

BIOCHEMICAL MODULATIONS: EXPLORING VARIATIONS IN MICE'S LIPID LANDSCAPE FROM DIVERSE PLANT OILS AND FATS

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Abstract: Fatty acids, known for their hydrocarbon chains spanning between 4 and 36 carbons, play a crucial role in various biological processes. Natural fats primarily consist of straight-chain fatty acids with an even number of carbon atoms. Fatty acid synthesis, or lipogenesis, predominantly occurs in the cytosol and is essential for several tissues, including the liver, kidneys, brain, and adipose tissue. The rate of lipogenesis is closely tied to an organism's nutritional status, increasing in well-fed animals with a high-carbohydrate diet and decreasing under caloric restriction or insulin deficiency, such as in diabetes mellitus.

Cholesterol, a key sterol, exists in animal tissues in the form of lipoproteins and free cells, or as esters in the bloodstream. Cholesterol esters serve as a common storage and transport form, while free cholesterol often transforms into bile acids. Unsaturated fatty acids within cell membrane phospholipids play a vital role in maintaining membrane fluidity.

High-Density and Low-Density Lipoproteins (HDL and LDL) are essential in transporting cholesterol to and from the liver. HDL, often referred to as "good cholesterol," aids in cholesterol exchange and its elimination in the bile. Clinical disorders related to fatty acid metabolism primarily involve oxidation processes, with triacylglycerides being the most prevalent lipid family, consisting of fatty acids and glycerol and serving as a major storage lipid.

Keywords: Fatty Acids, Lipogenesis, Cholesterol, Lipoproteins, Cell Membrane Fluidity

Introduction

Carboxylic acids with hydrocarbon chains spanning between (4 and 36) carbons are known as fatty acids. Natural fats consist of fatty acids which are typically straight chain variants with even number of the carbon atoms (Harwood and Robert, 1988). The cytosol is the primary pathway of de novo fatty acid synthesis (lipogenesis). A lot of tissues, like the liver, kidneys, lung, brain, adipose tissue, and mammary gland, have this system (24). Lipogenesis is regulated by the nutritional status of an organism. As a result, the rate becomes high in the well-fed animal whose food has a high carbohydrate content. It's depressed in the case when there is a caloric restriction, particularly on a high-fat diet, or in the case where there's an insulin deficiency, as in diabetes mellitus (DM) (47). Cholesterol represents the most common one of the steroids that are found in the animal tissues, where it is found in a form of the lipoproteins in either in free form in cells or combined as esters within blood circulation. Cholesterol esters represent the most common storage and transit forms of cholesterol, whereas free cholesterol is frequently transformed to bile acids (29). Unsaturated fatty acids in the cell membrane's phospholipids have a vital impact on maintaining membrane fluidity. High-Density and Low-Density Lipoproteins (HDL and LDL) are involved in transporting cholesterol to and from the liver. Because it may exchange cholesterol with other lipid carriers and carry it to the liver to be disposed in the bile, the HDL is known as the "good cholesterol" (49). The bulk of clinical disorders involving fatty acid metabolism are linked to oxidation mechanisms. Triacyl glycerides are the most prevalent lipid family in animals and plants, and they make up the majority of depot or storage lipids. It's made up of fatty acids and glycerol (8).

In industrialized nations, dietary lipids are responsible for roughly 25–45% of the total energy intake, and they have a significant impact on defining organoleptic qualities of foods, like consistency and palatability, as well as nutritional functions. Dietary lipids, with roughly 9 kcal/g of energy, are the most essential energetic nutritional component (43). Even though dietary (TGs) account for around 95% of total lipid consumption in terms of the composition, free fatty acids (FFA) and phospholipids that are normally found in their esterified form as TG, responsible for just 4–5%, in spite of their important role in various physiological functions (43). Cholesterol is generally found in its non-esterified form, making up roughly 3% of total lipid consumption.

