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The effect of hemp product consumption on blood fatty acid profiles and cardiovascular disease risk factors: results of a randomized, double-blind, crossover clinical trial

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Hemp seeds are high in polyunsaturated fatty acids (PUFAs) including gamma linolenic acid (GLA), stearidonic acid (SDA), alpha linolenic acid (ALA) and linoleic acid (LA). To date, limited evidence is available on hemp product consumption and particularly hemp seeds and oil in humans and its relation to cardiometabolic risk factors. The objective of present study was to examine the effects of hemp product consumption *versus* similar controls on circulating fatty acid profiles and cardiovascular disease (CVD) risk factors. A randomized, double-blinded, crossover trial with 30 normoglycemic adults (18–65 years) within a BMI range of 25–35 kg m^{-2} were included. Participants consumed both hemp products and controlled products over the course of 4 weeks each. As expected, ALA (18:3 n-3), GLA (18:3 n-6) and dihomo-γ-linolenic acid (DGLA, 20:3 n-6) were elevated after the hemp treatment than controls. Similarly, ALA, DGLA as well as eicosapentaenoic acid (EPA) levels were elevated after the hemp treatment than controls. No differences in serum lipid levels, glucose and insulin concentrations, blood pressure, or body composition were observed between treatments. Overall, consumption of hemp products modulated plasma and RBC fatty acids levels in a way which reflected the fatty acids these products are enriched in, without showing differences in major cardiometabolic risk factors. The present study demonstrated the human fatty acids profile response to consuming hemp products, novel functional foods rich in polyunsaturated fatty acids. The clinical trial registry number is NCT02400203 (https://clinicaltrials.gov).

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Introduction

Cannabis sativa L. is a herbaceous plant native to Central Asia and is broadly classified into drug-type (marijuana) and non-drug-type (hemp) varieties based on Δ^9-tetrahydrocannabinol content.[1] In recent years, hemp seeds and hemp-derived products have attracted increasing attention as functional food due to their distinctive and favorable fatty acid composition. Hemp seed oil is particularly rich in polyunsaturated fatty acids (PUFAs), providing substantial amounts of linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3), as well as smaller but physiologically relevant quantities of γ-linolenic acid (GLA, 18:3 n-6) and stearidonic acid (SDA, 18:4 n-3), fatty acids that are uncommon in most commonly consumed vegetable oils.[2–4] In addition, hemp seed oil contains tocopherols and polyphenolic compounds with antioxidant potential, further supporting its emerging role as a cardioprotective dietary fat source.

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide, accounting for approximately one third of global deaths.[5] Extensive epidemiological and clinical evidence demonstrates that dietary fat quality, rather than total fat intake alone, plays a critical role in modulating CVD risks. For instance, high dietary intake and circulating levels of LA and ALA are associated with improved cardiometabolic health and reduced risk of thrombotic and ischemic events,[6,7] while SDA intake may further counteract some CVD risk factors through its efficient conversion to long-chain n-3 fatty acids.[8]

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In contrast, diets characterized by high saturated fatty acid (SFA) content and low PUFA intake are consistently associated with increased CVD risk.[9,10] Moreover, an imbalanced dietary n-6/n-3 PUFA ratio has been linked to adverse cardiometabolic outcomes, whereas increased n-3 PUFA intake has been shown to reduce the risk of sudden cardiac death.[11] Beyond dietary intake, circulating fatty acid profiles measured in plasma as well as erythrocytes serve as promising biomarkers of fatty acid exposure, metabolism, and tissue incorporation, and are increasingly recognized as integrative indicators of cardiometabolic perturbations. Plasma fatty acids reflect more recent dietary intake, whereas erythrocyte fatty acid composition provides insight into longer-term fatty acid bioavailability and incorporation into cell membranes. Alterations in these fatty acid pools have been linked to established CVD risk factors, including dyslipidemia, hypertension, insulin resistance, and obesity based on findings from numerous human cohort studies and including those from our own group.[12–16]

Preclinical studies suggest that hemp seed and hemp oil consumption can beneficially modify fatty acid metabolism. Supplementation with hemp seed oil in maternal sows resulted in an altered n-6/n-3 PUFA profile with increased circulating ALA and SDA concentrations and EPA/DPA in piglets,[17] while feeding hemp seeds to laying hens increased hepatic LA, ALA, GLA, arachidonic acid (AA), and docosahexaenoic acid (DHA) levels and decreased the n-6/n-3 ratio.[18] Despite the growing popularity of hemp-based foods, well-controlled human intervention studies examining the effects of hemp product consumption on circulating fatty acid profiles and CVD risk factors remain limited. In particular, there is a lack of randomized clinical trials evaluating both plasma and erythrocyte fatty acid responses to hemp products in populations at elevated cardiometabolic risk.

