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Research Article

Comparative Study of Hepatic Fatty Acid Composition and Lipid Profile in Rats Fed Diets Rich in Palm Oil, Olive Oil, and Lard

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Abstract

The aim of our study was to determine the fatty acid (FA) composition of the liver, and the lipid profile of rats fed diets rich in palm oil, and to compare them with those of rats fed diets rich in olive oil and lard. Forty six-week-old male Wistar rats were divided into five groups (n = 8) and fed for 12 weeks with either a standard diet (385 kcal/100 g; 12% fat, 71% carbohydrates, and 17% protein) or a high-fat diet (525 kcal/100 g; 56% fat, 28% carbohydrates, and 16% protein). The lipids came exclusively from the oil tested in each group. Hepatic lipids were extracted using the Folch method and analysed by gas chromatography. Plasma lipid parameters (triglycerides, total cholesterol, HDL-C) were measured by automated spectrophotometry. Statistical analyses included Mann-Whitney tests (HFD vs. control comparison), Kruskal-Wallis tests (comparison between HFD groups), Spearman correlations, and multiple linear regressions. The results show that HF diets significantly increased final weight ($p = 0.003$) and visceral adipose tissue mass ($p = 0.0002$) compared to the control group. Olive oil induced significantly higher hepatic triglyceride accumulation (61.95 ± 27.26 mg/g, $p = 0.0275$), correlated with its richness in monounsaturated fatty acids (MUFAs), with a strong correlation between MUFAs in oils and their hepatic concentration ($\rho = 0.678$, $p < 0.001$). However, no significant effect was observed on plasma triglycerides, total cholesterol or HDL-C. These results suggest that the fatty acid composition of oils mainly influences hepatic metabolism without significantly altering blood lipids, probably due to powerful homeostatic mechanisms. The study highlights the need for a more nuanced nutritional approach, integrating the nature and stereospecificity of fatty acids according to the sn-2 hypothesis, as well as the presence of bioactive compounds that modulate gene expression. It paves the way for future research into the molecular mechanisms involved in lipid regulation and supports the development of personalised, mechanistically based dietary recommendations.

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Keywords

SFA, MUFA, HFD, sn-2 Hypothesis, Hepatic Triglycerides, Edible Oils, Lipid Metabolism, Nutrition, Cardiovascular Diseases

1. Introduction

Animal and vegetable fats and oils are composed of a mixture of triglycerides (TG) or triacylglycerols (TAG), whose structure consists of a glycerol backbone esterified with three fatty acids (FA). The positions at which fatty acids are bound to the glycerol backbone are designated sn-1, sn-2, and sn-3. Fatty acids may be saturated (SFA) or unsaturated (UFA), including monounsaturated (MUFA) and polyunsaturated (PUFA) types. They represent a major source of energy and essential compounds for the human body. Their quality, diversity, and quantity influence metabolic functions and affect cardiovascular risk, metabolic development, and the prevention of chronic diseases [1, 2].

From a public health standpoint, recommendations promote the consumption of unsaturated fats (mono- and polyunsaturated) over saturated fats, in order to limit the risk of obesity, diabetes, and cardiovascular diseases [3].

The lipid hypothesis, which proposed a link between saturated fatty acids, cholesterol, and cardiovascular disease (CVD), emerged in the 1950s [4-6]. From the 1990s onward, however, several epidemiological studies questioned this simplistic relationship between SFAs and CVD, as the correlation appeared weak or even absent [7].

Today, it is well established that the position of a fatty acid on the glycerol molecule determines its absorption. Pancreatic lipase hydrolyzes fatty acids at the sn-1 and sn-3 positions, producing 2-monoacylglycerols (2-MAG) and free fatty acids (FFA). In the presence of divalent ions (such as calcium or magnesium), the saturated fatty acids released from sn-1 and sn-3 form insoluble soaps that are not absorbed, whereas the fatty acid at the sn-2 position is absorbed in the form of a 2-monoglyceride by the enterocyte, enhancing its bioavailability [8-11].

Phospholipids are hydrolyzed by phospholipase A2, generating free fatty acids and lysophospholipids. Fatty acids with more than 14 carbon atoms in their chain are re-esterified within the enterocyte and circulate via the lymphatic pathway in the form of chylomicrons. Short- and medium-chain fatty acids are transported directly to the liver via the portal vein. Cholesteryl esters are hydrolyzed by pancreatic cholesterol esterase.

In this context, a detailed understanding of fatty acid metabolism, particularly in relation to their molecular structure, is essential. The sn-2 hypothesis suggests that fatty acids occupying the sn-2 position of triglycerides' glycerol are preferentially absorbed in the form of 2-monoglycerides, thereby conferring greater bioavailability to this fraction. This structural specificity influences digestion, tissue distribution, and the metabolic fate of lipids, all of which are crucial for assessing the true nutritional impact of various oils.

Human breast milk triglycerides provide a remarkable example of stereospecific positioning of fatty acids. Breast milk contains high amounts of fat, and the predominant fatty acid is palmitic acid (SFA), approximately 70% of which is esterified at the stereospecific sn-2 position of triglycerides, with oleic acid (MUFA) and linoleic acid (PUFA) preferentially occupying the sn-1 and sn-3 positions. The unique structural characteristics of human milk enable the infant to grow and develop on a diet rich in fats—especially saturated fats—while avoiding malabsorption of palmitic acid [12]. It must be noted that if palmitic acid were released from the sn-1 or sn-3 positions, it would form insoluble soaps in the presence of divalent ions, rendering these fats poorly absorbed in the intestine [8-11].

Thus, the positional distribution of fatty acids within triacylglycerols is at least as important as the fatty acid composition itself, conferring upon oils their characteristic "saturated" or "unsaturated" properties.

Palm oil, widely used in the food industry, exhibits a distinctive lipid composition: it is rich in saturated palmitic acid but also contains a substantial proportion of monounsaturated fatty acids, notably oleic acid, for which about 85% occupies the sn-2 position in triglycerides [10, 13]. In its crude form, palm oil is rich in carotenoids and contains both tocopherols and tocotrienols [14, 15], which provide specific antioxidant and nutritional advantages. These compounds (vitamins A and E) play protective roles against lipid oxidation and may modulate hepatic lipid metabolism [16-19]. However, refining leads to a significant loss of carotenoids [14].

