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Effect of dietary oils from various sources on carbohydrate and fat metabolism in mice

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Abstract

Background: Dietary oils differ in their fatty acid composition and the presence of additional microcomponents (antioxidants, etc.). These differences are thought to invoke different biochemical pathways, thus affecting fats and carbohydrates metabolism differently. Olive oil (OO) and soybean oil (SO) are common vegetable oils in the local cuisine. Peanuts oils of local varieties are viewed as potential sources of dietary vegetable oils, especially in the food industry.

Objective: We examined the effect of four different dietary vegetable oils on carbohydrate and lipid metabolism in mice. The selected oils were OO, high in oleic acid, extracted from cultivated high oleic acid peanut (C-PO), regular peanut oil (PO), and SO.

Design: In this study, 32 male C57BL/6J mice were randomly divided into four groups (n = 8 in each group) and were fed with four different diets enriched with 4% (w/w) dietary vegetable oils (OO, C-PO, PO, or SO). After 10 weeks, the mice were sacrificed. Western blot was used to examine proteins such as phospho-AMP-activated protein kinase (p-AMPK), acetyl-CoA carboxylase (ACC), cluster of differentiation 36 (CD36), and Sirtuin 1 (SIRT1), whereas real-time polymerase chain reaction (PCR) was used to examine the expression of sterol regulatory element-binding protein-1c (SREBP-1C), fatty acid synthase (FAS), glucose-6-phosphatase (G6Pase), and CD36 transcripts.

Results: In mice-fed SO, lipid accumulation was predominately in adipose tissue, accompanied a tendency decrease in insulin sensitivity. Mice-fed OO had lower plasma triglycerides (TG) and increased hepatic CD36 gene expression. The C-PO group presented lower messenger RNA (mRNA) levels in the liver for all examined genes: SREBP-1c, FAS, G6Pase, and CD36. There were no significant differences in weight gain, plasma cholesterol and high-density lipoprotein (HDL) cholesterol levels, hepatic ACC, SIRT1, AMPK, and CD36 protein levels or in liver function among the diets.

Discussion: It seems that as long as fat is consumed in moderation, oil types may play a lesser role in the metabolism of healthy individuals.

Conclusion: This finding has the potential to increase flexibility in choosing oil types for consumption.

Keywords: oleic acid; soybean oil; gene expression; peanut oil; D7 oil; triglycerides
resistance, non-alcohol fatty liver disease (NAFLD), and so on (2–5). Fat-rich diets not only lead to NAFLD but can also impair carbohydrates metabolism, manifesting in impaired glucose tolerance curves (6, 7), alterations in gut microbiota (8), and disrupt molecular systems responsible for signaling to the liver (9).

Studies suggest that the quality of fats, more than the quantity, has crucial effects on health (10, 11). Saturated fat intake is associated with increased cholesterol levels, changes in lipid profiles, and increased liver weight. In contrast, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) have been shown to positively impact plasma cholesterol levels in rats (2). Consumption of saturated fats can also lead to glucose intolerance in mice, whereas switching to a diet rich in MUFAs improves glucose tolerance (12).

Olive oil (OO), which is rich in MUFA (oleic acid), is considered to be beneficial for health (13). High-MUFA diets have been found to lower both plasma cholesterol and triacylglycerol concentrations (14). Significant associations between higher intakes of OO and reduced risk of all-cause mortality, cardiovascular events, and stroke have been documented in a systematic review and meta-analysis of cohort studies (15). Recently, the FDA recommended consuming oils with high levels of oleic acid to reduce cardiovascular disease (CVD) risk (16).

Risk factors associated with CVD development, such as dyslipidemia, impaired vascular function, and hypertension, can be improved with regular tree nut and peanut consumption (17), and therefore cultivating peanut varieties rich in oleic acid has become popular. The high concentration of oleic acid increases oil stability and decreases oxidation rates (18). In addition, the unique fatty acid profiles of these peanut cultivars are very similar to those of OO. This suggests that they might have similar effects on health. Peanut oils (PO), and especially refined POs, have been found to be safe for use, even among people who are allergic to peanuts (19). All of the above implies that there is potential for POs rich in oleic acid, to positively impact health.

Different types of oils can uniquely impact gene and protein expression in the liver, especially those related to carbohydrate and fat metabolism. Analysis of key genes and proteins can provide a good indication of the active biochemical pathways in the body. For example, sterol regulatory element-binding protein-1c (SREBP-1c) is a key regulatory transcription factor in fatty acid synthesis. It increases gene expression that promotes fatty acid synthesis, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (20). AMP-activated protein kinase (AMPK) is another important enzyme. It plays a role in cellular energy homeostasis (21). Activation of AMPK promotes catabolic pathways to generate more ATP (22). Other key metabolic genes and proteins are Sirtuin 1 (SIRT1), cluster of differentiation 36 (CD36), and glucose-6-phosphatase (G6Pase) (23–26).

We assumed that different types of oils, having different fatty acid profiles, would have varied metabolic effects on the body. The study aims to investigate the effects of different oils with unique fatty acid compositions on liver fatty acid metabolism in mice models. For this research we selected four different oils: OO and soybean oil (SO), which are common vegetable oils in the local cuisine and two peanuts oils of local varieties, which are viewed as potential sources of dietary vegetable oils, especially in the food industry; D7 oil – cultivated high oleic acid peanut oil (C-PO) and Hanoch oil – conventional Israeli PO.

