A LOW-CARBOHYDRATE PROTEIN CONTAINING BEDTIME SNACK TO CONTROL MORNING BLOOD GLUCOSE IN TYPE 2 DIABETES

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Abstract

In people with type 2 diabetes, liver insulin resistance leading to excess hepatic glucose production results in elevated fasting glucose. A bedtime snack is frequently recommended to improve morning glucose levels, yet there is little evidence supporting this recommendation. Moreover, it is not known what the optimal composition of such bedtime snacks should be.

PURPOSE: The primary purpose of this study was to determine whether a low-carbohydrate protein containing bedtime snack could reduce next morning fasting plasma glucose levels in people with type 2 diabetes when compared to a high-carbohydrate protein containing bedtime snack or a no bedtime snack condition. We also explored whether a low-carbohydrate protein containing snack consumed at bedtime would improve markers of insulin sensitivity and glucose control.

METHODS: Using a randomized crossover design, fifteen patients with type 2 diabetes completed three separate conditions: i) a low-carbohydrate protein containing bedtime snack (eggs), ii) a high-carbohydrate protein matched bedtime snack (low-fat yogurt); and iii) no bedtime snack. All conditions were three days in length and were isoenergetic.

RESULTS: Consuming a low-carbohydrate protein containing bedtime snack significantly reduced fasting plasma glucose ($P = 0.04$) and insulin ($P = 0.04$), improved quantitative insulin sensitivity check index (QUICKI; $P = 0.003$), and lowered nocturnal glucose assessed by continuous glucose monitoring (CGM; $P = 0.02$) when compared to a high-carbohydrate protein matched bedtime snack. There were no significant differences between the low-carbohydrate protein containing snack or high-carbohydrate protein-containing snack and the no bedtime snack condition.

CONCLUSIONS: Lower fasting glucose and improved markers of insulin sensitivity suggest that a low-carbohydrate protein containing bedtime snack (eggs) may be an easy to implement nutritional strategy to improve underlying disease pathophysiology in people with type 2 diabetes.

Lay Summary

Dieticians and popular nutrition websites often tell patients with type 2 diabetes to consume a bedtime snack containing protein to help control their morning blood sugars. Surprisingly, we were unable to find any scientific evidence to support this recommendation. In this study we compared a low-carbohydrate bedtime snack (eggs) to a higher-carbohydrate bedtime snack (yogurt) to see if one snack was better than the other at reducing blood sugars measured on the morning after participants consumed the different bedtime snacks. We made sure participants ate the exact same meals during the day and matched the protein and calorie content in both bedtime snacks. The results of our study showed that, in people with type 2 diabetes, eating the bedtime snack that was lower in carbohydrate (eggs) was better than eating a snack that is higher in carbohydrate (yogurt) at reducing morning blood sugar levels. Consuming a protein-containing snack that is low in carbohydrates, such as eggs, may be a good option for people with type 2 diabetes who struggle with high morning blood sugars.

Preface

The design of this research study was developed by Drs. Jonathan Little and Monique Francois. Research co-ordinators include myself and Courtney Chang. Blood samples were taken by Cody Durer, Jonathan Little and Etienne Myette-Cote. Analysis of blood markers was performed by Julianne Barry, Courtney Chang, Etienne Myette-Cote and myself. Statistical analyses were performed by Jonathan Little, Monique Francois and myself. I was responsible for the writing presented in this thesis with assistance provided by Jonathan Little and Monique Francois. Ethics for this thesis was approved by UBC clinical research ethics board, H17-01055.

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Dedication

This work is dedicated to my family.

Chapter 1: Introduction

1.0 Prevalence of Type 2 Diabetes

The World Health Organization (WHO) predicts that by the year 2030, diabetes will be the seventh leading cause of death worldwide (Mathers et al, 2006). Diabetes is a disease that does not discriminate, affecting adults and children in every country regardless of economic status, gender, age or ethnicity. The International Diabetes Federation (IDF) states that 1 in 11 adults suffers from diabetes accounting for over 425 million people worldwide (US Renal Data System, 2014).

Diabetes places a significant economic burden on societies accounting for over 12% of global healthcare expenditures amounting to over $725 billion dollars world-wide (Loke, 2018). The IDF projects that by the year 2045 we will see an 7% growth in diabetes healthcare expenditure having significant global economic impact (Loke, 2018).

Canada is not immune to the burden of diabetes. In 2015 is was estimated that over 9% of the population suffered from diabetes with the projection to rise to 12% in the year 2025 (Houlden, 2018). British Columbia's Primary Health Care Charter identifies the management of diabetes as a priority medical condition (Government of British Columbia, Ministry of Health, 2007). Despite having lower risk factors for developing diabetes, including obesity, prediabetes and lower incomes, BC has a higher concentration of people who are at greater risk. As such, it is expected the prevalence of this disease will increase to over 10% of the population by the year 2020 (a 37% increase from the year 2013) (Diabetes Canada, 2017).

Life expectancy is reduced by six years in those with diabetes over the age of 60 compared to matched controls (Mathers et al, 2006). It is estimated that high blood glucose is the third highest risk factor for premature death after smoking and high blood pressure (International

Diabetes Federation, 2017). Of those aged 20-79, diabetes accounts for over 10% of global all-cause mortality with half of these deaths occurring during the most productive years in those under 60 (International Diabetes Federation, 2017).

Diabetes is associated with significant morbidity and mortality. Adults with diabetes are at a two to three-fold increased risk of macrovascular events such as heart attack, stroke and heart failure (Loukine et al, 2015). Microvascular disease resulting from diabetes is the leading cause of kidney failure, non-traumatic amputation and blindness (US Renal Data System, 2014). Clearly, diabetes is a condition of high prevalence, cost, and priority in our society.

1.1 Type 2 Diabetes Etiology and Pathogenesis

Most people (~90%) with diabetes suffer from type 2 diabetes (T2D), which is characterized by hyperglycemia resulting from impaired insulin action and secretion (Loukine et al, 2015). Insulin resistance and β-cell dysfunction are key pathophysiologic triggers of type 2 diabetes, and both are often present many years prior to diagnosis (DeFronzo, 2009). In individuals without type 2 diabetes, glucose metabolism is regulated by a feedback loop that includes the β-cells located in the pancreas and insulin sensitive tissues (Kahn et al, 2014). The amount of insulin released to maintain glucose homeostasis is related to tissue insulin sensitivity (Kahn et al, 2014). When insulin resistance (i.e., reduced insulin sensitivity) develops, feedback to the β-cells ensures there is an increase in insulin secretion to maintain glucose tolerance or homeostasis (Kahn et al, 2014). In individuals with normal β-cell function, insulin resistance leads to hyperinsulinemia and the development of metabolic syndrome, but glucose levels remain normal (DeFronzo, 2009). If β-cell function deteriorates sufficiently, insulin resistance gives rise to insulin deficiency, hyperglycemia, and type 2 diabetes (DeFronzo, 2009) (Gerich, 2003).

The etiology of β-cell failure in type 2 diabetes is multifactorial (DeFronzo, 2009). Studies have shown a progressive age-related decline (Muller et al, 1996) and have linked several genes to

β-cell failure (Vaukonen et al, 1998). Evidence also indicates lipotoxicity, defined as an increase in plasma free fatty acid (FFA) concentrations, can impair β-cell function and consequently insulin secretion in genetically predisposed subjects (Kashayp et al, 2003) Additionally, a deficiency in glucagon like peptide-1 (GLP-1) and resistance to glucose-dependent insulinotropic polypeptide (GIP), two incretin hormones released from the small intestine that stimulate insulin secretion, have been shown to be major contributors to β-cell dysfunction. (DeFronzo, 2009).

Insulin resistance can occur in the presence of functional or failing β-cells (Gerich, 2003). Both the liver and muscle are severely resistant to insulin in individuals with type 2 diabetes (DeFronzo, 2009). In a study conducted by DeFronzo et al., a progressive rise in hepatic glucose production (HGP) was observed in subjects with diabetes who had fasting plasma glucose (FPG) levels >140 mg/dL (7.8 mmol/L); as the rate of basal HGP increased so did the FPG and these two factors were highly correlated ($r=0.85$, $P < 0.001$) (DeFronzo et al, 1989). These results indicate the presence of early, marked hepatic resistance to insulin action and that altered HGP plays a key role in maintaining the diabetic state (DeFronzo, 2009). Combined with insulin resistance, an elevation in circulating glucagon causes increases in both gluconeogenesis (the generation of glucose from non-carbohydrate substrates) and glycogenolysis (the breakdown of glycogen to glucose in the liver) to greatly enhance the rate of HGP in type 2 diabetes (DeFronzo, 2009).

Patients with type 2 diabetes also experience a progressive decline in glucose uptake by muscle, as insulin production decreases and insulin resistance increase. DeFronzo et al. (1985) used the hyperinsulinemic-euglycemic clamp technique coupled with hepatic and femoral venous catheterization to analyze the mechanism and sites of insulin resistance in non-obese patients with type 2 diabetes. Leg glucose uptake in response to insulin infusion was delayed and reduced by 45% in those with type 2 diabetes compared with healthy controls ($P < 0.01$)

(DeFronzo et al, 1985). Collectively, an increase in HGP and a reduction in peripheral glucose uptake are the major pathophysiological processes causing hyperglycemia in type 2 diabetes.

1.2 The Importance of Glycemic Control

Diabetes-associated complications can begin well before the onset of diagnoses. The “clock” for macrovascular and microvascular complications precedes the development of overt diabetes, such that at the time of diagnosis of type 2 diabetes, many individuals already have significant vascular disease (UKPDS, 1995). The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated a strong association between hemoglobin A1C levels and microvascular risk in patients with type 2 diabetes (UKPDS, 1999). An epidemiologic analysis found that each 1% decrease in HbA_{1c} level corresponded with a ~37% (33% to 41%, P < 0.0001) decrease in microvascular disease end points. Microvascular endpoints evaluated in the UKPDS included retinopathy requiring photocoagulation, vitreous hemorrhage, and or fatal or nonfatal renal failure (Stratton et al, 2000).

Epidemiologic analysis of UKPDS data also demonstrated an association between A1C levels and macrovascular risk in patients with T2D (UKPDS, 1999). Each 1% reduction in updated mean A1C was associated with reductions in risk of 21% for any endpoint related to diabetes (95% confidence interval 17% to 24%, *P* < 0.0001), 21% for deaths related to diabetes (15% to 27%, *P* < 0.0001) and 14% for myocardial infarction (8% to 21%, *P* < 0.0001) (Stratton et al, 2000). The authors concluded that in patients with type 2 diabetes the risk of complications was strongly associated with previous hyperglycemia. Any reduction in A1C is likely to reduce the risk of complications, with the lowest risk being in those with A1C values in the normal range (<6.0%) (UKPDS, 1999). These findings show that A1C is not only strongly associated with microvascular complication risk, but also associated with macrovascular complications.

Moreover, decreases in A1C contribute to a decreased risk of either type of complication (Stratton et al, 2000).

The Diabetes Canada 2018 guidelines recommend that individuals with T2D achieve an A1C of ≤ 7% to reduce the risk of microvascular disease and, if implemented early in disease course, CV complications (Imran et al, 2018). As A1C reflects the average blood glucose over the preceding 90-120 days (Imran et al, 2018), to achieve an A1C ≤7% people with diabetes should aim for a fasting plasma glucose (FPG) target of 4.0-7.0 mmol/L and a 2-hour PPG target of 5.0-10.0 mmol/L (Imran et al, 2018).

1.3 Fasting Plasma Glucose and the Dawn Phenomenon

Both fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) contribute to an individual's A1C (Imran et al, 2018) and each are directly correlated with the risk of macrovascular and microvascular complications (UKPDS, 1999). In 2003, Monnier *et al,* demonstrated a progressive shift in the contributions of fasting and postprandial hyperglycemia when patients progressed from moderated to high A1C. At an A1C of ~ 7-8% there was a relatively equal contribution of FPG and PPG to overall glycemic control assessed by A1C (Monnier et al, 2003). The contribution of post-prandial glucose excursions was more predominant in patients with lower A1C, whereas the contribution of fasting hyperglycemia increased in those with higher A1C (Monnier et al, 2003). Given the demands of the body to maintain euglycemia and the high glycemic demands of the brain on the body during a nocturnal fasted state, the average non-diabetic individual maintains a fasting glucose concentration of approximately 5 mmol/L (DeFronzo, 2009). In those with T2D there is an overproduction of glucose primarily by the liver (see above) and for an average 80 kg person, this amounts to the addition of an extra 25-30 g of glucose to the systemic circulation each night (DeFronzo et al, 1989). Fasting plasma insulin levels also appear to be increased during this time indicating

significant hepatic insulin resistance (i.e., the liver is still producing extra glucose despite insulin levels being high; (DeFronzo et al, 1989).