Olive oil is renowned for its high content of monounsaturated fatty acids, mainly oleic acid, distributed more uniformly across the sn-1, sn-2, and sn-3 positions, and is rich in tocopherols. Its nutritional value is well documented, particularly for its cardioprotective effects [20].

Lard, a traditional animal fat, differs by its higher proportion of saturated fatty acids and a positional distribution less favorable to the bioavailability of unsaturated ones. In lard, palmitic acid is found exclusively at the sn-2 position, with an unsaturated fatty acid at sn-3, while the fatty acid occupying sn-1 is highly variable [21].

Comparing these lipids would provide valuable insight into

how the composition and structure of triglycerides influence lipid metabolism.

The sn-2 hypothesis represents a central pillar for this investigation, offering meaningful implications for public health. An integrated analysis of molecular structure, antioxidant compounds, and lipid profiles would help to elucidate how oil composition and structuring affect hepatic lipid metabolism and nutritional health.

This study therefore aims to determine and compare the hepatic fatty acid composition and plasma lipid profile of rats fed diets rich in crude and refined palm oil, olive oil, and lard.

2. Materials and Methods

The study was conducted at the PhyMedExp laboratory, which is Unit 1046 of the INSERM (the French National Institute of Health and Medical Research) and is affiliated with the University of Montpellier, France, and the CNRS (the French National Centre for Scientific Research). It was conducted in collaboration with the Medical Biochemistry Laboratory of the Faculty of Medical Sciences at the Félix Houphouët-Boigny University in Abidjan, Côte d'Ivoire.

2.1. Study Framework and Animal Conditioning

Our study population consisted of forty (40) young male Wistar rats, aged 6 weeks, from the Laboratoires Janvier (Le Genest-St-Isle, France). The animal models were housed at the CECEMA (Centre for Breeding and Experimental Conditioning of Animal Models), at the University of Montpellier.

The CECEMA technical platform is a shared service of the University of Montpellier; it is part of the Réseau des animaleries de Montpellier (Montpellier Animal Facilities Network) and is intended for the housing of animals necessary for scientific research and teaching. The environment is highly controlled, and the animals housed and handled exhibit defined biological characteristics (health status, behavioral sensitivity, etc.) that must be consistently maintained to ensure the quality and long-term reliability of the experiments of experiments. The conditions for the layout and operation of the shared animal facility are defined by Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and decrees and orders from February 2013. The premises, with an area of 1881.62 m^2, include the conventional animal facility (1291.62 m^2) and the Contained Experimental Facility (ECE, A3L3) of 590 m^2.

Upon arrival, the animals were housed in individual cages under constant temperature and humidity conditions. The temperature was 20-22 ℃ and the humidity level was 45-50% with a standard 12-hour day/night cycle (8:00 p.m. to 8:00 a.m.). The animals were handled in accordance with the guidelines of the Animal Care Committee of the University of Montpellier and the NIH (National Institutes of Health). After a week of acclimatization, the rats were randomly divided into 5 groups of 8. They were placed, in pairs, in cages with free access to water and food. We favored an animal model that was not subjected to specific exercises but was also not restricted in its movements. For 12 weeks, the animals were fed one of five semi-purified diets, the composition of which is shown in table 1. Each group of rats was designated by the type of fat corresponding to its diet: Crude palm oil (CPO), refined palm oil (RPO), olive oil (OO), lard, and control. Rats were given free access to water and food during the whole experiment. The rats' food consumption was measured every two days, and their body weight was determined weekly. The guidelines of the University of Montpellier for the care and use of laboratory animals were followed, and all experimental procedures were approved by the Montpellier local ethics committee (Reference CEEA-LR-12002).

Animal conditioning and diet methodology were described by Gauze et al. [22].

Table 1. *Nutritional composition of different diets.*

INGREDIENTS (g/kg)	CONTROL	HIGH-FAT DIETS (HFD)			
		CPO	RPO	OO	LARD
Casein	165	200	200	200	200
Corn starch	443	234	234	234	234
Maltodextrin	144	80	80	80	80
Sucrose	100	53	53	53	53
Soybean oil	50	25	25	25	25
Crude palm oil	0	300	0	0	0
Refined palm oil	0	0	300	0	0
Extra virgin olive oil	0	0	0	300	0

INGREDIENTS (g/kg)	CONTROL	HIGH-FAT DIETS (HFD)			
		CPO	RPO	OO	LARD
Lard	0	0	0	0	300
Cellulose	50	50	50	50	50
Minerals (AIN-93M)	35	42	42	42	42
Vitamins (AIN-93M) *	10	12	12	12	12
L-Cystine	2	2,4	2,4	2,4	2,4
Choline chloride$	1,5	1,8	1,8	1,8	1,8
Total caloric value (kcal/g)	3,85	5,25	5,25	5,25	5,25
% carbohydrates	72	30	30	30	30
% protein	17	14	14	14	14
% fat	11	56	56	56	56

*: The vitamin E content is 25 IU/kg diet instead of 75 IU/kg diet in the various diets.
$: Choline bitartrate has been replaced by choline chloride to prevent the formation of kidney stones.

2.2. Diet and Fat

Rats were randomized into 5 groups of animals and fed 12 weeks one of the five following semi-purified diets shown in Table 1:

1) Control diet (Control), containing 5% lipids as soybean oil (11% of energy coming from lipids). The standard diet brought 385 kcal per 100 g of food, with 71% of energy coming from carbohydrates, 12% from fats, and 17% from proteins. This diet was used for the control group (CFP 71/12/17).
2) The four high-fat diets (HFD) had a caloric value of 525 kcal per 100 g of food, with 28% of the caloric intake coming from carbohydrates, 56% from fats, and 16% from proteins (CFP 28/56/16).
   a) HFD rich in CPO with 2.5% soybean oil and 30% CPO,
   b) HFD rich in RPO with 2.5% soybean oil and 30% RPO,
   c) HFD rich in OO with 2.5% soybean oil and 30% OO or
   d) HFD rich in Lard with 2.5% soybean oil and 30% Lard.

