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ORIGINAL CONTRIBUTION

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Lipaemia and liver composition in pregnant rats consuming olive oil and olive oil used for frying

Wachstum, Lipamie und Zusammensetzung der Leber trächtiger Ratten, die mit fritiertem Oel gefüttert wurden.

Summary The effect of the consumption of unused olive oil (polar content, 2 %; oleic acid, 78.9 mg/100 mg oil, and linoleic acid 7 mg/100 mg oil) and olive oil used discontinuously for frying potatoes 15 times (polar content, 9 %; oleic acid, 75.8 mg/100 mg oil and linoleic acid 6.2 mg/100 mg oil) was studied in pregnant rats with the aim of better understanding the relationship between the consumption of fat used in frying and lipid metabolism during periods of intense anabolism. Trials were performed in pregnant Wistar rats, divided into 2 groups and fed isocaloric diets in which the fat content (15 % wt/wt) consisted of unused olive oil (P1) or oil previously used for frying (P2), and the results were compared with those of nonpregnant rats fed unused olive oil (NP1) and olive oil used for frying (NP2). Pregnancy increased ($p<0.01$) food intake, body weight, weight gain, and food efficiency ratio (P2 vs NP2 and P1 vs NP1, respectively), but the treatment of oil included in the diets did not alter these parameters. Gestation significantly increased the serum triglyceride (TG) ($p<0.01$) and total cholesterol (TC) ($p<0.05$) concentrations and diminished that of phospholipids (PH) ($p<0.01$). A significant effect of the type of oil consumed and a pregnancy x oil interaction on Tg and PH levels was observed. The weight of the liver and its fat content increased significantly ($p<0.05$) as a result of pregnancy. Liver TC, TG, and PH increased (approximately 3 times the original values) during gestation, but no significant differences due to the intake of used or unused oil (P2 vs P1) were observed. The results indicate that the consumption of moderately altered olive oil, as the sole source of fat, does not alter the effect of pregnancy on the mothers' weight gain, lipaemia, and hepatic fat composition to any important degree.

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Zusammenfassung Um Informartionen über den Zusammenhang zwischen der Aufnahme von Fett, das zum Fritieren benutzt wurde, und dem Fetthaushalt während Perioden starken Körperaufbaus zu haben, wurde der Einfluß der Aufnahme von frischem Olivenöl (Gehalt polarer Verbindungen, 2 %; Ölsäure 78,9 mg/100 mg Öl, und Linolsäure 7 mg/100 mg Öl) und von Olivenöl, das 15 mal in Folge für das Fritieren von Kartoffeln benutzt worden war (Gehalt polarer Verbindungen 9 %; Ölsäure 75,8 mg/100mg Öl und Linolsäure 6,2 mg/100mg Öl) während der Gravidität, untersucht. Dazu wurden trächtige Wistar Ratten in zwei Gruppen geteilt, die beide eine isokalorische Diät bekamen, deren Fettanteil 15 % von frischem (unbenutztem) (P1) bzw. fritiertem (benutztem) (P2) Olivenöl stammte mit nicht trächtigen Ratten verglichen. Die Gravidität erhöhte ($p<0,01$) die Futteraufnahme, das Körpergewicht, die Gewichtszunahmen und die Futterverwertung. Die Ölqualität beeinflußte dagegen diese Parameter nicht. Während der Gravidität stiegen die Serumwerte der Triglyceride (TG) ($p<0,01$) und des Cholesterins (TC) ($p<0,05$) an, während die der Phosphatide (PH) sanken ($p<0,01$). Ein signifikanter Effekt der Ölqualität und eine Wechselwirkung zwischen Gravidität und Öl wurde für TG und PH festgestellt. Das Gewicht und der

Fettgehalt der Leber der trächtigen Ratten stiegen signifikant an ($p.<0.05$), Leber TC, TG und PH stiegen während der Gravidität (ungefähr um das 3-fache der Ausgangswerte), aber es traten keine signifikanten Unterschiede zwischen der Aufnahme von benutztem und nicht benutztem Öl (P2 vs P1) auf. Die Ergebnisse zeigen, daß die Aufnahme von leicht verdorbenem Olivenöl als alleinige Fettquelle der Nahrung keine besonderen Folgen für die Gravidität hat, was die Gewichtszunahme der Mütter und der Feten, die Lipämie und die Zusammensetzung des Leberfetts betrifft.

