University of Alberta

Feeding High Oleic Acid Canola Oil or Olive Oil Alters Inflammation, Insulin Resistance and Lipid Metabolism in a Rodent Model of Diet Induced Obesity

by

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Abstract

It is recommended that the majority of dietary fat be from monounsaturated fatty acids (MUFA) as their consumption has been shown to improve risk factors involved in the development of type 2 diabetes (T2D). Studies of the health benefits of MUFA have been performed almost exclusively using olive oil. The effects of consuming different sources of MUFA on immunological and metabolic health are unknown. A new cultivar of Canola is available that contains the same proportion of oleic acid as olive oil, however the positional distribution of fatty acids on triglyceride molecules likely differ. The aim of this study was to determine the efficacy of feeding MUFA using Canola oil on risk factors for T2D in an animal model of diet induced obesity (DIO). To determine this, DIO rats were fed either a low fat diet (LF; 12% w/w fat, 12% MUFA) high fat diet low in MUFA (HF; 20% w/w, 12% MUFA), a high fat diet containing olive oil (OO; 20% w/w, 30% MUFA), or a high fat diet containing high oleic acid Canola oil (HCO; 20 w/w, 30% MUFA) and the effect on immune function, insulin sensitivity and the fatty acid composition of structural and stored lipids were measured. Feeding LF led to greater inflammatory cytokine production and plasma glucose concentration following an OGTT with changes in phospholipid fatty acid composition compared to HF ($p<0.05$). Increasing MUFA in the diet resulted in a lower production of the inflammatory cytokines IL-6 and TNF-α by stimulated splenocytes ($p<0.05$); however OO resulted in a lower TNF-α production compared to HCO. Feeding OO resulted in a higher proportion of T-helper cells (CD4+) expressing activation markers (CD152, CD71) compared to feeding HF or HCO ($p<0.05$). Feeding different sources of MUFA did not alter glucose metabolism; however, increasing the amount of MUFA in the diet from Canola oil resulted in a significantly greater plasma insulin and glucose concentration compared to rodents fed the HF diet ($p<0.05$). Feeding different sources of MUFA did not affect the

fatty acid composition in the sn-2 position of triglycerides in the liver or perirenal adipose tissue. However, in spleen phospholipids, feeding OO led to significantly greater proportions of oleic acid and linoleic acid, compared to those fed HCO. Feeding HF resulted in a higher proportion of linoleic acid and lower oleic acid in spleen phospholipids compared to high MUFA diets. This suggests that both the source of MUFA (triglyceride species) as well as the overall content of MUFA and fat in the diet alters lipid metabolism and thereby could alter other physiological processes. In summary, feeding high MUFA diets lowered the pro-inflammatory response compared to a low MUFA diet; however, different sources of MUFA are not equivalent. Feeding MUFA from OO was more effective in dampening a pro-inflammatory response compared to HCO, despite having the same fatty acid composition. Future research will determine if this is due to changes in the fatty acid composition of phospholipids and triglycerides.

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Abbreviations

AA	Arachidonic acid	HDL	High density lipoprotein
APC	Antigen presenting cell	HF	High fat
AUC	Area under the curve	Ig	Immunoglobulin
BMI	Body mass index	IL	Interleukin
BSA	Bovine serum albumin	KRH	Krebs-Ringer-Hepes
CCM	Complete culture medium	LDL	Low density lipoprotein
CD	Cluster differentiation	LF	Low fat
CHO	Carbohydrate	LPS	Lipopolysaccharide
CLA	Conjugated linoleic acid	MAG	Monoacylglyceride
Con A	Concanavalin A	MHC	Major histocompatibility complex
CRP	C-Reactive protein	MLN	Mesenteric lymph node
CTLA4	Cytotoxic T lymphocyte antigen 4	MUFA	Monounsaturated fatty acid
DHA	Docoahexanoic acid	NK	Natural killer cell
DIO	Diet induced obesity	OGTT	Oral glucose tolerance test
EPA	Eicosapentanoic acid	OO	Olive oil
FAME	Fatty acid methyl esters	PAMP	Pathogen associated molecular patterns
FCS	Fetal calf serum	PANDA	Physical Activity and Nutrition for Diabetes in Alberta
GALT	Gut associated lymph tissue	PBMC	Peripheral blood mononuclear cell
GC	Gas chromatography	PBS	Phosphate buffered saline
GLP-1	Glucoagon like peptide-1	PC	Phosphtidylcholine
HCO	High oleic acid Canola oil	PKC	Protein kinase C

P/S	PUFA/SFA ratio	TLC	Thin layer chromatography
PUFA	Polyunsaturated fatty acid	T2D	Type 2 diabetes
SFA	Saturated fatty acid	TAG	Triacylglyceride
SF	Saturated fat	TG	Triglyceride
Sn	Stereospecific numbering	Treg	T-regulatory cell
Th	T-helper cell	VLDL	Very low density lipoprotein

Chapter 1: Introduction

Type 2 Diabetes

Prevalence and Incidence in Canada and Alberta

Diabetes prevalence continues to increase in Alberta, with similar trends for the rest of Canada, the US and even globally (Wild et al., 2004; Mokdad et al., 2001; Lipscombe and Hux, 2007). From 2005 to 2006, 1 in 17 Canadians, or 1.9 million in total were diagnosed with diabetes and the numbers are expected to rise. It was estimated that by 2012 2.8 million Canadians would be diagnosed with diabetes (Public Health Agency of Canada, 2009). In 2006-07 211, 168 new diabetes cases were diagnosed in individuals greater than one year old in Canada, with an incidence rate of 8.7 in 1000 for adults alone (Public Health Agency of Canada, 2009). In 2007, the number of Albertans with diabetes doubled from 1997. There were 15, 802 new cases of diabetes diagnosed in Alberta in 2007. Also of concern is the increasing diagnosis of type 2 diabetes (T2D) in both young adults and the population under 20 years of age. This has occurred in conjunction with the current obesity epidemic in both children and adults. From 1995 to 2007 the number of cases of individuals with diabetes under the age of 20 rose from 1466 to 2589 (Johnson et al., 2009a) and the incidence increased 144% during this time.

The cost of increasing diabetes prevalence will also cause additional economic burden in Alberta and Canada. It was reported that adults with diabetes had 70% more visits to a general practitioner, more specialist appointments, and more days spent in hospital compared to those without diabetes (Johnson et al., 2009b). This adds up to increased health care costs and a greater number of lost days of productivity. In 2009 the cost of diabetes accounted for 3.5% of public health care spending in Canada and this number is expected to rise as the population ages and obesity rates continue to increase (Canadian Diabetes Association, 2009). It was predicted that the economic burden of diabetes in Canada in 2010 would be approximately $12.2 billion dollars (Canadian Diabetes Association, 2009). The constant increase in diabetes prevalence and its high risk for developing complications will further tax the healthcare system and economy.

Complications of Type 2 Diabetes

Diabetes is a condition where insulin is either not secreted in sufficient amounts or cells do not respond appropriately to insulin (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). This leads to insulin resistance and hyperglycemia, conditions with severe physiological consequences if left unmanaged. Failure to manage diabetes by maintaining plasma glucose concentrations in the normal range can lead to serious complications. Overall mortality risk in individuals with diabetes is double the risk of mortality in the general population (Public Health Agency of Canada, 2009).

Macrovascular Complications

Individuals diagnosed with T2D are at greater risk for developing macrovascular complications including heart problems, hypertension, atherosclerosis, myocardial infarction, and stroke (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008; Public Health Agency of Canada, 2009). Cardiovascular disease risk is 2-4 times higher in those diagnosed with diabetes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008), accounting for 2/3 of deaths in people with diabetes. Poor glycemic control is associated with higher risk of developing macrovascular complications. A prospective cohort study of diabetes and the risk of developing related complications in the UK found that incidence of any endpoint, including incidence of macrovascular complications and mortality, increased with increasing HbA1C concentrations (Stratton et al., 2000). This association remained significant following adjustment for age, sex, ethnic group, systolic blood pressure, serum lipids, and smoking (Stratton et al., 2000). A 1% decrease in HbA1C was associated with a 21% decrease in risk for any diabetes related complication or death (Stratton et al., 2000). This provided strong evidence that plasma glucose control is necessary for the prevention of many of the complications of T2D.

Microvascular

Individuals with T2D are also at increased risk of microvascular complications, especially when plasma glucose concentration is poorly controlled. This includes kidney, eye, and nerve damage which can have adverse outcomes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008; Public Health Agency of Canada, 2009). Diabetic retinopathy is the cause of approximately 10,000 cases of blindness in the US and is the leading cause of vision loss in Canada (Fowler, 2008; Public Health Agency of

Canada, 2009). The risk of developing retinopathy greatly increases with hyperglycemia and hypertension (Fowler, 2008). It is suggested that hyperglycemia increases the formation of advanced glycosylate end products as well as exposure to oxidative stress, causing nerve damage to the eye (Fowler, 2008). Diabetes is also the leading cause of end stage renal failure in Canada (Public Health Agency of Canada, 2009). Like retinopathy, renal failure is associated with higher HbA1C and high blood pressure. Peripheral nerve damage due to neuropathy is another microvascular complication commonly encountered by people with T2D causing pain, numbness or sensory loss (Fowler, 2008). Some cases of neuropathy lead to amputation. Diabetes patients are at higher risk of developing foot ulcers due to neuropathy and vascular disease (Ramsey et al., 1999; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). Foot issues associated with diabetes is of major concern and special care is outlined in the Canadian Diabetes Association 2008 Clinical Practice Guidelines. Even minor trauma can lead to food ulcers, infections and amputation (Ramsey et al., 1999). Microvascular complications are associated with uncontrolled plasma glucose and managing plasma glucose at optimal concentrations is the best prevention of these complications (Fowler, 2008; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008).

Other

Another common diagnosis associated with diabetes is depression. Depression is diagnosed in diabetic patients more than in the general population and it has been reported that 15-20% of individuals with diabetes are diagnosed with depressive disorders compared to 2-9% in the non-diabetic population (Anderson et al., 2001). Like other complications of diabetes, this is associated with poorer self-care and glycemic control, as well as other complications of diabetes, lower quality of life, and increased health care costs (Anderson et al., 2001; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). Using a standardized scale to measure quality of life based on physical and mental outcomes, people with diabetes reported lower quality of life scores compared to a non-diabetic population while those with both diagnosed diabetes and depression had much lower scores compared to individuals with diabetes alone or individuals with no diagnosed diabetes (Goldney et al., 2004). Depression makes it more difficult for patients to gain self-efficacy in adequate self-care, such as maintaining a healthy diet and is associated with poorer glycemic control (Goldney et al., 2004).

Due to the complications discussed caused by consistently high blood glucose, individuals with T2D have a greater risk of morbidity and mortality than non-diabetics. It is clear that poorly controlled diabetes can significantly lower quality of life. An important outcome discussed in the 2008 Clinical Practice Guidelines for diabetes self-care is improved quality of life (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008; Goldney et al., 2004). Development and progression of complications associated with T2D increases time spent in the hospital, which may lower quality of life of the individual, and increases health care costs affecting both the individual and overall economy. Nutrition therapy is a key component of diabetes management and targets plasma glucose concentrations, blood pressure, dyslipidemia and weight loss, all important factors in preventing or delaying the onset of complications.

Risk Factors for Developing Type 2 Diabetes

The risk for developing T2D includes a combination of environmental and behavioural exposures coupled with a genetic predisposition (Tuomilehto et al., 2001). Some risk factors for developing T2D are increased fasting plasma glucose concentration, body mass index (BMI) in the overweight or obese range, inactivity, and family history of diabetes (Charles et al., 1991; Tuomilehto et al., 2001). Many of the risk factors associated with diabetes are potentially modifiable through lifestyle interventions involving both diet and physical activity. Weight loss and glycemic control through nutrition therapy is one of the main targets in the primary prevention of diabetes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). Studies involving lifestyle intervention trials have demonstrated that appropriate intervention involving diet and exercise can decrease the risk for developing diabetes significantly and can be effective in plasma glucose control (Knowler, 2002; Tuomilehto et al., 2001; Toobert et al., 2003). Factors that alter risk for developing diabetes include diet, weight, blood glucose, blood pressure, cholesterol and physical activity levels (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). In overweight or obese individuals, which accounts for 80 to 90% of T2D patients, weight loss of 5 to 10% initial body weight has been shown to improve risk factors for

developing diabetes itself as well as complications associated with diabetes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008).

Lifestyle Interventions and Type 2 Diabetes

The Diabetes Prevention Program was a randomized clinical trial comparing the effects of metformin with standard lifestyle advice, a placebo with standard lifestyle advice, and an intensive lifestyle intervention without drug therapy (Knowler, 2002). The standard lifestyle advice included written information on diet and exercise and an annual individual session focusing on a healthy lifestyle. The intensive lifestyle intervention included a weight loss goal of 7% initial body weight and weekly physical activity goals with group lessons on how to achieve this as well as individual sessions (Knowler, 2002). Participants were at high risk for developing T2D because of high fasting plasma glucose concentration, poor glucose tolerance and a BMI>24. With a 2.8 year average follow up, individuals who received the intensive lifestyle intervention had a 58% lower incidence for diabetes than the placebo group, while the metformin group had a 31% decreased incidence rate. The intensive intervention group, which was advised on a low calorie and low fat diet, were able to decrease daily caloric intake and weight significantly more than the other groups (Knowler, 2002). Similar results have been found in the Finnish Diabetes Prevention Program, with an intensive lifestyle intervention significantly lowering risk for developing T2D (Tuomilehto et al., 2001; Lindstrom et al., 2003; Lindstrom et al., 2006). A lifestyle intervention including detailed and individually tailored information about diet and exercise, goal setting, and regular visits with a nutritionist was effective in lowering risk for diabetes in overweight individuals with glucose intolerance (Tuomilehto et al., 2001). The fat recommendation for the intervention group was that 20% of total energy intake come from monounsaturated (MUFA) and polyunsaturated (PUFA) fat, and that saturated fat (SF) consist of less than 10% total energy intake (Uusitupa et al., 2000). Once weight goals were met, participants could increase fat to 25% energy if the excess was consumed as MUFA (Uusitupa et al., 2000). The control group received only general oral and written information about diet and exercise. After a mean follow up of 3.2 years, diabetes incidence was significantly lower in the intervention group (Tuomilehto et al., 2001). The intervention group also lost more weight, and had improved HDL cholesterol, triglycerides (TG) and blood pressure (Tuomilehto et al., 2001).

Positive results have also been observed in a lifestyle intervention based on the Mediterranean diet in post-menopausal women (Toobert et al., 2003). For this 6 month intervention trial women were instructed on a Mediterranean style diet by a registered dietitian. The diet was high in alpha-linolenic acid and MUFA and low in SF (Toobert et al., 2003). As olive oil was found to be unacceptable to participants Canola oil was used as a MUFA source. The oil supplied to participants contained 48% oleic acid, 14% saturated fat and some linoleic and linolenic acid (de Lorgeril and Renaud, 1994). The Mediterranean diet group was asked to replace butter and cream in their diet with olive or Canola oil fat sources (Toobert et al., 2007). Participants also received social support through group meetings as well as lessons for stress management (Toobert et al., 2003). The Mediterranean lifestyle group was compared to a group receiving usual care. Following the 6 month period participants in the intervention group had lower HbA1c, BMI and body weight compared to the usual care group and also had improved measures of quality of life (Toobert et al., 2003). Participants in the Mediterranean diet intervention lowered their total fat and saturated fat intake more than the group receiving usual care (Toobert et al., 2007). Intervention trials such as these with the Mediterranean diet have showcased the ability of nutrition and lifestyle interventions to lower risk for developing diabetes and improve diabetes outcomes when specifically tailored to the individual or target population.

Nutrition Therapy

The Canadian Diabetes Association 2008 Clinical Practice Guidelines recommends that individuals with T2D follow Eating Well with Canada's Food Guide and further breaks down nutrition goals by macronutrient as described in Table 1.

Table 1. Canadian Diabetes Association macronutrient recommendations (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008)

Nutrient	**Recommendation**
Carbohydrates	Consume 45-65% of energy as carbohydrates Carbohydrate choices should have a low glycemic index value Fiber intake should be 25 to 30 g/d. This can be attained by choosing more fruits and vegetables and replacing refined grains with whole grains (bread, pasta, rice etc.)
Protein	Consume 15-20% of energy as protein
Fat	Consume less than 35% energy as fat Saturated fat should not exceed 7% daily energy intake and trans fats intake from hydrogenated sources should be as little as possible Polyunsaturated fat intake should not exceed 10% daily energy intake, n-3 PUFA sources should be included Most dietary fat should be monounsaturated fat and saturated fat should be replaced with a MUFA source when possible

It has recently been observed that higher fat, high MUFA diets, providing energy from fat at the higher end of the recommendation, may have beneficial outcomes for diabetes management. This is discussed in a later section.

Summary

It is clear that diabetes prevention is important for both individuals and families affected by the disease as well as the economy. Lifestyle interventions are effective in improving the risk factors for developing diabetes and lowering the incidence of diagnosis. A

nutrient that may be effective in helping manage diabetes and the risk factors associated with the disease when incorporated into nutrition therapy is MUFA. The 2008 Canadian Diabetes Association clinical practice guidelines recommend that less than 35% of total energy come from fat. This recommendation is further broken down to consume less than 7% energy from SF and less than 10% PUFA. Most fat in the diet should be MUFA, while SF should be limited as much as possible.

Overview of the Immune System

The primary role of the immune system is to provide protection against infection by bacteria, fungi and parasites. In order to do this, immune cells distinguish self proteins from invading non-self proteins (reviewed by (Sakaguchi et al., 2008)). Immune cells also recognize transformed body cells, such as tumour cells and those with autoimmune tendencies and they are removed. Inappropriate immune responses sometimes occur, leading to allergy or autoimmunity (reviewed by (Sakaguchi et al., 2008)). Autoimmunity occurs when the immune system fails to distinguish self from non-self, and a response can occur either against a specific organ, for example beta cells of the pancreas in type I diabetes, or can occur systemically, such as in rheumatoid arthritis (Sinha et al., 1990). However, under normal conditions the immune system, made up of both innate and acquired components, functions to prevent and resolve infection.

Innate Immune System

The innate immune system provides protection against infection through the recognition of general pathogen markers. The first line defence mechanism of the immune system is external physical barriers including skin and mucus to prevent pathogens from entering the body and adhering to cells. Once a pathogen has entered the body, cells of the innate immune system provide a rapid response (Moser and Leo, 2010). The innate immune system is active at birth as it is non-specific and it does not require previous priming to antigens. Cells of the innate immune system include phagocytic cells such as macrophages, neutrophils, dendritic cells and some other cell types such as natural killer (NK) cells and mast cells (Moser and Leo, 2010). These cells can express co-stimulatory

molecules that communicate with cells of the adaptive immune system through antigen presentation (Janeway and Medzhitov, 2002; Fearon and Locksley, 1996).

The innate immune system includes cells that can recognize distinct but general characteristics of pathogens, known as pathogen associated molecular patterns (PAMP). One example of a PAMP is lipopolysaccharide (LPS), a molecule that is common between gram negative bacteria (Aderem and Ulevitch, 2000). Cells of the innate immune system have pattern recognition receptors (PRR) to recognize PAMPs. An example of PRRs is the toll-like receptor (TLR) family. TLR4 is the receptor expressed by macrophages and recognizes LPS (Janeway and Medzhitov, 2002). Activated TLRs stimulate the production of inflammatory cytokines such as IL-1 and IL-6, and increase the expression of major histocompatibility complex (MHC) which is involved in communicating with and activating cells of the acquired immune system (Janeway and Medzhitov, 2002). Dendritic cells are key antigen presenting cells (APC) that reside in non-lymph tissue but relocate to lymph tissue in response to inflammation (Fearon and Locksely, 1996). They express MHC, the receptor that binds antigen to present to the T-cell receptor, as well as B7, a molecule that binds CD28 on T-cells and provides the co-stimulatory signal required for T-cell activation (Lohr et al., 2003; Parker et al., 1993). Macrophages also express MHC and act as APCs. These cells are also able to act as phagocytic cells and engulf and destroy the pathogen by exposing it to reactive oxygen species and enzymes (Moser and Leo, 2010). Activated innate immune cells also produce chemokines to attract other cells to the site of infection, as well as cytokines, like tumor necrosis factor (TNF) and interleukins (IL), to aid in the destruction of the infecting pathogen (Moser and Leo, 2010).

Acquired Immune System

The adaptive immune system differs from the innate immune system in that it recognizes highly specific antigens and as such has a relatively slower response (Moser and Leo, 2010). T and B lymphocytes, or T and B cells, each have specific roles that are responsible for the adaptive immune response.

B-Cells

B-cells, the lymphocytes responsible for a humoral immune response, recognize antigens in circulation (Moser and Leo, 2010). They express and make immunoglobulins that specifically recognize one pathogen. When a B-cell binds an antigen for its given antibody or immunoglobulin it divides into plasma cells, which continue to make antibodies to bind the infecting pathogen, as well as memory cells which remain in the system after the infection is resolved to provide a faster response if the same pathogen is encountered again (Moser and Leo, 2010). B-cells express and produce IgM prior to activation by innate immune cells, such as dendritic cells, or helper T-cells (Hodgkin et al., 1990). T-cell dependant B-cell activation occurs by contact with a helper T-cell and exposure to cytokines produced by the T-cell, which induces an immunoglobulin isotype switch to produce IgG, IgA, or IgE (Wykes et al., 1998). These cells have a high affinity for antigens, however this response can take several days to develop (Cerutti et al., 2011). T-cell independent activation of B-cells by dendritic cells induces immunoglobulin isotype switches quickly; however, these immunoglobulins have a lower affinity for antigen relative to the T-cell dependant response (Cerutti et al., 2011).

T-Cells

Two major classes of T-lymphocytes, cytotoxic and helper T-cells, provide the cell mediated immune response. When a pathogen is intracellular, the infected cell displays MHC proteins that can be recognized by the T-cell receptor. Cytotoxic T-cells (CD3+CD8+) recognize MHC class I, which is expressed by nucleated body cells (Moser and Leo, 2010). When cytotoxic T-cells are bound and activated they secrete proteins that increase proteases present in the cell cytoplasm, leading to apoptosis (Moser and Leo, 2010). They also secrete cytokines to inhibit replication and aid in the destruction of the threatening pathogen (Moser and Leo, 2010). Helper T-cells (CD3+CD4+) recognize MHC class II, a protein expressed by antigen presenting cells of the immune system (Moser and Leo, 2010). Different classes of helper T-cells develop in response to an APC depending on cytokines, such as IL-12 and IL-4, and other soluble factors secreted by the APC (de Jong et al., 2002). Th1 cells are involved in the cell-mediated response and often have cytotoxic effects with the secretion of pro-inflammatory cytokines such as IL-2 and IFN-γ (Frostegard et al., 1999; Ferrick et al., 1995). Th2 cells often develop in response to a pathogen in blood and are involved in the activation of B-cells by providing a signal through contact, as well as by the production of cytokines (Ferrick et al., 1995;

Parker, 1993). T-helper cells also have a CD40 ligand, which interacts with B-cell CD40 to provide signals for activations (Parker, 1993). IL-4 is a cytokine that mediates the development of a Th2 response and down-regulates the immune response (Gagliardi et al., 2000). Both Th1 and Th2 cells are required for optimal immune function; however, imbalances are often involved in chronic conditions. In atherosclerotic plaques from humans, IL-2 and IFN-γ are found in the majority of plaques, with very little IL-4, suggesting the predominance of a Th1 response is involved in the development of atherosclerosis (Frostegard et al., 1999). In allergic response, a predominantly Th2 response is observed (Ngoc et al., 2005). Naive T-cells require antigen binding and a co-stimulatory molecule in order to be stimulated to mature and active T-cells (Moser and Leo, 2010). Activated T-cells can be identified by several cell surface markers including the transferrin receptor (CD71) which is required for the uptake of iron by cells and proliferation, the IL-2 receptor (CD25) which is expressed on activated cells and T-regulatory cells, and co-stimulatory molecules that are required for T-cell activation in conjunction with binding of the T-cell receptor such as CD28. Regulatory T-cells, which express both CD25 (the IL-2 receptor) and FoxP3, produce IL-10 and inhibit the immune response by blocking the activity of other immune cells and may have a beneficial role in chronic inflammation (Moser and Leo, 2010; Albers et al., 2005). Activated Tregs suppress both cytoxic and helper T-cells by suppressing IL-2 gene transcription and T-cell proliferation (Shevach et al., 2002). Tregs are important in preventing an autoimmune reaction and are increase in number in response to inflammatory stimuli (Fehervari and Sakaguchi, 2004; de Kleer et al., 2004; Taflin et al., 2011).

Each individual B or T cell expresses a receptor that is specific for only one antigen. Antigen binding stimulates proliferation of activated immune cells expressing receptors or antibodies for that single antigen. Some of these cells are maintained to propagate a much faster response to a second exposure and prevent future infection by the same pathogen (Moser and Leo, 2010). This is what gives the acquired immune system the valuable and defining characteristic of memory. The antigen-antibody complex formed leads to destruction or inactivation of the imposing antigen (Moser and Leo, 2010).

Assessment of Immune Function

As discussed above, the immune system is comprised of several tissues and cell types; therefore, there are many methods to assess immune function. Immune cells are found

systemically (blood, spleen), in lymph tissue and gut, as well as the upper airways (Albers et al., 2005). In blood, circulating inflammatory markers, such as acute reactive proteins (CRP, haptoglobin) and cytokines (IL-6 and TNF-α), can be measured. The mesenteric lymph nodes (MLN) are gut associated lymph tissue (GALT) and respond to antigens present in the gut (Kobayashi et al., 2004). Immune tissues differ in the make-up of immune cells present. For example, immune cells of the MLN are mainly T-cells, while the spleen is comprised of a mixture of B and T-cells (Mebius and Kraal, 2005). As such, using different immune tissues will provide information on different aspects/sites of immunity.

Immune function can be estimated by the ability of immune cells to respond to a challenge. This is measured by quantifying proliferation of stimulated immune cells, cytokine production by stimulated cells, as well as assessing the proportion and phenotypes of immune cells present in tissue. Different mitogens can be used to stimulate specific cell types. This includes, but is not limited to, LPS, Con A and anti-CD3/CD28. LPS stimulates TLR4 on macrophages and B-cell proliferation. Con A and CD3/CD28 activate T-cells, where CD3/CD28 directly binds the T-cell receptor and its required co-stimulatory molecule. Methods to assess immune function used in this thesis are discussed in in detail in Chapter 3.

Immune Changes in Obesity and Metabolic Syndrome

Chronic Inflammation

Normally the immune system responds to injury or invasion by pathogens rapidly and this becomes resolved in a short term acute immune response (Feghali CA and Wright TM, 1997). Often in chronic disease, several exposures, including environment, lifestyle, and physiological stresses characteristic of the disease can lead to chronic low grade inflammation (Nicklas et al., 2005; Pickup, 2004). Contributing factors to low grade inflammation include a combination of nutrition (inflammatory vs. anti-inflammatory promoting foods), age, smoking, stress, and others (Pickup, 2004). This chronic inflammation has long been associated with cardiovascular disease, and more recently T2D and obesity (Nicklas et al., 2005). Prolonged immune activation can lead to endothelial dysfunction, atherosclerosis, insulin resistance, as well as proteolysis and oxidative stress which increases risk for cardiovascular disease, stroke, congestive heart

failure, Alzheimer's disease, and osteoarthritis (Nicklas et al., 2005). Elevated markers of inflammation, such as C-reactive protein (CRP), IL-6 and TNF-α are predictive of cardiovascular disease and T2D (Theuma and Fonseca, 2003; Spranger et al., 2003). Acute phase protein production (CRP, haptoglobin) is stimulated by cytokines and is ongoing in many chronic diseases, with elevated acute phase protein concentrations observed in diabetes and cardiovascular disease (Pickup, 2004). Body weight is positively correlated with chronic inflammation and it has been shown that acute phase proteins, like CRP, and circulating concentrations of the inflammatory cytokine IL-6 are decreased with weight loss (Nicklas et al., 2005; Theuma and Fonseca, 2003). Conditions that may trigger chronic inflammation are obesity, in which there are higher levels of free fatty acids and a predisposition to insulin resistance, postprandial glycemia, increased oxidized lipoprotein concentrations, and infection (Theuma and Fonseca, 2003). All of these are conditions in which cytokine production is stimulated (Theuma and Fonseca, 2003).

It is thought that diabetes has an inflammatory pathogenesis similar to cardiovascular disease. T2D has been shown to be associated with altered immune function, and patients with T2D are at higher risk for infection than individuals who do not have T2D (Plotkin et al., 1996). This observation has been considered and possible alterations in immune function have been examined in people with T2D, mainly with the innate immune response and to a lesser extent the acquired immune response. This has been demonstrated in both rodent models and human studies.

Innate Immune System in Metabolic Syndrome

Chronic inflammation and oxidative stress in response to consistently elevated blood glucose concentrations may cause damage to β-cells, the insulin secreting cells of the pancreas (Donath et al., 2008). The requirement for increased glucose metabolism also leads to increased production of reactive oxygen species by mitochondria, stimulating inflammation and production of pro-inflammatory cytokines (reviewed by (Wellen and Hotamisligil, 2005)). Dyslipidemia, which is commonly observed in T2D patients, may also contribute to inflammation and β-cell function by increasing circulating cytokine concentrations and altering apoptosis through exposure to various free fatty acids (Donath et al., 2008).

Increased inflammation is observed in the acute phase response, which is stimulated by activation of the innate immune system. This is the source of stimulus for the production of many inflammatory cytokines (Gabay and Ushner, 1999). Acute phase proteins stimulate production of cytokines by macrophages and monocytes, including IL-1, IL-6, TNF-α, and IFN-γ (Gabay and Ushner, 1999; see Table 2). These cytokines are associated with obesity and diabetes risk (Theuma and Fonseca, 2003; Pickup, 2004). Adipocytes of obese individuals and those with T2D produce higher concentrations of the inflammatory cytokine TNF-α when compared with a lean, healthy population (Dandona et al., 2004; Esposito et al., 2002). Interestingly, neutralizing TNF-α can restore insulin sensitivity, further supporting the role of inflammation in the progression of diabetes. Increased plasma CRP and IL-6 concentrations have also been observed in obese individuals compared to lean individuals (Dandona et al., 2004).

High plasma glucose levels, as seen in people with diabetes and impaired insulin sensitivity, leads to greater oxidative stress which is a factor that may increase inflammation and beta cell damage (Dandona et al., 2004). In subjects with impaired glucose tolerance and control subjects with normal glucose tolerance, hyperglycemia was achieved using a clamp method for glucose infusion (Esposito et al., 2002). A plasma glucose concentration of 15 mmol/L was reached and maintained for 240 minutes, or 3 bolus infusions were given at 2 hour intervals. In all subjects hyperglycemia induced increased production of the cytokines TNF-α and IL-6 (Esposito et al., 2002). Subjects with impaired glucose tolerance had higher peaks for cytokine concentrations that did not return to basal levels as quickly as subjects with normal glucose tolerance. The glucose pulses had a more pronounced effect on cytokine production than constant hyperglycemia for an extended period of time. Glucose infusion with the addition of the antioxidant glutathione prevented any increase in cytokine production (Esposito et al., 2002). The ability of glutathione to prevent increases in cytokine production suggests that increased oxidative stress may provide the stimulus for cytokine production and inflammation. Also, elevated TNF-α and IL-6 concentrations have been associated with hyperglycemia and insulin resistance (Esposito et al., 2002). From the literature, one can conclude that conditions associated with metabolic syndrome and diabetes, such as obesity and insulin resistance, the innate immune system contributes to an inflammatory state through increased production of acute phase proteins, cytokines, and reactive oxygen species.

Acquired Immune System in Metabolic Syndrome

The acquired immune response in obesity and T2D has not been examined to the same extent as the innate immune and acute phase response. Some studies have examined cytokine production and T-cell function in rodents in conditions of obesity and metabolic syndrome.

An abnormal immune response has been observed in obese Zucker rats when compared to lean controls (Ruth et al., 2008; Plotkin et al., 1996). Splenocytes of obese animals produced greater concentrations of inflammatory cytokines including TNF-α, IL-1β, and IL-6 when stimulated with concanavlin A (Con A), a T-cell mitogen, in vitro (Ruth et al., 2008). IL-2, a cytokine that stimulates proliferation, was produced at lower concentrations in these obese animals compared to lean, suggesting an impaired proliferative response (Ruth et al., 2008). Also, a lower proportion of T-cells (CD3+) were observed in obese animals (Ruth et al., 2008). In a different study, T-cell lymphopenia was also observed in the blood, spleen and thymus of obese Zucker rats (Tanaka et al., 1998). The proportions of CD3+CD4+ and CD3+CD8+ cells decreased more rapidly in obese than lean rodents (Tanaka et al., 1998). Again, T-cell proliferation was lower in obese than lean animals. It was suggested that this may be due to hyperglycemia (Tanaka et al., 1998). Lower proportions of T-cells in obesity is a consistent finding in the literature, however studies examining changes in T-cell subset proportions have been less conclusive (Stentz and Kitabchi, 2003). It is often found that the concentration of cytotoxic (CD8+) cells are increased in obesity, with lower proportions of helper T-cells (CD4+) when compared to lean controls; this shift may favour an inflammatory response (Stentz and Kitabchi, 2003). As with humans, obese *fa/fa* rats were found to be more susceptible to infection at lower doses when exposed to *C. Albicans* compared to lean animals with the same exposures (Plotkin et al., 1996). In animal models, obesity altered T-cell function, warranting further investigation into altered adaptive immune function in metabolic syndrome and diabetes.

Dietary Fat in Immune Modifications

Dietary components effect inflammation and may be used to counter the inflammatory state observed in diabetes and obesity. One example that has been studied is specific dietary fatty acids, especially n-3 PUFA. A study comparing obese, leptin deficient

JCR:LA-cp rats fed a high or low dose of fish oil containing eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) to a group of obese and a group of lean rats fed a control diet containing no fish oil found differences in immune cell proportions as well as cytokine production between groups (Ruth et al., 2008). Isolated splenocytes from obese animals fed fish oil containing diets produced less IFN-γ compared to the control with no change in IL-2 production (Ruth et al., 2008). The high dose fish oil diet lead to lower production of IL-1β and higher concentrations of IL-10 in Con A stimulated splenocytes (Ruth et al., 2008). Splenocytes of rats fed fish oil contained a greater proportion of CD3+ T-cells, CD3+CD4+ helper T-cells and CD4+ with the activation marker CD28 (Ruth et al., 2008). According to this study feeding long chain n-3 PUFA was able to alter immune response through an altered T-cell response.

Vaccenic acid, a naturally occurring trans fatty acid, has also been examined in the JCR:LA-cp rat for its ability to alter inflammation. Lean and obese animals were fed a diet containing either 0% vaccenic acid or 1.5% vaccenic acid (Blewett et al., 2009). Compared to the lean animals, there was a greater proportion of total T-cells as well as cytotoxic (CD3+CD8+) and helper (CD3+CD4+) T-cells in splenocytes of obese animals (Blewett et al., 2009). Vaccenic acid lowered the proportion of naive helper T-cells compared to the control diet that did not contain vaccenic acid (Blewett et al., 2009). Vaccenic acid had a potentially anti-inflammatory effect, lowering the production of IL-2, IL-10 and TNF-α in response to Con A stimulation in spleen cells. In obese animals, feeding vaccenic acid lowered IL-2 and TNF-α to levels similar to lean controls (Blewett et al., 2009). Both Ruth and Blewett showed that feeding specific fatty acids was reflected in phospholipid or lipid raft fatty acid composition and that there was no effect of diet on plasma haptoglobin concentrations (Ruth et al., 2008; Blewett et al., 2009).

