Effects of Replacing Animal Protein with Plant Protein on Glycemic Control in Individuals with Diabetes

By

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for the degree of Master of Science

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ABSTRACT

The objective was to conduct a systematic review and meta-analysis (SRMA) of randomized controlled trials (RCTs) to assess the effect of replacing animal with plant protein on glycemic control in diabetes and to perform a cross-sectional study using baseline data from 5 RCTs to assess the relationship between replacing animal with plant protein on HbA1c in type 2 diabetes. The SRMA of 12 trials (n=240) showed diets emphasizing replacement of animal with major plant protein sources significantly lowered HbA1c, fasting glucose and fasting insulin compared with control diets. Our cross-sectional study (n=627) showed substitution of animal with plant protein was not associated with HbA1c change. Overall, the results suggest that replacement of animal with plant protein leads to modest improvements in glycemic control in diabetes, however research is needed to address the limitations of our SRMA and lack of agreement with the associations seen in our cross-sectional study.

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LIST OF ABBREVIATIONS

% E – Percent energy
%/d – Percent per day
2h-PG – 2-hour plasma glucose value
ADA – American Diabetes Association
AHS-2 - Adventist Health Study 2
AP – Animal protein
Apo-A1 – Apolipoprotein A1
Apo-B – Apolipoprotein B
BMI – Body mass index
C – Crossover
CAD – Coronary artery disease
CAN – Canada
CCHS - Canadian Community Health Survey
CDA – Canadian Diabetes Association
CHD – Coronary heart disease
CI – Confidence interval
CKD – Chronic kidney disease
CRP – C-reactive protein
CVD – Cardiovascular disease
DA – Dietary advice
DASH – Dietary Approaches to Stop Hypertension
DGAC – Dietary Guidelines Advisory Committee
DNK – Denmark
E3N study – Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale
EASD – European Association for the Study of Diabetes
EPIC study – European Prospective Investigation into Cancer and Nutrition
FAO - Food and Agriculture Organization
FPG – Fasting plasma glucose
g – grams
g/d – grams per day
GHF – Glomerular hyperfiltration
GI – Glycemic index
GFR – Glomerular filtration rate
GRC – Greece
HbA1c – Glycated hemoglobin
HDL-C – High-density lipoprotein cholesterol
HPLC – High-performance liquid chromatography
HR – Hazard ratio
Hs-CRP – high-sensitivity C-reactive protein
HOMA-IR – Homeostasis model assessment- estimated insulin resistance
HT – Hypertension
IDF – International Diabetes Federation
IHD – Ischemic heart disease
IRN – Iran
kg – kilograms
LDL-C – Low-density lipoprotein cholesterol
MD – Mean difference
M – Men
Met – Metabolic feeding control
MetS – Metabolic syndrome
MQS – Heyland Methodological Quality Score
N – Nephropathy
NA – Data not available
NCEP – National Cholesterol Education Program
NHANES – National Health and Nutrition Examination Survey
NO/cGMP – Nitric oxide/cyclic-guanosine-3′,5′-cyclic monophosphate
PREDIMED study – Prevención con Dieta Mediterránea
O – Overweight and/or obese
OGTT - Oral glucose tolerance test
OR – Odds ratio
P – Parallel
PDCAAS – Protein digestibility corrected amino acid score
PER – Protein efficiency ratio
PP – Plant protein
R – Retinopathy
RCT – Randomized controlled trial
RDA - Recommended dietary allowance
RR – Relative risk
SD – Standard deviation
SE – Standard error
SMD – Standardized mean difference
Supp – Supplemental feeding control
T1D – Type 1 diabetes
T2D – Type 2 diabetes
TC – Total cholesterol
UNU – United Nations University
USA – United States of America
USDA – U.S. Department of Agriculture
U.S. – United States
W – Women
WHO – World Health Organization
wk – weeks
y – years
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CHAPTER I – INTRODUCTION
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Type 2 diabetes (T2D) is a global health epidemic. According to the International Diabetes Federation (IDF), there were approximately 387 million adults (8.3%) living with diabetes in 2014, which is projected to increase by 53% by the year 2035. This increasing prevalence, which is exacerbated by increasing urbanization, sedentary lifestyles, and changes in the food environment, is alongside the increasing prevalence in obesity, lower limb amputation, kidney failure, and cardiovascular disease. In addition, diabetes is also a major economic burden, where it accounts for 5-10% of the total health care budget in many countries. Many epidemiological studies and randomized controlled trials (RCTs) have shown that T2D is largely preventable through diet and lifestyle modification, which has shown to be significantly more effective than the use of pharmacological agents. Following a plant-based diet, which encourages the intake of whole plant-based foods and discourages the intake of sources of animal protein and refined/processed foods, is one such modification that has shown to be beneficial for diabetes prevention and management.

More specifically, following a vegetarian or vegan diet, which is characterized as being high in major plant protein sources (i.e. soy, soy-derived foods, pulses and nuts) and deficient in animal protein, has been shown to be protective against the development of T2D and improve glycemic control in individuals with T2D. Studies looking at higher intakes of specific plant protein sources alone have also shown similar benefits. Diets high in animal protein, on the other hand, especially sources of red meat, are associated with an increased diabetes risk, do not show any glycemic control benefit, and have even been suggested to be considered as a risk factor for T2D. Furthermore, high intakes of animal protein have been shown to have negative impacts on our environment, which is a current growing global concern.

Despite this evidence, diabetes association guidelines (i.e. ADA and EASD) have not made any specific recommendations for replacing animal protein with plant protein or the intake of major plant protein sources for diabetes management. One exception to this is dietary pulses (e.g. beans, peas, chick peas, lentils), which have recently been recommended by the CDA in their most recent clinical practice guidelines for improving glycemic control in individuals with T2D. The evidence from RCTs looking at the effect of replacing animal with plant protein on glycemic control in individuals with diabetes remains inconsistent, where some have shown significant improvements in glycemic control, whereas others show no effect. In order to address this gap in knowledge and better understand the relationship between replacing animal with plant protein on glycemic control, this thesis work will present 1) the results of a systematic review and meta-analysis of RCTs on the effect of replacing sources of animal protein with major sources of plant protein on glycemic control in individuals with
diabetes (Chapter IV) and 2) the results of a cross-sectional study of baseline data from 5 RCTs on the association between replacing animal protein with plant protein on HbA1c in individuals with T2D (Chapter V).
CHAPTER II – LITERATURE REVIEW
CHAPTER II – LITERATURE REVIEW

2.1 DIABETES

Diabetes is a group of metabolic diseases characterized by the presence of hyperglycemia due to the body not being able to produce enough insulin, not being able to effectively respond to the action of insulin, or both\(^2\). Several classifications of diabetes exist, however, the majority fall into two broad categories: type 1 diabetes and type 2 diabetes\(^31\). Type 1 diabetes (T1D), previously referred to as insulin-dependent, juvenile or child-onset diabetes\(^32\), accounts for 5-10% of those with diabetes and is primarily a result of pancreatic \(\beta\)-cell destruction that usually leads to the body no longer being able to produce insulin\(^24, 31, 32\). Type 2 diabetes (T2D), previously referred to as non-insulin-dependent or adult-onset diabetes\(^32\), accounts for 90-95% of those with diabetes and is a heterogeneous disorder, with its main features consisting of reduced insulin sensitivity in various tissues and organs and a progressive decline in pancreatic \(\beta\)-cell function\(^24, 31, 33\). Both forms of diabetes have been increasing worldwide\(^34\), especially T2D, which has continued to increase in parallel with the dramatic rise in obesity\(^35\). This disease, which mostly affected adults, is now emerging in children and adolescents\(^35\) and has also become a major economic burden, with 5-10% of the total health care budget being used for T2D in many countries\(^2\).

2.1.1 PREVALENCE OF DIABETES

According to the International Diabetes Federation (IDF) in the year 2014 the global prevalence of diabetes was 8.3%, which is equivalent to approximately 387 million people living with diabetes. Among these individuals, the highest prevalence rates were reported in North America and the Caribbean (11.4% or approximately 39 million)\(^36\). In Canada, the most recent estimates from Statistics Canada report that 6.6% of the population living with diabetes, which is equivalent to approximately 2 million people\(^37, 38\). Similar estimates have been reported for the U.S. (9.3% or approximately 29.1 million people)\(^39\). The IDF projects that there will be a 53% increase in the number of people living with diabetes by the year 2035\(^36\).

2.1.2 PATHOPHYSIOLOGY OF DIABETES

Since T1D and T2D lead to hyperglycemia, individuals with either form can present with the following signs and symptoms: frequent urination (polyuria), excessive thirst (polydipsia), increased hunger (polyphagia), blurred vision, weight loss, lack of energy, frequent infections, slow-healing wounds, and tingling sensation or numbness in the hands or feet\(^4, 2, 31\).
T1D is defined as an auto-immune disease that is characterized by cellular-mediated destruction of the pancreatic β-cells, which usually leads to an absolute deficiency in insulin secretion\textsuperscript{31, 40}. The cause of T1D is not fully understood and continues to be debated, but is said to be multifactorial with both genetics and environment playing a role\textsuperscript{40}.

T2D on the other hand results from impaired insulin action and/or impaired insulin secretion\textsuperscript{2, 31}. The disease is also multifactorial and involves several organs and tissues, including the pancreas, liver, skeletal muscle, adipose tissue, the kidneys, the gastrointestinal tract and the brain\textsuperscript{33}. Although the precise mechanisms underlying the pathogenesis of T2D are not fully understood, there are several prevailing theories, some of which include: dysfunctional pancreatic β-cells, excessive accumulation of lipids, impaired fatty acid oxidation, a defect in insulin-mediated glucose uptake in skeletal muscle, and impaired sensing and response to hyperglycemia in the central nervous system due to genetic predisposition, physical inactivity, and obesity\textsuperscript{2, 33}.

2.1.3 DIABETES COMPLICATIONS AND MORTALITY

A consistent high level of circulating blood glucose over time in diabetes is associated with microvascular complications that affect the eyes (retinopathy), nerves (neuropathy), blood vessels (arterial stiffness), heart (CVD) and kidneys (nephropathy)\textsuperscript{1, 31}. In most high-income countries, diabetes is a leading cause of blindness, lower limb amputation, CVD, and kidney failure\textsuperscript{1}.

In terms of mortality, it was estimated that approximately 4.9 million people died globally from diabetes in the year 2014. Since estimating the number of deaths from diabetes can be somewhat challenging due to lack of data and underestimates made by existing health statistics, this number should be interpreted with caution\textsuperscript{1}. In Canada, only 3.1% of all deaths were attributed to diabetes in 2007, whereas 29.9% of individuals who died in 2008/09 had diabetes\textsuperscript{41}. This is because diabetes on its own does not usually lead to death directly, but the complications associated with diabetes do\textsuperscript{41}. Cardiovascular disease in particular is the leading cause of death in individuals with diabetes\textsuperscript{1}.

2.1.4 DIABETES RISK FACTORS

There are several risk factors associated with the development of T2D, which include: family history of diabetes (first-degree relative), increasing age (≥40 years), ethnicity (e.g. Aboriginal, African, Asian, Hispanic or South Asian descent), being overweight, having abdominal obesity, hypertension, low HDL cholesterol levels (<1.0 mmol/L in males and <1.3 mmol/L in females), elevated triglyceride levels (≥1.7 mmol/L), unhealthy diet, physical inactivity, or a history of gestational diabetes\textsuperscript{1, 31}. Having prediabetes, a term referring to impaired fasting glucose (fasting plasma glucose values between 5.6-6.9
mmol/L\textsuperscript{31}), impaired glucose tolerance (2-hour plasma glucose values from an OGTT between 7.8-11 mmol/L\textsuperscript{31}) or having an HbA1c value between 6.0% to 6.4%, also places individuals at high risk of developing T2D\textsuperscript{24,31}.

In terms of T1D, the risk factors are currently still being researched, but having a family member with T1D, environmental factors and exposure to some viral infections have been linked to an increased risk\textsuperscript{1,42}.

2.1.5 DIAGNOSIS OF DIABETES

There are multiple tests that can be used to diagnose diabetes. According to the CDA 2013 Clinical Practice Guidelines, in order to be diagnosed with diabetes an individual must have a fasting plasma glucose (FPG) value ≥7.0 mmol/L, HbA1c value ≥6.5% (in adults), 2-hour plasma glucose value (2h-PG) from a 75 g oral glucose tolerance test (OGTT) ≥11.1 mmol/L, or a random (any time of day) plasma glucose value ≥11.1 mmol/L\textsuperscript{24}.

At the time of diagnosis, it may be difficult to distinguish between T1D and T2D in certain situations. As a result, it may be useful to use physical signs of insulin resistance and autoimmune markers, such as anti-glutamic acid decarboxylase (GAD) or anti-islet cell antibody (ICA) antibodies, however, these have not been adequately studied as diagnostic tests\textsuperscript{24}.

2.1.6 MEASURES OF GLYCEMIC CONTROL

There are a number of advantages and disadvantages for each test used in the diagnosis of diabetes, which are summarized in Table 2.1. FPG, HbA1c, and 2h-PG from a 75 g OGTT each predict the development of retinopathy\textsuperscript{24}, whereas HbA1c is also a CVD risk factor and a better predictor of macrovascular events\textsuperscript{24,43,44}. In addition, FPG and 2h-PG from a 75 g OGTT have high day-to-day variability and FPG reflects glucose homeostasis at a single point in time. HbA1c, on the other hand, has low day-to-day variability, reflects long-term glucose concentration\textsuperscript{24}, and is considered the gold standard for assessing glycemic control\textsuperscript{45}, however it is poor for detecting pre-diabetes.
**Table 2.1** – Advantages and disadvantages of diagnostic tests for diabetes (adapted from 2013 CDA clinical practice guidelines)

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| **FPG** | • Established standard  
• Single sample  
• Fast, easy  
• Predicts microvascular complications | • High day-to-day variability  
• Sample not stable  
• Inconvenient (no caloric intake for at least 8 hours)  
• Reflects glucose homeostasis at a single point in time |
| **HbA₁c** | • Convenient  
• Single sample  
• Does not require fasting  
• Low day-to-day variability  
• Reflects long-term glucose concentration  
• Predicts microvascular complications  
• Better predictor of macrovascular disease than FPG or 2h-PG from a 75 g OGTT | • Expensive  
• Standardized, validated assay required  
• Altered by ethnicity and aging  
• Not for diagnostic use in children, adolescents, pregnant women or those with suspect T1D  
• Misleading in various medical conditions (e.g. hemoglobinopathies, iron deficiency, hemolytic anaemia, severe hepatic or renal disease) |
| **2h-PG from a 75 g OGTT** | • Established standard  
• Predicts microvascular complications | • Expensive  
• High day-to-day variability  
• Sample not stable  
• Inconvenient (requires continuous monitoring over 2-3 hours) |

FPG=fasting plasma glucose; OGTT=oral glucose tolerance test; 2h-PG=2-hour plasma glucose

### 2.1.7 PREVENTION AND MANAGEMENT OF DIABETES

Although there is currently no evidence of any interventions that can prevent or delay the development of T1D, prevention of T2D can largely be achieved by lifestyle modification through diet, physical activity and weight loss, which has shown to be more effective than pharmacological agents.

For individuals living with diabetes, achieving optimal glycemic control is an integral component in management of the disease. The CDA recommends a range of targets depending on an individual’s age, diabetes duration, risk of severe hypoglycemia, presence or absence of CVD, as well as expectancy of life. For most people with T1D and T2D, it is recommended that they target an HbA₁c level of ≤7.0%, which is consistent with those recommended by the ADA and EASD (general HbA₁c target level of <7%)³³,³⁶,³⁷,³⁸. Achieving such a target level can help reduce one's risk of microvascular complications, as well as macrovascular complications if implemented early in the disease. One such method that can help in achieving optimal glycemic control is nutrition therapy, which has been shown to lead to a 1-2% reduction in HbA₁c and reduce the use of diabetes medications.⁴⁹,⁵⁰,⁵¹
2.2 PLANT AND ANIMAL PROTEIN AND DIABETES

2.2.1 SOURCES OF PLANT AND ANIMAL PROTEIN IN THE DIET

Several sources of protein fall under the ‘Meat and Alternatives’ category of Health Canada’s Food Guide and the ‘Protein Food Group’ category of the USDA’s MyPlate. The major sources of plant protein under these categories consist of cooked legumes (i.e. pulses such as chickpeas and lentils; peanuts and soybeans), soy products (e.g. tofu, tempeh, texturized vegetable protein [TVP]), as well as nuts and seeds. Animal protein sources listed under these categories comprise of seafood (i.e. fish and shellfish), poultry (e.g. chicken and turkey), lean meat (e.g. beef, lamb and pork) and eggs. Although dairy products, such as milk, cheese, yogurt, and soymilk (a plant source) are not listed under the protein food group category, they are often consumed as a source of protein in some vegetarian diets (i.e. lacto-ovo vegetarian diet).

When comparing animal protein with plant protein sources most plant protein sources are lower in saturated fat and cholesterol, higher in fibre, free of heme iron, and are good sources of antioxidants and phytochemicals. Plant and animal protein also appear to differ in protein quality. Currently there are several different methods that can be used to evaluate protein quality, with each method consisting of its own limitations. In the U.S. and many other countries the protein digestibility corrected amino acid (PDCAAS) method has been used for over 20 years as the official method for determining protein quality, whereas in Canada the official method consists of a protein rating, which is based on the Protein Efficiency Ratio (PER). Since foods are frequently consumed in complement with one another, questions have been raised around the significance of using methods like the PDCAAS to assess the protein quality of single protein sources. Regardless of which score is used, however, meat, poultry, fish, eggs, dairy foods and soy protein are considered to be sources of high quality protein since they provide all 9 essential amino acids (referred to as complete protein sources), whereas protein found in legumes (other than soy), grains, nuts, seeds and vegetables can be deficient in one or more essential amino acids and are considered to be lower quality sources of protein (see Table 2.2 for a list of essential and non-essential amino acids). According to the American Dietetic Association and the Dietitians of Canada, however, consuming an assortment of plant foods over the course of a day can provide all the essential amino acids and when well planned, the typical protein intakes of lacto-ovo vegetarians and vegans appear to meet and exceed requirements. In addition, some plant protein sources have recognized health benefits, where both nuts and soy protein have been issued a health claim from the FDA and Health Canada, respectively, for their cholesterol lowering benefit.
There has been an increasing awareness and interest in plant-based diets over the last decade, which is evident by the many fast-food restaurants and university foodservice facilities that now offer a variety of meat free options\textsuperscript{55, 68} and by the significant growth in the U.S. market for meatless foods (e.g. meat analogs, vegetarian burgers, soymilk)\textsuperscript{55, 69}. This increased interest in eliminating animal protein from the diet is fueled by a number of reasons, where the most common ones include: health considerations, concern for the environment, economical reasons, ethical considerations/animal welfare factors and religious beliefs\textsuperscript{55}.

**TABLE 2.2** – List of essential and non-essential amino acids

<table>
<thead>
<tr>
<th>Essential amino acids\textsuperscript{a}</th>
<th>Nonessential amino acids\textsuperscript{b}</th>
<th>Conditional amino acids\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Alanine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Asparagine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Aspartic Acid</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Glutamic Acid</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Amino acids that cannot be produced by the body and must come from food intake\textsuperscript{70} (also referred to as indispensable amino acids)

\textsuperscript{b}Amino acids that can be produced by the body without food intake\textsuperscript{70} (also referred to as dispensable amino acids)

\textsuperscript{c}Amino acids that are usually not essential, but can be in times of illness and stress\textsuperscript{70}

**2.2.2 POPULATION LEVELS OF PLANT AND ANIMAL PROTEIN INTAKE IN THE U.S., CANADA AND EUROPE**

Based on analysis of data collected from across North America and Europe it appears that the major contributor to total protein intake is animal protein. Among plant protein intake, grain sources appear to be the greatest contributor, whereas intake of major plant protein sources, such as soy, pulses and nuts is low. According to data analyzed from NHANES 2007-2010, animal protein sources were the main contributors to total protein intake in the U.S. population, where poultry, meats and mixed dishes (consisting of meat, poultry and fish) were the top 3 contributors, contributing to 10\%, 9.5\% and 7.5\% of total protein intake, respectively\textsuperscript{62}. Grain sources such as breads, rolls, and tortillas were the 4th largest
contributors, contributing 6.4% of total protein intake, whereas other plant protein sources were one of the lowest contributors, contributing to 3.2% of total protein intake. Overall, this data is consistent with previous data analyzed from NHANES 2003-2006 and 1988-1991. It is important to note that although poultry and meats were the top 2 contributors to total protein intake, they contributed <4% to total daily energy intake, whereas breads, rolls, and tortillas were the most significant contributors to total daily energy intake, contributing 7%. Similar patterns of protein intake were reported from 27 centers across 10 countries in Europe participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which showed that animal protein was the greatest contributor to total protein intake across majority of the centers, contributing 55-73% of total protein, whereas plant protein accounted for 24-39%. Across all centers, cereals were the greatest contributors to plant protein. Although data on total plant and animal protein intake in Canada is limited, data analyzed from the 2004 Canadian Community Health Survey (CCHS) have reported intakes of specific plant protein sources, such as pulses and soy. In terms of pulses, it has been reported that only 13% of Canadians consume pulses on any given day, where daily intakes among consumers ranged from 13 g/d in the lowest quartile to 294 g/d in the highest quartile (less than 2 cups). Soy consumption has been reported to be even lower, where only 3.3% of Canadians consumed soy foods on any given day, with daily intakes ranging from 1.5 g/d among low consumers to 16.5 g/d among high consumers (1 serving tofu=150g= ¾ cup). Overall, these data appear to be consistent with the overall trends in protein intake within the U.S. and Europe.

2.2.3 GUIDELINES FOR PLANT AND ANIMAL PROTEIN CONSUMPTION

In terms of recommendations for total protein intake in individuals with diabetes, the 2013 CDA clinical practice guidelines state that there is no evidence to support that individuals with diabetes should alter their usual protein intake, which represents 15-20% of their total energy intake (1 to 1.5 g per kg per body weight per day). Similarly, the ADA states that the evidence on which to recommend an ideal amount of protein intake for optimizing glycemic control or improving one or more CVD risk measures are not conclusive. For individuals with diabetes and chronic kidney disease (CKD) the recommendations for total protein intake are inconsistent. The CDA states that it is important to consider targeting a level of intake that does not exceed the recommended dietary allowance (RDA) of 0.8 g per kg body weight per day and that the quality of protein intake must also be optimized in order to meet the essential amino acids requirements. The EASD, however, states that there is insufficient evidence on which to recommend protein restriction and protein type for individuals with diabetes and CKD. Similarly, the ADA does not recommend individuals with diabetes and CKD to reduce their protein intake.
intake below usual intakes since it does not appear to alter glycemic and cardiovascular measures or a decline in glomerular filtration rate (GFR)\textsuperscript{26}.

