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Erik Vandenboer

A Thesis

A thesis submitted in conformit with the requirements

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The acute effect of bovine whe protein fractions on blood glucose and insulin in rats

Erik VanDenboer

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Department of Applied Human Nutrition

Mount Saint Vincent Universit

The ingestion of whe protein (WP) leads to the reduction of postprandial blood glucose (BG) response paralleled with an increased level of insulin. However, the role of individual WP fractions: gl comacropeptide (GMP), β-Lactoglobulin (β-LG), and α-Lactalbumin (α-LA) remain unclear. The objective of this stud was to investigate the effect of WP, GMP, β-LG, and α-LA on BG and insulin response in rats. We h pothesized that due to the difference in their amino acid profile the effect of WP fractions on short-term BG control ma differ. Methods: A randomized repeated measures stud was conducted in rats fitted with jugular vein catheters and vascular access buttons (VAB). Nine male 10-week-old 275-300g Wistar Han rats were gavaged 350mg (allometricall scaled from a human dosage of 10g) of either intact WP, GMP, β-LG, α-LA, or a glucose control dissolved in 3ml of water after being fasted for 6h during da light. The use of a VAB allowed for the same rat to receive all five treatments in a random order, with a 4 h washout period between treatments. Blood was collected at 0, 15, and 30 min for insulin, and at 0, 15, 30, 60, 90, and 120 min for glucose. Whole blood was anal zed for glucose using a HemoCue 201 Glucose Anal zer, and plasma was anal zed for insulin using a wide range ELISA. The data were tested for normalit and anal zed using Two-Wa Repeated Measures ANOVA for the effect of time, treatment, and a treatment b time interaction. The data for the area under the curve (AUC) for 2h BG and 30 min insulin were anal zed with One-Wa Repeated Measures ANOVA. The differences between the treatments were assessed with Tuke -Kramer post hoc test. Results: There was an effect of treatment ($P<0.0001$), time ($P<0.0001$) and a treatment b time interaction ($P<0.0001$) over 120 min on BG response. Whe protein, GMP, β-LG, and α-LA resulted in significantl lower BG compared to glucose treatment at 15 and 30 min ($P<0.05$). Blood glucose AUC over 120 min was lower for WP, GMP, and α-LA compared to glucose treatment ($P<0.05$). There was an effect of treatment ($P=0.02$), time ($P<0.0001$) and a treatment b time interaction ($P=0.0002$) on insulin response over 30 min. Gl comacropeptide had a lower insulin response at 15 min compared to glucose and β-LG ($P<0.05$). Insulin AUC over 30 min was lower for GMP and α-LA compared to the glucose treatment ($P=0.003$). Conclusion: At the dose of 350mg WP, GMP, β-LG, and α-LA attenuated BG response at 15 and 30 min, while onl WP, GMP, and α-LA attenuated AUC over 120 min compared to the glucose control. GMP attenuated insulin response at 15 min compared to β-LG and the glucose control, and GMP and α-LA attenuated AUC over 30 min compared to the glucose control. This data suggest that WP fractions possess unique properties not observed in WP. Supported b the Natural Sciences and Engineering Research Council of Canada.

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List of Abbreviations

A$

α-LA	Alpha-lactalbumin
A1C	Gl cated Hemoglobin
7$	
BCAA	Branch Chain Amino Acids
β-LG	Beta-lactoglobulin
BMI	Bod Mass Inde
;$	
CACF	Carlton Animal Care Facilit
CRL	Charles River Laboratories
Q$	
DPP-4	Dipeptid l-peptidase-IV
1$	
FPG	Fasting Plasma Glucose
9$	
GDM	Gestational Diabetes
GIP	Glucose-dependent insulinotropic pol peptide
GLP-1	Glucagon-like peptide-1
GLUT	Glucose Transporter
GMP	Gl comacropeptide
@$	
LMIC	low- and middle-income countries
*$	
mTOR	Mechanistic target of rapam cin
J$	
OGTT	Oral Glucose Tolerance Test
.$	
PG	Plasma Glucose
PNP3M	PinPort Injector
!$	
T1D	T pe 1 Diabetes
T2D	T pe 2 Diabetes
T$	
VAB	Vascular Access Button
VABC	Vascular Access Button Cap

Chapter 1: Introduction

T pe 2 Diabetes (T2D) or diabetes mellitus is a heterogeneous metabolic disorder of h pergl cemia due to insufficient insulin secretion, resistance to insulin action, or a combination of both and is the current 7 leading cause of death globall (1,2). While incidence in Canada has remained fairl consistent, prevalence is increasing due to a growing population and increased life e pectanc (3).

While lifest le interventions and medication are useful in dela ing progression from pre-diabetes to T2D, the have proven unable to effectivel lower long-term incidence and prevalence, and alternative forms of treatment ma prove beneficial (4). E perimentation using milk proteins (casein and whe) have proven promising in their anti-h pergl cemic effects, et there is currentl insufficient research to make recommendations for their use (5). This research gap ma be partl addressed b this stud 's goal of e ploring the short-term blood glucose control effects of various milk protein fractions, as the mechanism and efficac of specific protein sub-fractions aiding normogl cemia regulation is unclear. Understanding the ph siological processes involved in the regulation of blood glucose in response to ingested food ingredients with biological activities ma provide direction for the development of therapeutic food products aimed at improving blood glucose control.

Chapter 2: Literature Review

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Typ 2 D t s M ll tus$

T pe 2 Diabetes mellitus (T2D) occurs when insulin is not produced in sufficient quantities and/or the bod is unable to properl use it due to insulin resistance, resulting in chronic h pergl cemia, subclinical inflammation, and long-term microvascular damage affecting the e es, kidne s, and nerves, and dramaticall increasing the risk of cardiovascular disease (6–).

2.1.1 Cl ss f c t n

There are three major classifications of diabetes: T pe 1 Diabetes (T1D) (~10% of cases), T2D (~90% of cases), and Gestational Diabetes (GD) (<1% of cases), and a fourth minor classification of other specific t pes, which result from a m riad of reasons (e.g., genetic defects of β-cell, genetic defects in insulin action, rate forms of immune-mediated diabetes, etc.) (6,9). T pe 1 Diabetes, formerl known as insulin-dependent diabetes occurs when the pancreas is unable to produce insulin and thus regulate plasma glucose (6). Destruction of β -cells occurs from cellular mediated auto-immune d sfunction (6). T pe 2 Diabetes, formerl known as insulin-independent diabetes, occurs when the bod loses sensitivit to insulin action and eventuall increased production is unable to compensate (6). Gestational Diabetes occurs during pregnanc and usuall resolves after pregnanc though T2D can develop (6).

2.1.2 D t s M ll tus Ov rv w

More than 130 genetic variants associated with t pe 2 diabetes, glucose levels, or insulin levels have been identified, however, the account for less than 15% of T2D heritabilit (10,11). This suggests that modifiable and non-modifiable risk factors, environmental risk factors, heterogeneit , gene-gene interactions, and epigenetics all contribute to T2D pathogenesis (12). The two major non-environmental modifiable risk factors are insufficient e ercise and metabolic s ndrome, which is at least three of following five: (1): elevated blood pressure (≥ 130/ 5 mmHg,) (2): an elevated fasting trigl ceride level (≥ 1.7 mmol/L), (3): a decreased high-densit lipoprotein cholesterol level (< 1.03 mmol/L for men and < 1.30 mmol/L for women), (4): an elevated fasting glucose level (≥ 6.1 mmol/L), and (5): abdominal obesit (waist circumference > 102 cm for men and > cm for women) (13–15). Major non-modifiable risk factors are a histor of h pergl cemia, prediabetes and/or gestational diabetes, famil histor , ethnicit , and age (14).

While the e act pathogenesis of T2D remains unclear a combination of genetic and environmental factors resulting in a worsening feedback c cle of impaired insulin sensitivit and a corresponding loss of β-cell function and mass appears to be a probable cause (16,17). β-cells are a central component of T2D, as impaired β-cell function in relation to increased insulin resistance is ultimatel responsible for T2D. β-cells, located in the pancreas inside islets of Langerhans, regulate blood glucose through the production and secretion of insulin. Glucose is transported inside β-cells b , primaril , Glucose Transporter (GLUT) 2 through facilitated diffusion (1). This eventuall results in an increase of ATP inside the β-cell through the metabolic breakdown of glucose eventuall resulting in o idative phosphor lation (19). The

increased amount of ATP inhibits ATP-sensitive potassium channels, which results in depolarization of voltage-dependent calcium channels resulting in increased calcium inside the β-cell (19). This increased calcium results in insulin secretion through calcium-mediated Insulin e oc tosis (1). While β-cells are able to temporaril alleviate decreased insulin sensitivit through increased insulin secretion, due to an increase in mass, this is a temporar stopgap as β-cells will eventuall fail to be able to meet insulin demands (16).

β-cell d sfunction results in progression from normal glucose tolerance to impaired glucose tolerance (pre-diabetes), which is a strong predictor for development of T2D (20,21). Glucose tolerance status is mainl determined b postprandial first phase insulin secretion (~10 min duration), however both first-phase insulin secretion and second-phase insulin secretion (~2-3 hours postprandial) are impaired in these tissues (22–24). Impaired glucose tolerance can progress to T2D within as little as a 3- ear time frame as insulin response begins to deteriorate at a quicker pace (25). However, β-cell d sfunction often begins as earl as 12 ears before T2D diagnosis (26). Insulin resistance is normall compensated for b an increase in insulin secretion and/or an increase in β-cell mass (27). Unless steps are taken to improve insulin sensitivit eventuall insulin resistance will worsen to the point where even a state of h perinsulinemia is incapable of effectivel causing the uptake of glucose b tissues, and will itself worsen insulin resistance (2). This deterioration of glucose-uptake function compounds into a cascade as increasing β-cell d sfunction causes chronic inflammation of the pancreatic islets further increasing d sfunction of β-cells and worsening insulin resistance (29). However, the d sfunction of β-cells is not total d sfunction, but rather limited to glucose-insulin interaction

as β-cells can still appear to retain their abilit to respond normall to amino acids and other hormones (30).

Insulin resistance often develops due to an increase in visceral fat and ectopic fat deposition in the liver and muscles (31,32). While the specific reason for insulin resistance has et to be elucidated chronic inflammation appears to pla a ke role in disrupting pathwa s of insulin action. This ma be through a process of failure of adipose tissue to sufficientl store fat resulting in in pro-inflammator mediators contributing to insulin resistance being released and free fatt acids being taken in b both skeletal muscle and the liver (33,34). E cess fat stored in skeletal muscle and the liver also promotes inflammation which further contributes to insulin resistance (33). Free fatt acids themselves ma have a role to pla earl on in insulin resistance as saturated fat can impact h pothalamus regulation and cause d sregulation between hunger and satiet cues matched to bod energ requirements (35). H pothalamus d sregulation results in increased weight gain through fat storage, which ma be the triggering event in the insulin resistance cascade, and thus a diet high in saturated fat, such as a "Western St le Diet" (high fat, high carboh drate, low protein, high amounts of red meat, and high amounts of processed food) ma potentiall pla a leading role in T2D progression (34,36).

The specific amount of bod fat that can lead to T2D differs b individuals, but a Bod Mass Inde (BMI) ≥ 30 km/m or abdominal obesit (waist circumference > 102 cm in men and cm in women) are primar risk factors (37). However, the fat threshold of when visceral and ectopic fat accumulation begins to cause insulin resistance is individualized and can occur at BMIs and waist circumferences both above and below these cutoffs (31). Weight loss has been shown to improve insulin sensitivit , though it appears less effective in long-standing T2D (3).

Ph sical activit has also been shown to increase insulin sensitivit (39). Currentl there is conflicting evidence on whether increased ph sical activit without weight loss is effective in increasing insulin sensitivit (39). While BMI is not t picall used in athletes as a measure of health due to high levels of ph sical activit and muscle mass American Football Linemen and Sumo Wrestlers are two unique t pes of athletes as high bod weight is essential for increased athletic performance, however, there is a striking difference between the two, as metabolic s ndrome in sumo wrestlers is relativel uncommon while in American Football Linemen is it quite pronounced (40,41). Bod imager of Sumo Wrestlers shows minimal abdominal visceral fat and mainl subcutaneous fat, and while bod imager of American Football Linemen has not been studied based upon the high prevalence of metabolic s ndrome it can be assumed that visceral fat deposition ma be more pronounced (40). While both t pes of athletes have strenuous ph sical e ercise routines and ver high dail caloric consumption (>5000 calories) the e act reason for the pronounced differences is unknown though dietar composition ma pla a role (40,41)

While, still unclear, a possible progression of the development of T2D ma follow this course: (1): first-phase insulin response is negativel affected as β-cell function fails to respond adequatel to increasing insulin resistance (β-cell function will continuall decrease over time, though the rate can be slowed significantl with treatment), (2): normal glucose tolerance progresses to impaired glucose tolerance as impaired β-cell function is still unable to adequatel respond to insulin resistance, (3): acute insulin response is further degraded as β-cell function continues to decrease and T2D develops, (4): anti-h pergl cemic medication is started, (5): gl cemic control on medication is lost, and (6): uncontrolled diabetes continues to

degrade β-cell mass and function and glucoto icit (e cessive glucose in the blood) and lipoto icit (e cessive fatt acids in the blood) increase the risk of other chronic diseases, with death from cardiovascular disease being the most probable outcome (24,29,42). While this progression often takes decades, and ma be possible to effectivel manage with a combination of nutrition and e ercise interventions alone, medication is usuall required (43).

While the ingestion of glucose results in insulin-mediated glucose transport to cells for use insulin works within a cadre of blood glucose regulating hormones (44). Decreasing blood glucose concentrations triggers pancreatic α-cells$to produce glucagon, which has an antagonistic effect to insulin and causes the liver to start releasing glucose from stored gl cogen (44). Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic pol peptide (GIP) are two gastrointestinal-secreted hormones known as incretins released in response to oral glucose intake that result in insulin secretion two- to three-times higher than via intravenous glucose administration alone (45,46). GIP and GLP-1 bind to their matching receptors in β-cells increasing the level of c clic adenosine monophosphate, which has a s nergistic effect on cellular insulin secretion (47). People with T2D frequentl have impaired e pression of GLP-1 and GIP in response to glucose intake furthering hindering β-cells abilit to function in the presence of insulin resistance (45). GLP-1 and GIP are regulated b dipeptid l-peptidase-IV (DPP-4) and DPP-4 inhibitors are regularl used in the management of T2D (4).

Overall, diabetes is increasingl becoming a fatal disease itself as it is currentl ranked as the 7 global cause of death and has increased globall b over 30% since 2006 (49). In addition to this, the strong associations between h pergl cemia and the risks of cardiovascular events (e.g. m ocardial infarction and stroke), microvascular events, and peripheral neuropath make the

development of treatment options a primar goal (42). Gl cemic control medications such as metformin, sulfon lureas, or insulin initiall prove effective in lowering A1C (gl cated hemoglobin) and FPG (fasting plasma glucose), but over time (t picall 4 to 10 ears) additional therap can be required, with almost half of people living with T2D needing additional treatment within 6 ears of diagnosis (26). Despite the initial effectiveness of sole-medications and later multiple-medications, managing weight gain must also be considered as sulfon lureas, thiazolidinediones, and insulin are associated with weight gain, whereas metformin and am lin analogs are weight neutral or associated with weight loss (50). The inabilit of medications to stop the progression of β-cell d sfunction and the increased risks of weight gain point towards other forms of treatment needing to be developed, such as dietar based interventions, which ma be a safer and more permanent intervention (4).

2.1.3 Typ 2 D t s D n s s

Diagnosis of T2D is based upon venous blood samples with a single positive test being acceptable for diagnosis ( ). The four accepted wa s to diagnosis diabetes are: (1): a fasting plasma glucose (FPG) ≥ 7.0 mmol/L after at least eight hours of no caloric intake, (2): a gl cated hemoglobin value (HbA1C) ≥ 6.5%, (3): a two hour plasma glucose (2hPG) value following an oral glucose tolerance test (OGTT) of 75 g of glucose ≥ 11.1 mmol/L, (4): and a random plasma glucose (PG) at an time of the da without regard to food intake *11.1 mm l/L* ( ). HbA1C is the preferred choice for diagnosing T2D as it can be measured at an point of the da without regards to fasting, as is required in a FPG test, or waiting, as is required in a 2hPG test, and is a reflection of the average PG over two to three months removing an potential da -to-da variabilit ( ).

