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Hypolipidemic Effect of Blended Oil in Hamster: Biochemical Analysis and Gene Expression Profiling

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Abstract Edible oils form an essential part of the modern diet. Cardiovascular disease (CVD) has become one of the leading causes of death worldwide. Dietary supplementation with certain edible oils may play a vital role in improving cardiovascular health and reducing the mortality rate due to heart disease. Palm oil (PO), sunflower oil (SFO), soybean oil (SBO) and blended oils (consisting of olive oil (OLO) with SFO or SBO), were prepared (65-69% MUFA) to provide higher amounts of MUFA. Animal experiments were carried out to find the effects of olive oil blends, by feeding hamsters diets containing 8% of either native or blends of oils for 60 days. Serum cholesterol levels were reduced by 27% and 29%, respectively, in hamsters given blended oils containing OLO/SFO and OLO/SBO compared to PO. Fecal cholesterol and thiobarbituric acid did not show a significant change when hamsters were given blends in comparison with PO. Body and liver weights were also not significantly affected. These studies indicated that the atherogenic potentials of PUFA-rich SFO and SBO can be significantly decreased by blending with an oil rich in MUFA, such as OLO, in appropriate amounts. These results illustrate that blended oil significantly affects the gene expression of lipoprotein and fatty acid transporter in hamster hepatocytes. Dietary blended oil up-regulated the mRNA levels of lipin, while it down-regulated lipoprotein and cytochrome P450s. The effects of blended oil on the aforementioned genes, except lipoprotein, could be extrapolated towards decreased LDL oxidation. These findings suggest that dietary blended oil and PO alter the expression of different genes associated with contact of lipoprotein in liver.

Keywords: *lipid metabolism, gene expression, palm oil, olive oil, blended oil*

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1. Introduction

Research on the consumption of dietary fats and oils has become an important topic. High fat content, together with the type of fat in the diet, has been blamed for causing conditions such as obesity, insulin resistance and metabolic syndrome X. Therefore, life-threatening conditions such as stroke and acute myocardial infarction can be directly related to either the fat content or fat type in the diet [1,2,3,4]. Cardiovascular diseases (CVD) have been reported to be a major cause of death in several parts of the world, and coronary disease has been found to be particularly high.

Dietary fats have received considerable attention as modifiers of risk factors for CVD. Blood lipid levels are positively connected with risk for CVD. Lower levels of blood lipids and cholesterol are generally observed in subjects who consume diets containing either low fat or higher amounts of polyunsaturated fatty acids (PUFA) than subjects consuming saturated fatty acids [5]. Increased consumption of novel dietary oils rich in monounsaturated fatty acids (MUFA) and α-linolenic acid may improve the fatty acid imbalance typical of modern Western diets, high in saturated fatty acids (SFA) and the n-6:n-3 fatty acid ratio [6]. Olive oil (OLO) is rich in MUFA, low in SFA and exhibits a low n-6:n-3 ratio. With enhanced oxidative stability, OLO is an attractive oil replacement for high SFA–high trans oil varieties currently used in the food industry. Furthermore, recommendations have been made to increase dietary n-3 fatty acid intake [6]. Dyslipidaemia, specifically, elevated LDL-cholesterol, is a primary risk factor in predicting CVD events and a major target of dietary intervention [7]. Previous studies have shown that MUFA-rich oils, e.g. olive, rapeseed, or high oleic varieties of sunflower or safflower oil, are hypocholesterolemic, as are PUFA-rich oils such as soybean, sunflower or safflower oil [8]. Apart from their fatty acid content, oils also provide a variety of micronutrients that may have beneficial effects on, amongst others, the cardiovascular system. Thus, both the fatty acid component and micronutrient component of oil may influence cardiovascular health.

To date, many studies have been done on edible oils and their effects on cardiovascular health. The effect of blend oil consumption on established biomarkers and gene expression related to CVD risk has been investigated in a

previous animal study [8]. The reduction of both circulating LDL-cholesterol levels and modulating established biomarkers are important in ameliorating CVD risk. However, few of these studies compared the effects of blend oils with each other. Therefore, the objectives of this study were to evaluate the efficacy of a blend of olive oil and sunflower oil or olive oil and soybean oil in modulating circulating lipids metabolism and hepatic tissue gene expression associated with CVD risk as compared with palm oil.

