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Lipid metabolism in experimental animals

By **Francisco J. Sánchez-Muñiz**[1] and **Carmen Cuesta**[2]

[1] Departamento de Nutrición y Bromatología I (Nutrición).
[2] Instituto de Nutrición y Bromatología (CSIC).
[1,2] Facultad de Farmacia. Universidad Complutense de Madrid. 28040-Madrid. Spain.

SUMMARY

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Publications are scarce in the way in chich metabolic processes are affected by the ingestion of heated fats used to prepare food. Similarly, studies measuring metabolic effects of the consumption on fried food are poorly known. The purpose of this presentation is to summarize information on frying fats and frying foods upon lipid metabolism in experimental animals. Food consumption is equivalent or even higher when oils or the fat content of frying foods are poorly alterated decreasing their acceptability when their alteration degree increase. After 4hr. experiment the digestibility and absorption coefficients of a single dosis of thermooxidized oils were significantly decreased in rats, however the digestive utilization of frying thermooxidized oils included in diets showed very little change in comparison with unused oils by feeding trials on rats. Feeding rats different frying fats induced a slight hypercholesterolemic effect being the magnitude of this effect related to the linoleic decrease in diet produced by frying. However HDL, the main rat-cholesterol carrier, also increased, thus the serum cholesterol/HDL-cholesterol ratio did not change. Results suggest that rats fed frying fats adapt their lipoprotein metabolism increasing the number of HDL particles. Deep fat frying deeply changed the fatty acid composition of foods, being possible to increase their n-9 or n-6 fatty acid and to decrease the saturated fatty acid contents by frying. When olive oil-and sunflower oil-fried sardines were used as the only protein and fat sources of rats-diets in order to prevent the dietary hypercholesterolemia it was provided that both fried-sardine diets showed a powerful check effect on the cholesterol raising effect induced by dietary cholesterol. The negative effect of feeding rats cholesterol plus bovine bile to induce hypercholesterolemia on some cell-damage markers such as lactate dehydrogenase, transaminases, alkaline phosphatase, was significantly check when olive oil- or sunflower oil-fired sardines were used in rats. However, gamma-glutamyltransferase increased when diets containing fried sardines from oils used several times for frying were consumed. In conclusion frying appears to be an useful tool to modify the fatty acid composition of food and the lipoprotein metabolism of consumers, however this culinary procedure has to be gently done to avoid high level of potential toxic compounds.

KEY-WORDS: Cell-damage markers – Digestion – Frying – Lipoprotein.

According to Grande (1) the main nutritional functions of fats are as a: (1) source of energy, (2) source of building materials for cellular structures, (3) source of essential fatty acids, (4) vehicle for fat-soluble vitamins and (5) factor in the control of serum lipids and lipoproteins.

New important functions are recently attributed to fats as a: factor in the control of immune response, in the control of vascular tone and reactivity and in the control and development of thrombogenesis and neoplasms of various locations (2-5). Furthermore, fat contributes to the palatability of the diet and has an important role in cooking and food processing (6).

Literature abounds on unheated fats and the numerous aspects related to their metabolism. In contrast, publications are scarce in the way in which metabolic processes are affected by the ingestion of heated fats which are commonly used to prepare food. Similarly, studies measuring metabolic effects of the consumption of fried food are poorly known. Yet most of the information available on the relationship to various pathologic conditions is derived from epidemiologic or experimental studies in which the lipid intake from the food consumed is calculated from raw food, unprocessed food, whereas most foods are usually consumed only after being subjected to various industrial and, especially, culinary processes. In the Mediterranean diet 50% or more of total fat intake is derived from the cooking fat, usually frying fats (7).

The purpose of this chapter is to summarize a body of research work on frying fats and frying foods and their effect upon lipid and lipoprotein metabolism in experimental animals. Our own research on each of these areas is contempled in this pool of information.

The composition of the diet influence the concentration of cholesterol and lipoproteins in the blood of man and experimental animals. It has been reported repeatedly that fats rich in saturated fatty acids raise serum cholesterol while oils rich in polyunsaturated fatty acids lowered it (1,8).

Studies with experimental animals show that rabbits fed cholesterol-free, semipurified diets respond to dietary fats in a similar way as do humans, at least in qualitative terms (9). However, according to some researchers the hypocholesterolemic effect of polyunsaturated fatty acids seems in human and rabbits is only found in rats when high-cholesterol diets are used.

