Effects of Dietary Pulses on
Lipid Risk Factors of Cardiovascular Disease and Oxidative Stress

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science
Graduate Department of Nutritional Sciences
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2013

ABSTRACT

The objective was to conduct a systematic review and meta-analysis of randomized feeding trials to assess the effect of dietary pulses (beans, chickpeas, lentils, peas) on established lipid targets of cardiovascular disease (CVD) and perform a secondary analysis of our randomized feeding trial to assess whether dietary pulses as a means of lowering the glycemic index offer further CVD protection by reducing oxidative stress. The meta-analysis of 26 trials (n=1013) found dietary pulse interventions significantly lowered LDL-C compared with isocaloric control interventions (mean difference=-0.17mmol/L [95% CI: -0.25, -0.09]; p<0.0001). No treatment effects were observed for Apo-B and non-HDL-C. Our feeding trial found no significant differences between the high-dietary pulse diet and high-fibre control diet on markers of oxidative stress, including thiobarbituric acid reactive substances (TBARS), conjugated dienes (CDs), and protein thiols. Overall, the results suggest dietary pulses reduce LDL-C but not oxidative stress as a means of reducing cardiovascular risk.

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LIST OF ABBREVIATIONS

CVD- Cardiovascular Disease
LDL-C- low-density lipoprotein cholesterol
CHD- Coronary Heart Disease
NHS- Nurses’ Health Study
AHS- Adult Health Study
USDA- United States Department of Agriculture
DASH- Dietary Approaches to Stopping Hypertension
ox-LDL-C- oxidized-LDL-C
Apo-B- apolipoprotein-B
Non-HDL-C- non-high density lipoprotein cholesterol
NHANES- National Health and Nutrition Examination Survey
BP- blood pressure
TC- total cholesterol
PAR- population attributable risk
FHS- Framingham Heart Study
CTT- Cholesterol Treatment Trialists
NCEP-ATP III- National Cholesterol Education Program- Adult Treatment Plan III
TG- Triglyceride
T2DM- type 2 diabetes mellitus
CCS- Canadian Cardiovascular Society
CDA- Canadian Diabetes Association
ADA- American Diabetes Association
FRS- Framingham Risk Score
NHS- Nurses’ Health Study
HPFS- Health Professional Follow-Up Study
HDL-C- high-density lipoprotein cholesterol
FAO/WHO- Food and Agriculture Organization of the United Nations/World Health Organization
AHA- American Heart Association
EASD- European Association for the Study of Diabetes
FDA- Food and Drug Association
EFSA- European Food Safety Authority
SCFA- short chain fatty acids
n- sample size
MDA- malondialdehyde
TBARS- thiobarbituric acid reactive substances
CD- conjugated dienes
CI- confidence intervals
PRISMA- Preferred Reporting Items for Systematic Review and Meta-Analyses
MQS- Methodological Quality Score
MD- mean difference
% E- percent energy
g/d- grams per day
SD- standard deviation
Ob- Obese
OW- Overweight
HC- Hypercholesterolemia
N- normal/healthy
Pre-MS- Pre-Metabolic Syndrome
IR- Insulin Resistant
IS- Insulin Sensitive
M- Men
W- Women
C- Crossover Design
P- Parallel Design
IP- inpatient
OP- outpatient
UR- unclear risk of bias
LR- low risk of bias
HR- high risk of bias
AHS- Adult Health Study
USDA- United States Department of Agriculture
GI- glycemic index
Haemoglobin A1c- HbA1c
CV- coefficient of variation
FBG- fasting blood glucose
ORAC- Oxygen Radical Absorbance Capacity
SFA- saturated fatty acid
MUFA- monounsaturated fatty acid
PUFA- polyunsaturated fatty acid
PREDIMED- Prevención con Dieta Mediterránea
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1. INTRODUCTION

Abnormal lipids have been identified as one of the most modifiable risk factors for cardiovascular disease (CVD), contributing to almost 50% of the population’s risk\(^1\). While statins are effective in reducing blood LDL-C\(^2\), major health organizations maintained that the initial and essential approach to CVD prevention and management is to modify dietary and lifestyle patterns\(^3\)-\(^6\). Studies have shown that 82% of coronary heart disease (CHD) may be prevented with healthier dietary and lifestyle choices\(^7\).

Dietary pulses (beans, chickpeas, peas, and lentils) have received particular attention for their ability to reduce CVD risk as part of low-glycemic index\(^8\), Dietary Strategies to Stop Hypertension (DASH)\(^9\), high-fibre\(^10\), Mediterranean\(^11\), and vegetarian\(^12\) dietary patterns. They are high in vegetable protein, fibre and have a low glycemic index\(^13\),\(^14\), all of which are associated with improved CHD outcomes\(^15\)-\(^17\). Dietary pulses are also high in 7S globulin, a protein which has been shown to increase uptake and degradation of LDL-C in human hepatic cells\(^18\). Small controlled feeding trials have demonstrated that diets which emphasize dietary pulses improved lipid levels\(^19\)-\(^30\). Taken together these data suggest that high dietary pulse intake may improve serum lipids.

Dietary pulses may offer further cardiovascular protection through reducing oxidized-LDL-C (ox-LDL-C). Dietary pulses are a good source of vegetable protein which has been shown to reduce the oxidation of LDL-C\(^31\),\(^32\). Reducing levels of ox-LDL-C is hypothesized to reduce the atherogenicity of LDL-C since ox-LDL-C is taken up more rapidly by the scavenger receptors of macrophages in the subendothelial arterial wall\(^33\)-\(^35\). Recent prospective cohort studies have reported the benefits of higher plant protein intakes on CVD risk\(^36\),\(^37\) and dietary patterns which may include dietary pulses such as low glycemic index\(^8\), DASH\(^9\), and Mediterranean\(^11\) have also been shown to reduce markers of oxidative stress. However except for one, few studies have directly assessed a high dietary pulse intake on oxidative damage\(^32\).
Despite these emerging benefits of dietary pulses, most major chronic disease prevention guidelines have not made specific recommendations for dietary pulse intake to reduce cardiovascular risk. Rather guidelines have encouraged the consumption of dietary pulses as part of a low-glycemic index\textsuperscript{8}, DASH\textsuperscript{9}, high-fibre\textsuperscript{10}, Mediterranean\textsuperscript{11}, or vegetarian\textsuperscript{12} diet. There is a need for higher quality evidence to support the lipid-lowering and oxidative stress mitigating effects of dietary pulses for guidelines development. To provide higher quality evidence on the effects of dietary pulses on lipids to reduce cardiovascular risk, the following thesis work will present: 1) the results of a systematic review and meta-analysis of randomized feeding trials of the effects of dietary pulse interventions on established lipid targets of CVD risk (LDL-C, Apo-B, and non-HDL-C) and 2) the results of a secondary endpoint analysis of a randomized feeding trial of the effects of a high dietary pulse diet as a means to lower the glycemic index compared to a high-fibre control diet on markers of oxidative stress.
2.1 CARDIOVASCULAR DISEASE

2.1.1 PREVALENCE

Cardiovascular disease (CVD) is a major public health concern. CVD is a class of diseases relating to the heart and vascular system including coronary heart disease (CHD), stroke, peripheral vascular disease, heart failure, rheumatic and congenital heart disease. According to the National Health and Nutrition Examination Survey (NHANES: 2007–2010), 83.6 million Americans (35.3%) were suffering from CVD in 2010 \(^{38}\) and 1.3 million Canadians were diagnosed in 2007 \(^{39}\). Specifically, the 2 most prevalent types of CVD, CHD and stroke, were diagnosed in 15.4 million (6.4%) and 6.8 million (2.4%) individuals, respectively \(^{38}\). Although the rate of CHD has declined over the decades (NHANES: 1971-2006), the prevalence of CHD is still high \(^{38}\). Similar trends have also been reported for Canada \(^{39}\). Despite recent advances in the understanding of CVD prevention, the prevalence of CVD still remains high.

2.1.2 COMPLICATIONS AND MORTALITY

CVD has serious long-term consequences. The pathogenesis of CVD is usually characterized by the development and progression of atheromatous plaques in the arterial wall \(^{40}\). Although atherogenesis begins as early as adolescence, CVD is considered a preventable disease \(^{7}\). Individuals with CHD have lower life expectancy because they are at high risk for myocardial infarctions (i.e. heart attacks), stroke, and heart failure, all of which increases the risk of long-term disability and mortality. In 2009, the overall death rate attributable to CVD was 236.1 per 100,000 or 1 in 3 deaths, and specifically CHD and stroke accounted for 1 in 6 deaths and 1 in 19 deaths, respectively \(^{38}\). Most individuals survive their first heart attacks \(^{38}\), however, re-occurring heart attacks can weaken the heart muscle and decrease the quality of life. It has been estimated that approximately every 34 seconds, 1 American has a coronary event, and approximately every minute, an American will die of one \(^{38}\). Taken altogether, the data suggest that the burden of CVD remains high.
2.1.3 RISK FACTORS

2.1.3.1 Coronary Heart Disease

Risk factors of CHD are characterized by the 10-year CHD Framingham Risk Score (FRS)\(^4\). The Risk Score predicts the risk of CHD based on non-modifiable risk factors including sex and age, and modifiable risk factors including diabetes, smoking, blood pressure (BP), total cholesterol (TC), and LDL-C levels\(^4\). It has been estimated that 80 to 90% of patients with CHD have at least 1 of the 4 modifiable risk factors of CHD and unhealthy dietary and lifestyle habits have been shown to account for more than 80% of coronary heart events\(^7\). The INTERHEART Study, a case-control study of 15 152 acute myocardial infarction cases and 14 820 controls from 52 countries, reported that diabetes contributed 9.9% of the population attributable risk (PAR), smoking at 35.7% (PAR), hypertension at 17.9% PAR, and abnormal lipids at 49.2% PAR\(^1\). In addition to these major risk factors, obesity, poor dietary patterns, physical inactivity, family history of CHD, and small LDL-C particles have been associated with an increase in CHD risk\(^3, 4\).

2.1.3.2 Stroke

Risk factors of stroke are characterized by the 10-year Stroke FRS\(^41\). Similar to the CHD risk score, risk factors for stroke include non-modifiable risk factors including sex and age, and modifiable risk factors including systolic BP, diabetes, smoking, CVD and atrial fibrillation status, left ventricular hypertrophy, and hypertension. Consequences of stroke have serious debilitating effects and it is one of the leading causes of long-term disability. Similar to those of CHD, other risk factors associated with stroke include obesity, poor dietary patterns, physical inactivity, and family history of stroke\(^41\).

2.2 LIPIDS AND CARDIOVASCULAR DISEASE

Abnormal lipids have been recognized as one of the most preventable risk factors for CVD. The pathogenesis of CVD is usually characterized by the development and progression of atherosomatous plaques in the arterial wall\(^40\). The Framingham Heart Study (FHS) was the first to correlate high lipid
levels with CHD incidence\textsuperscript{42}, and the INTERHEART Study has identified that almost 50% of acute myocardial infarctions can be prevented by modifying abnormal lipid levels\textsuperscript{1}. Further the success of statins, a class of drugs used to lower cholesterol levels by inhibiting the hepatic enzyme HMG-CoA reductase, have shown to lower CHD and stroke risk and \textsuperscript{43,44} the Cholesterol Treatment Trialists’ (CTT) Collaboration have also reported that every 1 mmol/L reduction of LDL-C can reduce CHD risk by 20\textsuperscript{45}. As a result of the overwhelming data supporting the role of abnormal lipids in heart health, major chronic disease guidelines including the National Cholesterol Education Program-Adult Treatment Plan III (NCEP-ATP III), Canadian Cardiovascular Society (CCS), Canadian Diabetes Association (CDA), and American Diabetes Association (ADA) have recognized the importance of lipids in heart health and have set lipid targets to lower the risk of CVD\textsuperscript{3,4,6,46}.

2.2.1 GUIDELINES

\textbf{Table 1. Summary of Lipid Targets by Major Chronic Disease Guidelines in Adults.}

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Primary Target</th>
<th>Secondary Target</th>
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</thead>
<tbody>
<tr>
<td>NCEP-ATP III (2004)\textsuperscript{4}</td>
<td>LDL-C</td>
<td>Non-HDL-C</td>
</tr>
<tr>
<td>CCS (2012)\textsuperscript{3}</td>
<td>LDL-C</td>
<td>Apo-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-HDL-C</td>
</tr>
<tr>
<td>CDA (2013)\textsuperscript{46}</td>
<td>LDL-C</td>
<td>-</td>
</tr>
<tr>
<td>ADA (2013)\textsuperscript{6}</td>
<td>LDL-C</td>
<td>-</td>
</tr>
</tbody>
</table>

Several chronic disease guidelines have set lipid targets for individuals to meet to lower cardiovascular risk. NCEP-ATP III, CCS, CDA, and ADA have set LDL-C as the primary treatment target to prevent and manage CVD risk. In addition, NCEP-ATP III has identified non-HDL-C as a secondary target and similarly, CCS recognize both Apo-B and non-HDL-C as secondary targets.
2.2.1.1 National Cholesterol Education Program- Adult Treatment Plan III (NCEP-ATP III)\(^4\)

**Table 2. Summary of Lipids Targets set by NCEP-ATP III.**

<table>
<thead>
<tr>
<th>Primary Target</th>
<th>Secondary Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C &lt;2.59mmol/L</td>
<td>Individuals with TG levels ≥2.26mmol/L, non-HDL-C &lt;3.37 mmol/L</td>
</tr>
</tbody>
</table>

NCEP-ATP III has set absolute lipid targets for all adults (≥20 years) regardless of their health status and number of CVD risk factors. An LDL-C level of <2.59mmol/L (100mg/dL) or is considered optimal. Individuals with triglyceride (TG) levels ≥2.26mmol/L (200mg/dL) or should also set their non-HDL-C targets at <3.37 mmol/L (130mg/dL).

2.2.1.2 Canadian Cardiovascular Society (CCS)\(^3\)

**Table 3. Summary of Lipid Targets set by CCS (2012).**

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Primary Target</th>
<th>Secondary Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (FRS ≥ 20%)</td>
<td>LDL-C ≤ 2 mmol/L or ≥ 50% decrease</td>
<td>Apo B ≤ 0.8 g/L, Non HDL-C ≤ 2.6 mmol/L</td>
</tr>
<tr>
<td>Intermediate (FRS 10- 19%)</td>
<td>LDL-C ≤ 2 mmol/L or ≥ 50% decrease</td>
<td>Apo B ≤ 0.8 g/L, Non HDL-C ≤ 2.6 mmol/L</td>
</tr>
<tr>
<td>Low (FRS &lt;10%)</td>
<td>LDL-C ≥ 50% decrease</td>
<td>-</td>
</tr>
</tbody>
</table>

CCS has recommended different lipid targets depending on one’s 10- year CHD Framingham Risk Score (FRS). At both the highest (FRS ≥20%) and intermediate (10-19%) risk levels, CCS recommends the LDL-C target to be set at ≤2 mmol/L (77.22mg/dL) or ≥50% decrease from baseline and the secondary targets for Apo-B is set at ≤0.8 g/L and non-HDL-C at ≤2.6 mmol/L (100.39mg/dL). At the lowest risk level (FRS <10%), the LDL-C target is set at ≥ 50% reduction from baseline with no defined targets for the secondary endpoints.

2.2.1.3 Canadian Diabetes Association (CDA)\(^6\)

**Table 4. Summary of Lipid Targets set by CDA (2013).**

<table>
<thead>
<tr>
<th>Primary Target</th>
<th>Secondary Target</th>
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<tbody>
<tr>
<td>LDL-C &lt;2.0mmol/L</td>
<td>Apo-B &lt;0.9g/L, Non-HDL-C &lt;2.6mmol/L</td>
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</table>
CDA recognizes fasting for >8 hours may be inappropriate for individuals with diabetes, especially if insulin is part of treatment. Under these circumstances, non-HDL cholesterol or Apo-B measurements should be used instead. The target for the primary endpoint, LDL-C, is set at ≤2.0 mmol/L (77.22mg/dL) for individuals with diabetes. Secondary lipid targets for individuals that cannot fast include a Apo-B target of <0.9 g/L and/or a non-HDL-C target of <2.6mmol/L (100.39mg/dL).

2.2.1.4 American Diabetes Association (ADA)⁶

Table 5. Summary of Lipid Targets set by ADA (2013).

<table>
<thead>
<tr>
<th>Primary Target</th>
<th>Secondary Target</th>
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<tbody>
<tr>
<td>Overt CVD, LDL-C &lt;1.8mmol/L</td>
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<tr>
<td>Non-overt CVD, LDL-C&lt;2.59mmol/L</td>
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</tbody>
</table>

ADA recommends that individuals with diabetes should measure their fasting lipid profile at least once a year, but those with a low-risk lipid profile at least every 2 years. A low risk lipid profile is defined as having levels of LDL-C <2.59mmol/L (100 mg/dL), HDL-C >1.30mmol/L (50 mg/dL), and TG 1.68mmol/L (150 mg/dL). For individuals without overt CVD, the target for LDL-C is set at <2.59mmol/L (100 mg/dL). For individuals with overt CVD, the target for LDL-C is set at <1.8 mmol/L (70 mg/dL). No secondary lipid target has been set.

2.3 OXIDATIVE STRESS AND CARDIOVASCULAR DISEASE

Oxidative damage to serum LDL-C may play a potentially important role in the etiology of CHD risk. It has been proposed that ox-LDL-C is more likely to be taken up by macrophages via scavenger receptors and deposited as atheromatous plaques as the macrophage dies and becomes foam cells, leading to increased incidence of cardiovascular events (Figure 1)⁴⁷.
Figure 1. Schematic Diagram of the Formation of Atheromatous Plaques via ox-LDL-C. Reprinted with permission from the Annual Review of Nutrition, Vol 25 © 2005 by Annual Reviews www.annualreviews.org. Abbreviations: LDL, low-density lipoprotein; MM-LDL, minimally modified-LDL; ox-LDL, oxidized-LDL; ROS, reactive oxygen species; SR-A scavenger receptor A; ROS, reactive oxygen species; RNS, reactive nitrogen species; M-CSF, macrophage colony stimulating factor.

