THE EFFECTS OF INTERMITTENT FASTING AND A HIGH PROTEIN DIET IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements For the Degree of Masters of Pharmacy

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Abstract

Intermittent fasting (IF) is a recently popularized meal timing strategy whereby individuals abstain continuously from any energy intake for 16 to 20 hours each day, subsequently condensing energy intake into a short period spanning 4 to 8 hours. We aimed to test the effects of intermittent fasting in 10 individuals with Type 2 Diabetes Mellitus in conjunction with recommendations to consume a high protein diet in a 6 to 8 week withdrawal study. This study consisted of three phases: baseline, intervention, and follow-up. During the 2-week baseline and intervention phases participants consumed meals at regular times. Biochemical, anthropometric, and physical activity measurements were taken at the end of each phase. Participants reported morning, afternoon and evening self-monitored blood glucose and fasting duration on a daily basis, in addition to completing a remote food photography diary three times within each study phase. Despite the short duration of the intervention phase, intermittent fasting led to significant decreases in weight, BMI, morning SMBG, and overall reductions in waist circumference, C-reactive protein, energy intake, carbohydrate intake, and fat intake. There were significant variations between participants in response to intermittent fasting in respect to changes in lipids and insulin sensitivity, which could not be explained by baseline biochemical or anthropometric measures, fasting duration, energy intake, or physical activity. Upon cessation of intermittent fasting, biochemical changes regressed towards baseline values during the follow-up period. Intermittent fasting was well tolerated by most participants, and no severe adverse events were noted. Morning nausea was the most common complaint, which abruptly ceased when medication timing was changed.

Acknowledgements

I would like to give special thanks to my academic supervisors, Dr. Terra Arnason and Dr. Kerry Mansell for their academic, moral, and financial support of me throughout the last two years, to my committee members for their insights on the nature of the project, and to Dr. Diane Martz for her advice on navigating the often confusing environment of graduate studies and research. The College of Graduate Studies and Research and the University of Saskatchewan Ethics Office have my personal gratitude for their financial support throughout my graduate studies. Lastly, a special thanks to Dr. Hasitha Welihinda for making time in his busy schedule to come to the university after work hours and on days off to provide medical consults to our participants.

Dedication

I would like to dedicate this thesis to a few people. First, to my brother Mike for always being the best example of what is to be an interesting, smart, and kind human being. To my parents for supporting me during the most difficult times in life. To Dr. Terra Arnason who, for whatever reason, decided to take a chance on a know-nothing 1st year kinesiology student 4 years ago.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AB: afternoon with beginning 14 days glucose data
ADRR: Average Daily Risk Rating
AE: afternoon with end 14 days glucose data
AM: afternoon with middle 14 days glucose data
BMI: Body Mass Index
CCR: Creatinine Clearance
CDA: Canadian Diabetes Association
CRP: C-Reactive Protein
DDSOM: Diabetes Dietary Satisfaction and Outcomes Measure
DLW: Doubly-Labelled Water Method
DM1: Diabetes Mellitus Type 1/Type 1 Diabetes
DM2: Diabetes Mellitus Type 2/Type 2 Diabetes
EB: evening with beginning 14 days glucose data
EE: evening with middle 14 days glucose data
EM: evening with middle 14 days glucose data
FPG: Fasted Plasma Glucose
FSIVGTT: Frequently Sampled Intravenous Glucose Tolerance Test
GFR: Glomerular Filtration Rate
GLP-1: Glucagon-like Peptide 1
HDL: High-Density Lipoprotein Cholesterol
HF: Hours Fasted
HFD: Hours Fasted Difference
HFP: Hours Fasted Percent Difference
HOMA-IR: Homeostasis Model Assessment of Insulin Resistance
HPD: High Protein Diet
HbA1c: Glycated Hemoglobin
IF: Intermittent Fasting
IFP: Inflection Points
IST: Insulin Suppression Test
KS: 2-Sample Kolmogorov-Smirnov Test
L: Linear model
LDL: Low-Density Lipoprotein Cholesterol
MB: morning with beginning 14 days data
ME: morning with end 14 days glucose data
MM: morning with middle 14 days glucose data
OGTT: Oral Glucose Tolerance Test
OLR: Ordinal Logistic Regression
Q/Quad: Quadratic model
QUICK1: Quantitative Insulin Sensitivity Check
RFPM: Remote Food Photography Method
RPG: Random Plasma Glucose
SI: Insulin Sensitivity
SMBG: Self-Monitored Blood Glucose
T/HDL: Total Cholesterol to High-Density Lipoprotein Cholesterol Ratio

TC: Total Cholesterol
TG: Triglycerides
VAS: Visual Analog Scales
WC: Waist Circumference
YPAS: Yale Physical Activity Survey
μ: Average/Mean
σ: Standard Deviation

CHAPTER 1
LITERATURE REVIEW

1.1 Introduction

As of 2010, diabetes mellitus (DM) afflicts more than 285 million adults between the ages of 20 to 79 worldwide, with this number expected to grow to 439 million adults by 2030 (1). Within Canada, 2.4 million individuals have been diagnosed with diabetes, with this number expected to rise to 3.7 million by 2019 (2). Diabetes contributes significantly to increased rates of cardiovascular disease, renal disease, retinopathy, and limb amputations within Canada (2). This translates into an increased cost burden on the Canadian health care system. Individuals afflicted with diabetes require three to four times the amount of medical resources compared to those without diabetes (2).

Diabetes can be categorized as Type 1, Type 2, or Gestational. The high incidence of diabetes is due primarily to Diabetes Mellitus Type 2 (DM2), which accounts for over 90% of all new diagnoses of diabetes (3). Diabetes Mellitus Type 1 (DM1) differs from DM2, as it is predominantly diagnosed in adulthood and often does not result in the complete and ongoing destruction of pancreatic beta cells. Roy Taylor has put forth the 'Twin Cycle Hypothesis' to explain the etiology of DM2 (4). Dr. Taylor postulates that the accumulation of hepatic and pancreatic fat is the primary driver of DM2, as it results in reduced hepatic and peripheral insulin sensitivity and diminished insulin secretion. These changes then trigger chronic uncontrolled hyperglycemia and further deterioration of hepatic and pancreatic function (4). Taylor attributes this accumulation of hepatic and pancreatic fat in DM2 to chronic positive energy balance (4). As such, the pathophysiology of DM2 arises from the triad of relative insulin deficiency, excess hepatic glucose output, and to a lesser extent, peripheral insulin resistance. DM1 is an

autoimmune disorder beginning primarily in childhood where pancreatic beta cells are destroyed by the innate immune system. DM1 and DM2 have very little in common as far as causal factors may explain; however, both conditions lead to chronic hyperglycemia and its accompanying comorbidities, such as renal, retinal, and vascular damage.

1.2 Health Markers in the Diagnosis and Management of DM2

1.2.1 Biochemical Measurements

1.2.1.1 Plasma Glucose and Glycemic control

A diagnosis of DM2 is achieved by direct and indirect assessment of circulating plasma glucose levels. There are three primary measures of glycemic control in DM2 – Fasting Plasma Glucose (FPG), Oral Glucose Tolerance Test (OGTT), and Glycated Hemoglobin (HbA1c). Plasma glucose is measured directly by FPG or an OGTT. The amount of circulating glucose in plasma after an 8h-14h overnight fast is defined as FPG. An OGTT is a measurement that reflects the amount of circulating glucose in plasma 2h after a 75g oral bolus of glucose is consumed. HbA1c reflects the percentage of glycated hemoglobin, which acts as an indirect measurement of average glucose levels of the preceding 3-month period. A diagnosis of DM2 is made when symptomatic hyperglycemia and any one of the following criteria are met: FPG > 7.0mmol/L, OGTT > 11.1mmol/L, or HbA1c > 6.5% (5). In the absence of symptomatic hyperglycemia, repeat testing on a separate day is performed. Lastly, a random plasma glucose (RPG) level > 11.1 mmol/L, which directly assesses circulating levels of glucose at a random time of day, can be used to justify additional testing methods but should not be used to confirm a diagnosis of DM2.

After diagnosis, most individuals with DM2 are advised to reach a HbA1c below 7.0%, FPG 4.0-7.0mmol/L, and post-prandial glucose levels of 5.0-10.0mmol/L, as this is associated with the reduction of micro- and macrovascular complications, and improved mortality outcomes (6–8). There is considerable debate that achieving a HbA1c < 6.5% is beneficial, as a recent meta-analysis of all major DM2 trials indicates that intensive diabetes treatment demonstrates limited benefits with significant increases in all-cause mortality and cardiovascular death end-points, as well as occurrences of severe hypoglycemia (9). Fortunately, clinicians are able to individualize treatment and glycemic targets for at-risk patients in order to reduce the risk of complications.

A fifth measure of glycemic control, Self-Monitored Blood Glucose (SMBG), is acquired when patients monitor their serum glucose levels with the use of a portable glucometer and logbooks. In rare circumstances, SMBG can be used in place of HbA1c to observe daily glycemic control when confounders compromise HbA1c measurements. However, in most circumstances SMBG remains as a separate and adjunctive tool of monitoring glycemic control alongside HbA1c (10). SMBG is an effective method to confirm and monitor the treatment of hypoglycemia in individuals with diabetes, and can be a modestly effective tool alongside behavioural modification for glycemic control – but only with exceptional adherence and supervision from a health care professional (10,11). With decreasing levels of HbA1c, postprandial glycemia becomes a larger proportion of average glucose levels, and SMBG can be used to accurately capture these glycemic excursions in well-controlled individuals with DM2 (12).

1.2.1.2 Insulin Resistance

Insulin resistance is a measure of the peripheral and hepatic incapacity to respond to circulating insulin, a primary feature of DM2. Peripheral and hepatic insulin resistance are independent and cumulative contributors to hyperglycemia in diabetes (13). Hepatic insulin resistance (via elevated hepatic glucose production) is one of the primary contributors to hyperglycemia in DM2, since peripheral tissues contribute approximately 20% of glucose disposal in the fasting state in those with DM2 (14). The restoration of hepatic insulin sensitivity and reduction in the rate of hepatic gluconeogenesis occurs during the reversal of DM2 from a hypocaloric diet (15).

The current gold standard in directly measuring insulin resistance is through the use of the hyperinsulinemic euglycemic clamp technique (16). The clamp technique involves a constant infusion of insulin of 5 to 120 $mU \cdot m^{-2} \cdot min^{-1}$ with boluses of dextrose infused at 5 to 10 minute intervals aided by a bedside glucose monitor to maintain euglycemia. Insulin sensitivity (SI) is then assessed by the formula $SI_{Clamp} = M/(G \times \Delta I)$ – where M is the rate of glucose disposal, G is the steady state blood glucose concentration, and ΔI represents the difference between fasting plasma insulin and steady state insulin (16). The hyperinsulinemic steady state suppresses hepatic gluconeogenesis, which demonstrates a direct comparison of the rate of peripheral glucose disposal relative to plasma insulin concentrations. Exogenous insulin from the hyperinsulinemic state controls for the effects of endogenous pro-insulin, giving a more direct view of insulin sensitivity (17). The main limitations of the hyperinsulinemic euglycemic clamp technique are time, labor cost, and lack of access to the general population. The supra-physiological levels of insulin remain a concern, as it may have systemic effects on glucose disposal and hepatic gluconeogenesis not present in normal physiological conditions.

Another direct test of insulin sensitivity is the Insulin Suppression Test (IST). IST involves a participant being administered an oral insulin suppressor followed by constant insulin and glucose infusion for 3 hours. The IST has similar limitations, validity, and reliability as the clamp technique but with fewer technical demands. However, the risk of hypoglycemia and hyperglycemia are increased in select populations during IST (16).

The only indirect method of assessing insulin resistance is the Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT). The FSIVGTT involves a single intravenously administered bolus of glucose followed by plasma glucose and insulin measurements taken continuously for 3 hours. The FSIVGTT oversimplifies glucose homeostasis and does not match the validity of direct methods, yet is as labor intensive as the direct methods (16).

Surrogate index measures of insulin resistance derived from measures of insulin and glucose in the fasting state include log fasting insulin, glucose/insulin ratio, Homeostasis Model Assessment (HOMA-IR), log HOMA-IR, and Quantitative Insulin Sensitivity Check Index (QUICK1). The HOMA-IR, calculated by ([fasting insulin (μU/ml)] × [fasting glucose (mmol/l)])/22.5, has the best agreement with the clamp technique in assessing insulin resistance in individuals with DM2 (18). Log fasting insulin and glucose/insulin ratio are the poorest measures of insulin resistance in individuals with DM2 (16). QUICK1 has been shown to be inferior to HOMA-IR with respect to their agreements with the clamp technique, but superior in reproducibility in individuals with DM2 (18). Unfortunately index and indirect measures suffer from confounded results due to the effect of pro-insulin on fasting insulin values (17).

1.2.1.3 Inflammation

Chronic low-grade inflammation has recently been implicated in the development of DM2, but the specific role of inflammation in the causation of DM2 has yet to be fully established (19). C-Reactive Protein (CRP), an acute phase protein, is the most widely used biomarker of inflammation. Elevated levels of CRP are indicative of non-specific systemic inflammation in multiple disease states. The synthesis of CRP occurs in the liver and is triggered by circulating cytokines released primarily by macrophages (20) as well as other immune cells (21). CRP then migrates towards and attaches to necrotic cells, where it exercises both pro- and anti-inflammatory effects (20). Interleukin-6 is primarily responsible for the synthesis of CRP (20). However, there are interactions between Interleukin-6, Interleukin-1-Beta, and other cytokines on the transcription and synthesis of CRP (20). Therefore, these cytokines are not as accurate as CRP for describing systemic inflammation or predicting the development of DM2 (22,23). Within individuals with DM2, elevated levels of CRP have been independently associated with the progression of nephropathy (24), elevated fasting glucose, insulin resistance, and vascular dysfunction (25). CRP is subsequently lowered by exercise (26,27), oral diabetes medications (28), and dietary therapy (29).

Other inflammatory biomarkers have been used in research settings to describe chronic inflammation in DM2. Yet do not have the evidence from large trials to establish relationships regarding the onset and progression of DM2 (19). These include interleukins, tumour necrosis factor-alpha, cortisol, ferritin, serum amyloid a, sialic acid, and a1-acid glycoprotein.

1.2.1.4 Chronic Kidney Disease

In 2009, 34% of Chronic Kidney Disease (CKD) cases were attributed to diabetes and individuals with diabetes were 12 times more likely than non-diabetics to be hospitalized with end stage renal disease (2). Glomerular Filtration Rate (GFR) is a measure of the amount of blood that passes through the glomeruli of the kidneys each minute (expressed as ml/min/1.73m^2), and is a common and effective measure for determining kidney function. As such, GFR is used to stratify CKD progression: Stage 1 or normal kidney function (GFR>89), Stage 2 or mild kidney damage (GFR 60-89), Stage 3 or moderate kidney damage (GFR 30-59), Stage 4 or severe kidney damage (GFR 16-29) and Stage 5 or end-stage renal disease (GFR < 16) (30). Creatinine is a standard marker of kidney function used commonly while monitoring CKD progression in DM2 (30) and is used in several formulas to estimate GFR. A static measure of plasma creatinine (when accompanied by age, sex, and race) is put into the Modification of Diet by Renal Disease equation (MDRD) and used to estimate GFR with sufficient accuracy (albeit a tendency to over-diagnose CKD) in both healthy populations and individuals with CKD when compared to measured iothalamate clearance (31). Additionally, a combination of urinary creatinine, urinary flow rate, and plasma creatinine over a 24-hour period can determine Creatinine Clearance (C_{CR}). C_{CR} refers to the amount of creatinine that is cleared from blood over a 24-hour period, which can be used as an indirect measure of GFR (32). C_{CR} is routinely estimated in standard medical laboratories by inputting static measures of serum creatinine, age, and bodyweight into the Cockgroft-Gault formula (33).

1.2.2 Anthropometric Measurements

1.2.2.1 Weight and Body Mass Index

Weight and Body Mass Index (BMI) are standard clinical tools used to classify patients as underweight, normal weight, overweight, or obese (34). Weight and BMI are used in many research and clinical settings to demonstrate the effect of lifestyle treatment on obesity and its comorbidities (7,35,36). BMI is defined as weight (kg)/height^2 (m), and is commonly used in research and clinical settings to classify adiposity and disease risk in patients (37). A BMI of < 18.5 kg/m^2 is considered underweight, >= 25.0 kg/m^2 as overweight, and >= 30.0 kg/m^2 as obese (34).

1.2.2.2 Waist Circumference

Waist Circumference (WC) is a measurement of central adiposity used to assess disease risk irrespective of weight or BMI. According to the Canadian Medical Association's guidelines on screening in overweight and obese patients, WC is an accurate estimator for the risk of the patient developing DM2, dyslipidemia, hypertension, and the metabolic syndrome (34). Waist circumference has superior validity compared to BMI in predicting individuals at risk for developing DM2, and superior validity compared to Waist-to-Hip ratio (37) .Waist circumference has been shown to decrease during weight loss from diet, exercise, and some medications in individuals with DM2, coinciding with improvements in glycemic control (7,35,36). A recent demonstration has shown that WC can be an extremely accurate predictor of the reversal of insulin resistance after bariatric surgery (38). Cut-offs for WC are >102cm for men and >88cm for women; dependent on ethnicity, age, and size (34).

1.2.2.3 Body Composition

Hydro-densitometry, where an individual is submersed underwater to measure body density and then an estimation of body fat is made using the Brozek or Siri Formula, is considered the gold-standard of body composition analysis (39). Dual X-Ray Absorptiometry (DEXA) is considered to be on par with the validity and reliability of Hydro-densitometry (39), but remains costly and requires technical expertise. Air-Displacement Plethysmography (ADP) is a commonly used method for analyzing body composition with accuracy comparable to Hydro-densitometry and DEXA, but with wider error ranges and imperfect correlations (39,40). Other methods of body composition, such as Bioelectrical Impendence Analysis (BIA) and Skinfold Measurements are more affordable, but suffer from considerable error and show questionable reliability and validity (41).

1.3 Capturing Dietary and Exercise Habits in DM2

1.3.1 Assessment of Physical Activity

Capturing physical activity habits for research purposes remains a significant challenge, with no methods showing clear superiority over others (42). Currently, there are several methods of assessing physical activity in free-living adults. Monitoring devices worn daily, such as two and three-dimensional accelerometers and pedometers are acceptable methods of tracking physical activity in free-living adults. Accelerometers are currently the gold standard in assessing physical activity in free-living adults (43), however they present many logistical hurdles in implementation, and may underestimate or omit certain types of physical activity (42). Effective implementation of accelerometry requires knowledgeable and sufficiently trained technicians, pre-experimental testing for validity and reliability of accelerometers, equipment and software

for analysis and data-acquisition, and most importantly, ideal participant compliance in order to implement these methods effectively.

A more simple and common form of measuring activity in free-living adults is through questionnaire. Although physical activity questionnaires have concerns with validity and reliability (43) they do show sufficient test-retest reliability (44,45). In particular, the Yale Physical Activity Survey (YPAS) has shown sufficient accuracy when compared to the doubly labelled water method (a measure of total caloric expenditure), outperforming even some monitoring devices in group analysis (43,46).

Importantly, the primary purpose of tracking physical activity in a repeated measures clinical trial is to control for outliers who may increase or decrease their physical activity throughout the study period. The YPAS has considerable test-retest reliability that is suitable for a repeated measures design with free living-adults (44,45).