Cuesta *et al.,* (10,11) found that after a 10-wk experimental period no significant variations were noted in the total, unesterified or esterified cholesterol or triglyceride levels of rats fed a diet containing 15%

palm olein in relation to those fed a diet containing 15% of olive oil.

When serum were fractioned in Very low density lipoproteins (VLDL), Low density lipoproteins (LDL) and High density lipoproteins (HDL) a significant drop was reported in the phospholipids of the three lipoproteins together with a significant rise in the total cholesterol/phospholipids ratio of the VLDL of rats fed palm olein when compared with group fed olive oil. The total cholesterol to protein ratio (a measure of the capacity for cholesterol transport in a lipoprotein) was not significantly affected by dietary saturation, a finding already obtained by other authors (12), although the total cholesterol/protein ratio of the VLDL and LDL of rats fed palm olein-diet was a 30% higher than that of the olive oil-fed group.

There is a plethora of literature describing the changes brought about by unused or unheated oils on lipid metabolism but little has as yet written about fats used in frying.

In an experiment in which rats were fed heated groundnut, palm, soya bean and sunflower oil for l3 weeks, Guillaumin *et al.,*. (13) found that the values for cholesterol, total lipids, triglycerides and free fatty acids were similar to the basal values for the rats fed the same oils unheated. According to Simko *et al.,*. (14) cooked animal fats and sunflower oil raise the level of beta-lipoproteins and serum cholesterol.

On comparing the ingestion of a fat rich in unsaturated fatty acids which had been heated repeatedly with the same fat unheated, Giani *et al.,*. (15) did not note any effects on plasma cholesterol although they did record a substantial decrease in triglycerides.

Tomassi (16) found that total cholesterol and triglycerides dropped when the polymerized, oxidized portion of soya bean oil used in frying was administered.

Kritchevsky and Tepper (17) administered diets for a period of 8 weeks which contained cholesterol and unused olive oil or corn oil, or olive oil and corn oil that had been heated at 215°C for 20 minutes. Consumption of the heated olive oil raised plasma cholesterol in relation to the level obtained with the unheated olive oil, in addition to which it increased triglycerides and decreased phospholipids. However, atheroma formation was much greater in the animals which consumed the used corn oil than in those fed the used olive oil.

We have conducted research in our laboratory into the way in which used frying fats affected lipid metabolism (18,19). Experiments were performed on male weanling Wistar rats weighing an average of 45 g which were split up into groups A, B, C and D. All the groups were fed «ad libitum» from weaning for l0 weeks and were administered the same semi-synthetic diet which only varied in the fat component which accounted for 15% of the diet. Two of the fats administered had been used beforehand 30 times to fry potatoes at 180°C; the volume of fat was kept constant a 5 litres and 100 grams of potatoes were added to each frying. Additionally, we should say that the fat was left to cool to ambient temperature after each frying.

Group A received unheated olive oil, Group B was fed the used olive oil, Group C was fed an unheated solid fat used in the food industry (palm olein) which was not excessively saturated and Group D was given the same solid fat used 30 times to fry potatoes.

Frying produced a decrease in the linoleic acid content of both fats, but higher in olive oil (3.8% vs 7.9%) than in the palm olein (7.1% vs 9.9%).

These variations in the fatty acid profile produced certain changes in lipidaemia and lipoproteinaemia which must also be considered from the point of view of the characteristics of lipid metabolism in the rat.

Consumption of the fat used 30 times to fry potatoes, whether it was the palm olein or the olive oil, produced similar effects on lipidaemia and lipoproteinaemia, summarized as follows: the more unsaturated fats when unheated, the more they increase total and esterified cholesterol when consumed, although they do not affect free cholesterol. According to Glomset and Norum (20), the great ability that rats have to esterify cholesterol is a mechanism which helps to control cholesterolaemia and is related to the increase in the cholesterol transported by HDL.

HDL have been described as lipoproteins which facilitate the clearance of triglyceride-rich lipoproteins. The ingestion of used frying fat also lowers the level of VLDL and so helps to regulate plasma cholesterol possibly by checking its synthesis or more probably by increasing liver uptake of VLDL, thereby checking subsequent cholesterol synthesis.