Key words Frying – growth – liver lipids – lipaemia – olive oil – pregnancy – rat

Schlüsselwörter Fritierung – Wachstum – Leberfett – Olivenöl – Schwangerschaft – Ratte

Introduction

Hyperlipaemia, characterized chiefly by hyper-triglyceridaemia, occurs during the late stages of gestation in humans (15, 22, 23). In both humans and rats, this condition is associated with an alteration of the endocrine system and a marked increase in maternal plasma lipids (24–26). The pregnant rat is a suitable model for studying human gestation while other species, including monkeys, rabbits and guinea pigs, become hypotriglyceridaemic, particularly in mid-gestation (30, 50).

The consequences of the intake of fatty acids on lipaemia and lipoproteinaemia in humans and laboratory animals have been well defined (6, 10, 20, 21, 40). Nevertheless, the effects of the consumption of oils and fats heated and used for frying on the lipoprotein metabolism of growing animals, or those in other periods of intense anabolism, are less well understood (11,13,43-45).

Polyunsaturated fatty acids, primarily linoleic acid, are known to be hypocholesterolemic (21, 31). The disappearance of polyunsaturated fatty acids (PUFA) during the frying process has been considered the primary cause of the hypercholesterolaemia observed in animals that consume oils used repeatedly for frying potatoes and which undergo a considerable degree of alteration (11, 13). Nevertheless, we have seen no studies which analyse the effect of the intake of heated fats on lipaemia in pregnant rats. Our research group (11, 13) reported higher levels of total and esterified cholesterol in rats fed different oils used for frying than in rats fed unused oils.

Frying has been considered to be a trans-fatty acid source. Mensink and Katan (31) reported that almost all trans-fatty acids make cholesterolemia rise. However, in well controlled frying studies the trans-fatty acid formation was scarce (41).

On the other hand, previous studies (5, 12, 16, 28, 46) have reported that many chemical reactions (e.g., oxidation, polymerization, hydrolysis, cyclization and isomerization) take place during frying, at the expense of degradation, principally of PUFA and monounsaturated fatty acids (MUFA), and lead to a complex mixture of volatile and non-volatile products. Some of these investigations note that as a consequence of the production of these products certain heated oils could present toxicity, possibly affecting development and growth (7, 13, 28, 35, 38-40, 45).

In consequence, there is a need to define the point at which the fats or oils have to be discarded and should not be used anymore for frying purposes or other food preparation. Although many methods are used for the evaluation of fat alteration, e.g., color index, acid value, peroxide value, iodine value, refraction index, K_{270}, the polar content determination is widely considered as one of the most specific methods to evaluate when an oil has to be discarded (5, 16).

The clasical analytical methods: color index, acid value, K_{270} have been condidered useful in the monitoring of oils used for frying, provided that initial values are available (29). They give high and significant correlations with polar content (29). Moreover, polar content displays a high and significant relationship with the altered methyl ester content (12).

Today it is condidered that either a fat or oil used for frying purposes must be discarded when its polar content is higher than 25 % (8). Dobarganes et al. (16) indicated that the determination of altered methyl esters has several advantages with regard to polar content determination because focuses the nutritional information about the amount of altered fatty acids in oil.

Taking into account the above and due to the high consumption of olive oil in Spain and other Mediterranean contries the present study was undertaken: to evaluate the alteration of unreplenished olive oil used 15 times to fry potatoes; to determine the consequences of the consumption of diets containing the aforementioned altered olive oil on food intake and weight gain in pregnant rats; to study the effects of the intake of the aforementioned altered olive oil on lipaemia and hepatic lipids in pregnant rats.

Materials and methods

Frying performance

Olive oil (Carbonell, Córdoba, Spain), and potatoes (Turia, Teruel, Spain) were purchased at a local supermarket. The oil was stored in the dark, below 15 °C, and used as purchased. Domestic deep-fat fryers with 3-l

aluminum vessels were used for frying. The proportion of food to frying oil during the repeated uses was kept at 500 g/3 l by emptying the oil of one of the fryers after each four uses into the other fryers to compensate for the oil absorbed. The oil was used for frying a total of 15 times. Potatoes, sliced ca 2 mm thick, were fried for 8 min. at an initial temperature of 180 °C. Further details of the frying procedure have been reported previously (12, 28, 44).

