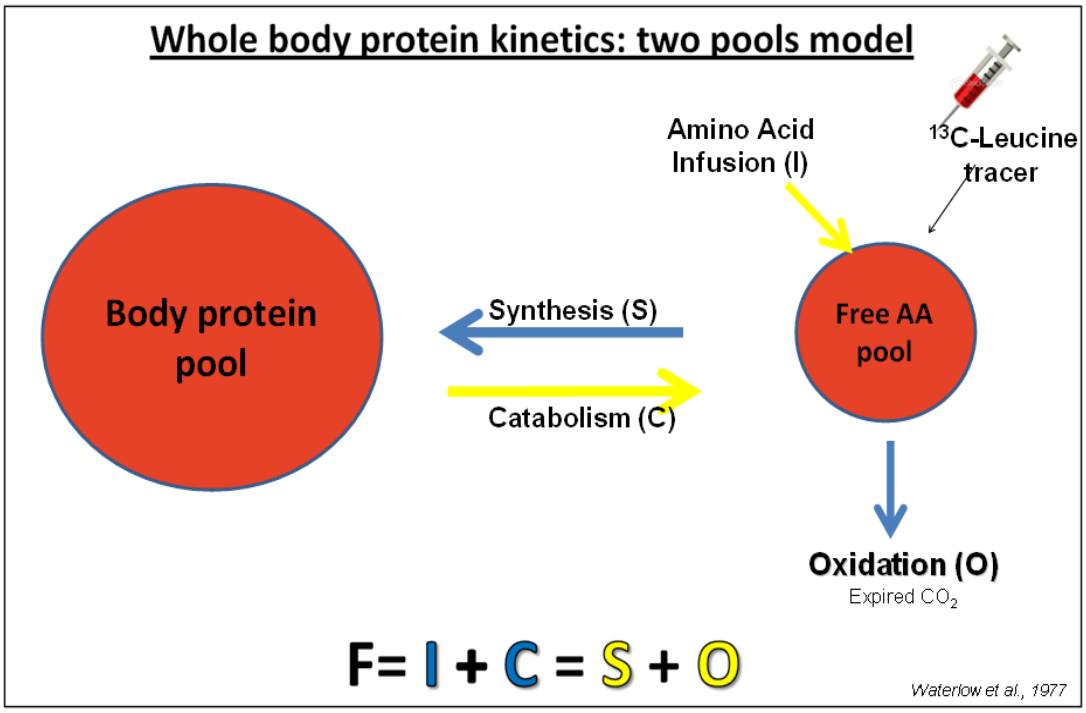
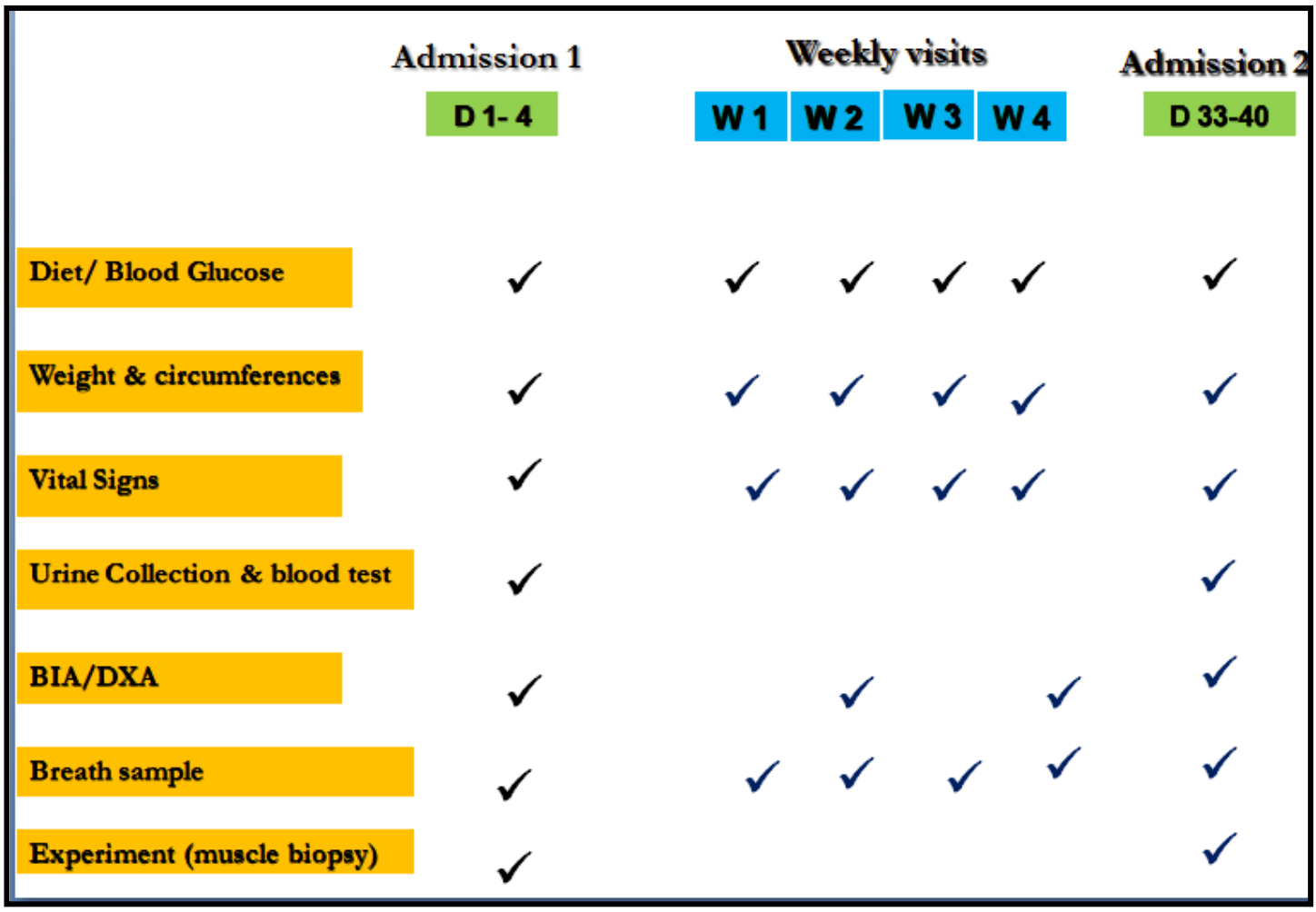


Figure 3. Measurements and tests timeline



# VI. Results

## 6.1. Subjects

Of the 48 adults screened, ten declined because they judged the study to be too time-consuming or had a time conflict and 32 did not meet the inclusion criteria. A total of six (3 men, 3 women) obese T2DM subjects, aged 58.8 ± 1.8 years and with 8.6 ± 4.1 years of known diabetes duration were enrolled in the study. Overall, five subjects were taking oral anti-hyperglycemic agents, either from three (n = 1), two (n = 2) or one classes per day (n = 2). At week 2, one participant was asked to discontinue his medication and another was given half the dose. On those requiring anti-hypertensive agents (n = 5), medications were discontinued at week three for two subjects and the dosage was decreased by 75% for the remaining person. Lipid lowering agents (n= 4) were maintained throughout the study except for one subject for whom the initial dosage was reduced by half at week 4.

## 6.2. Dietary intervention

At baseline, adiposity markers, including BMI, percent body fat and circumferences were above healthy reference values or cut-off-points in the classification of obesity (World Health Organization, 2009) (Table 2). As designed, the hypocaloric diet provided 60% of energy requirements, with 45% of energy derived from carbohydrate, 26% from protein (1.95 g/kg LBM/day) and 29% from fat (Table 2). Calories ranged from 1340 ± 82 kcal/d for women and 1614 ± 74.0 kcal/d for men, with an approximate additional deficit of 50 - 150 kcal/week based on REE measurement (Table 2). For the most part, water intake did not surpass 2 litres per day, except for one person who exceeded 3 litres per day during her first admission period, possibly due to her severe hyperglycemia. Dietary sodium was reduced to 1500 - 1800 mg/d, compared to 3240 mg for men and 2270 mg for women at baseline, estimated based on dietary recall. Normal baseline levels of potassium and calcium were maintained at 5 weeks.

## 6.3. Metabolic outcomes

Weekly changes in weight and circumferences are presented in Figure 4A. The small waist, umbilical waist and hip circumferences were all markedly reduced, respectively by 1.0 ± 0.3 cm, 1.4 ± 0.2 cm and 1.2 ± 0.3 cm.

At week 5 of energy restriction, weight decreased by 5.7% (-5.1 ± 0.5 kg), BMI by 1.7 ± 1.1 kg/m$^2$, fat mass by 11% (-3.9 ± 0.3 kg) and visceral adiposity by 25% (523.5 ± 145.1 g), and LBM was maintained (Table 3). Delta changes in body composition are presented in Figure 4B. Weight reduction in n = 5 was slightly above the 4.6% predicted weight loss, from energy deficit, based on 3500 kcal per pound ( which is equivalent to 1750 kcal per 0.5 kg) of adipose tissue. Only one person exceeded the predicted weight by 3 kg. After the study, three participants kept contact with the healthcare team by emails and returned to the centre for two follow-up visits. An additional weight loss of 4.5 kg was seen at 3 months for n = 3, which was maintained at 6 months for n = 2. The third participant had lost another 4.2 kg at his second visit. Partcipants were following the same dietary pattern, and one participant was still occasionally using formulas to replace one of her meals.

Participants were studied hyperglycemic at baseline; fasting plasma glucose concentration decreased by 29%, A1C by 14%, HOMA-IR by 54% and CRP by 50% at 5 weeks (Table 3). Lipid profile was significantly improved at 5 weeks, corresponding to reductions in triglycerides by 56% (-1.33 ± 0.37 mmol/L), TC by 28% (-1.43 ± 0.26 mmol/L), LDL concentrations by 32% (-0.66 ± 0.20 mmol/L); HDL did not change from Pre-intervention. Systolic blood pressure decreased by 13 ± 2 mmHg and the diastolic by 9 ± 4 mmHg. Pre-meals glycemia concentrations were all significantly lowered at the 5$^{th}$ week of energy-restriction (Figure 5). Fasting glycemia, lipid profile and blood pressure improvements were maintained at 3 months in those who returned for follow-ups.

## 6.4. Hormones, substrates, RQ and REE

Hormones and substrates concentrations, REE and RQ Pre- and at 5 weeks of energy restriction are presented in Table 4. Postabsorptive serum insulin decreased by 38%, glucagon by 18% and C-peptide by 38%. Baseline NEFA concentrations did not

change at 5 weeks. REE per LBM was maintained and the absolute REE was slightly decreased by 101 ±16 kcal (5%). At 5 weeks, baseline RQ decreased significantly by 7%. During the hyperinsulinemic clamp, C-peptide and glucagon were signicantly lower compared to Pre-intervention, respectively, by 52% and 19%, however the corresponding deltas vs. postabsorptive were not significantly different. In response to the hyperinsulinemic clamp, NEFA were suppressed to the same extent as in Pre- and amino acid infusion rate did not differ from the Pre-intervention study. Also, during the clamp, REE and RQ values did not differ from postabsorptive of the Pre-intervention study.

## 6.5. Leucine kinetics

Leucine kinetics are presented in Table 5. Postabsorptive whole-body protein kinetics showed a 12% reduction in leucine flux, 32% in oxidation, 6% in synthesis and net balance was less negative by 11 µmol/min, a value that can be extrapolated over 10 hours of fasting to spare 11 g of body protein. During the hyperinsulinemic clamp, protein flux increased by 31%, protein breakdown was suppressed by 12% and synthesis rate did not vary from postabsorptive; there was an insignificant rise in net anabolism.

## 6.6. Glucose kinetics

At 5 weeks of energy restriction, postabsorptive rates of EGP (Ra) and disposal (Rd) significantly decreased, respectively by 13% and 12%, and MCR was markedly increased by 24%; presented in Table 6. In response to the hyperinsulinemic clamp, the suppression of glucose $R_a$ by ~ 70% and the increase of glucose $R_d$ by ~ 72% did not differ from Pre, while glucose MCR was significantly higher (47%) and glucose infusion rate did not differ.

## 6.7. Surveys and questionnaires

At baseline, all participants reported that controlling diabetes with diet and medication were of equal importance and crucial for optimal diabetes management. Of these, two persons believed that diabetes requires special lifestyle accommodations, including a special diet and they thought that it was challenging to adopt

recommendations given by health professionals upon diagnosis. On average, participants scored 74% on the "Knowledge assessment questionnaires" (Appendix 13), whereby, they were not aware of the most common longterm diabetes-related complications and the frequency at which check-ups should be done to prevent co-morbidities. They also scored 80% on the "Self-efficacy diabetes management questionnaire" (Appenidx 14). According to the Baecke and MOSPA questionnaire, participants are considered sedentary at baseline with a score of 7.3 ± 0.4 for men and 7.2 ± 0.4 for women (< 100 min per week of low-intensity-exercise).

At 5 weeks, participants scored 86% on the knowledge questionnaire, which was significantly higher than the first assessment ($p = 0.003$). Men engaged in 30 - 60 min of moderately intense physical activities per day (biking or jogging) and women with no more than 60 min of low-intensity-exercise, mainly walking, at a frequency of 2 to 3 times per week (Appenidx 15). The duration and frequency of the scheduled activities were reported to be higher in most participants starting from week 3. Women reported being engaged in a 15 - 20 minutes of low intensity activity twice a week, which consisted of walking and men in a moderately intense exercice, such as 15 minutes stationary bike while watching TV or a 20 minutes of incline walking. The Baecke score were not significantly different at 5 weeks.

Table 3. Anthropometrics and metabolic outcome, Pre- and at 5 weeks of energy-restriction

| Characteristic | Pre-energy restriction | At 5 weeks of energy restriction | *p* |
|---|---|---|---|
| Weight (kg) | 88.8 ± 3.6 | 83.7 ± 3.3 | 0.001 |
| BMI (kg/m$^2$) | 30.6 ± 1.2 | 28.9 ± 1.1 | 0.001 |
| Fat Mass (kg) | 34.5 ± 2.8 | 30.6 ± 2.9 | <0.001 |
| Body Fat (%) | 39.0 ± 3.3 | 36.8 ± 3.8 | 0.012 |
| Visceral Adipose Tissue (kg) | 2.08 ± 0.51 | 1.51 ± 0.38 | 0.043 |
| LBM (kg) | 51.4 ± 4.0 | 50.2 ± 4.2 | 0.078 |
| Small Waist Circumference | 101.5 ± 3.6 | 100.5 ± 3.7 | 0.020 |
| Umbilical Waist Circumference | 111.6 ± 3.6 | 110.2 ± 3.6 | 0.002 |
| Hip Circumference (cm) | 115.1 ± 3.3 | 113.9 ± 3.2 | 0.024 |
| Waist-to-hip-ratio | 0.88 ± 0.02 | 0.88 ± 0.02 | 0.995 |
| BMC (kg) | 2.9 ± 0.2 | 2.9 ± 0.2 | 0.419 |
| Fasting plasma glucose | 8.3 ± 0.5 | 5.8 ± 0.3 | 0.003 |
| A1C (%) | 7.9 ± 0.5 | 6.7 ± 0.3 | 0.006 |
| HOMA-IR | 6.22 ± 0.69 | 2.77 ± 0.28 | 0.003 |
| CRP (mg/L) | 1.71 ± 0.46 | 0.86 ± 0.37 | 0.032 |
| Triglycerides (mmol/L) | 2.29 ± 0.57 | 0.97 ± 0.26 | 0.043 |
| Total Cholesterol (mmol/L) | 4.36 ± 0.34 | 2.93 ± 0.22 | 0.003 |
| LDL-Cholesterol (mmol/L) | 2.14 ± 0.31 | 1.49 ± 0.20 | 0.021 |
| HDL-Cholesterol (mmol/L) | 1.11 ± 0.05 | 1.01 ± 0.08 | 0.393 |
| Total Cholesterol/HDL | 3.90 ± 0.23 | 2.98 ± 0.27 | 0.006 |
| Systolic Blood Pressure (mm) | 123 ± 4 | 111 ± 4 | 0.042 |
| Diastolic Blood Pressure (mm) | 78 ± 3 | 69 ± 2 | 0.043 |

Data are mean ± SEM; A1C, Glycated hemoglobin; BMC, bone mineral content; BMI, body mass index; CRP, C-reactive protein; HOMA-IR: homeostatic model of assessment: insulin resistance; LBM, lean body mass. *$p < 0.05$ vs. Pre-energy restriction, measured by paired t-test.