1.3.2 Assessment of Dietary Intake

In Dr. Ioannidis' recent BMJ paper entitled *Implausible Results in Human Nutrition Research*, he states:

> "Nutritional intake is notoriously difficult to capture with the questionnaire methods used by most studies. A recent analysis showed that in the National Health and Nutrition Examination Survey, an otherwise superb study, for two thirds of the participants the energy intake measures inferred from the questionnaire are incompatible with life." (47)

Dr. Ioannidis' assertions are supported in validity and reliability tests for most written measures of dietary intake against the Doubly-Labelled Water method (DLW), the gold standard in measuring energy intake (48). In his paper, Dr. Ioannidis continues by berating even the most

technologically advanced methods of assessing dietary intake in free-living individuals, stating that they too have had abysmal results – supported by a single paper with limited scope (49). In his brief commentary Dr. Ioannidis overlooked the literature on a technique that has shown considerable efficiency and accuracy in capturing dietary intake, digital photography - namely the Remote Food Photography Method (RFPM). In 24h-recalls, written food diaries (weighed and un-weighed), and food frequency questionnaires, energy intake is consistently under-reported (up to -59% of actual energy intake) when compared to DLW in free-living adults (48). In comparison, the RFPM has consistently shown only modest or no under-reporting of energy intake in both laboratory and free-living situations up to six days in duration against meals with known caloric content and the DLW method, respectively (50,51). When free-living adults were sent customized prompts in order to remind them to take photographs of food regularly, RFPM was not significantly different from DLW (mean energy intake of -3.7% [p-value = 0.16] vs. DLW).

1.3.3 Assessment of Dietary Compliance, Hunger, and Satiety

Dietary intake records (as described in the previous section) are the general standard of assessing dietary compliance in most nutrition research. Satisfaction and tolerability of nutrition interventions remain greater challenges. In one study, a 30-item satisfaction questionnaire to assess dietary satisfaction was developed and tested prior to the study period. During validation, it was found that all items on the questionnaire correlated strongly with one simple visual analog scale (VAS):

"Rate your overall satisfaction with the way you are eating"

EXTREMELY DISLIKE 1 2 3 4 5 LIKE VERY MUCH

This visual analog scale was then adopted as the only measure of dietary satisfaction within that study, as it correlated strongly to adherence in participants (52). To date, only one questionnaire has been validated specifically for use in those with DM2 – the Diabetes Dietary Satisfaction and Outcomes Measure (DDSOM) (53). The DDSOM is a 47-item questionnaire assessing a range of perceptions using a 5-point VAS. Twenty-three items are dedicated to dietary satisfaction and ability to follow dietary recommendations, while the remaining items assess strategies for following dietary recommendations and the barriers to adhere to those meal plans. Satisfaction as determined by the DDSOM correlated strongly to adherence and lower HbA1c values in participants following meal plans and general dietary recommendations from dieticians (53). However, it remains to be seen if the DDSOM can be adapted to a broad range of dietary therapies, or simply standard medical nutrition interventions – as many of items on the questionnaire were specific to certain recommendations.

Hunger and satiety have been routinely measured in many dietary studies with the use of a VAS. VAS for hunger and satiety can be used to predict subsequent meal intake, act as a complementary measure when accounting for energy intake, can be manipulated in response to experimental changes in dietary habits, and show strong test-retest reliability within individuals in controlled settings (54–56). VAS for hunger and satiety can vary in their appearance and rating scale, although they are typically 100mm in length. Some VAS use numbers with corresponding descriptions while others use only descriptions with no corresponding numbers. Hunger and satiety are often measured by several VAS relating to hunger (prior to meal), stomach fullness (after meal), and motivation to continue eating (after meal) (55–58). It is important to note that these are purely subjective measures that are greatly impacted by social,

emotional, and cognitive factors and may not always accurately depict physiological hunger and satiety in free-living conditions as a result of an experimental dietary intervention.

1.4 Common Pitfalls of Medical Nutrition Interventions in DM2

Current guidelines for nutritional intervention in the management of individuals with DM2 come from the Canadian Diabetes Association (CDA), which promotes the use of the Canada Food Guide. As such, the CDA promotes the use of calorie and macronutrient counting, food grouping, portion control, and the glycemic index for the management of DM2 – all dietary practices that require a certain level of baseline knowledge, skills, and dedication that many people with DM2 are often unable to understand, acquire and implement (59). Even a dietary skill as fundamental as counting calories led to a significant increase in self-perceived psychological stress, and subsequently increased midday and evening cortisol levels – effects not seen when participants restricted calories unintentionally (60,61). Increasing cortisol levels through either cortisol administration (62) or psychological stress (63) have been shown to increase *ad lib* caloric intake. The effect of broad spectrum psychological stress on increased food intake is specifically enhanced in people intentionally trying to restrict food intake (64). An innumerable amount of practical barriers await individuals with DM2 who clearly understand nutrition recommendations.

There are additional barriers in the translation of nutrition guidelines for individuals who do not clearly understand nutrition. Behavioral obesity treatment applied by primary care physicians often do not result in significant weight loss (65). Considering health care workers may not have a more complete understanding of nutrition guidelines when compared to the

general population (66), it is understandable why such interventions are at best marginally effective.

Direct consultations with dieticians are more promising, as they have shown to lead modest reductions in fasting glucose and HbA1c (67). However, there are significant barriers that regularly prevent patients from attending diabetes self-management programs (68). Even when given a descriptive meal plan by a registered dietician, individuals with DM2 often do not follow these plans because of their difficulty to implement and because of the explicit restriction of food choices (69).

Individuals with DM2 list frustration, helplessness, unpredictable glycemic control, and continued disease progression as a primary barrier to motivation and continued adherence – despite adherence past recommendations (59). The outlook can be bleak for individuals with DM2. The UKPDS study showed that 75% of newly diagnosed individuals with DM2 required additional therapeutic interventions after nine years of intensive treatment with diet, insulin, sulfonylureas or metformin. Moreover, only 8-42% of individuals were able to achieve the glycemic targets at any point in the UKPDS study (7). One cannot blame individuals with DM2 for their negative outlook and inability to self-manage their disease. However, the current paradigm that emphasizes management over reversal is a likely cause of the inevitable progression of DM2.

Multiple studies have confirmed a relevant finding: DM2 is reversible in the majority of patients through diet alone. Previous trials with simple and aggressive recommendations (i.e. 600-1200 kcal/day with meal replacements and vegetables) that achieved moderate weight loss (15), or induced an acute energy deficit without significant weight loss (70) resulted in a complete reversal of DM2. These studies demonstrated that a very low-calorie diet is effective at

reversing DM2 but, with dropout rates in excess of 20%, leave much to be desired in terms of adherence and tolerability.

Simple nutritional interventions (interventions that do not require extensive training) have been used in the past to significantly improve outcomes in other disease states. The Lyon Diet Heart Study tested the effects of the Mediterranean Diet (explained in only a single 1-hour education session to participants) on cardiovascular outcomes in patients recovering from myocardial infarction and demonstrated a significant reduction in the re-occurrence of coronary events (71). It's important to note that the Mediterranean Diet as it was taught in this study required minimal patient education and guidance, did not advocate any skill based dietary habits (calorie counting, glycemic index, etc.) and only made very broad, sweeping, and generalized guidelines for dietary behaviors. Individuals with DM2 are in desperate need of similarly effective and simple nutritional interventions for the reversal and prevention of DM2, but that address specific issues in those with DM2.

1.5 Effects of Fasting

1.5.1 Prolonged Fasting, Diurnal Variations, and Dietary Intake in DM2

In the memoirs of Dr. George Cahill entitled "Fuel Metabolism in Starvation", which summarized a lifetime of medical research on starvation, fasting, and diabetes, Dr. Cahill notes:

> "We also fasted two type 2 diabetics, who differed from [healthy patients] by better nitrogen conservation. They were slightly more efficient, in keeping with the concept of James Neel (at Michigan) that type 2 diabetes may have been an evolutionary selective advantage in a starving population". (72)

These results were subsequently supported, in that individuals with DM2 were shown to produce more ketones in response to starvation, indicating that they were more efficient at producing the most dense and efficient energy substrate during starvation (73). Faiman & Moorhouse (73) also noticed a significant diurnal rhythm under fasting conditions now commonly referred to as "The Dawn Phenomenon", where a dramatic increase in fasted morning glucose levels is observed. The Dawn Phenomenon has since been observed in studies on individuals with DM2 (74). The Dawn Phenomenon is generally attributed to a morning increase in cortisol. An experimental administration of the cortisol inhibitor metyrapone resulted in a decrease of plasma cortisol and blood glucose during a 12h fasted morning test in those with DM2 (75), and hyperglycemia is routinely observed in those who suffer from hypercortisolism (76). It has also been observed that lean patients with DM2 and glucose intolerance had an enhanced sensitivity to cortisol, which contributed significantly to hyperglycemia (77). Although healthy non-diabetic individuals experience an increase in morning fasted cortisol, this is attenuated by an increase in insulin secretion in healthy individuals but not in those with DM2 (78,79). It has also been observed that cortisol administration suppresses peripheral and hepatic insulin sensitivity, even in healthy individuals (80). Given the interaction between glucose, insulin, and cortisol, is it reasonable to infer that time of day may affect the metabolic response to meal ingestion in individuals with DM2?

It has been routinely observed that healthy individuals are most insulin sensitive in the morning (81) and when fed breakfast (82), with worsening glucose tolerance during the evening (81,83). Despite the results of acute observational studies, randomized control trials in healthy individuals testing breakfast inclusion or omission have had mixed results (84). In those with DM2, one of the few trials controlling for total caloric intake demonstrated that consuming 70%

of daily calories after 1900h resulted in lower 24h insulin and glucose concentrations and improved insulin sensitivity, with no abnormal elevation in night time and morning fasted glucose on the following day (85). Individuals with DM2 studied with the hyperglycemic clamp showed a very clear diurnal rhythm: insulin sensitivity reached a peak at 7PM and a nadir in the morning at 8AM. Insulin sensitivity was inversely related to measures of cortisol and free fatty acid, which both showed clear diurnal rhythms as well (86). Additionally, it was shown that a snacking meal pattern (3 meals + 3 snacks a day) led to greater mean 24h serum glucose when compared to less frequent (3 meals per day) meal intake (85). However, 3 meals with snacks is a first line nutrition recommendation from the Canadian Diabetes Association for the management of DM2 (87). A previous trial has demonstrated the difficulty of managing morning hyperglycemia in individuals with DM2. It was shown that the largest glucose excursions occurred in between the time of breakfast and lunch in individuals with DM2 irrespective of BMI, HOMA, HbA1c, and B-cell function (88). These striking observations highlight that alternative meal timing strategies have the potential to greatly influence glucose levels and in turn impact the development of chronic complications in those with DM2 over that of the currently recommended dietary strategy in DM2 management.

1.5.2 Review of Intermittent Fasting Clinical Trials

One simple dietary intervention is Intermittent Fasting (IF), whereby caloric intake is restricted to a specific window of time followed by feeding within a restricted window of time. There are many variations of IF. One popularized method of IF restricts caloric intake for 18 to 20 hours per day with unrestricted zero-calorie water, coffee and tea intake permitted during this time. This method includes a 4-6 hour *ad libitum* feeding period, typically during midday or

evening, that emphasizes high protein intake. This version of IF is particularly interesting because meal intake occurs during the periods of time when those with DM2 reach a diurnal peak in insulin sensitivity, and fasting occurs when cortisol and free fatty acids are at their diurnal peaks. Similar protocols have been studied (57,58,89,90) in non-diabetic populations with beneficial effects on insulin-mediated glucose uptake, improved insulin inhibition of lipolysis, reduced basal cortisol levels, loss of body fat, and increases in the anti-diabetic hormone adiponectin – all in the absence of caloric restriction and/or weight loss. Under metabolic ward conditions, participants felt too full when trying to consume an entire day's worth of calories in the 4 hour feeding window, so much so that participants still lost a marginal amount of weight despite active encouragement from staff to eat more (57,58). This lends credibility to the hypothesis that IF would create a spontaneous energy deficit in free-living conditions when practiced consistently, a necessity for the reversal of DM2 (4). However, one study did note that morning FPG, glycemic control, and first phase insulin secretion during an OGTT worsened after 1 month of eucaloric IF (58). The health effects of IF in healthy individuals or those with DM2 have yet to be fully understood.

The popular media and the medical community have been recently discussing the potential of IF to prevent or treat cardiovascular disease and DM2 (91). However, only two trials have assessed the effects of IF in individuals with DM2. One multi-day randomized crossover trial compared the effects of three dietary intake patterns with equal energy intake, low-fat breakfast + lunch vs. low-carbohydrate breakfast + lunch vs. fasting + Mediterranean-style lunch (92). The Mediterranean lunch resulted in glucose, insulin, and triglyceride excursions comparable to the low-fat lunch, similar triglyceride excursions compared to the low-carbohydrate lunch, and enhanced GIP excursions compared to both the low-fat and low-

carbohydrate lunches. However, due to the extended morning fast, the fast + Mediterranean lunch condition resulted in overall decreases in glucose and insulin concentrations despite energy intake being equalized. Results of a 3-month randomized crossover IF trial on individuals with DM2 had participants consume their meals in the morning and early afternoon, while abstaining from caloric intake for the remainder of the day (93). Compared to a standard hypocaloric diet, the IF diet showed a superior decrease in HbA1c, superior response to an OGTT, as well as superior decreases in FPG and morning fasted glucagon that correlated strongly to a superior decrease in overall hepatic fat content. Although inconsistencies exist regarding the effects of eucaloric IF in healthy individuals, preliminary results for hypocaloric IF in individuals with DM2 seem promising.

1.6 High-Protein Diets in Weight Loss and Management of DM2

High-Protein Diets (HPDs) are not well defined in the literature, but remain highly popularized as integral parts of many commercial diets. Commonly used cut-offs for high protein intake are 1.5-2.0g/kg of lean body mass or 30% of daily energy intake (94–97). There is considerable evidence of the positive effects of HPDs. Improvements in metabolic risk factors, and decreases in abdominal fat mass, weight, and HbA1c have been observed with the use of HDPs by individuals with DM2 (94,95,97). HPDs have been shown to increase the retention of lean mass in individuals during a hypocaloric diet while improving cardiovascular risk markers compared to diets with standard and/or low levels of protein (98–100). The positive effects of HPDs have been primarily attributed to enhanced satiety and postprandial thermogenesis, with both mechanisms independently contributing to an overall negative energy balance (96). Despite the success of HPDs in clinical trials, one criticism of HDPs remains popularized.

The use of HPDs in the treatment of DM2 have been said to increase the risk of kidney damage due to an increased rate of nitrogen excretion (30). However, a recent trial has elucidated that GFR is not affected in individuals with DM2 and Stage 1-3 renal disease when dietary protein is increased to 30% of daily caloric intake during a hypocaloric diet (101). This trial showed that GFR improved with weight loss during a HPD. Given these results, little to no safety concerns remain regarding the use of HPDs for the treatment of DM2.

Increased protein intake in individuals with DM2 without severe renal disease is safe, promotes the reduction in *ad lib* calorie intake, improves satiety, retains lean mass, increases thermogenesis, and improves cardio-metabolic risk factors in DM2. Therefore, clinicians and dieticians should consider integrating higher protein intake into current nutrition guidelines and practices for individuals with DM2.

1.7 Effects of Coffee and Tea Consumption on Individuals with DM2

A common and overlooked aspect of clinically tested IF regimens are the allowance of zero calorie coffee and tea intake on metabolic risk markers in DM2. Although no clinical trials have been done to assess the long-term effects of increasing coffee and tea intake in individuals with DM2, there is an abundance of observational studies on the effects of coffee and tea intake on DM2, and several short-term trials assessing the acute effects of coffee and tea intake on metabolism in healthy individuals. The highest percentiles of coffee intake have been implicated in an inverse relationships with the development of DM2 (102), total mortality, cardiovascular disease, and stroke in those with DM2 (103,104), and weight (105). Acute coffee ingestion has been shown to increase metabolic rate, thermogenesis and restrict *ad libidum* energy intake (106,107), and increase antioxidant capacity (108). Acute coffee ingestion also promotes the

secretion of Glucagon-like Peptide 1 (GLP-1) (109), a pro-insulinogenic anti-hyperglycemic incretin which has therapeutic implications for the treatment of DM2 by suppressing hunger and restricting energy intake (110), as well as in vitro evidence that it may increase beta cell mass (111). However, long-term randomized clinical trials on coffee and tea intake are needed in order to confirm the results of these short-term and observational studies.

1.8 Risk of Hypoglycemia during Fasting – Evidence from Ramadan

Little to no information exists on the potential hypoglycemic effects of IF on individuals with DM2, particularly for those who are on glucose lowering medications. The study by Kahleova et al. did not report on the occurrence of hypoglycemia in relation to fasting duration or medication during IF (93). However, information on the risk of hypoglycemia during a prolonged fast is present in other practices that share similarities to IF.

Ramadan is an Islamic holy month whereby religious practitioners abstain from all food and liquid intake from sunrise to sunset for 29-30 consecutive days. Ramadan is markedly different compared to IF: it restricts all fluid intake during fasting hours (112), may disturb sleep patterns (113), has variable length and therefore metabolic effects dependent on time of year and location (114), and typically involves meal intake in both the early morning and late evening. However, it can be used to assess the risk of hypoglycemia during prolonged fasting, as individuals observing Ramadan fast for 12-20 hours consecutively each day. It has been shown that during this time, rates of hyperglycemia requiring hospitalization decline among those with DM2, while rates of hypoglycemia requiring hospitalization increase – the latter due primarily from the use of sulfonylureas, improper insulin administration, and hypoglycemic unawareness (115).

Current recommendations for individuals with diabetes during Ramadan that are relevant to those with DM2 practicing IF is the use of frequent self-monitoring of glucose levels, immediately breaking the fast if hypoglycemic (blood glucose <3.9 mmol), not fasting while ill, a medical assessment and patient education by a physician before beginning a month of fasting, altering the timing and method of administering medications during Ramadan, and finally, not engaging in Ramadan if certain medications are taken (see Table 1) (116). Considerable attention must be paid to the type of medication and health status of the individual before engaging in fasting.

Table 1.1 – Risk of Hypoglycemia from Diabetes Medications during Ramadan: Summary of results from Al-Arouj et al. (116)

Medication	**Recommendation**
Metformin	Metformin is generally considered safe, as the risk of hypoglycemia during Ramadan is relatively low.
Sulfonylureas	The use of sulfonylureas should be individualized for each patient. An adequate history of prior hypoglycemic occurrence should be considered before Ramadan. Certain sulfonylureas should be considered safe. The newer generation of these medications (gliclazide, glimepiride) have a lower risk of hypoglycemia. The use of chlorpropamide has a high chance of causing hypoglycemia and should not be used during prolonged fasts.
Glitazones	Insulin sensitizers are generally considered safe during Ramadan and present a low risk of hypoglycemia.
Short-Acting Insulin Secretagogues	Repaglinide and Nateglinide are generally considered safe, so long as they are only taken before meals during Ramadan.
Insulin	There is some evidence that the combination use of long acting insulin and short acting insulin can be used safely during Ramadan. However, this takes considerable attention on behalf of the individual and poses a greater risk of hypoglycemia compared to some oral agents.

CHAPTER 2
PURPOSE, OBJECTIVES, AND HYPOTHESIS

2.1 Purpose of Project

According to the Canadian Diabetes Association, nutritional interventions are considered an integral strategy for managing and preventing the complications that may arise in DM2 (29). With this in mind, Intermittent Fasting, a popularized nutritional intervention whereby energy intake is restricted for a pre-defined and extended period of time, may prove to be a beneficial addition to the current nutritional interventions commonly used in standard DM2 treatment. Individuals with DM2 have metabolic adaptations that may be of benefit during a fast, benefit from the creation of a spontaneous caloric deficit and increased protein, coffee and tea intake, and exhibit delayed diurnal insulin sensitivity. As such, IF with a high protein diet may be beneficial for those with DM2. If the IF method described previously proves to be an effective treatment for DM2, it may give those who have failed at implementing standard lifestyle interventions another chance at managing their diabetes. Intermittent Fasting may circumvent the use of costly surgeries or medications, while requiring little alteration in current exercise habits, food choices, and other aspects of lifestyle.