In terms of recommendations for specific sources of protein, public health guidelines in the U.S. and Canada, as well as international dietary guidelines issued by the World Health Organization (WHO) recommend daily intakes of major sources of plant protein, such as soy, soy-derived foods (e.g. tofu), pulses and nuts. Health Canada’s Food Guide recommends the intake of 2-3 servings of meat alternatives per day, such as beans, lentils, tofu, nuts and seeds. This is equivalent to 1½ -2¼ cups of beans or tofu per day and ½-¾ cup of nuts or seeds per day\textsuperscript{53}, which are similar to recommendations made by the USDA’s MyPlate\textsuperscript{78}. In a report of a joint WHO/FAO expert consultation, a minimum intake of 20 g/d of legumes (< 1 serving) was recommended in order to achieve adequate intakes of non-starch polysaccharides and to reduce overweight and obesity, CVD, and T2D risk\textsuperscript{79}. Diabetes association guidelines (i.e. CDA, ADA, and EASD), however, have not made any specific recommendations for replacing animal protein with plant protein or the intake of major sources of plant protein for achieving optimal glycemic control\textsuperscript{25, 26, 49}. One exception to this is dietary pulses (e.g. beans, peas, chick peas, lentils), which have recently been recommended by the CDA in their most recent clinical practice guidelines for improving glycemic control in individuals with T2D (based on Grade B, Level 2 evidence)\textsuperscript{49} and by the EASD which recommend at least 4 servings of legumes per week in order to help meet minimum requirements for fibre intake (Grade A evidence)\textsuperscript{25}. Although the ADA acknowledges the option of adopting a diet high in plant protein sources, such as a vegetarian or vegan diet, as an acceptable dietary pattern for diabetes management (Grade E evidence), they make no specific recommendations for the intake of major plant protein sources\textsuperscript{26}. Overall, the evidence on which most of these recommendations were made on has been assigned a low grade or is out dated. Therefore, the lack of data to support certain dietary recommendations, as well as the low-grade evidence that forms the basis of current recommendations in diabetes association guidelines calls for stronger evidence in this area.

\subsection*{2.2.4 PLANT-BASED DIETARY PATTERNS AND DIABETES}

Over the past few decades’ evidence from prospective cohort studies and RCTs have both highlighted the importance of lifestyle modification in the prevention and management of diabetes. Changes in lifestyle consisting of increased physical activity, healthy eating and weight loss have been shown to prevent 58\% of T2D cases, which is a greater reduction than that shown for pharmacologic agents (i.e. metformin)\textsuperscript{4}. Currently there are several well-known plant-based dietary patterns, such as Mediterranean, vegetarian or vegan, Portfolio and DASH, all of which encourage (to some degree)
higher intakes of whole plant-based foods when compared to a Western diet and discourage intakes of animal protein, as well as refined and processed foods. Many studies have shown that these plant-based dietary patterns are associated with a lower risk for various chronic diseases, such as metabolic syndrome (MetS), cardiovascular disease (CVD), cancer, and all-cause mortality. There have also been a number of studies looking at the relationship between plant-based dietary patterns, diabetes risk and glycemic control, which are summarized and discussed in the following two sections.

2.2.4.1 Plant-Based Dietary Patterns and Diabetes Risk in Prospective Cohort Studies

The most recent systematic reviews and meta-analyses of prospective studies looking at the association between following a Mediterranean diet and diabetes risk found that adherence to a Mediterranean diet was associated with a significant reduction in the risk of diabetes, one showing a 19% reduction and the other showing a 23% reduction. Large prospective cohort studies conducted in the Seventh-day Adventists, a population who tend to avoid smoking, alcohol and caffeine intake, have shown that vegetarians are associated with having a lower risk of diabetes in comparison to non-vegetarians. This was shown in Adventist Mortality Study, which followed over 25,000 white Seventh-day Adventists over a 21 year duration and the Adventist Health Study 2 (AHS-2), which followed over 40,000 men and women over a 2 year duration. The results of the latter study showed that the odds ratio (OR) for developing diabetes for vegans, lacto-ovo vegetarians and semi-vegetarians was 0.38 (95% CI: 0.24, 0.62), 0.62 (95% CI: 0.50, 0.76), and 0.49 (95% CI: 0.31, 0.76), respectively, in comparison to non-vegetarians. There has also been one systematic review and meta-analysis of prospective cohort studies looking at the DASH diet in relation to diabetes risk, which showed that diets assessed as high quality by the DASH score were associated with a 21% reduction in T2D risk. Overall, this evidence from prospective cohort studies shows that following a plant-based dietary pattern has beneficial implications for diabetes risk reduction.

2.2.4.2 Plant-Based Dietary Patterns and Glycemic Control in Controlled Dietary Trials

Several systematic reviews and meta-analyses of controlled dietary trials have been conducted looking at the effect of a plant-based dietary pattern on glycemic control in individuals at risk for or diagnosed with T2D. A systematic review and meta-analysis of 9 RCTs ≥ 4 weeks conducted in individuals with T2D (n=1178) showed that following a Mediterranean dietary pattern led to significant reductions in HbA1c (MD: -0.30; 95% CI: -0.46, -0.14), fasting glucose (MD=-0.72 mmol/l; 95% CI: -1.24 to -0.21) and fasting insulin (MD=-0.55 μU/ml; 95% CI: -0.81 to -0.29). These results were somewhat consistent with a network meta-analysis consisting of data from 8 controlled trials carried out in individuals at high risk for or diagnosed with T2D, which showed that the Mediterranean diet significantly reduced HbA1c when
compared to usual care but not when compared to other dietary treatments\(^9\). In addition, Mediterranean diets, when compared to low fat and low-carbohydrate diets, have shown to delay the need for antihyperglycemic medication in overweight individuals with newly diagnosed T2D\(^9\). Similarly, a systematic review and meta-analysis of 6 controlled trials ≥ 4 weeks (n=255) looking at the effect of vegetarian diets (majority of which were low-fat vegan diets) on glycemic control in middle-aged individuals with T2D showed an overall clinically significant reduction in HbA\(_1c\) (MD=-0.39; 95% CI: -0.62, -0.15) and a non-significant reduction in fasting glucose (MD=-0.36 mmol/L ; 95% CI: -1.04, 0.32) in comparison to comparator diets.\(^9\) In terms of the DASH diet, there has been one systematic review and meta-analysis of RCTs conducted in individuals with various health statuses (e.g. T2D, MetS, otherwise healthy, etc.) which showed following a DASH diet significantly reduced fasting insulin (MD: -0.15; 95% CI: -0.22, -0.08) and non-significant reductions in fasting glucose and HOMA-IR\(^10\). Lastly, a controlled trial looking at the effect of a modified portfolio diet in conjunction with medical management in individuals with T2D (n=30) after receiving by-pass surgery showed a non-significant reduction in both glucose and insulin concentrations in individuals after a 6-weeks\(^10\). Overall, these findings show that plant-based dietary patterns may be useful for the management of glycemia in individuals at risk for or diagnosed with T2D and appear to be consistent with prospective cohort studies showing benefits for diabetes risk.

2.2.5 PLANT PROTEIN AND DIABETES

2.2.5.1 Plant Protein And Diabetes Risk in Prospective Cohort Studies

There have been a number of prospective cohort studies that have looked at the association between total plant protein, as well as specific sources of plant protein, and diabetes risk. For total plant protein, three large prospective cohort studies have been conducted and show inconsistent findings. One of them, which was conducted using data from over 35 000 participants participating in the EPIC study over a follow-up duration of 10 years, showed that plant protein was not associated with diabetes incidence, whereas diets high in protein and animal protein were associated with an increased diabetes risk\(^10\). It is important to note, however, that the main contributors to plant protein intake in this study were bread (43%), fruit and vegetables (14%), and potatoes (9%), which are not considered to be major sources of plant protein\(^10\). The second study, which used data from over 85 000 women participating in the Nurses' Health Study over a follow-up duration of 20 years, assessed the relationship between low-carbohydrate diets and T2D risk and showed that low-carbohydrate diets high in plant sources of protein and fat modestly reduced T2D risk (RR=0.82; 95% CI: 0.71, 0.94), whereas low-carbohydrate diets high in animal sources of protein and fat did not show this benefit (RR=0.99; 95% CI: 0.85, 1.16)\(^8\). Similarly, the
most recent prospective cohort study, which was conducted in a diabetes-free cohort of over 92 000 women from the Nurses’ Health Study II and over 40 000 men from the Health Professionals Follow-up Study, showed that after a 4 year follow-up higher intakes of total protein and animal protein were associated with an increased T2D risk (HR= 1.07; 95% CI: 1.01, 1.17 and HR=1.13; 95% CI: 1.06, 1.21, respectively), whereas higher intake of plant protein was associated with a moderate decreased T2D risk (HR=0.91; 95% CI: 0.84, 0.98)\(^{103}\).

One systematic review and meta-analysis of prospective cohort studies has been conducted to assess the association between legume intake and diabetes risk. It consisted of only 2 studies, both of which showed different results. One of these studies was conducted in a cohort of over 60 000 middle-aged Chinese women over a follow-up duration of approximately ~5 years and showed that total legume intake was inversely associated with T2D risk when comparing the upper quintile of intake to the lower quintile of intake (RR: 0.62; 95% CI: 0.51, 0.74). In addition, when looking at different legume groups, soybean intake was inversely associated with T2D risk (RR: 0.53; 95% CI: 0.45, 0.62), whereas soy products (other than soy milk) and soy protein consumption (protein derived from soy beans and their products) were not\(^{14}\). The second study was also a large prospective cohort study consisting of data from over 35 000 older women participating in the Iowa Women's Health Study over a follow-up duration of 6 years and showed no significant association between intake of mature beans and T2D risk\(^{104}\). A more recent prospective cohort study conducted in a population of men and women in Mauritius (n=1421) over a 6 year follow-up duration showed that women with the highest tertile of pulse consumption had a reduced risk of developing abnormal glucose metabolism (OR: 0.52 [95% CI: 0.27, 0.99]) in comparison to those in the lowest tertile of consumption, which supports findings from the former study\(^{105}\).

Similarly, systematic reviews and meta-analyses of prospective cohort studies looking at association between nut intake and diabetes risk showed inconsistent results. Of the 4 most recent meta-analyses only one showed that nut intake was inversely associated with T2D risk (RR: 0.87; 95% CI: 0.81, 0.94)\(^{13}\), whereas three showed no significant associations between nut intake and T2D risk\(^{82, 83, 106}\).

In summary, prospective cohort studies looking at total plant protein and specific sources of plant protein show inconsistent findings in relation to diabetes risk, which may be due to the overall low consumption of these foods in the diet.

**2.2.5.2 Plant Protein and Glycemic Control in Controlled Dietary Trials**

There have been several systematic reviews and meta-analyses of RCTs looking at the effect of specific sources of plant protein on glycemic control. In terms of soy and soy products, two systematic reviews and meta-analyses of RCTs have been conducted, one including 24 trials (n=1518) in individuals
with various health statuses (e.g. otherwise healthy, obese/overweight, hypercholesterolemic, hypertensive, diabetes, etc.)\(^\text{107}\) and the other including 8 trials in individuals with T2D\(^\text{108}\). Both showed no overall effect on HbA\(_{1c}\), fasting glucose, fasting insulin, and/or HOMA-IR, however the direction of the effect favoured soy intake.\(^\text{107, 108}\)

In terms of dietary pulses, there have been a series of meta-analyses conducted by our group looking at the effect of incorporating dietary pulses into the diet alone or in the context of a low GI or high-fibre diet in individuals with and without diabetes\(^\text{11}\). Pulses alone (11 trials) were found to significantly lower fasting glucose (SMD = -0.82; 95% CI: -1.36, -0.27) and fasting insulin (SMD= -0.49; 95% CI: -0.93, -0.04) and in the context of a low-GI diet (19 trials) they were found to significantly lower glycosylated blood proteins (measured as HbA\(_{1c}\) or fructosamine, SMD= -0.28; 95% CI: -0.42, -0.14). In the context of a high-fibre diet (11 trials), they were also found to significantly lower glycosylated blood proteins (SMD = -0.27; 95% CI: -0.45, -0.09), as well as fasting glucose (SMD = -0.32; 95% CI: -0.49, -0.15)\(^\text{11}\). Findings from more recent RCTs show that the effect of legumes as part of a low GI diet in individuals with T2D (n=121) are consistent with these findings\(^\text{109}\), whereas the effect of a legume-enriched diet in first degree relatives of patients with diabetes (n=26) showed no significant effect on fasting glucose or HbA\(_{1c}\)\(^\text{110}\).

Lastly, in terms of tree nut intake, there have been two systematic reviews and meta-analyses conducted by our group, one in individuals with T2D (n=450)\(^\text{12}\) and one in individuals with at least one criterion of the MetS (n=2226)\(^\text{10}\). Both found significant improvements in fasting glucose (MD = -0.15 mmol/L; 95% CI: -0.27, -0.02 mmol/L and MD=-0.08 mmol/L ; 95% CI -0.16, -0.01 mmol/L, respectively)\(^\text{10, 12}\), as well as HbA\(_{1c}\) in individuals with T2D (MD = -0.07%; 95% CI:-0.10, -0.03%)\(^\text{12}\). Several other RCTs have been published since the conduct of these 2 meta-analyses, where majority appear to be consistent with our findings\(^\text{111-115}\).

Overall, incorporation of major plant protein sources into the diet appears to be beneficial for glycemic control.

**2.2.5.3 Plant Protein and Other Cardiometabolic Risk Factors in Controlled Dietary Trials**

Incorporation of major plant proteins into diet have also been shown to be beneficial for a number of other cardiometabolic risk factors. Systematic reviews and meta-analyses of RCTs show that nut consumption, dietary pulses and soy protein have beneficial effects on blood pressure\(^\text{116-118}\), measures of kidney function (i.e. serum creatinine and phosphorus concentrations, proteinura)\(^\text{119, 120}\), blood lipids\(^\text{10, 121-124}\), body weight\(^\text{125, 126}\), and inflammatory markers (i.e. CRP and hs-CRP)\(^\text{127}\). Overall, the results of these meta-analyses show strong support of plant proteins having beneficial effects on other cardiometabolic risk factors that affect diabetes prevention and management.
2.2.6 ANIMAL PROTEIN AND DIABETES

2.2.6.1 Animal Protein and Diabetes Risk in Prospective Cohort Studies

The consumption of animal protein sources and its impact on various health outcomes has received an increasing amount of attention over the last several years, especially in relation to its risk for various chronic diseases such as MetS\textsuperscript{128}, CVD\textsuperscript{129}, cancer\textsuperscript{130,131} and all-cause mortality\textsuperscript{132-134}. Some have even gone as far as to suggest that meat consumption be used as an identifiable risk factor for diabetes\textsuperscript{32}. The findings from several systematic reviews and meta-analyses of prospective cohort studies looking at various animal protein sources, such as red meat, dairy, fish, poultry, eggs and diabetes risk are summarized and discussed below.

In terms of meat consumption, there have been three systematic reviews and meta-analyses of prospective cohort studies conducted looking at association between meat consumption and T2D risk. The first meta-analysis by Aune et al. found that when comparing the highest to lowest categories of intake, red meat and processed meat were both associated with a higher T2D risk (RR: 1.21; 95% CI: 1.07, 1.38 and RR: 1.41; 95% CI: 1.25, 1.60, respectively), whereas total meat was not (RR: 1.17; 95% CI: 0.92, 1.48)\textsuperscript{15}. The remaining 2 meta-analyses specifically looked at intakes of unprocessed and processed red meat\textsuperscript{16,135}. The meta-analysis by Pan et al. found that unprocessed and processed red meat were both associated with a higher T2D risk (RR: 1.19; 95% CI: 1.04, 1.37 per 100 g/d and RR: 1.51; 95% CI: 1.25, 1.83 per 50 g/d, respectively)\textsuperscript{16}. The third meta-analysis by Micha et al. reported similar findings\textsuperscript{135}.

In addition, a meta-analysis consisting of over 50 000 Caucasians showed that a higher consumption of processed meat was associated with significantly higher fasting glucose levels and higher consumption of unprocessed meat was associated with significantly higher fasting glucose and insulin levels\textsuperscript{136}. Since the publication of these meta-analyses, several other prospective cohort studies have also been published, some of which are consistent with these findings whereas others are not. In terms of consistency, one of these studies, which analyzed data from 3 U.S. cohorts, found that in comparison to the group that had no changes in red meat intake, increasing intake of red meat by more than ½ serving per day was associated with a 48% higher risk in the subsequent 4-year period (HR: 1.30; 95% CI: 1.21, 1.41), whereas reducing meat intake by more than this amount was associated with a 14% lower risk (HR: 0.86; 95% CI: 0.80, 0.93)\textsuperscript{17}. In terms of inconsistency, three prospective cohort studies conducted in the Malmö Diet and Cancer study (n=27 140)\textsuperscript{137}, the Strong Heart Family Study (n=2001)\textsuperscript{138}, and the E3N study (n=66 118)\textsuperscript{139} found that higher intakes of processed red meat were associated with a higher risk of T2D, whereas unprocessed red meat was not. There was also one study, which was conducted in the Japan Public Health Center-based Prospective Study (n=63 849; follow-up= 5 years), that found total meat and total red meat were associated with a higher T2D risk in men but not in women (OR: 1.36
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[95% CI: 1.07, 1.73] and OR: 1.48 [95% CI: 1.15, 1.90], respectively) and that processed red meat was not associated with T2D risk in either men or women\textsuperscript{140}. Therefore based on the results of these meta-analyses and recent prospective cohort studies, it appears that red meat is associated with T2D risk, but it remains unclear whether there are differences between processed and unprocessed red meat.

In terms of dairy consumption, 4 systematic reviews and meta-analyses of prospective cohort studies have been conducted. The findings for total dairy intake are mixed, where three meta-analyses showed a significant inverse association between total dairy intake and T2D risk\textsuperscript{141-143} and one meta-analysis showed no significant association\textsuperscript{144}, which was also supported by a more recent prospective cohort study conducted in the The Malmö Diet and Cancer study\textsuperscript{137}. For specific sources of dairy, mixed findings were also reported for low-fat dairy and yogurt intake\textsuperscript{141-144}, whereas significant inverse associations were reported by 2 meta-analyses for cheese intake\textsuperscript{141,142} and no significant associations were reported for high-fat dairy or milk intake\textsuperscript{142,143}.

Similar to dairy, the findings from systematic reviews and meta-analyses of prospective cohort studies looking at fish intake have been mixed. In terms of overall fish/shellfish consumption, two systematic reviews and meta-analyses found no overall association between fish/shellfish intake and T2D risk\textsuperscript{145,146}. In another systematic review and meta-analysis, inconsistencies were found between their categorical and linear dose-response analyses, where no significant association was reported in their categorical analysis and a significant increased association was reported in their linear dose-response analysis\textsuperscript{147}. Evidence has also suggested that this association may be modified by geographical region and fish type. One meta-analysis found that fish intake was associated with an increased T2D risk in those studies conducted in the U.S. but not Europe, Asia or Australia\textsuperscript{148}, whereas another meta-analysis found that fish intake was inversely associated with T2D risk in those studies conducted in Asia (i.e. China and Japan) but not in Western countries (i.e. the U.S., U.K., Netherlands, Finland)\textsuperscript{146}. In terms of fish type, two meta-analyses found a significant inverse association between intake of fatty fish and T2D risk\textsuperscript{145,149} and one found no significant association with intake of lean fish and shellfish\textsuperscript{149}.

Lastly, prospective studies looking at egg and poultry consumption show mixed findings\textsuperscript{137,150,151,152} and no significant associations with T2D risk\textsuperscript{137,140}, respectively.

Overall, it appears that there is consistent evidence showing that red meat is associated with T2D risk, with inconsistencies regarding processed and unprocessed red meat, and mixed findings with regards to dairy, fish and egg consumption. Although evidence regarding poultry consumption is limited, it does not appear to be associated with T2D risk.
2.2.6.2 Animal Protein and Glycemic Control in Controlled Dietary Trials

Several RCTs have looked at the effect of animal protein consumption on various cardiometabolic risk factors, including glycemic control. In particular, there have been a number of trials that compared the effect of diets higher in beef, pork, dairy and egg to diets lower or deficient in these sources. The effect of varying amounts of lean beef was tested in a 5 week RCT conducted in hypercholesterolemic men and women (n=36) and showed no significant differences in fasting glucose and insulin when comparing one diet with the lowest amount of beef (20 g/d) to three diets higher in beef (28 g, 113 g and 153 g of beef per day, respectively) \(^{21}\). Similarly, the effect of a diet higher in pork (1 kg pork per week) was compared to a habitual diet in an RCT conducted in overweight adults (n=144) and showed no significant differences in fasting glucose and insulin after 3 months \(^{153}\). In terms of dairy, a meta-analysis of RCTs (20 studies; n=1677) showed that consumption of high or low fat dairy products had no significant effect on fasting glucose and HOMA-IR \(^{158}\). This was also supported by a more recent 6 week RCT conducted in postmenopausal women with abdominal obesity (n=27) that showed no significant between-group differences in fasting glucose, fasting insulin, and various insulin sensitivity indices \(^{154}\). Comparing diets higher in eggs to diets lower in eggs also showed no significant between-group differences in measures of glycemic control in 2 RCTs conducted in overweight or obese people with prediabetes or T2D \(^{19, 20}\). Therefore, based on the results of these studies, it appears that consuming higher amounts of animal protein sources such as beef, pork, dairy and eggs does not significantly alter glycemic control in individuals with one or more features of the MetS or T2D. This may be partly due to the fact that the control arms of these studies were consuming other sources of animal protein. For example in the study looking at higher intakes of beef, the control arm consumed a greater amount of poultry, pork and fish \(^{21}\). This is further supported by evidence from RCTs conducted in individuals with one or more features of the MetS or T2D that specifically compared the effect of consuming different types of animal protein sources (e.g. pork versus chicken versus fish) and showed, for the most part, no significant differences in fasting glucose \(^{155-157}\), fructosamine \(^{156}\) and/or fasting insulin \(^{155}\). However, when comparing a diet higher in animal protein to a diet higher in plant protein, RCTs have shown that diets higher in plant protein have beneficial effects on glycemic control in individuals with \(^{27, 158, 159}\) and without diabetes \(^{160, 161}\). Therefore, although RCTs show that various sources of animal protein do not alter glycemic control in individuals with or at risk for diabetes, majority of these studies consist of a comparator arm with an equivalent amount of protein from another animal protein source and are not consistent with findings from RCTs that compare animal protein with plant protein.
2.2.6.3 Animal Protein and Other Cardiometabolic Risk Factors in Controlled Dietary Trials

As mentioned in the previous section, many RCTs have been conducted looking at the effect of diets higher in specific animal protein sources on various cardiometabolic risk factors, such as body weight, blood pressure, blood lipids, kidney function, and inflammatory markers.

The findings for body weight are mixed. In terms of dairy intake, 2 systematic reviews and meta-analyses of RCTs showed that consumption of dairy products do not alter body weight, whereas subgroup analyses showed that in the context of energy restriction or short-term intervention (<1 year) they appeared to reduce body weight but had the opposite effect in ad libitum trials or long-term trials (≥1 year)\textsuperscript{162, 163}. A more recent meta-analysis showed that dairy consumption increased weight\textsuperscript{18}. In terms of other animal protein sources, an RCT conducted in overweight adults (n=144) showed that higher pork consumption resulted in significant reductions in measures of adiposity (i.e. body weight, BMI, waist circumferences, % body fat, fat mass and abdominal fat)\textsuperscript{153}, whereas another RCT comparing lean pork, beef and chicken found no significant differences in measures of adiposity between the 3 animal protein sources after 3 months in a group of overweight and obese middle aged men and women (n=49), with no changes within each arm either\textsuperscript{164}.

