Eight-week hempseed oil intervention improves fatty acid composition of erythrocytes phospholipids and omega-3 index in children and adolescent with primary hyperlipidemia

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Abstract

Children affected by primary hyperlipidemia have a high risk to develop cardiovascular diseases (CVDs) during adulthood. Several studies reported a positive association between the intake of polyunsaturated fatty acids (PUFA) and an improvement of lipid profile and CVD risk, thus dietary supplements may represent a potential strategy in the management of hyperlipidemia. In this context, the effectiveness of hempseed oil (HSO) rich in PUFA (particularly linoleic acid (LA) and α-linolenic acid (ALA)), in the modulation of hyperlipidemia has been poorly investigated.

The present pilot study aimed to explore the impact of HSO supplementation on serum lipid profile and fatty acid (FA) composition of red blood cells (RBCs) in children and adolescents with primary hyperlipidemia.

An 8-week parallel dietary intervention study was scheduled. Thirty-six hyperlipidemic probands (6-16 years) on diet therapy were randomized into 2 groups: 1- HSO group, receiving 3g of HSO providing 1.4 g of LA and 0.7 g/die of ALA; 2- control group, receiving only the dietary guidelines. Before and after the intervention, blood samples were collected and serum lipid profile, RBC fatty acid composition and omega-3-index were analyzed.

The 8 week supplementation with HSO significantly ($p<0.01$) reduced the RBCs content of total saturated and monounsaturated FA (-5.02 ± 7.94% and -2.12 ± 2.23%, respectively), while increased the levels of total n-3 and n-6 PUFA (+1.57 ± 1.96% and +5.39 ± 7.18%, respectively) and omega-3 index (+1.18 ± 1.42%) compared to control group. No significant effect was found for the serum lipid profile.

In conclusion, our findings seem to support the contribution of HSO supplementation in the improvement of RBC phospholipid composition and omega-3 index, while no effect was observed in the modulation of lipid profile. Further controlled studies are necessary for a complete comprehension of the effects of HSO in the modulation of hyperlipidemia and CVD risk on this and other target groups of population.

Keywords: Hempseed oil supplement, lipid profile, fatty acid composition of red blood cells, dietary intervention study, hyperlipidemic children

Introduction

Hyperlipidemia is a condition characterized by an excess of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and/or triglycerides (TG) in the blood.[1] Lifestyle habits, diet and genetic lipid disorders are recognized as the major determinants for the development and progression of hyperlipidemia.[2-3] Elevated levels of lipids and lipoproteins are strongly associated with atherosclerotic processes and cardiovascular diseases (CVDs) including coronary heart disease, stroke, and myocardial infarction.[1] The incidence of hyperlipidemia is gradually increasing, therefore the development of strategies aimed at the prevention/improvement of lipoprotein profile represents an outstanding chance for reducing the onset and progression of CVD. In this regard, several studies documented that a 1% reduction in LDL-C concentration has been associated with a decrease of 1-2% of CVD risk.[4-5]

Children and adolescent represent an important target group of population for CVD prevention programs. Diet and lifestyle represent the first line therapy for children despite a wide number of pharmacological and prospective studies have clearly documented the beneficial effects of statin on lipoprotein profile.[2,6] The Cardiovascular Health Integrated Lifestyle Diet (CHILD)-1 diet for children and adolescent recommends to decrease the amount of total fat (25-30% of total daily calories), saturated fat (<10% of daily kcal/estimated energy requirements), dietary cholesterol (<100 mg/1000 kcal and no more than 200 mg/day) and trans-fat (to avoid), while to increase the intake of MUFA and PUFA (up to 20% of total energy).[7] It is well know that saturated fatty acids (SFA) favor the raising of cholesterol concentration, while monounsatured and polyunsaturated fatty acids (MUFA and PUFA) contribute to its reduction.[8-9] Several meta-analysis of well controlled dietary and clinical trials clearly indicated that the quality of fat is more determinant than its total amount in the promotion of health.[10-13] Numerous studies have shown a significant reduction in LDL concentration when SFAs in a diet were substituted with unsaturated FAs.[14-16] Moreover, the replacement of 5% of the energy of SFAs with unsaturated fatty acids induced a 42% decrease in coronary heart diseases, while the replacement of 2% of energy deriving

from *trans*-fatty acids with that obtained from *cis*-fatty acid reduced up to 53% the same diseases.[17] Despite n-3 and n-6 PUFA share metabolic pathways, they exert a different effect. For example, n-6 FAs are involved in the reduction of LDL-cholesterol by increasing hepatic LDL receptor number and LDL turnover, while n-3 FAs are implicated in the decrease of TGs concentration in part through a reduction of very low-density lipoprotein synthesis in the liver, and in part by increasing the degradation of FAs and accelerating the clearance of TGs from the plasma.[18-19]

In the last years, a plethora of food supplements have been developed aiming to provide nutritional and/or physiological effect even if they should not be considered a diet replacement. Hempseeds oil is commonly used in dietary supplements for its fat composition rich in the essential FA omega-6 linoleic acid (LA, C18:3n-6) and n-3 PUFAs alpha-linolenic acid (ALA, C18:3n-3).[20] The ratio LA/ALA is between 2:1 and 3:1.[21] This proportion has been considered favorable since high amount of LA may reduce ALA conversion to n-3 LC-PUFA.[21-22] In fact LA can be converted into arachidonic acid (ARA) whereas ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with important impact on cardio-metabolic health.[23-24] Hempseeds oil is also rich in vitamin E and a number of minerals (e.g. potassium, magnesium, calcium, iron, and zinc) contributing to the CVD risk reduction.