2.4 DIET AND CARDIOVASCULAR DISEASE

2.4.1 DIET AND DRUGS

Many medications have become available for the prevention, management, and treatment of CVD events. While many are effective and safe, recent examples have not delivered the anticipated benefits. Statins have been shown to reduce CVD events, however these drugs are not well-tolerated by all for whom they are indicated, and a recent meta-analysis found statins did not reduce CVD mortality. Further fibrates, a class of hypocholesterolemic medications, was demonstrated in a meta-analysis to increase rates of all-cause mortality with no significant benefits for myocardial infarctions and stroke. Finally, a recent clinical drug trial of 25,673 high risk individuals for CHD given extended release of niacin vs. placebo found individuals given the drug treatment did not have lower rates of myocardial infarctions and stroke, but rather increased risk of developing diabetes complications.
lack of anticipated effects by drugs has reinforced the position of major health organizations that diet and lifestyle change remain the cornerstone of CVD prevention and management.

2.4.2 DIETARY PATTERNS AND CARDIOVASCULAR DISEASE

Diet has been recognized as an essential and preventive method of reducing cardiovascular risk\(^3\)\(^-\)\(^6\). Since Ancel Key's landmark publication of the Seven Countries Study which identified the influence of nutrition and diet on CHD risk\(^51\), there has been a renewed interest on understanding the effects of dietary and lifestyle patterns on heart health. In the past, nutrition studies have focused on a single nutrient component or food on cardiovascular risk, but more recently, the attention has shifted to focusing on the totality of the diet\(^52\). Diets such as DASH for blood pressure reduction\(^9\), Portfolio for lipid improvements\(^53\), and Mediterranean for CHD risk reduction\(^54\) have all shown greater improvements in CVD risk factors and events than any single nutrient component or food. In addition, the analysis of overall dietary patterns is more reflective of real-life conditions as individuals are likely to eat foods in the context of a meal and substitute one food for another rather than simply adding another food component to their diet\(^55\). For example, current recommendations advise to reduce total saturated fat intake to lower CVD. However, a majority of studies have reported that saturated fat intake is harmful only when substituting for polyunsaturated fatty acids (PUFAs), but there is no conclusive evidence of harm when substituting for carbohydrates or monounsaturated fatty acids (MUFAs)\(^56\). Food choices appear in clusters in diets and they reflective an overall pattern in the diet (eg. individuals with high saturated fat intake are less likely to have high PUFA intake)\(^55\). Given that there is strong collinearity between food intakes, focusing on encouraging a “healthy” dietary pattern may have more impact on the prevention and management of CVD risk\(^3\)\(^-\)\(^6\).

2.4.2.1 Prospective Cohort Studies

Prospective cohort studies have provided evidence that dietary patterns have important implications for CVD prevention and management. Two “basic” dietary patterns have been identified: 1)
Western pattern characterized by high consumptions of red and processed meat, butter, potatoes, refined grains, and high-fat dairy; 2) prudent pattern characterized by high consumptions of vegetables, fruits, legumes, seafood, and whole grain consumption. Although the Western diet is more prevalent in some developed countries, more developing countries are adapting to this dietary habit as their economic development becomes more integrated with the Western economy. In the Health Professional Follow-Up Study (HPFS: 1986-1994), after coronary risk factors were adjusted, the prudent diet was associated with a decreased risk of 24% for CHD compared to the lowest-quantile characterized as having the lowest adherence to the prudent diet. In contrast, the Western diet was associated with an increased risk of 46% for CHD when the extreme quantile was compared to the lowest. The difference in risk demonstrates the prudent diet offers an overall of 50% CHD risk reduction compared to the Western diet. Similar findings have also been reported by other prospective cohorts for both the prudent and Western diets. These findings are not surprising as the prudent diet is made up of foods and nutrient components that have been well associated with improved cardiovascular outcomes. There is evidence from dietary intervention trials suggestive that the combination of foods that are known to offer cardio-protection have additive effects when taken together. Other benefits of health have also been positively associated with greater adherence to the prudent diet including lower risks of CVD events, cancer, and total mortality.

2.4.2.2 Dietary Intervention Trials

Intervention trials have provided further support to strengthen the benefits of dietary patterns on cardiovascular risk. Studies of dietary patterns have shown greater reductions in CVD risk factors than isolated nutrients or foods. The Portfolio diet, for example, has 4 primary interventions: plant sterols, viscous fibre, soy protein, and nuts. Individually each of these foods has been recognized by the FDA to lower LDL-C. The combination of these foods in the same diet, however, has found significant serum LDL-C reductions to levels equivalent to the effects of early statin drug trials after 6 months of
intervention, an outcome greater than any of the foods consumed singly\textsuperscript{53}. The Dietary Approach to Stop Hypertension (DASH) diet characterized by a low salt diet by increasing consumptions of fruits and vegetables, nuts, whole grains, reduced low-fat dairy and red meat intakes has also shown similar benefits of dietary patterns over isolated foods but on blood pressure\textsuperscript{9}. More importantly, the Prevención con Dieta Mediterrànea (PREDIMED), a recent randomized feeding trial with a dietary pattern intervention design, has shown benefits on clinical cardiovascular outcomes\textsuperscript{54}. The PREDIMED study compared the control diet (dietary advice to reduce fat intake) to 2 intervention arms, a Mediterranean diet supplemented with either extra-virgin olive oil or mixed nuts. Combined together, the Mediterranean intervention diets showed a significant 30% reduction in the composite of myocardial infarction, stroke, and death from cardiovascular-cause events, after a median 4.8 years of follow-up. There is strong support demonstrating the effects of dietary patterns on cardiovascular risk factors and clinical hard-endpoints. These beneficial dietary pattern effects have been recognized by guidelines in the prevention and management CVD\textsuperscript{3,6}.

**2.5 PLANT-PROTEIN BASED DIET AND CARDIOVASCULAR DISEASE**

There is a growing interest in preventing cardiovascular risk using foods high in plant protein\textsuperscript{57,61}. Sources of high plant protein include fruits and vegetables, whole grains, soy, and dietary pulses. Diets high in plant-protein have been commonly been characterized in vegetarian and vegan diets, both of which have demonstrated significant reductions of both cardiovascular risk factors and overall risk in large prospective cohort studies such as the \textsuperscript{62,63}. Higher plant protein intake may offer cardio-protective effects because it displaces refined carbohydrate consumption, and is associated with higher fibre and lower saturated fat intakes\textsuperscript{37,57,64}. It is also likely that the different sources of plant protein may have an unique protein composition that further offers cardiovascular risk protection. For example, dietary pulses and soy are high in 7S globulin, a protein that has been shown to have hypocholesterolemic effects in humans\textsuperscript{18}. 


2.5.1 PROSPECTIVE COHORT STUDIES

Prospective cohort studies have primarily focused on the analysis of vegetable proteins and have reported a protective association against cardiovascular events. A study of a Finnish cohort (n=5133) found an inverse association between vegetables and CHD risk with the multivariate relative risks for CHD death at 0.66 (95% CI: 0.46, 0.96) for men and 0.66 (95% CI: 0.35-1.23) in women. Two of the largest prospective cohorts, the Nurses’ Health Study (NHS: 1976-1994) and Health Professionals’ Follow-up Study (HPFS: 1986-1994) have also shown reduced CVD events with higher vegetable and fruit intakes. Vegetables are high in many cardio-protective nutrients including fibre, potassium, flavonoids, and antioxidants, but are also a good source of protein which may have important cardiovascular implications. The Iowa Women’s Health Study of almost 30,000 post-menopausal women, reported a significant inverse relationship with vegetable protein intake and CHD deaths with greater displacement of carbohydrates or animal protein by vegetable protein intake. Contrary to this finding, the Adult Health Study (AHS) and a Japanese prospective cohort study, both of which did not assess for displacement of nutrients like the Iowa Women’s Health Study, found no significant associations with total vegetable protein intake and stroke. These findings appear to suggest the cardio-protective effects of vegetable proteins is greatest when it displaces carbohydrates or animal protein intakes; however there is still the possibility that high vegetable protein intake is protective against of CHD mortality but not stroke events. If the former is true, however, more studies are needed to better understand the association of vegetable protein intake and stroke risk when vegetable protein displaces other nutrient components in the diet.

Although soy protein has received particular attention for its hypolipidemic effects, the association of soy protein on cardiovascular events from prospective cohort studies have not been well-studied. To our knowledge, only one prospective cohort in Chinese women (n=75,000) has been published assessing soy protein intake and CHD events, which reported a 75% CHD event reduction with
~18 grams per day of soy protein compared to ~5 grams per day\textsuperscript{70}. Most of the cardio-protective effects of soy have been studied through dietary trials, however there is an urgent need for more studies to improve the existing evidence for the association of soy protein intake and hard endpoints such as myocardial infarction, stroke, and mortality.

2.5.2 DIETARY INTERVENTION TRIALS

With the exception of soy protein, there is a limited number of dietary intervention trials that have assessed the effects of other sources of plant protein on cardiovascular risk factors. The hypocholesterolemic effects of soy protein have been well documented\textsuperscript{69} and as a result, both Canada and US have issued health claims to encourage the consumption of soy protein to improve lipid levels to reduce CVD risk. Only one study has been identified to assess the effects for each of wheat gluten protein\textsuperscript{31}, barley protein\textsuperscript{71}, and total vegetable protein\textsuperscript{72} on cardiovascular risk factors. Both trials of wheat gluten and vegetable proteins have shown significant favourable on lipids with wheat gluten protein also showing improvements in ox-LDL-C when compared to a high fibre diet\textsuperscript{31, 72}. In contrast, barley protein consumption did not show favourable effects on lipids when compared to casein; however they may still be used to increase the overall intake of protein\textsuperscript{71}. These studies offer support for different sources of plant protein as a method of decreasing CVD risk; however there is a need for more studies to improve the existing evidence to support the effects of plant protein on CVD risk factors.

2.6 DIETARY PULSES AND CARDIOVASCULAR DISEASE

The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) define dietary pulses as: “dry seeds of leguminous plants which are distinguished from leguminous oil seeds by their low fat content.”\textsuperscript{73} They do not include soybeans or peanuts because both are high in fat content, or green beans or peas because these include both the seeds and pods of the
legume. They are high in plant protein, fibre, and have a low glycemic index, which have all been shown to improve cardiovascular outcomes8,13,19.

2.6.1 DIETARY PULSES IN GUIDELINES

Major health organizations have encouraged dietary pulse intake as part of a strategy to lower the risk of chronic diseases but none have directly made recommendations to increase dietary pulse consumption based on lipid-lowering effects and CVD prevention. Public health guidelines from Health Canada and the FAO/WHO encourage dietary pulse intake as a method of increasing fibre intake, and decreasing the risks of obesity, T2DM, and CVD74,75. Chronic disease association guidelines from the American Heart Association (AHA)76, as well as the American (ADA)6, Canadian (CDA)5, and European (EASD)77 Diabetes Associations encourage dietary pulse consumption as a means for improving cardiometabolic outcomes through reducing postprandial glycemia, reducing energy intake, lowering the GI, increasing dietary fibre and displacing cholesterol. In all cases, the evidence on which these recommendations is based has been assigned a low-grade, while the FAO/WHO (minimum of 20-g/d) and AHA (2-3-cups/wk) are the only associations to find sufficient evidence to quantify intake targets74,76. The Canadian Cardiovascular Society (CCS) did not make recommendations for dietary pulses; however it does recognize the LDL-C lowering effects of soy3. Neither Health Canada nor its American (FDA) or European (European Food Safety Authority [EFSA]) counterpart permits any pulse related health claims, and none of the major Canadian or American hypertension78,79 and dyslipidemia3,4 guidelines address dietary pulses in their recommendations. This situation, in which diabetes and heart association have made recommendations for dietary pulses but have assigned the recommendation as based on a low-grade of evidence and the lack of data to support health claims and other cardiometabolic recommendations, serves as a call for stronger evidence.
2.6.2 DIETARY PULSES AND CARDIOVASCULAR DISEASE

The association of dietary pulses and cardiovascular risk is difficult to ascertain. Prospective cohort and case-control studies have grouped dietary pulses with soybean and peanut products (collectively referred to as legumes) in the analyses. Collectively legumes have shown significant cardiovascular risk reduction with greater intakes; however unlike soybeans and peanuts, dietary pulses have a low fat content and are very likely to act differently than soybeans and peanuts in their cardio-protective effects. More likely, dietary pulses lower the risk of CVD through other nutritional components. The NHANES (1999-2002) study found that Americans who consumed approximately ½ cup dry beans or peas had higher intakes of fibre, vegetable protein, folate, zinc, iron and magnesium and lower intakes of saturated and total fat, all of which are cardio-protective. Dietary pulses are also high in 7S globulin which has been shown to have hypocholesterolemic effects. However to date, there has been no prospective cohort study that directly assessed dietary pulses only and CVD risk. There is a need for observational studies to better understand the relationship between high dietary pulse intake and heart health.

2.6.3 DIETARY PULSES IN DIETARY PATTERNS

Dietary pulses have been included in several dietary patterns. These diets have been shown to improve serum lipid levels, including the Portfolio, low-GI, DASH, high fibre, and vegetarian diets. Further the Mediterranean diet has been shown to improve the risk for composite of myocardial infarctions, stroke, and cardiovascular-cause deaths. However as dietary pulses were included with other dietary interventions in these studies, it is difficult to ascertain the independent effects of dietary pulses on cardiovascular risk factors and outcomes. Dietary intervention trials specific to assessing dietary pulses on cardiovascular risk are needed to better qualify and quantify its benefits.
2.6.4 DIETARY PULSES IN INTERVENTION TRIALS

2.6.4.1 Evidence from Meta-Analyses

Two previous meta-analyses have been conducted investigating the effects of dietary pulses (beans, chickpeas, lentils and peas) on lipids\textsuperscript{13, 83}. Both studies involving 15 trials estimated higher dietary pulse intakes for 2 to 16 weeks compared to control diets showed significant improvements in total cholesterol (TC) (=0.30 mmol/L, p<0.001; or ≈7.12%, p<0.05) and LDL-C levels (=0.20 mmol/L, p<0.001; or ≈6.16%, p<0.05) at doses of 50-440 grams per day (i.e. 1 to 10 175-g servings/week), while Anderson et al. also reported a significant TG reduction (16.6%, p<0.05)\textsuperscript{83}. A review of controlled feeding trials since these meta-analyses were published shows the results have remained largely consistent\textsuperscript{8, 20, 25}. Although both meta–analyses reported the effects of dietary pulses on LDL-C, neither considered other established lipid outcomes including Apo-B and non-HDL-C. Controlled feeding trials, however, have shown that consumption of dietary pulses reduce TC and VLDL-C levels\textsuperscript{22, 23, 25}, reductions which would be reflected in a lower non-HDL-C level. Future meta-analyses need to consider these established lipid targets to provide higher level of evidence for the development of clinical practice guidelines for the recommendations of dietary pulses.

2.6.4.2 Evidence from Randomized Feeding Trials in Humans

Clinical studies have shown regular consumption of dietary pulses can reduce serum TC and LDL-C but have little or no effect on HDL-C \textsuperscript{19-28, 30}. Few studies, however, have reported the effects of dietary pulses on other established lipid targets such as Apo-B and non-HDL-C.

The mechanism by which dietary pulses lower cholesterol levels is unclear; however, the effect can be broadly divided into 2 pathways: extrinsic and intrinsic. The extrinsic pathway proposes that dietary pulses reduce serum cholesterol by displacing saturated and trans fat intake. Americans who consumed approximately ½ cup dry beans or peas daily had lower intakes of saturated and total fat\textsuperscript{82}. While the effects of saturated and total fat on cardiovascular risk are conflicting, many human clinical
trials have shown a beneficial effect when saturated fat is replaced with polyunsaturated or monounsaturated fatty acid intake (PUFA or MUFA, respectively). Further, dietary pulses are high in soluble fibre which is known to bind to dietary cholesterol in the intestine and prevent its absorption. Possibly more importantly, dietary pulses are high in 7S globulins, a peptide which has been shown to increase uptake and degradation of LDL-C in human hepatic cells, and is also hypothesized to confer the favourable lipid effects of soybeans. Of the intrinsic pathway proposes that in addition to binding to dietary cholesterol, the soluble fibre in dietary pulses can bind to bile acids to prevent its re-absorption. Consequently, there is an increase in the production of bile acids by the liver, which decreases the liver pool of cholesterol and increases uptake of serum cholesterol by the liver thereby decreasing circulating cholesterol in the blood. Further fermentation of fibre in the colon produces short chain fatty acids (SCFA) such as propionate and butyrate, which are associated with inhibition of cholesterol synthesis.

2.6.5 DIETARY PULSES AND OXIDATIVE STRESS

There is suggestion that a high dietary pulse intake may reduce levels of ox-LDL-C. Foods high in plant protein such as wheat gluten have been shown to improve levels of oxidative stress in a dietary intervention trial. Dietary pulses are also high in plant protein; however, only one trial of 3 weeks in duration (n= 30) has assessed the effects of dietary pulses, at an intake of 130 grams per day, on oxidative stress, reporting no significant treatment differences when compared to an average American diet. Despite these findings, the study still reported a significant reduction of ox-LDL-C when comparing the 2 treatment arms (p= 0.035). The findings of this study suggest that high dietary pulse intake may still reduce levels of ox-LDL-C through a mechanism that is independent of oxidative stress. Possibly dietary pulses lower the levels of serum LDL-C, thereby reducing the availability of LDL-C for oxidation. Indeed several meta-analyses have demonstrated high dietary pulse intake lowers LDL-C
levels\textsuperscript{13,83}. However given the short duration and small sample size of this study, larger, longer, and high-quality clinical trials are needed to further understand the effects of dietary pulses on oxidative stress.