Both gluconeogenesis and glycogenolysis play a significant role in nocturnal and early morning fasting hyperglycemia (Boden et al, 1996). Gluconeogenesis contributes about half of the total HGP in humans following an overnight fast and is often regarded as the primary pathophysiological mechanisms responsible for the increase in fasting HGP in type 2 diabetes (Magnusson et al, 1992).

The early morning hours present a significant problem for patients with T2D and the term "DAWN Phenomenon" was coined in 1981 to describe early morning hyperglycemia and increased need for insulin to control blood glucose in the absence of food intake (Schmidt et al, 1981). A study by Monnier et al in 2013 demonstrated the DAWN phenomenon is independent of anti-hyperglycemic treatment and accounts for ~0.4% of a patient's total A1C (Monnier et al, 2013) therefore this morning period represents an important target for glycemic control.

1.4 Strategies to Reduce Fasting Plasma Glucose

Because morning hyperglycemia (i.e., the DAWN Phenomenon) adversely affects overall glycemic control, its detection and treatment are important components of diabetes care. Current approaches to lowering fasting plasma glucose are limited to treatment with glucose lowering medications and these typically do not correct the underlying pathophysiology of the disease (Cheng et al, 2013). The biguanide compound metformin, widely used as first line therapy to treat type 2 diabetes, may be useful for treating morning hyperglycemia (Hundal et al, 2000). Metformin reduces HGP by decreasing glycogenolysis and suppressing hepatic gluconeogenesis (Hundal et al, 2000). Therapeutic doses typically lead to a reduction in fasting plasma glucose of ~2-4 mmol/L with corresponding reductions in A1C by 1-2% largely

independent of age, weight and diabetes duration if some residual beta cell function remains (Bailey et al, 1996).

Thiazolidinediones (TZDs), represent another class of oral anti-hyperglycemic agent that can be used to improve insulin sensitivity and reduce lipolytic stimulation of hepatic gluconeogenesis (DeFronzo, 2009). However, the clinical use of TZDs is limited by concerns of adverse events, particularly weight gain, oedema, increase hospitalization for heart failure and bone fracture (DeFronzo, 2009).

Use of basal insulin has been shown to reduce fasting hyperglycemia and minimize the DAWN phenomenon (Porcellati et al, 2013) however, this comes at the cost of increased weight gain and risk of hypoglycemia. Exogenous insulin is also typically reserved for those with late-stage T2D with poor overall glucose control. Short-acting GLP-1 receptor agonists (such as exenatide and lixisenatide) primarily lower postprandial glucose and insulin concentrations by slowing down gastric emptying (Meier et al, 2012). Long-acting GLP-1 receptor agonist (such as albiglutide, dulaglutide, exenatide long acting release and liraglutide) lower blood glucose levels through stimulation of insulin secretion and reduction of glucagon levels (Meier et al, 2012) and therefore may help lower fasting glucose. However, given the cost and the route of administration (injection) most patients and healthcare providers find this option prohibitive.

Another viable target to reduce morning hyperglycemia would be altering glucagon secretion and action (Peterson et al, 2017). Antagonizing the action of glucagon has been effective in reducing hyperglycemia in rodent models however at this time data in humans is limited and available drugs targeting the glucagon system are not readily available (Lee et al, 2016). Thus, although many patients with T2D are treated with pharmacologic therapy, many of these options do not necessarily target fasting plasma glucose and their use can be prohibitive given the costs and undesirable side effects (Lipscombe et al, 2018).

Diet is a crucial component in the treatment of T2D - primarily to aid in weight loss. However, the optimal diet is hotly debated and long-term adherence to weight loss diets is notoriously low. Nutritional strategies that are easy to implement *and* target the pathophysiology of T2D represent an innovative treatment option. One potential dietary treatment strategy that in recent years has gained popularity in the press is the incorporation of a bedtime snack in order to reduce next morning fasting glucose ((Hamilton, 2017), (Saschin, 2017). Diabetes educators and other health care providers often advise patients to eat a bedtime snack to maintain blood sugar regularity and avoid morning hyperglycaemia; however, there is very little evidence examining whether this nutritional strategy is efficacious in people with type 2 diabetes. Furthermore, the composition of such a bedtime snack designed to lower next day fasting glucose has not been adequately studied.

1.5 Bedtime Snack Studies in T2D

The National Health and Nutrition Examination Survey (NHANES), 2013-2014, suggests one quarter of daily energy intake comes from the consumption of snacks (Center for Disease Control, 2017). Diabetes Canada 2018 Clinical Practice Guidelines recognize nutrition therapy as a means for improving glucose control and recommend that the inclusion of snacks in meal plans should be individualized "and balanced against the risk of weight gain" (Sivenpieper JL et al, 2018). On the one hand consuming too many calories after the evening meal has been associated with weight gain and glucose dyregulation (Morse SA et al, 2006), while on the other hand a bedtime snack may be a strategic option to help manage nocturnal and morning glucose (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001).

Logically, the meal in closest proximity to the overnight period (i.e., a bedtime snack or dinner) could have the most influence on next day morning glucose levels. However, despite the common recommendation given to type 2 diabetes patients to consume a snack before bed, little evidence exists to support this notion. To date, we are aware of four studies conducted in

individuals with type 2 diabetes (Table 1; (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). The first study, conducted by Axelsen et al (1997) had the aim of examining the effects of a large dose of slow release carbohydrates, in the form of uncooked cornstarch on nocturnal glucose metabolism and to assess the effects on morning fasting and post-prandial glucose. This study did not show an improvement in overall glucose control however, did see a suppression of nocturnal FFA and post-prandial IAUC after breakfast (Axelsen et al, 1997). A second study by Axelsen et al (1999) had the primary objective of determining whether bedtime carbohydrate intake might improve glucose tolerance to the next day's breakfast, a so called "second meal effect" where insulin levels from the preceding meal impact glucose tolerance of the next meal (Wolever et al, 1988). This study concluded that a bedtime meal containing uncooked cornstarch (a slow-release carbohydrate) improved next day breakfast glucose tolerance by ~20% when compared to placebo, while a bedtime meal consisting of white bread (a more rapidly absorbed carbohydrate) showed no difference when compared to placebo (Axelsen et al, 1999). A third study, conducted by Axelsen in 2000, looked at the ingestion of uncooked cornstarch on morning glycemic control and glycated hemoglobin (A1C). Additionally, this study assessed the effects of a low dose (~25g), and high dose (~45g) of an uncooked cornstarch bedtime meal on insulin sensitivity, postprandial glucose and triacylglycerol concentrations. Results demonstrated that the high-dose uncooked cornstarch bedtime meal significantly increased nocturnal blood glucose concentrations by 25% (2.1 ± 0.4 mmol/l) and insulin by 35% (30.6 ± 9.6 pmol/L) and suppressed FFA concentrations by 32% compared to starch-free placebo (low sugar fruit juice plus pectin). The low-dose (~25g) uncooked cornstarch bedtime meal improved fasting blood glucose by 12% but neither the low nor the high-dose uncooked cornstarch bedtime meal showed improvements in insulin sensitivity, postprandial triacylglycerol concentrations or A1C after 7 weeks. Therefore, this study suggested that a lower quantity of uncooked cornstarch could lower fasting plasma glucose whereas a high-dose spiked nocturnal glucose and insulin with no benefits on fasting

plasma glucose. The final study conducted by Dyer-Parziale (2001) examined the effects of ingesting a snack bar containing uncooked cornstarch (extend bar) at bedtime on nocturnal and morning glycemic excursions. Compared to placebo, consumption of the extend bar reduced mean blood glucose levels by ~14% at midnight (extend bar midnight value 7.11 ± 0.17 mmol/L vs placebo value 8.23 ± 1.78 mmol/L) and lowered fasting glucose by ~28% (extend bar, 6.34 ± 0.88 mmol/L vs placebo,8.81 ±1.68 mmol/L). Combined, the results of these studies suggest the ingestion of slowly digestible carbohydrates, in the form of raw uncooked cornstarch, may be an effective strategy to lessen the frequency of nocturnal and morning hyperglycemia in type 2 diabetes (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001).

Table 1: **Bedtime snack studies in type 2 diabetes**

Study	Subjects	Design	Interventions	Outcome
Axelsen 1997	N = 10 T2D, NIDDM	3 conditions (1d)	Uncooked cornstarch 106g CHO Equicaloric meal 58 g CHO (mixed meal) 17 g CHO (smaller mixed meal)	↑ plasma insulin levels, ↓ nocturnal FFA, ↓glucose IAUC after breakfast vs. mixed meals No change in FPG, fasting insulin or FFA
Axelsen 1999	N = 16 T2D Oral agents ± diet	Crossover 3 conditions (2d)	Uncooked cornstarch (~100 g, ~100% CHO) White bread (~100 g, ~100% CHO) Starch free placebo (pectin 0.033 g/kg BW, ~100% CHO)	↓ breakfast glycemic response by 21% vs. placebo No difference white bread vs. placebo
Axelsen 2000	HD, n=14 LD, n= 24 T2D	Two studies Study 1: HD, crossover, 7 weeks vs. placebo Study 2: LD compared to placebo, 7 weeks	High dose uncooked cornstarch (~45 g) Low dose uncooked cornstarch (~25 g) Starch free placebo (1.50 g pectin + fruit juice (~75J) + water	HD ↓ FFA by 32% HD ↓ breakfast glycemic response IAUC by 36% and increase C-peptide by 40% LD ↓ fasting blood glucose by 12% No change in insulin sensitivity
Dyer-Parziale 2001	13 patients T2D 8 with oral + insulin, 7 insulin	Crossover trial 2 conditions, 3 days	Extend bar [30 g CHO (5g uncooked cornstarch, 3 g protein, 3 g fat), 160 kcal] Placebo bar (30 g CHO, 3 g protein, 3 g fat)	↓ midnight glucose levels by ~14% with extend bar ↓ fasting glucose levels by ~28% with extend bar

CHO = carbohydrates, ↓ = decrease, ↑ = increase, FPG = fasting plasma glucose, FFA= free fatty acids, oral = oral anti-hyperglycaemic agents, insulin = exogenous insulin therapy, LD= low dose, HD = high dose, T2D = type 2 diabetes

1.6 Composition of Bedtime Snacks in Type 2 Diabetes

The above research supports the notion that food consumed before bed and the composition thereof can impact overnight and next morning (fasting and postprandial) glucose metabolism. However, all studies to date have examined the use of uncooked cornstarch (a “slow release” carbohydrate) as the functional bedtime food of choice. The use of a slow release carbohydrate makes sense over a rapidly digested carbohydrate to limit a nighttime glucose spike, which would seemingly be counterproductive for a bedtime snack (i.e., it does not make much sense to attempt to reduce next day fasting glucose with a strategy that spikes glucose the evening before). Nonetheless, uncooked cornstarch does cause a slight rise in glucose at night (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) which could negate any overall benefit on glycemic control even if it lowers fasting glucose. Uncooked cornstarch also does not represent a typical or "real-life" food option and it is questionable whether people with type 2 diabetes would be willing to consume this as a bedtime snack. Therefore, there seems to be a lack of scientific evidence assessing the consumption of "real-life" bedtime snacks on glucose control in type 2 diabetes. Despite the published literature exclusively examining uncooked cornstarch bedtime snacks, many websites and diabetes educators suggest their patients eat a snack containing protein at night to help control blood glucose. For example, the popular health website livestrong.com states: "A serving of protein should be eaten one to two hours before bedtime to stabilize blood sugar levels before the extended fasting period that occurs during sleep" (Hamilton, 2017).

The rationale for protein has not been tested in the aforementioned cornstarch studies but is supported by research suggesting that diets higher in protein have beneficial effects on weight loss, body composition and certain blood lipids (Hession et al, 2009). Additionally, the American Diabetes Association Standards of Medical Care 2018, suggests that in individuals with type 2 diabetes, ingesting protein appears to increase insulin response without increasing plasma

glucose concentration (American Diabetes Association, 2018). Additionally, individual amino acids, have been proposed as mediators with beneficial glucoregulatory effects; glycine and leucine can stimulate enhanced glucose disposal by increasing insulin secretion and modulating insulin signaling (Layman, 2004). In the longer-term, high protein low-carbohydrate diets have been shown to have positive effects on reducing cardiovascular risk factors and glycemic regulation, including fasting blood glucose and insulin responses (Layman et al, 2008). In a study conducted by Gannon and Nuttall (2004), a high protein, low-carbohydrate diet (30% PRO, 20% CHO, 50% FAT) was compared to a control diet (15% PRO, 55% CHO, 30% FAT) in men with type 2 diabetes over a 5-week period. Overnight fasting glucose concentrations decreased by 29% and hemoglobin A1C was reduced from a mean of 9.8% to 7.6% (Gannon & Nuttall, 2004). These data suggest that an increase in protein could help promote a decrease in carbohydrate to elicit the optimal metabolic response in those with T2D (Gannon & Nuttall, 2004). Whether a high-protein, low-carbohydrate bedtime snack can stimulate a reduction in overnight and fasting glucose in people with type 2 diabetes remains to be tested.