Table 2.1: Normal, Pre-diabetes, and T2D Blood Test Parameters( )

Blood Test	Normal	Pre-diabetes	T2D
FPG	< 6.1 mmol/L	6.1 - 6.9 mmol/L	≥ 7.0 mmol/L
HbA1C	< 6.0%	6.0% - 6.4%	≥ 6.5%
OGTT	< 7. mmol/L	7. - 11.0 mmol/L	≥ 11.1 mmol/L
PG	Unclear, further testing at health professional's discretion if close to T2D cutoff		≥ 11.1 mmol/L

Epigenetic changes (changes to gene e pression that do not involve DNA changes) appear to be at pla in T2D risk; while there is a genetic component to T2D etiolog , loci variation can onl e plain 10 – 15% of the hereditar component (20,51). The majorit of the appro imatel 100 loci that have been identified in influencing T2D development alter pancreatic islet function and not the insulin signaling pathwa (52). This is supported b e perimentation as meth lation of genes involved in pancreatic islet function in both clinical and animal studies results in either reduced or silenced e pression, hindering β-cell function (53–56) .

β-cell and α-cell mass are regulated in balance together through adolescence into adulthood and e ternal factors such as inadequate protein or caloric intake have been found to impair β-cell differentiation (57). This is notabl seen if malnutrition occurs during pregnanc resulting in reduced birth weight, as this can lead to permanent changes in pancreatic development and predispose an adult to T2D (5). This appears to be potentiall independent of weight later on in life as BMI was found to not be protective for T2D risk as for black women born with a low birth weight (<1500 g) (59). While a low birth weight is strongl associated with T2D risk

malnutrition during adolescence also appears to increase T2D risk as, short leg length, a marker

Perhaps the most striking evidence for lifest le epigenetic changes is in population groups, such as the Kitava of Papua New Guinea or the Xingu people of Brazil that, predominantl , live outside of a modern lifest le. These hunter-gather and horticulturist populations, respectivel , have significantl lower fasting insulin levels and improved insulin sensitivit at an older age compared to populations that follow a modern lifest le (69,70)

is impaired, it has been shown that a diet containing as much as 10% of total calories from sugar ma not negativel affect HbA1c in people with normogl cemia (74,75).

Allostatic load (the wear and tear on the bod caused b homeostatic adaptation processes in response to stress caused b a m riad of sources) results in the progression of beneficial short-term stress adaptation processes, such as adrenalin and cortisol secretion, becoming harmful as short-term stress adaptation processes become long-term (76–7). While intermediar outcomes such as h pertension, obesit , prediabetes, and blood lipid levels are all significant risk factors for developing T2D, the are impacted b various risk factors or e posure, which are themselves impacted b various environmental factors (79). Health services, ph sical activit resources, the safet -violence paradigm, access to amenities (e.g. grocer stores and recreation centres), walkabilit , urban sprawl, neighborhood condition, public transport access, and amount of green space are all environmental determinants that modulate risk factors or e posures based upon availabilit and/or access (79). These environmental determinants in turn ma result in risk factors such as: ph sical inactivit , unhealth diets, increased stress, inadequate sleep, fear, and isolation, and e posures such as: air pollution, noise pollution, and e cessive traffic (79). Current recommendations for minimizing risk and treating T2D focus primaril upon modif ing intermediar outcomes (h pertension, obesit , prediabetes, and blood lipid levels) through encouraging positive diet changes and increased ph sical activit (0). While these changes are certainl beneficial it should be recognized that the inherent individualistic nature of the western model of health ma be more effective at reducing

incidence of T2D though treating root causes (i.e. environmental determinants) through a social model of health (1).

Diabetes is becoming an increasingl global concern as 75% of people living with diabetes live in low- and middle-income countries (LMIC) (2). Estimates for individual t pes of diabetes do not e ist, however 90% of cases of diabetes are believed to be T2D (49). Data from 111 countries places the estimated persons living with diabetes globall at 415 million in 2015; with this e pected to increase to 642 million b 2040 (2). A larger stud pooling 751 studies and over 4 million adults from 146 countries found age-standardized diabetes prevalence rates to have increased from 4.3% in men and 5.0% in women 19 0 to 9.0% in men and 7.9% in women in 2014 (3). This group determined that the number of adults with diabetes globall has risen from 10 million in 19 0 to 422 million in 2014 due to a rise in prevalence, a rise in population growth, and a rise in life e pectanc (49, 3). Globall age-standardized diabetes prevalence in adults has either remained the same or worsened in ever countr , with the burden especiall affecting LMIC (3). While in Western Europe incidence has remained fairl consistent and prevalence has increased due to population growth and increased life e pectanc , in lower income countries both prevalence and incidence have increased with age-standardized prevalence in adult American Samoans being the highest at over 30% (3).

Diabetes resulted in a global 1.5 million deaths in 2012 and uncontrolled blood glucose resulted in another 2.2 million deaths through increased disease risk, primaril cardiovascular (49). Over 40% of these deaths occurred before the age of 70, with LMIC having the highest rates of diabetes death before the age of 70 (4). This is caused b a m riad of factors, and treatment

ma be directl impacted b the wide variation in cost for treating diabetes b countr and area-within-countr (4, 5).

While inadequate data makes historical comparisons difficult the prevalence of diabetes in Canada has been slowl but steadil increasing in Canada since at least the 1990s with 3.4% of Canadians 1 ears or older having T2D in 1994 compared to 4.5% in the 2000 and 7.5% in 2011 (6). While this can partiall be e plained b a diagnostic change in the FPG cut-off point for diabetes diagnosis from 7. mmol/L to 7.0 mmol/L and b increased testing as awareness of T2D increases(7) T2D prevalence continues on an upwards trend. In 2013 an estimated 3.0 million Canadians (.1%) lived with diagnosed diabetes (all t pes) (3). In 2016, 7.0% (~2.1 million) of Canadians 12 ears or older had diabetes compared to 7.3% (~2.3 million) in 2017 (, 9). Projections made in 2012 estimated 3.7 million Canadians would have diabetes b the 2019 (90). These figures are for onl diagnosed cases and do not account for the undiagnosed cases of diabetes, which were estimated to be 450,000 in the 2009 (90).

While the prevalence of diabetes has been increasing, age-standardized incidence has marginall decreased from 6.7 per 1000 in the 2004 ear to 6.3 in the 2014 ear (90). Reduced earl mortalit in people living with diabetes has resulted in greater morbidit ; in 2010 over 35% of Canadian adults with diabetes had two additional chronic conditions, and those 20-49 ears old saw a ph sician twice as often as those without diabetes (90,91). While diabetes itself does not result in death, it increases the risk of other diseases, especiall cardiovascular disease, as ~70% of all people with T2D will die from cardiovascular disease (90,92). These

complications and their corresponding increased medical s stem requirements is estimated to result in a cost of $15.36 billion over the 10- ear period of 2012 to 2022 (93).

T2D is a multifaceted problem and needs a multifaceted care plan including lifest le interventions and medications, however, the development of new nutritional interventions using food ingredients offers a novel wa to reduce postprandial gl caemia and ma reduce the economic burden of diabetes on societ .

2.7.1 Gluc s Tr nsp r t t n

Glucose transport proteins (GLUT) transport glucose into insulin-sensitive cells (94). GLUT1 and GLUT3 are insulin-independent, while GLUT 4 is insulin-dependent (94). GLUT2 is e pressed in β-cells and liver cells and serves as a transporter for glucose and also acts as a plasma glucose concentration sensor (95). As plasma glucose rises uptake b GLUT2 increases resulting in greater secretion of insulin (96). GLUT3 is mainl found in nerve tissue, especiall the brain (95). GLUT4 is mainl found inside muscle and adipose tissue and in the presence of insulin translocates to the cell membrane resulting in the uptake of glucose (94). Insulin resistance ma potentiall be caused b errors in GLUT4 e pression.

GLUT1 mediated glucose transportation results in glucose phosphor lation b he okinase I, while GLUT4 mediated glucose transportation results in glucose phosphor lation b he okinase II (97). He okinase I phosphor lation results in a glucose 6-phosphate that can be used in the he osamine pathwa , which negativel affects GLUT4, resulting in decreased insulin-dependent glucose transport (94). While both GLUT1 and GLUT4 are necessar for proper glucose control, overe pression of GLUT1 or reduced e pression of GLUT4 can both reduce insulin sensitivit

resulting in a greater risk of h pergl cemia (9). This has been observed in obese and T2D patients who have reduced GLUT4 mediated transportation (9). It appears that GLUT4 e pression is mainl decreased in adipose tissue, possibl from o idative stress, which ma be a triggering point for the development of insulin resistance in obese individuals (99,100).

2.7.2 Insul n s or t n

Insulin undergoes several stages of modification to arrive in its final form starting with cleavage of preproinsulin to proinsulin inside of β-cells (101). Proinsulin is composed of a 21 amino acid A chain, a 30 amino acid B chain, and a 30-35 amino acid C chain (102). Proinsulin is transported to the Golgi Apparatus where cleavage of the C chain results in the A and B chain forming the dipeptide hormone, insulin, and the C chain forming c-peptide, which retains bioactivit ; insulin and c-peptide are released in equimolar concentrations into the blood stream from the β-cell (101). This equimolar secretion serves as a basis for e amining the effects of various nutrients on hepatic insulin clearance, as ingestions of different foods ma result in similar insulin secretion amounts but vastl different insulin concentrations due to altered insulin clearance (103) This can result in much higher levels of insulin being present in the blood without increased insulin secretion and a greater blood glucose attenuation effect as has been observed in milk dair products (103–105).

2.7.3 Insul n c ntr l

Insulin control mainl depends upon the content of circulating glucose, amino acids, and fatt acids, as the are able to stimulate insulin secretion, however insulin secretion is also regulated b a wide variet of non-nutrient controllers such as glucagon, somatostatin, acet lcholine, and

dopamine (101). Nutrient and non-nutrient controllers work both s nergisticall and antagonisticall in a ver complicated set of interactions that are not full understood to effectivel control insulin secretion (101). β-cells abilit to regulate insulin secretion is critical in both an e cess and deficienc of nutrients to prevent both h pergl cemia and h pergl cemia, and this comple process, while centralized around glucose is a finel balanced act of control among the millions of β-cells inside the millions of islets of Langerhans (106)

Insulin clearance is an important part of insulin control and predominatel occurs in the liver, though the kidne s and muscle also contribute, where receptor-mediated uptake of insulin into hepatoc tes for degradation occurs. Insulin has a short half-life (4-6 minutes) appropriate for quick action in regulating blood glucose (107,10). Interestingl milk-based snacks appear to reduce hepatic insulin e traction resulting in a longer-half life and greater reduction in blood glucose than non-milk based snacks (103,109). $

2.8.1 Dietary Carbohydrates

In the presence of d sfunctional glucose regulation carboh drates that are absorbed at a slower rate are preferred due to a reduction in postprandial gl cemic fluctuation through reduced insulin demands (110). The quantit of digestible carboh drate a food contains is affected b various factors, including processing, ph sical form, fiber content, gelatinization, and fat content, as these can all influence how effectivel and/or quickl the carboh drate is absorbed (110). This is further affected b the degree of comple it of the carboh drate; mono- and di-saccharides are absorbed more quickl than more complicated oligosaccharides or pol saccharides, and what other nutrients are consume with the carboh drate (110).

This interaction between different t pes of carboh drates and gl cemic response can be described b the gl cemic inde , which is a comparison model for the gl cemic response induced b consumption of a certain food compared to that caused b the consumption of white bread or pure glucose (111,112). Meta-anal ses have found that in adults a low gl cemic inde diet ma have beneficial effects on HbA1c and lower incidence of h pogl cemic episodes, and ma have beneficial effects on insulin sensitivit in children (113,114).

The standard method for measuring the gl cemic inde of a food is to feed 10 or more health people 50 grams of digestible carboh drate of that food and measuring blood glucose over two hours (115). The same group of people must also perform a reference test where the will perform the same test but with 50 grams of glucose (115). Gl cemic inde is then calculated b dividing the blood glucose response for the test food b the reference food (115). However, the gl cemic inde b itself ma paint a misleading picture of what gl cemic response ma be e pected as the t pical amount of available carboh drate in a source of food ma be nowhere near 50 g. This can be seen in kiwis, which, while considered a medium-high gl cemic inde would require ingestion of ~10 kiwis to meet 50g, which is an e tremel unrealistic food portion (116,117). A potentiall more relevant measure of the e pected gl cemic response based upon a food's gl cemic inde is the gl cemic load (Table 2.2), which is determined b multipl ing the food's gl cemic inde b the amount of carboh drate this food contains and dividing b 100. This gives a more accurate representation of the gl cemic response a food can be e pected to have as foods can have a high gl cemic inde , but onl contain a small amount of available carboh drate such as in kiwis (111,116).

Table 2.2. E amples of Foods and their Gl cemic Load (11)

Low Gl cemic Load	Medium Gl cemic Load	High Gl cemic Load
Apple	Brown Rice	White Rice
Kiwi	Whole Grain Bread	White-flour pasta
Black Beans	Oatmeal	Sugar-sweetened beverages
Lentils	Pearled Barle	French Fries
Skim Milk	Bulgur	Baked Potato

2.8.2 D t ry F t

Dietar fat is strongl correlated with insulin resistance in both animals and humans and the most popular model for stud ing insulin resistance and/or T2D in animals is a high-fat diet (34,119,120). However, the harmful pro-inflammator effect of fats is dependent upon the t pe of fat as depending upon saturation level fats ma be harmful or beneficial (119,120). The evidence for saturated fat contributing to insulin r

for the rise of T2D in Canada. While the role obesit pla s in T2D is well founded and fat intake ma be a potential trigger leading to insulin resistance it does not appear to do so in a vacuum as e ercise or more importantl the lack of pla s a role as there is evidence for e ercise being protective of insulin sensitivit in a "Western St le" high fat diet (124–126).

While monosaturated fats appear to be more protective of insulin sensitivit than saturated fats results are equivocal as studies have found monosaturated fat intake to be more protective of insulin sensitivit but also to be associated with insulin resistance, though this ma potentiall be attributed to obesit complications and/or high total dietar fat content (120,127).

Pol unsaturated fats fall into two categories: omega-6 and omega-3; while omega-

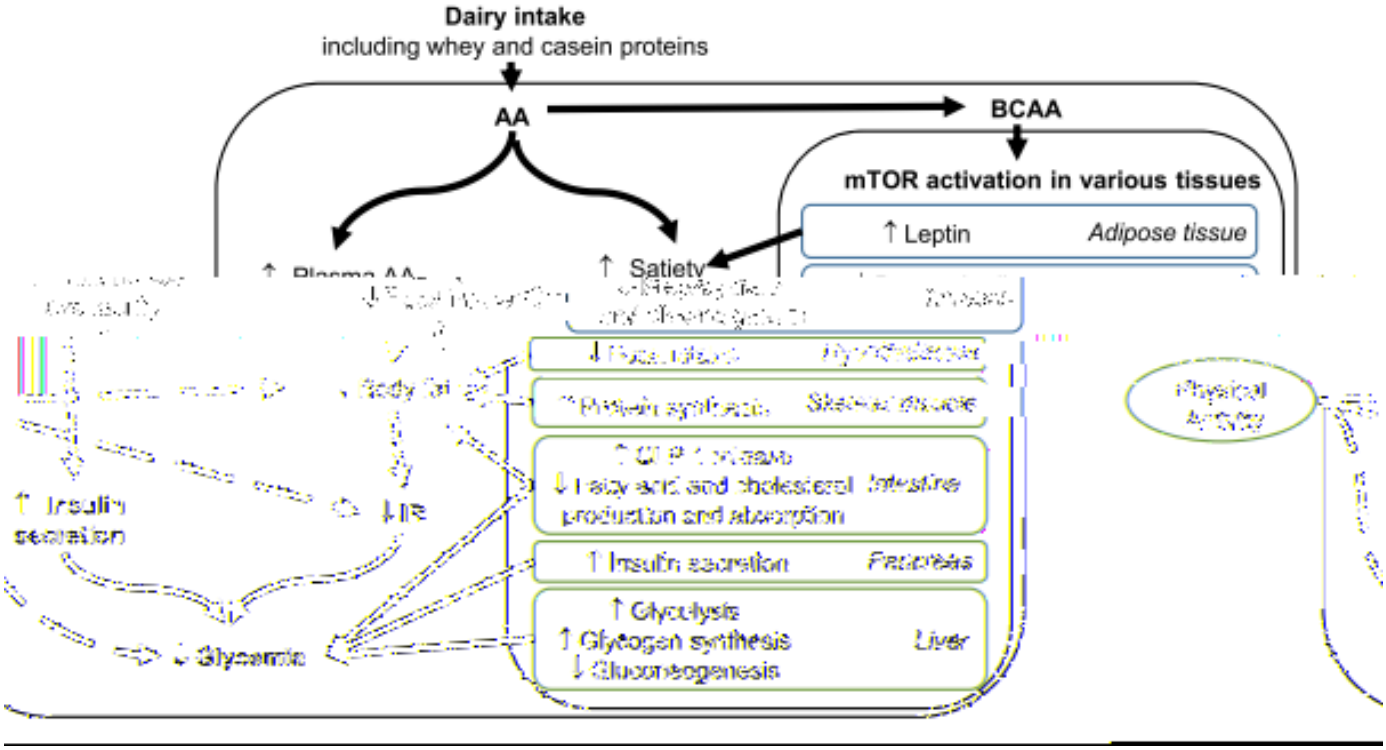
have a beneficial effect on blood glucose, but ver high protein diets over a longer period of time, especiall diets high in animal protein, appear to increase risk of T2D development (133–135). This parado also appears to remain consistent for individual amino acids as the branched-chain amino acids (BCAA) valine, leucine, and isoleucine appear to be effective in attenuating blood glucose response in the short-term, but diets high in BCAAs ma cause increased risk of T2D long-term (136,137). The e act reason for this is unclear as dietar protein acts as an insulin secretagogue aiding insulin secretion and helping reduce the blood glucose response after carboh drate ingestion (137). While once deaminated amino acids are available for gluconeogenesis it appears that onl a ver small portion (>10%) end up as circulating glucose and thus unlikel to significantl contribute to an increase in blood glucose (13). One possible reason for the potential harm of chronic high protein intake is the association of it with the "Western St le Diet", which along with an overall poorer qualit diet is often associated with limited e ercise, however there does appear to be a possible causal link with a high fat high animal protein diet as in the face of insulin resistance and/or a high fat diet protein catabolism is impaired (34,139,140).