The role of hempseeds oil in the modulation of hyperlipidemia and CVD risk has been evaluated in different animal models[25-29] demonstrating an improvement on hyperlipidemia and platelet aggregation, while only few studies have been performed in humans.[30-32] To the best of our knowledge, this is the first trial aimed to evaluate the effects of 8-week supplementation with a hempseeds oil on serum lipid profile and RBCs FA composition in children and adolescent with primary hyperlipidemia.

Material and methods

Subject enrollment

Fifty children and adolescents with primary hyperlipidemia were recruited among the pediatric patients cared at the Department of Health Science and Pediatrics of the University of Turin, after a screening for eligibility. The selection of the volunteers was performed based on the assessment of primary hyperlipidemia diagnosed, according to international standards, as previously reported.[33] Subjects were excluded from trial when presented secondary dyslipidemia; renal, endocrine and liver disorders or chronic diseases requiring drug treatment (i.e neurologic, oncohematologic disorders or intolerance to the hempseed oil). In addition, subjects with a body mass index over 85th percentile and smokers were excluded. Children were included only after demonstrating appropriate compliance with dietary instructions, provided by a trained nutritionist, in the previous 2 months. Recruited subjects and their families were trained by a nutritionist to adhere to a properly dietary regimen, evaluated through a weekly food diary during the study period.

Study design

The study was performed between January 2015 and October 2015. The study was an 8-week randomized, single blind, controlled, two-arm parallel-group. Thirty-six subjects were enrolled and randomly divided into two groups of 18 subjects each (**Figure 1**). The control group maintained the usual diet based on CHILD1 guidelines throughout the entire study period, while the test group introduced 3 g/day of a capsuled containing hempseed oil (HSO). For the entire duration of the intervention, all participants were encouraged to maintain their habitual diet and lifestyle habits (as assessed before enrollment) and to abstain from consuming any other kind of supplements and hempseed oil-based products. In order to check the compliance to the dietary instructions, subjects and their families were asked to fill in weekly food diaries. Daily energy and nutrient intake were analyzed with MètaDieta® Software (Me.Te.Da S.r.l., San Benedetto del Tronto, Italy) using

Italian Food Composition databases (data not shown). Compliance was also assessed by counting the eventual capsules returned.

At the beginning and at the end of the experimental period, all subjects enrolled underwent a medical examination early in the morning. Physical parameters including height, weight and blood pressure were measured. Fasting blood samples were drawn for the analysis of the lipid profile and FA composition of red blood cells (RBCs). The study was conducted according to the principles of the Declaration of Helsinki, and was approved by the ethics committee of the City of Health and Science University Hospital of Turin (EC:CS377). The trial was registered under ISRCTN.com (identifier no. ISRCTN12261900). All individuals involved in the trial, including doctors, research staff and parents of children, were aware of the specific product administered. The study was exhaustively explained to all participants and their parents signed an informed consent.

Intervention

The supplement AlfaLife®, produced by hempseed *Cannabis sativa* L., was prepared as gelatine-soft gel capsules and was provided by Freia Farmaceutici Srl (Milan, Italy). **Table 1** shows the nutritional composition of 100g hempseed oil according to the manufacturer's specification. Subjects received 4 capsules/day, providing 700 mg of α-linolenic acid (ALA) and 1400 mg of linolenic acid (LA), for 8 weeks. Capsules were ingested with water during lunch or dinner.

Anthropometric and physical evaluation

Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively (Wunder SA.BI. S.r.l. Italy), with the patients wearing hospital gowns and had bare feet. BMI was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). Systolic and diastolic blood pressure was measured twice with a mercury sphygmomanometer during the medical examination.

Serum lipid profile determination

Venous blood samples (2.5 ml) were drawn into vacutainer tubes containing silicon and

centrifuged at 1400 *x* g for 15 min at 4° C within 30 min. Serum was collected and immediately analyzed for the determination of the lipid profile. The levels of TC, HDL-C and TG were directly evaluated by an automatic biochemical analyzer (Olympus AU2700, Japan), while the LDL-C concentration was estimated using the Friedewald formula (LDL= TC-(HDL+TG/5). Non-high density lipoprotein cholesterol (non–HDL-C) was calculated as TC minus HDL-C.

RBCs separation

An aliquot of venous blood samples (2.5 ml) were drawn into vacutainer tubes containing K_2EDTA as anticoagulant and immediately centrifuged at 1400 *X* g for 15 min at 4° C. Plasma and white blood cells were removed, while RBCs were washed twice with a solution of sodium chloride (0.9%, w/v). Two aliquots (0.5 ml) of RBCs were stored at -80°C until the analysis.

