THE EFFECT OF A LOW-CARBOHYDRATE HIGH-FAT BREAKFAST ON GLYCEMIC CONTROL IN TYPE 2 DIABETES

by

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Abstract

Improving overall glycemia by targeting postprandial glucose spikes, particularly at the breakfast meal when insulin resistance is the highest in type 2 diabetes (T2D), may help to prevent diabetes complications. The purpose of this thesis was to determine whether consuming a low-carbohydrate high-fat breakfast was superior to a breakfast with the dietary guidelines recommended nutrient profile for improving postprandial and 24-hour blood glucose responses to mixed meals in individuals with T2D. Adults with physician diagnosed T2D (N=23, 59 ± 11y, A1c: 6.7 ± 0.6%, BMI: 31 ± 7kg/m2) completed two 24-h isocaloric intervention periods, in a random order. Participants consumed either i) a low-carbohydrate high-fat breakfast [LC-BF; <10% energy from carbohydrate (CHO), 85% energy from fat (FAT), and 15% energy from protein (PRO)], or ii) a breakfast with the dietary guidelines recommended nutrient profile (GL-BF; 55%CHO/30%FAT/15%PRO), with the same lunch and dinner provided (both 55%CHO/30%FAT/15%PRO). Continuous glucose monitoring assessed postprandial glucose responses to each meal (incremental area under the curve; iAUC) and the mean 24-h glucose during each intervention. The postprandial glucose excursion (3h iAUC) after the LC-BF was ~80% lower than the GL-BF ($p<0.01$). Overall postprandial hyperglycemia (measured as the sum of the 3h iAUC of breakfast, lunch and dinner), and glycemic variability (mean amplitude of glycemic excursions [MAGE]) were significantly reduced with the LC-BF compared to the GL-BF (3h iAUC: -100 ± 116 mmol/L•9h, $p<0.01$; MAGE: -0.4 ± 0.8 mmol/L•24h, $p=0.03$). However, the mean 24-h blood glucose was not significantly reduced (LC-BF: 7.2 ± 1.1 mmol/L vs. GL-BF: 7.5 ± 1.5 mmol/L, $p=0.09$). Restricting carbohydrate at breakfast reduces postprandial hyperglycemia in individuals with T2D. A low-carbohydrate high-fat breakfast may be a simple and effective strategy to reduce the development of diabetes complications in T2D and long-term interventions are warranted.

Preface

The design of this research study was developed by Dr. Jonathan Little and Dr. Monique Francois. Dr. Monique Francois and I completed the data collection. Participants with type 2 diabetes (T2D) were recruited through Valley Medical Laboratories, which runs the Kelowna Diabetes Program (KDP), and via poster advertisements and word of mouth. Ethical approval was obtained from the UBC Clinical Research Ethics Board (H16-00377) and the trial was registered in a publically accessible database (ClinicalTrials.gov Identifier: NCT02982330).

It is planned that the data from this thesis will be converted into a manuscript for submission for publication in a peer-reviewed scientific journal.

A portion of this thesis was presented as a poster at the Diabetes Canada conference, held in Edmonton, Alberta on November 1 to 4, 2017. The reference for the published abstract is: Chang, C., Francois, M., and Little, J. (2017). The effect of carbohydrate restriction at breakfast on glycemic control in type 2 diabetes. *Canadian Journal of Diabetes*, 41(5):S73. doi: 10.1016/j.jcjd.2017.08.204. Presented at the Diabetes Canada Conference on Nov. 3rd, 2017.

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To my parents, my partner and my siblings.

1 Introduction

1.1 Background

In type 2 diabetes (T2D) the body is resistant to the metabolic effects of insulin and the insulin response is blunted when carbohydrates (CHO) are consumed (Bell, 2001). Previous studies have shown that post-meal glucose spikes are linked to diabetes complications and diabetic neuropathy (Bell, 2001). In people with T2D, the largest post-meal spike tends to occur following the consumption of breakfast (Pedersen et al., 2016; Manders et al., 2006; van Dijk et al., 2011; Little et al., 2011). This large spike following breakfast is most likely due to insulin resistance being highest in the morning in people with T2D (Van Cauter et al., 1997) and because typical breakfast foods are relatively high in CHO (e.g., cereal, oatmeal, toast, fruit). Currently, the Diabetes Canada guidelines recommend consuming an even distribution of CHO throughout the day (Dworatzek et al., 2013). However, whether this distribution is optimal for preventing hyperglycaemia and improving glycemic control has not been adequately tested. Continuous glucose monitoring (CGM) devices provide researchers, healthcare providers and patients the ability to track changes in glucose over several days under free-living conditions (Zavalkoff & Polychronakos, 2002; Rodbard, 2016; Monnier et al., 2007; Klonoff, 2005; Vigersky & Shrivastav, 2017). CGM is therefore a useful tool for determining how manipulating the carbohydrate content of meals impacts glycemic regulation in attempt to optimize dietary recommendations in T2D. The overall aim of this MSc thesis project was to determine how reducing the CHO content of a breakfast meal affects 24-hour glucose control, assessed by CGM, in individuals with T2D.

1.2 Overview of Normal Glucose Homeostasis

Blood glucose concentration is determined by the balance between endogenous glucose production from the liver (i.e., glycogenolysis and gluconeogenesis), the appearance of exogenous glucose (from ingestion, digestion and absorption of CHO), and the uptake by tissues in the body (e.g., skeletal muscle, brain, adipose) (Nolan, 2011). Following ingestion of a meal, blood glucose levels increase and this triggers insulin release from the pancreatic beta-cells (DeFronzo, 2009). Incretin hormones released from the gut also serve to augment beta-cell insulin secretion. The immediate release of insulin from beta-cells suppresses glucagon secretion from juxtaposed pancreatic alpha-cells (Aronoff et al., 2004), which collectively function to reduce endogenous glucose production in the liver. The major action of the rise in circulating insulin is to increase the rate of glucose disposal into insulin sensitive tissues (e.g., skeletal muscle) in order to reduce blood glucose concentration (Nolan, 2011).

1.3 Insulin Signalling Pathway

Insulin signals peripheral tissues to increase the uptake of glucose through a complex signalling pathway involving the insulin receptor, insulin receptor substrate (IRS) 1 and 2, phosphatidyl-inositol-3 kinase (PI-3K), and Akt leading to translocation of the insulin-responsive glucose transporter 4 (GLUT4) protein from intracellular pools to the plasma membrane (see Figure 1) (Cartee, 2015). In this manner insulin signalling triggers glucose uptake into tissues by facilitated diffusion.

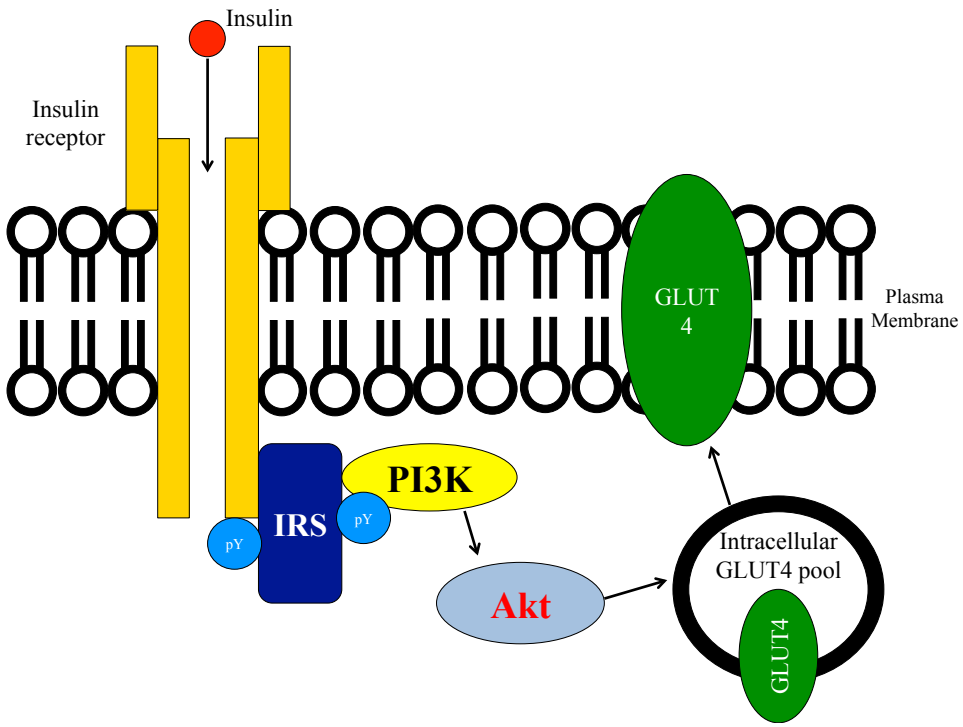

Figure 1. Summary of The Insulin Signalling Pathway for Glucose Uptake. When insulin binds to the insulin receptor, a series of phosphorylation events activates various signalling proteins ultimately resulting in translocation of GLUT4 from the intracellular pool to the plasma membrane. *Abbreviations*: IRS, insulin receptor substrate; PI-3K, phosphatidyl-inositol-3 kinase; GLUT4, glucose transporter 4 protein. [Adapted from Cartee, 2015].

1.4 Pathogenesis of Type 2 Diabetes (T2D)

T2D is characterized by decreased insulin sensitivity combined with beta-cell dysfunction (Cerf, 2013). This causes a pathological scenario where the level of insulin release from beta-cells is insufficient for the prevailing level of insulin sensitivity, leading to elevated levels of blood glucose (i.e., hyperglycemia) (DeFronzo, 2009). T2D is diagnosed when 1) fasting plasma glucose (no caloric intake for at least 8 hours) $\geq$7mmol/L, 2) glycated hemoglobin (HbA1c) $\geq$6.5%, or 3) plasma glucose two-hours following a 75g oral glucose tolerance test $\geq$11.1mmol/L (Goldenberg & Punthakee, 2013).

T2D is characterised by hyperglycemia in the fasting and fed state, due to deficiencies in both insulin secretion and action (DeFronzo, 2009). Specifically, beta-cell dysfunction in T2D is

characterized by a dramatic loss of first phase insulin secretion, which results in reduced suppression of hepatic glucose production following ingestion of a meal (Ceriello, 2010). Release of incretin hormones (i.e. GLP-1, GIP) is also reduced, causing further impairment in post-meal beta-cell insulin secretion (Cariello, 2010; Leahy, 2005). When combined with insulin resistance in peripheral tissues, blood glucose levels rise excessively high after a meal, a condition termed postprandial hyperglycemia (Leahy, 2005). Postprandial hyperglycemia is associated with increased oxidative stress, inflammation, and endothelial dysfunction, which mechanistically links impaired glucose regulation with cardiovascular disease risk in people with T2D (Ceriello, 2005; DeFronzo, 2009). Indeed, higher postprandial glucose (PPG) values are an independent risk factor for future cardiovascular events and all-cause mortality in T2D patients (Cavalot, 2013).