2. Materials and Methods

2.1. Materials

Refined vegetable oils (Palm oil; PO, Olive oil; OLO, Sunflower oil; SFO, Soybean oil; SBO) were obtained from the local market (Carrefour, Taiwan). Two oil blends were prepared as follows: The first blend (blended oil 1; BO1) was a mixture of OLO/SFO (7:3, w/w); and the second (blended oil 2; BO2), OLO/ SBO (8.6:1.4, w/w). Standards used for sterols characterization were purchased from Supelco (Bellefonte, PA, USA). All other chemicals used were of analytical grade. Liquid chromatographic grade solvents and reagents were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Gas Chromatographic Analysis of Fatty Acid Methyl Esters

Fatty acids were transesterified into fatty acid methyl esters (FAMEs) using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany), according to the procedure reported by Arens et al. FAMEs were identified on a Thermo TRACE 1310 equipped with flame ionization detector (FID) (Texas, USA) [9]. The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1mL was injected on a 30 m × 0.25 mm × 0.2 mm film, Supelco SP-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250°C. The initial column temperature was 100°C, programmed by 5°C/min temperature rise until 175°C was reached; this temperature was maintained for 10 min at 175°C, then increased incrementally at 88°C/min until it reached 220°C, at which it was maintained for 10 min. A comparison between the retention times of the samples with those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

2.3. Animal Experiment

The experimental protocol was approved by The National Laboratory Animal Center (NLAC) (Taipei, Taiwan). Syrian golden male hamsters weighing 95 ± 5 g were housed two to a cage in an air-conditioned room (22 ± 2°C) on a 12 h light cycle (7:00 a.m. to 7:00 p.m.). The diet was AIN 76 diet (Purina Mills, St. Louis, MO, USA). Hamsters consumed their food and water ad libitum. These animals were maintained according to the guidelines established in the Taiwan Government Guide for the Care and Use of Laboratory Animals.

2.4. Experimental Diets

Oil blends were incorporated into the experimental diets. The full composition of the experimental diets was as follows: starch 42%, casein 20%, fat 8%, cellulose 5%, minerals mixture 3.5%, DL-Methionine 0.3%, vitamin mixture 1%, choline 0.2% and cholesterol 0.2%. Table 1 presents the composition of minerals and vitamins in the diet used in this study. All groups (1–5) were fed a hypercholesterolemic diet (0.2% cholesterol) supplemented with the two oil blends (Table 1). Diets and water were freely available over the 2-month period and hamsters were weighed every week. All groups were fed the basal diet for 10 days as an adaptation period, and then fed the experimental diet for the remainder of the two months. At the end of the experiment, the hamsters were decapitated after overnight fasting, blood samples were collected in heparinized tubes, and the plasma was separated by centrifugation and stored at -20°C until assayed for blood triglyceride, cholesterol, and thiobarbituric acid-reactive substances (TBARS). Hepatocytes were isolated from the livers.

Table 1. Nutrient composition of experimental diets[1].

Ingredient (%)	PO	SBO	SFO	BO1	BO2
Starch	42	42	42	42	42
Casein	20	20	20	20	20
Sucrose	20	20	20	20	20
Palm oil	8	0	0	0	0
Soy oil	0	8	0	0	0
Sunflower oil	0	0	8	0	0
Blend oil 1	0	0	0	8	0
Blend oil 2	0	0	0	0	8
Cellulose	5	5	5	5	5
Minerals	3.5	3.5	3.5	3.5	3.5
Vitamins	1	1	1	1	1
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2	0.2
Cholesterol	0.2	0.2	0.2	0.2	0.2
Calorie (Kcal)	73.8	73.8	73.8	73.8	73.8

[1]Based on AIN-76 formula.

2.5. Biochemical Determinations

Blood samples were centrifuged at 2000g for 10 min at 4°C. Then, the plasma was removed for the respective analytical determinations. Plasma triglycerides and cholesterol were determined by enzymatic photocolorimetric methods using commercial kits (E. Merck, Darmstadt, Germany). Lipid peroxidation products in plasma were determined by reaction with thiobarbituric acid, measuring TBARS as the marker [10]. TBARS were determined by the spectrophotometric method, using 1,1,3,3-tetramethoxypropane to establish the standard curve.