Extraction and analysis of fatty acid composition of RBCs

The extraction and the analysis of FA composition of RBCs was performed according to the methods previously reported.[34,35]

Statistical Analysis

Based on previous studies,[36,37] the sample size (18 subjects) was considered sufficient to determine an improvement of the parameters related to hyperlipidemia and FA composition of RBCs. Results for each treatment are reported as the percentage change (obtained as differences between post- to pre-treatment) calculated for each variable considered. Variables were analyzed by one way ANOVA with treatment (HSO *vs* control group) as dependent factor. Data are reported as mean ± standard deviation (SD) or as mean changes (Δ, described as mean of single variation), with 95% confidence interval (CI). STATISTICA software (Statsoft Inc., Tulsa, OK, USA) was used to perform the statistical analysis. Significance of difference was set as $p \leq 0.05$.

Results

Characteristic of the subjects

In **Table 2** are reported the baseline characteristic of the enrolled subjects. A total of 36 hyperlipidemic (2 FH, 9 FCHL and 25 PHC) subjects (23 males and 13 females) successfully completed the intervention. The age of the subjects ranged between 6-15 years old. The levels of TC, LDL-C and non-HDL-C levels exceeded the 90th percentile for age and sex, while HDL-C values were in the normal range. All the subjects showed normal blood pressure levels and BMI (except for 8 subjects with borderline overweight). In addition, subjects showed an omega-3 index (sum of EPA + DHA) lower than 4%.

Energy and nutrients intake

Energy and nutrient intake at baseline and during the study period are reported in **Table 3**. The intake of hempseed oil significantly ($p<0.001$) increased total PUFA and omega-3 intake. No differences were observed in the control group.

Effect of intervention on anthropometric and biochemical parameters

In **Table 4** are reported the results on anthropometric and biochemical parameters. No significant effect of intervention on lipid profile, height, body weight, BMI and blood pressure parameters has been observed.

Effect of intervention on fatty acid composition of erythrocytes

Table 5 shows the changes in FA composition of RBCs between interventions. The supplementation with HSO significantly increased total n-3 PUFA (+1.57±1.96%, p=0.0001), total n-6 PUFA (+5.39±7.18%, p=0.0005), LC-n3 PUFA (+1.57±1.95%, p=0.0001), LC-n6 PUFA (+4.08±5.41%, p=0.0003), and the ratio n3/n6 (+0.05±0.06, p=0.0002) and LC-n3/n6 (+0.08±0.09, p=0.0002) compared to control treatment. In particular, HSO supplementation significantly

improved the levels of linoleic (+1.28±2.04%, p=0.013), arachidonic (+3.06±4.05%, p=0.0003), dihomo-γ-linolenic (+0.43±0.60%, p=0.0005), docosahexaenoic (+1.07±1.28%, p=0.0001), docosapentaenoic (+0.39±0.55%, p=0.0004), eicosapentaenoic (+0.11±0.15%, p=0.009) and adrenic acid (+0.59±0.93%, p=0.002) compared to control. A significant augmentation was also observed for omega 3-index in the group of subjects treated with the supplement (+1.18±1.42%, p=0.0001). Conversely, the intervention with HSO significantly reduced the levels of total SFA (-5.02±7.94%, p=0.0009) and total MUFA (-2.12±2.23%, p=0.0003). In particular, regarding SFA a significant decrease was observed for the content of myristic (-0.07±0.18%, p=0.029), pentadecanoic (-0.03±0.03%, p=0.006), palmitic (-3.27±4.96%, p=0.001), margaric (-0.10±0.24%, p=0.006), behenic (-0.45±0.45%, p<0.0001), tricosanoic (-0.06±0.06%, p=0.0001) and lignoceric (-0.54±1.92%, p=0.029) acids. Concerning MUFA, a significant decline for oleic (-1.14±1.53%, p=0.003), vaccenic (-0.10±0.12%, p=0.039) and nervonic (-0.83± 0.95%, p=0.007) acid was observed in the HSO group compared to control.

Discussion

To the best of our knowledge, the present study is the first designed to evaluate the effect of 8-week supplementation with HSO on markers related to lipid metabolism in children and adolescent with primary hyperlipidemia. We have shown that the regular intake of HSO was able to improve the RBCs FA composition, increasing the omega-3 index compared to control group. The HSO used in the present study was low in SFA and rich in PUFAs in line with other published data on HSO composition. The 3 g portion used in our study provided 1.4 g LA and 0.7 g ALA ensuring the coverage of requirements for this target group. In fact, the Italian reference intake values from the Italian Society of Human Nutrition for n-3 PUFA is 0.5–2% of total energy intake.[38] In addition, a daily intake of ALA corresponding to 0.5% of total calories has been recently reported as adequate intake (EFSA Scientific Opinion on Dietary Reference Values for Fats (EFSA Panel on Dietetic Products Nutrition and Allergies).[39]