Fatty acids are involved in the synthesis of inflammatory mediators, such as eicosanoids, and are an important component of cell membranes (reviewed by (Galli and Calder, 2009; Clandinin et al., 1991). Due to the many roles of fatty acids and their ability to alter membrane properties the effects of dietary fat on immune function is well studied, however the focus has been on select fatty acids such as PUFA and trans fat. It is also likely that MUFA are involved in these processes and have a distinct effect on immune function, though this has been less studied. The role of dietary fat in metabolism, cell structure and function as well as immune function is discussed in the following sections. As T2D is known to be a condition of chronic low grade inflammation, certain fatty acids

with anti-inflammatory effects may be beneficial in preventing the development and progression of T2D.

Summary

The immune system is affected by the presence of chronic disease like T2D and the prolonged exposure to inflammation can have detrimental effects. In the case of T2D, low grade chronic inflammation increases the risk of developing the disease as well as contributing to the development of complications, such as cardiovascular disease, in T2D patients. Changing the source of diet fat has been shown to modulate immune function and this may be beneficial in controlling blood glucose concentrations in T2D patients, thereby preventing the progression of complications associated with the disease. It may also be useful in diabetes prevention.

Table 2. Cytokines with a possible role in diabetes and inflammation

Cytokine	Source	Role
IL-1	Monocytes, B and T cells	In acute response increases prostaglandinE_2 synthesis and acute phase protein production Induces fever Involved in T-cell proliferation and activation
IL-2	Activated T-cells Th1 cytokine	Stimulates T-cell proliferation IL-2 receptor on T-cells is expressed as CD25 Increases MHC II expression and cytokine production
IL-6	Monocytes, Macrophages and T-cells	Stimulates acute phase protein production by the liver Activates T and B cells (stimulates B-cell antibody production) Increases IL-2 and IL-2 receptor expression Anti-inflammatory in acute inflammation Pro-inflammatory in chronic inflammation
IL-10	B and T cells (CD3+4+ and CD3+8+), Th2 cytokine	Inhibits IFN-γ production by T-cells Decreases T-cell proliferation and macrophage and NK cell activity Inhibits production of IL-1, IL-6, and TNF-α by monocytes/macrophages Regulatory, anti-inflammatory cytokine
TNF-α	Macrophages and monocytes	Stimulates IL-6 and the acute phase May be involved in insulin resistance (Dandona et al., 2004) Similar responses to IL-1 (ie. Fever)
IFN-γ	NK and Th1 cells (CD4+) and cytotoxic T-cell (CD8+)	Promotes the inflammatory response

*Reviewed by (Feghali and Wright, 1997)

The Importance of MUFA in the Diet of those with Insulin Resistance and Diabetes

The 2008 Canadian Diabetes Association clinical practice guidelines recommends that most fat in the diet come from MUFA , while limiting PUFA to less than 10% total energy intake and SF to less than 7% total energy intake. With rising prevalence of chronic disease such as T2D and cardiovascular disease dietary patterns have been examined for their contribution to increased or decreased risk for disease. One such pattern that has been of interest is that of Mediterranean populations. The Mediterranean diet pattern consists of high fruit, vegetable, and whole grain consumption contributing to its high fibre content as well as a low consumption of meat and dairy, leading to a low SF

intake (Willett et al., 1995). In contrast to diets recommended for weight loss and lowering risk factors for disease in Western countries, which have mainly been low in fat, the Mediterranean diet is characterized by a relatively high consumption of fat, due to the liberal use of olive oil, a rich source of MUFA (Willett et al., 1995). This dietary pattern has been well studied for the prevention and management of chronic diseases as populations who traditionally follow it have lower rates of obesity, cardiovascular disease and diabetes (Martinez-Gonzalez et al., 2008; Trichopoulou et al., 2003; Willett et al., 1995). MUFA as a component of olive oil has been attributed with many of the beneficial effects associated with a Mediterranean diet pattern. Dietary MUFA has been shown to improve several risk factors for developing heart disease including weight loss, blood glucose, blood lipids, insulin resistance, antioxidant capacity, inflammation, blood pressure and cardiovascular mortality (Ros, 2003). These effects have been studied almost exclusively with olive oil as the source of MUFA.

Disease Incidence

Epidemiological Studies

Lower incidence of cardiovascular disease and diabetes has been observed in Mediterranean countries (Martinez-Gonzalez et al., 2008; Trichopoulou et al., 2003; Sofi et al., 2010). This has been attributed to the dietary pattern in this region, which has since been tested in other populations with positive results (Paniagua et al., 2007; Rumawas et al., 2009; Esposito et al., 2010; Sofi et al., 2010). In the Nurse's Health Study, which followed 83,818 American women for 20 years and recorded diabetes incidence, higher intakes of nuts and peanut butter, which are rich sources of MUFA, was associated with significantly lower risk for developing T2D (Jiang et al., 2002). It has also been demonstrated that high MUFA diets low in SF can improve insulin sensitivity, an important factor in the prevention of diabetes (Rumawas et al., 2009; Brehm et al., 2009; Jiang et al., 2002; Esposito et al., 2010). Studies correlating adherence to a Mediterranean style diet that is high in MUFA also show its association with lower risk of developing cardiovascular disease, T2D and overall risk of mortality (Trichopoulou et al., 2003; Martinez-Gonzalez et al., 2008; Sofi et al., 2010; Esposito et al., 2010). Trichopoulou (2003) reported that increased adherence to a Mediterranean diet as measured by a Mediterranean diet score significantly lowered risk for overall mortality as well as risk of developing cardiovascular disease (Trichopoulou et al., 2003). A

Mediterranean diet score was assigned by giving points for increased servings of foods that follow a Mediterranean diet pattern, and taking points off for servings of food not included in the diet pattern such as dairy and red meat using a food frequency questionnaire. Increasing Mediterranean diet score adherence by just two points on a scale out of nine could lower risk of mortality by 25% (Trichopoulou et al., 2003). Compared to those with the lowest adherence (MDS <3 out of 9) the incidence rate of diabetes over 4.4 years was 0.41 for those with moderate adherence (score of 3-6), and 0.17 for those with the highest Mediterranean diet scores in a cohort of Spanish university graduates (Martinez-Gonzalez et al., 2008). Similarly, higher Mediterranean style dietary pattern scores in a US cohort was associated with lower incidence of metabolic syndrome during a 7 year follow up compared to those with lower scores (Rumawas et al., 2009). The associations found supporting the benefits of dietary MUFA in epidemiological studies such as the Nurse's Health Study are further supported with dietary intervention trials comparing high MUFA diets to diets high in other nutrients, commonly carbohydrates or saturated fat, and measuring their effects of risk factors for disease or efficacy in disease management.

Mechanistic Studies

Studies of diabetes incidence in animal models feeding high fat, high MUFA diets have not been performed. Many studies of other dietary fatty acids have shown that some specific fats, such as conjugated linoleic acid (CLA), n-3 long chain PUFA, and n-6 PUFA, have effects of the development of diabetes. In Zucker diabetic fatty rats and Zucker fa/fa rats, feeding CLA normalized blood glucose and improved oral glucose tolerance (reviewed by (Taylor and Zahradka, 2004)). Supplementing the diet with CLA also lowered fasting serum insulin concentrations and body weight compared to rats fed a control diet containing no CLA (Taylor and Zahradka, 2004). In rodents fed a high fat diet (60% energy fat) high in n-3 PUFA (19% fat from fish oil) insulin resistance was prevented in liver and muscle compared to rodents fed diets high in SF, MUFA or n-6 PUFA (Delarue J et al., 2004). In contrast, rodents fed a high fat diet (59% energy from fat) high in n-6 PUFA from safflower oil had impaired insulin action compared to those fed a low fat chow (16%) (Oakes et al.,1997).

While there are few animal studies of dietary fat and diabetes incidence, these studies illustrating the effects of different dietary fat sources on insulin function suggest that the type of fat present in the diet has a role in the development of T2D. Based on data from

human studies of dietary MUFA and animal studies of other dietary fats it is likely that MUFA may also play a role in modulating insulin sensitivity as well as T2D incidence.

Summary

Epidemiological studies strongly suggest lower cardiovascular disease and T2D incidence and prevalence in populations consuming a Mediterranean diet pattern. This has been observed in Mediterranean populations, as well as some Western populations where Mediterranean style diet pattern was assessed and correlated with disease outcomes. An important component of the Mediterranean diet is olive oil, a rich source of MUFA. It has been suggested that many of the beneficial health outcomes attributed to a Mediterranean diet is in part due to a high intake of MUFA. A high MUFA diet may lower diabetes prevalence by improving risk factors for developing the disease, such as blood lipids and insulin sensitivity.

Effect on Dyslipidemia

Epidemiological Studies

It is well established that dietary saturated fatty acids (SFA) have a negative impact on blood lipids and insulin sensitivity, increasing the risk for disease (Vessby et al., 2001; Roche et al., 1998; Welters et al., 2004). As such, it is recommended that SF is limited in the diet as much as possible, and often replaced with high quality carbohydrate. Based on outcomes from studies of the Mediterranean diet, it has been suggested that replacing saturated fat with monounsaturated fat may be equally, if not more, beneficial.

In the Framingham Heart study, a cohort study in the United States, greater adherence to a Mediterranean diet as measured by a given Mediterranean style dietary pattern score from food frequency questionnaires, was associated with lower serum TG and higher HDL cholesterol concentrations (Rumawas et al., 2009). Mediterranean diet score was also negatively associated with waist circumference (Rumawas et al., 2009). NHANES III data also supports these findings, with higher Mediterranean diet scores correlating with improved total cholesterol to high density lipoprotein (HDL) cholesterol ratios in males and females across all age groups, and lower TG in postmenopausal women (Carter et al., 2010). This was also observed in a cohort study using similar methods in a cohort of patients with T2D in southern Italy. Patients with higher Mediterranean diet scores in this population also had lower serum TG and higher HDL cholesterol concentrations than

those with lower scores (Esposito et al., 2009). These patients also had lower BMI, waist circumference, waist to hip ratio, and lower prevalence of metabolic syndrome when the Mediterranean diet scores were higher (Esposito et al., 2009).

A study of 226 overweight and obese subjects in Greece also found similar results showing increased adherence to a Mediterranean diet was associated with lower total cholesterol and low density lipoprotein (LDL) cholesterol levels compared to those who were not adhering (Paletas et al., 2010). This cohort was divided into 3 groups: those adhering to a Mediterranean diet, those whose diet was high in fat but not a Mediterranean diet patterns, and those whose diet was high in carbohydrate. The non-adhering groups were larger and the participants were younger compared to the adhering group. It has been reported that younger populations in the Mediterranean area may be adopting a more Westernized lifestyle, with lower consumption of vegetables, fruits, legumes and pasta and a greater intake of meat and sweets (Paletas et al., 2010). Metabolic syndrome prevalence was significantly higher in the non-adhering groups, with rates of 60.2% in the high fat group, 69.2% in the high carbohydrate group, and 27.3% in the Mediterranean diet group (Paletas et al., 2010).

Clinical Trials

The effects of feeding high MUFA diets on dyslipidemia have also been studied in clinical trials. Vessby et al (2001) compared a high MUFA diet to a high SFA diet to examine their effects on lipid metabolism. Consistent with other studies that altered dietary fat composition, it was found that fatty acid composition of blood lipids reflected the diet of the participant (Rivellese et al., 2008; Paniagua et al., 2007; Vessby et al., 2001). Participants in the SFA diet group experienced increased total and low density lipoprotein (LDL) cholesterol concentration while both were significantly decreased in participants on the high MUFA diet (Vessby et al., 2001). Half of the participants in each group also received a fish oil supplement while the rest received a placebo, this had some effect on cholesterol however the high MUFA diet in participants receiving a placebo significantly decreased plasma LDL cholesterol concentrations while there was no change in the SFA group receiving a placebo (Vessby et al., 2001). A different study comparing low, moderate, and high MUFA diets found that MUFA dose dependently lowered LDL cholesterol with no change in very low density lipoprotein (VLDL), HDL cholesterol or TG (Gill et al., 2003).

Rivellese et al (2007) conducted a similar study comparing high SFA diets to high MUFA diets in overweight and obese men with T2D. All other diet components such as carbohydrate and protein were similar; however the MUFA diet contained slightly less cholesterol. Butter was the main fat source for the saturated fat diet, while olive oil was used as the MUFA source. There was no difference between diets in weight, HDL concentrations or fasting TG (Rivellese et al., 2008). As found in other studies, fasting LDL cholesterol concentrations were lower in participants who followed a high MUFA diet compared to a high SFA diet (Rivellese et al., 2008). VLDL triglycerides were also lower following the MUFA diet compared to the SFA diet (Rivellese et al., 2008).

Whether to replace energy from SFA with carbohydrate or MUFA has been debated, especially in the case of T2D. A long term study comparing high MUFA to high carbohydrate (CHO) diets found supporting results for the beneficial effects of MUFA in the diet. Overweight and obese patients with well controlled T2D were placed in either a high MUFA diet group or high CHO diet group with similar saturated fat and protein content for 1 year, with some subjects participating in an 18 month follow up study (Brehm et al., 2009). The diets allowed for approximately 0.5 pound weight loss per week. At the end of the study there was no difference in weight loss between the groups and there was a significant increase in HDL in both diet groups (Brehm et al., 2009). There were no significant changes 18 months later in participants who took part in the extension study (Brehm et al., 2009). These findings suggest that a high MUFA diet is at least as effective as a high CHO, low fat diet for controlling T2D and lowering risk factors for further complications. In contrast, a short term diet study comparing a high MUFA, a high SFA, and a high CHO diet found that most lipid measures were not different after each diet, however the high CHO diet lowered fasting HDL compared to the other diets (Paniagua et al., 2007). Lowered HDL cholesterol with a high carbohydrate may be contraindicated and this may be a benefit of a high MUFA diet.

The benefits of high MUFA diets were further examined with two different levels of fat intake, either 40% energy from fat or 29% energy from fat (Egert et al., 2010). Both diets provided 51% of the fat as MUFA while other factors, including PUFA, SFA, fiber, carbohydrate, cholesterol, and antioxidants, were similar for both diets. After 4 weeks, both diets effectively decreased total cholesterol, LDL, LDL-oxidation, and LDL particle size compared to a 2 week wash in period where all subjects consumed a high SF diet (Egert et al., 2010). Despite HDL concentrations being lowered with both diets, the

LDL/HDL ratio improved. Both diets led to MUFA rich LDL particles. This may be beneficial as some studies have shown that particles with high MUFA content are less susceptible to oxidation, possibly due to the lower oxidative potential of oleic acid compared to PUFA (Wahle et al., 2004). Both diets improved many risk factors for diabetes by similar amounts, suggesting that, to some extent, the quality of macronutrients chosen may be of more importance than the quantity (Egert et al., 2010).

Mechanistic Studies

While somewhat limited, some animal trials have been done to examine the effects of high MUFA diets on blood lipids. Syrian golden hamsters were fed diets with the main source of fat coming from either palm stearin, cold pressed or refined rapeseed oil, virgin or refined olive oil, high oleic acid sunflower oil or high linoleic sunflower oil for five weeks (Trautwein et al., 1999). All diets lowered total plasma cholesterol compared to the palm stearin diet, which also significantly increased VLDL concentrations (Trautwein et al., 1999). Esterified cholesterol concentration in liver was highest in animals fed both olive oil diets and the high oleic acid sunflower oil diet. The cholesteryl esters (fatty acid composition) reflected the diet the animal received. It has been suggested that high MUFA diets shift cholesterol to storage form, increasing esters in the liver and lowering LDL concentrations in plasma (Trautwein et al., 1999). Another study used New Zealand rabbits as a model of athersclerosis to examine the effect of dietary fat on blood lipids. The fat sources for the experimental diets were fish oil, refined or virgin olive oil, or sunflower oil. There were also two control groups, one fed regular chow and one an atherogenic diet. Animals were fed their respective diets for 30 days (Ramirez-Tortosa et al., 1998). At the end of the feeding period, animals fed either olive oil diet, the fish oil diet or the sunflower oil diet had lower lipoprotein fractions compared to the atherosclerotic control (Ramirez-Tortosa et al., 1998). The olive oil and fish oil diets lowered lipoprotein lipids to a greater extent compared to the sunflower oil diet. The fish oil diet led to significantly greater levels of LDL oxidation, a risk factor for cardiovascular disease, while the other diets were closer to the non-athersclerotic control (Ramirez-Tortosa et al., 1998). Lower TG levels were also observed in a study of diabetic mice fed a high fat, high MUFA diet (38% fat, 26% MUFA) compared to a low fat diet (13% fat, 3% MUFA) (Kotake J et al., 2004).

Summary

Diets containing a high proportion of MUFA have positive outcomes on various blood lipid values and composition. Some studies report that high MUFA diets increase or have a neutral effect on HDL cholesterol levels (reviewed by (Garg, 1998)), while high CHO diets lower HDL concentrations (Garg, 1998; Paniagua et al., 2007). For this reason it has been suggested that replacing SF with MUFA rather than CHO has better blood lipid outcomes and is therefore a better recommendation. Both diets have been shown to consistently and effectively lower LDL cholesterol concentrations (reviewed by (Perez-Jimenez et al., 1995)). The use of dietary MUFA for improving blood lipid concentrations and composition is well supported. Other factors that may be affected by MUFA and improve risk factors for disease are insulin resistance and inflammation.

Effect on Insulin Sensitivity

Epidemiological Studies

High MUFA diets, as observed in Mediterranean populations, have also been associated with improved insulin sensitivity. Participants in the Framingham Heart Study Offspring cohort with higher Mediterranean style dietary pattern scores had lower fasting plasma glucose and improved HOMA-IR measures compared with those who had lower Mediterranean diet scores (Rumawas et al., 2009). The highest quintile of Mediterranean diet adherence also had lower incidence of metabolic syndrome over a 7 year follow up than those in the lower quintiles (Rumawas et al., 2009). Similar results were found in a cross sectional cohort study of diabetic patients in southern Italy where higher Mediterranean diet scores were associated with lower postprandial plasma glucose concentrations and less use of glucose lowering drugs (Esposito et al., 2009). While there was no difference in fasting insulin concentrations, Mediterranean diet score and MUFA:SFA ratio was negatively associated with HbA1c (Esposito et al., 2009). Improved fasting glucose, insulin and HOMA-IR were observed for normoglycemic participants with higher Mediterranean diet scores in the ATTICA cohort, which included participants living in Greece (Panagiotakos et al., 2007). When adjusted for confounding factors the associations disappeared in diabetic participants and those with impaired fasting glucose (Panagiotakos et al., 2007). In overweight and obese subjects in a Greek cohort Mediterranean diet adherence was associated with improved blood glucose

following an oral glucose tolerance test compared to those in the cohort whose diet did not adhere to a Mediterranean diet pattern (Paletas et al., 2010). According to NHANES III data, in a cohort of 13,197 participants aged 18-90 years old, there was a significant trend for increased Mediterranean diet score and improved serum insulin and HOMA-IR, suggesting improved insulin sensitivity with greater Mediterranean diet adherence (Carter et al., 2010). Overall, it appears that increasing adherence to a Mediterranean style diet has positive outcomes on insulin sensitivity.

Clinical Trials

Clinical trials involving feeding high MUFA diets have also shown beneficial outcomes for insulin sensitivity and glycemia. In the study by Vessby et al comparing a high MUFA diet to a high SFA diet, the SFA diet lowered insulin sensitivity while participants on the high MUFA diet had no change in insulin sensitivity from baseline (Vessby et al., 2001). A significant difference in insulin sensitivity index between the two diets was observed at the end of the study (Vessby et al., 2001). Fasting insulin was significantly decreased following the high MUFA diet by 5.8% (Vessby et al., 2001). In a long term trial comparing high MUFA to high CHO diets there was no difference in weight loss between the groups, and there was a significant reduction in blood pressure, HbA1C, fasting insulin and glucose, and insulin resistance with either diet (Brehm et al., 2009). This further shows the effectiveness of replacing energy from dietary SFA with MUFA in improving the risk factors for developing T2D.

The relevance of this to patients with T2D is especially highlighted in a study comparing a high MUFA, lower carbohydrate enteral formula to a high carbohydrate formula in both healthy volunteers and individuals with T2D. The high MUFA formula provided 49.3% energy from fat, with 70% of the fatty acids as oleic acid (Yokoyama J et al., 2008). The high carbohydrate formula provided 30.8% energy from fat with 26.5% of the fat as oleic acid. It was found that feeding the high MUFA formula lead to lower plasma glucose 30 minutes postprandially, as well as a lower area under the curve for glucose up to 180 minutes post meal in both healthy participants and those with diabetes compared to the high carbohydrate formula. Serum insulin was also lower in both groups fed the high MUFA formula (Yokoyama J et al., 2008). The high fat formula did not adversely affect lipid metabolism and the results from this trial suggest a high fat, high MUFA formula is more beneficial to those with diabetes than a high carbohydrate formula (Yokoyama J et al., 2008).

Paniagua (2007) hypothesized one mechanism by which MUFA may provide beneficial physiologic outcomes is by increasing glucagon like peptide-1 (GLP-1), a protein that is associated with lower blood glucose, possibly by restoring beta cell sensitivity. This was tested using a randomized crossover design with three 28 day periods. In each period participants consumed a diet either high in saturated fat, monounsaturated fat or carbohydrate. Again, olive oil was the source of MUFA. Fasting glucose and HOMA-IR, a measure of insulin sensitivity, were significantly improved following the MUFA diet period compared to the SFA or CHO periods (Paniagua et al., 2007). Feeding the CHO diet resulted in a postprandial area under the curve for glucose that was significantly higher compared to the MUFA and SFA diets (Paniagua et al., 2007). Fasting GLP-1 was similar between diets, however postprandial GLP-1 was lower following the CHO diet than the other two diets (Paniagua et al., 2007). A high carbohydrate diet may contribute to the stresses caused by high blood glucose, such as oxidative stress, and lead to insulin resistance. This would support the recommendation of a high MUFA diet.

Mechanistic Studies

Studies of insulin sensitivity and high MUFA diets have been carried out in animals and cell cultures to gain more mechanistic insight into the results observed in human trials. Improved insulin sensitivity was observed in rats fed diets with an olive oil or soybean oil fat source compared to an animal fat (high SFA) source or low fat control diet (Picinato et al., 1998). Following the 6 week study period there was no difference in body weight or food intake between any group, however the olive oil and soybean oil diet groups had lower basal serum glucose and improved glycemia following a glucose load compared to the animal fat and control dietary groups (Picinato et al., 1998). Blood insulin levels were also improved in the olive and soybean oil groups compared to the other diets following the glucose load (Picinato et al., 1998). Perfused islet cells from the animals fed the olive and soybean oil diets released more insulin at both a low and high glucose concentration than islets from animals fed the animal fat or control diet (Picinato et al., 1998). Insulin sensitivity was improved in rats on the olive oil and soybean oil diet compared to the other diets both in vitro and in vivo.

Additional evidence that MUFA may improve insulin sensitivity has been found in studies of islet cell cultures. A study of pancreatic beta cell cultures showed that MUFA may be protective, while SFA exposure increases beta cell death (Welters et al., 2004).

Different fatty acids were added to the insulin secreting rat beta cell line BRIN-BD11 to examine their individual and combined effects on apoptosis. The long chain saturated fatty acids palmitate and stearate caused a significant increase in cell death while oleate and palmitoleate did not change levels of apoptosis (Welters et al., 2004). Furthermore, in the presence of oleate or palmitoleate, cell death was attenuated in cells exposed to saturated fatty acids. This was also tested in human cultured islet cells where similar results were found. MUFA was protective against apoptosis and improved insulin function of cells following exposure to high glucose and palmitic acid, conditions that cause loss of beta cell function (Maedler et al., 2003).

Summary

Based on studies of dietary fat, it is accepted that SF has negative effects on insulin sensitivity. MUFA has recently been studied for its potential protective effects and study outcomes have, for the most part, been positive. While the results are not as conclusive for MUFA and it has not been as extensively studied, it is suggested that MUFA may protect beta cells in the pancreas, and have beneficial effects on insulin secretion and regulation. Overall, it appears that high MUFA and low SFA diets, as tested in clinical trials as well as through epidemiological studies of the Mediterranean diet, improve insulin sensitivity.

Effect on Immune Function

Inflammation

Although not well studied there are a few studies suggesting potential mechanisms for the effects of MUFA on inflammation and immune function. Estruch compared two different Mediterranean style diets, one with an olive oil MUFA source and another with a nut MUFA source, to a low fat diet in participants at high risk for developing T2D. Consuming the olive oil diet decreased CRP, a measure of systemic inflammation, while both of the Mediterranean diet groups had significantly lower plasma IL-6 concentrations compared to a low fat diet. This shows the potential for anti-inflammatory effect with consumption of a diet high in MUFA (Estruch et al., 2006). High fat diets with a high proportion of fat as MUFA did not induce weight gain and the diet was described by participants as more palatable than a low fat diet (Estruch et al., 2006). Mena et al also found a decrease in plasma IL-6 concentrations when comparing an olive oil or nut based

Mediterranean diet to a low fat diet. Consuming Mediterranean diets are consistently reported to lower IL-6 concentrations in plasma, indicating their ability to provide anti-inflammatory effects (Estruch et al., 2006; Mena et al., 2009; Dai et al., 2008). Furthermore, MUFA treatment of rat pancreatic cells improved viability of cells exposed to IFN-γ and IL-1β, two cytokines that are inflammatory and cause damage to beta cells (Welters et al., 2004). These studies suggest that high MUFA diets may protect against damage caused by the increased inflammation, as well as lower the inflammatory markers observed in obesity and diabetes.

T-Cell Function

It is well known that dietary fat has an effect on T-cell function. Men and women either at high risk for cardiovascular disease or diagnosed with T2D consuming high MUFA diets had a lower proportion of T-cells expressing activation markers and biomarkers of inflammation, compared to participants consuming a low fat diet (Mena et al., 2009). Other changes in immune cell responses have been observed with exposure to different types of fatty acids. Shaikh et al compared the effects of palmitic acid and oleic acid on human antigen presenting T2 cells expressing a mouse MHC I allele (Shaikh et al., 2008). Apoptosis was the main outcome measured and cells treated with the fatty acids were compared with cells cultured in BSA as a control (Shaikh et al., 2008). Antigen presenting cells (APC) were resistant to lysis when treated with palmitic acid compared to the bovine serum albumin (BSA) control. There was no effect for oleic acid compared to control cells. Also, the ability of APC to activate naïve T-cells was 45% for palmitic acid treated cells, significantly lower compared to 64% for BSA cells. There was no difference in oleic acid treated cells (70%) compared to the BSA control (Shaikh et al., 2008). Furthermore, palmitic acid treatment produced less MHC I surface levels than control cells and inhibited APC-cytotoxic T-cell conjugation. Oleic acid treated cells had less MHC I expression compared to the control, but not to the extent of palmitic acid treated cells (Shaikh et al., 2008). Interestingly, the immune effects of palmitic acid were mitigated in the presence of oleic acid in this study.

Many studies have shown that specific fatty acids, such as PUFA, SFA, vaccenic acid, and CLA have significant effects on immune functions including cytokine production and proportion of T cell populations in various tissues (Blewett et al., 2009; Wang et al., 2008; Ruth et al., 2008). It is therefore likely that MUFA also has the ability to alter

immune function however studies directly examining feeding diets high in MUFA on immune function, especially specific immune cell populations, are limited.

In addition to the previously discussed effects of MUFA and immune function, feeding olive oil or high oleic acid sunflower oil was shown to result in a lower proliferation of spleen and mesenteric lymph node lymphocytes following stimulation with Con A in animal feeding trials (reviewed by (Yaqoob, 2002; Wahle et al., 2004)). However, Con A stimulated peripheral blood mononuclear cells (PBMC) collected from men assigned to consume either a control diet that reflected a typical fatty acid composition of the United Kingdom population, or a diet enriched with olive oil for eight weeks did not differ in proliferation (Yaqoob and Calder, 1995). NK cell activity was suppressed in rats fed diets with an olive oil fat source for 10 weeks, compared to coconut or safflower oil (Yaqoob and Calder, 1995). This was also observed in a study where high oleic acid sunflower oil also produced suppressed NK cell activity, showing the effect was not due to other components in olive oil (Jeffery et al., 1996).

Summary

High MUFA diets, as shown by studies of the Mediterranean diet and olive oil, can improve risk factors for developing T2D such as dyslipidemia, insulin sensitivity, and inflammation. Studies of the Mediterranean diet report associations with lower circulating CRP and IL-6 (Estruch et al., 2006; Mena et al., 2009; Dai et al., 2008). Studies have also reported that MUFA treatment improves the viability of cells in culture when exposed to inflammatory cytokines (Welters et al., 2004). The benefits of high MUFA diets are strongly supported in the literature and the Canadian Diabetes Association now recommends that largest portion of the fat in the diet come from a MUFA source. The few studies conducted suggest that feeding oleic acid alters T-cell function in rodent models; however the same effect has not been seen in human studies (Yaqoob, 2002). This may be due to the high MUFA content fed in some animal feeding trials, the differences in tissues and biomarkers examined between animal and human studies, or that the majority of studies that look at high MUFA diets and immune function in humans have been short term. More studies of dietary MUFA and immune function, including T cell function, in both humans and animal models are required for more conclusive results. The preliminary findings in the literature in conjunction with the effects of other dietary FA, such n-3 PUFA, n-6 PUFA, and SFA, suggest that altering

dietary MUFA content will affect immune function, through cytokine production, acute phase proteins, and T lymphocyte function.

While there have been many reports of the positive physiological outcomes obtained by consuming high MUFA diets relative to other diets, another important aspect of the diet is that participants report the high fat diet to be more palatable than a low fat diet (McManus et al., 2001). This is important as it is more likely that the diet can be followed in the long term, a key aspect of diabetes management and weight loss followed by continued weight maintenance (McManus et al., 2001).

A possible mechanism through which dietary fat alters the physiological measures discussed is by changing the type of fat present in cell membranes, thereby altering cell structure, signalling and function (Field et al., 1985). MUFA can improve several risk factors for diabetes; however it has been tested mainly with olive oil or individual fatty acids. The structure of TG in different MUFA sources, such as Canola oil, may vary compared to olive oil and therefore may affect the health outcomes observed.

Dietary Fat, Triglycerides, and Storage

Triglyceride Structure

TG are the major type of fat in the diet and constitute a significant proportion of lipid consumed. They exist as three fatty acids esterified to a glycerol backbone (Fig 1). TG are named by stereospecific numbering (sn), with the fatty acid in the middle being in the sn-2 position and the outside positions being sn-1 and sn-3. Fatty acids in each position can vary by chain length and level of saturation, which alters the properties of the TG, including how it behaves in food as well as how the fat is absorbed, digested and metabolised once consumed. Different types of oils generally contain characteristic TG species. For example, most vegetable oils contain oleic acid (C18:1) or linoleic acid (C18:2) exclusively in the sn-2 positions, while SFA commonly occupy the sn-1 position and are not normally found in other positions (reviewed by (Karupaiah and Sundram, 2007; Hunter, 2001)). In olive and Canola oil the sn-2 position most commonly contains oleic acid, however oleic acid is also found in the other three positions (Aranda et al., 2004; Hunter, 2001).

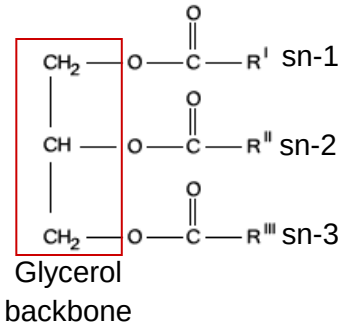

Figure 1. Structure of a triglyceride, where RI, RII and RIII can vary by chain length and degree of saturation

A new cultivar of Canola has been developed to produce oil with the same amount of oleic acid as olive oil. As previously discussed, olive oil rich diets are associated with improved health and lower risk factors for developing chronic disease, such as diabetes, and this is in part attributed to the high content of the MUFA oleic acid. Olive oil contains approximately 70% of its fatty acids as oleic acid, with about 50% of the TG species as triolein (oleic acid is present in all 3 positions). Oleic acid is the most abundant fatty acid found in the sn-2 position in olive oil, while there is very little linoleic or linolenic acid (Aranda et al., 2004; Bracco, 1994). Saturated fatty acids constitute about 13% of the total fatty acids present in olive oil (Aranda et al., 2004). In comparison, high oleic acid Canola oil also contains about 70% of the fatty acids as MUFA and only 7% as saturated fat (Canola Council of Canada, 2006). Also, in contrast to olive oil, traditional Canola oil contains more PUFA, such as linoleic and linolenic acid, and it is expected that the new Canola oil may have more of these fatty acids in the sn-2 position compared to olive oil. The TG species present in the newly developed Canola oil is unknown, and therefore may vary in its physiological effects compared to olive oil despite containing the same amount of oleic acid.

Lipid Digestion and Metabolism

Fatty acid positions on TG may play an important role in lipid digestion and metabolism as many enzymes, including lingual and pancreatic lipase, are specific for the sn-1 and -3 positions (Bracco, 1994; Mu and Hoy, 2004). These enzymes hydrolyze TG and result in two free fatty acids and an sn-2 monoglyceride (Bracco, 1994; Mu and Hoy, 2004).

Following hydrolysis in the small intestine long chain free fatty acids are transported into the enterocytes via a transporter mediated process while the 2-monoglyceride is believed to be absorbed through passive diffusion (Mu and Hoy, 2004). Once absorbed, dietary lipids are either oxidized for energy or stored (Hulbert et al., 2005). Short and medium chain fatty acids can be solubilised, absorbed and transported to the liver (Hunter, 2001). Long chain fatty acids have lower solubility and are transported through the lymph system in a chylomicron following processing in the enterocyte (Hunter, 2001). Prior to forming chylomicrons for systemic transportation, free fatty acids and 2-monoglycerides in the enterocyte are reassembled into TG. This process preserves the sn-2 fatty acid and is summarized in figure 2. TG are then combined with phospholipids, cholesterol and proteins to form chylomicrons to transport lipid in circulation to other tissue. An alternate fate of the 2-monoglyceride is its use as a backbone for new phospholipids (reviewed by (Karupaiah and Sundram, 2007)). Phospholipase A2 hydrolyzes fatty acids from membrane phospholipids, an enzyme that is also specific for the sn-2 position (Dennis, 2000). PUFA, which are commonly found in the sn-2 position of phospholipids are precursors for eicosanoid synthesis, molecules involved in inflammation and platelet aggregation (Dennis, 2000).

The specificity of enzymes involved in the metabolism of dietary TG highlight the possibility of altering physiological functions through dietary changes by changing the properties of fatty acids that cells of the body are exposed to. In a human trial, participants were given one of two diets with the same fat composition differing only by the distribution of palmitic acid on TG. It was found that the sn-2 fatty acid was conserved throughout metabolism (Zock et al., 1995). It has also been shown that varying fatty acids present in different positions can alter absorption in both animal models and human infants (Hunter, 2001). Examples of the effect of altering dietary lipid and TG structure on metabolic and physiological processes and their outcomes are discussed below.