CPO and CPO were supplied by SANIA, a company from Côte d'Ivoire, virgin OO was bought in a supermarket (these oils were chosen for their large current consumption) and the lard was provided by ALVA FOOD, a company based in Rezé, in the French city of Nantes. The high-fat diets were all supplemented with 2.5% soybean oil to maintain an adequate intake of linoleic acid and to avoid growth alterations resulting from linoleic acid deficiency. The detailed composition of the oils studied is given in Table 2.

Each rat was fed 25 g of food per day, or 8.125 g of fat per day for the HFD. The high-fat diets were all supplemented with 2.5% soybean oil to maintain an adequate intake of linoleic acid and prevent growth impairments due to linoleic acid deficiency. The diets were developed in accordance with the recommendations of the AIN-93 (American Institute of Nutrition) [23].

We used 325g of fat per 1000g of food. The HF (High Fat) diets were vacuum-packed in 1- to 2-kg bags in the form of a soft paste. The control diet was packaged in 5 to 10 kg bags in the form of kibble.

Table 2. *Fatty acid composition of fats used in diets.*

Fatty Acid %	Control*	CPO**	RPO**	OO**	Lard***
C14	0	0.52	0.53	0.03	1.5
C16	12.0	38.1	36.5	16.9	24.8
16:1n-7	0.60	0.13	0.15	2.01	3.1
C18	3.80	5.86	4.87	2.53	12?3

Fatty Acid %	Control*	CPO**	RPO**	OO**	Lard***
18:1n-9	19.7	43.2	46.4	58.0	45.1
18:1n-7	0	0.58	0.63	3.11	6
18:2n-6	55.4	10.0	9.5	15.6	9.9
18:3n3	8.0	0.28	0.16	0.63	1.1
C20	0.50	0.36	0.34	0.38	0
20:1n-9	0	0.13	0.15	0.17	0
20:1n-7	0	0.18	0.17	0.18	0
SFA	16.3	45.1	42.4	20.0	46.6
MUFA	20.2	44.4	47.7	63.6	43.3
PUFA	63.5	10.5	9.9	16.4	10.1
n-6	55.4	10.1	9.6	15.7	8.95
n-3	8.0	0.4	0.3	0.7	0.91
n-6/n-3	9.9	25.8	34.6	22.3	9.8

*: Composition of soybean oil according to Souci, Fachmann & Kraut, Food composition and nutrition tables. MedPharm, Scientific Publishers, Stuttgart, 2000, p. 188. ** Data obtained in the laboratory after analysis of CPO, RPO and OO used in this study. The composition of the oils was determined on a single sample (n=1). ***Source of lard: Anses. Ciqual 2017 nutritional composition table. Available at https://ciqual.anses.fr/

2.3. Extraction of Total Lipids from the Liver and Quantification of Fatty Acids in the Liver

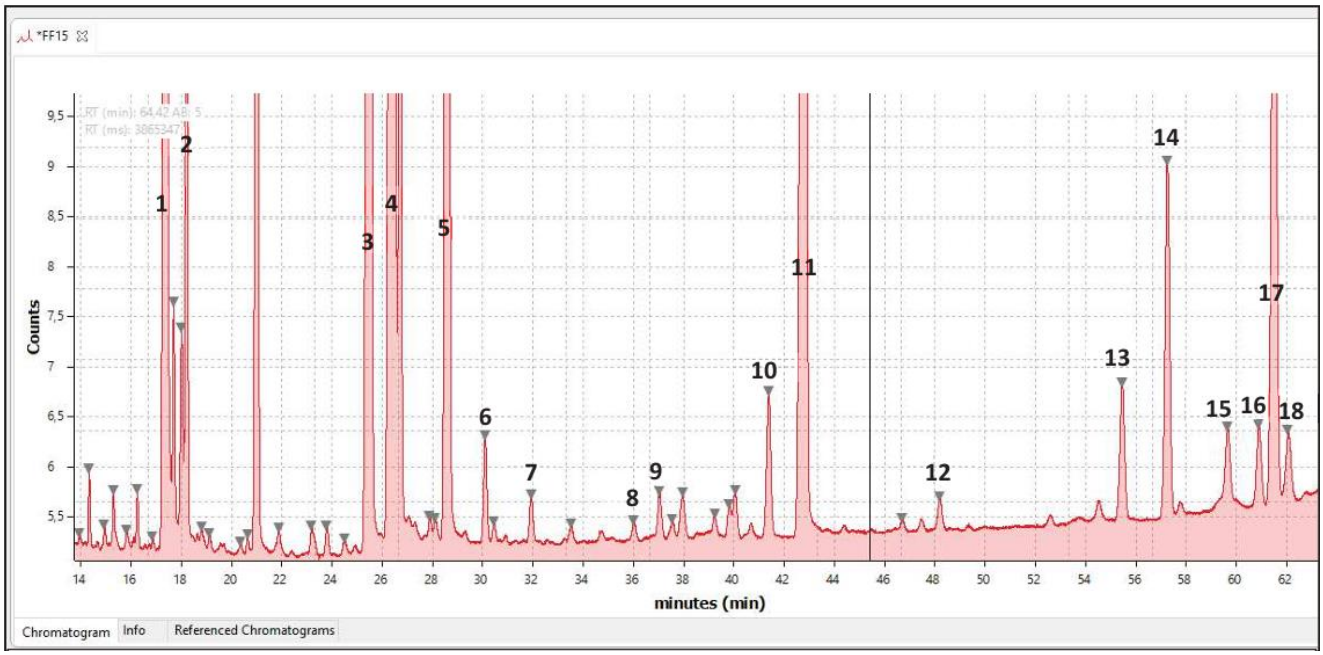

Figure 1. *Fatty acid profile of a rat liver fed a diet rich in CPO (1= palmitic acid (C16:0), 2 = Palmitoleic acide (C16:1n-7), 3 = Stearic acid (C18:0), 4 = Oleic acid (C18:1n-9), 5 = Linoleic acid (C18:2n-6), 6 = γ Linolenic (C18:3n-6), 7 = α Linoleic acid (C18:3n-3), 8 =Arachidic acid (C20:0), 9 = 11-Eicosenoic acid (C20:1n-9), 10 = Dihomo- γ -linolenic acid (C20:3n-6), 11 = Arachidonic acid (C20:4n-6), 12 = Behenic acid (C22:0), 13 = Docosatetraenoic acid (C22:4n-6), 14 = Docosapentaenoic acid (C22:5n-6), 15 = Clupanodonic acid (C22:5n- 3), 16 = Lignoceric acid (C24:0), 17 = Docosahexaenoic acid (C22:6n-3), 18 = Nervonic acid (C24:1n-9).*