Several intervention studies and meta-analysis has dealt with the effects of FAs on blood lipid and lipoprotein concentrations.[40-45] An association between the intake of a mixture of SFA and blood LDL cholesterol concentrations has been found in several studies.[46-48] In fact, SFAs have shown to affect plasma LDL-C by increasing the formation of LDL lipoproteins and by decreasing their turnover.[49] On the contrary, the replace of SFA with products rich in n-6 and n-3 PUFA seem to exert a multiplicity of functional activities including effects on plasma lipids and lipoproteins, eicosanoid metabolism, platelet lipid composition and function, and several other functions related to cardiovascular health.[14,50] However, the lipid lowering effect of increasing dietary ALA intake is controversial and the limited studies available on hyperlipidemic subjects make difficult the comparison among studies.[51] In a recent randomized, controlled trial in hypertrigliceridemic subjects, Dittrich et al.,[52] documented a significant reduction of TC and LDL-C levels after 10-week consumption of linseed oil (7.4 g/die of ALA). Similar findings were also observed after 6-week consumption of 11.4 g ALA/die from camelina oil in a group of hypercholesterolemic adult subjects.[53] Wilkinson and colleagues, reported a significant reduction of TC concentration, but also HDL-C, after 12 weeks of ALA-enriched diet (15 g ALA/die) from flaxseed oil in subjects with an atherogenic lipoprotein phenotype.[54] Zhao and coworkers found a significant reduction in the levels of TC, LDL-C and TG in hypercholesterolemic subjects when treated for 6 weeks with a diet rich in LA (12.6% En) and a diet rich in ALA (3.6% En) compared with the average American diet (6.5% En from LA and 0.8% En from ALA).[55] Conversely, ALA diet induced a significant reduction in the levels of HDL-C and apolipoprotein AI.[55]

Regarding normocholesterolemic subjects, Schwab *et al.*,[56] documented that the intake of 30 ml/day of HSO, providing 16.2 g LA and 6.6 g ALA, for 4 weeks failed to improve serum total and lipoprotein lipids in a group of healthy volunteers. In addition, it has been recently reported that several short term trials (6–12 weeks) showed no or inconsistent effects of 1.2–3.6 g/die ALA intake on blood lipids and lipoprotein in healthy individuals.[57-60]

The ratios HDL-C/TC and HDL-C/LDL-C are considered two important predictive values of CVD risk, more that single isolated parameter used independently.[61] We recently documented that 8-week intervention with 20-30g/day of hazelnuts (providing mainly MUFA, in particular LA) was able to provide an improvement of HDL/LDL ratio in a group of hyperlipidemic children.[35] On the contrary, in the present study we observed that 8 weeks of HSO supplementation significantly reduced the HDL/LDL ratio in the same group of target population. This effect was only detectable in the group of subjects supplemented with HSO, while no significant effect was observed in the control. This decrease is probably due to a slight, but not significant, reduction in the levels of HDL-C observed in HSO group. Such reduction is not surprising since, as above reported, several studies have shown a reduction of HDL-C concentrations following the intake of ALA.[38]

The FA composition of RBCs is considered an additional risk factor for the progression of atherosclerosis and coronary heart disease. This variable generally reflects the last three months of dietary fat intake, and it is thought to be a biomarker of the tissue FA status. Thus, the RBCs FA composition is postulated to better and earlier reflect lipid metabolism dysfunction, in respect to lipoprotein changes in blood serum, which are affected by the recent diet.[62,63]

Many studies conducted in adults found a correlation between altered FA composition in RBCs and coronary heart disease, arterial hypertension, dyslipidemia and other atherosclerosis-related diseases.[63,64] In the present study, the supplementation with HSO was able to improve the overall FA composition of RBC phospholipids by increasing the levels of total PUFA, n-3 and n-6 PUFA subclasses and n-3/n-6 PUFA ratio, while significantly reducing RBC levels of SFA and MUFA. Regarding SFA, while stearic acid (18:0) appears to have a neutral effect on LDL-C, lauric (12:0), myristic (14:0), and palmitic (16:0) acids are considered to be hypercholesterolemic.[49,65] In our experimental conditions the intake of HSO unaffected the levels of stearic acid, while significantly reduced the concentrations of lauric and palmitic acid.