**Materials and methods**

**Experimental animals, diets, and sample collection**

All experiments were performed within the Hebrew University of Jerusalem’s guidelines of the Authority for Biological and Biomedical Models and were approved by its Institutional Animal Care Ethics Committee. Male C57 BL/6 J mice, 6–7 weeks old, were purchased from Harlan Laboratories (Jerusalem, Israel). Thirty-two mice were randomly assigned to one of four groups that were fed a C-PO diet (extract from cultivation of Israeli peanut variety Einat, enriched with oleic acid, D7), PO diet (extract from cultivation of typical Israeli peanut variety, Hanoch), OO diet, or SO diet. All diets were based on the AIN-93 M diet, with a few modifications (Table 1). Main change was substitution of the SO used in standard AIN-93 M diet (27) with one of the other oils. None of the new chows was set as a control diet. Our goal was aimed to compare the effect of different oils rather than to compare the effect of the oils used in this study to normal oil (control oil).

Hanoch oil (PO) and D7 oil (C-PO) were extracted from peanuts of these cultivars by a cold press method using a KOMET Twin Screw Vegetable Expeller DD 8G device in the Dr. Ran Hovav Lab. SO and OO were purchased from the local supermarket. The chow for the four diets was prepared in our laboratory and was identical, except for the added oil. The mice were housed in a controlled environment (12/12 h light/dark cycle, 22–24°C) with ad libitum access to food and water. After 9 weeks on the diets, the mice fasted for 12 h and were subjected to an oral glucose tolerance test (OGTT). Before the OGTT, the mice were weighed and marked and were given d-glucose (3 g/kg body weight) by gavage. Glucose levels were monitored at 0, 30, 60, and 120 min after glucose loading. A glucometer-ACCU-CHEX (ROCH) was used to measure glucose levels in blood drawn from the tail tip. After 10 weeks on the experimental diets, the mice fasted for 12 h, their body weights were recorded, and they were sacrificed in random order by an isoflurane overdose. Blood was collected from the vena cava, and plasma was...
Obtained by centrifugation at 5600 × g at 4°C for 10 min, and stored at −20°C. Epididymal adipose tissue was removed, weighed, placed in liquid nitrogen, and stored at −75°C. Liver tissue was collected, weighed, minced in liquid nitrogen, and stored at −75°C.

**Lipid profiles**

Oils lipid profiles were determined by gas chromatography method. Total lipids were extracted from oils samples using a protocol adapted from the cold extraction procedure developed by Folch (28). For gas chromatography (GS) analysis, fatty acid methyl esters were generated. Lipid analysis was performed with a gas chromatograph (Agilent Technologies CA, USA) equipped with a fused-silica capillary column. Helium was used as the carrier gas. A known amount of C17:0 was added to the samples prior to extraction to determine the fatty acid concentrations. Peak identification was based on relative retention times of the standard.

**Blood parameters**

Analyses of plasma lipid profiles and the liver enzymes were performed by American Laboratories (Herzliya, Israel). Plasma samples were obtained by centrifugation of the blood as described above. In vitro test for the quantitative determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, cholesterol, and triglycerides (TG) in plasma were performed using Roche/Hitachi cobas c systems.

**Liver lipid content**

The liver lipid content was determined using the Folch (29) method for lipid extraction. A small fraction of liver tissue (~100 mg) was homogenized with a chloroform/methanol solution. Later, the samples were centrifuged at 800 × g for 10 min until phase separation was achieved. The upper, transparent phase was collected, and another phase separation was performed. The bottom lipid phase was separated, dried, and weighed.

**Plasma insulin levels**

Insulin plasma levels were determined using the Rat/Mouse Insulin ELISA Kit, Cat#EZRMI-13K (Merck Millipore, Darmstadt, Germany), according to the manufacturer’s protocol. Total insulin amounts were calculated using the manufacturer’s standard curve.

**RNA extraction and reverse transcription polymerase chain reaction analysis**

Total RNA was isolated from liver tissue by using the Tri-Reagent (Sigma-Aldrich, Rehovot, Israel), according to the manufacturer’s protocol. Complementary DNA was prepared with the qScript cDNA synthesis kit (Quanta BioSciences, Gaithersburg, MD, USA). Real-time polymerase chain reaction (RT-PCR) was performed with the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), with specific primers (Table 2). Quantitative changes in gene expression were

| Table 1. Diet composition of the four mice groups |
|------------------------------------------------|
| **Composition** | **Diets** | **Diets** | **Diets** | **Diets** |
|                | **C-PO**  | **PO**    | **OO**    | **SO**    |
|                | (kcal)    | (kcal)    | (kcal)    | (kcal)    |
|                | (g)       | (g)       | (g)       | (g)       |
| Casein         | 56        | 14        | 56        | 14        | 56        | 14        | 56        | 14        |
| l-methionine   | 0.72      | 0.18      | 0.72      | 0.18      | 0.72      | 0.18      | 0.72      | 0.18      |
| Cornstarch     | 198.28    | 49.57     | 198.28    | 49.57     | 198.28    | 49.57     | 198.28    | 49.57     |
| Maltodextrin 10| 50        | 12.5      | 50        | 12.5      | 50        | 12.5      | 50        | 12.5      |
| Sucrose        | 40        | 10        | 40        | 10        | 40        | 10        | 40        | 10        |
| Cellulose      | -         | 5         | -         | 5         | -         | 5         | -         | 5         |
| D7 oil         | 36        | 4         | -         | -         | -         | -         | -         | -         |
| Hanoch oil     | -         | -         | 36        | 4         | -         | -         | -         | -         |
| Olive oil      | -         | -         | -         | -         | 36        | 4         | -         | -         |
| Soybean oil    | -         | -         | -         | -         | -         | -         | 36        | 4         |
| Mineral mixAIN-76| -      | 3.5       | -         | 3.5       | -         | 3.5       | -         | 3.5       |
| Vitamin mixAIL-93-VX | - | 1       | -         | 1         | -         | 1         | -         | 1         |
| Choline chloride| -       | 0.25      | -         | 0.25      | -         | 0.25      | -         | 0.25      |
| BHT            | -         | 0.014     | -         | 0.014     | -         | 0.014     | -         | 0.014     |
| Total          | 381       | 100       | 381       | 100       | 381       | 100       | 381       | 100       |

BHT: butylated hydroxytoluene; C-PO, cultivated high oleic acid peanut oil; PO, peanut oil; SO, soybean oil; OO, olive oil.