2 Glucose Control in T2D

Given the link between elevated glucose levels and macro- and microvascular complications (Monnier & Colette, 2015), the primary treatment strategies in T2D focus on improving glucose control (Asif, 2014). Guidelines for treatment of hyperglycaemia aim to maintain optimal HbA1c levels, which is defined as ≤7% (Imran et al., 2013). HbA1c reflects the average blood glucose concentrations over the preceding 2-3 months (Nolan, 2011). However, there is a growing body of evidence suggesting that treatment strategies should not only focus on HbA1c levels but also focus on the "glucose triad" (Monnier et al., 2006). The glucose triad is comprised of three main elements: 1) HbA1c, an indication of satisfactory overall glucose control 2) fasting blood glucose, reflecting how well an individual is able to regulate blood glucose levels following an 8-12 hour fasting period and 3) postprandial glycemia (Monnier et al., 2008; Tay et al., 2015a). HbA1c and fasting glucose are widely emphasized in diabetes management. However, recent investigations support the notion that postprandial hyperglycaemia contributes more to overall daily hyperglycaemia in patients with better-controlled diabetes (i.e., HbA1c <7.3%) (Ceriello et al., 2014), whereas fasting glucose is the main contributor to overall hyperglycaemia with poorly controlled diabetes (HbA1c >9.3%) (Riddle, 2017; Monnier et al., 2006). Since individuals with poor glucose control (i.e., HbA1c >9.0%) are often excluded from lifestyle intervention studies, PPG may be a more appropriate outcome measure for the majority of research studies aimed at improving glucose control in T2D.

3 Postprandial Glucose and Glycemic Variability

3.1 Postprandial Glucose (PPG)

PPG level reflects blood glucose concentration following ingestion of a meal or glucose load and significantly contributes to glycemic variability (discussed below), especially in individuals with HbA1c levels below ~7% (Alssema et al., 2015; Ceriello, 2010). Elevated PPG excursions and acute hyperglycaemia influence risk markers of cardiovascular disease (CVD), such as retinal vascular reactivity and oxidative stress, leading to diabetes complications (Ceriello & Motz, 2004). Interestingly, a meta-analysis by Levitan and colleagues showed that high PPG levels in non-diabetic individuals had a 27% greater risk for CVD compared to individuals with low PPG levels (Levitan et al., 2004). Additionally, in comparison to HbA1c, higher PPG is strongly correlated with the progression of diabetic retinopathy (Shiraiwa et al., 2005). Collectively, the available evidence indicates that elevated PPG is a significant risk factor for diabetic complications.

PPG excursions have two main components: 1) the duration of the postprandial excursion, which contributes to sustained chronic hyperglycaemia and leads to production of reactive oxygen species (ROS) associated with the development of diabetes complications and CVD (Ceriello et al., 2008), and 2) the magnitude of postprandial rise, which contributes to glycemic variability or instability (Monnier et al., 2008). Growing evidence suggests that glycemic variability is an independent risk for diabetes complications over the long-term (Maurizi & Pozzilli, 2013; discussed below). Indeed, the International Diabetes Federation (IDF) has identified elevated PPG is an independent risk factor for the progression of diabetes complications with guidelines that PPG should be targeted through medical or dietary interventions in order to improve overall glycemic control and reduce glycemic variability (Ceriello et al., 2014).

3.2 Glycemic Variability

Although lower HbA1c levels are associated with reduced risk for diabetes complications, it has been shown that individuals with T2D may still be at risk for developing microvascular complications, regardless of achieving satisfactory HbA1c levels (Monnier et al., 2008; Tay et al., 2015a). This suggests that factors other than HbA1c may be influencing the risk for developing vascular complications in T2D. Because HbA1c reflects an average glucose level over a longer period of time, it does not give an indication of glycemic variability on an hour-to-hour or day-to-day basis, therefore limiting the assessment and optimal treatment methods for diabetes management (Schnell et al., 2017; Alssema et al., 2015). Glycemic variability is expressed by the amplitude, frequency and duration of fluctuations in glucose concentration, relative to mean blood glucose (Tay et al., 2015a). High PPG is a main contributor to glycemic variability (Monnier et al., 2008), though it is important to note that the acute fluctuations in daily blood glucose concentrations can be caused by factors other than PPG excursions (Tay et al, 2015a; Fysekidis et al., 2014) but also may be influenced by diabetes medications, sickness, stress or daily activities (such as exercise) (American Diabetes Association, 2010).

Glycemic variability is measured by many different metrics that reflect the overall glucose swings from multiple measurements taken throughout the day (Monnier, Collette & Owens, 2008). Early studies were based on repeated finger stick glucose values taken during waking hours whereas modern CGM technology (see below) now allows for more sophisticated and accurate glycemic variability measurements based on continuous glucose measurement across several days/weeks (Rodbard, 2016). These include standard deviation (SD) of glucose values and mean amplitude of glycemic excursions (MAGE), among other calculated metrics (Tay et al., 2015a). MAGE, calculated using CGM, is commonly used for assessing glycemic excursions (Maurizi & Pozzilli, 2013). This measure reflects major glucose fluctuations greater than one standard deviation above mean glucose, where higher MAGE readings reflect glycemic

instability and variability in blood glucose concentration (Schnell et al., 2017). MAGE has been regarded as one of the best methods for quantifying glycemic excursions over a 24-hour period (Zaccardi et al., 2009). The importance of glycemic variability as an important component of glucose control has been recognized by numerous medical organizations, but currently there are no firm guidelines for glycemic variability targets (Monnier et al., 2016; Tay et al., 2015a).

3.3 Targeting PPG and Glycemic Variability

The Diabetes Intervention Study showed that higher PPG excursions following breakfast predicted myocardial infarction and mortality in patients with newly diagnosed T2D (Hanefeld et al., 1996), whereas the San Luigi Gonzaga Study, a 5-year follow up study, showed that elevated PPG levels following lunch were a strong predictor of the occurrence of cardiovascular events (Cavalot et al., 2006). Given the links between elevated PPG and CVD, studies have attempted to target PPG as a form of diabetes management. Such studies include the STOP-Noninsulin-Dependent Diabetes Mellitus (STOP-NIDDM) and Hyperglycemia and Its Effect After Myocardial Infarction on Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus (HEART2D) trials. Although the STOP-NIDDM showed a 49% relative risk reduction of new cardiovascular events in patients with impaired glucose tolerance after approximately 3.3 years of treatment to reduce postprandial hyperglycemia (Chiasson et al., 2003), the HEART2D trial found that treatment of either postprandial or basal glucose using different insulin and pharmacological regimens led to similar HbA1c levels with no difference in risk for cardiovascular events following an acute myocardial infarction in individuals with T2D (Raz et al., 2009). It remains unknown whether targeting elevated PPG with lifestyle interventions, such as specific dietary strategies or exercise, can help prevent CVD. However, it is interesting to note that diets lower in glycemic index or glycemic load (two surrogate markers of PPG excursions)

tend to be associated with reduced cardiovascular mortality in observational studies and improved cardiovascular risk profile in intervention studies (Brand-Miller et al., 2007).

3.4 Postprandial Glucose Following Breakfast

CGM studies have shown that the post-meal glycemic excursion following breakfast is the highest glucose spike of the day (vanDijk et al., 2011; Preat et al., 2006; Gillen et al., 2012; Little et al., 2011; Monnier et al., 2002). It is interesting to note that these studies support the notion that despite being treated with oral glucose lowering medications and following healthy diets as defined by leading diabetes guidelines, individuals with T2D still spend many hours in hyperglycemia each day. For example, Preat et al. (2006) showed that individuals with T2D were still hyperglycemic (>10 mmol/l) for approximately 13 hours of the day, regardless of their continued use of oral medications and healthy diet parameters (Preat et al., 2006). Similarly, van Dijk and colleagues (2011) found that those with T2D were hyperglycemic (>10 mmol/L) for at least 9 hours per day (±4 hours), while individuals with well-controlled diabetes (HbA1c <7%) were still hyperglycemic for approximately 6 hours per day (vanDijk et al., 2011). This led the authors to speculate that oral blood glucose lowering medication did not seem to have a strong effect on lowering PPG (van Dijk et al., 2011). Interestingly, the STOP-NIDDM trial did suggest that the inclusion of acarbose following a meal could be beneficial for reducing cardiovascular risk in patients with glucose intolerance (Riddle et al., 2017), though it is widely accepted that acarbose causes unpleasant gastrointestinal discomfort that limit its therapeutic use (Derosa et al., 2015).

Collectively, the available data suggest that targeting elevated PPG is important for reducing CVD risk in T2D and highlight the need for non-pharmacological interventions that reduce PPG excursions. Breakfast appears to be the largest postprandial glucose spike, which is

related to the pathophysiology of T2D and also in free-living conditions likely related to the relatively high CHO content of typical breakfast meals. The use of CGM can allow for the assessment of exposure to hyperglycemia and glycemic excursions (Klonoff, 2005) over a period of several days under free-living conditions, which enables easy and accurate assessment of PPG (Avogaro, 2011). CGM may therefore be a useful tool to assess how different lifestyle strategies could impact the glycemic response to breakfast and other meals in individuals with T2D.

4 Diurnal Variation of Glycemic Control

4.1 Diurnal Glucose Variation in T2D

Previous studies have shown that there is a diurnal rhythm (i.e. a physiological or behavioural rhythm over a 24 hour period, generated endogenously or due to behavioural or environmental changes) in glucose metabolism and that this normal rhythm is disrupted in individuals with T2D (Qian & Scheer, 2016). Interestingly, in contrast to normoglycemic individuals, where glucose tolerance and insulin sensitivity are higher in the morning, those with T2D experience improvements in glucose tolerance throughout the day as insulin sensitivity improves from morning to evening (shown in Figure 2 below) (Van Cauter et al., 1997; Scheen & Van Cauter, 1998). This has been shown through oral glucose tolerance tests, mixed meal tolerance tests with tracers, intravenous glucose tolerance tests, and hyperinsulinemic-euglycemic clamp techniques [reviewed by Van Cauter et al., 1997; Qian & Scheer, 2016]. Although numerous studies have attempted to delineate the exact physiological mechanisms behind this occurrence, it still remains unclear as to why or how this happens [reviewed by Van Cauter et al., 1997; Qian & Scheer, 2016]. However, limited evidence in both human and animal studies have suggested the variation in diurnal glucose tolerance for individuals with T2D may be due to abnormalities in counterregulatory hormones (such as a surge in catecholamines, glucagon, growth hormone, incretins and cortisol), rate of insulin secretion/clearance, and/or rate of glucose production/utilization [reviews by Van Cauter et al., 1997; Qian & Scheer, 2016].