2.6.6 DIETARY PULSES AND OTHER CARIOVASCULAR RISK FACTORS

Regular dietary pulse consumption has also been shown to improve other risk factors of CVD including glycemia, body weight, and blood pressure. A meta-analysis of 11 trials reported that dietary pulses resulted in significant fasting blood glucose (standardized mean difference= -0.82 [95% confidence interval: -1.36 to -0.27]; \( p=0.03 \)) and insulin (standardized mean difference= -0.49 [95% confidence interval= -0.93 to -0.04]; \( p=0.03 \)) reduction\textsuperscript{87}. Early studies have shown high consumption of dietary pulses resulted in exceptionally low glycemic responses when fed to healthy volunteers\textsuperscript{14}, and in later experiments were demonstrated to possess a carbohydrate profile that was more slowly digested than that of other foods such as cereals\textsuperscript{88}. This property of slower absorption, which is common to the \( \alpha \)-glucosidase inhibitor class of medications such as acarbose suggest that dietary pulses are an important means of lowering the glycemic index of the diet and, by analogy with acarbose, CHD risk\textsuperscript{89}.

The review of the available evidence shows the data from experimental trials in humans are largely inconclusive for body weight, with the only meta-analysis published 10 years ago (n=11 trials) finding no effect of dietary pulses on body weight.\textsuperscript{83} However, since the meta-analysis has been published, several dietary intervention studies have reported significant weight loss with higher dietary pulse intake\textsuperscript{8,90}, suggesting that perhaps a beneficial body weight effect may have been missed in the meta-analysis due to the limited available studies. Further, a review of the few randomized trials conducted to-date is suggestive of a tendency for modest blood pressure-lowering effect\textsuperscript{8,20,25,90}. However, as a good source of fibre, dietary pulses would be expected to show a blood pressure lowering effect\textsuperscript{91}.
CHAPTER III- HYPOTHESIS, OBJECTIVES, AND RATIONALE
3. HYPOTHESIS, OBJECTIVES, RATIONALE

3.1 HYPOTHESIS

A diet high in dietary pulses will improve lipid risk factors of cardiovascular disease.

1. Diets emphasizing dietary pulses will improve levels of established lipid targets of cardiovascular risk including LDL-C, Apo-B, and non-HDL-C compared to control diets in a systematic review and meta-analysis of randomized feeding trials.

2. A high-dietary pulse diet as a means to lower the glycemic index of the diet will reduce oxidative stress markers including thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), and protein thiols compared to a high-fibre control diet in a secondary endpoint analysis of a randomized, controlled, parallel trial of 12 weeks in T2DM

3.2 OBJECTIVES

Overall objective: To investigate the effects of dietary pulses (beans, chickpeas, lentils, and peas) on lipid risk factors associated with increased CVD risk.

Specifically:

1. To assess the effects of dietary pulses on established lipid targets of cardiovascular disease in a systematic review and meta-analysis of randomized feeding trials.

2. To determine whether a diet high in dietary pulses, as a means to lower the GI of diet, compared to a high fibre diet will improve oxidative damage to serum lipids and proteins, as assessed by TBARS and CD in the LDL fraction and protein thiols in serum in a secondary endpoint analysis of a 12-week randomized feeding trial in T2DM.

3.3 RATIONALE

Although most major chronic disease prevention guidelines encourage the consumption of dietary pulses, few guidelines have made recommendations based on the direct lipid lowering or cardiovascular risk reduction benefits of dietary pulses. While 2 previous meta-analyses have assessed
the effects of dietary pulses on lipids\textsuperscript{13,83}, neither assessed the effects on established lipid targets nor did they include studies that were at least 3 weeks in duration, a requirement to satisfy the FDA criteria used in the scientific evaluation of lipid-lowering health claims\textsuperscript{92}. In all cases, the evidence on which the recommendations for dietary pulses are based on has been assigned a low-grade. Therefore, there is a need for higher quality evidence to support the lipid-lowering effects of dietary pulses for guideline development. Therefore a systematic review and meta-analysis assessing the effects of dietary pulses on established lipid targets of CVD was undertaken.

Dietary pulses may offer further cardiovascular protection through reducing oxidized-LDL-C (ox-LDL-C). Dietary pulses are a good source of vegetable protein and this nutritional property may provide further protection from CVD by reducing the oxidation of LDL-C\textsuperscript{31}. Further like soy, they are high in phenolics which may have beneficial effect on oxidative stress. Few feeding trials in humans have assessed the effects of dietary pulses on oxidative stress except for one small trial of 30 individuals\textsuperscript{32}. Therefore a human feeding trial comparing the effects of a high dietary pulse diet as a means to lower the GI to a high fibre comparator diet on oxidative stress was undertaken.
CHAPTER IV- THE EFFECT OF DIETARY PULSES ON ESTABLISHED LIPID THERAPEUTIC TARGETS OF CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS
4.1 CITATIONS

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THE EFFECT OF DIETARY PULSES ON ESTABLISHED THERAPEUTIC LIPID TARGETS OF CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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4.2 ABSTRACT

**Background:** Evidence from controlled feeding trials supports dietary pulses (beans, peas, chickpeas, and lentils) as a dietary intervention to improve dyslipidemia, but heart health guidelines have stopped short of ascribing specific heart-health benefits to dietary pulses or have graded the beneficial evidence as low. To improve the evidence on which dietary guidelines are based, we conducted a systematic review and meta-analysis to assess the effect of dietary pulses on established therapeutic lipid targets for cardiovascular risk reduction: LDL-C, Apo-B, and non-HDL-C.

**Methods and Results:** We searched MEDLINE, EMBASE, Cochrane, and CINAHL databases through Feb 11 2013 and included randomized controlled feeding trials of ≥3-weeks duration that compared a diet emphasizing dietary pulse intake with an isocaloric diet without pulses. Three independent reviewers extracted relevant data with disagreements resolved by consensus. Data were pooled by the generic inverse variance method using random effects models and expressed as mean differences (MD) with 95% confidence intervals (CI). Heterogeneity was assessed (Chi²) and quantified (I²). Study quality and risk of bias were assessed. Twenty-six isocaloric trials (n= 1013) satisfied the inclusion criteria. Diets emphasizing dietary pulses at a median dose of 130g/d (~1.5 servings) significantly lowered LDL-C compared with isocaloric control diets (MD= -0.17 mmol/L [95% CI: -0.25, -0.09]; p<0.0001). No treatment effects were observed for Apo-B and non-HDL.

**Conclusions:** Pooled analyses demonstrated that dietary pulses significantly improve LDL-C. The majority of trials, however, were short in duration and poor quality. There is a need for larger and higher quality trials.

**Clinical Trial Registration:** NCT01594567

**Abstract word count:** 247

**Key words:** systematic review, meta-analysis, dietary trials, legumes, dietary pulses, dyslipidemia, cardiovascular disease
4.3 INTRODUCTION

Abnormal blood concentrations of lipids and lipoproteins are one of the most important modifiable risk factors for cardiovascular disease (CVD). Although statins are effective in reducing blood LDL-C, major health organizations maintained that the initial and essential approach to CVD prevention and management is to modify dietary and lifestyle patterns.

Dietary non-oil-seed pulses are foods which have received particular attention for their ability to reduce CVD risk as part of low-glycemic index (GI), Dietary Strategies to Stop Hypertension (DASH), high-fibre, Mediterranean, and vegetarian dietary patterns. Dietary non-oil-seed pulses, including beans, peas, chickpeas, and lentils, have a low GI, are high in viscous soluble fibre, vegetable protein, and various bioactive compounds. Observational studies have shown legumes including dietary pulse consumptions are associated with CVD reduction and small controlled feeding trials have demonstrated that diets which emphasize dietary pulses improve LDL-C levels. Although most major chronic disease prevention guidelines encourage the consumption of dietary pulses as part of a dietary strategy, none of the guidelines have made recommendations based on the direct lipid lowering or cardiovascular risk reduction benefits. The focus of guidelines, such as those of the American Heart Association which recommends ≥4-5 servings/week, has been on the effects of dietary pulses to improve the diet quality in the management of diabetes and CVD risk. In all cases, the evidence on which recommendations are based has been assigned a low-grade. Dyslipidemia guidelines including those from the National Cholesterol Education Program (NCEP) adult treatment panel III (ATP III) and the Canadian Cardiovascular Society (CCS) do not address dietary pulses intake directly. There is a need for higher quality evidence to support the lipid lowering effects of dietary non-oil-seed pulses for guidelines development.

To improve the evidence on which dietary guidelines are based, we conducted a systematic review and meta-analysis of randomized controlled feeding trials on the effect of dietary non-oil-seed
pulses on established therapeutic lipid targets for cardiovascular risk reduction including LDL-C, Apo-B, and non-HDL-C.

4.4 METHODS

The Cochrane Handbook for Systematic Reviews of Intervention was used as a guideline for this meta-analysis. Reporting of results followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The review protocol is available at ClinicalTrials.gov (registration no. NCT01594567).

4.4.1 STUDY STRATEGY SELECTION

We searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and CINAHL through Feb 11 2013, to identify randomized controlled dietary trials of dietary non-oil-seed pulses. Table 1 shows the search strategy. Manual searches of references cited by published studies also supplemented the database search. No restriction was placed on language.

4.4.2 ELIGIBILITY CRITERIA

We included randomized trials that investigated the effect on LDL-C, Apo-B and non-HDL-C of diets high in dietary non-oil-seed pulses compared to control or usual diets matched for energy for a period of ≥3 weeks, a duration which satisfies the minimum follow-up requirement of the FDA used in the scientific evaluation of lipid-lowering health claims. Studies that only examined dietary non-oil-seed pulses (beans, chickpeas, lentils, and peas) were included; peanuts and soybeans were excluded because of their high oil content. We included interventions in which dietary pulses were not the sole intervention, provided that dietary pulses were the dominant intervention used to achieve intervention goals (n=3). Studies providing only dietary pulse extracts, such as bean extracts or isolated fibre supplements, were not eligible. Both control and treatment arms must have been matched for total energy (i.e. an isocaloric comparison). The selected endpoints included only those which have been
identified as therapeutic lipid targets in the NCEP-ATP III, 2012 CCS, 2012 American Diabetes Association (ADA), and 2013 Canadian Diabetes Association (CDA) guidelines.

The study by Anderson et al. was quasi-randomized. We attempted to reduce bias induced by non-randomization by re-analyzing this study by randomly assigning participants to either treatment or control, stratified by baseline total cholesterol and age, and treating the post-control and post-treatment arms as a parallel study design.

4.4.3 DATA EXTRACTIONS

Studies that met the inclusion criteria had their study characteristics and results extracted by 3 independent reviewers (VH, VHJ, and RJdS). As well, a 10-year CHD risk score was calculated for each study’s population using the Framingham risk equation. If information was missing for CHD risk prediction, participants were assumed to be at higher risk for that particular dimension (eg. assumed to be smokers, hypertensive, low HDL-C, and/or have family history of CHD).

The quality of each study was assessed using Heyland’s Methodological Quality Score (MQS). Studies could receive a maximum score of 13 points. Studies with scores of ≥8 were considered high quality. Points were awarded based on the quality of study methods, sample selection and follow-up, and intervention.

Studies were assessed for risk of bias using the Cochrane Risk of Bias Tool. Domains of bias assessed were sequence generation, allocation concealment, blinding, outcome data, and outcome reporting. Studies were marked as, high risk of bias when a 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.
4.4.4 TREATMENT OF MISSING DATA

When non-HDL-C was not reported, they were calculated from aggregate data using the formula: non-HDL-C = TC-HDL-C. A formula was used to calculate SD for non-HDL-C:

\[
\frac{1}{\sqrt{N}} \sqrt{s_{TC}^2 + s_{HDL}^2 + 2s_{TC-HDL}^2}
\]

; N denotes the total sample size; S denotes standard deviation; and as a reliable covariance \((s_{TC-HDL}^2)\) could not be estimated, it was assumed to be zero. Trials that did not report either change-from-baseline differences within or between treatments, or end-differences between treatments had these calculated from the available study data using standard formulae\(^97\). Authors were contacted, when possible, to request additional information (n= 3)\(^{20, 30, 90}\).

4.3.5 STATISTICAL ANALYSIS

Data were analyzed using Review Manager (RevMan) 5.0.25. Pooled analyses for isocaloric dietary pulse feeding trials were conducted using the Generic Inverse Variance method using random effects weighting. Mean endpoints of LDL-C, Apo-B and non-HDL-C were compared between dietary pulse arms and comparator arms. Data were expressed as mean differences (MD) with 95% CI. To mitigate the unit-of-analysis error from including trials with multiple intervention arms, we combined arms to create single pair-wise comparisons (n=1)\(^94\). To impute standard deviations for between-treatment differences in crossover trials, correlation coefficients between baseline and end-of-treatment values within each individual trial were derived from the reported within and between treatment SD according to a published formula\(^{101}\). These correlation coefficients were transformed into z-scores ± SD, meta-analyzed using inverse-variance weighing, and back transformed to derive a pooled correlation coefficient. A pooled correlation of 0.72 was used for LDL-C analysis but a correlation coefficient of 0.5 was assumed for non-HDL-C, owing to a lack of data, with sensitivity analyses done at 0.25 and 0.75. A two-sided p-value <0.05 was set as the level of significance for comparisons of MD.
The Q-statistic (Chi²) assessed and I² quantified the inter-study heterogeneity with significance set at p<0.10. An I² ≥50% indicated “substantial” heterogeneity and ≥75% indicated “considerable” heterogeneity⁹⁷. Sources of heterogeneity were explored using a priori subgroup analyses according to baseline cholesterol values, pulse dose and type, duration of follow-up, the difference in fibre content and saturated fat between the dietary pulses and control diet, study design (crossover or parallel), and study quality (MQS). A priori subgroup analyses were not conducted for Apo-B because only one study was included. A post-hoc analysis by sex (percentage of males) was also conducted. To determine if any single trial exerted an undue influence on the overall results, a sensitivity analysis was undertaken in which each individual study was removed from the meta-analysis and the effect size was re-calculated with the remaining studies.

Possible publication bias was assessed by visual inspection of funnel plots, and formally tested using Begg’s and Egger’s tests, with a P value <0.05 considered evidence of small-study effects.

4.5 RESULTS

4.5.1 SEARCH RESULTS

Figure 1 shows the flow of literature. The search identified 3002 reports, 2940 of which were determined to be irrelevant on review of the titles and abstracts. The remaining 62 reports were reviewed in full. A total of 22 reports providing data for 26 randomized trials were identified⁸,¹⁰, 19-30, 85, 86, 90, 93, 94, 99, 102, 103.

4.5.2 STUDY CHARACTERISTICS

Table 2 shows the characteristics of the 26 randomized trials (n=1013). Eight trials (31%) were conducted in hyperlipidemic participants, 3 trials (12%) in normolipidemic participants, and 15 trials (58%) in a combination of both. Median age of participants was 50.2 years with approximately equal number of men and women. Median baseline LDL-C was 3.50 mmol/L and non-HDL-C was 4.34 mmol/L. Studies had a median of 3 risk factors associated with increased CHD risk as defined by the Framingham
risk equation, implicating a moderate CHD risk (i.e. 10-year CHD risk ≤20%)⁴. Three trials were rated as CHD risk equivalent as according to NCEP-ATP III guidelines⁴ because they were conducted in individuals with Type 2 Diabetes⁸,²⁹,⁹⁹. All trials explicitly excluded individuals on lipid-lowering medications with the exception of 3 trials where it was unclear. Participants were from 6 countries: 11 trials were from the United States (42%), 6 trials from Australia (23%), 5 trials from Canada (19%), 2 trials from Spain (8%), and one trial each from Ireland and Iran (4%). Dietary pulse type included: beans (14 trials [54%]), peas (2 trials [8%]), chickpeas (2 trials [8%]), lentils (1 trial [4%]), and mixed pulses (8 trials [31%]). Dietary pulses were administered as flour in 3 trials (12%), as whole foods in 18 trials (69%), and mixed format (flour and/or whole foods) in 3 trials (12%) at a median dose of 130g/day (range: 50-377g/d).

Two trials did not report the form in which dietary pulses were administered as. The background diet consisted of 39-65% energy (E) from carbohydrate, 10-35% E protein, and 20-41% E fat with a median fibre and saturated fat intake of 20g/d (range: 13-47g/d) and 11%E (range: 5-15%E), respectively, in the comparator diets, and 26g/d (range: 17-53g/d) and 11%E (range: 5-15%E), respectively, in the dietary-pulse enriched diets. The method of increasing dietary pulses while maintaining caloric balance between arms differed across protocols: 15 trials (58%), replaced non-dietary pulse carbohydrates (eg. bread products, canned spaghetti, oat bran), specifically 7 trials replaced fibre, 5 trials (19%) replaced animal protein, 3 trials (12%) emphasized dietary pulses to achieve a low-glycemic diet, and 3 trials (12%) did not specify. Three trials (12%) were weight loss interventions designed to reduce total caloric intake by 30 or 35%. Five trials (19%) were metabolically-controlled (eg. all foods were provided), 17 trials (65%) were partially metabolically controlled (eg. test foods were provided), and 4 trials (15%) were not metabolically controlled (eg dietary advice was offered). Thirteen trials (50%) had a crossover design. Twenty-two trials (84%) were conducted in an outpatient setting, 2 trials (8%) in an inpatient setting, and 2 trials (8%) in a combination of the two. Median follow-up was 6 weeks (range: 3-52 weeks).
By the Heyland MQS, seven trials (27%) were of high quality (MQS ≥ 8). Poor description of randomization, absence of double-blinding, lack of intention-to-treat statistical analysis, and high dropout rates contributed to lower scores (Table 3). The Cochrane Risk of Bias Tool showed that 17 (77%) reports had unclear risk of bias and 5 reports (23%) had low risk of bias for sequence generation. Sixteen reports (73%) had unclear risk of bias and 6 reports (27%) had low risk of bias for allocation concealment. Nine reports (41%) had unclear risk of bias, 12 reports (54%) had low risk of bias, and 1 report (4%) had high risk of bias for blinding. Twenty-one reports (95%) were scored with low risk of bias for incomplete outcome data. Twelve reports (54%) scored unclear risk of bias for selective outcome reporting (Table 4). Funding of all trials was from: agency alone (50%); agency-industry sources (27%); industry alone (15%); and 2 trials were unclear of their funding sources.