The findings for blood pressure are mixed. Two RCTs looking at fish consumption showed that diets higher in certain types of fish (i.e. lean or white) significantly improved blood pressure in individuals with the MetS or CHD\textsuperscript{165, 166}, whereas one showed no effect in blood pressure in a group of older healthy adults\textsuperscript{167}. Diets higher in lean beef also showed benefits on blood pressure in normotensive men and women\textsuperscript{168}, whereas the effect of consuming lean pork in comparison to chicken and fish as the main protein source in the context of a DASH diet showed no significant between group differences in blood pressure but did show reductions within each arm in men and women with elevated blood pressure\textsuperscript{155}. Lastly, a systematic review and meta-analysis of RCTs showed that consumption of high or low fat dairy products had no significant effect on blood pressure\textsuperscript{18}.

The findings for blood lipids are also mixed. Diets higher in lean beef showed benefits on blood lipids (i.e. LDL-C, Apo-B) in hypercholesterolemic men and women\textsuperscript{21}. An RCT conducted in individuals with T2D and macroalbuminuria, showed that after 4 weeks non-HDL-C was significantly lower in a diet replacing red meat with chicken and in a lactovegetarian low-protein diet when compared to the usual diet\textsuperscript{156}. In terms of egg consumption, 2 RCTs conducted in individuals with prediabetes or T2D found that a high egg diet did not significantly alter most blood lipids (i.e. TC, LDL-C, triglycerides)\textsuperscript{19, 20}, however one found that higher egg consumption significantly increased HDL-C in comparison to the lower egg diet\textsuperscript{20}. In terms of diets higher in fish, one RCT conducted in older healthy adults and one in individuals with CHD\textsuperscript{167} found no significant effects on blood lipids, except for Apo-A1 which was significantly lower in
the control group of the study conducted in individuals with CHD\textsuperscript{165}. There was also an RCT conducted in individuals with the MetS that showed higher consumption of fish significantly lowering LDL-C\textsuperscript{166}. Lastly, a systematic review and meta-analysis of RCTs showed that consumption of high or low fat dairy products had no significant effect on blood lipids (i.e. LDL-C, HDL-C)\textsuperscript{18}, which was also supported by a more recent RCT\textsuperscript{154}.

In terms of kidney function, an RCT conducted in individuals with T2D and macroalbuminuria showed that after 4 weeks urinary albumin excretion rate was significantly lower in a usual diet replacing red meat with chicken and a lactovegetarian low-protein diet in comparison with just the usual diet\textsuperscript{156}.

Lastly, RCTs conducted in individuals with various health statuses (i.e. otherwise healthy, overweight and/or obese with and without glucose intolerance) show that diets higher in specific animal protein sources, such as dairy, red meat, dairy, and fish have no significant effect on markers of inflammation (i.e. CRP)\textsuperscript{18, 154, 157, 167, 169}.

Overall, the findings from RCTs indicate that the effects of animal protein across all the cardiometabolic risk factors discussed are inconsistent or lacking in evidence, with the exception of markers of inflammation, where several RCTs looking at various animal protein sources consistently showed no difference in effect.

2.2.7 REPLACING ANIMAL PROTEIN WITH PLANT PROTEIN AND DIABETES

2.2.7.1 Replacing Animal Protein with Plant Protein and Diabetes Risk in Prospective Cohort Studies

To date, there has been one prospective cohort study that specifically looked at the relationship between substituting animal with plant protein and diabetes risk. In a diabetes-free cohort of over 92 000 women from the Nurses’ Health Study II and over 40 000 men from the Health Professionals Follow-up Study (follow-up=4 years) substituting 5% energy from plant protein for animal protein was associated with an 18% reduced risk for T2D and substituting 1 serving per day of plant protein foods for animal protein foods was associated with 10-21% reduced risk for T2D\textsuperscript{103}. There has also been one cross-sectional study conducted in diabetes-free female participants from the Nurses’ Health Study (n=3690) looking at the association between substituting animal with sources of plant protein and HbA\textsubscript{1c}. They found no significant association between substituting one serving of total red meat with nuts or legumes and HbA\textsubscript{1c} but did show that substitution with poultry, fish, legumes, and nuts together was associated with significantly lower HbA\textsubscript{1c} (β ± SE: -0.031±0.015, P=0.04)\textsuperscript{170}. The findings from this study suggest that certain types of animal protein may affect glycemic control differently; however, more studies are needed to clarify these findings.
2.2.7.2 Replacing Animal Protein with Plant Protein and Glycemic Control in Controlled Dietary Trials

There have been a number of RCTs looking at the effect of replacing animal protein with plant protein sources on various cardiometabolic risk factors in individuals with diabetes and hypercholesterolemia, as well in individuals who are overweight or obese and otherwise healthy.

In terms of glycemic control, the findings are mixed. In individuals with diabetes, some RCTs have shown that the replacement of animal with plant protein improves markers of glycemic control, such as HbA1c, fasting glucose and fasting insulin, whereas other RCTs have shown no difference in effect (note: these studies will be discussed in greater detail in Chapter IV). Similarly, in individuals with hyperlipidemia, there have been some RCTs that have shown improvements in fasting glucose and fasting insulin, whereas others have shown no difference in effect. Lastly, RCTs conducted in overweight or obese individuals who were otherwise healthy have shown, for the most part, no significant effect on markers of glycemic control, however one recent study conducted in post-menopausal women with abdominal obesity (n=15; follow-up duration=4 weeks) showed that the diet replacing animal protein with soy protein resulted in significantly greater insulin sensitivity as measured by a frequently sampled intravenous glucose tolerance test (FSIGT) and the disposition index.

Overall, the effect of replacing animal with plant protein on glycemic control in individuals with various health statuses is not exactly clear. Therefore, a systematic review and meta-analysis of RCTs would be useful in quantifying and understanding how this replacement in the diet relates to markers of glycemic control in individuals with and at risk of developing diabetes.

2.2.7.3 Replacing Animal Protein with Plant Protein and Other Cardiometabolic Risk Factors in Controlled Dietary Trials

In terms of other cardiometabolic risk factors, there have been a number of RCTs which show that replacing animal with plant protein has beneficial effects on blood lipids (e.g. triglycerides, TC, LDL-C, HDL-C) in individuals with T1D or T2D with or without nephropathy, with the exception of one trial which showed no significant differences in blood lipids (i.e. TC, HDL-C, triglycerides) in individuals with T2D and microalbuminuria (n=17; follow-up=6 weeks). The beneficial effect of replacing animal with plant protein on blood lipids have been attributed to plant protein sources being higher in fiber, phytosterols, and isoflavones (in the case of soy protein). Similar beneficial effects on the lipid profile have also been reported for hyperlipidemic and obese individuals who are otherwise healthy. Replacement of animal with plant protein has also shown benefits on markers related to kidney function (e.g. urinary urea nitrogen, proteinuria, urinary creatinine, GFR, renal plasma flow) in individuals with T1D or T2D with or without nephropathy, with the exception of...
one trial which showed no significant differences in GFR, renal plasma flow, and albumin excretion rate in individuals with T2D and microalbuminuria (n=17; follow-up=6 weeks)\textsuperscript{30}. The beneficial effects on kidney function may be explained, to some extent, by improvements seen in blood lipids and glycemic control in these trials and differences in the amino acid profiles. Lastly, replacement of animal with plant protein has shown benefits on markers of inflammation (i.e. CRP, IL-6 and TNF-α) in individuals with T2D with or without nephropathy\textsuperscript{27,187}, which may be attributed to plant proteins being higher in nutrients that have shown anti-inflammatory potential (i.e. fiber, magnesium, and phenolic compounds, such as flavonoids and anthocyanins).

Overall, the findings from these RCTs suggest, for the most part, that replacing animal with plant protein may beneficial for blood lipids, kidney function and inflammation in individuals with diabetes.
CHAPTER III – RATIONALE AND OBJECTIVES

3.1 RATIONALE

Although plant protein sources have shown to be beneficial for lowering diabetes risk and improving glycemic control, diabetes association guidelines (i.e. ADA and EASD) currently do not make any specific recommendations for the intake of major plant protein sources (i.e. soy, soy-derived products and nuts) for optimal glycemic control. One exception to this is dietary pulses (e.g. beans, peas, chick peas, lentils), which have recently been recommended by the CDA in their most recent clinical practice guidelines for improving glycemic control in individuals with T2D. Animal protein, on the other hand, especially sources of red meat, is associated with an increased diabetes risk, shows no glycemic benefits, and has even been suggested to be considered as a risk factor for T2D.

Given this data on plant and animal protein, it still remains unclear whether replacing animal with plant protein would confer glycemic control benefits in individuals with diabetes. Current evidence from a small number of RCTs is inconsistent, where some have shown significant improvements in glycemic control, whereas others show no effect. Therefore, in order to gain a clearer understanding of this relationship and to address this knowledge gap we conducted a systematic review and meta-analysis of RCTs looking at the effect of replacing sources of animal protein with major sources of plant protein on HbA1c, fasting glucose, and fasting insulin in individuals with diabetes. We also conducted a cross-sectional study using baseline data from 5 RCTs conducted in individuals with T2D in order to assess the relationship between replacing animal with plant protein and HbA1c, the current clinical standard for assessing glycemic control, and to adjust for other nutrients that exist in whole food sources of plant and animal protein (i.e. available carbohydrates, fat, fibre, magnesium). Furthermore, the use of baseline data in these analyses would allow us to understand this relationship in the context of “real world” intakes in a sample of people with T2D.

3.2 OBJECTIVES

The overall objective is to investigate the effect of replacing animal protein with plant protein on glycemic control in individuals with diabetes.

Specific objectives include:

1. To assess the effect of replacing sources of animal protein with major sources of plant protein (i.e. soy, soy-derived products, pulses, nuts) on HbA1c, fasting glucose, and fasting insulin in individuals with diabetes in a systematic review and meta-analysis of RCTs.
2. To assess the association between replacing animal protein with plant protein on HbA1c in individuals with T2D in a cross-sectional study using baseline data from 5 RCTs conducted in Toronto, Canada.
CHAPTER IV – EFFECT OF REPLACING ANIMAL PROTEIN WITH PLANT PROTEIN ON GLYCEMIC CONTROL IN DIABETES: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS
EFFECT OF REPLACING ANIMAL PROTEIN WITH PLANT PROTEIN ON GLYCEMIC CONTROL IN DIABETES: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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4.1 ABSTRACT

**Aims:** Previous research on the effect of replacing sources of animal protein with plant protein on glycemic control has been inconsistent. To assess the evidence that this replacement may benefit glycemic control, we conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) on the effect of replacing animal protein with plant protein on glycemic control in individuals with diabetes.

**Methods:** We searched MEDLINE, EMBASE, and Cochrane databases through 26 August 2015. We included RCTs ≥ 3-weeks comparing the effect of replacing sources of animal protein with plant protein on HbA1c, fasting glucose (FG), and fasting insulin (FI). Two independent reviewers extracted relevant data, assessed study quality (Heyland Methodological Score) and risk of bias (Cochrane Risk of Bias Tool). Data were pooled by the generic inverse variance method and expressed as mean differences (MD) with 95% CIs. Heterogeneity was assessed (Cochran Q-statistic) and quantified (I²-statistic).

**Results:** Twelve trials (n = 240) met the eligibility criteria. Diets using major sources of plant protein to replace sources of animal protein at a level ≥35% of total protein per day significantly lowered HbA1c (MD= -0.16-% [95%-CI: -0.27, -0.05-%]), FG (MD= -0.53-mmol/L [95%-CI: -0.92, -0.13-mmol/L]) and FI (MD= -10.09-pmol/L [95%-CI: -17.31, -2.86-pmol/L]) compared with control arms. Most trials were small, short, and of poor quality.

**Conclusions:** Pooled analyses show that replacing sources of animal protein with plant protein leads to modest improvements in glycemic control in individuals with diabetes. Owing to uncertainties in our analyses there is a need for larger, longer, higher quality trials.

**Trial Registration:** ClinicalTrials.gov registration number: NCT02037321

**Keywords:** diabetes; glycemic control; plant protein; animal protein
4.2 INTRODUCTION

Diabetes association guidelines (i.e. American Diabetes Association, and European Association for the Study of Diabetes) do not currently recommend the intake of major sources of plant protein such as soy, soy-derived foods (e.g. tofu) and nuts for optimal glycemic control\textsuperscript{25,26}. One exception is dietary pulses (e.g. beans, peas, chick peas, lentils), which have recently been recommended by the Canadian Diabetes Association clinical practice guidelines for improving glycemic control in individuals with type 2 diabetes (T2D)\textsuperscript{49}. Plant proteins are a major component of vegan and/or vegetarian dietary patterns and have been shown in prospective cohort and cross-sectional studies in Seventh day Adventists to be associated with lower diabetes risk\textsuperscript{6,7} and all-cause mortality\textsuperscript{91}. Evidence from a systematic review and meta-analysis of RCTs also suggests that vegetarian diets may improve glycemic control in individuals with T2D\textsuperscript{9}. Furthermore, a recent prospective cohort study conducted in over 92 000 women from the Nurses’ Health Study II and over 40 000 men from the Health Professionals Follow-up Study found that substituting 5% energy from plant protein for animal protein was associated with an 18% reduced risk for T2D and substituting 1 serving per day of plant protein foods for animal protein foods was associated with 10-21% reduced risk for T2D\textsuperscript{103}. On the contrary, evidence from previous meta-analyses of prospective cohort studies\textsuperscript{15,16}, as well as more recent prospective cohort studies\textsuperscript{17} have shown that diets higher in animal protein, specifically in red meat, are associated with an increased incidence of T2D. A recent review has even suggested that meat consumption be considered as a risk factor for T2D\textsuperscript{22}. However, it is unclear whether the replacement of animal with plant protein would confer glycemic control benefits in individuals with diabetes. Evidence from RCTs remains inconsistent: some trials have shown replacement of animal with plant protein significantly improved glycemic control\textsuperscript{27,158}, whereas others have shown no effect\textsuperscript{30,174}. We therefore conducted a systematic review and meta-analysis of RCTs to synthesize the effect of replacing sources of animal protein with plant protein on glycemic control assessed by HbA\textsubscript{1c}, fasting glucose, and fasting insulin in individuals with diabetes.

4.3 METHODS

We followed the Cochrane Handbook for Systematic Reviews of Interventions for the planning and conduct of this meta-analysis\textsuperscript{188}. Reporting followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines\textsuperscript{189}. The review protocol is available at ClinicalTrials.gov (registration number: NCT02037321).
4.3.1 DATA SOURCES AND SEARCHES
We searched the databases MEDLINE, EMBASE, and the Cochrane Registry through 26 August 2015 using the search strategy shown in Supplemental Table 4.1. Manual searches of reference lists of review articles and included trials supplemented electronic database searches.

4.3.2 STUDY SELECTION
We included RCTs that compared a diet emphasizing the replacement of animal protein sources (e.g. meat, dairy, etc.) with major sources of plant protein (e.g. legumes, nuts, etc.) on HbA1c, fasting glucose, and/or fasting insulin to a control diet without this replacement matched for energy (isocaloric) for a follow-up duration ≥3 weeks in individuals with diabetes (T1D and/or T2D). Trials that consisted of a non-randomized treatment allocation, <3-weeks follow-up duration, non-isocaloric comparisons, lacked a suitable control (i.e. plant protein source in intervention arm did not replace an animal protein source present in the control arm), were not conducted in individuals with diabetes, or did not provide suitable endpoint data were excluded. No restrictions were placed on language.

4.3.3 DATA EXTRACTION AND QUALITY ASSESSMENT
Two investigators (EV, and one of SS, AN, VJ or AM) independently reviewed all reports that met the inclusion criteria. A standardized form was used to extract relevant information on trial characteristics and endpoint data. Trial characteristics included: sample size, participant characteristics (e.g. health status, sex, age, etc.), study setting, design (crossover or parallel), level of feeding control (metabolically controlled, partially metabolically controlled, or dietary advice), intervention arm (plant protein type), percent and grams per day of animal protein replaced with plant protein from total protein, control arm (animal protein type), food form (whole food or powder), macronutrient breakdown of background diet(s), energy balance (neutral, positive or negative), follow-up duration, and funding source type (agency, industry, or both). Where available, the mean±SD for baseline, end, and change from baseline values, as well as MD were extracted for the primary endpoints (HbA1c, fasting glucose, and fasting insulin). Missing SDs were calculated from other available data (95% CI, p-values, t or F statistics, SE) using standard formulae recommended by the Cochrane Collaboration. Authors were contacted to provide missing data.

The quality of each trial was assessed using the MQS. A maximum score of 13 points could be received on the basis of the trials methods, sample and intervention. Trials receiving scores of ≥8 and <8 were considered to be of higher and lower quality, respectively. Disagreements on MQS were resolved by consensus.
Trials were assessed for risk of bias using the Cochrane Risk of Bias Tool\textsuperscript{188}. Domains of bias assessed were sequence generation, allocation concealment, blinding, outcome data, and outcome reporting. Trials were marked as “high risk of bias” when the methodological flaw was likely to have affected the true outcome, “low risk of bias” if the flaw was deemed inconsequential to the true outcome, and “unclear risk of bias” when insufficient information was provided to permit judgment. All disagreements were resolved by consensus.

**4.3.4 DATA SYNTHESIS AND ANALYSIS**

Data for primary analyses were conducted using Review Manager (RevMan), version 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). The difference between the intervention and control arm’s change from baseline value was derived from each trial for the primary endpoints. If change from baseline values were not available, end-of-treatment values were used. In the case where we were not able to convert data into appropriate units (i.e. percent change from baseline values), individual patient data was requested\textsuperscript{175}. Paired analyses were conducted for all crossover trials\textsuperscript{191}. If SDs were missing for crossover trials and no other data were available for their derivation, and where we could not derive a calculated pooled correlation coefficient for imputing missing SDs (<5 trials reporting sufficient data) we assumed a correlation coefficient of 0.5, as it is a conservative estimate for an expected range of 0–1. A correlation coefficient of 0.5 was assumed in all three primary analyses due to insufficient data. Once the values were derived from each trial they were pooled and analyzed for each primary endpoint using the generic inverse variance method with random effects models. This approach was used even in the absence of statistically significant between-study heterogeneity, as they yield more conservative summary effect estimates in the presence of residual heterogeneity. Results were expressed as MD with 95% CIs. A two-sided p-value <0.05 was set as the level of significance.

Inter-study heterogeneity was tested using the Cochran Q-statistic and quantified using the I\textsuperscript{2}-statistic with a significance level set at p-value <0.10. An I\textsuperscript{2}<50%, I\textsuperscript{2}≥50% and I\textsuperscript{2}≥75% were considered to be evidence of “moderate”, “substantial” and “considerable” heterogeneity, respectively\textsuperscript{188}. Sources of heterogeneity were explored using sensitivity and subgroup analyses. To determine whether a single trial exerted an undue influence on the overall results, sensitivity analyses were performed in which each individual trial was removed from the meta-analysis and the effect size recalculated with the remaining trials. Sensitivity analyses were also undertaken using correlation coefficients of 0.25 and 0.75 to determine whether the overall results were robust to the use of an assumed correlation coefficient of 0.5. A priori subgroup analyses (categorical and continuous) were conducted for baseline values of...
HbA\textsubscript{1c}, fasting glucose, and fasting insulin within the intervention arm, plant protein type, animal protein type, percent and grams of animal protein replaced with plant protein (from total protein), absolute fiber and saturated fat intake within the intervention arm, difference in fiber and saturated fat intake between the intervention and control arm, change from baseline fiber and saturated fat intake within the intervention arm, health status (diabetes type), design, follow-up duration, and study quality (MQS). Post-hoc continuous or categorical subgroup analyses were conducted for isoflavone intake, body weight, sex, food form and risk of bias. A post-hoc analysis consisting of a piecewise linear meta-regression was performed using the mkspline function in order to identify a dose-threshold (breakpoints) for the continuous subgroup looking at percent animal protein replaced with plant protein (from total protein) on fasting glucose.

Publication bias was investigated by visual inspection of funnel plots, quantitative assessment using Egger and Begg tests, and Duval and Tweedie nonparametric “trim-and-fill” analyses, where a p-value <0.05 was considered evidence of small study effects.

All meta-regressions (a-priori and post-hoc), and publication bias analyses were conducted using STATA software, version 13.0 (StataCorp, College Station, TX) with a significance set at \( P < 0.05 \).

### 4.4 RESULTS

#### 4.4.1 SEARCH RESULTS

Figure 4.1 shows the literature search and selection process. We identified a total of 2554 reports, 2496 of which were determined to be irrelevant based on review of titles and abstracts. The remaining 58 reports were retrieved and reviewed in full, of which 45 were excluded. One of these reports was excluded for an irreconcilable discrepancy in the reporting of the results in two places in the same report\textsuperscript{185}. A total of 12 reports containing data on 12 trials in 240 participants with T1D (\( n = 21 \))\textsuperscript{159}, \textsuperscript{172} and T2D (\( n = 219 \))\textsuperscript{27, 30, 158, 171, 173-175, 186, 192, 193} met the eligibility criteria and were included in the analyses. Eight trials reported data for HbA\textsubscript{1c} (\( n = 133 \)), 10 for fasting glucose (\( n = 218 \)), and 5 for fasting insulin (\( n = 118 \)).