After twelve weeks of experimentation, rats that had been fasting for 16 hours were euthanized after anesthesia by intraperitoneal injection of pentobarbital (Ceva Santé Animale, Libourne, France). The livers were removed, and total liver lipids were extracted using the Folch technique [24]. The esterified FA extracts were stored at -20 ℃ for gas chromatography (GC) analysis. The liver analyses were carried out in collaboration with the Muscle Dynamics and Metabolism Laboratory within the mitochondrial and nutritional endocrinology team at INRA in Montpellier.

The fatty acids in the liver were quantified using a Focus GC chromatograph (Thermo Electron Corporation, Thermo Fisher Scientific), equipped with an AS-3000 injector. The analysis was performed using a Focus GC chromatograph (Thermo Electron Corporation, Thermo Fisher Scientific), equipped with an AS-3000 injector. One µl of the esterified fatty acid extract was heated to 220 ℃. The injector volatilizes the esterified fatty acids, which are carried by helium. The separation of the esterified fatty acids on the capillary column (Varian CPsil88) was carried out according to their respective polarity and boiling point. The different eluted compounds were detected at the column outlet using a flame ionization detector, with the temperature set at 220 ℃. The methyl esters were identified (Figure 1) by comparing their retention time with that of their commercial standards injected under the same conditions. The fractions were quantified using an internal standard, heptadecanoic acid, added in a known amount during the transesterification.

2.4. Blood Sampling and Lipid Parameter Measurement

Blood was sampled from the abdominal aorta using a heparinized syringe (sodium heparin, Panpharma SA Fougères, France) and divided between a heparinized tube and a dry tube. The tubes were centrifuged at 1000 g for 10 minutes at 4 ℃. Plasma and serum were aliquoted into cups and stored at -80 ℃. Using standard methods on a COBAS automated analyzer (Roche Diagnostics, France), plasma triglycerides (TG) were measured using the Fossati and Principe method (1982) [25] with the TRIGB reagent, total cholesterol was determined with the CHOL2 reagent (Cobas, Roche, France) according to the Allain et al. method (1974) [26] and HDL cholesterol was measured using the direct method (Sugiuchi et al., 1995) [27] with the HDLC3 reagent.

2.5. Statistical Testing

The statistical analysis was based on non-parametric tests. The Mann-Whitney statistical test was used to compare all HFD groups with the control group (named p1 (Ctrl/Fat) in the tables and figures). The Kruskal-Wallis statistical test was used to compare each HFD group named in the tables and figures: p2 (Fat/Fat). The significance threshold used was 5% ($p < 0.05$). The first level analysis of the results obtained concerns the comparison of the control diet with all high-fat diets, which reflects the effect of the quantity of fat. The second level concerns the differences among the high-fat diets themselves, which reflects the effect of the quality of fat.

Spearman's correlations tests were performed between the fatty acids in the oils, the fatty acids in the liver, and the lipid parameters of the liver and blood. Spearman's correlation test is a nonparametric test that is robust to outliers. It measures the strength and direction of the relationship between any two variables, based not on the values themselves, but on the ranks of those values. Spearman's rho coefficient ranges from -1 to +1: close to +1 indicates a strong positive monotonic relationship; close to 0 suggests that there is no monotonic relationship between the variables; close to -1 indicates a strong negative monotonic relationship, where one variable increase while the other decreases in a monotonic manner.

We first applied a simple regression model between oil-derived fatty acids and hepatic fatty acids. To refine the analysis, we then performed multiple regression tests including all variables to better account for potential interactions and confounding effects. Multiple regression tests are unique in their ability to explain or predict the variation of a continuous dependent variable based on several independent variables, which may be continuous or categorical. Unlike simple regression, which uses a single explanatory variable, multiple regression simultaneously incorporates several explanatory variables, making it possible to model complex relationships (as is the case in lipid metabolism) and assess the net effect of each variable independently of the others. Multiple regression therefore makes it possible to model the relationship between a target variable and several explanatory factors, estimate their respective contributions, and predict values. The interpretation of the multiple regression model is based on several key points: the estimated regression coefficients (or slope) indicate the expected average effect on the dependent variable for a change of one unit in an independent variable, with all other variables held constant, the coefficient of determination R^2 measures the proportion of the variance in the dependent variable explained by the model (The closer R^2 is to 1, the better the model fits the data; the p-values associated with the coefficients indicate whether each independent variable contributes significantly to explaining the dependent variable. The confidence interval (95% CI) gives a range of plausible values for a model parameter. It is a measure of uncertainty in the model estimate.

3. Results

3.1. Influence of Diet on Biometric Data in Rats

Table 3. Characteristics of Rats' biometric data.

	Control	CPO	RPO	OO	Lard	p1 (HF/Ctrl)	p2 (Fat/Fat)
Initial body weight (g)	209 ±12	203 ±4	204 ±5	207 ±5	208 ±7	NS	NS
Final body weight (g)	528 ±27a	609 ±40b	612 ±52b	588 ±49b	611±66b	0.003	NS
Visceral AT (g)	28.6±8.2a	44.7 ±9.1b	46.0 ±7.8b	42.7 ±10.5b	46.0 ±11.5b	0.0002	NS

Values are expressed as mean ± SD (n = 8). Values within the same row with different letters are significantly different. Rats were fed their respective diets for 12 weeks. CPO Crude palm oil; Refined palm oil: RPO, olive oil OO. p1 = p-value of a Mann-Whitney statistical test when comparing all high-fat (HF) diets and the control group (Ctrl). p2 = p-value of a Kruskal-Wallis statistical test when comparing CPO, RPO, OO, and Lard.