While long-term high animal protein diets appear to be linked to increased T2D risk the e act length of time and mechanism that contributes to an increased risk in unclear(141). Shorter length high protein diets and even individual high protein meals appear to attenuate blood glucose response compared to non-high protein alternatives (142–144). To further complicate matters high-protein diets when combined with weight loss have been found to reduce HbA1C b 0.5%, which is comparable to medication (142,144). However, whether the beneficial gl cemic effects are due to protein or weight loss is unclear as normal protein but energ

restricted diets with weight loss have found similar effects (145). High protein meals also seem to attenuate blood glucose response, however whether this is best done as a premeal or during a meal is unclear, and which protein source and amount is unclear (142,146–14).

While protein has been found to simulate insulin secretion BCAAs have also been found to stimulate insulin secretion without glucose intake being required (149). While the e act mechanisms remain to be discovered it is believed that a possible mechanism is b depolarizing calcium channels resulting in an influ of calcium initiating insulin secretion (101). BCAAs have also been found to promote GLP-1 secretion aiding insulin secretion (150). The main mechanism appears to be activation of the mechanistic target of rapam cin (mTOR) which contributes to a wide range of beneficial glucose regulating effects (Figure 2.1). However chronic activation of mTOR, which chronic levels of high BCAAs can do is predictive of insulin resistance, and thus there appears to be a fine line between BCAAs being beneficial and harmful (151–153). This points towards the use of BCAAs use as a supplemental food for improving blood glucose regulation rather than promoting a diet high in BCAAs. The use of milk proteins meets this criteria due to their high BCAA content and their abilit to attenuate blood glucose is well studied and shows promising results (103,146,154).

Figure 2. 2 Summar of the Processes of Dair Intake's Improved Blood Glucose Regulation (155)



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Bovine milk intake is positivel correlated with improved gl cemic control (156–159). This improvement is seen in adults, children, and with the use of both high-fat and low-fat dair products (156–159). A stud b Gheller et al., comparing dair and non-dair snacks matched for carboh drates (25 g) found the dair snack to significantl attenuate blood glucose response in children with lower blood glucose ($p<0.01$), increased insulin ($p<0.0001$), however no difference in C-peptide ($p=0.54$) was observed, which can be e plained b reduced hepatic insulin clearance b the dair snack ($p<0.01$) (103). A possible component of dair that improves gl cemic control is its unique proteins: whe and casein (160). Whe (0%) and casein (20%) are complete proteins with high contents of BCAAs (23% and 20% respectivel) (161).

Whe and casein are also characterized as fast and slow respectivel due to their rate of digestion (157). This has shown to increase satiet and improve gl caemic response, possibl due to a longer period of BCAA absorption (103,109). However, while casein's slower gastric empt ing appears to be more effective in reducing food intake and increasing satiet whe appears to be more pronounced at improving gl cemic response (162,163) a much lower blood glucose response (103).

Table 2.3. Bovine milk composition (164)

Composition (g/100 g)						
Water	Fat	Casein	Whe Protein	Lactose	Ash	Energ (kcal/100g)
7.3	3.9	2.6	0.6	4.6	0.7	66

2.1 .1 h y Pr t ns

Whe proteins are the proteins found in the whe portion after casein precipitation at pH 4.6 and 20° C has taken place (165). Precipitation does not result in a pure whe fraction as casein derived proteins such as gl comacropeptide (GMP) are present. Whe can further be broken down into sub-protein fractions including, but not limited to: beta-lactoglobulin, alpha-lactalbumin, lactoferrin, immunoglobulins, and gl comacropeptide(165). It is currentl thought that whe proteins are more likel to attenuate the blood glucose response compared to casein proteins (5). This has been shown through both insulin-dependent and insulin-independent

action (166). Through mechanisms et to be full understood whe appears to temporaril decrease hepatic insulin clearance resulting in improved insulin efficienc (103,166,167). Whe has also been found to increase GLP-1 and PPY secretion which inhibit gut motilit , slow gastric empt ing, and enhance glucose disposal (166,16 –171).

2.1 .2 B v n h y Pr t n Fr ct ns

Alph -l ct l um n (-LA) Alpha-lactalbumin composes about 25% of the total content of bovine whe protein and is routinel added to infant formula to create a nutrient profile closer to that of human milk (172). This 123 amino acid residue is composed of 22% BCAAs (165).

B t -l ct l ul n (β-LG) Beta-lactoglobulin represents about 50% of the total content of bovine whe protein and is a major contributor of the functional properties of whe protein, such as water binding (165,172). This 152 amino acid residue protein is composed of 25.3% BCAAs and has been found to aid in the immune response and promotion of cell proliferation (173).

Immun l ul ns (I G1, I G2, I A, n I M) Immunoglobulins show anti-microbial and anti-viral activit (172,174). E tensive research has been conducted in determining their use in promoting gastrointestinal results with promising results as both infection prevention and s mptom reduction has been seen (174)

Glyc m σ p pt (GMP) Gl comacropeptide is formed when k-casein is cleaved during cheese making para-k-casein and Caseinmacropeptide (CMP) (175,176). When CMP is gl cos lated it becomes GMP. Appro imatel 60% of CMP Is gl colsated into GMP with O-linked gl cans, which are influenced b stage of lactation and which of the 16 phenot pes of k-casein are present (176).

GMP is the onl naturall occurring protein that contains no phen lalanine and has been researched for use in the management of phen lketonuria with commercial products now available for use (177). GMP has 64 amino acid residues with ~23% of them being BCAAs (17). GMP appears to be effective at improving satiet and attenuating blood glucose (105,179,179).

L ct f rr n (LF) Lactoferrin is responsible for a wide range of health benefits. It serves as a non-heme iron binder and aids in iron absorption and transport. It also as anti-microbial and anti-viral roles and also appears to stimulate growth of beneficial bacteria in the intestinal tract (172). Lactoferrin consists of 6 9 amino acid residues with 1 .6% of them being BCAAs (165).

2.1 .3 C s n Pr t n Fr ct ns

Caseins compose the majorit of milk proteins in cow's milk (~ 0%) with four major t pes of casein. αs1-, αs2-, β-, and κ-casein being present (165). Caseins abilit to clot in the stomach and slow down digestion has been to improve satiet , but also to attenuate blood glucose (1 0,1 1). However, Caseins slower release of amino acids results in a less pronounced insulinotropic effect compared to whe (1 2).

Whe proteins appear to influence blood glucose regulation in a m riad of wa s, including dela ed gastric empt ing, and inducing insulinotropic effects, and incretin hormone stimulating effects (5). A variet of trials have e plored the potential of whe protein to improve gl cemic control, however wide ranges in dosing (4.5 to 90 grams), the form of protein administered, and time of treatment makes comparison between studies difficult (5). It appears that the amino acid profile of whe protein, which is higher in BCAAs ma be the reason wh whe

protein appears to be more effective at attenuating blood glucose response, though this does not completel e plain observed results (1 3). Whe protein b itself appears to be better at regulating blood glucose than other forms of dair , though overall increased intake of dair benefits blood glucose regulation (1 4). It has been found that whe results in a significantl lower glucose response in combination with a high-fat meal than casein does, though no differences in insulin, glucagon, or incretins were observed (163).

The potential application of whe protein use aiding blood glucose control is an attractive possibilit to deal with an increasingl dangerous problem. The risk diabetes poses to Canada is not disappearing, and it is quite likel that the risk will continue to increase not onl in Canada but globall . The development of novel treatment options needs to be completed in a timel manner to aid in risk reduction, and this work is a small step towards this.

While the term ‘’functional food” is gaining in popularit it is not a regulator term and has no agreed upon definition (1 5). The Academ of Nutrition and Dietetics describes functional foods as, “... whole foods and fortified, enriched, or enhanced foods having a potentiall beneficial effect on health when consumed as part of a varied diet on a regular basis, at effective levels.” (1 6). Dietitians of Canada describes functional foods as, “[… foods that offer unique health benefits that go be ond simpl meeting basic nutrient needs.”(1 5). These broad definitions are just a few amongst a m riad different governments and researchers have put forward, but overall the encompass the three central aspects of functional foods: nutritional function (must be a complete food and not just a specific nutrient), specific health benefits, and technological process (original food has been altered in some form) (1 7).

While functional food does not have a set definition it can be broken down into two groups: (1) foods with added bioactives and (2) foods with enhanced bioactives (1 5). Foods with added bioactives are food products that do not naturall contain the bioactive ingredient (e.g., orange juice with calcium, margarine with ph tosterols, or salt with thiamine. Foods with enhanced bioactives are food products that have been altered to have an increased level of a bioactive naturall present in the product (e.g. ogurt with increased probiotics or a flour mi with increased fibre from pulse inclusion).

The Canadian Food Inspection Agenc has mandator fortification and enrichment requirements creating functional foods (e.g. salt with iodine, milk with vitamin D, fruit juice with vitamin C, etc.) there are also a wide range of voluntar fortification and enrichment processes that are allowed (e.g. adding fluorine to bottled water) (1 5,1 7,1). While this process mainl on focuses upon adding single nutrients various health various claims (nutrient content, nutrient function, therapeutic claims, and disease risk reduction) can also be made based upon ingredients and the amount present in food (1 9). Regulations regarding claims are controlled b the Food and Drugs Act, and while there are usuall no specified regulations all claims are subject to Subsection 5(1) the must not be false, misleading, or deceptive (1 9). Nutrient content claims indicate the presence or amount of a nutrient but provide no specific information on benefits (e.g. “E cellent source of vitamin C”, “Ver high source of fibre”, “Low sodium” (1 9). Therapeutic claims provide a link between a substance within the food and a ph siological benefit it provides (e.g. “Coarse wheat bran and improved bowel function”) (1 9). Nutrient function claims are a subset of function claims and refer to an individual nutrient within a food (e.g. “Protein helps build and repair bod tissues” or “Calcium aids in the formation and maintenance of bones and

teeth”(1 9). A claim about improved gl caemic response can be made if the addition of ingredients enables “[A serving of stated size] of [name of product X] contains n grams of [name of ingredient Y]. This ingredient reduces the gl caemic response to this food” (190). In order for a food to make a gl caemic response claim it must be able to do so in a health individual in an acute dose (i.e. single dose) (190).

While Health Canada has regulations for the use of different claims there is little standardization and health claims ma impart a “halo effect” on consumers as products with health claims ma be viewed overall as being “healthier” than products without health claims (191).

This lack of standardized criteria for different claims or for simplified nutritional or general health messages on the front of food packages can be confusing for consumers. Similarl , the absence of core eligibilit criteria for disease risk-reduction and function claims has led to concerns that consumers ma be drawn onl to the claimed or highlighted product benefit, ignoring other potentiall less health aspects (13–17).

Functional foods are created from a wide variet of different foods and ingredients the use of bovine milk and/or its composites (e.g., whe protein) are some of the most popular choices (192). The use of bovine milk in the creation of infant formula represents one of its largest usages as the infant formula global market was valued at $50 billion USD in 2019 (193,194). The usage of whe protein is widespread as it has uses in both supplements and food products. Whe protein is commonl used in sports nutrition as a major component in whe -based protein powder; a global industr worth $10 billion USD in 201 (195). While the use of whe protein is widespread in food products two of its more popular uses is in “energ bars” and meal-

replacement beverages (196,197). High-protein milk beverages with added whe protein targeted at weightlifters can also be found on the market with more than twice the protein content (~20 grams-2 vs 9 grams per 250 ml) of comparable fluid bovine milk (19 ,199).

Rats and mice have been e tensivel used to stud diabetes and gl cemic control (200,201). This is possible due to humans and rats and mice having gastrointestinal s stems and gl cemic control hormones that are homologous in their function (200–202). A variet of models for stud ing diabetes and gl cemic control have been developed with major efforts taking place over the last 30 ears (200). Models for T1D have been developed using chemical induction, spontaneous autoimmune induction, genetic induction, and viral induction (200,203–206). Models for T2D have been developed using obesit -induced h pergl cemia, genetic β-cell d sfunction, or breeding to develop poor glucose tolerance (200,201,207,20).

Major milk proteins have been e tensivel studied in rats though data on whe and casein fractions is limited (209–214). In a stud b Gregersen et al., e amining diet supplementation of T2D rats and normogl cemic rats using whe , casein, and α-lactalbumin found all protein treatments attenuated the blood glucose response with a greater affect seen in T2D rats (209). A stud b Pezeshki et al., looked at the effects of casein, whe , and a combination of the two in diet-induced T2D rats for eight weeks found a reduction in food intake and blood glucose response compared to the control group, with whe showing the greatest effects (213). Interestingl whe also resulted in greater GLP-1 e pression strengthening the idea that whe proteins abilit to attenuate blood glucose is through both insulin-dependent and insulin-independent methods (213,215). The combination of whe and casein also resulted in a 170%

increase in muscle plasma membrane GLUT4, providing another possible reason for the improved blood glucose control seen with milk protein intake outside of increased stimulation of incretin and insulin secretion (213). Further support for whe containing unique properties contributing to glucose attenuation was found in an e periment b Aziz et al., comparing intact whe and comparable amino acid mi tures in health Wistar rats with whe proteins showing greater attenuation than the amino acid mi ture (212). Conclusions were drawn that bioactive peptides are formed during the digestion of whe protein that offer unique health benefits, which is supported elsewhere (1 4,209,212). A stud b Moura et al., stud looked at this closer b using a combination of BCAAs dipeptides found in whe compared to whe in Wistar rats (209). Evidence was found showing that some of potential benefits of whe post-e ercise ma be contributed to bioactive peptides found in whe and not onl in BCAA dipeptides (209). The use of milk proteins has shown positive results in rats in both T2D and normogl cemic animals. While e tensive research has been conducted on whole whe and whole casein ver little has been conducted on whe and casein sub-fractions, and e ploration ma highlight specific parts of whe and casein that are more beneficial for gl cemic regulation.

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Chapter 3: Rationale, Objectives, and Hypothesis

Diabetes is a growing global threat to health. Current treatment options have proven ineffective in substantiall reducing the incidence or prevalence of diabetes and novel treatment options are needed. There is a growing bod of work showing robust evidence that milk proteins ma be an effective method to aid in reducing loss of gl cemic control. The full e tent of how this is accomplished is unclear and investigating the individual fractions of whe ma offer greater insight into developing effective food products.

The objective of this stud is to investigate the acute effects of consumption of whe protein isolate, whe protein fractions (β-LG, α-LA, and GMP), and glucose in a water-based fluid matri on blood glucose and insulin in Wistar Han rats.

There will be a significant difference in blood glucose and insulin responses ($p<0.05$) between the treatments and between the treatments and glucose control over 120 minutes.

Chapter 4: Methodology

A randomized repeated-measures design was used to determine the acute effects of commercial whe protein isolate, GMP, α-LA, β-LG, and glucose in a water-based fluid matri in twelve Wistar-Han rats. Treatment order randomization (Appendi C) was done using software from random.org (eCOGRA certified). Ethics approval was obtained from the Dalhousie Universit Committee on Laborator Animals (Protocol #19-022).