#### 4.4.2 TRIAL CHARACTERISTICS

Table 4.1 shows the characteristics of the 12 included trials. Trials were conducted in outpatient settings across 5 countries: Iran (5 trials), U.S. (4 trials), and 1 trial each from Canada, Denmark and Greece. All trials were randomized and majority (83%) used a crossover design. Participants tended to be middle aged (median age: 62 years [range: 30–66 years]) with an approximately equal distribution of
men and women across trials (median % women: 49% [range: 0-78% women]) and overweight or obese (median BMI: 29 kg/m² [range: 23.8-35.1 kg/m²]). Most trials were conducted in individuals with T2D with the exception of 2 trials conducted in individuals with T1D. Half of the trials reported presence of microvascular complications (e.g. nephropathy, retinopathy) among participants. Median baseline HbA₁c, fasting glucose, and fasting insulin were 7.2% (range: 5.9-8.4%), 8.0-mmol/L (range: 6.7-10.4 mmol/L), and 70.2-pmol/L (56.3-134.2 pmol/L), respectively. Mean diabetes duration varied from ~1 to 10 years for those with T2D and 15 years or before the onset of 30 years of age for those with T1D; otherwise duration was undeclared. The majority of trials did not explicitly provide information on how diabetes was defined with the exception of 4 trials, in which T2D was defined as fasting plasma glucose ≥7-mmol/L or the use of oral glucose-lowering agents or insulin. Trials conducted in individuals with T2D had all participants treated with oral glucose-lowering agents (4 trials), some treated with either oral glucose-lowering agents, insulin or both (3 trials), all treated with insulin (1 trial), or all treated with diet and lifestyle alone (1 trial); otherwise information on the use of oral glucose-lowering agents or insulin was undeclared. All participants with T1D were treated with insulin (2 trials). Five trials required participants to keep their medications stable throughout the trial, while 4 trials reported no changes in medication use in most patients, otherwise it was undeclared. The majority of trials consisted of a partial replacement of animal with plant protein, with the exception of 2 trials which did a full replacement. The types of animal protein replaced with plant protein varied among the trials: 6 trials (50%) replaced mixed sources of animal protein with mixed sources of plant protein or sources of soy protein only; 4 trials (33%) replaced sources of dairy protein with sources of soy protein or almonds; and 2 trials (17%) replaced sources of red meat with pulses (e.g. lentils, chickpeas, peas and beans). The animal and plant protein sources were consumed in the form of whole foods in majority of the trials with the exception of 2 trials where the animal and plant sources exchanged for one another were in the form of isolated protein powders. The median percentage of animal protein replaced with plant protein from total protein was ~35%/d (range: 4-70%/d).
The background diets of the intervention arms consisted of 41–70% energy (E) from carbohydrate, 9–25% E from protein, and 20–40% E from fat with a median fiber and saturated fat intake of 22 g/d (range: 13–41 g/d) and 7.4% E (range: 3–10% E), respectively. The background diets of the control arms consisted of 41–69% E from carbohydrate, 9–26% E from protein, and 22–37% E from fat with a median fiber and saturated fat intake of 24 g/d (range: 7.7–42 g/d) and 8% E (range: 4–12% E), respectively. In terms of feeding control, 1 trial was metabolically controlled (i.e. all foods were provided), 8 trials were partially metabolically controlled (i.e. test foods/supplements were provided), and 2 trials were not metabolically controlled (i.e. dietary advice was provided); otherwise it was unclear. The median follow-up duration was 8-weeks (range: 4 weeks–4 years).

The majority of trials (92%) were considered to be of poor quality (MQS<8). Absence of double-blinding and a preselected or indeterminate sample selection contributed to lower scores (Supplemental Table 4). Majority of the trials (>75%) were judged as having a “low” or “unclear risk bias” in majority of the domains measured by the Cochrane Risk of Bias Tool. Five trials (42%) were considered “high risk of bias” due to incomplete outcome data (Supplemental Figure 4). In terms of sources of funding, 5 trials (42%) were funded by agency alone; 4 trials (33%) were funded by both agency and industry and for 3 trials funding information was not available.

### 4.4.3 HEMOGLOBIN A1C (HbA1c)

Figure 4.2A shows a forest plot of the pooled effect of replacing animal with plant protein on HbA1c in individuals with T1D and T2D. Diets emphasizing this replacement significantly lowered HbA1c in comparison to control diets (MD=−0.16% [95% CI: -0.27, -0.05%]; P=0.006), with no significant evidence of inter-study heterogeneity (I²=0%; P=0.55). Systematic removal of individual trials did not alter the results. Sensitivity analyses using different correlation coefficients in paired analyses of crossover trials (0.25 and 0.75) did not alter the significance of the pooled effect size.

Supplemental Table 4.3 and Supplemental Figure 4.2A-B show the results of continuous and categorical subgroup analyses for the effect of replacing sources of animal with plant protein on HbA1c. Meta-regression analyses did not reveal any statistically significant subgroup effects.

### 4.4.4 FASTING GLUCOSE

Figure 4.2B shows a forest plot of the pooled effect of replacing animal with plant protein on FG in individuals with T1D and T2D. Diets emphasizing this replacement significantly lowered fasting glucose in comparison to control diets (MD=−0.53 mmol/L [95% CI: -0.92, -0.13 mmol/L]; P=0.009) with considerable amount of inter-study heterogeneity (I²=82%; P<0.00001). Although heterogeneity was
significant, 8 of the 10 trials fell on the left side of the line of unity, which favours the replacement of animal with plant protein. Systematic removal of individual trials did not alter the results. Sensitivity analyses using different correlation coefficients in paired analyses of crossover trials (0.25 and 0.75) did not alter the significance of the pooled effect size.

Supplemental Table 4.4, Supplemental Table 4.6A-D and Supplemental Figure 4.3A-B show the results of continuous and categorical subgroup analyses for the effect of replacing animal with plant protein on fasting glucose. Two significant subgroups were identified in the categorical subgroup analyses (Supplemental Figure 4.3A). Evidence of effect modification was seen for animal protein type, where the fasting glucose reduction achieved by replacing mixed animal protein sources with plant protein was significantly greater than the fasting glucose reduction achieved by replacing dairy or red meat protein sources with plant protein (P=0.003). An effect modification was also seen with percent animal protein replaced with plant protein from total protein, where the fasting glucose reduction in trials that replaced ≥35% of animal protein with plant protein was significantly greater than the fasting glucose reduction when replacing <35% (P=0.025). No other subgroup analyses revealed statistically significant subgroup effects. Post-hoc analyses for the continuous subgroup looking at percent animal protein replaced with plant protein (from total protein) on fasting glucose using a piecewise linear meta-regression did not indicate a dose-threshold (Supplemental Table 4.6A-D).

4.4.5 FASTING INSULIN

Figure 4.2C shows a forest plot of the pooled effect of replacing animal with plant protein on fasting insulin in individuals with T2D. Diets emphasizing this replacement significantly lowered fasting insulin in comparison to control diets (MD=-10.09 pmol/L [95% CI: -17.31, -2.86 pmol/L]; P=0.006) with no significant evidence of inter-study heterogeneity (I^2=0%; P=0.41). Sensitivity analyses showed that removal of any one of the following 3 trials nullified the significance of the effect estimate: Gobert et al.173 (MD=-6.89 pmol/L [95% CI: -19.20, 5.42 pmol/L]; P=0.27; I^2=24%; P=0.27); Cohen et al.171 (MD=-8.68 pmol/L [95% CI: -17.90, 0.53 pmol/L]; P=0.06; I^2=22%; P=0.28) and Hosseinpour-Niazi et al.158 (MD=-3.89 pmol/L [95% CI: -15.61, 7.83 pmol/L]; P=0.52; I^2=0%; P=0.52). None of these trials included insulin-treated participants. Sensitivity analyses using different correlation coefficients of crossover trials showed that a correlation coefficient of 0.25 did not alter the significance of the pooled effect size, but a correlation coefficient of 0.75 changed the pooled effect size from significant to non-significant (MD=-7.14 pmol/L [95% CI: -16.69, 2.40 pmol/L]; P=0.14; I^2=38%; P=0.17).
Supplemental Table 4.5 and Supplemental Figure 4.4A-B show the results of continuous and categorical subgroup analyses for the effect of replacing animal with plant protein on fasting insulin. Meta-regression analyses did not reveal any statistically significant subgroup effects.

4.4.6 PUBLICATION BIAS

Figure 4.3A–C shows the funnel plots for each endpoint. Visual inspection of funnel plots revealed asymmetry for $HbA_1c$ and fasting glucose, suggesting study effects favouring the replacement of animal for plant protein. Egger tests revealed significant evidence of publication bias for $HbA_1c$ and approached significance for fasting glucose. Begg tests did not reveal significant evidence of publication bias for any of the endpoints. With one exception, these tests should be interpreted with caution, as most of them were based on <10 trials. Trim-and-fill analyses for $HbA_1c$ and fasting insulin did not identify any potentially missed studies due to publication bias (Supplementary Figures 4.5A, C), however, asymmetry in the funnel plot for fasting glucose was identified, and 1 additional study was “filled” in to mitigate publication bias. With the inclusion of the “filled” study, the MD for fasting glucose was not significantly altered (MD=−0.56 mmol/L [95% CI: -0.94, -0.18 mmol/L], P=0.004; Supplementary Figure 4.5B).

4.5 DISCUSSION

To the best of our knowledge this is the first systematic review and meta-analysis of RCTs to assess the effect of replacing sources of animal protein with major sources of plant protein on $HbA_1c$, fasting glucose, and fasting insulin in individuals with T1D and T2D. We included 12 RCTs looking at the effect of replacing animal with plant protein on these 3 endpoints in 240 predominantly middle-aged adults. Pooled analyses showed an overall significant modest lowering of $HbA_1c$ at ~0.16%, fasting glucose at ~0.53 mmol/L and fasting insulin at -10.09 pmol/L when using major sources of plant protein to replace sources of animal protein at a level ≥35% of total protein per day over a median follow-up duration of ~8 weeks.

4.5.1 RELATION TO OTHER STUDIES

There have been several systematic reviews and meta-analyses of RCTs looking at the effect of specific sources of plant protein (i.e. soy products, dietary pulses, and tree nuts) on glycemic control. In terms of soy products, 2 meta-analyses have been conducted, one in individuals with T2D and one in individuals with and without diabetes (e.g. healthy, hypercholesterolemia, metabolic syndrome, etc.)
Both found no overall significant effect on various glycemic endpoints, however, the direction of the effect favoured soy interventions\textsuperscript{107, 108}. Two meta-analyses looking at the effect of tree nuts have also been conducted, one in individuals with T2D\textsuperscript{12} and one in individuals with and without diabetes\textsuperscript{10}. Both found significant improvements in fasting glucose, as well as HbA\textsubscript{lc} in individuals with T2D\textsuperscript{12}. Lastly, there have been a series of meta-analyses conducted looking at the effect of incorporating dietary pulses into the diet alone or in the context of a low-GI or high-fibre diet in individuals with and without diabetes\textsuperscript{11}. Pulses alone were found to significantly lower fasting glucose and fasting insulin. In the context of a low-GI and high-fibre diet, pulses were found to significantly lower glycosylated blood proteins (measured as HbA\textsubscript{lc} or fructosamine), and fasting glucose in the context of a high-fibre diet\textsuperscript{11}.

Overall, the results of these meta-analyses appear to be consistent with our findings.

### 4.5.2 POSSIBLE MECHANISMS OF ACTION

Several potential mechanisms may explain the beneficial effects of replacing animal with plant protein on glycemic control. The reduction in body iron stores may be one such mechanism. As a pro-oxidant, iron catalyzes several cellular reactions that result in the production of reactive oxygen species, which increases oxidative stress and tissue damage, including damaging pancreatic \( \beta \)-cells\textsuperscript{60, 195}.

Prospective cohort studies have shown that increased heme iron intake, found only in animal protein sources\textsuperscript{196}, is significantly associated with an increased risk of T2D\textsuperscript{197, 198}, whereas non-heme iron intake, which is found in both plant and animal source foods\textsuperscript{196}, has been shown to be either inversely associated with\textsuperscript{198} or not associated with\textsuperscript{197} T2D incidence. This may be attributed to differences in bioavailability between non-heme and heme iron, as heme iron is associated with higher body iron stores\textsuperscript{199, 200}. Observational studies showed that serum ferritin, the storage form of iron, predicted the development of hyperglycemia\textsuperscript{201, 202} and T2D\textsuperscript{201, 203} and was found to be positively associated with insulin resistance\textsuperscript{201, 204, 205}. In addition, randomized trials have shown that the use of phlebotomy to reduce serum ferritin levels was associated with improved glucose tolerance in individuals with MetS\textsuperscript{206} and T2D\textsuperscript{207}. Another mechanism may relate to differences in amino acid profiles of animal and plant protein. Compared to animal proteins, plant proteins appear to be higher in L-arginine. Randomized trials have shown that long-term oral administration of L-arginine improves insulin sensitivity in individuals with T2D\textsuperscript{208, 209} and in vitro studies suggest that L-arginine promotes insulin secretion from pancreatic \( \beta \)-cells by stimulating electrical activity\textsuperscript{210-214}. Although our findings show improvements for fasting insulin, specific measures of insulin sensitivity were not explored in the present meta-analysis, where only one of the included trials looked at insulin resistance and showed non-significant reductions in HOMA-IR\textsuperscript{173}. Neither endpoint, however, is considered to be a good marker of peripheral insulin
sensitivity and thus further studies are warranted. Other potential mechanisms may involve a reduction in the glycemic index, as well as sodium and nitrites found in processed meats.

### 4.5.3 A-PRIORI AND POST-HOC SUBGROUP ANALYSES

Evidence of considerable heterogeneity was found in the primary pooled analysis for fasting glucose. Categorical subgroup analyses showed evidence of effect modification by animal protein type (P=0.003) and percent animal protein replaced with plant protein (from total protein) (P=0.025). Based on the residual I² for both of these subgroups (98.26% vs. 35.82%), it appears that a large portion of the heterogeneity is explained by the latter subgroup, which shows that trials replacing ≥35% of animal with plant protein had greater reductions in the mean difference for fasting glucose in comparison to those replacing <35%. Significant effect modification by this subgroup was not seen in our continuous subgroup analysis, supporting a non-linear relationship. In addition, post-hoc analyses on this subgroup did not identify a dose-threshold. Regarding the primary pooled analysis for fasting insulin, sensitivity analyses suggest that the primary pooled effect size is not robust when subject to removing certain individual trials from the pooled analysis, or when using a different correlation coefficient.

### 4.5.4 LIMITATIONS

Several limitations exist in the present meta-analysis. First, the majority of trials had small sample sizes. Second, it is uncertain whether the follow-up duration of included trials was sufficient to observe meaningful changes in glycemic control: while HbA₁c levels reflect blood glucose control in the preceding 3 months, the majority of trials (83%) were shorter than 12-weeks in duration. There was, however, no effect modification by follow-up duration in the subgroup analyses. Third, most trials (92%) were of poor study quality (MQS<8), however, no effect modification by study quality were observed in subgroup analyses. Fourth, most subgroup analyses were underpowered due to the limited number of available studies. Fifth, only a small number of trials (17%) focused on glycemic control endpoints as a primary outcome. Sixth, sensitivity analyses showed that the pooled effect estimate for fasting insulin was not robust.

### 4.5.5 IMPLICATIONS

The reductions seen in HbA₁c meets half the threshold proposed by the U.S. Food and Drug Administration for the development of new drugs for diabetes (≥ 0.3%) and therefore may or may not be clinically significant. It is important to note, however, that the glycemic benefits seen in our meta-analysis are in addition to the use of oral glucose-lowering agents by majority of individuals. Therefore,
replacing animal protein with major sources of plant protein may be one strategy that can be utilized in combination with medication to help improve and manage glycemic control in individuals with diabetes. Furthermore, the majority of RCTs in our meta-analysis used soy and soy-derived products to replace sources of animal protein, a food that is consumed by only 3.3% of Canadians on any given day, with daily intakes ranging from 1.5 g/d among low consumers to 16.5 g/d among high consumers (<1 serving)\(^7\). In general, this is consistent with the overall consumption of major plant protein sources in the North American population, which is very low relative to other sources of plant protein, such as grains\(^62,71-73\). Therefore, there is ample room in the diet to increase the intake of these sources.

4.5.6 CONCLUSIONS

In conclusion, the present systematic review and meta-analysis of RCTs found significant modest improvements in HbA\(_{1\text{c}}\), fasting glucose and fasting insulin in individuals with diabetes when using major sources of plant protein to replace sources of animal protein at a level \(\geq 35\%\) of total protein per day over a median duration of \(\sim 8\) weeks. Sources of uncertainty in our analyses include the estimate for fasting glucose, which was complicated by considerable heterogeneity but largely explained by our subgroup analysis that showed greater benefit in trials that replaced animal with plant protein at a level \(\geq 35\%\) of total protein and for fasting insulin, which was altered by sensitivity analyses. In order to address these sources of uncertainty and the limitations in our analyses, there is a need for larger, longer, higher quality RCTs designed to use other sources of plant protein, in addition to soy, to replace animal protein at a level \(\geq 35\%\) total protein with glycemic endpoints as a primary outcome. The inclusion of such RCTs in future meta-analyses will help guide the design of future RCTs in this area, as well as the development of nutrition recommendations and health claims.

4.6 ACKNOWLEDGEMENTS

We wish to thank Teruko Kishibe of Li Ka Shing’s International Healthcare Education Centre at St. Michael Hospital for her help in the development of the search strategy. Aspects of this work were presented as an abstract at the Banting & Best Diabetes Centre 26th Annual Scientific Day, May 8, 2015, Toronto, Ontario, Canada.

4.7 FUNDING

This work was supported by the Canadian Institutes of Health Research (funding reference number, 129920) through the Canada-wide Human Nutrition Trialists’ Network (NTN). The Diet, Digestive tract, and Disease (3-D) Centre, funded through the Canada Foundation for Innovation (CFI)
and the Ministry of Research and Innovation’s Ontario Research Fund (ORF), provided the infrastructure for the conduct of this project. EV was funded by a Canadian Institutes of Health Research (CIHR)-Fredrick Banting and Charles Best Canada Graduate Scholarship and the Banting and Best Diabetes Centre (BBDC)-Yow Kam-Yuen Graduate Scholarship in Diabetes Research. RPB and DJAJ were funded by the Government of Canada through the Canada Research Chair Endowment. None of the sponsors had a role in any aspect of the present study, including design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, approval of the manuscript or decision to publish.

4.8 CONFLICTS OF INTEREST

RJdS was funded by the CIHR Postdoctoral Fellowship Award and has received research support from the CIHR, the Calorie Control Council, the Canadian Foundation for Dietetic Research and the Coca-Cola Company (investigator initiated, unrestricted grant) and travel support from the World Health Organization (WHO) to attend group meetings. He has served as an external resource person to WHO’s Nutrition Guidelines Advisory Group and is the lead author of 2 systematic reviews and meta-analyses commissioned by WHO of the relation of saturated fatty acids and trans fatty acids with health outcomes. AJH hold a Tier II Canada Research Chair in Diabetes Epidemiology. RPB has received research funding from Bunge Ltd., travel support from Unilever and consultant fees from Kraft Foods and Mead Johnson. CWCK has received research support from the Advanced Foods and Material Network, Agrifoods and Agriculture Canada, the Almond Board of California, the American Pistachio Growers, Barilla, the California Strawberry Commission, the Calorie Control Council, CIHR, the Canola Council of Canada, the Coca-Cola Company (investigator initiated, unrestricted grant), Hain Celestial, the International Tree Nut Council Nutrition Research and Education Foundation, Kellogg, Kraft, Loblaw Companies Ltd., Orafti, Pulse Canada, Saskatchewan Pulse Growers, Solae and Unilever. He has received travel funding, consultant fees and/or honoraria from Abbott Laboratories, the Almond Board of California, the American Peanut Council, the American Pistachio Growers, Barilla, Bayer, the Canola Council of Canada, the Coca-Cola Company, Danone, General Mills, the International Tree Nut Council Nutrition Research and Education Foundation, Kellogg, Loblaw Companies Ltd., the Nutrition Foundation of Italy, Oldways Preservation Trust, Orafti, Paramount Farms, the Peanut Institute, PepsiCo, Pulse Canada, Sabra Dipping Co., Saskatchewan Pulse Growers, Solae, Sun-Maid, Tate and Lyle, and Unilever. He is on the Dietary Guidelines Committee for the Diabetes Nutrition Study Group of the European Association for the Study of Diabetes and has served on the scientific advisory board for the Almond Board of California, the International Tree Nut Council, Oldways Preservation Trust, Paramount Farms
and Pulse Canada. DJAJ has received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program through the Pulse Research Network, the Advanced Foods and Material Network, Loblaw Companies Ltd., Unilever, Barilla, the Almond Board of California, the Coca-Cola Company (investigator initiated, unrestricted grant), Solae, Haine Celestial, the Sanitarium Company, Orafti, the International Tree Nut Council Nutrition Research and Education Foundation, the Peanut Institute, the Canola and Flax Councils of Canada, the Calorie Control Council, the CIHR, the Canada Foundation for Innovation and the Ontario Research Fund. He has received an honorarium from the United States Department of Agriculture to present the 2013 W.O. Atwater Memorial Lecture. He received the 2013 Award for Excellence in Research from the International Nut and Dried Fruit Council. He received funding and travel support from the Canadian Society of Endocrinology and Metabolism to produce mini cases for the Canadian Diabetes Association. He has been on the speaker’s panel, served on the scientific advisory board, and/or received travel support and/or honoraria from the Almond Board of California, Canadian Agriculture Policy Institute, Loblaw Companies Ltd, the Griffin Hospital (for the development of the NuVal scoring system), the Coca-Cola Company, Saskatchewan Pulse Growers, Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, the International Tree Nut Council Nutrition Research and Education Foundation, the Peanut Institute, Herbalife International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Kellogg, Quaker Oats, Procter and Gamble, the Coca-Cola Company, the Griffin Hospital, Abbott Laboratories, the Canola Council of Canada, Dean Foods, the California Strawberry Commission, Haine Celestial, PepsiCo, the Alpro Foundation, Pioneer Hi-Bred International, DuPont Nutrition and Health, Spherix Consulting and WhiteWave Foods, the Advanced Foods and Material Network, the Canola and Flax Councils of Canada, the Nutritional Fundamentals for Health, AgriCulture and Agri-Food Canada, the Canadian Agri-Food Policy Institute, Pulse Canada, the Saskatchewan Pulse Growers, the Soy Foods Association of North America, the Nutrition Foundation of Italy (NFI), Nutra-Source Diagnostics, the McDougall Program, the Toronto Knowledge Translation Group (St. Michael's Hospital), the Canadian College of Naturopathic Medicine, The Hospital for Sick Children, the Canadian Nutrition Society (CNS), the American Society of Nutrition (ASN), Arizona State University, Paolo Sorbini Foundation and the Institute of Nutrition, Metabolism and Diabetes. JLS has received research support from the Canadian Institutes of Health Research (CIHR), Calorie Control Council, American Society of Nutrition (ASN), The Coca-Cola Company (investigator initiated, unrestricted), Dr. Pepper Snapple Group (investigator initiated, unrestricted), Pulse Canada, and The International Tree Nut Council Nutrition Research & Education Foundation. He has received travel funding, speaker fees, and/or honoraria from the American Heart Association (AHA),
American College of Physicians (ACP), American Society for Nutrition (ASN), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH), Canadian Diabetes Association (CDA), Canadian Nutrition Society (CNS), University of South Carolina, University of Alabama at Birmingham, Oldways Preservation Trust, Nutrition Foundation of Italy (NFI), Calorie Control Council, Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD), International Life Sciences Institute (ILSI) North America, International Life Sciences Institute (ILSI) Brazil, Abbott Laboratories, Pulse Canada, Canadian Sugar Institute, Dr. Pepper Snapple Group, The Coca-Cola Company, Corn Refiners Association, World Sugar Research Organization, and Società Italiana di Nutrizione Umana (SINU). He has consulting arrangements with Winston & Strawn LLP, Perkins Coie LLP, and Tate & Lyle. He is on the Clinical Practice Guidelines Expert Committee for Nutrition Therapy of both the Canadian Diabetes Association (CDA) and European Association for the study of Diabetes (EASD), as well as being on an American Society for Nutrition (ASN) writing panel for a scientific statement on sugars. He is a member of the International Carbohydrate Quality Consortium (ICQC) and Board Member of the Diabetes and Nutrition Study Group (DNSG) of the EASD. He serves an unpaid scientific advisor for the International Life Science Institute (ILSI) North America, Food, Nutrition, and Safety Program (FNSP). His wife is an employee of Unilever Canada. No competing interests were declared by EV, SES, VHJ, APN, AM, SBM, LL and RJ. There are no patents, products in development or marketed products to declare.