Importantly, unlike palm olein used in frying, the ingestion of olive oil used in frying significantly raises HDL-cholesterol which acts as a mechanism to halt the increase in cholesterolaemia.

Almost all the experiments carried out in the literature tend to agree with our results (18,19) as regards a rise in total cholesterol and a drop in plasma triglycerides.

Study of plasma lipoproteins revealed that they underwent slight changes only; an increase was noted in LDL-phospholipids of the groups which was fed the palm olein used for frying in relation to the group that was given the unused palm olein (18,19).

As has been previously presented (21) discontinuous frying of potatoes increase the polar content of sunflower oil used for frying, however the increase in the polar and thermoxidized compounds was much lower with a frequent turnover of fresh oil than without turnover.

In other experiments the effect on lipemia was determined in rats fed a diet with 15% of these sunflower oils that had been used in repeated fryings with or whitout turnover of frying oil (22,23). These

used oils contained 19.1% polar material, and 27.1% polar material, respectively. Serum triglycerides, phospholipids, total, free and esterified cholesterol levels in rats fed this diet for four weeks were compared to rats fed a control diet that contained 15% unused sunflower oil (5.1% polar material). All these data were also compared with the ones obtained from chow rats at the start of the experiment (Figure 1).

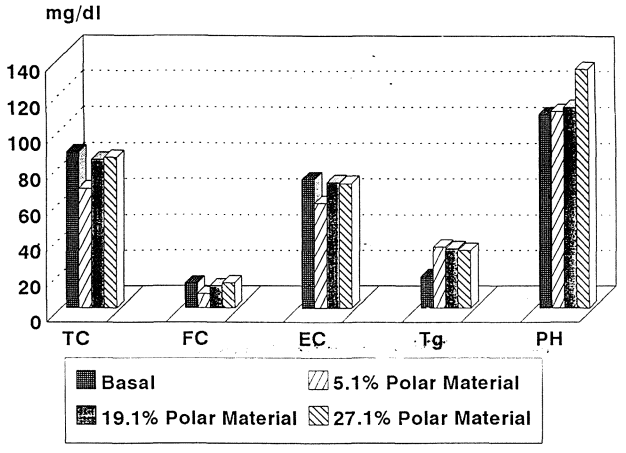

Figure 1
Serum lipids of rats fed diet containing 15% wt unused sunflower oil (5.1% polar material) or used sunflower oils containing (19.1% and 27.1% of polar material, respectively). TC: total cholesterol. FC: free cholesterol. EC: esterified cholesterol. Tg: triglycerides. PH: phospholipids.

Rats fed on diet containing a sunflower oil with a 5.1% polar material showed a decreased in Total cholesterol, esterified cholesterol and free cholesterol with respect basal values, and an increase of triglycerides; however, neither phospholipids nor the esterification index were modified. These data appear to be related to the effect of high amount of linoleic acid on total cholesterol and the effect of age on triglycerides.

No treatment effect was found on triglycerides levels. However, all forms of cholesterol were increased significantly in rats fed the diets containing used sunflower oils. These results suggest a different influence of the ingestion of oil with 5.1% of polar material that diets with 19.1% and 27.1% of polar material on cholesterol metabolism probably due to the decrease produced in linoleic acid during frying and because of the presence of thermooxidized compounds in the frying sunflower oils (22,23).

Since results on lipemia reflect changes on lipoproteinaemia, effects of these diets on plasma lipoprotein composition were evaluated. In this experiment the consumption of frying sunflower oils in respect to unused sunflower oil was followed by non noticeable effects on VLDL composition.

However, LDL of rats fed-frying used sunflower oils appear enriched in total and free cholesterol, as a possible consequence of the decreased intake of linoleic acid, as previously commented (Figure 2).