The saturated fatty acids (SFA) can be found in dairy products, deep-fried foods, processed meats (such as sausage, salami, and bacon), processed foods (such as cakes, snacks, and biscuits), and lards. SFAs can also be found in plant-based oils like the coconut and palm oil (11). Coconut oil usage has grown in popularity recently. In the United States, for example, oil consumption climbed by 34% in 2014 compared to 2004. The coconut oil popularity, especially cold-pressed coconut oil, has been largely owing to information regarding its healthpromoting characteristics, like the main SFA in coconut oil—lauric acid (12:0)—having pro-apoptotic and antiproliferative effects on breast cancer cells (25). The high amount of medium-chain triacylglycerols (MCTs) was also noted as a benefit of using coconut oil (26). MCTs enhanced thermogenesis and lowered food consumption, according to scientific studies

The widely known claim that coconut oil has anti-obesity qualities was not backed up by scientific research (6). Furthermore, to reduce the risks of metabolic disorders, dietary recommendations encourage the limitation of the intake of the SFA and replacing it with the poly-unsaturated fatty acids (PUFA). For instance, WHO and the American Dietary Guidelines 2015–2020 suggest limiting the intake of the SFA to no more than 10% of daily calories (USDA and HHS). According to the American Heart Association, limiting SFA intake to 5–6% of daily calories had positive effects upon people through lowering the LDLC (14).

Cardiovascular diseases (CVD) are a leading cause of mortality all over the world, according to the WHO (50). Free radicals are recognized to play catalytic role in CVD's etiopathogenesis, causing oxidative stress and lipid oxidative reactions to begin (33). Lipid peroxidation causes major changes in LDL and HDL (49). To lower the risk of CVD, lifestyle changes are frequently advocated. Nutritional factors are significant in the prevention of CVD, MS, and diabetes. Olive oil, which is high in poly/mono unsaturated fatty acids and antioxidants, lowers the risk of CVD (46). Olive oil, which is a key component of Mediterranean diet, is one of the healthiest available sources of the energy (7). Its many positive effects account for its preventive action against CVD. By reducing inflammatory processes, it enhances glucose metabolism (44), controls blood pressure and lipidemic profile, retains endothelium normal function, and lowers LDL cholesterol oxidation. Olive oil's preventive properties are due to its high content of ω 9 monounsaturated fatty acids (MUFA), particularly the oxidation-resistant oleic acid (44). According to the EFSA, it also includes high phenolic antioxidant levels like the hydroxy-tyrosol and its derivatives, strongly protecting against blood lipid oxidation (18). In the European Union, an olive oil can have a legally recognized health claim if it includes over 5mg of the derivatives of the hydroxytyrosol per 20g. Olive oil is more effective in lowering cholesterol levels and suppressing platelet activation than other sources of oleic acid, such as sunflower oil, highlighting the value of its phenolic makeup (27). When MUFAs are combined with phenolics, the levels of oxidized lipoproteins and hence the risk of CVD is dramatically reduced (27). Hydroxytyrosol, Tyrosol, and their esterified secoiridoid derivatives are among the phenolics found in olive oil that have anti-inflammatory and antioxidant activities. The aglycones of ligstroside and oleuropein, as well as their respective decarboxylateddialdehydic structures, are the most significant secoiridoids (7).

Material and methods

Animal care:

A total of thirty-six of male mice of four week and age $24 \text{ g} \pm 2$ were obtained from animal house of biotechnology research center, University of Nahrain-Baghdad-Iraq. Mice were kept in a 12 hrs light-dark cycle and a 25 ± 2 °C temperature.

Diet preparation and experimental design:

After weaning, male Swiss-Webster mice ($n = 36$) have been given standard growth diets for a period of four weeks. The animals have been then divided to six groups (6 mice in each one of the groups). Mice have been fed one of five different fats. The mice in the first group (control group) have been fed complete laboratory chow with 4.2% crude fat, 22% crude protein, 3.5% crude fiber, and 5.7% crude ash. The second group fed high fat diet of sunflower oil 20% wt/wt fat. Third group fed diet of corn oil 20 % wt/wt fat. Fourth group fed diet of coconut oil 20% wt/wt of fat. Fifth group fed diet of olive oil 20% wt/wt fat. Sixth group fed diet of beef tallow 20% wt/wt fat. Oils and beef tallow diets were prepared by adding 200 gm per kilogram fat (Mercer and Trayhurn, 1987).