As previously reported, HSO is an important source of LA that is metabolized to gamma-linolenic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6; DGLA) and arachidonic acid (20:4n-6; ARA). The conversion process is limited and less than 1% of LA is generally converted into ARA. The 8-week intake of HSO significantly increased the RBC levels of LA, in line with its high content in the oil, and the precursors DGLA, ARA and adrenic acid. Unexpectedly, and in contrast with other studies, the increased dietary intake of ALA was not associated with higher ALA concentration in RBC phospholipids with respect to baseline and control, but apparently stimulated the ALA pathway for its endogenous conversion into the LC-PUFA derivatives EPA, DPA and DHA, which increased after intervention. Our results are in line with few other human trials investigating the impact of increased ALA intake on RBC FA composition. In particular, Barceló-Coblijn *et al.*,[66] in a study conducted on subjects at high CVD risk, showed that 12-week supplementation with two different doses of flaxseed oil, rich in ALA (2.4 or 3.6 g ALA/die) led to a significant increased EPA, DHA and ALA in RBC phospholipids. The authors, by comparing multiple doses and different time-points, concluded that 2 weeks of treatment with 2.4 g ALA/die was sufficient to obtain alterations of n-3 PUFA in RBC phospholipids.[66] Comparable findings on the modulation of RBC FAs with flaxseed oil were also observed in patients with atherogenic lipoprotein phenotype treated with 15 g ALA/die for 6 and 12 weeks,[67] or in a more recent study conducted in hypertriglyceridemic subjects supplemented with 7.4 g ALA/die.[52] Only one study carried out in obese children concluded that daily ALA supplementation (1 g/die, 6-weeks) increased significantly n-3 FA composition of plasma lipids, while the effect on FA composition of RBCs was not evaluated.[68]

Omega-3 index, i.e. the total levels of EPA and DHA in RBCs, has been suggested as an additional biomarker of CVD risk, and a predictive parameter for morbidity and mortality from CVD.[62] The omega-3 index risk zones are as follow: high risk, <4%; intermediate risk, 4–8%; and low risk, >8%. Harris and von Schacky demonstrated that a low content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (<4%) in the RBC membranes is strongly associated with

coronary and cardiovascular diseases.[62] Since most of this class of compounds is contained in cell membranes, the index has been also calculated on RBC phospholipids and other cell types.[69] Most of the subjects enrolled shown very low omega-3 index at baseline (<3%). The intervention with HSO significantly raised the omega-3 index, due to an increase of the relative RBC contents of EPA and DHA, even if this index remained below the cut-off level of risk. On the contrary, we recently showed that 8-week intervention with hazelnuts failed to improve omega-3-index in the same group of hyperlipidemic children with a low omega-3 index.[35] This lack of effect was probably attributed to the low amount of n3-PUFA provided by hazelnuts. Egert and coworkers showed that the intake margarine fortified with ALA (4.4 g ALA/die) was not associated with an increase of omega-3 index (Egert et al. 2012). However, the subjects included in the trial were healthy and the omega-3 index was relatively high, indicating a good n-3 PUFA status (Egert et al. 2012).

In conclusion, this pilot intervention is the first documenting the potential beneficial effect of HSO supplementation in children and adolescents affected by primary hyperlipidemia. In particular, we were able to show that 8-week HSO supplementation induced an improvement of RBCs FA composition, reducing the quantity of SFA, increasing those of n-6 and n-3 PUFAs and improving the omega-3 index. On the contrary, HSO supplementation failed to reduce serum lipid profile compared to control group. Further randomized, placebo controlled trials in larger groups of subjects will be pivotal to ascertain the role of HSO supplementation in the modulation of hyperlipidemia in both the pediatric and adult target population.

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Conflict of interest

The authors declare no conflict of interest

Author's contribution

CDB and VD wrote the preliminary manuscript, performed statistical analysis and contributed to fatty acids analysis; FA contributed to patient diagnosis; EQ updated the database and monitored patients; PR critically revised the results and the manuscript; OG designed the study, revised biochemical data and reviewed the manuscript.

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525 **Table 1**-Nutritional composition of HSO supplement, according to the manufacturer specifications

526

Hempseed oil supplement	**100 g***	**Daily dose (4 capsules, 3 g)**
Energy, kcal	896	27
Carbohydrates, g	-	-
Protein, g	-	-
Total Fats, g	> 99.5	3
Total SFAs, g	10.4	0.3
Total MUFAs, g	11.8	0.4
Oleic acid, g	9.0	0.3
Total PUFAs, g	75.5	2.3
ALA	23.0	0.7
EPA	-	-
DHA	-	-
Stearidonic acid	1.1	0.03
LA	48.3	1.4
γ-linolenic acid	3.1	0.1
Cholesterol, g	-	-
Vitamin A, UI	9.5	0.3
Vitamin E, UI	14.9	0.4
Niacin (B3), mg	< 0.001	< 0.0001
Calcium, mg	9.8	0.3
Phosphorus, mg	0.013	< 0.001
Iron, mg	0.004	< 0.001
Magnesium, mg	0.004	< 0.001

527

528 Notes: ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HSO,

529 group treated with hempseed oil; LA, linoleic acid; MUFAs, monounsaturated fatty acids; PUFAs,

530 polyunsaturated fatty acids; SFAs, saturated fatty acids.