Table 4. Circulating hormone and substrates concentrations, REE and RQ, Pre- and at 5 weeks of energy-restriction, postabsorptive and during a hyperinsulinemic isoglycemic isoaminoacidemic clamp protocol

| | Pre- energy restriction | | At 5 weeks of energy restriction | | *p* | | |
|---|---|---|---|---|---|---|---|
| | Postabsorptive | Clamp | Postabsorptive | Clamp | Clamp effect | Intervention effect | Clamp × intervention |
| Serum Insulin (pmol/L) | 103 ± 13 | 502 ± 51 | 64 ± 6* | 456 ± 33 | < 0.001 | 0.077 | 0.829 |
| Plasma Glucagon (pmol/L) | 19 ± 3 | 16 ± 3 | 16 ± 3* | 13 ± 3* | 0.001 | 0.003 | 0.598 |
| Glucagon/Insulin ratio | 0.21 ± 0.05 | 0.03 ± 0.01 | 0.26 ± 0.04 | 0.03 ± 0.01 | 0.003 | 0.076 | 0.054 |
| Plasma C-peptide (pmol/L) | 684 ± 102 | 528 ± 100 | 421 ± 55* | 255 ± 44* | < 0.001 | 0.010 | 0.155 |
| Serum NEFA (µmol/L) | 520 ± 72 | 91 ± 14 | 573 ± 74 | 79 ± 8 | 0.001 | 0.756 | 0.636 |
| Plasma BCAA (µmol/L) | 376 ± 19 | 367 ± 21 | 392 ± 25 | 366 ± 21 | 0.148 | 0.644 | 0.201 |
| REE (kcal/d) | 1698 ± 68 | 1735 ± 90 | 1597 ± 81* | 1548 ± 98* | 0.755 | 0.003 | 0.053 |
| RQ | 0.82 ± 0.01 | 0.86 ± 0.01 | 0.76 ± 0.01* | 0.83 ± 0.01 | 0.006 | 0.004 | 0.093 |

Data are mean ± SEM; BCAA, branched-chain amino acids; REE, resting energy expenditure; RQ, respiratory quotient; NEFA, non-esterified fatty acids. *$p < 0.05$ vs. versus Pre-energy restriction, by paired t-test. Repeated-measures ANOVA were used for comparing the effect of the clamp, intervention, and interaction of these.

Table 5. Whole body leucine kinetics, Pre- and at 5 weeks of energy-restriction, postabsorptive and during a hyperinsulinemic isoglycemic isoaminoacidemic clamp protocol

| | Pre-energy restriction | | At 5 weeks of energy restriction | | *p* | | |
|---|---|---|---|---|---|---|---|
| μmol/ min | Postabsorptive | Clamp | Postabsorptive | Clamp | Clamp effect | Intervention effect | Clamp × intervention |
| Total $R_a$ (flux) | 147.4 ± 11.5 | 155.5 ± 9.0 | 129.9 ± 10.0* | 140.5 ± 7.2* | 0.027 | 0.002 | 0.141 |
| Oxidation | 33.7 ± 3.8 | 44.1 ± 2.3 | 22.9 ± 1.9* | 42.9 ± 2.9 | < 0.001 | 0.018 | 0.014 |
| Endogenous $R_a$ (breakdown) | 147.4 ± 11.5 | 120.0 ± 7.6 | 129.9 ± 10.0* | 104.5 ± 5.0* | 0.006 | 0.003 | 0.358 |
| Infusion Rate | na | 35.5 ± 2.6 | na | 36.0 ± 3.1 | na | na | na |
| Nonoxidative $R_d$ (synthesis) | 113.6 ± 8.0 | 111.4 ± 6.8 | 106.9 ± 8.2* | 97.6 ± 4.4* | 0.088 | 0.001 | 0.082 |
| Net Balance (synthesis – breakdown) | -33.7 ± 3.8 | -8.6 ± 2.1 | -22.9 ± 1.9* | -6.9 ± 2.5 | 0.003 | 0.014 | 0.007 |

Data are mean ± SEM; $R_a$: rate of appearance; $R_d$: rate of disposal. *$p < 0.05$ vs. Pre-energy restriction, by paired t-test. Repeated-measures ANOVA were used for comparing the effect of the clamp, intervention, and interaction of these.

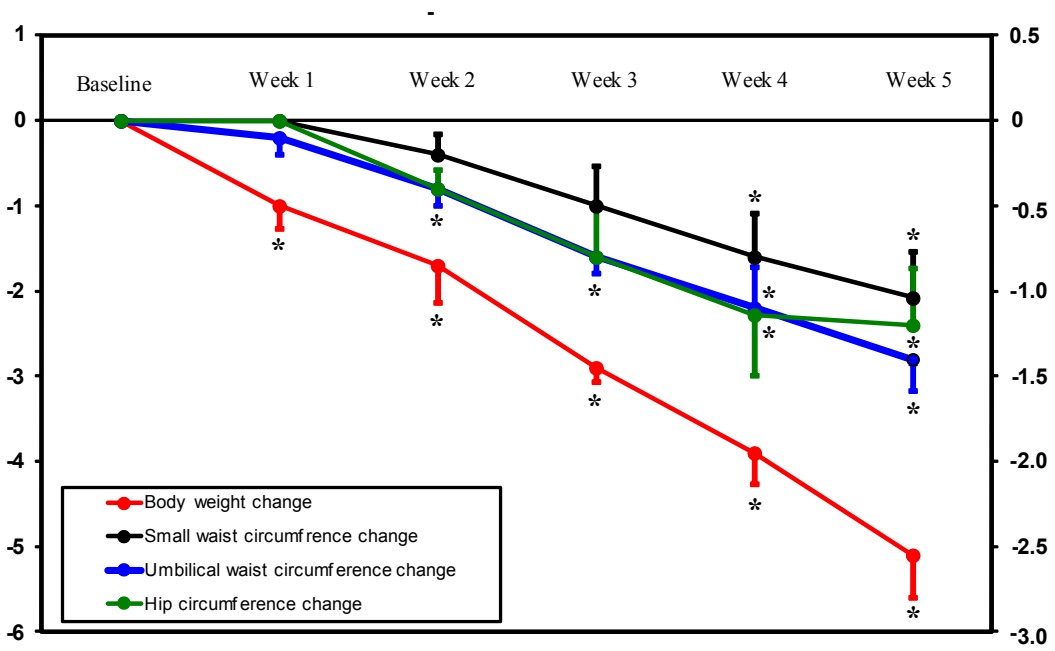
Table 6. Glucose kinetics, Pre- and at 5 weeks of energy-restriction, postabsorptive and during a hyperinsulinemic isoglycemic isoaminoacidemic clamp

| | Pre-Energy Restriction | | At 5 weeks of Energy Restriction | | *p* | | |
|---|---|---|---|---|---|---|---|
| | Postabsorptive | Clamp | Postabsorptive | Clamp | Clamp effect | Intervention effect | Clamp × intervention |
| Endogenous $R_a$ | 1023 ± 84 | 340 ± 89 | 891 ± 81* | 220 ± 32 | 0.001 | 0.062 | 0.902 |
| Infusion Rate | na | 1350 ± 109 | na | 1468 ± 201 | na | na | na |
| Total $R_d$ | 1057 ± 81 | 1706 ± 176 | 939 ± 73* | 1693 ± 234 | 0.019 | 0.204 | 0.261 |
| MCR | 129 ± 11 | 211 ± 19 | 160 ± 14* | 310 ± 49* | 0.017 | 0.029 | 0.068 |

Data are mean ± SEM, in µmol/ min; $R_a$: rate of appearance; $R_d$: rate of disposal; MCR: metabolic clearance rate (total glycemia/ Rd). *$p < 0.05$ vs. Pre-Energy restriction, by paired t-test. Repeated-measures ANOVA were used for comparing the effect of the clamp, intervention, and interaction of these.

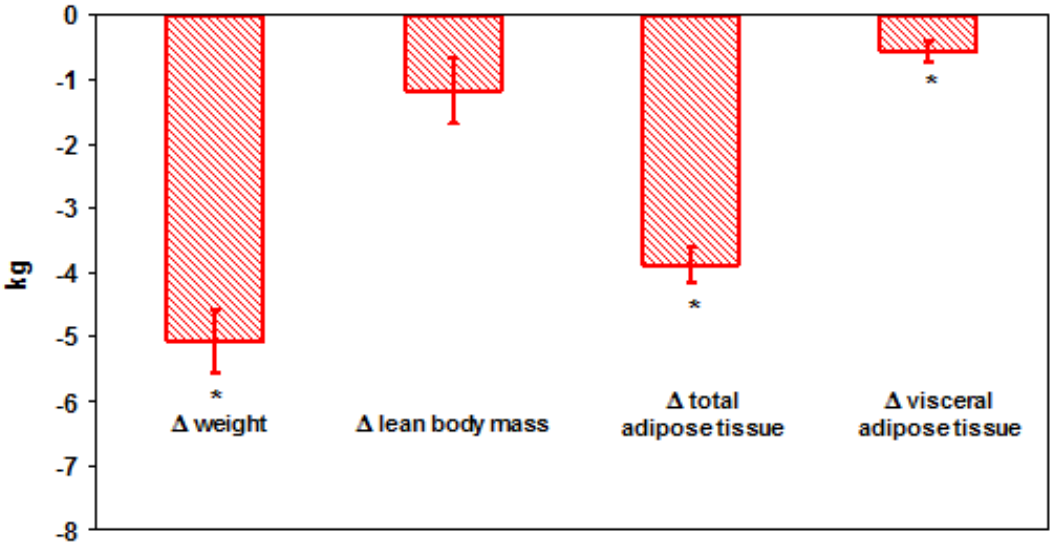
## Changes in body composition at 5 weeks of energy restriction

Figure 4A. Weekly change in weight and body circumferences



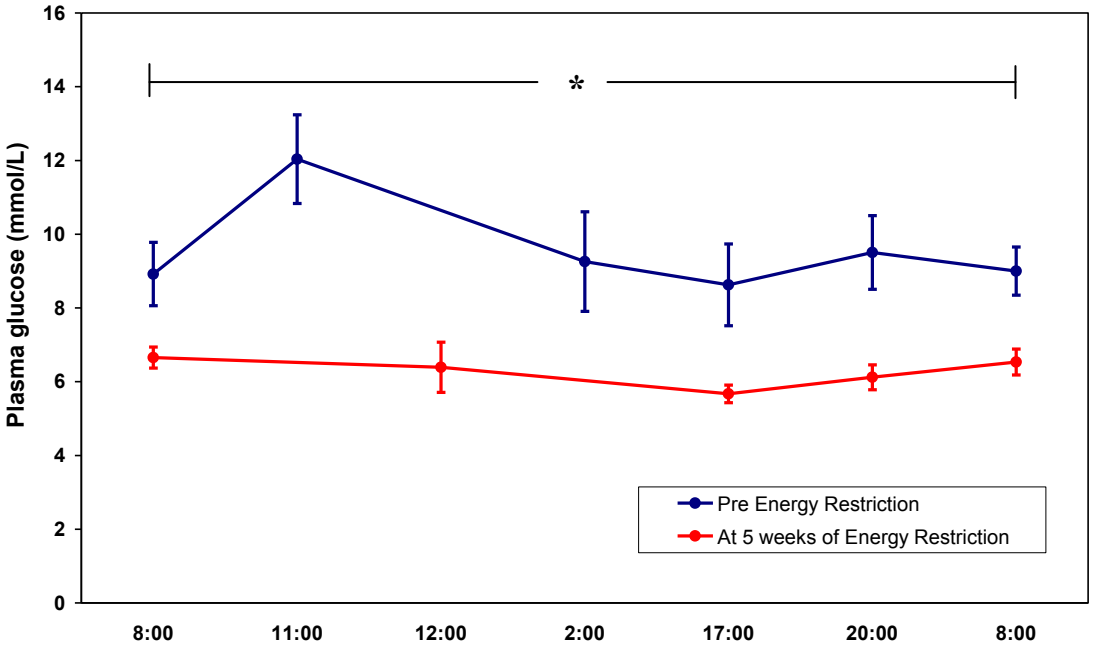

*$p < 0.05$ vs. baseline values, by paired t-test.

