THE EFFECTS OF HEMPSEED OIL AND HULLED HEMPSEED ON HEALTH BIOMARKERS

by

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ABBREVIATIONS

AA	Amino acid
ALA	Alpha linolenic acid
ANOVA	Analysis of variance
BMI	Body mass index
CE	Cholesteryl esters
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DGLA	dihomo-gamma linolenic acid
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
GC	Gas chromatography
GLA	Gamma linolenic acid
HDL	High density lipoprotein
LA	Linolenic acid
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acid
N-3	Omega-3 fatty acid
N-6	Omega-6 fatty acid
PUFA	Polyunsaturated fatty acid
PWV	Pulse wave velocity
RCFFN	Richardson Centre for Functional Foods and Nutraceuticals
SE	Standard error
SDA	Stearidonic acid
SFA	Saturated fatty acid
TC	Total cholesterol
TG	Total triglycerides

THC	Delta-9-tetrahydrocannabinol
VLDL	Very low density lipoprotein

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death in Canada (Heart & Stroke Foundation, 2015). Though, there has been significant progress in lowering the number of incidences over the last 60 yr, there are approximately 1.6 M Canadians living with heart disease or the effects from a stroke (Heart & Stroke Foundation, 2015). CVD is related to atherosclerosis, which is the process of plaque building up on the walls of the arteries (American Heart Association, 2016). CVD also includes other diseases such as heart failure, arrhythmia and heart valve problems (American Heart Association, 2016). The risk factors associated with CVD include smoking, physical inactivity, over-weight or obesity, high blood pressure, diabetes, stress or not consuming the daily recommendations for fruit and vegetables by having less than 5 servings/d (Heart & Stroke Foundation, 2015).

Diet is a critical preventative measure. In addition to consuming adequate amounts of fruits and vegetables, research has shown that polyunsaturated fatty acid (PUFAs) consumption has been shown to have a beneficial influence on risk factors for CVD (Mozaffarian et al., 2011). PUFAs such as the omega-6 (n-6) fatty acid, linoleic acid (LA) and the omega-3 (n-3) fatty acid, alpha-linolenic acid (ALA), are essential fatty acids (EFAs) to humans. These EFAs are not synthesized in the body, but need to be obtained through dietary sources. Some of these sources include a variety of seeds and some nuts. ALA can be converted into eicosapentaenoic acid (EPA), a precursor for

docosahexaenoic acid (DHA). Both EFAs, LA and ALA are needed from the diet, they both require the same delta 6-desaturase, delta 5-desaturase, and elongases enzymes for their further conversion into arachidonic acid and EPA. Therefore these enzymes are the rate-limiting step (Rodriguez-Leyva, 2010). The optimum ratios of these EFAs, however, are n-6:n-3 at 2:1-3:1 (Kaul et al., 2008). This optimal ratio is seen in hempseed. Additional to these previously mentioned PUFAs, hemp is also high in gamma linolenic acid (GLA) and stearidonic acid (SDA) (Rodriguez-Leyva et al., 2010). Interestingly, these fatty acids are biological metabolites during ALAs conversion to EPA and DHA (Rodriguez-Leyva et al., 2010).

Hemp is a member of the Cannabaceae family and belongs to the crop *Cannabis sativa L.* There are two main types of *Cannabis sativa L.,* the most common plant known is marijuana which contains delta-9-tetrahydrocannabinol (THC) (Rodriguez-Leyva et al., 2010). THC is present at concentrations of 1-20% and is the compound that gives the plant the psychoactive properties (Rodriguez-Leyva et al., 2010). The second type would be hemp, which is bred to contain THC levels of less than 0.3% and has no psychoactive properties (Rodriguez-Leyva et al., 2010).

Hemp has an excellent nutritional composition. Hemp contains the optimal ratio of n-6:n-3, 2:1-3:1, high protein levels of 24.8 g per 100 g, fibre levels of 27.6 g/100 g and vitamin E levels of 90 mg per 100 g. Hemp also has high levels of minerals such as phosphorus (1160 mg/100 g), potassium (859 mg/100 g), magnesium (483 mg/100 g), calcium (145 mg/100 g), iron (14 mg/100 g), zinc (7 mg/100 g) and the amino acid (AA) arginine at 3.1 g/100 g (Rodriguez-Leyva et al., 2010).

Research examining the benefits of hemp products on human health is limited as a result of the stigma that was previously associated with the plant. Hemp has been accepted world-wide since the 1800's for its many applications (Manitoba Agriculture, Food and Rural Development, *Industrial Hemp Production*). In 1938, Canada implemented an Opium and Narcotics Control Act which put an end to hemp production across North America (Manitoba Agriculture, Food and Rural Development). The stigma behind the crop prevented research and insight into this powerhouse plant, until the mid-1990s. Canada was able to breed the crop to contain low levels of THC at less than 0.3% and by 1998 the ban was lifted (Manitoba Agriculture, Food and Rural Development). Since 1998, industrial hemp has been gaining popularity in the health and nutrition world because of its excellent nutritional composition.

As a result of the limited data available on hemp, further research needs to be conducted to examine the potential health benefits that hemp may have to offer.

1.2 RATIONALE

Hempseed is known to have an excellent nutritional composition. It is a source of GLA, SDA, LA and ALA. These EFAs are known to show a positive effect in reducing the damaging health risks associated with CVD (Rodriguez-Leyva et al., 2010). Due to the lack of formal research studies conducted targeting these potential health benefits that hemp may have, the overarching goal of this study is to uncover some of the key areas to determine hemp's effects in modulating CVD risk factors.

1.3 OBJECTIVES

The research objectives of the study are to examine the effects of hulled hemp seeds and hemp oil on healthy, overweight individuals after a 4 wk supplementation period compared to a control product.

1. The primary objective will be to investigate the RBC fatty acid profile to determine if any changes are found after consumption of the hemp versus control treatment.
2. The secondary objective will examine the differences found in plasma fatty acid profile after consumption of the hemp versus control treatment.
3. The third objective will look into serum lipid and glucose concentration responses between consumption of hemp versus control treatments.
4. The final objective will determine if various measurements including anthropometry, body composition, blood pressure or arterial stiffness improve after consumption of hemp versus control treatment.

1.4 HYPOTHESIS

The research hypotheses of the study to be evaluated are:

1. That the consumption of hulled hempseed and hemp oil which is high in EFAs will improve the omega-3 index in the RBC of the participants.
2. Plasma fatty acid profiles will show great increase in the concentrations of GLA, SDA, LA and ALA after the hemp treatment.
3. After the consumption of the hemp treatment, serum lipids such as high density lipoprotein (HDL) levels will increase and low density lipoprotein

(LDL) levels will decrease and show overall lowering of total triglycerides (TG), total cholesterol (TC) and glucose levels.

4. The overall addition of incorporating more healthy fats into the diet will show an improvement in lowering body weight, increasing lean muscle and decreasing fat distribution throughout the body. The results will also include synergistically lowering blood pressure and improvement of arterial stiffness.

The following literature will bring awareness to the research currently available on hempseed. The literature will also examine both the clinical and animal studies and critically assess the research gaps.

CHAPTER TWO

LITERATURE REVIEW

2.1 CLINICAL STUDIES EXAMINING THE HEALTH IMPACTS AFTER HEMPSEED OIL CONSUMPTION

Hemp has been shown to have health improvements in individuals after short term consumption. Schwab et al. (2006) conducted one of the first studies which identified limitations in current research involving hempseed. Most studies did not have adequate control or placebo groups and did not control supplementation dosage. The studies also did not include diet records from the participants. As a result, this group designed their trial as a controlled, randomized, double-blinded, cross-over study to compare the effects of dietary hempseed oil and flaxseed oil on the serum lipid profile, fasting total and lipoprotein concentrations, plasma glucose and insulin concentrations as well as haemostatic factors in healthy volunteers. The study included 14 volunteers, 8 men and 6 females without chronic disease, BMI less than 30 kg/m2 and between the ages of 25-60 yr. Participants were randomly assigned to treatments and then crossed over to the other treatment after a four week wash out period, commencing the second phase. The study conducted by Schwab et al. (2006) consisted of two 4 wk phases with a 4 wk wash out period between the two phases. Participants were asked to come to the facility at the beginning and end of each intervention phase. During the intervention phases, participants were asked to consume 30 mL/d of the assigned oil. Results of the study showed differences in the effects on fatty acid composition of serum lipids. Hempseed oil had higher LA concentrations in serum cholesteryl esters (CE) and TG

when compared to the flaxseed oil treatment. Hempseed oil had higher concentrations of GLA. Both oil treatments had high levels of ALA but flaxseed oil had higher concentrations compared to the hempseed oil. Flaxseed oil also contained higher levels of EPA. Both hempseed and flaxseed oil consumption lowered serum TG concentrations but there were no effects on serum concentrations of CE or lipoprotein cholesterol, plasma glucose or insulin or haemostatic factors.

Thus, research indicates hemp consumption improves LA, GLA and ALA levels in circulation with 30 mL/d of hempseed oil for 4 wk and that the length of the treatment phase is an adequate amount of time to see the beneficial results. Future studies should incorporate these values into the experimental treatment dosages and study timeline. Research also reveals that consumption of hempseed oil has been shown to reduce TG levels in the circulation (Schwab et al., 2006).

Another study conducted by Kaul et al. (2008) had two objectives. The first objective was to determine if lower concentrations of supplemented PUFAs would be able to be distributed to a sample size that reflects the general population by administrating two, 1 g capsules/d and produce a significant effect on the biochemical parameters associated with CVD. The second objective was to compare the effects from three different PUFA sources, which consisted of fish oil, flaxseed oil and hempseed oil. The study design was a double-blinded, placebo-controlled trial using a daily dosage of two, 1 g capsules/day of the assigned PUFAs supplement. There were 88 participants who completed the study and the blood samples were collected on wk 0, 6 and 12 of the study. The results of the study showed that hempseed oil failed to produce a difference in levels of EPA, DHA, LA or ALA on wk 6 or 12. Hempseed

consumption had no significant effect on TC, TG, LDL or HDL levels. These results suggest that the dosage of two 1 g capsules/d failed to contain high enough levels of hempseed oil to show health benefits. The researchers suggested that ALA and LA may not be absorbed as well in the gastrointestinal tract as EPA and DHA. The researchers also suggested that there may be an intrinsic difference in the absorption of fatty acids.

The study shows that a higher dosage of hempseed may need to be incorporated in order to see a difference in the fatty acid profiles. Future studies should incorporate 30 mL/d of hempseed oil, as well as, two 30 g/d of seeds as the experimental treatment dosage. The limitations found in the previous hemp studies addressed by Schwab et al. (2006) provide great insight into how properly design a feeding trial.

In the study by Lemke et al (2015), the trial design followed a similar structure as the previously mentioned study. The study did not include a control group and only provided 15g Echium oil/day with the objectives being to find an increase in plasma EPA concentrations. The study consisted of two 6 week intervention periods with a minimum of a 2 week wash out period. Results of the study observed no difference in serum TG but a possible limitation may have been attributed to not having a control group to test the results against. Also, no significant difference was seen in the omega-3 index but a rise in EPA concentration in RBC phospholipids did occur.

These findings suggest that dosage may play a critical factor when determining intervention treatment. The study by Kaul et al. (2008) supports the idea or adequate dosage and future studies should look to blind the products by masking the hempseed oil and control oil by formulating the products into a similar salad dressing. Hempseed

oil, however, has a distinctive dark green colour that may be difficult to mask completely in a salad dressing.

2.2 ANIMAL STUDIES EXAMINING THE HEALTH IMPACTS AFTER HEMPSEED OIL CONSUMPTION

Two animal studies have examined hempseed oil's effect on platelet aggregation. The first study by Richard et al. (2006) involved a 12 wk feeding trial with rats. Eight rats were randomly assigned to each of the three groups. The first group contained 5% of grounded hempseed in the meal, the second group contained 10% of ground hempseed in the meal and the third was the control group which included 1% palm oil into the meal. The results of the study showed that the two groups that contained ground hempseed had an increase in LA and ALA levels which induced changes in the plasma fatty acid profiles. Also, platelet aggregation was greatly inhibited in hempseed-fed groups. The second study performed by Prociuk et al. (2008) examined platelet aggregation and plasma and lipid concentrations of hypercholesterolemic rabbits fed various treatments of hempseed meals. The first meal contained the control with 10% hempseed, the second meal contained the control with 10% partially dilipidated hempseed, the third meal contained the control with 0.5% cholesterol, the fourth meal contained 10% hempseed and 0.5% cholesterol and the final meal contained the control meal with 5% coconut oil. Results of the study showed no significant differences in platelet aggregation across treatments. Meals containing cholesterol appeared to have an increase in platelet aggregation. The interesting finding of the study was that the meal containing 10% hempseed and 0.5% cholesterol appeared to have a normalized

platelet aggregation rate. The authors suggested that these findings may be a result of the hempseed being present in the meal.