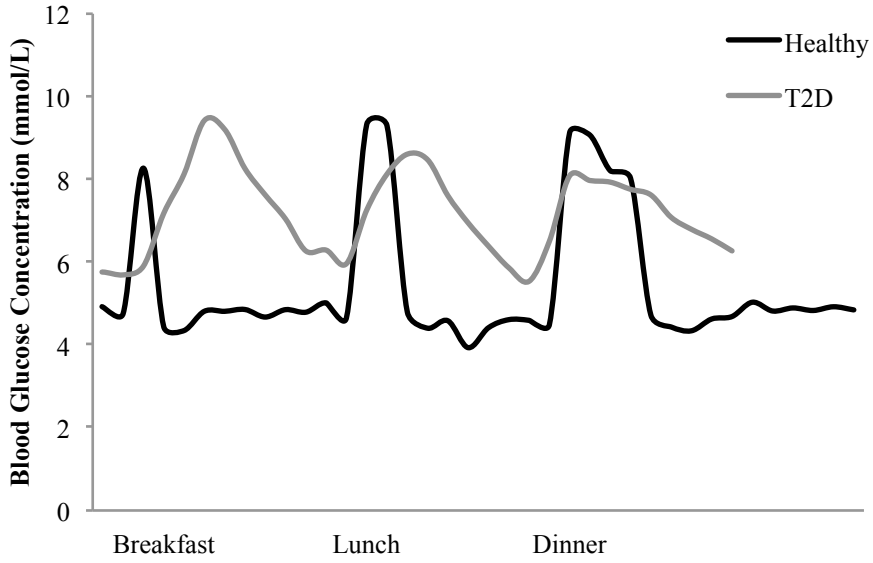

Figure 2. Diurnal Glucose Tolerance for Normoglycemic and T2D Individuals. In healthy individuals (black line) glucose tolerance and insulin sensitivity are highest in the morning and deteriorate across that day such that the largest postprandial glucose spike is typically seen after dinner. This diurnal variation is reversed in individuals with T2D (gray line) such that the largest postprandial glucose spike is typically seen in the morning. [Adapted from Van Cauter et al., 1992 & Pedersen et al., 2016].

4.2 The Dawn Phenomenon

The pathophysiology of T2D dictates that postprandial hyperglycemia is more prevalent following breakfast consumption (Preat et al., 2006; Van Cauter et al., 1997). In the early 1980's, Schmidt and colleagues introduced the "dawn phenomenon" concept, which stated that a spontaneous rise in plasma glucose was seen towards the end of the fasting period without ingestion of any dietary CHO in individuals with type 1 diabetes (Schmidt et al., 1981). This phenomenon was later shown to occur in individuals with T2D as well (Bolli & Gerish, 1984, Monnier et al., 2013). In normoglycemic individuals, blood glucose and plasma insulin levels are lower and remain constant throughout the night, and insulin secretion gradually increases prior to dawn to stop hepatic glucose production and prevent hyperglycemia (Porcellati et al., 2013). In T2D however, the insulin resistant liver will continue to produce glucose just before dawn in the

absence of insulin secretion (Schmidt et al., 1984). Overnight, the liver produces glucose at a faster rate in individuals with T2D (~2.5 mg/kg/min vs. ~2.0 mg/kg/min in normoglycemic individuals), as there is an impairment in the ability to suppress hepatic glucose production, even in the context of elevated fasting insulin levels (DeFronzo, 2009). Monnier et al. (2013) recently used CGM to demonstrate the presence of the dawn phenomenon in 248 individuals with T2D. It was shown that the dawn phenomenon continues through to the post-breakfast period leading to postprandial hyperglycemia as well as the highest post-meal glucose excursion of the day occurring at breakfast (Monnier et al., 2013). Thus, in contrast to healthy adults, individuals with T2D tend to experience the highest levels of blood glucose in the morning, particularly after the breakfast meal (Figure 2; Van Cauter et al., 1997). It seems logical to suggest that strategies aimed to reduce the glucose spike following breakfast could benefit people with T2D.

4.3 Effect of Breakfast Omission on Subsequent Meal Responses

Previous research has shown that dietary intake at one meal can influence the PPG response to subsequent meals in both normoglycemic and impaired glucose tolerant individuals (Fletcher et al., 2012). The second meal phenomenon dictates that the postprandial glucose excursion will be lower and insulin sensitivity will be higher at lunch when breakfast has previously been consumed. In contrast, glycemic responses are higher and insulin secretion impaired following lunch and dinner when breakfast is omitted (Jacubowicz et al., 2015). The improvement in postprandial glucose response and insulin sensitivity following lunch and dinner, when breakfast is consumed, is thought to occur via improved beta-cell responsiveness after the second meal, where insulin release is enhanced due to previous glucose exposure (Korsgaard & Colding-Jorgensen, 2006). Jacubowicz and colleagues (2015) confirmed this finding in a recent study and also demonstrated that this effect not only occurs following lunch but can also carry on to dinner (discussed below).

In 2009, Jovanovic and colleagues conducted a study to determine the mechanisms behind the second meal phenomenon (Jovanovic et al., 2009a). This study was conducted on 10 normoglycemic individuals on two separate days, in a randomized order. On one day participants consumed a standard breakfast (106g carbohydrates, 18g fat, 15g protein, 646 calories) and a standardized lunch (103g carbohydrates, 30g fat, 44g protein, 858 calories) separated by 4 hours on one of the experimental days. One the other day, breakfast was omitted but the same lunch was consumed. Blood samples and C magnetic resonance spectroscopy were used to measure changes in glucose concentration and postprandial muscle glycogen storage. Results from this study showed that when breakfast was consumed, the rise in plasma glucose following lunch was significantly reduced (0.9 ± 0.3 vs. 3.2 ± 0.3 mmol/L, $p < 0.01$), and postprandial muscle glycogen storage was improved by approximately 50% two hours following the lunch meal, which was then doubled by the five-hour mark (Jovanovic et al., 2009a). This study was one of the first to demonstrate the link between the second meal phenomenon and enhanced skeletal muscle glycogen synthesis, which is impaired in individuals with T2D (Jovanovic et al., 2009a). In another study by Jovanovic and colleagues (2009) it was shown that this phenomenon also occurs in individuals with T2D (Jovanovic et al., 2009b). When breakfast was consumed, individuals with T2D had a significantly reduced rise is plasma glucose concentration after lunch, compared to the day when no breakfast was consumed (0.68 ± 1.49 vs. 12.32 ± 1.73 mmol/L) (Jovanovic et al., 2009b).

Similarly, Jacubowicz and colleagues investigated the effect of breakfast omission in 22 patients with T2D who completed a two-day diet intervention in a random order, where three standardized meals were consumed on one day and two standardized meals on the other day. All test meals contained 20% fat, 54% carbohydrate and 26% protein, totaling 701 calories. Blood samples were collected at 8am on both test days and in 15-minute intervals following each meal for 180 minutes to assess postprandial plasma glucose. This study showed that, on the breakfast

omission day, area under the curve $(AUC)_{0\text{-}180}$ for plasma glucose, free fatty acids and glucagon were higher for both lunch (37, 41 and 15% higher respectively) and dinner (27, 30, and 12% higher, respectively) compared to the day where breakfast was consumed. Additionally, breakfast omission led to impaired insulin secretion (shown through the delay in peak insulin levels and reduced plasma insulin and c-peptide levels), and higher free fatty acid and glucagon levels after lunch and dinner (Jacubowicz et al., 2015). These findings extend those from Jovanovic and colleagues, and show that the glycemic response is worsened when breakfast is omitted, not only after lunch but also after dinner. Moreover, these findings emphasize the importance of breakfast consumption for glucose homeostasis across the day, and demonstrate that breakfast consumption can influence the glycemic response to subsequent meals. However, it still remains in question how the macronutrient composition at breakfast might affect the PPG response to subsequent meals.

4.4 Other Factors Influencing Diurnal Glucose Values

Presently, it is known that there is a diurnal glucose rhythm, which occurs over a 24-hour period and is generated as a consequence of physiological or behavioural changes (Qian & Scheer, 2016). However, it is not possible to distinguish whether, and to what degree, the diurnal rhythm is generated endogenously or due to behavioural (i.e. food intake/night eating, duration of fasting period/breakfast skipping, physical activity/sedentary time, sleep/wake cycle, sleep restriction/fragmentation) or environmental changes (i.e. 24 hour light or dark cycles) (Quian & Scheer, 2016; Green et al., 2008). Future studies are therefore needed to determine the underlying physiological mechanisms behind the diurnal rhythm, and how behavioural and environmental changes come into play.

5 Strategies to Reduce Postprandial Breakfast Glucose

5.1 Carbohydrate Restriction

It is well known that blood glucose concentration is highly influenced by CHO ingestion (Sheard et al., 2004), where the amount or type of CHO consumed is a significant determinant of PPG (Beulens et al., 2007). Thus, previous interventions have investigated the impact of different dietary strategies and CHO manipulation on glycemic outcomes in T2D patients (Tay et al., 2015a). For instance, Kang et al. examined the effect of CHO proportion at breakfast on PPG fluctuations in individuals with impaired glucose tolerance (N=55) compared to individuals with normal glucose tolerance (N=78). Individuals were assigned to one of three different groups based on their typical macronutrient intake [low-CHO (<45%CHO, n=40 NGT, 37 IGR), medium-CHO (45-65%CHO, n=139 NGT, 76 IGR) or high-CHO (>65%CHO, n=42 NGT, 41 IGR)]. Three-day CGMs were used to assess the glycemic response in all individuals. A positive correlation was seen between postprandial glucose fluctuations and increasing amounts of CHO ingested at each meal. Individuals with impaired glucose tolerance, assigned to the medium or high CHO breakfast groups, had significantly higher incremental AUC (iAUC), PPG excursions, PPG spikes and mean blood glucose levels. Additionally, the time it took for blood glucose levels to return to baseline following the medium or high carbohydrate means was significantly longer in the impaired glucose tolerance groups compared to the normal glucose tolerance individuals (Kang et al., 2013). These results indicate that consuming a larger amount of dietary CHO at breakfast leads to an increase in PPG and glycemic variability, whereas consuming a low-CHO breakfast meal seems to effectively curb PPG excursions and stabilize glucose levels throughout the day.

In a longer-term study conducted by Tay et al. it was shown that a low-CHO diet (14% total energy, 57 g/day; 28%PRO, 58%FAT) led to reductions in glycemic variability as well as

medication requirements, compared to a high CHO diet (53%CHO, 17%PRO and 30%FAT) in 115 individuals with T2D. Additionally, reductions in time spent in hyperglycemia, AUC, mean blood glucose level and HbA1c were seen following a 24-week nutrition and exercise protocol (Tay et al., 2014). After 52 weeks of following this protocol, further improvements were seen in glycemic control (HbA1c), fasting glucose, and glycemic variability, as well as a reduction in diabetes medication, weight, blood pressure and lipids (Tay et al., 2015b). These data support the notion that carbohydrate restriction at breakfast or throughout the day can lead to improvements in glycemic control and glycemic variability over the short and long term. However, these studies did not specifically address how the macronutrient manipulation at one meal (i.e. breakfast) affected the PPG response to subsequent meals.