1.7 Research overview, aims and hypothesis

1.7.1 Manipulating nutrition to reduce fasting plasma glucose

Despite popular advice from health care practitioners to consume a bedtime snack to help control fasting (and overnight) glucose, few studies exist in type 2 diabetes patients to support this recommendation. Consuming a bedtime snack seems reasonable as it represents the food consumption event in closest proximity to fasting yet limited studies supporting this nutritional strategy have been published. Of the limited research available, all investigations have used uncooked cornstarch as the experimental bedtime snack. Further research testing "real food" options are warranted. In this regard, a bedtime snack containing protein, without carbohydrates, might be the most logical choice as this would facilitate a modest rise in insulin (to potentially help inhibit HGP) but not cause an evening spike in glucose.

1.7.2 Aims

1. Primary Aim:

    To determine whether a low-carbohydrate protein containing bedtime snack can reduce the next morning fasting plasma glucose levels in people with type 2 diabetes when compared to a high-carbohydrate protein containing bedtime snack or a no bedtime snack condition.

2. Secondary Aims:

    a) To explore whether a low-carbohydrate protein containing snack consumed at bedtime will improve next day post breakfast glycemic response.

    b) To explore whether a low-carbohydrate protein containing snack consumed at bedtime can reduce nocturnal and 24-hour average glucose levels.

c) To explore whether a low-carbohydrate protein containing snack consumed at bedtime has an impact on insulin sensitivity and regulators of glucose metabolism.

1.7.3 Hypotheses

1. Primary hypothesis:

Consuming a low-carbohydrate protein containing bedtime snack will reduce next morning fasting plasma glucose levels when compared to a high-carbohydrate protein containing bedtime snack or a no bedtime snack condition.

2. Secondary hypothesis:

a) Consuming a low carbohydrate protein containing bedtime snack will reduce next day post-breakfast glucose response

2) Consuming a low carbohydrate protein containing bedtime snack will reduce nocturnal and 24-hour average glucose levels

3) Improvements in glucose will be accompanied by increased markers of insulin sensitivity

Chapter 2: Manuscript from thesis data

2.1 Manuscript Introduction

The American Diabetes Association (ADA) and Diabetes Canada (DC) guidelines recommend that individuals with type 2 diabetes achieve an A1C of ≤ 7% (American Diabetes Association, 2018) (Imran et al, 2018). As A1C reflects the average blood glucose over the preceding 90-120 days (Puntahakee et al, 2018), to achieve an A1C ≤7% people with diabetes should aim for a fasting plasma glucose (FPG) target of 4.0-7.0 mmol/L and a 2-hour PPG target of 5.0-10.0 mmol/L (Imran et al, 2018). The early morning hours present a significant problem for patients with type 2 diabetes and the term "DAWN Phenomenon" was coined to describe early morning hyperglycemia in the absence of food intake (Schmidt et al, 1981). Elevated fasting glucose is mainly driven by liver insulin resistance leading to excess hepatic glucose production (DeFronzo et al, 1989). Monnier et al (2013) demonstrated the DAWN phenomenon is independent of anti-hyperglycemic treatment and accounts for ~0.4% of a patient's total A1C (Monnier et al, 2013). Therefore, the morning period represents an important target for glycemic control.

Diet is a crucial component in the treatment of type 2 diabetes. Nutritional strategies that are easy to implement *and* target the pathophysiology of T2D represent an innovative treatment option. One potential dietary treatment strategy that has gained popularity in recent years is the incorporation of a bedtime snack to reduce next morning fasting glucose (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). Logically, the meal in closest proximity to the overnight period (i.e., a bedtime snack or dinner) could have the greatest influence on next day morning glucose levels. Diabetes educators and other health care providers often advise patients to eat a bedtime snack in order to maintain blood sugar regularity and avoid morning hyperglycaemia; however, there is very little evidence examining whether this nutritional strategy is efficacious in people with type 2 diabetes. Furthermore, the

composition of such a bedtime snack designed to lower next day fasting glucose has not been adequately studied.

Four studies have investigated the impact of a bedtime snack of uncooked cornstarch on next day glucose control (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). Dyer-Parziale (2001) examined the effects of ingesting a snack bar containing uncooked cornstarch at bedtime on nocturnal and morning glycemic excursions. Compared to placebo, consumption of the uncooked cornstarch bar reduced mean blood glucose levels by ~14% at midnight and lowered fasting glucose by ~28%. These findings suggest the ingestion of a bedtime snack may be an effective strategy to lessen the frequency of nocturnal and morning hyperglycemia in type 2 diabetes (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001).

Despite the published literature exclusively examining uncooked cornstarch bedtime snacks, many websites and diabetes educators suggest their patients eat a snack containing protein at night to help control blood glucose. Diets higher in protein may have beneficial effects on weight loss, body composition and certain blood lipids (Hession et al, 2009). In a study conducted by Gannon and Nuttall (2004), a high protein, low-carbohydrate diet (30% PRO, 20% CHO, 50% FAT) was compared to a control diet (15% PRO, 55% CHO, 30% FAT) in men with type 2 diabetes over a 5-week period. Overnight fasting glucose concentrations decreased by 29% and hemoglobin A1C was reduced from a mean of 9.8% to 7.6% (Gannon & Nuttall, 2004). These data suggest that an increase in protein could help promote a decrease in carbohydrate to improve overall glycaemic control in those with T2D. Whether a high-protein, low-carbohydrate bedtime snack can stimulate a reduction in overnight and fasting glucose in people with type 2 diabetes remains to be tested. The primary aim of this study was to determine whether a low-carbohydrate protein containing bedtime snack can reduce the next morning fasting plasma glucose levels in people with type 2 diabetes when compared to a high-carbohydrate protein

containing bedtime snack or a no bedtime snack condition. Secondary aims included examining the impact of different bedtime snacks on regulators of fasting glucose, and continuous glucose monitoring (CGM) determined changes in nocturnal glucose, next day post breakfast glycemic response, and 24-hour average glucose levels. We tested the overall hypothesis that a low-carbohydrate protein containing bedtime snack would be superior to a high-carbohydrate protein containing bedtime snack or a no bedtime snack condition for improving glucose control.

2.2 Methods and Materials

2.2.1 Participants

Sixteen patients with type 2 diabetes were recruited to participate in this randomized crossover trial. To be eligible, participants had to have a physician diagnosis of type 2 diabetes (hemoglobin A1C ≥6.5%) for at least 6 months, be between 30 and 80 years of age and be on stable glucose lowering medications for at least the last three months. Participants were excluded from the study if they were taking exogenous insulin, had an A1C of greater than 9%, were following a low or no carbohydrate diet, had suffered a major cardiovascular event within the past year (stroke or myocardial infarction) and/or were allergic/intolerant to eggs or dairy (see appendix C & D; screening forms). All subjects provided written informed consent (see appendix A; consent form). The study was approved by the University of British Columbia Research Ethics Board and the trial registered at ClinicalTrials.gov (NCT03207269). A total of 18 individuals were screened, 16 were randomized and 15 participants successfully completed the three conditions. Demographics and baseline characteristics are provided in Table 2.

Table 2: Baseline characteristics of participants N = 15

Baseline Characteristics		Mean (SD)
% Female		66.6
Age (years)		64(9)
A1C (%)		7.3(0.6)
Weight (kg)		87.1(17.6)
BMI (kg/m^2)		31.9(6)
SBP (mmHg)		129(4)
DBP (mmHg)		82(5)
Mean energy intake (kcals*)		2126(250)
Diabetes Duration (years)		9.4(5.7)
Medications	Metformin (n)	11
	Met + DPP4i (n)	1
	DPP4i (n)	1
	SGLT2i (n)	3
	Anti-hypertensive (n)	8
	Statin (n)	9

Values are means and standard deviations in brackets. A1C = glycated hemoglobin, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure. *Energy intake is calculated based on the Harris-Benedict formula and a Physical Activity Level of 1.4; Males (kcal/day)=(66.5+13.8W+5H-6.8A) X 1.4 and females (kcal/day)=(65.5+9.6W+1.8H-4.7A) X1.4; where W=weight in kg; H=height in cm; A=age in years) (Gerrior et al, 2006)

2.2.2 Experimental Protocol

Using a crossover design, participants completed three, 3-day interventions in a randomized counterbalanced order using a Latin Square Design to control for order effects (Figure 1).

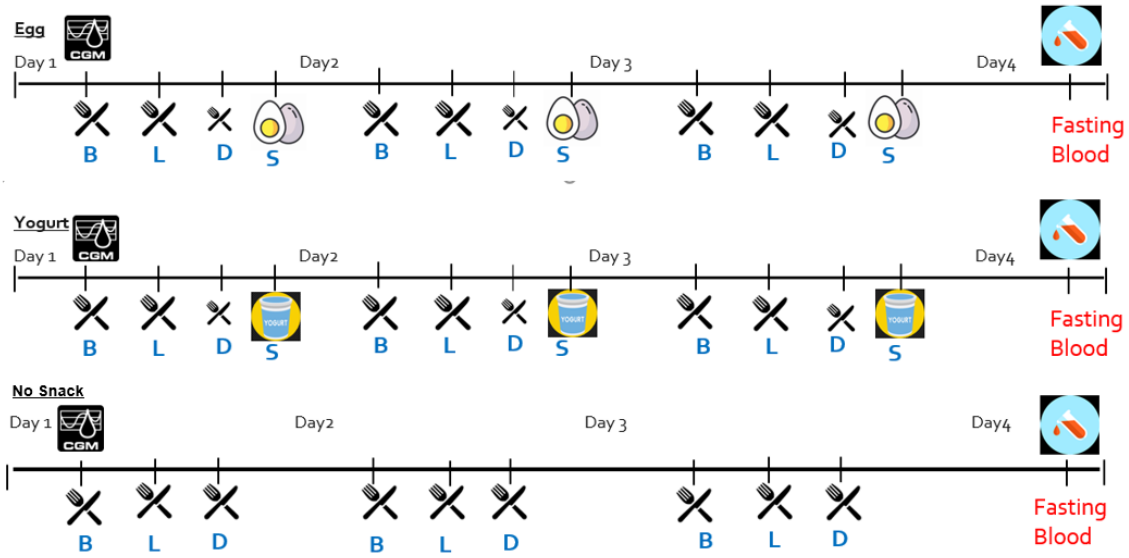

Figure 1: Randomized Crossover Design. Participants completed three, 3-day controlled dietary interventions with all food provided. Breakfast and lunch were identical on all days for all conditions. The dinner on the egg and yogurt conditions was reduced when compared to the no bedtime snack condition to maintain equal energy intake across all three interventions. The average of two fasting blood samples obtained on Day 4 following three days of each intervention was used to calculate the primary outcome of fasting plasma glucose and secondary outcomes of insulin sensitivity, glucoregulatory hormones, and free fatty acids. A continuous glucose monitor (CGM) was worn throughout each intervention. (B = Breakfast, L = Lunch, D = Dinner, S = Snack, Fasting blood = fasting blood samples taken)

Participants consumed a standardized isoenergetic diet for three days with either two cooked eggs, two containers of Greek yogurt or no snack (control) 30-45 minutes prior to bedtime. Three-day interventions were chosen based on previous bedtime snack studies (Axelsen et al, 2000) (Dyer-Parziale, 2001) and feasibility of facilitating a three-condition controlled diet crossover. The three conditions differed only in macronutrient composition of the dinner or bedtime snack with identical breakfast and lunch provided for all conditions. In each bedtime snack condition, the same food was provided for dinner, but the energy content of this meal was reduced by ~150 kcal to account for the energy in the bedtime snacks. Macronutrient profiles for each meal were in line with the Diabetes Canada Guidelines providing ~45% carbohydrate (low glycemic index foods), ~30% fat and ~25% from protein (Cheng et al, 2013). Blood glucose was

monitored continuously across each intervention period using continuous glucose monitor (CGM). CGM allows for moment to moment changes in blood glucose to be examined for several days, allowing the unique opportunity to assess glucose responses at different times of the day including fasting hyperglycemia in the morning, nocturnal glucose and postprandial glucose in response to meals. Glycemic variability is an indicator of glucose fluctuations throughout the day, including hypoglycemic and hyperglycemic episodes (Suh et al, 2015) and these are important determinants of diabetes pathophysiology (Weber & Schenll, 2009). Mean amplitude of glycemic excursion (MAGE) was employed to assess glycemic variability and is calculated based on the mean difference between consecutive peaks and troughs of differences greater than one standard deviation (SD) of mean glycemia (Czerwoniuk et al, 2011). Additionally, and to further determine glucose variability, we calculated the J-INDEX, a measure of the quality of glycemic control based on mean and standard deviations from the CGM data (Czerwoniuk et al, 2011).