Two animal studies have been conducted to examine the effects that hempseed may have on cardiac ischemic reperfusion injury. The first study by Al-Khalifa et al. (2007) examined how rats would recover from ischemia-reperfusion when fed various diets. Rats were assigned to one of the four diets which consisted of diet one containing 5% hempseed, diet two containing 10% hempseed, diet three containing 1% palm oil and diet four containing 10% dilipidated hempseed for the 12 wk study. What the researchers found was that there were no significant differences in the heart LA concentrations. The 10 % hempseed diet produced the greatest significant difference in ALA levels and all hempseed containing diets had a better recovery from ischemia-reperfusion when compared to the control diet. The other study conducted by Prociuk et al (2006) aimed to investigate the potential for hempseed to provide cardio-protective effects during ischemia-reperfusion injury. Rabbits were assigned to one of the six diets for 8 weeks. The first diet consisted of the control meal, the second meal contained 10% ground hempseed, the third meal 10% partially delipidated hempseed, the fourth meal contained 0.5% cholesterol, the fifth meal contained 0.5% cholesterol plus hempseed and the sixth meal contained 5% coconut oil. The results of the study showed no significant improvement in the hypothesized cardio-protective effects of hempseed during ischemia-reperfusion injury. There were also many limitations to the study, which included a small sample size and the use of rabbits which may not have emulated the action of these diet ingredients in humans. Also, the plasma fatty acids were extracted

from the hearts of the rabbits and placed into a standard perfusate that contained no PUFAs.

In a study by Gavel et al. (2011), researchers examined the potential for hempseed to inhibit atherosclerosis and the related vascular contractile dysfunction. The study involved 8 wk of dietary intervention. The various groups that the rabbits were assigned to included: the control diet, which consisted of regular rabbit chow, the control diet with 5% coconut oil, a control diet with 10% hempseed, 0.5% cholesterol, a control diet with both 10% hempseed and 0.5% cholesterol and a control diet with 10% delipidated hempseed. Results of the study showed that hempseed was not able to protect the vessels from plaque buildup, which is contradictory to the results seen by Prociuk et al. (2008). These findings showed that hempseed had no effect on atherosclerosis and vascular contraction. This study also contradicted the other research findings showing that hempseed supplementation increases plasma levels of PUFAs. The study may have needed to use a higher dose or a different study animal.

For the studies conducted by Richard et al. (2006), Prociuk et al. (2008), Al-Khalifa et al. (2007), Prociuk et al (2006) and Gavel et al. (2011), the incorporation of 10% hempseed has been shown to have health benefits of flaxseed. Although the animal studies have many limitations and limited results, some of the potential health benefits such as platelet aggregation and changes in the blood fatty acid profiles of the hempseed containing groups were present. The highest changes were seen in diets containing 10% hempseed.

A study conducted by Chichowska et al. aimed to examine the effects that hemp milk would have on weight gain of the body and internal organs in rats. Blood hormone

concentrations, various metabolic parameters and insulin and thyroid action were also investigated. The trial lasted 21 d and included 40 female rats. There were two experiments involved in the study. The first experiment assigned 8 rats to each of the three groups. The groups included a control group, hemp milk group and hemp milk and water group. The rats were administered the various experiment treatments at a concentration of 0.5 mL per 100 g of body weight. The control group rats were allowed free access to water. The second experiment followed the same procedures as in experiment one, only the control group rats were allowed access to hemp milk. The results of the study showed no difference in body weight, kidney and liver functions or improved thyroid gland and adrenals. The hemp milk did influence the endocrine system and changes in blood insulin were determined. Also, reduction of free cholesterol, TG and total and esterified cholesterol were seen. Interestingly, the study by Schwab et al. (2006) revealed no changes in plasma glucose or insulin concentrations.

Overall, the research available on hemp is limited. Previous studies provide a leading direction on adequate dosage and timeline for the study. Combining these findings, future studies should investigate further into the potential health benefits of hempseed oil and hulled hempseed.

2.3 SOYBEAN OIL COMPOSITION

Soybean oil is light in colour and can be easily masked with salad dressing ingredients such as balsamic vinegar and spices to help blind which oil the participants are consuming. Consuming the treatment oils in the form of a salad dressing help aid in masking hempseed oil's dark green colour. Soybean oil's fatty acid composition includes

high levels of LA, oleic acid and small amounts of ALA (Han et al., 2012). Incorporating a control treatment that is high in LA levels that match the high levels in hempseed oil allows to focus on the potential health benefits that the omega-3s contained in the hemp treatment. Interestingly, soybean oil also contains high levels of oleic acid. Oleic acid has been shown to have many cardio protective health effects including the prevention of ischemic cardiovascular disease, interaction with eicosanoid metabolism and influence platelet function (Cocchi et al., 2009).

2.4 POTENTIAL MECHANISMS AFFECTED BY OMEGA-3 INTAKE

Research has suggested that EPA is an important substrate for the production of eicosanoids, such as thromboxanes, resolvins and leukotrienes that promote anti-inflammatory properties (Calder, 2012). Additionally to anti-inflammatory properties, omega-3 intake may increase HDL and lower LDL levels through altering gene expression involved in TG and fatty acid synthesis (Jung et al., 2008). Platelet aggregation may be lowered by EPA substrate eicosanoids competing with AA substrate eicosanoids; AA eicosanoids promote inflammatory responses (Jung et al., 2008). Omega-3 fatty acids may help modulate growth signals or DNA sythensis which prevent smooth muscle proliferation (Jung et al., 2008).

CHAPTER THREE

MANUSCRIPT ONE:

THE EFFECT OF HULLED HEMP SEED AND HEMP OIL ON HEALTH BIOMARKERS

3.1 ABSTRACT

Diet is a critical preventative measure for cardiovascular disease (CVD). Previous research has found that polyunsaturated fatty acids (PUFAs) have been shown to reduce risk factors for CVD (Gavel et al., 2011, Gillingham et al. 2011, Kaul et al., 2008, Lemke et al., 2015, Mozaffarian and Wu., 2011, Rodriguez-Leyva et al., 2010., Schwab et al. 2006.). Hemp has an excellent nutritional composition and contains high levels of gamma linolenic acid (GLA), stearidonic acid (SDA), alpha linolenic acid (ALA) and linoleic acid (LA) (Rodriguez-Leyva et al., 2010). Currently, limited research is available on hemp seed and oil consumption. The objective of this study was to examine the effects of the omega-3 fatty acids contained in hempseed oil and hulled hempseed in 30 healthy, overweight individuals ages 18-65 yr. The study was a randomized, double-blinded with control, crossover human intervention trial. Results found that both ALA ($P = 0.01$) and eicosapentaenoic acid (EPA) ($P = 0.02$) concentrations were significantly higher after the consumption of the hemp treatment in the RBC. Plasma ALA ($P < 0.05$) and n-6 PUFAs ($P = 0.03$) concentrations were significantly higher after the consumption of the hemp treatment. However, docosahexaenoic acid (DHA) and total omega-3 PUFAs did not show a significant difference between treatments, nor did serum lipids, glucose metabolism or blood

pressure. There were no improvements seen in participants' blood pressure or degree of arterial stiffness. The DEXA scans revealed no effect of treatment on body composition over the course of the trial. Overall, results of the study suggest that the consumption of hemp led to a significant improvement in the fatty acid profile in the RBC and plasma. The clinical implications of these findings suggest that hemp consumption may help CVD risk factors through the improvement of the fatty acid profiles.

3.2 INTRODUCTION

Hemp contains high levels of stearidonic acid (SDA), gamma linolenic acid (GLA), alpha linolenic acid (ALA) and linolenic acid (LA) (Rodriguez-Leyva and Pierce, 2010). ALA and LA are essential fatty acids (EFAs) and must be obtained through the diet. ALA can be converted into eicosapentaenoic acid (EPA). EPA is beneficial to humans for many CVD risk factors, especially support of the arterial wall and the elasticity (Mozaffarian and Wu., 2011). DHA is important for the function of the brain as well as blood pressure (Mozaffarian and Wu., 2011). Both EFAs, ALA and LA require the same enzymes for their conversions such as, 6-desaturase, delta 5-desaturase and elongase enzymes. The activity of these enzymes is, therefore, the rate limiting step in the conversion into EPA (Rodriguez-Leyva et al., 2010). The optimum ratio of omega-6 (n-6), such as LA to omega-3 (n-3), such as ALA is 2:1. The optimum ratio of n-6:n-3 results in having the best conversion of rate into other fatty acids leading to the formation of EPA. Industrial hemp has been proven to have the optimum ratio at 2:1-3:1(Rodriguez-Leyva et al., 2010).

Research has shown that the consumption of PUFAs improve risk factors associated with cardiovascular disease (CVD) (Gavel et al., 2011, Gillingham et al. 2011, Kaul et al., 2008, Lemke et al., 2015, Mozaffarian and Wu., 2011, Rodriguez-Leyva et al., 2010., Schwab et al. 2006.). Risk factors associated with CVD include being over weight or obese, high blood pressure and diabetes (Heart & Stroke Foundation, 2015).

Therefore, the present study objective was to examine if consuming hempseed oil and hulled hempseed would show significant improvements in the RBC and plasma

fatty acid profiles within a group of healthy, overweight individuals. As a result of hemp's high n-6, n-3 and EFAs content, the study will investigate if consuming high amounts of these fatty acids will show changes within the fatty acid profile. Secondary outcomes of interest include changes in serum lipid and glucose levels. High density lipoprotein (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels have been linked as risk factors associated with CVD (Schwab et al., 2006). Research has shown that consuming more healthy fats lowers circulating LDL-C levels and raises HDL-C levels in the body (Mozaffarian and Wu., 2011). Additional secondary measurements planned include blood pressure, arterial stiffness, and obesity. DEXA scans will investigate if consuming hulled hempseed and hemp oil will significantly change total body fat, lean muscle and visceral adiposity.

3.3 EXPERIMENTAL METHODS

3.3.1 Subjects

Thirty metabolically healthy, overweight individuals with a body mass index (BMI) 25-35 kg/m2 and between the ages of 18-65 yr were included in the study. Both men and women were eligible and required the ability to provide written informed consent and comply with the trial guidelines. Participants were excluded from the study if they were pregnant or lactating, were smokers or consumed tobacco products in the previous six months. Exclusion criteria also included having a history of cancer, rheumatoid arthritis, chronic illness, cardiovascular problems, liver and kidney disease, inflammatory bowel disease, pancreatitis, gallbladder or biliary disease, neurological/ psychological disease, bleeding disorders, any platelet abnormalities, or gastrointestinal

disorders that could interfere with fat absorption. Individuals possessing high serum triglycerides (TG) >4.52 mmol/L, and/or LDL-C >6.5 mmol/L, hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg) and glucose levels > 6.1 mmol/L were excluded. Participants who consumed or planned to consume anticoagulants, were hypertensive or were taking lipid lowering medications, or were hypotensive were also excluded. Also, participants who had consumed omega-3 PUFA dietary supplements within the last 4 wk and reported consumption of more than two alcoholic drinks/d or history of alcoholism or drug dependence and use of any experimental medication within one month prior to starting the trial were excluded.

3.3.2 Experimental Design

The study was a double-blinded, randomized, cross-over design. Participants were recruited through advertisements in the newspaper Metro and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) website and email list. Follow-up on the potential participants included a telephone or email screening questionnaire which included questions regarding personal health information. The screening questionnaire determined the eligibility of potential participants. If the participant qualified, they were asked to attend the RCFFN for an in-person screening session.

During the in-person screening session, participants were asked to arrive at the RCFFN 10-12 h overnight fasted. A thorough explanation of the trial and consent forms were discussed, as well as signatures on all ethics approved documents were gathered. The restrictions of the study were discussed which included avoidance of foods containing high levels of n-3s including fatty fishes such as salmon, herring, mackerel

and various seeds, including flaxseed and chia seeds or other n-3 supplementation. Anthropometry measurements which included height, weight, hip and waist circumference and blood pressure, as well as a blood sample were made. Blood samples were processed on a Vitros 350 chemistry analyzer to determine glucose and lipid concentrations.

Each phase of the trial was 28 d. Participants were randomly assigned to a group which determined their sequencing of the trial. The trial included a 4 wk wash-out period between the two phases. Days 1, 27 and 28 were times where anthropometry measurements and blood samples were retained. The participants were asked to come to the RCFFN 10-12 h overnight fasted prior to their appointment. After day 1, the participants were only required to drop in weekly to pick up the treatment for the following week. Treatment products were double-blinded to appear similar in taste and visual appearance. Participants were required to consume two 30 g sachets of seeds and a single 30 g serving of salad dressing daily. Labelled cups were provided to ensure the proper amount was distributed daily.