5.2 Carbohydrate Distribution at Meals

Currently, the nutritional guidelines for diabetes management recommend consuming a moderate amount and even distribution of CHO throughout the day (Dworatzek et al., 2013). However, it has previously been shown that this distribution may not be optimal for glycemic control in T2D (Pearce et al., 2008). Pearce et al. examined the effect of CHO distribution on PPG, assessed by CGM. This study compared the effect of consuming a moderate amount of CHO throughout the day (~40% of total energy) versus consuming an even distribution of carbohydrate at each meal (70g CHO/each (3x) meal) or CHO loading at breakfast, lunch or dinner (125g CHO). Results from this study showed that peak PPG was only weakly related to CHO amount and glycemic load, which accounted for a mere 16-17% of variance in postprandial glycemic excursions. Interestingly, it was shown that consuming an even distribution of CHO throughout the day was not optimal for glycemic control. When the lunch meal was CHO loaded, $AUC_{20\text{-}h}$, PPG peak and time spent above 12 mmol/L values were lower in comparison to consuming an even distribution of CHO throughout the day or CHO loading at breakfast or

dinner (Pearce et al., 2008). These results indicate that consuming meals containing CHO at lunchtime may be the most beneficial for lowering PPG outcomes as compared to consuming an even distribution of CHO throughout the day. Larger scale studies and different CHO manipulations are warranted to determine whether this strategy is beneficial for improving glycemic control and reducing glycemic variability over the long-term.

5.3 Low-Carbohydrate Breakfast for Improving Glucose in T2D

As we were beginning data collection for this study, Pedersen and colleagues published a similar investigation to ours examining the effect of a low-CHO/high-fat breakfast on glycemic control in patients with T2D (Pedersen et al., 2016). In studies where carbohydrates are restricted, it is important to match the total energy intake of the manipulated meals so as to not confound interpretations due to an energy imbalance. This is most commonly accomplished by increasing the proportion of energy coming from fat to the low-carbohydrate meal condition(s) resulting in what can be described as a low-carbohydrate high-fat dietary approach. Pedersen et al. (2016) used CGM to track mean and peak blood glucose levels as well as time spent in a hyperglycaemic state (blood glucose >10 mmol/L) to compare conditions when participants consumed a low-carbohydrate high-fat breakfast versus a standard low-fat breakfast of equal energy content. It was shown that eliminating CHO from the breakfast meal in the low-carbohydrate high-fat breakfast condition decreased peak blood glucose following the meal, but did not affect the glucose response to a lunchtime meal or across a 24-hour period. Based on these findings, this study concluded that reducing daily CHO content by 33%, by avoiding CHO at breakfast, will likely not improve glycemic control in the absence of weight loss for people with T2D (Pedersen et al., 2016). However, there are several considerations worth noting in the authors' interpretations of their findings. First, the authors focused solely on 24-hour average glucose concentration and did not analyze PPG nor did they perform detailed analyses of

glycemic variability, which as mentioned above are key indices of glycemic control in patients with T2D. Given that numerous studies using CGM have reported that breakfast yields the highest PPG spike in individuals with T2D (Preat et al., 2006; Little et al., 2011; Gillen et al., 2012; Monnier et al., 2013; van Dijk et al., 2011; Manders et al., 2006) and there are independent associations between elevated PPG and cardiovascular mortality (Ceriello, 2005; DeFronzo, 2009; Cavalot et al., 2013) it would seem that limiting the post-breakfast spike would be of potential benefit to patients with T2D provided that a low-CHO breakfast did not negatively impact the subsequent lunch or dinner glucose responses. Over time, reducing the PPG response to breakfast could lead to an overall improvement in glycemic control (i.e., lower postprandial hyperglycemia and lower glycemic variability). Consuming CHO at lunch and dinner also aligns better with the altered circadian rhythms in T2D where insulin resistance is higher and insulin secretion is lower in the morning period (Van Cauter et al., 1997). Second, the authors did not base distribution of calories and foods on what participants normally eat, which may have led to changes in diurnal glucose rhythms within itself, therefore reducing external validity of the study. Finally, the study excluded individuals with an HbA1c between 7-8% as the investigators recruited and compared two groups of T2D patients; one with HbA1c <7% and one with HbA1c >8%, leaving it unknown what the impact of breakfast carbohydrate restriction on a large portion of T2D patients with HbA1c values in between 7 and 8%. With these data and considerations in mind, we hypothesize that avoiding CHO at breakfast will reduce the largest PPG spike of the day and will be beneficial for individuals with T2D by reducing overall PPG exposure and glycemic variability. Therefore, the primary purpose of this thesis is to examine the effect of consuming a low-CHO breakfast on PPG, glycemic variability and 24-hour blood glucose response to mixed meals in individuals with T2D.

6 Research Questions

i) Will a breakfast low in carbohydrate and high in fat be more effective than a breakfast with the dietary guidelines recommended nutrient profile (matched for caloric intake) for reducing glycemic response throughout the day in adults with type 2 diabetes (T2D)?

ii) Will a low-carbohydrate high-fat breakfast (LC-BF) improve postprandial glucose responses and reduce glycemic variability to a greater extent than a dietary guidelines breakfast (GL-BF), without worsening the glycemic response to isocaloric lunch and dinner meals?

7 Overall Objective

To determine whether a LC-BF is superior to a GL-BF for improving short-term glucose control in T2D.

7.1 Specific Objectives

1. To determine whether consuming a low-carbohydrate high-fat breakfast (LC-BF; <10%CHO/ 85%FAT/ 15%PRO) is superior to consuming an isocaloric breakfast with dietary guidelines recommended nutrient profile (GL-BF; 55%CHO/ 30%FAT/ 15%PRO) for improving postprandial glucose responses to mixed meals in individuals with T2D.
2. To determine whether the LC-BF will improve 24-hour glucose and glycemic variability when compared to the GL-BF.

8 Hypotheses

1. The LC-BF will lead to an overall reduction in the sum of postprandial glucose incremental area under the curve (iAUC) for breakfast, lunch and dinner, compared to the GL-BF day.
2. The LC-BF will lead to a greater reduction in 24-hour glucose levels and glycemic variability throughout the day compared to the GL-BF.
3. The LC-BF will not significantly worsen the postprandial glucose response to lunch or dinner.

9 Methods

9.1 Participants

Twenty-seven patients with physician diagnosed T2D, aged between 30 and 90 years, were recruited to perform two 24-hour experimental trials in a randomized order. This study was performed at the University of British Columbia Okanagan during the period of June 2016 to June 2017. All participants provided written informed consent, the study protocols were approved by the UBC Clinical Research Committee, and the trial registered at clinicaltrials.gov (NCT02982330). Participants were included if they had physician diagnosed T2D with stable medication and body mass for the preceding three months. Participants were excluded if they were taking exogenous insulin, regularly skipped breakfast, or had been diagnosed with type 1 diabetes or cardiovascular disease. Of the twenty-seven randomized, twenty-three participants successfully completed the two conditions. Baseline characteristics are shown in Table 1, and the Consort study flow diagram is presented as Figure 3.

Table 1. Baseline Characteristics of Participants (n=23)

Sex (F:M)	Age (y)	HbA_{1c} (%)	Body mass (kg)	BMI (kg/m^2)	Energy intake (kcals)	Blood pressure (mmHg)
12:11	59 ± 11	6.7 ± 0.6	88 ± 20	31 ± 7	1921 ± 387	124/79

Values are mean ± standard deviation. F = Females, M= Males, HbA_{1c} = Glycated hemoglobin, BMI = Body mass index

9.2 Experimental Protocol

Participants completed two 24-hour trials in a randomized crossover design. For one trial, a low-carbohydrate high-fat breakfast (LC-BF) was consumed, and on the other trial a breakfast providing the guidelines recommended macronutrient distribution (GL-BF) was consumed. The

two conditions differed only in the macronutrient composition of breakfast, with identical lunch and dinner meals between conditions. Macronutrient profiles for each provided lunch, dinner and GL-BF were based on the Canadian Diabetes Association Clinical Practice Guidelines (Dworatzek et al., 2013) providing ~55% carbohydrate (focusing on low glycemic index), ~30% fat, and ~15% protein, whereas the LC-BF consisted of <10% carbohydrate, ~85% fat, and ~15% protein. Breakfast options were standardized as an oat-based breakfast (GL-BF) or an egg omelette breakfast (LC-BF) (Table 2).

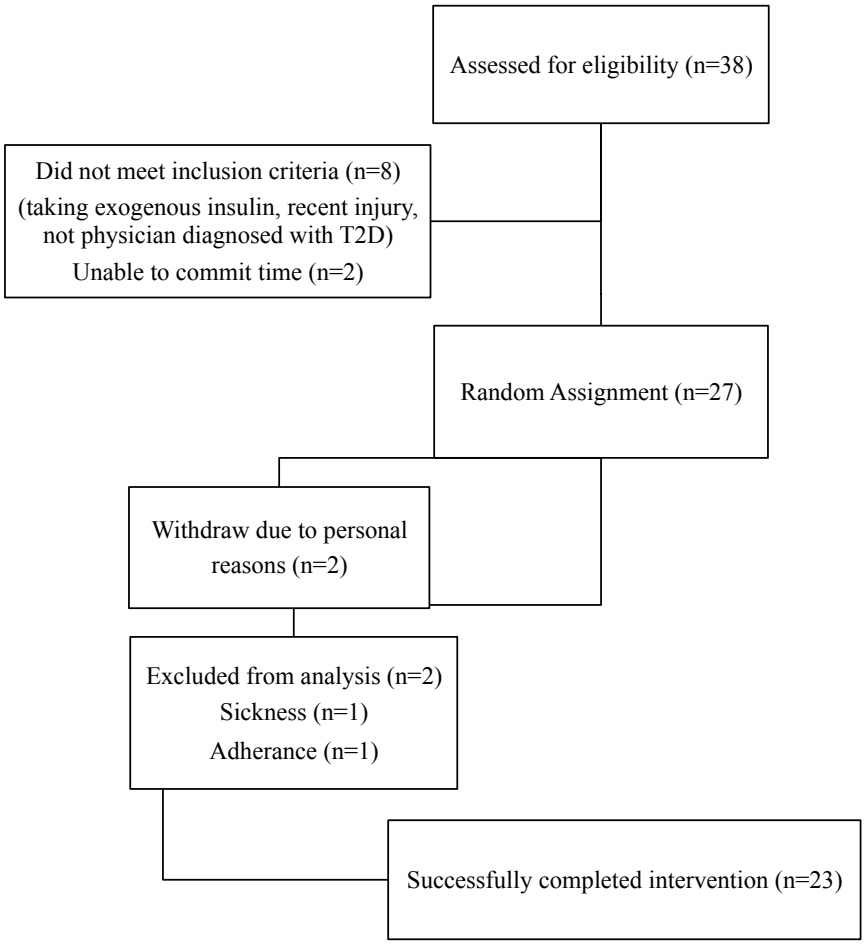

Figure 3. Consort Study Flow Diagram.