Animals and diet

Female virgin Wistar rats weighing approximately 160 – 165 g were obtained from the Instituto de Nutrición y Bromatología (CSIC-UCM, Madrid, Spain). The rats were kept in an environmentally controlled chamber at 22.3 ± 1.8 °C and 55-70 % humidity with a 12 h light-dark cycle. All the animals had free access to deionized water. Semi-synthetic diets (Table 1) were prepared according to the recommendations of the National Research Council for laboratory rats (36). Except for the quality of the oils (used or unused olive oil), both diets were designed to be similar in carbohydrate, protein, vitamin, fiber and mineral content. The energy densities of the diets were 1.417 kJ/100 g D.M. in the case of the diet containing unused oil, and 1.413 kJ/100 g D.M. in that of the diet with used oil. The characteristics of some lipid components of both diets are also set out in Table 1.

The study protocol was approved by the Spanish Interministerial Commission of Science and Technology and by an Internal Commission of the Faculty of Pharmacy of the Universidad Complutense of Madrid, Spain.

Experimental procedure

The rats were kept in stainless-steel cages with grid bottoms for a 5-day adaptation period. At the start of the study the animals weighed approximately 176 g. They were mated with adult males from 17.00 h to 9.00 h (2 males per female). The 24-h period immediately following the appearance of copulation plugs was regarded as day 1 of pregnancy. Those rats that did not become pregnant were used as controls.

Four groups of rats of 40 animals each, pregnant (P1 and P2) and nonpregnant (NP1 and NP2), consumed the same diet (with the exception made of the fat source) *ad libitum*, for 22 days. Unused olive oil constituted the fat source for the diet of groups P1 and NP1, whereas groups P2 and NP2 consumed a diet containing olive oil used 15 times to fry potatoes.

Rats were weighed twice a week, and food intake was recorded three times weekly. On days 0,14,18, and 22, after a 12 h fast, 10 randomized pregnant and nonpregnant rats from each diet group were anaesthetised using an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Blood was withdrawn by carotid puncture. Serum was separated from whole blood by centrifugation (1200 x *g* for 20 min. at 4 °C) within 30 min. of collection. The liver was removed and weighed.

Table 1 Composition of the experimental diets containing unused olive oil (diet 1) or olive oil used repeatedly for frying (diet 2)

Ingredient	Amount in diet n° 1 (g/100 g dry matter)	Amount in diet n° 2 (g/100 g dry matter)
Casein[1,2,3], according to a protein content of	13.8	13.9
Olive oil[4], according to a fat content of	14.9	14.8
Wheat starch	30.6	30.4
Mycrocrystalline cellulose[2]	5.0	5.0
Mineral premix[5]	3.3	3.3
Vitamin premix[6]	1.6	1.6
Sucrose[2]	ad 100	ad 100
Oleic acid	10.97±0.2[a]	10.12±0.6[b]
Linoleic acid	0.97±0.2[a]	0.83±0.6[b]
Polar content	0.3±0.1[a]	1.33±0.1[b]
Altered methyl esters	0.27±0.5[a]	0.74±0.6[b]