Hence, the objective of the present randomized, double-blind, crossover clinical trial was to investigate the effects of hemp product consumption on circulating fatty acid profiles in plasma and erythrocytes, as well as on established cardiovascular disease risk factors, in adults with overweight or obesity. This study aimed to provide controlled human evidence to clarify the cardiometabolic relevance of hemp-derived fatty acids and to establish circulating fatty acid signatures associated with hemp product intake.

Methods

Study design

This study was a randomized, double-blinded, two period cross-over design. Each treatment was 28 days followed by a 4-week wash-out period. The primary outcome was to determine changes in plasma as well as erythrocytes fatty acids profile and the secondary outcomes were to determine the changes in major CVD risk factors including lipids profile, blood pressure and arterial thickness, and fasting glucose and insulin. BMI, waist circumference and body composition were also measured.

Inclusion and exclusion criteria. Normoglycemic participants with a BMI between 25–35 kg m^{-2} and between the ages of 18–65 years were recruited for this study. Eligible participants were required 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, cardiometabolic perturbations, 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^{-1}, and/or LDL-C >6.5 mmol L^{-1}, hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg) and glucose concentrations >6.1 mmol L^{-1} 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 weeks and reported consumption of more than 2 alcoholic drinks per day or history of alcoholism or drug dependence and use of any experimental medication within one month prior to starting the trial were excluded. The study was approved by the ethical boards of University of Manitoba and was registered at https://clinicaltrials.gov under NCT02400203, and all participants provided written informed consent before the intervention.

Recruitment and screening. Participants were recruited through advertisements in the newspaper Metro and on the Richardson Centre for Food Technology and Research (RCFTR) website and email list. Potential participants were then briefly screened *via* telephone screening questionnaire which included questions related to the inclusion and exclusion criteria. The screening questionnaire determined the eligibility of potential participants. If a participant qualified, they were asked to attend the RCFTR for an in-person screening session. During the in-person screening session, participants were asked to arrive at the RCFTR after fasting 10–12 hours. Study protocol and consent forms, detailing the nature of the study, were provided to and discussed with potential participants. The restrictions of the study dietary intervention were discussed which included avoidance of foods containing high concentrations of n-3 fatty acids including fatty fishes such as salmon, herring, mackerel and various seeds, including flaxseed and chia seeds or other n-3 fatty acids supplementation. Participants read and signed the Informed Consent form before enrolling into the study. Then, anthropometry was measured including height, weight, hip and waist circumference and blood pressure. Blood samples were collected and processed on a Vitros 350 chemistry analyzer to determine glucose, insulin, and lipid concentrations.

Diet and treatment

The control treatment included 27 g of hulled sesame seeds with 3 g of lentil flakes incorporated to mimic the appearance

of hulled hempseed. The control treatment 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. Treatment products were produced by RCFTR staff outside the trial team and given to trial staff in a blinded fashion. Table 1 shows the recipe used for the control and the hemp treatment salad dressings. Hulled seeds were given in opaque sachets and salad dressings in plastic containers. Participants were required to consume two 30 g sachets of hulled seeds and a single 51.8 g serving of salad dressing daily, which contained 30 g of oil. Labelled cups were provided to ensure the proper amount was distributed daily. The salad dressing recipe was selected for flavor and color that would help mask any differences in treatment oils appearance and taste. During the participants' weekly visits, they were asked if there were any issues arising from consuming the treatment products and if they needed any further assistance and/or advise in ways to incorporate the treatment products into their diets. The fatty acid profiles of the soybean and hempseed oil used in the salad were determined by using the direct transesterification method and processed on the gas chromatograph (GC) using flame ionization.[19] Results of the fatty acid profile in the soybean and hempseed oils are presented in Table 2. The individual fatty acid concentration is calculated as peak area of the fatty acid divided by total area identified and expressed as g per 100 g. The sesame seeds and hempseed were sent to a third-party lab (Covance Lab. Madison, WI) to determine the macronutrient contents presented in Table 3.