4.5.3 GASTRO-INTESTINAL SYMPTOMS

Eleven trials provided self-reported gastrointestinal (GI) symptoms data. Four trials (36%) reported participants with upset stomach, 7 trials (64%) with flatulence, 6 trials (54%) with bloating, 1 trial with diarrhea and constipation each (9%), and 3 trials (27%) with increased stool frequency. Two trials compared GI symptoms to the comparator group but found no statistical differences across all symptoms except for increased flatulence in the comparator for 1 trial. Most studies reported that symptoms tended to improve over the course of the dietary pulse treatment. Only 2 trials had 1 to 2 participants reporting GI symptoms as a reason for study withdrawal.

4.5.4 EFFECT ON LDL-C

Twenty-one reports involving 25 comparisons (n= 686 intervention, 684 control) provided data on the effects of dietary pulses on LDL-C (Figure 2). Diets emphasizing dietary pulses significantly reduced LDL-C compared to control diets (MD= -0.17 mmol/L [95% CI: -0.25, -0.09]; p<0.0001) but showed significant evidence of inter-study heterogeneity (I² = 80%; p≤0.00001). Sensitivity analyses in
which each individual study was systematically removed and the effect size recalculated did not alter conclusions.

A priori subgroup analyses were carried out to test for possible modification of the effect of dietary pulses on LDL-C by study design characteristics. None of the subgroup analyses showed significant effects. In a post-hoc analysis by sex, we found greater LDL-C reduction as the ratio of males to females increased (β = -0.0036 [95% CI: -0.006, -0.001]; p = 0.012) (Figure 3 and Table 5).

4.5.5 EFFECT ON NON-HDL-C

Twenty reports involving 22 comparisons (n= 605 intervention, 605 control) provided data on the effects of dietary pulses on non-HDL-C (Figure 4). Diets emphasizing dietary pulses did not have a significant effect on non-HDL-C (MD= -0.09 mmol/L [95% CI: -0.19, 0.00]; p=0.06) but showed significant evidence of inter-study heterogeneity (I² = 98%; p≤0.00001). Sensitivity analyses showed when Abete et al.90 Anderson et al. (1984)19, Belski et al.20, Gravel et al.24, Hermsdorff et al.25, or Marinangeli et al. was removed, the pooled effect size became significant. The use of a correlation coefficient of 0.25 did not alter conclusions but a correlation coefficient of 0.75 resulted in a significant reduction in non-HDL-C.

A priori subgroup analyses were carried out to determine if the effect of dietary pulses on non-HDL-C differed according to study design characteristics. We found higher fibre intake in the dietary pulse compared to the control arm showed a significantly greater non-HDL reduction. None of the other subgroup analyses showed significance (Figure 5 and Table 5). Post-hoc analysis by sex also did not shown significant (Table 5).

4.5.6 EFFECT ON APOLIPROTEINS B

One trial met the inclusion criteria for Apo-B analysis. Isocaloric exchange of dietary pulses for another diet did not significantly alter Apo-B (MD= 0.02 [95% CI: -0.04, 0.08]) (Figure 6).
4.5.7 PUBLICATION BIAS

Inspection of funnel plots for evidence of publication bias revealed asymmetry favouring small studies with LDL-C reducing effects (Figure 7A). Egger’s test detected significant evidence of publication bias in LDL-C trials (p= 0.003) but Begg’s test did not show significant evidence of bias. Non-HDL-C showed no significant evidence of publication bias by visual inspection of funnel plot, Egger’s and Begg’s tests (Figure 7B).

4.6 DISCUSSION

We conducted a systematic review and meta-analysis of 26 randomized, controlled trials of the effect of dietary non-oil-seed pulses (beans, chickpeas, lentils and peas) on established therapeutic lipid targets for cardiovascular risk reduction in 1013 predominantly middle-age, normolipidemic or hyperlipidemic adults at moderate CHD risk, most of whom were not taking lipid lowering medications. Pooled analyses showed a significant LDL-C-lowering-effect of 0.17 mmol/L with a median dose of 130g/d of dietary pulses (~1.5 servings per day) over a median follow-up of 6-weeks. There was no significant effect of dietary pulses on Apo-B and non-HDL-C. Although some studies reported increased GI symptoms initially from the dietary pulse treatment, most reported that the effects subdued over the course of the study.

4.6.1 RESULTS IN RELATION TO OTHER STUDIES

This is the first systematic review and meta-analysis to report the effect of dietary pulse intake on all established lipids and lipoprotein CHD risk factors including LDL-C, Apo-B, and non-HDL-C. The observed LDL-lowering effect of dietary pulses is consistent with that of 2 previous meta-analyses\textsuperscript{13, 83}; however, our analysis was limited to randomized controlled trials with at least 3 weeks of follow-up duration in conformity with FDA guidelines\textsuperscript{92}.

We found a non-significant decreasing effect on non-HDL-C despite a significant reduction in LDL-C, suggesting a rise in VLDL-C. This contradictory result may be explained by elevated triglyceride
(TG) levels. Dyslipidemic guidelines suggest that when TG level is greater than 2.24 mmol/L (~200mg/dL)\(^4\) or 1.50 mmol/L (~133mg/dL)\(^5\), LDL-C and non-HDL-C no longer correlate well with each other. We found 13 trials (52%) in our analysis reported higher TG levels (≥2.24 mmol/L) and in a post-hoc analysis, individuals with normal levels of TG had greater reductions in LDL-C and non-HDL-C than those with high levels of TG (data not shown). However, we were unable to conduct a correlation analysis to examine the association of LDL-C and non-HDL-C stratified by TG levels; future analyses should consider this association.

The mechanism by which dietary pulses lower cholesterol levels is unclear; however there are several hypotheses that have been generated to explain this benefit. Dietary pulses reduce serum cholesterol by displacing saturated and trans fat intake. The 1999-2002 NHANES study found that Americans, who consumed approximately ½ cup dry beans or peas daily had lower intakes of saturated and total fat\(^8\). While the effects of saturated and total fat on cardiovascular risk are conflicting, many clinical trials have shown a beneficial effect when saturated fat is replaced with monounsaturated and polyunsaturated fatty acid intake (MUFA and PUFA, respectively)\(^8\). Further, dietary pulses are high in viscous fibre. Viscous fibre has been shown to trap bile acids in the intestine and prevent their absorption\(^8\), and human studies have demonstrated significant increases in fecal bile acid output related to LDL-C reduction\(^10\). Consequently, there is an increase in the production of bile acids by the liver, which decreases the liver pool of cholesterol and increases uptake of LDL-C by the liver thereby decreasing circulating LDL-C in the blood\(^2\). Further fermentation of fibre in the colon produces SCFA such as propionate and butyrate, which have been associated with inhibition of cholesterol synthesis in the liver\(^19\). Possibly of greater importance is the presence of a 7S globulin fraction, common to dietary pulses, which is consistent with this cholesterol reduction property\(^18\). It has been shown that peptides digested from this fraction, which may be absorbed intact, have a markedly inhibiting effect on Apo-B synthesis, the lipoprotein which influences hepatic cholesterol secretion.
4.6.2 INTER-STUDY HETEROGENEITY

We reported significant evidence of inter-heterogeneity in our LDL-C analysis; however none of our a priori subgroup analyses could explain the source of inter-study heterogeneity. To further investigate possible explanations, we conducted a post-hoc subgroup analysis by sex, where we treated the ratio of males to females from each study as a continuous variable. We found that studies conducted in all males tended to show a greater difference in LDL-C levels with dietary pulse intake compared to control\textsuperscript{10, 19, 21, 22, 85, 90}, than in the one study conducted in females only\textsuperscript{24}. Possible reasons why males may have shown a greater LDL-C reduction was because: 1) males tend to have poorer dietary patterns and respond more favourably to risk factors of CVD when dietary habits are healthier\textsuperscript{105}; 2) males of similar age have higher levels of LDL-C than pre-menopausal and post-menopausal women on hormone replacement therapy (HRT) and therefore LDL-C is more likely to be reduced in men\textsuperscript{4}; 3) compared to the total cholesterol fraction, males have higher LDL-C whereas females have higher HDL-C; the former is more responsive to dietary changes than the latter\textsuperscript{4} and; 4) as there is only one all-female study included, there may not be enough female-only studies for comparison. Although the subgroup analysis by sex showed reduction of inter-study heterogeneity, the level is still moderate and future analyses are needed to better understand the sources of heterogeneity.

The effect of dietary pulses on LDL-C should not be underestimated despite the high level of inter-study heterogeneity. The LDL-C effect translated to a ~5% reduction from baseline. This reduction in LDL-C equates to ~5-6% risk reduction in major vascular events (major coronary event, stroke, and coronary revascularization)\textsuperscript{45}. This is important especially in hypercholesterolemic patients who prefer dietary approaches to managing cholesterol levels or for those who cannot tolerate statin therapies. Further, the MDs of the majority of trials (23 of 25) fell within the 95% CI of the pooled estimate for LDL-C suggesting robustness in our data and increasing confidence in our conclusions. Therefore, while inter-
study heterogeneity was high in our pooled analysis of LDL-C, the effect estimate may still have meaningful clinical applications.

Our non-HDL-C findings were complicated by significant evidence of inter-study heterogeneity. In a previous systematic review and meta-analysis on the effect of dietary pulses on glycemic control, we reported significant effect modification by diabetes status, pulse type, dose, follow-up duration, study design, study quality, and baseline metabolic control\(^87\). In the current analysis, we only found a significant reducing non-HDL-C effect when the dietary pulse arm had greater fibre intake than the comparator. Diets high in fibre have been shown to reduce non-HDL-C\(^106\) and be inversely associated with CHD risk\(^15\). However, a substantial amount of inter-study heterogeneity remains unexplained. To further address inter-study heterogeneity, we conducted sensitivity analysis, where the removal of Abete et al.\(^90\), Anderson et al. (1984)\(^19\), Belski et al.\(^20\), Gravel et al.\(^24\), Hermsdorff et al.\(^25\), or Marinangeli et al.\(^102\) resulted in a significant non-HDL-C reduction. Each of these studies favoured the effect of the control treatment, but there was no common characteristics common amongst them that would lead us to believe that there is any bias in these analyses. Other sources of heterogeneity across trials need to be investigated when more trials are published.

4.6.3 LIMITATIONS

There are limitations to our work. First, the majority of studies were of low quality (MQS<8), short duration (<3 months), and did not report enough data to judge risk of bias. These observations reinforce the need for longer, better-designed clinical trials that report data more systematically and transparently to better understand the effect of dietary pulses on serum lipids. Second, only one trial reported on non-HDL-C values; therefore we had to calculate non-HDL-C and imputed its SD. To assess whether our method of SD imputation was reasonable, we conducted a sensitivity analysis, where we pooled reported SDs and substituted the calculated SD for trials that did not report these values. Our findings for non-HDL-C indicate that the second method of SD imputation did not alter our primary
conclusions, suggesting that our method of SD imputation is unlikely to have biased our conclusions. Third, only one trial provided Apo-B data. These deficiencies highlight the need for future trials to examine these endpoints as they are important biomarkers of cardiovascular health. Lastly, publication bias remains a possibility. We observed plot asymmetry favouring small studies with LDL-C reducing effects which was confirmed by a significant Egger’s test.

4.6.4 CONCLUSIONS

Our findings have implications for cardiovascular health. Dietary pulses resulted in a modest yet clinically meaningful reduction in the primary therapeutic lipid risk factor, LDL-C, showing a 0.17 mmol/L reduction (equivalent to ~5% reduction from baseline). This is important especially when combined as part of diets that have shown clinically impactful lipid-lowering that can potentially achieve upwards of 30% reductions in LDL-C\textsuperscript{53} and may be particularly helpful for those who prefer dietary approaches to managing cholesterol levels or for those who cannot tolerate statin therapies. The median pulse intake of \(\approx 1.5\) servings/day required to achieve this benefit supports a higher intake target than that proposed by the AHA of \(\geq 4-5\) servings/week. Achieving a high level of intake may prove a challenge in some Western countries, given that current U.S. level of intake is 0.2 serving/day. However, a dietary pulse intake of 130g/d is very reasonable as this level is currently consumed by many cultures without reports of side effects that would limit consumption. As the majority of these trials were conducted on a background heart-healthy NCEP-like diet, including \(>20-25\)g/d of fibre and \(<10\%E\) of saturated fat, these results can be considered in addition to the 5-10% LDL-C lowering expected from these diets alone\textsuperscript{53}. Whereas guidelines have largely focused on dietary pulses as part of a healthy dietary pattern in the prevention and management of diabetes and CVD, these data support lipid-lowering and cardiovascular risk reduction recommendations. As dietary pulses may have beneficial effects on other cardiometabolic risk factors including body weight, blood pressure, and glucose control\textsuperscript{87}, future systematic reviews and
meta-analyses should evaluate the effects of dietary pulses on these endpoints and others to address these factors that contribute to cardiovascular risk.

4.7 ACKNOWLEDGMENTS

We would like to thank Xavier Thobaut Gusmini and Júlio Medeiros Rego for their technical support.

Aspects of this work was presented at the 2012 Experimental Biology Conference, San Diego, CA, USA, April 21-25 2012, 15th Annual Canadian Society for Endocrinology and Metabolism/Canadian Diabetes Association (CSEM/CDA) Professional Conference and Annual Meetings, Toronto, ON, Canada, October 10-13 2012., and 53rd Annual Conference at the American College of Nutrition, Morristown, NJ, USA, November 14-17 2012.

4.8 SOURCE OF FUNDING

This work was funded by a Canadian Institutes of Health Research (CIHR) Knowledge Synthesis Grant (Funding Reference Number: 119797). VH was supported by an Ontario Graduate Scholar (OGS) award. RJD was funded by a Canadian Institutes for Health Research (CIHR) Postdoctoral Fellowship Award. DJAJ was funded by the Government of Canada through the Canada Research Chair Endowment. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

4.9 CONFLICTS OF INTEREST/DISCLOSURE

JLS has received several unrestricted travel grants from The Coca-Cola Company to present research at meetings and is a co-investigator on an unrestricted research grant from The Coca-Cola Company. JLS has also received travel funding and honoraria from Abbott Laboratories, Archer Daniels Midland, and the International Life Sciences Institute (ILSI) North America and Brazil; and research support, consultant fees, and travel funding from Pulse Canada. Spouse of JLS works for Unilever
Canada. RJD, JB, and CWCK are co-investigators on an unrestricted grant from The Coca-Cola Company. VV holds American (No. 7,326,404 B2) and Canadian (No. 2,410,556) patents for the use of viscous fibre blend in diabetes, metabolic syndrome and cholesterol lowering. VV currently holds grant support for ginseng research from the Canadian Diabetes Association, Canada and the National Institute of Horticultural & Herbal Science, RDA, Korea. CWCK has served on the scientific advisory board, received research support, travel funding, consultant fees, or honoraria from Pulse Canada, Barilla, Solae, Unilever, Hain Celestial, Loblaws Inc., Oldways Preservation Trust, the Almond Board of California, the International Nut Council, Paramount Farms, the California Strawberry Commission, the Canola and Flax Councils of Canada, and Saskatchewan Pulse Growers. CWCK also receives partial salary funding from research grants provided by Unilever, Loblaw’s, and the Almond Board of California. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg’s, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg’s, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials
Network (AFMNet), Loblaw, Unilever, Barilla, Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg’s, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. Dr Jenkins' wife is a director, VV is the vice-president and part owner, and LC is a clinical study coordinator of Glycemic Index Laboratories, Toronto, Ontario, Canada. VH, VHJ, AA, SBM, MD, FMS, AMB, PKE, RPB, RGJ, and LAL have no declared conflicts of interest related to this paper.
### 4.10 Tables

**Table 1.** Search Strategy for Studies Assessing the Effect of Dietary Pulses on Lipids in Randomized, Controlled Feeding Trials*.