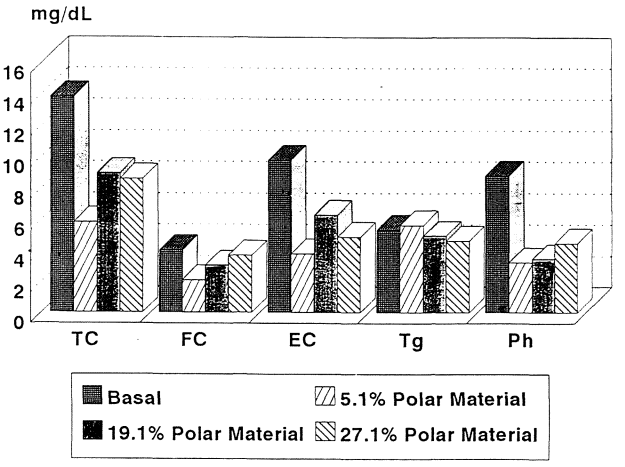

Figure 2
Low density lipoprotein lipids of rats fed diet containing 15% wt unused sunflower oil (5.1% polar material) or used sunflower oils containing (19.1% and 27.1% of polar material, respectively). TC: total cholesterol. FC: free cholesterol. EC: esterified cholesterol. Tg: triglycerides. PH: phospholipids.

The results in HDL, showed the same trend observed in whole serum (Figure 3) and were in agreement with those from other studies in rat fed oils used for fryings (18,19) and are in accordance with the key role that HDL plays on rat lipid metabolism. As it is well known; HDL fraction accounts for the 70-80% of lipoprotein pattern in rats, while VLDL represent only the 15-20% and LDL the 6-10%.

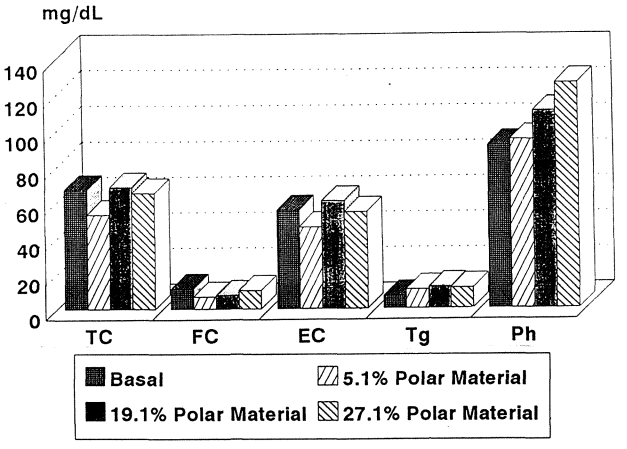

Figure 3
High density lipoprotein lipids of rats fed diet containing 15% wt unused sunflower oil (5.1% polar material) or used sunflower oils containing (19.1% and 27.1% of polar material, respectively). TC: total cholesterol. FC: free cholesterol. EC: esterified cholesterol. Tg: triglycerides. PH: phospholipids.

Steinberg (24) pointed out that lecithin-cholesterol acyltransferase (LCAT) is a key component of the reverse cholesterol transport. In the rat lipid metabolism, HDL accept free cholesterol from VLDL and further it is converted to the ester form by the action of LCAT, an enzyme whose substrate is HDL. Glass *et al.,*. (25) and Steinberg (24) have shown a

great hepatic uptake of cholesterol ester from HDL in the rat. This is a mechanism by which cholesterol may be delivered to the liver. Thus, it would be expected that an accelerated esterification rate in HDL would protect rat against an increase in plasma cholesterol.

Results also shown that the relative lipid composition of serum HDL in rats fed the unused or used sunflower oils did not differ significantly. Thus, the increase in the amount of cholesterol carried by HDL in rat fed used oils could be due to an increase in the number of serum HDL particles. According to Steinberg (24), this fact would guarantee an increased reverse cholesterol transport to the liver and prevent the cholesterol rise in serum.

Although the fat of the diets account for a 15%, they have a different level of thermoxidative alteration, thus the amount of thermoxidative compounds ingested were significantly different in each respective rat group (Figure 4). Peroxidized lipoproteins (lipoproteins containing peroxidized fatty acids) are not cleared from plasma by cell receptor for nonperoxidized lipoproteins and do not participate in the down-regulation of this lipoprotein receptor or the endogenous synthesis of cholesterol removal by scavenger cell receptor in the liver. It may be possible that peroxidized lipids in diets containing high polar compounds levels could be absorbed and incorporated into lipoproteins and, therefore, these compounds would not participate in the nonperoxidized clearance pathway, possibly explaining the higher total, LDL and HDL cholesterol levels in rats fed those diets (Figures 1-3).