3.3.3 Experimental Treatment Products

Participants consumed both the control treatment and hemp treatment over the course of the trial. The control treatment included 27 g of sesame seeds with 3 g of lentil flakes incorporated afterwards to mimic the appearance of hempseed. The salad dressings base oil was soybean oil. The hemp treatment included 30 g of hulled hempseed and the salad dressing base oil was hemp oil. If participants were having any issues with flavour or acidity then the ingredients were modified to accommodate any

concerns. **Table 1.** shows the recipe used for the control and hemp treatment, Participants recorded in a food journal what time of day they consumed the treatment products and recorded any other food that was consumed with the treatment products. During the participants' weekly drop ins, they were asked if there were any issues arising from consuming the treatment products and if they needed any further assistance in ways to incorporate the treatment products into their diets. The fatty acid profile of the soybean and hempseed oil used in the salad was determined by using the one-step fatty acid extraction method and processed on the gas chromatography (GC) linked with flame ionization. Results of the fatty acid profile in the soybean and hempseed oils are presented in **Table 2.** The soybean seeds and hempseed were sent to a third party lab to determine the macronutrient contents which are presented in **Table 3.**

3.3.4 Study Timeline

Participants arrived at the RCFFN for baseline measurements and to pick up the first week of treatment. The baseline measurements included anthropometry measurements, such as height, weight, hip, and waist circumference. Blood pressure and arterial stiffness were recorded, as well as a DEXA scan for body composition, which includes visceral adiposity. Day 1 was the start day of the phase. A blood sample was retained and processed within 2 h of the blood draw and stored in a -80℃ freezer until further analysis.

After day 1 of the phase, participants returned to the RCFFN weekly to pick up their treatment for the following week. Wk 4 was the final week of the intervention

period. On day 27 and 28 participants were asked to arrive at the RCFFN 10-12 h overnight fasted as they were endpoint days for both phases. A blood sample was required on both endpoint days and all other baseline measurements were performed on either day 27 or 28 depending on the participants' availability or preference.

Participants then entered a minimum 4 wk wash out period before entering phase 2 of the trial. During the washout period participants were asked to follow the same n-3 food restrictions discussed prior to commencing the study and asked to maintain regular dietary habits. The same procedures during phase one continued during phase two of the study.

3.3.5 BMI, Blood pressure, arterial stiffness and DEXA

On day 1, 27 and 28 of both treatment phases body mass index (BMI), blood pressure, arterial stiffness, and DEXA scans were performed. BMI was calculated using the participants' weight and height measurements (National Heart, Blood and Lung Institute, *Assessing your weight and health risk*).

$$BMI = \text{Weight of the person (Kg)} / (\text{Height of the person (M)})^2$$

Blood pressure was determined on an electronic blood pressure reader. Measurements were performed in triplicate and the last two readings were averaged to determine the blood pressure value. Arterial stiffness was measured through pulse wave analysis (PWA) on the Mobil-O-Graph. A DEXA scan was conducted to determine

the lean muscle, fat and visceral adiposity levels. An electric scale was used to measure body weight of the participants.

3.3.6 Blood sampling, serum lipid analysis and glucose analysis

Within one hour of the samples being drawn, they were centrifuged at 3000 rpm at 4℃ for 20 min. Plasma and RBC were collected. All tubes were placed in a -80℃ freezer until further analysis.

Serum lipid analysis was performed to measure plasma TG, TC, LDL-C, HDL-C and very low density lipoprotein (VLDL) cholesterol levels. LDL-C was determined using the Friedewald equation (Martin et al., 2013).

3.3.7 RBC fatty acid profile analysis

The internal standard was C17:0 at 1mg/mL in chloroform. Approximately 0.5 g of RBC were weighed out into individual, glass test tubes. Methanol, internal standard, toluene and acetyl chloride were added to the test tubes. Each test tube was flushed with nitrogen and capped tightly. Samples were put in an oven set to 80℃ for one h. After, 6% potassium carbonate was added and the samples were centrifuged at 3000 rpm, at 4℃ for 5 min. The top layer was removed. Samples were flushed with nitrogen until completely dried. Hexane was added into the GC vials. The sample was transferred into an insert and stored in a -80℃ freezer until further analysis.

GC vials were removed from -80℃ and set at room temperature for approximately one h prior to being analyzed. Samples were then analyzed on a 30 m

column GC with flame ionization detector. The oven was programmed for 70℃, the flow was 32.0 mL/min and the injector and detector were set to 250℃ and 270℃, respectively.

The n-3 index was determined by combining the amount of EPA and DHA present in the RBC (von Schacky et al., 2014). The n-3 index is important because prior studies have shown that having higher levels of EPA and DHA lower risk factors associated with CVD (von Schacky et al., 2014).

3.3.8 Plasma fatty acid profile analysis

Samples were removed from the -80℃ freezer and thawed at room temperature for approximately one h before beginning extraction methods, using the same method as RBC extraction. The samples were placed in a -80℃ freezer after the plasma extraction procedures were completed. The samples were then thawed at room temperature for approximately one h and analyzed on the GC.

3.3.9 Statistical analysis

Statistical analysis was performed with PROC POWER using SAS 9.2 (SAS Inc). Results of the study are shown as means ± standard error (SE). The effects of dietary treatments used a mixed model analysis of variance (ANOVA) procedure, with repeat measures. Treatment, sex, sequence, treatment*sex, treatment*sequence were assigned as fixed factors and participant ID was assigned as a random factor. The results were determined by comparing the results from day 28 from phase 1 to the

results on day 28 from phase 2. The second set of results were determined by calculating the percent change during each phase then comparing the results between the hemp treatment and control treatment. Statistical significance was set at $P<0.05$ for all outcome measurements.

3.4 RESULTS

3.4.1 Subject characteristics

Baseline characteristics are presented in **Table 4.** Overall 30 participants, 13 males and 17 females, completed the study. All participants were overweight or obese (BMI - 28.9 ± 2.9 kg/m²). Overall 7 participants dropped out of the study. Four participants had troubles incorporating the treatments into their daily schedule; 1 participant was worried about the increase in fat intake from the intervention products, 1 participant had to leave the study for personal reasons and 1 participant had adverse effects during the control treatment phase. Compliance was determined in the remaining 30 participants who completed the study based on the results seen in their RBC and plasma levels.

3.4.2 BMI, blood pressure, arterial stiffness and DEXA

No significant differences were observed between the hemp and control treatment for the total body weight. Results are presented in **Table 5. and Table 6.** No significant differences were found between the hemp and control treatments in BMI.

Blood pressure values revealed there were no significant differences between the hemp and control treatment in either systolic or diastolic blood pressure. The PWV

showed no significant difference in the hemp treatment as well as the augmentation index. DEXA scans showed no significant differences in visceral fat, lean muscle or body fat.

3.4.3 Serum lipid and glucose concentrations

The fasting serum and glucose concentrations are presented in **Table 7. and Table 8.** There were no significant differences between the hemp and control treatment in total cholesterol, triglyceride, HDL-C, LDL-C and VLDL concentrations. There were no significant differences in glucose concentrations measured between both phases.

3.4.4 Red blood cell fatty acids

The results of the RBC fatty acid concentrations are presented in **Table 9. and Table 10.** There were no significant differences measured between treatments in saturated fatty acid levels (SFA), except for an increase in C15:0 (day 28 $P = 0.01$ and percent change $P = 0.01$) and C24:0 (day 28 $P = 0.01$) levels after consumption of the control treatment when compared to the hemp treatment**.** As expected, there was a significant increase in C18:3n3 (ALA) (day 28 $P = 0.01$ and percent change $P = <0.01$) and C20:5n3 (EPA) (day 28 $P = 0.02$ and percent change $P = <0.01$) levels after consumption of the hemp treatment when compared to the control treatment. Overall, the total n-3 PUFA, which includes C18:3n3, C18:4n3 (SDA), C20:3n3, C20:5n3, C22:6n3 (DHA) and the n-3 index, did not show a significant difference between the treatments when comparing day 28 and percent change. C22:5n3 showed no significant differences when comparing day 28 of each treatment but showed a significant increase

in percent change during the hemp phase (percent change $P < 0.02$). There was an increase in C20:3n3 level after the consumption of the control treatment when comparing day 28 of each phase (day 28 $P < 0.05$), however, there were no significant changes seen in percent change**.** This may correlate with findings that after consumption of the control treatment, the omega-3 index was slightly higher when compared to the hemp treatment when comparing results from day 28 (hemp 4.6% versus control 4.7%)**.** However, there was no significant difference in the n-6 to n-3 ratio or total PUFA levels. Additionally, C20:3n6 (dihomo-gamma linolenic acid, dGLA) levels increased (day 28 $P < 0.05$) after consumption of the hemp treatment but no differences where found in percent change. C20:4n6 found no significant findings when comparing day 28 results of each phases but showed significant differences in percent change results (percent change $P < 0.04$) during the hemp phase. There were no other significant differences in n-6 PUFAs between treatment groups**.** Interestingly, there were no significant differences between treatments in monounsaturated fatty acid levels (MUFA), except for an increase in levels of C24:1 (day 28 $P = 0.02$) when comparing day 28 results of both phases after the consumption of the soy treatment**.**

3.4.5 Plasma fatty acids

Results for plasma fatty acid concentrations are presented in **Table 11. and Table 12.** No significant differences were observed between the hemp treatment and control treatment in total SFA. Nor were significant differences seen between the groups for individual SFA which includes C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C20:0**.** Interestingly, no significant differences were observed between the hemp treatment and

control treatment in total MUFAs, as well as individual MUFAs including C14:1, C17:1, C18:1, C20:1, C22:1, with the exception of C16:1 showing significant differences in percent change (percent change $P = 0.04$) but no significant findings when comparing day 28 of each phase. Overall, after the consumption of the hemp treatment, significant differences in total PUFAs (day 28 $P = 0.01$ and percent change $P = 0.04$), as well as n-6 PUFAs (day 28 $P = 0.03$) were observed. N-6 PUFAs including C18:3n6 (GLA) ($P < 0.05$) and C20:3n6 ($P < 0.05$) also showed a significant decline after consumption of the hemp treatment, however, the remaining n-6 PUFAs showed no differences between treatments (C18:2n6 (LA), C20:2n6, C20:4n6 and C22:4n6). The overall n-6 to n-3 ratio showed no significant difference. Total n-3 PUFAs showed no significant differences between the two treatments and there were no significant differences amongst individual n-3 PUFAs (C20:5n3, C22:5n3 and C22:6n3), with the exception of a significant increase in C18:3n3 ($P < 0.05$) after the hemp treatment.

3.5 DISCUSSION

The present trial is the first free living clinical study designed to specifically target potential health benefits from consumption of hempseed oil and hulled hemp seeds. Interestingly, the RBC and plasma concentrations revealed after the consumption of hempseed oil and hulled hemp seeds, there were significant changes in the fatty acid profiles. In the RBC data, there was an improvement in C20:5n3 (EPA), C18:3n3 (ALA) and C20:3n6, (DGLA) levels after consumption of the hemp treatment. RBCs reflect the long term lipid storage throughout the body. Increases were seen in ALA in both the RBC and plasma which was not unexpected, as the hemp oil contains such high levels

of ALA (~20%). This also confirms participants' compliance during the study. ALAs conversion pathway's final conversion step is into EPA. After the hemp treatment, there were also significant increases in EPA but not DHA levels. Also, there were significant increases in DGLA in both the RBC and plasma fatty acid data, which is the fatty acid GLA is converted into during the ALA conversion into EPA (Arab et al, 2003). These results show that C18:3n6 (GLA), which manifested significant increases after the hemp treatment within the plasma fatty acid data, may have been converted into DGLA before being stored within the RBC.

Overall, no improvements in the RBC n-3 index or in the RBC and plasma fatty acid n-6:n-3 ratio were seen after hemp consumption. The plasma fatty acid revealed a significant increase in the n-6 PUFA concentrations after the hemp treatment; this was a result from the improvement in GLA and DGLA. Additionally, the plasma fatty acid profile showed significant increases in total PUFAs concentrations after the hemp treatment. The DEXA scans performed at the beginning and end of each phase did not reveal any changes in lean muscle to body fat ratios amongst participants.

Blood pressure and arterial stiffness showed no significant differences between the hemp and control group. However, research has shown that LA consumption may lower blood pressure. A previous study conducted, had participants consume four, 23 g servings/d of LA (Rodriguez-Leyva et al., 2010). The study revealed that blood pressure was lowered after a 4 wk intervention period (Rodriguez-Leyva et al., 2010). Hemp contains high levels of LA (approximately 55%) and other EFAs but LA was not targeted as an individual fatty acid to examine. Another possibility may be that since soy contains high levels of LA (approximately 53%), both treatments may have had similar impacts.