Thirty-two mice were randomly assigned to one of four groups that were fed a C-PO diet, PO diet, OO diet, or SO diet. PO and C-PO were extracted from peanuts of these cultivars. All diets were based on the AIN-93 M diet with a few modifications.
Table 2. List of primers used in RT-PCR

| Name          | Reverse                  | Forward                  |
|---------------|--------------------------|--------------------------|
| β-actin       | 5’-GGGTTGTGAAGTGTCAAA-3’ | 5’-CTAAGGCAACCGTGAAAAG-3’ |
| CD36          | 5’-AAGGCGATGGCCTGAAAGA-3’ | 5’-TCTCCTGACATTGGCAGGCT-3’ |
| FAS           | 5’-GGGTGGTTCCATTAATCTCAT-3’ | 5’-CTAGAAACTTTCCAGAAATTTCC-3’ |
| G6pase        | 5’-AAGAGATGCAGAGGACCA-3’  | 5’-ACTCCAGCATGTACCGGAAG-3’ |
| SREBP-1c      | 5’-GATAGGGTGGCCTGAGTG-3’  | 5’-GATCAAAGAGGAGCCAGTG-3’ |

FAS, fatty acid synthase.

determined by normalizing against β-actin and by using the delta-delta-Ct (ddCt) algorithm (30).

**Protein extraction and western blotting**

Total liver tissue protein was extracted using a lysis buffer containing: 20 mM Tris-HCl (pH 7.4), 145 M NaCl, 10% glycerol, 5 mM EDTA, 1% Triton X-100, 0.5% NP-40, 100 mM phenylmethylsulfonyl fluoride (PMSF), 200 mM NaN3, 5 mM NaF, and 1% protease inhibitor cocktail. Lysates were centrifuged at 8500 × g for 15 min at 4°C, and the protein concentration was determined by the Bradford method with bovine serum albumin used as a standard. The samples were subjected to sodium dodecyl sulfate polyacrylamide gel (12%) electrophoresis (SDS-PAGE), after which proteins were transferred onto nitrocellulose membranes. Blots were then incubated with primary antibodies: anti-rabbit adenosine monophosphate-activated protein kinase (AMPK), antirabbit phosphorylated adenosine monophosphate-activated protein kinase (pAMPK), antirabbit acetyl-CoA carboxylase (ACC), antirabbit phosphorylated acetyl-CoA carboxylase (pACC) (Cell Signaling Technology, Beverly, MA, USA), antirabbit monoclonal CD36, antirabbit polyclonal Sirtuin 1 (SIRT1) (Abcam, Cambridge, UK), and antimouse β-actin (BD Biosciences, San Jose, CA, USA) and then, after several washes, with secondary goat antirabbit antibodies (Jackson Immuno Research Laboratories, West Grove, PA, USA). The immune reaction was detected by enhanced chemiluminescence, with bands being quantified by densitometry and expressed as arbitrary units.

**Statistical analyses**

Values are presented as means ± SE. Analysis of variance (one-way ANOVA) and the Tukey–Kramer honest significant difference post hoc test were used to compare means. When comparing significant differences between only two groups, a Student’s t-test was applied. All data were tested for normal distribution prior to applying any statistical test. The significance level was $P < 0.05$ for all analyses, unless otherwise specified. Connecting letters report was used to display the results of the Tukey–Kramer test ($P > 0.05$). Results that share, or are connected by, the same letter do not differ statistically. Results that are not connected by a common letter do differ statistically. Notations of double letters, like ‘a/b’, stand for two levels of significance ‘a’ and ‘b’. Asterisks were used for significant differences in the Student’s t-test. There is no special meaning for number of asterisks used. JMP 14 Pro software (SAS Institute, Cary, NC, USA) was used for the statistical analyses.

**Results**

**Oil lipid profiles**

The fatty acid compositions of different oils used in this study are presented in Table 3. Both the C-PO and OOs were rich in MUFA (mainly oleic acid), 77.9 and 82.8%, respectively. The SO was rich in PUFA (mainly linoleic acid, $ω6$), 55.6%. The linoleic acid/ω6-linolenic ratio ($ω6/ω3$) ratio was lowest in OO (6.8%) and highest in the PO (361%). It should be noted that although both C-PO and OO have similar high oleic acid content, the $ω6/ω3$ ratio in C-PO is about 6.5 higher than in OO.

**Bodyweight and food consumption**

Weight gain was similar throughout the experiment (Fig. 1A). Similar food intake was observed among all groups of mice regardless of diet composition (Fig. 1B). No statistically significant differences were found among the groups.

**Oral glucose tolerance test**

There were no significant differences in glucose levels among the mice fed different diets at baseline and the endpoint of the OGTT. However, intergroup differences were observed at 30 and 60 min. Glucose clearance in the OO group was fastest, while mice-fed SO had the slowest clearance rates (Fig. 2C, D). Also, a tendency (trend) for higher area under the curve (AUC) of OGTT was observed for the SO diet group (23,050 ± 641) when compared to the AUC of the OO diet group (20,991 ± 732) or to the PO group (20,882 ± 387) using the Student’s t-test ($P < 0.07$). This implies slower glucose clearance in the SO diet group compared to the PO and OO diet groups.