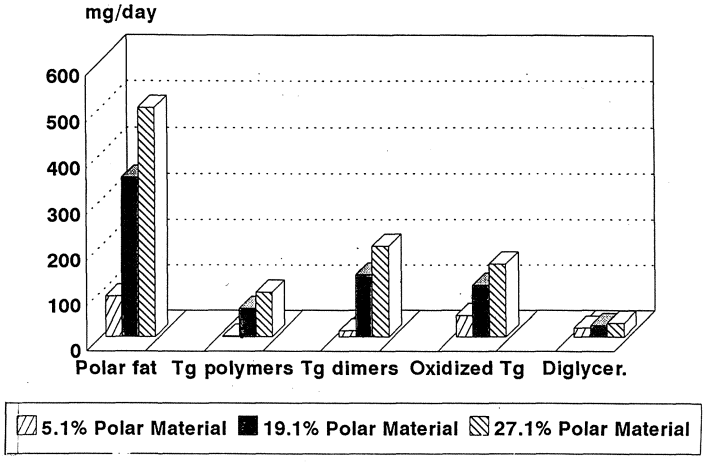

Figure 4
Thermoxidized compounds ingested by rats fed diet containing 15% wt unused sunflower oil (5.1% polar material) or used sunflower oils containing 19.1% and 27.1% of polar material, respectively. Tg: triglicerides. Diglycer.: diglycerides.

During frying interations among components of food and the culinary fat used take place (26). These exchanges and interactions imply that the concentration of some specific fatty acids in the food deeply change.

As it is shown in Figure 5 frying of 600-700 g of sardines (whose head, scales, visceras and backbone were removed were opened into a fan shape, floured in wheat flour) in 3L of oil or fat at 180ºC for 4 minutes produced an exchange between the fat in the sardines and the frying media, which caused significant changes in the fatty acid composition and in the n-6/n-3 ratio of the oily fish.

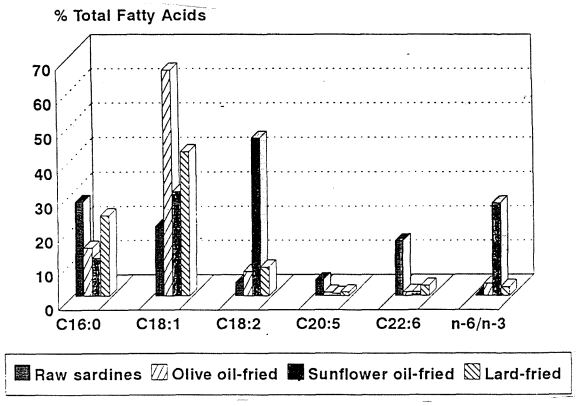

Figure 5
Major fatty acid composition of raw and fried sardines in olive oil, sunflower oil and lard. n-6/n-3: n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids ratio.

The existence of different kinetics for the various fatty acids is shown in this study, where the relative saturated fatty acid content of the sardines decreased when frying in olive or sunflower oils. Oleic acid increased about 50% when fried in sunflower oil, 100% when in lard and 200% in olive oil. Linoleic acid increased about 12 times with sunflower oil . The levels of PUFA n-3 represented by eicosapentaenoic acid (EPA) and docosahexahenoic acid (DHA) decreased in all cases (26).

Oily fish, due to its PUFA n-3 content, have been shown to produce hypolipemic effect (27-29), however this effect would be modified due to the changes produced in this kind of fish when frying.

To test the hypolipemic effect of fried sardines the following experiment was carried out: Growing Wistar rats (approximatly 65 g at the outset) were divided in homogeneous groups. Two groups were fed a mixture of sardines fried in an olive oil that had been used 1 and 2 times or 8-10 times to fry sardines, respectively. Other two groups were fed with a mixture of fried sardines from the 1st and 2nd fryings or from the 8-10th frying in sunflower oil, respectively. These different fried sardines were used as a combined source of protein and fat. Other groups were fed with defatted casein supplemented with 2g/Kg DL-methionine, and olive oil or sunflower oil, respectively. Diets contained roughly 150 g/Kg dry matter (DM) of protein and 170 g/Kg of lipids (fat plus cholesterol). Cholesterol (20 g/Kg) plus 0.5 g/Kg DM bovine bile was used as a serum cholesterol-raising agent (Figure 6).