Animals were split into four groups of three (Group 1, Group 2, Group 3, Group 4). After the acclimatization period on da eight Group 1 and Group 2 received their treatments and were then allowed to rest until da three. On da two Group 3 and Group 4 received treatments and were allowed to rest until da four. All groups had had 6-hour da light fasts (Figure 4.1) but were offset b 3 hours to allow for sufficient time for data collection. While 12-hour fasts are more common there is an increasing bod of work showing that 6-hr fasts can be appropriate (201,216,217). A 6-hour da light fast was decided upon based upon literature and a discussion with the CACF veterinarian, Dr. Sue Pearce, who e pressed concern over a 12-hour fast with onl a 4 -hour rest period.

Figure 4.1 1-Da 1-Group Data Collection Visual $

A sample size of n=7 Wistar Rats was determined to be sufficient to determine a 15% difference based upon an α = 0.05 and β = 0.0 . Previous studies also show this to be an appropriate number of animals for this t pe of stud (21 –220). Attrition rates are unknown for this methodolog , so twelve Wister-Han rats were ordered from Charles River Laboratories (CRL) to account for possible attrition (Wilmington, Massachusetts, USA). The Wistar-Han rat is a general multipurpose model suitable for this t pe of e perimentation (221). Rats lwa on

minimal discomfort, and ph sical restraint is kept to a minimum. Upon the arrival the animals were be fitted with a vascular access button cap (VABC) to prevent the VAB from being damaged. Animals had their tails marked for identification purposes and were housed as d ads at 20-22 C on a 12-hour dark/light c cle at the Carlton Animal Care Facilit (CACF) at Dalhousie Universit . The abilit to house catheterized rats as d ads is a relativel novel concept enabled using the VAB fitted with a VABRC. T picall , catheterized rats must be housed individuall which increases the stress of the animal as rats are ver social (222–224). The animals were fed standard rodent chow (Purina, St. Louis, MO, USA), and allowed seven da s to acclimatize before e perimentation began. During this acclimatization period the animals were monitored and pla ed with dail to habituate them to human handling. Pla ful handling, such as tickling, has been found to reduce rats fear of humans, which results in better animal welfare, easier handling, and a lower stress response to gavaging (223,225,226). Catheter maintenance was performed according to CRL instructions, with animal's having their catheters routinel flushed to aid in prolonging catheter patenc (227). Catheters were flushed b removing the locking solution (500 IU/ml in 50% gl cerol) (SAI Infusion Technologies, Lake Villa, IL, USA) using a sterile 1 mL Leur slip tip s ringe (BD, Franklin Lakes, NJ, USA) attached to a PinPort Injector (PNP3M) (Instech Laboratories, Pl mouth Meeting, PA, USA). The PNP3m is required to access the VAB and allows for eas withdrawal and injection and improves the duration of catheter patenc ; an access to the VAB requires the use of a PinPort Injector. Once the locking solution was removed the catheter was flushed with twice the catheter dead volume of sterile saline (~100 uL) and then locked with the a volume equal to the catheter dead volume (~50 uL) of locking solution (227). This procedure was done when the animals arrived and at Da 3 and Da 6 of the acclimatization period.

While a wide range of different milk protein doses have been studied a 10 g human dose has been found to be effective in significantl attenuating blood glucose from a single intake (154,215,220). This dosage is in alignment with Health Canada's "Draft guidance Document on Food Health Claims Related to the Reduction in Post-Prandial Gl caemic Response", which requires the serving size to be proportional to the intended intake method (i.e., as a snack or meal) (190). Solutions based upon a 10% wt/vol were tested to ensure suitabilit for gavaging. There currentl e ists an % wt/vol milk protein drink and thus there is alread a commerciall available comparable basis for this (22).

The 10 g dosage was converted to an animal equivalent dose (AED) b using allometric scaling. This was done b using a correction factor (k) which is an estimate of the average bod weight (kg) of a species divided b its average bod surface area (m) (229). The average bod weight of a human is given as 60 kg, the k value is 7, and the bod weight of the rats is 0.3 kg (229). Thus, the AED for the rats used in this stud is:

AED = Human does (mg / kg) × K ratio

AED = ((10 g * 1,000 mg/g) / 60 kg) 7

AED = 1,166.67 mg/kg

AED = 1,166.67 mg/kg *0.3 kg

AED = 350 mg

Treatments were prepared at the Centre of Applied Research at Mount Saint Vincent Universit . In addition to the milk proteins (Table 4.1) a dose of 350 mg of glucose will also be used to compare the magnitude of effect blood glucose response and insulin action. Further e periments are aimed to e plore this relationship in closer detail once a basis is established. This is appro imatel half of what an oral glucose tolerance test is in rodents (2g/kg) and is well tolerated (166). Treatments were prepared using purified water at 32 C to ensure treatments were properl dissolved/suspended. To ensure the treatments are not too viscous to flow through the gavaging needle each dose was dissolved/suspended in 3 mL of water and then drawn up into a 3mL BD Luer-Lok Tip S ringe (BD, Franklin Lakes, NJ, USA) creating a 10% wt/vol solution, which were then frozen until use where the be thawed and brought to room temperature before the were administered. This volume is safe for gavaging and poses no risk to the animals (230).

Table 4.1 Treatment Information

Treatment	Manufacturer	Product Information	Dosage Calculation
Whe Protein Isolate	Agropur Dair Cooperative (Saint-Hubert, QC, Canada)	BiPRO 9500 Whe Protein Isolate	7% As Is Protein 350/ 7 = 402.3 mg
Gl comacropeptide	Agropur Dair Cooperative (Saint-Hubert, QC, Canada)	BiPRO GMP 9000	93.6% As Is Protein 350/.936 = 373.93
α-lactalbumin	Agropur Dair Cooperative (Saint-Hubert, QC, Canada)	BiPRO Alpha 9000 Alpha-Lactalbumin	92.5% As Is Protein 350/.925 = 37 .4 mg
β-lactoglobulin	MilliporeSigma (St. Louis, MO, USA)	B-lactoglobulin from bovine milk >99%	99% As Is Protein 350/.99 = 353.5 mg
Glucose	MilliporeSigma (St. Louis, MO, USA)	De trose	100% As Is Glucose 350/1 = 350 mg

Treatments were administered b oral gavage immediatel after blood was collected at time point 0. While alternative oral feeding methods have been developed their protocols require the use of a sweetening agent, which would impact blood glucose, and are imprecise in the dosage administered (231–233). While gavaging does pose risks several steps were taken to minimize this. The use of a disposable plastic gavaging needle (FTP-15-7 , Instech Laboratories, Inc., PA,

USA) not onl reduces the risk of esophagus perforation but also reduces stress levels which ma occur with repeated gavaging (234,234–237). The use of a 3mL dose has been found to be both appropriate for testing and safe (230,23). Repeated gavaging with metal gavaging needles has been found to potentiall cause weight loss; animals were weighed the animal at each session (234). Finall , the most important wa to reduce gavaging-related risks is technician proficienc ; supervised training was undergone to ensure gavaging techniques are safe and satisfactor (237,23).

The normal method of gavaging a rat is to hold the animal b the scruff of the neck and to insert the gavage needle in a vertical fashion, however the placement of the VAB prevents this, and the animal was wrapped in a towel to hinder mobilit and gavaged horizontall (239). To ensure the gavage needle was placed properl it was measured before gavaging (239).

Blood was

Table 4.2 Blood collection timing and volume

Collection Time Point	Blood Glucose (uL)	Insulin (uL)	Total Amount Collected (uL)
0	10	0	90
15	10	0	90
30	10	0	90
60	10		10
90	10		10
120	10		10

The use of a VAB allows for repeated blood collection without the use of anesthesia as blood collection is pain free. This is a unique blood collection method in rodents and allows for a smaller number of animals to be used with comparisons to be made within the same subject. To collect blood the VABC was removed and the VAB was cleaned with an alcohol pad. A PNP3M fitted to a 1 mL BD Luer-Lok Tip S ringe (BD, Franklin Lakes, NJ, USA) was used to unplug the catheter b drawing up the locking solution in the catheter until blood first appeared in the s ringe. If the first attempt failed, the animal was repositioned as it is possible the catheter was crimped or

slightl blocked. If this fails a small volume of saline (5-10 uL) using a sterile PNP3M and s ringe was injected into the catheter to rinse it. Once the catheter was unplugged blood was collected using a sterile PNP3M and s ringe according to the blood collection timing and volume in Table 4.2. The catheter was flushed with double the catheter's dead volume (100 uL) and locked with an equivalent amount of locking solution (50 uL).

Blood glucose was anal zed using the HemoCue 201+ (HemoCue AB, Ängelholm, Sweden) as it is fast, accurate, and needs a ver small volume of whole blood to measure glucose (242). Blood collected for blood glucose anal sis was immediatel emptied onto a sterile piece of parafilm as it is a h drophobic surface, and the blood formed a droplet enabling the use of a cuvette. A HemoCue Microcuvette (HemoCue AB, Ängelholm, Sweden) was used to draw up 5 uL of whole blood, which was inserted into the HemoCue 201+ for anal sis. Readings were given in mg/dl which was then converted to mmol/L b dividing b 1 (243).

Blood collected for plasma insulin was immediatel transferred to a Microvette 100 K3E tube (Sarstedt AG & Co. KG, Nümbrecht, German), inverted 10 times, and then centrifuged at 2,500 g @ 3 C for 10 minutes using a refrigerated centrifuge (VWR International, PA, USA). Blood plasma formed a supernatant which was then e tracted using a pipettor and aliquoted as two ~20 ul samples into two 0.5 ml microtubes (Sarstedt AG & Co. KG, Nümbrecht, German). The samples were then be placed in a microtube bo and frozen at - 0 C for later anal sis.

Plasma Insulin was anal zed using Ultra-Sensitive Rat Insulin ELISA (Cr stal Chem, IL, USA) (Appendi D). Samples were run in duplicate with the mean value being used for anal sis.

Group 1 and Group 3 had their food removed at 7 am, while Group 2 and Group 4 had their food removed at 10 am. Group 1 and Group 3 started data collection at 1 pm, while Group 2 and Group 4 started data collection at 4 pm. At time -15 min the catheters were flushed as internal piloting showed this improved data collection b preparing the catheters and animals for the coming e periment. A timeline from receiving the animals to the stud endpoint can be found in Table 4.3.

Table 4.3 Data Collection Timeline (Animals shown as R1-R12)

Q2M	9/) : C$ V$	4#33+) O$V	!/#2(6#0($$				
			W5@=	X5@9	9 * .	Y "#M$. /)(#+0	9, : ') 3#
1-7 (Acclimatization)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1	1	R3		R2	R1	
	2	1	R5			R4, R6	
9	3	1	R9	R7	R		
	4	1		R11		R10, R12	
10	1	2		R1			R2, R3
	2	2		R5			R4, R6
11	3	2				R7, R	R9

	4	2		R10, R12	R11		
12	1	3	R2	R3			R1
	2	3		R4, R6			R5
13	3	3		R	R9		R7
	4	3	R10				R11
14	1	4	R1	R2	R3		
	2	4	R6		R4, R5		
15	3	4	R7			R9	R
	4	4			R10	R11	R12
15	1	5			R1	R2, R3	
	2	5	R4		R6	R5	
16	3	5	R	R9	R7		
	4	5	R11, R12				R10

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Data anal sis was conducted using GraphPad Prism version .0 (GraphPad Software, CA, USA). Results are e pressed as mean ± standard deviation. Values of $P \leq 0.05$ were considered statisticall significant. A two-wa Repeated Measures ANOVA was performed with a Tuke - Kramer post hoc test of the effects of treatment, time, and treatment b time. Area under the curve for insulin and glucose were calculated using the trapezoid method and assessed for

significance using a One-wa ANOVA with Tuke ’s-Kramer post-hoc test (244). The effect of se and age was not modeled as all subjects are the same age and male. Session effect did not need to be included in the model as no effect was found. $

This stud has been reviewed and approved b the Universit Committee on Laborator Animals at Dalhousie Universit (Protocol 19-022). Animal training on the care and use of laborator animals (Appendi A) and specific technical procedures for rats (Appendi B) was obtained. Animals are not capable of giving consent and thus the 3 Rs (Replacement, Reduction, and Refinement) are necessar to ensure the risk for harm is minimized. This has been accounted for b using the minimum number of animals to ensure valid results accounting for attrition, a reduced fasting time to allow for quicker recover , and the use novel catheter technolog to minimize discomfort to the animals. Animals were monitored dail to check on their health status. Upon completion of the stud animals were repurposed for training and for a second e periment. Animals no longer patent at the end of the stud were anesthetized with isoflurane and euthanized with carbon dio ide.

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Chapter 5: Results

5.1

Nine 10-week-old male Wistar Han Rats with an average weight of 32 $\pm$ 19 g (Table 5.1) were used. There was no significant weight loss during the duration of the stud (Table 5.2).

Table 5.1 Mean Weight (g) $\pm$ SD of Rats Throughout the Duration of the stud

Animal Number	Average weight (g) $\pm$ SD
Rat 1	310 $\pm$ 9
Rat 4	329 $\pm$ 9
Rat 6	319 $\pm$ 12
Rat 7	363 $\pm$ 12
Rat	336 $\pm$ 5
Rat 9	293 $\pm$ 5
Rat 10	345 $\pm$ 10
Rat 11	331 $\pm$ 10
Rat 12	327 $\pm$ 13

Table 5.2 Weight (g) of Rats Throughout the Duration of the Stud

Animal Number	Session Weight (g)				
	1	2	3	4	5
Rat 1	297	305	310	31	322
Rat 4	317	324	327	336	341
Rat 6	303				

There was an effect of treatment (P<0.0001), time (P<0.0001) and a treatment b time interaction (P<0.0001) on blood glucose over 120 min. Whe protein, GMP, β-LG, and α-LA resulted in significantl lower blood glucose compared to the glucose treatment at 15 and 30 min (P<0.05) (Figure 5.1). No statistical differences were observed at 0, 60, 90, and 120 min. $

Table 5.3 Mean Blood Glucose b Treatment over 120 min

Treatment	Mean Blood Glucose mmol/l ± SD					
	0 min	15 min	30 min	60 min	90 min	120 min
Whe	7.6 ± 0.5	6. ± 0.5	6.7 ± 0.3	7.0 ± 0.4	7.0 ± 0.3	6.7 ± 0.4
Glucose	7.5 ± 0.6	.5 ± 0.8	7.9 ± 0.6	7.1 ± 0.5	7.1 ± 0.5	7.1 ± 0.4
GMP	7.4 ± 0.3	7.1 ± 0.5	6.9 ± 0.4	6.9 ± 0.3	6.9 ± 0.4	6.8 ± 0.2
β-LG	7.5 ± 0.6	6.9 ± 0.6	6.9 ± 0.6 $	7.2 ± 0.5	7.0± 0.3	6.9 ± 0.2
α-LA	7.2 ± 0.2	6.4 ± 0.3 $	6.5 ± 0.6 $	6.7 ± 0.4	6.6 ± 0.3	6.6 ± 0.5

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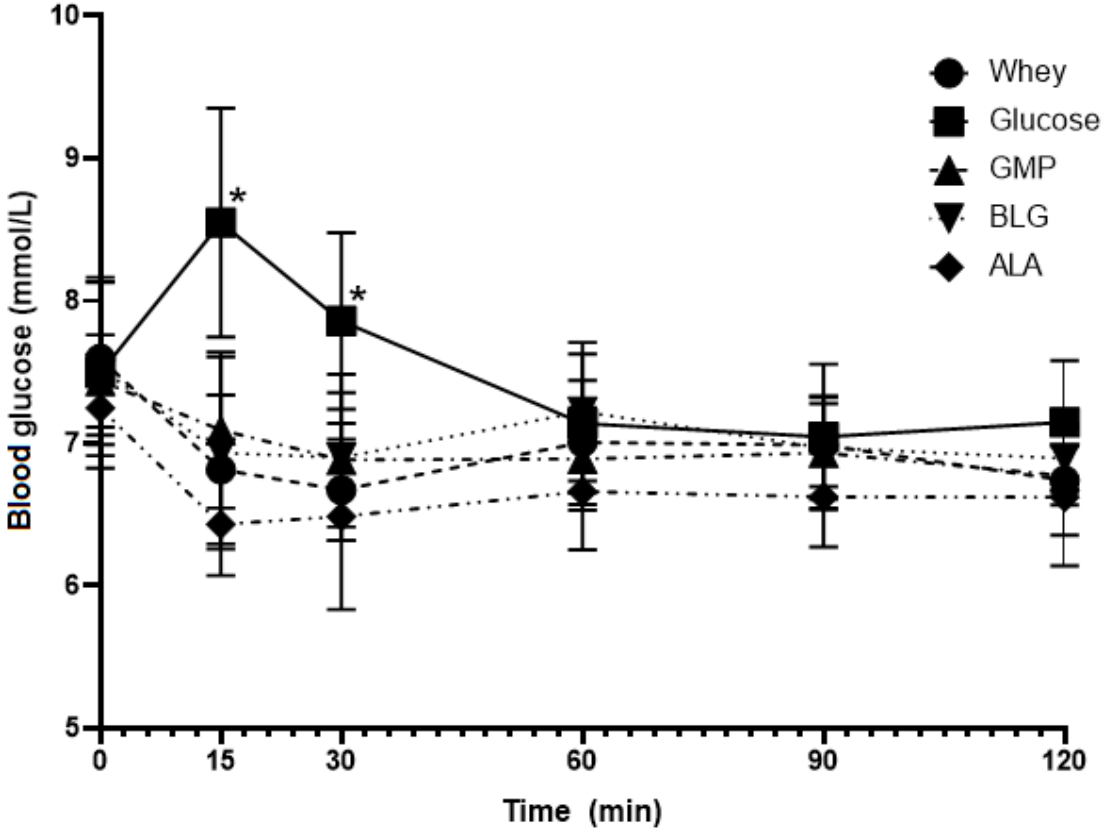
Figure 5.1 Mean Blood Glucose b Treatment over 120 min