[1]Acid casein (89.4 % fresh matter, 7.1 moisture) plus 0.2 % DL methionine
[2](Central Ibérica de Drogas, S.A. Madrid. Spain)
[3]D,L-methionine (E. Merck AG, Darmstadt, Germany)
[4]Olive oil (Carbonell. Andújar, Jaén. Spain)
[5]Mineral premix (E. Merck. A.G. Darmstadt, Germany). Composition (in mg/Kg dry weight of food): KI, 0.21; Na_2SeO_3, 0.24; $Na_2CrO_4.4H_20$, 1.58; NaF, 2.43; $CuSO_45H_20$, 24.72; $ZnCO_3$, 25.5; $MnSO_4H_20$, 169.2; $FeSO_47H_20$, 199.0; $MgCO_3$, 769.7; NaCl, 906.3; $MgSO_47H_20$, 2,250; $Na_2PO_42H_20$, 2,930; KCO_3H, 6,100; $CaPO_4H.2H_20$, 8,590; KPO_4H_2, 8200; $CaCO_3$ 10,000
[6]Vitamin mixture (E. Merck A.G. Darmstadt, Germany). Composition (in mg/Kg dry weight of food): Choline dihydrogen citrate, 1,111; folic acid, 1.11; niacin, 22.22; calcium pantothenate, 8.88; riboflavin, 3.33; thiamine, 4.44; pyridoxine HCl, 6.66; retinyl palmitate, 4,400 IU; _-tocopheryl acetate, 33.33 IU; cholecalciferol 22O IU; menadione, 0.055
[5,6]According to National Research Council (1978)
[a,b]Values (mean ± SD of three analyzed samples) in the same row bearing different superscripts are significantly different (Student *t*-test, p.<0.05)

Fatty acid analysis

Samples of the oils were saponified using 0.5 N sodium hydroxide, then methylated following the method of Metcalfe et al. (32). The non-altered and altered fatty acid methyl esters of olive oils were separated by means of silicagel columns (E. Merck Darmstadt, Germany) as previously described (12). The separation of the nonpolar and polar fractions was checked by thin-layer chromatography on 0.5 mm thick 60 F 250 silica gel plates (20 x 20 cm glass). Polar and nonpolar fractions were diluted

Table 2 Food intake and body weight gain of pregnant (P1 and P2) and non-pregnant (NP1 and NP2) rats fed semi-synthetic diets containing unused olive oil and olive oil and olive oil used repeatedly for frying, respectively.

	Pregnant unused olive	Pregnant used oilve	Non-pregnant unused olive	Non-pregnant used olive	Pregnancy effect	Oil effect	Pregnancy-oil interaction
Total food intake (g dry matter)	310.5±11.2	292.9±13.5	260.3±25.0	268.1±23.7	<0.01	NS	<0.01
Body weight (g)	276.7±18.0	272.8±15.0	210.3±9.9	206.8±11.0	<0.01	NS	<o.01
Weight gain (g)	100.7±6.1	95.6±6.3	36.2±7.2	35.9±7.2	<0.01	NS	<0.01
Food efficiency ratio *	0.32±0.05	0.33±0.04	0.14±0.02	0.13±0.03	<0.01	NS	<0.01

Results are the mean ± SD for groups of 10 animals. Pregnancy and oil effects were studied by two way analysis of variance and multiple comparison test. * Food efficiency ratio: 100 x Weight gain (g)/food intake (g dry matter).

50 times (wt/vol) in hexane/diethyl ether 80:20 (vol/vol). Samples were applied as 20 µl spots with a 705 Hamilton microsyringe. Plates were developed with hexane/diethyl ether/acetic acid 80:20:1 (vol/vol/vol) in a lined tank for ca. 25 min. (ca. 17 cm) and then removed, letting the solvent evaporate. The spots were visualized by coating the plates with iodine vapors (5).

The non-altered methyl ester profile was analysed by gas chromatography on a Hewlett-Packard 5890 Series II chromatograph (Palo Alto, California) fitted with a flame ionization detector (FID). Analyses were made on steel column packed with 10 % Supelcoport 2330 on 100/120 Chromosorb WAW 2 m x 1/8 in (≈ 3.2 mm) (Supelco, Barcelona, Spain). The temperature of the column was held for 8 min. at 170 °C and then increased to 240 °C at the rate of 2 °C/min. The temperature of the injector was 250 °C and that of the detector, 300 °C. Sample size was 0.5 µl. The peak areas were measured using a Hewlett-Packard 3396 Series II integrator. The fatty acids were identified by comparing their relative and absolute retention times with those of commercial standards analysed under the same experimental conditions. Chromatographic standards were from Sigma (St. Louis, Missouri).