All intervention diets were isoenergetic, providing the same number of calories using the estimated daily energy requirement for each participant based on the Harris-Benedict equation (Gerrior et al, 2006). A minimum of four days washout was required between each condition. To provide a high level of dietary intake control, increase compliance with dietary interventions, and ensure participants consumed the same energy and macronutrients during each condition, all food was provided for participants to consume in free-living conditions (Jones et al, 2013). Individualized meal plans were developed based on participants' food preference and allergies. All meals were provided by a local meal preparation service to ensure standardized diets for each participant (www.mealprep4U.com). Snacks were provided for consumption between breakfast and lunch, and between lunch and dinner to meet daily energy requirements (45% carbohydrate, 27% fat, 30.5% protein, see Table 3).

Table 3: Sample menu for a moderately active female, 57 years with BMI of 30.5

Meal	CHO (g)	Fat (g)	Protein (g)	Energy (kcal)
Breakfast - Lemon/raspberry waffles Oat flour (300 g) Protein powder (62.5g) Flax seed (12.5 g) Egg whites (125 g) Raspberries (75 g) Almond milk unsweetened (250 ml) Baking powder (5 ml) Cinnamon (5 ml) Lemon zest (5 ml)	60.4	6.2	54.2	**514**
Snack Solo™ bar Carrots 200 g	 26 20	 7 0.4	 11 1.8	 **200** **82**
Lunch Mexi/Cali Bowl Ground turkey (154g) Lettuce (250 g) Corn (56 g) Black beans (56 g) Tomato (56 g) Onion (5 g) Garlic (0.25 g) Guacamole (15 ml) Shredded cheese (28 g)	42.8	18.1	41.5	**507**
Snack Nut bar (40 g) Carrots (200 g)	 16 20	 15 0.5	 6 1.8	 **200** **82**
Dinner* Greek Chicken Chicken (140 g) Tomato (200 g) Peppers (56 g) Zucchini (56 g) Onions (5 g) Lactose free Greek yogurt tzatziki (28g) Lemon potatoes (112 g) Dressing (olive oil, lemon juice) (15 ml)	47	15.8	42.9	**502**
Totals (g)	**232.2**	**63**	**159.2**	**2087**

**In the bedtime snack conditions dinner was reduced to 350 kcal to account for the energy content of the egg or yogurt snacks. Two eggs (10.67 g fat, 12.38 g protein, 1.13 g carbohydrate; 150 kcal); two containers of yogurt (0.33 g fat,12.38 g protein, 24.38 g carbohydrate; 150 kcal)*

Body mass, height, blood pressure waist and hip circumference were measured prior to study commencement. For each 3-day testing period participants reported to the laboratory on the

morning of day 1 for insertion of the CGM (Medtronic, iPro™2 professional CGM with Enlite™sensor) and to pick up their controlled diet with portion sizes and meal timing prescribed and recorded in logbooks. Food was pre-packaged and labelled in individual containers. Participants returned food containers and completed food logs to monitor compliance. In the event that a participant did not consume all the food provided in the first condition (n=4), subsequent conditions were adjusted accordingly to ensure each condition was iso-caloric. Daily physical activity was minimized during the testing periods and was monitored using an wrist-worn activity tracker (FitBit Flex 2™) and logbook. Medications were kept constant during all experimental conditions and recorded in logbooks provided to participants (see appendix D). The CGM sensor was inserted on day 1 and was removed on the morning of day 4 of each condition. Participants were instructed to take four capillary glucose measurements per day for CGM calibration (prior to breakfast, lunch, dinner and bedtime). On day 4 of each condition, fasting blood samples (two samples taken 15 minutes apart) were taken from each participant.

2.2.3 Continuous blood glucose monitoring

Data from the CGM were downloaded and integrated with glucose calibrations using Medtronic Carelink software (Medtronic Inc.) before being exported to excel for analyses. Glycemic variability (MAGE and J-INDEX) and mean glucose across each 24-hour period (starting immediately before breakfast) were analyzed using the online EasyGV platform (EasyGV, Oxford, UK). Post-breakfast glycemic response (3-hour area under the curve (AUC)) and AUC for 24-hour glucose was assessed using the trapezoid method (Le Floch et al, 1990). Nocturnal glucose was calculated from the time of consumption of the bedtime snack until one hour prior to the next morning pre-breakfast finger stick calibration with sleep verified by the activity monitor data.

2.2.4 Blood Measures

2.2.4.1 Metabolic markers

Venous blood samples were obtained by venipuncture using the BD Vacutainer® Push Button Blood Collection Set with 2 mL BD™ P800 Blood Collection System tubes. Two blood samples were taken 15 minutes apart to account for the pulsatile secretion of insulin (Hellman, 2009). Plasma was prepared by centrifugation at 1200 X g at 4°C for 10 minutes. All samples were stored at -80°C for batch analyses. Glucose (Pointe Scientific, G5717-120 Glucose) was measured by the hexokinase method and free fatty acids (FFAs) were measured by colorimetric assay (HR Series NEFA-HR(2), Wako Chemicals, Texas, USA) according to manufacturer's instructions using a clinical chemistry analyzer (Chemwell 2910, Awareness Technologies). All samples were analyzed in duplicate with an average coefficient of variation (CV) <4% between duplicates.

C-peptide, GLP-1, glucagon, leptin and insulin were analyzed by multiplex immunoassay (HDIAB34KPMX5BK, Human Diabetes MAG Premix 5 Plex Millipore, Massachusetts, USA) and read on a MAGPIX™ Bio-Plex® reader (BioRad, Hercules, CA, HDIAB-34K-PMX5). Thawed plasma was centrifuged at 10,000 g for 10 minutes at 4°C to remove any debris prior to analyses in duplicate. The average CV for duplicates was <6%. Results were analyzed using Bio-Plex® Manager 6.1 Software.

Glucose and insulin were used to calculate the quantitative insulin sensitivity check index (QUICKI) (Katz et al, 2000).

2.2.5 Statistical Analyses

Data were analyzed using SPSS Statistics (version 25). Normality was assessed by visual inspection of histograms and residual plots. Non-normal data was natural log transformed for analyses. The primary outcome of fasting plasma glucose was measured as an average of the two fasting blood samples taken on day 4 of each intervention and analyzed by repeated

measures ANOVA followed by Sidak post hoc tests. Summary CGM data (24-hour average AUCs, nocturnal average glucose, 3-h post-breakfast AUCs) and fasting blood hormones were analyzed similarly. Statistical significance was set at $P < 0.05$ and Cohen's d effect sizes were calculated for pairwise comparisons.

Sample size estimation was based on the effect size for a clinically relevant difference in FPG. Glucose lowering medications are approved by Health Canada based on their ability to lower A1C by ~0.5-1.5% mmol/L. An A1C reduction of 0.5% equates to 0.8 mmol/L in average blood glucose (American Diabetes Association, 2008). We therefore chose 0.8 mmol/L as the smallest clinically relevant difference in glucose to calculate sample size. Using data from a type 2 diabetes study in our lab [N=24, (Barry et al, 2016)], where FPG had a mean of 7.8 mmol/L and SD of 0.8 mmol/L, with a correlation among repeated measures of r=0.5, a 0.8 mmol/L difference in FPG the calculated effect size for Cohen's d=1.0. G*Power software calculated that 13 participants would be needed to achieve 90% power to detect this clinically relevant difference between bedtime snack interventions (two sided, $\alpha < 0.05$). Accounting for 15% dropout or missing samples we aimed to recruit 16 individuals with type 2 diabetes.

2.3 Results

Of the 16 patients who were eligible and enrolled in the study, 16 were randomized. Fifteen patients completed all three conditions with one male participant failing to complete the study due to an inability to comply with the prescribed diet. This participant was removed from analyses resulting in final data being available for 15 participants.

2.3.1 Fasting plasma glucose and nocturnal CGM glucose

Changes in fasting plasma glucose are shown in Figure 2A. A one-way repeated measures ANOVA was significant [$F(2, 28) = 4.44$, $P = 0.02$]. Partial eta squared was 0.24. Post-hoc comparisons revealed that the mean fasting plasma glucose for the egg condition (7.17 ± 0.2 mmol/L) was significantly lower than the mean fasting plasma glucose for the yogurt condition

(7.55 ± 0.21 mmol/L; *P* = 0.04, d of 0.68). However, there were no significant differences found between the no bedtime snack condition (7.47 ± 0.26 mmol/L) and either egg or yogurt.

Changes in nocturnal CGM glucose are shown in Figure 2B. A one-way repeated measures ANOVA was significant [F (2, 28) =4.28, *P* = 0.02]. Partial eta squared was 0.23. Post-hoc comparisons revealed that the mean nocturnal CGM glucose for the egg condition (7.61 ± 0.24 mmol/L) was significantly lower than the mean nocturnal CGM glucose for the yogurt condition (8.17 ± 0.32 mmol/L; *P* = 0.02, d of 0.94). However, there were no significant differences found between the no bedtime snack condition (7.87 ± 0.27 mmol/L) and either egg or yogurt.

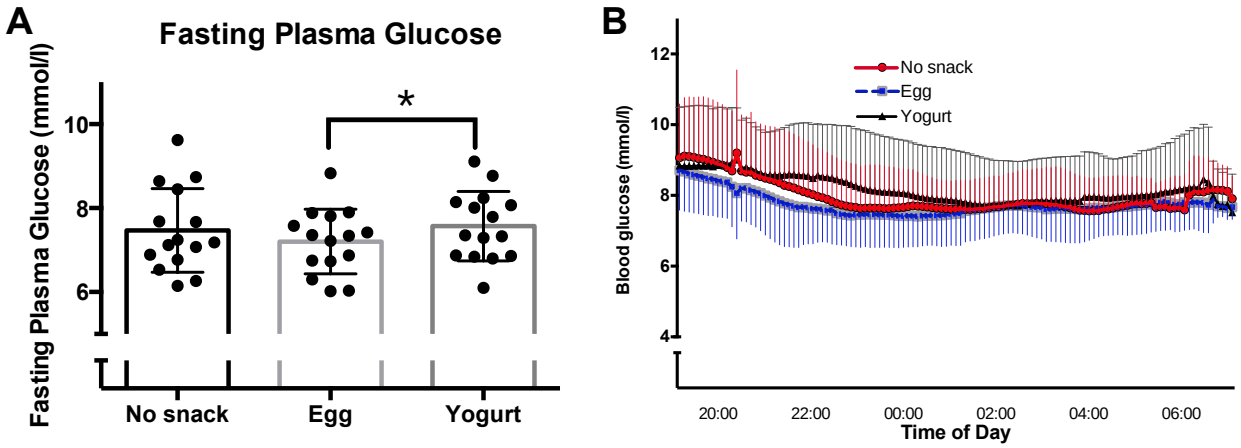

Figure 2: Changes in fasting plasma glucose (A) and nocturnal CGM glucose (B) following egg, yogurt and no snack conditions.

One-way repeated measures ANOVA revealed a significant main effect for bedtime snack condition (*P* < 0.05 for both). Nocturnal glucose taken from time of bedtime snack consumption to one-hour pre-breakfast in panel B. * *P* <. 0.05 in the egg vs. the yogurt bedtime snack condition.

2.3.2 Fasting plasma insulin, C-peptide and QUICKI

Changes in fasting plasma insulin are shown in Figure 3A. A one-way repeated measures ANOVA was significant [F (2, 28) =4.78, *P* = 0.02]. Partial eta squared was 0.26. Post-hoc comparisons revealed that the mean fasting plasma insulin for the egg condition (110.7 ± 51.7 pmol/L) was significantly lower than the mean fasting plasma insulin for the yogurt condition

(127.9± 55.6 pmol/L; *P* = 0.04, d of 0.45). However, there were no significant differences found between the no bedtime snack condition (116.2 ± 49.6 pmol/L) and either egg or yogurt.

Changes in fasting plasma C-peptide are shown in Figure 3B. A one-way repeated measures ANOVA was significant [F (2, 28) =7.29, *P* = 0.003]. Partial eta squared was 0.34. Post-hoc comparisons revealed that the mean fasting plasma C-peptide for the egg condition (744.1 ± 283.1 nmol/L) was significantly lower than the mean fasting plasma C-peptide for the yogurt condition (833.0 ± 294.5 nmol/L; *P* = 0.02, d of 0.48). However, there were no significant differences found between the no bedtime snack condition (M=794.0 ± 266.1 nmol/L) and either egg or yogurt.

Changes in QUICKI are shown in Figure 3C. A one-way repeated measures ANOVA was significant [F (2, 28) =7.39, *P* = 0.003]. Partial eta squared was 0.35. Post-hoc comparisons revealed that QUICKI for the egg condition (0.30 ± 0.03) was significantly higher than mean QUICKI for the yogurt condition (0.29 ± 0.23; *P* = 0.01, d of 0.50). However, there were no significant differences found between the no bedtime snack condition (0.29 ± 0.02) and either egg or yogurt.