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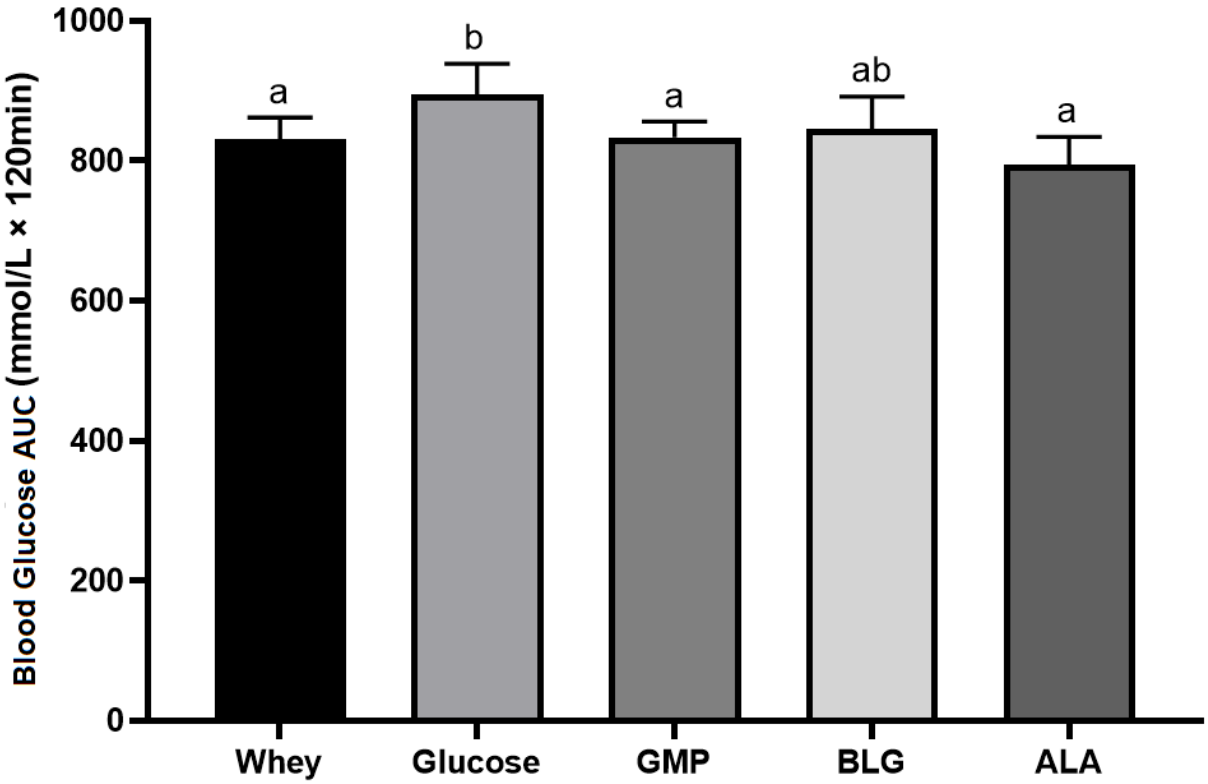
Blood glucose AUC over 120 min was significantl lower for whe protein, GMP, and α-LA compared to glucose treatment (P<0.05) (Figure 5.2). There was no significant difference between glucose and β-LG (P=0.3). There was no significant different between whe protein, GMP, and α-LA (p>0.05).$

Figure 5.2 Blood Glucose AUC over 120 min



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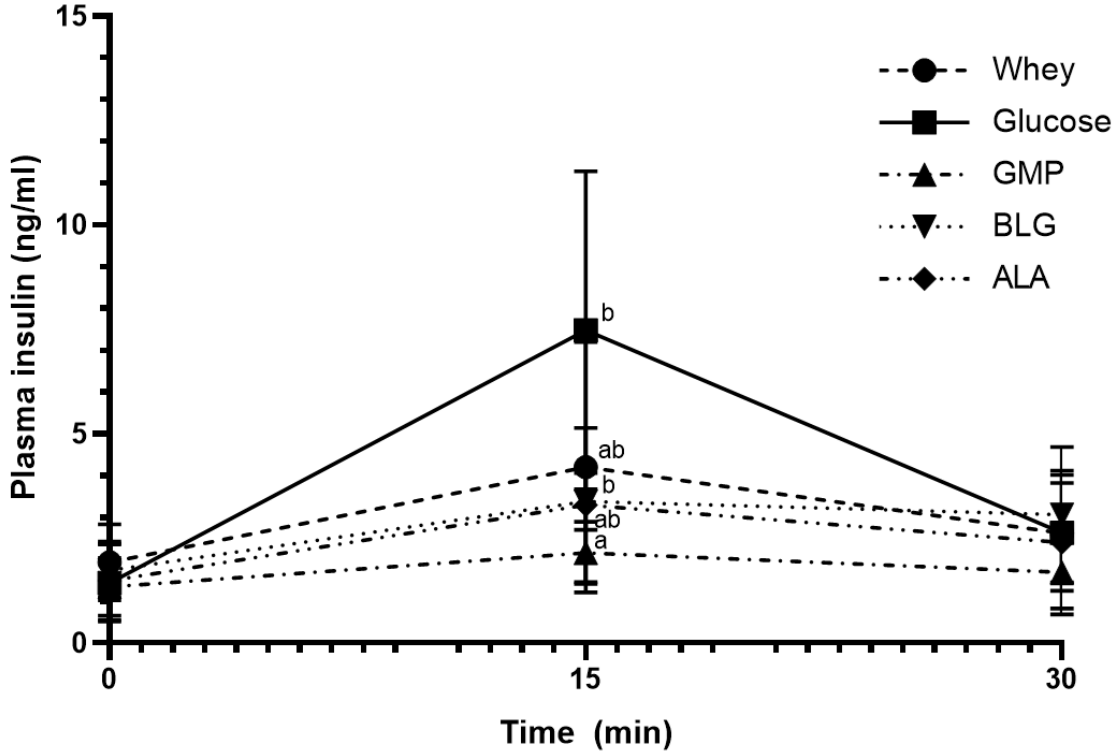
There was an effect of treatment (P=0.02), time (P<0.0001) and a treatment b time interaction (P=0.0002) on insulin response over 30 min (Figure 5.3). Gl comacropeptide had a lower insulin response at 15 min compared to glucose and β-LG (P<0.05). There was no significant difference between glucose and β-LG (p=0.07). There was no significant difference between α-LA and whe (p=0.93).

Table 5.4 Mean Plasma Insulin b Treatment over 30 min

Treatment	Mean Plasma Insulin ng/ml $\pm$ SD		
	0 min	15 min	30 min
Whe	1.9 ± 0.9	4.2 ± 2.8	2.6 ± 1.1
Glucose	1.4 ± 0.9	7.5 ± 3.7	3.5 ± 1.3
GMP	1.3 ± 0.7	2.2 ± 0.7	2.2 ± 0.7
β-LG	1.7 ± 0.6	3.4 ± 0.6	3.4 ± 1.5
α-LA	1.5 ± 0.9	3.3 ± 1.7	3.3 ± 1.6

Figure 5.3 Mean Plasma Insulin b Treatment over 30 min



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Insulin AUC over 30 min was significantl lower for GMP and α-LA compared to the glucose treatment (P=0.003) (Figure 5.4). There was no significant difference between whe , GMP, β-LG, and α-LA (P>0.05). There was no significant difference between whe , glucose, and β-LG (p>0.05).

Figure 5.4 Plasma Insulin AUC over 30 min

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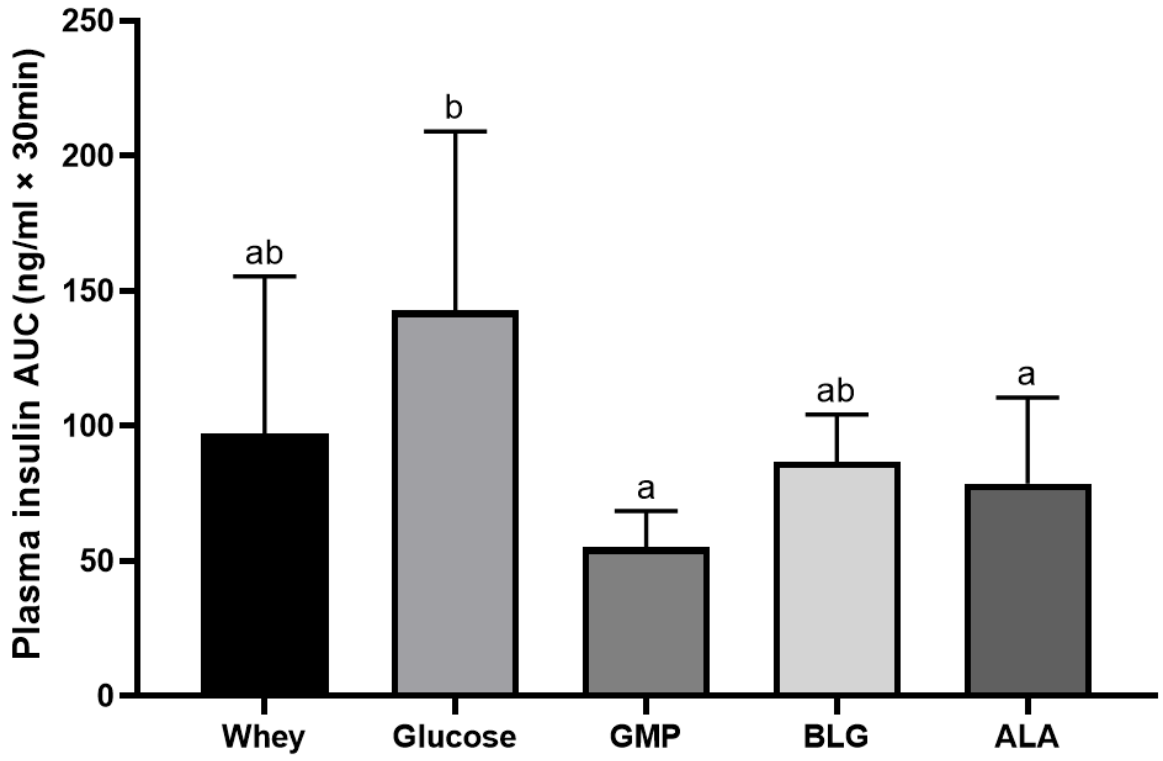

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Chapter 6: Discussion

6.1 Novelty of Methodology

The findings in this stud support the h pothesis that there is a significant difference in blood glucose response between α-LA, β-LG, whe protein, and GMP compared to the glucose control (Figure 5.1). To the author's knowledge this is the first stud evaluating the effects of whe , GMP, β-LG, α-LA and glucose on blood glucose control and insulin secretion in Wistar rats, and, to the author's knowledge, this is also the first stud e amining the effects of oral gavage in rats fitted with a VAB.

While catheterization of rats for stud ing hormones and other compounds in blood is not a new technique either anesthesia, movement restriction, or single housing must be emplo ed, and prolonged catheter patenc is difficult to maintain (245–247). The unique model deplo ed for this e periment allowed for group housing, improving animal welfare, and allowed for pain-free withdrawal of blood samples in conscious animals. The abilit to perform a repeated measurement e periment in rats that involved blood withdrawal is rare due to ethical considerations limiting blood collection methods unless catheterized. The emplo ed methodolog not onl conserves the number of animals required but should provide more biologicall accurate data.

Findings from this stud support previous evidence showing milk proteins abilit to reduce blood glucose response (1 2,1 4,24 ,249). At 15- and 30-minutes blood glucose response was significantl ($p<0.05$) lower in the whe protein, GMP, α-LA, and β-LG treatments compared to the glucose treatment (Figure 5.1). Blood glucose AUC over 120 min was also significantl lower

for whe protein, GMP, and α-LA ($p<0.05$), however there was no significant difference ($p=0.3$) between glucose and β-LG. This was une pected as β-LG has the highest BCAA profile of whe protein fractions, and BCAA have been shown to cause insulin secretion independent of the presence of glucose, which would suggest that glucose response for β-LG should be lower than observed (149). A possible e planation for this can be e plained b the observed insulin secretion (Figure 5.3) and insulin AUC (figure 5.4) where no significant difference was observed between β-LG and glucose, which shows the significant abilit of β-LG to invoke insulin secretion, however, milk protein appears to attenuate the blood glucose response through both insulin-dependent and insulin-independent methods (166). While β-LG caused the largest rise in insulin AUC of the three whe -protein fractions its inabilit to attenuate blood glucose AUC (Figure 5.2) points to an inabilit to trigger insulin-independent methods of blood glucose regulation. Whe protein and BCAAs have been found to stimulate incretin secretion, which aid in blood glucose regulation, suggesting that digestion of β-LG ma result in unique peptides that lack the bioactivit seen in whe protein (150,213). The unique effects of amino acids activit on β-cells is not limited to BCAAs as bioactive peptides also appear to be encr pted in milk proteins and to pla a role in glucose regulation (36,160,1 4,209,250). These bioactive peptides have been found to reduce gastric empt ing and to facilitate greater insulin and incretin secretion (1 2).

Interestingl , while whe protein did attenuate blood glucose response at 15- and 30-minutes (Figure 5.1) and blood glucose AUC (Figure 5.2) no significant difference between whe protein and glucose was found regarding mean insulin (Figure 5.3) and insulin AUC (Figure 5.4). This data suggests that whe protein's abilit to attenuate blood glucose ma be more closel tied to its abilit to increase insulin secretion compared to α-LA and GMP, which were also able to

attenuate blood glucose (Figure 5.3) and blood glucose AUC (Figure 5.4) compared to the glucose treatment, however the were able to do so with a significant reduction in insulin AUC (Figure 5.4).

Previous work on α-LA and GMP supports their unique function compared to whe protein. A stud comparing a variet of treatments, including whe , casein, whe with GMP, and α-LA found α-LA to be 40% more satiating and to reduce energ intake b 20% compared to casein and whe in health adult humans (105). The data found in this stud supports this as α-LA's abilit to attenuate blood glucose ma be due to its unique structure and/or its abilit to produce unique bioactive peptides providing enhanced insulin-independent blood glucose regulator effects.

This data also supports GMPs abilit to reduce blood glucose in humans to a greater degree than whe protein (104). A stud conducted b Hoefle et al., in prediabetic humans found that 50 g of GMP consumed in tandem with 50 g of maltode trin attenuated 4-hr AUC b 1 % compared to 50 g of maltode trin alone (104). This was significantl higher ($p = 0.04$) than a 50 g whe protein and 50 g maltode trin treatment (11% AUC reduction) (104). Hoefle et al., also found that GMP attenuated blood glucose through insulin-independent pathwa s as AUC was 34% lower in the GMP treatment compared to the whe protein treatment; the also found that c-peptide and GIP were higher in the whe protein treatment but unaffected in the GMP protein treatment, suggesting that a potential, as of et, undiscovered method of blood glucose control ma be present (104).