Polar content

The total polar fraction of the oils was determined by the silica column chromatographic method of Waltking and Wessels (49), with the following modification: An accurately weighed sample of 1 ± 0.01 g of each oil was dissolved in 20 ml hexane/diethyl ether, 87:13 (vol/vol) in the case of the unused oil, and 90:10 (vol/vol) when used oil was analysed. The same proportion of hexane/diethyl ether was used to fill the column and to elute the nonpolar fraction, in order to obtain a sharper separation (12). Two samples each of the unused olive oil and the oil used for frying fifteen times were analysed.

The purity of the polar and nonpolar fractions was checked by thin-layer chromatography, as mentioned with relation to methyl esters in the fatty acid analysis section.

Serum lipid analysis

Total cholesterol (TC) was determined according to the method of Allain et al. (2), triglycerides (Tg) were measured following the Buccolo et al. technique (9) and phospholipids (PH) were calculated using the Takayama et al. procedure (47). The variance coefficients were <4 % in all cases.

Liver lipid analysis

Approximately 0.5 g of liver tissue was homogenized in chloroform-methanol (2:1, vol/vol). The homogenate was centrifuged and decanted, and the pellet was reextracted with chloroform-methanol (2:1). The pooled extracts were then purified twice, according to the method of Folch et al. (18), dry-evaporated under nitrogen atmosphere and redissolved in isopropanol/water (95:5, vol/vol). TC, Tg, and PH were determined according to the enzymatic methods previously indicated for serum lipid analysis. Hepatic TC, Tg, and PH values were determined in pregnant rats only.

Statistical analysis

The results are expressed as mean ± SD. Data from semi-synthetic diets were compared using an unpaired Student's t-test, and results were considered significant at $p<0.05$ (17). The effect of diet (oil type), gestation, and their possible interaction on body weight, weight gain, food efficiency ratio, and serum lipids and liver composition (Tables 2-4) were analysed by analysis of variance (two way-ANOVA). When significant interactions were found, the influence of the day of gestation in separate groups or the effect of oil type was tested by one-way analysis of variance followed by a multiple comparison test. The effect of diet and day of gestation and their interaction on hepatic liver lipids (Table 5) were analysed by two way-ANOVA.

Table 3 Serum lipids (mg/dl) of pregnant and non-pregnant fed semi-synthetic diets containing unused olive oil and olive oil used repeatedly for frying

			DAY 0	DAY 14	DAY 18	DAY 22	Pregnancy effect	Oil effect	Pregnancy-oil interaction
TRYGLICERIDES (mg/dl)	Pregnant	Unused oil	--	63.4±5.4^{a}*	85.3±9.6^{b}**	302.7±10.2^{c}***	<0.01	<0.01	<0.01
		Used oil	--	57.6±2.9^{a}	70.0±10.7^{b}	348.6±11.1^{c}			
	Non pregnant	Unused oil	57.2±9.7^{a}	51.2±8.9^{a}	88.4±3.9^{b}**	87.1±12.0^{b}			
		Used oil	54.2±8.4^{a}	54.3±9.0^{a}	73.7±9.5^{b}	75.0±11.0^{b}			
PHOSPHOLIPIDS (mg/dl)	Pregnant	Unused oil	--	85.8±9.7^{b}***	81.1±8.1^{b}	88.1±3.4^{b}	<0.01	<0.01	<0.05
		Used oil	--	114.2±11.2^{a}	82.8±5.6^{b}	83.1±9.3^{b}			
	Non pregnant	Unused oil	120.5±9.5^{a}	136.2±10.6^{a}**	132.0±8.5^{a}**	144.2±9.8^{b}			
		Used oil	110.5±6.9^{a}	115.9±7.9^{a}	105.6±3.1^{a}	130.2±9.9^{b}			
CHOLESTEROL (mg/dl)	Pregnant	Unused oil	--	59.8±5.9^{a}	62.2±5.4^{a}	80.2±10.9^{b}	<0.05	NS	NS
		Used oil	--	66.1±7.9^{a}	57.0±8.3^{a}	90.3±9.2^{b}			
	Non pregnant	Unused oil	54.4±3.9^{a}	58.7±7.9^{a}	57.3±7.4^{a}	54.8±6.0^{a}			
		Used oil	56.6±4.1^{a}	58.2±8.6^{a}	59.5±7.2^{a}	64.1±8.0^{a}			

Results are mean ± SD for groups of 10 animals. Effect of pregnancy and type of oil consumed were studied by two ways analysis of variance and multiple comparison tests.
*,**,***: p<0.05, p<0.01, p<0.001 between P1 and P2 groups or NP1 and NP2 group at the same study day (one way analysis of variance and multiple comparison test). Data for the same lipid bearing a different superscripts are significantly different (one way analysis of variance and multiple comparison test p.<0.05)

Results

As shown in Table 1, total polar content and altered methyl esters were 4.5 and 3.0 times higher in the used oil diet than in the unused oil one (p<0.05). The concentrations of oleic and linoleic acid were significantly higher (p<0.05) in diet 1 than in diet 2.