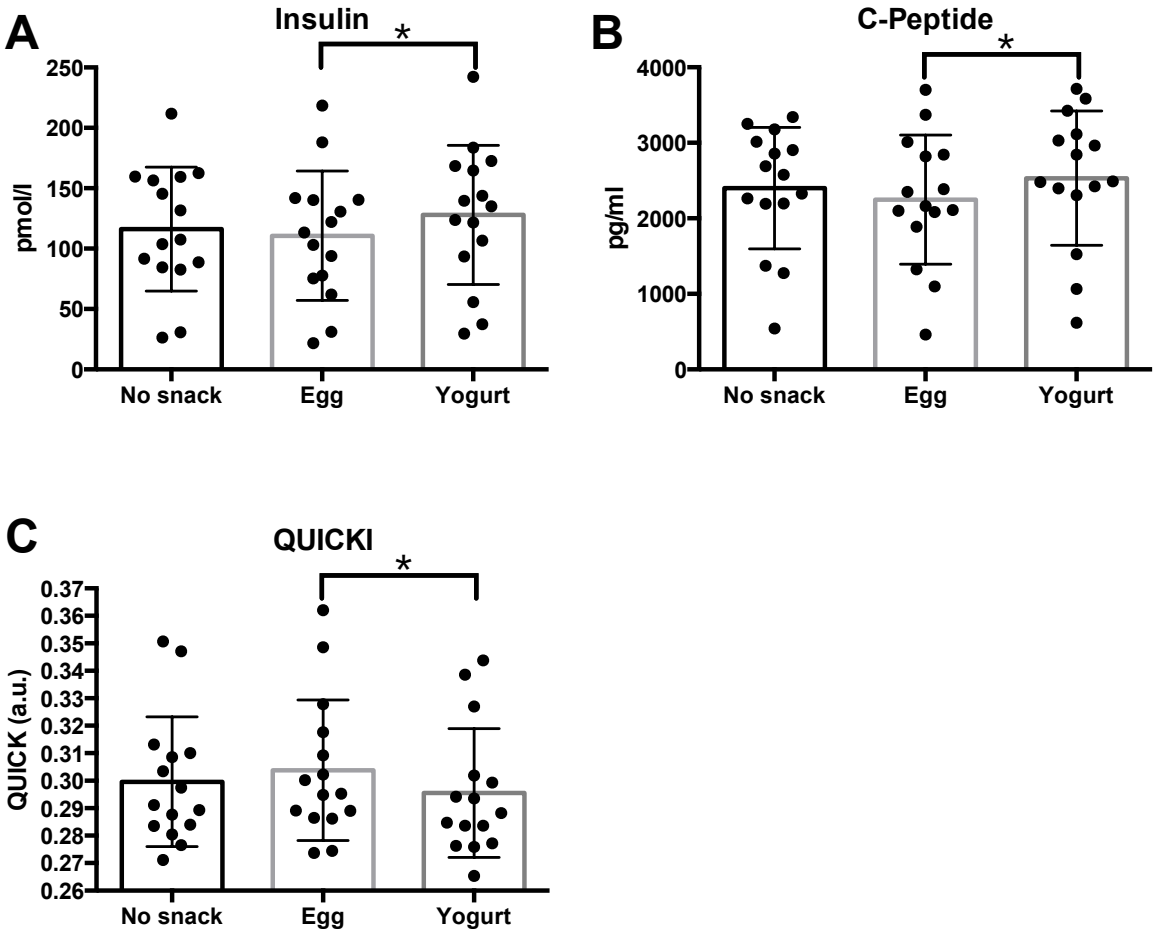

Figure 3: Changes in fasting plasma insulin (A), fasting plasma C-peptide (B) and QUICKI (C) following egg, yogurt and no snack conditions. There was a significant main effect for bedtime snack condition ($P < 0.05$) *significant decrease in mean fasting plasma insulin, C-peptide and QUICKI for the egg vs. the yogurt bedtime snack condition ($P < 0.05$).

2.3.3 Mean 24-hour CGM AUC and mean 3-hour post-breakfast CGM AUC

A one-way repeated measures ANOVA was conducted to compare mean 24-hour CGM AUC from three bedtime snack conditions (egg, yogurt, control). There were no significant main effects on 24-hour CGM AUC, [F (2,28) =0.48, p=0.63]. Changes in mean 3-hour post-breakfast CGM glucose AUC are shown in figure 3. A one-way repeated measures ANOVA was conducted to compare mean 3-hour post-breakfast CGM glucose AUC from three bedtime

snack conditions (egg, yogurt, control). There were no significant main effects on mean 3-hour post-breakfast CGM glucose AUC, [F (2,28) =0.19, p=0.83]. See table 4.

2.3.4 Variables associated with glucose metabolism, glycemic variability and activity

Changes in fasting plasma leptin are shown in table 4. A one-way repeated measures ANOVA was significant [F (2, 28) =5.3, *P* = 0.01]. Partial eta squared was 0.22. Post-hoc comparisons revealed that the mean fasting plasma leptin concentration for the egg condition was significantly lower than following the no snack condition (*P* = 0.04, d of 0.20). However, there were no significant differences found between the yogurt condition and either egg or no snack. There were no differences between conditions with respect to other variables associated with glucose metabolism, glycemic variability and activity (steps per day) (see table 4).

Table 4: Other variables associated with glucose metabolism, glycemic variability and activity

Variable	NS	Egg	Yogurt	ANOVA	NS vs Egg	NS vs YG	Egg vs YG
Leptin (ng/ml)	11.5 (5.8)	10.4 (5.3)	11.2 (5.3)	0.01	0.04	0.99	0.05
Glucagon (pg/ml)	131 (36)	124 (32)	136 (42)	0.14	-	-	-
GLP-1 (pg/ml)	53.7 (50.1)	57.4 (54.8)	63.6 (54.8)	0.20	-	-	-
FFA (mmol/L)	0.60 (0.14)	0.56 (0.11)	0.61 (0.15)	0.39	-	-	-
MAGE (mmol/L)	4.1 (1.9)	4.2 (1.7)	3.7 (1.7)	0.47	-	-	-
J-INDEX	34.0 (10.5)	32.6 (7.0)	33.8 (9.9)	0.75	-	-	-
24H AUC	12205 (1517)	12015 (1435)	12303 (1864)	0.48	-	-	-
3H AUC	1819 (226)	1781 (314)	1805 (254)	0.19			
Steps/day	7400 (1073)	7633 (1061)	8367 (1301)	0.23	-	-	-

Data are presented as mean and standard deviations (SD). *P*-values are shown for the overall one-way repeated measures ANOVA and for the Sidak post-hoc tests. Refer to the text for effect sizes. NS = no snack, egg = low-carbohydrate protein containing bedtime snack, yogurt (YG) = high-carbohydrate protein containing bedtime snack; leptin, glucagon, GLP-1, FFA (fasting blood glucose sample means); MAGE, J-INDEX, 24-hour (24H) AUC and 3-hour (3H) AUC (CGM data); steps/day (average steps from FitBit™).

2.4 Discussion

The results of this study demonstrate that consuming a low-carbohydrate protein containing bedtime snack significantly reduced fasting plasma glucose and insulin, improved QUICKI, and lowered nocturnal CGM glucose when compared to a high-carbohydrate protein containing bedtime snack in individuals with type 2 diabetes. Despite the differences between bedtime snack conditions, neither the low-carbohydrate protein containing bedtime snack nor the high-carbohydrate protein containing bedtime snack were significantly different from a no bedtime snack condition (which was matched for total daily energy content). This suggests that eating either bedtime snack was not superior to consuming no snack at bedtime, although clearly the low-carbohydrate protein containing bedtime snack was not worse than no snack. There were

no differences between any of the bedtime snack conditions on other glycemic control outcomes assessed by CGM, nor were there any differences in fasting FFA.

2.4.1 Egg bedtime snack lowers fasting plasma glucose and insulin

The morning period represents an important target for glycemic control. Monnier et al (2013) demonstrated the DAWN phenomenon is independent of anti-hyperglycemic treatment and accounts for ~0.4% of a patient's total A1C (Monnier et al, 2013). Previous studies have shown the composition of bedtime snack foods and the timing of consumption can impact next morning fasting glucose metabolism (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). Specifically, these studies demonstrated the consumption of slowly digestible carbohydrates in the form of raw uncooked cornstarch, eaten as a bedtime snack (last meal before bed), effectively lessen the frequency of morning hyperglycemia in type 2 diabetes (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). Although the use of a "slow release carbohydrate" in the form of raw uncooked cornstarch makes sense over a rapidly digested carbohydrate to limit an overnight glucose spike, the uncooked cornstarch did cause an increase in nocturnal glucose concentration (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000). Increased nocturnal glucose as a result of a bedtime snack seems counterproductive if improved morning glucose comes at the cost of inducing a glucose spike at night, which could seemingly negate any overall benefit on glycemic control.

Our study demonstrated, for the first time, that a low-carbohydrate protein containing "real-life" snack (two eggs) consumed at bedtime reduced morning fasting plasma glucose in individuals with type 2 diabetes when compared to a higher carbohydrate matched protein bedtime snack (low-fat yogurt). This represents an important finding given many health care professionals have been advising individuals with type 2 diabetes to consume a snack containing protein prior to bedtime to maintain "blood sugar regularity" without evidentiary support (Kalergis et al, 2003). Our findings suggest that a low-carbohydrate protein containing snack, such as eggs, could be

a rational suggestion for a bedtime snack in people with type 2 diabetes. The rationale for a low-carbohydrate protein containing bedtime snack is supported by research that shows ingesting protein preserves insulin responses without a corresponding increase in plasma glucose concentrations (Nuttall et al, 1984).

Elevated fasting plasma glucose concentrations are a hallmark of type 2 diabetes and a result of elevated hepatic glucose production resulting from liver insulin resistance (DeFronzo et al, 1989). Given HGP is a product of both gluconeogenesis and glycogenolysis (Boden et al, 1996), it is plausible the low-carbohydrate protein containing bedtime snack led to an increase in liver insulin sensitivity which decreased hepatic glucose production and the resultant fasting plasma glucose. The significantly higher QUICKI scores (and lower fasting plasma insulin and C-peptide) in the egg bedtime snack condition compared to yogurt support this notion. Given type 2 diabetes is characterized by hyperglycemia resulting from impaired insulin action and secretion (Loukine et al, 2015). An improvement in insulin sensitivity (QUICKI score), calculated from fasting plasma glucose and insulin values, is a good indicator that the low-carbohydrate bedtime snack condition led to an improved liver insulin sensitivity and reduced hepatic glucose production.

2.4.2 Bedtime snacks impact overnight/nocturnal glucose

In a previous study by Dyer-Parziale (2001) the ingestion of a snack bar containing uncooked cornstarch at bedtime reduced nocturnal glucose assessed as the blood glucose level at a single timepoint during the night (midnight). Compared to placebo, consumption of the uncooked cornstarch bar reduced mean blood glucose levels at midnight by ~14% (Dyer-Parziale, 2001). Using CGM in our study allowed us to monitor a diurnal glucose profile in "free living" conditions and as such, we saw a significant difference in mean nocturnal CGM glucose values between the low-carbohydrate protein containing bedtime snack and the high-carbohydrate protein containing bedtime snack. This finding makes sense since the high-

carbohydrate bedtime snack led to a spike in glucose at bedtime shortly after consumption without any evidence of rebound hypoglycemia. This is similar to prior work by Axelsen and colleagues (1999) who also showed an increase in nocturnal glucose with a "rapid release" bedtime carbohydrate" snack (white bread). It appears that such a night time glucose spike as a result of a bedtime snack containing carbohydrates is suboptimal for next morning glycemic regulation when compared to a low-carbohydrate bedtime snack.

2.4.3 Regulators of glucose metabolism

Previous bedtime snack studies have demonstrated an improvement in glucose tolerance at breakfast despite an increase in nocturnal glucose concentrations (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000). In contrast, our study did not show benefit in 3-hr post prandial breakfast CGM glucose AUC. It has been hypothesized previously that this so called "second meal effect" was the result of increased insulin levels from the preceding meal and its subsequent impact on glucose tolerance of the next meal (Wolever et al, 1988). However, others have suggested the mechanism underlying the observed "second meal effect" is via suppression of plasma FFA allowing for improved muscle insulin sensitivity (Jovanovic et al, 2009). Although we did not measure FFA throughout the nocturnal period, we did measure fasting FFA and found no significant difference between conditions, which could indicate that post-prandial breakfast glucose tolerance is not affected unless FFA are lowered. However, it is important to note that breakfast glucose responses were not worsened with either bedtime snack compared to the no bedtime snack condition.

2.5 Strengths and Limitations

Compliance of study participants to assigned dietary interventions is critical to ensure validity of outcomes. Generally, participant compliance to full feeding trials is good at more than 90% (Jones et al, 2013). By providing food for participants to consume in "free living" conditions and

developing meal plans according to individual preference we maintained a high rate of compliance.