2.6. Low-Density Lipoprotein (LDL) Oxidation Assay

Blood from hamsters were collected in a centrifuge tube and centrifuged (2100g for 15 min) to obtain serum. The LDL fraction (density 1.020-1.055 g/mL) was isolated by density centrifugation in an ultracentrifuge using a 70 Ti rotor (Beckman, Palo Alto, CA) [11]. After centrifugation

the main lipoproteins (VLDL, LDL, and HDL) were separated from each other. Isolated LDL was dialyzed against 10 mM PBS overnight at 4°C in the dark, and purged with nitrogen before use. The cholesterol content of the isolated LDL samples was determined with the CHOD-PAP enzymatic test kit (Merck, Darmstadt, Germany) and diluted with PBS (5 mM) to give a final cholesterol concentration of 150 µg/mL [12]. The diluted LDL (100 µL) was incubated with 10 µL serum and 130 µL PBS (5 mM) in the presence of 10 µL of 125 µM CuSO4 (the final concentration was 5 µM). The kinetics of LDL oxidation in the initiation, propagation, and termination processes were obtained by monitoring the absorbance of conjugated diene formation at 232 nm with a multidetection microplate reader (Synergy HT, BIO-TEC, Atlanta, GA) at 15 min intervals at 30°C.

2.7. Affymetrics Genechip and Genespring Analyses

Liver RNA was extracted using a reagent (Trizol; Invitrogen Corp., Carlsbad, CA, USA) and then a kit (RNeasy Mini; Qiagen GmbH, Hilden, Germany). The purified RNA was quantified by measuring the optical density at 260 nm using a spectrophotometer (ND–1000; Nanodrop Technologies, Inc., Wilmington, DE, USA) and quality was confirmed using a bioanalyzer (Bioanalyzer 2100; Agilent Technologies, Inc., Santa Clara, CA, USA). Briefly, 0.5 µg of total RNA was amplified using a kit (Fluorescent Linear Amplification Kit; Agilent) and labeled with Cy3–CTP (CyDye, Perkin–Elmer, Fremont, CA, USA) during the in vitro transcription process. Two micrograms of Cy–labeled cRNA was fragmented to an average size of about 50–100 nucleotides by incubating it with fragmentation buffer at 60°C for 30 min. Fragmented labeled cRNA was then hybridized to an 8× 60–k microarray (Mouse Genome Oligo Microarray Kit; Agilent) at 60°C for 17 h, and then scanned at 535 nm (Microarray Scanner; Agilent) [13]. The scanned images were analyzed using commercial software (Feature Extraction 9.5.3; Agilent), as were the microarray data (GeneSpring GX 7.3.1; Agilent). Chip intensities of six microarrays were normalized to equal signal distribution using per-chip normalization at the 75th percentile. The resulting 42,305 genes probes were selected for differential expression; 1,190 differentially expressed genes were selected using 1.0–fold changes and the Significance Analysis of Microarray (SAM) method with a false–discovery rate (FDR) threshold of 0.05 [14].

2.8. Statistical Analysis

Serum of 8 hamsters was pooled before analysis. All samples were extracted in triplicate. Biochemical data were analyzed by ANOVA analysis of variance, and differences were considered statistically significant at $P<0.05$.

For gene expression studies, all samples were extracted and analyzed in triplicate. The scanning data were analyzed by Affymetrix software (GeneSpring GX), and the average value of each experiment was scaled to 100 in order to compare directly any of the experimental data. All scaled array data were exported as Microsoft Excel files and evaluated by using Student's t-test. Fold data were calculated from the average ratio of the signal values of blend oil 2 to those of the control group. A gene was considered to have been up-regulated if all signal values of blend oil 2 group were more than 50, the average increase was more than 2-fold, and the difference had a p-value < 0.05. A gene was considered to have been down-regulated if all signal values of the control group were more than 50, the average decrease was less than 0.5-fold, and the difference had a p-value <0.05. Data were analyzed by ANOVA and Tukey's analysis of variance, and differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Fatty Acid Composition of Dietary Lipids

Analysis of the lipids in the diets showed that PO-containing diets had 38.9% of saturated fatty acids; SFO, SBO, BO1 and BO2 diets had 16.0%, 9.7%, 13.4% and 15.1% of saturated fatty acids, respectively (Table 2). BO1 and BO2 were rich in MUFA and contained 63.8% and 67.19% of 18:1. One of the objectives of this study was to prepare oil blends having higher proportions of oleic acid (18:1 n-9). OLO, after blending with SFO and SBO, provided 18.1% and 15.2% of 18:2, respectively. The SFA:MUFA:PUFA ratio of PO is 1:1.2:0.3, that of SBO is 1:1.6:3.7, and that of SFO is 1:3.9:5.4, showing that none of the oils used in the study contain fatty acids in the proportions desired by nutritionists (high MUFA). When OLO was blended with SFO or SBO in selected amounts, the SFA:MUFA:PUFA ratios of the resulting blends were in the proportion of 1:4.9:1.6 for BO1 (OLO+SFO) and 1:4.6:1 for BO2 (OLO+SBO). These oil blends were then fed to hamsters for a period of 60 days.