Determination of serum total cholesterol (T.chol):

The T.chol concentration was evaluated with the use of the enzymatic approach (33) using the commercially obtainable kit (bio-Merieux). The value of the T.chol is spectro-photometrically specified at 500nm.

Determining the serum HDL-c: Level of the HDL-c has been measured with the use of the enzymatic approach (6) by using the (bioMerieux) kit.

This approach's idea is precipitating lipoproteins and chylomicrons of the low density lipoprotein and VLDL through the addition of the phosphotungstic acid in presence of the ion of magnesium. Supernatant which has been produced after centrifuging included the HDL from which phospholipids and cholesterol may be found. HDL was specified spectro-photometrically at 500nm.

Determining the serum triglycerides (TG):

The total concentration of the serum TG was assessed through the use of the enzymatic approach of Precipel and Fossati, (16) by using the Bio-Merieux kit. The TG total serum concentration had been specified at 500nm.

Determining the serum VLDL-C:

The VLDL was specified based on the classical equation of (17). $\text{VLDL-c (mg.dl}^{-1}) = 0.2 \times \text{TG (mg.dl}^{-1})$.

Determining the serum LDL-c:

The serum LDL was specified based on Friedewald's equation $\text{LDL-c} = \text{T.Chol.} - (\text{VLDL-c} + \text{HDL-c})$.

Determining the serum total protein (TP):

Serum TP was measured with the use of the colorimetric approach according to Tietz, (42). By commercially available kits (Randox). This kit depends on Biuret method to determine total protein in serum, cupric ion, in alkaline medium interact with the bond of the protein peptide, which results in forming a coloured complex.

Determination of the concentration of serum Albumin:

Albumin concentration in the serum was determined with the use of the colorimetric approach by commercially available kit (CliniChem). This approach has been based upon certain bromo-cresol green (BCG) binding, an anionic dye, and protein in the pH of the acid for forming a complex of the green color. The color intensity, which is produced, is proportionate with the sample's albumin concentration.

Determination of Globulin concentration:

Total globulin level was computed through the subtraction of the Albumin from the total protein (21). $\text{Globulin concentration (g/dl)} = \text{TP (g/dl)} - \text{Albumin (g/dl)}$.

Measurement of serum Malondialdehyde (MDA):

The MDA's concentration in the serum was specified based on the approach of Buege and Aust method. MDA is produced from the breakdown of the poly-unsaturated fatty acids and it plays the role of a suitable peroxidation reaction index. The approach of the thiobarbituric acid (TBA) was utilized for the estimation of MDA, reacting with the TBA and resulting in pink color read at λ max 535 nm (5).

Measurement of serum Glutathione GSH:

The serum thiol concentration was measured based on Ellman approach (15).

Statistical analyses:

The data analyses were carried out through the utilization of the SPSS for the Windows, v22. Data have been represented in the form of mean \pm standard deviation (SD). The normality test of Shapiro–Wilk was utilized for the determination of whether the researched parameters were following the gaussian distribution.

Bonferroni Post Hoc test for several comparisons were applied after the tests of the analysis of variance (ANOVA).

The levels of the association were analyzed with the use of the Pearson's analysis of correlation. A $p < 0.05$ value has been viewed as significant (14).

Results and discussion

Table (1) lists a comparison of the concentrations of the serum TC, HDL, LDL, TG and VLDL in the healthy and supplement groups in mice. There has been a significant increase ($p < 0.050$) in serum TC and LDL in mice that have been treated by sunflower oil (G2), corn oil (G3) and beef tallow (G6) compared with control healthy group (G1). In addition to that, there were lower level significantly in TC and LDL ($p < 0.050$) ($p < 0.050$) in mice treated coconut oil (G4) and olive oil (G5) compared with healthy control group (G1).