Food intake, body weight gain, and food efficiency ratios (weight gain/food intake) are presented in Table 2. Both pregnant rat groups (P1 and P2) displayed greater food intake and body weight gain and higher food efficiency ratios than the control groups (NP1 and NP2). A significant interaction between pregnancy and oil type was found for total food intake, body weight, weight gain, and food efficiency ratio.

Table 3 sets forth the serum lipid values of the pregnant rats and control animals. TC levels increased significantly in groups P1 and P2, especially on day 22 of gestation. TC levels were not modified in groups NP1 and NP2. PH levels were affected by gestation (p<0.01) and the type of oil consumed (p<0.01). This value was hardly change with time in group P1, whereas in group P2 a significant decrease was noted. A significant increase (p<0.05) was detected in groups NP1 and NP2 on day 22 of the study (Table 3). At days 14 and 18 NP1 rats gives higher PH values than NP2 rats. Tg concentrations were affected by pregnancy (p<0.01) and type of oil consumed (p<0.01), and increased throughout gestation. At day 22 P2 rats display significantly higher Tg levels (p<0.001) than P1 rats Tg levels of the control animals also increased significantly (p<0.05). Moreover, a significant interaction between pregnancy and oil type (p<0.01) on Tg was found.

The effects of the consumption of used and unused olive oil on liver weight and composition in the different groups are summarised in Table 4. Liver weight was influenced by pregnancy (p<0.01). The hepatosomatic index, moisture, and protein contents of the hepatic tissue (data not shown) were not significantly affected by pregnancy or oil type. Table 4 indicates that pregnancy did affect (p<0.01) the hepatic fat content, which increased significantly during gestation (p<0.05).

Table 5 shows that hepatic Tg, PH, and TC values increased throughout gestation in both groups of pregnant rats, but were not affected (with the excepcion of TC as mg/g fat) by the type of oil consumed. Hepatic PH levels (e.g., mg/g liver) remained unchanged until the last stage of gestation, in which a significant increase occurred.

Discussion

The results of polar content, as a representative measure of the total alteration of the oil, and those of altered fatty acid methyl esters, indicate that a moderate alteration

Table 4 Analytical data of the livers of pregnant and non-pregnant rats fed semi-synthetic diets containing unused olive oil and olive oil used repeatedly for frying

Study days			Day 0	Day 18	Day 22	Pregnancy effect	Oil effect	Pregnancy-oil interaction
Total weight (g)	Pregnant	Unused oil	5.5±2.2[a]	7.5±2.9[b]	7.7±2.7[b]	<0.01	NS	<0.01
		Used oil	5.6±1.3[a]	7.6±2.2[b]	7.9±1.6[b]			
	Non pregnant	Unused oil	5.4±1.9	ND	5.9±0.7			
		Used oil	5.8±0.9	ND	5.6±0.7			
Fat (% DM)	Pregnant	Unused oil	3.9±1.2[a]	4.9±1.7[b]	5.6±2.0[b]	<0.01	NS	NS
		Used oil	4.0±1.3[a]	4.9±2.0[b]	5.0±1.8[b]			
	Non pregnant	Unused oil	3.8±1.3	ND	4.0± 2.4			
		Used oil	3.9±1.5	ND	4.0±1.7			

Results are mean± SD for groups of 10 animals. ND: not determined. Effect of pregnancy and type of oil consumed were studied by two way analysis of variance and multiple comparison tests. Data in the same row bearing different superscripts are significantly different

Table 5 Hepatic liver data of pregnant rats fed semisynthetic diets containing unused olive oil (P1) and olive oil used repeatedly for frying (P2)