Of interesting note is the relativel recent use of GMP as a protein source in people who suffer from phen lketonuria (PKU), a disease where the amino acid phen lalanine is unable to be

broken down which ultimatel impacts neurotransmitter release causing impaired cognitive function (251). T pe 2 Diabetes prevalence appears to be similar in those with PKU to the general population (~10%) (252). Unfortunatel there is currentl no data available on commerciall available GMP products and their impact on blood glucose response in people with PKU, but this stud 's findings offer a potential reason for further investigation into this.

In summar , all protein treatments attenuated blood glucose at 15- and 30-minutes compared to the glucose control, however onl whe protein, GMP, and α-LA attenuated blood glucose AUC response. At 15 minutes GMP had a significantl lower insulin response compared to the rest of the treatments, and GMP and α-LA had a significantl lower insulin AUC compared to the rest of the treatments. These results show that whe protein fractions have unique health properties not observed in whe protein, especiall GMP, and require further stud to properl understand the observed effects.$

There are several limitations of this stud . Using an animal model alwa s has limitations as work done in animals still requires human confirmation, however b ingesting rather than injecting the treatments, using rats without genetic defects, and using a dosage realistic for human consumption a more valid comparison can be reasonabl e pected (253). The use of an equivalent dosage of 10 g appears to be adequate based on previous research comparing the effects of var ing whe protein treatments, but it is possible that this is not the ideal dosage (166,215). It was selected to portra a human dosage more accuratel and to prevent a higher dosage providing an overpowering effect (146,220). While the number of animals was calculated to be sufficient these data suggest that this ma be untrue for e amining hormonal response.

Chapter 7: Future Directions

This stud ’s findings show the potential application of whe protein fractions in the development of functional foods aimed at attenuating the blood glucose response after food ingestion. Finding the minimum effective dose where both significant and clinicall relevant reduction in blood glucose occurs is a necessar ne t step. While GMP and α-LA showed the best abilit to attenuate glucose with the minimum amount of insulin secretion it is possible the dosage of β-LG and whe protein was too low to see a similar outcome and a higher dosage ma show equivalent results. Work should also be conducted on seeing the effects of the treatments and glucose ingestion simultaneousl and work should be conducted looking at the effects of the treatments as a pre-meal before the ingestion of glucose. Methodolog should also be refined to enable for a greater amount of blood to be withdrawn to enable the anal sis of more gastrointestinal hormones (e.g., c-peptide, GIP, GLP-1). With the techniques developed through this stud this is possible if a greater number of animals are used to account for higher attrition due to a longer stud protocol. Special attention should be paid to GMP, which appears to be equall valid at attenuating blood glucose response as whe protein, α-LA, and β-LG, but with a much lower secretion of insulin which ma be beneficial in the face of insulin resistance.

Chapter 8: Conclusion

Whe protein fractions possess unique properties on blood glucose control that are not observed in whole whe protein and are worth of continued stud . This stud also showed the use of oral gavage in rats fitted with a novel catheterization s stem to be a viable method for testing blood parameters in response to food intake.

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References

1. Diabetes Canada. Type 2 diabetes [Internet]. DiabetesCanadaWebsite. [cited 2019 Jun 25]. Available from: https://www.diabetes.ca/about-diabetes/type-2

2. Holt RI, Cockram C, Flyvbjerg A, Goldstein BJ. Textbook of diabetes. John Wiley & Sons; 2017.

3. Canada PHA of. Diabetes in Canada [Internet]. Government of Canada. 2017 [cited 2019 Jun 3]. Available from: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/diabetes-canada-highlights-chronic-disease-surveillance-system.html

4. Committee DCCPGE, others. Diabetes Canada 2018 clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes. 2018;42(Suppl 1):S1–325.

5. Hidayat K, Du X, Shi B-M. Milk in the prevention and management of type 2 diabetes: the potential role of milk proteins. Diabetes/Metabolism Research and Reviews. 2019;35(8):e3187.

6. Diabetes Canada. What is diabetes? [Internet]. DiabetesCanadaWebsite. [cited 2020 Nov 24]. Available from: https://www.diabetes.ca/about-diabetes/what-is-diabetes

7. Herder C, Bongaerts BWC, Rathmann W, Heier M, Kowall B, Koenig W, et al. Association of Subclinical Inflammation With Polyneuropathy in the Older Population: KORA F4 study. Diabetes Care. 2013 Nov 1;36(11):3663–70.

8. Diabetes Canada. Diabetes Canada cliion: KORA7 8DeKO7e8Deg7 8DeKDe7u8D7on8DetheC,e7 8De7p8De7Ol8Dt

16. Scheen A. Pathophysiology of type 2 diabetes. Acta Clinica Belgica. 2003;58(6):335–41.

17. Alsahli M, Gerich JE. Abnormalities of Insulin Secretion and β-Cell Defects in Type 2 Diabetes. In: Richard, I.G, Golt, C.S.C, Flyvbjerg, A., Goldstein, J., editors. Textbook of Diabetes. 4th ed. Blackwell Publishing Ltd; 2010.

18. Boland BB, Rhodes CJ, Grimsby JS. The dynamic plasticity of insulin production in β-cells. Molecular Metabolism. 2017 Sep 1;6(9):958–73.

19. Fu Z, Gilbert ER, Liu D. Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. Curr Diabetes Rev. 2013 Jan 1;9(1):25–53.

20. Cnop M, Vidal J, Hull RL, Utzschneider KM, Carr DB, Schraw T, et al. Progressive loss of β-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. Diabetes care. 2007;30(3):677–82.

21. Schulze MB, Hu FB. PRIMARY PREVENTION OF DIABETES: What Can Be Done and How Much Can Be Prevented? Annual Review of Public Health. 2005;26(1):445–67.

22. van Haeften TW, Pimenta W, Mitrakou A, Korytkowski M, Jenssen T, Yki-Jarvinen H, et al. Relative contributions of β-cell function and tissue insulin sensitivity to fasting and postglucose-load glycemia. Metabolism. 2000 Oct 1;49(10):1318–25.

23. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Invest. 1984 Oct 1;74(4):1318–28.

24. Festa A, Williams K, D'Agostino R, Wagenknecht LE, Haffner SM. The natural course of β-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study. Diabetes. 2006;55(4):1114–20.

25. Ferrannini E, Nannipieri M, Williams K, Gonzales C, Haffner SM, Stern MP. Mode of onset of type 2 diabetes from normal or impaired glucose tolerance. Clinical Diabetology. 2004;5(2):105–12.

26. Group UKPDS. U.K. Prospective Diabetes Study 16: Overview of 6 Years' Therapy of Type II Diabetes: A Progressive Disease. Diabetes. 1995 Nov 1;44(11):1249–58.

27. Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin Resistance and Insulin Secretory Dysfunction Are Independent Predictors of Worsening of Glucose Tolerance During Each Stage of Type 2 Diabetes Development. Diabetes Care. 2001 Jan 1;24(1):89–94.

28. Shanik MH, Xu Y, Škrha J, Dankner R, Zick Y, Roth J. Insulin Resistance and Hyperinsulinemia: Is hyperinsulinemia the cart or the horse? Diabetes Care. 2008 Feb 1;31(Supplement 2):S262–8.

29. Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Ehses JA. Islet inflammation in type 2 diabetes: from metabolic stress to therapy. Diabetes care. 2008;31(Supplement 2):S161–4.

30. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;52(1):102–10.

31. Gaggini M, Saponaro C, Gastaldelli A. Not all fats are created equal: adipose vs. ectopic fat, implication in cardiometabolic diseases. Horm Mol Biol Clin Investig. 2015 Apr;22(1):7–18.

32. Neeland IJ, Ross R, Després J-P, Matsuzawa Y, Yamashita S, Shai I, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. The Lancet Diabetes & Endocrinology. 2019 Sep;7(9):715–25.

33. Dali-Youcef N, Mecili M, Ricci R, Andrès E. Metabolic inflammation: Connecting obesity and insulin resistance. Annals of Medicine. 2013 May 1;45(3):242–53.

34. Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids Health Dis [Internet]. 2015 Sep 29 [cited 2020 Nov 30];14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4587882/

35. Yue JTY, Lam TKT. Lipid sensing and insulin resistance in the brain. Cell Metab. 2012 May 2;15(5):646–55.

36. Ortega Á, Berná G, Rojas A, Martín F, Soria B. Gene-Diet Interactions in Type 2 Diabetes: The Chicken and Egg Debate. International Journal of Molecular Sciences. 2017 Jun;18(6):1188.

37. Duclos M. Osteoarthritis, obesity and type 2 diabetes: The weight of waist circumference. Annals of Physical and Rehabilitation Medicine. 2016 Jun 1;59(3):157–60.

38. Clamp LD, Hume DJ, Lambert EV, Kroff J. Enhanced insulin sensitivity in successful, long-term weight loss maintainers compared with matched controls with no weight loss history. Nutr Diabetes. 2017 Jun;7(6):e282.

39. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans. BMJ Open Sport Exerc Med [Internet]. 2017 Mar 1 [cited 2020 Nov 19];2(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5569266/

40. Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and Pathogenesis of Visceral Fat Obesity. Obesity Research. 1995;3(S2):187s–94s.

41. Buell JL, Calland D, Hanks F, Johnston B, Pester B, Sweeney R, et al. Presence of Metabolic Syndrome in Football Linemen. J Athl Train. 2008 Nov 1;43(6):608–16.

42. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000 Aug 12;321(7258):405–12.

43. American Diabetes Association. Nutrition principles and recommendations in diabetes. Diabetes care. 2004;27(suppl 1):s36–s36.

44. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Experimental & Molecular Medicine. 2016 Mar;48(3):e219–e219.

45. Nauck MA, Meier JJ. Incretin hormones: Their role in health and disease. Diabetes, Obesity and Metabolism. 2018;20(S1):5–21.

46. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig. 2010 Apr 22;1(1–2):8–23.

47. Tengholm A. Cyclic AMP dynamics in the pancreatic β-cell. Ups J Med Sci. 2012 Nov;117(4):355–69.

48. Nadkarni P, Chepurny OG, Holz GG. Regulation of Glucose Homeostasis by GLP-1. Prog Mol Biol Transl Sci. 2014;121:23–65.

49. Naghavi M, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, Abera SF, et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet. 2017 Sep 16;390(10100):1151–210.

50. Cheng V, Kashyap SR. Weight considerations in pharmacotherapy for type 2 diabetes. Journal of obesity. 2011;2011.

51. Bernstein D, Golson ML, Kaestner KH. Epigenetic control of β-cell function and failure. Diabetes research and clinical practice. 2017;123:24–36.

52. Franks PW, McCarthy MI. Exposing the exposures responsible for type 2 diabetes and obesity. Science. 2016;354(6308):69–73.

53. Hall E, Dayeh T, Kirkpatrick CL, Wollheim CB, Nitert MD, Ling C. DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets. BMC medical genetics. 2013;14(1):76.

54. Volkmar M, Dedeurwaerder S, Cunha DA, Ndlovu MN, Defrance M, Deplus R, et al. DNA methylation profiling identifies epigenetic dysregulation in pancreatic islets from type 2 diabetic patients. The EMBO Journal. 2012 Mar 21;31(6):1405–26.

55. Dhawan S, Georgia S, Tschen S, Fan G, Bhushan A. Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. Developmental cell. 2011;20(4):419–29.

56. Huang H-P, Tsai M-J. Transcription factors involved in pancreatic islet development. J Biomed Sci. 2000 Jan 1;7(1):27–34.

57. Hill DJ. Nutritional programming of pancreatic β-cell plasticity. World journal of diabetes. 2011;2(8):119.

58. Desai M, Crowther NJ, Lucas A, Hales C. Organ-selective growth in the offspring of protein-restricted mother. The British journal of nutrition. 1996 Nov 1;76:591–603.

59. Ruiz-Narváez EA, Palmer JR, Gerlovin H, Wise LA, Vimalananda VG, Rosenzweig JL, et al. Birth Weight and Risk of Type 2 Diabetes in the Black Women's Health Study: Does Adult BMI Play a Mediating Role? Dia Care. 2014 Sep;37(9):2572–8.

60. Johnston LW, Zinman B. Short Leg Length, a Marker of Early Childhood Deprivation, Is Associated With Metabolic Disorders Underlying Type 2 Diabetes. DIABETES CARE. 2013;36:8.

61. Ng S-F, Lin R, Laybutt D, Barres R, Owens J, Morris M. Chronic high-fat diet in fathers programs ?-cell dysfunction in female rat offspring. Nature. 2010 Oct 1;467:963–6.

62. Dunn GA, Bale TL. Maternal High-Fat Diet Promotes Body Length Increases and Insulin Insensitivity in Second-Generation Mice. Endocrinology. 2009 Nov 1;150(11):4999–5009.

63. Veenendaal MVE, Painter RC, Rooij S de, Bossuyt PMM, Post J van der, Gluckman PD, et al. Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. BJOG: An International Journal of Obstetrics & Gynaecology. 2013;120(5):548–54.

64. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. European Journal of Human Genetics. 2002 Nov;10(11):682–8.

65. Ortega-Azorín C, Sorlí JV, Asensio EM, Coltell O, Martínez-González MÁ, Salas-Salvadó J, et al. Associations of the FTO rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern

75. Nadeau J, Koski KG, Strychar I, Yale JF. Teaching subjects with type 2 diabetes how to incorporate sugar choices into their daily meal plan promotes dietary compliance and does not deteriorate metabolic profile. Diabetes Care. 2001 Feb;24(2):222–7.

76. Adler NE, Stewart J. Health disparities across the lifespan: Meaning, methods, and mechanisms: Health disparities across the lifespan. Annals of the New York Academy of Sciences. 2010 Feb;1186(1):5–23.

77. McEwen BS. Stressed or stressed out: What is the difference? J Psychiatry Neurosci. 2005 Sep;30(5):315–8.

78. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Hormones and Behavior. 2003 Jan;43(1):2–15.

79. Dendup T, Feng X, Clingan S, Astell-Burt T. Environmental risk factors for developing type 2 diabetes mellitus: a systematic review. International journal of environmental research and public health. 2018;15(1):78.

80. Canada PHA of. Type 2 diabetes [Internet]. Government of Canada. 2018 [cited 2019 Jun 15]. Available from: https://www.canada.ca/en/public-health/services/diseases/type-2-diabetes.html

81. Yuill C, Crinson I, Duncan E. Key concepts in health studies. Sage; 2010.

82. Ogurtsova K, Fernandes JD da R, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Research and Clinical Practice. 2017 Jun 1;128:40–50.

83. Zhou B, Lu Y, Hajifathalian K, Bentham J, Di Cesare M, Danaei G, et al. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4·4 million participants. The Lancet. 2016 Apr 9;387(10027):1513–30.

84. World Health Organization. Diabetes Fact Sheet [Internet]. 2018 [cited 2019 Jun 21]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes

85. Moucheraud C, Lenz C, Latkovic M, Wirtz VJ. The costs of diabetes treatment in low- and middle-income countries: a systematic review. BMJ Global Health. 2019 Feb 1;4(1):e001258.

86. Hosseini Z, Whiting SJ, Vatanparast H. Type 2 diabetes prevalence among Canadian adults - dietary habits and sociodemographic risk factors. Appl Physiol Nutr Metab. 2019 Oct;44(10):1099–104.

87. Wareham NJ, O'Rahilly S. The changing classification and diagnosis of diabetes. BMJ. 1998 Aug 8;317(7155):359–60.

88. Government of Canada. Diabetes, 2016 [Internet]. Statistics Canada. 2017 [cited 2019 May 7]. Available from: https://www150.statcan.gc.ca/n1/pub/82-625-x/2017001/article/54859-eng.htm

89. Government of Canada. Diabetes, 2017 [Internet]. Statistics Canada. 2018 [cited 2019 May 7]. Available from: https://www150.statcan.gc.ca/n1/pub/82-625-x/2018001/article/54982-eng.htm

90. Pelletier C, Dai S, Roberts K, Bienek A, others. Report summary Diabetes in Canada: facts and figures from a public health perspective. Chronic diseases and injuries in Canada. 2012;33(1).

91. Gregg EW, Cheng YJ, Srinivasan M, Lin J, Geiss LS, Albright AL, et al. Trends in cause-specific mortality among adults with and without diagnosed diabetes in the USA: an epidemiological analysis of linked national survey and vital statistics data. The Lancet. 2018;391(10138):2430–40.

92. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart Disease and Stroke Statistics—2011 Update: A Report From the American Heart Association. Circulation [Internet]. 2011 Feb [cited 2020 Nov 24];123(4). Available from: https://www.ahajournals.org/doi/10.1161/CIR.0b013e3182009701

93. Anja B, Laura R. The cost of diabetes in Canada over 10 years: applying attributable health care costs to a diabetes incidence prediction model. Health promotion and chronic disease prevention in Canada: research, policy and practice. 2017;37(2):49.