Figure 4B. Delta body composition at 5 weeks of intervention



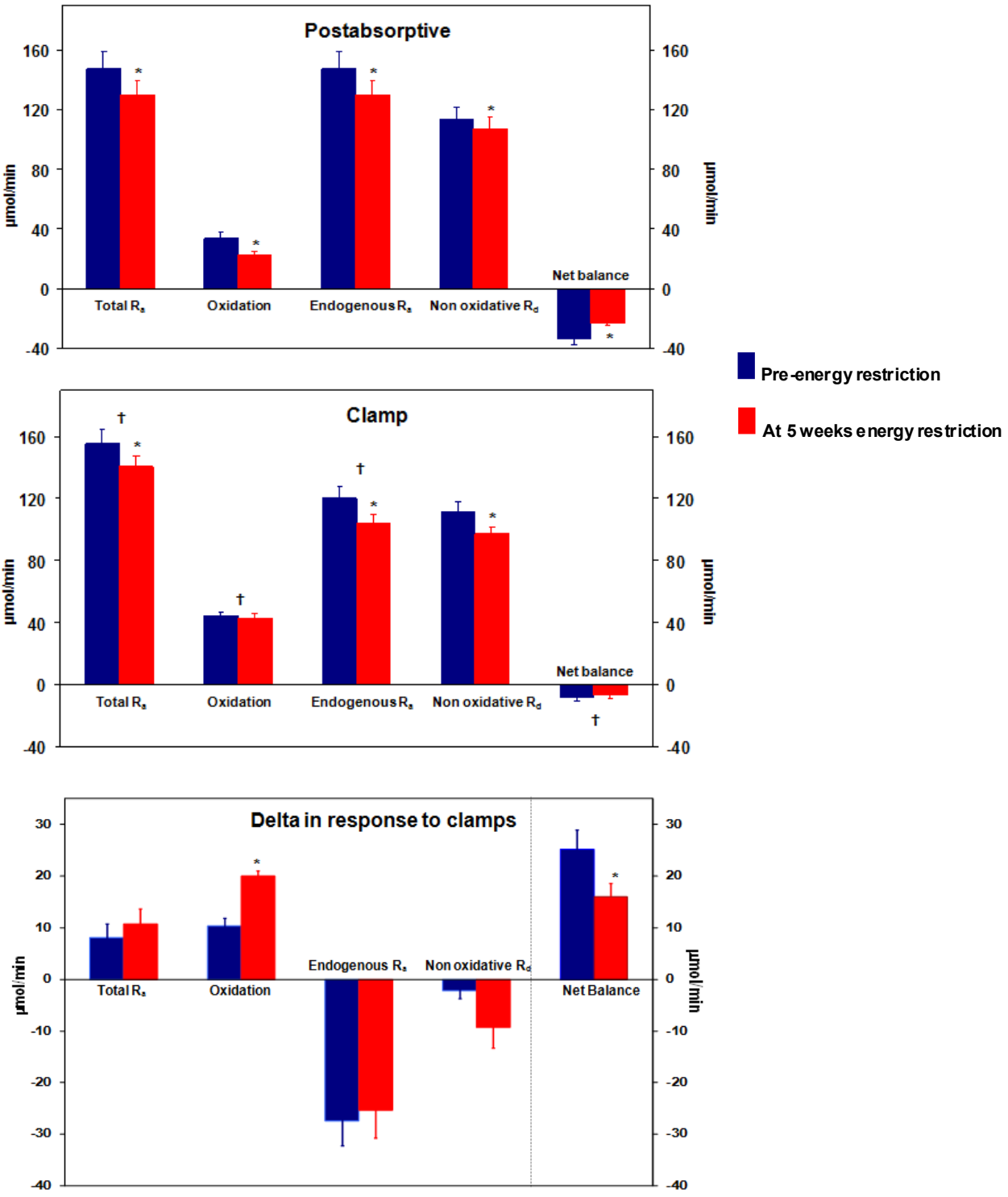

*$p < 0.05$, by paired t-test

Figure 5. Comparison of capillary glucose concentrations during hospitalizations, Pre- and at 5 weeks period of energy-restriction



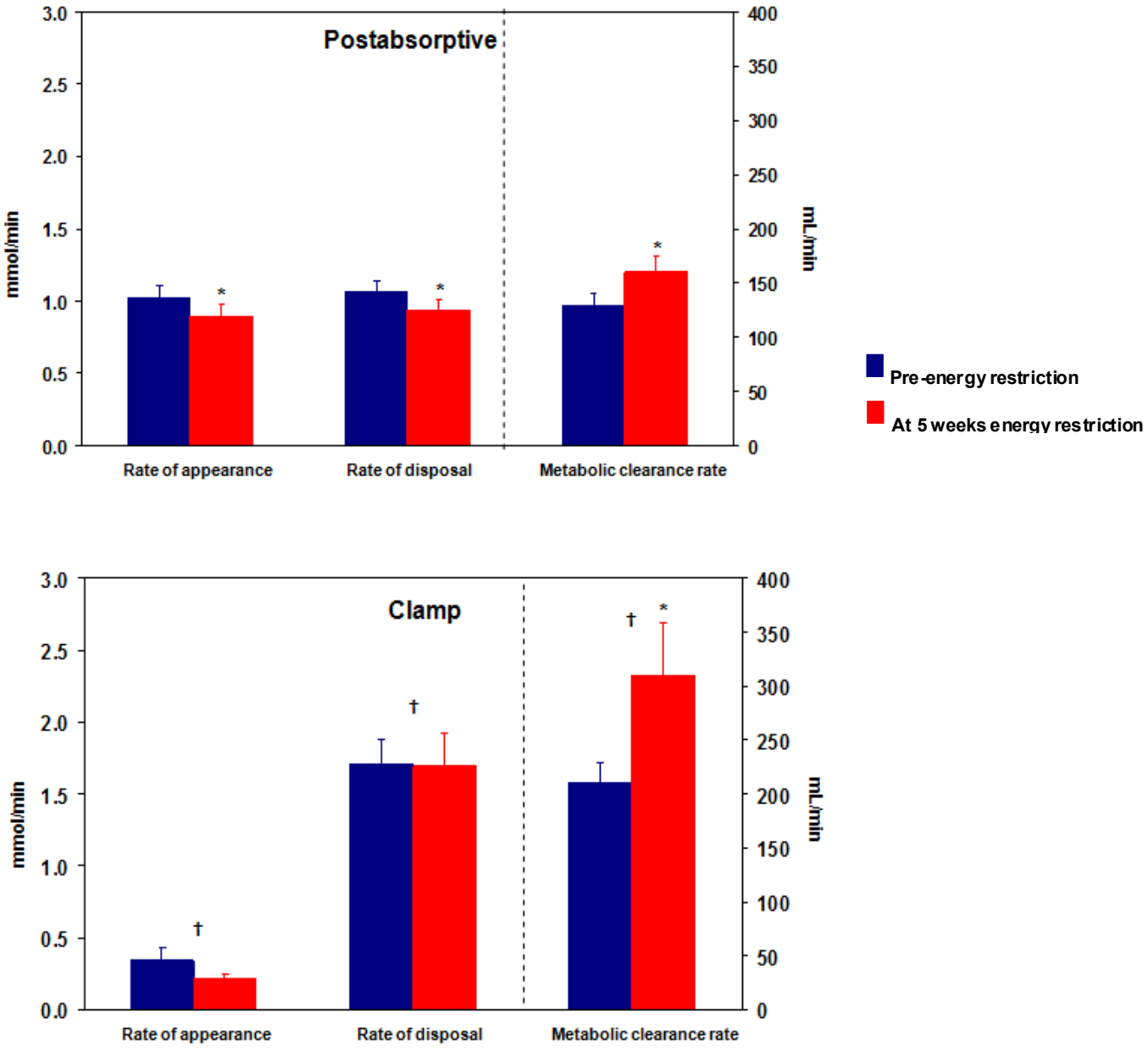

*$p < 0.05$ vs. Pre-energy restriction, by paired t-test.

Figure 6. Leucine kinetics at baseline and at 5 weeks of energy-restriction




*$p < 0.05$ vs. Pre-energy restriction, by paired t-test; †$p < 0.05$, clamp effect by repeated measures ANOVA

Figure 7. Glucose kinetics at baseline and at 5 weeks of energy-restriction




*$p < 0.05$ vs. Pre-energy restriction, by paired t-test; †$p < 0.05$, clamp effect by repeated measures ANOVA

# VII. Discussion

In the present study, 5 weeks of 40% energy-restriction with maintained abundant dietary protein in sedentary obese subjects with T2DM, resulted in weight reduction mainly attributed to total and visceral fat mass loss, sparing of lean mass, and substantial improvement in glycemic control and CVD risk factors. With normalization of fasting plasma glucose and a decrease in fasting plasma insulin, the following was seen: 1) postabsorptive whole-body protein turnover was reduced along with rates of oxidation, as assessed using L-[1-$^{13}$C] leucine; 2) in response to the hyperinsulinemic clamp, whole-body leucine flux increased, protein breakdown was suppressed to the same extent as at baseline, protein synthesis was yet again not stimulated and oxidation rates were the same as in Pre-intervention, such that there was no improvement in the anabolic response of protein to insulin; 3) postabsorptive glucose turnover was reduced, and MCR increased 4) in response to the hyperinsulinemic clamp, suppression of endogenous glucose production and increase in total glucose disposal rates were the same as in Pre-intervention, while MCR increased. Based on the self-reported physical actvity questionnaires, participants were expressing an interest to increase their activity levels by the third week, however, the changes in activity levels during the 5 weeks were non-significant and thus could not have been a major player in the outcomes observed.

## 7.1. Clinical and metabolic outcomes

### 7.1.1. Body weight and composition

Overall, body weight and circumferences (i.e. small waist, umbilical waist and hip) were markedly reduced at 5 weeks, mainly attributed to fat loss, while LBM was conserved. It should be noted that the preservation of LBM can be secondary to sufficient dietary protein maintained throughout the study and evenly distributed across daily meals, as proposed by Paddon-Jones et al. (2010). Visceral adiposity was also significantly reduced. The rate and total weight loss from this study complies with the 5 - 10 % reduction of body weight (i.e. loss of 0.5 - 1.0 kg per week) recommended by the CDA CPGs to improve glycemic control and cardiovascular disease risk factors (Wharton et al., 2013).

Previous studies on energy-restriction and meal replacements have reported comparable losses of 6% of initial body weight at 12 weeks, in obese T2DM subjects (Yip et al., 2001; Heymsfield et al., 2003; Birnkworth et al., 2004; Cheskin, et al., 2008). In line with this study, preserving LBM and reducing fat mass during energy restriction was also reported by other diet protocols which provided no less than 25% of total energy from protein (Franz, 2002; Trumbo et al., 2002; Farnsworth et al., 2003). On the other hand, comparable reduction of visceral adiposity was reported with a very-low-calorie-diet followed over 28 days (Vazquez et al., 1995). Greater decreases in waist circumference were reported in the current study compared to similar dietary interventions (1300 - 1600 kcal/d) with a reduction of 0.6 - 0.8 cm/month (Heilbronn et al., 1999; Esposito et al., 2003; McLaughlin et al., 2003; Toobert et al., 2003; Cheskin, et al., 2008; Krebs et al., 2012). This may be explained by the greater control of participants' dietary intake in our study with the provision of ready-to-eat meals, liquid formulas as well as customized dietary plan.

It has been previously reported that weight loss is more difficult to achieve in individuals with T2DM (Hensurd, 2001), especially fat loss (Baker et al., 2012). A study by Baker et al. (2012) compared the effect of weight loss on body composition in obese subjects with or without T2DM, after completing a very-low-energy diet program for 24 weeks. Despite equal weight loss in the two groups at 5 years (-8.5 ± 1.3 vs. -9.4 ± 1.2 kg), a lower fat reduction per unit of weight loss was seen in the T2DM group (-5.5 ± 0.8 vs. -6.8 ± 1.2 kg reduction in adipose tissue). One possible explanation for this difference is the anabolic effect of higher circulating insulin concentrations in diabetic subjects, which could promote adipose tissue storage (Hales & Randle, 1963). Additionally, the greater fat reduction in the nondiabetic group with similar weight loss suggests greater loss of lean tissue in diabetes (Park et al., 2009). It has also been shown that people with diabetes have a greater loss of body water during energy restriction, possibly related to a decrease in the sodium retention mediated by hyperinsulinemia (Peters et al., 2002). Sex differences can also impact the change in body composition during energy restriction. It has been shown that men experience in general greater weight reduction (~ 25%) (Heilbronn et al., 1999; Mustajoki & Pekkarinen, 2001; Baker et al., 2012) and slightly higher reduction in waist circumference (0.5 ± 1 cm) (Heilbronn et al., 1999), compared to women. Sex differences were not statistically significant in our study, possibly due to the small sample size.

The actual weight-loss was comparable to that predicted from the daily energy deficit in 5 subjects (-0.4 ± 0.5 kg), one having exceeded it by -3.0 kg; indicating that subjects adhered well to the prescribed dietary intervention. This also confirms that the observed weight loss of ± 5 kg was induced by the dietary restriction given that activity levels differed among participants throughout the study. The greater weight loss in the first patient from the current study is related to the antihypertensive medication (Coversyl plus, is a combination of hydrochlorothiazide and ACE) that he was taking and was only stopped at his fourth week of energy-restriction. The hydrochlorothiazide component of this medication, a class of diuretics, reduces fluid and sodium reabsorption by the kidneys, resulting in increased urine output (Davis et al., 1992). One of the serious side effects of diuretics is excessive loss of body water, leading to dehydration. When measuring LBM with DXA, changes in lean mass are not distinguished from those of water loss. Given the fact that LBM is 73% water (Franssila-Kallunki et al., 1992), water loss may be mistaken for a loss of lean tissue. In fact, when the estimated excess of water loss was taken into account, the rate of weight loss of this subject did not differ from that of the mean of the group. Previous studies have documented the effect of medications combined with energy restriction on weight changes. A study by Davies et al. (1989) noted that diuretics' effects on weight change were most pronounced in subjects assigned to a weight loss diet (i.e.VLED, 780 kcal/d) compared to a low sodium-high potassium diet, over 8 weeks or compared to beta-blockers, another type of anti-hypertensive medications. The reduction in LBM was attributed to increased diuresis and reduction in total body water.

### 7.1.2. Cardiometabolic outcome and glycemic control

#### 7.1.2.1. Diabetes outcome

At 5 weeks of energy restriction, fasting plasma glucose (FPG) was normalized for all participants and A1C was < 6.5% in three patients and < 7.5% in the other three, with an average reduction of 1.9 ± 0.3%. The greatest decrease in FPG and A1C were seen in those with the highest baseline levels, and those whose diagnosis was less than 2 years.

Similar dietary interventions (diets of 1600 kcal/day) associated with a weight loss of 5.7% have shown improvement in glycemic control. Because energy restriction automatically leads to weight loss, it is difficult to determine which of these two factors contribute to this

improvement. However, 55% of the total reduction in FPG occurred by day 7, when only one-fifth of weight loss had been achieved. In addition, no correlation was found between weight loss and the improvement in FPG, which supports studies that have shown that energy restriction improves glycemic control independent of weight loss (Henry et al., 19852; Heilbron et al., 1999).

The reduction in FPG may be explained by the glucose/NEFA cycle. High NEFA concentrations are associated with a decrease in glucose uptake and oxidation via inhibition of pyruvate dehydrogenase and phosphofructokinase (Heilbronn et al., 1999). Previous weight loss trials have shown that a reduction in fat mass is related to a reduction in circulating NEFA, and improved insulin sensitivity (Heilbronn et al., 1999). However in our study fasting NEFA were still elevated at 5 weeks possibly because of the ongoing energy-deficit state. A study by Wolever et al. (2008) found that lower postprandial glucose levels were associated with a low-GI diet. Hence, the high fibre and low-GI diet that was advocated in this study, may have also contributed in lowering A1C.

Comparable nutritional interventions using meal replacements have shown similar reductions in A1C at 12 weeks, of 0.8 ± 1.1% reductions (Yip et al., 2008) and 1.2 % ± 0.8% (Ditschuneit et al., 1999). On the other hand, assigning subjects to either a low-fat-diet (≤ 30% from fat) (Barbara et al., 2002; Gulbrand et al., 2002; May, 2008) or a high-protein-diet (28 - 30% energy from protein) (Brinkworth et al., 2004; Krebs et al., 2012) was associated with 1 - 3% reduction in A1C at 1 year. Furthermore, intensive lifestyle intervention, which consisted of a combination of dietary plans, meal replacements and educational sessions reduced A1C by 6.7% at 1 year (Pi-Sunyer, et al., 2007). Another study has shown a 3% decrease in A1C with the Mediterranean lifestyle program at 24 weeks (Toobert, et al., 2003). Hence, the significant improvement in A1C level reported in the current study is attributed to the high adherence to the dietary plan with meal replacements and the provided ready-to-eat-meals replicating the Mediterranean style diet (low saturated and high in MUFA, high in fibre and low in GI). Since activity levels were not markedly different from week 1, it suggests that physical activity contributed little to total weight loss in this study, albeit it may have favoured maintenance of LBM.