Study procedures. Participants who were eligible were randomly assigned to a sequence of the two interventions by clinical trial coordinator who opened a numbered sealed envelope that contained the sequence. The order of sequences, AB or BA, in the envelopes was created in advance using the rand(0) function in excel in groups of 4 or 6, separate sets of envelopes were made for males and females, a total of 24 each were made. Participants were instructed to fast 10–12 hours prior to study visits. On days 1, 27 and 28 of both treatment periods, blood samples were drawn and measurements, including BMI, blood pressure, arterial stiffness, and dual-energy X-ray absorptiometry (DEXA), were conducted. Fasting blood samples were collected *via* venipuncture by trained phlebotomists or registered nurses. Blood samples were centrifuged at 3000 rpm at 4 °C for 20 min within 1 hour of being drawn. Plasma, serum, and RBC samples were aliquoted into microtubes. All microtubes were placed in a −80 °C freezer until further analysis.

BMI was calculated using the participants' weight and height measurements. An electric scale was used to measure body weight of the participants. Individuals removed shoes and heavy cloths prior to weighing. For height measurements individuals stood with their scapula, buttocks and heels against a wall, the neck was held in a natural non-stretched position and the head was held straight. Participants' systolic and diastolic blood pressures were measured in triplicate, on the non-dominant arm in a sitting position using a validated oscillometric blood pressure monitor (BP760CAN, Omron, Burlington, Ontario, Canada). Participants were required to rest for 5–10 min before taking the measurement. Pulse wave velocity and augmentation index were measured on the non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS Client Server Software (IEM GmbH, Stolberg, Germany) according to the manufacturer's protocol. Body composition including body fat percentage, and visceral adipose tissue mass were assessed using DEXA

Table 1 Salad dressing recipe for hemp and control treatment

Ingredients	Hemp	Control
Balsamic vinegar (g)	11	11
Chopped, dried parsley flakes (g)	0.5	0.5
Fresh lemon juice (g)	6	6
Chopped, fresh garlic cloves (g)	2.5	2.5
Crumbled, dried basil (g)	0.5	0.5
Dried oregano (g)	0.5	0.5
Salt (g)	0.5	0.5
Pepper (g)	0.3	0.3
Hempseed (g)	30	NA
Soybean oil (g)	NA	30
Total (g)	**51.8**	**51.8**

The recipe is presented in daily portions.

Table 2 Fatty acid profile of hempseed oil and soybean oil

	Hemp	Soy
SFA (%)	9.93	15.4
C16:0	6.5	10.8
C18:0	1.96	3.78
C20:0	0.86	0.32
MUFA (%)	9.61	23.9
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
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
n-6 PUFA (%)	60.93	53.6
n-3 PUFA (%)	19.7	6.89
n-6 : n-3 ratio	3.09	7.7

Fatty acid percentages are based on averages between two samples. ND = non-detectable.

Table 3 Macronutrients of hulled hempseed and sesame seeds

	Hulled hempseed	Sesame seeds with lentil flakes
Calories (kcal)	172	191
Calories from fat (kcal)	119	144
Fat (g)	13.3	16
Total carbohydrates (g)	3.54	4.2
Total dietary fiber (g)	0.78	2.45
Protein (g)	9.69	7.5

The values are presented per 30 g of serving.

(Lunar Prodigy Advance, GE Healthcare, Mississauga, Ontario, Canada). After day 1, the participants were required to drop in weekly to pick up the treatments for the following week, which continued during both study periods. During the washout period, participants were asked to follow the same n-3 fatty acids food restrictions discussed prior to commencing the study and asked to maintain regular dietary habits.

Assessments

Plasma insulin, lipid, and glucose analysis. Plasma insulin was measured by radioimmunoassay (RIA) in duplicate using Millipore Human Insulin Specific RIA Kits (125 I-Insulin). Plasma total cholesterol (TC), HDL cholesterol, triglycerides, and glucose concentrations were determined by automated enzymatic methods on a Vitros-350 chemistry analyzer (ortho-Clinical Diagnostics). LDL-cholesterol concentrations were calculated by the Friedewald equation.[20]

Fatty acid profile analysis. Plasma and RBC fatty acids were extracted and methylated using a direct transesterification method.[19] Briefly, 1.6 ml methanol was initially added into the sample (500 µl plasma or 0.5 g RBC) followed by adding 75 µl of 1 mg ml^{-1} methyl *cis*-10-heptadecenoate (Sigma Aldrich, USA) as the internal standard. Then, adding 400 µl toluene and 200 µl acetyl chloride into the sample while mixed on a vortex. A ten-second nitrogen flash was also applied to each sample followed by incubation at 80 °C for 1 hour. After cooling to room temperature, 5 ml of 6% K_2CO_3 was added into the tube and followed by centrifugation at 2500 rpm for 5 minutes. The top layer was transferred into the GC vial and stored at −80 °C for further analysis.