The purpose of this clinical trial is to evaluate the short-term biochemical and behavioral effects of a popularized version of IF on free-living adults with DM2 given minimal education.

2.2 Objectives and Aims

Objective 1

Observe the biochemical and clinical effects of Intermittent Fasting on individuals with Diabetes Mellitus 2.

Specific Aim 1

Reduce fasting plasma glucose, SMBG, C-reactive protein, HOMA-IR, and waist circumference.

Specific Aim 2

Observe the effects on creatinine clearance and weight.

Objective 2

Observe the behavioral effects of Intermittent Fasting on energy intake, macronutrient intake, compliance and tolerability.

Specific Aim 1

Record hours fasted, energy intake, macronutrient composition, and coffee/tea intake.

Specific Aim 2

Assess the tolerability and perception of IF in participants via written questionnaires.

Specific Aim 3

Assess appetite, hunger, and satiety through the use of visual analog scales.

2.3 Hypothesis

We hypothesize that a short-term period of IF in individuals with DM2 will lead to improvements in glycemic control, inflammatory biomarkers, and insulin sensitivity, while demonstrating compliance and tolerability.

CHAPTER 3
METHODS

3.1 Participant Recruitment and Eligibility

3.1.1 Power Analysis and Recruitment

A power analysis was performed, which found that 33 participants were necessary to detect a 3cm change in waist circumference, a 0.75mmol change in C-reactive protein, or a 0.75mmol change in fasting blood glucose with a power of 80% and 95% confidence. We recruited participants from posters placed in general practitioners' offices in the Saskatoon Health Region, Royal University Hospital, and the University of Saskatchewan. Advertisements were also placed in classified advertisements in newspapers and Kijiji. A contact number and e-mail address was displayed on all advertisements. Upon contact from participants, the M.Sc.(c) explained the study in detail over phone, confirmed that prospective participants met inclusion/exclusion criteria, and acquired verbal consent prior to the first meeting and receiving signed consent. Ten participants with DM2 were recruited for the study.

3.1.2 Inclusion/Exclusion Criteria

Individuals with a diagnosis of DM2 (confirmed by fasting glucose >7.0mmol, HbA1c > 6.5%, or OGTT > 11.0mmol) and between the ages of 18-65 were eligible to enroll in this study. Certain medical conditions were excluded from enrollment, such as the presence of ischemic heart disease or heart failure, chronic inflammatory diseases, chronic infections, moderate to severe renal disease (GFR<45), uncontrolled hypertension or hypoglycemic unawareness as these complications may have increased the likelihood of adverse events. Participants with DM2

on glyburide or insulin were excluded from the study due to their increased risk of hypoglycemia, while Dr. Arnason and Dr. Mansell assessed others on a case-by-case basis.

3.2 Study Design

Consenting participants that met inclusion criteria engaged in a six or eight week withdrawal study testing the effects of intermittent fasting. The duration was dependent on the time of recruitment. Initially the study was advertised as a 6-week study, and after 5 participants were recruited the duration of the study was increased to 8 weeks to extend the period of IF. However, the study was changed back to its original 6-week duration in an attempt to increase recruitment. In total, 7 participants completed the 6-week study, and 3 participants completed the 8-week study. After participants met study requirements and signed consent, they were educated on study procedures by a M.Sc.(c) in Pharmacy. Dr. Welihinda or Dr. Arnason then educated participants on the risk and management of hypoglycemia during IF, and acquired a complete family and medical history.

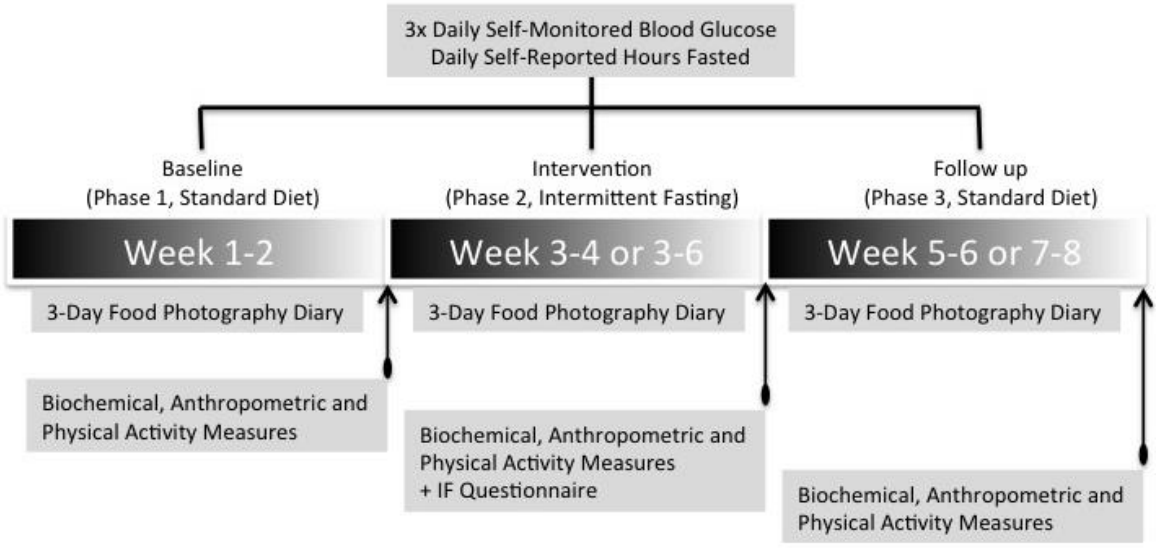


Figure 3.1 – Study Design

During the study, participants engaged in normal dietary patterns (breakfast, lunch and dinner) during weeks 1-2 (baseline) and 5-6 or 7-8 (follow-up) (Figure 1). For weeks 3-4 or 3-6 (intervention) participants followed the IF meal timing pattern of daily fasts for 18-20h per day, followed by a 4-6h feeding periods (see Figure 1.). *Ad libidum* zero-calorie coffee and tea intake during fasting hours were permitted, and an emphasis was placed on high protein foods during the feeding periods for weeks 3-4/3-6. In order to educate participants on high protein food, they were shown images of high-protein foods and given a list of high protein foods with their corresponding protein content by serving size.

3.3 Data Collection and Endpoints

3.3.1 Self-Reporting: Hours Fasted, Self-Monitored Blood Glucose, Remote Food Photography, and Visual Analog Scales

Throughout all study phases participants reported SMBG with the use of a glucometer and logbook that was provided to them by study staff. Participants were instructed to measure blood glucose with their glucometers three times per day: morning (fasted), afternoon (random) and evening (random). In the same logbook, they also kept a diary of total consecutive hours fasted each day. In each of the three time periods (baseline, intervention, follow-up) participants completed a random 3-day food diary using the Remote Food Photography Method (RFPM) (117), in tandem with a visual analog scale for satiety and hunger ratings (54). During these days, participants received customized text message prompts by study staff to ensure compliance with RFPM, in addition to text messages with numbered visual analog scales so they may rate perceived hunger and satiety before and after meals. Participants responded to all text prompts to

confirm that they had adhered to RFPM, as well as sent images of their food (before and after consumption to capture food waste) and the scores of their visual analog scales.

3.3.2 Biochemical and Anthropometric Measurements

Participants underwent fasted blood draws at the Royal University Hospital blood draw clinic on the first and last days of the intervention phase, as well as on the last day of the follow-up phase to determine the acute and sustained effects of IF on glycemic control and insulin resistance (FPG, Fasting Insulin, HOMA-IR), kidney function (Creatinine, Creatinine Clearance), and inflammation (CRP). On each of these days, participants also underwent anthropometric measurements (Height, Weight, BMI, Waist Circumference), as well as the YPAS to control for variations in physical activity. Additional measures, which are not the focus of this thesis, were also collected for ferritin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglycerides (TG).

3.4 Statistical Analysis

All statistical procedures were performed on SPSS v. 22 and STATA v. 13. Data preparation was done using Excel 2011 and STATA v. 13. Significance was set at alpha = 0.05 (95% confidence) for all tests. Trends were identified through visual inspection.

3.4.1 Repeated Measures ANOVA

Ten individuals (9 female, 1 male) completed three blood draws, and the intervention and follow-up periods. Nine individuals (8 female, 1 male) completed the lead-in phase and all SMBG logs, 1 of which submitted incomplete logs of daily hours fasted (HF) for the lead-in and

follow-up phases. Missing means from incomplete logs of SMBG and HF or missing values from blood draws or clinical measurements required for Repeated Measures ANOVA were imputed with the following formulas:

$$Baseline = x1, Intervention = x2, Follow\text{-}up = x3$$

$$x1 = -(\mu\Delta x1\text{-}2 * x2) + x2$$

$$x2 = (\mu\Delta x1\text{-}2 * x1) + x1$$

$$x3 = (\mu\Delta x2\text{-}3 * x2) + x2$$

Data that did not pass the sphericity assumption were interpreted using the Greenhouse-Geisser correction, and data that showed large violations of normality were interpreted with Friedman's test.

3.4.2 Self-Monitored Blood Glucose Data Preparation

During construction of the regression models for SMBG, imputation was not performed. The participant who did not complete the lead-in phase was excluded from the analysis; only participants who completed full SMBG logs for every study phase were included. To account for variance in the length of baseline, intervention, and follow-up phases, participants' logs were equalized at 42 days (14 days for each phase). The equalization procedure was done in a stepwise manner until each study phase contained 14 days:

1) If phase x = 14 days no adjustments were made
2) If phase x > 14 days, days with no data were removed.
3) If phase x > 14 days after Step 2: Days with incomplete data were removed. In the event of a tie (i.e. two days had equal amounts of missing data), the day closest to the beginning or end of a phase was removed to foster independence between experimental conditions.

4) If phase x > 14 days after Step 3: Days at the beginning or end of a phase were removed to foster independence between study phases.
5) If phase x < 14 days, days were added to the end of that phase and left blank.

Three individuals completed an intervention phase with an average duration of 26 days. Because of the significantly larger sample size, the equalization procedure above could not be fully applied without possibly compromising the validity of the regression model. In order to account for any possible variance that occurred due to a result of the prolonged intervention, 3 data sets for morning, afternoon, and evening SMBG (M, A, E) were constructed using the beginning (B), middle (M), and last (E) 14 days of the intervention phase as input for the group means used in the regression models. Afterwards, the equalization procedure was implemented until each phase consisted of 14 days.

3.4.3 Self-Monitored Blood Glucose Regression Models

After data preparation, the group means and standard deviations of days 1 through 42 were calculated individually for three daily measurements: fasted Morning (M), random Afternoon (A), and random Evening (E) SMBG measurements. Additionally, three group means and standard deviations were created for each data set generated: Beginning (B), Middle (M), and End (E). This generated 9 regression models (MB, MM, ME; AB, AM, AE; EB, EM, EE) for both the means and standard deviations (μMB, σMB; μMM, σMM; μME, σME; μAB, σAB: μAM σAM; μAE, σAE; μEB, σEB; μEM, σEM; μEE, σEE) – 18 models in total. Inflection points of quadratic equations were calculated using the formula [$f^{1}(y)$ of $C + Ax + Bx^2 = 0$] rounded down to the nearest full integer. Regressing group means and standard deviations

individually led to the creation of models that could be used to interpret the effect of IF on mean group SMBG, as well as the effect of IF on the variability of group SMBG.

3.4.4 Two-Sample Kolmogorov-Smirnov and Ordinal Logistic Regression Tests

After regression with SMBG data, the two-sample Kolmogorov-Smirnov (KS) test and Ordinal Logistic Regression (OLR) were used to further explore the effects of the experimental intervention as well as the direct impact of fasting on SMBG. Cut-offs for OLR were created using standard guidelines for diabetic fasting and random blood glucose (7.0mmol/L and 11.1mmol/L respectively) (6), with an additional arbitrary stratification of hyperglycemia reflecting the midpoint (9.05mmol/L) for morning, afternoon, and evening SMBG values (Table 2). No category was created to represent hypoglycemic events, as none were recorded throughout the duration of the study. For both the KS test and OLR, full non-equalized SMBG data sets were used.

Table 3.1 – Cut-offs for Ordinal Logistic Regression

Cut-off	Definition
1	<= 7.0mmol/L (Normal Fasting Glucose)
2	7.0mmol/L - 9.05mmol/L (Fasting Hyperglycemia/Random normoglycemia)
3	9.05mmol/L - 11.1mmol/L (Fasting Hyperglycemia/Random normoglycemia)
4	=> 11.1mmol/L (Random Hyperglycemia)

The KS test compared the individual distributions of baseline, intervention, and follow-up phases separately for morning, afternoon, and evening SMBG. Two variables were created for OLR: Hours Fasted Difference (HFD) and Hours Fasted Difference by Percent (HFP):

HFD = Hours Fasted – Average Hours Fasted during baseline

HFP = (HFD/Average Hours Fasted during baseline)*100

OLR was used to elucidate the effect of HFD and HFP on morning, afternoon, and evening SMBG between phases that were shown to have different SMBG distributions with the KS test.

3.5 Ethical Considerations

Privacy and confidentiality are paramount when conducting any human biomedical research. Any paper files are stored in a locked filing cabinet in Dr. Arnason's secure clinical office in Royal University Hospital. All digital data was completely de-identified, and a key to the de-identified data is kept on paper and stored alongside other paper files in Dr. Arnason's office. Patient contact information was stored on the personal password protected and encrypted mobile phone of the M.Sc. (c) under pseudonyms, and all text messages and images were promptly deleted after being sent and/or recorded. Participants were instructed not to include themselves or any identifiers in images or messages sent over text message and e-mail.

All research data will be stored for a period of five years. Paper data is stored in the locked filing cabinet within Dr. Arnason's office, and all digital data will be stored on a USB drive locked within Dr. Arnason's office.

Ethics were obtained in March 2014 from the Research Ethics Office at the University of Saskatchewan.

CHAPTER 4
RESULTS

4.1 Baseline Characteristics of Participants

10 participants completed the study, with 7 individuals completing the 6-week study and 3 individuals completing the 8-week study. The mean age of participants was 53.8 years old (Table 4.1). All participants were on metformin, and most were on other non-diabetic medications. Herbals and supplement use was present in 6 patients at baseline. Only two participants were on other diabetic medications in addition to metformin (liraglutide and a sulfonylurea), and only one participant was an occasional/active smoker (Table 4.2). There were significant variations between participants at baseline in several key measures such as weight, BMI, ferritin, triglycerides, HOMA-IR, CRP, and fasting insulin (Table 4.3). At baseline, participants had confirmed DM2, and on average were obese (BMI > 30.0kg/m^2), with waist circumferences above cut-off values (>88cm in women, >102cm in men).

Table 4.1 – Baseline Characteristics of 10 Participants

Measure	Mean ± Standard Deviation
Age	53.8 ± 9.11 years old
Weight	100.6 ± 21.75 kg
BMI	36.9 ± 8.29 kg/m^2
Waist Circumference	109.6 ± 11.1 cm
Daily Hours Fasted	11.6 ± 1.9 hours/day

Table 4.2 – Baseline Smoking, Medication, and Supplement use

	Currently using
Metformin	10 (10)
Sulfonylureas	1 (10)
Other diabetic medications	1 (10)
Other non-diabetic medications	8 (10)
Herbals and Supplements	6 (10)
Active Smokers	1 (10)

4.2 Biochemical and Anthropometric Changes

Significant differences from baseline to intervention were noted in weight (-1.395kg, p = 0.009) and BMI (-0.517, p = 0.013), with non-significant differences in WC (-1.75cm, p = 0.083). CRP lowered from baseline to intervention in 8 of 10 participants, however, a large magnitude increase in CRP in a single participant during IF rendered the results non-significant. From baseline to follow-up there were non-significant decreases in weight (-1.120kg, p = 0.078), and BMI (-0.417kg/m^2, p = 0.083), while all other parameters had a tendency to regress to baseline values during the follow-up period. No significant differences were observed in any other parameters between any time points (Table 4.4).

Table 4.3 - Descriptive Statistics and Assumptions for Biochemical and Anthropometric Parameters

Outcome	**Measure**	**Baseline**	**Intervention**	**Follow-up**	**Sphericity**	**Residual Normality**
Ferritin (μg/L)	Mean	118.30	123.70	118.30	**No**	**No**
(RR: 20-120)	Std. Dev	98.48	96.57	92.66		
T/HDL	Mean	3.52	3.44	3.57	Yes	Yes
(RR: < 3.5)	Std. Dev	0.73	0.97	1.08		
LDL (mmol/L)	Mean	2.51	2.38	2.43	Yes	**No**
(RR: 2.2-3.4)	Std. Dev	0.76	0.95	0.82		
HDL (mmol/L)	Mean	1.31	1.32	1.31	Yes	Yes
(RR: 0.9-2.4)	Std. Dev	0.20	0.27	0.30		
Triglycerides (mmol/L)	Mean	1.57	1.52	1.72	Yes	Yes
(RR: 0.6-2.3)	Std. Dev	0.91	0.74	0.90		
TC (mmol/L)	Mean	4.53	4.39	4.52	Yes	Yes
(RR: 4.2-5.2)	Std. Dev	0.77	0.99	1.05		
C-Reactive Protein (mg/L)	Mean	4.31	3.97	4.06	Yes	Yes
(RR: < 1.0)	Std. Dev	3.80	3.71	3.47		
Fasting Insulin (pmol/L)	Mean	129.29	116.57	116.06	Yes	Yes
(RR: 43.0-194.0)	Std. Dev	62.21	44.38	57.43		
Glucose (mmol/L)	Mean	7.45	7.61	7.93	Yes	Yes
(RR: 3.6-6.0)	Std. Dev	1.52	1.02	1.58		
HOMA-IR	Mean	6.91	6.45	6.56	Yes	Yes
N/A	Std. Dev	3.00	2.39	3.04		
Creatinine (μmol/L)	Mean	63.80	63.40	62.40	Yes	Yes
(RR: 45-90)	Std. Dev	5.90	6.48	7.46		
CC_r (mL/min)	Mean	149.01	147.90	151.50	Yes	Yes
(RR: > 90)	Std. Dev	46.98	51.35	53.32		
Weight (kg)	Mean	100.6	99.21	99.48	Yes	Yes

	Std. Dev	21.75	21.33	21.48		
BMI (kg/m^2)	Mean	36.90	36.38	36.48	Yes	Yes
(RR: < 25.0)	Std. Dev	8.29	8.10	8.14		
Systolic BP (mmHg)	Mean	130.00	127.00	128.50	Yes	Yes
(RR: 90-130)	Std. Dev	17.80	21.39	14.28		
Diastolic BP (mmHg)	Mean	80.50	79.78	81.70	Yes	Yes
(RR: 60-90)	Std. Dev	13.20	15.66	12.25		
Waist Circumference (cm)	Mean	109.55	107.80	107.50	**No**	Yes
(RR: <88cm, <102cm)	Std. Dev	11.08	11.09	10.86		
Daily Hours Fasted	Mean	11.61	16.82	11.52	Yes	Yes
N/A	Std. Dev	1.89	1.18	2.01		
Days In Phase	Mean	15.10	18.20	15.8	N/A	N/A
N/A	Std. Dev	1.97	5.69	4.10		
μMorning SMBG (mmol/L)	Mean	8.16	7.73	8.09	**No**	Yes
N/A	Std. Dev	1.28	1.823	1.39		
μAfternoon SMBG (mmol/L)	Mean	7.47	7.1659	7.02	Yes	Yes
N/A	Std. Dev	0.99	1.16	0.90		
μEvening SMBG (mmol/L)	Mean	8.71	8.58	8.76	Yes	Yes
N/A	Std. Dev	1.87	1.89	1.68		