The current study is in line with others, which reported that weight loss is associated with improved insulin sensitivity of glucose (Savage et al., 1979; Gumbiner et al., 1990) in

obese patients with T2DM, based on HOMA-IR, a surrogate for insulin resistance (Gougeon et al., 2008). In this study, glucose and insulin concentrations were significantly reduced, such that HOMA-IR decreased by 54% at 5 weeks. However, measured with the hyperinsulinemic clamp, no improvement in insulin sensitivity was found, in a setting where energy restriction was ongoing.

#### 7.1.2.2. Blood pressure

Blood pressure was significantly decreased and normalized in all subjects. Anti-hypertensive medications were discontinued for n=2 and the dose was reduced for n= 1. This is consistent with other findings which reported reduction in blood pressure during weight loss in subjects with (Reisin et al., 1978; Howell, 1982; Andersson et al., 1984) or without hypertension (Rocchini et al., 1989) and independently of salt intake (Tuck et al., 1981).

Comparable improvements were shown with weight reduction in obese T2DM, using meal replacements (Ditschuneit et al., 1999, Yip et al., 2001), low salt diet (~ 1500 mg) (Toobert, et al., 2003; Pi-Sunyer, et al., 2007) or a low fat / high fibre diet (DASH diet), with high consumption of fruits, vegetables and whole grains and low in total and saturated fat) (Puska et al., 1983; Modan et al., 1985; He & Whelton, 1999; Streppel et al., 2005; Whelton et al., 2005). Hence, recommending participants to reduce their salt intake and providing prepared meals low in sodium and elevated in potassium, calcium and phosphorus, may have contributed to the reduction in blood pressure in the current study, in addition to the effect of weight loss. Other studies have shown similar reduction in blood pressure with increased physical activity, even at low intensity levels (e.g., exercise at less than 45% of maximum aerobic power) (Bjorntorp, 1982; Warburton et al., 2006). Participants from the present study were encouraged to reduce their sedentary time. Based on participants' activity journals, it was noted that they did not increase or change their time spent on moderate to high intensity activty levels, such that the contribution of physical activty in reducting blood pressure was minor in this study.

A common characteristic to diabetes, obesity and hypertension is the insulin-resistant condition identified by fasting hyperinsulinemia (Modan et al., 1985; Rocchini et al., 1989). Elevated concentrations of insulin were observed in both hypertensive obese (Lucas et al., 1985; Manicardi et al., 1986) and non obese subjects (Rose et al., 1986; Ferrannini et al.,

1987). In addition, abnormal cell membrane cation transport was documented with elevated intracellular sodium and reduced potassium levels in T2DM (Resh et al., 1982; Moore, 1983). This was not the case of participants from this study, since they all had normal potassium levels at baseline, and their blood pressure was controlled with anti-hypertensive medications (n = 4). Hyperinsulinemia has been ascribed a pathogenetic role in hypertension at the cellular, vascular and tissue level. Given the regulatory role of insulin in cell membrane cation transport, insulin resistance is associated with abnormal cellular sodium efflux and potassium influx (Modan et al., 1985; Klimes, et al., 1989) which results in increased intracellular sodium concentration (DeFronzo, 1981; Hall, 1993). Thus, even though potassium levels did not change from baseline, improvement in insulin concentration may still explain the reduction in blood pressure, regardless of the level. The improvement in blood pressure is thought to be dependent on the magnitude of change in insulin resistance (Rocchini et al., 1989; Landsberg, 1992; Steinberger & Daniels, 2003).

Another line of evidence that supports the role of insulin in hypertension concerns the kidneys, whereby, insulin reduces sodium excretion by enhancing sodium reabsorption by the tubular. This was documented under normal physiological conditions in healthy individuals and in insulin-dependent diabetic subjects (Atchley et al., 1933; Miller et al., 1954). Therefore, the hyperinsulinemia-mediated anti-natriuretic effect which increases renal sodium retention and extracellular fluid volume expansion may have been alleviated by the reduction in insulin levels reported at 5 weeks (Hall et al., 1993). Insulin also acts on the renin-angiotensin system (RAS), by stimulating renin (the extracellular volume regulator, released by kidney cells in response to low sodium levels or low blood volume). Hyperinsulinemia has also been associated with increased renin levels in obese T2DM subjects by overactivating the RAS, which contributes to the pathogenesis of hypertension (Kalupahana & Moustaid-Moussa, 2012; Underwood, et al., 2013). Weight loss-related reduction in plasma insulin levels, has been associated with a reduction of plasma renin levels (Tuck et al., 1981; Kimura et al., 1987). This may have also occurred in the current study but was not measured.

Another mechanism by which insulin can potentially elevate blood pressure is by activating the sympathetic nervous system, to enhance norepinephrine secretion (Hall et al., 1993). Norepinephrine is a potent vasoconstrictor which acts on the kidneys, to reduce the

blood flow and increase sodium reabsorption (Rose et al., 2000). Epidemiological studies have shown that weight loss was associated with a reduced sympathetic nervous system activity and norepinephrine; however, this was not tested in the current study.

#### 7.1.2.3. Lipoproteins

TG, TC and LDL were significantly reduced at 5 weeks, and HDL was maintained at baseline levels. The greatest reductions in lipids levels occurred in those with the highest baseline levels. The reduction in fasting insulin and improved glycemic control at 5 weeks may explain the improvements in the lipid profile. The resistance to the action of insulin on lipoprotein lipase in peripheral tissues (enzyme in charge of lipolysis) contributes to high levels of LDL and TG (Grundy, 1990). Additionally, hyperinsulinemia enhances hepatic very-low-density-lipoprotein (VLDL) synthesis, the precursors of LDL and TG, and may directly contribute to their increased plasma concentrations (Grundy, 1990).

Trials using meal replacements in T2DM were less stringent in lipid profile improvements, with a reduction of 3% in TC and 9% in TG, 7.5% increase in HDL level and 9% in LDL at 34 weeks (Cheskin et al., 2008). In contrast with our study, Yip et al. (2001) reported lower improvements in lipid profile at 12 weeks, following a low fat diet with one meal replacement per day or an exchanged dietary plan based on ADA recommendations: 7% vs. 4% reduction in TC, 7% vs. 6% in LDL, 14% vs. 8% in TG, and 3% vs. 4% in HDL levels, with no difference between the two groups.

Similar low fat dietary intervention plans (< 30% from fat and 28% from protein) have reported comparable findings at 8 weeks, with a reduction in TC by 16%, LDL by 3%, TG by 23% without altering HDL levels (Parker et al., 2008). Another dietary-plan based study by Heilbronn, et al. (1999) evaluated the type of dietary fat on lipid profile. In line with our results, the low fat/high MUFA (monounsaturated fat) diet (i.e. 30% from fat and 49% from CHO) induced higher reductions in LDL (17% vs. 10%), TC (22% vs. 10%) and TG (32% vs. 8%) and a lower reduction in HDL (1.2% vs. 11%) levels compared to the low fat diet (9% from fat and 70% from CHO) at 12 weeks (Heilbronn, et al., 1999). The reduction in TG levels in the lower fat group may be due to the lower CHO and saturated fat intake, also seen in studies using low CHO diets (mean CHO 4-45%) (Kirk et al, 2008) or low refined CHO diets (kodama et al., 2009) in T2DM. In fact, high saturated fat diet and refined CHO

trigger the overproduction of VLDL-TG and LDL by the liver and reduce the activity of LDL receptors, causing lower LDL clearance from the circulation (Grundy, 1990). Hence, the reduction in LDL and TG from our study is most likely attributed to the low saturated fat intake.

High MUFA diet is another mean to TG reduction, via enhanced clearance of VLDL and LDL by the liver (Grundy, 1990). High MUFA consumption was not emphasized in our study, but the reduction in total fat consumption was; participants were not recommended to consume nuts and seeds and were asked to limit olive oil consumption to 2 tablespoons per day, as recommended by Canada's Food Guide (Health Canada, 2011).

Lifestyle intervention studies were also able to demonstrate similar improvements in the lipid profile. Results from the Look Ahead study (Pi-Sunyer, et al., 2007) showed a 4% increase in HDL, 11% reduction in LDL and 25% in TG levels at 1 year of an intensive lifestyle intervention compared to a structured educational intervention.The reduction in LDL and increase in HDL levels were witnessed by by Järvi et al. (1999), when assigning subjects to a low-GI energy-restricted diet (i.e. 16% calories from protein, 28% from fat, and 55% carbohydrate) for 3 weeks. Low-GI diet was also emphasized in this study, and the impact on increasing HDL levels might have been with longer duration of the diet. The reduction effect of low-GI on LDL may be attributed to the high fibre content (De Natale et al., 2009), since a high fibre diet is associated with reduced dietary cholesterol absorption in the intestines (Brown et al., 1999). In this study, elevated dietary fibre was related with the high consumption of vegetables as well as low GI food in the provided meals. Another important factor that contributed to the improvement in lipid profile may be related to the Mediterranean and DASH diets (Psaltopoulou et al., 2004; Esposito et al., 2010), which were also reproduced in the food choices of the provided meals. The reduction in TG, LDL and TC and the increase the HDL levels, may be explained by the fact that these two dietary patterns advocate a low saturated and high polyunsaturated fat consumption (i.e. MUFA and omega-3) (i.e. high consumption of seeds, nuts and moderate of oils) in combination with high GI/high fibre and low refined CHO intake (i.e. high intake of fruits and vegetables and whole-grain) (Azadbakht et al., 2011; Ajala et al., 2013).

Evidence-based nutrition practice guidelines for dyslipidemia (Academy of Nutrition and Dietetics, 2013) promote a cardio-protective eating pattern which consists of 25 - 30% of

calories from fat, with < 7% from saturated and trans fatty acid, and the majority of dietary fat from unsaturated sources. Ideally, cholesterol intake should not exceed 200 mg/d. As evidenced by clinical trials, this eating pattern may reduce TC by 7 - 21%, LDL by 7 - 22% and TG by 11 - 31%. Our results support these recommendations. Hence the improvements in the lipid profile were attributed to the dietary intervention and to the improvement in diabetes outcome (i.e. lower fasting insulin and glycemia levels).

#### 7.1.2.4. C-reactive protein

Fasting C-reactive protein (CRP), an acute-phase protein secreted in response to inflammatory conditions (Nesto, 2003; Lambert et al., 2004; Berg et al., 2005), was significantly lowered at 5 weeks (0.85 ± 0.09 mg/L) from elevated baseline levels.

High plasma levels co-exist with high adiposity and insulin resistance, in obese and hyperinsulinemic individuals (Festa et al., 2001; Lambert et al., 2004; McLaughlin et al., 2009). Lambert et al. (2010) found that one standard deviation increase in BMI was associated with a 52% increase in CRP concentrations. This association is also significant with other adiposity measures, including waist and hip circumferences and total body fat (TBF) (Greenfield et al., 2004). Even though, there was a decrease in CRP levels and body fat in all 6 subjects, there was nosignificant correlation between the two.

The contributory mechanism interplaying obesity-related inflammation and high CRP levels, is attributed to pro-inflammatory cytokines (i.e. interleukin 6, IL-6, IL-1 and tumor necrosis factor-α, TNFα) released by adipose tissue (Clement et al., 2004; Berg et al., 2005; Compher & Badellino, 2008; Després et al., 2008; Moschen et al., 2010). Weight loss reduces the inflammatory state by different means, including diminished gene expression of pro-inflammatory markers in adipose tissue and monocytes and reduced stimulation of immune cells mediating CRP secretion (Kopp et al., 2003; Clement et al., 2004; Viardot et al., 2010). A 6% weight reduction achieved either by a 12-week caloric restriction (900 - 1400 kcal/d) or 28 days of very low calorie diet (i.e. 800 kcal/d) (Clement et al., 2004) was associated with 35 - 45% reduction in inflammatory markers release (i.e. IL-6). Although those pro-inflammatory markers were not measured in our study, the reduction in CRP levels implies a reduction in them as well. A systematic review presenting the impact of different lifestyle interventions (dietary and /or physical activity) on CRP levels reported comparable reduction

in CRP levels and weight loss to our study, whereby, for each 1 kg of weight loss, the mean change in CRP level was -0.13 mg/L (Selvin et al., 2007).

Evidence-based studies show a positive relationship between CRP concentrations and fasting insulin concentrations, which tend to decrease with weight loss. McLaughlin,et al. (2002) compared the impact of a 3-month caloric restricted diet (providing 40% of the estimated daily caloric need) in 38 obese insulin resistant or non-resistant women and found that CRP concentrations fell in parallel with day-long integrated plasma insulin concentration but only in the insulin resistant group. Our results are comparable to those reported by McLaughlin et al. (2002) showing 27% decreases in fasting plasma glucose and 31% in CRP concentrations in the insulin resistant group. Other studies have shown that variations in CRP are modulated by changes in insulin resistance and or/compensatory hyperinsulinemia, independently of obesity (McLaughlin, et al. 2002; Pradhan et al., 2003; Compher & Badellino, 2008; Lambert et al., 2010). Given the small sample size of this study, no significant associations were observed between fasting levels of CRP, insulin and glucose, and which may also be asociated with difference in baseline levels within the group.

Another factor contributing to high CRP levels is elevated blood glucose levels. Hyperglycemia has been shown to trigger the release of inflammatory cytokines from monocytes (a type of leukocyte contributing to the body's immune system) (Devaraj et al., 2005). This approach was interpreted by exposing endothelial cells to high glucose concentrations, which induced an increase in oxidative stress and apoptosis (Risso et al., 2001), and in turn, pro-inflammatory responses and greater release of CRP (Leiter et al., 2005). Thus, the reduction in CRP in our study is also attributed to the lower fasting glucose levels at 5 weeks.

The effect of weight loss on reducing CRP levels is used to measure improvements in cardiovascular morbidities implicated in the atherosclerotic process and is therefore a significant metabolic outcome of this study (Brinkworth et al., 2004).

## 7.2. Substrates, hormones REE and RQ

### 7.2.1. C-peptide and insulin

C-peptide concentrations were significantly reduced from fasting baseline levels and more so in response to hyperinsulinemia, at 5 weeks of energy restriction. Simultaneous

measurement of insulin and C-peptide concentrations in peripheral blood is commonly used to assess both β-cell secretion of insulin and hepatic insulin extraction. This approach is based on the fact that C-peptide is co-secreted with insulin in an equimolar ratio and has a constant peripheral clearance (Waldhäusl, et al., 1979; Polonsky et al., 1984; Van Cauter et al., 1992; Wauters et al., 2003). Unlike the liver being the major site of insulin extraction, the extraction of C-peptide by the liver is negligible. Conequently, the ratio of C-peptide (I:CP) to insulin is an estimate of hepatic insulin extraction and indirectly hepatic sensitivity to the action of insulin (Wauters et al., 2003). Given the fact that both fasting C-peptide and insulin concentrations were parallely reduced, I:CP ratio was not different from Pre-intervention. This suggests that hepatic clearance did not differ (Slabber et al., 1994).