Table 2. Example Menu (62y, moderately active female, BMI=27.1)

	Cals (kcals)	CHO (g)	FAT (g)	PRO (g)
LC-BF				
Omelette with: 2 eggs 3.5 Tbsp. whipping cream 1/2 c. shredded cheddar cheese 1 c. spinach 1 tsp. margarine (for frying) 1 c. coffee w/ 1 Tbsp. 10% cream	633	4.6	55.3	29.3
GL-BF				
Breakfast Parfait with: 1/2 c. oats 3/4 c. sliced banana 1/2 c. blueberries 100 g. low-fat yogurt 100 g. Greek yogurt 1 Tbsp. + 2 tsp. pumpkin seeds 1 c. coffee w/ 3 Tbsp. 1% milk	628	82.3	20.4	28.9
Lunch				
Turkey Sandwich with: 2 slices whole grain bread 1 Tbsp. margarine 75 grams turkey 3 outer leaf lettuce (romaine) 6 cherry tomatoes 6 baby carrots 1 large apple 6 cashew nuts	513	67.5	16.6	23.5
Dinner				
650mL butternut squash soup 2/3 c. cubed chicken breast 10 whole wheat crackers 37 g. marble cheese 1 large apple	940	120.6	31.1	44.4
Daily Average	**(kcals)**	**(%)**	**(%)**	**(%)**
LC-BF	2086	35.6	45.8	18.6
GL-BF	2081	52.1	29.4	18.5

LC-BF = low carbohydrate breakfast, GL-BF = guidelines breakfast, cals = calories, kcal = kilocalories, g = grams, CHO = carbohydrates, PRO = protein, Tbsp. = tablespoon, c = cups, tsp. = teaspoon

First, participants completed a three-day food log in order to generate individualized meal plans based on food preferences and normal caloric distribution for meals. Calories were matched between conditions for each meal within participants (Table 3). Energy requirements for the day were calculated using the Harris-Benedict formula and Physical Activity Level 1.4 (Gerrior et al., 2006; Males**:** RMR [kcal/day] = 66.4730 + 13.7516W + 5.0033H – 6.7750A and Females: RMR [kcal/day] = 665:0955 + 9.5634W + 1.8496H - 4:6756A, where W = weight in kilograms; H = height in centimeters; A = age in years). Participants were provided with all food items, as well as meal preparation instructions, for six meals (three meals per day), with the timing of meals standardized between trials (meals were separated by at least three hours). A logbook was provided for participants who were instructed to record the timing of their meals, any changes made to their prescribed meal plan, daily physical activity and to record capillary glucose measurements for CGM calibration. The primary outcome of postprandial glucose was assessed using CGM (iPro®2 Professional, Medtronic Inc.). The CGM sensor (Enlite™ Sensor, Medronic Inc.) was inserted the day before the first condition and removed 24 hours after the second condition. Participants were also instructed to take four capillary glucose measurements per day for CGM calibration (before breakfast, lunch, dinner and bedtime).

9.3 Self-Reported Appetite Ratings

To explore subjective ratings of hunger, fullness, and desire to eat something sweet or savoury, Visual Analog Scales (VAS) were used. Before and after each meal participants rated each of the following four questions by marking vertically on a horizontal line with descriptive anchors on either side (‘not at all’ to ‘extremely’): 1) How hungry do you feel; 2) How full do you feel; 3) How strong was your desire to eat savoury foods; and 4) How strong was your desire to eat sweat foods. The VAS scores were converted to a 0-100 scale, as previously described (Flint et al., 2000; Rebello et al., 2016).

9.4 Analyses

Data from the CGM were downloaded and integrated with four capillary glucose calibrations using the Carelink software (Medtronic Inc.) before being exported to Excel for analyses. Glycemic variability and mean glucose across each 24-h period (starting immediately before breakfast) were analyzed using the online EasyGV platform (EasyGV, Oxford, UK). Postprandial hyperglycemia, for both total (24-hour) and meals (3-hour) area under the curve (AUC) and incremental AUC (iAUC), was assessed using the trapezoid method (Le Floch et al., 1990). Total AUC describes glycemic control incorporating basal blood glucose levels, whereas iAUC largely represents the glycemic response to meals (Le Floch et al., 1990).

9.5 Statistical Analyses

Statistical analyses were performed using SPSS 24.0 (SPSS, Chicago, Illinois). Data were assessed for normality using histograms and Q-Q plots and transformed using natural log or square root transformation prior to analyses if necessary. For all summary CGM variables (sum of 3h post-meal glucose iAUC, 24h mean, 24h AUC, 24h iAUC, MAGE, SD, maximal glucose, minimal glucose) data were analyzed using paired t-tests. The primary outcome was overall postprandial hyperglycemia, defined as the sum of the 3h post-meal iAUC. Additionally, repeated measures ANOVA (2 conditions X 3 time points; breakfast, lunch & dinner) was used to examine the postprandial responses at each meal. Separate repeated measures ANOVAs were used to assess hunger/satiety scores before (pre) and after (post) meals. Significant interactions were followed up with paired-sample t-tests between conditions. Sample size was calculated a priori in order to detect a 20% reduction (effect size, Cohen's d = 0.72) in the sum of 3-hour post-meal iAUC based on means and standard deviations from previous CGM studies conducted in our laboratory. It was estimated that 23 paired observations would be needed to detect a 20%

difference in iAUC with 90% power and effect size of 0.72 assuming a conservative correlation between repeated measures of r=0.5 (calculated using G*Power v3). A 20% reduction was considered clinically relevant based on previous studies showing that commonly-prescribed glucose-lowering medications lead to a ~20% reduction in PPG (Goldstein et al., 2007). Effect sizes for pairwise comparisons were calculated using Cohen's *d*.

10 Results

Summary CGM curves for the LC-BF and GL-BF conditions are shown in Figure 4A. Full CGM data analyses for all variables are shown in Table 3.

10.1 Postprandial Blood Glucose

The primary outcome of the sum of the 3-h post-meal glucose iAUC was significantly reduced by -100 ± 116 mmol/L•9h in the LC-BF condition compared to the GL-BF ($p<0.01$, 95% CI: -151.4, -48.6, Figure 4A & 4B). 24-h iAUC was also lower by -173.5 ± 361 mmol/L with the LC-BF compared to the GL-BF ($p<0.05$, 95% CI: -333.4, -13.6). Total 24-h AUC was not significantly different between the LC-BF and GL-BF conditions (Table 3). The 3-h postprandial iAUC, mean and peak glucose responses to each meal were significantly different between the LC-BF and GL-BF conditions (Condition X Time interactions: $p<0.01$, Table 3). Compared to GL-BF, the LC-BF reduced the 3-h blood glucose after breakfast (by -1.4 ± 1.3 mmol/L, $p<0.01$, 95% CI: -1.94, -0.80) but not after lunch (0.1 ± 1.3 mmol/L, $p=0.65$, 95% CI: -0.47, 0.74) or dinner (0.0 ± 1.1 mmol/L, $p=0.91$, 95% CI: -0.44, 0.50).

10.2 Glycemic Variability

The 24-h MAGE in the LC-BF condition was significantly lower (by -0.4 ± 0.8 mmol/L, $p=0.03$, 95% CI: -0.7, -0.04, Figure 4C) when compared to the GL-BF. The SD of blood glucose across 24-h with the LC-BF was also significantly lower (by -0.2 ± 0.4 mmol/L, $p=0.01$, 95% CI: -0.41, -0.07, Table 3) compared to the GL-BF.

10.3 24-hour Average and Peak Blood Glucose

Mean 24-h blood glucose was not significantly different between the LC-BF (7.2 ± 1.1 mmol/L) and GL-BF (7.5 ± 1.5 mmol/L) conditions (p=0.09, Table 3). However, the peak blood glucose was significantly reduced by 0.9 mmol/L (p=0.02, 95% CI: -1.56, -0.167, Table 3).

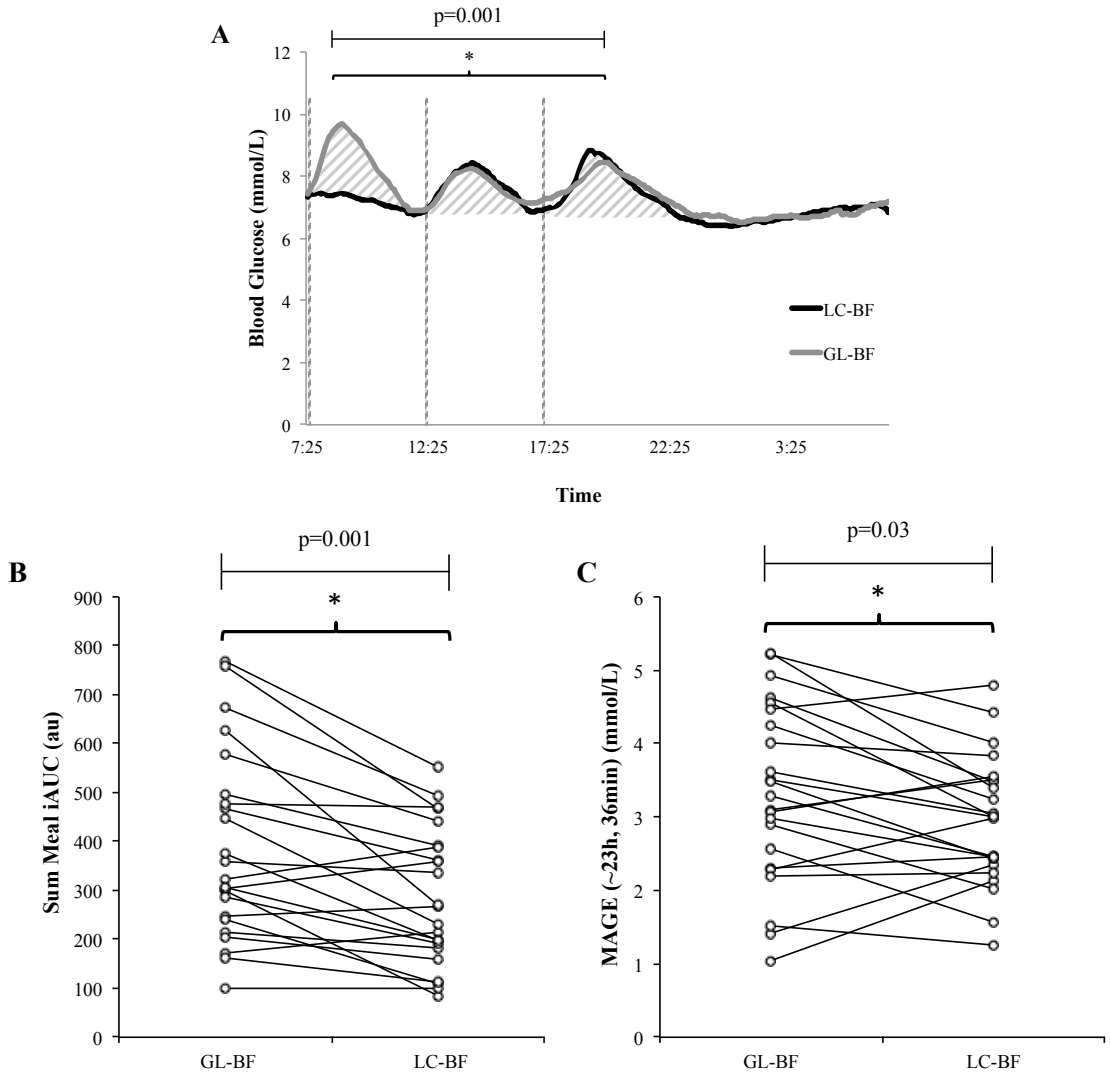

Figure 4. CGM Summary and Individual Data for Sum Meal iAUC and MAGE. A) Continuous blood glucose data (n=23) for 24-h with a low-carbohydrate high-fat breakfast (LC-BF; black line) compared to guidelines breakfast (GL-BF; grey line). Striped shaded area indicates the sum of 3-h post-meal incremental area under the curve (iAUC), which was significantly lower in the LC-BF versus the GL-BF condition. **B)** Sum 3-h postprandial iAUC individual data. **C)** MAGE individual data. Abbreviations: LC-BF = low carbohydrate breakfast, GL-BF = guidelines breakfast, iAUC = incremental area under the curve, MAGE = mean amplitude of glycemic variability. P-values refer to paired t-tests between conditions.