T/HDL = Total cholesterol:HDL ratio, HDL = High-Density Lipoprotein, LDL = Low-Density Lipoprotein, TC = Total Cholesterol, HOMA-IR = Homeostasis Model Assessment, CC_r = Creatinine Clearance, BMI = Body Mass Index, BP = Blood Pressure, μ = average, SMBG = Self-Monitored Blood Glucose, RR = Reference Range

Table 4.4 – Percent Change Between Study Phases for Biochemical and Anthropometric Parameters

Outcome (Δ= % change)	Measure	Baseline to Intervention	Intervention to Follow-up	Baseline to Follow-up
ΔFerritin	Mean	+9.85%	-3.06%	+5.99%
	Std. Dev	19.60	8.70	19.88
ΔT/HDL	Mean	-3.03%	+5.08%	+1.16%
	Std. Dev	12.12	17.20	16.49
ΔLDL	Mean	-6.34%	+5.95%	-3.10%
	Std. Dev	18.05	17.90	12.54
ΔHDL	Mean	+0.85%	+0.37%	+0.13%
	Std. Dev	10.18	19.49	14.89
ΔTriglycerides	Mean	+5.31%	+19.58%	17.96%
	Std. Dev	37.75	33.25	33.93
ΔTC	Mean	-3.56%	+3.36%	-0.90%
	Std. Dev	10.16	10.46	9.38
ΔC-Reactive Protein	Mean	-5.75%	+47.18%	+23.95%
	Std. Dev	37.49	155.07	119.96
ΔInsulin	Mean	-3.38%	+0.095%	-7.64%
	Std. Dev	35.05	31.43	36.49
ΔGlucose	Mean	+3.89%	+3.69%	+7.10%
	Std. Dev	11.43	12.60	13.24
ΔHOMA-IR	Mean	+1.70%	+3.96%	-0.10%
	Std. Dev	42.88	35.01	42.72
ΔCreatinine	Mean	-0.50%	-1.28%	-2.19%
	Std. Dev	7.25	9.56	6.81
ΔCC_r	Mean	-0.94%	+1.97%	+0.67%
	Std. Dev	8.11	9.52	9.20
ΔWeight	Mean	-1.36%	+0.27%	-1.10%
	Std. Dev	1.02	1.12	1.31
ΔBMI	Mean	-1.35%	+0.26%	-1.10%
	Std. Dev	1.02	1.12	1.31
ΔSystolic BP	Mean	-2.95%	+0.62%	-2.31%
	Std. Dev	6.24	7.30	6.40
ΔDiastolic BP	Mean	-2.31%	+2.58%	+0.98%
	Std. Dev	14.24	5.90	12.08
ΔWaist Circumference	Mean	-1.60%	-0.26%	1.85%
	Std. Dev	1.98	1.78	3.13
ΔDaily Hours Fasted	Mean	+30.74%	-46.19%	-1.72%
	Std. Dev	11.58	26.88	13.65
ΔμMorning SMBG	Mean	-8.54%	+5.07%	-1.35%
	Std. Dev	22.10	9.67	10.76

ΔμAfternoon SMBG	Mean	-5.05%	-1.76%	-6.32%
	Std. Dev	10.18	7.40	8.34
ΔμEvening SMBG	Mean	-2.02%	+1.82%	+1.17
	Std. Dev	11.16	10.82	6.86

T/HDL = Total cholesterol:HDL ratio, HDL = High-Density Lipoprotein, LDL = Low-Density Lipoprotein, TC = Total Cholesterol, HOMA-IR = Homeostasis Model Assessment, CC_r = Creatinine Clearance, BMI = Body Mass Index, BP = Blood Pressure, μ = average, SMBG = Self-Monitored Blood Glucose

4.3 Self-Monitored Blood Glucose

Nine participants self-reported fasted morning, random afternoon, and random evening SMBG with the use of a provided glucometer and logbook throughout all study phases. Among these participants, adherence to SMBG reporting was highest for fasted morning readings with only 7 missing data points, followed by random afternoon and evening with 48 and 53 data points missing among all participants, respectively. Throughout the study duration, participants recorded a total of 409 morning fasted, 368 random afternoon, and 363 random evening SMBG readings.

4.3.1 Regression Models of Self-Monitored Blood Glucose

SMBG Regression Models were built using the means from 9 participants who completed full SMBG logs. All 18 data sets and their residuals passed the normality assumptions necessary for simple linear regression using Shapiro-Wilk (Table 4.5) and visual inspection of histograms and Q-Q plots with the exception of the residuals from the linear models for μEB and σME ($p < 0.05$). All models were clear of autocorrelation, which was confirmed via analysis of residual distributions and ACF graphs.

Table 4.5 – Regression Assumptions for Self-Monitored Blood Glucose

	Data			Residuals		
	Statistic	df	P-value	Statistic	df	P-Value
μMB-Q	.958	42	.130	.972	42	.396
σMB-Q	.978	42	.593	.972	42	.393
μAB-L	.977	42	.558	.980	42	.642
σAB-L	.981	42	.713	.973	42	.407
μEB-L	.948	42	.054	**.947**	**42**	**0.49**
σEB-L	.986	42	.876	.981	42	.711
μMM-Q	.981	42	.717	.989	42	.958
σMM-Q	.964	42	.206	.964	42	.203
μAM-L	.971	42	.345	.967	42	.261
σAM-L	.971	42	.354	.966	42	.234
μEM-L	.977	42	.531	.973	42	.426
σEM-L	.983	42	.770	.989	42	.947
μME-Q	.977	42	.538	.983	42	.203
σME-Q	.968	42	.280	**.941**	**42**	**.032**
μAE-L	.950	42	.066	.954	42	.087
σAE-L	.975	42	.473	.969	42	.295
μEE-L	.988	42	.929	.988	42	.935
σEE-L	.968	42	.288	.983	42	.774

Q = Quadratic model, L = Linear model, μ = average, σ = standard deviation, MB = morning with beginning 14 days data, MM = morning with middle 14 days glucose data, ME = morning with end 14 days glucose data, AB = afternoon with beginning 14 days glucose data, AM = afternoon with middle 14 days glucose data, AE = afternoon with end 14 days glucose data, EB = evening with beginning 14 days glucose data, EM = evening with middle 14 days glucose data, EE = evening with last14 days glucose data, df = degrees of freedom

Table 4.6 contains the full set of quadratic and linear models of SMBG generated with their accompanying statistical information. All quadratic models for μMorning SMBG were significant ($p < 0.0005$) and showed moderate to weak R^2 values with distinct nadirs during the IF phase, indicating a decrease in average fasted morning SMBG during the IF intervention. All quadratic models for σMorning SMBG were significant ($p < 0.0005$) and showed moderate R^2 values with distinct peaks in the IF

phase, indicating an increase in SMBG variability during the IF intervention. All models for μAfternoon SMBG showed trends of decreasing SMBG levels throughout the entire study period ($p = 0.108, 0.133, 0.158$), but R^2 values indicated the models were weak. Many models for μAfternoon became weaker once non-significant coefficients were dropped (data not shown). σAfternoon SMBG showed weak accordance with quadratic models, giving some indication that the variability of afternoon SMBG may have increased marginally during the IF phase. Models for μEvening SMBG had no accordance with any linear or non-linear models (all $p > 0.1$, data not shown). However, σEvening SMBG were significant (all $p < 0.05$), indicating that variability decreased linearly over the entire study period, but these models were relatively weak ($R^2 < 0.15$). Inflection points, which are representative of either the peak or nadir of the quadratic models, were calculated to lie within or immediately after the intervention phase (Days 14-28). In summation, mean morning SMBG values decreased during IF and regressed towards baseline during follow-up (Figure 4.1), while morning SMBG variability increased as a result of IF (Figure 4.2). Mean afternoon SMBG, mean evening SMBG, and afternoon SMBG variability did not significantly change over the study period, but evening SMBG variability decreased linearly throughout the entire study's duration (Figure 4.3). These models suggest decreased glycemic variability in the evening, and more variable but lower mean fasted morning glycemia.

Table 4.6 - Self-Monitored Blood Glucose Regression Models

Measure	*Model*	*R*	R^2	*F-stat.*	*P-value*	*Coefficients*	*P-values*	*IFP*
μMB	Quad	0.664	0.441	15.376	< 0.0005	$8.728 - 0.072Day + 0.002Day^2$	All < 0.0005	18
σMB	Quad	0.699	0.488	18.617	< 0.0005	$0.787 + 0.085Day - 0.002Day^2$	All < 0.0005	21
μMM	Quad	0.593	0.352	10.581	< 0.0005	$8.714 - 0.066Day + 0.001Day^2$	All < 0.0005	33
σMM	Quad	0.706	0.498	19.332	< 0.0005	$0.752 + 0.097Day – 0.002Day^2$	All < 0.0005	24
μME	Quad	0.537	0.288	7.903	0.001	$8.67 – 0.056Day + 0.001Day^2$	All < 0.0005	28
σME	Quad	0.692	0.479	17.942	< 0.0005	$0.815 + 0.088Day – 0.002Day^2$	All < 0.0005	22
μAB	Linear	0.251	0.063	2.698	0.108	$7.642 – 0.01Day$	< 0.0005, 0.108	
σAB	Linear	0.286	0.082	3.552	0.067	$1.860 – 0.013Day$	< 0.0005, 0.067	
	Quad	0.414	0.171	4.022	0.026	$1.475 + 0.040Day – 0.001Day^2$	< 0.0005, 0.139, 0.047	20
μAM	Linear	0.236	0.056	2.354	0.133	$7.668 – 0.01Day$	< 0.0005, 0.133	
σAM	Linear	0.259	0.067	2.865	0.098	$1.876 – 0.013Day$	< 0.0005, 0.098	
	Quad	0.395	0.156	3.604	0.037	$5.297 + 0.045Day – 0.001Day^2$	< 0.0005, 0.049, 0.0132	23
μAE	Linear	0.222	0.049	2.075	0.158	$7.658 – 0.01Day$	< 0.0005, 0.158	
σAE	Linear	0.293	0.086	3.756	0.06	$1.897 – 0.014Day$	< 0.0005, 0.06	
	Quad	0.424	0.179	4.262	0.021	$1.478 + 0.043Day – 0.001Day^2$	< 0.0005, 0.129, 0.041	22
μEB	None							
σEB	Linear	0.319	0.102	4.525	0.040	$2.663 – 0.016Day$	< 0.0005, 0.04	
μEM	None							
σEM	Linear	0.333	0.111	5.001	0.031	$2.781 – 0.018Day$	< 0.0005, 0.031	
μEE	None							
σEE	Linear	0.329	0.108	4.850	0.033	$2.687 – 0.017Day$	< 0.0005, 0.033	

Quad = Quadratic model, Linear = Linear model, μ = average, σ = standard deviation, MB = morning with beginning 14 days data, MM = morning with middle 14 days glucose data, ME = morning with end 14 days glucose data, AB = afternoon with beginning 14 days glucose data, AM = afternoon with middle 14 days glucose data, AE = afternoon with end 14 days glucose data, EB = evening with beginning 14 days glucose data, EM = evening with last 14 days glucose data, EE = evening with middle 14 days glucose data, IFP = Inflection Points

Figure 4.1 – Mean Morning Fasted Self-Monitored Blood Glucose

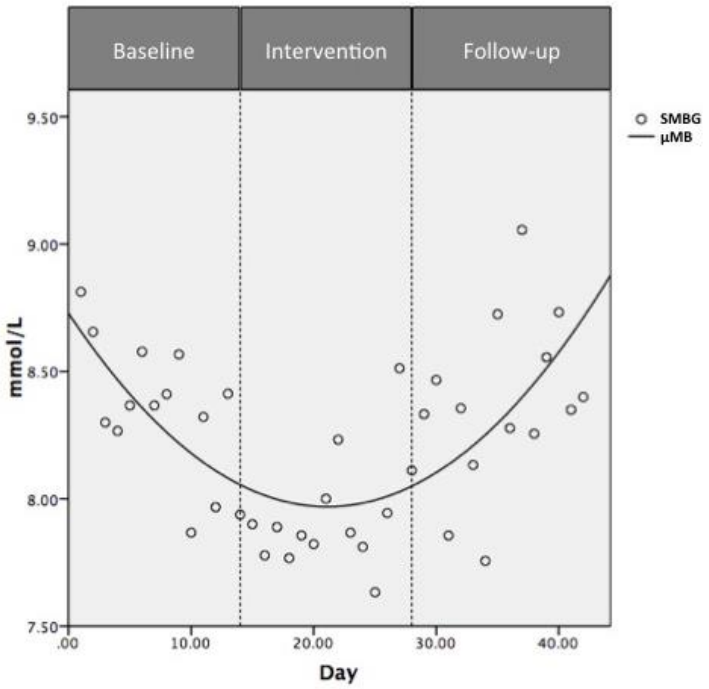


Figure 4.2: Morning Fasted Self-Monitored Blood Glucose Variability

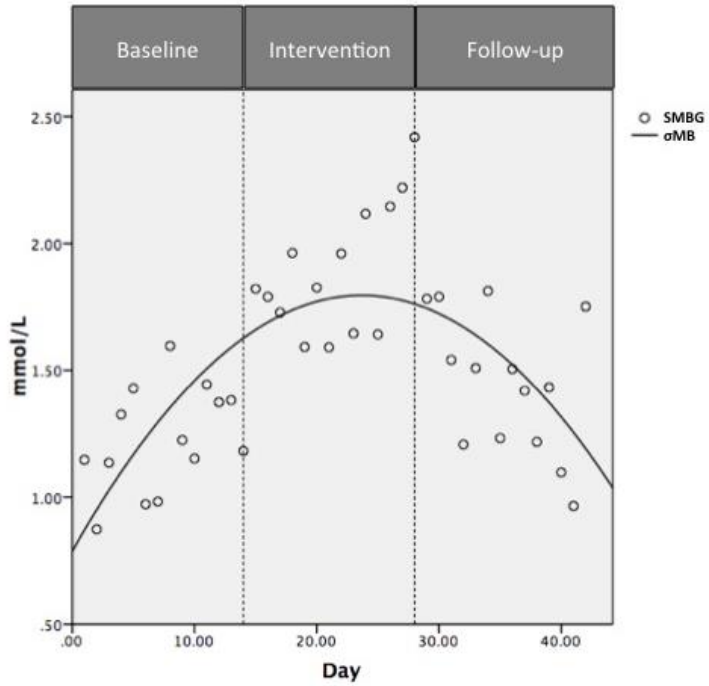
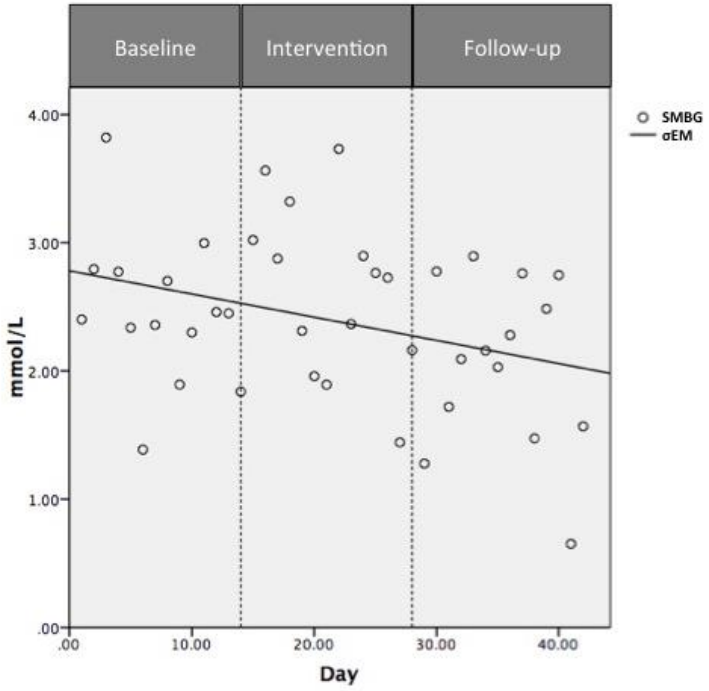


Figure 4.3: Evening Random Self-Monitored Blood Glucose Variability



4.3.2. Self-Monitored Blood Glucose Distributions

The Kolmogorov-Smirnov (KS) test was used to analyze data from all 9 individuals who had completed their full SMBG logs. Unlike the regression models, the KS test used the non-equalized data sets, as non-equalized and equalized data sets showed good agreement and non-equalized data sets made full use of all the collected data.

Results of the KS test indicated that the distribution of morning SMBG was different between baseline and intervention phases ($p = 0.002$) and between intervention and follow-up phases ($p = 0.003$), but there was no difference detected between baseline and follow-up phases ($p = 0.55$). There was a significant difference between intervention and follow-up phases for evening SMBG distributions ($p = 0.044$), but not between any other phases (all $p > 0.1$). No

significantly different distributions were detected between any phases for afternoon SMBG (all $p > 0.1$).

In order to investigate the nature of the difference in distributions seen in the KS test, raw counts and percentages for categories used in OLR were tabulated for each phase. For morning SMBG, there was a 20.3% percent increase in the occurrence of SMBG < 7.0 mmol/L, a 6.3% increase in the occurrence of SMBG => 11.1 mmol/L, and a 26.6% decrease in the occurrence of SMBG 7.0-11.1 mmol/L from baseline to intervention phase (Table 4.7). This confirmed that the increase in variability seen in morning SMBG regression models (section 4.3.1) was due primarily to an increase in the frequency of normoglycemia and a decrease in the frequency of hyperglycemia (7.0 - 11.1mmol/L) during the intervention phase. This also confirms that the decrease in mean fasted morning SMBG seen in the regression models (section 4.3.1) was valid, and the increase in SMBG variability was due primarily to an increase in normoglycemia (SMBG < 7.0mmol/L).

For evening SMBG, there was a 17.7% increase in the frequency of SMBG 7.0 – 11.1 mmol/L, a 14.7% decrease in SMBG < 7.0 mmol/L, and a 2.9% decrease in SMBG => 11.1 mmol/L from intervention to follow-up (Table 4.8). Even though no significant changes in mean evening SMBG were found in regression models (section 4.3.1), this suggests that the decrease in evening SMBG variability was likely due to an overall worsening of glycemic control during the follow-up period. However, since mean evening SMBG regression models in Section 4.3.1 found no significant change in mean evening SMBG and the KS test found no difference between the distributions of baseline and follow-up evening SMBG, we cannot make any conclusive statements on IF's impact on evening glycemic control.