Our study employed a combination of methods to assess food consumption and monitor activity level. Log books were used to monitor meal timing, food quantity consumed and ensure glucose calibration. Additionally, daily correspondence between participants and study coordinators ensured accountability and compliance to meals and protocols. Extra attention and enforcement were employed in non-compliant situations (of which there were two). In both cases (which occurred in different participants), the evening snack was not consumed (one in the egg condition and the other in the yogurt condition) so CGM data on this evening and the following day was removed from analyses.

While the short study length improved compliance with study protocols, the three-day study duration is not long enough to determine the long-term impacts on A1C or other cardiometabolic risk factors. To determine the long-term effects of consuming a low-carbohydrate protein containing bedtime snack, longer duration studies examining changes in A1C and vascular outcomes will be needed.

While the protein and energy content were kept constant in both bedtime snack conditions, the manipulation of carbohydrate meant that fat content of the bedtime snacks was different. It therefore becomes difficult to untangle the isolated impact of carbohydrate and fat on glucose metabolism and study outcomes. Additionally, although we matched for protein content there is evidence that protein quality/source can play a role in metabolic action and as such, it is possible the amino acid composition of the low-carbohydrate bedtime snack of eggs had an impact on glucose metabolism. Eggs contain ~8.5% leucine and ~20% glycine (Millward et al, 2008) and both have been shown to stimulate enhanced glucose disposal by increasing insulin secretion and modulating insulin signaling (Layman, 2004). Our goals were to examine "whole

food" but future studies could explore how individual protein sources or amino acids consumed at bed influence glycemic control.

2.6 Significance of findings

This study demonstrated that a low-carbohydrate protein containing snack (eggs) consumed before bed reduced fasting plasma glucose, lowered fasting plasma insulin and C-peptide, improved QUICKI, and reduced nocturnal CGM glucose when compared to a higher-carbohydrate matched protein bedtime snack (yogurt) in people with type 2 diabetes. Because diet is a crucial component in the treatment of type 2 diabetes and nutritional strategies that are easy to implement *and* target the pathophysiology of type 2 diabetes represent a practical means of improving glucose metabolism, these findings represent important observations for therapeutic consideration. Since patients with type 2 diabetes are often advised to consume snacks before bed, future studies exploring low-carbohydrate protein-containing bedtime snacks may be warranted to examine longer-term impacts on glycemic control and diabetes-related outcomes.

Chapter 3: Conclusion

In this thesis, a low-carbohydrate protein containing snack (eggs) consumed before bed i) reduced fasting plasma glucose; ii) lowered fasting plasma insulin, C-peptide and leptin; iii) improved QUICKI; and iv) reduced nocturnal CGM glucose when compared to a higher-carbohydrate matched protein bedtime snack (low-fat yogurt) in people with type 2 diabetes. These data are in support of our primary hypothesis that consuming a low-carbohydrate protein containing bedtime snack could reduce next morning fasting plasma glucose when compared to a high-carbohydrate protein containing bedtime snack. Contrary to our secondary hypotheses the low-carbohydrate protein containing bedtime snack did not reduce the next day post-breakfast glucose response (3-hour post prandial breakfast CGM glucose AUC) or 24-hour average glucose levels (24-hour CGM glucose AUC) when compared to our other conditions. The following discussion and conclusion will expand upon the findings from this thesis, place them into context, address their significance, and provide suggestions for future research.

3.1 Effect of a bedtime snacks on fasting plasma glucose

Our study demonstrated, for the first time, a low-carbohydrate protein containing "real-life" snack (two eggs) consumed at bedtime reduced morning fasting plasma glucose in individuals with type 2 diabetes when compared to a higher carbohydrate matched protein bedtime snack (low-fat yogurt). These data supported our primary hypothesis, which was based on previous research demonstrating a bedtime snack containing uncooked cornstarch ("slow release carbohydrate") significantly reduced fasting plasma glucose when compared to rice flour matched carbohydrate “placebo” (Dyer-Parziale, 2001) or starch-free lower energy (Axelsen, 2000) bedtime snacks in individuals with type 2 diabetes.

Reducing FPG is important in the management and treatment of type 2 diabetes. The Diabetes Canada 2018 guidelines make the specific recommendation for those with T2D - the

achievement of A1C <7% should be accompanied by FPG targets between 4-7 mmol/L (Imran et al, 2018). This recommendation is based on evidence demonstrating that both FPG and postprandial glucose contribute to an individual's A1C (Imran et al, 2018) and each are directly correlated with the risk of macrovascular and microvascular complications (UKPDS, 1999) (Stratton et al, 2000).

Research by Monnier et al (2013) demonstrated the DAWN phenomenon (morning hyperglycemia) was independent of anti-hyperglycemic treatment and accounted for ~0.4% of a patient's total A1C. Therefore, maintaining tight glycemic control in the morning hours is critical to managing disease progression and outcomes. In our study the low-carbohydrate protein containing bedtime snack resulted in a ~5% decrease in fasting plasma glucose when compared to the high-carbohydrate protein containing bedtime snack. Previous studies using uncooked cornstarch as a bedtime snack demonstrated a 12-28% reduction in FPG (Dyer-Parziale, 2001) (Axelsen et al, 2000) when compared to different carbohydrate containing comparator bedtime snacks. Although the difference in our study was statistically significant it was not of the same magnitude as the "uncooked cornstarch" studies. Possible reasons for this include a higher baseline A1C of participants in other studies (Dyer-Parziale, 2001), lack of reporting on the content of dinner or other meals ((Dyer-Parziale, 2001), and in the case of Axelsen (2000) the bedtime snacks did not appear to be iso-energetic (data was not included). Regardless, our study demonstrated a medium-to-large effect size (Cohen's d=0.68) comparing the egg to the yogurt snack, suggesting that the findings were robust. Furthermore, the findings that several measures of fasting glucose metabolism were all improved in the same positive direction (lower FPG, lower insulin, lower C-peptide, higher QUCKI) lends support to a true effect of the egg bedtime snack compared to yogurt.

We did not show a difference between the low-carbohydrate protein containing bedtime snack and the no-snack condition on fasting glucoregulatory measures. This is perhaps due to "no snack" at bedtime resulting in an extended fasting period between dinner and morning. The

extended fasting period without the intake of food may be marked by an increase in the use of FFA and/or liver glycogen to meet the body's nocturnal energy demands. Consequently, a surge in early morning glucose may be differentially affected by the increased fasting time when no bedtime snack is consumed. Testing this idea would have required an additional condition involving a “late” dinner (or no dinner with a “large” bedtime snack) such that total daily energy intake and timing from last night time meal to fasting blood sample was matched.

3.2 Effects of a bedtime snack on CGM outcomes

In our study we did not see any difference in next day post breakfast glucose response (3-hour post prandial breakfast CGM glucose AUC) or 24-hour average glucose levels (24-hour CGM glucose AUC, MAGE, J-INDEX) between any of the bedtime snack conditions.

These results are contrary to previous studies by Axelsen (1997, 1999, 2000) which all showed a decrease in breakfast glycemic response or a "second meal effect" of consuming a cornstarch bedtime snack. This may be in part due to the composition of breakfast given in all these previous studies. The work of Axelsen included a fat rich breakfast (40% fat) whereas our breakfast was relatively high in protein and low in fat. Evidence in individuals with type 2 diabetes has shown the ingestion of fat may attenuate the glycemic response to carbohydrate and decrease glucose levels as a result of inhibiting gastric emptying (Gentilcore et al, 2006) whereas the higher protein content in our breakfast may have impacted glycemic responses through augmented insulin secretion (Manders et al, 2014). In order to exclusively examine the impact of bedtime snacks on next morning glycemic responses, a more direct assessment of glucose tolerance (e.g., oral glucose tolerance test, intravenous glucose tolerance test) with less confounding meal variables would need to be performed. Such glucose tolerance tests isolate for the impact of glucose but lack external validity as people typically consume mixed meals and not pure glucose for breakfast.

Using CGM in our study allowed us to monitor a daytime and night time glucose profile in "free living" conditions and as such, we saw a significant difference in mean nocturnal CGM glucose values between the low-carbohydrate protein containing bedtime snack and the high-carbohydrate protein containing bedtime snack. This was perhaps not surprising as the high-carbohydrate bedtime snack led to a spike in glucose at bedtime shortly after consumption with no apparent hypoglycemic rebound. This is similar to the prior work by Axelsen and colleagues (1999) who showed an increase in nocturnal glucose with a "rapid release" bedtime carbohydrate snack was consumed. It appears from our study that a night time glucose spike resulting from a bedtime snack containing carbohydrates is suboptimal for next morning glycemic regulation when compared to a low-carbohydrate bedtime snack, at least in the context of comparing eggs to yogurt. Future work should consider how different carbohydrate, fat, and protein containing foods impact nocturnal *and* fasting glucose parameters in type 2 diabetes in order to optimize bedtime snacks.

In a previous study by Dyer-Parziale (2001) the ingestion of a snack bar containing uncooked cornstarch at bedtime reduced nocturnal glucose assessed as the blood glucose level at midnight. Compared to placebo, consumption of the uncooked cornstarch bar reduced mean blood glucose levels at midnight by ~14% (Dyer-Parziale, 2001). Our study saw a 7% difference in nocturnal CGM glucose between the low-carbohydrate protein containing bedtime snack and the high-carbohydrate protein containing snack which translated to a large effect size of 0.9 (Cohen's d=0.94). Using CGM was an advantage in our study as it allowed participants to sleep in their own bed and measure glucose every 5 min throughout the day/night, as opposed to selecting a single timepoint (i.e., midnight) for an overnight blood draw.

There were no effects of the bedtime snacks on glycemic variability (MAGE or J-INDEX) or 24-hour glucose assessed by CGM. Glycemic variability is an indicator of glucose fluctuations throughout the day, including hypoglycemic and hyperglycemic episodes (Suh et al, 2015). Recent evidence suggests glycemic variability is involved in the pathogenesis of vascular

complications of diabetes (Weber & Schenll, 2009). Our study was short in duration and therefore it is likely a longer study may be needed to show differences in these outcomes as a result of bedtime snack interventions. In addition, despite impacting fasting glucose and improving markers of insulin sensitivity, the impact of consuming a bedtime snack may not be enough to result in overall changes to 24-hour CGM glucose outcomes.

3.3 Effects of a bedtime snack on other variables associated with glucose metabolism

Our study demonstrated, for the first time, a low-carbohydrate protein bedtime snack (two eggs) reduced morning fasting plasma insulin and C-peptide concentrations in individuals with T2D when compared to a higher carbohydrate protein matched bedtime snack (low-fat yogurt). This is an important finding given previous bedtime snack studies showed no impact on these outcomes (Axelsen et al, 1997) (Axelsen et al, 1999) or did not measure fasting plasma insulin or C-peptide concentrations (Axelsen et al, 2000) (Dyer-Parziale, 2001). Assessment of the quantitative insulin sensitivity check index (QUICKI) showed an improvement in this marker of insulin sensitivity for the low-carbohydrate protein containing bedtime snack condition when compared to the high-carbohydrate protein containing bedtime snack. C-peptide, insulin and QUICKI all demonstrated medium effect sizes (d = 0.45 - 0.50). Both gluconeogenesis and liver glycogenolysis play a significant role in nocturnal and early morning fasting hyperglycemia (Boden et al, 1996) and these are often regarded as the primary pathophysiological mechanisms responsible for the increase in fasting plasma glucose in type 2 diabetes (Magnusson et al, 1992). Finding nutritional strategies that are easy to implement and address the pathophysiology of the disease are paramount. The changes in these markers of liver insulin resistance (insulin, C-peptide and QUICKI) demonstrate the impact of our bedtime snack on important pathophysiological mechanisms of T2D. Glucagon, released by the alpha cells in the pancreas, is the primary stimulator of hepatic glucose production and evidence indicates that hyperglucagonemia is related to elevated fasting glucose in type 2 diabetes (Aronoff et al,

2004). There were no effects of any bedtime snack condition on fasting glucagon, suggesting that the lowering of fasting plasma glucose was related to liver insulin sensitivity as opposed to alpha cell glucagon release or sensitivity. It is likely that the liver responds much faster to nutritional manipulations than steady state release of glucagon from alpha cells.

Another interesting finding not seen previously in other bedtime snack studies was the difference in leptin between bedtime snack conditions. Leptin levels were reduced in the low-carbohydrate protein containing bedtime snack condition when compared to the no bedtime snack condition. This was the only difference seen between bedtime snack and no bedtime snacks conditions and, to our knowledge, the first time that a bedtime snack has been shown to impact leptin in type 2 diabetes. Leptin in considered a satiety hormone and is produced by adipose tissue (Klok et al, 2007) to inhibit hunger. Given that leptin levels are correlated to insulin and glucose (Boden et al, 1996) it is possible that the lower leptin in the egg condition was related to lower fasting glucose and insulin. Given the complex interplay between fasting glucose, insulin, FFA (lipolysis) and leptin (Klok et al, 2007), it is plausible that foods consumed in the evening could impact leptin release. Alternatively, leptin may just be responding to the changes in insulin and glucose that were a result of changes in liver insulin resistance.