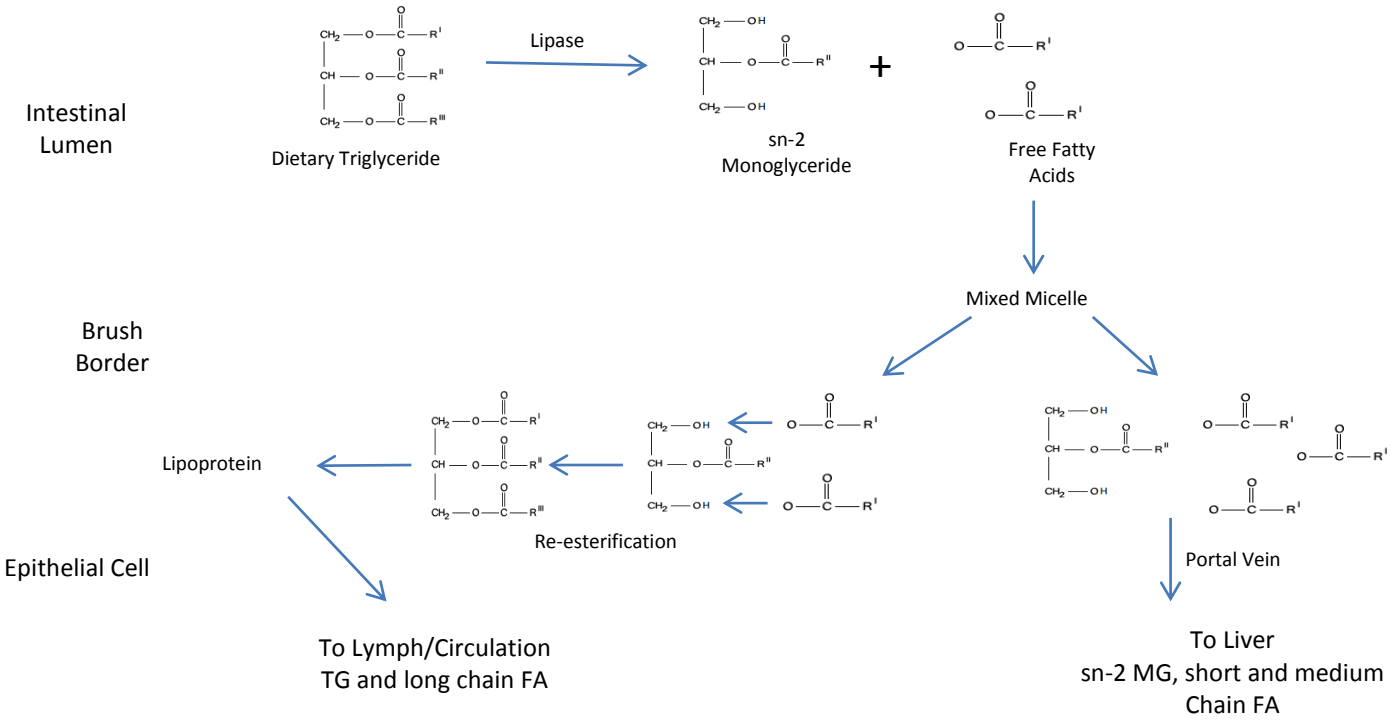

Figure 2. Absorption pathways for sn-2 monogylycerides and free fatty acids

Dietary Lipids, Storage and Changes in Cell Structure and Function

Studies examining lipids and health risk have commonly looked at total MUFA, PUFA, and SFA content, while fewer studies have looked at specific fatty acid content and TG species. These factors could have important implications for health as the stereospecific position of certain fatty acids can alter their metabolic fate and have physiological consequences such as changes to blood lipid composition, fat storage, cell membrane structure (ie. immune cell membranes), which in turn alters function. It has been shown in animals and humans that the composition of dietary fat is reflected to some extent in cell membrane lipid composition (Prades et al., 2003; Clandinin et al., 1991). For example, feeding olive oil in rats increased oleic acid content in erythrocyte phospholipids compared to rodents fed diets high in n-3 PUFA from fish oil or linoleic acid from corn oil (Rao et al., 1993). Changes in cell membrane composition are associated with changes in the function of proteins and signals in the membrane. Cell membranes contain receptors, ion channels, and enzymes and are involved in the regulation of second messengers and gene expression (Prades et al., 2003). A change to any of these factors has the potential to also change the functional properties of a membrane. For example, feeding n-3 PUFA regulates T-cell function by increasing incorporation of these fatty acids into the cell membrane and effecting membrane fluidity (reviewed by (Prescott, 2004)). Increasing n-3 PUFA in the cell membrane also limits the ability of antigen presenting cells to bind and activate T-cells (Prescott, 2004). It is now well established in animal and human studies that dietary fatty acids contribute to the fat composition of adipose tissue, with positive correlations between dietary fat intake and adipose TG and phospholipid composition (Field et al., 1985). These differences have been seen in both structural and storage lipids, further implicating their potential for changing membrane function (Field et al., 1985). Immune cell fatty acid composition is also affected by diet and increasing dietary EPA and DHA is known to increase their content in immune cells at the expense of n-6 PUFA (Calder, 2009). Arachidonic acid (AA), EPA, and DHA inhibit T-cell proliferation and IL-2 production, possible immunosuppressive effects, in cell culture and animal models (Calder, 2009). The extent of the effect of diet on the composition of fat varies for different tissues and classes of lipids (Hulbert et al., 2005). Some possible mechanisms of changing membrane function include altering the ability of proteins (ie. enzymes) to move within membranes, the ability of lipid to move laterally or flip-flop within the membrane (fluidity), and the movement of acyl chains on membrane phospholipids (Clandinin et al., 1991; Calder,

2009). As all of these properties contribute to membrane function it is likely that changes to dietary fat intake that cause changes in membrane lipid composition can alter physiological processes, such as immune function. One example of this is observed in which changes were found in enterocyte membrane phospholipids with high oleic acid diets in rodents compared to rodents fed a semi-purified control diet containing linoleic acid (Vazquez et al., 2000). Animals fed either an olive oil diet (10% OO) or a high oleic sunflower oil diet (10% HOSO) both had the expected increased oleic acid and lower proportions of stearic and linoleic acid in the brush border membrane (Vazquez et al., 2000). There was no difference in total phospholipid content between the control and the olive group, however the HOSO group had a lower membrane concentration of phosphatidylethanolamine and a higher content of phosphatidylcholine (PC) and a higher PC/sphingomyelin ratio (Vazquez et al., 2000). It is possible that the differences observed between oils with similar fatty acid compositions were the result of positional variations in TG species between the oils. Membrane fluidity was higher in both high MUFA diets and significant decreases in sucrase and maltase activity were observed in the MUFA groups compared to the control. This illustrates dietary changes reflected in membranes and function (Vazquez et al., 2000). Changes to the fatty acid composition of the brush border membrane for both HOSO and olive oil were similar, suggesting that changes were caused by oleic acid, not other components of the oil (Vazquez et al., 2000).

Effect of Lipid Metabolism on Blood Lipids

The effects of varied TG structure on fat absorption and metabolism has been studied, most often comparing the effect of SFA in the sn-2 position using structured TG or comparing interesterified oils to native oils. Total fat absorption is affected in rats and piglets fed diets containing TG with varied structure, with greater absorption of fat when the sn-2 position is occupied by a SFA (Mattson, 1959; Bracco, 1994; Kubow, 1996). While in animal models and human infants it is clear that stereospecific positioning of fatty acids plays a role in the efficiency of lipid absorption, its role in adult human lipid metabolism is less clear. Adult humans without underlying conditions should efficiently absorb fat; however, the metabolic fate of absorbed fat may still react to various TG species. Zock et al (Zock et al., 1995) found that varying the proportion of palmitic acid in different stereospecific positions on TG in diets with a constant amount of palmitic

acid, did not alter serum TG or cholesterol concentrations in adult men and women. It is notable that the sn-2 fatty acid was maintained throughout metabolism (Zock et al., 1995). Four studies reviewed by Hunter found no effect on blood lipid parameters, including TG, total cholesterol, HDL cholesterol and LDL cholesterol, in humans with a diet containing randomized fat compared to native fats (Hunter, 2001). Randomized fats undergo interesterification, a process that allows all fatty acids to be distributed equally throughout the sn-1, -2, or -3 positions of TG (Karupaiah and Sundram, 2007). Native fats contain TG that are present in natural oils and fats, where each oil contains characteristic TG species. The studies included male and female participants that were either normocholesterolemic or hypercholesterolemic (Hunter, 2001). It is possible that the short term period used in these studies (21 to 35 days) is not long enough to observe an effect. Furthermore, varied amounts of fat as a proportion of total energy as well as examining different fatty acids in the diet could present different outcomes (Hunter, 2001). While the effect of TG structure on total blood lipid parameters appears to have a minimal effect in adult humans, there is evidence that it may affect blood lipid composition and clearance. After feeding rats diets containing the same fatty acids but in different combinations of TG positions, it was found the fatty acids in chylomicrons also varied (Redgrave et al., 1988). More stearic acid was present in chylomicrons after the consumption of the same amount of stearic acid in a 1,3-dioleoyl-2-stearoyl glycerol TG compared to a 1,2-dioleoyl-3-stearoyl glycerol TG (Redgrave et al., 1988). Slower removal of chylomicron remnants from circulation was also observed in animals that received lipids with palmitic acid predominantly in the sn-2 position compared to oleic acid in the sn-2 position (Redgrave et al., 1988). Slower clearance of chylomicrons may pose an increased risk for atherosclerosis and vascular disease. More evidence is needed to make conclusions about the possible effects of specific TG species on lipid metabolism and storage and disease risk.

Immune Function

Lipids and membrane structure also play an important role in immune function. The immune related effects of varying amounts of dietary PUFA have been studied extensively and it is clear that altering PUFA has metabolic ramifications that cause changes to immune function; however, the effects of other fats, such as MUFA, are less

studied (Kew et al., 2003). Exposures to different fatty acids alters cell membrane phospholipid composition, immune cell proliferation, antibody and cytokine production, as well as immune cell activation and cell populations present (Kew, 2003; Calder, 2009; Jeffery, 1997).

In a study of PBMC fatty acid composition it was found that immune cell proliferation and cytokine production was correlated with fatty acids present in membrane phospholipids (Kew et al., 2003). Rat splenocytes treated with unsaturated fatty acids proliferate less following stimulation with Con A compared to cells treated with other fatty acids (Yaqoob et al., 1998). This was supported in a study by Jeffery et al where animals fed diets with the greatest proportion of oleic acid had a greater proportion of oleic acid in lymphocytes and proliferated less with Con A stimulation compared to animals fed diets lower in oleic acid and higher in linoleic acid (Jeffery, 1997). As previously discussed, dietary lipids are reflected in cell membrane lipids. This may be a mechanism for stimulating or inhibiting proliferation after stimulation and therefore altering immune function.

Dietary fat has also been shown to alter antibody and cytokine production by immune cells, important markers of inflammation and in some cases disease risk. In PBMCs, cytokine production was correlated with the fatty acids present in membrane phospholipids. For example, greater proportions of stearic acid, α-linoleic acid, AA and some other n-6 PUFA correlated with greater production of IL-2, IFN-γ, and IL-4 (Kew et al., 2003).

Different fatty acid exposures are also capable of altering immune cell populations and the proportion of activated immune cells. In splenocytes from rats fed a high fat diet there were lower proportions of cytotoxic T cells and lower NK cell activity compared to spleen cells of rats fed a low fat diet (Yaqoob et al., 1998). Lower NK cell activity was also observed in lymphocytes of rats fed high oleic acid diets compared to animals fed diets lower in oleic acid (Jeffery, 1997).

There is evidence that changing dietary lipid composition alters the pool of fatty acids that cells are exposed to with the potential to change function. The focus of research in this area mainly examines single fatty acids, such as oleic acid, rather than TG species; however TG structure may play a key role in phospholipid composition and the fatty acids that cells are exposed to thereby altering immune function. One mechanism by

which the composition of membrane phospholipids may alter immune function is by effecting cell signalling.

Cell Signaling

Dietary lipids have been shown to play a role is in altering the proteins and their ability to be activated in the cell membrane. Examples of this include membrane G proteins and protein kinase C (PKC). Following 4 weeks on a high fat (30% energy) diet with virgin olive oil as the main fat source a group of elderly patients with T2D as well as normoglycemic patients all living in the same long term care residence had increased proportions of oleic acid and decreased proportions of PUFA in erythrocyte membranes compared to baseline measures where residents' main fat source was sunflower oil (Perona et al., 2007). Lower proportions of SFAs were observed in phospholipids, as well as higher MUFA/PUFA and MUFA/SFA ratios in the phospholipids of both groups. The amount of membrane G proteins and PKC were significantly lower in the diabetic group compared to the control group at baseline, with olive oil significantly decreasing G proteins in both groups and PKC in the control group (Perona et al, 2007). Another observation from this study was that feeding virgin olive oil increased the amount of DHA in the membranes of patients with T2D. This is possibly due to changes to delta 5 and 6 desaturase activity, the enzymes involved in the metabolism of essential fatty acids to DHA and EPA. Increased membrane PUFA/SFA increases membrane fluidity, which likely affects membrane function by altering lipid and protein interactions. G proteins are involved in insulin secretion from beta cells by regulating signal transduction by cAMP second messengers (Robertson et al., 1991; Escriba et al., 1997). G protein localization and activity is associated with protein-lipid interactions, therefore changes to fatty acids in membranes that in turn alter their properties can potentially change G protein activity and insulin secretion (Escriba et al., 1997).

It has been suggested that increased proportion or amount of oleic acid and decreased linoleic acid in the diet limits competition for the delta 6 desaturase pathway, the metabolic path for synthesizing the n-3 fatty acids EPA and DHA from alpha linolenic acid and arachidonic acid from linoleic acid (Rao et al., 1993). Western diets contain a high n-6:n-3 ratio which may favour the formation of the inflammatory metabolites of arachidonic acid, while lowering linoleic acid in the diet could increase EPA and DHA, favouring anti-inflammatory metabolites (Giugliano et al., 2006).

Another example of a physiological change in response to dietary fat can be seen in the adenylate cyclase signal transduction pathway (reviewed by (Clandinin et al., 1991)). Adenylate cyclase is regulated by a membrane receptor, which when bound stimulates cAMP (reviewed by (Clandinin et al., 1991)). Changing the proportions of oleate and linoleate in the diet changed the acyl tails present on phospholipids, causing changes to adenylate cylase activity, an enzyme stimulated by glucagon (Neelands and Clandinin, 1983). Increases in dietary oleic acid dose dependently increased proportions in the membrane as well as increased the amount of stimulated adenylate cyclase. In contrast, more n-6 PUFA in the membrane was associated with decreased activity (Clandinin et al., 1991). Another important membrane bound receptor is the insulin receptor. Feeding diets with different PUFA/SFA (P/S) ratios has been shown to alter adipose tissue membranes as well as insulin sensitivity (Clandinin et al., 1991). Increasing PUFA in the diet also increased phospholipid PUFA. Cells with higher PUFA content bound significantly more insulin, while insulin binding decreased with increased cell SFA (Clandinin et al., 1991). Dietary fat induces several changes which can alter physiological responses including: de novo phospholipid synthesis, and transfer of acyl chains by phosphlipase, acyltransferase and methyltransferase (reviewed by (Clandinin et al., 1991)). Changes to plasma membrane and immune cell phospholipids in response to dietary lipid intake have been shown in the brain, testes, muscle, small intestine, colon, spleen and mesenteric lymph nodes (Clandinin et al., 1991).

The current literature suggests that it is possible for changes in TG structure between oils with the same proportions of fatty acid to affect metabolism and in turn other physiologically processes. This includes blood lipid composition, membrane composition and function in many tissues, and immune function. This is important as many of these changes have implications for developing chronic disease. Much of the current literature in this area focuses on type of fat (MUFA, PUFA, or SFA) or specific fatty acids. Some studies have looked at the effects of TG species however the focus has been on those with a sn-2 SFA. Different TG species may alter the metabolic fate and biological effects of dietary fatty acids and further investigation into various fatty acids, including MUFA, in specific TG positions is worthwhile. Oils with similar fatty acid compositions, like olive oil and high oleic acid Canola oil, could potentially have varying health effects as the TG composition of the new Canola oil is unknown.

Diet Induced Obesity

Different animal models have been used in the study of obesity, including genetically obese models and models that become obese when fed a high energy diet (Augustine and Rossi, 1999; Li et al., 2008). Genetic models of obesity allow a specific gene to be targeted, such as the leptin receptor or production of leptin (Augustine and Rossi, 1999), while DIO models can be polygenic and the development of obesity depends on both environment and genetics (Li et al., 2008). The Sprague Dawley rat is an outbred rodent model that is susceptible to developing obesity when fed a palatable, high energy diet (Levin et al., 1997). Sprague Dawley rats have been shown to develop two phenotypes, where rats either develop DIO on a high energy diet, or are diet resistant and grow at the same rate as those fed a chow diet (Levin et al., 1997). As in human obesity, both genetics and environment play a role in obesity development in Sprague Dawley rats and leads to the development of hyperinsulinemia, insulin resistance and other characteristics of metabolic syndrome (Levin et al., 1997, Li et al., 2008). This is beneficial compared to genetic models, such as the ob/ob mouse which is leptin deficient or the leptin receptor deficient fa/fa Zucker rat, as single gene mutations causing obesity in humans occurs only in a small population (Speakman et al., 2008). Through the use of knockout and genetic models, very specific physiologic pathways and molecules related to obesity can be studied to provide further insights into the development of obesity and possible treatments (Speakman et al., 2008). A limitation to these models is that the observed outcomes in obese models can be due to either the gene mutation itself or other complications associated with obesity (Speakman et al., 2008). For example, a leptin deficiency is known to modulated inflammation and immune function (Fantuzzi and Faggioni, 2000); therefore, alteration in immune function changes could be a combination of leptin deficiency as well as the development of obesity. The Sprague Dawley DIO model has similarities to the development and outcomes of human obesity that make it useful for examining outcomes related to obesity, diabetes, and the metabolic syndrome.

Summary

Incidence of T2D in Canada has been increasing at an alarming rate. As such, interventions are needed that target specific populations to prevent both the development and progression of the disease. Uncontrolled blood glucose concentrations in individuals

with T2D leads to adverse consequences, including significantly increased risk of macrovascular and microvascular complications as well as overall mortality. It has been shown that carefully tailored diet intervention programs can be effective in lowering risk of developing diabetes and improving weight loss and blood glucose control.

T2D is characterized by chronic low grade inflammation. Increased exposure to inflammatory molecules may be one mechanism involved in the development of complications associated with diabetes. The effects of dietary fat on inflammatory processes have been studied, mainly examining the effect of PUFA. Through evidence provided by epidemiological studies, especially in populations consuming a Mediterranean style diet, MUFA has also been found to provide favourable health effects that lower the risk of developing diabetes and cardiovascular disease. In these epidemiological studies, high MUFA diets have been associated with lower concentrations of circulating CRP and IL-6, important acute phase proteins that are associated with chronic disease risk. The influence of MUFA on T cell function is not clear; however, animal studies feeding high MUFA diets or cell culture studies with a MUFA treatment report that both T and B lymphocyte function is reduced. It is also likely that MUFA provides anti-inflammatory effects, however this has not been well studied. Studies examining the benefits of high MUFA diets have almost exclusively used olive oil as a MUFA source.

A new high oleic acid Canola oil with the same proportion of oleic acid as olive oil may provide the same health benefits as olive oil, however different TG species may be present in the oil. Due to differences in the metabolism of TG and fatty acids, specifically the sn-2 fatty acid, different immune and metabolic outcomes may be observed in feeding the new Canola oil compared to olive oil. This has not been previously studied and the TG species present in the high oleic acid Canola oil has not been characterized. If the new oil is found to be similar to olive oil it would be an acceptable substitute to Albertans for olive oil in a diet intervention based on the Mediterranean diet pattern.

Table 3. Studies of disease and dietary factors affecting immune function

Author	Subjects	Duration	Intervention	Outcomes
Plotkin 1996	6 week old Zucker rats Obese fa/fa and lean	18 weeks	Free access to chow to develop obesity Pairs of obese and lean rats injected with C. albicans at various doses	Obese rats were more susceptible to infection at lower doses Obese rats had lower phagocytic function
Esposito 2002	CNT (n=20) had normal glucose tolerance, no HT, and normal lipids IGT (n=12) subjects had no CV complications	Single time point	Clamp – plasma glucose raised to 15 mmol/L and maintained for 240 min by 30% glucose infusion at varying rates Glucose pulse – 3 bolus infusions at 2 h intervals Glutathione – pulses + glutathione Plasma TNF alpha, IL-6, IL-18	(Background: IL-6 increased risk of developing T2D, IL-6 and TNF alpha higher in people with T2D) Hyperglycemia induced increases in cytokines in all subjects, peaks were higher in IGT subjects and took longer to return to basal Glutathione prevented any increase from basal in both groups Pulses had more of an effect than continual hyperglycemia, and the glutathione response suggests cytokines increase due ox High glucose increases cytokine production TNF and IL-6 have also been shown to induce hyperglycemia and insulin resistance
Ruth 2008	Zucker rats Fa/fa obese and lean N=10/group	8 weeks	Diets containing 8.5% fat with either: Control (0% w/w CLA), 0.4% w/w c9t11, 0.4% t10c12, or a mix (0.4% each of c9t11 and t10c12)	Splenocytes of obese rats fed control produced ↑ IgA, IgM, IgG, IL-1β, IL-6, and TNF-α Splenocytes of obese rodents fed t10c12 CLA produced ↓TNF-α and IFN-γ (LPS) ↓ IL-2 produced by splenocytes of obese rats compared to lean

				CLA was incorporated into phospholipids of CLA fed rats
Tanaka 1998	34 fa/fa obese Zucker rats 33 lean Zucker rats	38 weeks 5 weeks to determine genetically obese rodents from leans	T-cells in blood, spleen and thymus measured at different time points to observe effect of obesity and age	8 weeks – reduced T-cells in blood of obese rodents compared to lean 11 weeks – lower T-cell numbers in spleen and thymus of obese rats compared to lean and lower T-cell proliferation Overall, T-cell lymphopenia observed in obese rodents
Ruth 2009	Obese JCR:LA-cp, n=10/group Lean JCR:LA-cp fed control only, n=5	3 weeks	Rodents fed either: Control (0% EPA+DHA) Low FO (0.8% EPA+DHA) High FO (1.4% EPA+DHA)	Splenocytes of obese rodents produced ↓ IFN-γ and IL-1β when stimulated Both FO diets ↓ IFN-γ and IL-4, IL-2 was not different (Con A) HFO ↓ IL-β and ↑IL-10 FO fed rats had more DHA in lipid rafts
Blewett 2009	8 week old lean and obese JCR:LA-cp rats N=20/group	3 weeks	Rodents fed diets containing either: 0% vaccenic acid (VA; control) or 1.5% w/w VA	Obese rats fed control had ↑ spleen macrophages, total T-cells, and cytoxic and helper T-cells, ↑ IL-6 production with Con A compared to leans MLN cells of obese rodents had ↓ T-cells, cytoxic T-cells and ↑CD25+ cells and produced ↑ IL-2, IL-10, IFN-γ and TNF-α (Con A) Feeding VA ↓ IL-2, IL-10, and TNF-α produced by spleen compared to control In MLN feeding VA led to IL-2 and TNF-α production similar to lean animals

Table 4. Studies of MUFA and T2D risk

Author	Subjects	Duration	Intervention	Outcomes
Martinez-Gonzalez 2008	13380 participants in Spain with no diabetes	Average follow up 4.4 years	FFQ and Mediterranean diet adherence assessed at baseline T2D cases recorded throughout study follow up period	↑ Mediterranean diet score associated with lower diabetes risk Compared to low adherence, incidence ratio for moderate adherence was 0.41, and 0.17 for high adherence
Trichopoulou 2003	22043 participants in Greece	44 month follow up	FFQ taken and Mediterranean diet score assessed Morbidity and mortality incidence recorded throughout	↑ Mediterranean diet score was associated with lower risk of mortality
Paniagua 2007	11 insulin resistant subjects, non DB No drugs/insulin BMI>25	3 28 day periods, one for each diet	Compared high SFA, MUFA or CHO Randomized cross over OO provided MUFA source	GLP-1 lowers blood glucose and may restore beta cell sensitivity Again, plasma lipid reflective of diet Fasting glucose was lower with MUFA and CHO than SFA, fasting insulin was unchanged HOMA-IR was lower for MUFA than SFA and CHO Lipid measures same for each diet, except HDL was lowered in CHO diet (Fasting)

				Postprandial glucose AUC was higher with CHO Postprandial TG AUC was lower for CHO than MUFA and SFA Fasting GLP-1 was similar between diets, but postprandial GLP-1 was lower with CHO than MUFA and SFA Fatty acid composition of fasting plasma PL is a marker of fat intake weeks to months prior to sample (22)
Rumawas 2009	Framingham Offspring Cohort 2730 participants with no diabetes	7 year average follow up	Mediterranean diet adherence assessed by FFQ Measures of metabolic syndrome compared between 5th (1991-1995) and 7th examination (1998-2001)	↑ Mediterranean diet adherence was associated with ↓ HOMA-IR, waist circumference, fasting plasma glucose, plasma triglycerides and ↑HDL
Jiang 2002	83818 participants from the Nurse's Health Study	16 years average follow up	New diabetes cases were recorded and associated with different levels of nut consumptions (never, <once/week, 1-4/week, >5)	↑ Nut consumption associated with ↓ risk of developing diabetes
Taousi 2002	5 week old Wistar rats	4 weeks	Rats fed either either high fat (58% energy from fat)	Feeding n-6 PUFA ↓ GLUT4 and the insulin

			diets: High n-3 PUFA High n-6 PUFA Diets induced hyperglycemia and hyperinsulinemia to measure and compare insulin signalling	receptor in muscle while n-3 PUFA had no effect
Carter 2010	13197 participants in NHANES III aged 18-90 years	6 years	FFQ and 24-hour recall used to assess Mediterranean diet adherence	↑ Mediterranean diet adherence was associated with improved total cholesterol to HDL ration, serum insulin, HbA1C, HOMA-IR, CRP and ↑HDL
Esposito 2009	901 outpatients with T2D in Italy	2001-2007, measures were cross sectional	Measured HbA1C, and pre- and postprandial plasma glucose concentration Mediterranean diet score assessed	↑ Mediterranean diet adherence associated with ↓ BMI, waist circumference, HbA1C, and postprandial plasma glucose concentration
Paletas 2010	226 overweight or obese participants	Single time point	Measured diet history, anthropometrics, fasting blood glucose concentration, and Mediterranean diet adherence	↑ Mediterranean diet adherence was associated with ↓ incidence of metabolic syndrome, LDL cholesterol and postprandial plasma glucose concentration ↑ Prevalence of metabolic syndrome in

				Greece is associated with ↓ adherence to a Mediterranean diet
Vessby 2001	162 healthy participants	3 months	Participants received diets to maintain weight either high is SF (17% SF, 14% MUFA) or MUFA (8% SF, 23% MUFA) Both diets provided 38% energy from fat	Consuming high SF diet ↓ insulin sensitivity and ↑ LDL cholesterol Consuming high MUFA diet ↑ insulin sensitivity and ↓ LDL
Gill 2003	35 hypercholesterolemic participants	6 weeks	Participants received one of the following diets providing 30% energy from fat: Low MUFA (7.8 % MUFA) Moderate MUFA (10.3%) High (13.7%)	MUFA dose dependently ↓ LDL cholesterol
Rivellese 2008	11 T2D men Overweight/obese A1C~7%	3 weeks run-in 3 weeks/diet	High MUFA vs. High SFA Other components similar, lower cholesterol in MUFA Sources: Butter vs. OO	LDL FA composition following diet reflected diet No change in weight, HDL or fasting TGs Lower LDL with MUFA Postprandial response for TG, insulin, glucose, and FFA not different Apo-B48 in CM lower, but not significant, for MUFA Apo B48 in large VLDL decreased faster in MUFA, continue to increase in SFA Greater HSL and LPL

				activity in adipose following MUFA diet
Brehm 2009	T2D, relatively well controlled Overweight/obese N=124 (29 dropped out) 36 for extension	1 year + 18 month follow up for extension study	High MUFA diet vs. High CHO Similar SFA and pro Diets allowed for moderate weight loss ~ ½ lb/week	No difference in weight loss Significant reduction in BP, A1C, fasting insulin and glucose, and insulin resistance and increase in HDL for both groups No significant changes 18 months later – participants were able to maintain positive health outcomes achieved during the initial study period
Egert 2010	37 healthy volunteers, BMI <27	2 week wash in with high fat, high SFA diet 4 weeks on study diet	High fat, high MUFA vs. Low fat, high MUFA MUFA 51% of fat High fat – 40% energy Low fat – 29% energy PUFA, SFA, fiber, carb, cholesterol, antioxidants similar for both diets MUFA source: rapeseed and high oleic sunflower oil, high MUFA margarine	Both diets decreased TC, LDL, HDL, LDL oxidation, plasma oxLDL, LDL size compared to wash in No difference in plasma TAG Even though HDL was lowered, the LDL/HDL ratio still improved Both diets led to MUFA rich LDL particles oxLDL correlates with LDLc, no difference in ratio of oxLDL/LDLc Amount of fat and CHO

				may not be as important as type and quality
Trautwein 1999	Syrian golden hamsters	5 weeks	Animals were fed diets providing 5% energy from fat Fat was provided by either unrefined or refined rapeseed oil, unrefined or refined olive oil, sunflower oil (↑ PUFA), or palm stearin (↑SF)	Feeding SF ↑ total cholesterol, MUFA and PUFA ↓ total cholesterol Lowest total cholesterol was observed in animals fed either rapeseed diet and high PUFA MUFA rich fats ↑ oleate in liver cholesteryl esters
Ramirez-Tortosa 1998	Rabbits N=36	Received atherogenic diet for 50 days and experimental diets for following 30 days A control group received regular chow for the initial 50 days	Following induction of atherosclerosis animals were randomized to receive a diet containing 98.25% standard chow and 1.75% fat from: fish oil, refined olive oil, virgin olive oil, or sunflower oil A control group did not get the atherogenic diet	Atherogenic diet ↑ plasma cholesterol and TG but ↓ with experimental diets Fish oil ↑ LDL oxidation susceptibility Sunflower oil, virgin olive oil, and refined olive oil were similar to control for LDL oxidation MUFA and n-3 PUFA diet improved blood lipids
Kotake 2004	db/db mice (genetically diabetic) and non-diabetic mice	1 week	Diabetic and non-diabetic mice received a diet containing either 38% energy from fat (26% from MUFA) or 13%	In non-diabetic mice ↑MUFA ↓ serum TG compared to low fat In diabetic mice ↑MUFA ↓serum glucose

			energy from fat (3% MUFA)	concentration and improved impaired glucose tolerance
Panagiotakos 2007	1514 men and 1528 women aged 18-89 years Either with T2D/impaired fasting glucose or normoglycemic	Single time point	FFQ used to assess Mediterranean diet adherence Measured HOMA-IR	Normoglycemic participants in the upper range for Mediterranean diet adherence had 7% lower plasma glucose, 5% lower plasma insulin, and 15% lower HOMA-IR Diabetic/impaired fasting glucose participants had similar results but it did not remain significant after adjustments for age, sex, weight, physical activity, smoking, hypertension, and cholesterol
Yokoyuma 2008	Patients with T2D (n=12) and healthy participants (n=10)	Single time point	Randomized crossover study Participants received 250 ml of either a ↑ MUFA enteral formula or a ↑ CHO enteral formula after a 12 hour fast. Blood glucose and insulin was measured at various time points following consumption of the formula. There was a 7	↑ MUFA formula ↓ post prandial plasma insulin and glucose concentrations in all subjects compared to the ↑CHO formula

			day washout and participants received the other formula	
Picianato 1998	Male rats aged 18-22 days	6 weeks	Rats were fed diets enriched to 10% energy fat with either SF, n-6 PUFA from soybean oil, MUFA from olive oil or a diet containing a balance of all oils	Feeding olive oil and soybean oil ↑ glucose oxidation and ↑ insulin secretion both in vivo and in isolated pancreatic islet cells GLUT2 content ↑ after feeding OO and ↓ with SF feeding
Welters 2004	insulin-secreting rat β-cell line BRIN-BD11		Several fatty acids were used to determine their effect on beta cell apoptosis and cytokine production Including SFAs, oleate, and palmitoleate (measured viability of the cell culture with each FA)	LCSFAs palmitate and stearate caused significantly increase in cell death Oleate and palmitoleate were well tolerated, these monounsaturated FAs also attenuated cell death in the presence of SFAs No change in proliferation MUFA also improved cell viability in cells exposed to IFN-gamma and IL-1beta
Maedler 2003	Human cultured islet cells		Exposed cells to saturated, unsaturated or a mixture	MUFA protective against apoptosis MUFA improved insulin function of beta cells when following

				exposure to high glucose and palmitic acid causing loss of function
Estruch 2006	772 participants with ↑ cardiovascular disease risk 55-80 years of age	3 months	Participants were randomized to consume a diet that was either: Low fat Mediterranean diet with an olive oil MUFA source (1 L olive oil/week) Mediterranean diet with a nut MUFA source (30 g/day)	Both Mediterranean diets ↓ plasma glucose, systolic blood pressure, and total cholesterol to HDL ratio Only olive oil ↓ CRP
Mena 2009	102 participants with T2D or >3 risk factors for cardiovascular disease	3 months	Serum inflammatory markers were measured at baseline and 3 months Participants were randomized to consume a diet that was either: Low fat Mediterranean diet with an olive oil MUFA source Mediterranean diet with a nut MUFA source	Both Mediterranean diets ↓CD49d (adhesion molecule) and CD40 (pro-inflammatory ligand), IL-6 and soluble intercellular adhesion molecule-1 A low fat diet had no effect on CD49d or CD40 Only olive oil ↓ CRP
Dai 2008	345 male twins	Single time point	Mediterranean diet assessed by FFQ Plasma IL-6 and CRP concentrations measured	Mediterranean diet adherence was associated with ↓ IL-6 but not CRP Within pair diet adherence was significantly associated with IL-6, 1 unit

				difference in diet score was associated with a 9% ↓ in IL-6
Shaikh 2008	Human T2 cells, transporter associated with antigen processing deficient and expressing Kb – the mouse MHC I allele		Palmitic acid vs. Oleic acid Assessed apoptosis, apoptosis index calculated using difference in apoptosis of treated cells and BSA cells Peptide loaded to MHC I	Lipid overload – accumulation of FA in non-adipose tissue APC were resistant to lysis when treated with PA (dose dependant) vs. BSA, no effect of OA Ability of APC to activate CD8+ naive T-cells: less activation with PA(45%) vs. BSA (64%) - SIG, more activation with OA (70%) vs. BSA – not sig PA treated cells had lower MHC I surface levels, OA lowered MHC I levels but to a lesser extent PA inhibited APC-CTL conjugation dose dependently, no effect for OA OA treatment was protective in presence of PA PA=impaired T-cell activation and antigen presentation

Chapter 2: Rationale and Objectives

The prevalence of T2D has increased in conjunction with the growing obesity epidemic, with approximately 1 in 17 Canadians diagnosed with the disease as of 2007 and an estimated 2.8 million cases by 2010. Diet is a key factor in diabetes prevention and recommendations are outlined in the Canadian Diabetes Association clinical practice guidelines (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). The PANDA (Physical Activity and Nutrition for Diabetes in Alberta) project is aimed at developing and implementing a lifestyle intervention program for Albertans with T2D, which will help in glycemic control and prevent complications associated with the disease. Based on positive outcomes of studies of the Mediterranean diet (Martinez-Gonzalez et al., 2008; Trichopoulou et al., 2003; Willett et al., 1995), the nutrition guidelines for PANDA will follow a Mediterranean diet pattern tailored specially for Albertans. The fat content of the Mediterranean diet is higher in MUFA and lower SF than commonly consumed by North Americans (Willett et al., 1995). A diet higher in MUFA has been reported to improve several risk factors associated with the development or complications of T2D including body weight, blood glucose concentration, blood lipids, insulin resistance, antioxidant capacity, blood pressure and inflammation (Ros, 2003). Furthermore, populations that traditionally consume a high MUFA, low SF diet, such as the Mediterranean diet, have lower incidence of cardiovascular disease and T2D (Martinez-Gonzalez et al., 2008; Trichopoulou et al., 2003). Based on data supporting a high MUFA diet the CDA recommends that most dietary fat come from a MUFA source in the most recent Clinical Practice Guidelines (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008).