Table 4.7 – Morning Self-Monitored Blood Glucose by Phase

SMBG (mmol/L)	Baseline	Intervention	Follow-up
< 7.0	17 (13.8%)	57 (34.1%)	18 (15.1%)
7.0 - 9.05	64 (52.0%)	68 (40.7%)	59 (49.6%)
9.05 - 11.1	41 (33.3%)	30 (18.0%)	39 (32.8%)
=> 11.1	1 (0.8%)	12 (7.1%)	3 (2.5%)
Total	123	167	119

Table 4.8 – Evening Self-Monitored Blood Glucose by Phase

SMBG (mmol/L)	Baseline	Intervention	Follow-up
< 7.0	27 (24.5%)	42 (27.6%)	13 (12.9%)
7.0 - 9.05	31 (28.1%)	50 (32.9%)	42 (41.6%)
9.05 - 11.1	30 (27.3%)	30 (19.7%)	29 (28.7%)
=> 11.1	22 (20.0%)	30 (19.7%)	17 (16.8%)
Total	110	152	101

4.3.3 Relationship between Hours Fasted and Self-Monitored Blood Glucose

Ordinal Logistic Regression (OLR) was performed using data from the 8 individuals who had completed their full SMBG logs and daily hours fasted logs. Similar to the KS test, OLR models used the non-equalized data sets for the reasons mentioned in Section 4.3.2. In order to explore the relationship between the increase in hours fasted and SMBG, Hours Fasted

Difference (HFD) and Hours Fasted Percent (HFP) were calculated for baseline and intervention phases only.

HFD and HFP OLR models showed a significant association between HFD and HFP with morning SMBG (Chi-Square Likelihood Ratio = 8.36, p = 0.004 and Chi-Square Likelihood Ratio = 9.37, p = 0.002, respectively) but not for afternoon or evening SMBG (all p > 0.1). OLR models demonstrated that with increasing HFD and HFP, there was a significant increase in the probability of morning SMBG < 7.0 mmol/L and a significant decrease in the probability of morning SMBG > 9.05 mmol/L (Figure 4.4. and 4.5). The largest effects were observed for SMBG < 7.0 mmol/L and SMBG 9.05 – 11.1 mmol/L, which were inversely related.

Figure 4.4 – Morning Self-Monitored Blood Glucose as a function of Hours Fasted Difference (HFD)

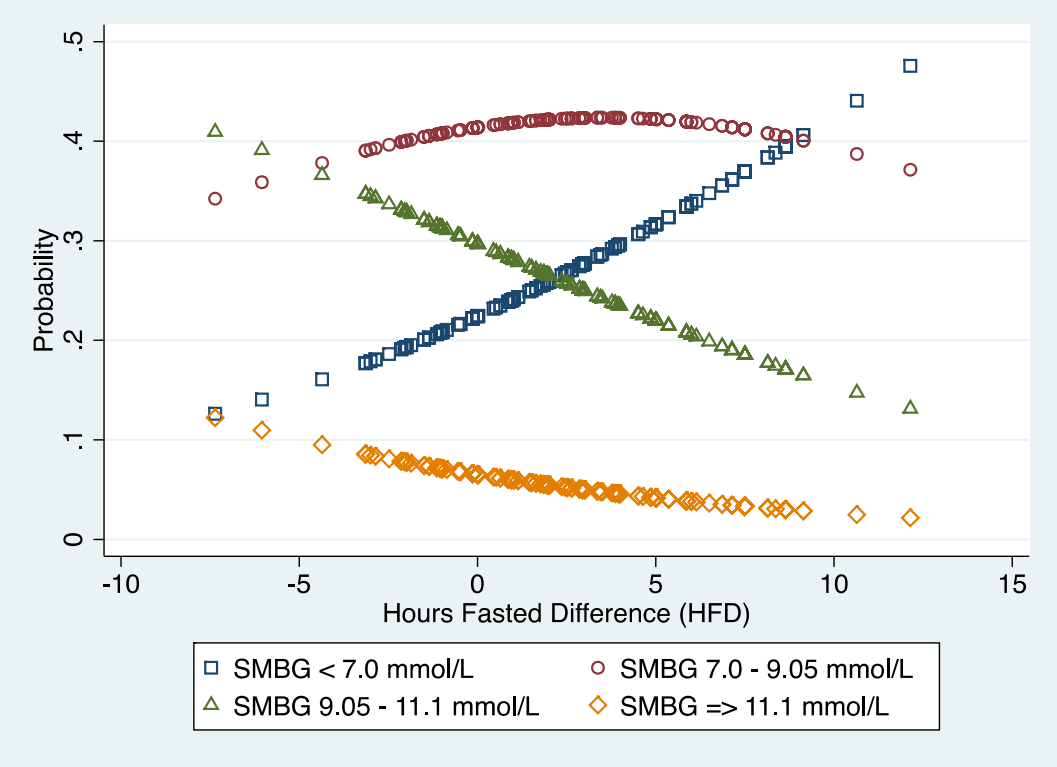
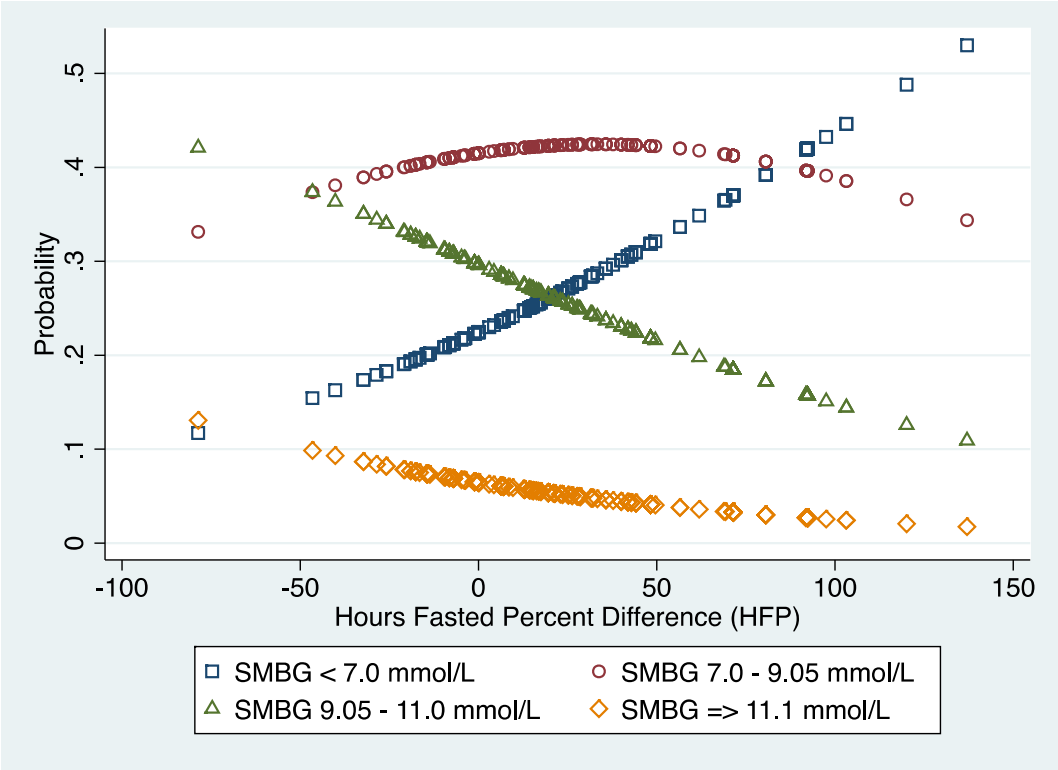


Figure 4.5 – Morning Self-Monitored Blood Glucose as a function of Hours Fasted Percent Difference (HFP)



The follow-up phase was excluded from the results above because any after-effects from IF on SMBG (either positive or negative) may have distorted the relationship between hours fasted and SMBG. Exploratory analyses were run with data from follow-up phases included, which strengthened the relationships observed above (data not shown).

Ordinal Logistic Regression of HFD and HFP OLR previous SMBG regression findings that mean morning SMBG levels decreased as a result of the IF intervention, and that improvements in morning SMBG were primarily a function of extended daily fasting durations relative to average baseline fasting duration. As such, the magnitude of the IF intervention was strongly correlated to improvements in SMBG.

4.4 Diet Composition, Physical Activity, Hunger and Satiety

There was a significant increase in self-reported daily hours fasted among all participants (+5.22 hours, $p < 0.0005$), but most participants did not regularly meet the study recommendations of 18-20h fasts each day (mean 16.82 ± 1.18). Five participants partook in the Remote Food Photography Method (RFPM) and Yale Physical Activity Survey (YPAS). Food photography data was lost (due to an unknown cause) for one participant during phase 3 – as such, that participant was omitted from any analysis using data from phase 3. Although no statistically significant differences were detected in energy or macronutrient intake between any phases, this was likely due to the small sample size (Table 4.9). Visual inspection of the data demonstrated discernable trends in lower energy (-298.63 kcal/day), carbohydrate (- 47.87 g/day), and fat (-23.2 g/day) intake during the intervention phase when compared to baseline. During the follow-up phase, energy intake decreased further from baseline (-498.64 kcal/day), and carbohydrate and fat intake remained suppressed compared to baseline (-42.04 g/day and -30.33 g/day, respectively). However, this may have been due to loss of data from one participant who had the highest calorie intake among the group in baseline and intervention phases. Protein intake did not change throughout the study, but did increase as a percentage of total energy intake during the intervention phase. Physical activity, as measured by the YPAS energy expenditure adjustment and activity index, increased during the intervention phase (+1856.3 kcal/week, +7.8 activity index units) and subsequently decreased in the follow-up phase, but there was no agreement between energy expenditure and activity index (-2450.0 kcal/week, +3.4 activity index units).

Table 4.9 – Calculated Energy and Macronutrient Intakes by Phase (5 participants)

		Baseline	**Intervention**	**Follow-up**
Energy Intake (kcal/day)	Mean	1904.3	1605.7	1510.5*
	Std. Dev	404.1	375.5	755.4*
Protein Intake (g/day)	Mean	94.2	93.2	79.4*
	Std. Dev	26.6	26.1	30.7*
Carbohydrate Intake (g/day)	Mean	190.6	142.7	164.2*
	Std. Dev	58.5	62.1	93.9*
Fat Intake (g/day)	Mean	86.9	63.6	60.9*
	Std. Dev	16.6	25.2	35.5*
Physical Activity (kcal/week)	Mean	4922.3	6778.56	4329.0
	Std. Dev	3774.4	4329.5	3440.8
Physical Activity (units)	Mean	38.4	46.2	50.0
	Std. Dev	18.3	14.0	27.2
Daily Hours Fasted	Mean	11.61	16.82	11.52
	Std. Dev	1.89	1.18	2.01

*Data from 4 Participants

Visual Analog Scales for hunger and satiety were not reported due to insufficient reporting by study participants. There were large gaps in the data, with several participants reporting hunger and satiety for less than 20% of meals recorded via food photography.

4.5 Intermittent Fasting Questionnaire

Ten participants completed their questionnaires on the IF regimen. Two participants remarked increased energy levels during the day. Three individuals commented on improved health during the intervention (related to SMBG). One participant expressed being unmotivated to engage in evening exercise due to hunger concerns. Three participants reported nausea with IF, but with a re-adjustment of medication timing nausea was no longer present in two of the participants. Six participants said they would continue with the IF regimen after the completion of the study, in a full or modified capacity (i.e. every other day or reduce fasting hours). Three participants said they would discontinue fasting entirely once the study was completed. The most

consistent complaint in the study was related to not being able to meet protein intake recommendations. Only two individuals remarked that their social life was negatively impacted by the intervention, while two other individuals commented that fasting was difficult when others were snacking or eating. Most individuals reported no effect on social life, and only one individual reported a lack of support from family and friends. Two individuals commented that they enjoyed not having to prepare breakfast/lunch. Four individuals said they would recommend IF to others, while five abstained from commenting. One individual left brief comments indicating that IF was tolerable, but gave no further information on any of the questions.

4.6 Individual Results

Individual results for each participant can be found in Appendix A-J. Individual results were reported in order to emphasize the variability of biochemical responses observed across participants. 8/10 participants lost >0.5kg as a result of IF and 5/10 participants continued to lose weight during the follow-up phase. 3/10 participants regained the equivalent of the weight lost during the follow-up phase. 7/10 participants saw a decrease in waist circumference as a result of IF, with 4/7 losing >3cm. 5/10 participants decreased waist circumference during the follow-up phase as well, and 3/10 participants had a higher WC after follow-up when compared to baseline. 4/9 participants had statistically significant decreases in morning SMBG as a result of IF, with 3/4 showing concavity, and one participant showing a constant linear decrease throughout the study. 3/9 participants had statistically significant increases in morning SMBG during IF, with two exhibiting convexity and the 3rd participant showing a constant linear increase. The remaining participants showed no significant changes in SMBG, however, one participant did show visual signs of decreases in morning SMBG. 2/9 participants showed decreases in

afternoon SMBG as a result of IF (one concave, one linear). One participant showed a linear increase in afternoon SMBG over the entire study duration. 3/9 participants showed significant changes in evening SMBG, one showing a concave decrease, one a convex increase, and a third a linear increase of SMBG throughout the study period. It is incredibly important to highlight individual results, as not a single participant in the study closely mimicked mean changes across all biochemical parameters. It is worth noting that individual results were variable, and that there was often little correlation between changes in SMBG, fasting hours, triglycerides, and insulin sensitivity (Table 4.10).

Table 4.10 – Individual Changes in Fasting Hours, Triglycerides and Glycemic Control, Baseline to Intervention

Participant	Fasting Hours (%)	Triglycerides (%)	HOMA-IR (%)	Morning SMBG	Afternoon SMBG	Evening SMBG
1	N/A	49.44	65.26	N/A	N/A	N/A
2	21.39	12.68	66.31	Decrease	Decrease	None
3	48.55	-3.03	-47.57	Decrease	None	None
4	20.02	-21.88	9.28	Increase	Decrease	None
5	N/A	-1.69	-38.62	None	None	None
6	28.93	-14.12	-28.94	Increase	None	Increase
7	41.67	85.06	-6.71	Decrease	None	Decrease
8	12.23	3.85	-11.99	Increase	Increase	Increase
9	27.78	-50.14	43.76	None	None	None
10	45.37	-7.09	-33.76	Decrease	None	None

One participant had remarkably poor and consistent results. The participant in question was the only participant with a diagnosis of hypothyroidism upon initiation of the study, and was the only patient to show negative outcomes across all measured parameters, despite decreases in weight (which was all regained in follow-up). During IF, this participant had increases in LDL (+4.3%), TC (+1.9%), TG (+3.8%), CRP (+92.3%), waist circumference (+1.0%), morning

SMBG, afternoon SMBG, and evening SMBG, with decreases in HDL (-7.3%) and small irregular decrease in HOMA-IR (-12.0%). This participant also rated IF very poorly on her questionnaire and frequently complained of nausea and lack of appetite. It has been found in previous studies that fasting can reduce triiodothyronine and increase reverse triiodothyronine concentrations in healthy subjects (118), and reduce triiodothyronine receptor capacity in fasting animals (119). Given these results, future IF studies should be cautious when recruiting participants with hypothyroidism and should measure changes in thyroid hormones.

CHAPTER 5
DISCUSSION

5.1 Safety, Tolerability, and Comprehension

One of the primary concerns when planning the study was the safety of the patients and minimizing the risk of hypoglycemia. At the time, we had little data on the risk of hypoglycemia during extended fasts in individuals with DM2. Luckily, not a single case of confirmed hypoglycemia was reported during the study, and only one case of symptomatic hypoglycemia that did not meet criteria for hypoglycemia (SMBG > 4.0 mmol/L) was reported.

The majority of participants enjoyed IF, with very few finding the intervention difficult. Enjoyment of IF seemed to be tied strongly to self-perceived improvements in SMBG readings. Participants that did not find IF tolerable indicated that food temptation and social life were challenging, and typically did not see improvements in SMBG. Two of three individuals who reported nausea during IF still saw the intervention as enjoyable and successful after medication timing was altered. Several individuals commented on the ease of the diet, and a reduction in stress compared to regular meal times that require food preparation. Individuals who prepared their own meals, and not those who consumed pre-prepared meals (either packaged, prepared in a restaurant, or prepared by a family member) reported more satisfaction with IF. This may indicate that IF is an ideal dietary intervention to apply in individuals who bear sole responsibility for meal preparation and have limited time for meal preparation.

As mentioned in Section 1.4, individuals with DM2 are in need of simple and accessible nutrition interventions that can be easily taught and do not require baseline knowledge and skills. In our study, IF was taught to all the participants in a single session lasting only 15-30 minutes. Logbooks indicated that participants understood the intervention and closely adhered to the

fasting recommendations. However, the high protein aspect of the dietary intervention was problematic. Participants who completed the food photography diaries did not show a significant increase in their protein intake above baseline, and did not meet the 1.5g/kg target. However, participants did show sharp declines in carbohydrate and fat intake, but not protein intake, which may have been a result of the recommendations to consume high protein foods. Compared to the IF guidelines, participants required more education on how to increase protein intake, such as lists of high protein foods and photographs of high protein meals. IF alone, without the high protein diet, proved to be an easy and accessible intervention that required minimal time for instruction and had little barriers to comprehension and application.

5.2 Comparison to other Intermittent Fasting Studies

Our study was unique in several regards. It was the first study on IF to capture a fluid transition (i.e. no washout period) from normal dietary conditions to IF, as well as a follow-up period to observe the after effects of IF. It was also the first IF study to observe weight loss without applying controls on calorie consumption. Past studies have applied IF either eucalorically (57,58), hypocalorically (93), or did not observe weight loss (89,90). Our study was also the first on IF to track either daily SMBG or daily hours fasted and make inferences on the relationship between those measures. Lastly, it was one of the first nutrition studies to effectively make use of the Remote Food Photography Method, which has superior validity compared to standard measures of dietary intake (50,51).

Kahleova et al. (93) provides the most similar study to our own, having applied an IF regimen to individuals with DM2 over a 3 month period in a cross-over fashion. This study showed similar trends in weight and waist circumference reduction compared to our own study.

However, this study showed statistically significant improvements in fasting insulin and lipids with IF, whereas our study showed no statistically significant changes due to strikingly different responses in lipids (HDL, LDL, TC, TG) and HOMA-IR between participants that did not correlate to changes in SMBG, hours fasted, energy intake, or baseline biochemical and anthropometric measurements. The intervention applied by Kahleova et al. differed in that participants were explicitly instructed to consume meals during the morning and early afternoon, as opposed to our own intervention where patients self-selected meal times - frequently opting for afternoon and evening meals instead. This difference may have effected biochemical results via feeding entrainment (120,121) or through currently unknown biological mechanisms.

Results from four previous trials in healthy individuals provide insight on the potential of feeding entrainment to impact biochemical outcomes (57,58,122–124). Keim et al. demonstrated, using a randomized crossover design, that women consuming the majority of their calories at night had superior fat-free mass retention and fat mass reduction during a hypocaloric diet when compared to women consuming the majority of their calories in the morning (122) – supporting our anthropometric findings. However, neither lipids nor insulin sensitivity were measured in that study.

Farshchi et al. tested the effects of breakfast inclusion and breakfast omission on healthy women, showing that breakfast omission led to increased fasting LDL, and a larger area under the curve of insulin in response to a morning test meal (123) (neither of which are considered beneficial shifts) – despite no changes in weight, waist circumference, body fat percentage, or resting energy expenditure. However, dietary records in that study indicated that participants consumed less energy when breakfast was included vs. excluded. This contrasts the results of our

study, where participants saw improvements in anthropometric parameters (Weight, BMI, Waist Circumference), and also consumed less energy during IF compared to standard meal patterns.

Papers published by Stote et al. and Carlson et al. on healthy normal weight individuals showed that IF (with one large evening meal only) increased fasting glucose, triglycerides, LDL, HDL and total cholesterol, and worsened insulin sensitivity in response to a morning oral glucose tolerance test – despite leading to reductions in weight, fat mass, and cortisol levels, with no changes in hematologic variables (57,58). Given that reductions in fat mass, weight, and waist circumference are typically accompanied by improvements in insulin sensitivity and lipids, it is reasonable to question if the positive effects of loss of fat mass on morning fasting lipids, insulin and glucose are masked or confounded by the effects of feeding entrainment to morning meal exclusion.