94. Ebeling P, Koistinen HA, Koivisto VA. Insulin-independent glucose transport regulates insulin sensitivity. FEBS Letters. 1998 Oct 9;436(3):301–3.

95. Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. :7.

96. Kellett G, Brot-Laroche E, Mace O, Leturque A. Sugar Absorption in the Intestine: The Role of GLUT2. Annual review of nutrition. 2008 May 1;28:35–54.

97. Postic C, Leturque A, Printz RL, Maulard P, Loizeau M, Granner DK, et al. Development and regulation of glucose transporter and hexokinase expression in rat. Am J Physiol. 1994;

98. Pendergrass M, Koval J, Vogt C, Yki-Jarvinen H, Iozzo P, Pipek R, et al. Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. Diabetes. 1998 Mar 1;47:387–94.

99. Garvey WT, Maianu L, Zhu J-H, Brechtel-Hook G, Wallace P, Baron AD. Evidence for Defects in the Trafficking and Translocation of GLUT4 Glucose Transporters in Skeletal Muscle as a Cause of Human Insulin Resistance. :10.

100. Boden G, Homko C, Barrero CA, Stein TP, Chen X, Cheung P, et al. Excessive caloric intake acutely causes oxidative stress, GLUT4 carbonylation, and insulin resistance in healthy men. Sci Transl Med. 2015 Sep 9;7(304):304re7.

101. Holt RIG, Cockram C, Flyvbjerg A, Goldstein BJ. Textbook of Diabetes. John Wiley & Sons; 2016. 1108 p.

102. Norman AW, Henry HL. Chapter 6 - Pancreatic Hormones: Insulin and Glucagon. In: Norman AW, Henry HL, editors. Hormones (Third Edition) [Internet]. San Diego: Academic Press; 2015 [cited 2021 Aug 21]. p. 109–39. Available from: https://www.sciencedirect.com/science/article/pii/B9780080919065000069

103. Gheller BJ, Gheller M, Li A, Nunes F, Anini Y, Glanville NT, et al. Effect of dairy and nondairy snacks on postprandial blood glucose regulation in 9–14-year-old children. Applied Physiology, Nutrition, and Metabolism. 2019;44(10):1073–80.

104. Hoefle AS, Bangert AM, Rist MJ, Gedrich K, Lee Y-M, Skurk T, et al. Postprandial metabolic responses to ingestion of bovine glycomacropeptide compared to a whey protein isolate in prediabetic volunteers. Eur J Nutr. 2019 Aug;58(5):2067–77.

105. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, Westerterp KR, Engelen MPKJ, Brummer R-JM, et al. A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. Clin Nutr. 2009 Apr;28(2):147–55.

106. Da Silva Xavier G. The Cells of the Islets of Langerhans. J Clin Med. 2018 Mar 12;7(3):54.

107. Duckworth W, Bennett R, Hamel F. Insulin Degradation: Progress and Potential 1. Endocrine reviews. 1998 Nov 1;19:608–24.

108. Gerich JE. Is Reduced First-Phase Insulin Release the Earliest Detectable Abnormality in Individuals Destined to Develop Type 2 Diabetes? Diabetes. 2002 Feb 1;51(suppl 1):S117–21.

109. Brandon Gheller. The Effect of Dairy and Non-dairy Snack Products on Glycemic Regulation in Over Weight and Obese Boys [Internet]. Mount Saint Vincent University; 2014 [cited 2021 Aug 21]. Available from: http://ec.msvu.ca:8080/xmlui/bitstream/handle/10587/1341/BrandonGhellerMScAHNThesis2014.pdf?sequence=1

110. Russell WR, Baka A, Björck I, Delzenne N, Gao D, Griffiths HR, et al. Impact of Diet Composition on Blood Glucose Regulation. Critical Reviews in Food Science and Nutrition. 2016 Mar 11;56(4):541–90.

111. Yalçın T, Al A, Rakıcıoğlu N. The effects of meal glycemic load on blood glucose levels of adults with different body mass indexes. Indian J Endocrinol Metab. 2017;21(1):71–5.

112. Jenkins D, Wolever T, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. The American journal of clinical nutrition. 1981;34(3):362–6.

113. Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev. 2009 Jan 21;(1):CD006296.

114. Schwingshackl L, Hobl LP, Hoffmann G. Effects of low glycaemic index/low glycaemic load vs. high glycaemic index/ high glycaemic load diets on overweight/obesity and associated risk factors in children and adolescents: a systematic review and meta-analysis. Nutr J. 2015 Aug 25;14(1):87.

115. 14:00-17:00. ISO 26642:2010 [Internet]. ISO. [cited 2020 Nov 30]. Available from: https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/04/36/43633.html

116. Richardson DP, Ansell J, Drummond LN. The nutritional and health attributes of kiwifruit: a review. Eur J Nutr. 2018;57(8):2659–76.

117. Diabetes Canada. glycemic-index-food-guide.pdf [Internet]. Diabetes Canada. [cited 2020 Nov 30]. Available from: https://guidelines.diabetes.ca/docs/patient-resources/glycemic-index-food-guide.pdf

118. Atkinson FS, Foster-Powell K, Brand-Miller JC. International Tables of Glycemic Index and Glycemic Load Values: 2008. Diabetes Care. 2008 Dec 1;31(12):2281–3.

119. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clinical Nutrition. 2004 Aug 1;23(4):447–56.

120. Lovejoy JC. The influence of dietary fat on insulin resistance. Curr Diab Rep. 2002 Oct 1;2(5):435–40.

121. Rivellese A. Effects of omega-3 fatty acids on carbohydrate and lipid metabolism in healthy people: The KANWU study. In: Diabetologia. SPRINGER-VERLAG 175 FIFTH AVE, NEW YORK, NY 10010 USA; 2000. p. A3–A3.

122. Canada H. Do Canadian Adults Meet Their Nutrient Requirements Through Food Intake Alone? [Internet]. Health Canada. 2010 [cited 2020 Dec 1]. Available from: https://www.canada.ca/en/health-canada/services/food-nutrition/food-nutrition-surveillance/health-nutrition-surveys/canadian-community-health-survey-cchs/canadian-adults-meet-their-nutrient-requirements-through-food-intake-alone-health-canada-2012.html

123. Government of Canada SC. Percentage of total energy intake from fat, by dietary reference intake age-sex group, household population aged 1 and over, Canadian Community Health Survey (CCHS) - Nutrition, Canada and provinces [Internet]. 2017 [cited 2020 Dec 3]. Available from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310076901

124. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans. BMJ Open Sport Exerc Med [Internet]. 2017 Mar 1 [cited 2020 Dec 1];2(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5569266/

125. Van Proeyen K, Szlufcik K, Nielens H, Pelgrim K, Deldicque L, Hesselink M, et al. Training in the fasted state improves glucose tolerance during fat-rich diet. J Physiol. 2010 Nov 1;588(Pt 21):4289–302.

126. Munch-Andersen T, Olsen DB, Søndergaard H, Daugaard JensR, Bysted A, Christensen DL, et al. Metabolic profile in two physically active Inuit groups consuming either a western or a traditional Inuit diet. International Journal of Circumpolar Health. 2012 Jan 31;71(1):17342.

127. Imamura F, Micha R, Wu JHY, de Oliveira Otto MC, Otite FO, Abioye AI, et al. Effects of Saturated Fat, Polyunsaturated Fat, Monounsaturated Fat, and Carbohydrate on Glucose-Insulin Homeostasis: A Systematic Review and Meta-analysis of Randomised Controlled Feeding Trials. PLoS Med [Internet]. 2016 Jul 19 [cited 2020 Dec 1];13(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4951141/

128. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health Implications of High Dietary Omega-6 Polyunsaturated Fatty Acids. J Nutr Metab [Internet]. 2012 [cited 2020 Dec 1];2012. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335257/

129. Simopoulos AP. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases: Experimental Biology and Medicine [Internet]. 2008 Jun 1 [cited 2020 Dec 1]; Available from: https://journals.sagepub.com/doi/10.3181/0711-MR-311?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

130. Balić A, Vlašić D, Žužul K, Marinović B, Bukvić Mokos Z. Omega-3 Versus Omega-6 Polyunsaturated Fatty Acids in the Preve

140. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance. Cell Metabolism. 2009 Apr 8;9(4):311–26.

141. Fan M, Li Y, Wang C, Mao Z, Zhou W, Zhang L, et al. Dietary Protein Consumption and the Risk of Type 2 Diabetes: ADose-Response Meta-Analysis of Prospective Studies. Nutrients. 2019 Nov 15;11(11):2783.

142. Ajala O, English P, Pinkney J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. Am J Clin Nutr. 2013 Mar 1;97(3):505–16.

143. Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr. 2003 Oct;78(4):734–41.

144. Parker B, Noakes M, Luscombe N, Clifton P. Effect of a High-Protein, High-Monounsaturated Fat Weight Loss Diet on Glycemic Control and Lipid Levels in Type 2 Diabetes. Diabetes Care. 2002 Mar 1;25(3):425–30.

145. Larsen RN, Mann NJ, Maclean E, Shaw JE. The effect of high-protein, low-carbohydrate diets in the treatment of type 2 diabetes: a 12 month randomised controlled trial. Diabetologia. 2011 Apr 1;54(4):731–40.

146. Akhavan T, Luhovyy BL, Brown PH, Cho CE, Anderson GH. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. Am J Clin Nutr. 2010 Apr 1;91(4):966–75.

147. Jakubowicz D, Froy O, Ahrén B, Boaz M, Landau Z, Bar-Dayan Y, et al. Incretin, insulinotropic and glucose-lowering effects of whey protein pre-load in type 2 diabetes: a randomised clinical trial. Diabetologia. 2014 Sep 1;57(9):1807–11.

148. Jakubowicz D, Wainstein J, Landau Z, Ahren B, Barnea M, Bar-Dayan Y, et al. High-energy breakfast based on whey protein reduces body weight, postprandial glycemia and HbA1C in Type 2 diabetes. The Journal of Nutritional Biochemistry. 2017 Nov 1;49:1–7.

149. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014 Dec;10(12):723–36.

150. Chen Q, Reimer RA. Dairy protein and leucine alter GLP-1 release and mRNA of genes involved in intestinal lipid metabolism in vitro. Nutrition. 2009 Mar 1;25(3):340–9.

151. Neishabouri SH, Hutson SM, Davoodi J. Chronic activation of mTOR complex 1 by branched chain amino acids and organ hypertrophy. Amino Acids. 2015 Jun;47(6):1167–82.

152. Hagiwara A, Nishiyama M, Ishizaki S. Branched-chain amino acids prevent insulin-induced hepatic tumor cell proliferation by inducing apoptosis through mTORC1 and mTORC2-dependent mechanisms. J Cell Physiol. 2012 May;227(5):2097–105.

153. Yoon M-S, Choi CS. The role of amino acid-induced mammalian target of rapamycin complex 1(mTORC1) signaling in insulin resistance. Exp Mol Med. 2016 Jan;48(1):e201–e201.

154. Akhavan T, Luhovyy BL, Panahi S, Kubant R, Brown PH, Anderson GH. Mechanism of action of pre-meal consumption of whey protein on glycemic control in young adults. The Journal of Nutritional Biochemistry. 2014 Jan 1;25(1):36–43.

155. Chartrand D, Da Silva MS, Julien P, Rudkowska I. Influence of Amino Acids in Dairy Products on Glucose Homeostasis: The Clinical Evidence. Canadian Journal of Diabetes. 2017 Jun 1;41(3):329–37.

156. Tong X, Dong J-Y, Wu Z-W, Li W, Qin L-Q. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr. 2011 Sep;65(9):1027–31.

157. Lee M, Lee H, Kim J. Dairy food consumption is associated with a lower risk of the metabolic syndrome and its components: a systematic review and meta-analysis. Br J Nutr. 2018 Aug 28;120(4):373–84.

158. Abargouei AS, Janghorbani M, Salehi-Marzijarani M, Esmaillzadeh A. Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. International journal of obesity. 2012;36(12):1485–93.

159. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. Eur J Nutr. 2013 Feb 1;52(1):1–24.

160. Muro Urista C, Álvarez Fernández R, Riera Rodriguez F, Arana Cuenca A, Téllez Jurado A. Review: Production and functionality of active peptides from milk. Food sci technol int. 2011 Aug 1;17(4):293–317.

161. Chartrand D, Da Silva MS, Julien P, Rudkowska I. Influence of Amino Acids in Dairy Products on Glucose Homeostasis: The Clinical Evidence. Canadian Journal of Diabetes. 2017 Jun 1;41(3):329–37.

162. Abou-Samra R, Keersmaekers L, Brienza D, Mukherjee R, Macé K. Effect of different protein sources on satiation and short-term satiety when consumed as a starter. Nutrition Journal. 2011 Dec 23;10(1):139.

163. Mortensen LS, Hartvigsen ML, Brader LJ, Astrup A, Schrezenmeir J, Holst JJ, et al. Differential effects of protein quality on postprandial lipemia in response to a fat-rich meal in type 2 diabetes: comparison of whey, casein, gluten, and cod protein. Am J Clin Nutr. 2009 Jul 1;90(1):41–8.

164. Jenness R. Composition of milk. In: Fundamentals of dairy chemistry. Springer; 1988. p. 1–38.

165. Farrell Jr HM, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, et al. Nomenclature of the proteins of cows' milk—Sixth revision. Journal of dairy science. 2004;87(6):1641–74.

166. Akhavan T, Luhovyy BL, Panahi S, Kubant R, Brown PH, Anderson GH. Mechanism of action of pre-meal consumption of whey protein on glycemic control in young adults. The Journal of nutritional biochemistry. 2014;25(1):36–43.

167. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin: pitfalls and limitations. Diabetes. 1984;33(5):486–94.

168. Van Den Hoek AM, Heijboer AC, Corssmit EP, Voshol PJ, Romijn JA, Havekes LM, et al. PYY3–36 reinforces insulin action on glucose disposal in mice fed a high-fat diet. Diabetes. 2004;53(8):1949–52.

169. Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Niijima A. Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. American Journal of Physiology-Endocrinology And Metabolism. 1996;271(5):E808–13.

170. Allen JM, Fitzpatrick ML, Yeats JC, Darcy K, Adrian TE, Bloom SR. Effects of peptide YY and neuropeptide Y on gastric emptying in man. Digestion. 1984;30(4):255–62.

171. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, et al. Glucagon-like peptide 1

182. Hidayat K, Du X, Shi B. Milk in the prevention and management of type 2 diabetes: The potential role of milk proteins. Diabetes Metab Res Rev [Internet]. 2019 Nov [cited 2021 Aug 22];35(8). Available from: https://onlinelibrary.wiley.com/doi/10.1002/dmrr.3187

183. Nilsson M, Holst JJ, Björck IM. Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. The American Journal of Clinical Nutrition. 2007 Apr 1;85(4):996–1004.

184. Luhovyy BL, Akhavan T, Anderson GH. Whey Proteins in the Regulation of Food Intake and Satiety. Journal of the American College of Nutrition. 2007 Dec 1;26(6):704S-712S.

185. Duncan AM, Dunn HA, Stratton LM, Vella MN. Translating knowledge into dietetic practice: a Functional Foods for Healthy Aging Toolkit. Appl Physiol Nutr Metab. 2014 May;39(5):600–3.

186. Hasler CM, Brown AC, American Dietetic Association. Position of the American Dietetic Association: functional foods. J Am Diet Assoc. 2009 Apr;109(4):735–46.

187. Bigliardi B, Galati F. Innovation trends in the food industry: The case of functional foods. Trends in Food Science & Technology. 2013 Jun;31(2):118–29.

188. Whitfield K. Trial of Thiamine Supplementation in Cambodia - Full Text View - ClinicalTrials.gov [Internet]. [cited 2020 Dec 11]. Available from: https://clinicaltrials.gov/ct2/show/NCT03616288

189. Government of Canada CFIA. Health claims on food labels [Internet]. 2014 [cited 2020 Dec 11]. Available from: https://www.inspection.gc.ca/food-label-requirements/labelling/industry/health-claims-on-food-labels/eng/1392834838383/1392834887794

190. Health Canada. Draft Guidance Document on Food Health Claims Related to the Reduction in Post-Prandial Glcaemic Response. Health Canada; 2013.