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<thead>
<tr>
<th>DATABASE</th>
<th>SEARCH PERIOD</th>
<th>SEARCH</th>
</tr>
</thead>
</table>
| MEDLINE  | 1948 to week 2 of February 2013 | 1. (Fabaceae or lentil$ or chickpea$ or bean$ or pea$ or legume$ or leguminous) and (lipid$ or cholesterol$ or apolipoprotein B or hyperlipidemia or lipaemia). mp  
2. Limit to animals  
3. 1 not 2  
4. Limit to Clinical Trials, Clinical Trial, ALL  
5. Limit to Clinical Trial  
6. Limit to Controlled Clinical Trial  
7. Limit to Randomized Controlled Trial |
| EMBASE Classic and EMBASE | 1947 to Week 6 of 2013 | 1. (Fabaceae OR lentil$ OR chickpea$ OR bean$ OR pea$ OR legume$ OR leguminous) AND (lipid$ OR cholesterol$ OR apolipoprotein B OR hyperlipidemia OR lipaemia). mp  
2. Limit to Animals and Animal Studies  
3. 1 not 2  
4. Limit to Clinical Trial  
5. Limit to Randomized Controlled Trial  
6. Limit to Controlled Clinical Trial |
| The Cochrane Library | 1991 to February 13 2013 | 1. (Fabaceae OR lentil$ OR chickpea$ OR bean$ OR pea$ OR legume$ OR leguminous) AND (lipid$ OR cholesterol$ OR apolipoprotein B OR hyperlipidemia OR lipaemia). mp |
| CINAHL | 1982 to February 13 2013 | 1. (lentil$ OR chickpea$ OR bean$ OR pea$ OR legume$ OR leguminous) AND (lipid$ OR VLDL OR apolipoprotein B OR hyperlipidemia OR lipaemia) |

* The original search was conducted on May 9 2011 for MEDLINE, EMBASE, and CINAHL; and June 13 2011 for the Cochrane Library; updated searches for all databases were performed on December 2 2011, April 9, October 2 and 29, November 13 2012, and February 13 2013.
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<th>BASELINE (SD)</th>
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<th>MEDICATION LE]</th>
<th>DESIGN</th>
<th>SETTING</th>
<th>FREEING CONTROL]</th>
<th>ENERGY BALANCE**</th>
<th>PULSE DOSE (pmm/day)**</th>
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<td>OP</td>
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<tr>
<td>Anderson et al. 1990–1 [85]</td>
<td>6 HC (8M/7W)</td>
<td>64.2±4.2</td>
<td>-</td>
<td>4</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~113</td>
</tr>
<tr>
<td>Anderson et al. 1990–3 [81]</td>
<td>9 HC (8M/3W)</td>
<td>57.9±9.9</td>
<td>-</td>
<td>4</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~113</td>
</tr>
<tr>
<td>Anderson et al. 1990–1 [80]</td>
<td>19 HC (19M/0W)</td>
<td>54±9</td>
<td>-</td>
<td>4</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~152</td>
</tr>
<tr>
<td>Belink et al. 2011 [20]</td>
<td>93 OW/Ob (52M/41W)</td>
<td>~46±6.10</td>
<td>3.3±0.76 (3.29±0.77)</td>
<td>4</td>
<td>No  P</td>
<td>OP</td>
<td>Australia</td>
<td>Partial</td>
<td>Negative (30% E)</td>
<td>~50</td>
</tr>
<tr>
<td>Cobia et al. 1990 [21]</td>
<td>20 HC (12M/8W)</td>
<td>5.0±3.75</td>
<td>-</td>
<td>5</td>
<td>No  C</td>
<td>OP</td>
<td>Australia</td>
<td>Partial</td>
<td>Neutral</td>
<td>~377</td>
</tr>
<tr>
<td>Duwe et al. 1997 [22]</td>
<td>5 N (3M/2W)</td>
<td>58.4±17.9</td>
<td>4.45±0.63 (4.24±0.62)</td>
<td>2</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~130</td>
</tr>
<tr>
<td>Finley et al. 2007–9 [23]</td>
<td>45 N (20M/20W)</td>
<td>~37±4.11</td>
<td>-</td>
<td>3</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~130</td>
</tr>
<tr>
<td>Gennelly et al. 2017 [105]</td>
<td>53 N (3M/2W)</td>
<td>majority of participants</td>
<td>30±50</td>
<td>-</td>
<td>3</td>
<td>P</td>
<td>OP</td>
<td>Ireland</td>
<td>Partial</td>
<td>~59</td>
</tr>
<tr>
<td>Grewal et al. 2010 [24]</td>
<td>114 Pre MS (8M/134W)</td>
<td>~51±8.6</td>
<td>3.8±0.87 (3.91±0.90)</td>
<td>2</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~81</td>
</tr>
<tr>
<td>Hermosof et al. 2011 [25]</td>
<td>20 CS (17M/3W)</td>
<td>36±8</td>
<td>3.5±0.60 (4.32±0.62)</td>
<td>3</td>
<td>No  P</td>
<td>OP</td>
<td>Spain</td>
<td>Non-Novab</td>
<td>Negative (30% E)</td>
<td>~138</td>
</tr>
<tr>
<td>Hodgson et al. 2010 [30]</td>
<td>74 OW/Ob (20M/48W)</td>
<td>~57±7.9</td>
<td>3.26±0.31 (3.18±0.75)</td>
<td>3</td>
<td>No  P</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~64</td>
</tr>
<tr>
<td>Jenkins et al. 2012 [8]</td>
<td>121 T2D (51M/70W)</td>
<td>~59.1±12.8</td>
<td>3.00±0.12 (2.93±0.56)</td>
<td>3</td>
<td>No  P</td>
<td>OP</td>
<td>Canada</td>
<td>Non-Novab</td>
<td>Neutral</td>
<td>~196</td>
</tr>
<tr>
<td>Jimeno-Cruz et al. 2004 [98]</td>
<td>8 T2D</td>
<td>51±5</td>
<td>-</td>
<td>4</td>
<td>No  C</td>
<td>OP</td>
<td>USA</td>
<td>Non-Novab</td>
<td>Neutral</td>
<td>--</td>
</tr>
<tr>
<td>Mackay et al. 1982 [28]</td>
<td>39 HC (22M/17W)</td>
<td>~47±28.60</td>
<td>4.0±0.53 (4.3±0.55)</td>
<td>4</td>
<td>-  C</td>
<td>OP</td>
<td>New Zealand</td>
<td>Partial</td>
<td>Neutral</td>
<td>80</td>
</tr>
<tr>
<td>Mariangeli et al. 2011 [102]</td>
<td>23 OW/Ob (7M/16W)</td>
<td>~52±10.6</td>
<td>3.8±0.62 (3.7±0.62)</td>
<td>3</td>
<td>No  C</td>
<td>OP</td>
<td>Canada</td>
<td>Metab</td>
<td>Neutral</td>
<td>~138</td>
</tr>
<tr>
<td>Pittaway et al. 2006 [27]</td>
<td>47 (30M/17W)</td>
<td>53±9.8</td>
<td>-</td>
<td>5</td>
<td>No  C</td>
<td>OP</td>
<td>Australia</td>
<td>Partial</td>
<td>Neutral</td>
<td>~140</td>
</tr>
<tr>
<td>Pittaway et al. 2007 [28]</td>
<td>27 (30M/17W)</td>
<td>50±6.1</td>
<td>-</td>
<td>5</td>
<td>No  C</td>
<td>OP</td>
<td>Australia</td>
<td>Partial</td>
<td>Neutral</td>
<td>~140</td>
</tr>
<tr>
<td>Shams et al. 2008 [29]</td>
<td>30 T2D</td>
<td>50±3±6.8</td>
<td>3.69±0.44 (4.53±0.34)</td>
<td>3</td>
<td>No  C</td>
<td>OP</td>
<td>Pan</td>
<td>Partial</td>
<td>Neutral</td>
<td>~50</td>
</tr>
<tr>
<td>Winnam et al. 2007 [33]</td>
<td>23 HC (10M/13W)</td>
<td>45±9±10.6</td>
<td>3.47±0.37 (3.3±0.59)</td>
<td>1</td>
<td>No  C</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~50</td>
</tr>
<tr>
<td>Winnam et al. 2007 [34]</td>
<td>16 Midry IR (7M/9W)</td>
<td>43±12</td>
<td>3.37±0.42 (3.3±0.42)</td>
<td>2</td>
<td>No  C</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~50</td>
</tr>
<tr>
<td>Zhong et al. 2010 [10]</td>
<td>36 IS (7M/29W)</td>
<td>53±6±7.6</td>
<td>3.34±0.96 (3.3±0.32)</td>
<td>4</td>
<td>No  C</td>
<td>OP</td>
<td>United States</td>
<td>Partial</td>
<td>Neutral</td>
<td>~250</td>
</tr>
</tbody>
</table>

*Ob= Obese; OW= Overweight; HC= Hypercholesterolemia; T2D= Type 2 Diabetes; N= normal/healthy; Pre-MS= Pre-Metabolic Syndrome; IR= Insulin Resistant; IS= Insulin Sensitive; M= Men; W= Women; "* not reported; **= calculated values
†Values listed in the following order: LDL-C (mmol/L), Apo-B (g/L), non-HDL-C (mmol/L); Value for the control arm is reported first followed by value for the treatment arm; some endpoints combined baseline control and treatment arms together and, therefore, reported only one value; For Winham et al.*, B denotes Bean Arm and P Pea Arm
‡Framingham 10-year CHD Risk Factor Score Assessment. For a more conservative approach, participants were assumed to be smokers, hypertensive, have low HDL-C, and/or have family history of CHD if not reported. Risk score for men is reported first followed by risk score for women.
§C= Crossover Design; P= Parallel Design; The study by Winham et al.** had a crossover design with one control arm and 2 treatment arms (beans and peas). To mitigate unit-of-analysis error, we combined the 2 groups to create a single pair-wise comparison, which we conservatively analyzed as a parallel trial for the overall analysis.
### Table 2. Study Characteristics Table.

<table>
<thead>
<tr>
<th>PULSE FORARM</th>
<th>TYPE OF PULSE</th>
<th>COMPARATOR</th>
<th>DIET (CHO: PROTEIN: FAT % E)</th>
<th>FIBRE (g/g)%</th>
<th>SATURATED FAT (mg/g)%</th>
<th>FOLLOW-UP</th>
<th>MOS§§</th>
<th>FUNDING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>Mixed</td>
<td>No Pulses</td>
<td>52:19:32</td>
<td>20:17</td>
<td>~9</td>
<td>8 weeks</td>
<td>7</td>
<td>Agency/Industry</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>Oat-Bran</td>
<td>43:44:20:36:37</td>
<td>47:48</td>
<td>~14 15</td>
<td>3 weeks</td>
<td>8</td>
<td>Agency/Industry</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>No Pulses</td>
<td>43:19:38</td>
<td>13 23</td>
<td>~12</td>
<td>3 weeks</td>
<td>6</td>
<td>Agency/Industry</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>No Pulses</td>
<td>43:19:38</td>
<td>13 23</td>
<td>~14</td>
<td>3 weeks</td>
<td>6</td>
<td>Agency/Industry</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>No Pulses</td>
<td>43:19:38</td>
<td>13 23</td>
<td>~11</td>
<td>3 weeks</td>
<td>6</td>
<td>Agency/Industry</td>
</tr>
<tr>
<td>Whole</td>
<td>Mixed</td>
<td>No Pulses</td>
<td>60:17:23</td>
<td>19 24</td>
<td>~6 7 weeks</td>
<td>8 Agency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>Chicken Soup</td>
<td>41:48:15:17:36:41</td>
<td>~18:17  ~17:24</td>
<td>-</td>
<td>~12 weeks</td>
<td>6</td>
<td>Agency</td>
</tr>
<tr>
<td>Whole</td>
<td>Peas</td>
<td>Cornflakes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6 weeks</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Whole</td>
<td>Mixed</td>
<td>No Pulses</td>
<td>50:51:19:31:33</td>
<td>18:5 26:68</td>
<td>5:40.9 5:40.3</td>
<td>8 weeks</td>
<td>8</td>
<td>Agency</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>High Gl</td>
<td>51:54:26:27:18:23</td>
<td>30 51</td>
<td>9</td>
<td>3 weeks</td>
<td>9</td>
<td>Agency</td>
</tr>
<tr>
<td>Flour</td>
<td>Peas</td>
<td>White Flour</td>
<td>55:15:30</td>
<td>19 19</td>
<td>8</td>
<td>4 weeks</td>
<td>6</td>
<td>Industry</td>
</tr>
<tr>
<td>Whole/FLOUR</td>
<td>Chickpea</td>
<td>Whole Wheat</td>
<td>43:44:17:18:34</td>
<td>29:10:8 28:17:5</td>
<td>~14 14</td>
<td>5 weeks</td>
<td>5</td>
<td>Agency</td>
</tr>
<tr>
<td>Whole</td>
<td>Lentils</td>
<td>No Pulses</td>
<td>48:52:18:19:29:31</td>
<td>23:6 29:36</td>
<td>-</td>
<td>6 weeks</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>Chicken</td>
<td>50:51:18:34</td>
<td>~18 11:1</td>
<td>11:1 12:1</td>
<td>4 weeks</td>
<td>3</td>
<td>Agency</td>
</tr>
<tr>
<td>Whole</td>
<td>Beans</td>
<td>Chicken</td>
<td>50:51:18:34</td>
<td>~18 11:1</td>
<td>11:1 12:1</td>
<td>4 weeks</td>
<td>3</td>
<td>Agency</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INTERPRETATION</th>
</tr>
</thead>
</table>

**Inpatient; OP= outpatient**

¶ metab denotes all foods were provided; partial if test food or some meals were provided; and non-metab if no foods were provided

**based on cooked weight; dry weight was converted to wet weight by multiplying 2.75**

††Mixed refers to more than one type of dietary pulses being studied

‡‡ Value for the control arm is reported first followed by value for the treatment arm

§§ MOS= Heyland Methodological Score; Score of ≥8 denotes higher quality

††† For Abeyesekara et al. analysis was for n= 80⁹⁶; Belski et al. age is based on n=131⁰⁵; Gravel et al. n=132⁴⁴; Marinangeli et al. n=29ⁱ⁰⁲

¶¶ Dietary Pulses were provided in 1 of 2 forms: 1) Whole, where intact dietary pulses were consumed; 2) Flour, where dietary pulses were ground to a powder form and was incorporated into baked foods

***Neutral denotes weight-maintaining diet; Negative weight-reduction diet (% energy restricted from baseline diet reported in brackets)
### Table 3. Study Quality Assessment by Using the Heyland MQS<sup>100</sup>.

| Study                          | Methods† | Sample‡ | Intervention§ | MQS  
|-------------------------------|----------|---------|----------------|------
|                               | Randomization | Blinding | Analysis | Selection | Compatability | Follow-up | Protocol | Co-interventions | Crossovers | (n/13) |
| Gormley et al. 1979 [103]     | 1        | 0       | 0       | 0          | 1      | 1          | 0        | 0        | 1                | 0           | 3     |
| Anderson et al. 1984 [19]     | 1        | 0       | 2       | 0          | 1      | 1          | 1        | 1        | 2                | 0           | 8     |
| Anderson et al. 1990 [85]     | 1        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 6     |
| Cobiac et al. 1990 [21]       | 1        | 0       | 2       | 0          | 1      | 1          | 1        | 1        | 2                | 0           | 8     |
| Mackay et al. 1992 [26]       | 1        | 0       | 0       | 0          | 1      | 0          | 1        | 2        | 0                | 0           | 5     |
| Duane et al. 1997 [22]        | 1        | 0       | 2       | 0          | 1      | 1          | 1        | 1        | 2                | 0           | 8     |
| Jimenez-Cruz et al. 2004 [99] | 1        | 0       | 2       | 1          | 1      | 1          | 1        | 2        | 0                | 0           | 9     |
| Pittaway et al. 2006 [27]     | 1        | 0       | 0       | 0          | 1      | 1          | 0        | 1        | 2                | 0           | 5     |
| Pittaway et al. 2007 [28]     | 1        | 0       | 0       | 0          | 1      | 1          | 0        | 1        | 2                | 0           | 5     |
| Finley et al. 2007 [23]       | 1        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 6     |
| Winham et al. 2007 [93]       | 2        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 7     |
| Winham et al. 2007-COM [94]   | 1        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 6     |
| Shams et al. 2008 [29]        | 1        | 0       | 2       | 0          | 1      | 1          | 1        | 0        | 2                | 0           | 7     |
| Abete et al. 2009 [90]        | 1        | 0       | 2       | 1          | 1      | 1          | 0        | 1        | 1                | 0           | 7     |
| Gravel et al. 2010 [24]       | 2        | 0       | 0       | 1          | 1      | 0          | 0        | 2        | 0                | 0           | 6     |
| Hodgson et al. 2010 [30]      | 2        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 7     |
| Zhang et al. 2010 [10]        | 1        | 0       | 0       | 0          | 1      | 0          | 1        | 2        | 0                | 0           | 5     |
| Belski et al. 2011 [20]       | 2        | 1       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 8     |
| Hermensdorf et al. 2011 [25]  | 1        | 0       | 2       | 0          | 1      | 1          | 1        | 1        | 2                | 0           | 8     |
| Marinangeli et al. 2011 [102] | 1        | 0       | 0       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 6     |
| Abeysekara et al. 2012 [86]   | 2        | 0       | 0       | 1          | 1      | 0          | 1        | 1        | 0                | 0           | 6     |
| Jenkins et al. 2012 [8]       | 1        | 0       | 2       | 1          | 1      | 0          | 1        | 2        | 0                | 0           | 8     |

*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<sup>100</sup>.

† 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.
Table 4. Risk of Bias Assessment by Using the Cochrane Risk of Bias Tool*.

<table>
<thead>
<tr>
<th>Bias Category</th>
<th>Low risk of bias</th>
<th>Unclear risk of bias</th>
<th>High risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random sequence generation (selection bias)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allocation concealment (selection bias)‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blinding of participants and personnel (performance bias)§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete outcome data (attrition bias)¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective reporting (reporting bias)¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other bias**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Studies were rated UR if insufficient information was given to assess risk; LR if the study design is likely to have little influence over the true outcome; HR if the study design is likely to have an influential effect on the true outcome.

†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 and/or participants.

¶Incomplete outcome data assessed whether missing outcome data affected true outcome.

¶¶Selected outcome reporting assessed whether investigators pre-registered trial and/or specified primary and secondary outcomes.