Blood serum lipid and glucose concentrations in response to hemp feeding revealed similar findings to previous research in that no changes were seen after the consumption of the hemp treatment. These results may be due to the short length of the study. Perhaps, if the present trial went beyond 28 days of consuming the hemp treatment there may have been significant changes in TC, TG, HDL, LDL or glucose levels.

3.6 CONCLUSION

Overall, the present study has shown that after a 4 wk period of consuming hulled hempseed and hemp oil in healthy, overweight individuals, improvements in ALA, GLA, DGLA and EPA levels in RBC and plasma concentrations within the body were observed. The study did not, however, show any changes in BMI, blood pressure, arterial stiffness or body composition. Nor did the study reveal any changes in the serum lipids, including TG, TC, LDL-C or HDL-C levels. Due to the rise in EFAs, this research shows the importance of diet and the role it plays on health. This discovery will hopefully lead into further investigations into the potential health benefits that hemp may have on risk factors associated with CVD.

Table 1. Salad dressing recipe for hemp and control treatment

Ingredients	1 day portion (grams)	7 day portion / week supply (grams)
Balsamic vinegar	11	77
Chopped, dried parsley flakes	0.5	3.5
Fresh lemon juice	6	42
Chopped, fresh garlic cloves	2.5	17.5
Crumbled, dried basil	0.5	3.5
Dried oregano	0.5	3.5
Salt	0.5	3.5
Pepper	0.3	2.1
Hempseed oil or soybean oil	30	210
Total	51.8	362.6

Table 2. Fatty acid profile of hempseed oil and soybean oil

	Hemp	Soy
SFA	9.93	15.4
C12:0	0.02	ND
C14:0	0.04	0.08
C15:0	0.02	0.04
C16:0	6.5	10.8
C18:0	1.96	3.78
C20:0	0.86	0.32
MUFA	9.61	23.9
C14:1n-9	0.01	0.01
C16:1n-7	0.12	0.1
C17:1n-7	0.02	0.06
C18:1n-9	9.02	23.5
C20:1n-11	0.43	0.25
C22:1n-13	ND	ND
PUFA	79	60.7
C18:2n-6	55.7	53
C18:3n-6	4.53	0.561
C18:3n-3	18.2	6.89
C18:4n-3	1.5	ND
C20:2n-6	0.07	0.03
C20:3n-6	0.01	ND
C20:4n-6	ND	ND
C20:5n-3	ND	ND
C22:4n-6	0.15	ND
C22:5n-3	ND	ND
C22:6n-3	ND	0.12
n-6 PUFA	60.7	53.6

n-3 PUFA	18.2	7.04
n-6:n-3 Ratio	3.33	7.63

Fatty acid values are based on averages between two samples.
ND= Non-detectable

Table 3. Macronutrients of hulled hempseed and sesame seeds

	Hulled hempseed (per 30 g serving)	Sesame seeds with lentil flakes (per 30 g serving)
Calories (Kcal)	172	191
Calories from Fat (Kcal)	119	144
Fat (g)	13.3	16
Total Carbohydrates (g)	3.54	4.2
Total Dietary Fibre (g)	0.78	2.45
Protein (g)	9.69	7.5

Macronutrient information obtained through COVANCE, a third party lab. Certificate of Analysis provided in *Appendix Three.*

Table 4. Baseline characteristics

	Mean
Age (yr)	49.23 ± 14.52
Weight (kg)	84.27 ± 12.92
BMI (kg/m2)	28.94 ± 2.9
Waist circumference (cm)	97.97 ± 12.8
Systolic pressure (mmHg)	120.37 ± 16.28
Diastolic pressure (mmHg)	78.60 ± 9.48
Glucose (mmol/L)	5.43 ± 0.56
Cholesterol (mmol/L)	5.14 ± 1.08
Triglycerides (mmol/L)	1.31 ± 0.65
dHDL (mmol/L)	1.41 ± 0.33
LDL (mmol/L)	3.14 ± 0.95
VLDL (mg/dL)	23.17 ± 11.56

Values are expressed as mean ± SE

Table 5. BMI, blood pressure, arterial stiffness and DEXA on day 28 after start of hemp and control treatment

	Hemp	Soy	P Value
BMI (kg/m2)	29.29 ± 0.54	29.07 ± 0.54	0.13
Blood Pressure			
Systolic (mmHg)	120.77 ± 3.16	124.51 ± 3.16	0.28
Diastolic (mmHg)	80.29 ± 1.62	80.2 ± 1.62	0.95
Arterial Stiffness			
Augmentation Index 75%	17.87 ± 2.68	14.95 ± 2.69	0.25
Pulse Wave Velocity (m s(-1))	9.33 ± 1.57	7.29 ± 1.58	0.38
DEXA			
Visceral Fat (lbs)	2.94 ± 0.26	2.76 ± 0.26	0.09
Lean Muscle (lbs)	68.62 ± 2.68	67.52 ± 2.68	0.43
Fat (lbs)	60.09 ± 0.83	60.29 ± 0.83	0.13
Total Body Weight (kg)	86.05 ± 1.79	85.28 ± 1.79	0.08

Values are expressed as mean ± SE
mean ± SE after hemp treatment consumption and mean ± SE after control treatment consumption are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 6. Percent change of BMI, blood pressure, arterial stiffness and DEXA from before and after consumption of hemp and control treatment

	Hemp	Soy	P Value
BMI (kg/m2)	0.31 ± 0.33	-0.12 ± 0.33	0.37
Blood Pressure			
Systolic (mmHg)	-34.21 ± 0.84	-33.54 ± 0.84	0.44
Diastolic (mmHg)	-33.36 ± 1.07	-34.85 ± 1.07	0.29
Arterial Stiffness			
Augmentation Index 75%	32.79 ± 52.19	57.18 ± 58.32	0.8
Pulse Wave Velocity (m s(-1))	37.54 ± 30.51	-4.29 ± 34.98	0.38
DEXA			
Visceral Fat (lbs)	4.81 ± 4.33	2.07 ± 4.33	0.67
Lean Muscle (lbs)	0.01 ± 0.53	-0.81 ± 0.53	0.31
Fat (lbs)	0.98 ± 0.54	0.27 ± 0.54	0.37
Total Body Weight (kg)	0.26 ± 0.32	-0.12 ± 0.32	0.38

Values are expressed as mean ± SE
mean ± SE percent change of Day 1 to Day 28 of hemp treatment and mean ± SE percent change of Day 1 to Day 28 of control treatment are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 7. Serum lipid analysis and glucose concentrations on day 28 after start of hemp and control treatments

	Hemp	Soy	P Value
Glucose (mmol/L)	5.4 ± 0.11	5.31 ± 0.11	0.42
Total Cholesterol (mmol/L)	5.03 ± 0.2	5.13 ± 0.2	0.24
Triglyceride (mmol/L)	1.27 ± 0.14	1.16 ± 0.14	0.15
HDL (mmol/L)	1.39 ± 0.05	1.43 ± 0.05	0.06
LDL (mmol/L)	3.06 ± 0.17	3.17 ± 0.17	0.11
VLDL (mmol/L)	22.4 ± 2.45	20.44 ± 2.47	0.17

Values are expressed as mean ± SE
mean ± SE after hemp treatment consumption and mean ± SE after control treatment consumption are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 8. Percent change of serum lipid analysis and glucose concentrations from before and after consumption of hemp and control treatment

	Hemp	Soy	P Value
Glucose (mmol/L)	1.63 ± 1.91	-1.34 ± 1.91	0.28
Total Cholesterol (mmol/L)	-4.1 ± 1.83	-2.99 ± 1.83	0.7
Triglyceride (mmol/L)	-8.05 ± 5.62	-7.46 ± 5.62	0.91
HDL (mmol/L)	0.79 ± 1.81	3.05 ± 1.81	0.39
LDL (mmol/L)	-4.74 ± 3.01	-4.31 ± 3.01	0.93
VLDL (mmol/L)	-8.97 ± 5.81	-7 ± 5.81	0.72

Values are expressed as mean ± SE
mean ± SE percent change of Day 1 to Day 28 of hemp treatment and mean ± SE percent change of Day 1 to Day 28 of control treatment are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 9. RBC fatty acid concentrations on day 28 after start of hemp and control treatment

	Hemp	Control	P Value
SFA	42.6 ± 0.29	42.9 ± 0.29	0.5
C10:0	0.03 ± 0.01	0.05 ± 0.01	0.53
C12:0	0.07 ± 0.04	0.02 ± 0.04	0.35
C14:0	0.37 ± 0.07	0.42 ± 0.07	0.64
C15:0	0.27 ± 0.03	0.35 ± 0.03	0.01
C16:0	23.7 ± 0.23	23.9 ± 0.23	0.69
C18:0	16.1 ± 0.23	16 ± 0.23	0.57
C20:0	0.18 ± 0.02	0.2 ± 0.02	0.41
C22:0	0.8 ± 0.05	0.79 ± 0.05	0.81
C24:0	1.04 ± 0.09	1.28 ± 0.09	0.01
MUFA	16.6 ± 0.27	17 ± 0.27	0.19
C14:1n-9	0.12 ± 0.02	0.1 ± 0.02	0.4
C16:1n-7	0.78 ± 0.08	0.74 ± 0.08	0.72
C17:1n-7	0.5 ± 0.06	0.5 ± 0.06	0.98
C18:1n-9	12.8 ± 0.17	12.9 ± 0.17	0.35
C18:1n-7	1.17 ± 0.04	1.16 ± 0.04	0.76
C22:1n-13	0.03 ± 0	0.03 ± 0	0.96
C24:1n-15	0.94 ± 0.08	1.17 ± 0.08	0.02
PUFA	40.8 ± 0.3	40 ± 0.3	0.08
C18:2n-6	12.3 ± 0.21	12.5 ± 0.21	0.28
C18:3n-6	0.09 ± 0.01	0.07 ± 0.01	0.17
C18:3n-3	0.36 ± 0.02	0.27 ± 0.02	0.01
C18:4n-3	0.59 ± 0.07	0.55 ± 0.07	0.62
C20:2n-6	0.23 ± 0.01	0.23 ± 0.01	0.83
C20:3n-6	1.83 ± 0.06	1.59 ± 0.06	<0.05
C20:4n-6	14.9 ± 0.27	14.4 ± 0.27	0.11

C20:3n-3	0.08 ± 0.03	0.17 ± 0.03	<0.05
C20:5n-3	0.78 ± 0.05	0.7 ± 0.05	0.02
C22:2n-6	0.04 ± 0.01	0.03 ± 0.01	0.85
C22:4n-6	2.94 ± 0.09	2.8 ± 0.09	0.12
C22:5n-6	0.38 ± 0.02	0.399 ± 0.02	0.38
C22:5n-3	2.47 ± 0.1	2.36 ± 0.1	0.13
C22:6n-3	3.81 ± 0.18	3.99 ± 0.18	0.26
n-6 PUFA	32.7 ± 0.34	32 ± 0.34	0.09
n-3 PUFA	8.08 ± 0.21	8.01 ± 0.21	0.78
n-6:n-3 Ratio	4.13 ± 0.13	4.09 ± 0.13	0.77
n-3 Index	4.59 ± 0.2	4.69 ± 0.2	0.58

Values are expressed as mean ± SE
mean ± SE after hemp treatment consumption and mean ± SE after control treatment consumption are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 10. Percent change of RBC fatty acid concentrations from before and after consumption of hemp and control treatment

	Hemp	Control	P Value
SFA	3.04 ± 0.96	3.82 ± 0.1	0.62
C10:0	114.44 ± 0.51	168.06 ± 0.55	0.51
C12:0	415.16 ± 259.98	61.64 ± 027.74	0.37
C14:0	51.34 ± 33.05	61.99 ± 34.22	0.83
C15:0	32.62 ± 19.62	105.61 ± 19.93	0.01
C16:0	6.52 ± 1.88	6.1 ± 1.94	0.86
C18:0	2.21 ± 1.45	0.73 ± 1.5	0.49
C20:0	26.38 ± 11.98	22.43 ± 12.39	0.83
C22:0	-2.02 ± 9.05	-3.42 ± 9.32	0.89
C24:0	1.95 ± 10.54	3.17 ± 10.88	0.93
MUFA	-8.23 ± 1.95	-4.75 ± 2	0.19
C14:1n-9	66.42 ± 395.53	740.58 ± 409.57	0.25
C16:1n-7	10.69 ± 16.46	12.87 ± 17.04	0.92
C17:1n-7	36.38 ± 35.67	77.51 ± 36.92	0.41
C18:1n-9	-7.41 ± 1.3	-4.97 ± 1.35	0.18
C18:1n-7	-2.69 ± 4.73	-6.44 ± 4.9	0.61
C22:1n-13	-2.91 ± 7.71	-9.2 ± 6.97	0.5
C24:1n-15	-10.44 ± 9.18	-4.11 ± 9.49	0.6
PUFA	1.21 ± 1.02	-1.16 ± 1.05	0.09
C18:2n-6	5.5 ± 1.99	7.96 ± 2.05	0.32
C18:3n-6	33.47 ± 21.4	19.37 ± 22.15	0.67
C18:3n-3	74.77 ± 11.62	6.45 ± 12.03	<0.05
C18:4n-3	27.77 ± 31.54	55.84 ± 32.93	0.43
C20:2n-6	20.95 ± 6.7	17.14 ± 6.94	0.69
C20:3n-6	6.21 ± 3.16	-1.86 ± 3.27	0.1
C20:4n-6	1.72 ± 1.95	-3.48 ± 2.02	0.04