Similar dietary interventions as the current study have reported comparable decline in C-peptide concentrations in overweight and obese subjects, under an energy restricted diet with low-glycemic load (i.e. 43% CHO, 30% fat) (Pereira et al., 2004). Comparable reductions in fasting levels of insulin and C-peptide levels were documented with 11% weight reduction at 3 weeks of a very low calorie diet, suggesting increased insulin secretion. The reduction in C-peptide in response to high insulin levels was suggested to be associated with an increased hepatic clearance (Jimenez et al., 1987; Hale et al., 1988; Gama & Marks, 1989; Svendsen et al., 2012).

It has been previously shown that reduction in fat mass is positively associated with reduction in C-peptide levels, which may be secondary to improvement in insulin resistance (Pouliot et al., 1994). We did not find any significant association between delta fat mass and delta C-peptide or delta insulin levels, given the large variation within subjects and the small sample size.

### 7.2.2. Glucagon

We found a significant reduction in glucagon concentrations at both fasting and during the hyperinsulinemic clamp at 5 weeks of energy-restriction compared to baseline, but no difference in the response to the clamp. This implies that there was no change in the suppression of glucagon secretion in response to high insulin levels. In response to the clamp, there was a borderline significant effect on the glucagon-to-insulin ratio (G/I) ($p$ = 0.05), which is due to improvements in fasting insulin and glucagon concentrations.Therefore, the

index of tissue sensitivity to insulin, referred by the G/I ratio (DeFronzo et al., 1979) did not improve in response to high insulin levels.

Previous observations in T2DM subjects, documented that glucagon suppression was blunted (Aronoff et al., 1977) and fasting glucagon levels were two times higher in obese subjects and those with T2DM compared to a control group (Iannello et al., 1997). The reduction in fasting glucagon, resulting from the intervention, is correlated with the significant improvement in fasting insulin concentration and the increase in MCR, as well as with the improvement in insulin resistance based on HOMA-IR measurements. The lower glucagon level contributed to the normalization of fasting glucose levels reported at 5 weeks. A 4 weeks of a very low calorie diet (i.e. 500 kcal including 50 g CHO) assigned to obese subjects with or without T2DM (Savage et al., 1979), reported comparable reduction in fasting plasma glucose and glucagon levels to this study.

Improvement in hormones concentrations in response to dietary interventions are also assessed by measuring the magnitude of changes in gut hormones. Under normal physiological conditions, a reduction in glucagon is secondary to the increase in GLP-1, which is a gut hormone that inhibits glucagon release (Drucker, 2002). Therefore, it would have been interesting to measure gut hormones in order to determine their effect on substrates and glucose regulatory hormones (i.e. insulin and glucagon).

### 7.2.3. REE, respiratory quotient (RQ) and NEFA

Consistent with other findings (Bessard et al., 1983; Baba et al., 1999; Leibel et al., 1995), the decrease in REE at 5 weeks was no longer significant when expressed per kg of LBM or kg of body weight, explained by the small change in body composition (i.e. unchanged LBM and mobilized fat mass) (Piatti et al., 1994; Leibel et al., 1995; Good paster et al., 2003). This decrease in REE, however if maintained would be equivalent to less than 400 g of weight gain per month during an isoenergetic diet. A positive association was found between the magnitude in sensation of hunger and the reduction in REE during weight loss (Leibel et al., 1995; Westerterp-Plantenga et al., 2009). Participants from the current study did not report any change in their sensation of hunger. Some studies reported that diet composition has an effect on REE during weight-loss, whereby, REE was markedly lowered with a low-fat high protein (>36 % of energy from protein) (Piatti et al., 1994; Baba et al.,

1999) and a low-glycemic load diets (Pereira et al., 2004). Other studies conducted also in obese T2DM subjects found no effect of diet composition on REE, when comparing a protein intake of 27 % versus 16 %, and suggested that REE reduction was secondary to energy restriction rather than the macronutrient composition of the diet (Luscomber et al., 2002; Luscomber et al., 2003).

Higher fasting RQ at baseline and blunted increase in RQ during a hyperinsulinemic clamp was also reported by other studies that compared T2DM subjects with insulin-sensitive subjects (Galgani et al., 2008). The reduction of fasting RQ at 5 weeks, illustrates an interventional effect of the diet and /or the weight loss. This reduction corresponded to a greater fat oxidation, and suggests that more fat is used as substrate. Findings from this study complement the knowledge of reduced substrates availability secondary to the energy-deficit state, wherein the body adapts by using the energy in a more efficient manner; a process referred to as neuroendocrinological adaptation (Chan et al., 2003).

Elevated plasma nonesterfied fatty acids (NEFA) at baseline, possibly a consequence of impaired insulin-related suppression of lipolysis (Lannello et al., 1998), were not changed at 5 weeks. The absence of change in NEFA, also seen during a 4-week VLED intervention in T2DM (Gougeon et al., 1994), suggests that the increase in lipolysis is sustained, but in a setting where the use of fat as fuel increased as indicated by a decline in RQ. In response to the clamp high insulin levels, NEFA suppression rate was maintained to the same extent as Pre-intervention. By contrast with other studies that showed strong association between changes in insulin sensitivity (based on HOMA-IR) and NEFA levels ($p < 0.01$) in 60 obese resistant women (Esposito & al., 2003), this study was not able to report such correlations at 5 weeks, even with greater improvements in HOMA-IR and fasting insulin levels; which may be due to the small sample size and to the fact that particpants were in an energy deficit state.

## 7.3. Leucine and glucose kinetics

### 7.3.1. The postabsorptive state

#### 7.3.1.1. Leucine kinetics

This is the first study that attempted to assess concurrently the impact of improved fasting glycemia and insulin, secondary to energy restriction and weight loss on the kinetics of protein and glucose metabolism in T2DM. Postabsorptive whole-body protein turnover

and rates of oxidation decreased, resulting in a lesser negative net balance. BCAA concentrations did not change from Pre-intervention.

Whole body leucine kinetic results were retained for n = 5. The first participant had to be excluded from the analysis, for the following reason. He was originally consuming a low protein diet (based on his 24-hour recall), and the higher protein intake resulted in a positive nitrogen balance during his first admission and lower whole-body protein turnover rates compared to the results of the other 5 participants. During his second admission at 5 weeks of intervention, his nitrogen balance as per the other participants was at equilibrium, but his protein turnover rates had increased compared to the others in whom it had decreased. Therefore, to ensure abundant protein intake at baseline, all subjects were selected according to a protein intake of 15% of energy and were provided with a high protein meal replacement formula, for 5 days before entering the study.

In this study, the postabsorptive leucine kinetics measured prior to energy restriction are comparable to those reported by Pereira et al. (2008) in poorly controlled T2DM, where leucine flux positively correlated with fasting insulin levels. Other studies done in moderately hyperglycemic obese subjects with T2DM, have also shown accelerated protein flux and higher breakdown using 24h fed-fasted $^{15}$N-glycine method (Gougeon et al., 1994; Gougeon et al., 1997; Gougeon et al., 2008). At 5 weeks of energy restriction, fasting glucose and insulin decreased, as well as, all parameters of leucine kinetics, consistent with previous studies where a better glucose control by medications and/or dietary intervention improved protein metabolism (Gougeon et al., 1994; Gougeon et al., 1997; Gougeon et al., 1998;). When glycemia was normalized with exogenous insulin (Gougeon et al., 1997; Gougeon et al., 1998), or after following 28-days of a VLDL (i.e. 410 kcal with 93 g of protein per day) (Gougeon et al., 1994), an improvement in protein kinetics was documented (i.e. lower flux, breakdown and synthesis), towards values approaching those of the nondiabetic group. The significant reduction of breakdown levels (~ 12%) and to a lower extent of synthesis (~ 6%) and that of oxidation, were translated by a 11 μmol/min less negative net balance. This value was extrapolated over 10 hours of fasting and which represents a sparing of 11 g of body protein. Hence, the reduction in flux, a measure of protein breakdown, in presence of lower fasting insulin and normalized fasting glucose suggests an improvement in the insulin sensitivity of protein metabolism, secondary to the dietary intervention and weight loss.

Significant decreases from isoenergic feeding in protein synthesis were also documented by other energy-restricted intervention, including 4 weeks of VLED (Gougeon et al., 1994) and low energy diet (50% of their maintenance energy with 1.5 g/kg/d protein) (Gougeon et al., 2000), both tested with the 60-h oral $^{15}$N-glycine method. These studies illustrate another possible explanation of lower leucine kinetics at the postabsorptive state. A physiological adaptation to reduced caloric intake may have occurred, as proposed by Umpleby et al. (1990), where the body adapts to use proteins more efficiently. By reducing the turnover, proteins are spared (i.e. lower breakdown and lower oxidation) and energy-requiring processes are reduced (i.e. decreased protein synthesis). The latter is supported by a significant reduction in REE not completely explained by changes in body composition and LBM. Confirmedly, decreased RQ at 5 weeks indicates a higher utilization of fat as substrate. Given that nitrogen equilibrium was maintained throughout the 5 weeks, suggests that appropriate protein intake was provided to maintain LBM, while selectively mobilizing fat. This approach was suggested by Gougeon et al. (2000), reporting maintenance of LBM with 5 - 6% weight loss at 28 weeks of low energy diet (50% of their maintenance energy with 1.5 g/kg/d protein). The even distribution of proteins throughout the three meals and one snack may have also contributed to maintaining nitrogen balance at equilibrium despite energy restriction (Paddon-Jones et al., 2010).

As previously reported by Gougeon et al. (2000), elevated protein flux was positively associated with greater visceral adiposity, REE, and insulin resistance of glucose (Gougeon et al., 2000). Given the reduction in flux, HOMA-IR, REE and fasting plasma glucose noted in this study, no significant correlation was found, possibly due to the small sample size.

#### 7.3.1.2. Glucose kinetics

Postabsorptive EGP measured prior to energy restriction is comparable to those reported by Umpleby et al. (1999) on T2DM. At 5 weeks, EGP and Rd were decreased (decreased glucose turnover) and MCR was significantly increased. This is mainly attributed to weight-loss and to the lower fasting glycemia.

The hyperglycemia in T2DM has been attributed to enhanced rate and output of EGP (Consoli et al., 1989; Boden et al., 2001); this may suggest that the lower fasting blood glucose at 5 weeks is secondary to lower EGP. Consoli et al. (1987) used a $^{3}$H-glucose and a

$^{14}$C-acetate tracer methodology to determine EGP and gluconeogenic rates, and reported higher levels in T2DM compared to the lean group. The authors concluded that fasting hyperglycemia was due to accelerated gluconeogenesis, induced by increased substrate availability (high levels of amino acids in T2DM). Based on these findings, the ongoing hypocaloric state may have contributed to the lower substrate availability for endogenous Ra, which resulted in lower fasting glucose levels.

### 7.3.2. Hyperinsulinemic isoglycemic isoaminoacidemic clamp

#### 7.3.2.1. Leucine kinetics

The response to the hyperinsulinemic clamp did not differ from that of Pre-intervention, whereby, leucine flux increased, protein breakdown was suppressed, while oxidation and synthesis rate did not change from postabsorptive, resulting in no improvement in net anabolism. Also, amino acid infusion rate was not different from Pre-intervention. These findings are consistent with observations made by Pereira et al. (2008) in poorly controlled T2DM. It should be noted that subjects are still in an energy-deficit mode when studied at 5 weeks, which may explain why synthesis did not improve and neither did the anabolic response. Results from the present study, showing no change in response to insulin differ from those of the fed-fasted 24h state (Gougeon et al., 1997; Gougeon et al., 1998). The latter was associated with a decrease in breakdown greater than in synthesis, leading to an increase in net anabolism, when glycemia was normalized by intensive insulin therapy.

Under normal physiological conditions, oxidation is suppressed by insulin while net anabolism is increased (Adegoke et al., 2009). In the current study, oxidation was increased as was net anabolism, and this was because amino acids were infused to maintain postabsorptive concentrations. Compared to the Pre-intervention hyperinsulinemic state, there were no differences in the increase in oxidation levels or that of net anabolism. The latter which remained negative, did not become positive as has been observed in non-diabetic healthy subjects after the intervention despite improvements in other markers of insulin resistance. When conducting the same protocol in healthy subjects, there was an increase in flux and synthesis, decrease in breakdown and reverse in net balance without altered oxidation (Adegoke et al., 2009). In the current study, the absence of an increase in synthesis appears to be the best marker of insulin resistance of protein metabolism.

#### 7.3.2.2. Glucose kinetics

Despite the improvement in glycemia and the 51% decrease in HOMA-IR, glucose Rd in response to the hyperinsulinemic clamp was not significantly different from that of Pre-intervention, nor was insulin-mediated suppression of EGP. However, MCR was significantly higher by ~ 51%. Both differed from what is observed in normal subjects using the similar clamp approach (Olasunkanmi et al., 2009).The lack of insulin-induced changes in Ra and Rd are consistent with observations made by Pereira et al. (2008) on uncontrolled T2DM; suggesting that participants from our study remain insulin resistant, despite normalized glycemic levels. Hence, the infused amino acids are possibly compensating for the lower availability of glucose as substrate for oxidation, as well as the lower availability of amino acids from catabolism at both postabsorptive and in response to hyperinsulinemia. Given the fact that the same amino acids were infused with a lower fasting glucose and insulin, at 5 weeks and that Ra and Rd were not different from Pre-intervention clamp, MCR increased. This may suggest that the body is using the infused substrates more efficiently. Although postabsorptive glucose and protein metabolism improved, the sensitivity of protein metabolism to insulin did not, but was not deteriorated. This may potentially be reversed by a longer duration of energy-deficit or observed soon after during weight and euglycemic maintenance state.

## 7.4. Strengths and Limitations

The validated clamp and tracer techniques are considered the gold standard technique for measuring insulin sensitivity of protein and glucose metabolism *(*DeFronzo et al., 1979; Chevalier et al., 2004; Legro, Castracane & Kauffman, 2004). Each participant acted as his/her own control, meaning studied at their own glycemic level. This was done in order to mimic the real world situation. Furthermore, minimizing confounders was also done to limit discrepancy of the results, such as smoking, coffee consumption, and other factors considered in the exclusion criteria. Providing a high-protein formula before the onset of the study was done to ensure that protein intake would be at 15% of energy and not differ during energy restriction. A higher certainty of adherence to the protocol was allowed by admitting participants twice to the hospital, which also helped them to adapt at a faster rate to the energy-restricted diet. For empowering adherence to diet at home, participants were also

provided with detailed dietary plans, liquid formula to replace both a meal and a snack, ready-to-heat frozen vacuumed meals for supper and key food items to minimize variations in dietary food choices (i.e. for the cereal brand). Furthermore, weekly assessments helped participants track their progress and stay motivated and more importantly allowed for close monitoring of their dietary compliance and diabetes control. According to Savage et al. (1979), a period of at least 4 weeks was needed to maintain a stable fasting glucose in obese T2DM subjects, using a very low calorie diet (i.e. 500kcal/d). However, in the current study, glycemic normalization was noted with less stringent energy restriction.