Studies examining the beneficial health effects of increasing the MUFA content of the diet have been performed almost exclusively using olive oil. Canola oil is also a rich source of the MUFA oleic acid. Traditional Canola oil contains approximately 60% of its fatty acids as oleic acid compared to 70% in olive oil (Canola Council of Canada, 2006;Aranda et al., 2004). Both oils contain triolein as a main TG species. Recently, a new high oleic acid cultivar of Canola has been developed with the same proportion of oleic acid as olive oil. Due to the same MUFA content in the oils, the new high oleic acid Canola oil may be predicted to have the same health benefits as olive oil. However, this translation may not be quite as straight-forward as it has been shown using other oils, as

differences in TG species may alter metabolism (reviewed by (Karupaiah and Sundram, 2007)). The TG species distribution in the new Canola is currently unknown. Based on the composition of traditional Canola oil, it would be expected that the high oleic acid Canola oil would contain a large proportion of triolein as well as TG containing linoleic and linolenic acid. In olive oil triolein constitutes approximately 51% of the TG species present, while 20 to 30% of the TG contain the saturated fatty acid palmitic acid in the sn-1 position with oleic acid occupying the other positions (Aranda et al., 2004). Although the two oils contain the same amount of oleic acid, the component commonly attributed with the health benefits observed in olive oil rich diets, it is likely there are differences in the TG species present in the oils.

Enzymes involved in lipid digestion, including lingual and pancreatic lipase, cleave fatty acids specifically at the sn-1 and sn-3 positions, releasing two free fatty acids and a 2-monoglyceride (Bracco, 1994; Mu and Hoy, 2004). Each component can then be metabolized through different pathways depending on the properties of the free fatty acids and monoglyceride (Mu and Hoy, 2004; Hunter, 2001). Properties that dictate the metabolic path utilized include fatty acid chain length and degree of unsaturation (Hunter, 2001). Fatty acids either directly enter the liver via the portal vein or enter lymph esterified into TG as a component of chylomicrons (Hunter, 2001). The sn-2 fatty acid of a monoglyceride is often maintained throughout metabolism. Because of these differences it is possible that dietary oils containing different TG species could lead to varying metabolic outcomes, even with a similar fatty acid profile.

Specific dietary fatty acids are known to alter immune function (Kew, 2003;Yaqoob, 1998;Jeffery, 1997) and this is one aspect that may be affected by the different TG species between the olive and high oleic acid Canola oil. It is also likely that TG storage in the liver and adipose tissue may be altered, specifically the sn-2 position fatty acid. Furthermore, phospholipid fatty acid composition could be affected, with implications for cell membrane structure and therefore, cell signalling and function (Clandinin et al., 1991; Escriba et al., 1997).

Increasing prevalence of T2D in Alberta and Canada has been steadily increasing, which has implications for lower quality of life in diabetes patients as well as an increased burden on the economy with increased health care costs (Johnson et al., 2009b).

Preventing both the development of the disease in high risk populations as well as preventing further complications in individuals diagnosed with diabetes is extremely important. A nutrition toolbox based on a Mediterranean style diet pattern tailored to Albertans is being developed as part of a lifestyle intervention for people in Alberta with diabetes. Canola oil is well accepted in an Albertan diet because it is locally produced, making it relatively inexpensive, and has a milder flavour compared to olive oil. The new high oleic acid Canola oil may have similar health benefits as olive oil, and as such would make a Mediterranean style diet more attainable for people living in Alberta.

Objectives

The objective of this study is to determine whether high MUFA diets that have the same fatty acid content but differ in TG species (e.g. stereospecific position of fatty acids on TG) have similar metabolic effects that could alter risk for developing T2D in a rodent model of diet induced obesity (DIO). To address this, two high fat diets containing different MUFA sources, either olive oil or high oleic acid Canola oil, were fed. A high fat diet containing a lower proportion of fat as MUFA, with a greater proportion of SFA and PUFA was used to examine and compare the effects of the amount of MUFA and fatty acid composition on the desired outcomes. A lower fat, high carbohydrate diet with the same fat composition as the low MUFA and high PUFA and SFA diet was used to examine the effect of the amount of fat on metabolic and immune outcomes.

Objective 1: To determine whether feeding high MUFA diets with an olive oil or high oleic acid Canola oil MUFA source have similar effects on acquired immune function in a DIO model compared to feeding a diet higher in PUFA and SFA. T and B cell function were assessed by measuring ex vivo cytokine production and proliferation following mitogen stimulation and by measuring immune cell phenotypes and distribution present in peripheral immune tissue (spleen) and gut associated lymph tissue (GALT), as represented by mesenteric lymph nodes (MLN).

Objective 2: To determine if feeding high oleic acid Canola has similar glycemic effects as feeding olive oil in an animal model of DIO with insulin resistance. The effect on glycemic response was assessed by determining plasma glucose and insulin concentrations after an oral glucose challenge

Objective 3: To determine and compare the fatty acid composition and positional distribution of TG and phospholipids in the intestine, splenocytes, and major storage tissues (liver and adipose tissue) as a possible mechanism for changes in immune function and insulin sensitivity. We also characterized and compared fatty acid composition and TG sn-2 fatty acids in olive oil and high oleic acid Canola oil.

Outline

Chapter 1 was a literature review to discuss the current literature in the area of dietary MUFA, its association with T2D risk and immune function, and the potential for triglyceride species composition of oils to impact lipid metabolism rather than the fatty acid composition alone. It is apparent in the literature that knowledge gaps exist in the study of dietary MUFA, particularly its effect on immune function.

Chapter 2 provided rationale for completing the current study based on the current literature. It also outlines the objectives that will be addressed in this experiment.

Chapter 3 describes the methods that were used to address the objectives of the experiment.

Chapter 4 provides the results obtained from the experiment.

Chapter 5 contains the discussion of the study outcomes in the context of the current literature, the possible meaning of the results and the application of them to current knowledge of MUFA and T2D risk and the direction of future studies.

Chapter 3: Methods

Animals and Diets

Male Sprague Dawley rats (n=24, received at 8 weeks of age, experiment 1; n=32, received at 6 weeks of age, experiment 2) were obtained from Charles River Laboratories (Montreal, Quebec, Canada) and housed 2 per cage with 12/12-hour light/dark cycle and free access to water. Procedures were reviewed and approved by the Committee of Animal Policy and Welfare of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and conducted in accordance with the Canadian Council on Animal Care guidelines.

Four nutritionally adequate diets were designed for this experiment. Two diets were used to induce obesity for 3 weeks; a standard high fat diet providing 40% calories from fat (TD.84172, Harlan Teklad, Madison WI, with 20% w/w oil) (n=42) and a low fat high carbohydrate diet (LF) providing 25% calories from fat (TD.06206, Harlan Teklad, Madison WI, with 12% w/w oil) (n=14). Diet compositions are described in Table 5. Following the initial 3 weeks where rats were fed the high fat control diet (HF), animals in the high fat diet group were randomized to either continue on the high fat control diet or to receive one of two high MUFA diets; where 30% of the fat was replaced by either olive oil (OO) or high oleic acid Canola oil (HCO). The oil composition and the macronutrient composition of the four experimental diets are presented in Table 6 and 7. The actual fatty acid composition, analyzed by gas liquid chromatography (GC) (Blewett et al., 2009) is presented in Table 8. All diets met the essential fatty acid requirements of the rodent, had similar P/S ratios and were vitamin E and phenol balanced. Animals received their respective diet for an additional four weeks.

Table 5. Composition of experimental diets (g/kg)

	Diet	
	LF	HF
Casein	317.6	337.5
L-Methionine	2.94	3.13
Dextrose, monohydrate	275	261
Cornstarch	264	250
Cellulose	58.8	62.5
Fat*	120	200
Mineral Mix, Bemhart-Tomarelli (170750)	59.8	63.6
Sodium Selenite (0.0445% in sucrose)	0.353	0.375
Manganese Sulfate, monohydrate	0.28	0.30
Vitamin Mix, AOAC (40055)	11.8	12.5
Inositol	7.35	7.81
Choline Chloride	1.62	1.72

*See Table 5 for fatty acid composition

Table 6. Oil composition of experimental diets (%Fat provided by oil)

	Diet		
	LF&HF	OO	HCO
Sunflower Oil	39	23	24
Flax Oil	4	4	1
Tallow	57	40	45
Olive Oil	-	33	-
High n-9 Canola*	-	-	30

*High n-9 Canola oil was provided by Bunge Nexera

Table 7. Macronutrient composition of experimental diets (%Energy)

	Diet	
	HF	LF
Carbohydrate	39	49
Fat	38	25
Protein	23	26

Table 8. Fatty acid composition of the experimental diets (g/100 g fatty acids)

	Diet		
	HF & LF	OO	HCO
16:0	15	16	13
16:1n9	0.35	0.28	0.3
18:0	45	36	37
18:1n9	14	27	27
18:1c11	0.23	1.1	1.2
18:2n6	23	17	18
20:0	0.39	0.39	0.47
18:3n3	2.3	1.9	1.9
20:3n6	0.38	0.25	0.3
22:4n6	0	0.1	0.04
Total SFA	60	52	51
Total PUFA	25	20	20
Total MUFA	14	28	29
PUFA:SFA	0.4	0.4	0.4

Insulin and Glucose

An oral glucose tolerance test (OGTT) was performed one week prior to euthanasia. Animals were fasted overnight and weighed prior to the OGTT procedure. Approximately 50 µl blood was collected from the tail vein in a heparinized tube (Thermo Fisher Scientific, Ottawa, ON), transferred to an Eppendorf tube (Thermo Fisher Scientific) and stored on ice. Blood was also collected into a glucometer (Accu-chek Compact Plus, Roche, Laval, QC) strip to measure blood glucose concentration. The glucose dose per animal was calculated as 1 g glucose/kg body weight. Following the initial blood collection glucose was administered orally. Blood samples for the glucometer and insulin assay were taken at 10, 20, 30, 60, 90 and 120 minutes after the glucose was given. Blood samples for insulin were centrifuged to reserve plasma in Eppendorf tubes and frozen at -80°C until analysis for insulin. The insulin assay was performed using a commercially available kit (Alpco, Salem, NH, USA).

Lymphocyte Isolation

Following four weeks on the experimental diets animals were euthanized using a CO_2 chamber. At necroscopy, blood, intestine, epididymal fat pads, peri-renal fat, liver,

spleen and mesenteric lymph nodes were collected aseptically, weighed and stored at -80°C until later analysis.

Nylon mesh screens were sterilized in 70% ETOH overnight. Splenocytes and MLN cells were isolated by pushing cells through the screens using the barrel of a sterile syringe in 5g/L BSA (Sigma-Aldrich, Oakville, ON, Canada) in Krebs-Ringer-Hepes (KRH) buffer. Each sample was centrifuged at 1000 rpm for 10 minutes to pellet cells (Jouan, Perkin Elmer, Woodbridge, ON, Canada). Splenocytes were treated with lysis buffer (ACK; 155 mM NH4Cl, 0.1mM EDTA, 10mM KHCO3, pH 7.4 (Simga Aldrich, Oakville, ON)) to lyse red blood cells and centrifuged. After discarding the supernatant the samples were washed with 10 ml of KRH buffer, resuspended and centrifuged again. Fifteen ml complete culture medium (CCM; RPMI 1640 with antibiotic and antimycotic, 5% v/v fetal calf serum (FCS; Invitrogen, Burlington, ON, Canada)) was added to the pellet of both MLN and spleen samples, resuspended and counted on a heamacytometer (Thermo Fisher Scientific, Ottawa, ON, Canada) using 20 µl of the cell suspension and 20 µl of trypan blue dye (Sigma).

Cell Phenotyping

An immunofluorescence assay was used to determine cell phenotypes. V-well plates were pre-conditioned with 4g/L FCS (Invitrogen) in phosphate buffered saline (PBS) for 30 minutes. An aliquot of each sample (100,000 to 400,000 cells) was added to each well and washed by adding 200 µl buffer followed by centrifugation. A 20 µl aliquot of fluorescent labelled antibody was added to the wells according to the template (antibodies by well: CD3/28/8/4, CD152/25/8/4, CD71/8/4, CD3/45RA/27, CD284/68/11, CD161/3, CD28/80/152, IgM, IgG, IgA, CD3/25/FoxP3/4) and incubated for 30 minutes in refrigerator. The cell subsets identified by each antibody are described in Table 9. The plate was washed twice with 200 µl buffer and centrifuged. The supernatant was discarded and the remaining sample vortexed. Two hundred µl 1% w/v paraformaldehyde (Thermo Fisher Scienfic) was added to each well. The proportion of cells that were positive for each marker was determined by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA, US) according to the relative fluorescence intensity using Cell-Quest software (Becton Dickinson, San Jose, CA, US). One, two and three colour immunofluorescent analysis was performed.

Table 9. Cell subsets identified by antibody labels

Antibody	Cells Identified
CD3	T-cells
CD45RA (BD Bioscience, Mississauga, ON)	B-cells
CD27	TNF receptor
CD68 (Cedarlane, Burlington, ON)	Monocytes/macrophages
CD284 (Abcam, Cambridge, MA, US)	Toll like receptor 4
CD11 (BD Bioscience)	Integrins, involved in cell adhesion
CD3-/CD161+ (CD161; BD Bioscience)	NK cells
CD80/CD28	T-cell co-stimulatory signal (ligand to CD28), present on activated B-cells
CD8	Cytotoxic T-cell, binds MHC class I
CD4	Helper T-cell, binds MHC class II
CD152 (BD Bioscience)	Cytotoxic T-lymphocyte antigen 4 (CTLA4) T-cell inhibitory signal
CD25	IL-2 receptor
CD71 (Cedarlane)	Transferrin receptor, activation marker on proliferating T-cells
IgM (BD Bioscience)	Primary response antibody
IgG (BD Bioscience)	Secondary response antibody
IgA (Cedarlane)	Antibody involved in mucosal immunity
CD28+CD3+CD8+ CD28+CD3+CD4+	CD28 stimulatory signal, activated T-cell marker
CD4/CD25/FoxP3	T-regulatory cells

*All antibodies supplied by eBioscience (San Diego, CA, US) unless otherwise indicated, all sources were mouse anti-rat

Proliferation and Mitogen Stimulation

The ability to produce cytokines by splenocytes and MLN cells was determined following mitogen stimulation of isolated splenocytes and mesenteric lymph nodes using Con A (MP Biomedicals, Montreal PQ), LPS (Sigma, Oakville, ON) and CD3/CD28 (eBioscience, San Diego, CA, US). This was done in two separate experiments, using a low dose of Con A (2.5 µg/ml) in the first experiment and a high dose of Con A (5µg/ml) in the second experiment. The concentration of LPS (100 µg/ml) and CD3/CD28 (5 µg/ml /5µg/ml) did not change. Three mls of cells at a concentration of $1.25x10^6$cells/ml

per cytokine for each sample and each mitogen was prepared. Mitogen (300 µl) was added to each sample at the specified concentration. For unstimulated cells 300 µl CCM per tube was added.

Following the addition of mitogen, cell suspensions were incubated for 48 hours at 37°C and 5% CO_2. To stop the reaction, tubes were centrifuged at 1500 rpm for 10 minutes and the supernatant collected and stored at -80°C until analysis for cytokines.

Commercial ELISA kits (eBioscience, San Diego, US) were used to measure the concentration of IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ concentration in supernatants following the manufacturer instructions. Amount of ^{3}H-thymidine (GE Healthcare, Mississauga, ON, Canada) uptake by cells was measured to estimate theability of cells to proliferate in response to mitogen stimulation. The cell suspension (200 µl) was added to wells of sterile U-bottom plates and 20 µl of mitogen was added in triplicate (Con A, LPS, CD3/CD28, unstimulated). Eighteen hours prior to harvesting, 10 µl ^{3}H-thymidine was added to each well. Cells were harvested the next day and counted using TopCount (Canberra Packard Canada, Mississauga, ON, Canada). Proliferation was expressed as a stimulation index calculated as the ratio of the amount of ^{3}H-thymidine incorporated by stimulated cells divided by the amount of ^{3}H-thymidine incorporated by cells incubated in the absence of mitogens (unstimulated).

Serum haptoglobin concentration was measured by ELISA using a commercial kit (Alpco, Salem, US) following the manufacturer's instructions. This is an acute phase protein used as a marker of systemic inflammation, similar to CRP.

Spleen and Jejunum Phospholipid Fatty Acid Composition

To determine the effect of dietary fat on spleen and jejunum phospholipid fatty acids a modified Folch method was used for lipid extraction (Field 1988). Briefly, potassium chloride (0.8 mL, 0.25% KCl) was added to spleen and gut samples and the mixture was transferred to a glass vial. Another 0.8 mL was used to rinse the plastic vial that contained the sample and transferred to the glass vial. In sequence 0.8 mL methanol, 2.0 mL chloroform:methanol (1:1), 2.7 mL chloroform and 2.5 mL chloroform:methanol (2:1) was added to the glass vial. Samples were vortexed and stored in the refrigerator overnight. The next day the bottom layer was taken off with a glass pipette and

transferred to smaller glass vials and dried under nitrogen on a heating block. Dried samples were stored at -35°C after flushing with nitrogen.

To separate phospholipids, a G-plate solvent was mixed using 160 mL petroleum ether, 40 mL diethyl ether, and 2 mL acetic acid. G-plates were heated at 110°C for 1 hour. Hexane (100 µl) was added to each sample and spotted on the G-plate and the G-plate was placed in a tank with solvent (Layne 1996). Once the solvent was approximately 1 cm from the top of the plate it was removed, dried and sprayed with ANSA. Under UV light the phospholipid band was identified and scraped into a tube using a razor blade. Samples were stored at -35°C until methylation for GC analysis.

For identification of phospholipid fatty acids, silica scraped bands were methylated by adding 1 ml of BF_3 and 2 mL of hexane to each sample. Samples were then capped, vortexed and placed in a sand bath for 1 hour at 110°C. They were then refrigerated overnight. The next day the hexane layer was taken off using a glass pipette and transferred to a GC vial. Samples were dried under nitrogen. Once dry, 75 µl of hexane was added to spleen samples and 500 µl hexane was added to gut samples and fatty acids were analyzed by automated gas-lipid chromatography (Agilent Technologies, Mississauga, ON, Canada) using a 100 m CP-Sil 88 fused capillary column (Varian Inc., Mississauga, ON, Canada) as previously reported (Cruz-Hernandez et al., 2004). All chemicals used for phospholipid fatty acid analysis were purchased from Thermo Fisher Scientific (Ottawa, ON, Canada).

Extraction of Total Lipids from Rat Liver and Perirenal Tissue and Purification of TAG

Lipids were extracted from rat liver and perirenal tissues by homogenizing the tissue in phosphate buffered saline (pH 7.4; 1 ml per 500 mg of tissue). Twelve volumes of chloroform/methanol (2:1, v/v) and two volumes of 1M calcium chloride were added to the resulting homogenate, mixed thoroughly, and then centrifuged to induce a phase separation. The organic phase was extracted into a fresh tube, and the remaining homogenate was re-extracted in a similar fashion with eight volumes of chloroform. The extracts were combined and the solvent was evaporated under nitrogen. Triacylglycerol (TAG) was purified from the extract by applying 50 mg of the total lipid extract (in chloroform) to a 20x20 cm glass-backed silica gel G thin layer chromatography (TLC)

plate that had been activated overnight at 100°C (Machery Nagel DC FergiplattenSil G-25). The plates were developed half-way in chloroform/methanol/acetic acid/water (60:30:3:1 v/v), dried, and developed fully in hexane-diethyl ether (80:20 v/v). The lipids were visualized by spraying with primuline (0.05% in 80:20 v/v acetone: water) and viewing under ultraviolet light. Spots corresponding to TAG species were identified based on comparison to a triolein standard. Sections of silica containing TAG were scraped and extracted as previously described, with up to 4 ml chloroform/methanol (2:1 v/v) and 2 ml 0.9% sodium chloride, then re-extracted with chloroform. The solvent was then evaporated under nitrogen and the extract was resuspended in a known volume of chloroform.

Analysis of the Positional Distribution of Acyl Groups at the sn-2 Position of TAG

The positional distribution of acyl groups at the *sn*-2 position of TAG was determined by partial pancreatic lipase digestion, followed by analysis of the resulting *sn*-2 monoacylglycerols (MAG). Approximately 5 mg of purified TAG were added to a borosilicate tube and evaporated under nitrogen. To this residue, 1.35 ml of incubation medium (0.05% deoxycholate, 2.2% $CaCl_2$, 1MTris, pH 8.0) were added along with a stir bar, and the mixture was equilibrated to 40°C with constant stirring. The lipase reaction was initiated by the addition of 100 µl of pancreatic lipase (Porcine Type 2 Lipase, Sigma-Aldrich, Oakville, ON), followed by 5 min of incubation at 40°C with vigorous stirring. The reactions were quenched by the addition of 2 ml chloroform/methanol (2:1 v/v). Negative control reactions were quenched prior to the addition of the lipase. The resulting organic phase was extracted into a fresh tube, and the reaction mixture was extracted a second time with 2 ml of chloroform/methanol (2:1 v/v). The resulting lipid extracts were evaporated under nitrogen, resuspended in 100 µl chloroform, then spotted on a boric acid (10%) impregnated TLC plate (Uniplate Silica G, Analtech), which had been activated overnight at 100°C. The plate was developed in hexane/diethyl ether/acetic acid (70:30:1 v/v). Spots corresponding to *sn*-2 MAG (using 15:0-MAG as a standard) were visualized with primuline spray as above and scraped into fresh tubes. Fatty acid methyl esters (FAMEs) were prepared by adding 1.2 ml 5% (w/v) sodium methoxide to the silica containing *sn*-2 MAG and incubating at room temperature for 30 min. The reaction was quenched with 1 ml water and the FAMEs were extracted with 2 x

2 ml of hexane. The FAMEs extract was evaporated under nitrogen and resuspended in iso-octane for GC/MS analysis. Total lipid and purified TAG that was not subjected to lipase digestion were also methylated and analyzed by GC/MS.

GC/MS was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5975B Inert XL Mass Selective Detector running in electron impact ionization (EI) mode. Chromatographic separation of the FAMEs was achieved using a DB-23 column (Agilent Technologies, 30 m x 0.25 mm x 0.25 μm) with helium as the carrier gas (constant flow mode, 40 cm/s) and the following temperature program: initial temperature 90°C, ramping at 10C/min to 180°C, followed by a 5 min hold, then ramping at 5°C/min to 230°C and held for 5 min, for a total run time of 29 min. The inlet was operated in splitless mode at 290°C, the MS transfer line was 250°C, the ion source was 230°C and the MS detector was 150°C. Mass spectral data was acquired in scan mode (30-350amu), and chromatographic peaks were identified based on comparison with external FAMEs standards (Std 421A & Std 96, including EPA and DHA; Nu-Chek Prep, Elysian, MN) and the NIST05 mass spectral database.

Statistical Analysis

Statistical analysis was performed using the SAS (Statistical Analysis System; version 9.1 SAS Institute Inc., Cary, NC, USA) or SPSS (Statistical Package for Social Sciences; PASW Statistics 18, IBM, Armonk, NY, USA) statistical software. Means are expressed as ± the SEM. The significance level in this study is $p<0.05$. Experiments were performed in two separate cohorts. Differences between cohorts within the same diet group were determined using a univariate ANOVA to account for a random block variable. If block differences were observed, Tukey's test was used to perform post hoc analysis to determine differences between diet groups. Where no significant differences between blocks were determined, data was analysed as a one-way ANOVA for a diet effect with post hoc analysis using least square means to determine differences between groups. When data sets were not normally distributed the data was log transformed. If a normal distribution was determined following the log transformation statistical analysis were performed as described above. When a normal distribution was not obtained using a log transformation, non-parametric statistical analysis was performed using a Kruskal-Wallace test to determine if differences existed between diet groups.

Chapter 4: Results

Triglyceride sn-2 Composition of Diets

Although the OO and HCO diets had the same overall fatty acid composition, differences were observed in the TG sn-2 fatty acid composition. The OO diet had a greater proportion of oleic acid in the sn-2 position (60%), compared to the HCO diet (50%; Table 10). The OO diet had 30% linoleic acid and lower total PUFA in the sn-2 position compared to the HCO diet, which consisted of 37% linoleic acid and 41% PUFA. The triglyceride sn-2 position fatty acid composition of the HF and LF diets consisted of 60% PUFA and 30% MUFA.

Animal Characteristics

Following 3 weeks consuming either a low fat (12% w/w) or high fat (20% w/w) diet to induce obesity and 4 weeks feeding experimental diets there were no significant differences between groups in body weight or spleen weight (Table 11). Liver weight was significantly lower in rats fed the OO diet ($p<0.05$) compared to LF (Table 11). The liver weight from rats fed the HCO diet did not differ significantly from the OO group or the two control fat diets (high and low). Number of splenocytes per gram spleen was greater in OO (509 ± 78) fed animals compared to LF (269 ± 27) and HF (308 ± 45) ($p<0.05$). Splenocytes/g in rats fed the HCO diet did not differ significantly from the OO group or two control fat diets (Table 11).

OGTT

Following the OGTT, the time to reach peak plasma glucose concentration differed between animals fed different diets. Rodents fed LF had a plasma glucose peak 10 minutes following the glucose challenge (Fig 3a) as well as a significantly higher glucose AUC (Fig 4a), compared animals fed HF in which plasma glucose peaked 30 minutes following the OGTT. In response to this, rodents fed LF also had a greater insulin peak compared to those fed HF (Fig 3b); however the overall AUC for plasma insulin was not significantly different between HF and LF fed animals (Fig 4b). In comparison, plasma glucose concentrations in rats fed HCO and OO peaked 20 minutes following the glucose

challenge. Plasma insulin concentration reached a peak at 10 minutes following the OGTT in rodents fed LF, HF, and OO; however, was slower to rise in rodents fed HCO. Animals consuming the HCO diet had a significantly greater AUC for both plasma insulin and glucose following an OGTT than those fed the HF diet. Plasma insulin and glucose concentrations for animals fed the OO diet were not significantly different from the HCO diet group or either of the control fat groups.

Immune Function

^{3}H-Thymidine Incorporation

There was no diet effect on ^{3}H-thymidine incorporation by splenocytes following stimulation with low dose Con A or LPS (Table 12). Splenocyte proliferation in animals fed the LF and HF diet was not significantly different following stimulation with high dose Con A or CD3/CD28 (Table 12). In animals fed OO, splenocyte stimulation index was not significantly different from those fed HF following high dose Con A stimulation, however it was significantly higher compared to splenocytes of rats fed HCO. Splenocytes of OO fed rats had significantly higher stimulation indicies following CD3/CD28 stimulation compared to those fed both HF and HCO.

Immune Cell Phenotypes

Spleen

There was no diet effect on the proportion of total T-cells (CD3+) or the proportion of T-cells (CD8+ and CD4+) expressing CD25. There was also no diet effect for the proportion of cells that were CD3+CD27+, CD68+CD11+ or IgM+ (Table 13). However, feeding a HF diet compared to a LF diet resulted in a low proportion of CD3+ cells that were CD8+ and a lower percent of CD8+ cells that were CD152+ and CD71+ (Table 13, $P<0.05$). Feeding HF also resulted in a lower proportion of CD3-CD161+ splenocytes compared to LF.

There were also significant differences between both high MUFA diets and HF; as well as between OO and HCO. Rats from both high MUFA diets had a higher proportion of NK cells (CD3-CD161+) in spleen and more IgG+ cells compared to HF (Table 13,

p<0.05). Feeding OO, but not HCO resulted in a higher %CD4+CD152+ and CD71+ cells, and CD8+CD152+ cells compared to the HF (Table 13, p<0.05). Feeding HCO but not OO resulted in a lower proportion of B cells (CD45RA+) and a higher proportion of CD68+CD284+. There was a lower proportion of CD4+ cells expressing CD152 and CD71, as well as a lower proportion of IgG+ cells and a higher proportion of IgA+ cells when HCO was fed compared to feeding OO (Table 13, p<0.05).

MLN

There was no diet effect on the proportion of cells that were CD3+CD27+ or IgG+, as well as on the proportion of CD4+ or CD8+ cells expressing CD25. Feeding a HF diet compared to a LF diet resulted in a lower percent of CD8+ cells that were CD71+ and a lower proportion of B cells (CD45RA+), CD68+CD11+ cells and IgM+ cells (Table 14, p<0.05) and a lower % CD3+ cells that were CD4+CD28+ (Fig 6, p<0.05).
Feeding either MUFA diet compared to the HF resulted in a lower % CD3+ cells that were CD4+CD28+ (Fig 6, p<0.05). However, feeding OO, but not HCO a higher percent of CD8 cells that were CD71+ and a higher proportion of IgA+ cells (Table 14, p<0.05) and a higher percent of CD4+ cells that were CD25foxp3+ (Fig 5, p<0.05). A lower proportion of T cells, CD3+CD8+ cells, %CD3 that were CD8+CD28+, and proportion of CD68+CD284+, CD68+CD11+ and IgA+ cells was observed after feeding HCO, but not OO (Table 14 and Fig 6, p<0.05).

Cytokine Production by Con A Stimulated Splenocytes

Low Stimulation (2.5μg/ml Con A)

Following stimulation of splenocytes with 2.5 μg/ml Con A significant diet effects were observed. Splenocytes of rodents fed HF produced a significantly greater concentration of IL-10 compared to feeding LF (Fig 7, p<0.05); however, the production of other measured cytokines was not different between those fed HF and LF. Feeding OO led to a significantly lower production IL-6, IL-10, TNF-α and IFN-γcompared to feeding HF (Fig 7, p<0.05). Feeding OO also led to a significantly lower production of TNF-αand IFN-γcompared to feeding HCO; however they were not different in the production of IL-1β, IL-2, IL-6 or IL-10.

High Stimulation (5 µg/ml Con A)

Following stimulation of splenocytes with 5 µg/ml Con A, splenocytes of rats fed HF produced significantly lower concentrations of IL-1β compared to feeding LF (Fig 7, $p<0.05$). No other significant diet effect on measured cytokines was observed between LF and HF. Compared to feeding HF, stimulated splenocytes of OO fed rats produced significantly lower concentrations of TNF-α (Fig 7, $p<0.05$). Cytokine production by splenocytes did not differ between rodents fed HCO or OO.

Cytokine Production by Con A Stimulated MLN Cells

Low Stimulation (2.5 µg/ml)

Stimulation of MLN cells with 2.5 µg/ml Con A did not result in significant differences in cytokine production between rodents fed HF or LF. Feeding OO, however, led to a significantly lower production IL-6 and IL-10 compared to feeding HF (Fig 8, $p<0.05$). Feeding either high MUFA diet (HCO and OO) also did not lead to significant differences in cytokine production.

High Stimulation (5 µg/ml)

MLN cells of rodents fed HF produced significantly more IL-2 and less IL-6 compared to those fed LF following stimulation with 5 µg/ml Con A (Fig 8, $p<0.05$). Compare to HF, feeding OO led to a significantly greater production of IL-6 by stimulated MLN cells (Fig 8, $p<0.05$). MLN cells from animals fed either OO or HCO did not differ from each other in the production of measured cytokines following stimulation; however both high MUFA diets led to a lower production of IFN-γ compared to feeding HF.

Cytokine Production by CD3/CD28 (5 µg/ml/5 µg/ml) Stimulated Splenocytes

Splenocytes of rodents fed HF produced lower concentrations of IL-6 compared to those fed LF following stimulation with CD3/CD28 (Fig 9, $p<0.05$). Feeding either OO or HF did not lead to significant differences in cytokine production. Feeding OO and HCO did not lead to significant differences in the production of IL-2, IL-6, and IFN-γ by stimulated splenocytes; however, splenocytes of rodents fed OO produced lower concentrations of IL-10 and TNF-α compared to those fed HCO (Fig 9, $p<0.05$).

Cytokine Production by LPS Stimulated Splenocytes

Feeding LF or HF did not lead to differences in the production of measured cytokines following stimulation with LPS. Splenocytes of animals fed OO produced less IL-10 and TNF-α compared to feeding HF (Fig 10, $p<0.05$). Feeding OO or HCO did not lead to significant differences in cytokine production following stimulation with LPS. Significant immune changes in both spleen and MLN cells are summarized in Table 15.

Plasma Haptoglobin

There was no significant difference in plasma haptoglobin concentrations between the HF and LF diet (Fig 11). Animals fed the OO diet had greater plasma haptoglobin concentration (19.8 ng/ml± 1.8, $p<0.05$) compared to those fed the LF (12.5 ng/ml ± 1.9) or HF (10.5 ng/ml ± 2.9) diets (Fig 11). The plasma haptoglobin concentration of HCO (16.5 ng/ml ± 2.0) fed animals was not significantly different from the OO animals or either of the control fat diets.

Tissue Lipid Analysis

Spleen Total Phospholipid Fatty Acids

HF and LF

Compared to the high fat diet, rats fed a low fat, high carbohydrate diet had a significantly greater proportion of palmitic acid and a lower proportion of stearic acid and linoleic acid in spleen phospholipids (Fig 12, $p<0.05$). LF fed rodents also had lower proportions of total PUFA and total n-6 PUFA, and a greater proportion of MUFA in spleen phospholipids compared to HF (Table 16). There were no significant differences between proportions of oleic, arachidonic, α-linoleic or docosahexanoic acid between animals fed the HF or LF diet.

High MUFA diets

Rats fed the high MUFA diets had a significantly greater proportion of oleic acid and lower proportion of linoleic acid in spleen phospholipids compared to animals fed HF (Fig 12, $p<0.05$). Rats fed either high MUFA diet also had a lower proportion of total SF,

and a higher proportion of total MUFA compared to feeding HF (Table 16, $p<0.05$). Feeding MUFA from HCO led to a significantly lower proportion of oleic acid, total MUFA, and linoleic acid in spleen phospholipids compared to the OO MUFA source; however there were not significant increases in other fatty acids with HCO compared to OO. There was a significantly greater proportion of SF in the spleen of HCO fed animals compared to OO (Table 16, $p<0.05$). There were no significant differences in the proportion of arachidonic, α-linoleic, or docosahexanoic acid between animals fed either the OO or HCO diet. Feeding the HCO diet led to a significantly greater proportion of arachidonic acid in spleen phospholipids compared to those fed the HF diet (Fig 12, $p<0.05$).