Table 2. Fatty acid composition of experimental oils

	PO	SBO	SFO	BO1	BO2
C8:0	0.03	—	—	—	—
C10:0	0.04	—	—	—	—
C12:0	0.32	—	—	—	—
C14:0	1.05	0.07	0.06	0.02	—
C16:0	33.66	6.41	10.52	11.76	11.86
C16:1	0.23	0.1	0.06	0.71	0.72
C18:0	3.43	2.99	4.75	3.24	3.22
C18:1	47.32	37.08	24.22	63.8	67.19
C18:2	13.07	51.65	51.92	18.14	15.18
C18:3	0.17	0.26	6.14	1.35	1.24
C20:0	0.25	0.15	0.41	0.42	0.33
SFA (%)	38.8	9.6	15.7	15.3	15.1
MUFA (%)	47.6	37.2	24.3	65.0	67.9
PUFA (%)	13.3	51.9	52.6	19.5	16.4

3.2. Effect of Dietary Oils on Growth Parameters

The amounts of food consumed by hamsters in the different treatment groups were comparable. There was no significant change in the food efficiency ratio, measured by gain in body weight relative to the amount of food consumed. Finally, there were no significant changes in the weights of liver or fat pads in the hamsters fed the different diets (Table 3).

Table 3. Body weights, liver weights and epididymal fat pad weights from hamsters fed experimental diets[1]

Diet	Initial body weight (g)	Final body weight (g)	Liver weight (g)	Fat pads weight (g)	Diet consumption (g/day)
PO	97.9 ± 8.0	125.8 ± 12.6	55.5 ± 0.97	2.11 ± 0.37	4.61±0.52
SBO	98.6 ± 6.6	122.3 ± 6.6	4.88 ± 0.53	1.96 ± 0.40	4.59±0.62
SFO	97.6 ± 7.9	121.3 ± 8.3	4.97 ± 0.67	1.76 ± 0.35	4.68±0.30
BO1	95.0 ± 8.5	124.2 ± 119	4.47 ± 0.70	1.85 ± 0.55	4.84±0.62
BO2	96.8 ± 4.9	122.0 ± 10.7	4.77 ± 0.47	1.80 ± 0.43	4.75±0.53

[1] Means with the same letter in the same column are not significantly different. Each value represents mean ± SD; n= 10-12.

3.3. Fatty Acid Composition of Dietary Lipids

The type of fat consumed altered the cholesterol concentration in serum. Hamsters fed PO had a serum TC concentration of 241.6 mg/dL (Table 4), while those fed SFO and SBO had serum cholesterol concentrations of 190.4 and 205.6 mg/dL, respectively. Thus, 14.9 and 21.2% decrease in serum cholesterol concentration was observed in hamsters given SFO and SBO compared to those fed PO. Compared to those fed PO, LDL-C decreased by 21.2% in hamsters fed SFO and by 41.7% in hamsters fed BO2. Similarly, TG was decreased by 26.2% (SFO) and 39.9% (BO2). Endpoint LDL:HDL-cholesterol ratios were increased after the consumption of the SBO diet (3.45) and SFO diet (3.90) by 0.58% and 13.7%, respectively, compared with the PO (3.43). LDL: HDL-cholesterol ratios were also increased after the consumption of the BO1 diet (4.27) and BO1 diet (4.62) by 24.5 % and 34.7 %, respectively, compared with the PO (3.43).

3.4. Effect of Blended Oils on Lipid Parameters in Serum

Hamsters fed BO1 had a serum cholesterol concentration of 175.0 mg/dL, which was 27.6% lower than in hamsters given PO (Table 4). When BO2, with a balanced fatty acid composition, was fed to hamsters, the serum cholesterol was found to be 171.1 mg/dL, which was 30.8% lower than in hamsters fed PO. Serum LDL-C and TG concentrations were also lower in hamsters fed blended oils as compared to hamsters given PO. Specifically, serum LDL-C and TG concentrations were 35.0 and 31.6% lower in hamsters fed BO1, and 41.7 and 39.9% less in those given BO2, compared to the hamsters given PO (Table 4).

Table 4. Concentrations of serum lipids and ratio of HDL:LDL in hamsters fed various diets.