**Bodyweight, adipose, and liver tissues weight**

By the end of the experiment, body weight, fasting glucose levels, and liver tissue weight were similar among the groups. However, adipose tissue weight varied between the diets ($P < 0.05$), 0.48 ± 0.03 for the C-PO group and 0.78 ± 0.09 for the SO group (Table 4).
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Liver and adipose tissue weight to body weight ratio

The SO diet group had significantly lower liver tissue weight-to-body weight ratio (3.70 ± 0.05) (Fig. 3A) and the highest adipose tissue weight-to-body weight ratio (3.29 ± 0.45) (Fig. 3B), \( P < 0.05 \), while the C-PO diet group had the highest liver tissue weight-to-body weight ratio (4.04 ± 0.07) (Fig. 3A) and the lowest adipose tissue weight-to-body weight ratio (2.04 ± 0.10) (Fig. 3B), \( P < 0.05 \).

Lipid accumulation in the liver

Lipid accumulation in the liver tissue, also known as ectopic lipid accumulation (Fig. 4) was lowest in the SO diet

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**Table 3.** Fatty acid composition of the four oils used*

| Fatty acids                      | % Area |
|---------------------------------|--------|
| C-PO                            | PO     | OO    | SO    |
| Pentadecylic acid (C15:0)       | 0.000  | 0.000 | 0.000 | 0.017 |
| Palmitic acid (C16:0)           | 5.682  | 10.236| 10.481| 10.670|
| Palmitoleic acid (C16:1 cis-9)  | 0.079  | 0.050 | 0.750 | 0.078 |
| Margaric acid (C17:0)           | 0.085  | 0.096 | 0.052 | 0.091 |
| Stearic acid (C18:0)            | 2.491  | 3.456 | 3.079 | 4.334 |
| Oleic acid (C18:1 cis-9)        | 81.111 | 49.198| 76.931| 25.210|
| Vaccenic acid (C18:1 trans-11)  | 0.501  | 0.416 | 2.008 | 1.413 |
| Linoleic acid (C18:2 cis-9,12)  | 3.056  | 29.778| 4.312 | 50.509|
| Linolealid acid (C18:2 trans-9,12) | 0.000  | 0.000 | 0.000 | 0.070 |
| \( \alpha \)-Linolenic acid (C18:3 cis-9,12,15) | 0.069  | 0.082 | 0.631 | 4.974 |
| Arachidic acid (C20:0)          | 1.211  | 1.514 | 0.428 | 0.533 |
| Paullinic acid (C20:1 cis-11)   | 1.499  | 0.897 | 0.264 | 0.259 |
| Eicosadienoic acid (C20:2 cis-11,14) | 0.000  | 0.018 | 0.000 | 0.043 |
| Mead acid (C20:3 cis-8,11,14)   | 0.020  | 0.019 | 0.018 | 0.037 |
| Behenic acid (C22:0)            | 2.239  | 2.365 | 0.128 | 0.563 |
| Erucic acid (C22:1 cis-13)      | 0.114  | 0.049 | 0.000 | 0.000 |
| Tricosyl acid (C23:0)           | 0.039  | 0.037 | 0.025 | 0.062 |
| Lignoceric acid (C24:0)         | 1.353  | 1.325 | 0.058 | 0.200 |
| Squalene                        | 0.043  | 0.033 | 0.629 | 0.000 |
| Linoleic acid/\( \alpha \)-Linolenic ratio (\( \omega \)6/\( \omega \)3) | 44.553 | 361.178| 6.835 | 10.155|
| Total saturated fat              | 13.099 | 19.028| 14.252| 16.469|
| Total trans fat                  | 0.501  | 0.416 | 2.008 | 1.483 |
| Total MUFA                       | 82.803 | 50.194| 77.945| 25.547|
| Total PUFA                       | 3.145  | 29.897| 4.961 | 55.563|

C-PO, cultivated high oleic acid peanut oil; PO, peanut oil; SO, soybean oil; OO, olive oil; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*Profiles of the four oils were determined by GC using C17:0 as a standard.

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**Fig. 1.** Effects of various oils on bodyweight and food consumption. Male C57 BL/6 J mice aged 6–7 weeks were fed a cultivated high oleic acid peanut oil (C-PO), peanut oil (PO), olive oil (OO), or soybean oil (SO) diet for 10 weeks. (A) Bodyweight was measured weekly for 10 weeks. (B) Average food consumption was measured during a 12-day period. All values are means ± standard error of the means (SEM), \( n = 8 \).
Effect of the oils on serum lipids and liver biomarkers

Serum lipids and liver biomarkers are shown in Table 5. There was no significant difference in the AST levels between the diets, and there was no correlation between ALT and alkaline phosphate levels, although significant intergroup differences were found. In addition, plasma lipid profiles showed a similarity in total cholesterol and high-density lipoprotein (HDL) cholesterol levels among all the diet groups. However, TG levels were lowest (64.67 ± 3.47 mg/dL) in the OO group (P < 0.05).
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Effect of the oils on mRNA gene expression involved in carbohydrate and lipid metabolism

mRNA levels of SREBP-1c, FAS, G6Pase, and CD36 genes were significantly lower for the C-PO diet group. In addition, SREBP-1c and CD36 mRNA levels were significantly higher in the OO diet group (Fig. 5).