4.9 AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CWCK DJAJ JLS. Analyzed the data: EV SES VHJ AG AM RjdS JLS. Wrote the paper: EV JLS. Interpretation of the data: EV SES VHJ AG AM RjdS AJH RPB LAL RJ. Critical revision of the article for important intellectual content: EV SES VHJ AM RjdS AJH RPB SBM LAL RJ CWCK DJAJ JLS. Final approval of the article: EV SES VHJ AM RjdS AJH RPB SBM LAL RJ CWCK DJAJ JLS. Obtaining of funding: CWCK DJAJ JLS. Administrative, technical, or logistic support: SBM VHJ AM. Collection and assembly of data: EV SES AG AM. Guarantors: CWCK DJAJ JLS.
### 4.10 TABLES

**Table 4.1 – Characteristics of included randomized controlled trials**

<table>
<thead>
<tr>
<th>Study, Year (Reference)</th>
<th>Study Type</th>
<th>Participants</th>
<th>Mean Age (SD or Range), y</th>
<th>Mean Body Weight or BMI (SD or Range)b</th>
<th>Settingc</th>
<th>Design</th>
<th>Feeding Controld</th>
<th>Plant Protein typee</th>
<th>Animal Protein typee</th>
<th>Amount AP replaced with PPf</th>
<th>Diet (P:C:F)g</th>
<th>Energy balance</th>
<th>Follow-up</th>
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<tr>
<td>Kontessis et al, 1995 (159)</td>
<td></td>
<td>9 T1D (7W, 2M)</td>
<td>32 (20–48)b</td>
<td>23.8 (20.6-27.8) kg/m²b</td>
<td>OP, GRC</td>
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<td>Stephenson et al, 2005 (172)</td>
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<td>12 T1D+GHF (6W, 6M)</td>
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<td>79.0 (5.9) kg</td>
<td>OP, USA</td>
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<td>Anderson et al, 1998 (192)</td>
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<td>8 T2D+N,O,HT (M only)</td>
<td>68 (18.4)</td>
<td>111 (66.8) kg</td>
<td>OP, USA</td>
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<td>Hermansen et al, 2001 (174)</td>
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<td>20 T2D+R (6W, 14M)</td>
<td>63.6 (7.5)</td>
<td>88.7 (11.9) kg</td>
<td>OP, DNK</td>
<td>C</td>
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<td>Wheeler et al, 2002 (30)</td>
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<td>17 T2D+N (3W, 14M)</td>
<td>56 (12.4)</td>
<td>102.3 (21.4) kg</td>
<td>OP, USA</td>
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<td>Azadbakht et al, 2008 (27)</td>
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<td>41 T2D+N,R (23W, 18M)</td>
<td>61.9 (11.8)</td>
<td>71 (9) kg</td>
<td>OP, IRN</td>
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<tr>
<td>Azadbakht et al, 2009 (186)</td>
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<td>14 T2D+N (4W, 10M)</td>
<td>62.5 (12.1)</td>
<td>70.6 (10.3) kg</td>
<td>OP, IRN</td>
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**SYSTEMATIC REVIEW AND META-ANALYSIS**

44
Table 4.1 (continued) – Characteristics of included randomized controlled trials

<table>
<thead>
<tr>
<th>Study, Year (Reference)</th>
<th>Participants</th>
<th>Mean Age (SD or Range), y</th>
<th>Mean Body Weight or BMI (SD or Range)a</th>
<th>Settingc</th>
<th>Design</th>
<th>Feeding Controld</th>
<th>Plant Protein typee</th>
<th>Animal protein typee</th>
<th>Amount AP replaced with PPf</th>
<th>Diet (P:C:F)g</th>
<th>Energy balance</th>
<th>Follow-up</th>
<th>MQSh</th>
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<tbody>
<tr>
<td>Gobert et al, 2010 (173)</td>
<td>29 T2D (13W, 16M)</td>
<td>60.1 (9.64)</td>
<td>83.4 (10.9) kg</td>
<td>OP, CAN</td>
<td>C</td>
<td>Supp</td>
<td>Soy</td>
<td>Milk</td>
<td>~34% (40 g/d)</td>
<td>~23:45:32</td>
<td>~23:44:33</td>
<td>Neutral</td>
<td>8 wk</td>
<td>7</td>
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<td>Cohen et al, 2011 (171)</td>
<td>13 T2D (6W, 7M)</td>
<td>66 (8.1)</td>
<td>101.1 (29.6) kg</td>
<td>OP, USA</td>
<td>P</td>
<td>Supp</td>
<td>Almonds</td>
<td>Cheese</td>
<td>NA (~6 g/d)</td>
<td>NA</td>
<td>Neutral</td>
<td>12 wk</td>
<td>7</td>
<td>Agency</td>
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<tr>
<td>Miraghajani et al, 2013 (175)</td>
<td>25 T2D+N (15W, 10M)</td>
<td>51 (10)</td>
<td>76.5 (13.6) kg</td>
<td>OP, IRN</td>
<td>C</td>
<td>DA</td>
<td>Soy</td>
<td>Milk</td>
<td>~18% (~13.3 g/d)</td>
<td>14:46:40</td>
<td>13:50:37</td>
<td>Neutral</td>
<td>4 wk</td>
<td>6</td>
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<tr>
<td>Abd-Mishani et al, 2014 (193)</td>
<td>21 T2D (18W, 6M)</td>
<td>61.7 (6)</td>
<td>74.5 (7.1) kg</td>
<td>OP, IRN</td>
<td>C</td>
<td>DA</td>
<td>Pulses</td>
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<td>~17% (~13.3 g/d)</td>
<td>15:55:30</td>
<td>15:55:30</td>
<td>Neutral</td>
<td>8 wk</td>
<td>4</td>
</tr>
<tr>
<td>Hosseinpour-Niazi et al, 2014 (158)</td>
<td>31 T2D+O (24W, 7M)</td>
<td>58.1 (33.4)</td>
<td>27.7 (3.34) kg/m²</td>
<td>OP, IRN</td>
<td>C</td>
<td>DA</td>
<td>Pulses</td>
<td>Red meat</td>
<td>~17% (~13.3 g/d)</td>
<td>14:54:33</td>
<td>15:52:34</td>
<td>Neutral</td>
<td>8 wk</td>
<td>6</td>
</tr>
</tbody>
</table>

AP=animal protein; C=crossover; CAN=Canada; DA=dietary advice; DNK=Denmark; GHF=glomerular hyperfiltration; GRC=Greece; HT=hypertension; IRN=Iran; M=men; Met=metabolic feeding control; MQS=Heyland Methodological Quality Score; N=nephropathy; NA=data not available; O=overweight and/or obese; P=parallel; PP=plant protein; R=retinopathy; Supp=supplemental feeding control; T1D=type 1 diabetes; T2D=type 2 diabetes; USA=United States of America; W=women; wk=weeks; y=years

aBaseline body weight (kg) values. Baseline BMI values (kg/m²) are only reported when no data on weight were available.
bReported as a median value.
cCountries are abbreviated using three letter country codes (ISO 3166-1 alpha-3 codes).
dMetabolic feeding control (Met) was the provision of all meals, snacks, and study supplements consumed during the study under controlled conditions. Supplement feeding control (Supp) was the provision of study supplements only. Dietary advice (DA) is the provision of counseling on the appropriate test and control diets.
Plant and animal protein types refer to the specific sources of plant proteins prescribed by the study to replace a specific source(s) of animal protein. If prescribed plant or animal protein type was not specified by the study it was assumed that the protein type consisted of mixed sources. For example, Kontessis et al. referred to their intervention arm as a “vegetable protein diet” and their control arm as an “animal protein diet”; therefore it was assumed that the intervention and control arm consisted of mixed sources of plant and animal protein, respectively. All protein sources prescribed in each study were in whole food form with the exception of Hermansen et al. and Gobert et al. which prescribed isolated protein powders. Any study with an intervention arm prescribing plant protein exclusively rather than partial substitution of animal for plant protein is indicated in parentheses as “PP only”.

Numbers in this column represent the amount of plant protein that was prescribed by the study to replace animal protein. Numbers not in parentheses represent the percent of total protein replacing animal protein with plant protein. Numbers in parentheses represent the amount of plant protein prescribed/amount of animal protein replaced in grams per day. Numbers preceded by “~” were calculated using relevant data provided by the study.

Numbers in this column indicate the designed percent energy breakdown from protein:carbohydrate:fat reported from each study. If these values were not available or provided, the measured percent energy breakdown from the end of the study were used. Numbers preceded by “~” were calculated using relevant data provided by the study.

Trials with a MQS score ≥8 were considered to be of higher quality.

Agency funding is that from government, university, or not-for-profit health agency sources. None of the trialists declared any conflicts of interest with the exception of Stephenson et al. and Hermansen et al.

Both intervention and control arm were designed to contain 1 gram of protein per kilogram body weight per day. Intervention arm consisted of plant protein exclusively and the control arm consisted of ~70% animal protein and 30% plant protein. The intervention arm was also provided animal fat supplements, as well as calcium and phosphate tablets.

Nine daily soy food intake options were provided: soy patties; soy pasta; soy chocolate beverage; chocolate, vanilla, or plain silk soy milk; lemon or chocolate soy bars; roasted soy nuts, or frozen edamame.

Both intervention and control arm were designed to contain 1 gram of protein per kilogram body weight per day. Fifty percent of the protein in the soy protein intervention arm was in the form of a beverage, meat analogue patties, or ground meat analogue, whereas 50% of the protein in the animal protein control arm was in the form of ground beef with a specified fat content and cow milk.

Both intervention and control arm were provided with their respective powders and instructed to mix half of their daily allotted amount in 250ml of water before breakfast and half before their evening meal. The powder provided in the intervention arm also contained 20g/d of soy cotyledon fiber and a high isoflavone content (minimum 165 mg/d), whereas the powder provided in the control arm contained 20 g/d of cellulose.

Intervention arm consisted of plant protein exclusively (62% soy-based), where major protein foods included tofu, textured vegetable protein, soy milk, and legumes. The control arm consisted of 60% animal protein and 40% plant protein, where major protein foods included beef, poultry, fish and milk.

73% of participants in this study had retinopathy. Both intervention and control arm were designed to contain 0.8 grams of protein per kilogram body weight per day. The intervention arm consisted of 35% soy protein (in the form of textured soy protein), 30% other plant protein and 35% animal protein and the control arm consisted of 70% animal protein and 30% plant protein.

The intervention arm consisted of 35% soy protein (in the form of textured soy protein), 30% other plant protein and 35% animal protein and the control arm consisted of 70% animal protein and 30% plant protein.

SYSTEMATIC REVIEW AND META-ANALYSIS
Women in this study were postmenopausal. The powder provided in the intervention arm also consisted of 88 mg/d isoflavones (65% genistein, 31% daidzein, 4% glycitein). Participants supplemented their habitual diets with the powders and were provided with multiple examples of ways to consume them but were encouraged to reconstitute them with water with the option of adding Nestle flavor packets. In order to avoid excess protein and calcium intakes, participants were counseled to replace foods like milk, cheese, and lunch meat with the powders.

Both intervention and control arm were designed to contain 0.8 grams of protein per kilogram body weight per day. The intervention and control arm consisted of 1 glass of soy and cow’s milk (240 mL each) per day, respectively.

Both intervention and control arm were prescribed a Therapeutic Lifestyle Change (TLC) diet. The intervention arm was the same as the control arm but participants were advised to replace 2 servings of red meat with different types of cooked legumes such as lentils, chickpeas, peas and beans 3 days per week. Half a cup of cooked legumes was considered to be one serving of red meat.
4.11 FIGURES

Figure 4.1 – Flow diagram depicting the literature search and selection process
**Figure 4.2A** – Forest plot of RCTs investigating the effect of replacing sources of animal with plant protein in individuals with diabetes on HbA1c. Pooled effect estimates for each subgroup and overall effect are represented by the diamonds. Data are expressed as weighted mean differences (MD) with 95% CIs, using the generic inverse-variance method with random effects models. Paired analyses were applied to all crossover trials. Inter-study heterogeneity was tested by the Cochran Q-statistic and quantified by $I^2$ at a significance level of $P < 0.10$. 

### Subgroup and Study (Reference)

<table>
<thead>
<tr>
<th>Subgroup (Reference)</th>
<th>Year</th>
<th>Participants, $n$</th>
<th>Weight, %</th>
<th>Mean Difference [95% CI] in HbA1c, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 2 Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anderson et al. (192)</td>
<td>1998</td>
<td>8</td>
<td>1.3</td>
<td>-0.70 [-1.68, 0.28]</td>
</tr>
<tr>
<td>Hermansen et al. (174)</td>
<td>2001</td>
<td>20</td>
<td>2.9</td>
<td>-0.30 [-0.96, 0.36]</td>
</tr>
<tr>
<td>Wheeler et al. (30)</td>
<td>2002</td>
<td>17</td>
<td>47.7</td>
<td>-0.10 [-0.26, 0.06]</td>
</tr>
<tr>
<td>Gobert et al. (173)</td>
<td>2010</td>
<td>29</td>
<td>24.6</td>
<td>-0.06 [-0.29, 0.17]</td>
</tr>
<tr>
<td>Cohen et al. (171)</td>
<td>2011</td>
<td>13</td>
<td>19.0</td>
<td>-0.30 [-0.56, -0.04]</td>
</tr>
<tr>
<td>Miraghajani et al. (175)</td>
<td>2013</td>
<td>25</td>
<td>2.2</td>
<td>-0.36 [-1.12, 0.39]</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>97.9</td>
<td></td>
<td></td>
<td>-0.15 [-0.26, -0.03]</td>
</tr>
<tr>
<td>Heterogeneity:</td>
<td>Tau² = 0.00; Chi² = 3.93, df = 5 ($P = 0.56$); $I^2 = 0%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect:</td>
<td>$Z = 2.54$ ($P = 0.01$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type 1 Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kontessis et al. (159)</td>
<td>1995</td>
<td>9</td>
<td>0.9</td>
<td>-0.30 [-1.49, 0.89]</td>
</tr>
<tr>
<td>Stephenson et al. (172)</td>
<td>2005</td>
<td>12</td>
<td>1.2</td>
<td>-0.90 [-1.95, 0.15]</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>2.1</td>
<td></td>
<td></td>
<td>-0.64 [-1.43, 0.15]</td>
</tr>
<tr>
<td>Heterogeneity:</td>
<td>Tau² = 0.00; Chi² = 0.55, df = 1 ($P = 0.46$); $I^2 = 0%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect:</td>
<td>$Z = 1.59$ ($P = 0.11$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>133</td>
<td>100</td>
<td></td>
<td>-0.16 [-0.27, -0.05]</td>
</tr>
<tr>
<td>Heterogeneity:</td>
<td>Tau² = 0.00; Chi² = 5.93, df = 7 ($P = 0.55$); $I^2 = 0%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect:</td>
<td>$Z = 2.75$ ($P = 0.006$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for subgroup differences:</td>
<td>Chi² = 1.45, df = 1 ($P = 0.23$), $I^2 = 31.1%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup and Study (Reference)</td>
<td>Year</td>
<td>Participants, n</td>
<td>Weight, %</td>
<td>Mean Difference [95% CI] in Fasting Glucose, mmol/L</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------</td>
<td>-----------------</td>
<td>-----------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td><strong>Type 2 Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hermansen et al. (174)</td>
<td>2001</td>
<td>20</td>
<td>6.4</td>
<td>-0.40 [-1.65, 0.85]</td>
</tr>
<tr>
<td>Azadbakht et al. (27)</td>
<td>2008</td>
<td>41</td>
<td>7.0</td>
<td>-1.44 [-2.61, -0.28]</td>
</tr>
<tr>
<td>Azadbakht et al. (186)</td>
<td>2009</td>
<td>14</td>
<td>3.5</td>
<td>0.45 [-1.45, 2.35]</td>
</tr>
<tr>
<td>Gobert et al. (173)</td>
<td>2010</td>
<td>29</td>
<td>14.9</td>
<td>0.12 [-0.33, 0.57]</td>
</tr>
<tr>
<td>Cohen et al. (171)</td>
<td>2011</td>
<td>13</td>
<td>9.3</td>
<td>-0.50 [-1.41, 0.41]</td>
</tr>
<tr>
<td>Miraghaejani et al. (175)</td>
<td>2013</td>
<td>25</td>
<td>8.1</td>
<td>-0.18 [-1.22, 0.85]</td>
</tr>
<tr>
<td>Abd-Mishani et al. (193)</td>
<td>2014</td>
<td>24</td>
<td>13.6</td>
<td>-0.33 [-0.89, 0.22]</td>
</tr>
<tr>
<td>Hosseinpour-Niazi et al. (158)</td>
<td>2014</td>
<td>31</td>
<td>17.1</td>
<td>-0.51 [-0.79, -0.24]</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td>79.9 -0.34 [-0.63, -0.06]</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.04; Chi² = 9.67, df = 7 (P = 0.21); I² = 28%
Test for overall effect: Z = 2.38 (P = 0.02)

| **Type 1 Diabetes**            |      |                 |           |                                                   |
| Kontessis et al. (159)         | 1995 | 9               | 18.6      | -1.00 [-1.01, -0.99]                              |
| Stephenson et al. (172)        | 2005 | 12              | 1.5       | -3.55 [-6.66, -0.44]                             |
| **Subtotal (95% CI)**          | 20.1 |                 |           | -1.78 [-4.09, 0.53]                              |

Heterogeneity: Tau² = 2.00; Chi² = 2.59, df = 1 (P = 0.11); I² = 61%
Test for overall effect: Z = 1.51 (P = 0.13)

| **Total (95% CI)**             | 218  | 100             |           | -0.53 [-0.92, -0.13]                             |

Heterogeneity: Tau² = 0.21; Chi² = 50.62, df = 9 (P < 0.00001); I² = 82%
Test for overall effect: Z = 2.63 (P = 0.009)
Test for subgroup differences: Chi² = 1.47, df = 1 (P = 0.23), I² = 32.1%

**Figure 4.2B** – Forest plot of RCTs investigating the effect of replacing sources of animal with plant protein in individuals with diabetes on fasting glucose. Pooled effect estimates for each subgroup and overall effect are represented by the diamonds. Data are expressed as weighted mean differences (MD) with 95% CIs, using the generic inverse-variance method with random effects models. Paired analyses were applied to all crossover trials. Inter-study heterogeneity was tested by the Cochran Q-statistic and quantified by I² at a significance level of P <0.10.
**Figure 4.2C** – Forest plot of RCTs investigating the effect of replacing sources of animal with plant protein in individuals with diabetes on fasting insulin. Pooled effect estimates for each subgroup and overall effect are represented by the diamonds. Data are expressed as weighted mean differences (MD) with 95% CIs, using the generic inverse-variance method with random effects models. Paired analyses were applied to all crossover trials. Inter-study heterogeneity was tested by the Cochran Q-statistic and quantified by $I^2$ at a significance level of $P < 0.10$. 

<table>
<thead>
<tr>
<th>Subgroup and Study (Reference)</th>
<th>Year</th>
<th>Participants, $n$</th>
<th>Weight, %</th>
<th>Mean Difference [95% CI] in Fasting Insulin, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hermansen et al. (174)</td>
<td>2001</td>
<td>20</td>
<td>14.6</td>
<td>6.00 [-12.91, 24.91]</td>
</tr>
<tr>
<td>Gobert et al. (173)</td>
<td>2010</td>
<td>29</td>
<td>19.7</td>
<td>-11.72 [-28.00, 4.56]</td>
</tr>
<tr>
<td>Cohen et al. (171)</td>
<td>2011</td>
<td>13</td>
<td>0.4</td>
<td>-29.17 [-138.07, 79.73]</td>
</tr>
<tr>
<td>Miraghajani et al. (175)</td>
<td>2013</td>
<td>25</td>
<td>3.3</td>
<td>2.50 [-37.46, 42.46]</td>
</tr>
<tr>
<td>Hosseinpour-Niazi et al. (158)</td>
<td>2014</td>
<td>31</td>
<td>62</td>
<td>-13.89 [-23.07, -4.71]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>118</td>
<td>100</td>
<td></td>
<td>-10.09 [-17.31, -2.86]</td>
</tr>
</tbody>
</table>

Heterogeneity: $\text{Tau}^2 = 0.00$; $\text{Chi}^2 = 3.98$, df = 4 ($P = 0.41$); $I^2 = 0$
Test for overall effect: $Z = 2.74$ ($P = 0.006$)
Figure 4.3A - Publication bias funnel plot for HbA1c. The solid line represents the pooled effect estimate expressed as the weighted mean difference for each analysis, the dashed lines represent pseudo-95% confidence limits, and the circles represent effect estimates for each included study. P-values displayed in the top right corner of each funnel plot are derived from quantitative assessment of publication bias by Egger and Begg tests set at a significance level of P<0.05.
**Figure 4.3B** - Publication bias funnel plot for fasting glucose. The solid line represents the pooled effect estimate expressed as the weighted mean difference for each analysis, the dashed lines represent pseudo-95% confidence limits, and the circles represent effect estimates for each included study. P-values displayed in the top right corner of each funnel plot are derived from quantitative assessment of publication bias by Egger and Begg tests set at a significance level of P<0.05.
Figure 4.3C - Publication bias funnel plot for fasting insulin. The solid line represents the pooled effect estimate expressed as the weighted mean difference for each analysis, the dashed lines represent pseudo-95% confidence limits, and the circles represent effect estimates for each included study. P-values displayed in the top right corner of each funnel plot are derived from quantitative assessment of publication bias by Egger and Begg tests set at a significance level of P<0.05.
### Supplemental Table 4.1 – Search strategy*

<table>
<thead>
<tr>
<th>Database</th>
<th>Search period</th>
<th>Search terms</th>
</tr>
</thead>
</table>
| MEDLINE  | 1946 to August 2015 | 1. exp Diet, Vegetarian/  
2. vegetarian.mp.  
3. vegan.mp.  
4. exp Vegetable Proteins/  
5. (vegetable* adj1 protein*).mp.  
6. (plant* adj1 protein*).mp.  
7. (plant* adj1 food*).mp.  
8. (plant* adj1 based).mp.  
9. exp Fabaceae/  
10. exp Soybean Proteins/  
11. soy*.mp.  
12. tofu*.mp.  
13. natto*.mp.  
14. tempeh*.mp.  
15. miso*.mp.  
16. lentil*.mp.  
17. bean*.mp.  
18. legume*.mp.  
19. peanut*.mp.  
20. (meat* adj1 analog*).mp.  
21. lactooovo*.mp.  
22. lacto-ovo*.mp.  
23. ovolacto*.mp.  
24. ovo-lacto*.mp.  
25. lactoveg*.mp.  
26. lacto-veg*.mp.  
27. ovo-veg*.mp.  
28. ovo-veg*.mp.  
29. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28  
30. omnivor*.mp.  
31. (conventional adj3 diet*).mp.  
32. (normal adj3 diet*).mp.  
33. (regular adj3 diet*).mp.  
34. (mixed adj3 diet*).mp.  
35. exp Meat/  
36. exp Eggs/  
37. exp Egg Proteins, Dietary/  
38. exp Dairy Products/  
39. exp Milk/  
40. exp Milk Proteins/  
41. (meat* adj1 protein*).mp.  
42. (meat* adj1 product*).mp.  
43. (animal* adj1 protein*).mp.  
44. (animal* adj1 product*).mp.  
45. (fish* adj1 protein*).mp.  
46. (fish* adj1 product*).mp.  
47. (poultry adj1 protein*).mp.  
48. (poultry adj1 product*).mp.  
49. (chicken* adj1 protein*).mp.  
50. (chicken* adj1 product*).mp.  
51. (egg* adj1 protein*).mp.  
52. (egg* adj1 product*).mp.  
53. (milk adj1 protein*).mp.  
54. (milk adj1 product*).mp.  
55. (dairy adj1 protein*).mp.  
56. (dairy adj1 product*).mp.  
57. 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56  
58. OGTT.mp.  
59. exp Hemoglobin A, Glycosylated/ |
60. hba1c.mp.
61. fructosamine*.mp.
62. insulin*.mp.
63. glycemia.mp.
64. exp Glucose/
65. exp Hyperglycemia/
66. hyperinsulin*.mp.
67. dysglycemia.mp.
68. gly* albumin.mp.
69. exp Diabetes Mellitus/
70. metabolic syndrome.mp.
71. HOMA*.mp.
72. 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or
71
73. 29 and 57 and 72
74. limit 73 to animals
75. limit 74 to human
76. 74 not 75
77. 73 not 76
78. 77 not (exp infant formula/ or exp milk, human/)