Table-1-Effect of various oil and fat on lipid profile in mice

Parameter	G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	P value
TC (mg/dl)	138.77 (3.98)	168.44 (6.65)	156.88 (4.55)	132.21 (2.34)	129.77 (5.89)	177.54 (5.87)	$\leq 0.05z$
TG (mg/dl)	220.78 (8.11)	222.65 (4.32)	218.98 (3.09)	223.55 (4.89)	219.45 (5.65)	217.78 (5.11)	≤ 0.05
HDL-C (mg/dl)	78.34 (7.93)	77.84 (1.33)	76.66 (2.89)	79.63 (4.88)	80.12 (5.89)	76.03 (3.73)	≤ 0.05
VLDL-C (mg/dl)	44.156 (2.21)	44.53 (3.32)	43.796 (5.32)	43.91 (7.21)	43.89 (2.66)	43.556 (4.32)	≤ 0.05
LDL-C (mg/dl)	16.276 (1.33)	46.07 (3.95)	36.424 (5.43)	8.67 (1.21)	5.73 (09.32)	57.954 (6.79)	≤ 0.05

This is the first work to look at the impacts of different fats on lipid profiles in mice. Food consumption has been equal in all of the groups and was unaffected by the fat intake type, making it easier to compare the biological effects of the various diet components. Total cholesterol levels are much higher in the corn, sunflower, and beef tallow categories. The high-fat diet had no effect on triglyceride levels in any

of the groups. This is consistent with recent findings that olive oils have no effect on serum triglyceride levels (31).

We investigated the impacts of an oil mix (vegetable oil and beef tallow) on lipid metabolism in the mice in this work through simulating high-fat dietary patterns. The beef tallow diet produced the fattest mass, according to our findings. The vegetable oil (corn and sunflower oil) diet, at the same time, caused cholesterol metabolism problems even in people with the lowest fat mass. The capability for storing fat might be linked to the dietary fat type that is consumed instead of the overall number of calories consumed (40). SFA is a factor in obesity; studies show that eating edible beef tallow, high in SFA, causes more body fat accumulations compared to safflower oil that is high in n-6 fatty acids (37). Decreased thermogenesis and lower oxygen consumption induce body fat accumulations in the SFA-rich diets. SFA-rich diets impact membrane fatty acid composition. The metabolic rates are changed, which causes a drop in metabolic rate, in conjunction with altering membrane phospholipids (37).

According to studies, unusually high serum LDL-C levels are the major cause of hypercholesterolemia (22). Low levels of HDL-C and high LDL-C levels are linked to an increased CVD risk (22). The HDL-C/LDL-C ratio represents a useful indication for determining CVD risk, and it is more sensitive compared to TC and TG in predicting CVD risk. The ratio of HDL-C to LDL-C in mice fed olive oils was considerably lower compared to mice fed beef tallow. Those findings suggest that consuming vegetable oils (corn, sunflower, and soybean oils) increases risks of CVD when compared with other oils. The percentage of MUFAs in the diet could alter cholesterol metabolism. When put to comparison with SFO diet, Duavyet al. (13) found that consuming MUFA-rich olive oil lowers the levels of the serum LDL-C (13). Even though the current research found comparable outcomes, the mechanisms underlying such findings must be examined further.

People use a variety of different approaches to lose fat body mass, such as utilizing coconut oil supplement (COS) (6). COS was shown to have a variety of health advantages, including hypolipemic, hypocholesterolemic, antioxidant, antiplaque, and antidiabetic qualities in studies. COS is high in medium-chain lauric acid, which transforms to monolaurin, which is easily absorbed, digested, and utilized by the body, resulting in less fat deposition (1). COS was found to lower LDL-C and raise HDL-C in our research. Coconut oil contains medium-chain TGs, which are quickly absorbed into the bloodstream after consumption.

Effect of total protein on supplement group

Table (2) shows significances differences in total protein on supplement group. There is decrease significane in G2, G3 and G6 treated with sunflower, corn oil and beef tallow respectively, in total protein and albumin compared with control group. While there is no significance differences in G4 treated with coconut oil, in total protein and albumin compared with control group. Also there is increase significances in G5 treated with olive oil in total protein and albumin compare with control group.