Table 4. Final body weight, tissue weight and fasting glucose*

|                  | C-PO     | PO       | OO       | SO       |
|------------------|----------|----------|----------|----------|
| Final body weight (g) | 23.1 ± 0.4 | 23.4 ± 0.5 | 24.4 ± 0.6 | 23.7 ± 0.4 |
| Fasting glucose level (mg/dL) | 162.0 ± 9.6 | 159.5 ± 10.8 | 153.6 ± 7.4 | 138.6 ± 8.8 |
| Adipose tissue (g) | 0.48 ± 0.03b | 0.54 ± 0.04ab | 0.56 ± 0.08ab | 0.78 ± 0.09ab |
| Liver weight (g)  | 0.92 ± 0.02 | 0.90 ± 0.03 | 0.97 ± 0.04 | 0.88 ± 0.02 |

C-PO, cultivated high oleic acid peanut oil; PO, peanut oil; SO, soybean oil; OO, olive oil.
*Male C57BL/6J mice aged 6–7 weeks were fed a C-PO, PO, OO, or SO diet for 10 weeks. All values are means ± SEM, n = 6–8. Data marked with different letters (a, b) are significantly different (P < 0.05).

Fig. 3. Effect of diets on liver and adipose tissue weight-to-total body weight ratio. Male C57BL/6J mice aged 6–7 weeks were fed either a cultivated high oleic acid peanut oil (C-PO), peanut oil (PO), olive oil (OO), or soybean oil (SO) diet for 10 weeks. (A) Liver weight to body weight ratio. (B) Adipose tissue to body weight ratio. All values are means ± SEM, n = 7–8. Data marked with different letters (a, b) are significantly different (P < 0.05).

Fig. 4. Effect of diets on lipid accumulation in the liver. Male C57BL/6J mice aged 6–7 weeks were fed a cultivated high oleic acid peanut oil (C-PO), peanut oil (PO), olive oil (OO), or soybean oil (SO) diet for 10 weeks. The lipid content was determined using the Folch lipid extraction method from 100 mg liver tissue. All values are means ± SEM, n = 7–8. Data marked with different letters (a, b) are significantly different (P < 0.05).

Effect of different oils on protein expression levels relevant to carbohydrate and lipid metabolism

The p-ACC/ACC ratio and protein levels of SIRT1 and CD36 showed no significant differences between the groups. The p-AMPK/AMPK ratio showed no significant differences using a Tukey’s Kramer test. However, there was a significant difference (P < 0.05) between the C-PO diet and the other diets when analyzed using the Student’s t-test.

Discussion

Metabolic syndrome is a cluster of conditions that occur together, increasing risk of heart disease, stroke, and type 2 diabetes. These conditions include increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or TG levels.

This study aimed to evaluate the effect of different oils with different fatty acid profiles on specific metabolic responses in the metabolism of carbohydrates and fats in mice for better understanding effect of dietary oils on metabolic syndrome. The specific oils studied were the sole source of fat in the diet and the only modified variable among the diets.

Linoleic acid and α-linolenic acid are precursors for essential fatty acids ω6 and ω3 correspondingly. Lipid analysis showed that the linoleic acid to α-linolenic acid ratio was 44.2% for C-PO (peanuts with high oleic content),
It has been reported that high ω6-to-ω3 ratio promotes the pathogenesis of many deceases, including CVDs (31). In contrast, increasing ω3 levels and decreasing ω6-to-ω3 ratios are beneficial in the prevention and treatment of coronary artery disease (31). The ω6-to-ω3 ratio was 6.5 times higher for the C-PO compared to OO. This may be the reason for the differences between the oils and their effect on metabolism. It should be noted that both C-PO and OOs have overall similar lipid profiles and both contain high concentrations of oleic acid.

In this study, all the mice had similar food consumption levels, and weight gain was similar for all groups. However, compared to the other three diets, SO led to higher adipose tissue weight gain, higher adipose tissue weight-to-body weight ratio, and lower liver tissue weight-to-body weight ratio.

Table 5. Effect of the diets on serum lipid profiles and liver biomarkers

|                      | C-PO        | PO          | OO          | SO           |
|----------------------|-------------|-------------|-------------|--------------|
| Triglycerides (mg/dL)| 82.67 ± 6.01b | 89.80 ± 4.02a | 64.67 ± 3.47ab | 90.80 ± 9.01a |
| Cholesterol (mg/dL)  | 121.60 ± 6.21 | 117.33 ± 6.43 | 118.67 ± 5.81 | 115.40 ± 2.25 |
| HDL cholesterol (mg/dL)| 106.22 ± 4.05 | 110.00 ± 4.77 | 110.45 ± 5.06 | 109.40 ± 3.37 |
| Alk Phos (U/L)       | 100.20 ± 2.96c | 82.17 ± 2.48b | 86.00 ± 4.28b | 77.60 ± 3.44b |
| AST (U/L)            | 52.80 ± 7.56 | 53.00 ± 3.00 | 49.33 ± 6.46 | 44.60 ± 2.77 |
| ALT (U/L)            | 16.40 ± 2.62a | 24.33 ± 2.36b | 17.20 ± 0.58ab | 16.00 ± 1.14b |
| Insulin (ng/mL)      | 1.09 ± 0.06 | 1.25 ± 0.11 | 1.43 ± 0.24 | 1.15 ± 0.04 |

C-PO, cultivated high oleic acid peanut oil; PO, peanut oil; SO, soybean oil; OO, olive oil; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

*aMale C57 BL/6 J mice aged 6–7 weeks were fed a C-PO, PO, OO, or SO diet for 10 weeks. All values are means ± SE, n = 3–6. Data marked with different letters (a, b) are significantly different (P < 0.05).
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weight ratio, as well as lower lipid accumulation in the liver tissue. This suggests that the SO metabolic pathways led to fat accumulation in adipose tissue rather than in liver tissue. This effect could be attributed to the unique SO lipid profile which is rich in PUFA (mainly ω6) and poor in MUFA compared to other oils. In contrast, in the OO group, liver tissue weight-to-body weight ratio and lipid accumulation in the liver tissue were the highest, suggesting that OO promotes lipid accumulation in the liver, as was also found by other studies (32).