This is a pilot study, thus the statistical power was small and may not be sufficient to observe differences in some parameters and undergo associations and regression analyses between changes in diabetes-related parameters, anthropometrics and kinetic results. Out of these 6 participants, one was excluded from body composition data analysis, for two reasons, because he was taking a diuretic, which increased his total weight loss beyond the predicted and overestimated the loss of LBM. The medication should have been stopped a week prior to the first admission. He was also excluded from the protein kinetics quantification due to a low protein diet at baseline that may have contributed to reversal in kinetics and exaggeration of his results and lean tissue loss comparing to the other participants. Recruitment was difficult because of the length and invasive nature of the study as well as the stringent inclusion criteria especially for persons who are morbidly obese (BMI > 30 $kg/m^2$). One possible option would have been not to mandate the biopsies on the experiment day.

Although the liquid meal replacement Ensure® was necessary to ensure adequate energy and protein intake of the isoenergetic diet, it is still considered to be rich in simple sugars (18g per 250 ml) and thus may have contributed to hyperglycemia prior to the initiation of the energy restricted regimen. However, the usage of Ensure® was important in order to allow for comparison with previous studies undertaken in the same laboratory. Another limitation to our results was due to the fact that not all vacuumed ready-to-eat meals were standardized (i.e. some vary in weight), which may have engendered some variation in the estimation of subjects' overall intake, but still maintained an energy deficit. One possible limitation is related to the nitrogen balance data, which could have been underestimated since the urine collection was done for 3 days on the first admission and less than 6 days for all

participants on the second admission. This may have been prevented by asking subjects to start their urine collection at home 4 days prior to admission.

The significance of the results can be improved by a larger sample size, which may had allowed assessing a sex effect. Adding a control group, such as an elderly T2DM group can be interesting as it provides more insight on protein kinetics to study the impact of the hypocaloric diet with sufficient dietary proteins on both age and obesity related sarcopenia (Apovian, 2010; Li & Heber, 2012). A control group of non or pre-diabetic obese adults would have also permitted comparisons in improvements in early onset of insulin resistance in relation to protein and glucose kinetics with the protocol studied (Savaga et al., 1979). One last comment that is worth mentioning is in relation to the length of the dietary intervention on improving kinetics of protein metabolism. A longer intervention period may be necessary to reverse insulin resistance of protein metabolism, measured by the gold standard. Additionnally, the improvement in protein anabolism in T2DM may potentially be seen after weight-loss, during an isoenergetic weight-maintening diet.

# VIII. Conclusion

The provision of 60% of energy needs with maintained typical abundant dietary protein, conserved muscle mass and mobilized fat mass in obese subjects with T2DM. This nutritional therapy was associated with clinically significant improvements in cardiovascular risk factors and glycemic control. Improvements were maintained at 3 months post-study and were accompanied with additional weight loss, which suggest promising long-term benefits and greater self-management empowerment following this intervention. During energy-deficit, postabsorptive glucose and protein metabolism improved at 5 weeks, but insulin sensitivity of protein metabolism did not, but was not deteriorated. Thus, abundant protein appears to preserve body proteins in the face of energy deficit and insulin resistance, and may be considered in the management of sarcopenic obesity.

After a thorough literature search, this study is the first to measure insulin sensitivity of protein and glucose metabolism during energy restriction in T2DM using the gold standard of methodologies, i.e. the hyperinsulinemic isoglycemic isoaminoacidemic clamp. In light of the current findings, more research is needed to convey understanding of the cellular mechanisms implicated in mixed muscle protein synthesis in T2DM. Assessing insulin sensitivity during an isoenergetic weight-maintaining diet post weight-loss is to be determined. Other interventions may be more beneficial in improving insulin sensitivty of protein anabolism, such as physical activity (Snowling & Hopkins, 2006; Gordon et al., 2009; Bassil & Gougeon, 2013). Regular exercise has been shown to induce an increase in muscle mass and strength along with a reduction in body fat in T2DM subjects. Bassil & Gougeon (2013) suggested that the effect of physical activity on improving muscle wasting in T2DM is endorsed by an improvement in blood flow and insulin responsiveness. Exercice training is also associated with a more favourable skeletal muscle fibre type composition (Simoneau et al., 1985), a determinant of the action of insulin (Hickey et al., 1995; Tanner et al., 2002).

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# Appendix 1. Ethics approval



**Revision To An Approved Study**

| Review in progress | |
|---|---|
| Study ID: | 817 |
| Study ref number | BMA-05-024 |
| MUHC Principal Investigator: | Rejeanne Gougeon |
| Study title: | Insulin Sensitivity of Protein in Type 2 Diabetes Mellitus |
| Current study status: | Approved |
| Report status: | Review in progress |
| Reference Number | RAS-817-2139 |
| Revision number | 4 |
| The proposed revision takes effect as of: | 04/23/2012 |
| What is the proposed revision type(s) | Amendment/Study modification |
| Indicate the study documents(s) affected by this revision: | - Protocol<br>- Consent/Assent document(s)<br>- Advertisement material |

If required, provide any comments about the proposed revision:

In T2 diabetes with high protein intake, normalizing hyperglycemia with medication did not improve the blunted protein anabolic response to insulin. Low protein intake in hyperglycemic T2D did not worsen that blunted response although protein synthesis was lower during a simulated fed state. So we opted not to study low protein diets in normoglycemic diabetic persons. Rather we chose to test whether euglycemia following fat loss through a 5-week low energy, high protein diet improve insulin sensitivity of glucose and protein metabolism. My study is funded by CIHR until 2013. Last year I had to submit a progress report in which I suggested this change in protocol and specific objective and it was approved. Therefore, we submit this amendment to test a low caloric diet in obese T2D persons (n=20) using the same tracer methodology during postabsorptive and hyperinsulinemic clamps with muscle biopsies.

Append any new study documents (with changes highlighted) resulting from this revision:

Saved Documents :

| Name |
|---|
| Poster_w_hikers_english[2]_revised (JR).doc |
| Poster_w_hikers-FRE[1]_revised (JR).doc |
| BMA 05 024 FORMULAIRE DE CONSENTEMENT1er mars 2012perte pondérale.doc |
| BMA 05 024 Consent Form English March 1 2012.doc |

# Appendix 2. Patient Consent Forms



**CONSENT FORM**

**McGILL UNIVERSITY HEALTH CENTRE**

**Royal Victoria Hospital: Clinical Investigation Unit**

***INSULIN SENSITIVITY***
***OF PROTEIN METABOLISM IN TYPE 2 DIABETES MELLITUS***

Your participation in this study: You have been invited by Dr. Réjeanne Gougeon to take part in a study of the effect of diabetes on how well insulin maintains your body proteins. This study takes five (5) and a half weeks. The first three (3) days and the last week of the study will be spent in the hospital.

1) Screening: To be sure the study will be safe for you and that you are a good candidate, the following will be done first. You will have an interview, a physical examination, standard blood and urine tests, chest X-ray and electrocardiogram (ECG). A suitable time will then be set for you to have your body composition and your resting metabolic rate measured. The screening tests will take about 4 hours to complete. If the tests confirm that you should enter the study, you will then be admitted to the Clinical Investigation Unit (CIU) of the Royal Victoria Hospital.

2) Special Diet: You will be asked to stop your diabetes medications for at least three (3) days prior to admission, unless your usual blood glucoses are high. For the first 3 days at the hospital (pre-weight loss intervention), you will consume the following diet: a breakfast with cereal and milk, then the rest of your food will be a liquid formula diet that contains all necessary nutrients in amounts exactly suited to your needs. Consequently, from day 4 till the end of the study, at home and during the last week at the hospital, you will consume the following weight reducing diet: a breakfast of your choice calculated by a dietitian to be adequate in calories and protein, a lunch and a snack provided as a meal replacement formula and for supper, a ready-to-cook balanced meal that we will provide. Once a week, you will be visiting the Crabtree Laboratory, to pick up the food, sufficient for the following week. It is important that you drink plenty of water while you are on the study.

3) Activity: During the whole period of the study, you will be asked to maintain your habitual activities.

4) Data Collection: You will be asked to collect all your urine in a suitable container provided for you. It is important for the study that these urine collections be carefully and completely done. You will be weighed each morning in sleep wear after passing urine. Every day, you will take your temperature and record your water intake. You will test your blood glucose four (4) to six (6) times daily. It will never be allowed to rise to very high levels. If required to keep your glucose controlled, insulin injections

Consent form
MUHC: Royal Victoria Hospital
Department of Medicine
Title: Insulin Sensitivity of Protein Metabolism
In Type 2 Diabetes Mellitus REB BMA-05-024
Version date: March 1, 2012

other catheter will be in a vein of the opposite arm. It will be attached to an intravenous bag with water and salt. This solution will carry in the test substances. Tiny, trace amounts of radioactive (tritiated) glucose and of the safe stable isotope, $^{13}$C-leucine, will be given for the duration of the test. The amount of radioactive material received will be about 250 uCi (9.25 MBq). It represents a radiation exposure similar to that received from a standard X-ray of the chest. The dose is minimal and disappears totally from the body (mainly urine) within 2 1/2 weeks of administration. Stable isotopes occur naturally and are so safe that they are even used for study in infants, children and pregnant women. You will first receive at both studies, an amount of 15 mL of tritiated glucose and 5.9 mL of labeled $^{13}$C-leucine followed by a continuous infusion of both at 0.11 mL/min. You will be asked to drink a small amount of water with sodium $^{13}$C-bicarbonate (the same stable isotope). Insulin will be infused for the last 2½ hours. As well a mixture of amino acids (protein) solution and glucose solution will be infused to ensure that your blood amino acid and glucose levels remain at levels that occur as during fasting followed by as after a meal. Blood samples of 1 mL (one-fifth of a teaspoon) will also be taken every 5 minutes, and of 12.5 mL every 30 minutes. Between 2 and 2 ½ , 4½ and 5 hrs, blood samples will increase to every 10 minutes. The total blood volume will be 420 mL, which represents a blood donation.
Your resting metabolic rate will be measured twice during the insulin study. In addition, several times during the test you will be asked to give breath samples by blowing two breaths into a tube connected to a plastic bag.

9) Muscle biopsies: The procedure used is a standard one for studies and diagnosis. It is even done in athletes, who exercise immediately after the procedure. This procedure will be done on days 4 and 39. Two (2) samples will be taken at different times, (8:30 and 12:00) from the large muscle on the outer side of your thigh. The skin and tissue under it will be anesthetized (like a dentist's "freezing") before a skin incision of 0.7 cm (about the width of a pencil) is made. A needle (hollow cylinder of 5 mm diameter) will be inserted into your muscle to remove a piece of about one tenth of a gram (the size of a small pill). Once the biopsy is obtained the skin will be held together with sterile strips of adhesive tape and a protective dressing will be applied on top of this. Firm pressure will be applied to the area for 10 minutes to prevent bruising. Because of the local anesthetic, you should feel no pain during the study.

10) Risks: The risks of muscle biopsy are considered more than minimal. You may feel some pain in the thigh following the study and for a few days, from the muscle biopsies. This should be controlled by acetaminophen (Tylenol®). There is a chance that the small scars on your skin will not disappear completely. The radiation from the DEXA and the labeled glucose is considered negligible. There is a risk of both high and low blood sugars but your blood glucose will be monitored to keep it stable to reduce the risk to minimum. The risks involved in consuming the diet and in blood sampling are considered to be minimal. You may experience some changes in your bowel movements with the diet, but special attention will be paid to minimize them. There may be slight pain or discomfort while having blood tests with a slight risk of bruising. The amount of blood drawn during the whole study will not exceed that in an ordinary blood donation. There is a small risk of dehydration if you do not drink enough fluid to match the amount the urine passed during the diet period. This will be carefully monitored by the research team. Your study will be supervised by experienced nutritionists and

Consent form
MUHC: Royal Victoria Hospital
Department of Medicine
Title: Insulin Sensitivity of Protein Metabolism
In Type 2 Diabetes Mellitus REB BMA-05-024
Version date: March 1, 2012

may be given. You will be asked to call the physician if you feel uncomfortable or your blood glucose level is over 15 mmol/L. From day 4 to the last week, you will be weighed once a week when picking up the food at the hospital.

5) Body Composition and Resting Metabolic Rate: your amounts of body fat, muscle and other tissues will be measured by several methods. 1) Bioelectrical impedance analysis. (BIA) is a safe and painless method that involves passing a weak electrical current via wires attached to your right hand and foot. 2) Four skinfold measurements are performed with a pair of calipers gently squeezing the skin of the front and back of upper arm, back and abdomen. 3) Circumference measurements are performed with a measuring tape around the arm, chest, waist, hip and thighs. 4) Dual x-ray Absorptiometry (DEXA) uses an X-ray analysis that distinguishes the different tissues (fat, bone or muscle). It involves lying down on a narrow bed for about 20 minutes while the bed is passed through a scanner. Several views of your body will be taken. For the abdominal views, you will be asked to hold your breath for 15 to 25 seconds. You may experience discomfort by lying down in the same position for the duration of the test, the noise inside the scanner, or being asked to hold your breath. You will not be asked to have this test if you do not tolerate confined spaces. 5) Your resting metabolic rate is measured while you lie comfortably and quietly in bed. You will have a large transparent hood that is open to the air of the room, over your upper chest and head, for about 20 minutes. This measures your breathing as you rest. This is called "indirect calorimetry". Methods 2, 4 and 5 will be done during both stays at the hospital, while method 1 and 3 measurements will be measured once a week.

6) Gluconeogenesis Test: This test requires that you take deuterated water [water that containes a stable isotope (not radioactive)] to measure the body's production of sugar. This test will start on day 11. For this, you will eat the same diet until 4:45 PM. There will be no food after this, until the next day. At 9:45, 10:15, 10:45, 11:15 PM, you will drink a 40 mL (X 4) or 5 ounces of water with deuterium. For the rest of the night, you may drink deuterated water diluted at 0.5%. A blood sample (25 mL will be collected at 9:45 AM the next day for this test. Deuterated water has caused slight, tolerable and short-lived dizziness in some people, but is otherwise the same as drinking ordinary water. This test will be done twice, i.e. day 3, before weight loss intervention and on day 38, after the weight loss intervention.

7) Behaviour Modification Counselling: During your stay in hospital, you may choose to have individual sessions with Dr. Gougeon for training in behaviour modification. This will be for your benefit, to learn how to make changes in your lifestyle.

8) Study of the effects of insulin: On days 4 and 39, the study of the effects of insulin on body protein will be performed at the Crabtree Laboratory of the Nutrition Centre which is located in the hospital. It will last approximately 6 hours. On the morning of the test, while you are fasting and resting comfortably in bed, the physician will insert two intravenous catheters in the veins of your arm and hand. Your hand catheter will allow for repeated painless blood samples to be taken. This catheter will be in a vein on the back on your hand or wrist. The hand will be placed in a warming box at 65$^0$ C to make the veins dilate so the blood in the vein will be similar to that of an artery. This is not a painful or uncomfortable procedure because the hand becomes used to the warm temperature. The

Consent form
MUHC: Royal Victoria Hospital
Department of Medicine
Title: Insulin Sensitivity of Protein Metabolism
In Type 2 Diabetes Mellitus REB BMA-05-024
Version date: March 1, 2012

doctors, who will also remain available to you until your return to your usual state. At this time, there will be a follow-up of your diabetes management by the research team.