The final weight of the rats fed the HF diet was found to be significantly higher than that of the rats fed the control diet (p=0.003). This trend was also observed with visceral fat (p=0.0002). The above table shows that the animals that were on high-fat diets responded well: they all gained weight uniformly and all accumulated visceral fat. Thus, the diets were effectively taken by all the animals, which ensures reproducibility and consistency between the five groups. These results indicate that the quantity of oil influences weight and adipose tissue, but not on the quality of fat.

3.2. Fatty Acid Composition of Hepatic Lipid Extracts and Hepatic TG Values

After 12 weeks of dieting, no significant difference was observed between the FA composition of liver extracts from the control diet and that of high-fat diets (HFD). This suggests that the amount of fat has no impact on the FA composition of the liver (see Table 4).

Table 4. Fatty acid composition of hepatic lipid extracts and hepatic TG values.

	Control	CPO	RPO	OO	Lard	p1 (HF/Ctrl)	p2 (Fat/Fat)
SFAs %	37,5 ±0,56	40,1 ±0,69a	38,8 ±1,04a	28,0 ±0,83b	35,5 ±0,81a	NS	< 0.0001
MUFAs %	24,2 ±0,84	29,9 ±1,10a	30,7 ±1,66a	45,3 ±0,91b	33,3 ±1,51a	NS	< 0.0001
PUFAs %	38,3 ±0,99	30,0 ±0,77a	30,5 ±0,79ab	26,7 ±0,45c	31,2 ±0,82b	NS	< 0.0001
Liver TG, mg/g	31.54±9.89	29.11±16.84a	29.38±16.87a	61.95±27.26b	38.92±11.79a	NS	0,0275

SFA: Saturated fatty acids; UFA: Unsaturated fatty acids; MUFA: Monounsaturated fatty acids. Values assigned different letters are significantly different.

The impact of oil quality was assessed by comparing the HFD diets with each other (Table 4 and Figure 2). The composition of liver lipid extracts from rats fed the olive oil-rich diet was significantly lower in SFAs and PUFAs than that of the other rat groups (p<0.0001). However, there was no significant difference between refined palm oil PUFAs and lard's PUFAs. MUFAs content of liver extracts from rats fed the OO-rich diet was significantly higher than in the other diets (p<0.0001). These results suggest that oil quality influences the fatty acid composition of hepatic lipid extracts and hepatic TG values, but not the amount of fat.

3.3. Lipid Profile

No significant difference was observed between the lipid profile of the control group and that of the high-fat diets. This means that the quantity of fat had no impact on the lipid profile. In terms of oil quality, the lipid profile of PO rich diets was not significantly different from that of diets rich in olive oil and lard (Table 5 and Figure 2). There is therefore no significant difference between the lipid profile of animals fed

PO and those fed olive oil and lard.

Table 5. Plasma lipid profile.

	Control	CPO	RPO	OO	Lard	p1 (HF/Ctrl)	p2 (Fat/Fat)
Blood TG, mM	1.18 ±0,55	0.99 ±0,30	1.21 ±0,60	1.01 ±0,31	1.01 ±0,42	NS	NS
Total Cholesterol, mM	2.00 ±0.41	1.75 ±0.42	1.89 ±0.46	1.72 ±0.28	1.55 ±0.26	0.078	NS
Cholesterol HDL, mM	1.49 ±0.30	1.34 ±0.24	1.8 ±0.36	133 ± 0.20	1.06 ± 0.60	NS	NS

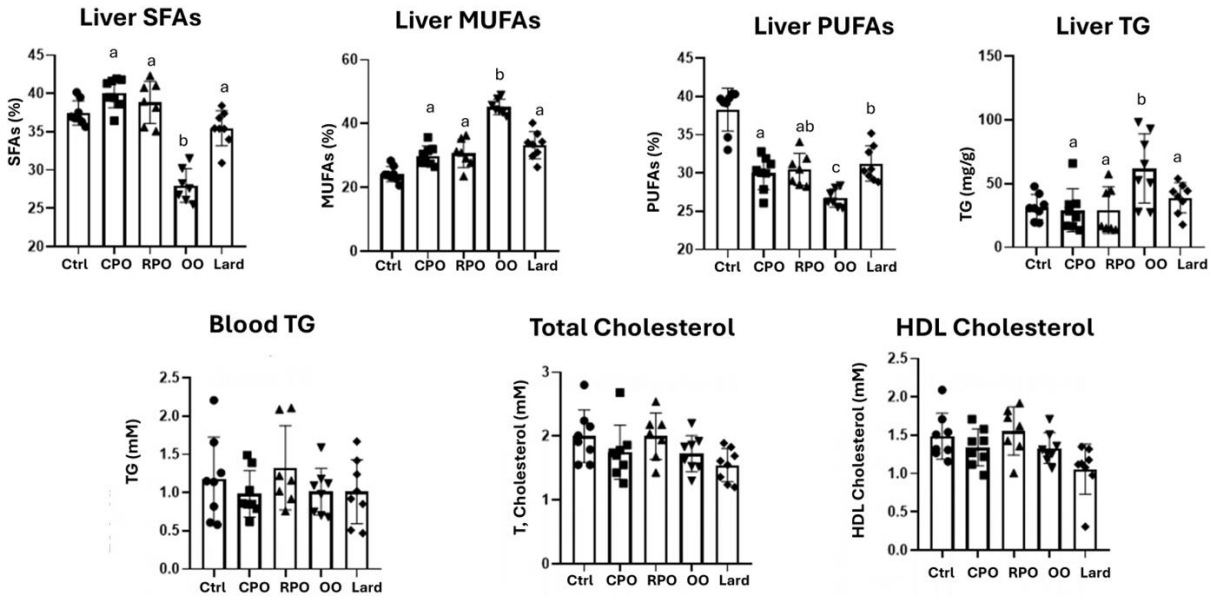

Figure 2. Liver and blood lipid profile of rats according to dietary lipid source.