Plasma cholesterol and HDL cholesterol levels were similar for all groups. Interestingly, plasma TG levels were lower in the OO group compared to other groups. This may indicate that in OO-rich diets, TG are more likely transported to various tissues such as liver, adipose, and muscle tissues. The C-PO group had lower TG levels compared to the PO and SO groups. Both olive and C-POs are rich in oleic acid. Previous studies have shown that oleic acid lowers cholesterol levels in plasma (33, 34). There is no consensus regarding oleic acid’s effect on plasma TG levels. Some studies have shown that oleic acid lowers plasma TG levels (15), while in other studies this effect was not observed (34).

Analysis of the liver enzymes indicates that there were no significant pathologies in any of the groups, and the diets had similar effects on the liver. This is considered to be normal in diets with 4% (w/w) fat.

Both fasting glucose levels and blood insulin levels were found to be similar among the groups. However, glucose plasma clearance rates varied. Sixty minutes after glucose intubation, plasma glucose levels were significantly higher in SO-fed mice compared to other diets. Also, a trend was seen for the AUC value (t-test, P < 0.07), when comparing the SO group to the PO or OO groups. These findings

Fig. 6. Effect of the oils on protein expression levels relevant to carbohydrate and lipid metabolism. Male C57 BL/6 J mice aged 6–7 weeks were fed a cultivated high oleic acid peanut oil (C-PO), peanut oil (PO), olive oil (OO), or soybean oil (SO) diet for 10 weeks. Protein expression levels were determined using the western blot method on liver tissue, using actin levels to normalize the values. (A) p-AMPK/AMPK proteins level ratio. *A significant difference (P < 0.05) of the C-PO diet compared to other diets in a Students’ t-test. (B) p-ACC/ACC protein level ratios, (C) Sirtuin 1 (SIRT1) relative protein levels, (D) cluster of differentiation 36 (CD36) relative protein levels. All values are mean ± SEM.
suggest that mice fed a SO diet were less sensitive to insulin, which may lead to slower glucose clearance from the blood compared to other diets. This is in agreement with previous results, suggesting SO, which is rich in PUFAs, is elevating insulin resistance in mice (35), thus increasing risk for metabolic syndrome.

We also evaluated the effect of the oils used in this study on the expression of several genes. mRNA levels of the G6Pase gene were significantly lower for the C-PO group in comparison to other groups. However, there was no significant difference in the plasma glucose levels after an overnight fast among the groups. G6Pase is a liver and kidney membrane-bound enzyme that plays an important role in providing glucose during starvation. Starvation increases the level of the G6Pase mRNA expression by about 30% (26). It is possible that the C-PO enhances satiation and inhibits starvation pathways in comparison to other oils, explaining the lower G6Pase mRNA levels.

mRNA levels of the SREBP-1c gene was also found to be significantly lower for the C-PO group in comparison to other diets. SREBP-1c is a transcription factor that regulates lipid homeostasis. It controls expression of various enzymes required for endogenous cholesterol, fatty acid (FA), triacylglycerol, and phospholipid synthesis (36), including FAS and ACC enzymes (both these proteins are involved in fatty acids synthesis). Therefore, a correlation is expected between SREBP-1c mRNA levels and ACC and FAS expression with lower SREBP-1c levels leading to lower ACC and FAS expression. The ACC protein is active when not phosphorylated. It appears that lower active levels of ACC lead to a higher p-ACC/ACC protein ratio and lower fatty acid content in the liver. However, the p-ACC/ACC protein ratio was similar to the other groups, as well as liver lipid weight which was not different from the other groups. As expected, FAS mRNA levels were significantly lower for the C-PO group than for other groups. FAS plays a central role in de novo lipogenesis. Our results have shown that C-PO led to significantly reduce the mRNA FAS expression compared to the other diets including OO which is also high oleic oil. This may indicate that C-PO has potential to reduce the lipogenesis and protect against fatty liver development. We believe further examination is required.

No significant differences were observed in p-AMPK/AMPK protein ratio among the groups or in the activity levels of the ACC protein. However, a Student’s t-test revealed significant differences in AMPK levels between the C-PO and PO groups (P < 0.05). AMPK plays a role in cellular energy homeostasis as well as in SREBP-1c regulation. When cell energy is decreased, there is an increase in the active form of AMPK enzyme (p-AMPK) resulting in an increased p-AMPK/AMPK protein ratio. An increase in the p-AMPK/AMPK protein ratio decreases cellular anabolic processes and increases catabolic processes. Therefore, decreases in SREBP-1c gene expression and ACC activity were expected.

SIRT1 relative protein levels were similar among the groups. SIRT1 deacetylates proteins. AMPK enhances SIRT1 activity (via synthesis of NAD+), thus promoting protein deacetylation. SIRT1 also activates AMPK through LKB1 deacetylation. In fact, AMPK and SIRT1 create a negative feedback loop by affecting each other’s activation (37, 38). Therefore, a correlation in their expression is expected. However, no significant difference was found in SIRT1 levels between the C-PO and PO groups (Student’s t-test), as was observed for AMPK levels.