C20:3n-3	1384.36 ± 921.13	2024.58 ± 892.68	0.49
C20:5n-3	15.48 ± 8.54	-7.78 ± 8.7	<0.05
C22:2n-6	69.32 ± 110.73	192.88 ± 99.77	0.41
C22:4n-6	0.65 ± 11.99	16.26 ± 12.42	0.38
C22:5n-6	91.5 ± 32.46	16.97 ± 34.08	0.1
C22:5n-3	4.9 ± 3.61	-3.14 ± 3.69	0.02
C22:6n-3	-5.78 ± 4.89	-4.97 ± 5.02	0.87
n-6 PUFA	2.7 ± 1.41	0.53 ± 1.45	0.21
n-3 PUFA	-2.39 ± 3.01	-5.9 ± 3.1	0.29
n-6:n-3 Ratio	8.24 ± 3.96	10.35 ± 4.08	0.65
n-3 Index	-3.72 ± 4.76	-5.98 ± 4.86	0.59

Values are expressed as mean ± SE
mean ± SE percent change of Day 1 to Day 28 of hemp treatment and mean ± SE percent change of Day 1 to Day 28 of control treatment are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 11. Plasma fatty acid concentrations on day 28 after start of hemp and control treatment

	Hemp	Control	P Value
SFA	43.73 ± 0.84	45.14 ± 0.84	0.21
C10:0	0.11 ± 0.04	0.06 ± 0.04	0.4
C12:0	0.07 ± 0.01	0.08 ± 0.01	0.43
C14:0	0.52 ± 0.03	0.54 ± 0.03	0.65
C15:0	0.22 ± 0.01	0.21 ± 0.01	0.31
C16:0	28.85 ± 0.82	30.6 ± 0.82	0.09
C18:0	13.78 ± 0.31	13.52 ± 0.31	0.48
C20:0	0.07 ± 0.01	0.06 ± 0.01	0.18
MUFA	14.61 ± 0.96	14.46 ± 0.96	0.9
C14:1n-9	0.08 ± 0.03	0.04 ± 0.03	0.38
C16:1n-7	1.92 ± 0.77	0.88 ± 0.77	0.35
C17:1n-7	0.06 ± 0	0.06 ± 0	0.36
C18:1n-9	12.42 ± 0.42	13.31 ± 0.42	0.08
C20:1n-11	0.11 ± 0.07	0.19 ± 0.07	0.45
C22:1n-13	0.7 ± 0.06	0.56 ± 0.06	0.08
PUFA	41.79 ± 0.45	40.48 ± 0.45	0.01
C18:2n-6	24.59 ± 0.46	24.63 ± 0.46	0.95
C18:3n-6	0.2 ± 0.02	0.08 ± 0.02	<0.05
C18:3n-3	0.7 ± 0.05	0.49 ± 0.05	<0.05
C18:4n-3	0.05 ± 0	0.05 ± 0	0.41
C20:2n-6	0.19 ± 0.01	0.2 ± 0.01	0.81
C20:3n-6	3.26 ± 0.13	2.46 ± 0.13	< 0.05
C20:4n-6	9.22 ± 0.29	8.74 ± 0.29	0.06
C20:5n-3	0.34 ± 0.32	0.18 ± 0.3	0.76
C22:4n-6	0.16 ± 0.02	0.2 ± 0.02	0.08
C22:5n-3	0.61 ± 0.05	0.61 ± 0.05	0.98
C22:6n-3	2.16 ± 0.13	2.38 ± 0.13	0.17

n-6 PUFA	37.62 ± 0.44	36.52 ± 0.44	0.03
n-3 PUFA	4.17 ± 0.19	3.96 ± 0.19	0.39
n-6:n-3 Ratio	10.68 ± 1.39	10.26 ± 1.39	0.84

Values are expressed as mean ± SE
mean ± SE after hemp treatment consumption and mean ± SE after control treatment consumption are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

Table 12. Percent change of plasma fatty acid concentrations from before and after consumption of hemp and control treatment

	Hemp	Control	P Value
SFA	0.83 ± 4.48	6.66 ± 4.48	0.39
C10:0	76.45 ± 74.91	-5.05 ± 73.66	0.45
C12:0	97.52 ± 57.35	9.37 ± 57.24	0.31
C14:0	5.31 ± 6.92	2.37 ± 6.92	0.76
C15:0	9.56 ± 8.65	-3.57 ± 8.58	0.33
C16:0	-1.77 ± 70.24	98 ± 70.24	0.33
C18:0	32.66 ± 22.17	5.75 ± 22.17	0.4
C20:0	21.11 ± 20.25	2.78 ± 21.66	0.43
MUFA	-14.38 ± 3.8	-12.02 ± 3.8	0.68
C14:1n-9	113.29 ± 42.02	32.93 ± 44.36	0.23
C16:1n-7	-0.47 ± 10.21	-25.55 ± 10.21	0.04
C17:1n-7	-72.89 ± 101.57	59.08 ± 101.57	0.52
C18:1n-9	-9.58 ± 2.94	-4.29 ± 2.94	0.21
C20:1n-11	-7.82 ± 86.33	69.68 ± 87.47	0.54
C22:1n-13	4.13 ± 9.63	-23.7 ± 9.63	<0.05
PUFA	7.61 ± 1.56	4.03 ± 1.56	0.04
C18:2n-6	14.12 ± 2.83	17.7 ± 2.83	0.38
C18:3n-6	162.8 ± 40.92	-19.44 ± 40.92	<0.05
C18:3n-3	115.82 ± 48.61	88.67 ± 48.61	0.72
C18:4n-3	38.82 ± 103.93	186.68 ± 103.93	0.33
C20:2n-6	9.16 ± 9.04	0.01 ± 9.24	0.5
C20:3n-6	16.94 ± 41.34	49.04 ± 41.34	0.59
C20:4n-6	3.93 ± 88.35	119.29 ± 88.35	0.36
C22:4n-6	-22.69 ± 8.51	-26.83 ± 8.97	0.72
C22:5n-3	-3.07 ± 9.99	-23.89 ± 9.99	0.17
C22:6n-3	-19.18 ± 5.16	-15.35 ± 5.47	0.63

n-6 PUFA	10.31 ± 1.69	7.69 ± 1.69	0.25
n-3 PUFA	-0.55 ± 6.52	-16.68 ± 6.52	0.15
n-6:n-3 Ratio	51.23 ± 23.03	45.71 ± 23.03	0.87

Values are expressed as mean ± SE
mean ± SE percent change of Day 1 to Day 28 of hemp treatment and mean ± SE percent change of Day 1 to Day 28 of control treatment are compared using ANOVA
P Values less than 0.05 have significant findings between the two treatments

CHAPTER FOUR

CONCLUSION

4.1 SUMMARY AND IMPLICATIONS

The present study showed consistency with previous research that the fatty acid composition of hempseed oil and hulled hempseed can be used to increase concentrations of ALA, GLA, DGLA and EPA within the body through dietary consumption. Hempseed is high in EFAs such as ALA, LA and SDA that can be converted within the body into EPA. After the consumption of the hemp treatment, there were increases in ALA, EPA and dGLA in the RBC. Additionally, plasma levels showed increases in ALA levels. The present study did not, however, show that the consumption of the hemp treatment improved serum lipids, including TC, TG, LDL-C or HDL-C or glucose concentrations. Nor were differences in BMI, lean muscle or body fat observed. Also, no changes were observed in blood pressure or arterial stiffness. The present study provides evidence that dietary consumption of nutrient rich foods and EFAs will help improve the overall nutrition of individuals.

4.2 LIMITATIONS AND FUTURE DIRECTIONS

Overall, the present study was an excellent stepping stone into the discovery of hempseed oil and hulled hemp seed health benefits. The study revealed an adequate dosage of treatment of 30 ml/d of hempseed oil and two, 30 g sachets of hempseeds. This dosage was sufficient to see changes in the fatty acid profile in both the RBC and plasma concentrations, as well as ease of incorporating into daily dietary consumption.

A limitation to the present study may be that the length of the interventions phases may not have been long enough to see optimal efficacy. There was enough time to detect changes within the RBC and plasma levels, but not enough time to detect changes within the body including BMI, blood pressure, arterial stiffness, serum lipids (TC, TG, LDL-C and HDL-C) or glucose concentrations.

Another limitation of the present study may include the diverse participant group. Future studies should consider narrowing the age range and focusing on target age groups. Also, the free living aspect of the study may be another limitation. There was no control over what participants consumed outside of the RCFFN, consciously or unconsciously. A future study may want to consider a full feeding trial to have complete control over the participants' diet and if not provide a full feeding trial then ensure compliance by examining the final results. If the participants are consuming a product high in specific fatty acids, then the participants' RBC and plasma concentrations should depict these values.

4.3 FINAL CONCLUSION

Cardiovascular disease is one of the leading causes of death in North America. Research has suggested that PUFAs may reduce some of the potential risk factors related to CVD. As a result of industrial hemp being banned in Canada, research into the potentially nutritional plant products such as hemp has been limited. Hemp contains high levels of SDA, GLA, ALA and LA which all assist in the ALA conversion pathway into EPA and DHA. This discovery will hopefully lead to further investigations into the potential health benefits that hemp may have on risk factors associated with CVD.

4.4 REFERENCES

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APPENDICES

APPENDIX 1

ETHICS APPROVAL FOR HEMP STUDY

UNIVERSITY OF MANITOBA | Biosafety Advisory Committee

Office of the Chair
Dr. Donald Smyth
Faculty of Medicine
A201 Chown Bldg
Tel: (204) 789-3356

Biosafety Offic
Steven Ch
T248 Basic Science Buildi
Winnipeg, Manito
Canada R3E 0T
Tel: (204) 789-36

Biosafety Project Approval Certificate

Principal Investigator: Dr. Peter Jones
Grant Agency: Manitoba Harvest & Agri-Food Research and Development Initiative
Grant Title: Hemp product consumption and human health trials
Certificate Number: BPAC 14.109

Date: October 3, 2014

FOR EHSO/BSAC CHAIR OFFICE USE ONLY

Certificate approval status:

- [x] Approved
- [x] Conditional approval – funds withheld subject to the conditions given below corrections reviewed and approved by Chair/BSO
- [ ] Hold – funds withheld until conditions below are met corrections reviewed and approved by BSAC
- [ ] Pre-approved – funds released by the BSAC chair pending committee review and approval
- [ ] Rejected – resubmission will not be accepted see below

Certificate approval conditions:
The lab supervisor Rebecca Mollard must be listed on your biosafety permit. Please submit an amendment and ensure she is trained. In Block 3 you must identify specific labs in RCFFN where the work will be conducted and those rooms must be included in your biosafety permit. Please clarify if you are preparing whole plasma and red blood cells in a biosafety cabinet or a fume hood. Aerosol generating procedures with biological agents are to be carried out in an approved biosafety cabinet. .

Rooms 163 and 245 on permit SCC Nov 24, 2014

Please respond to the Biosafety Advisory Committee Chair c/o the Biosafety Officer at T248 Basic Science

The signature of the Biosafety Advisory Committee Chair below confirms that this application has been appropriately reviewed by the Biosafety Advisory Committee and that the containment level and that safety procedures identified are appropriate to the work proposed in this application.