The histograms show the means (n = 7-8), expressed as mean ± SD (n = 8). Rats were fed their respective diets for 12 weeks. CPO Crude palm oil; Refined palm oil: RPO, olive oil OO. Mann-Whitney statistical test when comparing all high-fat (HF) diets and the control group (Ctrl). Kruskal-Wallis statistical test when comparing CPO, RPO, OO, and Lard. Fatty acids are expressed as a percentage, and triglycerides, total cholesterol, and HDL cholesterol are expressed in mM. Different letters indicate significant differences.

3.4. Integrated Analysis of the Relationships Between the FA Profile of Oils, Liver Extracts, and the Plasma Lipid Profile

Table 6. Spearman correlation tests between oil FAs, liver FAs, and lipid profile.

Variables	ρ	p-value
FA in oils and FA in liver		
SFAs	0.131	0.432
MUFAs	0.678	< 0.001*
PUFAs	0.223	0.178
FA in oils and TG in liver		

Variables	ρ	p-value
SFAs	-0.061	0.712
MUFAs	0.214	0.191
PUFAs	-0.129	0.432
FA in oils and Blood TG		
SFAs	-0.077	0.643
MUFAs	-0.012	0.942
PUFAs	-0.079	0.632
FA in oils and Total Cholesterol		
SFAs	-0.358	0.025*
MUFAs	-0.029	0.860
PUFAs	0.087	0.596
FA in oils and HDL-Cholesterol		
SFAs	-0.366	0.022*
MUFAs	0.067	0.684
PUFAs	0.056	0.737
FA in liver and TG in liver		
SFAs	-0.716	< 0.001*
MUFAs	0.636	< 0.001*
PUFAs	-0.407	0.011*
FA in liver and Blood TG		
SFAs	0.066	0.696
MUFAs	-0.067	0.688
PUFAs	0.011	0.947
Liver TG and Blood TG		
TG	-0.117	0.478

The correlation between oil FAs and liver FAs is significant only with MUFAs (ρ=0.678; p < 0.001).

There is no correlation between oil FAs and hepatic TG.

There is a significant correlation between liver FAs and liver TG. SFA (ρ=-0.716; p < 0.001) and PUFA (ρ=-0.407; p=0.011) are negatively correlated with hepatic TG, while MUFA are positively correlated with hepatic TG (ρ=-0.636; p<0.001).

There is no correlation between fatty acids in oils and blood TG, no correlation between fatty acids in the liver and blood TG, no correlation between TG in the liver and blood TG.

There is a significant negative correlation between SFA and total cholesterol (ρ =-0.358; p=0.025) and HDL-cholesterol (ρ =-0.366; p=0.022).

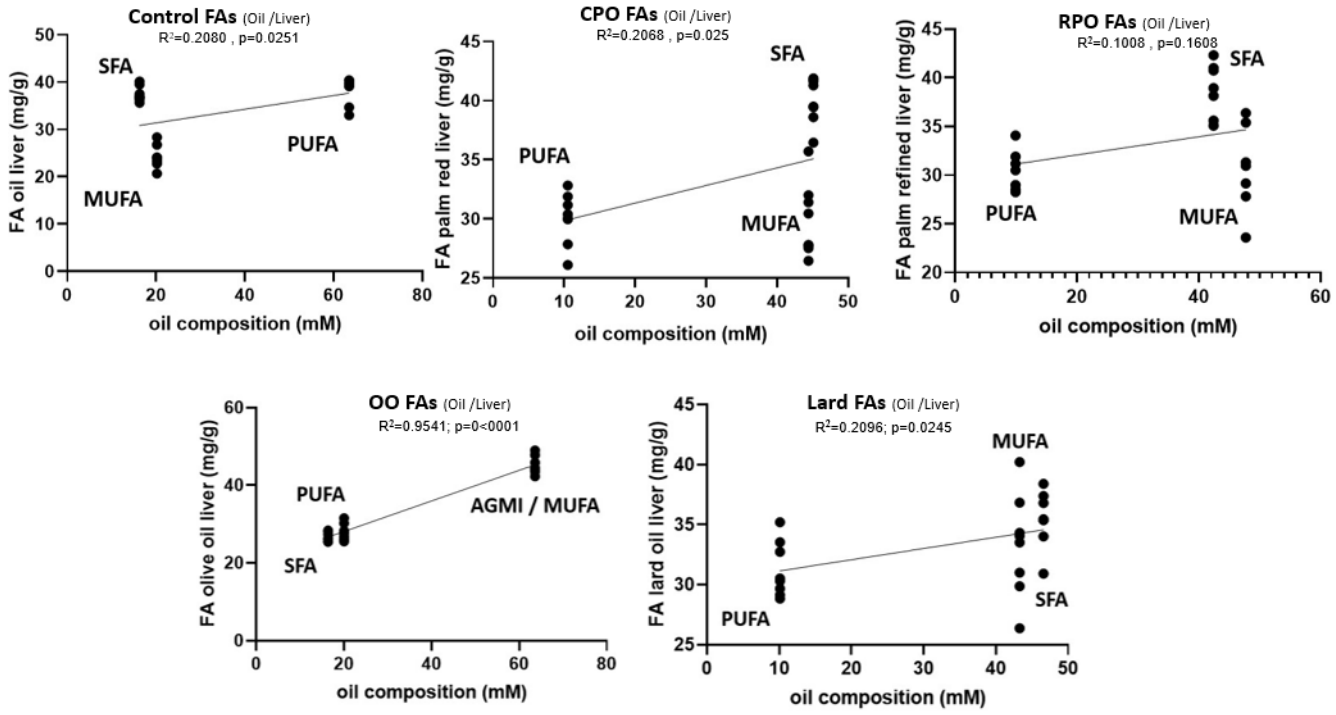

Figure 3. *Simple regression between oil composition and hepatic fatty acids according to oil type.*

All oils, except for refined palm oil (RPO, p>0.05), significantly influence liver fatty acid levels (Figure 3). A very strong correlation exists between the fatty acid composition of olive oil and liver fatty acid levels (R^2 = 0.9541; p < 0.0001), suggesting that olive oil has a major and highly significant impact on liver fatty acid levels, greater than that of refined palm oil (R^2 = 0.2068, p = 0.025), lard (R^2 = 0.2096, p = 0.0245), and soybean oil (Control: R^2 = 0.2080, p = 0.0251). The significant correlation between oil-derived fatty acids and hepatic fatty acids observed for MUFAs (ρ = 0.678; p < 0.001) in Table 6 suggests that this strong association may be attributed to the high MUFA content of olive oil.