11) Benefits: You will benefit directly if you opt to have diet and activity counseling, which will help you on the long term to maintain healthy eating habits. Additionally, you will be experiencing some weight loss and that may lead to a better control of your diabetes (improvement in your blood tests results i.e. lipid profile and glucose levels). It is hoped that the information obtained from your contribution will lead to a better understanding of the abnormal protein metabolism in diabetes. This should show how the protein requirements differ in diabetes, and to help define optimal intakes for weight maintenance and reduction.

Any questions you may have about your diabetes, the diet and the study results will be answered by contacting Dr. Réjeanne Gougeon at (514) 843-1665.
Both Dr. Errol B. Marliss at (514) 843-1665 or pager (514) 406-1746 or Dr. José A. Morais at (514) 843-1665 or pager (514) 406-0163 will be available to answer questions regarding your diabetes control and the muscle biopsy.
Should you have any question regarding your rights as a research subject, you may contact the Patient Representative of the Royal Victoria Hospital at (514) 934-1934, local 35655.

12) Adherence: It is important to comply with the study protocol in order to measure the impact of weight loss. Therefore, adherence to the meal instructions as well as the consumption of the meals and the liquid formula are necessary. If you have any concern, you can always discuss it with the dietitian. Also, it is important to visit the Crabtree Laboratory once a week (the date will be assigned), in order to take the necessary measurements as well as be provided with the diet.

13) Confidentiality: The data obtained will be treated confidentially and it will not be possible to identify you personally by name or otherwise in any publication of the results, or in presentations at scientific meetings. As part of normal research practice it is important that information related to the study can be checked for accuracy. It may be necessary for Health Canada (Health Protection Branch), other regulatory agencies, and the McGill University Health Centre Quality Assurance for research to review the information obtained from study documents and your medical records. In such circumstances, confidentiality will be maintained at all times.

You will receive $35 for the morning screening visit, and will be covered for all transportation costs, which include: the first screening visit, and every Wednesday over four weeks for food pick-up and $400 for the full study that includes the first 3 days and last week in the hospital. These payments are to compensate for your time and loss of potential income during the 39 days of the study.

Appendix 3. Internal Advertising Posters

L'Institut de recherche
du Centre universitaire de santé McGill
The Research Institute
of the McGill University Health Centre

# Type 2 Diabetes Study

**The McGill Nutrition & Food Science Centre** is seeking **men and women** for a research study looking at how diabetes and weight loss affect blood glucose levels and the state of protein in the body. (This is not a trial for a new medication)


**Principal Investigator: Réjeanne Gougeon, PhD**

**You are eligible if you:**

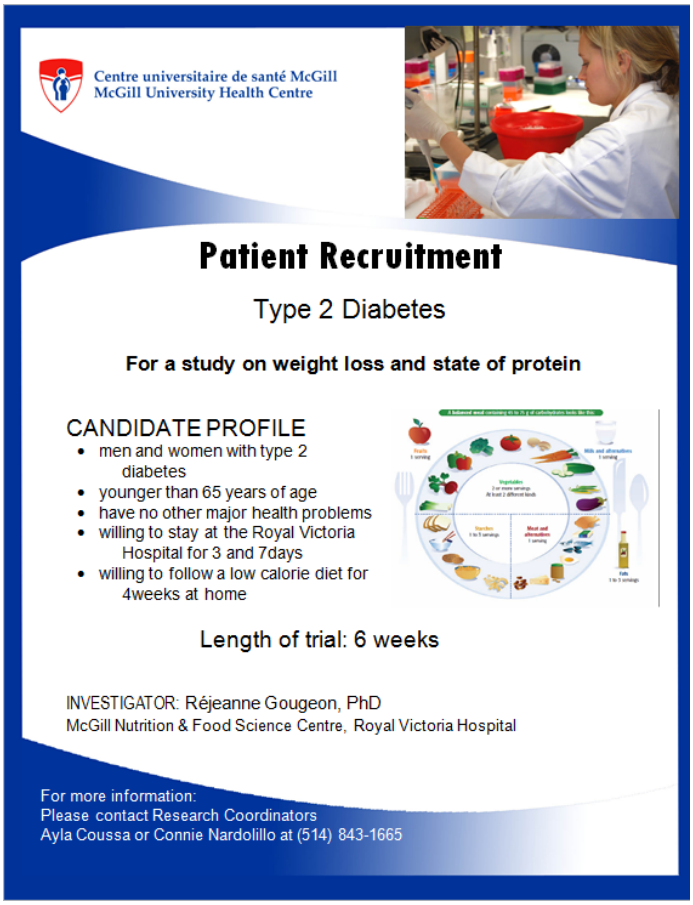
- **have type 2 diabetes**
- **are younger than 65 yrs of age**
- **have no other major health problems**
- **are willing to stay at the Clinical Investigation Unit of the Royal Victoria Hospital for 3 and 7d**
- **are willing to follow a low calorie diet for 4 weeks at home**

**For more information call: Connie Nardolillo or Ayla Coussa, study coordinators at 514-843-1665 between 9 a.m. and 5 p.m.**

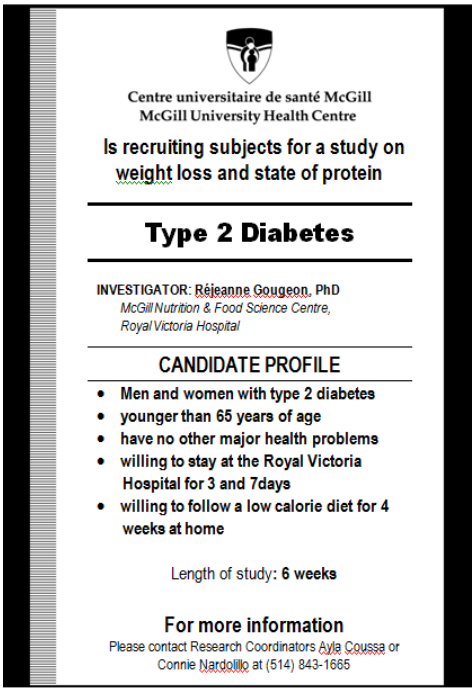
Benefits of participating in the study include knowing your energy requirements, getting dietary and lifestyle counseling, losing weight with provided meal replacements and ready-to-cook meals and knowing the results of your blood tests. You will be compensated for your participation!

Type 2 Diabetes Study – MUHC 514-843-1665

# Appendix 4. External Advertising Posters




# Appendix 5. Newspaper Advertising



# Appendix 6. Phone screening questionnaire

## Questionnaire for potential subjects

Date:_____Protocol:_____
___

Name:_____Phone#:_____
___

Age:_____ Weight:_____ Height:_____
BMI:_____

1. Currently in another study? □ Yes - End date?
_____
□No

2. Previous participation? □Yes
□No

3. Student? □Yes
□ No

4. University/Faculty
_____

5. Stable weight for 6 months? □Yes
□No
□Don't know

6. Allergies (Food/ Medications)? □ Yes
□ No

7. Smoker? □Yes
□No

8. Vegetarian (red meat, eggs, cheese, milk, tofu, vegetables, fish, poultry)?
□ Yes
□No - How much red meat? _____

9. Drink alcohol (wine, beer)? □Yes – Less than 3 drinks/day?
_____
□No

9. Medicare card? □Yes
□No

10. RVH card? □Yes
□No

11. Any diseases within the family? □ Yes
_____
□ No

12. Type 2 DM? □Yes - For how many years?
_____
- Medication (dosage, frequency)?
_____
_____
□No

13. Glycemic control:

| | Date & Value | Date & Value | Date & Value | Date & Value | Date & Value |
|---|---|---|---|---|---|
| Hemo A1C | | | | | |
| BS Monitoring | | | | | |

14. ♀ Post-menopausal? □ Yes - Last period?
_____
□ No - Regular periods?

15. Surgery/Hospitalization/ Accidents? □Yes – Why? When?
_____
_____
_____
_____
_____
□No

16. Blood transfusions? □Yes – Why? When?
_____
_____
_____
□ No

17. Other Medical Conditions & Medications

| Medical condition | How many yrs? | Current medications (Dosage, frequency) |
|---|---|---|
| Diabetes complications | | |
| Heart/High blood pressure | | |
| Thyroid | | |
| Anemia/Fe deficiency | | |
| Kidney | | |
| Liver | | |
| Respiratory | | |
| Cancer | | |
| Cholesterol/Lipids | | |
| Psychiatric | | |
| Other: (including HIV, Hep) | | |
| | | |
| | | |

18. Herbs & Vitamins? Over the Counter Pills? □ Yes – Which ones?
_____
_____
_____
□ No

19. Activities/Exercise:
_____
_____
_____
_____

20. Where did you hear about the study?
□Poster - Where? _____
□Newspaper - Which one? _____
□Other _____

Comments:
_____
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Appendix 7. Pre-intervention, Isoenergetic weight-maintenance diet

**Protein Metabolism in Type 2 Diabetes**

**Diet (Day1)**

**Date:**

**Subject:** **TOTAL: 8 bottles Ensure + 3.5 bottles Glucerna**

**8:00am**

- **Blood Glucose Monitoring**
- **Cereals, Milk, 2 bottles of Ensure**

**11:00am**

- **Blood Glucose Monitoring**
- **1 bottle of Ensure and 1.5 bottles Glucerna**

**2:00pm**

- **Blood Glucose Monitoring**
- **1 bottle of Ensure and 1 bottle Glucerna**

**5:00pm**

- **Blood Glucose Monitoring**
- **2 bottles of Ensure**

**8:00pm**

- **Blood Glucose Monitoring**
- **2 bottles of Ensure and 1 bottle Glucerna**

- **VOID, take note of time:_____**
- **At 8:00 am: Cereals, Milk, meal replacement (Ensure/Glucerna)**
- **Begin 24hr urine collection (Mon. to Tues.)**
- **Measure water intake.**

Appendix 8. Meal replacements formula for energy-restriction diet

| | Boost Diabetic | Glucerna Diabetic |
|---|---|---|
| Format | 237ml per bottle | 237ml per bottle |
| Nutrition fact | Kcal: 190kcal<br>Fat: 7g, w-3: 0.3g, w-6: 1.2g<br>CHO:16g<br>Fibre: 2g<br>Sugar: 2g<br>Protein:16g<br>Ca: 350mg<br>Vit D: 100IU | Kcal: 225kcal<br>Fat: 8.2g w-3: 0.41g w-6: 2.4g<br>CHO: 26.7g<br>Fibre: 3.1g<br>Sugar: 4.4g<br>Protein: 11.3g<br>Ca: 25%<br>Vit D: 26% |


Appendix 9. Weekly energy-restricted dietary plan

## Dietary Plan week 2 (Thursday July 11th –Thursday 18th) Mr. T

| Thursday | Friday | Saturday | Sunday | Monday | Tuesday | Wednesday |
|---|---|---|---|---|---|---|
| 1 Glucerna<br>1 milk (1%)<br>1/2cup of kashi<br>1 medium egg<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>1 milk (1%)<br>1/2cup of kashi<br>1 medium egg<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>¾ c yogurt (0%)<br>1/2cup of kashi<br>1 slice of cheese<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>¾ c yogurt (0%)<br>1/2cup of kashi<br>1 medium egg<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>¾ c yogurt (0%)<br>1/2cup of kashi<br>1 slice of cheese<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>1 milk (1%)<br>1/2cup of kashi<br>1 medium egg<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) | 1 Glucerna<br>¾ c yogurt (0%)<br>1/2cup of kashi<br>1 slice of cheese<br>1 toast<br>½ cup berries (raspberries or blueberries or strawberries) |
| 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) | 1 BOOST<br>+<br>1 BOOST<br>+ green salad (NO OIL) |
| **MEAL 1: Pork**<br>**+ grilled veggies** | **MEAL 2: Salmon**<br>**+ salad:** 1cup lettuce + 5 cherry tomatoes + 1tsp balsamic + 1tsp olive oil | **MEAL 3: Beef**<br>**+ salad (or veggies)** | **MEAL 4: Shrimps**<br>**+ salad (or veggies)** | **MEAL 5: Chicken**<br>**+ salad (or veggies)** | **MEAL 6: Veal**<br>**+ salad (or veggies)** | **MEAL7: Caille**<br>**+ salad (or veggies)** |
| 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 Clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) | 1small yogurt (0%)<br>+<br>**1** Fruit (can be an orange or 2 clementine or 1 apple or 3 small plums or 1 large plum or ½ cup berries) |

# Appendix 10. Nutrition facts of vaccumed meals provided during energy-restriction

**Sliced chicken, sweet potatoes and brussels sprouts**

## Nutrition Facts

Serving Size (295g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 340 | Calories from Fat 80 |
| | **% Daily Value*** |
| **Total Fat** 9g | **14%** |
| Saturated Fat 4.5g | **23%** |
| Trans Fat 0g | |
| **Cholesterol** 100mg | **33%** |
| **Sodium** 390mg | **16%** |
| **Total Carbohydrate** 31g | **10%** |
| Dietary Fiber 5g | **20%** |
| Sugars 11g | |
| **Protein** 34g | |

Vitamin A 6% • Vitamin C 80%
Calcium 10% • Iron 15%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Veal with mushrooms, celery, asparagus and red potato**

## Nutrition Facts

Serving Size (386g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 320 | Calories from Fat 40 |
| | **% Daily Value*** |
| **Total Fat** 4.5g | **7%** |
| Saturated Fat 1.5g | **8%** |
| Trans Fat 0g | |
| **Cholesterol** 115mg | **38%** |
| **Sodium** 400mg | **17%** |
| **Total Carbohydrate** 38g | **13%** |
| Dietary Fiber 7g | **28%** |
| Sugars 15g | |
| **Protein** 33g | |

Vitamin A 15% • Vitamin C 60%
Calcium 10% • Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Trout with spinach, wild rice and green beans**

## Nutrition Facts

Serving Size (254g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 380 | Calories from Fat 100 |
| | **% Daily Value*** |
| **Total Fat** 12g | **18%** |
| Saturated Fat 5g | **25%** |
| Trans Fat 0g | |
| **Cholesterol** 150mg | **50%** |
| **Sodium** 490mg | **20%** |
| **Total Carbohydrate** 34g | **11%** |
| Dietary Fiber 4g | **16%** |
| Sugars 3g | |
| **Protein** 35g | |

Vitamin A 20% • Vitamin C 8%
Calcium 10% • Iron 10%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Beef with green peppers, yellow beet and green peas**

## Nutrition Facts

Serving Size (200g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 360 | Calories from Fat 90 |
| | **% Daily Value*** |
| **Total Fat** 10g | **15%** |
| Saturated Fat 3.5g | **18%** |
| Trans Fat 0g | |
| **Cholesterol** 85mg | **28%** |
| **Sodium** 460mg | **19%** |
| **Total Carbohydrate** 30g | **10%** |
| Dietary Fiber 5g | **20%** |
| Sugars 1g | |
| **Protein** 37g | |

Vitamin A 10% • Vitamin C 15%
Calcium 2% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Cajun shrimp with pasta and asparagus**