Methylated fatty acid samples were analyzed by gas chromatography (GC, Varian 430) by using a 30 m × 0.25 mm column (Agilent Technologies, Inc). Fatty acids in samples were identified based on the retention time of internal standard and a mix of standard known fatty acids. Concentrations of each fatty acid were expressed as percentage of total identified fatty acids. The RBC long chain n-3 fatty acid content was determined by combining the amount of EPA, DPA, and DHA present in the RBC.

Statistical analysis

Sample size was determined using a power calculation performed using PROC POWER (SAS 9.2, SAS Inc). Estimated changes in erythrocyte EPA fatty acid was selected as primary outcome and LDL-C was selected as secondary outcome based on our previous study,[21] a trial involving ALA supplementation from flax oil, a n = 30 had a power = 0.996 to detect a difference in EPA fatty acids of 0.56% and power = 0.611 to detect a difference of 0.22 mmol L^{-1} in LDL-C, with α = 0.05 and correlation factor of repeated measures set at 0.8 using a 2-sided test. The estimated changes in fatty acids and LDL-C were taken as half the effect of the canola/flax treatment which provided a dose of 20 g d^{-1} ALA (the current trial would delivered 12.27 g d^{-1} ALA and 0.56 g d^{-1} SDA). The highest standard deviations for the fatty acid and cholesterol treatments from the previous study were selected as the estimates of variance for the power calculation. This sample size also provided moderate power (power = 0.611) to detect a clinically relevant difference of 0.22 mmol L^{-1} in LDL-C as a secondary outcome. To account for potential attrition, 37 participants were enrolled, resulting in 30 participants completing the study, which met the predefined sample size requirement for the primary outcome.

The outcomes are reported as means ± standard error (SE). Effects of dietary treatments compared the endpoint outcome value of each treatment period using a mixed model analysis of variance procedure (PROC MIXED, SAS 9.2, SAS Inc), with repeat measures. Treatment, sex, sequence, treatment × sex, treatment × sequence were assigned as fixed factors and participant ID was a repeated factor. Normality of data was observed and checked visually based on the plot of residuals. Statistical significance was set at $P < 0.05$ for all outcome measurements.

Results

Participant characteristics

Baseline demographic characteristics of participants are presented in Table 4. The trial procedures are outlined in Fig. 1. Overall, 37 participants were enrolled in the study and 30 participants completed with 13 males and 17 females. A total of seven participants dropped out of the study. Four participants had trouble incorporating the treatments into their daily diet; one participant was worried about the increase in fat intake from the intervention products: one participant had to leave the study for personal reasons, and one participant reported gastrointestinal symptoms related to the salad dressing during the control treatment period.

Plasma and RBC fatty acids profile

Results for plasma fatty acid concentrations is presented in Table 5. There were no differences in total SFA and monosaturated fatty acids (MUFA) between hemp and control treatments. As expected, total PUFA was increased after hemp treatment compared to the control (P = 0.01). Specifically, GLA (C18:3n-6), ALA (C18:3n-3), DGLA (C20:3n-6) concentrations were found to be higher in hemp treatment than in control (all

Table 4 Baseline characteristics

Characteristic	Mean
Age (year)	49.23 ± 14.52
Weight (kg)	84.27 ± 12.92
BMI (kg m^{-2})	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^{-1})	5.43 ± 0.56
Cholesterol (mmol L^{-1})	5.14 ± 1.08
Triglycerides (mmol L^{-1})	1.31 ± 0.65
dHDL (mmol L^{-1})	1.41 ± 0.33
LDL (mmol L^{-1})	3.14 ± 0.95

Values are expressed as mean ± SD.