** Other bias assessed other sources of bias that are not listed in the Cochrane Risk of Bias Tool. Only Abeyesekara et al.⁸⁶ is listed as the dietary pulse arm was given both food and dietary advice throughout the study, but the control arm was simply told to keep following their usual dietary habits.
Table 5. Subgroup analyses by *a priori* dichotomous categorization for LDL-C and non-HDL-C.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Range</th>
<th>No. of Trials</th>
<th>N</th>
<th>β (95% CI)</th>
<th>Residual $I^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDL-C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A Priori Subgroup</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline LDL</td>
<td>2.27-5.32 mmol/L</td>
<td>17</td>
<td>773</td>
<td>-0.10 (-0.42, 0.21)</td>
<td>83.6</td>
<td>0.482</td>
</tr>
<tr>
<td>Dose</td>
<td>50-377 grams/day</td>
<td>24</td>
<td>949</td>
<td>0.00 (0.00, 0.00)</td>
<td>65.4</td>
<td>0.316</td>
</tr>
<tr>
<td>Difference in Fiber Intake*</td>
<td>-3-24 grams/day</td>
<td>25</td>
<td>992</td>
<td>-0.01 (-0.02, 0.01)</td>
<td>80.4</td>
<td>0.256</td>
</tr>
<tr>
<td>Difference in Saturated Fat Intake†</td>
<td>-1-2 % Energy</td>
<td>17</td>
<td>724</td>
<td>-0.01 (-0.22, 0.20)</td>
<td>78.8</td>
<td>0.920</td>
</tr>
<tr>
<td>Duration</td>
<td>3 weeks-1 year</td>
<td>25</td>
<td>960</td>
<td>0.01 (0.00, 0.02)</td>
<td>80.3</td>
<td>0.240</td>
</tr>
<tr>
<td><strong>Post-hoc Subgroup</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Males</td>
<td>0-100%</td>
<td>23</td>
<td>946</td>
<td>-0.004 (-0.01, -0.00)</td>
<td>53.1</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Non-HDL-C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A Priori Subgroup</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline non-HDL-C</td>
<td>2.99-6.70 mmol/L</td>
<td>16</td>
<td>762</td>
<td>0.01 (-0.31, 0.28)</td>
<td>98.8</td>
<td>0.929</td>
</tr>
<tr>
<td>Dose</td>
<td>50-377 grams/day</td>
<td>21</td>
<td>861</td>
<td>0.00 (0.00, 0.00)</td>
<td>98.1</td>
<td>0.519</td>
</tr>
<tr>
<td>Difference in Fiber Intake*</td>
<td>-3-23 grams/day</td>
<td>21</td>
<td>816</td>
<td>-0.03 (-0.06, 0.00)</td>
<td>98.3</td>
<td>0.024</td>
</tr>
<tr>
<td>Difference in Saturated Fat Intake†</td>
<td>-1-2 % Energy</td>
<td>15</td>
<td>660</td>
<td>0.05 (-0.23, 0.34)</td>
<td>97.9</td>
<td>0.706</td>
</tr>
<tr>
<td>Duration</td>
<td>3 weeks-1 year</td>
<td>22</td>
<td>869</td>
<td>0.01 (-0.01, 0.03)</td>
<td>97.6</td>
<td>0.289</td>
</tr>
<tr>
<td><strong>Post-hoc Subgroup</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Males</td>
<td>0-100%</td>
<td>19</td>
<td>783</td>
<td>0.00 (0.00, 0.01)</td>
<td>98.1</td>
<td>0.446</td>
</tr>
</tbody>
</table>

Abbreviations: N denotes the number of participants in each subgroup; residual $I^2$ was reported as a percent value and significance as a P-value with p < 0.01 as significant.

*Difference in fibre intake compared fibre intake of the dietary pulse arm with control at the end of the study. If fibre intake at the end of the study was not provided, intended fibre intake (as specified in the study protocol) for each arm was used for calculation.

† Difference in saturated fat intake compared saturated fat intake of the dietary pulse with the control at the end of the study. If saturated fat intake at the end of the study was not provided, intended saturated fat intake (as specified in the study protocol) for each arm was used for calculation.
3001 reports identified
1006 MEDLINE (through Feb 11 2013)
1060 EMBASE (through Feb 11 2013)
881 Cochrane Library (through Feb 11 2013)
35 CINAHL (through Feb 11 2013)

2940 reports excluded on basis of title and/or abstract
1266 duplicate reports
14 animal or in vitro studies
1 book chapter
5 commentaries or editorials
82 case reports
151 review papers
256 observational studies
1112 studies with no dietary pulse intervention
8 studies with dietary pulse extracts
10 studies with unsuitable endpoints
8 acute or short-term studies
27 co-intervention trials where dietary pulses were not emphasized

61 reports reviewed in full

39 reports excluded
1 review paper
3 studies with no dietary pulse intervention
2 studies with dietary pulse extracts
7 studies with unsuitable endpoints
1 hypocaloric study
3 acute or short-term studies
12 co-intervention trials where dietary pulses were not emphasized
1 study with soy in control group
4 studies were not randomized
2 studies lacked control group
1 study reported in unusable statistics
1 cannot be retrieved
1 provided incorrect data and authors could not be contacted

22 reports (26 trials) included in the meta-analysis (n= 1013)

**Figure 1.** Flow of the literature search.
**Figure 2.** Forest plot of trials investigating the effect of isocaloric exchange of dietary pulses for control diets on LDL-C. A pooled effect estimate is shown as a diamond. Paired analyses were applied to all crossover trials. Data are mean differences (MD) with 95% CI, where p-values are for Generic Inverse Variance random effects models. Inter-study heterogeneity was tested by Cochrane’s Q ($I^2$) at a significance level of $P<0.10$ and quantified by $I^2$, where $I^2 \geq 50\%$ is considered to be evidence of substantial heterogeneity and $\geq75\%$, considerable heterogeneity.
### DIETARY PULSES AND LIPIDS

**Figure 3.** Forest plots of subgroup analyses for dichotomous variables investigating the effect of isocaloric exchange of dietary pulses for other adequate comparators on LDL-C in all participants. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled effect estimate for the overall (total) analysis. The residual $I^2$ value indicates the inter-study heterogeneity unexplained by the subgroup. Pair-wise between-subgroup mean differences with 95% CI (mmol/L) for comparator were as follows: (1 vs. 2) -0.23 (-0.78, 0.31), (1 vs. 3) -0.44 (-1.11, 0.22), (1 vs. 4) -0.52 (-1.09, 0.04), (1 vs. 5) -0.16 (-0.60, 0.28), (2 vs. 1) 0.23 (-0.31, 0.78), (2 vs. 3) -0.21 (-0.90, 0.48), (2 vs. 4) -0.29 (-0.89, 0.31), (2 vs. 5) 0.07 (-0.41, 0.55), (3 vs. 1) -0.07 (-0.41, 0.55), (3 vs. 2) 0.44 (-0.22, 1.11), (3 vs. 4) -0.08 (-0.79, 0.63), (3 vs. 5) 0.28 (-0.33, 0.89), (4 vs. 1) 0.52 (-0.04, 1.09), (4 vs. 2) 0.29 (-0.31, 0.89), (4 vs. 3) 0.08 (-0.63, 0.79), (4 vs. 5) 0.36 (-0.15, 0.87), (5 vs. 1) 0.16 (-0.28, 0.60), (5 vs. 2) -0.07 (-0.55, 0.41), (5 vs. 3) -0.28 (-0.89, 0.33), (5 vs. 4) -0.36 (-0.87, 0.15).
Figure 4. Forest plot of trials investigating the effect of isocaloric exchange of dietary pulses for control diets on non-HDL-C. A pooled effect estimate is shown as a diamond. Paired analyses were applied to all crossover trials. Data are mean differences (MD) with 95% CI, where p-values are for Generic Inverse Variance random effects models. Inter-study heterogeneity was tested by Cochrane’s Q ($I^2$) at a significance level of P < 0.10 and quantified by $I^2$, where $I^2 \geq 50\%$ is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity.
### DIETARY PULSES AND LIPIDS

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Level</th>
<th>Trials</th>
<th>Participants</th>
<th>Mean Difference (95% CI) in non-HDL-C, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Within Subgroups</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>869</td>
<td></td>
<td>-0.09 (-0.19 to 0.00)</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4.14 mmol/L</td>
<td>6</td>
<td>421</td>
<td></td>
<td>-0.02 (-0.42 to 0.39)</td>
</tr>
<tr>
<td>≥4.14 mmol/L</td>
<td>10</td>
<td>341</td>
<td></td>
<td>-0.11 (-0.42 to 0.20)</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans (1)</td>
<td>11</td>
<td>317</td>
<td></td>
<td>-0.21 (-0.51 to 0.08)</td>
</tr>
<tr>
<td>Chickpeas (2)</td>
<td>2</td>
<td>74</td>
<td></td>
<td>-0.21 (-0.83 to 0.40)</td>
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<tr>
<td>Lentils (3)</td>
<td>1</td>
<td>30</td>
<td></td>
<td>-0.34 (1.20 to 0.52)</td>
</tr>
<tr>
<td>Peas (4)</td>
<td>3</td>
<td>92</td>
<td></td>
<td>-0.16 (0.66 to 0.34)</td>
</tr>
<tr>
<td>Mixed (5)</td>
<td>6</td>
<td>379</td>
<td></td>
<td>0.09 (-0.26 to 0.44)</td>
</tr>
<tr>
<td>Dietary Fibre Intake</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;28 grams/day</td>
<td>11</td>
<td>277</td>
<td></td>
<td>-0.01 (-0.30 to 0.27)</td>
</tr>
<tr>
<td>≥28 grams/day</td>
<td>10</td>
<td>539</td>
<td></td>
<td>-0.18 (-0.45 to 0.09)</td>
</tr>
<tr>
<td>Saturated Fat Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7% E</td>
<td>2</td>
<td>48</td>
<td></td>
<td>0.42 (0.20 to 1.05)</td>
</tr>
<tr>
<td>≥7% E</td>
<td>13</td>
<td>612</td>
<td></td>
<td>-0.13 (-0.38 to 0.11)</td>
</tr>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100 grams/day</td>
<td>9</td>
<td>460</td>
<td></td>
<td>-0.05 (-0.25 to 0.15)</td>
</tr>
<tr>
<td>≥100 grams/day</td>
<td>12</td>
<td>401</td>
<td></td>
<td>-0.05 (-0.24 to 0.15)</td>
</tr>
<tr>
<td>Design</td>
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<tr>
<td>Crossover</td>
<td>11</td>
<td>322</td>
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<td>-0.25 (-0.49 to -0.01)</td>
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<tr>
<td>Parallel</td>
<td>11</td>
<td>454</td>
<td></td>
<td>0.05 (-0.22 to 0.32)</td>
</tr>
<tr>
<td>MQS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>15</td>
<td>568</td>
<td></td>
<td>-0.14 (-0.38 to 0.10)</td>
</tr>
<tr>
<td>≥8</td>
<td>7</td>
<td>301</td>
<td></td>
<td>-0.09 (-0.42 to 0.24)</td>
</tr>
</tbody>
</table>

**Figure 5.** Forest plots of subgroup analyses for dichotomous variables investigating the effect of isocaloric exchange of dietary pulses for other adequate comparators on non-HDL-C in all participants. Point estimates for each subgroup level (diamonds) are the pooled effect estimates. The dashed line represents the pooled effect estimate for the overall (total) analysis. The residual I² value indicates the inter-study heterogeneity unexplained by the subgroup. Pair-wise between-subgroup mean differences with 95% CI (mmol/L) for comparator were as follows: (1 vs. 2) -0.21 (-1.06, 0.64), (1 vs. 3) -0.09 (-1.12, 0.96), (1 vs. 4) -0.27 (-1.04, 0.50), (1 vs. 5) -0.52 (-1.20, 0.17), (2 vs. 1) 0.21 (-0.64, 1.06), (2 vs. 3) 0.12 (-0.93, 1.18), (2 vs. 4) -0.06 (-0.85, 0.73), (2 vs. 5) -0.30 (-1.01, 0.40), (3 vs. 1) 0.09 (-0.96, 1.13), (3 vs. 2) -0.12 (-1.18, 0.93), (3 vs. 4) -0.18 (-1.18, 0.81), (3 vs. 5) -0.43 (-1.36, 0.50), (4 vs. 1) 0.27 (-0.50, 1.04), (4 vs. 2) 0.06 (-0.73, 0.85), (4 vs. 3) 0.18 (-0.81, 1.18), (4 vs. 5) -0.25 (-0.86, 0.37), (5 vs. 1) 0.52 (-0.17, 1.20), (5 vs. 2) 0.30 (-0.40, 1.01), (5 vs. 3) 0.43 (-0.50, 1.36), (5 vs. 4) 0.25 (-0.37, 0.86).
### Study Pulses, n Control, n Mean Difference (95% CI) in Apo-B (g/L)

<table>
<thead>
<tr>
<th>Study</th>
<th>Pulses, n</th>
<th>Control, n</th>
<th>Mean Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravel et al. 2010 [24]</td>
<td>60</td>
<td>54</td>
<td>0.02 [-0.04, 0.08]</td>
</tr>
</tbody>
</table>

Test for overall effect: $Z = 0.67$ ($P = 0.50$)

**Figure 6.** Forest plot of a trial investigating the effect of isocaloric exchange of dietary pulses for control diet on Apo-B. An effect estimate is shown as a diamond. The datum is mean difference (MD) with 95% CI, where the $P$ value is for Generic Inverse Variance random effects model.
Figure 7. Funnel plot investigating publication bias and effect of small study effects in clinical trials with isocaloric exchange of dietary pulses for other adequate comparators on LDL-C (7A) and non-HDL-C (7B) in all participants. The solid line represents the pooled effect estimate expressed as the weighted mean difference for each analysis. Dashed lines represent pseudo-95% confidence limits.
CHAPTER V- EFFECT OF A HIGH DIETARY PULSES, LOW GLYCEMIC INDEX DIET ON OXIDATIVE STRESS MARKERS IN TYPE 2 DIABETES
EFFECT OF A HIGH DIETARY PULSES, LOW GLYCEMIC INDEX DIET ON OXIDATIVE STRESS MARKERS IN TYPE 2 DIABETES

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5.1 ABSTRACT

Background: Diets high in vegetable protein such as dietary pulses (beans, chickpeas, lentils, and peas) have been inversely associated with coronary heart disease (CHD). Whether dietary pulses reduce CHD by reducing oxidative stress is unclear. We conducted a secondary analysis of a randomized controlled clinical trial to assess the effect of a diet emphasizing dietary pulses on oxidative damage in type 2 diabetes (T2DM).

Methods and Results: This secondary analysis compared 113 T2DM participants who had serum available and completed either 12 week dietary intervention trial high in dietary pulses, which was used to lower the glycemic index of the diet, or high in wheat fibre diets in a randomized parallel design. Markers of oxidative stress were measured using thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CDs) in the LDL fraction, and protein thiols in serum. There were no treatment differences in oxidative stress markers, although pooling data from both treatments showed a significant inverse correlation between changes in dietary pulse intake with changes in CDs (r=-0.225; p=0.016), suggesting a higher dietary pulse intake is associated with improved oxidative stress status. Estimated glucose excursions, however, were not significantly associated with markers of oxidative damage.

Conclusions: This secondary analysis of a randomized controlled trial did not detect treatment differences in markers of oxidative damage although dietary pulse intake tended to be inversely related to one marker of oxidative stress. Appropriately powered intervention trials of dietary pulses on oxidative damage as the primary outcome are warranted.

Clinical Trial Registration: NCT01063361

Abstract Word Count: 237

Keywords: clinical trial, legumes, dietary pulses, oxidative stress, oxidative damage, cardiovascular disease
5.2 INTRODUCTION

Diets emphasizing vegetable proteins have been shown to reduce coronary heart disease (CHD) mortality risk when substituting for carbohydrates or animal protein in the Iowa Women’s Study\(^2\). Similarly, human dietary intervention trials have shown high plant protein intake that replaced carbohydrate improve lipid levels\(^3\),\(^7\). These findings are suggestive that foods that are high in vegetable proteins may offer cardio-protective effects. However other than soy proteins\(^6\), and a study on wheat gluten\(^3\) and barley\(^7\), few studies have explored other food sources of plant protein, or assessed the mechanism by which vegetable protein may have to reduce cardiovascular risk.

Dietary non-oil-seed pulses (beans, peas, chickpeas, and lentils) are a good source of vegetable protein and they have been shown to reduce CHD risk factors, including LDL-C and systolic blood pressure (SBP)\(^8\),\(^2\). One cup of dietary pulses, on average, can provide 16.3 grams of the 50 grams of daily protein intake as recommended by United States Department of Agriculture (USDA)\(^10\). Similar to soy, dietary pulses contain the 7S globulin peptide which has been shown to increase uptake and degradation of LDL-C in human hepatic cells\(^18\). However, the effect of dietary pulses on ox-LDL-C, an emerging risk factor of CVD, is unclear. Dietary patterns such as Dietary Strategies to Stop Hypertension (DASH)\(^10\), low glycemic index (GI)\(^10\), and Mediterranean\(^1\) diets, which may contain dietary pulses, have been shown to reduce markers of oxidative stress. We have therefore assessed the effect of a low-GI diet specifically emphasizing dietary pulses on oxidative stress markers in a secondary analysis of participant serum from a trial of pulse consumption in type 2 diabetes (T2DM).

5.3 METHODS

This trial has been registered at clinicaltrials.gov (number, NCT01063361). Details of the methods and results of the original study of 121 participants have been reported previously\(^8\).
5.3.1 PARTICIPANTS

Participants were recruited from newspaper and public transport advertisements as well as hospital clinics. A total of 131 eligible participants were randomized (Figure 1). Eligible participants had an existing diagnosis of T2DM for at least 6 months, were taking a stable dose of oral anti-hyperglycemic agents for at least the previous 2 months, and had hemoglobin A1c (HbA1c) values that were between 6.5% to 8.5% of total hemoglobin both at initial screening and at the visit 1 week prior to commencing the study. No participants had clinically significant cardiovascular, renal, or liver disease or a history of cancer. The present analysis focuses on the 113 participants who completed the study, and had serum samples available for analysis for oxidative damage markers at weeks 8, 10, or 12.