CD36 mRNA levels were higher in the OO group and lower in the C-PO group (P < 0.05). Generally, gene expression is a good indicator of protein expression in the body. Therefore, it was expected that the CD36 protein level would be impacted accordingly. Yet, there was no significant difference in the CD36 protein levels between the groups. We can only speculate about the lack of this correlation that both transcription and translation may be coordinately regulated, whereas there is additional regulation at the level of protein degradation. To the best of our experience and knowledge, there is not always a correlation between the level of expression mRNA and protein level of many genes including as we reported in the case of CD36 (39). It is possible that OO consumption inhibits mRNA translation, which could explain normal protein levels despite higher mRNA levels. Also, in our experiment, there was no correlation found between CD36 protein levels and liver lipid accumulation. CD36 protein levels were similar between the four diets. However, there were differences found in liver lipid accumulation among the groups. Studies have associated CD36 deficiency with defective fatty acid and glucose metabolism in hypertensive rodents (25, 40). In addition, studies have shown that CD36 overexpression enhances free fatty acid (FFA) uptake by hepatocytes (41, 42). All of our diets had standard fat percentage (as recommended by AIN-93 M diet). Hence, lipids accumulation in the liver can be attributed to other mechanisms, rather than CD36 expression. In this work, we show that the oils affected CD36 mRNA expression differently, with the C-PO group the expression being the lowest. We speculate that C-PO may lead to improved metabolic syndrome and carbohydrate metabolism. Further research is required.

C-PO and OO have similar lipid profiles (MUFA, PUFA, and saturated fat). They both are high in oleic acid which is considered to be beneficial in carbohydrate and lipid metabolism. Notably, our findings were different for both oils. This may be attributed to different linoleic/α-linolenic acid ratios or possibly related to other components in the oils. For example, studies show that extra
Accumulation in liver, which maybe should be taken into consideration in people with fatty liver. However, it should be noted that fatty acid metabolism in mice differs from humans; therefore, some of the results seen in mice might not have the same effect on humans and further investigation is required. Finally, for C-PO, a new peanut variety, further investigation is suggested, as it can become an additional dietary source of oleic acid.

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References

1. Cinti S. The adipose organ at a glance. DMM 2012; 5(5): 588–94. doi: 10.1242/dmm.009662
2. Laguna-Camacho A. Influence on adiposity and atherogenic lipaemia of fatty meals and snacks in daily life. J Lipids 2017; 2017: n.p. doi: 10.1155/2017/1375342
3. Herieka M, Erridge C. High-fat meal induced postprandial inflammation. Mol Nutr Food Res 2014; 58(1): 136–46. doi: 10.1002/mnfr.201300104
4. Hernandez EA, Kahl S, Seelig A, Begovatz P, Irmler M, Kupriyanova Y, et al. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. J Clin Investig 2017; 127(2): 695–708. doi: 10.1172/jci89444
5. Mirmiran P, Amirhamidi Z, Eftehah HS, Bahadoran Z, Azizi F. Relationship between diet and non-alcoholic fatty liver disease: a review article. Iran J Public Health 2017; 46(8): 1007–17. PMID: 28984701
6. Moreno-Fernández S, García-Rimón M, Vera G, Astier J, Landrier JF, Miguel M. high fat/high glucose diet induces metabolic syndrome in an experimental rat model. Nutrients 2018; 10(10): 1502. doi: 10.3390/nu10101502
7. Hsu M-C, Wang M-E, Jiang Y-F, Liu H-C, Chen Y-C, Chiou C-H. Long-term feeding of high-fat plus high-fructose diet induces isolated impaired glucose tolerance and skeletal muscle insulin resistance in miniature pigs. Diabetol Metab Syndr 2017; 9: 81. doi: 10.1186/s13098-017-0281-6
8. Velázquez KT, Enos RT, Bader JE, Sougiannis AT, Carson MS, Chatzistamou I, et al. Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. World J Hepatol 2019; 11(8): 619–37. doi: 10.4254/wjh.v11.i8.619
9. Huang YY, Gusdon AM, Qu S. Nonalcoholic fatty liver disease: molecular pathways and therapeutic strategies. Lipids Health Dis 2013; 12: 171. doi: 10.1186/1476-511X-12-171
10. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med 2011; 364(25): 2392–404. doi: 10.1056/NEJMoa1014296
11. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Archiv Intern Med 2009; 169(7): 659–69. doi: 10.1001/archinternmed.2009.38
12. Lamping KG, Nuno DW, Coppey LJ, Holmes AJ, Hu S, Ottman CL, et al. Modification of high saturated fat diet with n-3 polyunsaturated fat improves glucose intolerance and vascular dysfunction. Diabetes Obes Metab 2013; 15(2): 144–52. doi: 10.1111/dom.12004
13. Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)-a review. Int J Mol Sci 2012; 13(3): 3291–340. doi: 10.3390/ijms13033291
14. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. Lipids Health Dis 2014; 13: 154. doi: 10.1186/1476-511x-13-154
15. Kris-Etherton PM, Pearson TA, Wan Y, Hargrove RL, Moriarty K, Fishell V, et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr 1999; 70(6): 1009–15. doi: 10.1093/ajcn/70.6.1009
16. Gottlieb S. Statement from FDA Commissioner Scott Gottlieb, M.D., on a new qualified health claim for consuming oils with high levels of oleic acid to reduce coronary heart disease risk FDA Statement [19 November 2018]. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/UCM626210.htm.
17. Coates AM, Hill AM, Tan SY. Nuts and cardiovascular disease prevention. Curr Atheroscler Rep 2018; 20(10): 48. doi: 10.1007/s11883-018-0749-3
18. Zainuddin A, Parkányiová J, Parkányiová L, Pokorny J, Sakurai H. Comparison of oxidative resistance of traditional and high-oleic peanut oils in emulsions. Czech J Food Sci 2018; 22: 136–9. doi: 10.17221/10637-CJFS

19. Hourihane JO, Bedwani SJ, Dean TP, Warner JO. Randomised, double blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. BMJ 1997; 314(7087): 1084–8. doi: 10.1136/bmj.314.7087.1084

20. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Investig 2002; 109(9): 1125–31. doi: 10.1172/jci15593