Table 7. *Multiple linear regression analysis between the FA composition of diets, liver FAs, and lipid profile.*

Variables	Slope	R^2	*p*-value	95% CI
FA in oils and FA in liver				
SFAs	0.159	0.206	0.004*	[0.05; 0.27]
MUFAs	0.446	0.676*	< 0.001*	[0.34; 0.55]
PUFAs	0.156	0.588*	< 0.001*	[0.11; 0.20]
FA in oils and TG in liver				
SFAs	-0.435	0.076	0.088	[-0.94; 0.07]
MUFAs	0.614	0.172	0.009*	[0.17; 1.07]
PUFAs	-0.102	0.011	0.532	[-0.43; 0.23]
FA in oils and Blood TG				
SFAs	-0.001	0.001	0.848	[-0.01; 0.01]
MUFAs	-0.003	0.010	0.552	[-0.01; 0.01]
PUFAs	0.002	0.007	0.604	[0.00; 0.01]
FA in oils and Total Cholesterol				
SFAs	-0.006	0.039	0.227	[-0.01; 0.01]

Variables	Slope	R^2	*p*-value	95% CI
MUFAs	-0.006	0.045	0.193	[-0.01; 0.01]
PUFAs	0.005	0.071	0.100	[0.00; 0.02]
FA in oils and HDL-Cholesterol				
SFAs	-0.005	0.049	0.173	[-0.01; 0.01]
MUFAs	-0.003	0.017	0.429	[-0.01; 0.01]
PUFAs	0.003	0.052	0.165	[0.00; 0.01]
FA in liver and TG in liver				
SFAs	-3.400	0.559*	< 0.001*	[-4.42; -2.38]
MUFAs	1.930	0.478*	< 0.001*	[1.24; 2.62]
PUFAs	-1.958	0.163	0.012*	[-3.45; -0.46]
FA in liver and Blood TG				
SFAs	0.013	0.019	0.410	[-0.01; 0.05]
MUFAs	-0.007	0.014	0.476	[-0.02; 0.02]
PUFAs	0.006	0.004	0.718	[-0.02; 0.04]
Liver TG and Blood TG				
TG	-0.003	0.028	0.307	[-0.01; 0.01]

Regression analyses show that monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in oils have a significantly positive association with FA levels in the liver: MUFAs ($R^2 = 0.676$, $p < 0.001$) and PUFAs ($R^2 = 0.588$, $p < 0.001$). Saturated fatty acids (SFAs) have a weaker but significant association with liver FAs ($R^2 = 0.206$, $p = 0.004$).

Regarding hepatic triglycerides (TG), only MUFAs show a weak but significant positive association (R^2=0.172, $p = 0.009$), while correlations between oil FAs and blood TG or total/HDL cholesterol are not significant.

In the liver, SFAs show a strong negative association with hepatic TG ($R^2 = 0.559$, $p < 0.001$), while MUFAs display a significant positive relationship with hepatic TG ($R^2 = 0.478$, $p < 0.001$). There is a weak but significant positive association between PUFAs and hepatic TG (R^2=0.163, $p = 0.012$).

No significant link was observed between hepatic FAs and blood TG, nor between liver TG and blood TG.

4. Discussion

This study aimed to compare the impact of different high-fat diets, composed of oils with contrasting lipid profiles (palm oil, olive oil, and lard), on the metabolism of rats fed for twelve weeks by determining liver fatty acid composition, hepatic triglycerides (TG), and blood lipid profile (TG, TC, HDL-C).

The results suggest that the fatty acid composition of dietary oils significantly influences the hepatic lipid profile, particularly via MUFAs, which promote an increase in liver triglycerides. This selective incorporation of fatty acids in the liver could reflect the metabolic specificity of MUFAs in hepatic lipid accumulation. Olive oil, rich in MUFAs, therefore causes a greater accumulation of hepatic triglycerides, which could have implications for hepatic lipid metabolism and the risk of steatosis. The SFAs' link to a decrease in hepatic TG underscores their distinct metabolic role.

The fatty acid composition of dietary oils was found to have no significant relationship with blood triglycerides, nor with total or HDL cholesterol, suggesting that these dietary fats primarily impact liver composition but not directly blood lipids.

This observation calls for reflection on the methodological limitations and potential limiting factors of the study, the relevance of fatty acid composition as a predictor of triglyceride levels, and alternative mechanisms that regulate this parameter.

Several limiting factors must be considered. The limited sample size reduces statistical power and increases inter-individual variability, which may mask real but modest differences. The twelve-week duration, while adequate for inducing experimental obesity, may remain relatively short for observing chronic alterations in lipid metabolism. In addition, there is the inherent limitation of the animal model, since the regulation of triglyceride-rich lipoproteins differs between rats and humans, thus restricting the generalizability of the results.

The lack of a significant relationship between the fatty acid

composition of oils and plasma TG levels could be explained by the complexity of fat metabolism. The liver and peripheral tissues possess powerful homeostatic mechanisms that maintain TG levels within a relatively stable range. VLDL secretion by the liver (the main source of circulating triglycerides) and TG clearance by lipoprotein lipase are adjusted according to intake, which mitigates the direct impact of dietary fat quality. Furthermore, hepatic production and secretion of VLDL are strongly modulated by insulin sensitivity, carbohydrate intake, and overall energy balance rather than by the type of dietary fat consumed [28, 29]. De novo endogenous lipogenesis can further mask the effects of dietary fatty acids by synthesizing triglycerides from non-lipid precursors such as glucose. Other mechanisms must be considered to explain the regulation of plasma TG. Mitochondrial oxidation of fatty acids, by increasing energy consumption, can reduce the availability of substrates for triglyceride synthesis. Furthermore, inflammation associated with the accumulation of visceral adipose tissue alters insulin sensitivity and disrupts lipid regulation. Finally, bioactive compounds present in certain oils, such as carotenoids in red palm oil, tocopherols, and polyphenols in olive oil (also present in palm oil) [10, 13-15], can modulate the expression of key lipid metabolism genes, such as PPARα or SREBP-1c, and indirectly influence triglyceride levels [17, 18].