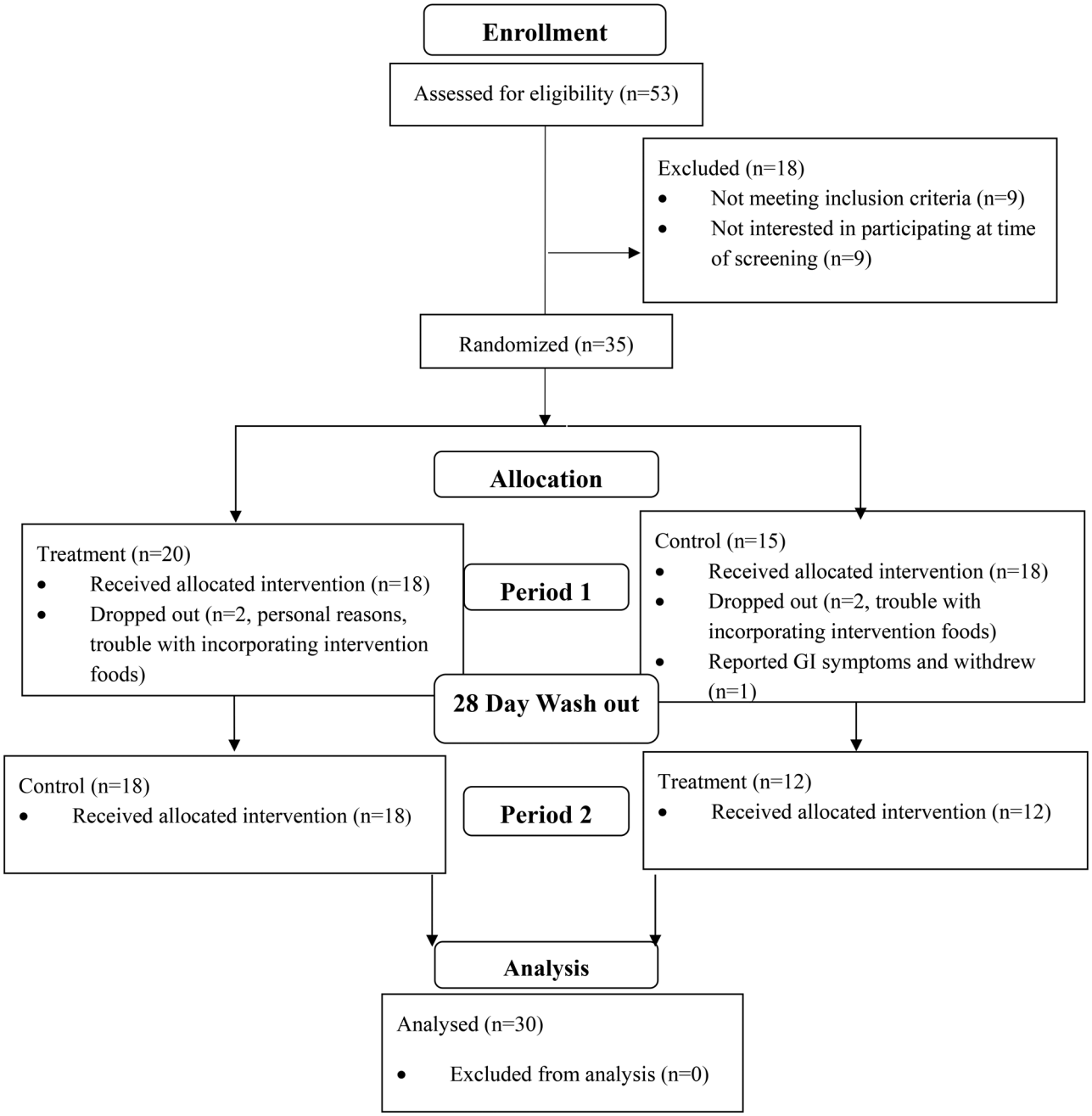

Fig. 1 CONSORT flowchart of the trial; GI: gastrointestinal.

$P < 0.05$). The results of the RBC fatty acid concentrations are presented in Table 6. Similar to what we observed in plasma, there were no major differences in SFA, MUFA between the treatments. In terms of individual PUFAs, ALA (C18:3n-3, $P = 0.01$), EPA (C20:5n-3, $P = 0.02$) as well as DGLA (C20:3n-6, $P < 0.05$) concentrations were found to be higher in hemp treatment than in control.

Blood pressure, arterial stiffness and DEXA measures

Blood pressure measurement were not different between the hemp and the control treatments for either systolic or diastolic blood pressure. The PWV and the augmentation index showed no differences after consumption of hemp treatment compared to control. No differences were seen in visceral fat, body fat percentage, or BMI across treatments. Results are presented in Table 7.