	DAY 0		DAY 18		DAY 22				
	P1	P2	P1	P2	P1	P2	Days gestation effect	Oil effect	Days gestation-oil interaction
CHOLESTEROL									
mg/g liver	1.8±1.3[a]	1.7±1.1[a]	3.5±1.5[b]	3.4±0.3[b]	4.3±0.8[b]	4.8±0.7[b]	<0.01	NS	<0.01
mg/total liver	9.4±0.3[a]	9.6±0.2[a]	28.9±3.1[b]	19.9±2.9[b]	32.9±1.3[bc]	37.4±1.2[c]	<0.01	NS	<0.01
mg/g fat	51.5±7.4[a]	39.6±6.5[a]	71.2±4.2[b]	69.4±4.5[b]	76.6±5.4[b] *	97.1±8.3[c]	<0.01	<0.05	<0.01
TRIGLYCERIDES									
mg/g liver	10.4±3.1[a]	12.6±4.7[a]	16.5±9.1[ab]	15.7±4.2[ab]	24.9±7.6[b]	23.7±2.9[b]	<0.01	NS	<0.01
mg/total liver	69.4±0.6[a]	73.1±5.1[a]	135.3±8.7[b]	93.5±3.5[b]	191.2±20.4[c]	186.2±15.3[c]	<0.01	NS	<0.01
mg/g fat	499.9±12.9[a]	506.5±15.0[a]	395.9±6.2[b]	320.4±8.1[b]	294.6±9.9[c]	288.7±11.7[bc]	<0.01	NS	<0.01
PHOSPHOLIPID									
mg/g liver	16.7±3.2[a]	18.9±4.0[a]	18.8±3.8[a]	20.3±4.6[a]	35.2±3.0[b]	30.7±3.8[b]	<0.05	NS	NS
mg/total liver	89.9±5.6[a]	109.6±3.2[a]	154.7±1.1[b]	120.6±0.9[b]	270.3±3.8[c]	240.6±6.8[c]	<0.05	NS	NS
mg/g fat	490.6±10.2[a]	454.0±9.9[a]	383.5±6.9[b]	414.1±7.5[b]	628.8±9.0[c]	614.2±6.7[c]	<0.01	NS	NS

Results are mean± SD for groups of 10 animals. Effect of day of gestation and type of oil consumed were studied by two way analysis of variance and multiple comparison tests. Data in the same row for the same dietary treatment, bearing different letters are significantly different, *: $p<0.05$ between P1 and P2 at the same study day (one way analysis of variance and multiple comparison tests)

occurs in olive oil used for frying potatoes 15 times. This alteration is related to the decrease in the oleic and linoleic acid contents of the oil. These data coincide with those obtained from other oils after their successive but discontinuous use in frying (11, 12, 28). However, the olive oil displayed a lesser degree of degradation than that observed in other oils used for frying (5, 12, 41). This fact suggests that olive oil performs quite well in the potato-frying process.

Naim et al. (35) suggested that food intake is primarily influenced by factors such as taste, smell, and texture. Oxidation and hydrolysis of the fat affects its palatability, spoiling the taste of the food. Nevertheless, in the present study, the consumption of the diet including used frying oil was not significantly lower that that of the other diet, either in pregnant or nonpregnant animals.

Billek (7) found that the smell and taste of fats containing more than 25 % polar material was acceptable, but that fats were considered unacceptable and deteriorated when their polar content surpassed 30 %. In the present study, the polar content of the used olive oil was 9 mg/100 mg oil, at least partially explaining the good acceptance of the diet in which it was included.

Pregnancy in both rats and humans is characterized by increased food intake and substantial weight gain (1, 14, 27, 34, 42, 48). As the mammalian fetus is not able to oxidise free fatty acids (1, 25), fetal growth is markedly dependent upon the availability of exogenous or endogenous glucose. High-fat feeding alters glucose metabolism during periods of increased glucose demand by inducing a decrease in the glucose turnover rate (4, 23, 25) and a state of insulin resistance characterised by a decreased insulin sensitivity of glucose transport and lipolysis in adipose tissue (14, 15, 20, 25). This state of insulin resistance would explain the lower body weight gains seen in this study with respect to those noted by other authors (27,34).