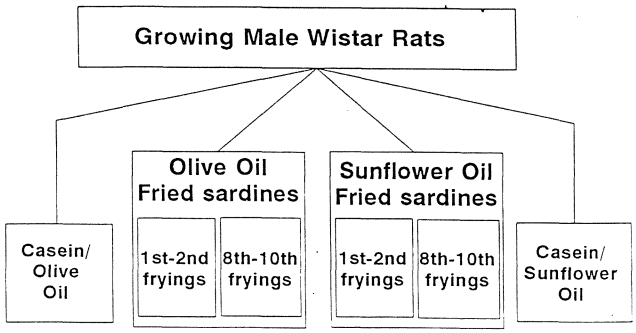

Figure 6
Experimental design. Fried sardines were used as the only source of protein and fat of semisynthetic diets. All diets contained cholesterol and bovine bile as hypercholesterolemic agent.

Except in the groups of sunflower oil fried sardines, total cholesterol increased with regard to its respective basal value after 4-weeks experiment (Table I). Therefore, the cholesterol-raising effect of diets in groups containing casein (13.9 mmol/L and 18.2 mmol/L, respectively) was more noticeable than in sardine groups (about 1 mmol/L, or less). The hypercholesterolemic effect of cholesterol-casein-diets concurs with others. Durand *et al.,* (30) found that replacing half the olive oil in the diet by sardine oil suppressed and even reversed the hypercholesterolemic effect of the diet. The hypocholesterolemic effect of fried sardine-diets could be attributed to its PUFA n-3 content. According to Nestel (27) fish oils may modify the cholesterol-raising effect of dietary cholesterol reducing out flow of VLDL from the liver.

Table I

Serum cholesterol and triglyceride changes in rats fed the experimental diets containing casein plus olive-oil (Diet 1), a mixture of sardines fried in olive-oil in the first and second occasions of use (Diet 2), a mixture of sardines fried in olive-oil in the 8th to 10th occasions of use (Diet 3), casein plus sunflower oil (Diet 4),a mixture of sardines fried in sunflower oil in the first and second occasions of use (Diet 5), a mixture of sardines fried in sunflower oil in the 8th to 10th occasions of use (Diet 6). (Adapted from Sánchez-Muniz *et al.,* 32, 33)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Basal Serum Cholesterol (mmol/l)	2.36 (0.18)	2.43 (0.34)	2.40 (0.09)	2.41 (0.24)	2.28 (0.32)	2.65 (0.35)
Final Serum Cholesterol (mmol/l)	16.25 (2.93)b	3.39 (0.23)**a	3.36 (0.28)**a	20.64 (2.94)b	2.71 (0.19)++	3.07 (0.39)++
Basal Serum Triglycerides (mmol/l)	0.77 (0.21)	1.00 (0.20)	0.90 (0.18)	0.98 (0.15)	1.16 (0.25)	1.05 (0.19)
Final Serum Triglycerides (mmol/l)	0.97 (0.09)a	0.42 (0.08)*a	0.41 (0.05)*a	1.25 (0.15)a	0.47 (0.02)+a	0.50 (0.10)+a

Results are Mean (SEM) for groups of six animals. Asterisks indicate statistical differences between diet 1 and diets 2 or 3 (*, $p<0.05$; **, $p<0.01$). Croisses indicate statistical differences between diet 4 and diets 5 or 6 (+, $p<0.05$; ++, $p<0.01$). Values bearing a letter were significantly different with respect to its respective basal value (a, $p<0.05$; b, $p<0.01$).

Triglyceride levels were lower in sardine diets than in casein diets containing olive oil or sunflower oil. Moreover, in sardine diets triglyceride level decreased with regards to its respective basal value after 4-week experiment (Table I). These results are in agreement with the lowering effect of fish oil indicated elsewhere in the literature (27-29). However, in casein diets triglyceride levels increased after 4 weeks. These results are also in agreement with others (30). Rats fed 15 wt% Max EPA for 2 weeks, had a 40% lower concentration in plasma triglycerides than those fed the same amount of safflower oil; the rates of VLDL-triglyceride and of VLDL-apoprotein B production were significantly reduced by large dosis of fish oils (27,29). In rats liver, additional factors such as diminished fatty acid synthesis and increased fatty acid oxidation have been demonstrated (31).