3.4 Limitations

In our study, we chose to match the protein and energy content in both bedtime snack conditions. Consequently, the levels of carbohydrate and fat differed between the egg and yogurt bedtime snacks. Therefore, it becomes difficult to determine if altered carbohydrate or fat (or both) impacted study outcomes. Furthermore, it is possible that differences in amino acid profile and/or digestibility of the protein sources resulted in different responses as previous work has indicated that these factors can influence glycemic regulation (Layman, 2004). In order to increase the internal validity and attempt to control for these factors (e.g., provide food

preparations that are matched for as many variables as possible) would require custom food manufacturing and also would decrease external validity.

Both males and females were recruited for this study and we had more females volunteer to participate. However, the study was not designed to explore sex differences and we were underpowered to do so. Most of the women (66.6%) in our study were post-menopausal, which should have reduced differences in sex hormones, but it is currently unclear how sex or menopausal status may influence how bedtime snacks impact glucoregulation. Future studies should consider whether sex influences metabolic responses to bedtime snacks in type 2 diabetes.

Participants in this study were provided with meals to increase adherence to study protocol. However, it might be contended that by providing meals we were manipulating more than the bedtime snack. For example, we may have provided healthier or different foods that participants were not accustomed to eating, and it is likely that prescribing and providing all foods reduced eating opportunities (snacks and social situations) that may have normally occurred in the day.

Although CGM was used to assess glucose control across the entire 3-day protocol, the analyses of fasting plasma glucose, hormones, and FFA were limited to fasting blood samples obtained on day 4 of the protocol. To account for the pulsatile secretion of insulin (and other hormones) and reduce variability (Juh et al, 2002), we obtained two fasting blood samples and took the average. However, the lack of venous blood samples in the nocturnal period and post-breakfast preclude making any conclusions regarding how the bedtime snack interventions impacted insulin or FFA during these periods. Given that Axelson's studies of bedtime snacks report influences on nocturnal FFA and have linked these to changes in fasting or post-breakfast glucose (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) further

mechanistic research should explore nocturnal metabolism in greater detail after bedtime snacks with differing carbohydrate, fat, and protein contents.

Despite these limitations, a congruent medium-to-large effect size on the primary outcome (fasting plasma glucose) and medium effect sizes on key insulin sensitivity markers (QUICKI, fasting insulin, fasting C-peptide) was detected between the egg and yogurt bedtime snacks, which provides increased confidence that the bedtime snack manipulations did indeed have an effect on glycemic regulation.

3.5 Future Research

To optimize a bedtime snack, one needs to consider not only the composition of the selected food but the quantity as well. Our goals were to examine "real-life food" but future studies should explore different parameters related to food composition and quantity.

3.5.1 Protein source

Individual protein sources or amino acids influence glycemic control (Layman, 2004). Both leucine and glycine (Millward et al, 2008) have been shown to stimulate enhanced glucose disposal by increasing insulin secretion and modulating insulin signaling (Layman, 2004). Protein sources are also digested at different rates (e.g., whey is digested quickly whereas casein is digested more slowly – (Hoffman et al, 2004). Manipulating the protein source in a bedtime snack would prove useful in assessing its impact on glycemic variables.

3.5.2 Carbohydrate source

Previous studies looking at bedtime snack interventions all employed a "slow release carbohydrate" in the form of uncooked cornstarch (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). These studies all saw some improvement in glucose control ("second meal effect" or improved fasting glucose) (Axelsen et al, 1997) (Axelsen et al, 1999) (Axelsen et al, 2000) (Dyer-Parziale, 2001). Another factor for

consideration is fibre given previous research demonstrates a link between soluble fibre and reductions in A1C and fasting plasma glucose in diabetes (Chen et al, 2016).

Therefore, future research should consider testing a bedtime snack containing protein with a small amount of carbohydrate in the form of "slow release carbohydrate", perhaps in the form of soluble fibre. Alternatively, it could be possible to optimize a cornstarch-based bar where the carbohydrate content was lowered but protein was added to potentially achieve the same insulin response.

3.5.3 Fat source

Research has demonstrated that low-carbohydrate high-fat diets can positively impact glucose control (Snorgaard et al, 2017) and, in patients with type 2 diabetes, can improve insulin sensitivity (Boden et al, 2005). Manipulating the fat composition of a bedtime snack, on the background of an overall low-carbohydrate diet, could be an interesting area for future research. With respect to bedtime snacks, it is possible that the type of fatty acid (monounsaturated vs. polyunsaturated vs. saturated) and/or the source (animal vs. plant) could influence the results, but this would likely require a longer-term study.

3.5.4 Micronutrients

There is some evidence to suggest that individuals with diabetes are deficient in certain minerals, namely potassium, magnesium, zinc and chromium (Chehade et al, 2009). It is difficult to ascertain from the current research the role these might play in diabetes and therefore consideration should be given to evaluating their impact on glucose metabolism. For example, it may be possible to augment the effects of a bedtime snack by adding micronutrients or a bedtime snack could be an opportune time to supplement with micronutrients of concern for a person with type 2 diabetes.

3.5.5 Glycemic index

Guidelines from the ADA and DC (American Diabetes Association, 2018) (Imran et al, 2018) all recommend choosing low glycemic foods as their carbohydrates of choice. These

recommendations are based on research suggesting that replacing high glycemic index foods with low glycemic index has a positive impact on glycemic control in people with diabetes (Brand-Miller et al, 2003). When devising the composition of an ideal bedtime snack consideration should be given to the glycemic index of the selected food.

3.5.6 Quantity of a bedtime snack

Two of the bedtime snack studies we discussed previously employed a high dose of uncooked cornstarch as their choice food source (Axelsen et al, 1997) (Axelsen et al, 1999). Both studies showed the decrease in next morning glucose tolerance came at the cost of an increase in nocturnal glucose (Axelsen et al, 1997) (Axelsen et al, 1999). Therefore, the optimal quantity of food should be an important consideration when making decision about the bedtime snack intervention. Understanding the size of a bedtime snack is an important determinant of snack composition.

3.5.7 Other possible considerations if bedtime snacks are promoted for type 2 diabetes

In recent years, there has been growing interest in the impact of pre-sleep protein (i.e., a bedtime snack) on increasing overnight muscle protein synthesis in order to promote muscle hypertrophy (for athletes) or prevent muscle loss (for older adults) (Trommelen et al, 2016). Given that many people with type 2 diabetes can be classified as having sarcopenic obesity (low muscle mass on a background of a high fat mass) (Stenholm et al, 2008) (Trierweiler et al, 2018), it is possible that a protein-containing bedtime snack may serve a dual purpose of lowering fasting glucose and helping to preserve muscle mass in older adults with type 2 diabetes. Such a combination might be beneficial in the long-term for improving overall cardiometabolic health and mobility, especially in elderly type 2 diabetes patients.

In contrast, the inclusion of a bedtime snack could have potential negative effects by extending the “eating window” (i.e., the number of hours per day that food is consumed) for someone with type 2 diabetes. A recent study found that five weeks of time-restricted feeding, specifically limiting the calories that are consumed late in the day, can have positive effects on insulin

sensitivity and beta-cell function in people with prediabetes (Sutton et al, 2018). The promotion of bedtime snacks is seemingly in contrast to this early time-restricted feeding concept. It may be interesting in future studies to compare isoenergetic early-time restricted feeding with a strategic low-carbohydrate protein containing bedtime snack in people with type 2 diabetes.

3.6 Summary

The results of this preliminary investigation are promising for individuals with type 2 diabetes who may be looking for an easy to implement nutritional strategies that impacts the pathophysiology of their disease. This study demonstrated that a low-carbohydrate protein containing snack (eggs) consumed before bed reduced fasting plasma glucose, lowered fasting plasma insulin and C-peptide, improved QUICKI, and reduced nocturnal CGM glucose when compared to a higher-carbohydrate matched protein bedtime snack (yogurt). Because diet is a crucial component in the treatment of type 2 diabetes these findings represent important observations for therapeutic consideration, specifically with regards to the timing and composition of foods that can be recommended. We recognize the bedtime snack employed in this study may not be suitable for all individuals with type 2 diabetes and therefore it will be necessary in future studies to examine other "real-food" or potentially “engineered” bedtime snacks in order to optimize bedtime snack choices. Our findings provide the first evidence, to our knowledge, that supports the common recommendation that patients with type 2 diabetes can help control their morning blood glucose levels by consuming a snack containing protein before bed and the results suggest that this protein containing snack should also be low in carbohydrate. Future studies exploring low-carbohydrate protein-containing bedtime snacks are warranted to examine longer-term impacts on glycemic control and diabetes-related outcomes.

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Appendices

Appendix A: Participant information and consent form

Participant Information and Consent Form

Title: Bedtime Snack Study

Principal Investigator: Jonathan Little, PhD
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Phone: (250) 807-9876

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Sponsor: Egg Farmers of Canada and Egg Nutrition Center.

Emergency Telephone Number:
Dr. Jonathan Little, 24-hour emergency contact number; 250 878 6893

1. INVITATION

You are invited to participate in a research study on bedtime snacking. This study is looking for people with type 2 diabetes between the ages of 30-80 years. People treated with insulin are not eligible.

2) 2. YOUR PARTICIPATION IS VOLUNTARY

Your participation is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled to or are presently receiving. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. The researchers have a duty of care to all subjects and will inform you of any information that may affect your willingness to remain in the study.

If you wish to participate in this study, you will be asked to sign this form. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

3. WHO IS CONDUCTING THE STUDY?

The study is being conducted by Dr. Jonathan Little, who is an Assistant Professor in the School of Health and Exercise Sciences at UBC Okanagan. This study is sponsored through a grant from The Egg Farmers of Canada to Dr. Little and a young investigator grant from the Egg Nutrition Centre provided to Ms Francois.

4. BACKGROUND

Uncontrolled blood sugar puts people with type 2 diabetes at high risk of cardiovascular disease and other diabetes related complications. Often people with type 2 diabetes are told to eat a snack before bed to help control high blood sugar levels across the night and into the morning. However, it is not known what the best snack is to control morning blood sugar levels without causing an increase in overnight blood glucose levels.

More recently, **continuous glucose monitors (CGM)** have given researchers a wider lens to examine different aspects of glucose control. People wear these small devices to measure glucose in the body every 5 minutes. The CGM can be worn for many days in a row.

5. WHAT IS THE PURPOSE OF THE STUDY?

This study seeks to examine the effect of consuming two different bedtime snacks compared to no bedtime snack, on morning blood glucose levels as a means to control overall blood glucose in type 2 diabetes.
A secondary purpose is to determine if certain bedtime snacks help to control blood glucose levels at the next meal (breakfast).

6. WHO CAN PARTICIPATE IN THIS STUDY?

You may be able to participate in this study if:

- You are between the age of 30-80 years.
- You have been diagnosed with type 2 diabetes for at least 6 months.
- You have been on stable glucose-lowering medications for at least the last 3 months.
- You have an A1C between 6.5-9%.
- You are able to understand and comply with study requirements (e.g., attend visits in the morning and eat the meals that will be provided to you).

7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You will not be eligible to participate in this study:

- If you are allergic to eggs or intolerant to components in dairy (e.g., lactose)
- If you are currently pregnant or planning on becoming pregnant in the next 3 months

- You are currently taking insulin
- If your most recent hemoglobin A1C result was over 9.0%
- You have had a recent heart attack or stroke (within the last year)

8. WHAT DOES THE STUDY INVOLVE?

This study involves at total of 7 visits to the laboratory at the University of British Columbia Okanagan, Kelowna. These visits are divided into one baseline visit (first visit) and six experimental visits; two for each of the three experimental conditions. For each condition you will visit the laboratory at the start (day 1) and end (day 4) and will consume a slightly different diet each time. Each condition will be separated by seven days.

<u>Visit 1</u>. Determining your eligibility (~1-hour lab visit).

- **Initial Meeting.** You will come to the Exercise Metabolism and Inflammation Laboratory (**EMIL**) on the University of British Columbia Okanagan campus. We will discuss any questions or concerns you may have about the study. If you agree to participate, we ask that you sign this consent form before any study procedures are done. Then you will complete an eligibility questionnaire. Questions include general information about your health, medications, and current diet (allergies, food preferences). We will also measure your body mass, waist circumference and blood pressure.
- A food frequency questionnaire, allergies and dislikes will also be collected for the food you will be provided. The food provided will be based on the current Canadian Diabetes Guidelines and your caloric needs calculated using the previously validated Harris Benedict equation (uses weight, age and activity factor). This visit will take 1 hour.