Marked changes in lipid metabolism take place in mammals during pregnancy and lactation. Late in the gestation period, hypertriglyceridaemia characterizes fed (4, 22, 33) and fasting (23, 25) rats. During pregnancy, an adaptation to prolonged fasting occurs at an accelerated rate, and involves enhanced fat mobilization and associated triglyceridaemia. Hypertriglyceridaemia in pregnancy could be of significance to fetal growth and development, and its mechanism is of interest (4, 27). During pregnancy and in the fed state, triglycerides from the diet, as well as from endogenous sources, enter the circulation in increased amounts. Different authors (4, 20, 22, 33) report that pregnant rats exhibit significantly higher plasmatic concentrations of PH, Tg, and TC than virgin controls.

The effect of consuming oil used repeatedly in frying on lipaemia has previously been studied in rats (11, 13, 43). Our research group (11,43) note that the consumption of diets containing oils used several times for frying induces a moderate increase in plasma TC levels. On day 22, NP2 rats tended to display higher TC and lower Tg levels than NP1 rats.

The increase of serum Tg levels in the nonpregnant rats during the study may be due to the high fat content of the diets (14, 15, 19, 34). Before starting the experiment, rats were fed with a chow diet containing 3.5 % fat, while through the experiment they consumed a 15 % fat-diet.

However, information on the effect of the consumption of such oils on pregnant rat lipaemia is scarce. In the current study, PH levels in group P2 animals were considerably lower than those of group P1 individuals. In contrast, nonpregnant rats (groups NP1 and NP2) displayed increased PH levels. These differences may be due to a modification in the synthesis of hepatic phospholipids, as well as to their inclusion in lipoproteins and/or to a modification in their subsequent metabolism. Some authors (24, 37) have suggested that there may be specific pools of diacylglycerol that are used preferentially for phosphatidylcholine synthesis rather than for the synthesis of phosphatidylethanolamine or triacylglycerol.

Coinciding with our results, Montes et al. (33) found that with respect to serum lipids, no changes in the Tg or TC levels were seen on day 14, but that on day 22 these levels were significantly higher than those of the nonpregnant controls. However, in contrast with the data from the present study, hardly any modification in the serum PH levels was noted by those authors (33).

In accordance with the classic findings reported in the bibliography (3, 15, 23, 33), the livers of the pregnant rats increased in weight, as gestation progressed and body weight increased (Table 4). The data of this study confirm that the consumption of diets with scarcely or moderately altered oil does not appear to affect the liver. In accordance with our data, Rodríguez et al. (40) did not find any significant difference in the liver weight and hepatosomatic index of rats fed olive oil or palm oil used in frying. However, Potteau et al. (38, 39) reported that the consumption of oils used for frying at high temperatures or over long periods of time produces an increase in liver weight. Sánchez-Muniz et al. (44, 45) have noted that rats fed sardines fried in sunflower oil and olive oil used several times for this purpose displayed a higher hepatosomatic index than those fed sardines fried in the first or second usage of the sunflower oil and olive oil, respectively. Potteau et al. (38) indicated that the consumption of oils used repeatedly for frying produced an increased hepatosomatic index.

Our findings in regard to the greater hepatic fat content seen in the pregnant rats agree with those of other studies (3, 33).

TC, Tg, and PH in liver increased 2-3 fold after 22 days of gestation with independently of the oil included in the diet. These data contrast with those of Montes et al. (31) which showed a decrease in mg/g liver Tg and

TC. These authors suggest that their results were in line with a more efficient hepatic Tg release due to reduced activity of lipoprotein lipase in late gestation. However, Montes et al (33) used Purina rat chow which contained 4.5 % fat by weight, while in our experiment rats were fed a diet containing 15 % fat by weight. Thus, the effect of the dietary fat level on liver lipids during gestation should be tested in future studies.

In conclusion, the results of our study indicate that the consumption of a moderately altered oil as the only source of fat hardly has repercussions on the body weight, lipaemia, and hepatic fat composition of pregnant rats. Future studies should investigate the effect of the consumption of more polyunsaturated and/or altered oils on those parameters during pregnancy.

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