Jejunal Total Phospholipid Fatty Acids

Jejunal phospholipids from rodents fed LF had a greater proportion of palmitic acid, stearic acid and total SF compared to rodents fed high fat diets (Fig 13 and Table 17, $p<0.05$). Compared to HF fed animals, those fed LF also had lower proportions of total PUFA and total n-6 PUFA. Animals fed high MUFA diets had a greater proportion of oleic acid and total MUFA in jejunal phospholipids compared to those fed HF or LF, and a lower proportion of linoleic acid compared to those fed HF (Fig 13 and Table 17, $p<0.05$). Animals fed LF had a significantly greater proportion of α-linolenic acid in jejunal phospholipids compared to those fed other diets. There were no significant differences in arachidonic or docosahexanic acid among diets. No significant differences between feeding HCO and OO were observed.

Liver and Perirenal Adipose Triglyceride sn-2 Fatty Acids

LF and HF

Feeding the LF diet compared to the HF diet resulted in a greater proportion of oleic acid and palmitic acid in the sn-2 position of TG and a lower proportion of stearic and the PUFA, α-linolenic, α-linoleic acid, arachidonic acid and DHA in liver (Fig 14, $p<0.05$). In perirenal adipose tissue, feeding LF compared to HF diet resulted in greater proportion of oleic acid and palmitic acid in the sn-2 postion of TG and a lower proportion of the PUFA, α-linolenic and α-linoleic acid (Fig 15, $p<0.05$)

High MUFA and HF

Feeding high MUFA diets, compared to the HF control diet resulted in a lower the proportion of sn-2 fatty acids in liver TG being PUFA (linoleic acid, linolenic acid, DHA and AA) and stearic acid a higher proportion being oleic acid (Fig 14, $p<0.05$). In perirenal fat, feeding the high MUFA diets compared to the HF resulted in a lower proportion of the sn-2 fatty acid in TG being stearic acid and PUFA (LA and ALA for the HCO only) and a higher proportion being oleic acid (Fig 15, $p<0.05$). Feeding different sources of MUFA, either HCO or OO, did not lead to changes in sn-2 fatty acids in TG in either of these tissues.

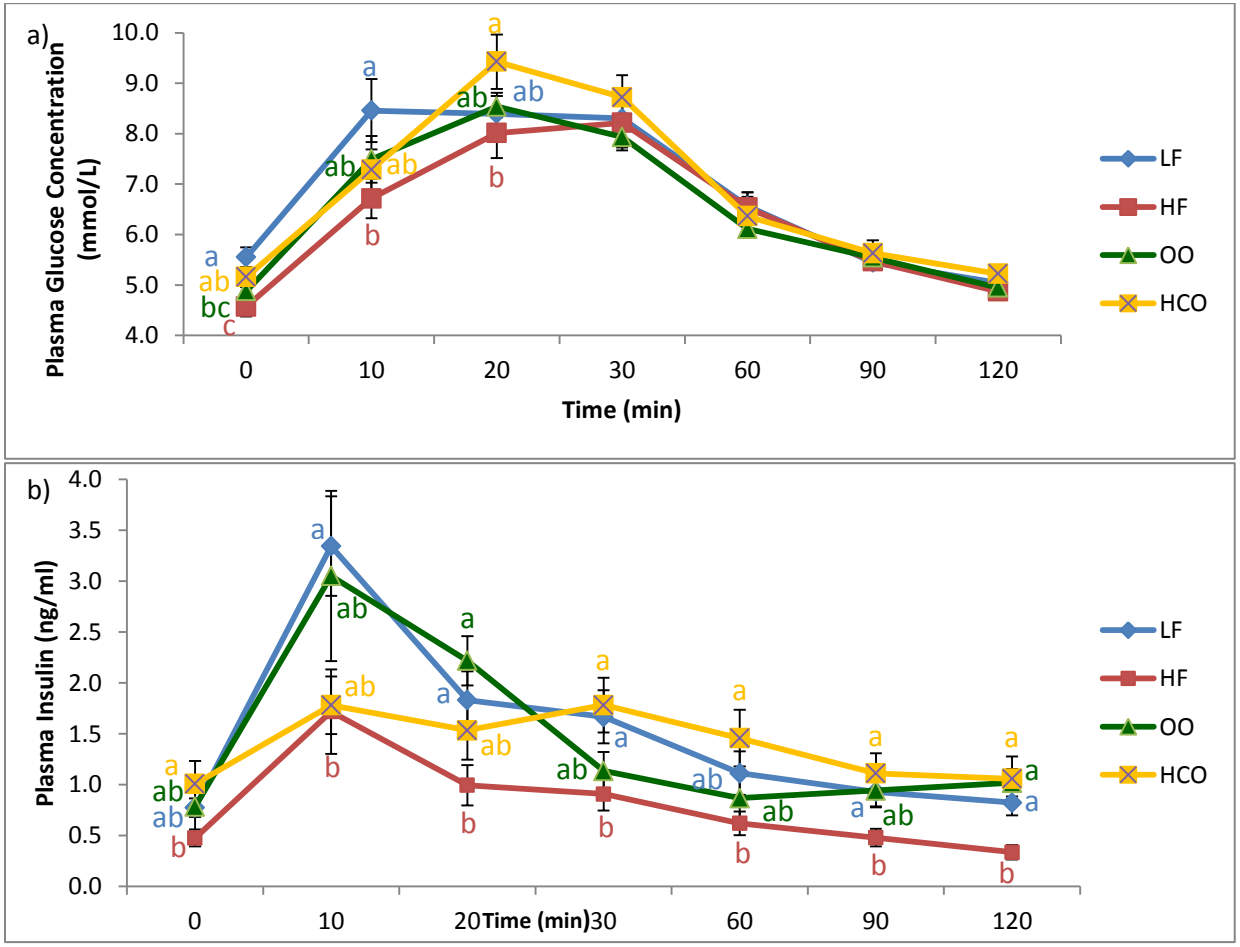

Figure 3. Plasma concentration of a) glucose and b) insulin for each time point following an OGTT

Mean ± SEM. Means within a time point that do not share a common superscript are significantly different ($p<0.05$)

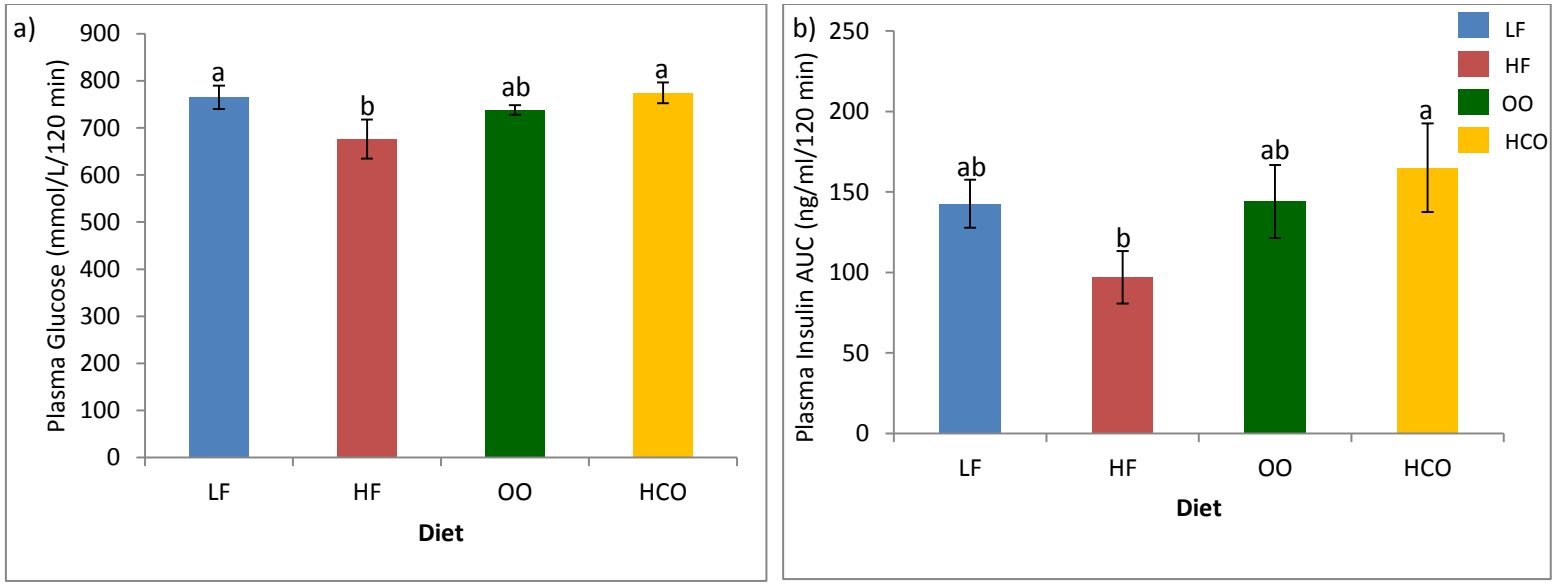

Figure 4. Area under the curve for a) plasma glucose concentration and b) plasma insulin concentration following an OGTT with collection over of 120 minutes

Mean ± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

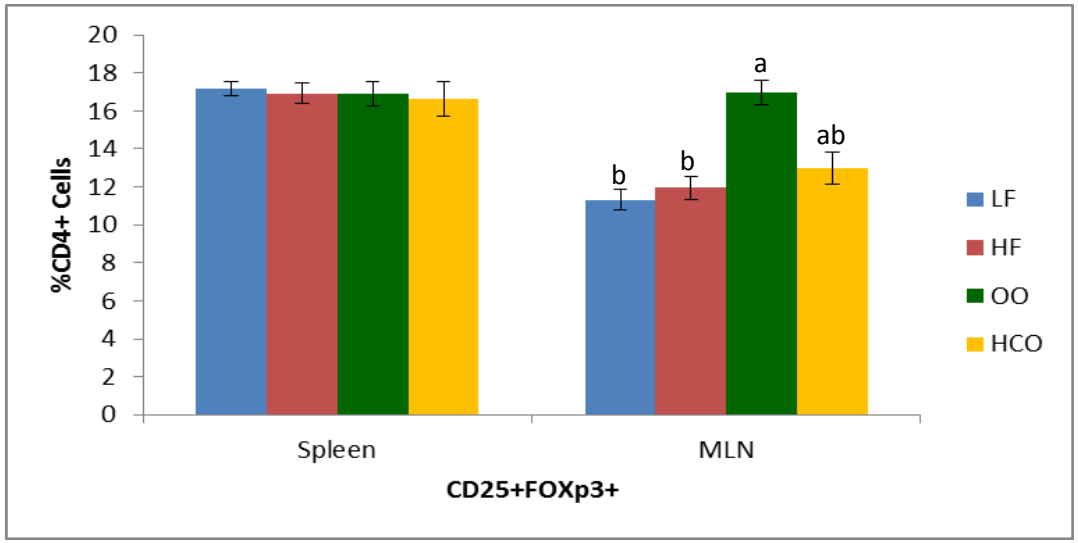

Figure 5. CD25+FOXp3+ splenocytes and MLN cells as a proportion of total CD4+ cells

Mean ± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

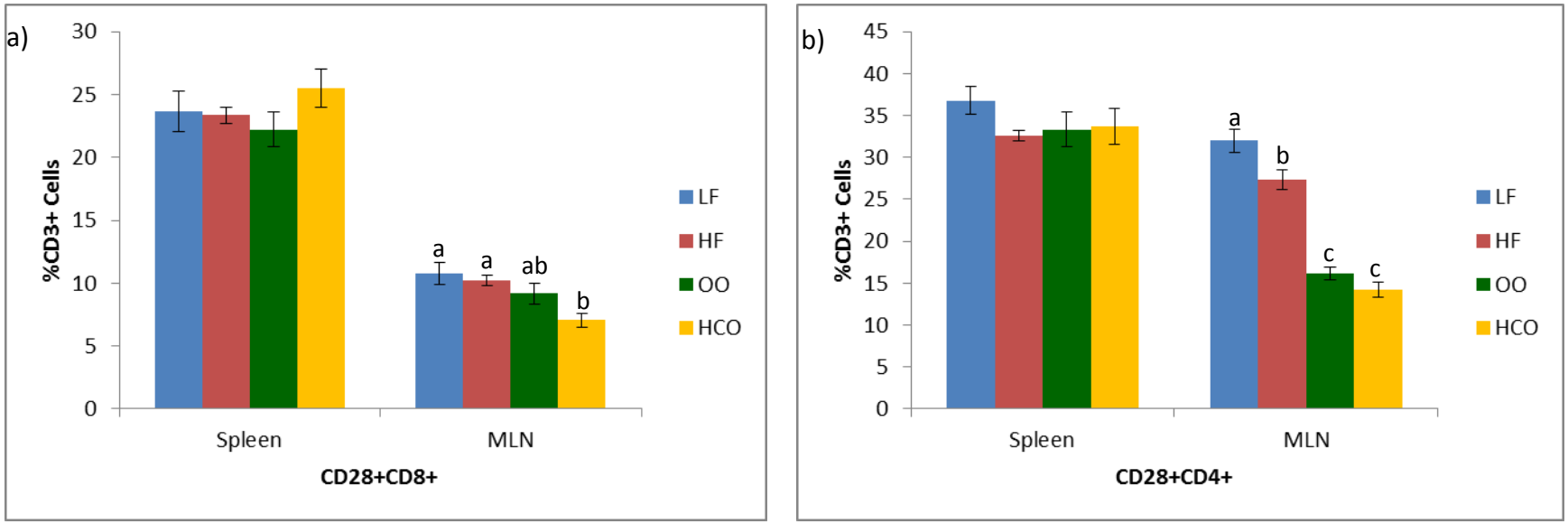

Figure 6. Spleen and MLN cells that are a) CD28+CD8+ and b) CD28+CD4+ as a proportion of total CD3+ T-cells
Mean ± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

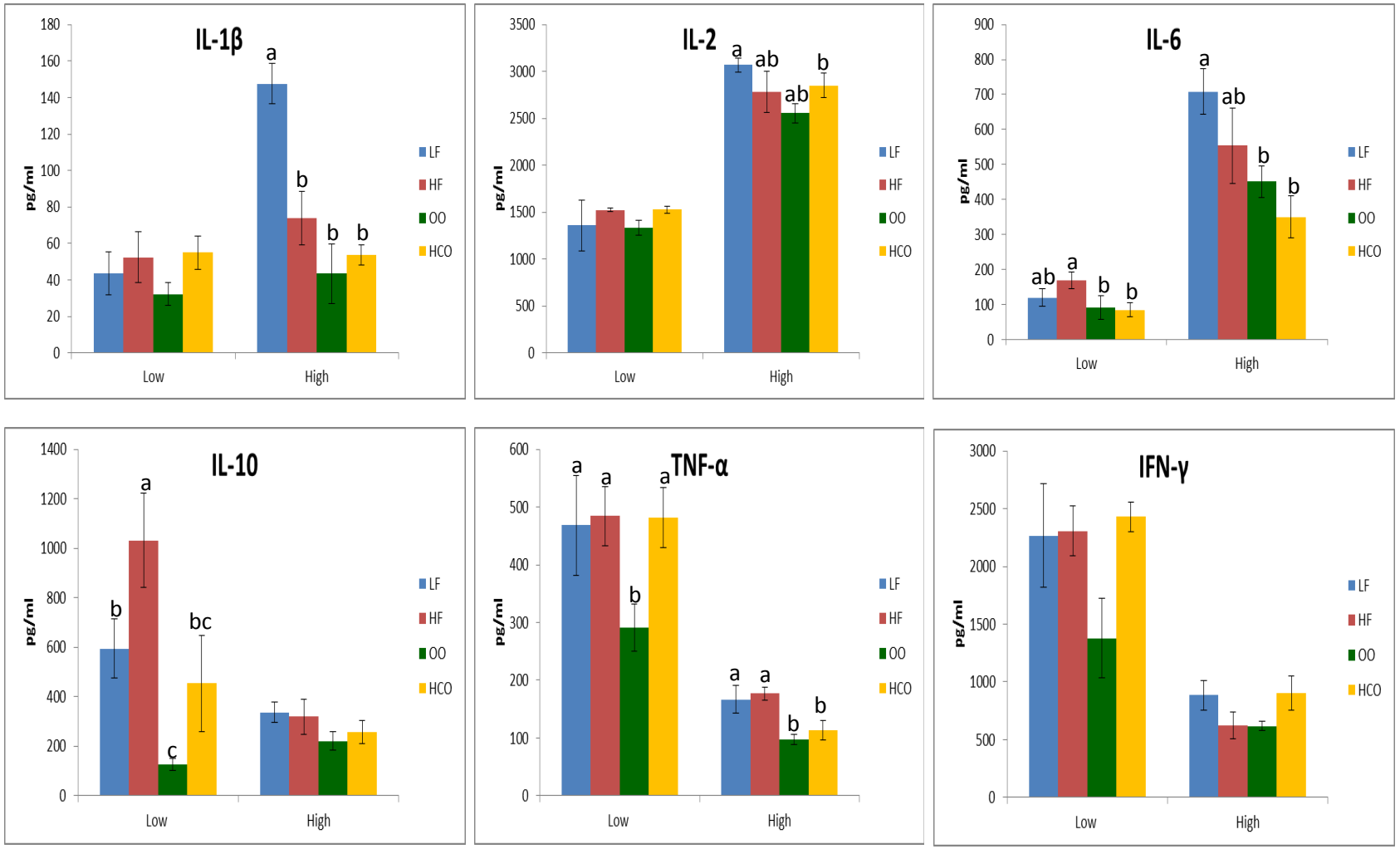

Figure 7. Cytokine production in response to low (2.5 µg/ml) and high (5 µg/ml) Con A stimulation in splenocytes
Mean ± SEM. Means within a Con A condition that do not share a common superscript are significantly different ($p<0.05$)

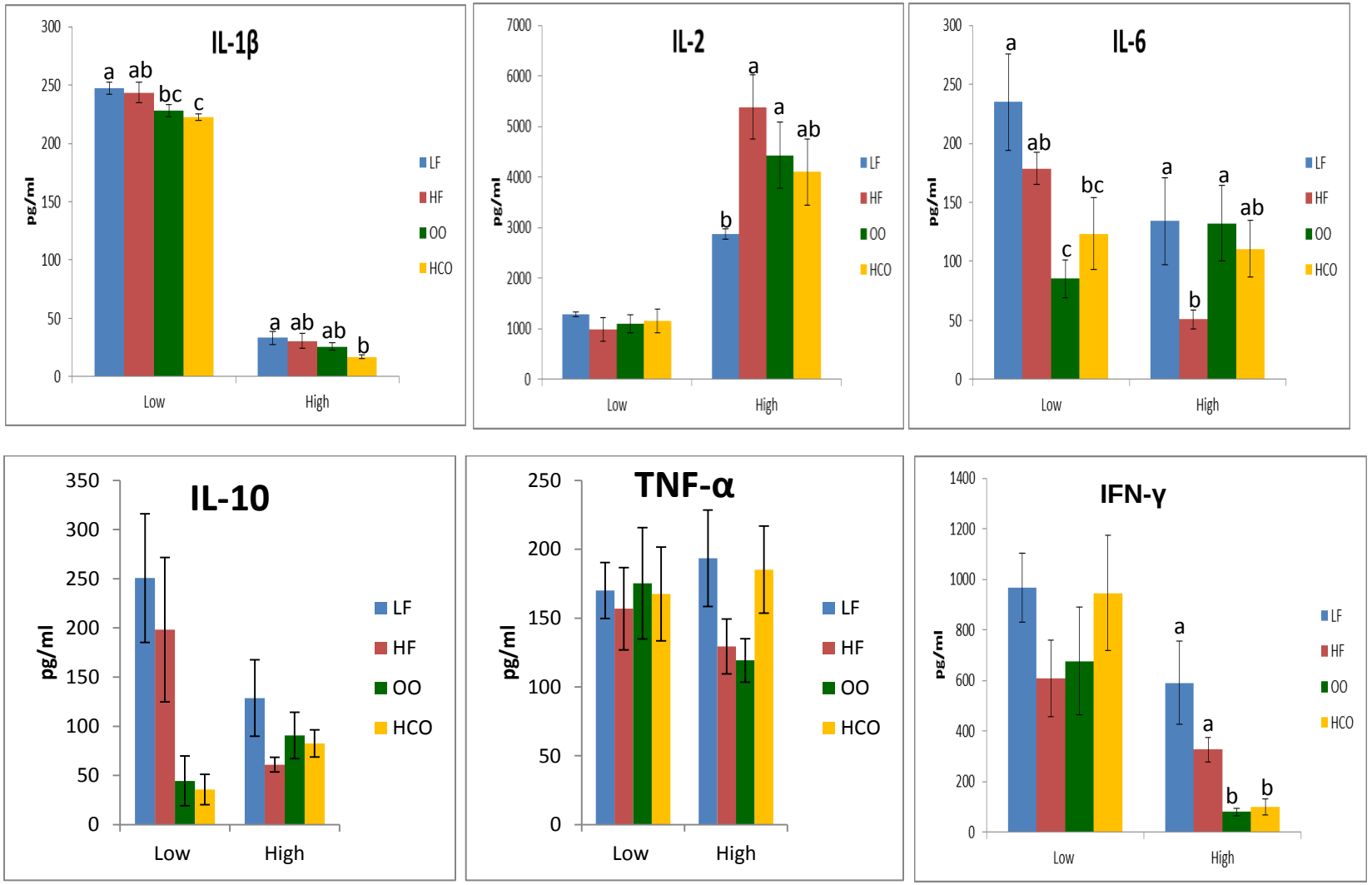

Figure 8. Cytokine production in response to low (2.5 µg/ml) and high (5 µg/ml) Con A stimulation in MLN cells
Mean ± SEM. Means within a Con A condition that do not share a common superscript are significantly different ($p<0.05$)

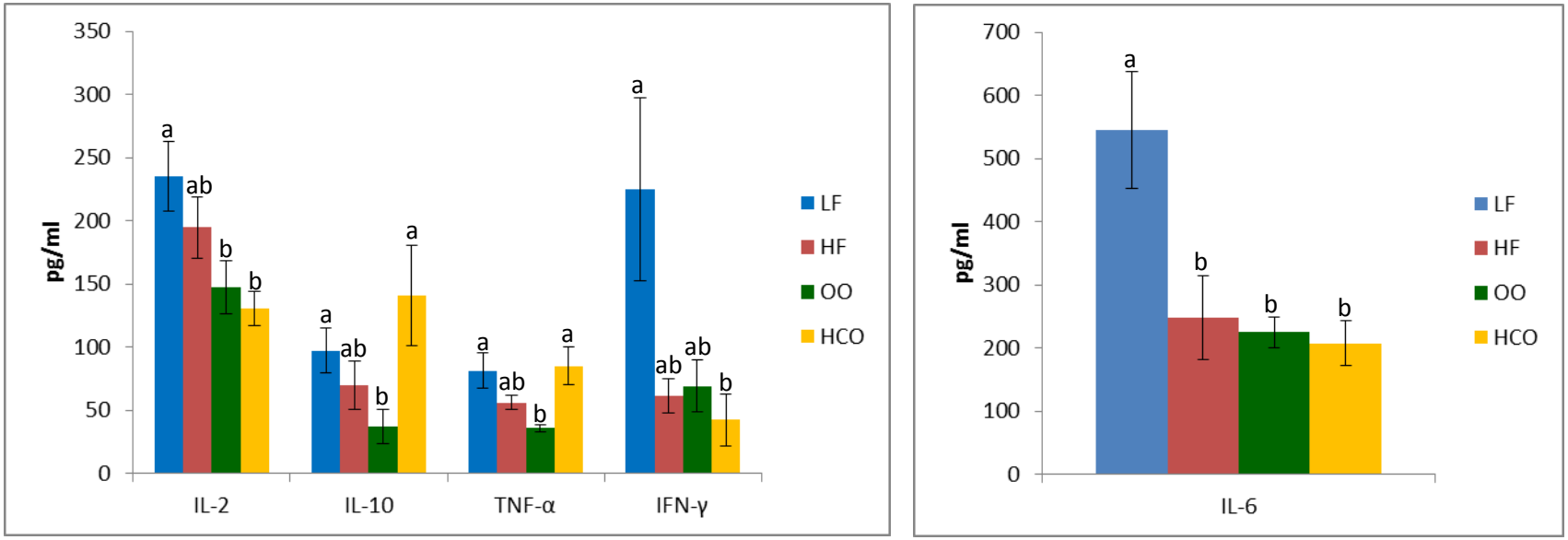

Figure 9. Cytokine production in response to CD3/CD28 stimulation (5 µg/ml/5 µg/ml) in splenocytes
Mean ± SEM. Means within a CD3/CD28 condition that do not share a common superscript are significantly different ($p<0.05$)

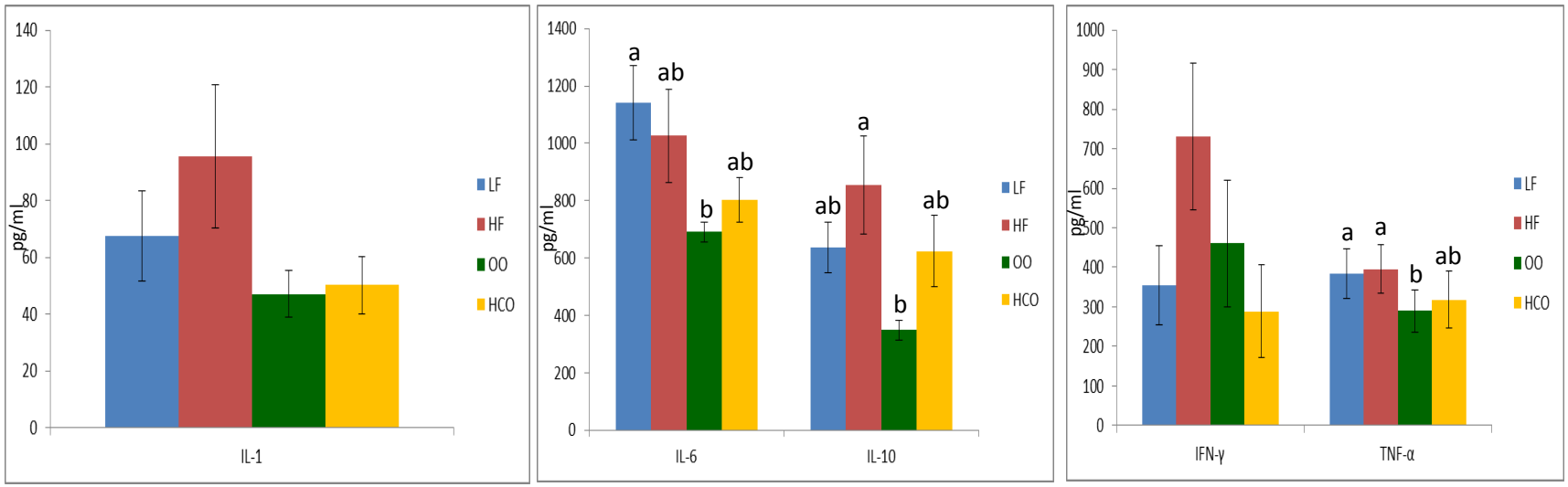

Figure 10. Cytokine production in response to LPS stimulation (100 µg/ml) in splenocytes
Mean ± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

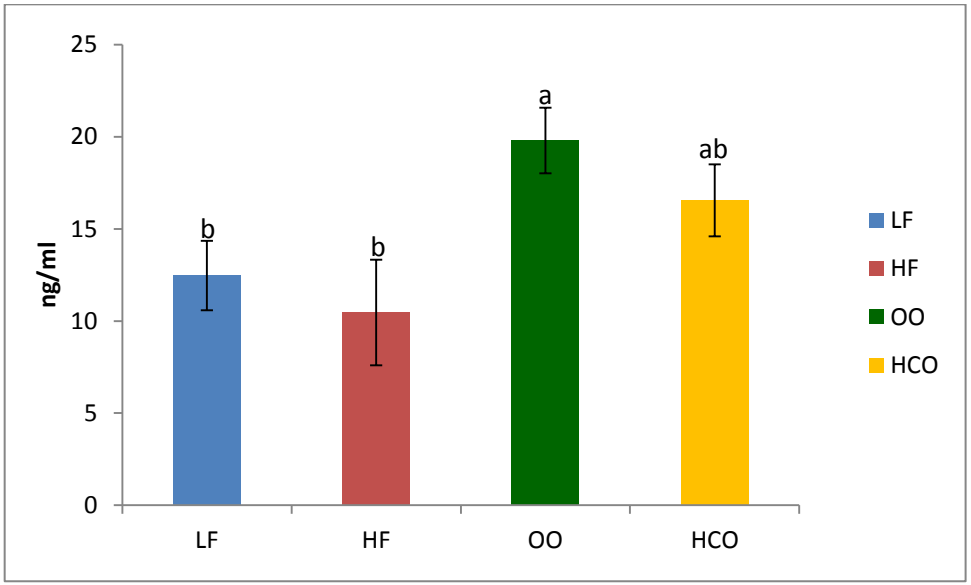

Figure 11. Plasma haptoglobin concentration (ng/ml)
Mean± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

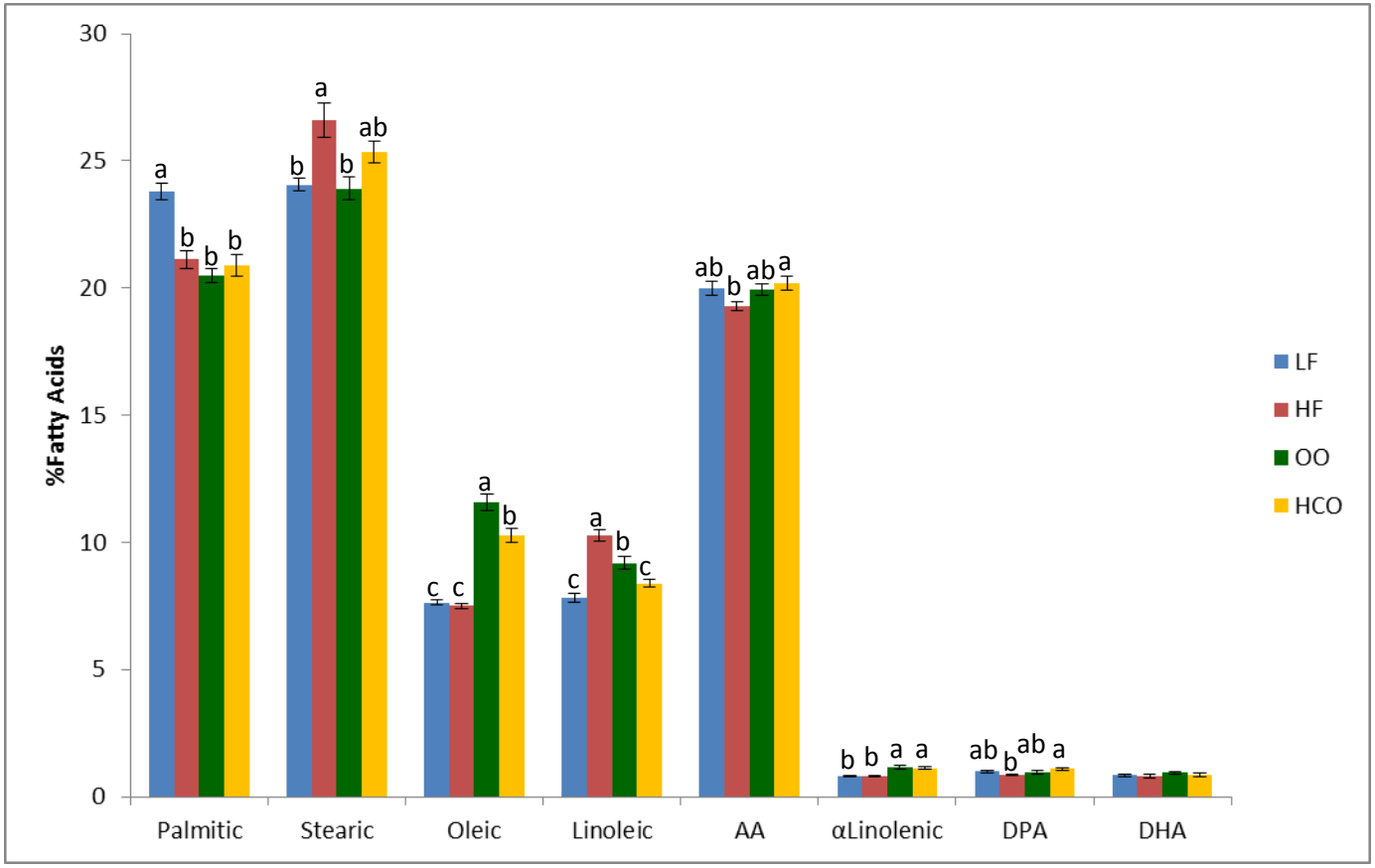

Figure 12. Spleen phospholipid fatty acid composition as a proportion of total fatty acids
Mean ± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

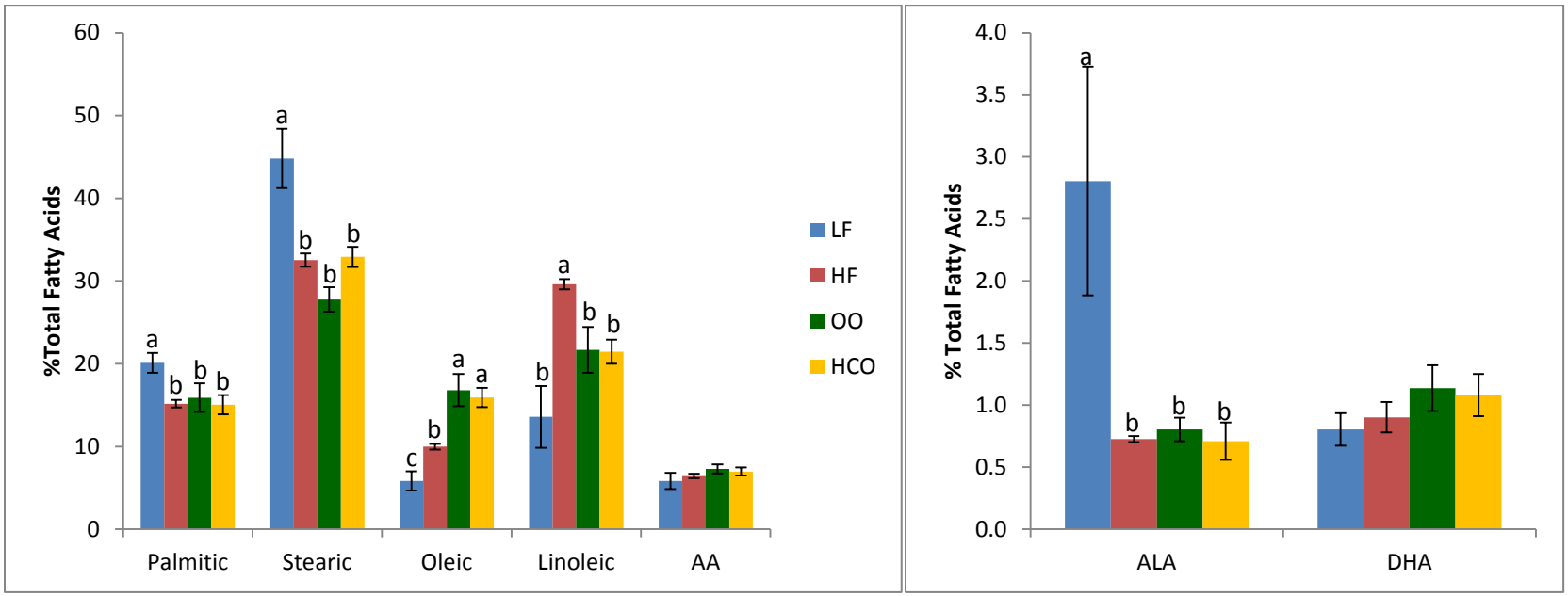

Figure 13. Jejunum phospholipid fatty acid composition as a proportion of total fatty acids
Mean± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