5.3.2 PROTOCOL

The study followed a randomized, parallel design with 2 treatment arms of 3 months’ duration consisting of 1) a low-GI diet emphasizing dietary pulse consumption (high dietary pulse diet), and 2) a high wheat fibre diet (high fibre diet). Eligible participants were randomized by a statistician based on sex and HbA1c value (HbA1c value ≤7.1% or >7.1% of total hemoglobin). Neither the dietitians nor the participants were blinded, but equal emphasis was placed on healthiness of both diets.

Participants attended the research centre for screening and at week −1, baseline (week 0), and weeks 2, 4, 8, 10, and 12 of the study. Participants were considered as completers of the study if they made their last visit at week 8, 10, or 12. At each visit, anthropometric outcomes (body weight and waist circumference) were measured and a fasting blood sample was taken. If HbA1c values rose above 8.5% of total hemoglobin on 2 successive occasions, and could not be explained by specific circumstances, participants were withdrawn from the study and referred back to their physician.

The study was approved by the research ethics board of St. Michael’s Hospital and the University of Toronto, Toronto, Ontario, Canada. Written consent was obtained from all participants.
5.3.3 DIETARY INTERVENTION

The emphasis of the treatment diet was to consume low GI foods as achieved primarily through increased consumption of dietary pulses. The target dietary pulse consumption was 1 cup per day (approximately 190 g per day, or 2 servings per day) of cooked beans, chickpeas or lentils. While a high fibre diet was achieved by consumption of whole wheat and whole grain carbohydrate foods (whole wheat breakfast cereals, breads, brown rice, etc). Participants were provided with a checklist of 15-grams carbohydrate portions of recommended foods and the quantities they were expected to consume daily. Seven-day food records covering the week prior to each visit were discussed with the dietitian and assessed for adherence to prescribed dietary interventions.

5.3.4 BIOCHEMICAL ANALYSIS

Fasting HbA1c, glucose, and lipids were measured a fresh sample in the hospital routine clinical laboratory. Measures of oxidative damage were made in batches at the end of the study on serum from fasting blood samples stored at -70°C. Oxidative stress was measured as thiobarbituric acid reactive substances (TBARS), (CV=5.0%) and conjugated dienes (CDs) (CV=3.6%) in the LDL-C fraction by the methods of Jentzsch et al. and Ahotupa et al., respectively. Total protein thiols were also measured on stored serum as a marker of oxidized plasma proteins by the method of Hu et al. (CV=2.3%).

Diets were assessed for macronutrients, fatty acids, cholesterol, fibre and glycemic index using ESHA food processor software (version 10.9.0), a program based on USDA data and international GI tables.

5.3.5 STATISTICAL ANALYSIS

The primary outcome of the original study was HbA1c. The present analysis focused on oxidized products in serum of participants who had sufficient serum at both zero and week 8, 10, or 12 (n=113).

The treatment differences in markers of oxidative stress were assessed using an unpaired 2-tailed Student’s t-test. Pearson correlations and partial correlations were undertaken on pooled data
from both treatment arms to determine the relation of change in oxidized products to measures of study outcomes and change in dietary pulse intake.

We indirectly assessed the impact of postprandial glycemic excursions on oxidative damage by stratifying participants into 4 groups based on their changes in fasting blood glucose (FBG) and HbA1c (See Table 1 for classification of participants). If participants showed no changes in both FBG and HbA1c, then they were excluded from the analysis (n = 2). A 2-sample t-test compared participants in the lowest glucose excursion quadrant with those in the highest glucose excursion quadrant, and with those in the other 3 quadrants combined.

All analyses were carried out using SAS software (version 9.3). Results are expressed as absolute mean changes from baseline with 95% confidence intervals (CI), unless otherwise noted. Significance was set at p< 0.05.

5.4 RESULTS

Fifty-eight individuals (91%) in the high fibre diet and 55 individuals (82%) in the high dietary pulse diet completed the study and provided blood samples for oxidative stress measurements (Figure 1). There were no significant differences between the 2 treatment arms at baseline (Table 2). Dietary variables were not significantly different between treatment groups at baseline. There were, however, significant treatment differences in the dietary variables as change from baseline (Table 3). Notably, the dietary pulse arm had greater changes in dietary pulse, percent energy from dietary protein, vegetable protein, and fibre intake than the fibre diet (p<0.05). There was a greater reduction and lower GI at the end of the study on the dietary pulse than the high-fibre arm. In contrast, the dietary pulse diet had smaller changes in percent energy from saturated fat (SFA) and polyunsaturated fatty acid (PUFA) intakes, although SFA was still lower and PUFA still higher at the end of the study than the fibre diet.

HbA1c was reduced significantly in the treatment compared to control as was SBP. The effects have been repeated previously.

HbA1c was reduced significantly in the treatment compared to control as was SBP. The effects have been repeated previously.

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5.4.1 OXIDATIVE STRESS MARKERS

There was no significant baseline or treatment difference in markers of oxidative stress: TBARS, CDs, and protein thiols (Table 4). However in the pooled analysis, change in dietary pulse intake was inversely correlated with change in CDs (Figure 2 and Table 5).

Pooled data from both treatments showed significant positive associations between changes in TBARS and CDs with changes in LDL-C, TC:HDL-C and TG (Table 5). A positive correlation was also found between change in CDs and change in diastolic blood pressure (DBP) and mean arterial pressure (MAP), while change in protein thiols was positively correlated with heart rate reduction. Partial Pearson correlations adjusting for change in body weight did not change results except for the correlation between change in protein thiols with change in LDL-C, changing from non-significant to significant after adjustment (Table 5).

5.4.2 GLYCEMIC EXCURSION

Both treatment arms were pooled to assess the effects of estimated postprandial glycemic excursions on markers of oxidative stress. Within each quadrant, we did not find significant changes in any of the oxidative stress markers from baseline except for quadrant C where it showed improvements of protein thiol level (p= 0.0014).

We further assessed whether participants with different levels of glycemic excursion would effect levels of oxidative stress markers. Comparing participants with the lowest estimated glycemic excursions (Group D) with those with the highest estimated glycemic excursions (Group A), there were no significant differences on TBARS, CDs, and protein thiols. Nor was there a significant effect on oxidative stress markers when participants with the lowest glycemic excursion was compared to those in all other groups combined (Group D vs. combined Groups A, B, and C).

5.5 DISCUSSION

Our results indicated a diet high in plant protein from dietary pulses (beans, chickpeas, lentils, peas) has no greater reduction on markers of oxidative stress than a high fibre comparator diet in 113
T2DM participants after 12 weeks of intervention. However, an inverse correlation was found between change in dietary pulse intake and change in CDs, suggesting higher intakes of dietary pulses improved levels of CDs. Lower estimated glycemic excursions did not significantly affect levels of oxidative stress markers.

5.5.1 RELATIONS TO OTHER STUDIES

To our knowledge, no randomized trials have assessed the effects of diets high in plant protein on oxidative stress. Most dietary intervention studies have focused on investigating specific food sources of plant protein such as soy\textsuperscript{31, 113}, wheat gluten\textsuperscript{31}, and barley\textsuperscript{71}. In comparison to these results, our analysis of dietary pulses did not show improvements to oxidative damage. However, we did observe a significant inverse relationship between dietary pulse intake and CDs. Although a high dietary pulse diet may not reduce oxidative stress, there is still evidence suggesting a high dietary pulse intake may reduce levels of ox-LDL-C\textsuperscript{32}. A previous weight-loss trial in 30 obese individuals found a diet high in dietary pulses compared to a standard American diet significantly reduced levels of ox-LDL-C without reducing levels of oxidative stress as measured by malondialdehyde (MDA) and 8-isoprostane F2\alpha\textsuperscript{32}. These results suggest dietary pulses may reduce levels of ox-LDL-C by reducing the number of LDL-C particles that are available for oxidation. Indeed, 2 previous meta-analyses have shown that diets emphasizing dietary pulses significantly reduce LDL-C levels compared to control diets\textsuperscript{13, 83}. Furthermore, dietary pulses are high in 7S globulin, a protein that has also been shown to increase uptake and degradation of LDL-C in humans\textsuperscript{18}. Consequently, lower levels of LDL-C available for oxidation after dietary pulse consumption may reduce CVD risk by lowering LDL-C particle size.

We found no effect of estimated glucose excursion on markers of oxidative stress in our pooled analysis of both treatment arms. The lack of an effect is unexpected. Dietary pulses have a low-GI and some are high in protein and fibre, both of which lower postprandial blood glucose, a factor which has been strongly associated with oxidative stress\textsuperscript{109, 114, 115}. Previous randomized dietary trials of low-GI
diets have shown reductions in oxidative stress markers\textsuperscript{109}. It is possible that our study did not find significant changes in oxidative stress markers with glycemic excursion reductions because our measurements were calculated and indirect; we used a combination of changes in FBG and HbA1c levels as a proxy for changes in postprandial glycemia. In addition, the use of a healthy control group that is high in fibre (i.e. a positive control group), may have minimized the opportunity to see treatment differences in our analysis since whole wheat lignans and other phenolics have antioxidant properties\textsuperscript{116}.

5.5.2 LIMITATIONS

There are several limitations to our work. First and most importantly, the present analysis on oxidative stress levels represents a secondary analysis. The trial was designed to provide good power for measures of HbA1c\textsuperscript{8} which may not have had sufficient power to detect significant treatment differences in markers of oxidative damage. Second, we did not adjust for antioxidants found in foods and supplementations. The use of Oxygen Radical Absorbance Capacity (ORAC) values to measure the antioxidant capacity of foods is discouraged by the USDA, citing reasons that the values were tested in vitro and the antioxidant effects cannot be confidently extrapolated to human physiology\textsuperscript{117}. The use of antioxidant supplements was not adjusted for in our analysis because the frequency of use was not different between the 2 treatment arms at baseline and at the end of the trial (data not shown). Third, there are limitations to the methods we used to measure TBARS, CDs, and protein thiols. Each of these oxidative stress markers has its own inherent limitations including measurement and may not be representative of overall oxidative stress levels because they are rapidly degraded in vivo\textsuperscript{110-112}. Further our methods of oxidative stress measurement may not be sensitive enough to measure levels of oxidative stress markers in participants’ serum. For examples, while we reported TBARS values in the range between 0.217- 0.294 \(\mu\)mol/L and measurements can be made between 0.1 to 2.1 \(\mu\)mol/L, measurements of TBARS are most accurate at levels >0.3\(\mu\)mol/L\textsuperscript{110}. Taking together all these issues associated with measuring oxidative stress, we tried to increase the sensitivity of our analysis by using 3
different methods of measurement of oxidative stress. We found no treatment differences on oxidative stress regardless of the methods used. Last, although our original study did not show a significant LDL-C reduction, we still reported a significant non-HDL reduction, suggesting that a high dietary pulse intake may still improve cardiovascular risk by reducing serum atherogenic lipids.

5.6 CONCLUSIONS

A diet high in vegetable protein from dietary pulses (beans, chickpeas, lentils, and peas) of approximately 213 grams per day does not appear to reduce markers of oxidative damage when compared to a high fibre diet in individuals with T2DM. Although our secondary analysis did not show treatment differences in measures of oxidative stress, meta-analyses have reported high dietary pulse intake can still reduce CVD risk by improving levels of LDL-C. As our analysis was secondary in nature and the methods of measuring oxidative stress were relatively insensitive, we cannot rule out a potential oxidative stress benefit of high vegetable protein diets. Therefore further large, long-term, high-quality trials powered to assess the effect of high vegetable protein diets on oxidative stress are needed. To identify research gaps and provide a more precise estimate of the effect of vegetable proteins on these endpoints, our group has undertaken a series of systematic reviews and meta-analyses of randomized trials. Results from this project will guide our understanding of the role of vegetable protein in cardiovascular risk reduction.

5.7 SOURCE OF FUNDING

This work was supported by ABIP through the PURENet and the Saskatchewan Pulse Growers. VH was supported by an Ontario Graduate Scholar (OGS) award. RJD was funded by a Canadian Institutes for Health Research (CIHR) Postdoctoral Fellowship Award. DJAJ was 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, or approval of the manuscript.

5.8 CONFLICTS OF INTEREST/DISCLOSURE

JLS has received several unrestricted travel grants from The Coca-Cola Company to present research at meetings and is a co-investigator on an unrestricted research grant from The Coca-Cola Company. JLS has also received travel funding and honoraria from Abbott Laboratories, Archer Daniels Midland, and the International Life Sciences Institute (ILSI) North America and Brazil; and research support, consultant fees, and travel funding from Pulse Canada. Spouse of JLS works for Unilever Canada. RJD, JB, and CWCK are co-investigators on an unrestricted grant from The Coca-Cola Company. CWCK has served on the scientific advisory board, received research support, travel funding, consultant fees, or honoraria from Pulse Canada, Barilla, Solae, Unilever, Hain Celestial, Loblaws Inc., Oldways Preservation Trust, the Almond Board of California, the International Nut Council, Paramount Farms, the California Strawberry Commission, the Canola and Flax Councils of Canada, and Saskatchewan Pulse Growers. CWCK also receives partial salary funding from research grants provided by Unilever, Loblaw’s, and the Almond Board of California. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg’s, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg’s, Quaker
Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla, Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg’s, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. Dr Jenkins’ wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada.
### 5.9 TABLES

**Table 1. Definitions of quadrant groups for glucose excursion analysis.**

<table>
<thead>
<tr>
<th>Quadrant Group</th>
<th>Definition</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>- no change in FBG and an increase in HbA1c, or - decrease in FBG and no change in HbA1c, or - decrease in FBG and an increase in HbA1c</td>
<td>High postprandial glucose response</td>
</tr>
<tr>
<td>B</td>
<td>- increase in FBG and an increase HbA1c</td>
<td>Intermediate postprandial glucose response</td>
</tr>
<tr>
<td>C</td>
<td>- decrease in FBG and a decrease in HbA1c</td>
<td>Intermediate postprandial glucose response</td>
</tr>
<tr>
<td>D</td>
<td>- no change in FBG and a decrease in HbA1c, or - increase in FBG and no change in HbA1c, or - increase in FBG and a decrease in HbA1c</td>
<td>Low postprandial glucose response</td>
</tr>
</tbody>
</table>
**Table 2. Baseline Characteristics of Participants.**

<table>
<thead>
<tr>
<th>No. of Participants (%)</th>
<th>High Wheat Fibre Diet (n= 58)</th>
<th>Low-Gl Legume Diet (n= 55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>62 (7.7)</td>
<td>58 (10.2)</td>
<td>0.070</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (47)</td>
<td>31 (56)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31 (53)</td>
<td>24 (44)</td>
<td>0.348</td>
</tr>
<tr>
<td>Race/ethnicity European</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>10 (17)</td>
<td>6 (11)</td>
<td>0.422</td>
</tr>
<tr>
<td>East Indian</td>
<td>16 (28)</td>
<td>15 (27)</td>
<td>1.000</td>
</tr>
<tr>
<td>European</td>
<td>16 (28)</td>
<td>13 (24)</td>
<td>0.671</td>
</tr>
<tr>
<td>Far Eastern</td>
<td>4 (7)</td>
<td>6 (11)</td>
<td>0.521</td>
</tr>
<tr>
<td>Other whites/white</td>
<td>5 (9)</td>
<td>10 (18)</td>
<td>0.170</td>
</tr>
<tr>
<td>Others</td>
<td>7 (12)</td>
<td>5 (9)</td>
<td>0.763</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>82.4 (17.0)</td>
<td>87.0 (20.4)</td>
<td>0.194</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>30.0 (5.6)</td>
<td>31.7 (6.8)</td>
<td>0.141</td>
</tr>
<tr>
<td>Current smokers</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>HbA1c value, % of total hemoglobin, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 7.1%</td>
<td>30 (52)</td>
<td>26 (47)</td>
<td>0.708</td>
</tr>
<tr>
<td>&gt;7.1%</td>
<td>28 (48)</td>
<td>29 (53)</td>
<td></td>
</tr>
<tr>
<td>Duration of DM, mean (SD)</td>
<td>8.6 (6.6)</td>
<td>9.3 (6.5)</td>
<td>0.557</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mmol/L</td>
<td>2.4 (1.0)</td>
<td>2.1 (0.7)</td>
<td>0.136</td>
</tr>
<tr>
<td>Non-HDL-C, mean (SD), mmol/L</td>
<td>2.0 (1.0)</td>
<td>1.6 (0.3)</td>
<td>0.016</td>
</tr>
<tr>
<td>TC:HDL-C, mean (SD)</td>
<td>3.5 (0.9)</td>
<td>3.7 (1.0)</td>
<td>0.229</td>
</tr>
<tr>
<td>Triglycerides, mean (SD), mmol/L</td>
<td>1.3 (0.7)</td>
<td>1.6 (1.1)</td>
<td>0.043</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>56 (97)</td>
<td>51 (93)</td>
<td>0.430</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>22 (38)</td>
<td>17 (31)</td>
<td>0.553</td>
</tr>
<tr>
<td>Thiazolodinedione</td>
<td>7 (12)</td>
<td>10 (18)</td>
<td>0.435</td>
</tr>
<tr>
<td>DPP-4 Inhibitor</td>
<td>8 (14)</td>
<td>5 (9)</td>
<td>0.559</td>
</tr>
<tr>
<td>Meglitinides (nonsulfonylurea)</td>
<td>3 (5)</td>
<td>3 (5)</td>
<td>1.000</td>
</tr>
<tr>
<td>α-Glucosidase inhibitor</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>0.235</td>
</tr>
<tr>
<td>Cholesterol Lowering medications</td>
<td>38 (66)</td>
<td>42 (76)</td>
<td>0.222</td>
</tr>
<tr>
<td>Blood pressure medications</td>
<td>35 (60)</td>
<td>41 (75)</td>
<td>0.115</td>
</tr>
</tbody>
</table>
# Table 3. Daily Nutritional Profile at Baseline and End of Study†