<u>Visit 2</u>. Inserting the continuous glucose monitor (CGM) (~30-minute lab visit).

The day before each three-day intervention period a CGM will be inserted, and will remain inserted throughout the three-days to measure your blood sugar. A CGM is a small device that measures your blood glucose every 5 minutes. You will wear the CGM for the length of the study (3 days X 3 conditions, total of 9 days). The small CGM sensor will be placed on the skin of your abdomen by a person trained by the CGM manufacturer. The CGM sensor has a small filament that is inserted under your skin with a small needle (less than 1 cm long). The needle is then removed and only the flexible filament remains under your skin. Tape will be placed over the CGM to hold it in place. A picture of the CGM is shown in the figure below. The CGM insertion should take no more than 5 minutes. During this lab visit you will be given a small booklet containing important information about the CGM, pedometer, and food log. You will also be given standardized meals to follow for the next 3 days and wear an activity monitor (Fitbit).

Figure: A continuous glucose monitor.

Note: Only the flexible filament is inserted under the skin. →

Interventions:

You will be given all the food you are supposed to eat for the each 3-day intervention as well as a menu and preparation instructions. You will be asked to adhere to the diets and record any changes. Specifically, you will be given 3 days' worth of breakfasts, lunches and dinners, as well as snacks and desserts. Your job is to stick to this diet for 3 days, adhering as close as possible to the menu plan and instructions. The foods have been selected specifically for your diet and anything you "add in" on your own could impact the calories that have been carefully calculated. For this reason, we ask you not to supplement the diet with any of your own additional foods. We will also ask that you don't drink alcohol, pop/soda, or juices during the 3-day period (including the night before the trial). You will be allowed to drink additional water and coffee (without sugar) over the 3-day period provided you record your intake and match this for each condition.

Fitbit:

A Fitbit, or wrist based (looks like a bracelet) activity tracker will be used to monitor and compare activity and sleep patterns (i.e., steps per day & hours of sleep) between the three intervention periods. Importantly, you should not change your typical activity and sleep levels for each intervention. You will not have to interact with the FitBit or the associated "app" and no personal identifiers will be used when collecting this data.

The three diet conditions are:
Egg Bedtime Snack: You will be provided with two eggs for each of the three evenings to be consumed within 30 min before going to bed.
Yogurt Bedtime Snack: You will be provided with 150 ml of yogurt for each of the three evenings to be consumed within 30 min before going to bed.
Control Condition: You will consume the standardised meals with no evening snack. Please make sure you stop eating dinner before 7:30pm.
*The order of the three conditions will be randomized. This means that the order you will receive the different diet conditions will be determined at random (like picking a number from a hat).

Finally, you'll have to take your capillary blood glucose (finger prick) every day for 3 days. This will be done 5 min before each meal and 15 min before bed with a handheld glucose monitor that we will provide you. We will be using those glucose values to calibrate the CGM.

<u>Visit 3</u>: Fasting blood and CGM removal (~30-minute lab visit).

i. After fasting for at least 10 hours (no food or drink; except water for 10 hours before your visit) your CGM will be removed, the area cleaned with an alcohol swab and a fasting blood sample (2 teaspoons or 10 millilitres) will be obtained

from your arm vein using a needle. A trained phlebotomist will obtain the blood sample.

You will repeat Visits 2 and 3 for the next two conditions. Therefore Visit 4 and 6 and Visit 5 and 7 will be identical to Visits 2 and 3 except that you will consume the different diet in between.

Please note: Blood samples collected will only be used by the research team to determine your blood glucose concentration, blood hormone levels and inflammatory responses. There will be no blood banking or genetics research done using these samples. They will be identified by a number/code only with no personal identifiers. Before analyses they will be stored in a freezer located in the laboratory (ASC 288) of Dr. Little who will be responsible for them for the duration of the study. The lab is accessed by Salto card only for authorized personnel. A freezer inventory log is kept for all samples in this lab on a password protected computer. Any leftover blood samples will be destroyed following the conclusion of research.

Summary of Visits and Timeline.

This study consists of three, three day conditions each separated by one week. You will be provided with a calendar to keep track of your visits to the laboratory.
The following is an outline of what will happen for each condition:

Day 1	Day 2	Day 3	Day 4
Lab Visit: CGM insertion Standardized meals	Standardized meals; no lab visit	Standardized meals, no lab visit	**Lab Visit**: Fasting blood sample in the morning, CGM return

WHAT ARE MY RESPONSIBILITIES?

1) Please show up to your appointments at the correct time.
2) Consume all standardized meals to the best of your ability.
3) Record any changes that you make to your provided food and menu plan.
4) Check your capillary glucose 4 times per day (i.e., finger prick glucose; supplies provided).
5) Report any noticeable changes in your health status (e.g., sickness, cold).

10. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?

Blood sampling: The blood sample procedures are similar to what you would experience at a doctor's office, hospital or medical laboratory. The needle is inserted under completely sterile conditions, however there is a risk of infection. There is also chance of bleeding if adequate pressure is not maintained upon removal of the needle. This may cause some minor discomfort and could result in bruising/skin discolouration that could last for up to a few weeks. There is also remove risk that trauma to the vessel wall could

result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication in our laboratory after several hundred venous blood sampling procedures. But the risk of pain and infection will be minimized by using an experienced trained phlebotomist, and of course by ensuring the needle is inserted and removed under completely sterile conditions. Any complications beyond mild redness/inflammation or slight soreness should be reported to the researcher or a medical doctor. These risks are *Extremely Rare (less than 1%)*

Continuous glucose monitoring: There is a risk of infection and an extremely low risk of bruising/skin discoloration from the insertion of the micro needle that could last for up to a few days. The insertion of the CGM is done under sterile conditions by a trained researcher and we have never experienced any infections or bruising in hundreds of CGM insertions. There is also a small risk of bleeding at the site of insertion. The skin of some individuals is sensitive to adhesives used in tape or bandaids and can get red or itchy when the CGM is attached with the medical tape used. There may be redness, irritation, soreness, rash, or tenderness in the area where the tape was applied after removal of the CGM but this will usually disappear after a few days. If you experience pain or discomfort related to the CGM you can remove it at anytime, consult the study staff to have it removed, or contact your healthcare provider for assistance. Removal involves taking off the adhesive, similar to removing a bandaid.

The CGM is approved for continual wear for up to 6 days. You should not go into a hot tub (sauna, whirlpool) while wearing the CGM nor should you undergo an MRI scan while wearing the CGM.

No pharmacological agents will be given in this study, so there is no risk of drug interactions with your current medications. It is not possible to know all of the risks that may happen in a study. The researchers have taken all reasonable safeguards to minimize any known risks to a study participant.

11. WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING?

You may not benefit directly from participating in this study. We hope this study will help us better understand how certain snacks and the timing of those snacks affect glucose levels. You will receive information on how your blood glucose responds to bedtime snacking. You are not expected to receive any other benefits from participating in this study.

If there are any findings that may be of interest or importance to you or your primary care physician (e.g., low or high blood glucose values during the day or night) you will be provided with this data and encouraged to discuss with your primary care physician.

12. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request

the withdrawal of your information or blood samples collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know.

13. CAN I BE ASKED TO LEAVE THE STUDY?

If you are not able to follow the requirements of the study or for any other reason, the researchers may withdraw you from the study.

14. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of UBC, and UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

Primary Care Physician(s) /Specialist(s) Notification

Please indicate, by checking the applicable box, whether you want us to notify your primary care physician(s) or specialist(s) of your participation in this study. This is not a consent to release medical information.

___Yes, I want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study. My primary care physician(s) and/or specialist(s) name(s) is/are: _____

The name of the medical clinic I attend is: _____

Subject Initials: _____

____No, I do not want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study.
Subject Initials: _____

____I do not have a primary care physician or specialist.
Subject Initials: _____

15. AFTER THE STUDY IS FINISHED

Future Contact

___ Yes: If future research opportunities present themselves, the researcher may contact me as a potential research subject.

16. WHAT HAPPENS IF SOMETHING GOES WRONG?

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else, and you do not release the study doctors or participating institutions from their legal and professional responsibilities.

In case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and that the following person can then be contacted for further information: Dr. Jonathan Little – 250-807-9876 (office) or 250-878-6893 (24 hr mobile)

If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

17. WHAT WILL THE STUDY COST ME?

Reimbursement*:* There are no costs to you for participating in this study. Complimentary research participant parking is available upon request should you need to park on campus. You will receive free meals for a total of nine (3 days X 3 conditions) days during the study.

18. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Jonathan Little at *250 878-6893 (24hr) or* Office: 250-807-9876.

19. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT?

If you have any concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

21. SIGNATURES

Subject Consent

My signature on this consent form means:

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Participant's Signature	Printed name	Date

Signature of Person Obtaining Consent

_____	_____		
Signature of Person Obtaining Consent	Printed name	Role	Date

Investigator Signature (Optional)

_____	_____	_____
Investigator Signature	Printed name	Date

My signature above signifies that the study has been reviewed with the study subject by me and/or by my delegated staff. My signature may have been added at a later date, as I may not have been present at the time the subject's signature was obtained.

Appendix B: Phone Screening

PHONE SCREENING

Persons interested in taking part in the study are to complete this form with the Study Coordinator by phone. Start by asking if they have any questions about the study?

1. Have been diagnosed with type 2 diabetes? Y N

   If yes: How long ago? _____ (include if more than 6 months)

2. Are you between 30-70 years of age Y N

   If yes: What is your month and year of birth? (mm/yr) _____

3. Do you currently take insulin? Y N

4. (Circle: Male Female)
   If female: When was your last menstrual cycle? _____

5. Would you be able to visit our lab at the University of British Columbia Okanagan Campus for 1 hour on two separate occasions within 3 days of each other? Y N

6. To the best of your knowledge, have you ever suffered from any serious medical problems other than type 2 diabetes? (For example, heart attack or stroke) Y N
   If so, please specify.

   _____

   _____

Instructions:

-Provide participant with directions to lab for *full screening* and/or *baseline assessment.*

-Ask if they have any other questions about the study or if they would like to receive an information sheet by e-mail before the next visit.

-Remind participant to bring results with recent A1C and Lipids (HDL-C, LDL-C Total-C, TG) within last 6 months and serum creatinine within last year to the *Baseline Assessment* visit.
-Remind participants that they will be asked about their medications (some have lists they can bring).

Appendix C: Participant Screening and Medical Information Form

PARTICIPANT SCREENING AND MEDICAL INFORMATION FORM

Persons interested in taking part in the study are to complete this form with the Study Coordinator.

1. Have been diagnosed with type 2 diabetes? Y N

   If yes: How long ago? _____ (include if more than 6 months)

2. Are you between 30-80 years of age Y N

   If yes: What is your date of birth? (mm/yr) _____

3. Do you currently take insulin? Y N

4. (Circle: Male Female)
   If female: When was your last menstrual cycle? _____

5. Has your diabetes medication been changed in the last 3 months? Y N
   (Note: If medication not stable, we could wait for stability before including)

6. Please list any medications you are taking for diabetes, blood pressure, or cholesterol?

   _____

7. Please list other medications if applicable?
   If so, please specify. (Note: this includes Valium®, aspirin, antacids, vitamins)

   _____

   (Note: treatment with corticosteroids are not eligible for this study)

8. To the best of your knowledge, have you ever suffered from any serious medical problems other than type 2 diabetes? (For example, heart attack or stroke) Y N
   If so, please specify.

   _____

(Although not reasons for exclusion, particular note should be made of polycystic ovary syndrome (PCOS), Cushing's syndrome, musculoskeletal limitations, chronic obstructive pulmonary disease, heart disease such as possible cardiac deficiencies including arrhythmia and other heart conditions, high blood pressure, epilepsy, glaucoma, Parkinson's, hypo/hyperthyroidism and blood disorders such as anemia.)

9. Do you have any other known allergies, including to certain foods? Y N
If so, please specify.

---

10. Do you have any other dietary restrictions?
If so, please specify.

---

11. Do you smoke more than one cigarette (cigar or other) per day?
Y N

12. Do you consume alcohol? Y N
If so, how many drinks do you consume per week on average? _____

13. Within the last 12 months, have you experienced alcohol or substance abuse?
Y N

Appendix D: Logbook for participants

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 eat only the food we provide, record any food you cannot eat and/or change.
- Please take your finger stick blood glucose readings and enter the results into both this log book.

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).

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:_____

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

Meal	**Time**	**Food and Drink changes (be as specific as possible)**	**Physical Activity**
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

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	Food and Drink changes (be as specific as possible)	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

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	Food and Drink changes (be as specific as possible)	Physical Activity
Breakfast			
Lunch			
Dinner			
Snack (if applicable)			

CGM – Finger prick blood glucose.
DAY FOUR

Date: _____

	Time	Blood glucose
Calibration 1 Before breakfast		