531

532

Table 2- Baseline characteristic of the study population

Variables	Values
Age, years	11.8 ± 2.36
Weight, kg	49.2 ± 16.3
Height, cm	150.7 ± 14.5
BMI, kg/m^2	21.1 ± 4.02
SBP, mmHg	107.6 ± 11.04
DBP, mmHg	67.1 ± 8.79
Serum lipid profile	
CT, mg/dL	209.0 ± 36.4
LDL-C, mg/dL	136.4 ±34.1
HDL-C, mg/dL	56.8 ± 13.4
Non-HDL-C, mg/dL	154.2 ± 36.8
HDL/LDL-C ratio	2.08 ± 1.76
TG, mg/dL	87.9 ± 42.9
FA composition in RBCs	
Total SFA, %	54.02 ± 7.86
Total MUFA, %	20.25 ± 2.36
Total PUFA, %	25.73 ± 9.51
n-3, %	3.40 ± 2.30
n-6, %	20.62 ± 6.88
n-3/n-6 ratio	0.14 ± 0.08
LC-PUFA n-3	3.31 ± 2.31
LC-PUFA n-6	10.84 ± 5.33
LC n-3/n-6, ratio	0.30 ± 0.08
Omega-3 index	2.52 ± 1.83
EPA, %	0.27 ± 0.15
DHA, %	2.26 ± 1.70

Notes: BMI, body mass index; SBP, systolic blood pressure, DBP, diastolic blood pressure, HDL-C, high-density lipoprotein cholesterol; HSO, group treated with hempseed oil; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides, FA, fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids (C ≥20, double bonds ≥3); MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n-6 PUFAs, omega-6 PUFAs; n-3 PUFAs, omega-3 PUFAs; RBC, red blood cell; SFAs, saturated fatty acids; Omega-3 index: sum of EPA + DHA.

Table 3. Daily energy and nutrient intake at baseline and changes after 8 weeks intervention with hampseed oil or control treatment

Variables	Control (*n*=18)		HSO (*n*=18)				
	Baseline	Week 8	Baseline	Week 8	P_T	P_t	$P_{T x t}$
Energy (kcal)	1089.8 ± 239	1125.5 ± 147	1015.4 ± 198.6	1055.4 ± 181.7	0.177	0.297	0.953
Protein (% of E)	16.2 ± 2.9	16.0 ± 2.9	16.1 ± 1.9	15.5 ± 2.4	0.683	0.305	0.557
Carbohydrate (% of E)	52.5 ± 5.2	50.1 ± 5.9	53.2 ± 4.2	53.2 ± 4.7	0.189	0.234	0.230
Total fat (% of E)	31.5 ± 4.1	33.9 ± 5.8	30.7 ± 3.8	34.6 ± 5.2	0.999	0.007	0.495
SFA (% of E)	9.8 ± 2.2	10.1 ± 2.1	9.1 ± 1.7	10.2 ± 2.5	0.646	0.078	0.252
MUFA (% of E)	14.5 ± 2.9	16.0 ± 5.0	13.7 ± 2.9	15.0 ± 3.8	0.270	0.146	0.866
PUFA (% of E)	3.3 ± 0.8	3.3 ± 0.7	3.4 ± 0.8	5.7 ± 0.9*	<0.001	<0.001	<0.001
ω-3 (% of E)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	1.2 ± 0.1*	<0.001	<0.001	<0.001
ω-6 (% of E)	2.2 ± 0.8	2.1 ± 0.5	2.3 ± 0.8	2.1 ± 0.5	0.825	0.636	0.805
Fibers (g)	9.6± 2.5	9.8 ± 2.5	9.6 ± 2.3	10.4 ± 3.5	0.668	0.268	0.492
Cholesterol (mg)	127.0 ± 39.1	131.7 ± 54.2	116.4 ± 34.7	124.0 ± 43.0	0.461	0.441	0.854

Notes: HSO, group treated with hempseed oil; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; ω-3, omega-3 fatty acids; ω-6, omega-6 fatty acids. Values are expressed as mean ± SD. *Significantly different as compared to baseline and control group

549 **Table 4-** Changes in blood pressure, body weight, BMI, and lipid profile in participants with
550 primary hyperlipidemia evaluated after 8 weeks of supplementation with hempseed oil or control
551 treatment
552

Variables	**Control (n=18)**	**HSO (*n*=18)**	**P value**
	Δ (95% CI)	Δ (95% CI)	
Weight (kg)	0.46 (-0.01; 0.93)	0.64 (-0.21; 1.49)	0.679
BMI kg/m^2)	-0.05 (-0.29; 0.19)	-0.03 (-0.39; 0.33)	0.907
SBP (mmHg)	2.22 (-2.87; 7.31)	-3.33 (-9.77; 3.07)	0.150
DBP (mmHg)	-0.89 (-5.29; 3.51)	-3.33 (-9.71; 3.06)	0.490
TC	-6.2 (-19.7; 7.2)	-4.5 (-13.6; 4.6)	0.824
TG	-6.3 (-24.1; 11.6)	16.0 (-3.6; 35.6)	0.085
HDL-C	-2.56 (-6.49; 1.37)	-1.94 (-5.34; 1.46)	0.806
LDL-C	-4.94 (-13.7; 3.81)	-14.2 (-15.2; -13.2)	0.156
HDL/LDL	0.02 (-0.04; 0.07)	0.04 (-0.01; 0.09)	0.479
Non-HDL-C	-6.3 (-17.6; 4.9)	3.4 (-31.2; 37.9)	0.577