Biosafety Advisory Committee Chair: [redacted] Date: 30 March 2015

January 6, 2014 SCC

Appendix One: Ethics Approval For Hemp Study

P126-770 Bannatyne Avenue
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UNIVERSITY OF MANITOBA | Bannatyne Campus
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Faculty of Agriculture and Food Sciences	HS 18264 (B2014:115)
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** June 3, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE: Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Manitoba Harvest	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: May 26, 2015	**BREB receipt date of Documents:** June 1, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Amendment per REB Amendment Form		May 22, 2015
Consent and Assent Form(s):		
Research Participant Information and Consent FormTrial 2(not labelled as Trial 2 on consent document)	4.0	May 22, 2015
Research Participant Information and Consent FormTrial 1 - Part 1	4.0	May 22, 2015
Research Participant Information and Consent FormTrial 1- Part 2	4.0	May 22, 2015
Other:		

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

Appendix One: Ethics Approval For Hemp Study

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UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Faculty of Agriculture and Food Sciences	B2014:115
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** April 1, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE: Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Manitoba Harvest	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: March 23, 2015	BREB receipt date of Documents: March 27, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Protocol	2.0	March 23, 2015
Consent and Assent Form(s):		
Research Participant Information and Consent FormTrial 2(not labelled as Trial 2 on consent document)	3.0	March 23, 2015
Research Participant Information and Consent FormTrial 1 - Part 1	3.0	March 23, 2015
Research Participant Information and Consent FormTrial 1- Part 2	3.0	March 23, 2015
Other:		

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

Appendix One: Ethics Approval For Hemp Study

UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS
Research Ethics Board

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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES

Full Board Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Faculty of Agriculture and Food Sciences	B2014:115
BREB MEETING DATE:	**APPROVAL DATE:**	**EXPIRY DATE:**
October 27, 2014	January 9, 2015	October 27, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		
N/A		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
	Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS:	
Manitoba Harvest	

Submission Date(s) of Investigator Documents:	REB Receipt Date(s) of Documents:
October 6 and December 18, 2014	October 6 and December 22, 2014

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Protocol	1.0	October 6, 2014
Consent and Assent Form(s):		
Research Subject Information and Consent Form Trial 2(not labelled as trial 2 on consent document)	2.0	December 19, 2014
Research Subject Information and Consent Form- Trial 1 Part 1	2.0	December 19, 2014
Research Subject Information and Consent Form- Trial 1- part 2	2.0	December 19, 2014
Other:		
Appendices 1-15	1.0	October 6, 2014

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the research study/project named on this ***Certificate of Final Approval*** at the ***full board meeting*** date noted above and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

Appendix One: Ethics Approval For Hemp Study

UNIVERSITY OF MANITOBA | Biosafety Advisory Committee

Office of the Chair
Dr. Donald Smyth
Faculty of Medicine
A201 Chown Bldg
Tel: (204) 789-3356

Biosafety Offic
Steven C
T248 Basic Science Buildi
Winnipeg, Manitc
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Tel: (204) 789-36

Biosafety Project Approval Certificate

Principal Investigator: Dr. Peter Jones
Grant Agency: Manitoba Harvest & Agri-Food Research and Development Initiative
Grant Title: Hemp product consumption and human health trials
Certificate Number: BPAC 14.109

Date: October 3, 2014

FOR EHSO/BSAC CHAIR OFFICE USE ONLY

Certificate approval status:

- [x] Approved
- [x] Conditional approval – funds withheld subject to the conditions given below corrections reviewed and approved by Chair/BSO
- [ ] Hold – funds withheld until conditions below are met corrections reviewed and approved by BSAC
- [ ] Pre-approved – funds released by the BSAC chair pending committee review and approval
- [ ] Rejected – resubmission will not be accepted see below

Certificate approval conditions:

The lab supervisor Rebecca Mollard must be listed on your biosafety permit. Please submit an amendment and ensure she is trained. In Block 3 you must identify specific labs in RCFFN where the work will be conducted and those rooms must be included in your biosafety permit. Please clarify if you are preparing whole plasma and red blood cells in a biosafety cabinet or a fume hood. Aerosol generating procedures with biological agents are to be carried out in an approved biosafety cabinet. .

Rooms 163 and 245 on permit SCC Nov 24, 2014

Please respond to the Biosafety Advisory Committee Chair c/o the Biosafety Officer at T248 Basic Science

The signature of the Biosafety Advisory Committee Chair below confirms that this application has been appropriately reviewed by the Biosafety Advisory Committee and that the containment level and that safety procedures identified are appropriate to the work proposed in this application.

Biosafety Advisory Committee Chair: [redacted] Date: 30 March 2015

January 6, 2014 SCC

Appendix One: Ethics Approval For Hemp Study

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UNIVERSITY OF MANITOBA | Bannatyne Campus
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Faculty of Agriculture and Food Sciences	HS18264 (B2014:115)
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** June 30, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS:	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: June 25 and June 30, 2015	**BREB receipt date of Documents:** June 26 and June 30, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Consent and Assent Form(s):		
Other: Flyer 1	V. 1	June 25, 2015

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

Appendix One: Ethics Approval For Hemp Study

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. This amendment is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

Appendix One: Ethics Approval For Hemp Study

P126-770 Bannatyne Avenue
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UNIVERSITY OF MANITOBA | Bannatyne Campus
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Faculty of Agriculture and Food Sciences	HS18264 (B2014:115)
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** August 4, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Manitoba Harvest	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: August 4, 2015	**BREB receipt date of Documents:** August 4, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Revised Protocol	V. 3	July 30, 2015
Consent and Assent Form(s):		
Research Subject Information and Consent Form - Trial 1 Part 2	V. 5	July 30, 2015
Research Participant Information and Consent Form - Trial 2 (NOT labelled as Trial 2 on Consent Document)	V. 5	July 30, 2015
Other:		

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

Appendix One: Ethics Approval For Hemp Study

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

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3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Ian Maclean, Ph.D.
Acting Chair, Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

Appendix One: Ethics Approval For Hemp Study

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada, R3E 0W3
Telephone: 204-789-3255
Fax: 204-789-3414

UNIVERSITY OF MANITOBA | Research Ethics - Bannatyne
Office of the Vice-President (Research and International)

ANNUAL APPROVAL EXPIRY REMINDER NOTIFICATIONS & COMPLIANCE REQUIREMENTS Bannatyne Campus Research Ethics Board (BREB & HREB)

In an effort to improve compliance with Annual Study Status Reporting and to ensure continuing review and approval of study projects are completed prior to the study expiry, the Bannatyne Campus Research Ethics Board (Biomedical Research Ethics Board and Health Research Ethics Board) will begin sending out "Annual Approval Expiry Reminder Notifications" to all investigators who have active studies registered with the Biomedical and/or Health Research Ethics Boards.

Per TCPS[2] "research is subject to continuing research ethics review from the date of initial REB approval throughout the life of the project." If an Investigator wishes to continue a research project beyond the expiry date listed on the most current Certificate of Approval, the Investigator must submit, an Annual Study Status Report to the applicable Research Ethics Board (REB). The report must be received by the Bannatyne Campus REB Office PRIOR to expiration date listed on your last Certificate of Approval.

REMINDER NOTICATIONS
Beginning in August of 2015, Research Investigators will begin to receiving email notifications 60, 30, and 14 days prior to expiration and upon expiration of their REB-approved research protocols. These reminder notifications will alert you of the pending REB approval expiry of your active research project with the goal of reminding you that an Annual Study Status Report on the project is due if you are wishing to continue with your project beyond the study's expiry date. Email notifications will be sent to the primary (principal) investigator and the identified primary study contact as indicated on the submitted REB application form.

To ensure smooth implementation, roll-out of these reminder notices will be staggered between the two boards:

Biomedical Research Ethics Board (BREB)	-	Commencing Monday, August 10th, 2015
Health Research Ethics Board (HREB)	-	Commencing Monday, September 8th, 2015

ENFORCEMENT OF FAILURE TO COMPLY GUIDELINES
As of **January 1st, 2016**, the Bannatyne Campus Research Ethics Board will begin strictly adhering to and enforcing Section 6.0 of the University of Manitoba Bannatyne Campus Annual Approval/Continuing Review Guidelines - "Failure to Submit an Annual Study Status Report by the Expiry Date". Investigators are strongly encouraged to read and familiarize themselves with these guidelines which can be found on the REB website at: http://umanitoba.ca/faculties/health_sciences/medicine/ethics/annual_review.html

If you have any questions, please contact the REB Coordinator at:

Shelly Rempel-Rossum
Email: [redacted]
Phone: [redacted]

Appendix One: Ethics Approval For Hemp Study

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada, R3E 0W3
Telephone : 204-789-3255
Fax: 204-789-3414

UNIVERSITY OF MANITOBA | Bannatyne Campus
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and Richardson Centre for Functional Foods and Nutraceuticals/Agriculture and Food Services	HS18264 (B2014:115)
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** August 20, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Hemp Product Consumption and Human Health Trials
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Manitoba Harvest	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: August 18, 2015	**BREB receipt date of Documents:** August 20, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Consent and Assent Form(s):		
Other: Treatment Journal (Appendix 16) including Easy Ways to Eat Your Seeds		August 18, 2015

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

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Appendix One: Ethics Approval For Hemp Study

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Sincerely,

Ian Maclean, Ph.D.
Acting Chair, Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

APPENDIX TWO

CORRESPONDING FORMS FOR HEMP STUDY

ADVERTISEMENT FOR HEMP STUDY

Poster, Version 1
October 6th, 2014

UNIVERSITY OF MANITOBA

Your Health Begins With The Food You Eat!

The University of Manitoba is looking for participants for a study examining the effects of hemp food product consumption on:

- **Blood lipids**
- **Blood Pressure**
- **Physical activity**
- **Glucose metabolism**

For more information please call
(204) 480-1042
Visit us online at: www.rcffn.ca

Richardson Centre for Functional Foods and Nutraceuticals 196 Innovation Drive • University of Manitoba • Winnipeg, MB • R3T 6C5

Dr. Peter Jones, Principal Investigator

Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042	Nutritional Study (204) 480-1042

STUDY CONSENT FORMS

RCFFN preliminary trial screening consent

You have expressed an interest in participating in a study at the Richardson Center for Functional Foods and Nutraceuticals (RCFFN). You have been invited to have your health assessed to determine if you meet the requirements of the study.

The clinical coordinator team will assess your cholesterol level, medical history, body measurements, and your availability over the next 2 years. Depending on your results you will be offered the opportunity to participate in a study.

To allow the necessary information to be obtained, you agree to provide fasting blood samples (approximately 10 ml or 2 teaspoons) for the measurement of blood cholesterol, blood glucose and other biomarkers.

Prior to taking part in any study, you will be given the specific study consent form to read and sign if you are still interested in participating.

The blood is taken from a vein in the forearm, as is usually done during a blood test. Some known risks, although rare, are associated with placing a needle into a vein. These include the possibility of infection, perforation or penetration of the needle through the vein, and bleeding, pain, or bruising at the site.

I understand I can withdraw from this process at any time at my discretion.

Participant's Signature	Participant's Name (please print)	Date
Investigator's Signature (or Clinical Coordinator)	Investigator's Name (please print) (position)	Date

Appendix Two: Study Consent Forms

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: FREE Living Hulled HEMP Seed and Oil (FREEHEMP) Trial

Protocol number: B204:115 (3)

Investigator: Peter J.H. Jones, PhD
Richardson Centre for Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive
Winnipeg, Manitoba R3T 6C5
Phone: [redacted]

Sponsors: **Manitoba Harvest,** 69 Eagle Dr, Winnipeg, MB R2R 1V4

Instructions

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. Should you decide to participate in this clinical study, please be advised that you are not allowed to participate in any other study for the duration of this clinical study. The study doctor and institution are receiving professional fees and financial support to conduct this study.

Purpose of study

Appendix Two: Study Consent Forms

FREE Living Hulled HEMP Seed and Oil (FREEHEMP) Trial

The primary purpose of this trial is to assess the clinical responses to hemp products, specifically hulled hemp seeds and hemp oil, compared to a control products, sesame seeds and soybean oil, on blood levels of fats and lipoproteins, as well as cardiovascular disease (CVD) biomarkers. A total of 30 participants will participate in this study. Please be aware that all hemp products used in this trial have 0% tetrahydrocannabinol (THC), the active ingredient in cannabis.

Study procedures

Pre-screening procedures

If you agree to take part in this study, you will be asked to give a fasting (nothing to eat or drink 12 hours before the test) blood sample (approximately two teaspoons or 10ml) to measure your blood lipid levels and additional biochemistry parameters. To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, smoking habits, exercise, your health, if you are on any medications and your eating habits. Your height, weight, waist circumference and blood pressure will also be measured.

Intervention study procedures

The study will consist of 2 phases of 28 days each during which you will consume your assigned treatment foods. Consumption of treatment foods will be from days 1 to 28. There will also be a washout period of 4 weeks between the 2 treatment phases where you can consume your habitual diets. The entire study is designed to take 12 weeks from start to completion. During each study phase, you will be provided with prepackaged, shelf-stable sachets of seeds [2 sachets (30g per sachet) per day] and salad dressings [30g of oil (in individually packaged in daily containers) per day].