Lipids, glucose, and insulin concentrations

The fasting glucose, insulin, and lipid concentrations are presented in Table 8. No differences were seen between hemp and control treatments in total cholesterol, triglyceride, HDL-C, or

Table 5 Plasma fatty acid percentages on day 28 of hemp and control treatments

Measure	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.

Table 6 RBC fatty acid percentages on day 28 after start of hemp and control treatment

Measure	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
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
C20:5n-3	**0.78 ± 0.05**	**0.7 ± 0.05**	**0.02**
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

Values are expressed as mean ± SE.

LDL-C concentrations. There were no changes in glucose and insulin concentrations measured across periods.

Table 7 BMI, blood pressure, arterial stiffness and DEXA on day 28 after start of hemp and control treatment

Measures	Hemp	Soy	*P*-Value
BMI (kg m^{-2})	29.29 ± 0.54	29.07 ± 0.54	0.13
Systolic blood pressure (mmHg)	120.77 ± 3.16	124.51 ± 3.16	0.28
Diastolic blood pressure (mmHg)	80.29 ± 1.62	80.2 ± 1.62	0.95
Augmentation index (%)	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
Visceral fat (lbs)	2.94 ± 0.28	2.87 ± 0.28	0.57
Body fat (%)	36.5 ± 0.92	36.2 ± 0.92	0.31

Values are expressed as mean ± SE.

Discussion

This randomized, double-blind, crossover clinical trial investigated the effects of hemp product, specifically the hemp seeds and hemp oil consumption on circulating fatty acid profiles and major cardiovascular disease (CVD) risk factors in adults with overweight or obesity. The principal finding of this study is that daily consumption of hulled hempseed and hempseed oil for four weeks significantly increased the relative abundance of some beneficial fatty acids, namely ALA, GLA, DGLA, and EPA in plasma and erythrocyte membranes respectively, without adversely modulating major cardiometabolic risk markers, including blood lipids, blood pressure, arterial stiffness and/or body composition.

The observed enrichment of plasma as well as RBC ALA, GLA, DGLA, and EPA following hemp product consumption indicates effective absorption, metabolism, and incorporation of hemp-derived polyunsaturated fatty acids into cell membranes. Erythrocyte fatty acid composition reflects longer-term dietary exposure and tissue bioavailability rather than short-term intake,[22,23] these findings provide robust evidence that hemp products can favorably modify systemic fatty acid pool. In particular, the increase in EPA is noteworthy, as EPA has well-established anti-inflammatory and cardioprotective properties and has been associated with reduced obesity-related metabolic dysfunction and cardiovascular mortality.[8,24] In our present study, the elevation of EPA observed is likely attributable to, at least in part, the unique fatty acid composition of hemp products, characterized by relatively high ALA and GLA content and a lower LA/ALA ratio compared with many conven-

Table 8 Serum lipid analysis, glucose and insulin concentrations on day 28 after start of hemp and control treatments

	Hemp	Soy	*P*-Value
Glucose (mmol L^{-1})	5.4 ± 0.11	5.31 ± 0.11	0.42
Fasting insulin (μU ml^{-1})	17.83 ± 1.36	18.1 ± 1.36	0.78
Total cholesterol (mmol L^{-1})	5.03 ± 0.2	5.13 ± 0.2	0.24
Triglyceride (mmol L^{-1})	1.27 ± 0.14	1.16 ± 0.14	0.15
HDL (mmol L^{-1})	1.39 ± 0.05	1.43 ± 0.05	0.06
LDL (mmol L^{-1})	3.06 ± 0.17	3.17 ± 0.17	0.11

Values are expressed as mean ± SE.

tional vegetable oils. This fatty acid milieu may facilitate endogenous conversion of ALA to EPA by reducing competitive inhibition from excessive linoleic acid.[25,26] In contrast, DHA levels remained unchanged, which is consistent with prior evidence demonstrating limited conversion of ALA to DHA in humans, particularly over short intervention periods. DGLA and EPA are known precursors to anti-inflammatory eicosanoids and series-1 prostaglandins, which are implicated in improved vascular function and reduced inflammatory tone.[27] Elevated circulating or membrane-bound DGLA has been associated with lower triglyceride concentrations and reduced cardiovascular mortality in observational studies,[24,28] while EPA has demonstrated cardiovascular benefits independent of DHA in both epidemiological and interventional settings. Thus, the fatty acid shifts observed in this study are directionally consistent with a more favorable cardiometabolic profile, even in the absence of measurable short-term changes in clinical endpoints.