191. Canada H. Canadian Consumer Perspectives on Food Fortification [Internet]. aem. 2004 [cited 2020 Dec 11]. Available from: https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/vitamins-minerals/canadian-consumer-perspectives-food-fortification-executive-summary.html

192. Bhat H, Bhat ZF. Milk and Dairy Products as Functional Foods: A Review [Internet]. Science Alert. [cited 2020 Dec 11]. Available from: https://scialert.net/fulltext/?doi=ijds.2011.1.12

193. Infant Formula Market Size, Growth, Share | Industry Analysis [2027] [Internet]. [cited 2020 Dec 11]. Available from: https://www.fortunebusinessinsights.com/industry-reports/infant-formula-market-101498

194. Enfamil® Infant | Mead Johnson Nutrition [Internet]. [cited 2020 Dec 9]. Available from: https://www.hcp.meadjohnson.com/products/routine-care-products/enfamilreg-infant/

195. Statista. Whey protein: global market size 2018 & 2024 [Internet]. Statista. [cited 2020 Dec 11]. Available from: https://www.statista.com/statistics/728005/global-whey-protein-market-size/

196. SlimFast Advanced Nutrition Smoothie Mixes | SlimFast [Internet]. [cited 2020 Dec 9]. Available from: https://slimfast.com/products/advanced-smoothie-mixes/

197. Salted Caramel Cashew Flavor [Internet]. Clif Bar & Company. [cited 2020 Dec 9]. Available from: https://shop.clifbar.com/products/clif-whey-protein-salted-caramel-cashew

198. Chocolate | Milk2Go Sport Pro [Internet]. www.milk2go.com. [cited 2020 Dec 9]. Available from: https://www.milk2go.com:443/en/pro-sport/pro-chocolate

199. Health Canada. Canadian Nutrient File [Internet]. 2012 [cited 2020 Dec 11]. Available from: https://food-nutrition.canada.ca/cnf-fce/report-rapport.do

200. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012 Jun;166(3):877–94.

201. Bowe JE, Franklin ZJ, Hauge-Evans AC, King AJ, Persaud SJ, Jones PM. Assessing glucose homeostasis in rodent models. Journal of Endocrinology. 2014 Sep 1;222(3):G13–25.

202. Hryn VH, Kostylenko YP, Yushchenko YP, Lavrenko AV, Ryabushko OB. General comparative anatomy of human and white rat digestive systems: a bibliographic analysis. Wiad Lek. 2018;71(8):1599–602.

203. Yang Y, Santamaria P. Lessons on autoimmune diabetes from animal models. Clin Sci (Lond). 2006 Jun;110(6):627–39.

204. Dufrane D, van Steenberghe M, Guiot Y, Goebbels R-M, Saliez A, Gianello P. Streptozotocin-Induced Diabetes in Large Animals (Pigs/Primates): Role of GLUT2 Transporter and β-cell Plasticity. Transplantation. 2006 Jan 15;81(1):36–45.

205. Mathews CE, Langley SH, Leiter EH. New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation. 2002 Apr 27;73(8):1333–6.

206. van der Werf N, Kroese FGM, Rozing J, Hillebrands J-L. Viral infections as potential triggers of type 1 diabetes. Diabetes Metab Res Rev. 2007 Mar;23(3):169–83.

207. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes. 1988 Sep;37(9):1163–7.

208. Goto Y, Kakizaki M, Masaki N. Production of spontaneous diabetic rats by repetition of selective breeding. Tohoku J Exp Med. 1976 May;119(1):85–90.

209. Moura CS, Lollo PCB, Morato PN, Risso EM, Amaya-Farfan J. Bioactivity of food peptides: biological response of rats to bovine milk whey peptides following acute exercise. Food & Nutrition Research [Internet]. 2017 Mar 7 [cited 2021 Aug 23]; Available from: https://foodandnutritionresearch.net/index.php/fnr/article/view/1156

210. Toedebusch RG, Childs TE, Hamilton SR, Crowley JR, Booth FW, Roberts MD. Postprandial leucine and insulin responses and toxicological effects of a novel whey protein hydrolysate-based supplement in rats. 2012;10.

211. Drummond E, Flynn S, Whelan H, Nongonierma AB, Holton TA, Robinson A, et al. Casein Hydrolysate with Glycemic Control Properties: Evidence from Cells, Animal Models, and Humans. Journal of Agricultural and Food Chemistry [Internet]. 2018 Apr 11 [cited 2021 Aug 23]; Available from: https://pubs.acs.org/doi/pdf/10.1021/acs.jafc.7b05550

212. Aziz A, Anderson GH, Giacca A, Cho F. Hyperglycemia after protein ingestion concurrent with injection of a GLP-1 receptor agonist in rats: a possible role for dietary peptides. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005 Sep 1;289(3):R688–94.

213. Pezeshki A, Fahim A, Chelikani PK. Dietary Whey and Casein Differentially Affect Energy Balance, Gut Hormones, Glucose Metabolism, and Taste Preference in Diet-Induced Obese Rats. The Journal of Nutrition. 2015 Oct 1;145(10):2236–44.

214. Gregersen S, Bystrup S, Overgaard A, Jeppesen PB, Sonderstgaard Thorup AC, Jensen E, et al. Effects of Whey Proteins on Glucose Metabolism in Normal Wistar Rats and Zucker Diabetic Fatty (ZDF) Rats. Rev Diabet Stud. 2013;10(4):252–69.

215. Akhavan T, Luhovyy BL, Brown PH, Cho CE, Anderson GH. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. The American journal of clinical nutrition. 2010;91(4):966–75.

216. Sun C, Li X, Liu L, Canet MJ, Guan Y, Fan Y, et al. Effect of fasting time on measuring mouse blood glucose level. 2016 Feb 29;9:4186–9.

217. Jensen T, Kiersgaard M, Sørensen D, Mikkelsen L. Fasting of mice: a review. Lab Anim. 2013 Oct 1;47(4):225–40.

218. Baena M, Sangüesa G, Dávalos A, Latasa M-J, Sala-Vila A, Sánchez RM, et al. Fructose, but not glucose, impairs insulin signaling in the three major insulin-sensitive tissues. Sci Rep. 2016 May 19;6(1):26149.

219. Aziz A, Anderson GH, Giacca A, Cho F. Hyperglycemia after protein ingestion concurrent with injection of a GLP-1 receptor agonist in rats: a possible role for dietary peptides. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005 Sep 1;289(3):R688–94.

220. Drummond E, Flynn S, Whelan H, Nongonierma AB, Holton TA, Robinson A, et al. Casein hydrolysate with glycemic control properties: evidence from cells, animal models, and humans. Journal of agricultural and food chemistry. 2018;66(17):4352–63.

221. Charles River Laboratories. Wistar Han IGS Rat [Internet]. [cited 2021 Aug 2]. Available from: https://www.criver.com/products-services/find-model/wistar-han-igs-rat?region=3611

222. Weiss O, Segev E, Eilam D. “Shall two walk together except they be agreed?” Spatial behavior in rat dyads. Anim Cogn. 2015 Jan;18(1):39–51.

223. Canadian Council on Animal Care. CCAC guidelines: Rats. 2020;110.

224. Brudzynski SM, Ociepa D. Ultrasonic vocalization of laboratory rats in response to handling and touch. Physiology & behavior. 1992;52(4):655–60.

225. Cloutier S, LaFollette MR, Gaskill BN, Panksepp J, Newberry RC. Tickling, a Technique for Inducing Positive Affect When Handling Rats. J Vis Exp. 2018 May 8;(135):57190.

226. Cloutier S, Wahl KL, Panksepp J, Newberry RC. Playful handling of laboratory rats is more beneficial when applied before than after routine injections. Applied Animal Behaviour Science. 2015 Mar 1;164:81–90.

227. Charles River Laboratories. Vascular Catheterizations Handling Instructions [Internet]. [cited 2021 Aug 2]. Available from: https://www.criver.com/sites/default/files/resources/VascularCatheterizationsHandlingInstructions.pdf

228. Saputo. Milk2Go Sport [Internet]. www.milk2go.com. 2020 [cited 2019 Jun 26]. Available from: https://www.milk2go.com:443/en/sport

229. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016 Mar;7(2):27–31.

230. Diehl K-H, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001 Jan;21(1):15–23.

231. Atcha Z, Rourke C, Neo AH, Goh CW, Lim JS, Aw C-C, et al. Alternative Method of Oral Dosing for Rats. Journal of the American Association for Laboratory Animal Science. 2010;49(3):9.

232. Voluntary Oral Administration of Losartan in Rats. Journal of the American Association for Laboratory Animal Science. 2015;54(5):8.

233. Wheeler TL, Eppolito AK, Smith LN, Huff TB, Smith RF. A novel method for oral stimulant administration in the neonate rat and similar species. Journal of Neuroscience Methods. 2007 Jan;159(2):282–5.

234. Murphy SJ, Smith P, Shaivitz AB, Rossberg MI, Hurn PD. The Effect of Brief Halothane Anesthesia during Daily Gavage on Complications and Body Weight in Rats. 2001;40(2):4.

235. Arantes-Rodrigues R, Henriques A, Pinto-Leite R, Faustino-Rocha A, Pinho-Oliveira J, Teixeira-Guedes C, et al. The effects of repeated oral gavage on the health of male CD-1 mice. Lab Anim. 2012 May;41(5):129–34.

236. Jones CP, Boyd KL, Wallace JM. Evaluation of Mice Undergoing Serial Oral Gavage While Awake or Anesthetized. Journal of the American Association for Laboratory Animal Science. 2016;55(6):6.

237. Brown AP, Dinger N, Levine BS. Stress produced by gavage administration in the rat. Contemp Top Lab Anim Sci. 2000 Jan;39(1):17–21.

238. Refining procedures for the administration of substances. Lab Anim. 2001 Jan 1;35(1):1–41.

239. Boughter W. Guide to Oral Gavage for Mice and Rats [Internet]. [cited 2021 Aug 14]. Available from: https://www.instechlabs.com/blog/guide-to-oral-gavage-for-mice-and-rats

240. Donovan J, Brown P. Blood Collection. Current Protocols in Neuroscience [Internet]. 2005 Oct [cited 2021 Aug 14];33(1). Available from: https://onlinelibrary.wiley.com/doi/10.1002/0471142301.nsa04gs33

241. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1(2):87–93.

242. SegerhagEllen, GybergViveca, IoannidesKyriacos, JenningsCatriona, RydénLars, StagmoMartin, et al. Accuracy of a Simplified Glucose Measurement Device—The HemoCue Glucose 201RT. Diabetes Technology & Therapeutics [Internet]. 2015 Sep 10 [cited 2021 Aug 18]; Available from: https://www.liebertpub.com/doi/abs/10.1089/dia.2014.0354

243. Editor. Blood Sugar Converter [Internet]. Diabetes. 2019 [cited 2021 Aug 18]. Available from: https://www.diabetes.co.uk/blood-sugar-converter.html

244. Yeh S-T. Using Trapezoidal Rule for the Area under a Curve Calculation. :5.

245. Thrivikraman KV, Huot RL, Plotsky PM. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. Brain Res Brain Res Protoc. 2002 Oct;10(2):84–94.

246. Feng J, Fitz Y, Li Y, Fernandez M, Cortes Puch I, Wang D, et al. Catheterization of the Carotid Artery and Jugular Vein to Perform Hemodynamic Measures, Infusions and Blood Sampling in a Conscious Rat Model. J Vis Exp. 2015 Jan 30;(95):51881.

247. Luca TD, Szilágyi KL, Hargreaves KA, Collins KS, Benson EA. Improving the Patency of Jugular Vein Catheters in Sprague–Dawley Rats by Using an Antiseptic Nitrocellulose Coating. J Am Assoc Lab Anim Sci. 2018 Sep;57(5):520–8.

248. Law M, Huot PSP, Lee YT, Vien S, Luhovyy BL, Anderson GH. The effect of dairy and nondairy beverages consumed with high glycemic cereal on subjective appetite, food intake, and postprandial glycemia in young adults. Appl Physiol Nutr Metab. 2017 Nov;42(11):1201–9.

249. Eussen SJPM, van Dongen MCJM, Wijckmans N, den Biggelaar L, Oude Elferink SJWH, Singh-Povel CM, et al. Consumption of dairy foods in relation to impaired glucose metabolism and type 2 diabetes mellitus: the Maastricht Study. Br J Nutr. 2016 Apr 28;115(8):1453–61.

250. Jakubowicz D, Froy O. Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. The Journal of Nutritional Biochemistry. 2013 Jan 1;24(1):1–5.

251. Brown CS, Lichter-Konecki U. Phenylketonuria (PKU): A problem solved? Molecular Genetics and Metabolism Reports. 2016 Mar 1;6:8–12.

252. Trefz KF, Muntau AC, Kohlscheen KM, Altevers J, Jacob C, Braun S, et al. Clinical burden of illness in patients with phenylketonuria (PKU) and associated comorbidities - a retrospective study of German health insurance claims data. Orphanet Journal of Rare Diseases. 2019 Jul 22;14(1):181.

253. Kowalski GM, Bruce CR. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. American Journal of Physiology-Endocrinology and Metabolism. 2014;307(10):E859–71.

Appendices

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DALHOUSIE UNIVERSITY
Inspiring Minds

TO: Erik Vandenboer

CERTIFICATION #: **2018-104**

The above is your certification number for successfully completing the exam **"Introduction to the Care and Use of Laboratory Animals"** based on the following material:

- On-line review of CCAC's ***Core Stream*** AND ***Animals Housed in Vivaria Stream*** modules

Completion of the exam fulfills the initial Canadian Council on Animal Care (CCAC) requirement for the National Institutional Animal User Training Program (NIAUT).

Please retain this number and provide it with any protocols submitted to the University Committee on Laboratory Animals for review.

Date: June 15, 2018

Jennifer Devitt
Training Coordinator
jennifer.devitt@dal.ca
494-8507

DALHOUSIE UNIVERSITY
Inspiring Minds

PRACTICAL TRAINING CERTIFICATION

This is to certify that

Erik Vandenboer

has participated in the practical training session :

THE RAT:
RECOMMENDED TECHNICAL PROCEDURES

- *Video presentation covering humane handling and restraint methods, sexing of adult and neonatal rats, identification of individual animals, anesthesia techniques, and*
- [illegible]
- [illegible]
- [illegible]
- [illegible]
- [illegible]
- [illegible]

Dated : April 11, 2001 @

Jennifer Devitt

Jennifer Devitt
Training Coordinator
jennifer.devitt@dal.ca
494-3849

Appendix C.

Rat #	Session				
	1	2	3	4	5
R1	WP	β-LG	Glu	α-LA	GMP
R2	GMP	Glu	α-LA	β-LG	WP
R3	α-LA	Glu	β-LG	GMP	WP
R4	WP	Glu	β-LG	GMP	α-LA
R5	α-LA	β-LG	Glu	GMP	WP
R6	WP	Glu	β-LG	α-LA	GMP
R7	β-LG	WP	Glu	α-LA	GMP
R	GMP	WP	β-LG	Glu	α-LA
R9	α-LA	Glu	GMP	WP	β-LG
R10	WP	β-LG	α-LA	GMP	Glu
R11	β-LG	GMP	Glu	WP	α-LA
R12	WP	β-LG	Glu	GMP	α-LA

CATALOG# 90060

Ultra Sensitive rat Insulin ELISA Kit

Catalog number

Ultra Sensitive Rat Insulin ELISA 90060

Intended use

A high quality enzyme immunoassay for the quantification of rat insulin in fluid, plasma, and serum.

Test principle

Crystal Chem's Ultra Sentitive Rat Insulin ELISA Kit is based on a sandwich enzyme immunoassay using only a 5 µL sample to produce same day results. The kit can be run using an ultrasensitive low range, wide range, or high range screening method to yield a wide dynamic range with just one kit.

Specifications

Sample Types	Serum, Plasma, and Fluid
Assay Time	Same Day Procedure
Range	Low Range: 0.1-6.4 ng/mL Wide Range: 0.1 - 12.8 ng/mL High Range: 1 - 64 ng/mL
Sample Size	5 µL
Sensitivity	0.05 ng/mL
Precision	CV < 10%

Specificity

Rat Insulin 100%
Mouse Insulin 100%*
Human IGF-I ..... Not detected
Human IGF-II ..... Not detected

*Can vary from lot to lot. See insert in kit.

Highlights

- ✓ Kits use only 5 µL sample
- ✓ Very sensitive (0.05 ng/mL)
- ✓ Run different ranges using the same kit
- ✓ Works with multiple sample types
- ✓ Complete the full test in < 3.5 hours

Summary of protocol

Add 95 µL diluent with 5 µL sample

↓

Incubate 2 hours at 4 °C

↓

Wash plate

↓

Add 100 µL conjugate solution

↓

Incubte 30 minutes at room temperature

↓

Wash plate

↓

Add 100 µL substrate solution

↓

Incubate for 40 minutes (low/wide range)
or 10 minutes (high range)

↓

Add 100 µL stop solution

↓

Measure OD at 450/630 nm

See kit insert or email us for a complete protocol