Table 2-effect of various oil and fat on total protein

Parameters	G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	P value
Total protein g/dl	5.9±0.9	4.2±1.2	4.6±1.4	5.5±1.1	6.8±1.6	4.6±1.8	≤0.05
Albumin g/dl	4.2±0.4	3.8±0.9	3.6±0.8	4.3±1.1	4.9±1.3	3.5±1.1	≤0.05

Globulin g/dl	1.7±0.4	0.4±0.6	1.0±0.5	1.2±0.3	1.9±0.6	1.1±0.6	≤0.05
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The present results showed that total protein was significantly higher in G5 with olive oil. The important point here is that the reduced total protein can occur following a decrease in albumin (41). Based on the study findings, changes in serum total protein were consistent with changes in albumin. As the most abundant plasma protein that is made in the liver, albumin is the source of the body's amino acids and contributes to the maintenance of osmotic pressure. Additionally, albumin carries various substances such as bilirubin, calcium, and long -chain fatty acids. Albumin can absorb toxic heavy metals and drugs and thus counteract their toxic effects (12). Total protein, globulin, and albumin are three major indices used to control and monitor the course of diseases, immune system disorders, and hepatic and renal dysfunctions (30). The results showed that the addition of of olive oil to the of mice significantly increased total protein and albumin compared to the control group. It can be concluded that the increasing plasma albumin level improves the distribution of active ingredients of this antioxidant in mice blood.

Effect of various oil and fat on lipid peroxidation

Table (3) show significances differences in malondialdehyde (MDA) and glutathione (GSH). There is increase significances $P \leq 0.05$ in MDA in G2, G3 and G6 treated with sunflower oil, corn oil and beef tallow respectively, and decrease significance $P \leq 0.05$ in G5 treated with olive oil compared with control group. Also there is decrease significance $P \leq 0.05$ in GSH with G2, G3 and G6 and increase significance $P \leq 0.05$ in G5 treated with olive oil compared with control group.

Table 3-Effect of various oil and fat on MDA and GSH.

Parameters	G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	P value
MDA ($\mu\text{mol/L}$)	1.66±0.3	2.1±0.4	1.97±0.4	1.71±0.2	1.57±0.3	2.2±0.4	≤0.05
GSH ($\mu\text{mol/L}$)	197±11	159±22	162±23	188±19	211±24	166±31	≤0.05

As with ROS generation and imbalances in pro-oxidant/antioxidant status leads to oxidative stress and thus toxicity.(20) Production of free radicals causes lipid peroxidation and subsequently leads to cell membrane damage and inhibition of enzyme production/reduction in activity, reduction in cell function, and eventually cell death.(23) In this study, Sunflower oil, corn oil and beef tallow exposure significantly increased lipid peroxidation. In our study, olive oil (OO) significantly decrease MDA level.. This study also showed reduction in cellular GSH following sunflower oil, corn oil and beef tallow exposure. GSH is as an important cellular antioxidant and essential cofactor for As methylation.(32). During As exposure, GSH, with reduction in H_2O_2 , and lipid hydroperoxide play a protective role in cell damage.(36) Most of the proteins are synthesized in the liver and damage to this organ reduces production of proteins especially enzymes. Some compounds in sunflower, corn and beef tallow leads to interaction with sulfhydryl groups of proteins/enzymes, inhibition of cellular respiration in mitochondria, producing reactive forms, reactive oxygen species (ROS), and hence oxidative stress.(38).Olive oil have polyphenolic compounds and flavonoids can scavenge free radicals.(1). In this study, OO significantly protects liver against oxidative stress induced by metabolic pathway, probably due to its antioxidant and free radical scavenging properties of olive oil. Olive oil have bioactive compounds such as polyphenols, hydrocarbons, phytosterols, and triterpenes.(39).

These phenolic compounds of olive oil have potentially advantageous biological effects such as antimicrobial, antioxidant, and anti-inflammatory properties.(34). Flavonoids by maintaining hepatocellular membrane stability prevent from cellular leakage and enhance hepatic regeneration.(2). In addition, olive oil with polyphenol-rich compounds and a rich source of fats is able to reverse metabolic disorders and reduce coronary heart disease and cancers. Hydroxytyrosol and oleuropein from phenolic compounds of olive oil play a role in its taste and stability. In addition, these phenolic compounds with intense antioxidants have healthful effects (1). .

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