553
554 Notes: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-
555 C, high-density lipoprotein cholesterol; HSO, group treated with hempseed oil; LDL-C, low-
556 density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
557 Values are expressed as mean changes (Δ) calculated considering post-treatment variations
558 (calculated as differences between post- to pre-treatment), with 95% confidence interval (CI min;
559 max).
560
561

562 **Table 5-** Changes in FA composition of RBC phospholipids in participants with primary hyperlipidemia evaluated after

563 8 weeks of supplementation with hempseed oil or control treatment

564

FA composition of RBC phospolipids (%)	**Control (*n*=18)** Δ (95% CI)	**HSO (*n*=18)** Δ (95% CI)	**P value**
Total SFAs	+3.10 (0.50; 5.70)	-5.02 (-8.97; -1.07)*	0.0009
Total MUFAs	+0.40 (-0.30; 1.09)	-2.12 (-3.23; -1.01)*	0.0003
Total PUFAs	-3.50 (-6.62; -0.37)	+7.14 (2.48; 11.81)*	0.0003
Total PUFAs n-3	-0.83 (-1.51; -0.16)	+1.57 (0.60; 2.55)*	0.0001
Total PUFAs n-6	-2.40 (-4.69; -0.10)	+5.39 (1.82; 8.96)*	0.0005
PUFAs n-3/n-6	-0.02 (-0.04; 0.00)	+0.05 (0.02; 0.09)*	0.0002
Total LC-PUFAs n-3	-0.83 (-1.50; -0.16)	+1.57 (0.60; 2.53)*	0.0001
Total LC-PUFAs n-6	-2.08 (-3.93; -0.24)	+4.08 (1.39; 6.76)*	0.0003
LC-PUFAs n-3/n-6	-0.02 (-0.04; 0.01)	+0.08 (0.04; 0.13)*	0.0002
Omega-3 index	-0.65 (-1.19; -0.10)	+1.18 (0.47; 1.88)*	0.0001
SFAs			
14:0 (myristic acid)	+0.10 (-0.02; 0.23)	-0.07 (-0.16; 0.03)*	0.029
15:0 (pentadecanoic acid)	+0.02 (0.00; 0.04)	-0.03 (-0.06; 0.00)*	0.006
16:0 (palmitic acid)	+1.58 (0.10; 3.07)	-3.27 (-5.73; -0.80)*	0.001
17:0 (margaric acid)	+0.16 (0.02; 0.31)	-0.10 (-0.22; 0.02)*	0.006
18:0 (stearic acid)	+0.34 (-0.19; 0.87)	-0.40 (-1.92; 1.12)	0.340
20:0 (arachidic acid)	+0.02 (-0.11; 0.16)	-0.11 (-0.19; -0.02)	0.092
22:0 (behenic acid)	+0.22 (0.02; 0.41)	-0.45 (-0.67; -0.22)*	< 0.0001
23:0 (tricosanoic acid)	+0.04 (0.00; 0.08)	-0.06 (-0.09; -0.03)*	0.0001
24:0 (lignoceric acid)	+0.61 (0.13; 1.10)	-0.54 (-1.49; 0.41)*	0.029
MUFAs			
16:1n-9 (hypogeic acid)	+0.003 (-0.012; 0.018)	-0.01 (-0.03; 0.01)	0.296
16:1n-7 (palmitoleic acid)	+0.05 (-0.05; 0.15)	-0.02 (-0.04; 0.00)*	0.146
18:1n-9 (oleic acid)	+0.20 (-0.23; 0.63)	-1.14 (-1.90; -0.38)*	0.003
18:1n-7 (vaccenic acid)	-0.02 (-0.07; 0.03)	-0.10 (-0.15; -0.04)*	0.039
20:1n-9 (eicosenoic acid)	-0.03 (-0.10; 0.05)	-0.03 (-0.06; 0.00)	0.958
24:1n-9 (nervonic acid)	0.19 (-0.39; 0.77)	-0.83 (-1.30; -0.35)*	0.007
n-6 PUFAs			
18:2n-6 (LA)	-0.26 (-0.96; 0.44)	+1.28 (0.26; 2.29)*	0.013
18:3n-6 (γ-linolenic acid)	+0.002 (-0.013; 0.017)	+0.01 (-0.02; 0.05)	0.506
20:2n-6 (EDA)	-0.04 (-0.10; 0.05)	+0.03 (-0.01; 0.07)	0.057
20:3n-6 (DGLA)	-0.19 (-0.37; 0.02)	+0.43 (0.13; 0.72)*	0.0005
20:4n-6 (ARA)	-1.60 (-3.03; -0.17)	+3.06 (1.05; 5.07)*	0.0003
22:4n-6 (adrenic acid)	-0.31 (-0.63; 0.01)	+0.59 (0.13; 1.05)*	0.002
n-3 PUFAs			
18:3n-3 (ALA)	-0.003 (-0.022; 0.015)	+0.01 (-0.02; 0.04)	0.496
20:5n-3 (EPA)	-0.18 (-0.32; 0.04)	+0.11 (0.03; 0.18)*	0.009
22:5n-3 (DPA)	-0.02 (-0.08; 0.04)	+0.39 (0.12; 0.66)*	0.0004
22:6n-3 (DHA)	-0.63 (-1.15; -0.10)	+1.07 (0.43; 1.71)*	0.0001