Diet[1]	Serum Triglycerol	Serum Cholesterol (mg/dL)	Serum LDL-C[2]	Serum HDL-C	HDL/LDL
PO	168.1 ± 44.0^{a}	241.6 ± 27.1^{a}	52.8 ± 9.3^{a}	168.3 ± 37.6^{a}	3.4 ± 0.7^{a}
SBO	141.5 ± 41.6^{ab}	205.6 ± 16.1^{b}	42.2 ± 7.9^{ab}	140.4 ± 27.8^{ab}	3.5 ± 0.8^{a}
SFO	124.1 ± 20.5^{bc}	190.4 ± 23.3^{bc}	38.3 ± 6.8^{b}	128.2 ± 31.4^{b}	3.9 ± 0.9^{ab}
BO1	114.9 ± 9.6^{bc}	175.0 ± 25.5^{c}	34.3 ± 5.5^{bc}	139.9 ± 20.7^{ab}	4.3 ± 1.2^{ab}
BO2	101.1 ± 19.2^{c}	171.1 ± 25.2^{c}	30.8 ± 7.1^{c}	135.7 ± 28.1^{b}	4.6 ± 1.6^{b}

[1] Means with the same letter in the same column are not significantly different. Each value represents mean ± SD; n= 10-12.
[2] LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.

3.5. LDL Oxidation and TBAR of Blended Oils

Two antioxidant activity assays were applied to blood samples from hamsters fed the experimental diets. The Cu2+-induced human LDL oxidation method was used to evaluate the inhibitory effect of blended oil on LDL oxidation in serum. Both blended oils and trolox prolonged the LDL oxidation lag phase as compared to LDL control (Figure 1). The ΔTlag values (defined as the difference in lag phase between the control and the sample) for LDL oxidation were 874.8 min for BO1 and 857.8 min for BO2, an increase of 81.9 and 78.3%, respectively, compared to PO (Table 5). On the basis of these results, it is evident that blended oils show enhanced antioxidant activity in vitro. The concentrations of TBARS were also analyzed in the plasma (Figure 2). TBARS levels in the blended oil samples were not significantly different than the PO.

Table 5. Mean lag phase in LDL oxidation in serum of hamsters on different experimental diets[1]

Groups	LDL lag time (min)[1]
Palm oil (PO)	481.0 ± 18.5^{a}
Soybean oil (SBO)	540.0 ± 27.1^{ab}
Sunflower oil (SFO)	575.5 ± 36.3^{b}
Blended oil 1 (BO1)	874.8 ± 19.5^{c}
Blended oil 2 (BO2)	857.8 ± 22.8^{c}

[1] Means with the same letter are not significantly different. Each value represents mean ± SD; n= 10-12.

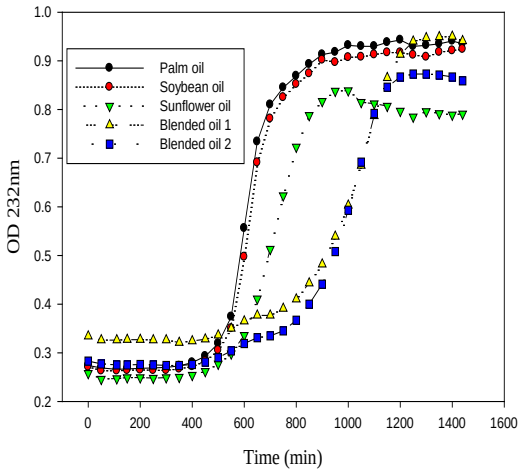

Figure 1. Time course of Cu^{2+}- induced oxidation of LDL in different groups of test hamsters

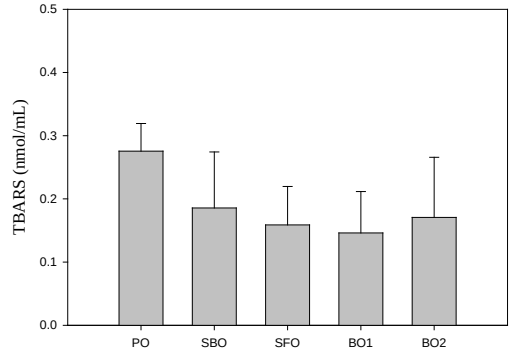

Figure 2. Thiobarbituric acid reactive substances (TBARS) concentrations in plasma of hamsters fed different experimental diets