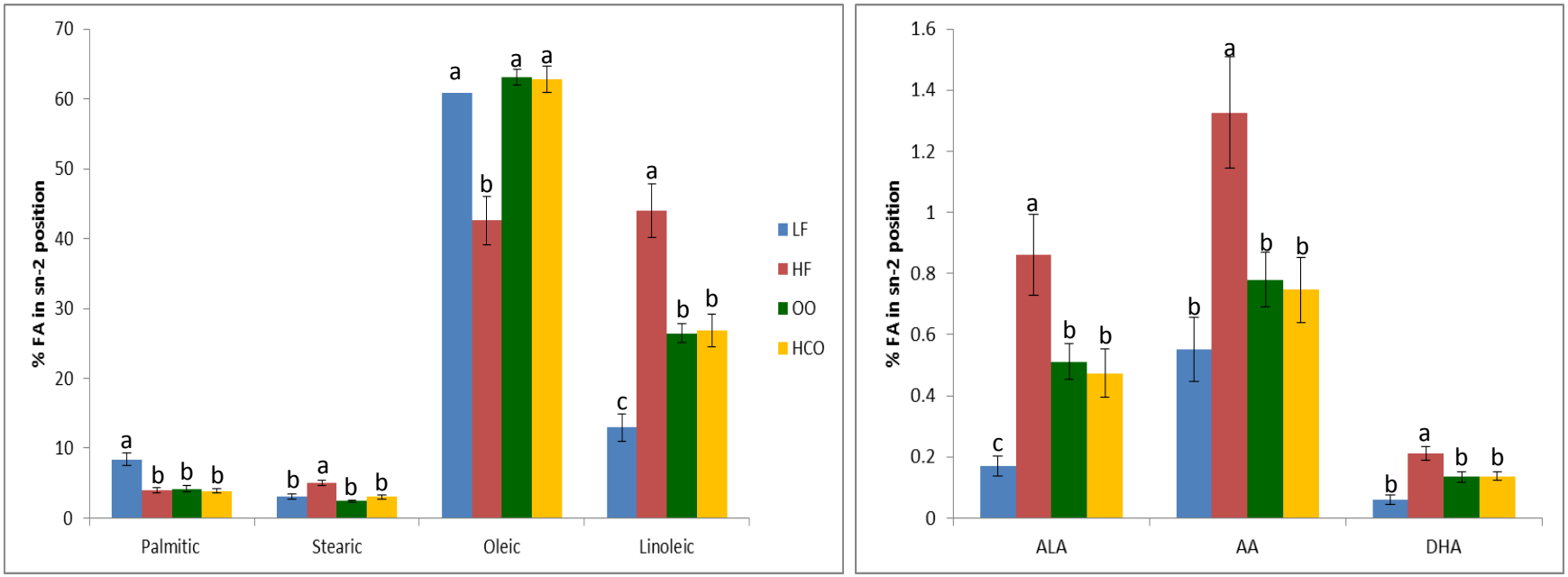

Figure 14. Proportion of sn-2 fatty acids in liver triglycerides
Mean± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

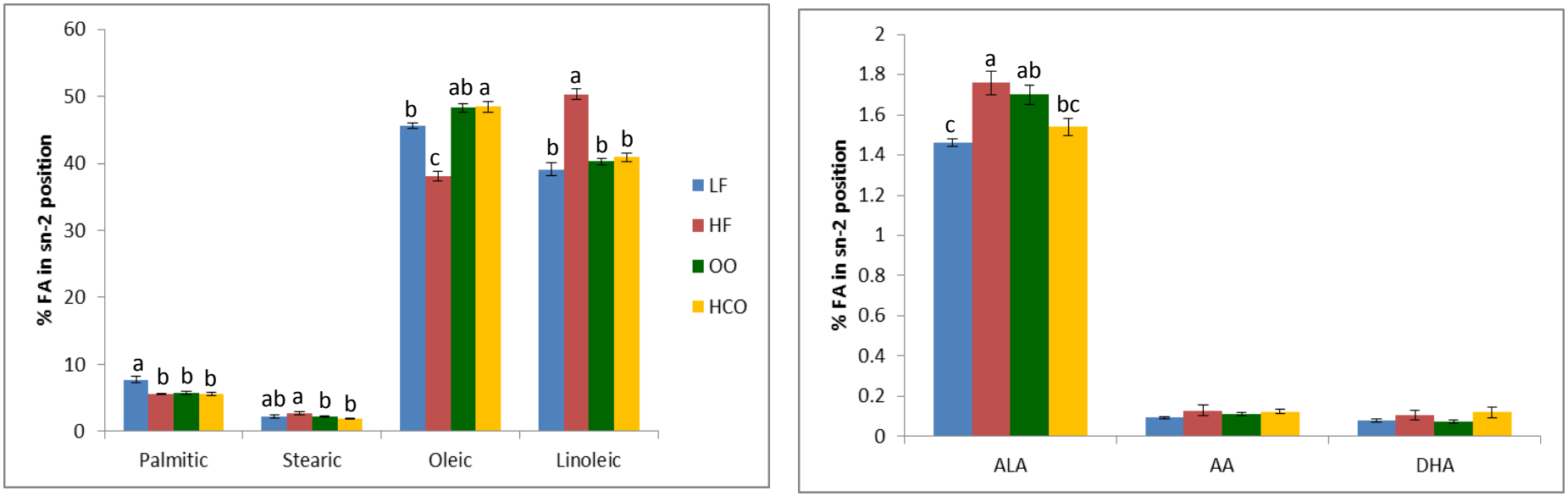

Figure 15. Proportion of sn-2 fatty acids in perirenal adipose tissue triglycerides
Mean± SEM. Means that do not share a common superscript are significantly different ($p<0.05$)

Table 10. Sn-2 fatty acid composition of experimental diets (%total sn-2 fatty acids)

	Diet		
	HF/LF	OO	HCO
Palmitic	0.89	1.07	1.00
Palmitoleic	0.04	0.47	0.09
Stearic	8.60	5.90	8.66
Oleic	29.94	60.01	49.72
Linoleic	55.83	29.69	37.11
α-Linolenic	4.57	2.76	3.28
Eicosanoic	0.12	0.10	0.14
SFA	9.49	6.97	9.67
PUFA	60.53	32.54	40.53
MUFA	29.98	60.48	49.81

Table 11. Animal characteristics

	Diet			
	LF	HF	OO	HCO
Body Weight (g)	495.6 ± 7.1	485.1 ± 12.7	493.3 ± 11.3	498.1 ± 8.4
Spleen Weight (g)	0.9 ± 0.05	1.0 ± 0.05	0.9 ± 0.04	1.0 ± 0.03
Spleen Weight mg/g Body Weight	1.9 ± 0.08	2.0± 0.07	1.9± 0.08	2.0± 0.07
Liver Weight (g)	19.4 ± 0.6^{a}	19.1 ± 0.7^{ab}	16.4 ± 0.8^{b}	17.6 ± 0.7^{ab}
Liver Weightmg/g Body Weight	39.0± 0.9	39.5 ± 1.0	33.8 ± 1.9	35.5 ± 1.4
Total Splenocytes in spleen (x 10^{6})	295 ± 52^{b}	256 ± 29^{b}	482 ± 72^{a}	358 ± 48^{ab}
Splenocytes (x 10^{6})/g	308 ± 45^{b}	269 ± 27^{b}	509 ± 78^{a}	378 ± 49^{ab}
n/group	14	14	14	14

Mean ± SEM. Within a row, means that do not share a common superscript are significantly different ($p<0.05$)

Table 12. Stimulation index as a ratio of ^{3}H-thymidine uptake by stimulated to ^{3}H-thymidine uptake by unstimulated splenocytes(n=8/group) following mitogen stimulation with low dose Con A (2.5 µg/ml), high dose Con A (5µg/ml), CD3/28 (5µg/ml of each CD3 and CD28), and LPS (100 µg/ml)

	Diet			
	LF	HF	OO	HCO
Con A SI Low (n=6)	5.0 ± 2.5	6.2 ± 2.0	9.8 ± 2.2	6.1 ± 2.1
Con A SI High (n=8)	15.5 ± 4.2^{ab}	10.7 ± 4.0^{ab}	18.0 ± 4.7^{a}	6.9 ± 2.0^{b}
CD3/CD28 SI (n=8)	7.3 ± 2.8^{ab}	4.6 ± 1.4^{b}	11.3 ± 1.4^{a}	3.5 ± 0.9^{b}
LPS SI (n=8)	1.1 ± 0.3	1.6 ± 0.3	1.6 ±0.2	1.0 ± 0.2

Mean ± SEM. Within a row, means that do not share a common superscript are significantly different ($p<0.05$)

Table 13. Immune cell phenotypes present in isolated splenocytes as a proportion of total cells unless otherwise indicated (n=14*)

	LF	HF	OO	HCO
CD3+ Total	43 ± 2	43 ± 1	41 ± 1	42 ± 1
%CD3 CD4+	50 ± 3^{b}	53 ± 2^{ab}	59 ± 2^{a}	57 ± 1^{ab}
%CD3 CD8+	58 ± 2^{a}	44 ± 1^{b}	48 ± 1^{b}	46 ± 1^{b}
CD3+CD27+	38 ± 3	36 ± 2	36 ± 1	40 ± 2
%CD4 CD152+	3.4 ± 0.2^{b}	3.3 ± 0.2^{b}	7.5 ± 1.5^{a}	2.9 ± 0.4^{b}
% CD8 CD152+	15 ± 1^{a}	12 ± 1^{b}	15 ± 1^{a}	13 ± 1^{ab}
%CD4 CD71+	10 ± 1^{b}	12 ± 1^{b}	20 ± 3^{a}	11 ± 1^{b}
%CD8 CD71+	30 ± 2^{a}	26 ± 1^{b}	30 ± 1^{ab}	27 ± 1^{ab}
%CD4 CD25+	25 ± 1	25 ± 1	26 ± 1	25 ± 1
%CD8 CD25+	9.3 ± 0.3	9.8 ± 0.4	9.6 ± 0.5	11.9 ± 1.2
CD45RA Total	54 ± 3^{a}	54 ± 2^{a}	53 ± 2^{ab}	48 ± 2^{b}
CD68+CD11+	3.5 ± 0.7	2.7 ± 0.8	2.7 ± 1.0	3.2 ± 1.1
CD68+CD284+	6.7 ± 0.9^{ab}	5.6 ± 1.0^{b}	6.4 ± 1.4^{ab}	7.6 ± 1.3^{a}
CD3-CD161+	5.5 ± 1.0^{b}	4.1 ± 1.1^{c}	8.1 ± 1.3^{a}	8.3 ± 1.2^{a}
IgM+	57 ± 1	57 ± 1	55 ± 1	55 ± 1
IgG+	5.6 ± 0.2^{c}	6.0 ± 0.4^{c}	8.6 ± 0.4^{a}	7.2 ± 0.5^{b}
IgA+	11 ± 1^{a}	8.7 ± 1.2^{ab}	7.3 ± 2.0^{b}	10 ± 1^{a}

Mean ± SEM. Means within a row that do not share a common superscript are significantly different ($p<0.05$)

*CD3CD27, CD45RA, CD68CD11, CD68CD284+, CD68CD284-, CD3-CD161+, IgA (n=8)

%CD4CD152, %CD8CD152, %CD4CD71, %CD8CD71, IgG (n=6)

Table 14. Immune cell phenotypes present in isolated MLN cells as a proportion of total cells unless otherwise indicated (n=14*)

	LF	HF	OO	HCO
CD3+ Total	58 ± 1^{ab}	63± 1^{a}	58± 2^{ab}	57± 2^{b}
%CD3 CD4+	68± 1	68± 1	71± 1	71± 1
%CD3 CD8+	29± 1^{ab}	30± 1^{a}	29± 1^{ab}	26± 1^{b}
CD3+CD27+	57± 1	54± 1	55± 1	54± 2
%CD4 CD71+	6.3 ± 0.5^{b}	7.6 ± 0.4^{ab}	9.4 ± 1.7^{a}	7.8± 0.7^{ab}
%CD8 CD71+	15± 2^{a}	11± 1b^{c}	14± 3^{ab}	8.2 ± 0.5^{c}
%CD4 CD25+	22 ± 1	21 ± 1	23 ± 1	20 ± 1
%CD8 CD25+	11 ± 1	10 ± 1	11 ± 1	8.5 ± 0.7
CD45RA Total	29 ± 1^{a}	24± 1^{b}	32± 6^{ab}	27± 2^{ab}
CD68+CD11+	1.8 ± 0.2^{a}	1.2 ± 0.1^{b}	1.4 ±0.2^{ab}	0.8 ±0.1^{c}
CD68+CD284+	3.9 ± 0.3^{a}	4.3 ± 0.3^{a}	4.7 ± 0.4^{a}	2.6 ± 0.3^{b}
IgM+	43± 1^{a}	39± 1^{bc}	40± 2^{ab}	35± 1^{c}
IgG+	3.2 ± 0.4	2.3 ± 0.2	2.8 ± 0.4	2.8 ± 0.1
IgA+	7.2 ± 0.7^{a}	8.5 ± 0.9^{a}	9.7 ± 1.3^{a}	4.6 ± 0.6^{b}

Mean ± SEM. Means within a row that do not share a common superscript are significantly different ($p<0.05$)

*%CD8CD71, CD3CD27, CD45RA, CD68CD11, CD68+CD284+, CD68+CD284-, and IgA (n=8), IgG (n=6)

Table 15. Summary of Immune Changes

	Spleen	MLN
Compared to HF, feeding LF:	- ↑ IL-1β - ↑ IL-6 (CD3/CD28) - ↓ IL-10 - ↑ %CD3CD8+ - ↑ CD3-CD161+ - ↑ %CD8CD152+ - ↑ %CD8CD71+	- ↓ IL-2 - ↑IL-6 - ↑%CD3CD28+CD4+ - ↑ %CD8CD71+ - ↑ CD45RA - ↑ CD68+CD11+ - ↑IgM
Compared to HF, feeding ↑MUFA:	- ↓ IL-6 - ↓ IL-10 - ↓ TNF-α - ↑ CD3-CD161+ - ↑IgG	-↓ IL-10 - ↓ IFN-γ - ↓ %CD3CD28+CD4+
Compared to HF, feeding OO:	- ↓ IL-6 - ↓ IL-10 (Con A, LPS) - ↓TNF-α (Con A, LPS) - ↑ %CD4CD152+ - ↑ %CD8CD152+ - ↑ %CD4CD71 - ↑ IgG - ↑ CD3-CD161+	- ↓ IL-6 (low Con A) - ↑ IL-6 (high Con A) - ↓ IL-10 - ↓ IFN-γ - ↓%CD3CD28+CD4+ - ↑ %CD4CD25+FoxP3+ - ↑ %CD8CD71+
Compared to OO, feeding HCO:	- ↑ IL-10 (CD3/CD28) - ↑ TNF-α (CD3/CD28) - ↓%CD4CD152+ - ↓%CD4CD71+ - ↓ IgG - ↑IgA	- ↓ %CD8CD71+ - ↓ CD68+CD11+ - ↓ CD68+CD284+ - ↓IgM - ↓ IgA

*Cytokine production in response to Con A stimulation unless otherwise stated

↑ indicates a greater production of cytokine, or proportion of immune cells ($p<0.05$)

↓ indicates a lower production of cytokine, or proportion of immune cells ($p<0.05$)

Table 16. Spleen phospholipid fatty acid composition (g/100 g fatty acids)

	Diet							
	LF		HF		OO		HCO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:00	25.03	0.34^{a}	22.04	0.34^{b}	21.27	0.26^{b}	21.66	0.44^{b}
16:1n9	0.27	0.01	0.28	0.01	0.24	0.05	0.27	0.01
18:00	25.32	0.25^{b}	27.78	0.67^{a}	24.84	0.47^{b}	26.31	0.43^{ab}
18:1n9	8.04	0.10^{c}	7.83	0.10^{c}	12.00	0.33^{a}	10.65	0.27^{b}
18:1c11	2.90	0.09^{a}	1.54	0.07^{c}	1.85	0.09^{b}	2.01	0.10^{b}
18:2n6	8.23	0.19^{c}	10.71	0.23^{a}	9.53	0.24^{b}	8.69	0.14^{c}
20:0	0.39	0.01	0.42	0.03	0.35	0.01	0.42	0.02
18:3n3	0.84	0.04^{b}	0.82	0.03^{b}	1.20	0.06^{a}	1.16	0.04^{a}
20:2n6	0.97	0.06^{b}	1.33	0.05^{a}	1.20	0.04^{a}	1.01	0.04^{b}
20:3n6	1.50	0.09	1.74	0.05	1.49	0.03	1.56	0.04
20:4n6	21.03	0.26^{a}	20.11	0.17^{b}	20.71	0.24^{a}	20.94	0.27^{a}
22:4n6	0.83	0.04^{ab}	0.94	0.06^{a}	0.65	0.04^{c}	0.71	0.05^{bc}
22:5n3	1.05	0.05^{ab}	0.87	0.03^{b}	0.97	0.08a^{b}	1.12	0.05^{a}
22:6n3	0.88	0.04	0.84	0.08	0.96	0.03	0.89	0.07
Total SFA	50.74	0.21^{a}	50.24	0.46^{a}	46.45	0.53^{c}	48.40	0.55^{b}
Total PUFA	38.05	0.35^{c}	40.11	0.41^{a}	39.46	0.28^{ab}	38.68	0.53^{bc}
Total MUFA	11.22	0.19^{c}	9.65	0.10^{d}	14.09	0.29^{a}	12.92	0.19^{b}
n-6 PUFA	32.56	0.41^{b}	34.83	0.43^{a}	33.59	0.39^{ab}	32.91	0.41^{b}
n-3 PUFA	2.76	0.08^{bc}	2.53	0.13^{c}	3.14	0.08^{a}	3.17	0.13^{ab}

Mean $\pm$ SEM. Means within a row that do not share a common superscript are significantly different ($p<0.05$)

Table 17. Jejunum phospholipid fatty acid composition (g/100 g fatty acids)

	Diet							
	LF		HF		OO		HCO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16:0	20.82	1.21^{a}	15.53	0.45^{b}	16.48	1.73^{b}	15.49	1.15^{b}
16:1n9	0.00	0.00	0.37	0.23	0.36	0.04	0.00	0.00
18:0	46.38	3.58^{a}	33.30	0.79^{b}	28.78	1.48^{b}	33.88	1.21^{b}
18:1n9	6.03	1.16^{c}	10.22	0.34^{b}	17.42	1.94^{a}	16.41	1.16^{a}
18:1c11	0.77	0.06	0.71	0.02	1.32	0.04	1.05	0.02
18:2n6	14.06	3.73^{b}	30.33	0.62^{a}	22.45	2.78^{b}	22.10	1.47^{b}
20:0	0.00	0.00^{b}	0.48	0.01^{a}	0.98	0.32^{a}	0.72	0.22^{a}
18:3n3	2.90	0.92^{a}	0.74	0.02^{b}	0.83	0.09^{b}	0.73	0.15^{b}
20:2n6	0.00	0.00	0.00	0.00	1.19	0.49	0.92	0.26
20:3n6	6.05	0.97^{a}	6.58	0.28^{b}	7.55	0.54^{b}	7.18	0.49^{b}
22:4n6	1.59	0.32	0.51	0.01	0.97	0.38	0.00	0.00
22:5n3	0.57	0.17	0.32	0.06	0.48	0.17	0.40	0.05
22:6n3	0.83	0.13	0.92	0.12	1.18	0.18	1.11	0.17
Total SFA	67.19	4.24^{a}	49.30	1.04^{b}	46.23	3.30^{b}	50.10	1.83^{b}
Total PUFA	26.00	7.21^{c}	39.40	1.17^{a}	34.66	2.01^{ab}	32.44	1.25^{bc}
Total MUFA	6.80	1.25^{b}	11.30	0.24^{b}	19.11	2.25^{a}	17.46	1.19^{a}
n-6 PUFA	21.70	3.53^{c}	37.41	0.86^{a}	32.17	2.13^{ab}	30.20	1.24^{b}
n-3 PUFA	4.31	1.05	1.99	0.41	2.49	0.20	2.24	0.04

Mean $\pm$ SEM. Means within a row that do not share a common superscript are significantly different ($p<0.05$)

Chapter 5: Discussion

It is currently recommended that the majority of dietary fat in the diet of those with diabetes, metabolic syndrome and in the healthy population be from a MUFA source, such as olive or Canola oil. High MUFA diets are associated with improving several risk factors for diabetes, including dyslipidemia, blood glucose concentration and insulin resistance, as well as with lowering incidence of cardiovascular mortality (reviewed by (Ros, 2003)). Dietary lipid composition plays a role in modulating immune function (Yaqoob et al., 1998; Kew et al., 2003; Clandinin et al., 1991); therefore it is likely that increasing dietary MUFA would also alter immune function. The purpose of this study was to compare the effect of feeding two high MUFA diets with the same fatty acid composition but differing in TG species composition on risk factors for developing diabetes, including the acquired immune response and insulin sensitivity, in a DIO model. The high MUFA diets were also compared to two other diets, one containing a lower proportion of fat as MUFA and more PUFA and SFA (HF), and the other containing a lower proportion of energy from fat. Fatty acid composition of storage tissues (adipose and liver) and phospholipids from the spleen and jejunum were compared as a possible mechanism for the observed changes in immune function and insulin sensitivity. Furthermore, the sn-2 fatty acid composition of TG was determined in both the dietary oils and fat storage tissues. Modifying the amount and relative proportion of MUFA in the diet of obese Sprague Dawley rats altered the proportion and the ability of immune cells to respond to stimuli. These changes were independent of a diet effect on weight as there were no significant differences in weight among animals in different diet groups. Furthermore, the diets effectively induced obesity in these rats, as the expected weights of male rats of the same age (13-15 weeks) are reported to be from 350-425 g while the rats in this study were approximately 500 g (Charles River 2001; Alemaan, 1999; Lillie 1996). Rodents fed the HF diet were more insulin sensitive compared to those fed LF; however, no significant difference was observed between the two high MUFA diets. These changes were accompanied by altered phospholipid composition in the spleen and jejunum, as well as in TG sn-2 fatty acids in storage suggesting a possible mechanism for the observed outcomes. The results of this study suggest that altering either dietary fat composition, amounts or TG fatty acid composition (TG species) while maintaining overall fatty acid composition does have varying effects on diabetes risk by altering inflammation, acquired immune cell function, and insulin sensitivity.

Effect of changing the level of fat in the diet, while maintaining the fatty acid composition

Rodents fed a diet rich in simple carbohydrate (dextrose and corn starch) and lower in fat had splenocytes and MLN cells that had a more pro-inflammatory cytokine response to stimulation with Con A and CD3/CD28, both T-cell mitogens, compared to those fed HF. The HF diet replaced a proportion of the simple carbohydrate with fat of an identical composition. The pro-inflammatory response was observed in that both cell types produced greater concentrations of the pro-inflammatory cytokine IL-6 after Con A stimulation in MLN and CD3/CD28 stimulation of splenocytes (Table 15). Consistent with a higher inflammatory cytokine response to T cell stimulation, splenocytes from the LF rats also produced more IL-1β and less IL-10 after Con A stimulation.

The effects of feeding different combinations of dietary fat and carbohydrates, by altering both composition and amounts on inflammation have been studied; however, almost all of these studies are in conjunction with weight loss which is known to impact immune function (Heilbronn et al., 2001; Esposito et al., 2003; Sharman and Volek, 2004). A positive correlation was found between plasma hs-CRP concentrations of participants in the Women's Health Study and dietary glycemic load (which would be higher in the LF diet fed in this study), with the correlation being even stronger in overweight participants (Liu et al., 2002). It was concluded that quickly absorbed carbohydrates may contribute to the development of chronic inflammation; however, because of the study design used this finding cannot indicate causation. Another study compared a high fat diet (35% energy from fat) to a high carbohydrate diet (15% energy from fat) where the participants consumed their respective diets to strictly maintain weight (Kasim-Karakas et al., 2006). The weight maintenance period was followed up with an ad libitum diet period where most participants lost weight and had lower concentrations of plasma inflammatory mediators regardless of which diet group they were in. This finding is relatively consistent throughout the literature (Nicklas et al., 2005; Theuma and Fonseca, 2003). Without weight loss, there was a trend for increasing IL-6 and CRP plasma concentrations in subjects consuming a high carbohydrate diet group; however, it was not found to be significant (Kasim-Karakas et al., 2006). Sorensen et al attempted to compare a high sucrose diet to a lower carbohydrate diet by replacing the sucrose with artificial sweetener. Consuming a diet where artificial sweetener replaced simple carbohydrates resulted in lower plasma haptoglobin and transferrin; however, there were

significant differences in body weight change between groups at the end of the study (Sorensen et al., 2005). It is known that body weight has a considerable effect on plasma inflammation biomarkers (Theuma, 2003;Nicklas et al., 2005), therefore energy intake was a confounding factor in this study and it was not possible to distinguish the effect of nutrients on inflammation from weight loss. Esposito et al (Esposito et al., 2002) examined the effect of short term acute hyperglycemia using an IV glucose infusion to achieve increased blood glucose concentrations. The increase in blood glucose was accompanied by increases in plasma IL-6 and TNF-α concentrations suggesting that high carbohydrate meals increase inflammation in the short term. Based on this finding, along with the etiology of T2D, it is logical that a high sucrose diet would exacerbate inflammation; however, because many of these studies also examine weight loss and due to the difficulties of examining one nutrient in a diet it is difficult to discriminate the effect of sucrose and simple carbohydrates from total energy intake. In our study, plasma haptoglobin, an acute phase protein and marker of inflammation, changes were not observed between rodents fed HF and LF, although spleen and MLN T-cell function was altered in response to changes in the level of fat in the diet. There were significantly greater concentrations of some inflammatory cytokines produced by immune tissue of rodents fed LF compared to HF (Table 15); however, no significant difference in plasma haptoglobin was observed in the HF and LF diets, which is similar to the findings of the study by Kasim-Karakas et al.

Along with an increase in the production of pro-inflammatory cytokines after stimulation, feeding the low fat diet also resulted in a greater proportion of cytotoxic T-cells (%CD3CD8+) in spleen compared to those fed HF. Both spleen and MLN cells of animals fed LF had a significantly greater proportion of cytotoxic T-cells expressing activation markers such as CTLA4 (CD152) and the transferrin receptor (CD71), which is expressed by proliferating cells. The higher proportion of CD8+ cells (cytotoxic T cells) in spleen and the higher proportion of these cells expressing activation markers, particularly the transferrin receptor (CD71) likely contributed to this greater production of inflammatory cytokines, such as IL-6. CD8+ cells commonly produce a type 1 cytokine response (reviewed by (Mosmann and Sad, 1996)); however, significant differences in IFN-γ production, a cytokine representative of the type 1 response, were not observed.

Several studies have shown that altering either energy provided by fat or fatty acid composition of the diet has a greater effect on Th1 cytokines than Th2 cytokines. Studies of the effects of fatty acids on immune function and the production of inflammatory mediators have examined IL-2 production and proliferation; however, other markers have not been well studied. Also, as with many studies of lipids and immune function, the focus has been n-3 PUFA and not MUFA. An altered Th1 response was observed in a study by Wallace et al (Wallace et al., 2001) by varying the fat composition of diets fed to rodents. Splenocyte proliferation and mitogen stimulated cytokine production was compared between rodents fed a low fat diet (starch and sucrose carbohydrate source) compared to several high fat diets consisting of either a coconut, safflower or fish oil lipid source (Wallace et al., 2001). Rodents fed either the safflower or fish oil diets had splenocytes that produced less IFN-γ (Th1) and proliferated less with Con A stimulation compared to the low fat or coconut oil diet; however, IL-2 production was the same for all groups. This is consistent with our findings that IL-2 production by splenocytes did not differ in response to diet. There was also no difference in IL-4 production, a Th2 cytokine, between rodents fed different diets. A different study compared feeding a wheat based diet to a soy based diet in non-obese diabetic mice in the prediabetic stage (Flohe et al., 2003). The cereal based diet shifted the production of cytokines towards a Th1 response, increasing TNF-α and IFN-γ, with no change in IL-10 or TGF-β (Th2 cytokines) in the gut. The cereal based diet appeared to be more pro-inflammatory and a significantly greater proportion of the cereal fed mice developed diabetes compared to the soy based diet. Based on these studies, it appears that Th1 cytokines are more susceptible to dietary changes than Th2 cytokines. While there appeared to be some trends for greater production of IFN-γ and TNF-α by immune cells of rodents fed LF compared to those fed HF, the results were not significant.

In MLN, a primarily T-cell tissue, despite the higher IL-6 response, feeding LF resulted in a lower concentration of IL-2, a key cytokine required for proliferation. This is suggestive of altered T-cell proliferative function. This is interesting as MLN cells of animals fed LF also contained a greater proportion of helper T-cells expressing CD28 (Fig 6), a protein required for co-stimulation of the T-cell receptor (CD3), and thereby necessary for T-cell activation. T-cell dysfunction in obesity has been demonstrated in a mouse model of DIO. Mice injected with an influenza virus developed a greater number of CD8+ memory T-cells; however, they were less functional and poorly maintained,

compared to lean mice fed a low fat diet (Karlsson et al., 2010). A study comparing high fat fed obese mice and lean mice found an imbalance in Th1 type inflammatory cells and Foxp3+ regulatory cells that favoured inflammation in obese mice (Winer et al., 2009; Sell and Eckel, 2010). These studies further support the obesity induced shift towards a pro-inflammatory immune response and possibly T-cell dysfunction. T-cell dysfunction has been well characterized in genetic models of obesity (Ruth et al., 2008; Plotkin et al., 1996; Tanaka et al., 1998); however, this has been less studied in models of DIO.

The MLN of LF fed animals had a greater proportion of B-cells as well as cells expressing IgM compared to animals fed HF. There was also a greater proportion of CD68+CD11+ cells in these rats. CD68 is a marker for macrophages and monocytes, while CD11 is an integrin protein present on activated cells. Activated macrophages are involved in the activation of B-cells and production of immunoglobulins (Triantafilou, 2002). There were, however, no differences in cytokine production following LPS stimulation, a mitogen that acts mainly on B-cells as well as innate immune cells, between rodents fed LF and HF. B-cell activation by LPS is dependent on TLR4 (CD284) (Janeway and Ulevitch, 2000). There were no significant differences in the proportion of splenocytes or MLN cells expressing TLR4 between rodents fed HF or LF, which may have contributed to a similar LPS cytokine response. Several studies have shown an increase in CD11+ macrophages and monocytes in obese humans and animals in adipose tissue and blood (Sell and Eckel, 2010; Wu et al., 2010). CD11 is involved in T-cell activation and expansion (Sell and Eckel, 2010; Wu et al., 2010), which is consistent with the greater proportion of activated T-cells observed in this study.

A greater proportion of splenocytes were NK cells in rodents fed LF compared to those fed HF. NK cells are highly cytotoxic and have the ability to distinguish normal cells from abnormal cells through recognition of MHC class I (Moretta and Moretta, 2004). NK cells are activated by type 1 cytokines and can stimulate B-cells to synthesize immunoglobulins (reviewed by (Perussia, 1996; Vivier et al., 2008)). The presence of more B-cells, and possibly more NK cells, likely contributed to the greater proportion of IgM+ B-cells.

The results of the present study suggest that immune cells from GALT (i.e. MLN) of rodents fed LF may be more susceptible to the observed production of pro-inflammatory cytokines and possibly the generation of dysfunctional cells compared to immune cells

from animals fed HF as they were more activated and produced greater concentrations of cytokines with a lower concentration of the proliferative hormone IL-2.

As observed in other studies of dietary lipid composition, the fatty acids present in tissue reflected the fatty acids in the diet. As expected, feeding LF led to greater proportions of palmitic acid and oleic acid in the sn-2 position of liver and perirenal adipose TG, compared to feeding HF as these fatty acids are the end product of lipid synthesis in cells. When a greater proportion of the diet consisted of fat, the tissue fatty acid content was more similar to the diet, in this case exhibiting greater proportions of linoleic acid and stearic acid. Changes in spleen and jejunal phospholipids were also observed with greater proportions of palmitic acid and less linoleic acid in cells from both tissues of animals fed LF compared to those fed HF. In the jejunum only, rodents fed LF had a greater proportion of stearic acid and a lower proportion of oleic acid in phospholipids compared to those fed HF. Overall, feeding LF led to lower proportions of MUFA and PUFA in the spleen and a greater proportion of SF and lower PUFA in the jejunum (Tables 16 and 17). It is likely that these changes are involved in the observed differences in immune function as it has been well established that PUFA are involved in membrane function and cell signalling. A possible mechanism for this change is altered membrane fluidity caused by changes to the proportion of PUFA and SFA present in membrane phospholipids. Increasing the saturation of phospholipid fatty acids limits membrane fluidity while unsaturation increases membrane fluidity (Field et al., 1988; Clandinin et al., 1991; Calder, 2009). The degree of saturation of membrane phospholipid fatty acids is altered by diet fatty acid composition. The effect of MUFA in immune cell phospholipids is not well studied; however, it has been shown that increasing AA, EPA and DHA in immune cell phospholipids inhibits IL-2 production and T-cell proliferation in cell culture and in animal feeding studies (reviewed by (Calder, 2009)). Jeffery et al (Jeffery, 1997) showed that rat splenocytes treated with unsaturated fatty acids proliferated less when stimulated with Con A compared to other fatty acids. Other studies have shown that increases in the proportion of the saturated fatty acid stearic acid in PBMC membranes is correlated with increased production of IL-2, IFN-γ, and IL-4 (Kew et al., 2003). The effects of SF and the degree of unsaturation in membrane phospholipids observed in our study is consistent with the literature as feeding LF led to splenocytes that had significantly lower proportions of PUFA, jejunal cells with significantly more SFA and less PUFA, as well as a shift towards a pro-inflammatory response compared to feeding HF. The resulting

immune cell composition from feeding HF may lead to cells with greater membrane fluidity which effects protein and receptor function, and in turn alters the immune response.

Another aspect of metabolism that may have been altered by membrane phospholipid composition due to the level of dietary fat is insulin sensitivity. In rodents fed LF, plasma glucose concentration peaked at 10 minutes following a glucose challenge compared to 30 minutes in rodents fed HF. LF fed animals also had a significantly greater plasma glucose AUC value compared to HF. In response to higher plasma glucose, rodents fed LF also had a significantly greater plasma insulin peak concentration compared to HF. Several mechanisms have been observed in the literature whereby dietary lipids alter glucose metabolism. In this study membrane lipid composition was determined as a possible mechanism for differences in glucose metabolism.