565

566 Notes: ALA, α-linolenic acid; ARA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA,

567 docosapentaenoic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; HSO, group treated with hempseed oil; LA, linoleic

568 acid; LC-PUFAs, long chain polyunsaturated fatty acids (C ≥20, double bonds ≥3); MUFAs, monounsaturated fatty acids; PUFAs,

569 polyunsaturated fatty acids; n-6 PUFAs, omega-6 PUFAs; n-3 PUFAs, omega-3 PUFAs; RBC, red blood cell; SFAs, saturated fatty

570 acids. Omega-3 index: sum of EPA + DHA.

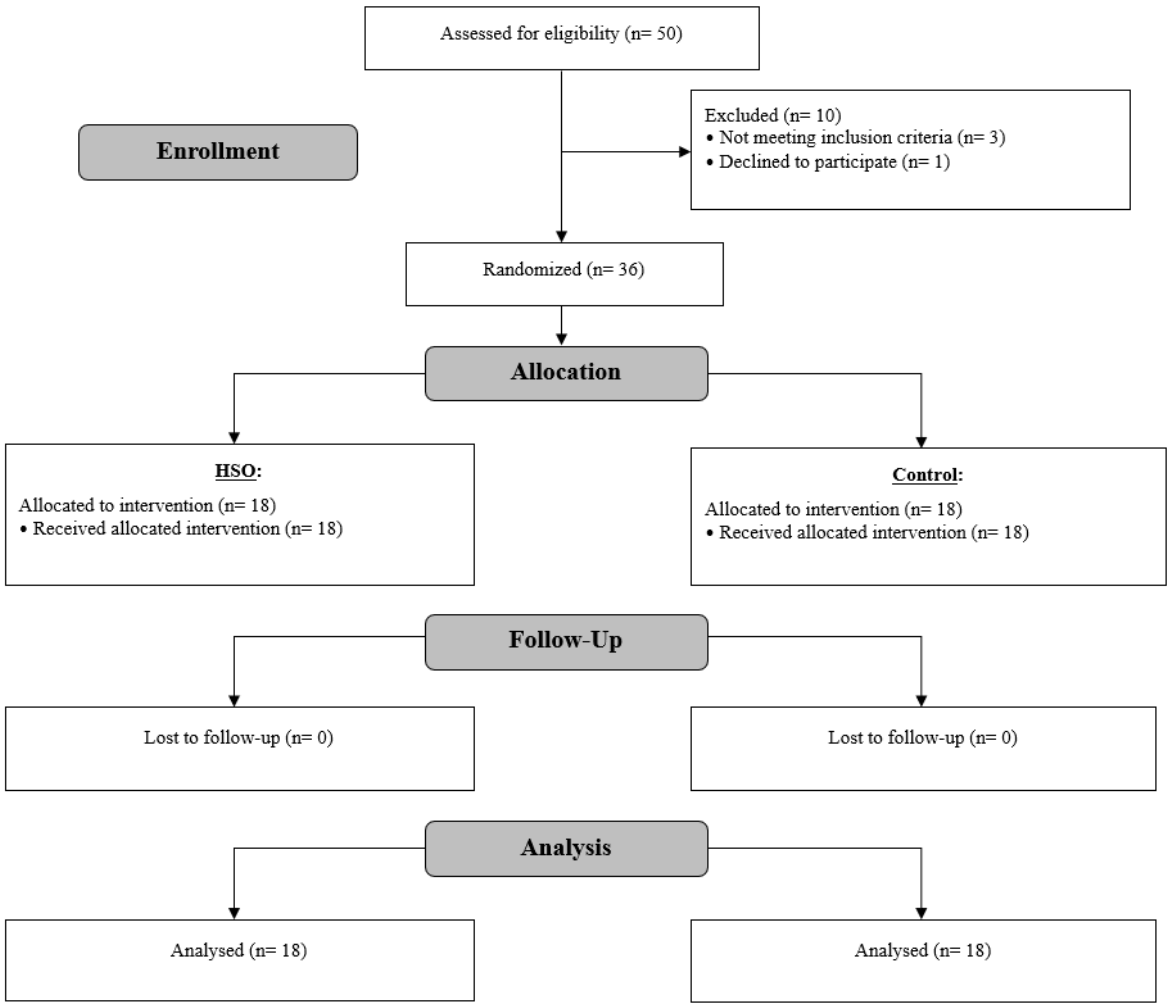
571 Values are expressed as changes (Δ) calculated considering post-treatment variations (calculated as differences between post- to

572 pre-treatment), with 95% confidence interval (CI min; max). *Data within the same row differ significantly ($p<0.05$).

573 **Figure 1-** CONSORT study flow-chart showing the process of patient selection and enrollment, allocation to the three

574 study groups, and rate of subjects completing the study.

575 HSO, group receiving hempseed oil treatment; Control, group receiving control treatment



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