3.6. Hepatic and Fecal Triglycerol and Cholesterol Levels

Hepatic and fecal triglycerol and cholesterol levels were measured at the end of each treatment phase (Table 6). Liver cholesterol concentrations were reduced when the subjects consumed the BO1 diet (42.9 mg/g) and the BO2 diet (44.4 mg/g) compared with the PO control (62.5 mg/g), a 31.4% and 29.0% reduction, respectively. Similarly, liver triglycerol concentrations were reduced after the consumption of the BO1 diet (7.2 mg/g) and the BO2 diet (7.5 mg/g) compared with the PO (13.11 mg/g), a 45.2% and 43.2% reduction, respectively. However, no significant differences were observed in endpoint or percentage change from PO in liver triglycerol concentrations between the SFO and SBO diets. In addition, no differences in fecal cholesterol concentrations were observed between the treatment groups (PO, SBO, and SFO). With respect to percentage change from baseline, no differences were observed for fecal TG concentrations between the treatment groups. Fecal triglycerol concentrations were induced after the consumption of the BO1 diet (11.7 mg/g) and the BO1 (13.1 mg/g) as compared with the PO diet (8.5 mg/g). BO1 induced fecal cholesterol concentrations by 37.0%, and BO2 by 52.8%, compared with the PO diet.

Table 6. Liver and fecal cholesterol and triglyceride contents in the different groups of test hamsters[1]

	Liver (mg/g)		Fecal (mg/g)	
	Cholesterol	Triglycerol	Cholesterol	Triglycerol
PO	62.5 ± 15.1^{a}	13.1 ± 0.7^{a}	22.8 ± 1.7^{a}	8.5 ± 0.4^{a}
SBO	59.2 ± 4.5^{a}	12.4 ± 1.7^{a}	20.3 ± 5.4^{a}	9.1 ± 1.4^{ab}
SFO	55.8 ± 7.6^{a}	10.1 ± 1.6^{b}	23.9 ± 3.2^{a}	9.7 ± 1.0^{bc}
BO1	42.9 ± 8.9^{b}	7.2 ± 1.3^{c}	25.4 ± 3.0^{a}	11.7 ± 0.4^{c}
BO2	44.4 ± 7.3^{b}	7.5 ± 1.2^{c}	25.6 ± 3.9^{a}	13.1 ± 1.1^{d}

[1]Means with the same letter in the same column are not significantly different. Each value represents mean ± SD; n= 10-12.

3.7. Gene Expression

A major objective of this study was to elucidate the manner in which blended oils regulate gene expression. We sought to determine whether the gene expression of transporters was affected specifically in the liver.

Using a DNA microarray, we observed that the refined oils induced changes in expression of genes for metabolic enzymes with various physiological activities. Both refined oils and blended oils caused a large alteration in the gene expression of CYP 450s, as well as the gene expression of various transporters such as SLC, APO and ABC transporters. Notably, dietary refined oils decreased the gene expression of many cytochrome P450 superfamilies of enzymes related to steroid metabolism and synthesis of cholesterol, steroids and other lipids. Dietary blended oils also profoundly affected lipid metabolism, up- or down-regulating more than 2-fold many genes related to lipid metabolism and synthesis of cholesterol, steroids and other lipids (Table 7). Many miscellaneous genes with diverse functions were also up– or down regulated by the blended oils (Table 7).

The composition of dietary fatty acid modified the mRNA levels of many genes encoding enzymes involved in lipid oxidation and transportation. The increases in the expression of various genes listed in Table 7 were stronger with blended oils than refined oil (PO). However, blended oils were equally effective in down-regulating other CYPs (CYP4A10, CYP51, CYP5R3), solute carrier (SLC25A25) and ATP binding cassette (ABCD3 and ABCC5) genes. Blended oil decreased to a similar degree the expression of APOB, APOA4 and CORT, involved in the degradation of cholesterol, steroids and lipids metabolism. In contrast, they increased the expression of FABP4 and LIPIN, which catalyze many reactions involved in lipid metabolism.

In summary, these results illustrate that blended oil significantly affects the gene expression of lipoprotein and fatty acid transporter in hamster hepatocytes. Dietary blended oil up-regulated the mRNA levels of lipin, while it down-regulated APOB, CORT, APOA4 and CYP51. The effects of blended oil on LDL-C and HDL-C were not counteracted by dietary refined oil. The effects of blended oil on the aforementioned genes, except lipoprotein, could be extrapolated towards decreased LDL oxidation. These findings suggest that dietary blended oil and PO alter the expression of different genes associated with contact of lipoprotein in liver.