Table 3. Summary of Continuous Glucose Monitoring (CGM) Data

	GL-BF	LC-BF	P-value	Cohen's d
24-h total AUC (mmol/L•24-h)	10610 ± 2221	10223 ± 1633	0.08	-0.48
24-h iAUC (mmol/L•24-h)	540 ± 477	366 ± 289*	0.03	-0.55
Sum meal iAUC (mmol/L•9-h)	390 ± 196	290 ± 143*	0.001	-0.96
MAGE (mmol/L)	3.3 ± 1.2	2.9 ± 0.9*	0.03	-0.53
24-h mean glucose (mmol/L)	7.5 ± 1.5	7.2 ± 1.1	0.14	-0.39
24-h SD (mmol/L)	1.3 ± 0.5	1.0 ± 0.3*	<0.001	-0.72
24-h peak glucose (mmol/L)	10.9 ± 2.5	10.0 ± 1.6*	0.02	-0.65
24-h min glucose (mmol/L)	5.8 ± 1.1	5.5 ± 0.9	0.16	-0.31
PPG Breakfast (mmol/L)				
3-h mean glucose	8.8 ± 2.0	7.4 ± 1.3*	<0.001	-1.19
3-h peak glucose	10.5 ± 2.4	8.1 ± 1.5*	<0.001	-1.75
3-h min glucose	7.0 ± 1.7	6.7 ± 1.3	0.16	-0.32
3-h AUC	1583 ± 368	1335 ± 244*	<0.001	-1.19
3-h iAUC	136 ± 78	35 ± 40*	<0.001	-1.86
PPG Lunch (mmol/L)				
3-h mean glucose	7.8 ± 2.1	8.0 ± 1.2	0.48	0.18
3-h peak glucose	9.3 ± 2.5	9.3 ± 1.6	0.94	0.01
3-h min glucose	6.4 ± 1.6	6.5 ± 0.9	0.59	0.14
3-h AUC	1415 ± 374	1454 ± 223	0.46	0.19
3-h iAUC	113 ± 75	118 ± 90	0.71	0.08
PPG Dinner (mmol/L)				
3-h mean glucose	8.2 ± 1.8	8.2 ± 1.5	0.91	0.02
3-h peak glucose	10.0 ± 3.3	9.5 ± 1.8	0.44	-0.18
3-h min glucose	6.4 ± 1.4	6.6 ± 1.4	0.38	0.19
3-h AUC	1473 ± 334	1476 ± 269	0.93	0.02
3-h iAUC	136 ± 90	135 ± 83	0.98	-0.01

GL-BF = guidelines breakfast, LC-BF = low carbohydrate breakfast, AUC = area under the curve, iAUC = incremental area under the curve, MAGE = mean amplitude of glycemic excursions, SD = standard deviation, PPG = postprandial glucose, mmol/L = millimoles per litre

10.4 Self-Reported Appetite Ratings

Hunger, satiety, desire for sweets, and desire or savoury foods assessed before (pre) and after (post) each meal are presented in Table 4. Pre-meal hunger demonstrated a significant condition X time interaction (p=0.03) with post-hoc pairwise comparison testing between conditions showing lower hunger before dinner in the LC-BF compared to the GL-BF (p=0.03; Figure 5A). Post-meal hunger, and pre- and post-meal fullness did not differ between conditions (all p>0.11). Desire to eat sweet foods tended to be lower in the LC-BF condition compared to the GL-BF condition (Main effect condition: p=0.06, Figure 5B). The post-meal desire to eat sweet foods, and pre- and post-meal desire for savoury foods did not significantly change across time or differ between conditions (all p>0.17).

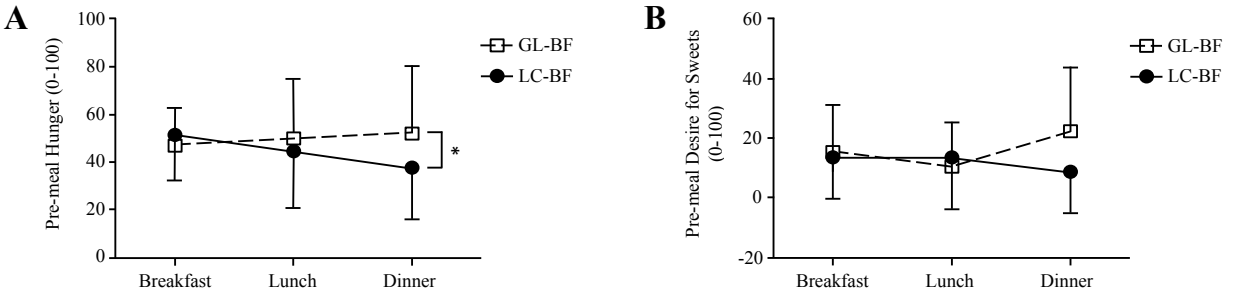

Figure 5. Self-Reported Ratings of Satiety. A) Self-reported ratings of pre-meal hunger (n=13). **B)** Self-reported ratings of pre-meal craving for sweets (n=13). A significant condition X time interaction was detected for pre-meal hunger (p=0.03). *P<0.05 pairwise comparison between GL-BF and LC-BF at the dinner time point.

Table 4. Self-Reported Ratings of Satiety

	GL-BF	LC-BF
Breakfast		
Pre-meal hunger	47 ± 15.6	51.1 ± 18.3
Post-meal hunger	12.4 ± 19.3	14 ± 19.1
Pre-meal fullness	21.7 ± 15.3	23.1 ± 23.3
Post-meal fullness	65.7 ± 19.8	54.7 ± 29.7
Pre-meal desire for savoury	26.4 ± 25.1	27.5 ± 21
Post-meal desire for savoury	21.7 ± 24.5	20.9 ± 21.8
Pre-meal desire for sweets	15.1 ± 15.8	13.7 ± 14.3
Post-meal desire for sweets	10.2 ± 15.7	9.6 ± 15.2
Lunch		
Pre-meal hunger	50 ± 24.6	44.8 ± 24.7
Post-meal hunger	14.2 ± 18	16.8 ± 22.5
Pre-meal fullness	15.4 ± 12.9	24.2 ± 23.7
Post-meal fullness	52.2 ± 25.5	63.5 ± 19.9
Pre-meal desire for savoury	34.3 ± 20.3	31.6 ± 20.7
Post-meal desire for savoury	22.8 ± 22.6	26.6 ± 23.5
Pre-meal desire for sweets	10.4 ± 14.6	13.5 ± 17.6
Post-meal desire for sweets	12.4 ± 17	18.4 ± 20.3
Dinner		
Pre-meal hunger	51.9 ± 28.4	37.6 ± 21.8*
Post-meal hunger	9.6 ± 11.9	10.2 ± 11.6
Pre-meal fullness	23.4 ± 18.3	36.3 ± 22.5
Post-meal fullness	55.5 ± 16.9	62.9 ± 20.5
Pre-meal desire for savoury	39.6 ± 28.6	30.2 ± 20.2
Post-meal desire for savoury	18.4 ± 22.1	17.6 ± 19.8
Pre-meal desire for sweets	22.3 ± 21.3	8.8 ± 14
Post-meal desire for sweets	12.1 ± 16.8	9.9 ± 14.2

Values are mean ± standard deviation of n = 13 individuals per group. Units of measurement reported as percentage**,** p values determined via paired t-tests. GL-BF = guidelines breakfast, LC-BF = low-carbohydrate breakfast. A significant meal X time interaction was found for pre-meal hunger ($P<0.05$). *$P<0.05$ versus dinner pre-meal hunger in GL-BF condition.

11 Discussion

The present study shows that consuming a low-carbohydrate high-fat breakfast (LC-BF) lowers postprandial hyperglycemia and glycemic variability across the subsequent 24 hours. In addition, ratings of pre-meal hunger and desire to eat sweet foods later in the day were reduced in the LC-BF condition. These potential benefits of a LC-BF were realized when compared to an isocaloric mixed macronutrient breakfast based on what is typically recommended; i.e., low in fat and moderate in carbohydrate (Dworatzek et al., 2013; American Diabetes Association, 2004). Previous studies have shown that an overall low-carbohydrate high-fat diet lowers hyperglycemia, blood lipids and improves body composition over several weeks/month (Boden et al., 2005; Forsythe et al., 2008; Samaha et al., 2003; Saslow et al., 2014; Volek et al., 2004; Westman et al., 2008); however long-term compliance to restrictive dietary interventions are poor (Pagoto & Appelhans, 2013). Here, we provide evidence that a low-carbohydrate high-fat breakfast may be a simple and effective strategy to reduce hyperglycemia in individuals with T2D. However, intervention studies are warranted to determine the long-term impact on glycemic control measures, cardiovascular risk factors, and other health outcomes.

11.1 Low-Carbohydrate High-Fat Breakfast Lowers Postprandial Hyperglycemia

The postprandial glucose response to breakfast was reduced by 74% when carbohydrates were restricted to less than 10% of breakfast caloric intake. This is in agreement with previous studies (Ceriello et al., 1999; Kang et al., 2013; Pedersen et al., 2016), highlighting the effectiveness of carbohydrate restriction to limit postprandial hyperglycemia in T2D. The present findings and those of others (Clark et al., 2006; Pedersen et al., 2016) show that there is no carryover effect of a LC-BF on the postprandial response to lunch or dinner. Pedersen et al. (2016) have previously proposed that restricting carbohydrates at breakfast might lead to a

subsequent worsening of the lunch and dinner responses, as this has been seen with breakfast omission (Jacubowicz et al, 2015). However this does not appear to be the case as there were no differences between lunch and dinner between the LC-BF and GL-BF conditions. Therefore, much of the effect for reducing overall postprandial hyperglycemia in our study (i.e., sum of iAUC; Figure 4B) can be attributed to reducing the immediate postprandial glycemic response to breakfast with no evidence of a LC-BF worsening glucose responses to lunch or dinner.