As previously discussed, altered dietary fat alters the function and activity of membrane proteins and glucose uptake, through altering membrane lipid composition and therefore membrane fluidity, gene regulation and transcription (Field et al., 1988; Wild et al., 1997; Zhou et al., 1997). Glucose absorption is transporter mediated and requires membrane proteins such as the GLUT proteins in cells and sodium coupled transporters in the intestine (Bell et al., 1990). For example, studies have shown that lipids alter Na+/glucose co-transporter (SGLT-1) activity (Wild et al., 1997). Diabetic rats fed diets high in saturated fat had a greater expression of the transporter in the small intestine and increased glucose uptake, while feeding PUFA lowered glucose uptake in the intestine and exhibited lower amounts of the SGLT-1 protein and mRNA (Wild et al., 1997). PUFA have also been shown to increase glucose uptake, improve insulin sensitivity (Field et al., 1990), and extend the time that GLUT4 remains at the membrane (Gasperikova et al., 1997), while diets high in saturated fat limit the translocation of GLUT4 to the cell membrane and are associated with reduced expression of GLUT4 and insulin signalling (Clarke, 2000). In the current study, it was observed that rodents fed LF had significantly greater SFA and a lower proportion of PUFA in jejunum membrane phospholipids which may have led to an increased and more rapid glucose uptake from the intestine. While the diets had the same proportion of fat as PUFA and SFA, the HF diet provided more overall fat and therefore more PUFA as well as SFA and had a greater incorporation of PUFA and lower incorporation of SFA into membrane phospholipids.

Feeding OO did not lead to significant differences in glucose metabolism compared to feeding HF. Interestingly, compared to rodents fed HF, those fed OO had the same proportion of SF and PUFA in jejunal membrane phospholipids. The literature suggests that PUFA and SFA has significant effects on insulin sensitivity and glucose metabolism (Field et al., 1990; Wild et al., 1997; Clarke, 2000). In contrast to this, animals fed HCO had significantly higher plasma insulin and glucose AUC values compared to HF. Rats fed HCO had the same proportion of SFA and a lower proportion of PUFA in jejunal membrane phospholipids compared to those fed HF.

Other possible mechanisms by which glucose metabolism is altered by dietary fat composition and content are suggested in the literature. The delayed rise in plasma glucose in rodents fed three high fat diets (HF, OO, and HCO) may be related to delayed gastric emptying and subsequent slowed glucose transport in the epithelium (Collier and O'Dea, 1983; Joannic et al., 1997; Radulescu et al., 2009). Consuming fat prior to a high carbohydrate meal has been shown to slow gastric emptying and digestion in the small intestine, thereby slowing the glycemic response (Collier and O'Dea, 1983; Gentilcore et al., 2006). Radulescu et al. (2009) compared the effect of consuming either lard, safflower oil, or olive oil with potato on the glycemic response in humans. In this same study, although there was no difference in the area under the curve between diets, the rise in plasma glucose was slower and the peak was lower in the three high fat diets than for a low fat high carbohydrate meal (Radulescu et al., 2009).

Fatty acids may also be involved in the transcription of proteins that regulate insulin secretion, such as peroxisome proliferator-activated receptor γ (PPAR-γ) and uncoupling proteins (UCP) (Ito et al., 2004; Oberkofler et al., 2009). Exposure to free fatty acids alters PPAR-γ activation and leads increased transcription of UCP-1, a protein involved in suppressing insulin secretion (Ito et al., 2004). While HCO and OO contained the same proportion of fatty acids, the difference in the triglyceride species composition may have altered the free fatty acids that cells were exposed to, thereby having variable gene transcription outcomes. In this study, feeding HF led to a lower insulin response and slower glycemic response compared to feeding LF. Fewer differences were observed with feeding OO, while feeding HCO led to higher plasma insulin and glucose AUC values compared to HF. Altering dietary fat, including both fatty acid and TG species composition, may have led to differences in glucose metabolism and insulin secretion through an effect on the rate of digestion and absorption of glucose, glucose transport, or

gene regulation; however, these were not examined in the current study. This study suggests that the effect of dietary fat on membrane lipid composition likely has an important role in glucose metabolism.

Feeding a high carbohydrate diet led to a greater production of inflammatory cytokines and greater activation of CD8+ T-cells compared to a high fat diet. As well, differences in fatty acid storage in TAG and changes to phospholipid composition were observed. In this rodent model of DIO, replacing simple carbohydrates (dextrose and starch) with a diet containing 38% energy from fat, which also contained a larger amount of MUFA, appeared to result in a lower pro-inflammatory cytokine response after T-cell stimulation, compared to feeding the high carbohydrate diet (25% energy from fat, 15% fat as MUFA). This is important as obesity is a pro-inflammatory condition where chronic inflammation can lead to many complications and comorbidities including diabetes and cardiovascular disease (Navarro-Gonzalez and Mora-Fernandez, 2008; Dandona et al., 2004). Using dietary fat in proportions at the upper end of the Acceptable Macronutrient Distribution Range (20-35% energy as fat) to replace simple carbohydrate in the diet may be beneficial in this aspect, especially in the case of diabetes where carbohydrate metabolism is impaired. The MUFA content of the HF and LF diets may also contribute to the inflammatory responses observed rather than the total fat and carbohydrate composition alone, as the HF diet consisted of a greater proportion of MUFA relative to the LF diet. This is supported in the comparison of the HF diet to the high MUFA diets. Using diet to mediate inflammation could lower the risk for developing diabetes or lower the risk of complications in individuals already diagnosed with diabetes. As shown in these experiments, the type of fat, and more specifically the source of fat, also impacts immune function.

Feeding a high MUFA diet, either with an olive oil or high oleic acid Canola oil source, appeared to have less pro-inflammatory effects compared to feeding HF, a diet containing more SFA and PUFA and less MUFA. These effects include lower production of IL-6, TNF-α and IFN-γ by stimulated immune cells from rodents fed high MUFA diets. Splenocytes and MLN cells of rodents fed OO or HCO also produced significantly less IL-10 compared to those fed HF (Table 15). These effects may be most important in obesity and diabetes as these conditions are associated with a chronic inflammatory state characterized by greater circulating concentrations of IL-6 and TNF-α (Dandona et al., 2004; Esposito et al., 2002; Theuma and Fonseca, 2003). IFN-γ is representative of a

Th1 cytokine response which has pro-inflammatory effects; therefore a decrease in the production of this cytokine in cells from high MUFA fed rats suggests a shift away from the Th1 response. IL-10 is a regulatory cytokine that has a role in reducing the production of IL-6. While lower IL-10 production appears to be contrary to a less pro-inflammatory response, it may be that there was a lower stimulus for IL-10 production due to lower IL-6 production in the MUFA fed rats. While a lower Th1 response, characterized by lower concentrations of IFN-γ, is suggestive of a lower pro-inflammatory response, it is important to note that a shift towards a Th2 response may also have negative implications as it is associated with immune conditions such as allergy (Ngoc et al., 2005); however, Th2 cytokines, such as IL-4, were not measured in the current study. In some cases a lowered Th1 response may contribute to a change in the balance in the Th1 and Th2 type response and could have beneficial effects. For example, an imbalance was shown in a study of C57BL/6 mice fed a high fat diet to induce obesity, where a shift towards a Th1 response was observed (Strissel et al., 2010). At 12 weeks adipose tissue of high fat fed mice had greater IL-12 expression, a cytokine involved in inducing the Th1 response, as well as IFN-γ (Strissel et al., 2010). These mice also exhibited a down regulation of the Th2 cytokines IL-10 and IL-4, suggesting that obesity may alter Th1/Th2 balance, favouring the Th1 immune response (Strissel et al., 2010). Feeding high fat, high MUFA diets may alleviate the Th1 response in obesity.

MLN cells consisted of a greater proportion of activated T-helper cells (%CD3CD4+CD28+) in rats fed high MUFA diets compared to rats fed HF (Fig 6). Splenocytes from rats fed high MUFA diets also consisted of a greater proportion of NK cells (CD3-CD161+) and IgG expressing cells. In obesity, greater numbers of CD8+ cells and lower numbers of CD4+ have been observed (Stentz and Kitabchi, 2003). Ruth et al (Ruth et al., 2009) found that feeding fish oil high in n-3 PUFA in a rodent model of obesity helped to alleviate these effects. Animals fed fish oil had a greater proportion of CD3+ cells, along with a greater proportion of helper T-cells and helper T-cells expressing CD28 compared to obese animals fed a diet that was higher in n-6 PUFA (Ruth et al., 2009). Although the MUFA diets did not alter total T-cells compared to feeding HF, feeding MUFA had similar effects to feeding n-3 PUFA on the proportion of activated helper T-cells. Diets containing fish oil have been well characterized as having beneficial anti-inflammatory effects (Ruth et al., 2009; Calder, 2009). It has been hypothesized that changes to immune function following changes to dietary fatty acid

composition are the result of changes to cell membrane composition (Clandinin et al., 1991; Ruth et al., 2008). This has been studied extensively with PUFA but very little work has been published on the potential effects of high MUFA diets. By increasing dietary n-3 PUFA, n-3 PUFA is also increased in immune cell membranes (Ruth et al., 2009). Interestingly, feeding high MUFA diets also increased the incorporation of n-3 fatty acids in splenocyte phospholipids compared to those fed HF in our study (Table 16). It has been hypothesized that by increasing dietary MUFA and thereby displacing linoleic acid, competition for the enzyme Δ6 desaturase is limited (Rao et al., 1993). This enzyme is required for the synthesis of the anti-inflammatory n-3 metabolites EPA and DHA; however, the same enzyme is also used to metabolise n-6 PUFA, which are consumed in much larger amounts in a Western diet and favour more pro-inflammatory metabolites (Rao et al., 1993). Our results suggest that feeding MUFA may have some similar immune effects to feeding n-3 PUFA, possibly through the same mechanism, although to a lower extent. Given the recommendation to consume most dietary fat from a MUFA source, further investigation into the effects of MUFA on immune function would be worthwhile.

Lowering total dietary fat, regardless of type, increases NK cell activity while increasing dietary lipid suppresses NK cell activity (reviewed by (De Pablo and De Cienfuegos, 2000)). Fish oil and olive oil have been shown to lower NK cell activity to a greater extent compared to SFA and n-6 PUFA (reviewed by (De Pablo and De Cienfuegos, 2000). The results observed in this study are not consistent with these findings as we found a greater proportion of NK cell numbers in the spleen of rodents fed high MUFA diets. It is, however, possible that there could still be an effect of feeding MUFA on NK activity as this was not measured in the current study. The greater proportion of cells expressing IgG suggests a greater activation of B-cells in rodents fed high MUFA diets. In the primary immune response B-cells express IgM, and a class switch to IgG is stimulated by contact with a T-helper cell or dendritic cell as well as the presence of cytokines (Isakson et al., 1982; Cerutti et al., 2011). The increase in activated T-helper cells in rodents fed high MUFA diets may be consistent with this finding. Th1 cells can be involved in activating B-cells; however, not as effectively as Th2 cells (Parker 1993). Activity of Th2 cells was not assessed in this study. Future studies on the effects of MUFA on the immune system should measure Ig produced by stimulated immune cells

of animals fed high MUFA diets, as well as IL-4 production as a measure of Th2 cell activation. In the current study Ig expression was measured on unstimulated cells.

There were no differences between the two high MUFA diets in insulin and glucose metabolism. This could be due to smaller changes in membrane phospholipid composition between the two diets relative to comparing LF and HF. No changes to jejunum phospholipids were observed, and no changes in total PUFA, n-3 PUFA and n-6 PUFA were observed between HCO and OO fed animals. Although there was a significantly greater proportion of oleic and linoleic acid in splenocytes of rodents fed OO compared to HCO, this was not associated with a difference in insulin sensitivity.

There were several differences in cytokine production by stimulated immune cells of animals fed either OO or HCO compared to HF; however, fewer differences in immune cell phenotypes were observed. Activated helper T-cells can produce either a pro-inflammatory type 1 response (Th1) or a type 2 response (Th2) (reviewed by (Mosmann and Sad, 1996)). It is possible that feeding high MUFA diets may inhibit the production of Th1 type cytokines, such as TNF-α and IFN-γ. These results are similar to what has been observed with feeding n-3 PUFA (Ruth et al., 2009), which limits the pro-inflammatory immune response. This has also been observed with feeding vaccenic acid (Blewett et al., 2009). Obese rodents fed these diets had similar T-cell subset proportions to lean animals as well as lowered pro-inflammatory cytokine production (Ruth et al., 2009; Blewett et al., 2009). This supports findings of the benefits of a high MUFA diet and that these diets may be useful in modulating immune function. While both high MUFA diets exhibited these effects, the two diets were not equal in all aspects of immune function that were measured in this study.

Feeding high MUFA from OO as compared to the high fat diet

Cytokine production by stimulated immune cells from rats fed HCO and OO displayed few differences; however, there were several differences in immune cell phenotypes presented by cells from OO fed rats compared to both HF and HCO fed rats. Splenocytes of rodents fed OO had a greater proportion of T-cells (CD8+ and CD4+) expressing CTLA4, a regulatory protein that inhibits T-cell activation, as well as a significantly greater proportion of T-cells expressing the transferrin receptor (%CD4CD71+)

compared to HF (Table 13). Also, MLN cells of rodents fed OO had a greater proportion of T-regulatory cells (Treg, %CD4CD25+FoxP3+, Fig 5).

Rodents fed OO produced significantly lower concentrations of several pro-inflammatory cytokines compared to feeding HF (IL-6, TNF-α, IFN-γ). Interestingly, rodents fed OO exhibited a significantly greater proportion of activated immune cells compared to all other diets. Cell phenotypes were quantified in unstimulated immune cells, while cytokine production was measured following mitogen stimulation. It is possible that because cells were already activated in an unstimulated state, they did not become further stimulated to produce cytokines with the addition of a mitogen. Furthermore, CTLA4 is a regulatory protein that provides negative feedback to the activated T-cell (Alegre et al., 2001). The increased proportion of cells expressing CTLA4, especially CD4+ cells, may inhibit signalling to T-cells to produce inflammatory cytokines. The increased proportion of Tregs present also supports this, as they also produce a regulatory signal to limit the production of inflammatory mediators. It is likely that CD4+ cells expressing CTLA4 are, in fact, Tregs (Dieckmann et al., 2005), suggesting that feeding olive oil may induce increased Treg activity. Treg activity also increases in response to inflammation, which would be consistent with the significant increase in plasma haptoglobin that was observed with feeding OO compared to HF. Tregs have in important suppressive role in the immune system and a deficiency of Tregs is associated with autoimmune conditions (Fehervari and Sakaguchi, 2004); however, increased Treg numbers are observed in inflammatory conditions (de Kleer et al., 2004; Taflin et al., 2011). The significant increase in plasma haptoglobin concentration in rats fed OO compared to those fed HF was not expected given the numerous positive health outcomes, including lower plasma CRP and IL-6 concentrations, that have been associated with consuming olive oil (Estruch et al., 2006; Mena et al., 2009; Dai et al., 2008), and the lower production of pro-inflammatory cytokines by immune cells observed in this study. The greater concentration of plasma haptoglobin observed in rats fed OO may be related to the greater proportion of immune cells expressing activation markers in OO fed animals compared to those fed HF. It is also possible that the different immune tissues examined have different responses to varying proportions of dietary MUFA. Although splenocytes and PBMCs both represent the peripheral immune system, it may be interesting to examine the phospholipid fatty acid composition of circulating immune cells from

rodents fed different proportions of MUFA to determine a possible mechanism for the observed differences.

It is well established that varying the fatty acid composition of the diet alters immune function (Kew et al., 2003; Yaqoob et al., 1998; Ruth et al., 2008; Blewett et al., 2009). Olive oil has been of interest as a useful lipid source in total parenteral nutrition (TPN) because of its effects on immune function relative to soybean oil (n-6 PUFA), which is traditionally provided in TPN lipid emulsions. TPN involves the intravenous delivery of nutrition to bypass the gastrointestinal tract when it is non-functional. Splenocytes of rodents fed an olive oil based lipid emulsion (12% energy) had a greater number of cells expressing CD25, the IL-2 receptor involved in T-cell activation, compared to rodents fed a soybean oil based emulsion (Moussa et al., 2000). Furthermore, expression of CD25 was correlated with the amount of oleic acid present in spleen phospholipids (Moussa et al., 2000). Another study that compared TPN lipid emulsions with an olive oil base to a soybean oil base found that PBMCs from healthy volunteers exposed to varying concentrations of the high n-6 PUFA lipid dose dependently lowered splenocyte proliferation and CD4 and CD8 cell activation while the olive oil based lipid had no effect (Granato et al., 2000). Both lipids, however, decreased the production of TNF-α and IFN-γ by LPS stimulated PBMCs (Granato et al., 2000). These effects are consistent in the literature and it has been suggested that olive oil plays a role in inhibiting the production of pro-inflammatory mediators while maintaining immune function through cell activation (reviewed by (Wanten and Calder, 2007)). The increase in T-cell activation as determined by the expression of the transferrin receptor (CD71) in the current study, after feeding OO, is similar to findings for feeding n-3 PUFA. Feeding fish oil to sedentary rats increased the proportion of splenocytes expressing CD71 compared to rodents fed a diet consisting of more n-6 PUFA (Robinson and Field, 1998). Our findings were in agreement with the literature in that feeding OO led to increased lymphocyte activation compared to feeding HF, a diet higher in PUFA and SFA. Feeding olive oil may induce a shift away from a pro-inflammatory cytokine profile through increased activity of regulatory immune cells. This was shown by greater proportions of suppressive cells such as Tregs in MLN and cells expressing CTLA4 in the spleen of animals fed OO compared to those fed HF as well as a lower production of pro-inflammatory cytokines. The effect of olive oil on regulatory immune functions has not

been characterized; however induction of Tregs with EPA has been observed (Iwami et al., 2011).

Feeding MUFA from different sources

Although the HCO and OO diets contained the same proportion and amounts of fatty acids, as well as other diet components, there were differences in immune response. Stimulated immune cells of animals fed HCO produced significantly more IL-10 and TNF-α compared to those fed OO following stimulation with CD3/CD28, a mitogen that specifically targets the T-cell receptor.

There were also several differences in cell phenotypes between immune cells from animals fed HCO and OO. Rodents fed OO had a greater proportion of splenocytes that were CD4+CD152+ and CD4+CD71+. Additionally a greater proportion of activated macrophages (CD68+ expressing either CD11 or CD284) were present in the MLN of OO fed rats compared to HCO, as well as a greater proportion of cytotoxic T-cells expressing the transferrin receptor (Table 15). Activated monocytes and macrophages are associated with obesity; however, they are also involved in T-cell activation, which is consistent with our observations.

As previously discussed, B-cell activation by LPS can occur indirectly through the activation of innate immune cells by TLR4 which was expressed on a greater proportion of MLN cells in rodents fed OO compared to HCO. B-cells also present antigens to T-cells and thereby activate them (Moser and Leo, 2010). It is possible that altering dietary MUFA, both the amount and position on TG, may alter the activity of antigen presenting cells, thereby altering acquired immune cell activation and cytokine production rather than a direct effect on T or B-cells. Differences in immunoglobulin positive B-cells were also observed between the two high MUFA diets. In the spleen, rodents fed OO had a greater proportion of cells expressing IgG and lower proportion of cells expressing IgA compared to those fed HCO. In the MLN cells, a greater proportion of cells expressing both IgM and IgA in rodents fed OO compared to HCO were observed. IgG is expressed following an immune response, and the observation in the spleen of OO fed animals could be related to the greater proportion of activated T-helper cells. The greater proportion of cells expressing IgA in the MLN is also consistent with a greater proportion of activated T-helper cells and macrophages expressing the activation markers CD11 and

CD284, as these are responsible for presenting antigens to activate the B-cell and induce an Ig class switch. The switch to IgA in MLN but not the spleen in OO fed animals may be related to the proximity of MLN to the gut, where IgA has a specific role in immunity of the epithelium (Woof and Kerr, 2006). The greater proportion of cells expressing IgG in the spleen and IgA in the MLN, as well as a greater proportion of cells expressing TLR4 suggests that feeding OO leads to greater activation of both B and T-cells and may affect the interaction and activity of APC and T-helper cells.

Feeding either HCO or OO led to a lower production of inflammatory cytokines by immune cells compared to feeding HF; however, a greater effect was observed in OO fed animals. The most striking difference observed between feeding the two different MUFA sources was found in immune cell phenotype. There was no significant difference in IL-2 production; however, following high dose Con A (5 μg/ml) and CD3/CD28 stimulation splenocytes from rats fed OO had a higher rate of proliferation (measured by the rate of incorporation of 3H-thymidine) than those fed HCO. Obesity is reported to induce a constant inflammatory state and leads to impaired immune function (Nicklas et al., 2005; Pickup, 2004). In consequence, when presented with a challenge the immune system does not respond as well. The higher proportion of activated cells and greater proliferation with the same amount of IL-2 suggests a better immune response along with a lower production of inflammatory cytokines following OO feeding compared to HCO and HF in DIO rats.

One study found that feeding both safflower oil and fish oil in a DIO model lowered the production of IFN- γ and splenocyte proliferation (Con A) compared to feeding coconut oil or a low fat diet (Wallace et al., 2001). IL-2 production was also lowered with safflower and fish oil diets; however, IL-2 and IFN-γ mRNA was not different between safflower oil, coconut oil, or the low fat diet but fish oil fed animals had significantly lower mRNA levels for both IL-2 and IFN-γ (Wallace et al., 2001). This study suggests that different oils effect cytokine production and proliferation at different stages of cytokine synthesis, with fish oil affecting transcription and other oils having effects further down the pathway. This is an excellent example of how different dietary fats alter immune function. It may be that observed changes between feeding HCO or OO are due to changes in membrane receptors, creating a different response with similar cytokine production.

This study was designed to determine if feeding oils that differed in TG species but having the same overall fatty acid profile could alter the metabolism of fatty acids and effect change in acquired immunity and insulin sensitivity. The OO and HCO diets did differ in their triglyceride species composition of the sn-2 position. MUFA made up 60% of the fatty acids in the sn-2 position of OO triglycerides and 50% in HCO, while PUFA made up 33% of the triglyceride sn-2 fatty acids in OO, and 41% in HCO. As observed in studies of dietary fat and cell membrane lipid composition, the tissue fatty acid composition was altered in the direction of the dietary fat composition in both TG from liver and perirenal adipose tissue as well as in spleen and jejunum phospholipids. Animals fed either HCO or OO had a significantly greater proportion of oleic acid in these tissues compared to HF or LF, while HF fed animals had a larger proportion of linoleic acid (Fig 12-15). Feeding either MUFA source did not affect the storage of fatty acids as there was no observed difference in the sn-2 position of fatty acids on TG from the liver or perirenal adipose tissue. However, OO fed rats had significantly greater proportions of both oleic and linoleic acid in spleen phospholipids, a functional component of the cell membrane, compared to those fed HCO. Furthermore, rodents fed HCO had a greater proportion of total SF and a lower proportion of total MUFA in splenocytes phospholipids. This supports the hypothesis that these oils are metabolized differently as both diets contained the same proportions of oleic and linoleic acid, as well as other fatty acids. As previously discussed, SF in cell membranes is a factor in membrane fluidity. This observed difference in lipid incorporation into membrane phospholipids may have contributed to the differences in immune function observed between HCO and OO. It was hypothesized that HCO and OO may have different metabolic outcomes due to differences in TG species and the stereospecificity of fatty acids in TG within the oils, although both MUFA sources contained the same proportions of fatty acids. It is possible that MUFA in the sn-2 position of dietary triglycerides is incorporated into phospholipids to a greater extent and has a greater effect in mitigating the pro-inflammatory response. A strength of this study was that the experimental diets were blends of oils to ensure they contained the same fatty acids and they were balanced for vitamin E and phenols. This allowed for a focus on outcomes due to different TG species altering metabolism of lipids and thereby altering the effects on immune function.

Rats fed HCO had significantly greater AUC values for both plasma insulin and glucose concentration over a 120 minute period as well as greater plasma insulin and glucose

concentration peak following an OGTT compared to those fed HF (Fig 4). Plasma insulin and glucose AUC for rodents fed OO fell in between HF and HCO and was not significantly different from either group. This suggests that rats fed HCO were more glucose intolerant and were insulin resistant compared to rats fed HF. High MUFA diets that have been studied typically use an olive oil source and have shown beneficial effects on insulin sensitivity. Improved insulin sensitivity has been associated with the Mediterranean diet in epidemiological studies (Rumawas et al., 2009; Esposito et al., 2009; Panagiotakos et al., 2007), as well as with high MUFA diets in clinical trials (Vessby et al., 2001; Yokoyama J et al., 2008) and animal studies (Picinato et al., 1998). This result from feeding the HCO diet appears to be contrary to what is observed in high MUFA diets in the literature; however high oleic acid Canola oil and its effect on insulin sensitivity has not been studied. This should be further examined as this is an oil that is recommended as healthy based on the MUFA content.

Summary

Based on the findings of this study as well as the current literature in the area, it appears that adding olive oil as the major source of MUFA in the diet may lessen the pro-inflammatory response of the immune system in obesity, similar to what has been reported when obese animals are fed diets that are high in long chain n-3 PUFA. Furthermore, olive oil was more effective in shifting the immune system towards an anti-inflammatory/resolution response compared to diets high in n-6 PUFA and SFA, while the response of cells from rats fed the high oleic acid Canola oil fell somewhere in between olive oil and n-6 PUFA in its ability to inhibit the production of pro-inflammatory mediators. The finding that different sources of MUFA affect the immune response with different potencies is novel. MUFA have previously been grouped together as being equivalent. While attention has been paid to n-3 PUFA, it is well established, for example, that EPA, DHA and ALA have different effects on the immune response; the Canadian Diabetes Association recommends most fat in the diet be consumed as MUFA, regardless of the source. Interestingly, feeding the two high MUFA diets led to differences in fatty acid incorporation into spleen phospholipids suggesting that the metabolism of lipids is altered not only by the specific fatty acids present in the diet but also their stereospecific positioning on TG. Notably, splenocyte phospholipids of rodents fed OO contained a greater proportion of oleic acid, linoleic acid and total MUFA

while those fed HCO had a greater proportion of total SF. SF composition and PUFA such as linoleic acid have been shown to alter membrane fluidity, which is associated with changes in the function of proteins within the membrane, including the insulin receptor and receptors related to immune function. MUFA has not been well studied in this regard; however it is likely that this also affects the cell membrane properties and the function of proteins contained there. There was no observed difference in glucose metabolism between the two high MUFA diets; however, HCO fed animals appeared to be more insulin resistant than rodents fed HF. Because high oleic acid Canola oil and insulin sensitivity has not been previously studied and is not in agreement with the literature on high MUFA diets this result should be further investigated.

A large project is currently underway involving the creation of the 'Alberta Diet', a diet developed specially for people living in Alberta with diabetes. This diet is based on the Mediterranean diet; however, foods that are locally available and accepted by the target population are being incorporated into the diet to ensure its cultural acceptability. Recently, a new high oleic acid Canola oil was developed with the same proportion of oleic acid as olive oil, a characteristic component of the Mediterranean diet. It was hypothesized that this new oil could present the same health benefits as olive oil and would be a more accessible and affordable substitute for olive oil in the Alberta Diet. While the high oleic acid Canola oil had some beneficial immune effects such as lowering IL-6 production by immune cells, it was not as effective in creating a lessened pro-inflammatory response after stimulation when compared to olive oil. Furthermore, rodents fed HCO appeared to have impaired insulin sensitivity compared to those fed OO. It would be worthwhile to compare the effects of HCO and OO on other diabetes risk factors that have been shown to be improved with olive oil. One study has examined this and found improved LDL-cholesterol and total cholesterol in a human trial that compared diets containing 36% energy from fat with either a high oleic acid Canola fat source or Western diet based fat source (high in saturated fat) (Gillingham et al., 2011). This study did not find any differences in inflammatory markers, including circulating IL-6, CRP and adhesion molecules, between the two diets. Participants consumed their respective diets for a period of 28 days (Gillingham et al., 2011). It may be interesting to examine the effects of high oleic acid Canola oil on immune function in humans in a longer term trial as well as examine the effect on insulin and glucose metabolism. A gap in the literature currently exists in regard to the study of dietary MUFA, MUFA in cell

membrane phospholipids and its effect on membrane fluidity, and on immune function. Studies in these areas are needed, especially with the recommendation to consume most dietary fat as MUFA. It would be interesting to investigate the effect of feeding MUFA in an obese model and compare it to a lean control model. Furthermore, T-cell function and immune function should be characterized in diet induced obese models and compared to a lean model. This has been characterized in genetically obese models; however the effect of obesity and diet on T-cell function in DIO models is unclear. Further examination of the effects of oils containing the same fatty acid composition but differing in TG species should also be performed as study in this area is limited. Dietary fat recommendations are based on total MUFA, PUFA, SF, and to some extent the type of PUFA; however, recommendations do not consider the source of these lipids. This study shows that different sources of the same fatty acids do, in fact, have significant effects on fatty acid incorporation into phospholipids, which was associated with changes in immune function, insulin sensitivity and potentially other physiologic outcomes.

Literature Cited

Aderem, A., and R. J. Ulevitch. 2000. Toll-like receptors in the induction of the innate immune response. Nature 406(6797):782-787.

Albers, R., J. M. Antoine, R. Bourdet-Sicard, P. C. Calder, M. Gleeson, B. Lesourd, S. Samart∅n, I. R. Sanderson, J. Van Loo, F. W. Vas Dias, and B. Watzl. 2005. Markers to measure immunomodulation in human nutrition intervention studies. British Journal of Nutrition 94(03):452-481.

Alegre, M. L., K. A. Frauwirth, and C. B. Thompson. 2001. T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol 1(3):220-228.

Alemaan, C. L., R. M. Mas, I. Rodeiro, M. Noa, C. Hernandez, R. Menendez, and R. Gamez. 1998. Reference database of the main physiological parameters in Sprague-Dawley rats from 6 to 32 months. Lab Anim 32(4):457-466.

Anderson, R. J., K. E. Freedland, R. E. Clouse, and P. J. Lustman. 2001. The Prevalence of Comorbid Depression in Adults With Diabetes. Diabetes Care 24(6):1069-1078.

Aranda, F., S. Gomez-Alonso, R. M. Rivera del -lamo, M. D. Salvador, and G. Fregapane. 2004a. Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: Comparison with other Spanish cultivars. Food Chemistry 86(4):485-492.

Augustine, K. A., and R. M. Rossi. 1999. Rodent mutant models of obesity and their correlations to human obesity. Anat. Rec. 257(2):64-72.

Bell, G. I., T. Kayano, J. B. Buse, C. F. Burant, J. Takeda, D. Lin, H. Fukumoto, and S. Seino. 1990. Molecular biology of mammalian glucose transporters. Diabetes Care 13(3):198-208.

Blewett, H. J., C. A. Gerdung, M. R. Ruth, S. D. Proctor, and C. J. Field. 2009. Vaccenic acid favourably alters immune function in obese JCR:LA-cp rats. British Journal of Nutrition 102(04):526-536.

Bracco, U. 1994. Effect of triglyceride structure on fat absorption. Am J Clin Nutr 60(6):1002S-11009.

Brehm, B. J., B. L. Lattin, S. S. Summer, J. A. Boback, G. M. Gilchrist, R. J. Jandacek, and D. A. D'Alessio. 2009. One-Year Comparison of a High Monounsaturated Fat Diet With a High-Carbohydrate Diet in Type 2 Diabetes. Diabetes Care 32(2):215-220.

Calder, P. C. 2009. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins, Leukotrienes and Essential Fatty Acids 79(3-5):101-108.

Canadian Diabetes Association. 2009. The prevalence and cost of diabetes. http://www.diabetes.ca/documents/about-diabetes/Prevalanceand Cost_09.pdf.

Canadian Diabetes Association Clinical Practice Guidelines Expert Committee. 2008. Canadian Diabetes Association 2008 clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes 32(Suppl 1):S1-S201.

Canola Council of Canada. 2006. Canola Quick Facts. http://www.canola-council.org/facts_trade.aspx.

Carter, S. J., M. B. Roberts, J. Salter, and C. B. Eaton. 2010. Relationship between Mediterranean Diet Score and atherothrombotic risk: Findings from the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. Atherosclerosis 210(2):630-636.

Cerutti, A., I. Puga, and M. Cols. 2011. Innate control of B cell responses. Trends in Immunology 32(5):202-211.

Charles, M. A., A. Fontbonne, N. Thibult, J. M. Warnet, G. E. Rosselin, and E. Eschwege. 1991. Risk factors for NIDDM in white population: Paris prospective study. (noninsulin-dependent diabetes mellitus). Diabetes. 40(7): 796-799.

Clandinin, M. T., S. Cheema, C. J. Field, M. L. Garg, J. Venkatraman, and T. R. Clandinin. 1991. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J. 5(13):2761-2769.

Clarke, S. D. 2000. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. British Journal of Nutrition 83(Supplement S1):S59-S66.

Collier, G., and K. O'Dea. 1983. The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein. The American Journal of Clinical Nutrition 37(6):941-944.

Cruz-Hernandez, C., D. Zeyuan, Z. Jianqiang, A. Hill, M. Yurawecz, P. Delmonte, M. Mossoba, M. Dugan, and J. Kramer. 2004. Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. Journal of AOAC International 87(2):545-562.

Dai, J., A. H. Miller, J. D. Bremner, J. Goldberg, L. Jones, L. Shallenberger, R. Buckham, N. V. Murrah, E. Veledar, P. W. Wilson, and V. Vaccarino. 2008. Adherence to the Mediterranean Diet Is Inversely Associated With Circulating Interleukin-6 Among Middle-Aged Men: A Twin Study. Circulation 117(2):169-175.

Dandona, P., A. Aljada, and A. Bandyopadhyay. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in Immunology 25(1):4-7.

de Jong, E. C., P. L. Vieira, P. Kalinski, J. H. N. Schuitemaker, Y. Tanaka, E. A. Wierenga, M. Yazdanbakhsh, and M. L. Kapsenberg. 2002. Microbial Compounds Selectively Induce Th1 Cell-Promoting or Th2 Cell-Promoting Dendritic Cells In Vitro with Diverse Th Cell-Polarizing Signals. The Journal of Immunology 168(4):1704-1709.

de Kleer, I., L. R. Wedderburn, L. S. Taams, A. Patel, H. Varsani, M. Klein, W. de Jager, G. Pugayung, F. Giannoni, G. Rijkers, S. Albani, W. Kuis, and B. Prakken. 2004. CD4+CD25bright Regulatory T Cells Actively Regulate Inflammation in the Joints of Patients with the Remitting Form of Juvenile Idiopathic Arthritis. The Journal of Immunology 172(10):6435-6443.

de Lorgeril, M., and S. Renaud. 1994. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 343(8911):1454.

De Pablo, M. A., and G. A. De Cienfuegos. 2000. Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78(1):31-39.

Delarue J, LeFoll C, Corporeau C, and Lucas D. 2004. N-3 long chanin polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? Reproduction nutrition development 44(3):289-299.