EMBASE 1947 to August 2015
1. exp vegetarian diet/
2. exp vegetarian/
3. vegetarian*.mp.
4. vegan*.mp.
5. exp vegetable protein/
6. (vegetable* adj1 protein*).mp.
7. (plant* adj1 protein*).mp.
8. (plant* adj1 food*).mp.
9. (plant* adj1 based).mp.
10. exp Fabaceae/
11. soy*.mp.
12. tofu*.mp.
13. natto*.mp.
14. tempeh*.mp.
15. miso*.mp.
16. lentil*.mp.
17. bean*.mp.
18. legume*.mp.
19. peanut*.mp.
20. (meat* adj1 analog*).mp.
21. lactoovo*.mp.
22. lacto-ovo*.mp.
23. ovolacto*.mp.
24. ovo-lacto*.mp.
25. lactoveg*.mp.
26. lacto-veg*.mp.
27. ovo-veg*.mp.
28. ovo-veg*.mp.
29. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or
16 or 17 or 18 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
30. exp omnivore/
31. omnivor*.mp.
32. (conventional adj3 diet*).mp.
33. (normal adj3 diet*).mp.
34. (regular adj3 diet*).mp.
35. (mixed adj3 diet*).mp.
36. exp Meat/
37. exp egg/
38. exp dairy product/
39. (meat* adj1 protein*).mp.
40. (meat* adj1 product*).mp.
41. (animal* adj1 protein*).mp.
42. (animal* adj1 product*).mp.
43. (fish* adj1 protein*).mp.
44. (fish* adj1 product*).mp.
45. (poultry adj1 protein*).mp.
46. (poultry adj1 product*).mp.
47. (chicken* adj1 protein*).mp.
48. (chicken* adj1 product*).mp.
49. (egg* adj1 protein*).mp.
50. (egg* adj1 product*).mp.
51. (milk adj1 protein*).mp.
52. (milk adj1 product*).mp.
53. (dairy adj1 protein*).mp.
54. (dairy adj1 product*).mp.
55. 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54
56. exp oral glucose tolerance test/
57. OGTT.mp.
58. exp hemoglobin A1c/
59. hba1c.mp.
60. fructosamine*.mp.
61. insulin*.mp.
62. exp glucose blood level/
63. glycemia.mp.
64. exp glucose/
65. 'impaired fasting glucose'.mp.
66. hyperglycemia.mp.
67. 'impaired glucose tolerance'.mp.
68. hyperinsulin*.mp.
69. dysglycemia.mp.
70. 'gly* albumin'.mp.
71. exp diabetes mellitus/
72. exp insulin dependent diabetes mellitus/
73. exp non insulin dependent diabetes mellitus/
74. exp pregnancy diabetes mellitus/
75. exp metabolic syndrome X/
76. HOMA*.mp.
77. 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76
78. 29 and 55 and 77
79. limit 78 to (animals and animal studies)
80. 78 not 79
81. 80 not (exp breast milk/ or exp infant formula/)

Cochrane Central Register of Controlled Trials through to 26 August 2015
1. exp Diet, Vegetarian/
2. vegetarian*.mp.
3. vegan*.mp.
4. exp Vegetable Proteins/
5. (vegetable* adj1 protein*).mp.
6. (plant* adj1 protein*).mp.
7. (plant* adj1 food*).mp.
8. (plant* adj1 based).mp.
9. exp Fabaceae/
10. exp Soybean Proteins/
11. soy*.mp.
12. tofu*.mp.
13. natto*.mp.
14. tempeh*.mp.
15. miso*.mp.
16. lentil*.mp.
17. bean*.mp.
18. legume*.mp.
19. peanut*.mp.
20. (meat* adj1 analog*).mp.
21. lactoovo*.mp.
22. lacto-ovo*.mp.
23. ovolacto*.mp.
24. ovo-lacto*.mp.
25. lactoveg*.mp.
26. lacto-veg*.mp.
27. ovo-veg*.mp.
28. ovo-veg*.mp.
29. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
30. omnivor*.mp.
31. (conventional adj3 diet*).mp.
32. (normal adj3 diet*).mp.
33. (regular adj3 diet*).mp.
34. (mixed adj3 diet*).mp.
35. exp Meat/
36. exp Eggs/
37. exp Egg Proteins, Dietary/
38. exp Dairy Products/
39. exp Milk/
40. exp Milk Proteins/
41. (meat* adj1 protein*).mp.
42. (meat* adj1 product*).mp.
43. (animal* adj1 protein*).mp.
44. (animal* adj1 product*).mp.
45. (fish* adj1 protein*).mp.
46. (fish* adj1 product*).mp.
47. (poultry adj1 protein*).mp.
48. (poultry adj1 product*).mp.
49. (chicken* adj1 protein*).mp.
50. (chicken* adj1 product*).mp.
51. (egg* adj1 protein*).mp.
52. (egg* adj1 product*).mp.
53. (milk adj1 protein*).mp.
54. (milk adj1 product*).mp.
55. (dairy adj1 protein*).mp.
56. (dairy adj1 product*).mp.
57. 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or
43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56
58. OGTT.mp.
59. 'oral glucose tolerance test'.mp.
60. exp Hemoglobin A, Glycosylated/
61. hba1c.mp.
62. fructosamine*.mp.
63. insulin*.mp.
64. glycemia.mp.
65. exp Glucose/
66. exp Hyperglycemia/
67. hyperinsulin*.mp.
68. dysglycemia.mp.
69. gly* albumin.mp.
70. exp diabetes mellitus/
71. metabolic syndrome.mp.
72. HOMA*.mp.
73. 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or
71 or 72
74. 29 and 57 and 73
75. 74 not (exp infant formula/ or exp milk, human/)

* For all databases the original search was 19 December 2013; updated searches were performed 10
November 2014 and 26 August 2015
### Supplemental Table 4.2 – Study quality assessment using the Heyland Methodological Quality Score (MQS)*

<table>
<thead>
<tr>
<th>Study, Year (Reference)</th>
<th>Design</th>
<th>Sample</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Randomization (n/2)</td>
<td>Blinding (n/1)</td>
<td>Analysis (n/2)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------------</td>
</tr>
<tr>
<td>Kontessis et al, 1995 (159)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Stephenson et al, 2005 (172)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anderson et al, 1998 (192)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hermansen et al, 2001 (174)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Wheeler et al, 2002 (30)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Azadbakht et al, 2008 (27)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Azadbakht et al, 2009 (186)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gobert et al, 2010 (173)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cohen et al, 2011 (171)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Miraghejani et al, 2013 (175)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abd-Mishani et al, 2014 (193)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hosseinpour-Niazi et al, 2014 (158)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

MQS=Heyland Methodological Quality Score

*The Heyland MQS assigns a score of 0 or 1 or from 0 to 2 over 9 categories of quality related to study design, sampling procedures, and interventions, for a total of 13 points. Trials that scored ≥8 were considered to be of higher quality.

Randomization was scored 2 points for being randomized with the methods described, 1 point for being randomized without the methods described, or 0 points for being neither randomized nor having the methods described. Blinding was scored 1 point for being double-blind or 0 points for “other.” Analysis was scored 2 points for being intention-to-treat; all other types of analyses scored 0 points.

Sample selection was scored 1 point for being consecutive eligible or 0 points for being preselected or indeterminate. Sample comparability was scored 1 point for being comparable or 0 points for not being comparable at baseline. Follow-up was scored 1 point for being 100% or 0 points for <100%.

Treatment protocol was scored 1 point for being reproducibly described or 0 points for being poorly described. Co-interventions were scored 2 points for being described and equal, 1 point for being described but unequal or indeterminate, or 0 points for not being described. Treatment crossovers (where participants were switched from the control treatment to the experimental treatment) were scored 2 points for being <10%, 1 point for being >10%, and 0 points for not being described.
Supplemental Table 4.3 – Continuous *a priori* & post-hoc subgroup analyses for HbA1c

<table>
<thead>
<tr>
<th>Subgroup category</th>
<th>Range</th>
<th>No. of Trials</th>
<th>N</th>
<th>β [95% CI]</th>
<th>Residual I²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5.9-8.5 %</td>
<td>8</td>
<td>133</td>
<td>-0.04 [-0.27, 0.19]</td>
<td>0.00%</td>
<td>0.695</td>
</tr>
<tr>
<td>Percent AP replaced</td>
<td>4-70 %/d</td>
<td>7</td>
<td>120</td>
<td>0.00 [-0.01, 0.01]</td>
<td>0.00%</td>
<td>0.844</td>
</tr>
<tr>
<td>Grams AP replaced</td>
<td>2.4-64 g/d</td>
<td>8</td>
<td>133</td>
<td>0.00 [-0.00, 0.01]</td>
<td>0.00%</td>
<td>0.294</td>
</tr>
<tr>
<td>Absolute fiber intake</td>
<td>12.6-41 g/d</td>
<td>6</td>
<td>112</td>
<td>0.00 [-0.02, 0.02]</td>
<td>0.00%</td>
<td>0.751</td>
</tr>
<tr>
<td>Between arm Δ in fiber intake</td>
<td>-1.4-4.9 g/d</td>
<td>6</td>
<td>112</td>
<td>-0.04 [-0.23, 0.15]</td>
<td>0.00%</td>
<td>0.611</td>
</tr>
<tr>
<td>Within arm Δ in fiber intake</td>
<td>-1.8-15 g/d</td>
<td>3</td>
<td>61</td>
<td>-0.00 [-0.52, 0.52]</td>
<td>56.60%</td>
<td>0.995</td>
</tr>
<tr>
<td>Absolute SF intake</td>
<td>7.6-9.7 %E</td>
<td>3</td>
<td>58</td>
<td>0.02 [-4.33, 4.37]</td>
<td>56.60%</td>
<td>0.958</td>
</tr>
<tr>
<td>Between arm Δ in SF intake</td>
<td>-3.6-0 %E</td>
<td>3</td>
<td>58</td>
<td>0.16 [-1.57, 1.89]</td>
<td>3.07%</td>
<td>0.453</td>
</tr>
<tr>
<td>Within arm Δ in SF intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isoflavone intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body weight</td>
<td>79-111 kg</td>
<td>7</td>
<td>124</td>
<td>0.00 [-0.02, 0.02]</td>
<td>14.86%</td>
<td>0.970</td>
</tr>
<tr>
<td>Sex</td>
<td>0-77.8 %W</td>
<td>8</td>
<td>133</td>
<td>-0.00 [-0.01, 0.01]</td>
<td>0.00%</td>
<td>0.632</td>
</tr>
</tbody>
</table>

AP=animal protein; E=energy; N=number of subjects; No.=number; SF=saturated fat; W=women

\(^{a}\)Total number of trials=8

\(^{b}\)β is the slope derived from subgroup analyses on meta-regression analyses and represents the treatment effect of replacing animal protein with plant protein for each subgroup. The residual I² value indicates heterogeneity unexplained by the subgroup. Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control (C) arm (T-C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E-B). * Statistically significant between subgroups (P<0.05).
Supplemental Table 4.4 – Continuous *a priori* & post-hoc subgroup analyses for fasting glucose

<table>
<thead>
<tr>
<th>Subgroup category</th>
<th>Range</th>
<th>No. of Trials</th>
<th>N</th>
<th>β [95% CI]</th>
<th>Residual I²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.7-10.4 mmol/L</td>
<td>9</td>
<td>209</td>
<td>-0.35 [-0.80, -0.10]</td>
<td>24.18%</td>
<td>0.109</td>
</tr>
<tr>
<td>Percent AP replaced</td>
<td>4-70 %/d</td>
<td>9</td>
<td>205</td>
<td>-0.01 [-0.03, 0.01]</td>
<td>56.00%</td>
<td>0.187</td>
</tr>
<tr>
<td>Grams AP replaced</td>
<td>6-50 g/d</td>
<td>10</td>
<td>218</td>
<td>-0.01 [-0.03, 0.02]</td>
<td>68.19%</td>
<td>0.502</td>
</tr>
<tr>
<td>Absolute fiber intake</td>
<td>12.6-41 g/d</td>
<td>8</td>
<td>181</td>
<td>0.03 [-0.04, 0.10]</td>
<td>68.38%</td>
<td>0.361</td>
</tr>
<tr>
<td>Between arm Δ in fiber intake</td>
<td>-7.6 g/d</td>
<td>8</td>
<td>181</td>
<td>0.04 [-0.14, 0.22]</td>
<td>80.26%</td>
<td>0.576</td>
</tr>
<tr>
<td>Within arm Δ in fiber intake</td>
<td>-1.8-15 g/d</td>
<td>5</td>
<td>116</td>
<td>0.04 [-0.29, 0.37]</td>
<td>71.20%</td>
<td>0.729</td>
</tr>
<tr>
<td>Absolute SF intake</td>
<td>2.6-9.7 %E</td>
<td>5</td>
<td>127</td>
<td>-0.02 [-0.80, 0.77]</td>
<td>65.75%</td>
<td>0.953</td>
</tr>
<tr>
<td>Between arm Δ in SF intake</td>
<td>-3.6--0.6 %E</td>
<td>5</td>
<td>127</td>
<td>1.00 [-0.32, 2.33]</td>
<td>28.52%</td>
<td>0.095</td>
</tr>
<tr>
<td>Within arm Δ in SF intake</td>
<td>-3.3--0.2 %E</td>
<td>4</td>
<td>96</td>
<td>0.97 [-1.96, 3.89]</td>
<td>69.72%</td>
<td>0.291</td>
</tr>
<tr>
<td>Isoflavone intake</td>
<td>56-165 mg/d</td>
<td>4</td>
<td>104</td>
<td>0.00 [-0.47, 0.05]</td>
<td>68.87%</td>
<td>0.868</td>
</tr>
<tr>
<td>Body weight</td>
<td>70.6-102.3 kg</td>
<td>8</td>
<td>178</td>
<td>0.01 [-0.07, 0.09]</td>
<td>47.63%</td>
<td>0.756</td>
</tr>
<tr>
<td>Sex</td>
<td>28.6-77.8 %W</td>
<td>10</td>
<td>218</td>
<td>-0.01 [-0.04, 0.01]</td>
<td>69.77%</td>
<td>0.244</td>
</tr>
</tbody>
</table>

AP = animal protein; E = energy; N = number of subjects; No. = number; SF = saturated fat; W = women

*a* Total number of trials = 10

β is the slope derived from subgroup analyses on meta-regression analyses and represents the treatment effect of replacing animal protein with plant protein for each subgroup. The residual I² value indicates heterogeneity unexplained by the subgroup. Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control (C) arm (T-C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E-B). * Statistically significant between subgroups (P < 0.05).
Supplemental Table 4.5 – Continuous *a priori* & post-hoc subgroup analyses for fasting insulin

<table>
<thead>
<tr>
<th>Subgroup category</th>
<th>Range</th>
<th>No. of Trials</th>
<th>N</th>
<th>$\beta$ [95% CI]</th>
<th>Residual $I^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>56.3-134.2 pmol/L</td>
<td>5</td>
<td>118</td>
<td>0.18 [-0.65, 1.02]</td>
<td>6.36%</td>
<td>0.536</td>
</tr>
<tr>
<td>Percent AP replaced</td>
<td>4-34 %/d</td>
<td>4</td>
<td>105</td>
<td>0.22 [-2.34, 2.78]</td>
<td>36.54%</td>
<td>0.744</td>
</tr>
<tr>
<td>Grams AP replaced</td>
<td>2.5-50 g/d</td>
<td>5</td>
<td>118</td>
<td>0.31 [-0.45, 1.08]</td>
<td>0.00%</td>
<td>0.284</td>
</tr>
<tr>
<td>Absolute fiber intake</td>
<td>19.2-41 g/d</td>
<td>4</td>
<td>105</td>
<td>0.62 [-2.68, 3.91]</td>
<td>31.50%</td>
<td>0.506</td>
</tr>
<tr>
<td>Between arm $\Delta$ in fiber intake</td>
<td>-1.2-4.5 g/d</td>
<td>4</td>
<td>105</td>
<td>-1.37 [-12.13, 9.39]</td>
<td>28.58%</td>
<td>0.638</td>
</tr>
<tr>
<td>Within arm $\Delta$ in fiber intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Absolute SF intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Between arm $\Delta$ in SF intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Within arm $\Delta$ in SF intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isoflavone intake</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body weight</td>
<td>76.3-96.1 kg</td>
<td>4</td>
<td>87</td>
<td>0.44 [-7.30, 8.18]</td>
<td>0.00%</td>
<td>0.830</td>
</tr>
<tr>
<td>Sex</td>
<td>30-77.4 %W</td>
<td>5</td>
<td>118</td>
<td>-0.30 [-0.92, 0.33]</td>
<td>0.00%</td>
<td>0.226</td>
</tr>
</tbody>
</table>

AP=animal protein; E=energy; N=number of subjects; No. =number; SF=saturated fat; W=women

*a* Total number of trials=5

$\beta$ is the slope derived from subgroup analyses on meta-regression analyses and represents the treatment effect of replacing animal protein with plant protein for each subgroup. The residual $I^2$ value indicates heterogeneity unexplained by the subgroup. Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control(C) arm (T-C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E-B). * Statistically significant between subgroups (P<0.05).
Supplemental Table 4.6A-D – Post-hoc piecewise linear meta-regression analyses for the continuous subgroup looking at percent animal protein replaced with plant protein from total protein on fasting glucose

A – Dose-threshold=20%
Residual $I^2=61.29$
$P=0.466$

<table>
<thead>
<tr>
<th>Dose ranges</th>
<th>β [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20%</td>
<td>0.00 [-0.13, 0.13]</td>
<td>0.999</td>
</tr>
<tr>
<td>≥20%</td>
<td>-0.01 [-0.04, 0.02]</td>
<td>0.279</td>
</tr>
</tbody>
</table>

B – Dose-threshold=30%
Residual $I^2=49.03$
$P=0.339$

<table>
<thead>
<tr>
<th>Dose ranges</th>
<th>β [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30%</td>
<td>0.00 [-0.06, 0.08]</td>
<td>0.831</td>
</tr>
<tr>
<td>≥30%</td>
<td>-0.02 [-0.05, 0.01]</td>
<td>0.196</td>
</tr>
</tbody>
</table>

C – Dose-threshold=35%
Residual $I^2=47.76$
$P=0.330$

<table>
<thead>
<tr>
<th>Dose ranges</th>
<th>β [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35%</td>
<td>0.00 [-0.05, 0.06]</td>
<td>0.930</td>
</tr>
<tr>
<td>≥35%</td>
<td>-0.02 [-0.06, 0.01]</td>
<td>0.200</td>
</tr>
</tbody>
</table>

D – Dose-threshold=40%
Residual $I^2=49.08$
$P=0.369$

<table>
<thead>
<tr>
<th>Dose ranges</th>
<th>β [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40%</td>
<td>-0.00 [-0.05, 0.05]</td>
<td>0.983</td>
</tr>
<tr>
<td>≥40%</td>
<td>-0.02 [-0.07, 0.02]</td>
<td>0.290</td>
</tr>
</tbody>
</table>

β is the slope derived from the piecewise linear meta-regression analyses and represents the treatment effect on fasting glucose for doses above and below each dose-threshold representing percent animal protein replaced with plant protein from total protein. The residual $I^2$ value indicates heterogeneity unexplained by each dose-threshold.
Supplemental Figure 4.1 – Cochrane Risk of Bias Graph. Risk of bias graph consists of the review authors’ judgments about each risk of bias item presented as percentages across all included studies. Random Sequence Generation assessed whether the method of randomization was described. Allocation concealment assessed whether investigators could tell which treatment participants were going to be randomized to. Blinding of participants and personnel assessed whether the study was blinded to investigators, study personnel/outcome assessors, and/or participants. Incomplete outcome data assessed whether missing outcome data affected the true outcome. Selected outcome reporting assessed whether all of the studies pre-specified outcomes (primary and secondary) of interest have been reported in a pre-specified way.
Supplemental Figure 4.2A – Categorical *a priori* subgroup analyses for HbA1c. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual I^2 value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for plant protein type were as follows: 0.12 [-0.43, 0.68] (1 vs. 2); 0.19 [-0.47, 0.84] (1 vs. 3); -0.06 [-0.69, 0.56] (3 vs. 2). Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control (C) arm (T–C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E–B). AP=animal protein; N=number of participants; MQS=Heyland Methodological Quality Score; SF=saturated fat. *Statistically significant between subgroups (P<0.05)
**Supplemental Figure 4.2B** – Categorical risk of bias subgroup analyses for HbA1c. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual $I^2$ value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for sequence generation were as follows: 0.23 [-0.12, 0.58] (2 vs. 1); -0.58 [-1.99, 0.83] (3 vs. 1); 0.81 [-0.58, 2.19] (2 vs. 3). Pairwise between-subgroup mean differences (95% CIs) for blinding of participants, personnel, and outcome assessors were as follows: 0.12 [-0.32, 0.57] (2 vs. 1); -0.14 [-1.20, 0.92] (3 vs. 1); 0.27 [-0.83, -1.36] (2 vs. 3). N=number of participants; ROB=risk of bias. *Statistically significant between subgroups (P<0.05)
Supplemental Figure 4.3A – Categorical *a priori* subgroup analyses for fasting glucose. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual I² value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for plant protein type were as follows: -0.69 [-1.88, 0.50] (1 vs. 2); -0.50 [-2.39, 1.39] (1 vs. 3); -0.56 [-1.76, 0.64] (1 vs. 4); -0.19 [-2.02, 1.63] (3 vs. 2); -0.13 [-1.23, 0.96] (4 vs. 2); 0.06 [-1.77, 1.89] (4 vs. 3). Pairwise between-subgroup mean differences (95% CIs) for animal protein type were as follows: 0.94 [0.46, 1.41] (2 vs. 1); 0.53 [0.18, 0.87] (3 vs. 1); 0.41 [-0.15, 0.97] (2 vs. 3). Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control (C) arm (T–C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E–B). AP=animal protein; N=number of participants; MQS=Heyland Methodological Quality Score; SF=saturated fat. *Statistically significant between subgroups (P<0.05)
Supplemental Figure 4.3B – Categorical risk of bias subgroup analyses for fasting glucose. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual I² value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for sequence generation were as follows: 0.48 [-0.26, 1.23] (2 vs. 1); -2.84 [-6.70, 1.01] (3 vs. 1); 3.32 [-0.54, 7.19] (2 vs. 3). Pairwise between-subgroup mean differences (95% CIs) for blinding of participants, personnel, and outcome assessors were as follows: 0.28 [-0.72, 1.27] (2 vs. 1); 0.51 [-1.39, 2.41] (3 vs. 1); -0.23 [-2.13, 1.68] (2 vs. 3). Pairwise between-subgroup mean differences (95% CIs) for incomplete outcome data were as follows: 0.11 [-1.43, 1.65] (1 vs. 2); 0.45 [-1.23, 2.13] (1 vs. 3); -0.34 [-1.50, 0.82] (3 vs. 2). Pairwise between-subgroup mean differences (95% CIs) for selective outcome reporting data were as follows: 0.74 [0.06, 1.43] (1 vs. 2); 0.62 [-0.40, 1.64] (1 vs. 3); 0.12 [-0.90, 1.14] (2 vs. 3). N=number of participants; ROB=risk of bias. *Statistically significant between subgroups (P<0.05)
Supplemental Figure 4.4A – Categorical *a priori* subgroup analyses for fasting insulin. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual I² value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for plant protein type were as follows: -26.15 [-268.74, 216.45] (3 vs. 2); -10.87 [-56.82, 35.08] (4 vs. 2); 15.28 [-227.65, 258.21] (4 vs. 3). Absolute intakes represent intakes within the treatment arm. Between arm differences represent the difference between the treatment (T) and control (C) arm (T–C). Within arm differences represent the difference between end (E) and baseline (B) values within the treatment arm (E–B). AP=animal protein; N=number of participants; MQS=Heyland Methodological Quality Score; SF=saturated fat. *Statistically significant between subgroups (P<0.05)
**Supplemental Figure 4.4B** — Categorical risk of bias subgroup analyses for fasting insulin. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled estimate for the overall (total) analysis. The residual $I^2$ value indicates heterogeneity unexplained by the subgroup. Pairwise between-subgroup mean differences (95% CIs) for blinding of participants, personnel, and outcome assessors were as follows: -20.35 [-274.86, 234.16] (1 vs. 2); -31.67 [-302.72, 239.38] (1 vs. 3); 11.32 [-88.11, 110.75] (3 vs. 2). N=number of participants; ROB=risk of bias. *Statistically significant between subgroups (P<0.05)
Supplemental Figure 5.5A – Funnel plot for trim-and-fill analysis of HbA1c. The horizontal line represents the pooled effect estimate expressed as a mean difference, the diagonal lines represent the pseudo-95% CIs of the mean difference and the clear circles represent effect estimates for each included.
Supplemental Figure 5.5B — Funnel plot for trim-and-fill analysis of fasting glucose. The horizontal line represents the pooled effect estimate expressed as a mean difference, the diagonal lines represent the pseudo-95% CIs of the mean difference and the clear circles represent effect estimates for each included study while back squares represent "imputed" studies.