One final piece of evidence comes from Thomas et al., which offers unique insight on how regular eating patterns may effect lipid and glucose excursions during meal times (124). In that study, the investigators tested the acute effects of breakfast skipping or breakfast inclusion on lunchtime biochemical parameters in two groups – regular breakfast eaters and regular breakfast skippers. What they found was that regular breakfast eaters, when forced to skip breakfast, had greater insulin and free fatty acid excursions in response to lunch and decreased fat oxidation after lunch compared to days where they consumed both breakfast and lunch. In contrast, regular breakfast skippers did not experience irregular biochemical responses at lunch after breakfast omission or breakfast inclusion.

There is currently little to no feeding entrainment research on humans, but evidence from animal studies suggest that central and peripheral biological clocks are altered as a result of shifts in feeding schedule (120,121), and that these shifts may impact endogenous lipid and glucose

biosynthesis (125). There is currently no evidence in humans regarding the effects of feeding entrainment on various morning fasted or 24-hour biochemical readings in any outcomes that were measured in this study. Moreover, there is little to no indication if these alterations in biological clock regulation are harmful or beneficial to human health in regards to clinical endpoints.

Given these results, there many potential inferences that could be made to explain the discrepancy between the observed results in healthy individuals and those with DM2. IF with morning meals may lead to metabolic improvements vs. IF with evening meals. IF may affect healthy individuals differently than individuals with DM2. IF may exert different biochemical and anthropometric effects under eucaloric conditions compared IF in hypocaloric conditions. The response to IF may have large inter-individual variances, skewing the results of independent IF studies. Deviating from regular temporal meal consumption patterns may impact biochemical outcomes. Lastly, all or some of these factors may interact. Further research is warranted to discern the true effects of IF from those of feeding entrainment, energy balance, and health status.

5.3 Challenges

5.3.1 Recruitment

The most difficult challenge in the study was undoubtedly recruitment, and this led to severely underpowered results. This comes as no surprise, since other studies have found that more than half of studies never meet recruitment targets (126). Although online advertisements proved effective in garnering large view counts (+10,000 views over the study duration) and hundreds of e-mail and telephone inquiries, they were not sufficient to meet adequate targets for

participant enrollment. Advertisements placed in medical clinics around Saskatoon were marginally effective, only providing a small number of telephone consults.

In the initial exclusion criteria for the first phase of the study, individuals on any sulfonylureas were excluded from the study. This was overwhelmingly the primary barrier to recruitment, and omitted many interested potential participants during initial telephone consults. This was the case despite acquiring the potential participants' approval to contact their primary physician to request a change in medication for the duration of the study. Despite these efforts, not a single physician ever replied to the request and none of these participants were recruited for the study. After we became aware of the new study by Kahleova et al. (93), which had DM2 participants on sulfonylureas complete a 3 month trial of IF with no reports of adverse side-effects, we had sufficient evidence to justify the recruitment of participants on new generation sulfonylureas (i.e. Gliclazide). Unfortunately, this wasn't enacted until the last months of study recruitment.

Another hurdle in recruitment was the lack of recruitment infrastructure. We were declined support from local diabetes nutrition programs, which would have served as an ideal center for recruitment and participant education. This may have been due to the stark contrast between conventional nutrition interventions for people with DM2 (which promote regular meal and snack intake) and IF, as it has been found that studies that investigate clinical interventions that are unfamiliar to clinical collaborators are more likely to encounter recruitment problems (127). For the first half of recruitment we had no physicians seeking patients for us, and when some physicians were eventually enlisted in helping with recruitment very few actually referred patients. Future nutrition studies should have both the co-operation of local DM2 nutrition programs, as well as physicians who routinely see DM2 patients with more integral roles in the

study, such as principle investigator or co-principle investigator. Future studies of low-risk lifestyle interventions should seek to incentivize physicians (and other health care workers) not in principle study roles to aid in recruitment.

5.3.2 Data Analysis

Very few papers have been published on the analysis of SMBG data. Several statistical techniques exist for analyzing blood glucose from continuous glucose monitoring (CGM). However, SMBG data presents unique problems related to missing data and non-continuity that cannot be addressed with statistical techniques developed for CGM. One technique for analyzing SMBG data, called the Average Daily Risk Range (ADRR), is only suitable for predicting major hypo- or hyper-glycemia (defined as <3.9mmol/L and >10.0mmol/L, respectively). ADRR uses 3-5 daily SMBG measurements over the course of 1 month to establish blood glucose variability, which it then transforms into a risk index to predict future occurrences of hypo- and hyper-glycemia (128–130). ADRR is only representative of extreme SMBG data, and does little to explore smaller deviations or describe the distribution of SMBG data. ADRR would have provided an inadequate measure of SMBG in our study, since no hypoglycemic and very few hyperglycemic events occurred throughout the entire study period. Lastly, ADRR does not reflect blood glucose targets for individuals with diabetes.

SMBG data presents a unique problem for statistical analysis, particularly for non-linear time-series regression. Statistical and non-statistical outliers disproportionately skew the regression analysis by contributing more to the sum of squares, effecting the regression line and subsequent R^2 value. Statistical and non-statistical outliers are extremely common in SMBG logs, and should not be omitted. A standard, albeit misguided, procedure for dealing with outliers

is deletion, but because they are biologically relevant events in SMBG logs these large deviations should be retained for analysis. An additional problem lies in standard weighting schemes used to enhance the accuracy of regression models that do not adequately address issues with SMBG logs. In SMBG data, deviations from the normal glucose range create primarily right tails (hyperglycemia). Hyperglycemic events are generally more common than hypoglycemic events in T2DM, and are further from ideal glucose ranges. For example:

*(**Event:*** |Normal FPG – ***Event FPG***| = Absolute Difference in FPG*)*

Extreme Hypoglycemic event: | 5.0mmol/L – **2.0mmol/L** | = 3.0mmol/L

Mild Hypoglycemic event: | 5.0mmol/L – **3.0mmol/L** | = 2.0mmol/L,

Mild hyperglycemic event: | 5.0mmol/L – **11.0mmol/L** | = 6.0mmol/L

Extreme Hyperglycemic event: | 5.0mmol/L – **18.0mmol/L** | = 13.0mmol/L

This uneven distribution makes SMBG data difficult to properly weight in a biologically relevant manner. Most nonlinear weighting procedures operate under the pretence that deviation increases as the Y value (i.e. glucose) increases. In the case of SMBG logs, as Y increases the deviation may decrease in size largely due to smaller tails (i.e. extreme data points are closer to the mean, making the distribution more narrow). The specific combination of statistical procedures (Regression, KS test, OLR) described in the methods section was developed in order to address these concerns.

5.4 Strengths and Weaknesses

5.4.1 Internal Validity

As explored in the previous section, there is little research on human feeding entrainment, its interaction with diurnal rhythms, its influence on fasting morning biochemical

parameters, and its impacts on health or lipid and glucose homeostasis. As such, it is difficult to interpret the observed alterations in fasting glucose, insulin, and lipids that occurred as a result of IF. However, anthropometric changes were in line with previous studies of IF, and the nature of our study design allowed us to clearly observe both inter- and intra-individual anthropometric changes, which were largely positive. The study design, paired with the daily self-tracking of daily hours fasted and SMBG, elucidated the underlying relationship between time spent fasting and morning SMBG. However, the lack of relationship between daily hours fasted and afternoon and evening SMBG may have been due to a lack of internal validity, namely the inability of the study design to account for meal ingestion around afternoon and evening SMBG readings. The distributions of afternoon and evening SMBG were markedly wider and right tailed compared to morning SMBG. This comes as no surprise, since random glucose measurements are by nature more stochastic than fasted morning glucose, given that they may or may not be influenced by recent food intake. Lastly, the inclusion of a follow-up phase provided additional insights into the nature of IF. The convex and concave models for morning SMBG suggest that any alteration (whether positive or negative) in glucose homeostasis is not permanent and quickly reverses upon cessation of IF. Likewise, most biochemical and anthropometric parameters regressed towards baseline values (albeit sustaining some of the effects of IF) during the follow-up period, which may have been due to the lack of clinically significant weight loss (5-10% of bodyweight), some of which was regained during the follow-up phase.

The poor self-reporting of visual analog scales prohibited a reliable analysis of the effects of IF on hunger and satiety, and alternative means should be used in future free-living studies to gauge hunger and satiety, such as post-hoc hunger and satiety ratings. Other studies that successfully used visual analog scales to record hunger and satiety did so via staff attendance at

meal times (57). Self-reporting of SMBG readings and continuous fasting hours was significantly better than expected in most participants, and could become an integral part of future observational research. Coffee and tea intake was poorly reported by most participants, as such, it was impossible to determine any effects coffee or tea intake may have had on study participants.

5.4.2 External Validity

Due to the small sample size in this study, external validity was severely compromised. The results of a study on 10 individuals with DM2 cannot be extrapolated to apply to the millions of heterogeneous individuals with DM2. The primary significant results, namely reductions in weight and BMI, did not reach levels that would be considered clinically relevant (i.e. 5-10% decrease in body weight). Participants did significantly reduce their weight, but the magnitude was clinically insignificant, as most participants did not change their BMI classification.

Extrapolations from this study are limited to short-term biochemical and anthropometric changes. External validity in biomedical research is achieved primarily through research on long-term health outcomes and clinical endpoints (mortality, cancer, cardiovascular events, etc.), which are beyond the scope of this study. Establishing external validity for the effects of IF will be accomplished by future trials that are larger in scope and well funded. Ultimately, this study will function as preliminary research used to inform future clinical trials.

5.4.3 Biases and Conflicts of Interest

The investigators of this study claim no conflicts of interest. However, two study investigators, Mr. Bowen and Dr. Welihinda, had at one point prior to the study practiced the IF meal timing pattern for a period of >2 years each. At no point during the recruitment, data collecting, or data analysis was Mr. Bowen practicing IF, but Dr. Welihinda had continued IF throughout the duration of the entire study. No other study investigators claimed biases or conflicts of interest.

5.5 Future Directions

Before further IF research is conducted, investigators should aim to conduct more basic research on the interaction between feeding entrainment and 24-hour diurnal biochemical patterns. One study, that tested the effects of 2 days per week of very low energy dieting, serves as an example of the type of controls that must be implemented (131). In that study, investigators captured data on triglycerides and HOMA-IR over five consecutive mornings, with two mornings being during low-energy days, and three mornings of ad lib caloric intake. It was discovered that HOMA-IR and triglyceride levels undulated in tandem with energy intake, with both lowering during low-energy days and rising back to baseline levels during high-energy days. Since caloric undulation occurs over the span of a single day during IF, future investigators should take blood samples from participants at regular intervals for a full 24-hours and record any fluctuations in cardiometabolic risk factors before and after the intervention period. Relying entirely on morning fasted readings to determine the effect of IF may result in missing significant biochemical changes that occur during peak fasting hours. Future IF investigators should also keep in mind the results by Thomas et al. (124) by capturing regular meal time habits

before the beginning of the study, and then use that data to sub-divide participants in post-hoc analyses.

Given that most biochemical parameters regressed towards baseline in our study, future studies should include longer intervention periods until clinically significant weight loss is achieved (>10% of body weight) to see if changes in glucose homeostasis, lipids, and inflammation are sustained or improve during follow-up periods.

Due to the lack of association between the IF intervention and afternoon and evening random SMBG, future studies could acquire larger sample sizes to account for the additional noise in random SMBG measurements, or by collecting information on meal and snack times in tandem with SMBG data to directly explore for effects of food intake on SMBG during IF.

5.6 Conclusion

A short bout of Intermittent Fasting in a small group of individuals with DM2 led to significant group decreases in weight, BMI, and Morning SMBG. Non-significant group decreases in waist circumference and CRP were also observed. There was a significant association between the percent and total increases in fasting hours during the intervention phase and low morning SMBG. However, large variances in response were noted between participants, which may have been due to a larger magnitude change in daily hours fasted during the IF phase. After IF was ceased most parameters regressed to baseline values. IF was well tolerated in most individuals and had minimal to no side effects in most participants when medication timing was adjusted. In the five participants who measured food intake and physical activity, IF led to an overall decrease in caloric intake as measured by food photography, and had inconsistent changes in physical activity as measured by YPAS. It is probable that the observed benefits of IF

on anthropometric parameters were a product of a diet-mediated energy deficit, but the cause of the large variance observed between participants in biochemical parameters remains unknown.

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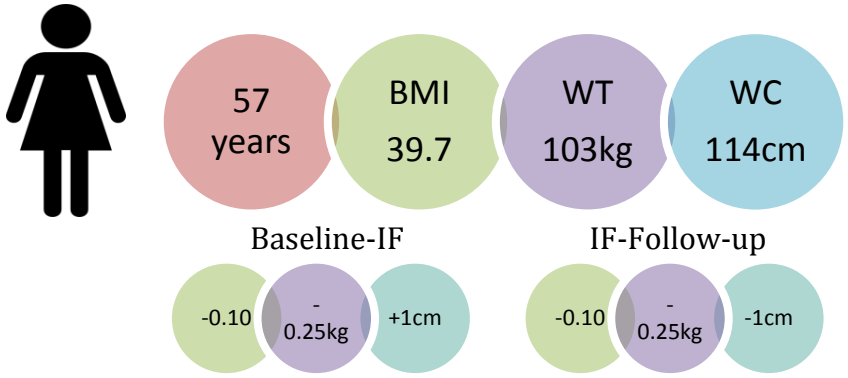
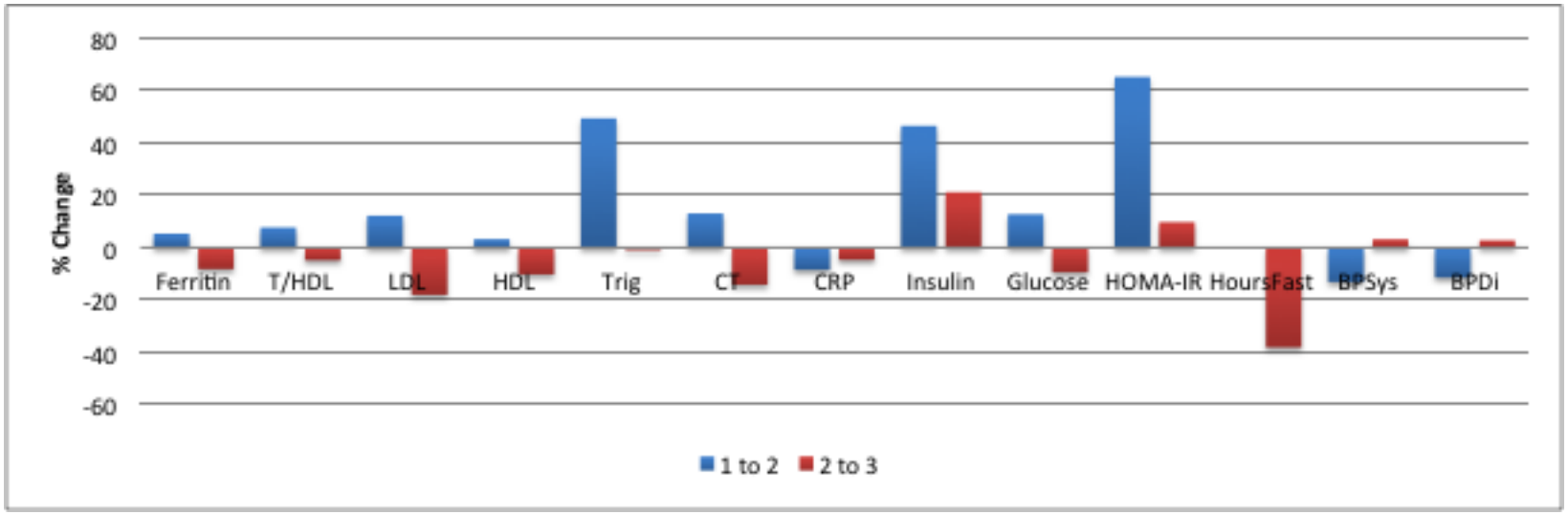
7. Appendix

A. Participant 1





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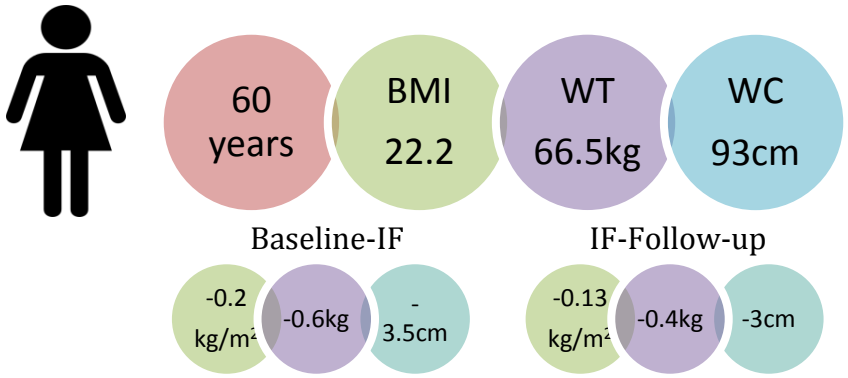

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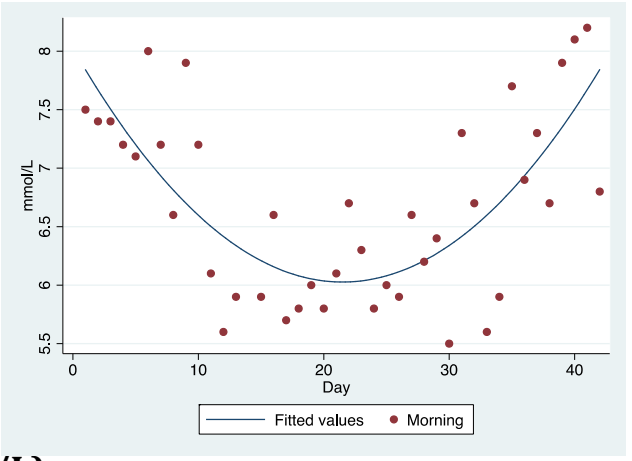
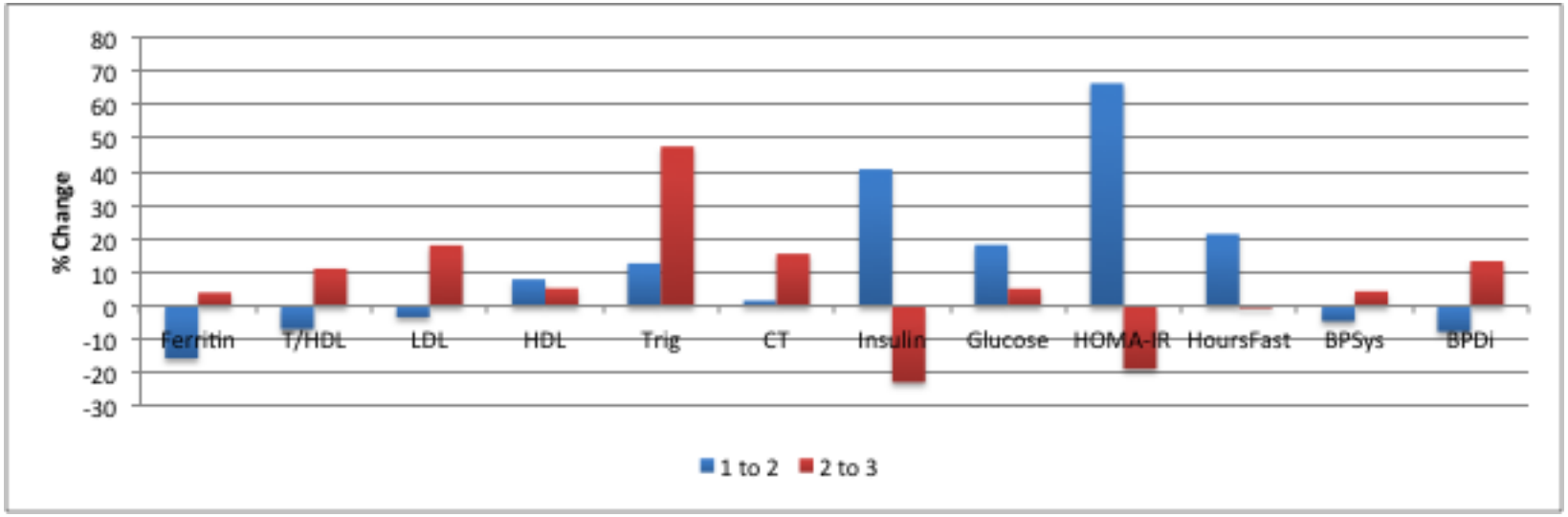
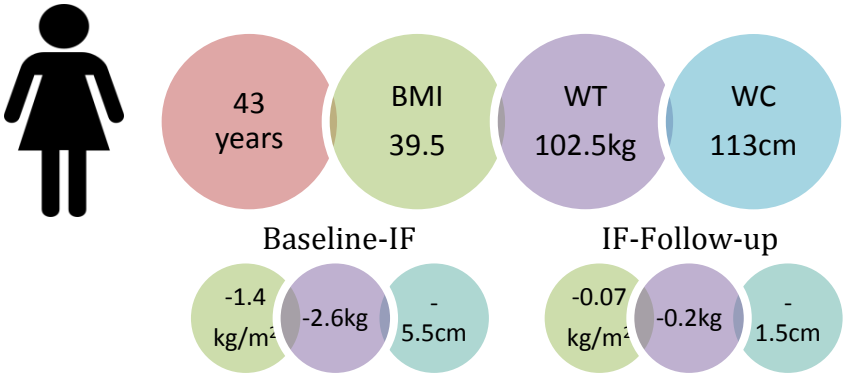