Despite these biologically meaningful changes in both plasma and erythrocyte fatty acids, no significant effects were observed on BMI, body composition, blood pressure, arterial stiffness, or plasma lipid concentrations. This is not unexpected since structural vascular changes, lipid remodeling, and body composition adaptations typically require longer intervention durations, larger sample sizes, or populations with overt metabolic dysregulation. Consistent with this interpretation, major anthropometric parameters, hs-CRP, blood pressure, fasting glucose and insulin concentrations, and HOMA-IR were also not significantly altered following MUFA- or PUFA-rich dietary interventions relative to high-carbohydrate or high-dairy-fat diets in our previous full-diet randomized controlled crossover trial.[12]

Similarly, several randomized controlled trials have reported that hemp seed and/or hempseed oil supplementation does not significantly modify circulating lipid parameters, including total cholesterol, triglycerides, HDL-C, and LDL-C,[29–31] findings that are concordant with the present results. In addition, population-based evidence suggests that short-term (≥2 weeks) supplementation with n-3 PUFAs does not substantially affect total cholesterol levels *per se*.[32] In contrast, some pre-clinical studies have reported improvements in atherosclerosis or insulin sensitivity following hemp product supplementation in animal models,[33–35] while reductions in total and LDL cholesterol have also been observed in selected human cohorts.[30] Discrepancies across studies likely reflect differences in species, baseline metabolic status, intervention dose, duration, and overall dietary context. Importantly, we emphasize that no single food or isolated dietary component is expected to induce substantial improvements in cardiometabolic health in isolation, particularly in the absence of broader dietary pattern optimization and lifestyle modification. Cardiovascular risk is shaped by the integrated effects of food matrix interactions, habitual diet quality, physical activity, and metabolic health. In this context, the absence of short-term changes in clinical risk markers does not diminish the biological relevance of the observed shifts in circulating fatty acid profiles, which may represent early and necessary metabolic adaptations that precede longer-term cardiometabolic benefits.

To the best of our knowledge, this was first human clinical study that directly investigate the beneficial effects of hemp seeds and hemp oil on major cardiometabolic risk factors in overweight and obese patients. A key strength of this study is the use of erythrocyte fatty acid composition as a primary outcome, providing an objective and biologically relevant marker of fatty acid exposure and incorporation. Exclusion of participants who had recently consumed n-3 supplements minimized background variability and strengthened attribution of observed changes to the intervention. However, we also admit that limitations should be acknowledged. Specifically, the intervention duration may have been insufficient to detect changes in downstream cardiometabolic outcomes. Dietary intake outside the intervention products was not quantitatively controlled, which may have attenuated detectable effects. Additionally, the relatively modest sample size limited statistical power to detect small but potentially meaningful changes in secondary outcomes. Finally, the study population consisted of overweight or obese adults from a single geographic region, which may limit generalizability.

Overall, the present study showed that 4-week consumption of hulled hempseed and hemp oil in overweight individuals increased ALA, GLA, DGLA and EPA relative percentages in plasma and RBC respectively, demonstrating effective incorporation of hemp-derived polyunsaturated fatty acids into long-term lipid pools. These changes occurred without adverse effects on lipid metabolism, vascular function, and/or body composition. Collectively, these findings support the metabolic safety of hemp products and highlight their potential utility as dietary sources of polyunsaturated fatty acids for improving circulating fatty acid profiles. Longer-term studies are warranted to determine whether these favorable changes translate into clinically meaningful cardiovascular benefits.

Author contributions

Y. S., K. J., D. M. prepared the manuscript. A. C. and A. S. L. conducted the clinical trial and analysis and contributed to the manuscript. R. M., D. M. designed the study protocol, selection criteria of participants, sought funding, ethical

approval and reviewed the manuscript. Y. S., X. L. and D. M. revised the manuscript. R. M. and D. M. supervised the staff and conduct of the clinical trial. All authors contributed to, have read, made critical revisions, and approved the final manuscript before submission.

Conflicts of interest

The authors declare no conflict of interests.

Data availability

The data for this article is primarily based on analyses from human randomized controlled trial and securely stored at the University of Manitoba with ethical implications. Deidentified data will be made available upon request to the corresponding author.

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