Dennis, E. 2000. Phospholipase A2 in Eicosanoid Generation. Am. J. Respir. Crit. Care Med. 161(2):S32-S35.

Dieckmann, D., H. Plottner, S. Dotterweich, and G. Schuler. 2005. Activated CD4+CD25+ T cells suppress antigen-specific CD4+ and CD8+ T cells but induce a suppressive phenotype only in CD4+ T cells. Immunology 115(3):305-314.

Donath, M. Y., D. M. Schumann, M. Faulenbach, H. Ellingsgaard, A. Perren, and J. A. Ehses. 2008. Islet Inflammation in Type 2 Diabetes. Diabetes Care 31(Supplement 2):S161-S164.

Egert, S., M. Kratz, F. Kannenberg, M. Fobker, and U. Wahrburg. 2010. Effects of high-fat and low-fat diets rich in monounsaturated fatty acids on serum lipids, LDL size and indices of lipid peroxidation in healthy non-obese men and women when consumed under controlled conditions. European Journal of Nutrition:1-9.

Escriba, P. V., A. s. Ozaita, C. Ribas, A. Miralles, E. Fodor, T. Farkas, and J. s. A. Garcia-Sevilla. 1997. Role of lipid polymorphism in G protein-membrane interactions: Nonlamellar-prone phospholipids and peripheral protein binding to membranes. Proceedings of the National Academy of Sciences of the United States of America 94(21):11375-11380.

Esposito, K., F. Nappo, R. Marfella, G. Giugliano, F. Giugliano, M. Ciotola, L. Quagliaro, A. Ceriello, and D. Giugliano. 2002. Inflammatory Cytokine Concentrations Are Acutely Increased by Hyperglycemia in Humans: Role of Oxidative Stress. Circulation 106(16):2067-2072.

Esposito, K., A. Pontillo, C. Di Palo, G. Giugliano, M. Masella, R. Marfella, and D. Giugliano. 2003. Effect of Weight Loss and Lifestyle Changes on Vascular Inflammatory Markers in Obese Women. JAMA 289(14):1799-1804.

Esposito, K., M. I. Maiorino, C. Di Palo, D. Giugliano, and for the Campanian Postprandial Hyperglycemia Study Group. 2009. Adherence to a Mediterranean diet and glycaemic control in Type 2 diabetes mellitus. Diabetic Medicine 26(9):900-907.

Esposito, K., M. I. Maiorino, A. Ceriello, and D. Giugliano. 2010. Prevention and control of type 2 diabetes by Mediterranean diet: A systematic review. Diabetes Research and Clinical Practice 89(2):97-102.

Estruch, R., M., Martinez-Gonzalez, D. Corella, J. Salas-Salvado, V. Ruiz-Gutierrez, M. a. I. Covas, M. Fiol, E. Gomez-Gracia, M. C. Lopez-Sabater, E. Vinyoles, F. Aros, M. Conde, C. Lahoz, J. Lapetra, G. Saez, E. Ros, and for the PREDIMED Study Investigators. 2006. Effects of a Mediterranean-Style Diet on Cardiovascular Risk Factors. Annals of Internal Medicine 145(1):1-11.

Fantuzzi, G., and R. Faggioni. 2000. Leptin in the regulation of immunity, inflammation, and hematopoiesis. Journal of Leukocyte Biology 68(4):437-446.

Fearon, D. T., and R. M. Locksley. 1996. The Instructive Role of Innate Immunity in the Acquired Immune Response. Science 272(5258):50-54.

Feghali CA, and Wright TM. 1997. Cytokines in acute and chronic inflammation. Frontiers in bioscience 2:d12-d26.

Fehervari, Z., and S. Sakaguchi. 2004. CD4+ Tregs and immune control. Journal of Clinical Investigation 114(9):1209-1217.

Ferrick, D. A., M. D. Schrenzel, T. Mulvania, B. Hsieh, W. G. Ferlin, and H. Lepper. 1995. Differential production of interferon-[gamma] and interleukin-4 in response to Th1- and Th2-stimulating pathogens by [gamma][delta] T cells in vivo. Nature 373(6511):255-257.

Field, C. J., A. Angel, and M. T. Clandinin. 1985. Relationship of diet to the fatty acid composition of human adipose tissue structural and stored lipids. Am J Clin Nutr 42(6):1206-1220.

Field, C. J., E. A. Ryan, A. B. Thomson, and M. T. Clandinin. 1988. Dietary fat and the diabetic state alter insulin binding and the fatty acyl composition of the adipocyte plasma membrane. Biochem. J. 253(2):417-424.

Flohe, S. B., H. E. Wasmuth, J. B. Kerad, P. E. Beales, P. Pozzilli, R. B. Elliott, J. P. Hill, F. W. Scott, and H. Kolb. 2003. A wheat-based, diabetes-promoting diet induces a Th1-type cytokine bias in the gut of NOD mice. Cytokine 21(3):149-154.

Fowler, M. J. 2008. Microvascular and Macrovascular Complications of Diabetes. Clinical Diabetes 26(2):77-82.

Frostegard, J., A. K. Ulfgren, P. Nyberg, U. Hedin, J. Swedenborg, U. Andersson, and G. K. Hansson. 1999. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis 145(1):33-43.

Gabay, C., and I. Ushner. 1999. Acute-Phase Proteins and Other Systemic Responses to Inflammation. New England Journal of Medicine 340(6):448-454.

Gagliardi, M. C., F. Sallusto, M. Marinaro, A. Langenkamp, A. Lanzavecchia, and M. T. De Magistris. 2000. Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. Eur. J. Immunol. 30(8):2394-2403.

Galli, C., and P. C. Calder. 2009. Effects of Fat and Fatty Acid Intake on Inflammatory and Immune Responses: A Critical Review. Annals of Nutrition and Metabolism 55(1-3):123-139.

Garg, A. 1998. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. The American Journal of Clinical Nutrition 67(3):577S-582S.

Gentilcore, D., R. Chaikomin, K. L. Jones, A. Russo, C. Feinle-Bisset, J. M. Wishart, C. K. Rayner, and M. Horowitz. 2006. Effects of Fat on Gastric Emptying of and the Glycemic, Insulin, and Incretin Responses to a Carbohydrate Meal in Type 2 Diabetes. Journal of Clinical Endocrinology & Metabolism 91(6):2062-2067.

Gill, J. M., J. C. Brown, M. J. Caslake, D. M. Wright, J. Cooney, D. Bedford, D. A. Hughes, J. C. Stanley, and C. J. Packard. 2003. Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics: dose-dependent effects on LDL. Am J Clin Nutr 78(1):47-56.

Gillingham, L., S. Harris-Janz, and P. Jones. 2011. Dietary Monounsaturated Fatty Acids Are Protective Against Metabolic Syndrome and Cardiovascular Disease Risk Factors. Lipids 46(3):209-228.

Giugliano, D., A. Ceriello, and K. Esposito. 2006. The Effects of Diet on Inflammation: Emphasis on the Metabolic Syndrome. Journal of the American College of Cardiology 48(4):677-685.

Goldney, R. D., P. J. Phillips, L. J. Fisher, and D. H. Wilson. 2004. Diabetes, Depression, and Quality of Life. Diabetes Care 27(5):1066-1070.

Granato, D., S. P. Blum, C. Rossle, J. Le Boucher, A. Malnoe, and G. Dutot. 2000. Effects of Parenteral Lipid Emulsions With Different Fatty Acid Composition on Immune Cell Functions In Vitro. Journal of Parenteral and Enteral Nutrition 24(2):113-118.

Gsperikova, D., I. Klimes, T. Kolter, P. Bohov, A. Maassen, J. Eckel, M. T. Clandinin, and E. Sebokova. 1997. Glucose Transport and Insulin Signaling in Rat Muscle and Adipose Tissue Effect of Lipid Availabilitya. Annals of the New York Academy of Sciences 827(1):144-157.

Heilbronn, L. K., M. Noakes, and P. M. Clifton. 2001. Energy Restriction and Weight Loss on Very-Low-Fat Diets Reduce C-Reactive Protein Concentrations in Obese, Healthy Women. Arterioscler Thromb Vasc Biol 21(6):968-970.

Hodgkin, P., L. Yamashita, R. Coffman, and M. Kehry. 1990. Separation of events mediating B cell proliferation and Ig production by using T cell membrane and lymphokines. The Journal of Immunology 145(7):2025-2034.

Hulbert, A. J., N. Turner, L. H. Storlien, and P. L. Else. 2005. Dietary fats and membrane function: implications for metabolism and disease. Biological Reviews 80(1):155-169.

Hunter, J. E. 2001. Studies on effects of dietary fatty acids as related to their position on triglycerides. Lipids 36:655-668.

Isakson, P. C., E. Pure, E. S. Vitetta, and P. H. Krammer. 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. The Journal of Experimental Medicine 155(3):734-748.

Ito, E., S. Ozawa, K. Takahashi, T. Tanaka, H. Katsuta, S. Yamaguchi, M. Maruyama, M. Takizawa, H. Katahira, K. Yoshimoto, S. Nagamatsu, and H. Ishida. 2004. PPAR-γ overexpression selectively suppresses insulin secretory capacity in isolated pancreatic islets through induction of UCP-2 protein. Biochemical and Biophysical Research Communications 324(2):810-814.

Iwami, D., K. Nonomura, N. Shirasugi, and M. Niimi. 2011. Immunomodulatory effects of eicosapentaenoic acid through induction of regulatory T cells. International Immunopharmacology 11(3):384-389.

Jeffery N.M., Cortina M, Newsholme EA, and Calder PC. 1997. Effects of variations in the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. British Journal of Nutrition 77(5):805-823.

Jeffery N.M., Yaqoob P, Mewsholme EA, and Calder PC. 1996. The effects of olive oil upon rat serum lipids levels and lymphocyte functions appear to be due to oleic acid. Annals of Nutrition and Metabolism 40(2):71-80.

Jiang, R., J. E. Manson, M. J. Stampfer, S. Liu, W. C. Willett, and F. B. Hu. 2002. Nut and peanut butter consumption and risk of type 2 diabetes in women. JAMA 288:2554-2560.

Joannic, J. L., S. Auboiron, J. Raison, A. Basdevant, F. Bornet, and B. Guy-Grand. 1997. How the degree of unsaturation of dietary fatty acids influences the glucose and insulin responses to different carbohydrates in mixed meals. The American Journal of Clinical Nutrition 65(5):1427-1433.

Johnson, J.,and S. U.Balko. 2009a. Epidemiological trends of diabetes in Alberta. in Alberta Diabetes Atlas 2009. Johnson JA, ed. Institute of Health Economics, Edmonton.

Johnson, J., D.M.Rabi, A. L.Edwards, and S.U.Balko. 2009b. Diabetes and Health Care Utilization in Alberta. in Alberta Diabetes Atlas 2009. Johnson JA, ed. Institute of Health Economics, Edmonton.

Karlsson, E. A., P. A. Sheridan, and M. A. Beck. 2010. Diet-Induced Obesity in Mice Reduces the Maintenance of Influenza-Specific CD8+ Memory T Cells. The Journal of Nutrition 140(9):1691-1697.

Karupaiah, T., and K. Sundram. 2007. Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: a review of their nutritional implications. Nutrition & Metabolism 4(1):16.

Kasim-Karakas, S. E., A. Tsodikov, U. Singh, and I. Jialal. 2006. Responses of inflammatory markers to a low-fat, high-carbohydrate diet: effects of energy intake. The American Journal of Clinical Nutrition 83(4):774-779.

Kew, S., T. Banerjee, A. M. Minihane, Y. E. Finnegan, C. M. Williams, and P. C. Calder. 2003. Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25-72 y. Am J Clin Nutr 77(5):1278-1286.

Knowler. 2002. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. New England Journal of Medicine 346(6):393-403.

Kobayashi, H., S. Miura, H. Nagata, Y. Tsuzuki, R. Hokari, T. Ogino, C. Watanabe, T. Azuma, and H. Ishii. 2004. In situ demonstration of dendritic cell migration from rat intestine to mesenteric lymph nodes: relationships to maturation and role of chemokines. Journal of Leukocyte Biology 75(3):434-442.

Kotake J, Y. Tanaka, Umehara N, Miyashita A, Tsuru T, Hikida S, and Mizote H. 2004. Effects of a high-monounsaturated fat diet on glucose and lipid metabolisms in normal and diabetic mice. Journal of Nutritional Science and Vitaminology 50(2):106-113.

Kubow, S. 1996. The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis. J Nutr Biochem 7:530-541.

Levin, B. E., A. A. Dunn-Meynell, B. Balkan, and R. E. Keesey. 1997. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 273(2):R725-R730.

Li, S., H. Y. Zhang, C. C. Hu, y. Lawrence, K. E. Gallagher, A. Surapaneni, S. T. Estrem, J. N. Calley, G. Varga, E. R. Dow, and Y. Chen. 2008. Assessment of Diet-induced Obese Rats as an Obesity Model by Comparative Functional Genomics. Obesity 16(4):811-818.

Lillie, L. E., N. J. Temple, and L. Z. Florence. 1996. Reference values for young normal Sprague-Dawley rats: weight gain, hematology and clinical chemistry. Human & Experimental Toxicology 15(8):612-616.

Lindstrom, J., P. Ilanne-Parikka, M. Peltonen, S. Aunola, J. G. Eriksson, K. Hemio, H. Hamalainen, P. Harkonen, S. Keinanen-Kiukaanniemi, M. Laakso, A. Louheranta, M. Mannelin, M. Paturi, J. Sundvall, T. T. Valle, M. Uusitupa, and J. Tuomilehto. 2006. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. The Lancet 368(9548):1673-1679.

Lindstrom, J., A. Louheranta, M. Mannelin, M. Rastas, V. Salminen, J. Eriksson, M. Uusitupa, and J. Tuomilehto. 2003. The Finnish Diabetes Prevention Study (DPS). Diabetes Care 26(12):3230-3236.

Lipscombe, L. L., and J. E. Hux. 2007. Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995-2005: a population-based study. The Lancet 369(9563):750-756.

Liu, S., J. E. Manson, J. E. Buring, M. J. Stampfer, W. C. Willett, and P. M. Ridker. 2002. Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. The American Journal of Clinical Nutrition 75(3):492-498.

Lohr, J., B. Knoechel, S. Jiang, A. H. Sharpe, and A. K. Abbas. 2003. The inhibitory function of B7 costimulators in T cell responses to foreign and self-antigens. Nat Immunol 4(7):664-669.

Maedler, K., J. Oberholzer, P. Bucher, G. A. Spinas, and M. Y. Donath. 2003. Monounsaturated Fatty Acids Prevent the Deleterious Effects of Palmitate and High Glucose on Human Pancreatic Cell Turnover and Function. Diabetes 52(3):726-733.

Martinez-Gonzalez, M., C. d. l. Fuente-Arrillaga, J. M. Nunez-Cordoba, F. J. Basterra-Gortari, J. J. Beunza, Z. Vazquez, S. Benito, A. Tortosa, and M. Bes-Rastrollo. 2008. Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. BMJ 336(7657):1348-1351.

Mattson, F. H. 1959. The absorbability of stearic acid when fed as a simple or mixed triglyceride. J Nutr 69:338-342.

McManus, K., L. Antinoro, and F. Sacks. 2001. A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. International Journal of Obesity & Related Metabolic Disorders 25(10):1503.

Mebius, R. E., and G. Kraal. 2005. Structure and function of the spleen. Nat Rev Immunol 5(8):606-616.

Mena, M. P., E. Sacanella, M. Vazquez-Agell, M. Morales, M. Fito, R. Escoda, M. Serrano-Martinez, J. Salas-Salvado, N. Benages, R. Casas, R. M. Lamuela-Raventos, F. Masanes, E. Ros, and R. Estruch. 2009. Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. The American Journal of Clinical Nutrition 89(1):248-256.

Mokdad, A. H., B. A. Bowman, E. S. Ford, F. Vinicor, J. S. Marks, and J. P. Koplan. 2001. The Continuing Epidemics of Obesity and Diabetes in the United States. JAMA 286(10):1195-1200.

Moretta, L., and A. Moretta. 2004. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J 23(2):255-259.

Moser, M., and O. Leo. 2010. Key concepts in immunology. Vaccine 28(Supplement 3):C2-C13.

Mosmann, T. R., and S. Sad. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunology Today 17(3):138-146.

Moussa, M., J. Le Boucher, J. Garcia, J. Tkaczuk, J. Ragab, G. Dutot, E. Ohayon, J. Ghisolfi, and J. P. Thouvenot. 2000. In vivo effects of olive oil-based lipid emulsion on lymphocyte activation in rats. Clinical Nutrition 19(1):49-54.

Mu, H., and C. K. Hoy. 2004. The digestion of dietary triacylglycerols. Prog in Lipid Res 43:105-133.

Navarro-Gonzalez, J. F., and C. Mora-Fernandez. 2008. The Role of Inflammatory Cytokines in Diabetic Nephropathy. Journal of the American Society of Nephrology 19(3):433-442.

Neelands, P. J., and M. T. Clandinin. 1983. Diet fat influences liver plasma-membrane lipid composition and glucagon-stimulated adenylate cyclase activity. Biochem. J. 212(3):573-583.

Ngoc, L., D. Gold, A. Tzianabos, O. Arther, S. Weiss, and J. Celedon. 2005. Cytokines, allergy and asthma. Current Opinion in Allergy and Clinical Immunology 5(2):161-166.

Nicklas, B. J., T. You, and M. Pahor. 2005. Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. CMAJ 172(9):1199-1209.

Oakes, N. D., G. J. Cooney, S. Camilleri, D. J. Chisholm, and E. W. Kraegen. 1997. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 46(11):1768-1774.

Oberkofler, H., M. Hafner, T. Felder, F. Krempler, and W. Patsch. 2009. Transcriptional co-activator peroxisome proliferator-activated receptor (PPAR) co-activator-1 is involved in the regulation of glucose-stimulated insulin secretion in INS-1E cells. Journal of Molecular Medicine 87(3):299-306.

Paletas, K., E. Athanasiadou, M. Sarigianni, P. Paschos, A. Kalogirou, M. Hassapidou, and A. Tsapas. 2010. The Protective Role of the Mediterranean Diet on the Prevalence of Metabolic Syndrome in a Population of Greek Obese Subjects. J Am Coll Nutr 29(1):41-45.

Panagiotakos, D. B., N. Tzima, C. Pitsavos, C. Chrysohoou, A. Zampelas, D. Toussoulis, and C. Stefanadis. 2007. The Association between Adherence to the Mediterranean Diet and Fasting Indices of Glucose Homoeostasis: The ATTICA Study. J Am Coll Nutr 26(1):32-38.

Paniagua, J. A., A. G. de la Sacristana, E. Sanchez, I. Romero, A. Vidal-Puig, F. J. Berral, A. Escribano, M. J. Moyano, P. Perez-Martinez, J. Lopez-Miranda, and F. Perez-Jimenez. 2007. A MUFA-Rich Diet Improves Posprandial Glucose, Lipid and GLP-1 Responses in Insulin-Resistant Subjects. J Am Coll Nutr 26(5):434-444.

Parker, D. C. 1993. T Cell-Dependent B Cell Activation. Annual Review of Immunology 11(1):331-360.

Perez-Jimenez, F., A. Espino, F. Lopez-Segura, J. Blanco, V. Ruiz-Gutierrez, J. L. Prada, J. Lopez-Miranda, J. Jimenez-Pereperez, and J. M. Ordovas. 1995. Lipoprotein concentrations in normolipidemic males consuming oleic acid-rich diets from two different sources: olive oil and oleic acid-enriched sunflower oil. Am J Clin Nutr 62:769-775.

Perona, J. S., O. Vogler, J. M. Sanchez-Dominguez, E. Montero, P. V. Escriba, and V. Ruiz-Gutierrez. 2007. Consumption of Virgin Olive Oil Influences Membrane Lipid Composition and Regulates Intracellular Signaling in Elderly Adults With Type 2 Diabetes Mellitus. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 62(3):256-263.

Perussia, B. 1996. The Cytokine Profile of Resting and Activated NK Cells. Methods 9(2):370-378.

Picinato, M. C., R. Curi, U. F. Machado, and A. R. Carpinelli. 1998a. Soybean- and olive-oils-enriched diets increase insulin secretion to glucose stimulus in isolated pancreatic rat islets. Physiology & Behavior 65(2):289-294.

Pickup, J. C. 2004. Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes. Diabetes Care 27(3):813-823.

Plotkin, B. J., D. Paulson, A. Chelich, D. Jurak, J. Cole, J. Kasimos, J. R. Burdick, and N. Casteel. 1996. Immune responsiveness in a rat model for type II diabetes (Zucker rat, fa/fa): susceptibility to Candida albicans infection and leucocyte function. J Med Microbiol 44(4):277-283.

Prades, J. S., S. R. S. Funari, P. V. Escriba, and F. Barcelo. 2003. Effects of unsaturated fatty acids and triacylglycerols on phosphatidylethanolamine membrane structure. Journal of Lipid Research 44(9):1720-1727.

Prescott S.L., and P.C. Calder. 2004. N-3 polyunsaturated fatty acids and allergic disease. Current Opinion in Clinical Nutrition and Metabolic Care 7(2):123-129.

Public Health Agency of Canada. 2009. Report from the National Diabetes Surveillance System: Diabetes in Canada 2009. http://www.phac-aspc.gc.ca/publicat/2009/ndssdic-snsddac-09/pdf/report-2009-eng.pdf .

Radulescu, A., Y. Hassan, M. C. Gannon, and F. Q. Nuttall. 2009. The Degree of Saturation of Fatty Acids in Dietary Fats Does Not Affect the Metabolic Response to Ingested Carbohydrate. Journal of the American College of Nutrition 28(3):286-295.

Ramirez-Tortosa, M. C., C. M. Aguilera, J. L. Quiles, and A. Gil. 1998. Influence of dietary lipids on lipoprotein composition and LDL Cu2+-induced oxidation in rabbits. Biofactors 8(1/2):79.

Ramsey, S. D., K. Newton, D. Blough, D. K. McCulloch, N. Sandhu, G. E. Reiber, and E. H. Wagner. 1999. Incidence, outcomes, and cost of foot ulcers in patients with diabetes. Diabetes Care 22(3):382-387.

Rao, C., E. Zang, and B. Reddy. 1993. Effect of high fat corn oil, olive oil and fish oil on phospholipid fatty acid composition in male F344 rats. Lipids 28(5):441-447.

Redgrave, T. G., D. R. Kodali, and D. M. Small. 1988. The effect of triacyl-sn-glycerol structure on the metabolism of chylomicrons and triacylglycerol-rich emulsions in the rat. J Biol Chem 263:5118-5123.

Rivellese, A. A., R. Giacco, G. Annuzzi, C. De Natale, L. Patti, L. Di Marino, V. Minerva, G. Costabile, C. Santangelo, R. Masella, and G. Riccardi. 2008. Effects of monounsaturated vs. saturated fat on postprandial lipemia and adipose tissue lipases in type 2 diabetes. Clinical Nutrition 27(1):133-141.

Robertson, P., E. Seaquist, and T. F. Walseth. 1991. G proteins and modulation of insulin secretion. Diabetes 40(1): 1-6.

Robinson, L. E., and C. J. Field. 1998. Dietary Long-Chain (n-3) Fatty Acids Facilitate Immune Cell Activation in Sedentary, but not Exercise-Trained Rats. The Journal of Nutrition 128(3):498-504.

Roche, H. M., A. Zampelas, J. M. Knapper, D. Webb, C. Brooks, K. G. Jackson, J. W. Wright, B. J. Gould, A. Kafatos, M. J. Gibney, and C. M. Williams. 1998. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. Am J Clin Nutr 68(3):552-560.

Ros, E. 2003. Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. Am J Clin Nutr 78(3):617S-6625.

Rumawas, M. E., J. B. Meigs, J. T. Dwyer, N. M. McKeown, and P. F. Jacques. 2009. Mediterranean-style dietary pattern, reduced risk of metabolic syndrome traits, and incidence in the Framingham Offspring Cohort. The American Journal of Clinical Nutrition 90(6):1608-1614.

Ruth, M. R., S. D. Proctor, and C. J. Field. 2009. Feeding long-chain n-3 polyunsaturated fatty acids to obese leptin receptor-deficient JCR:LA-cp rats modifies immune function and lipid-raft fatty acid composition. British Journal of Nutrition 101(09):1341-1350.

Ruth, M. R., C. G. Taylor, P. Zahradka, and C. J. Field. 2008. Abnormal Immune Responses in fa/fa Zucker Rats and Effects of Feeding Conjugated Linoleic Acid. Obesity 16(8):1770-1779.

Sakaguchi, S., T. Yamaguchi, T. Nomura, and M. Ono. 2008. Regulatory T Cells and Immune Tolerance. Cell 133(5):775-787(Abstr.)

Sell, H., and J. Eckel. 2010. Adipose tissue inflammation: novel insight into the role of macrophages and lympocytes. Current Opinon in Clinical Nutrition and Metabolic Care 4(13):366-370.

Shevach, E. M., R. A. DiPaolo, J. Andersson, D. M. Zhao, G. L. Stephens, and A. M. Thornton. 2006. The lifestyle of naturally occurring CD4+CD25+Foxp3+ regulatory T cells. Immunological Reviews 212(1):60-73.

Shaikh, S. R., D. Mitchell, E. Carroll, M. Li, J. Schneck, and M. Edidin. 2008. Differential Effects of a Saturated and a Monounsaturated Fatty Acid on MHC Class I Antigen Presentation. Scandinavian Journal of Immunology 68(1):30-42.

Sharman, M. J., and J. S. Volek. 2004. Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men. Clin. Sci. 107(4):365-369.

Sinha, A., Lopez MT, and McDevitt HO. 1990. Autoimmune diseases: the failure of self tolerance. Science 248(4961):1380-1388.

Sofi, F., R. Abbate, G. F. Gensini, and A. Casini. 2010. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. The American Journal of Clinical Nutrition 92(5):1189-1196.

Sorensen, L. B., A. Raben, S. Stender, and A. Astrup. 2005. Effect of sucrose on inflammatory markers in overweight humans. The American Journal of Clinical Nutrition 82(2):421-427.

Speakman, J., C. Hambly, S. Mitchell, and E. Krol. 2008. The contribution of animal models to the study of obesity. Lab Anim 42(4):413-432.

Spranger, J., A. Kroke, M. Mahlig, K. Hoffmann, M. M. Bergmann, M. Ristow, H. Boeing, and A. F. H. Pfeiffer. 2003. Inflammatory Cytokines and the Risk to Develop Type 2 Diabetes. Diabetes 52(3):812-817.

Stentz, F., and E. Kitabchi. 2003. Activated T Lymphocytes in Type 2 Diabetes: Implications From in Vitro Studies. Current Drug Targets 4(6):493-503.

Stratton, I. M., A. I. Adler, H. A. Neil, D. R. Matthews, S. E. Manley, C. A. Cull, D. Hadden, R. C. Turner, and R. R. Holman. 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321:405-412.

Strissel, K. J., J. DeFuria, M. E. Shaul, G. Bennett, A. S. Greenberg, and M. S. Obin. 2010. T-Cell Recruitment and Th1 Polarization in Adipose Tissue During Diet-Induced Obesity in C57BL/6 Mice. Obesity 18(10):1918-1925.

Taflin, C., B. Favier, J. Baudhuin, A. Savenay, P. Hemon, A. Bensussan, D. Charron, D. Glotz, and N. Mooney. 2011. Human endothelial cells generate Th17 and regulatory T cells under inflammatory conditions. Proceedings of the National Academy of Sciences 108(7):2891-2896.

Tanaka, S. i., F. Isoda, T. Yamakawa, M. Ishihara, and H. Sekihara. 1998. T Lymphopenia in Genetically Obese Rats. Clinical Immunology and Immunopathology 86(2):219-225.

Taylor, C. G., and P. Zahradka. 2004. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. The American Journal of Clinical Nutrition 79(6):1164S-1168S.

Theuma, P., and V. Fonseca. 2003. Inflammation and emerging risk factors in diabetes mellitus and atherosclerosis. Current Diabetes Reports 3(3):248-254.

Toobert, D. J., R. E. Glasgow, L. A. Strycker, M. Barrera, J. L. Radcliffe, R. C. Wander, and J. D. Bagdade. 2003. Biologic and Quality-of-Life Outcomes From the Mediterranean Lifestyle Program. Diabetes Care 26(8):2288-2293.

Toobert, D. J., R. E. Glasgow, L. A. Strycker, M. Barrera, D. P. Ritzwoller, and G. Weidner. 2007. Long-term effects of the Mediterranean lifestyle program: a randomized clinical trial for postmenopausal women with type 2 diabetes. International Journal of Behavioural Nutrition and Physical Activity 4(1).

Trautwein, E. A., D. Rieckhoff, A. Kunath-Rau, and H. F. Erbersdobler. 1999. Replacing Saturated Fat with PUFA-Rich (Sunflower Oil) or MUFA-Rich (Rapeseed, Olive and High-Oleic Sunflower Oil) Fats Resulted in Comparable Hypocholesterolemic Effects in Cholesterol-Fed Hamsters. Annals of Nutrition & Metabolism 43(3):159-172.

Trichopoulou, A., T. Costacou, C. Bamia, and D. Trichopoulos. 2003. Adherence to a Mediterranean Diet and Survival in a Greek Population. New England Journal of Medicine 348(26):2599-2608.

Tuomilehto, J., J. Lindstrom, J. G. Eriksson, T. T. Valle, H. Hamalainen, P. Ilanne-Parikka, S. Keinanen-Kiukaanniemi, M. Laakso, A. Louheranta, M. Rastas, V. Salminen, S. Aunola, Z. Cepaitis, V. Moltchanov, M. Hakumaki, M. Mannelin, V. Martikkala, J. Sundvall, and M. Uusitupa. 2001. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. New England Journal of Medicine 344(18):1343-1350.

Uusitupa, M., A. Louheranta, and J. Lindstrom. 2000. The Finnish Diabetes Prevention Study. British Journal of Nutrition 83:S137-S142.

Vazquez, C. M., R. Zanetti, C. Santa-Maria, and V. Ruiaz-Gutierrez. 2000. Effects of Two Highly Monounsaturated Oils on Lipid Composition and Enzyme Activities in Rat Jejunum. Bioscience Reports 20(5):355-368.

Vessby, B., M. Uusitupa, K. Hermansen, G. Riccardi, A. A. Rivellese, L. C. Tapsell, C. Nalsen, L. Berglund, A. Louheranta, B. M. Rasmussen, G. D. Calvert, A. Maffetone, E. Pedersen, I. B. Gustafsson, and L. H. Storlien. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU study. Diabetologia 44(3):312-319.

Vivier, E., E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini. 2008. Functions of natural killer cells. Nat Immunol 9(5):503-510.

Wahle, K., D. Caruso, J. Ochoa, and J. Quiles. 2004. Olive oil and modulation of cell signaling in disease prevention. Lipids 39(12):1223-1231.

Wallace, F. A., E. A. Miles, C. Evans, T. E. Stock, P. Yaqoob, and P. C. Calder. 2001. Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. Journal of Leukocyte Biology 69(3):449-457.

Wang, Y., J. Lu, M. R. Ruth, S. D. Goruk, M. J. Reaney, D. R. Glimm, D. F. Vine, C. J. Field, and S. D. Proctor. 2008. Trans-11 Vaccenic Acid Dietary Supplementation Induces Hypolipidemic Effects in JCR:LA-cp Rats. J. Nutr. 138(11):2117-2122.

Wanten, G. J., and P. C. Calder. 2007. Immune modulation by parenteral lipid emulsions. The American Journal of Clinical Nutrition 85(5):1171-1184.

Wellen, K. E., and G. S. Hotamisligil. 2005. Inflammation, stress, and diabetes. J Clin Invest 115(5):1111-1119.

Welters, H. J., M. Tadayyon, J. H. B. Scarpello, S. A. Smith, and N. G. Morgan. 2004. Mono-unsaturated fatty acids protect against [beta]-cell apoptosis induced by saturated fatty acids, serum withdrawal or cytokine exposure. FEBS Letters 560(1-3):103-108.

Wild, G. E., R. Turner, L. G. S. Chao, J. Faria, M. Keelan, M. T. Clandinin, and A. B. R. Thomson. 1997. Dietary lipid modulation of Na+/glucose co-transporter (SGLT1), Na+/K+ ATPase, and ornithine decarboxylase gene expression in the rat small intestine in diabetes mellitus. The Journal of Nutritional Biochemistry 8(12):673-680.

Wild, S., G. Roglic, A. Green, R. Sicree, and H. King. 2004. Global Prevalence of Diabetes. Diabetes Care 27(5):1047-1053.

Willett, W. C., F. Sacks, A. Trichopoulou, G. Drescher, A. Ferro-Luzzi, E. Helsing, and D. Trichopoulos. 1995. Mediterranean diet pyramid: a cultural model for healthy eating. The American Journal of Clinical Nutrition 61(6):1402S-1406S.

Winer, S., Y. Chan, G. Paltser, D. Truong, H. Tsui, J. Bahrami, R. Dorfman, Y. Wang, J. Zielenski, F. Mastronardi, Y. Maezawa, D. J. Drucker, E. Engleman, D. Winer,

and H. M. Dosch. 2009. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 15(8):921-929.

Woof, J. M., and M. A. Kerr. 2006. The function of immunoglobulin A in immunity. J. Pathol. 208(2):270-282.

Wu, H., X. D. Perrard, Q. Wang, J. L. Perrard, V. R. Polsani, P. H. Jones, C. W. Smith, and C. M. Ballantyne. 2010. CD11c Expression in Adipose Tissue and Blood and Its Role in Diet-Induced Obesity. Arterioscler Thromb Vasc Biol 30(2):186-192.

Wykes, M., A. Pombo, C. Jenkins, and G. G. MacPherson. 1998. Dendritic Cells Interact Directly with Naive B Lymphocytes to Transfer Antigen and Initiate Class Switching in a Primary T-Dependent Response. The Journal of Immunology 161(3):1313-1319.

Yamada, K., P. Hung, K. Yoshimura, S. Taniguchi, B. Ou Lim, and M. Sugano. 1996. Effect of Unsaturated Fatty Acids and Antioxidants on Immunoglobulin Production by Mesenteric Lymph Node Lymphocytes of Sprague-Dawley Rats. Journal of Biochemistry 120(1):138-144.

Yaqoob, P., and P. C. Calder. 1995. The effects of dietary lipid manipulation on the production of murine T cell-derived cytokines. Cytokine 7(6):548-553.

Yaqoob, P., J. A. Knapper, D. H. Webb, C. M. Williams, E. A. Newsholme, and P. C. Calder. 1998. Effect of olive oil on immune function in middle-aged men. The American Journal of Clinical Nutrition 67(1):129-135

Yaqoob, P. 2002. Monounsaturated fatty acids and immune function. European Journal of Clinical Nutrition 56(8):S9.

Yokoyama J, Someya Y, Yoshihara R, and Ishii H. 2008. Effects of High-monounsaturated Fatty Acid Enteral Formula versus High-carbohydrate Enteral Formula on Plasma Glucose Concentration and Insulin Secretion in Healthy Individuals and Diabetic Patients. The Journal of International Medical Research 36:137-146.

Zhou, Y. P., and V. E. Grill. 1994. Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93(2):870-876.

Zock, P. L., J. H. M. de Vries, N. J. de Fouw, and M. B. Katan. 1995. Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans. Am J Clin Nutr 61:48-55.