Imputed MD accounting for publication bias:
-0.56 [95% CI: -0.94, -0.18]
P-value: 0.004
Supplemental Figure 5.5C – Funnel plot for trim-and-fill analysis of fasting insulin. The horizontal line represents the pooled effect estimate expressed as a mean difference, the diagonal lines represent the pseudo-95% CIs of the mean difference and the clear circles represent effect estimates for each included.
CHAPTER V – ASSOCIATION BETWEEN SUBSTITUTING ANIMAL PROTEIN WITH PLANT PROTEIN AND HEMOGLOBIN A1C IN TYPE 2 DIABETES: A CROSS-SECTIONAL STUDY
ASSOCIATION BETWEEN SUBSTITUTING ANIMAL PROTEIN WITH PLANT PROTEIN AND HEMOGLOBIN A1C IN TYPE 2 DIABETES: A CROSS-SECTIONAL STUDY

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5.1 ABSTRACT

Aims: It remains unclear whether replacing animal with plant protein would confer glycemic control benefits in individuals with T2D. Current evidence from observational studies is lacking and findings from RCTs are inconsistent. Therefore, we conducted a cross-sectional study to assess the effect of substituting animal with plant protein on HbA1c in individuals with T2D.

Methods: We analyzed baseline data from 5 RCTs conducted in a group of middle-aged men and women with T2D (n=627) in Toronto, Canada. A multivariate nutrient density model was used to model the effect of substituting total plant protein for animal protein on HbA1c. A spearman correlation was used to validate protein intake by correlating total protein intake with urinary urea measurements.

Results: Substituting total plant protein for animal protein was not associated with a difference in HbA1c concentrations. Total protein intake correlated with urinary urea measurements ($r_s=0.397; P<0.0001$).

Conclusions: This cross-sectional study showed that replacement of animal protein with total plant protein was not associated with HbA1c. Prospective cohort studies and large, high quality RCTs are warranted in order to address the limitations in our study and to further clarify the relationship between substituting animal protein with major sources of plant protein and glycemic control in individuals with diabetes.

Keywords: diabetes; HbA1c; plant protein; animal protein
5.2 INTRODUCTION

Type 2 diabetes is largely preventable through diet and lifestyle modification, which has been shown to be significantly more effective than the use of pharmacological agents\textsuperscript{4}. For individuals living with diabetes, achieving optimal glycemic control is an integral component in management of the disease. Nutrition therapy is one such method that can help with the management of diabetes, where it has been shown to lead to a 1-2% reduction in HbA\textsubscript{1c}\textsuperscript{49} and reduce the use of diabetes medications\textsuperscript{50,51}.

Diets high in major plant protein sources (i.e. soy, soy-derived foods, pulses and nuts) and deficient in animal protein, such as a vegetarian or vegan diets, have been shown to be protective against the development of T2D\textsuperscript{6-8} and beneficial for glycemic control in individuals with T2D\textsuperscript{9}. Prospective cohort studies and RCTs looking at higher intakes of specific plant protein sources alone have also shown similar benefits\textsuperscript{10-14}. Diets high in animal protein, especially in sources of red meat, are associated with an increased diabetes risk\textsuperscript{15-17} and have even been suggested to be considered as a risk factor for T2D\textsuperscript{22}. Given these data on plant and animal protein, it still remains unclear whether replacing animal protein with plant protein would confer glycemic control benefits in individuals with diabetes. Current evidence from observational studies is lacking and findings from RCTs are inconsistent\textsuperscript{27-30}.

Therefore, in order to assess the relationship between replacing animal protein with plant protein and HbA\textsubscript{1c}, the current clinical standard for assessing glycemic control\textsuperscript{45}, we have undertaken a cross-sectional study using baseline data from 5 RCTs conducted in individuals with T2D. More specifically, this study will allow us to assess this relationship while adjusting for other nutrients that exist in whole food sources of plant and animal protein (i.e. available carbohydrates, fat, fibre, magnesium) and to understand this relationship in the context of “real world” intakes in T2D.

5.3 METHODS

Baseline data were obtained from 5 RCTs conducted at the Clinical Nutrition and Risk Factor Modification Centre at St. Michael’s Hospital (SMH) in Toronto, Canada. All 5 trials were approved by the research ethics board of SMH and the University of Toronto, and written consent was obtained from all participants at the time of recruitment. The trials were registered on ClinicalTrials.gov (registration numbers: NCT00410722, NCT01063361, NCT01348568, NCT00438698, NCT01063374). Details of the methods for 4 of the 5 original trials have previously been reported\textsuperscript{109,222-224}.

5.3.1 STUDY POPULATION

Participants were recruited by placing advertisements in local newspapers, the subway, hospital clinics and/or distribution of similar advertisements to diabetes education programs. A total of 672
unique individuals with dietary, anthropometric and biomarker data were included in the analysis. Eligible participants consisted of men and post-menopausal women diagnosed with T2D for at least 6 months, were on a stable dose of antihyperglycemic medications for at least the previous 3 months, had an HbA1c value between 6.5% to 8.5% at screening and baseline, and were free of any clinically significant cardiovascular, renal (serum creatinine >150µmol/L) or liver (serum alanine aminotransferase >3 times greater than the upper limit of the normal) disease, or a history of cancer.

5.3.2 ASSESSMENT OF DIETARY INTAKE

Participants provided food records covering the previous 7 days before their clinic visit at baseline. Diet records were analyzed using a computer program (ESHA Food Processor SQL, version 10.9.0; ESHA, Salem, OR) based on U.S. Department of Agriculture data and international GI tables using the bread scale with additional GI measurements made on local foods (Glycemic Index Laboratories).

5.3.3 BIOCHEMICAL ANALYSES

Blood and urine were analyzed at the SMH Core Lab and University of Toronto, FitzGerald Building. In two of the trials HbA1c was analyzed within 2 days using whole blood collected in EDTA Vacutainer tubes and measured by a designated high-performance liquid chromatography (HPLC) method (Tosoh G7 Automated HPLC Analyzer; Tosoh Bioscience, Grove City, OH) with a coefficient of variation (CV) of 1.7%. In the remaining three trials, HbA1c was analyzed within 24 hours using whole blood collected in EDTA Vacutainer tubes by a turbidometric inhibition latex immunoassay (TINIA Roche Diagnostics) with a CV between assays of 3% to 4%. Urea from 24-hour urine collection was measured using an enzymatic method (SYNCHROLINX LX System).

5.3.4 STATISTICAL ANALYSES

Distributions of all continuous variables were assessed for normality by visual inspection of distribution and probability plots. Variables that were not normally distributed underwent natural log transformations. Descriptive statistics for continuous variables were summarized as means ± SDs and categorical variables were summarized using frequencies and percents.

For the primary analysis a multivariate nutrient density model was used to evaluate the relationship between the main predictor variable: substitution of animal protein with total plant protein and the main outcome variable: HbA1c. We used seven models adjusted for different sets of covariates in our analyses. Model 1 was adjusted for total plant protein, available carbohydrate, total fat, alcohol,
and total energy (all continuous). Model 2 was adjusted for Model 1 covariates and age (continuous). Model 3 was adjusted for Model 2 covariates and sex (male or female). Model 4 was adjusted for Model 3 covariates, BMI (continuous) and waist circumference (continuous). Model 5 was adjusted for Model 4 covariates, smoking (current or not current), diabetes duration (continuous), ethnicity (African, European, Far Eastern, Indian/South Asian, or other whites/Caucasian), and number of antihyperglycemic medications used (0, 1, 2, 3, or 4). Model 6 was adjusted for Model 5 covariates, magnesium intake (continuous), fibre intake (continuous), and GI (continuous). Model 7 was adjusted for Model 6 covariates and for the type of method used to analyze HbA\textsubscript{1c} (immunoassay or HPLC). The estimate ± SE is reported for each model and represents the estimated change in HbA\textsubscript{1c} associated with a 1% energy increase derived from substituting animal protein with plant protein.

In order to validate protein intake reported from the 7-day food records, urea from 24 hour urinary output was correlated with total protein intake. Since the two variables were not normally distributed, a spearman correlation was used to assess the relationship between the two variables. All analyses were carried out using SAS software (version 9.4). Significance was set at P< 0.05.

5.4 RESULTS

5.4.1 PARTICIPANT CHARACTERISTICS

Table 5.1 shows the characteristics of the 627 middle-aged, overweight or obese participants with T2D. There were approximately an equal number of men and women. The mean diabetes duration was 8 years and majority of participants were using at least 1 antihyperglycemic medication. Approximately half of the participants met the recommended target levels for HbA\textsubscript{1c} (≤7.0%\textsuperscript{46}). Most participants were of European (46%) or Indian/South Asian (22%) descent. The mean percentage of daily energy derived from animal and plant protein was 12% and 7.2%, respectively (Table 5.2). Majority of the animal protein consisted of protein from red meat, poultry and dairy (~3% E from each) and the majority of plant protein consisted of protein from grain sources (4.2% E), where greater than 50% of the grain sources were derived from breads (whole grain and white bread).

5.4.2 SUBSTITUTION OF ANIMAL PROTEIN WITH TOTAL PLANT PROTEIN AND HbA\textsubscript{1c}

Table 5.3 shows the estimates for change in HbA\textsubscript{1c} associated with a 1% energy increase derived from substituting animal protein with plant protein in 7 models adjusted for different sets of covariates. The most fully adjusted model showed no significant association between substituting animal with total plant protein and HbA\textsubscript{1c} (Model 7; P=0.126).
5.4.3 PROTEIN VALIDATION

Figure 5.1 shows the results of the spearman correlation analysis conducted to validate protein intake, which showed that total protein intake was moderately correlated with 24 hour urinary urea output ($r_s=0.397; P<0.0001; n=444$).

5.5 DISCUSSION

To the best of our knowledge this is the first cross-sectional study to assess the relationship between substituting animal protein with plant protein and HbA1c in individuals with T2D. Our analysis of 627 middle-aged men and women showed that substituting animal protein with total plant protein was not associated with HbA1c.

5.5.1 RELATION TO OTHER STUDIES AND POTENTIAL EXPLANATIONS

Our findings appear to be consistent with a previous cross-sectional study conducted in diabetes-free female participants from the Nurses’ Health Study (n=3690), which did not show a significant association between substituting one serving of total red meat with nuts or legumes and HbA1c, but did show that substitution with poultry, fish, legumes, and nuts together was associated with significantly lower HbA1c ($\beta \pm SE: -0.031\pm0.015, P=0.04$)\(^{170}\). The lack of association between substituting animal protein with total plant protein intake and HbA1c in our analysis may be due to the overall low consumption of major plant protein sources (soy, pulses and nuts/seeds) by participants in our study, which together contributed to <2% of total energy intake. This low level of consumption is reflected in the overall pattern of protein intake by participants, which showed that 60% of total protein intake consisted of animal protein, with the remaining protein consisting of >50% from grain sources and only 18% from soy, pulses, and nuts/seeds, which is consistent with the overall pattern of protein intake reported for the North American population\(^{71,72}\). As a result, it is possible that the range of intake in our study may not be large enough to detect an overall difference and that with higher intakes of major plant protein sources the association between substituting animal protein with plant protein and HbA1c may change. This is supported by evidence from RCTs showing beneficial effects on glycemic control when substituting animal protein with major plant protein sources in individuals with T2D\(^{171}\). In addition, the lack of association between substituting animal protein with total plant protein and HbA1c may also be due to the high intake of plant protein for grain sources, which may be a marker of high GI refined carbohydrates. This is supported by evidence from prospective cohort studies and RCTs showing that consumption of higher GI foods is associated with increased T2D risk\(^{229-231}\) and poorer glycemic
control outcomes in individuals with diabetes\textsuperscript{216,217}, respectively. Therefore, due to the heterogeneous nature of plant and animal protein sources, it would be useful to compare substitutions of different animal protein sources with major plant protein sources in order to gain a better understanding of whether substitution of specific sources is more beneficial than overall substitution.

Furthermore, it is possible that our findings could be influenced by incomplete adjustment resulting from measured residual confounding, which may include measurement errors from 7 day food records. Misreporting of dietary intake is a major issue in dietary studies as it introduces error in the estimation of energy intake and nutrients. Several cross-sectional and prospective studies using weighed food records have been shown to underestimate energy intake by approximately 18\% and the prevalence of under reporters in these studies was 33\%\textsuperscript{232}. The extent of misreporting by individuals in our study is not clear. Our protein validation analysis showed a moderate correlation between reported daily protein intake and urea measured from 24 hour urinary output ($r_s=0.397$). However, since there may be a large within-subject variation in daily nitrogen excretion, repeat collections of consecutive 24 hour urinary output samples are required to validate protein intake\textsuperscript{232,233}, which was not possible in the current study since this was an exploratory analysis using baseline data from 5 RCTs.

### 5.5.2 LIMITATIONS

There are several limitations in our study that require consideration when interpreting our findings. First, this was a cross-sectional analysis and therefore causal relationships cannot be inferred. Second, the extent of misreporting by individuals in our analysis is not clear and may potentially contribute to measured residual confounding. Third, since we were not able to adjust for other relevant covariates (e.g. physical activity, socioeconomic status, etc.) it is possible that unmeasured residual confounding may also exist in our analysis. Fourth, the adjusted $R^2$'s for the main analysis was very small ($R^2 \leq 0.05$), suggesting that the relationship is not strongly linear. Fifth, given the low intakes of major plant protein sources it is possible that there was not a large enough range of intake to detect an overall difference between substituting animal protein with plant protein and HbA\textsubscript{1c}.

### 5.5.3 CONCLUSIONS

Overall, the results of our cross-sectional study show that replacing animal protein with total plant protein is not associated with HbA\textsubscript{1c} in a group of middle-aged men and women with T2D. Given the overall low consumption of major plant protein sources in the general population and the heterogeneous nature of plant and animal protein sources, there is a need for prospective cohort studies and large RCTs looking at the replacement of different types of animal protein with major plant...
protein sources on markers of glycemic control in individuals with diabetes in order to help address the limitations in our study and further clarify our findings.

5.6 FUNDING

This work was supported by the Canadian Institutes of Health Research, Canola Council of Canada, Agriculture and Agri-Food Canada, Loblaw Companies (Canada), Barilla (Italy), International Tree Nut Council Nutrition Research and Education Foundation, Peanut Institute, ABIP through the PURENet and the Saskatchewan Pulse Growers. EV was funded by a Canadian Institutes of Health Research (CIHR)-Fredrick Banting and Charles Best Canada Graduate Scholarship and the Banting and Best Diabetes Centre (BBDC)-Yow Kam-Yuen Graduate Scholarship in Diabetes Research. RPB and DJAJ were funded by the Government of Canada through the Canada Research Chair Endowment. None of the sponsors had a role in any aspect of the present study, including design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, approval of the manuscript or decision to publish.

5.7 CONFLICTS OF INTEREST

RJdS was funded by the CIHR Postdoctoral Fellowship Award and has received research support from the CIHR, the Calorie Control Council, the Canadian Foundation for Dietetic Research and the Coca-Cola Company (investigator initiated, unrestricted grant) and travel support from the World Health Organization (WHO) to attend group meetings. He has served as an external resource person to WHO’s Nutrition Guidelines Advisory Group and is the lead author of 2 systematic reviews and meta-analyses commissioned by WHO of the relation of saturated fatty acids and trans fatty acids with health outcomes. AJH hold a Tier II Canada Research Chair in Diabetes Epidemiology. RPB has received research funding from Bunge Ltd., travel support from Unilever and consultant fees from Kraft Foods and Mead Johnson. JLS has received research support from the Canadian Institutes of health Research (CIHR), Calorie Control Council, American Society of Nutrition (ASN), The Coca-Cola Company (investigator initiated, unrestricted), Dr. Pepper Snapple Group (investigator initiated, unrestricted), Pulse Canada, and The International Tree Nut Council Nutrition Research & Education Foundation. He has received travel funding, speaker fees, and/or honoraria from the American Heart Association (AHA), American College of Physicians (ACP), American Society for Nutrition (ASN), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH), Canadian Diabetes Association (CDA), Canadian Nutrition Society (CNS), University of South Carolina, University of Alabama at Birmingham, Oldways Preservation Trust, Nutrition Foundation of Italy (NFI), Calorie Control
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Council, Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD), International Life Sciences Institute (ILSI) North America, International Life Sciences Institute (ILSI) Brazil, Abbott Laboratories, Pulse Canada, Canadian Sugar Institute, Dr. Pepper Snapple Group, The Coca-Cola Company, Corn Refiners Association, World Sugar Research Organization, and Società Italiana di Nutrizione Umana (SINU). He has consulting arrangements with Winston & Strawn LLP, Perkins Coie LLP, and Tate & Lyle. He is on the Clinical Practice Guidelines Expert Committee for Nutrition Therapy of both the Canadian Diabetes Association (CDA) and European Association for the study of Diabetes (EASD), as well as being on an American Society for Nutrition (ASN) writing panel for a scientific statement on sugars. He is a member of the International Carbohydrate Quality Consortium (ICQC) and Board Member of the Diabetes and Nutrition Study Group (DNSG) of the EASD. He serves an unpaid scientific advisor for the International Life Science Institute (ILSI) North America, Food, Nutrition, and Safety Program (FNSP). His wife is an employee of Unilever Canada. DJAJ has received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program through the Pulse Research Network, the Advanced Foods and Material Network, Loblaw Companies Ltd., Unilever, Barilla, the Almond Board of California, the Coca-Cola Company (investigator initiated, unrestricted grant), Solae, Haine Celestial, the Sanitarium Company, Orafti, the International Tree Nut Council Nutrition Research and Education Foundation, the Peanut Institute, the Canola and Flax Councils of Canada, the Calorie Control Council, the CIHR, the Canada Foundation for Innovation and the Ontario Research Fund. He has received an honorarium from the United States Department of Agriculture to present the 2013 W.O. Atwater Memorial Lecture. He received the 2013 Award for Excellence in Research from the International Nut and Dried Fruit Council. He received funding and travel support from the Canadian Society of Endocrinology and Metabolism to produce mini cases for the Canadian Diabetes Association. He has been on the speaker's panel, served on the scientific advisory board, and/or received travel support and/or honoraria from the Almond Board of California, Canadian Agriculture Policy Institute, Loblaw Companies Ltd, the Griffin Hospital (for the development of the NuVal scoring system), the Coca-Cola Company, Saskatchewan Pulse Growers, Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, the International Tree Nut Council Nutrition Research and Education Foundation, the Peanut Institute, Herbalife International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Kellogg, Quaker Oats, Procter and Gamble, the Coca-Cola Company, the Griffin Hospital, Abbott Laboratories, the Canola Council of Canada, Dean Foods, the California Strawberry Commission, Haine Celestial, PepsiCo, the Alpro Foundation, Pioneer Hi-Bred International, DuPont Nutrition and Health, Spherix Consulting and WhiteWave Foods, the Advanced
Foods and Material Network, the Canola and Flax Councils of Canada, the Nutritional Fundamentals for Health, AgriCulture and Agri-Food Canada, the Canadian Agri-Food Policy Institute, Pulse Canada, the Saskatchewan Pulse Growers, the Soy Foods Association of North America, the Nutrition Foundation of Italy (NFI), Nutra-Source Diagnostics, the McDougall Program, the Toronto Knowledge Translation Group (St. Michael's Hospital), the Canadian College of Naturopathic Medicine, The Hospital for Sick Children, the Canadian Nutrition Society (CNS), the American Society of Nutrition (ASN), Arizona State University, Paolo Sorbini Foundation and the Institute of Nutrition, Metabolism and Diabetes. No competing interests were declared by EV, CI, and VHJ. There are no patents, products in development or marketed products to declare.