These results are consistent with scientific literature. In rats, several studies have shown that high-fat diets, regardless of their nature, induce weight gain and adipose tissue accumulation, but that plasma TG remain relatively stable [30-32]. However, a mouse study comparing high-fat diets (HFDs) with 41% lipids for 8 weeks, comparing olive oil, palm oil, and hybrid palm oil, showed lower hepatic fat accumulation in the olive oil diet than in the palm oil diet [33]. Another study conducted on rats fed a cholesterol-enriched diet for three months, along with 0.5 ml/kg of palm or olive oil, showed that palm and olive oils exhibited antihyperlipidemic and hypocholesterolemic effects. The improvement of antioxidant defenses, the reduction of inflammation and lipid peroxidation, and the modification of hepatic FAS mRNA expression are the main mechanisms by which palm and olive oils exert their beneficial effects. [34].

The complexity of lipid metabolism underscores the importance of incorporating into nutritional recommendations a nuanced approach that goes beyond simply classifying oils as "good" or "bad." It is essential to consider not only the nature of fatty acids but also their molecular positioning, as suggested by the sn-2 hypothesis, which influences their absorption and tissue incorporation. Furthermore, the bioactive compounds present in certain oils play a substantial role in modulating the expression of key genes involved in lipid metabolism [16-19].

International guidelines recommend reducing SFA consumption while increasing PUFA and MUFA intake to mitigate cardiovascular risk. However, data concerning MUFAs and the risk of cardiovascular disease remain contradictory [35]. A recent review analyzed current knowledge and gaps in the literature regarding MUFAs and the risk of cardiovascular disease, focusing on consumption, the different types of MUFAs, and their concentration in adipose tissue as a biomarker of endogenous exposure, raising concerns about a possible higher risk of cardiovascular disease [36].

This more nuanced approach would help avoid simplistic generalizations, such as the systematic stigmatization of palm oil or the exclusive valorization of olive oil, by recognizing the complexity of fat metabolism and the combined effects of the different components of oils. It also paves the way for future research aimed at better understanding the underlying molecular and physiological mechanisms, including the impact of fatty acid sn-2 positions on the regulation of hepatic and plasma triglycerides, as well as the role of bioactive compounds present in oils.

Thus, our work contributes to refining nutritional strategies for the prevention of metabolic and cardiovascular diseases by offering a more integrated and mechanistic view of lipid metabolism, which could guide more effective and personalized dietary recommendations.

5. Conclusion

The results of this study highlight that the fatty acid composition of dietary oils significantly influences hepatic metabolism, particularly via monounsaturated fatty acids (MUFAs), which promote triglyceride accumulation in the liver. Conversely, the lack of a marked effect on plasma lipids underscores the robustness of the homeostatic mechanisms regulating triglyceride levels and suggests that the liver is a sentinel organ more sensitive to the quality of dietary fats than the bloodstream.

These observations have important implications for nutritional recommendations. They call for moving beyond simplistic classifications of fats as "good" or "bad" and adopting a more integrated approach that considers not only the fatty acid profile but also their molecular positioning. The integration of the sn-2 hypothesis provides further mechanistic insight. This structural specificity thus influences the hepatic metabolic dynamics of dietary lipids as well as the presence of bioactive compounds that modulate gene expression. The proposed approach would allow for better targeting of nutritional interventions, particularly in the prevention of hepatic steatosis and associated metabolic disorders.

From a scientific perspective, this work contributes to a deeper understanding of the complexity of lipid metabolism by highlighting the interaction between the quality of dietary fats, hepatic regulatory mechanisms, and systemic factors such as insulin sensitivity, inflammation, and de novo lipogenesis. It thus paves the way for new research aimed at exploring in greater detail the molecular mechanisms involved, including the role of nuclear receptors (PPARα, SREBP-1c), the impact of fatty acids according to their stereospecific configuration, and the synergistic effects of bio-

active compounds present in oils.

In short, this study argues for a revision of current nutritional paradigms by integrating the complexity of fat metabolism and promoting personalized dietary recommendations based on a mechanistic understanding of the interactions between nutrients and metabolism.

Abbreviations

CECEMA	Centre for Breeding and Experimental Conditioning of Animal Models (Centre d'Elevage et de Conditionnement Expérimental des Modèles Animaux)
CNRS	French National Centre for Scientific Research (Centre National de Recherche Scientifique)
CPO	Crude Palm Oil
Ctrl	Control
CVD	Cardiovascular Disease
ECE	Contained Experimental Facility (Etablissement Confiné d'Expérimentation)
FA	Fatty Acid
FFA	Free Fatty Acid
GC	Gaz chromatography
CFP	Carbohydrates / Fats / Proteins
HDL	Hight density lipoprotein
HFD	Hight Fat diet
INRA	the National Institute for Agricultural Research (Institut National de Recherche Agronomique)
INSERM	French National Institute of Health and Medical Research (Institut National de la Santé et de la Recherche Médicale.)
MAG	Monoacylglycerol
MUFA	Monounsaturated Fatty Acid
OO	Olive Oil
PUFA	Polyunsaturated Fatty acid
RPO	Refined Palm Oil
SFA	Saturated Fatty acid
TAG	Triacylglycerol
TG	Triglyceride
UFA	Unsaturated Fatty Acid
VLDL	Very Low Density Lipoprotein

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Charles Coudray: Methodology, Supervision

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Conflicts of Interest

The authors certify that there is no conflict of interest with respect to this manuscript.

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