<table>
<thead>
<tr>
<th></th>
<th>High-Fibre Diet (n=58)</th>
<th>Low-GI Pulse Diet (n=55)</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy, kcal/d</strong></td>
<td>Baseline: 1605 (1502-1708)</td>
<td>End: 1442 (1340-1545)*</td>
<td>Baseline: 1742 (1638-1845)</td>
</tr>
<tr>
<td></td>
<td>Pulse intake, g/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.4 (32.1-58.8)</td>
<td>14.4 (8.6-20.3)*</td>
<td>55.4 (38.8-72.0)</td>
</tr>
<tr>
<td><strong>Total fat, %E</strong></td>
<td>32.9 (31.4-34.4)</td>
<td>28.5 (26.9-30.0)*</td>
<td>33.1 (31.5-34.7)</td>
</tr>
<tr>
<td><strong>SFA, %</strong></td>
<td>9.4 (8.8-10.0)</td>
<td>8.7 (8.1-9.4)</td>
<td>10.3 (9.6-11)</td>
</tr>
<tr>
<td><strong>MUFA, %E</strong></td>
<td>12.7 (11.8-13.5)</td>
<td>10.5 (9.8-11.3)*</td>
<td>12.7 (11.9-13.5)</td>
</tr>
<tr>
<td><strong>PUFA, %E</strong></td>
<td>7.2 (6.7-7.7)</td>
<td>5.8 (5.3-6.2)*</td>
<td>6.7 (6.2-7.2)</td>
</tr>
<tr>
<td><strong>Diet Cholesterol, mg/1000kcal</strong></td>
<td>142.9 (127.3-158.4)</td>
<td>154.4 (133.8-175.0)</td>
<td>141.0 (126.2-155.8)</td>
</tr>
<tr>
<td><strong>Protein, %E</strong></td>
<td>19.5 (15.2-17.8)</td>
<td>21.2 (20.3-22.2)*</td>
<td>19.8 (19.0-20.6)</td>
</tr>
<tr>
<td><strong>Plant Protein, %E</strong></td>
<td>8.12 (7.59-8.66)</td>
<td>7.60 (7.14-8.06)</td>
<td>7.99 (7.48-8.51)</td>
</tr>
<tr>
<td><strong>Carbohydrates, %E</strong></td>
<td>46.4 (44.6-48.1)</td>
<td>48.6 (46.7-50.5)*</td>
<td>46.0 (46.7-50.5)</td>
</tr>
<tr>
<td><strong>Fibre, g/1000kcal</strong></td>
<td>16.5 (15.2-17.8)</td>
<td>18.4 (17.2-19.6)*</td>
<td>15.9 (14.4-17.4)</td>
</tr>
<tr>
<td><strong>Alcohol, %E</strong></td>
<td>2.5 (1.3-3.7)</td>
<td>3.7 (2.2-5.3)</td>
<td>2.2 (0.8-3.6)</td>
</tr>
<tr>
<td><strong>Glycaemic Index§</strong></td>
<td>78.6 (77.2-80.0)</td>
<td>81.7 (80.5-82.8)*</td>
<td>80.0 (78.4-81.6)</td>
</tr>
<tr>
<td><strong>Glycaemic Load</strong></td>
<td>145.0 (134.7-155.3)</td>
<td>140.9 (131.9-151.0)*</td>
<td>161.8 (148.4-175.3)</td>
</tr>
</tbody>
</table>

Abbreviations: SFA denotes saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid
* Significant difference within treatment by 2-tailed unpaired t test (p<0.05)
† Values are expressed as means with their 95% confidence intervals
‡ P values represent the significance of differences between treatment as changes from baseline by 2-tailed paired t test (p<0.05)
§ Based on the GI bread scale
Table 4. Mean Study Measurements and Significance of Treatment Differences on Oxidative Stress Markers‡.

<table>
<thead>
<tr>
<th></th>
<th>High-Fibre Diet (n=58)</th>
<th></th>
<th>Low-GI Pulse Diet (n=55)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
<td>Mean difference</td>
<td>Baseline</td>
</tr>
<tr>
<td>TBARS (μmol/mmol LDL-C)</td>
<td>0.254 (0.227, 0.282)</td>
<td>0.242 (0.216, 0.268)</td>
<td>-0.012 (-0.030, 0.006)</td>
<td>0.249 (0.219, 0.279)</td>
</tr>
<tr>
<td>CD (μmol/mmol LDL-C)</td>
<td>29.013 (26.205, 31.821)</td>
<td>28.873 (25.827, 31.920)</td>
<td>-0.139 (-1.320, 1.040)</td>
<td>31.074 (27.481, 34.667)</td>
</tr>
<tr>
<td>Protein Thiols (μM)</td>
<td>390.64 (373.59, 407.68)</td>
<td>422.82 (405.67, 438.98)</td>
<td>32.18 (13.00, 51.37)*</td>
<td>396.78 (376.10, 417.46)</td>
</tr>
</tbody>
</table>

Abbreviations: TBARS denote thiobarbituric acid reactive substances; CD conjugated dienes

*Significant difference within treatment by 2-tailed paired t test (p<0.05)

†P values represent the significance of differences between treatment as changes from baseline by 2-tailed paired t test (p<0.05)

‡ Values are expressed as means with their 95% confidence intervals
Table 5. Associations between Changes in Oxidative Stress Markers and Changes in Study Outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlations</th>
<th>Partial Correlations†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>∆TBARS (μmmol)</td>
<td>∆CD (μmmol)</td>
</tr>
<tr>
<td>ΔPulse Intake (grams/day)</td>
<td>-0.018</td>
<td>-0.225*</td>
</tr>
<tr>
<td>ΔSBP (mm Hg)</td>
<td>-0.084</td>
<td>0.099</td>
</tr>
<tr>
<td>ΔDBP (mm Hg)</td>
<td>0.006</td>
<td>0.238*</td>
</tr>
<tr>
<td>ΔHR (beats per min)</td>
<td>0.154</td>
<td>0.135</td>
</tr>
<tr>
<td>ΔWC (cm)</td>
<td>0.084</td>
<td>0.031</td>
</tr>
<tr>
<td>ΔFBG (mmol/L)</td>
<td>0.017</td>
<td>0.062</td>
</tr>
<tr>
<td>ΔHbA1c (%)</td>
<td>-0.012</td>
<td>0.035</td>
</tr>
<tr>
<td>ΔLDL-C (mmol/L)</td>
<td>0.250*</td>
<td>0.297*</td>
</tr>
<tr>
<td>ΔTC:HDL-C</td>
<td>0.253*</td>
<td>0.307*</td>
</tr>
<tr>
<td>ΔTG (mmol/L)</td>
<td>0.306*</td>
<td>0.400*</td>
</tr>
<tr>
<td>ΔTBARS (μmmol)</td>
<td>-</td>
<td>0.109</td>
</tr>
<tr>
<td>ΔCD (μmmol)</td>
<td>0.109</td>
<td>-</td>
</tr>
<tr>
<td>ΔThiols (μM)</td>
<td>0.027</td>
<td>-0.021</td>
</tr>
</tbody>
</table>

*Significance by Pearson and Partial correlations (p<0.05)
†Partial Correlations adjusted for change in body weight
5.10 FIGURES

Figure 1. Flowchart of Participants through the Trial.
Figure 2. Correlation of Change in Pulse intake (ΔPulse Intake) and Change in Conjugated Dienes (ΔCDs). Analysis was conducted with Pearson’s Correlation (p=0.016).
6.1 OVERALL DISCUSSION

Two studies have been undertaken to assess the effects of dietary pulses (beans, chickpeas, lentils, and peas) on oxidative stress and lipid measures associated with cardiovascular risk. Our systematic review and meta-analysis found that high dietary pulse consumption compared to control diets reduced LDL-C by 0.17 mmol/L or 5% from baseline, but found no significant effects on secondary lipid targets of CVD risk including Apo-B and non-HDL-C. Moreover, although our intervention trial, which emphasized dietary pulse intake as a means to lower glycemic index, did not significantly reduce oxidative stress markers compared to a high fibre control diet, an inverse correlation was observed between dietary pulse intake and one of the oxidative stress markers. Taken together, our results suggest that a diet high in dietary pulses may improve cardiovascular risk through lowering levels of LDL-C.

Our results support previous findings from other studies. Our systematic review and meta-analysis of 26 trials found dietary pulse intervention compared to control diets reduced LDL-C by 0.17 mmol/L. These results are in agreement with 2 previous meta-analyses assessing dietary pulse intakes which have also reported significant LDL-C reductions\textsuperscript{13}, \textsuperscript{83}. Similarly, our results for oxidative stress markers are congruent with previous findings. A previous human feeding trial of 30 obese participants on a high dietary pulse diet compared to a typical American diet had also found no differences in oxidative damage using measurements of malendialdehyde (MDA) and isoprostane-F2\alpha. Despite these findings, the investigators still reported significant reductions of serum ox-LDL-C\textsuperscript{32}. Our feeding trial suggests that high dietary pulse intake may not directly reduce levels of oxidative stress, however, they may still reduce levels of ox-LDL-C by lowering the number of LDL-C particles available for oxidation as suggested by our meta-analysis. Consequently, lower levels of LDL-C available for oxidation after dietary pulse consumption may reduce CVD risk by lowering LDL-C particle size.
Several hypotheses have been generated in understanding the mechanism of action with respect to the lipid-lowering effects of dietary pulses. Dietary pulses are high in fibre and have a low-glycemic index, both of which are associated with reduced lipid levels and CHD risk\textsuperscript{15,118}. Possibly, more important is that dietary pulses and common to all legumes including soy and peanuts, is that they are all high in a protein called 7S globulin. This protein fraction has been considered responsible for the hypolipidemic effect of soy proteins\textsuperscript{18}. Prospective cohort studies have shown high intakes of legumes (dietary pulse and soy/peanuts) are associated with reduced risk of CHD and stroke\textsuperscript{80,81} but prospective cohort study has been conducted to date to assess the association of dietary pulse intake on cardiovascular events. More studies are needed to understand whether higher pulse intake can lower the risk of CVD hard endpoints such as myocardial infarctions, stroke, and CVD-related mortality.

6.2 LIMITATIONS

6.2.1 SYSTEMATIC REVIEW AND META-ANALYSIS

Limitations of our systematic review and meta-analysis include: 1) the majority of studies were of low quality (MQS<8), short duration (<3 months), and did not report enough data to judge risk of bias; 2) the majority the non-HDL-C values had to be calculated and SDs imputed. 3) only one trial provided Apo-B data. These observations reinforce the need for longer, better-designed clinical trials that report data more systematically and transparently to better understand the effect of dietary pulses on serum lipids as they are important biomarkers of cardiovascular health.

6.2.2 DIETARY INTERVENTION TRIAL

Limitations of our dietary intervention trial include: 1) the analysis on oxidative stress levels represents a secondary analysis which was not sufficiently powered to detect significant treatment differences in markers of oxidative damage; 2) adjustments for antioxidants found in foods and supplementations were not possible. The use of Oxygen Radical Absorbance Capacity (ORAC) values to measure the antioxidant capacity of foods is discouraged by the USDA, citing reasons that the values
were tested in vitro and the antioxidant effects cannot be confidently extrapolated to human physiology\textsuperscript{117}. The antioxidant supplements was not adjusted for in our analysis because the frequency of use was not different between the 2 treatment arms at baseline and at the end of the trial (data not shown); 3. we did not directly measure levels of ox-LDL-C but used markers of oxidative stress (TBARS, conjugated dienes, and protein thiols) as surrogates. As these markers are rapidly degraded \textit{in vivo}, they may not be representative of the level of ox-LDL-C in circulation. There is a need for future trials to improve the existing evidence on the effects of dietary pulses on ox-LDL-C.

\textbf{6.3 MECHANISM OF ACTION}

The mechanism by which dietary pulses lower cholesterol levels is unclear; however, the effect can be broadly divided into 2 pathways: extrinsic and intrinsic. The extrinsic pathway proposes that dietary pulses reduce serum cholesterol by displacing saturated and trans fat intake\textsuperscript{57, 82}. High saturated and trans fat intake has been associated with animal protein intake which has also been associated with increased cardiovascular risk\textsuperscript{119}. Possibly more importantly, dietary pulses are high in 7S globulins, a peptide which has been shown to increase uptake and degradation of LDL-C in human hepatic cells, and is also hypothesized to confer the hypocholesterolemic effects of soybeans\textsuperscript{18}. Dietary pulses are also high in soluble fibre which is known to bind to dietary cholesterol in the intestine and prevent its absorption\textsuperscript{85, 86}. Of the intrinsic pathway proposes that in addition to binding to dietary cholesterol, the viscous soluble fibre in dietary pulses can bind to bile acids to prevent its re-absorption. Consequently, there is an increase in the production of bile acids by the liver, which decreases the liver pool of cholesterol and increases uptake of serum cholesterol by the liver thereby decreasing circulating cholesterol in the blood\textsuperscript{22}. Further fermentation of fibre in the colon produces short chain fatty acids (SCFA) which have been associated with inhibition of cholesterol synthesis\textsuperscript{19, 23}.
6.4 CLINICAL IMPLICATIONS

Dietary patterns that emphasize dietary pulses can lower the risk for CVD. Although we did not show a significant difference on oxidative stress marker with higher dietary pulse intake, our systematic review and meta-analysis found a significant LDL-C reduction in diets that emphasize dietary pulse intake. Our data also reinforce the evidence from and support existing recommendations for heart healthy dietary patterns of which pulses are a key component: Portfolio, DASH, and Mediterranean. The median dietary pulse intake in our meta-analyses was of 130g/day (~1.5 servings/day), a higher intake target than that proposed by the AHA of ≥4-5 servings/week. Achieving a high level of intake may prove a challenge in some Western countries, given that current U.S. level of intake is 0.2 serving/day. However, a dietary pulse intake of ~1.5 servings/day is very reasonable as this level is currently consumed by many cultures without reports of side effects that would limit consumption. As the majority of these trials were conducted on a background heart-healthy NCEP-like diet, including >20-25g/d of fibre and <10%E of saturated fat, these results can be considered in addition to the 5-10% LDL-C lowering expected from these diets alone. Whereas guidelines have largely focused on dietary pulses as part of a healthy dietary pattern in the prevention and management of diabetes and CVD, these data support lipid-lowering and cardiovascular risk reduction recommendations.

6.5 FUTURE DIRECTIONS

Future research is needed. The reducing effects of dietary pulses on LDL-C has been shown in our and 2 previous meta-analyses; therefore future analyses should focus on assessing the effects of dietary pulses on other established lipid endpoints. More importantly, human feeding trials measuring Apo-B is needed. Our analysis had only identified one trial that met the inclusion criteria; therefore it is likely we were under-powered to accurately detect the effects of dietary pulses on Apo-B. Secondly, there is an urgent need for further large, long-term, high-quality trials to assess the effect of high vegetable protein diets on oxidative stress and other major cardiovascular risk factors including body...
weight, glycemic control, lipids, and blood pressure. To identify research gaps and provide a more precise estimate of the effect of vegetable proteins on these endpoints, our group has undertaken a series of systematic reviews and meta-analyses of randomized trials. Results from this project will guide our understanding of the role of vegetable protein in cardiovascular risk reduction. Lastly, although dietary intervention trials have shown favourable effects of dietary pulses on biomarkers of CVD risk, no prospective cohort studies have assessed specifically the association of dietary pulses on hard endpoints of CVD. Most observational studies have reported high legume intakes which include dietary pulses and soy or peanuts, have favourable effects on CVD risk13, 83, but none have assessed legume intake independent of soy or peanuts. More studies are needed to understand the effects of dietary pulses on CHD or stroke risk. Lastly, the role of ox-LDL-C in human cardiovascular health is uncertain. Although animal and cell culture studies have supported the hypothesis of ox-LDL-C and CVD risk35, large human clinical trials supplementing dietary patterns with anti-oxidants have reported no improvements on incidence of CHD and stroke120, 121. Consequently, no major dyslipidemic guidelines including NCEP-ATP III and Canadian Cardiovascular Society (CCS) have recognized and set target goals for serum ox-LDL-C or have made recommendations for anti-oxidant supplements for reducing cardiovascular risk3, 4. The role of ox-LDL-C needs to be more directly assessed in relation to cardiovascular outcomes.
CHAPTER VII- CONCLUSIONS
7.1 CONCLUSIONS
Dietary patterns that emphasize dietary pulses (beans, chickpeas, lentils, and peas) improve cardiovascular risk by reducing LDL-C levels but not oxidative stress.

This thesis work demonstrated the following:

1. In a systematic review and meta-analysis of 26 randomized trials (n= 1013), diets emphasizing dietary pulses significantly lowered LDL-C compared with isocaloric control diets (mean difference= -0.17 mmol/L [95% CI: -0.25, -0.09]; p<0.0001). No treatment effects were observed for Apo-B and non-HDL-C.

2. In a secondary endpoint analysis of a feeding trial in 113 participants, there were no significant differences between a high dietary pulse diet as a means to lower the glycemic index to a high fibre comparator diet on markers of oxidative stress including thiobarbituric acid reactive substances (TBARS), conjugated dienes (CDs), and protein thiols.
CHAPTER VIII- REFERENCES
8.1 REFERENCES


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