11.2 Low-Carbohydrate High-Fat Breakfast Lowers Glycemic Variability

Glycemic variability (frequency and magnitude of 24-hour glucose oscillations) as assessed by MAGE and 24-hour SD was significantly reduced when a LC-BF was consumed compared to a GL-BF. The MAGE gives an index of within-day fluctuations in glucose that are greater than one SD, and is regarded as the most comprehensive marker of intraday glycemic variability (Monnier et al., 2008; Tay et al., 2015a). Interestingly, the reduction in MAGE in the present study agrees with a previous study reporting a significant reduction in glycemic variability with the use of the drug acarbose (Derosa et al., 2015). A reduction in glycemic variability may be cardioprotective as hyperglycemic excursions are known to be proatherogenic by stimulating reactive oxygen species and inflammatory cytokine production that contribute to the development of cardiovascular disease (Brownlee, 2005). For example, in individuals with T2D, a meal high in carbohydrates (causing hyperglycemia) increases the susceptibility of LDL to oxidation (Ceriello et al., 1999) and has been shown to impair vascular endothelial function (Ceriello et al., 2005). Additionally, a meal containing equal amounts of carbohydrate and fat significantly impairs endothelial function, whereas a low-carbohydrate meal alone does not (Ceriello et al., 2002). Indeed, oscillating blood glucose is more deleterious for promoting oxidative stress (Monnier et al., 2006) and predicting future cardiovascular risk than constant hyperglycemia (Ceriello et al., 2008). These data highlight the importance of reducing

postprandial hyperglycemia and glycemic variability in individuals with T2D. The current study expands on previous work by using CGM analyses to show that postprandial hyperglycemia and glycemic variability are reduced over a 24-hour period after a LC-BF is consumed.

11.3 Low-Carbohydrate High-Fat Breakfast May Restore Diurnal Glucose Rhythms

Generally, an even distribution of macronutrients across the day is recommended in T2D (American Diabetes Association, 2004; Gillen & Tapsell, 2006). However, it is currently unclear whether this recommendation is optimal for glucose control in patients with T2D or for all cardiometabolic health outcomes. The hyperglycemic response to breakfast is the largest and most prevalent in individuals with T2D (van Dijk et al, 2011). Indeed, the present study showed that by reducing hyperglycemia at breakfast the 24-hour peak glucose was reduced. Therefore, restricting carbohydrates at breakfast appears to be a simple and effective strategy to reduce the magnitude and prevalence of hyperglycemia across the day. However, it is important to note that the same may not be true for individuals without T2D. In healthy adults, markedly higher responses to carbohydrates are seen in the evening (Carroll & Nestel, 1973, Saad et al., 2012), which is likely related to the opposite diurnal variation in glucose tolerance and insulin sensitivity seen in healthy adults compared to individuals with T2D (reviewed by Van Cauter et al. 1997; Qian & Scheer, 2016). In the healthy state, glucose tolerance and insulin sensitivity are highest in the morning whereas in T2D this is reversed, contributing to the large postprandial glucose spike after breakfast (Manders et al., 2006; van Dijk et al., 2011; Little et al., 2011). Therefore, the optimal timing of carbohydrates may depend on an individuals' degree of glycemic control. Kessler et al. (Kessler et al., 2017) showed that four weeks of consuming high-carbohydrate meals until 13:30 followed by low-carbohydrate meals between 16:30 to 22:00 resulted in a 7.9% decrease in the whole-day glycemic response, compared to the inverse sequence of meal composition in individuals with impaired glucose tolerance. Indeed,

carbohydrate consumption in the evening is linked to the metabolic dysregulation that occurs with late feeding in shift workers (Morgan et al., 2012). However, these data are in contrast to some findings from normoglycemic overweight/obese adults which show that glycemic control is improved when carbohydrates are eaten at dinner (Alves et al., 2014). Moreover, in obese individuals consuming carbohydrates mainly at dinner has been shown to lead to more pronounced weight loss and reduced hunger (Sofer et al., 2011). Clearly further research regarding potential diurnal rhythms and carbohydrate consumption is warranted. Our findings of reduced postprandial hyperglycemia and lower glycemic variability with carbohydrate restriction at breakfast may apply to those with T2D where glycemic outcomes are most important.

11.4 Low-Carbohydrate High-Fat Breakfast May Reduce Hunger Later in The Day

Research on the satiating effects of carbohydrate versus fat is conflicting (Blundell et al., 1993; Cecil et al., 1999, Cotton et al., 1994), however, most studies have only looked at the immediate response to a single meal and not how manipulating carbohydrate or fat at the breakfast meal impacts subsequent meals. Our design allowed us to determine how changing only breakfast might impact hunger and satiety later in the day when identical lunch and dinner meals were consumed. The rapid weight loss seen with a low-carbohydrate high-fat diet is purported to be attributed to, in part, appetite suppression (Erlanson-Albertsson & Mei, 2005). Our findings of lower hunger at dinner, after consuming a LC-BF, could be interpreted to indicate that such a strategy could lead to reduced energy intake in people with T2D. Interestingly, cravings for sweet foods followed the same trend as hunger, showing evidence of reduced cravings for sweets later on in the day (Main effect condition, $p=0.06$). These findings of lower hunger, and potentially lower cravings for sweets, may help inform additional ad libitum studies to determine whether consuming a LC-BF can curb hunger and therefore help promote weight loss in T2D. Notably, Gibbons and colleagues have previously shown that ghrelin, GLP-1

and PYY levels increase significantly following a low-carbohydrate high-fat meal, where ghrelin and GLP-1 levels were associated with short-term improvements in appetite control and reduced feelings of hunger (Gibbons et al., 2013). Therefore, it may be beneficial to measure ghrelin, GLP-1 and PYY levels throughout the day, in association with feelings of hunger and satiety, following a low-carbohydrate high-fat breakfast in future studies.

11.5 Strengths and Limitations

The use of CGM was a strength in the present study because it enabled analyses of 24-hour glucose profiles, postprandial glucose excursions and glycemic variability under free-living conditions, which could not have otherwise been done with the use of capillary glucose measurements alone. Another strength of this study was the use of individualized meals plans, which were tailored to the participant food preferences and typical meal sizes, as opposed to providing a generalized meal plan for all volunteers. We believe this may have contributed to the high rate of participant adherence to the study parameters and demonstrates utility for potential implementation in future research and clinical applications.

Although glucose control is the major clinical target in T2D, blood lipids are also important cardiovascular risk factors. The present study used CGM in free-living volunteers to measure glucose but unfortunately we did not have serial blood samples to measure postprandial triglycerides or free fatty acids, which may impact cardiovascular risk mechanisms independent of glucose (Ceriello et al., 2004). It is possible that the LC-BF could have differentially impacted blood lipids throughout the day and future work should explore how this diet manipulation influences other aspects of metabolism in addition to glucose control. Insulin levels and gastric emptying were also not measured in the current study, which limited the ability to determine how the different breakfasts impacted mechanisms related to postprandial glucose control. Additionally, the negative impacts of high PPG and glycemic variability have been attributed to

ROS, inflammation and endothelial dysfunction, which elevate CVD risk (Ceriello, 2005; Monnier et al., 2008). These mechanistic aspects of CV risk were not directly assessed in the present study through venous blood sampling and/or vascular function measures so it remains undetermined whether lowering PPG and glycemic variability impacted these outcomes. Furthermore, given that hunger/satiety assessments were an exploratory outcome and focus was placed on following the diet plan exactly as prescribed, the hunger/satiety measures were perhaps not prioritized by the participants, which resulted in a smaller sample size for these measures due to incomplete and missing data. Lastly, the external validity may have been reduced because participants were asked to consume relatively large meals with at least 3-5 hours in between in order to accurately measure 3-h PPG. In reality individuals may break up meals into smaller portions and consume snacks between them.

12 Conclusions

A LC-BF significantly reduces the largest glucose spike of the day, improves overall postprandial hyperglycemia, and lowers glycemic variability in individuals with T2D. The inclusion of a low-carbohydrate high-fat breakfast meal in T2D patients may be a practical and easy way to target the large morning glucose spike, when insulin resistance is the highest and glucose tolerance the lowest, without worsening glycemic responses to subsequent meals. The results of our study suggest potential benefits of altering macronutrient distribution throughout the day such that carbohydrates are restricted at breakfast with a balanced lunch and dinner rather than consuming an even distribution and moderate amount of carbohydrates throughout the day. Further testing is needed to determine if, over the long term, this meal pattern lowers cardiovascular risk markers and diabetes complications. The encouraging preliminary findings showing lower hunger later in the day following a LC-BF also indicates this approach could have wider implications for weight loss, but this will require further research.

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Appendices

Appendix A: CGM, Diet and Physical Activity Logbook

CGM, Diet and physical activity Logbook

Continuous glucose monitoring (CGM) Instructions for participants

This is your CGM and diet log book for the days that you will be wearing the CGM. Please read and use the following instructions. If you have any problems or questions please call:

_____on _____

WHAT TO DO WHILE WEARING YOUR DEVICE:

- You should continue your normal daily activities while wearing the device.
- The device (but not the monitor) is waterproof and can be worn in the bath or shower.
- Please continue to eat your normal diet. You will need to record what you ate and when you ate it in this log book.
- Please take your finger stick blood glucose readings and enter the results into both this log book and the monitor.

WHAT NOT TO DO WHILE WEARING YOUR DEVICE:

- Although the device that is attached to your stomach (sensor) is waterproof, we ask that you please do not go in a hot tub while wearing the CGM.
- It is important that you do not take any Tylenol or other acetaminophen-containing products while wearing the device. This is because they interfere with the sensors ability to measure blood sugar.

WHEN TO TAKE BLOOD SUGAR FINGER PRICKS.

- For each day that you wear the CGM the times that you need to take a finger stick blood glucose are recorded on each day in the log book.
- You need to take these measures at least 3 times a day in order to calibrate the CGM.
    - Finger pricks will be taken before breakfast, lunch and dinner (an additional one can be taken before bed time if you want).

HOW TO TAKE A BLOOD SUGAR FINGER PRICK.

1) Wash your finger with warm water and soap and dry it with a towel. If you can not access a tap at the time, clean your finger with an alcohol wipe.

2) Put a new strip into the glucose meter inserting the end with black and white stripes so that you can't see the stripes anymore. Put the meter aside. The meter will be ready for drop of blood when the drop signal flashes:

3) Using the blue lancet, sliding the light blue button back until it clicks. The lancet it now ready to use.
4) Press the end of the lancet against the side of your finger and press the small light blue button.
5) You need to create a blood drop about this size ●. Gently message at the base of the finger to create the blood drop. DO NOT squeeze your finger near the site.
6) Touch and hold the drop of blood to the end of the strip with the thin yellow stripe on the top edge. Blood will be drawn into the stripe. Keep holding the drop of blood to the top edge of the test strip until the confirmation window is full.
7) **Record this reading into your logbook.**
8) Dispose of the strip. DO NOT dispose of the lancet. The lancet provided will last for time you are wearing the CGM.

CGM
EXAMPLE DAY

NOTE: This is an example of what your log book will look like. You DO NOT have to follow these times exactly.

	Time	Blood glucose
Calibration 1 Before breakfast	7:30 AM	6.5
Calibration 2 Before lunch	11:30 AM	7.5
Calibration 3 Before bedtime	9:30 PM	8.7

NOTE: Please be as specific as possible when entering your additional food data. Use the examples below.