***CRP: baseline -> IF -> follow-up (0.5 -> 0.4 -> 2.3, mmol/L)**



C. Participant 3



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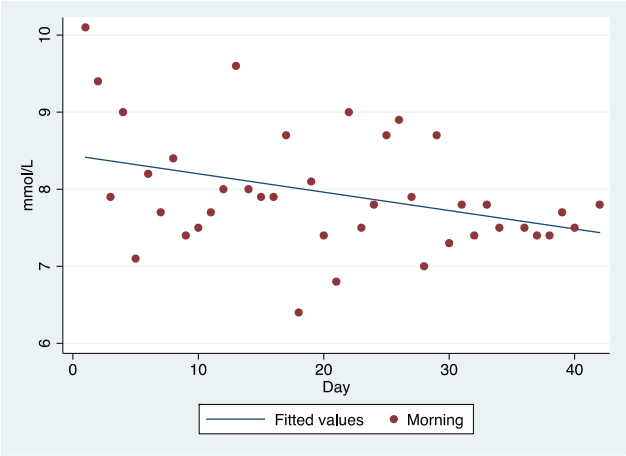
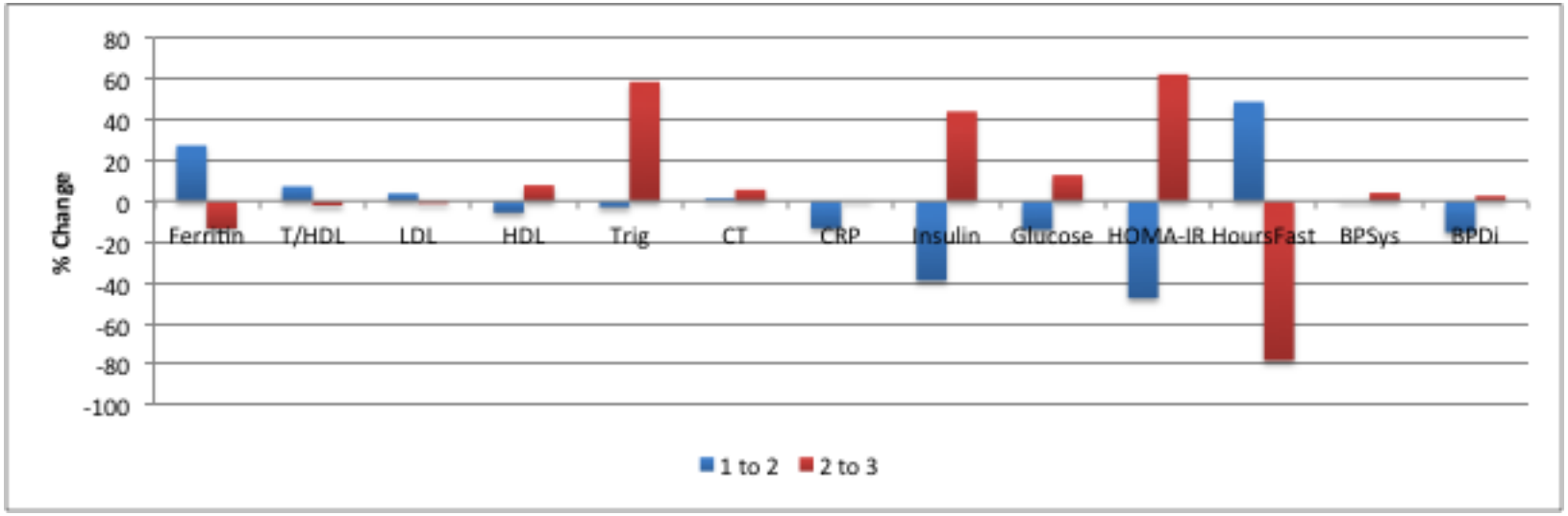
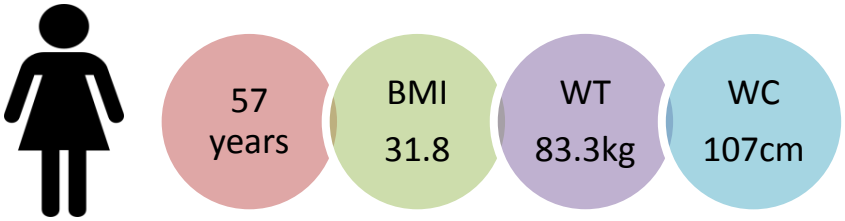




D. Participant 4



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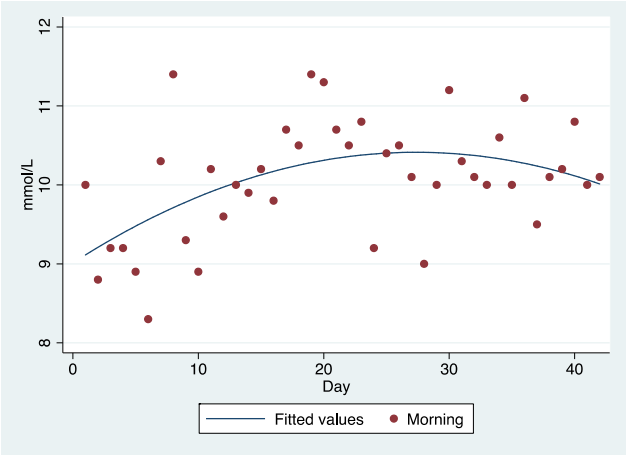

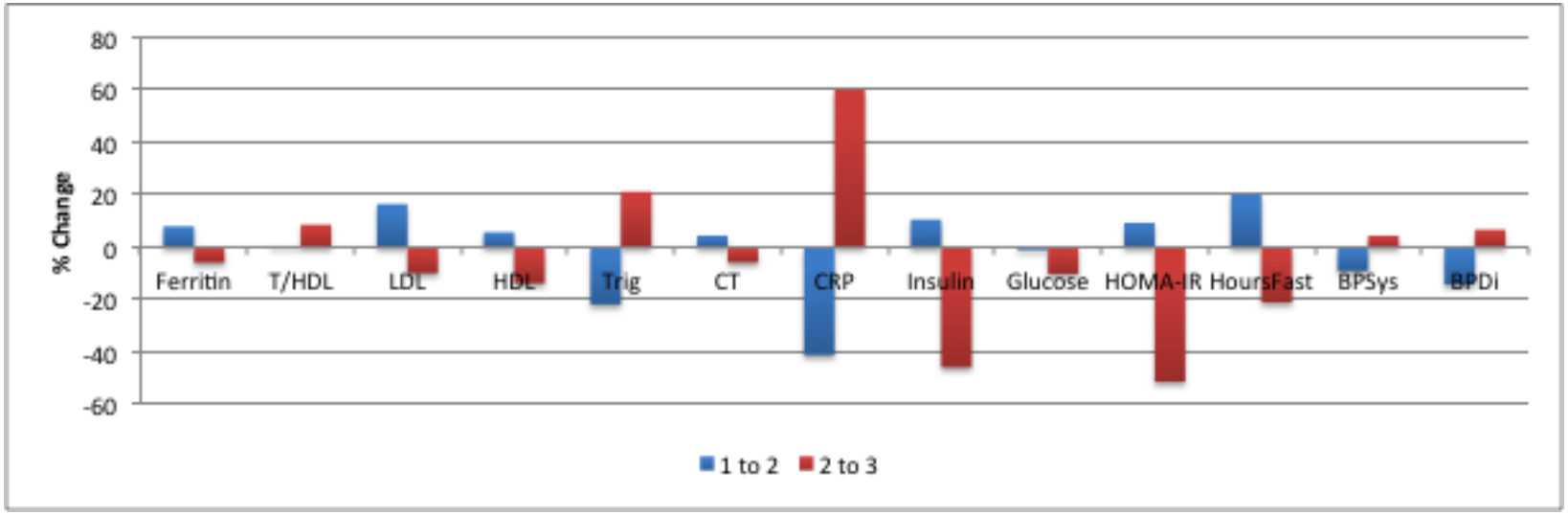

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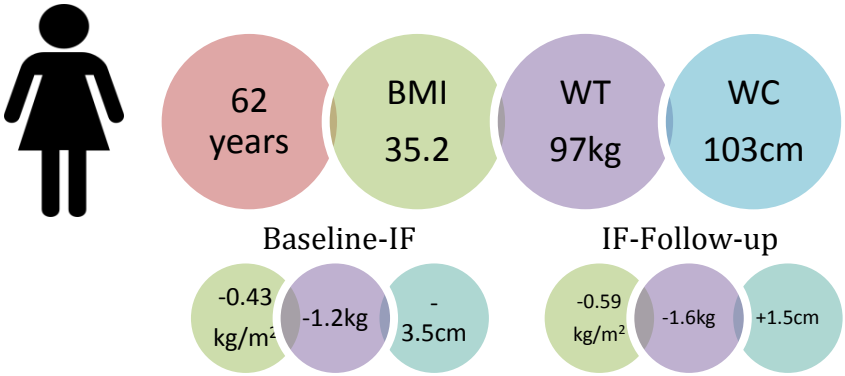

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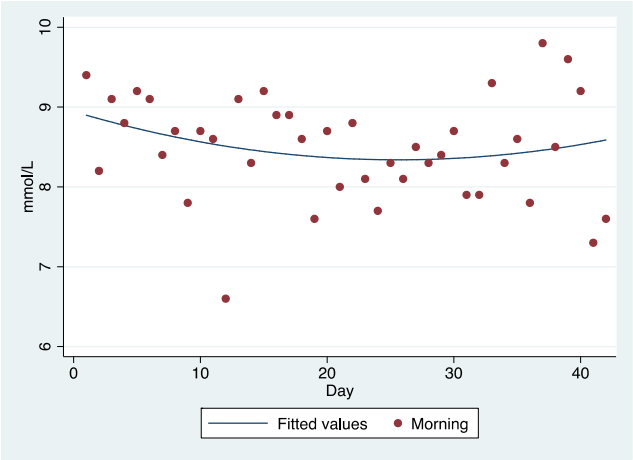

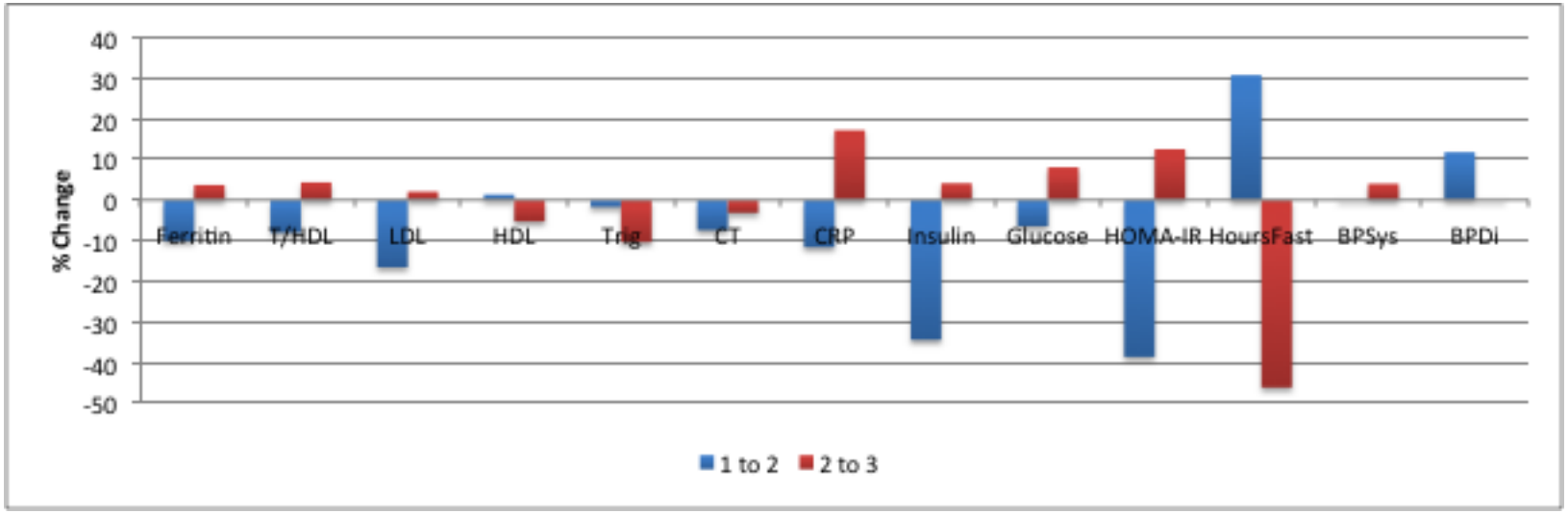

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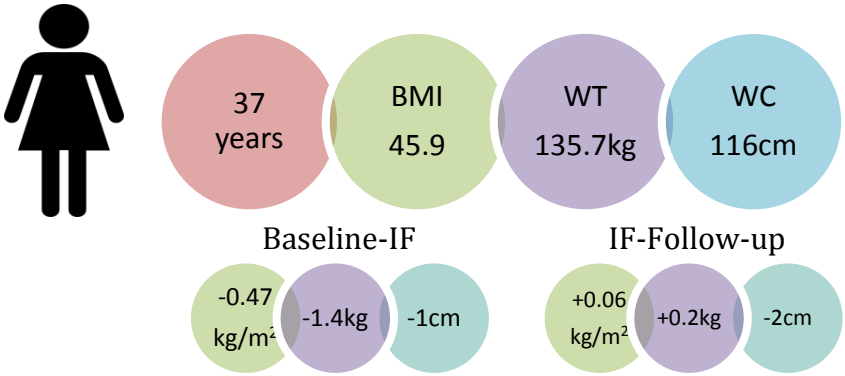

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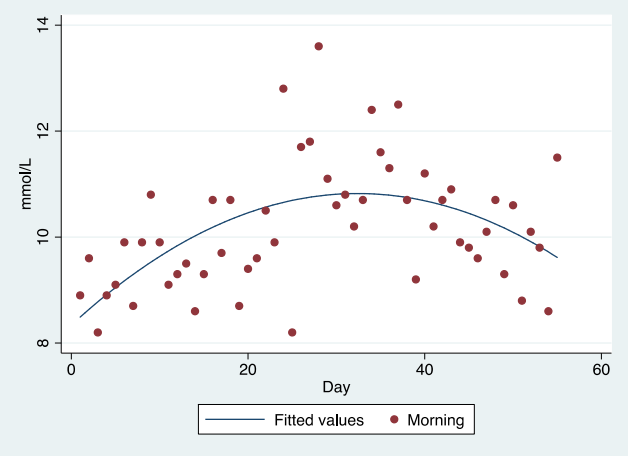
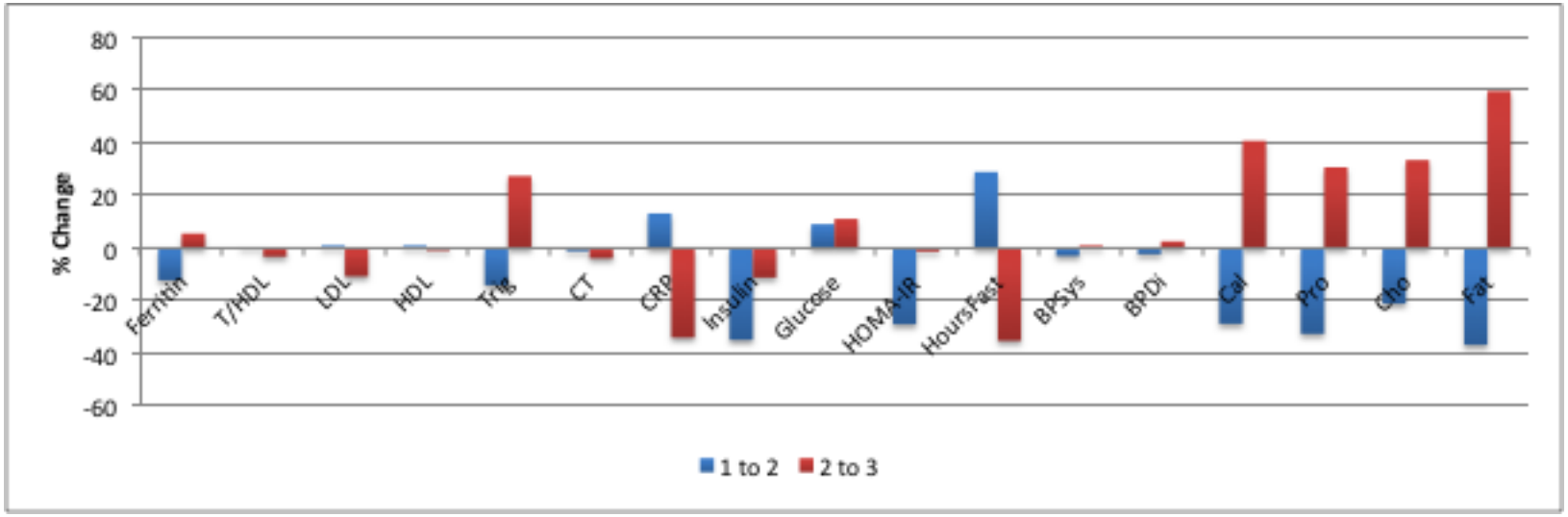
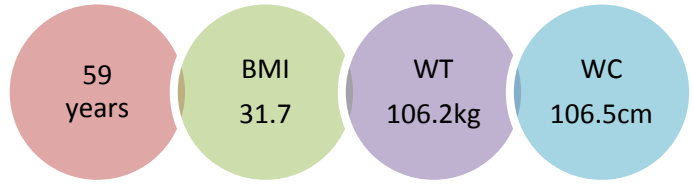
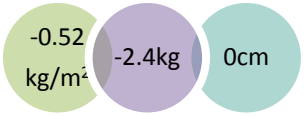