## Nutrition Facts

Serving Size (274g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 320 | Calories from Fat 35 |
| | **% Daily Value*** |
| **Total Fat** 4g | **6%** |
| Saturated Fat 0g | **0%** |
| Trans Fat 0g | |
| **Cholesterol** 225mg | **75%** |
| **Sodium** 450mg | **19%** |
| **Total Carbohydrate** 35g | **12%** |
| Dietary Fiber 2g | **8%** |
| Sugars 4g | |
| **Protein** 34g | |

Vitamin A 20% • Vitamin C 15%
Calcium 10% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Minced chicken with sherry vinegar, barley, leek onion and red pepper**

## Nutrition Facts

Serving Size (231g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 310 | Calories from Fat 50 |
| | **% Daily Value*** |
| **Total Fat** 6g | **9%** |
| Saturated Fat 2.5g | **13%** |
| Trans Fat 0g | |
| **Cholesterol** 85mg | **28%** |
| **Sodium** 300mg | **13%** |
| **Total Carbohydrate** 30g | **10%** |
| Dietary Fiber 6g | **24%** |
| Sugars 2g | |
| **Protein** 34g | |

Vitamin A 10% • Vitamin C 140%
Calcium 6% • Iron 15%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

**Pork garlic with parsley, sweet potato, zucchini and orange pepper**

## Nutrition Facts

Serving Size (291g)
Servings Per Container

**Amount Per Serving**

| | |
|---|---|
| **Calories** 350 | Calories from Fat 80 |
| | **% Daily Value*** |
| **Total Fat** 9g | **14%** |
| Saturated Fat 3g | **15%** |
| Trans Fat 0g | |
| **Cholesterol** 100mg | **33%** |
| **Sodium** 340mg | **14%** |
| **Total Carbohydrate** 30g | **10%** |
| Dietary Fiber 5g | **20%** |
| Sugars 13g | |
| **Protein** 35g | |

Vitamin A 25% • Vitamin C 130%
Calcium 6% • Iron 15%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

| | | Calories: | 2,000 | 2,500 |
|---|---|---|---|---|
| Total Fat | Less than | | 65g | 80g |
| Saturated Fat | Less than | | 20g | 25g |
| Cholesterol | Less than | | 300mg | 300mg |
| Sodium | Less than | | 2,400mg | 2,400mg |
| Total Carbohydrate | | | 300g | 375g |
| Dietary Fiber | | | 25g | 30g |

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Appendix 11. Daily activity log during energy-restriction diet

Please list the different activities that you do during the day (**including sitting, watching TV, reading, biking, walking, etc)**

| Date | Time | Type | Duration |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

Appendix 12. Poster presentation, CDA Vascular Conference, Montreal October 2013

# Metabolic and Body Composition Responses to a Moderate Energy Restricted, Abundant Protein Diet in Type 2 Diabetic Adults

Ayla Coussa, Errol B. Marliss, Stéphanie Chevalier, José A. Morais, Marie Lamarche, Michael Tsoukas and Réjeanne Gougeon

Crabtree Nutrition Laboratories, MUHC, Royal Victoria Hospital, Montréal, Québec

## Background

- Protein metabolism is abnormal in type 2 diabetes mellitus (T2D).
- Insulin resistance of protein anabolism occurs concurrently with that of glucose.
- Normalizing glycemia did not improve protein anabolic response.
- We showed that abundant protein supply can compensate for this resistance.
- Hypoenergetic diets without increased % protein might worsen protein loss.
- Optimal protein intake during energy restriction in T2D is not established.

## Hypotheses

An energy restricted diet with maintained typical abundant protein intake, appropriately distributed, will decrease insulin resistance of glucose metabolism, protect muscle mass and prevent deterioration of protein metabolism.

## Methods

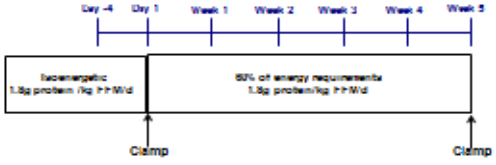
Participants: n=6 (3M, 3W); 59 ± 2 y; T2D duration: 9 ± 4 y; 0-2 oral antihyperglycemic agents; conventionally controlled, mild comorbidities.
Daily diet: 3 equal meals, 1 pre-prepared + meal replacement formula provided; 26% protein, 45% CHO and 29% fat of 60% energy needs.

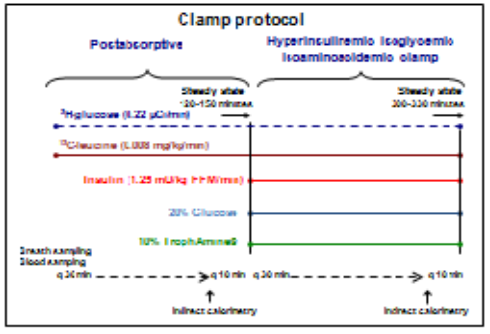
Study protocol

**Admission 1**
Anthropometrics, BP
Chest-X-ray, ECG, DEXA, BIA
Blood and 24H urine

**Weekly visits**
Anthropometrics, BP and BIA
Diet and medication adjustment

**Admission 2**
Anthropometrics, BP
DEXA, BIA, Blood and 24H urine



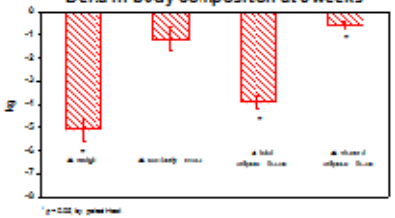

## Results

Anthropometrics and metabolic outcome

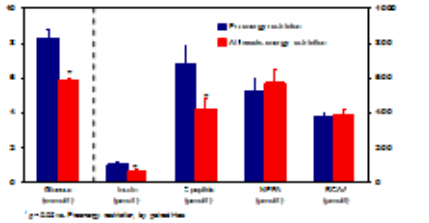
| | Pre-energy restriction | At 5 weeks of energy restriction | p |
|---|---|---|---|
| Body composition | | | |
| Weight (kg) | 88.8 ± 3.6 | 83.7 ± 3.3 | 0.031 |
| Body mass index (kg/m²) | 30.8 ± 1.2 | 29.0 ± 1.1 | 0.031 |
| Waist circumference (cm) | 111.6 ± 3.6 | 110.2 ± 3.6 | 0.022 |
| Hip circumference (cm) | 116.1 ± 3.1 | 113.9 ± 3.2 | 0.024 |
| Lean body mass (kg) | 51.4 ± 4.0 | 50.2 ± 4.2 | 0.078 |
| Fat mass (kg) | 34.5 ± 2.8 | 30.8 ± 2.9 | <0.001 |
| Body fat (%) | 39.0 ± 3.3 | 36.8 ± 3.8 | 0.012 |
| Metabolic outcome | | | |
| Fasting plasma glucose (mmol/L) | 8.3 ± 0.5 | 5.8 ± 0.3 | 0.003 |
| A1C (%) | 7.3 ± 0.5 | 6.7 ± 0.3 | 0.006 |
| HOMA-IR | 6.22 ± 0.69 | 2.77 ± 0.28 | 0.003 |
| Triglycerides (mmol/L) | 2.29 ± 0.57 | 1.97 ± 0.26 | 0.063 |
| Total cholesterol (mmol/L) | 4.36 ± 0.34 | 3.93 ± 0.22 | 0.033 |
| LDL-cholesterol (mmol/L) | 2.14 ± 0.31 | 1.49 ± 0.20 | 0.021 |
| HDL-cholesterol (mmol/L) | 1.11 ± 0.05 | 1.01 ± 0.06 | 0.323 |
| Total cholesterol/HDL | 3.93 ± 0.21 | 3.98 ± 0.27 | 0.936 |
| CRP* (mg/L) | 1.71 ± 0.46 | 3.86 ± 0.37 | 0.032 |

Data are mean ± SEM; CRP: C-reactive protein; HOMA-IR: homeostatic model of assessment: insulin resistance.
*p < 0.05 vs. Pre-energy restriction, by paired t-test

Delta in body compositon at 5 weeks




Postabsorptive substrate and hormone concentrations

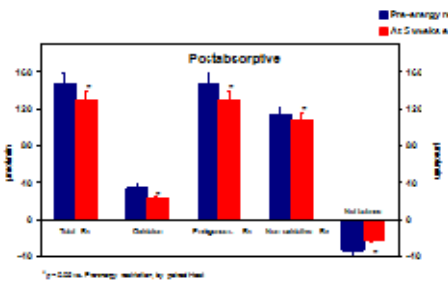



Substrate and hormone concentrations in response to clamp

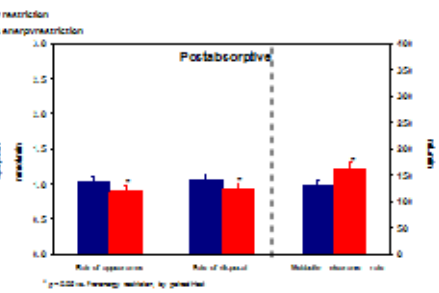
| | Pre-energy restriction | | At 5 weeks of energy restriction | |
|---|---|---|---|---|
| | Postabsorptive | Clamp | Postabsorptive | Clamp |
| Serum insulin (pmol/L) † | 103 ± 13 | 502 ± 11 | 84 ±6* | 456 ± 33 |
| Plasma Glucagon (pmol/L) † | 12 ± 3 | 16 ± 3 | 16 ±3* | 13 ± 3* |
| Plasma C-peptide (pmol/L) † | 684 ± 102 | 528 ± 100 | 421 ± 55* | 255 ± 44* |
| Serum NEFA (µmol/L) † | 523 ± 72 | 91 ± 14 | 573 ± 74 | 75 ± 8 |
| Plasma BCAA (µmol/L) † | 375 ± 19 | 387 ± 11 | 392 ± 25 | 366 ± 21 |

Data are mean ± SEM; BCAA, branched-chain amino acids.
* p < 0.05 vs. Pre-energy restriction, by paired t-test
† p < 0.025 clamp effect, by repeated-measures ANOVA

Leucine kinetics




Glucose kinetics




## Summary

At 5 weeks of excellent adherence to energy restriction:
- LEAN MASS LOSS WAS MINIMAL, most weight lost was fat
- POSTABSORPTIVE:
  - ➢Glycemia near normal, insulin, C-peptide and HOMA-IR decreased
  - ➢Glucose turnover decreased, metabolic clearance rate (MCR) increased
  - ➢Protein turnover and oxidation decreased. Net protein balance less negative, resulting in overnight sparing of ~ 11 g. No change in BCAA
- HYPERINSULINEMIC CLAMP:
  - ➢Glucose: no change in suppression of production and in increase of disposal; and increase MCR
  - ➢Protein: leucine flux increased, protein breakdown suppressed, synthesis unchanged from postabsorptive, resulting in no improvement in net anabolism.

## Conclusions and Implications

In T2D participants with conventional diabetes management, this diet resulted in:
- Improved postabsorptive glycemia and HOMA-IR, conserved lean and mobilized fat mass, without WORSENING protein metabolism.
- Improvement in CV risk factors.

This approach could possibly help prevent age and T2D related progressive lean tissue loss, even during weight reduction.

## Acknowledgements

Technical assistance from Concettina Nardolillo, Ginette Sabourin and Daniel White. All the participants for their generosity and CIHR for funding this study.

# Appendix 13. Diabetes knowledge questionnaire

| | Questions | Yes | No | Don't Know |
|---|---|---|---|---|
| 1 | Eating too much sugar and other sweet foods is a cause of diabetes. | | | |
| 2 | The usual cause of diabetes is lack of effective insulin in the body. | | | |
| 3 | Diabetes is caused by failure of the kidneys to keep sugar out of the urine. | | | |
| 4 | Kidneys produce insulin. | | | |
| 5 | In untreated diabetes, the amount of sugar in the blood usually increases. | | | |
| 6 | If I am diabetic, my children have a higher chance of being diabetic. | | | |
| 7 | Diabetes can be cured. | | | |
| 8 | A fasting blood sugar level of 210 is too high. | | | |
| 9 | The best way to check my diabetes is by testing my urine. | | | |
| 10 | Regular exercise will increase the need for insulin or other diabetic medication. | | | |
| 11 | There are two main types of diabetes: Type 1 (insulin-dependent) and Type 2 (non-insulin dependent). | | | |
| 12 | An insulin reaction is caused by too much food. | | | |
| 13 | Medication is more important than diet and exercise to control my diabetes. | | | |
| 14 | Diabetes often causes poor circulation. | | | |
| 15 | Cuts and abrasions on diabetes heal more slowly. | | | |
| 16 | Diabetics should take extra care when cutting their toenails. | | | |
| 17 | A person with diabetes should cleanse a cut with iodine and alcohol. | | | |
| 18 | The way I prepare my food is as important as the foods I eat. | | | |
| 19 | Diabetes can damage my kidneys. | | | |
| 20 | Diabetes can cause loss of feeling in my hands, fingers and feet. | | | |
| 21 | Shaking and sweating are signs of high blood sugar. | | | |
| 22 | Frequent urination and thirst are signs of low blood sugar. | | | |
| 23 | Tight elastic hose or socks are not bad for diabetics. | | | |
| 24 | A diabetic diet consists mostly of special foods. | | | |

*Source: Starr County*

This product was adapted from the DKQ "Diabetes Knowledge Questionnaire," - Garcia and Associates for the diabetes self management project at Gateway Community Health Center, Inc. with support from the Robert Wood Johnson Foundation® in Princeton, NJ.

# Appendix 14. Self-efficacy diabetes management questionnaire

Paciente: _____ MR#:_____
Promotora: _____ Fecha: _____

How confident are you that you can,

1. do all the things necessary to manage your condition on a regular basis?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

2. keep stress and worry from interfering with the things you want to do?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

3. follow your meal plan when you have to prepare or share food with other people who do not have diabetes?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

4. choose the appropriate foods to eat when you are hungry (for example, snacks)?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all Confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

5. exercise at least 15 to 30 minutes a day, 4 to 5 most days of the week?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

6. know what to do when your blood sugar level foes higher or lower than it should be?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

7. judge when the changes in your health mean your should visit the doctor?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

8. control your diabetes so that it does not interfere with the things you want to do?

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not at all confident | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Completely confident |

This product was developed by the Advancing Diabetes Self Management project at La Clinica de La Raza, Inc. in Oakland, CA with support from the Robert Wood Johnson Foundation® in Princeton, NJ.

Appendix 15. Physical activity assessment questionnaire

PANDA
Physical Activity & Nutrition for Diabetes in Alberta

Participant ID: _____

**Physical Activity Adherence**

Considering a **7-Day period** (a week), how many times on average do you do the following kinds of exercise for **more than 15 minutes.**

| | Times Per Week |
|---|---|
| A. STRENUOUS PHYSICAL ACTIVITY<br>(heart beats rapidly, sweating)<br><br>(e.g., running, jogging, hockey, soccer, squash, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling, vigorous aerobic dance classes, heavy weight training) | _____ |
| B. MODERATE PHYSICAL ACTIVITY<br>(not exhausting, light perspiration)<br><br>(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing) | _____ |
| C. MILD PHYSICAL ACITIVITY<br>(minimal effort, no perspiration)<br><br>(e.g., easy walking, yoga, archery, fishing, bowling, lawn bowling, shuffleboard, horseshoes, golf, snowmobiling) | _____ |

Considering a **7-Day period** (a week), how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. Often 2. Sometimes 3. Never/rarely