Meal	Time	Food and drink (Be as specific as possible)	Physical Activity
Breakfast	8:00 AM	Breakfast provided (no leftover).	15 minutes starting at 8:30am.
Snack **Please include brand names of food	11:00 PM	**WHAT TO WRITE:** ½ medium banana; 1 slice whole wheat bread; 1 cup milk (2%); 1 ½ cup cereal (Multigrain Cheerios) **WHAT NOT TO WRITE:** Banana, bread, milk, cereal	Not applicable.

CGM - Finger prick blood glucose.
DAY ONE

Insertion date: _____ Insertion time: _____ RA initial:_____

CGM Serial Number: _____

Meal	Time	Blood glucose
Calibration 1 1 h after insertion		
Calibration 2 Before lunch		
Calibration 3 Before dinner		
Calibration 4 (if necessary) Before bedtime		

Meal	Time	Any Food/Diet Changes	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

Total # steps per day: _____

CGM - Finger prick blood glucose.
DAY TWO

Date: _____

	Time	Blood glucose
Calibration 1 Before breakfast		
Calibration 2 Before lunch		
Calibration 3 Before dinner		
Calibration 4 (if necessary) Before bedtime		

Meal	Time	Any Food / Diet Changes	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

Total # steps per day: _____

CGM - Finger prick blood glucose.
DAY THREE

Date: _____

	Time	Blood glucose
Calibration 1 Before breakfast		
Calibration 2 Before lunch		
Calibration 3 Before dinner		
Calibration 4 (if necessary) Before bedtime		

Meal	Time	Any Food / Diet Changes	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

Total # steps per day: _____

CGM - Finger prick blood glucose.
DAY FOUR

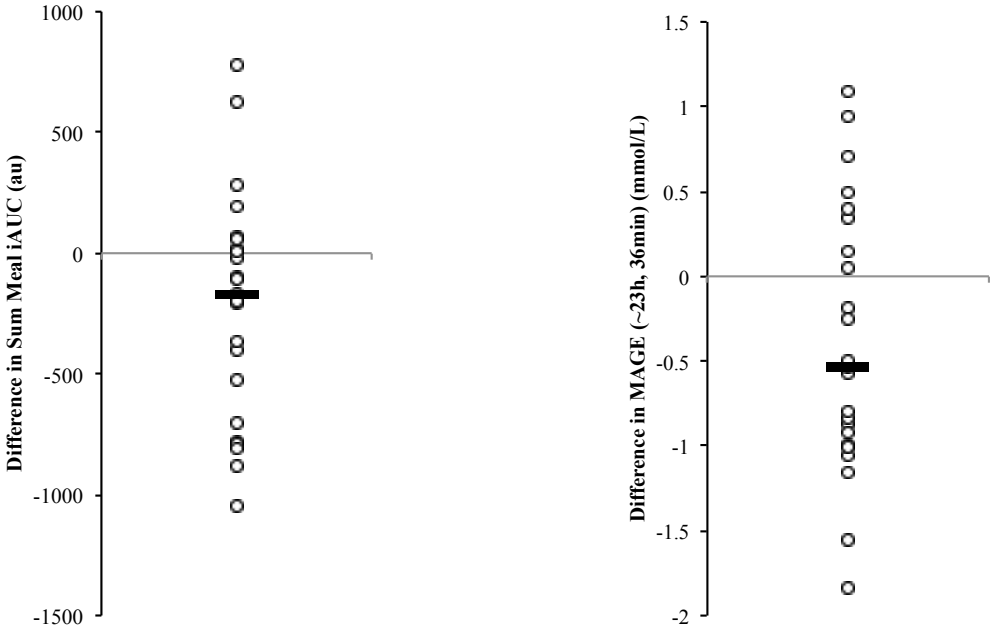
Date: _____

	Time	Blood glucose
Calibration 1 Before breakfast		
Calibration 2 Before lunch		
Calibration 3 Before dinner		
Calibration 4 (if necessary) Before bedtime		

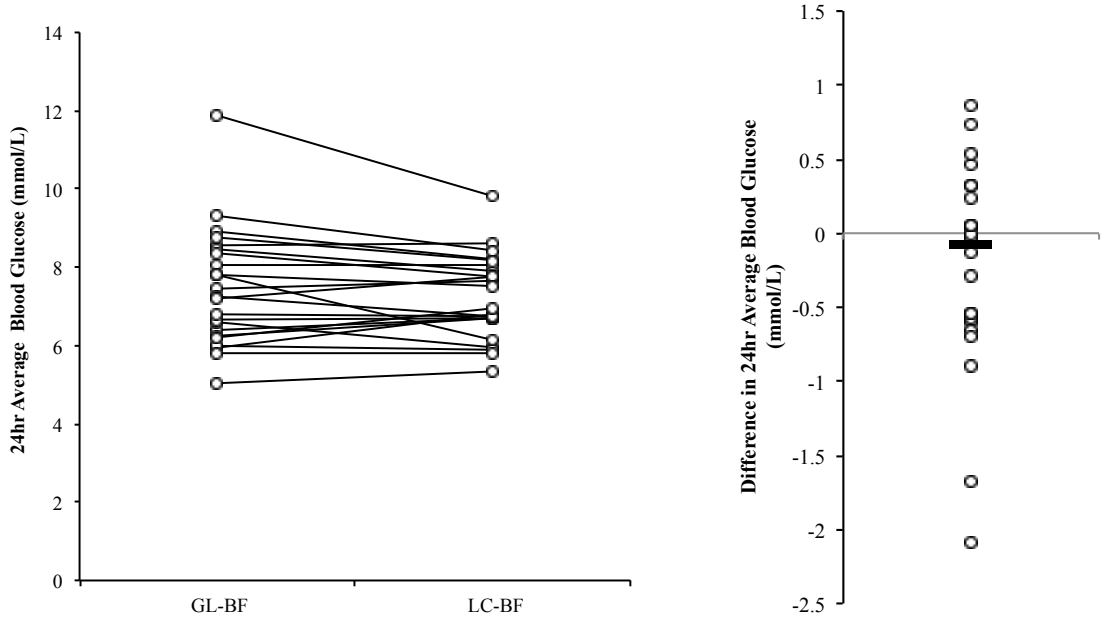
Meal	Time	Any Food / Diet Changes	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

Total # steps per day: _____

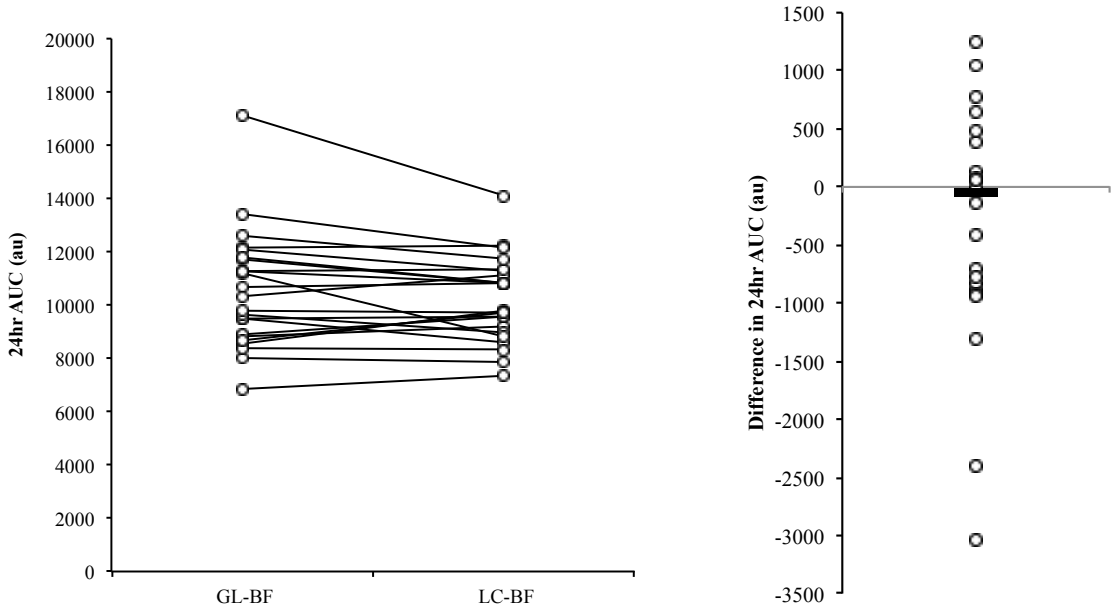
Appendix B: Figures with individual data



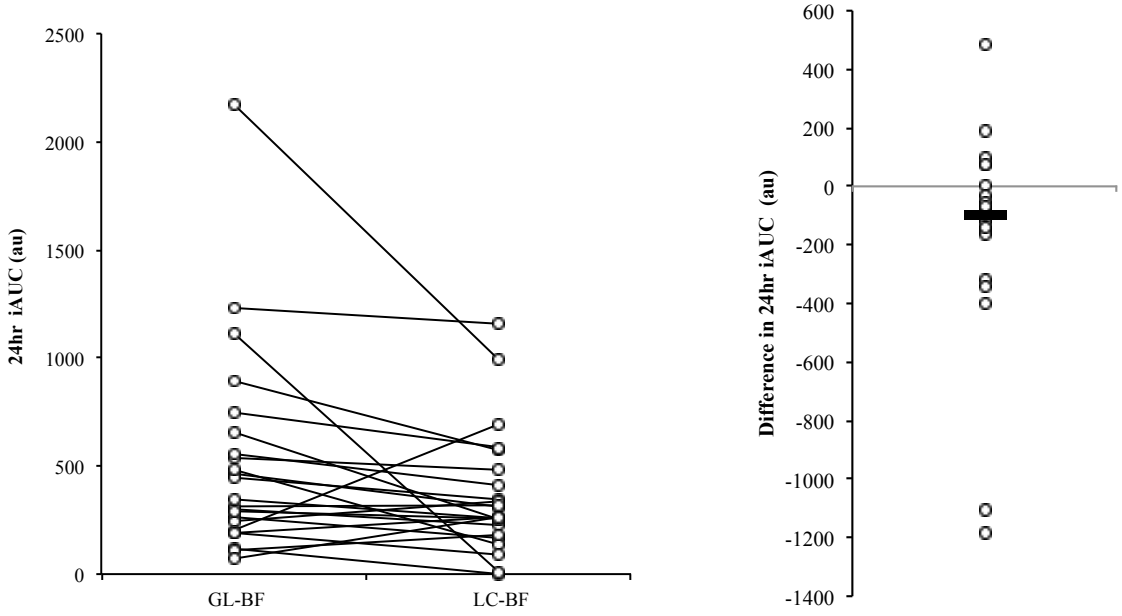
Difference in Sum Meal Incremental Area Under the Curve (iAUC; left) and Mean Amplitude of Glycemic Excursions (MAGE; right) between the guidelines breakfast and low-carbohydrate high-fat breakfast conditions.



24-hour average blood glucose between conditions



24-hour area under the curve (AUC) between conditions



24-hour incremental area under the curve (iAUC) between conditions