Table 7. Microarray analyses of Mus musculus genes for lipid metabolism transporters whose expressions were differentially expressed by blended oil (BO2)

Gene Symbol	Genbank Accession	Gene name	Fold change
UCP1	NM_009463	uncoupling protein 1 nuclear gene encoding mitochondrial protein	12.12
FABP4	NM_024406	fatty acid binding protein 4, adipocyte	9.16
MEST	NM_008590	mesoderm specific transcript	4.26
LPIN1	NM_015763	lipin 1, transcript variant 2	3.33
CYP2A4	NM_009997	cytochrome P450, family 2, subfamily a, polypeptide 4	2.15
RARB	NM_011243	retinoic acid receptor, beta	2.05
APOB	NM_009693	Apob apolipoprotein B	-3.85
CYP51	NM_020010	cytochrome P450, family 51	-2.83
CROT	NM_023733	carnitine *O*-octanoyltransferase	-2.24
APOA4	NM_007468	apolipoprotein A-IV	-1.00
ABCD3	NM_008991	ATP-binding cassette, sub-family D	-1.56
ABCC5	NM_013790	ATP-binding cassette, sub-family C, member 5	-1.20
CYP4A10	NM_010011	cytochrome P450, family 4, subfamily a, polypeptide 10	-1.74
CYB5R3	NM_029787	cytochrome b5 reductase 3	-1.05
RAF1	NM_029780	v-raf-leukemia viral oncogene 1	-1.07
OCIAD2	NM_026950	OCIA domain containing 2	-1.06
HES1	NM_008235	hairy and enhancer of split 1	-1.56
SFRS1	NM_173374	splicing factor, arginine/serine-rich 1	-1.33
NR0B2	NM_011850	nuclear receptor subfamily 0, group B, member 2	-1.32
SLC25A25	NM_146118	solute carrier family 25, member 25	-1.08

4. Discussion

The present results are the first to demonstrate the lipid lowering efficacy of low-SFA and PUFA diets blended with OLO. Blended oils comprising olive oil (OLO) and sunflower oil (SFO) or soybean oil (SBO) with SFA:MUFA:PUFA ratio of 1:4.6:1 and PUFA:SFA ratio of 1:1.6 enriched with nutraceuticals were prepared. Compared with the PO control, we observed substantial decreases in TC and LDL-cholesterol for both the OLO+SFO (BO1) and the OLO+SBO (BO2) diets after 60 days. Our examination of the lipid-lowering action of PUFA-rich vs. MUFA-rich diets supports the notion that MUFA-rich diets reduce TC and LDL-cholesterol concentrations compared to PUFA-rich diets. Compared with the PO diet, the BO1 diet and BO2, both higher in dietary MUFA content, tended to reduce TG concentrations; however, due to large individual variation, while the differences compared to PO were significant, the differences of BO1 compared to SBO alone and BO2 compared to SFO alone were not significant. More detailed studies to parse the contribution of SBO or SFO versus the OLO on TG concentrations may be war rented.

The ability of blended oil (high MUFA) to reduce TC and LDL-cholesterol, as well as to preserve HDL-cholesterol, is of particular interest since to date the ability of blended oils to modulate blood lipids has not been assessed. Furthermore, it has previously been reported that not all MUFA-rich oils elicit the same effects on plasma cholesterol concentrations, suggesting the importance of other oil-derived fatty acid and non-lipid components [15,16]. Reports suggest that flaxseed oil or teaseed oil interventions fail to modify TC and LDL-cholesterol levels when compared with other dietary interventions [17,18]. However, these results could be confounded by the use of MUFA and n-6 PUFA dietary controls. Limited work has directly compared dietary OLO with MUFA-rich oils. In the present study, substitution of 70% olive oil in the blended oil treatment groups was effective in further reducing TC compared with the PO group.

The HDL:LDL ratio is valuable in evaluating CVD risk across many populations. As well, non-HDL-cholesterol provides a single measure of the atherogenic apo B containing lipoproteins and can thus provide a tool for cardiovascular risk assessment [19,20]. Generally, dietary strategies replacing SFA or PUFA with MUFA result in a reduction in plasma TC and LDL-cholesterol and a parallel decrease in plasma HDL-cholesterol concentrations. Previous studies administering high doses of high MUFA oil to hypercholesterolaemic subjects have observed reductions in HDL-cholesterol levels [21,22,23,24]. Notably, in our study, HDL:LDL ratios were increased in response to the blending diets compared with the PO control, an improvement in an important CVD risk factor. After the consumption of the blended oil diet, non-HDL-cholesterol levels decreased to a greater extent than the SBO, SFO diet and the PO control. Therefore, the additive effects of oleic acid in the blended oil diet may have provided additional hypolipidaemic effects that extend beyond those incurred by the SBO or SFO diet alone. In conclusion, the present study investigates effects of OLO blended oil on serum lipids and other markers of CVD risk. When SBO and SFO blended with olive oil effectively reduced serum TC and LDL cholesterol compared with a PO. Substitution of dietary fats common to the SBO and SFO with MUFA oil is a feasible option to target dietary recommendations and risk factors for CVD.