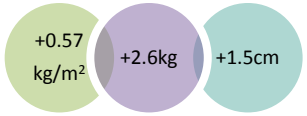
G. Participant 7



Baseline to IF



IF-Follow-up



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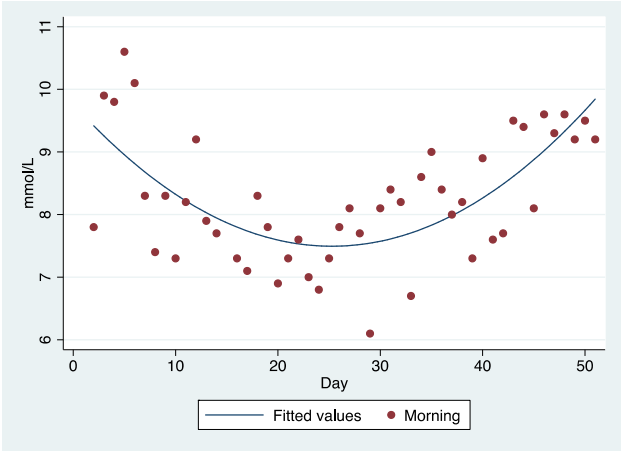
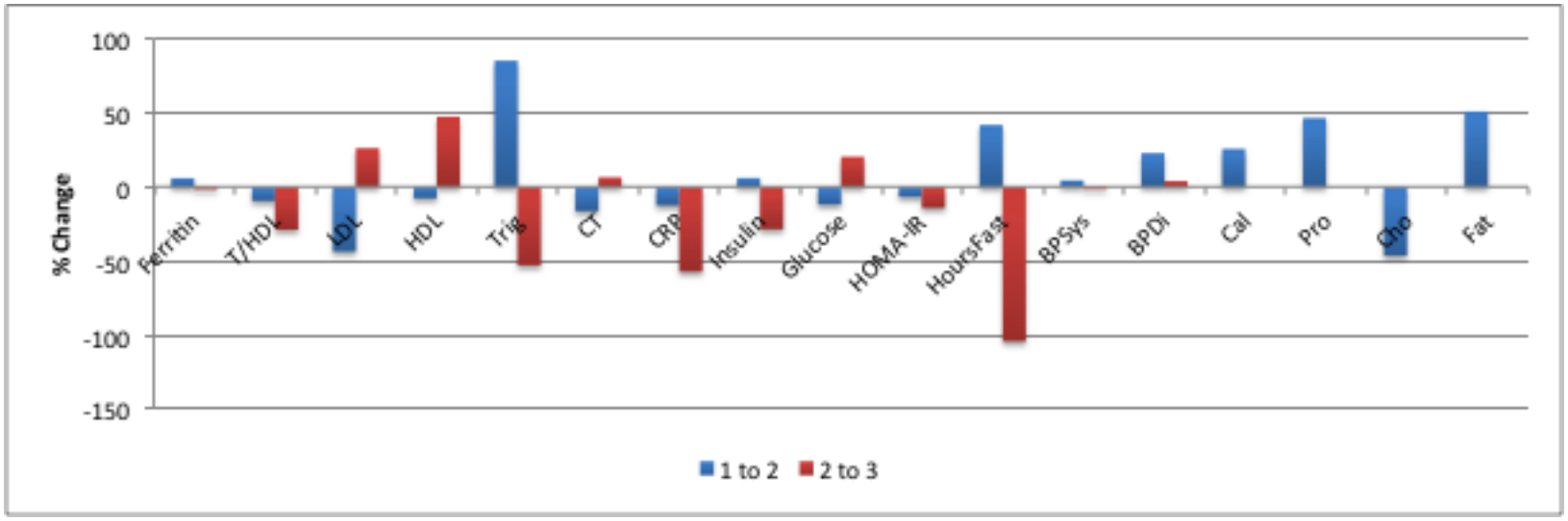
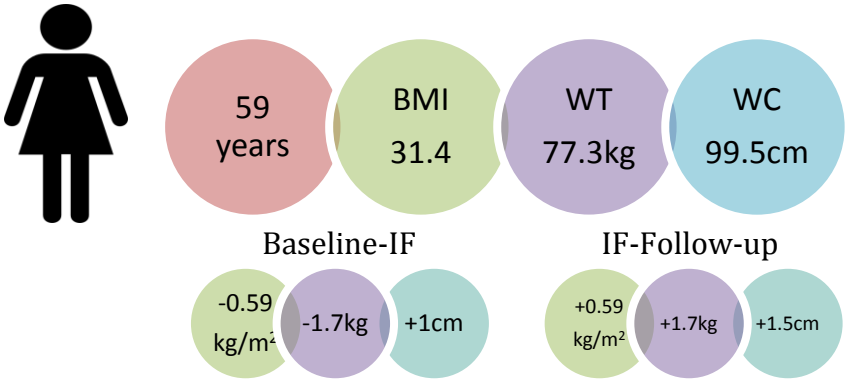




H. Participant 8



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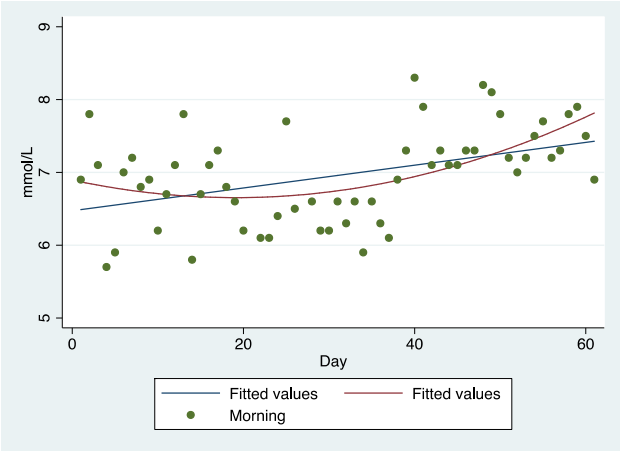
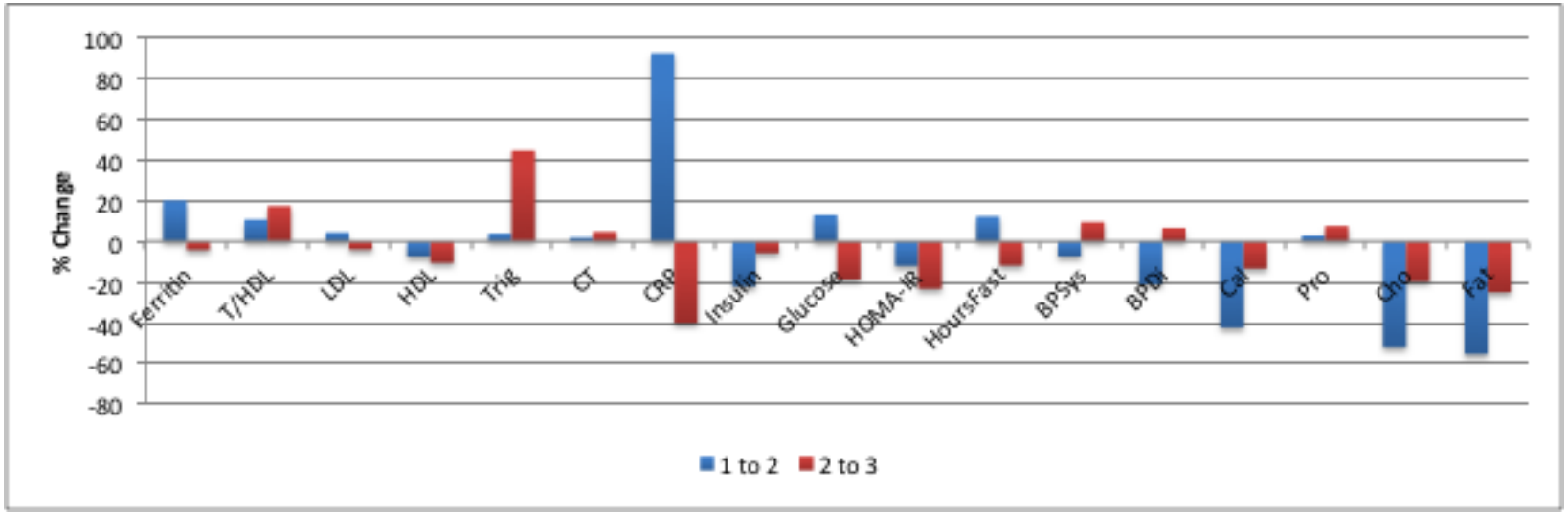
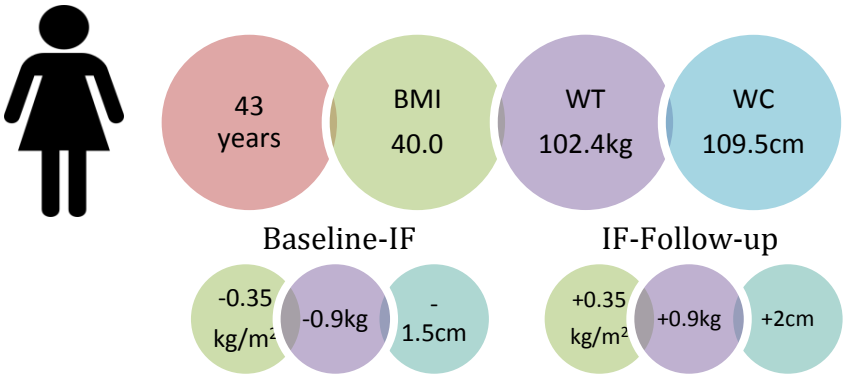




I. Participant 9



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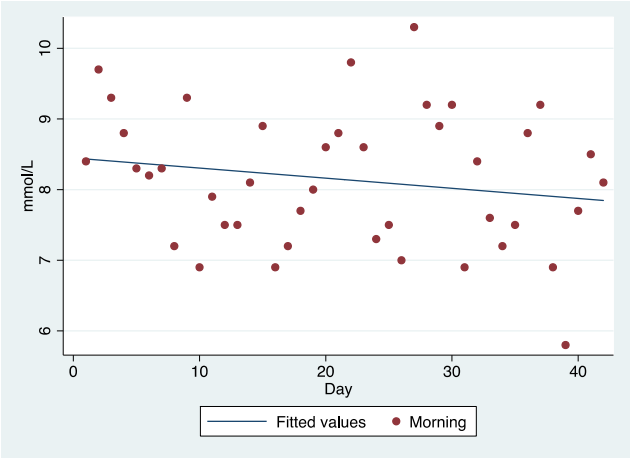
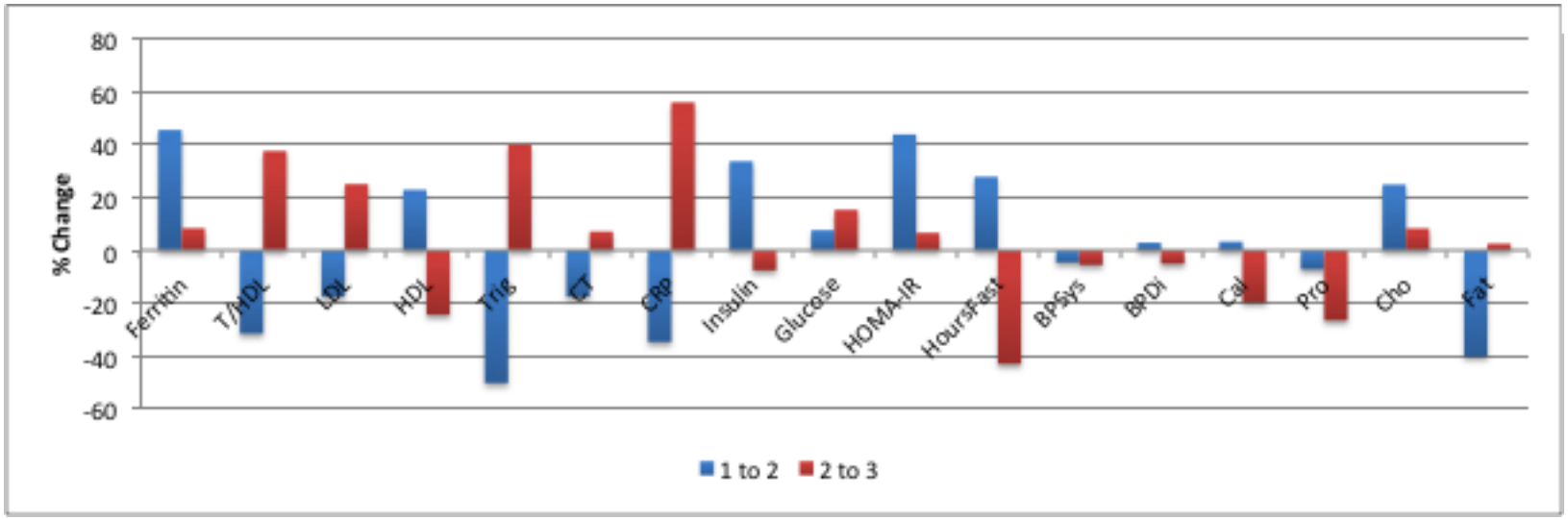
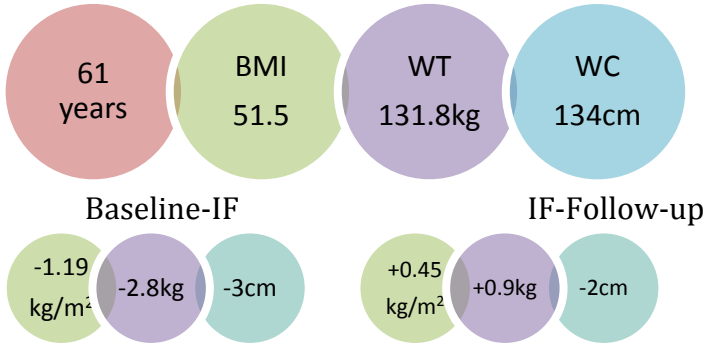




J. Participant 10



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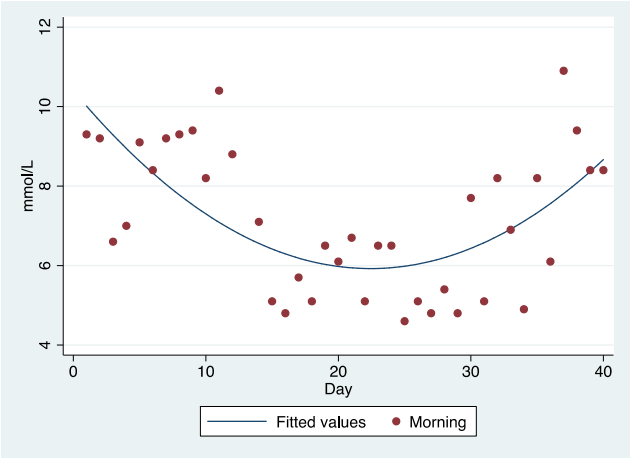

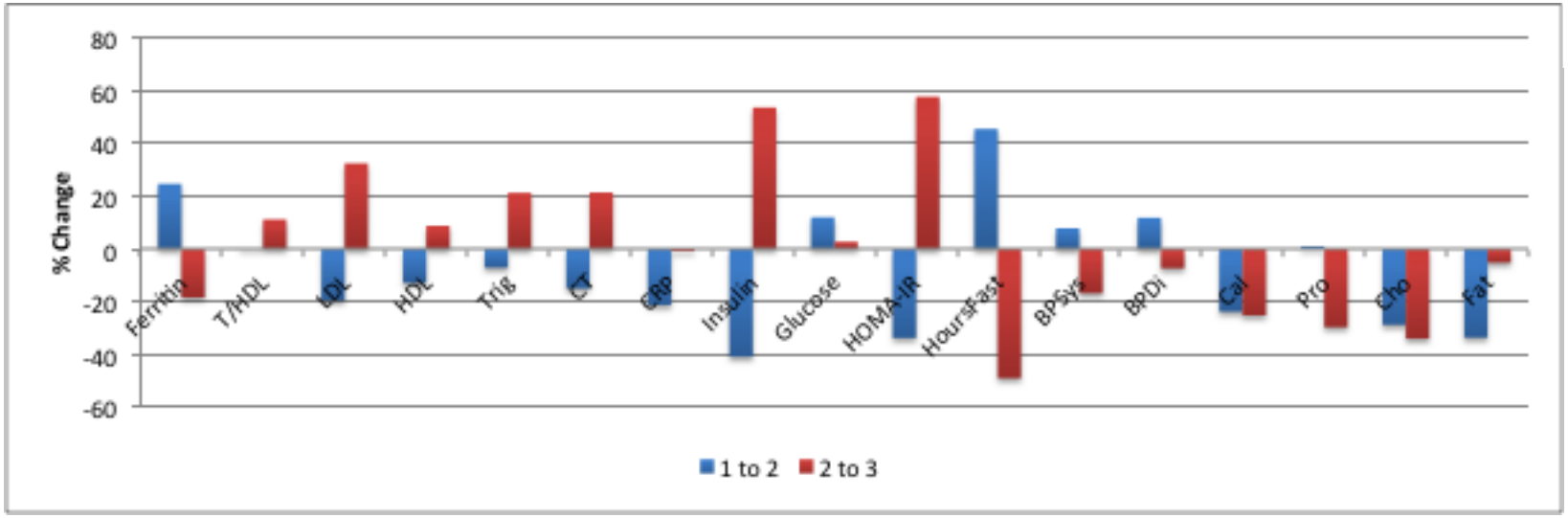

K. Photo Food Diary Examples

Dinner (Before)

Dinner (After)

Breakfast

Lunch

K. List of High Protein Foods

Ground meats
- ½ cup Beef – 16g Pro
- ½ cup Pork – 16g Pro
- ½ cup Chicken – 17g Pro
- ½ cup Turkey – 18g Pro

Patties
- 4oz Beef Patty – 30g Pro
- 4oz Chicken Patty – 28g Pro
- 4oz Turkey Patty – 24g Pro
- 4oz Pork Sausage Patty – 16g

Beef Lean cuts or wild meats
- 1 oz Round Steak – 8g
- Deck of cards – 24g Pro

Beef Fatty cuts
- 1 oz Ribeye Steak – 7g
- Deck of cards – 21g Pro

Sausage
- 1 oz Breakfast Sausage – 5g Pro
- Deck of cards – 15g Pro
- 1oz Pork Sausage – 3g Pro
- Deck of cards – 9g Pro

Fish
- 1 oz Salmon or northern pike/walleye – 7g
- Deck of cards – 21g

Chicken (breast)
- 1 oz – 9g Pro
- Deck of cards – 27g Pro

Chicken (other)
- Thigh (1 full) – 21g Pro
- Drumstick (1 full) – 15g Pro
- Wing (1 full) – 5g Pro

Pork
- Loin (chops) – 8g/oz
- Deck of cards – 24g
- 1 Chop (150g) – 43g
- Ham (Processed) – 5g/oz
- Deck of cards – 15g

Bacon (1 strip) – 3g

Dairy

1 cup Milk – 8g Pro
1 cup Greek Yogurt – 24g Pro
1 cup Plain Yogurt – 14g Pro
½ cup cheese (cheddar, mozza, asiago, brie) – 15g Pro
½ cup feta cheese – 10g Pro

Beans

1 cup black beans cooked – 15g Pro
1 cup kidney beans cooked – 16g Pro

Tofu

¼ Block – 7g Pro

Lentils

1 cup – 52g Pro

Veggie Burger

3oz Patty – 11g Pro