You will be instructed by clinical coordinators to integrate the study products into your daily meals and to avoid other dietary sources of n-3 fatty acids, such as flax, chia, camelina, krill and fish. You will also be required to eat one sachet of hulled seeds in the morning and once in the evening, and to consume the dressing throughout the day, for each 4 week treatment period. You will be asked to continue their habitual diets, while avoiding large dietary sources of n-3 fatty acids, throughout the treatment and washout periods. You will be instructed to maintain the same level of physical activity and alcohol intake throughout the study period, you will also be given activity monitors to wear on

your waist during the treatment periods. The activity monitors will measure your physical activity levels and sleep quality. The study coordinator will contact you weekly via telephone or email to monitor treatment adherence and to answer any questions or concerns you might have. Background dietary intakes will be measured at day 1 and day 28 using food frequency questionnaires and 3-day food recall. Questionnaires on effects of the interventions including side effects will also be completed at the end of each intervention period.

This study is a double-blind design, which means that neither you nor the clinical study staff will know which treatment you will be receiving in each phase. You will receive both treatments, however, it will be unknown the order you will be given in what phase. In the unlikely event of an emergency, this information will be made available.

During days 1, 27 and 28 of each of the treatment phases of the trial, fasting blood samples (approximately 4 teaspoons or 20 ml will be taken on each blood draw day) will be obtained for assessment of blood fatty acid composition, lipid profile, glucose, and biomarkers, including hormones such as insulin, related to the control your blood sugar and appetite. Inflammatory biomarkers will also be measured in the blood samples. Each blood test will take approximately 5 minutes.

We will measure the amount of fat in your body using a procedure called dual energy x-ray absorptiometry (DXA). These analyses will be performed 4 times in total during the study, once at the beginning of each phase and once at the end of each phase. For this procedure, you will need to lie in a horizontal position for about 5-10 minutes while the scan arm passes from your head to your feet. The radiation from this test is very low dosage (equivalent to approximately 1 day of natural background radiation). The dosage is 1000 times less than the limit for trivial exposure. You will be asked not to wear anything metal (metal may affect bone density values which will affect body composition calculations). In addition, you will need to ensure that you will not undergo barium tests/ exams, or a nuclear medicine scan or injection with an x-ray dye within two weeks prior to your DXA scan. If you are female and are not post-menopausal you will be asked to take a pregnancy test prior to beginning the study and subsequently before each DXA scan. To measure abdominal subcutaneous and visceral fat, the DXA scan you will undergo will be used to measure this.

On days 1 and 28 of each phase we will also measure your body weight, hip and waist circumference, take your blood pressure in conjunction with pulse wave velocity (PWV) and augmentation index (AI) to assess blood vessel function, using a Mobil-O-Graph, in triplicate, and you will be asked to fill out gastro-intestinal tolerability questionnaires.

Any change in your health status and medications at any point during the study needs to be reported to the study investigators.

No alcoholic beverages are to be consumed within 48 hours prior to blood draws during the study periods. No caffeinated beverages consumption within 12 hours prior to blood draws during the study periods.

Risks and discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part. Some known risks, although rare, are associated with placing a needle into a vein. These include the possibility of infection, perforation or penetration of the needle through the vein, and bleeding, pain, and/or bruising at the site.

Benefits

You may not benefit from participation in this research; however, the study should contribute to a better understanding of the effect of consumption of hemp products through incorporation into diet on blood fat and lipoprotein concentrations, as well as CVD biomarkers. You will also receive access to your test results when they become available.

Costs

All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment that you will receive.

Remuneration for participation

You will receive up to a maximum of $200.00 at completion of this study for your time and inconvenience of the study schedule. This amount will be divided into 2 portions. You will receive $100 after completion of period 1 and another $100 after completion of period 2. If you withdraw early from the study, you will receive an appropriate pro-rated fraction of this amount.

In addition, the treatment and control products such as hulled hemp seeds, hemp oil, hulled sesame seeds and salad dressing provided during the study will be free of cost for you.

Appendix Two: Study Consent Forms

Alternatives

You are not obligated to participate in this study. The study coordinators and principal investigator will answer any questions you have about the experimental group of this study.

Confidentiality

Personal records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The RCFFN staff involved with your care may review/copy medical information that may reveal your identity. The Biomedical Research Ethics Board at the University of Manitoba may also review your research-related records for quality assurance purposes. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the RCFFN. The Principal Investigator will maintain the codes linking your personal information under lock and key.

You will be assigned a participant code. The coding system of the study for participant identification will be MHC of followed by a three-digit number. The three-digit number will be based on chronological order of participant selection. The identification codes corresponding to the study participants will be on the written documents which will only be available to the RCFFN staff. The documents linking participant identification to their code will be kept in a locked filing cabinet in a locked office.

Study samples will be stored in the freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be used for any additional analyses, nor stored for any longer than 5 years, nor shared with any other group, other than is indicated in the protocol, without your specific consent.

Voluntary participation/withdrawal from the study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your other medical care.

Your participation in this study may be terminated without your consent by the study coordinators, physician or principal investigator. The study staff will withdraw you if he/

she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you decide to participate, you will agree to cooperate fully with the study visit schedule, and will follow the study staff's instructions.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially close.

Medical care for injury related to study

In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should notify the principal investigator or study coordinator or go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form or releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, the principal investigator or study coordinator will be contacted, who will inform you of the results.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study staff.

Investigator:	Dr. Peter Jones	Tel No.	[redacted]
Coordinator:	Dr. Dylan Mackay	Tel No.	[redacted]

For questions about your rights as a research participant, you may contact:

The Biomedical Research Ethics Board, University of Manitoba at 204-789-3389

Do not sign this consent form unless you have a chance to ask questions and have received satisfactory answers to all of your questions.

This study is registered on a publicly available Registry Databank at Clinicaltrials.gov, the trial identifier is NCT02400203. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://ClinicalTrials.gov. This website will not include

Appendix Two: Study Consent Forms

information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Consent

I agree to allow the study doctor to inform my family doctor that I am participating in this study or to obtain information regarding my medical history.

Yes No

1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the clinical trial (research study) described above.

2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose and duration of the trial, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.

3. I agree to cooperate fully with the study coordinator and the principal investigator and will tell them if I experience any side effects, symptoms or changes in my health.

4. I am free to withdraw from the study at any time, for any reason, and without prejudice to my future medical treatment.

5. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.

6. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature:_____ Date: _____

Printed name of above:_____

Appendix Two: Study Consent Forms

FREE Living Hulled HEMP Seed and Oil (FREEHEMP) Trial

I confirm that I have explained the purpose, duration etc. of this clinical trial, as well as any potential risks and benefits, to the participant whose name and signature appears above. I confirm that I believe that the participant has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature:_____ Date: _____

Printed name of above:_____ Study role:_____

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

STUDY SCREENING QUESTIONNAIRE

ID: _____ DATE (DD/MM/YYYY): _____

Part: _____ Study: _____

Screening Questionnaire

(NOTE: After you are recruited for the study, you will be assigned an ID# which will be used on your forms and data throughout the study.)

Sex : ☐ **M** ☐ **F** **DOB:**

AGE: ____ **HEIGHT:** ____ **WEIGHT:** ____ **BMI:** ____

WAIST CIRCUMFERENCE: ____ **HIP CIRMUFERENCE:** ____

BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)

ARM: ☐ **LEFT** ☐ **RIGHT**

TYPE OF DEVICE: ☐ **Mercury** ☐ **Automatic**

10 min Systolic BP (mmHg) = ____ 10 min Diastolic BP (mmHg) = ____
13 min Systolic BP (mmHg) = ____ 13 min Diastolic BP (mmHg) = ____
16 min Systolic BP (mmHg) = ____ 16 min Diastolic BP (mmHg) = ____
Avg. Systolic BP (mmHg) = ____ **Avg. Diastolic BP (mmHg) =** ____
Heart rate / min = HR(1): ____ HR(2): ____ HR(3): ____ HR(mean)= ____

BLOOD SAMPLES: CHECKLIST

Did you eat or drink anything in the last 12 hours? ☐ **Yes** ☐ **NO**

Have you consumed alcohol or a product containing alcohol in the last 48 hours? ☐ **Yes** ☐ **NO**

In the last 24 hours, have you done intensive exercise? ☐ **Yes** ☐ **NO**

Appendix Two: Study Screening Questionnaire

ID: _____ DATE (DD/MM/YYYY): _____

Part: _____ Study: _____

BLOOD SAMPLES:	☐ LEFT ARM	☐ RIGHT ARM

BLOOD SAMPLES:	☐ **Yes**	☐ **NO**
EXTRA BLOOD SAMPLE (Keep Frozen)	☐ **Yes**	☐ **NO**

Nurse's Initials: _____

Comments:

_____

_____

Participation in Athletics/Exercise:

ACTIVITY	**HOW OFTEN?**	**HOW LONG? (HOURS)**

Do you usually eat breakfast? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Health Status:

Do you have diabetes? ☐ **YES** ☐ **NO**

Do you have any other major disease or condition? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Are you taking any medication? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Appendix Two: Study Screening Questionnaire

ID: _____ DATE (DD/MM/YYYY): _____

Part: _____ Study: _____

Are you taking any dietary supplements, vitamins and / or minerals, homeopathic remedies and other natural health products (probiotics, medicinal plants, omega-3)? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Do you have any reactions to any foods? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Are you on a special diet? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Have you recently lost or gained weight? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Do you smoke? ☐ **YES** ☐ **NO**

How many alcoholic beverages do you consume per day? _____ **Per week?** _____

MEASUREMENT DAY JOURNAL

Subject Code _____

Study Phase _____ Treatment _____

Start Date _____ End Date _____

SECTION 1: START AND END WEIGHT & HEIGHT

Day 1: Weight (kg) _____ Staff Initials _____

Height (cm) _____

Day 28: Weight (lbs) _____ Staff Initials _____

Height (cm) _____

SECTION 2: WAIST CIRCUMFERENCE

Instructions: *Waist circumference is measured at either the natural waist, i.e., smallest circumference of the abdomen, or midway between the lowest rib and iliac crest. Hip circumference is measured around the maximum circumference of the buttocks. For women this is usually at groin level. For men it is normally about 2-4 inches below the navel. The measuring tape should be snug around the body, but not pulled so tight that it is constricting.*

Day 1:

Waist -

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) waist circumference measurement: _____

Hip -

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) hip circumference measurement: _____

Day 28:

Waist -

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) waist circumference measurement: _____

Appendix Two: Measurement Day Journal

Hip -

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) hip circumference measurement: _____

SECTION 3: SEATED BLOOD PRESSURE (MOBILOGRAPH)

Has the subject consumed caffeine, used any medication or eaten any food in the last 12 hours and/or exercised in the past 2 hours? □ Yes* □ No

*If yes, subject needs to be rescheduled for a blood pressure measurement.

Instructions: *The subject should be instructed to relax as much as possible; ideally, at least 5 minutes should elapse before the first reading is taken. Apply cuff to non-dominant arm. After applying the cuff, the subject must be quiet and remain continuously seated without legs crossed for 5 minutes. Instruct the subject not to talk during the reading. Wait 1 minute after each reading before taking the next reading.*

Day 1:

a. First blood pressure measurement: ___/___ (SBP/DBP)

b. Second blood pressure measurement: ___/___ (SBP/DBP)

c. Third blood pressure measurement: ___/___ (SBP/DBP)

d. Average (2nd and 3rd) blood pressure measurement: ___/___ (SBP/DBP)

Day 27 or 28:

a. First blood pressure measurement: ___/___ (SBP/DBP)

b. Second blood pressure measurement: ___/___ (SBP/DBP)

c. Third blood pressure measurement: ___/___ (SBP/DBP)

d. Average (2nd and 3rd) blood pressure measurement: ___/___ (SBP/DBP)

Appendix Two: Measurement Day Journal

SECTION 4: BLOOD COLLECTION

DAY 1

Date (DD/MM/YY) _____ Time (HH:MM) _____

Did the subject fast? Yes ___ / No ___ Feeling ill? _____

Caffeine in last 12 hrs? Yes ___ / No ___ If yes, when _____

Alcohol in last 48 hrs? Yes ___ / No ___ If yes, when _____

Arm Right ___ / Left___

Collect:

1 X 4 ml serum (red/yellow tube)	____	(day 1, 27, 28)
1 X 10 ml plasma EDTA (purple top)	____	(day 1, 27, 28)
1 X 6 ml plasma EDTA (purple top)	____	(day 1, 27, 28)

Have all blood samples been obtained? Yes ___ / No** ___

** *If No, please state reasons why:* _____

_____

_____

_____

Blood Draw: Excellent ___ Good ___ Difficult ___ Very Difficult ___

Comments: _____

_____

_____

_____

Blood Taker: _____

Appendix Two: Measurement Day Journal

SECTION 4: BLOOD COLLECTION (continued)

DAY 27

Date (DD/MM/YY) _____ Time (HH:MM) _____

Did the subject fast? Yes ___ / No ___ Feeling ill? _____

Caffeine in last 12 hrs? Yes ___ / No ___ If yes, when _____

Alcohol in last 48 hrs? Yes ___ / No ___ If yes, when _____

Arm Right ___ / Left___

Collect:

1 X 4 ml serum (red/yellow tube)	____	(day 1, 27, 28)
1 X 10 ml plasma EDTA (purple top)	____	(day 1, 27, 28)
1 X 6 ml plasma EDTA (purple top)	____	(day 1, 27, 28)

Have all blood samples been obtained? Yes ___ / No** ___

*** If No, please state reasons why:* _____

_____

_____

_____

Blood Draw: Excellent ___ Good ___ Difficult ___ Very Difficult ___

Comments: _____

_____

_____

_____

Blood Taker: _____

Appendix Two: Measurement Day Journal

SECTION 4: BLOOD COLLECTION (continued)

DAY 28

Date (DD/MM/YY) _____ Time (HH:MM) _____

Did the subject fast? Yes ___ / No ___ Feeling ill? _____

Caffeine in last 12 hrs? Yes ___ / No ___ If yes, when _____

Alcohol in last 48 hrs? Yes ___ / No ___ If yes, when _____

Deuterium water provided?* Yes ___ / No ___ ***Only required on day 41 of each phase***

Arm Right ___ / Left___

Collect:

1 X 4 ml serum (red/yellow tube)	____	(day 1, 27, 28)
1 X 10 ml plasma EDTA (purple top)	____	(day 1, 27, 28)
1 X 6 ml plasma EDTA (purple top)	____	(day 1, 27, 28)

Have all blood samples been obtained? Yes ___ / No** ___

*** If No, please state reasons why:* _____

_____

_____

_____

Blood Draw: Excellent ___ Good ___ Difficult ___ Very Difficult ___

Comments: _____

_____

_____

_____

Blood Taker: _____

Appendix Two: Measurement Day Journal

SECTION 5: DXA

To be completed by study staff:

BASELINE DXA

DAY: 1 (± 2 days of Day 1)

Date (DD/MM/YY): _____ Time of scan (HH:MM): _____

Since screening has the volunteer had any radiation exposure (e.g. x-rays, radiotherapy, CT scan)? Yes __ / No __

Date & details: _____

_____

_____

FEMALES ONLY:

Possibility of being pregnant Yes __ / No __

Has a pregnancy test been offered? Yes __ / No __

Has a test been taken? Yes __ / No*** __ Test Results (circle): **Negative / Positive**

Result Confirmation:

Volunteer signature:_____ Study staff signature:_____

***If no test please explain why?

_____

_____

Check:

Jewelry removed (including glasses): Yes __ / No __

Spine straight: Yes __ / No __

Comments:_____

_____

_____

Staff Initials:_____

SECTION 5: DXA (continued)

To be completed by study staff:

ENDPOINT DXA

DAY: 27 28 (please circle one)

Date (DD/MM/YY): _____ Time of scan (HH:MM): _____

Since screening has the volunteer had any radiation exposure (e.g. x-rays, radiotherapy, CT scan)? Yes __ / No __

Date & details: _____

_____

_____

FEMALES ONLY:

Possibility of being pregnant Yes __ / No __

Has a pregnancy test been offered? Yes __ / No __

Has a test been taken? Yes __ / No*** __ Test Results (circle): **Negative / Positive**

Result Confirmation:

Volunteer signature:_____ Study staff signature:_____

***If no test please explain why?

_____

_____

Check:

Jewelry removed (including glasses): Yes __ / No __

Spine straight: Yes __ / No __

Comments:_____

_____

_____

Staff Initials:_____

Appendix Two: Measurement Day Journal

SECTION 6: OTHER INFORMATION

Day 28

1. In the past 28 days, has your exercise level changed? ☐ Yes ☐ No
   If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

2. Have you taken any prescription or non-prescription drugs in the past 28 days? ☐ Yes ☐ No
   If Yes, specify: description _____ amount _____
   _____ _____

3. Have you taken any vitamins, minerals or other supplements in the past 28 days? ☐ Yes ☐ No
   If Yes, specify: description _____ amount _____
   _____ _____

4. Have you been ill in the past 28 days? ☐ Yes ☐ No
   If Yes, describe illness: _____

5. If you were ill in the past 28 days, did your eating change as a result? ☐ Yes ☐ No
   If Yes, describe: _____

6. Have you experience any fluctuations in mood in the past 28 days? ☐ Yes ☐ No
   If Yes, describe: _____

7. Has your energy level changed in the past 28 days? ☐ Yes ☐ No
   If Yes, was it: ☐ More Energy ☐ Less Energy

Appendix Two: Measurement Day Journal

ADVERSE EVENTS

Description	Start Date	Intensity*	Outcome	Date Ended	Diet Related?

*Mild: An event that is easily tolerated by the volunteer, causing minimal discomfort and not interfering with everyday activities
Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
Severe: An event which is incapacitating and prevents normal everyday activities

Appendix Two: Measurement Day Journal

STUDY COMMENTS & PROTOCOL DEVIATIONS

Date (DD/MMM/YY)	Comments	Study Personnel Initials

Appendix Two: Measurement Day Journal

STATUS SUMMARY

☐ Volunteer completed the study

☐ Volunteer withdrew from the study Date of withdrawal: _____

REASON FOR WITHDRAWAL. Tick appropriate box(es):

☐ Informed consent withdrawn by the volunteer
☐ Failure to comply with study requirements
☐ Investigator decision to withdraw volunteer

Provide rationale for withdrawal (if applicable):

_____

_____

_____

_____

☐ Volunteer's final results sent

☐ Statement supplier form completed

Investigator Initials _____

WAYS TO INCORPORATE YOUR SEEDS HANDOUT

EASY WAYS TO EAT YOUR SEEDS!

The contents of these packets are the perfect addition to meal replacement bars, cereal, porridge, salads, pesto, pasta, dressings, sauces, smoothies, desserts and side dishes. Below are some examples:

- Sprinkle over your favourite yogurt.
- Sprinkle over your favourite lunch or dinner salad.
- Sprinkle over oatmeal, porridge, or cereal with breakfast.
- Combine with granola, fruit, and yogurt for a tasty parfait!
- Toss into your stir-fry or favourite Asian dish.
- Add to your favourite fruit or green smoothie.
- Sprinkle on top of a hearty bowl of chili.
- Add to hummus, guacamole, or your other favourite dips to go with chips/crackers.
- Sprinkle over spaghetti, fettucine, or other pasta dishes.
- Add to your burrito, fajita, or taco.
- Can be a topping on baked potatoes, wild rice dishes, casseroles, and other staple sides.

and more!!

APPENDIX THREE

ADDITIONAL DOCUMENTS FOR HEMP STUDY

COVANCE

Report Number: 1274259-0
Report Date: 13-Jul-2015
Report Status: Final

Certificate of Analysis

University of Manitoba
196 Innovation Drive
Winnipeg Manitoba R3T 6C5 Canada

Sample Name:	**Natural Hemp Hearts (Raw Shelled Hemp Seeds)**	**Covance Sample:**	**4095810**
Project ID	UNIV_MANIT-20150701-0002	**Receipt Date**	01-Jul-2015
PO Number	Charge/VISA	**Receipt Condition**	Ambient temperature
Sample Serving Size	30 g	**Login Date**	01-Jul-2015
		Storage Condition	-70 (+/- 10) Degrees Celsius
		Online Order	10

Analysis	Result	Result per Serving
Calories		
Calories	574 Cal/100g	172 Cal/Serving size
Calories from Fat		
Calories	398 Cal/100g	119 Cal/Serving size
Fat by Acid Hydrolysis		
Fat	44.2 g/100g	13.3 g/Serving Size
Carbohydrates		
Total Carbohydrates	11.8 g/100g	3.54 g/Serving Size
Total Dietary Fiber		
Total Dietary Fiber	2.61 g/100g	0.783 g/Serving Size
Protein (N x 6.25) Dumas Method		
Protein	32.3 g/100g	9.69 g/Serving Size
Ash		
Ash	5.57 g/100g	1.67 g/Serving Size
Moisture		
Moisture	6.17 g/100g	1.85 g/Serving Size

Method References	Testing Location
Ash (ASHM_S:8)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 923.03, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Calories (CALC:9)	**Covance Laboratories - Madison**
Code of Federal Regulations, Title 21, Part 101.9, pp. 24-25.	
Calories from Fat (CFAT:8)	**Covance Laboratories - Madison**
Code of Federal Regulations, Title 21, Part 101.9, pp. 24-25.	
Carbohydrates (CHO:11)	**Covance Laboratories - Madison**
United States Department of Agriculture, "Energy Value of Foods", Agriculture Handbook No. 74, pp. 2-11, (1973).	

COVANCE

Report Number: 1274259-0
Report Date: 13-Jul-2015
Report Status: Final

Certificate of Analysis

University of Manitoba
196 Innovation Drive
Winnipeg Manitoba R3T 6C5 Canada

Method References	Testing Location
Fat by Acid Hydrolysis (FAT_AH_S:6)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 922.06 and 954.02, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 933.05. (Modified)	
Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 925.32. (Modified)	
Moisture (M100T100_S:8)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 925.09 and 926.08, AOAC INTERNATIONAL, Gaithersburg, MD, USA,(2005). (Modified).	
Protein (N x 6.25) Dumas Method (DGEN_S:11)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 968.06 and 992.15, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Total Dietary Fiber (TDFL_S:20)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL 18th Ed., Method 991.43, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	

Testing Location(s)	Released on Behalf of Covance by
Covance Laboratories - Madison	**Lori Ross - Associate Director**

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375 x4170

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2918.01

These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Covance.

Appendix Three: Additional Documents for Hemp Study

COVANCE

Report Number:	**1273270-0**
Report Date:	**10-Jul-2015**
Report Status:	**Final**

Certificate of Analysis

University of Manitoba
196 Innovation Drive
Winnipeg Manitoba R3T 6C5 Canada

Sample Name:	**Control Treatment (Raw Untoasted Sesame Seeds Organic Fresh Green Lentil Hulls)**	**Covance Sample:**	**4095811**
Project ID	UNIV_MANIT-20150701-0002	**Receipt Date**	01-Jul-2015
PO Number	Charge/VISA	**Receipt Condition**	Ambient temperature
Sample Serving Size	30 g	**Login Date**	01-Jul-2015
		Storage Condition	-70 (+/- 10) Degrees Celsius
		Online Order	10

Analysis	Result	Result per Serving
Calories		
Calories	635 Cal/100g	191 Cal/Serving size
Calories from Fat		
Calories	479 Cal/100g	144 Cal/Serving size
Fat by Acid Hydrolysis		
Fat	53.2 g/100g	16.0 g/Serving Size
Carbohydrates		
Total Carbohydrates	14.0 g/100g	4.20 g/Serving Size
Total Dietary Fiber		
Total Dietary Fiber	8.17 g/100g	2.45 g/Serving Size
Protein (N x 6.25) Dumas Method		
Protein	25.0 g/100g	7.50 g/Serving Size
Ash		
Ash	3.31 g/100g	0.993 g/Serving Size
Moisture		
Moisture	4.45 g/100g	1.34 g/Serving Size

Method References	Testing Location
Ash (ASHM_5:8)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 923.03, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Calories (CALC:9)	**Covance Laboratories - Madison**
Code of Federal Regulations, Title 21, Part 101.9, pp. 24-26.	
Calories from Fat (CFAT:8)	**Covance Laboratories - Madison**
Code of Federal Regulations, Title 21, Part 101.9, pp. 24-26.	
Carbohydrates (CHO:11)	**Covance Laboratories - Madison**
United States Department of Agriculture, "Energy Value of Foods", Agriculture Handbook No. 74, pp. 2-11, (1973).	

COVANCE

Report Number: 1273270-0
Report Date: 10-Jul-2015
Report Status: Final

Certificate of Analysis

University of Manitoba
196 Innovation Drive
Winnipeg Manitoba R3T 6C5 Canada

Method References	Testing Location
Fat by Acid Hydrolysis (FAT_AH_8:8)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 922.06 and 954.02, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 933.05. (Modified)	
Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 925.32. (Modified)	
Moisture (M100T100_8:8)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 925.09 and 926.08, AOAC INTERNATIONAL, Gaithersburg, MD, USA,(2005). (Modified).	
Protein (N x 6.25) Dumas Method (DGEN_8:11)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 968.06 and 992.15, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	
Total Dietary Fiber (TDFL_8:20)	**Covance Laboratories - Madison**
Official Methods of Analysis of AOAC INTERNATIONAL 18th Ed., Method 991.43, AOAC INTERNATIONAL, Gaithersburg, MD, USA, (2005). (Modified)	

Testing Location(s)	Released on Behalf of Covance by
Covance Laboratories - Madison	**Lori Ross - Associate Director**

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