5.8 AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: DJAJ JLS. Analyzed the data: EV CI VHJ RJdS JLS. Wrote the paper: EV JLS. Interpretation of the data: EV CI VHJ RJdS AJH RPB DJAJ JLS. Critical revision of the article for important intellectual content: EV CI VHJ RJdS AJH RPB DJAJ JLS. Final approval of the article: EV CI VHJ RJdS AJH RPB DJAJ JLS. Obtaining of funding: DJAJ JLS. Administrative, technical, or logistic support: CI VHJ. Collection and assembly of data: EV CI. Guarantors: DJAJ JLS.
### Table 5.1 – Participant Characteristics

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<th>Characteristic</th>
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<tr>
<td><strong>n</strong></td>
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</tr>
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<td><strong>Age (years)</strong></td>
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<tr>
<td><strong>Sex</strong></td>
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<tr>
<td>Female</td>
<td>265 (42)</td>
</tr>
<tr>
<td>Male</td>
<td>362 (58)</td>
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<tr>
<td><strong>Race/Ethnicity</strong></td>
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<td>African</td>
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<td>European</td>
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<tr>
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<td>46 (7)</td>
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<tr>
<td>Indian/South Asian</td>
<td>139 (22)</td>
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<td>Othera</td>
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<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>30 ± 6</td>
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<tr>
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<td>105 ± 13</td>
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<td><strong>Current smokers</strong></td>
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<tr>
<td><strong>Duration of diabetes (years)</strong></td>
<td>8 ± 6</td>
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<tr>
<td><strong>HbA1c (%)</strong></td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>≤7.1%</td>
<td>364 (58)</td>
</tr>
<tr>
<td>&gt;7.1%</td>
<td>263 (42)</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
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<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>4.1 ± 1.0</td>
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<td><strong>LDL-C (mmol/L)</strong></td>
<td>2.3 ± 0.9</td>
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<td><strong>HDL-C (mmol/L)</strong></td>
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</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.5 ± 0.9</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>124 ± 14</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>72 ± 9</td>
</tr>
<tr>
<td><strong>No. of antihyperglycemic medications</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>1</td>
<td>323 (52)</td>
</tr>
<tr>
<td>2</td>
<td>225 (36)</td>
</tr>
<tr>
<td>3</td>
<td>73 (12)</td>
</tr>
<tr>
<td>4</td>
<td>4 (0.6)</td>
</tr>
</tbody>
</table>

No.=number

*a* White or Caucasian

*b* n=498

*c* n=565

*d* n=626

*Antihyperglycemic medications consisted of metformin, sulfonylurea, thiazolidinedione, DPP-4 inhibitors, meglitinides (nonsulfonylurea), and/or α-glucosidase inhibitors.*
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1827.8 ± 498.2</td>
</tr>
<tr>
<td>Available carbohydrate (%E)</td>
<td>42.7 ± 7.4</td>
</tr>
<tr>
<td>Total fat (%E)</td>
<td>32.6 ± 6.5</td>
</tr>
<tr>
<td>Total Protein (%E)</td>
<td>19.2 ± 3.4</td>
</tr>
<tr>
<td>Plant protein (%E)</td>
<td>7.2 ± 1.8</td>
</tr>
<tr>
<td>Grain (%E)</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td>Potato (%E)</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Pulse (%E)</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>Soy (%E)</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Nut/Seed (%E)</td>
<td>0.6 ± 0.7</td>
</tr>
<tr>
<td>Other (%E)</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Animal protein (%E)</td>
<td>12 ± 4.0</td>
</tr>
<tr>
<td>Red meat (%E)</td>
<td>2.7 ± 2.5</td>
</tr>
<tr>
<td>Poultry (%E)</td>
<td>2.9 ± 2.5</td>
</tr>
<tr>
<td>Seafood (%E)</td>
<td>1.2 ± 1.4</td>
</tr>
<tr>
<td>Egg (%E)</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>Dairy (%E)</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>Other (%E)</td>
<td>1.7 ± 1.8</td>
</tr>
<tr>
<td>Alcohol (%E)</td>
<td>1.7 ± 3.3</td>
</tr>
<tr>
<td>Fibre (g/1000 kcal)</td>
<td>14.4 ± 5.0</td>
</tr>
<tr>
<td>Magnesium (g/1000 kcal)</td>
<td>196 ± 49.3</td>
</tr>
<tr>
<td>Glycemic Index</td>
<td>56.3 ± 4.1</td>
</tr>
</tbody>
</table>

%E = percent energy

*aThis includes any remaining sources of plant or animal protein (i.e. fruits and vegetables, gluten added to various foods, etc.)
Table 5.3 – Estimates for change in HbA\textsubscript{1c} associated with a 1% energy increase derived from substituting animal protein with total plant protein\(^*\)

<table>
<thead>
<tr>
<th>Model(^a)</th>
<th>n</th>
<th>Estimate ± SE (%)</th>
<th>Adjusted-\textit{R}\textsuperscript{2}</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>627</td>
<td>0.01 ± 0.01</td>
<td>-0.002</td>
<td>0.492</td>
</tr>
<tr>
<td>2</td>
<td>627</td>
<td>0.01 ± 0.01</td>
<td>0.013</td>
<td>0.382</td>
</tr>
<tr>
<td>3</td>
<td>627</td>
<td>0.01 ± 0.01</td>
<td>0.015</td>
<td>0.485</td>
</tr>
<tr>
<td>4</td>
<td>498</td>
<td>0.04 ± 0.02</td>
<td>0.026</td>
<td>0.033</td>
</tr>
<tr>
<td>5</td>
<td>487</td>
<td>0.04 ± 0.02</td>
<td>0.035</td>
<td>0.031</td>
</tr>
<tr>
<td>6</td>
<td>487</td>
<td>0.03 ± 0.02</td>
<td>0.044</td>
<td>0.117</td>
</tr>
<tr>
<td>7</td>
<td>487</td>
<td>0.03 ± 0.02</td>
<td>0.044</td>
<td>0.126</td>
</tr>
</tbody>
</table>

\(n=\text{sample size}\)

\(^a\text{Model 1 was adjusted for total plant protein, available carbohydrate (continuous), total fat (continuous), alcohol (continuous), and total energy (continuous). Model 2 was adjusted for Model 1 covariates and age (continuous). Model 3 was adjusted for Model 2 covariates and sex (male or female). Model 4 was adjusted for Model 3 covariates, BMI (continuous) and waist circumference (continuous). Model 5 was adjusted for Model 4 covariates, smoking (current or not current), diabetes duration (continuous), ethnicity (African, European, Far Eastern, Indian/South Asian, or other), and number of antihyperglycemic medications used (0, 1, 2, 3, or 4). Model 6 was adjusted for Model 5 covariates, magnesium intake (continuous), fibre intake (continuous), and GI (continuous). Model 7 was adjusted for Model 6 covariates and for the type of method used to analyze HbA\textsubscript{1c} (immunoassay or HPLC).}\)

\(^*\text{Note: Data are presented using non-log transformed variables}\)
5.10 FIGURES

Figure 5.1 – Correlation between Total Protein Intake and Urea Output

$r_s = 0.397$
$P < 0.0001$
6.1 OVERALL DISCUSSION

We conducted two studies to investigate the effect of replacing animal protein with plant protein on glycemic control in individuals with diabetes. Our systematic review and meta-analysis of RCTs found that replacing animal protein with major plant protein sources (i.e., soy, soy-derived products, pulses, and nuts) modestly improved glycemic control in individuals with T1D and T2D, where our pooled analyses showed a statistically significant reduction in HbA1c, fasting glucose, and fasting insulin by 0.16%, 0.53 mmol/L and 10.09 pmol/L, respectively. Our cross-sectional study showed that substituting animal protein with total plant protein independent of other nutrients (i.e., available carbohydrates, fat, fibre, magnesium) was not associated with HbA1c in a group of middle-aged men and women with T2D. Taken together our results suggest that (1) replacing animal protein with major plant protein sources leads to modest improvements in glycemic control in individuals with diabetes and (2) due to the limited amount of data in this area, future research is warranted to address the limitations of our systematic review and meta-analysis and lack of agreement with the associations seen in our cross-sectional study.

Although both studies are the first to assess the replacement of animal with plant protein on glycemic control in individuals with diabetes, our results support findings from previous studies related to this area of study. In terms of our systematic review and meta-analysis, there have been several systematic reviews and meta-analyses of RCTs looking at the effect of specific sources of plant protein on glycemic control. Three of these meta-analyses were conducted by our group, which found that incorporation of nuts or pulses into the diet led to significant improvements in measures of glycemic control in individuals with and without diabetes10-12. There have also been two meta-analyses that looked at the effect of soy and soy-derived products, which showed non-significant reductions in measures of glycemic control107, 108. Overall these results are consistent with the findings of our meta-analysis. In terms of our cross-sectional study, our results were somewhat consistent with a previous cross-sectional study conducted in diabetes-free women participating in the Nurses’ Health Study, which assessed the relationship between substituting one serving of red meat with alternative protein sources such as poultry, fish, legumes, and nuts and HbA1c. Similar to our findings, substituting a serving of red meat with nuts or legumes alone were not associated with HbA1c, but substitution with poultry, fish, legumes, and fish together was associated with significantly lower HbA1c (β ± SE: -0.031±0.015, \( P=0.04 \))170. This inconsistency between the results of RCTs and epidemiological studies may be due to the fact that there is a low consumption of major plant protein sources in the North American diet.
relative to animal protein and protein from other plant sources (i.e. grains)\textsuperscript{62,71-73}. Due to the low range of intakes, this makes it difficult to detect an overall difference between substituting animal protein with major sources of plant protein and HbA\textsubscript{1c} in individuals with diabetes. Furthermore, the high intake of protein from grain sources may be contributing to the lack of association between substituting animal with total plant protein and HbA\textsubscript{1c}, since it may be a marker for refined carbohydrates. This is consistent with systematic reviews and meta-analyses of prospective cohort studies showing that higher GI is associated with higher T2D risk\textsuperscript{229-231} and RCTs showing that higher GI is associated with higher HbA\textsubscript{1c} levels in individuals with T2D\textsuperscript{234,235}.

6.2 LIMITATIONS

6.2.1 SYSTEMATIC REVIEW AND META-ANALYSIS

Limitations of our systematic review and meta-analysis include: 1) most of the trials had small sample sizes (50% <20 participants); 2) the majority of trials (83%) were shorter than 12-weeks, which may not be a sufficient follow-up duration to observe meaningful changes in glycemic control\textsuperscript{220}; 3) the majority of trials (92%) were of low quality (MQS<8); 4) most subgroup analyses were underpowered due to the limited number of available studies; 5) only a small number of trials (17%) focused on glycemic control endpoints as a primary outcome; and 6) sensitivity analyses showed that the pooled effect estimate for fasting insulin was not robust.

6.2.2 CROSS-SECTIONAL STUDY

Limitations of our cross-sectional study include: 1) since this is an observational study causal relationships cannot be inferred; 2) the extent of misreporting by individuals is not clear and may potentially contribute to measured residual confounding in our analysis; 3) we were not able to adjust for other relevant covariates (e.g. physical activity, socioeconomic status, etc.) and therefore it is possible that there may also be unmeasured residual confounding in our analysis; 4) the adjusted $R^2$ for the main analysis was very small ($R^2 \leq 0.05$), suggesting that the relationship between substituting animal with plant protein and HbA\textsubscript{1c} is not strongly linear; and 5) given the low intakes of major plant protein sources it is possible that there was not a large enough range of intake to detect an overall difference between substituting animal protein with plant protein and HbA\textsubscript{1c}.

Overall, the limitations of these 2 studies reinforce the need for more trials that are larger, longer and of higher quality with a specific focus on glycemic endpoints as a primary outcome to better understand the effect of replacing animal protein with plant protein on glycemic control in individuals with diabetes.
6.3 MECHANISMS OF ACTION

There are several potential mechanisms that may explain the beneficial effects of replacing animal protein with major plant protein sources on glycemic control. The reduction in body iron stores may be one such mechanism. Prospective cohort studies have shown that increased heme iron intake, found only in animal protein sources, is associated with a significantly higher risk of T2D, whereas non-heme iron intake, which is found in both plant and animal protein sources, has been shown to be either inversely associated with or not associated with the incidence of T2D. This may be attributed to differences in bioavailability between non-heme and heme iron, where heme iron is associated with higher body iron stores. Furthermore, observational studies have shown that serum ferritin, the storage form of iron, predicted the development of hyperglycemia and T2D and was found to be positively associated with insulin resistance, whereas randomized trials have shown that the use of phlebotomy to reduce serum ferritin levels was associated with improved glucose tolerance in individuals with metabolic syndrome and T2D. Overall, the negative impact of iron on glycemic control may be due to its pro-oxidant activities, where it catalyzes several cellular reactions that result in the production of reactive oxygen species, which in turn increases oxidative stress and tissue damage, including damage to the pancreatic β-cells.

Another mechanism may relate to differences in the amino acid profiles between animal and plant protein. Compared to animal proteins, plant proteins appear to be higher in L-arginine (Figure 6.1A-B). Randomized trials have shown that long-term oral administration of L-arginine improves insulin sensitivity in individuals with T2D and in vitro studies suggest that L-arginine promotes insulin secretion from pancreatic β-cells by directly depolarizing the plasma membrane at a neutral pH and only in the presence of glucose. Specifically, the electrogenic transport of L-arginine into the β-cell via the amino acid transporter mCAT2A has been shown to lead to an increase in intracellular Ca²⁺ concentration through depolarization of the plasma membrane and activation of voltage-dependent calcium channels, which in turn stimulates insulin secretion (see Figure 6.2). However, other mechanisms have also been proposed (i.e. the NO/cGMP pathway). Given these findings, it is uncertain whether the arginine levels typically found in dietary proteins would also have a significant effect on insulin secretion. Furthermore, although our findings show improvements for fasting insulin, specific measures of insulin sensitivity were not explored in our meta-analysis, where only one of the included trials looked at HOMA-IR and found non-significant reductions. Neither endpoint, however, is considered to be a good marker of peripheral insulin sensitivity and therefore further studies are warranted.
### Figure 6.1A – Amino Acid Profiles of Different Plant Protein Sources

<table>
<thead>
<tr>
<th>AMINO ACID ( % of total protein for 100g serving)</th>
<th>Soybeans</th>
<th>Chickpeas</th>
<th>Lentils</th>
<th>Kidney beans</th>
<th>Navy beans</th>
<th>Almonds</th>
<th>Walnuts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>1.215</td>
<td>0.959</td>
<td>0.898</td>
<td>1.200</td>
<td>1.215</td>
<td>0.997</td>
<td>1.116</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.150</td>
<td>7.122</td>
<td>7.251</td>
<td>8.489</td>
<td>8.505</td>
<td>6.970</td>
<td>7.682</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.984</td>
<td>6.693</td>
<td>6.984</td>
<td>7.001</td>
<td>6.318</td>
<td>2.686</td>
<td>2.784</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.215</td>
<td>1.309</td>
<td>0.854</td>
<td>1.303</td>
<td>1.349</td>
<td>0.740</td>
<td>1.550</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.915</td>
<td>1.343</td>
<td>1.308</td>
<td>0.934</td>
<td>0.923</td>
<td>1.021</td>
<td>1.366</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.526</td>
<td>5.361</td>
<td>4.933</td>
<td>5.894</td>
<td>5.723</td>
<td>5.353</td>
<td>4.668</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.587</td>
<td>2.483</td>
<td>2.672</td>
<td>2.364</td>
<td>2.394</td>
<td>2.128</td>
<td>2.666</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.688</td>
<td>2.754</td>
<td>2.816</td>
<td>2.745</td>
<td>2.503</td>
<td>2.548</td>
<td>2.567</td>
</tr>
<tr>
<td>Serine</td>
<td>5.571</td>
<td>5.045</td>
<td>4.612</td>
<td>6.275</td>
<td>5.820</td>
<td>4.313</td>
<td>6.133</td>
</tr>
</tbody>
</table>

- Non-essential amino acids (glutamic & aspartic acid) abundantly found in all proteins
- Most abundant amino acid found in plant protein source (with the exception of glutamic & aspartic acid)
Figure 6.1B – Amino Acid Profile Of Animal Protein Sources

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>Beef</th>
<th>Chicken</th>
<th>Cod fish</th>
<th>Salmon</th>
<th>Eggs</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>0.657</td>
<td>1.261</td>
<td>1.121</td>
<td>1.120</td>
<td>1.216</td>
<td>1.273</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.551</td>
<td>4.905</td>
<td>4.608</td>
<td>4.607</td>
<td>5.453</td>
<td>5.182</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.604</td>
<td>2.600</td>
<td>2.961</td>
<td>2.960</td>
<td>3.116</td>
<td>2.636</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.291</td>
<td>1.048</td>
<td>1.073</td>
<td>1.073</td>
<td>2.321</td>
<td>0.606</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.950</td>
<td>4.037</td>
<td>3.903</td>
<td>3.903</td>
<td>5.310</td>
<td>5.182</td>
</tr>
<tr>
<td>Valine</td>
<td>4.963</td>
<td>5.177</td>
<td>5.151</td>
<td>5.149</td>
<td>6.097</td>
<td>6.545</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.465</td>
<td>6.762</td>
<td>5.983</td>
<td>5.983</td>
<td>6.002</td>
<td>2.848</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.190</td>
<td>3.726</td>
<td>2.943</td>
<td>2.944</td>
<td>2.369</td>
<td>3.030</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.079</td>
<td>5.838</td>
<td>6.049</td>
<td>6.046</td>
<td>5.564</td>
<td>3.394</td>
</tr>
</tbody>
</table>

- Non-essential amino acids (glutamic & aspartic acid) abundantly found in all proteins
- Most abundant amino acid found in plant protein source (with the exception of glutamic & aspartic acid)
Other potential mechanisms that may explain the effects of replacing animal protein with plant protein on glycemic control involve a reduction in the glycemic index, the sodium and nitrite content found in processed meats, reduced intake of saturated fat, advanced glycation end products (AGEs), phytoestrogens found in soy, and higher fiber intake. It has also been suggested that the negative impact of meat intake may be mediated by its effects on body weight, inflammatory biomarkers, visceral adiposity, and intracellular lipid concentrations.

With regards to the results of our cross-sectional study, the lack of association between substituting animal with major plant protein sources may be due to the low intake of soy, pulses and nuts/seeds in our analysis, which together contributed to <2% E. This is consistent with North American and European dietary intake patterns, which show that sources of animal protein are the major contributors to total protein intake and that protein from grain sources are the major contributors to total plant protein intake. As a result, these low ranges of intake makes it difficult to detect an overall difference between substituting animal protein with major sources of plant protein and HbA1c in individuals with diabetes. Furthermore, the lack of association may be a result of grain protein being a marker of refined carbohydrates, where higher GI has been shown to be associated with an increased T2D risk and poorer glycemic control in individuals with diabetes.

Figure 6.2 – Model for the regulation of insulin secretion in the β-cell stimulated by glucose and amino acids. Reprinted with permission from Diabetes, Vol 55 © 2006 by the American Diabetes Association http://diabetes.diabetesjournals.org.
Therefore, the findings from both of our studies suggest that if major plant protein sources were consumed at higher levels of intake there may be less of an inconsistency between the results reported from observational studies and those reported from RCTs.

6.4 CLINICAL IMPLICATIONS

Higher consumption of major sources of plant protein appears to be beneficial for the management of diabetes. Our data support the evidence from existing recommendations for diabetes management that recommend following plant-based diets, such as the Portfolio, vegetarian or vegan, Mediterranean, and DASH diet, of which soy, soy-derived products, pulses and/or nuts are key components. In our meta-analysis, the median percent of animal protein replaced with plant protein from total protein was approximately ~35% per day, which is equivalent to approximately 29 grams of protein per day for a middle-aged, overweight or obese adult with diabetes. This amount of protein is found in approximately 3 servings of pulses or tofu, which meets the recommended intakes made by public health guidelines for intakes of meat and alternatives. Achieving such intakes, however, may be challenging in North America as well as Europe, given that current intake levels of major plant protein sources are low. In addition, it has been shown that although individuals with diabetes are willing to follow a plant-based diet, very few actually do. This may be in part due to a gap in translation from knowledge to practice, where most health care providers appear to be aware of plant-based diets for the management of diets, but the level of practice in implementing them is low. Overall, our data support that a partial or full substitution of animal with plant protein may be beneficial in the management of with glycemic in individuals with diabetes, however further research is needed to clarify our findings.

6.5 FUTURE DIRECTIONS

Future research is needed to confirm the results of our two studies. First, our systematic review and meta-analysis revealed that majority of existing RCTs consist of small sample sizes (50% <20 participants), short follow-up duration (83% <12 weeks), are of low-quality (92% MQS<8) and do not focus on measurements of glycemic control as a primary outcome. Therefore, there is an urgent need for larger, longer, higher quality RCTs to assess the effect of replacing whole food sources of animal with plant protein on measurements of glycemic control as a primary outcome in individuals with diabetes. The inherent limitations of our cross-sectional study also support the need for such trials. Second, there is only one prospective cohort study that has assessed the association between replacing animal with plant protein on hard endpoints (i.e. development of diabetes), whereas most have looked at either
plant-based dietary patterns or specific plant protein sources. Therefore, more studies are needed to understand how replacement of animal with plant protein in the diet affects diabetes risk. Third, our cross-sectional study provided further support for the fact that consumption of major plant protein sources is low, which makes it difficult to detect a difference in HbA1c when substituting animal protein with plant protein. Fourth, given the heterogeneous nature of plant and animal protein sources, we suggest comparing substitutions of different animal protein sources with major plant protein sources in order to gain a better understanding of whether it is more beneficial to substitute specific sources of animal protein versus overall animal protein. Fifth, given the increasing amount of evidence that have shown plant-based dietary patterns are associated with reducing the risk of diabetes, metabolic syndrome, CVD, cancer, and all-cause mortality and plant protein sources such as soy, pulses and/or nuts showing beneficial effects on glycemic control, blood pressure, kidney function, blood lipids, body weight, and inflammation, it is clear that there is a gap between knowledge and translation. Therefore, there is a need to focus on strategies that will promote consumption of plant-based diets in those with or at risk for diabetes. As a first step in addressing these future directions, our group has undertaken a series of systematic reviews and meta-analyses of RCTs to look at the effect of replacing animal with plant protein on other cardiometabolic risk factors, such as blood lipids and measures of kidney function, which will help us gain a better understanding of the effects of replacing animal with plant protein in the management of diabetes. Overall, given the global diabetes epidemic and mounting concern for the protection of our environment, it will remain important to implement life-style modification strategies that will also positively impact our environment. The replacement of animal protein with plant protein in our diet may serve as one potential strategy.
CHAPTER VII – CONCLUSIONS
CHAPTER VII – CONCLUSIONS

Replacing animal protein with major sources of plant protein (i.e. soy, soy-derived products, pulses and nuts) leads to modest improvements in glycemic control in individuals with diabetes, however research is needed to address the limitations of our systematic review and meta-analysis and lack of agreement with the associations seen in our cross-sectional study.

Overall, this thesis demonstrated the following:

1. In a systematic review and meta-analysis of 12 RCTs (n=240), diets emphasizing a replacement of animal protein with major sources of plant protein significantly lowered HbA1c, fasting glucose and fasting insulin in comparison with control diets in individuals with diabetes.

2. In a cross-sectional study using baseline data from 5 RCTs conducted in a sample of middle-aged men and women with T2D (n=627), substitution of animal protein with total plant protein was not associated with HbA1c.
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