When SFO and SBO were used as a source of dietary fat, the serum lipid profiles were hardly affected in comparison with those fed PO. This may be due to the fact that the influence of MUFA may have been counteracted by the dominant presence of SFA and PUFA in the diet. However, when the 18:1 from the OLO was supplemented into SFO and SBO triglycerides, a significant hypolipidemic effect was observed. It can be deduced from the present study that BO1 showed a moderate change in cholesterol-lowering ability, but a significant reduction in TG level was observed. In the case of BO2, a significant reduction in serum cholesterol and TG was observed in comparison with animals given PO. SFO is MUFA- and PUFA-rich oil and, on its own, showed a good hypocholesterolemic effect, but when present in combination with OLO as a blend at a 1:2.3 ratio, it showed a marginal effect. However, soybean oil effectively lowered cholesterol when present with OLO at a 1:6 ratio. This indicates that SFO concentration may have to be increased further in the blends in order to achieve a cholesterol-lowering effect. Our studies have shown that the serum lipoprotein composition in animals has improved upon feeding the blends of oils containing OLO with SFO or SBO, in comparison with animals given native PO. Thus, the atherogenic potential of PO can be reduced by blending it with SFO or SBO, which contribute to improving lipoprotein composition when consumed as a blend.

Essential fatty acids (EFA), which are not made by the body, must be obtained through food. Two essential fatty acids, linoleic and alpha-linolenic, are required for normal body function. These two essential fatty acids are found principally in oil seeds, such as soybeans and sunflower oil [25]. In our present study, the lipoprotein compositions of the serum have shown an increase in EFA status for the groups fed with blends of BO1 and BO2 in comparison with native PO, which indicates that these oil blends may help to overcome EFA deficiency. Moreover, the feasibility of incorporating OLO, SFO and SBO into typical diets requires further consideration. In order to maintain total fat energy intake, it is crucial to target fat substitution v. fat supplementation of the diet. Therefore, increased compliance with dietary recommendations and targeting a reduction in CHD risk would be possible by replacing a proportion of commonly used dietary oils and spreads with SFO and SBO alone or blended with olive oil.

n-3 and n-6 fatty acids may inhibit natural ligand binding of oxysterols to nuclear receptor LXR, resulting in a reduction of SREBP-1c transcription. This effect can be eliminated by deletion or mutation of the LXR regulated promoter region of SREBP-1c [26]. However, fish oil fed rats showed a suppression of hepatic SREBP-1c target genes, but no change in expression of genes directly regulated by LXR such as CYP 450 and ATP1CYP7A1, ABCG5, or ABCG8 [27]. In addition, hepatocytes treated with eicosapentaenoic acid inhibited SREBP-1c controlled genes both in the absence and the presence of a synthetic LXR agonist. The inhibition of LXR may be an indirect effect of PUFA stimulation of PPAR transcription factors. Stimulation of FXR enhances the expression of a short heterodimer protein, which has a negative feedback effect

on LXR activity [28]. This study was to investigate the effects of MUFA/PUFAs on the expression of lipid metabolism-related genes to unveil potential genes and pathways affecting lipid metabolism.

5. Conclusions

Many kinds of nutrient and cholesterol transporters in the liver have been identified as uptake and efflux transport systems. Various transporters have been classified as ATP-binding cassette (ABC) transporters or solute carriers (SLCs) based on sequence similarity by the Mouse Gene Nomenclature Committee.

Recently, novel dietary oils with modified fatty acid profiles have been manufactured to improve fatty acid intakes and reduce CVD risk. Our objective was to evaluate the efficacy of novel palm oil (PO), sunflower oil (SFO), soybean oil (SBO) or blended with olive oil (BO1 or BO2), on modulating lipid metabolism and thus CVD risk factors. In conclusion, blended oils (BO1 and BO2) are cardioprotective through lipid-lowering effects, and may also induce hypolipidemia by reducing plasma cholesterol. This study has identified metabolic pathways and key regulators that may respond differently to blended oil depending on mentalism transcription factors and efflux transport system. Further studies are required, but the data suggest that it may be possible, through future genetic selection programs, to identify families better adapted to alternative diet formulations.

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