21. Choi YJ, Lee KY, Jung SH, Kim HS, Shim G, Kim MG, et al. Activation of AMPK by berberine induces hepatic lipid accumulation by upregulation of fatty acid translocase CD36 in mice. Toxicol Appl Pharmacol 2017; 316: 74–82. doi: 10.1016/j.taap.2016.12.019

22. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol 2011; 13(9): 1016–23. doi: 10.1038/nchb2329

23. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. The glucose-6-phosphatase complex of PGC-1alpha and SIRT1. Nature 2005; 434(7029): 113–8. doi: 10.1038/nature03354

24. Chen H, Liu X, Chen H, Cao J, Zhang L, Hu X, et al. Role of SIRT1 and AMPK in mesenchymal stem cell differentiation. Ageing Res Rev 2014; 13: 55–64. doi: 10.1016/j.arr.2013.12.002

25. Praveneec M, Landa V, Zidek V, Musilova A, Kozadoa L, Qi N, et al. Transgenic expression of CD36 in the spontaneously hypertensive rat is associated with amelioration of metabolic disturbances but has no effect on hypertension. Physiol Res 2003; 52(6): 681–8. doi: 10.1152/physiogenomics.00083.2011

26. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochem J 2002; 362(Pt 3): 513–32. doi: 10.1042/0264-6021:3620513

27. Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; 123(11): 1939–51. doi: 10.1093/jn/123.11.1939

28. Meslati-Stahy R, Mida K, Argov-Argaman N. Size-dependent lipid content of bovine milk fat globule and membrane phospholipids. J Agric Food Chem 2011; 59(13): 7427–35. doi: 10.1021/jf10373j

29. Eggers LF, Schwudke D. Liquid extraction: Folch. In: Wenk MR, ed. Encyclopedia of lipidomics. Dordrecht, Netherlands: Springer; 2016, pp. 1–6.

30. Pabinger S, Rödiger S, Kriegner A, Vierlinger K, Weinhäusel A. A survey of tools for the analysis of quantitative PCR (qPCR) data. BDQ 2014; 1(1): 23–33. doi: 10.1016/j.bdq.2014.08.002

31. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 2008; 233(6): 674–88. doi: 10.3181/0711-nr-311

32. Ferramosca A, Savy V, Zara V. Olive oil increases the hepatic triacylglycerol content in mice by a distinct influence on the synthesis and oxidation of fatty acids. Biosci Biotechnol Biochem 2008; 72(1): 62–9. doi: 10.1271/bbb.70369

33. Natali F, Siculella L, Salvati S, Gnoni GV. Oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells. J Lipid Res 2007; 48(9): 1966–75. doi: 10.1194/jr. M700051-JLR200

34. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985; 26(2): 194–202. PMID: 3989378

35. Deo P, Evans JR, Dhahbi J, Chellappa K, Han DS, Spindler S, et al. Soybean oil is more obesogenic and diabetogenic than coconut oil and fructose in mouse: potential role for the liver. PLoS One 2015; 10(7): e0132672. doi: 10.1371/journal.pone.0132672

36. Eberle D, Hegarty B, Bossard P, Ferre P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 2004; 86(11): 839–48. doi: 10.1016/j.biochi.2004.09.018

37. Zendedel E, Butler AE, Atkin SL, Sahbekar A. Impact of curcumin on sirtuins: a review. J Cell Biochem 2018; 119(12): 10291–300. doi: 10.1002/jcb.27371

38. Vancura A, Nagar S, Kaur P, Bu P, Bhagwat M, Vancurova I. Reciprocal regulation of AMPK/SNF1 and protein acetylation. Int J Mol Sci 2018; 19(11): E3314. doi: 10.3390/ijms19113314

39. Luiken JJ, Arumugam Y, Dyck DJ, Bell RC, Pelsers MM, Turcotte LP, et al. Increased rates of fatty acid uptake and plasma-lamellar fatty acid transporters in obese Zucker rats. J Biol Chem 2001; 276(44): 40567–73. doi: 10.1074/jbc.M100052200

40. Atimn TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, et al. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. Nat Genet 1999; 21(1): 76–83. doi: 10.1038/5013

41. Wilson CG, Tran JL, Erion DM, Vera NB, Febbraio M, Weiss EJ. Hepatocyte-specific disruption of Cd36 attenuates fatty liver and improves insulin sensitivity in HFD-fed mice. Endocrinology 2016; 157(2): 790–85. doi: 10.1210/en.2015-1866

42. Krammer J, Digel M, Ehehalt F, Stremmel W, Füllekrug J, Dettman JD, et al. Transgenic expression of CD36 in the spontaneously hypertensive rat is associated with amelioration of metabolic disturbances but has no effect on hypertension. Physiol Res 2003; 52(6): 681–8. doi: 10.1152/physiogenomics.00083.2011

43. Pérez-Jiménez F, Ruano J, Perez-Martinez P, Lopez-Segura F, Hettiarachchi ML, Badana J, et al. Triglycerides in human hepatoma cells. Int J Med Sci 2011; 8(7): 599–614. doi: 10.7150/ijms.8.599

44. Perez-Jimenez F, Ruano J, Perez-Martinez P, Lopez-Segura F, Lopez-Miranda J. The influence of olive oil on human health: not a question of fat alone. Mol Nutr Food Res 2007; 51(10): 1199–208. doi: 10.1002/mnr.200600273

45. Meidan E, Kolesnikov Y, Tirosh O. High fat diets composed of palm stearin and olive oil equally exacerbate liver inflammatory damage and metabolic stress in mice. Mol Nutr Food Res 2018; 62(13): e1700915. doi: 10.1002/mnr.201700915

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