Our results provide that in comparison with the casein diets, fried sardine diets showed a powerful check effect on the cholesterol-raising effect induced by dietary cholesterol.

Some enzymes were selected and tested as markers of tissue functionality, since 1.– rats were fed with fried sardines from different number of fryings that contained fat with different level of thermoxidized compounds (26) and 2.– different degree of hypercholesterolemia and hepatomegalia were found in rats fed casein or fried sardine diets (32,33). Such marker enzymes: Lactate dehydrogenase (LDH), α-Hydroxybutirate dehydrogenase (α-HBDH), aspartate amino transferase (AST), alanine amino transferase

(ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GammaGT) would be useful to study the possible effects of the consuption of cholesterol-enriched fried sardine diets, containing altered or non altered fat (32,33).

Regarding the enzyme activities, the tendency to decrease serum levels in rats fed fried sardines in relation to the cholesterol-enriched casein diets (above all in the case of sunflower oil) suggests a protective role of cholesterol-enriched fried sardine diets (Table II). Thus, these data may be due to PUFA n-3 of fried sardines, since a decrease of both liver cell injury and disease progression in chronic liver disease due to PUFA n-3 has been described (34), and would be related to a protective effect of PUFA n-3 by modifying tromboxane A_2 production increase observed in hypercholesterolemic animals (3,27). The lower PUFA n-6 intake might produce less arachidonic acid saturation in cell membranes, leading to a decreased eicosanoid production response to several stimuli by decreasing the incidence and severity of some diseases (3). γ-GT is defined as a biliary tract enzyme, which is released as a consequence of drug or toxic agents treatment (35,36). Leonard *et al.,*. (37) found very low serum γ-GT activities in rats, but it could be a good indicator of liver injury . The findings in γ-GT levels might be linked to a damage of biliary tract in rats fed with diets containing fried sardines in oils used several times, with especial mention of sunflower oil (Table II). Further studies are required to elucidate to what extent diets containing different oil-fried-sardines could be employed as protector of cell damage under these experimental conditions.

Table II

Lactate dehydrogenase (LDL), -hydroxybutyrate dehydrogenase (-HBDH), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GammaGT) in rats fed the experimental diets containing casein plus olive-oil (Diet 1), a mixture of sardines fried in olive-oil in the first and second occasions of use (Diet 2), a mixture of sardines fried in olive-oil in the 8th to 10th occasions of use (Diet 3), casein plus sunflower oil (Diet 4),a mixture of sardines fried in sunflower oil in the first and second occasions of use (Diet 5), a mixture of sardines fried in sunflower oil in the 8th to 10th occasions of use (Diet 6). (Adapted from Sánchez-Muniz *et al.,* 30, 31)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
LDH IU/l	779.5 (155.9)	595.8 (116.0)	561.6 (61.3)	3165.2 (586.8)	561.5 (100.5)++	535.0 (75.7)++
α-HBDH IU/l	172.8 (31.0)	124.7 (24.2)	116.0 (10.5)	1153.8 (138.7)	113.7 (20.6)++	109.7 (16.7)++
AST IU/l	78.5 (10.7)	73.7 (9.6)	104.0 (9.3)	214.8 (24.7)	95.5 (13.1)	99.3 (6.7)
ALT IU/l	43.0 (4.6)	33.3 (7.2)*	27.2 (7.1)*	ND	25.8 (3.3)	29.3 (0.9)
ALP IU/l	734.5 (36.1)	617.8 (49.5)	530.4 (26.6)*	ND	ND	ND
GammaGT IU/l	2.7 (0.4)	1.3 (0.5)	14.4 (1.0)**	0.2 (0.2)	4.8 (0.8)+	25.3 (1.7)++

Results are Mean (SEM), and calculated at 30° C. Asterisks indicate statistical differences between diets 1 and diets 2 or 3 (*, $p<0.05$; **, $p<0.01$). Croisses indicate statistical differences between diet 4 and diets 5 or 6 (+, $p<0.05$; ++, $p<0.01$). ND, not determined.

In conclusion frying appears to be an important useful tool to modify the fatty acid composition of food and the lipoprotein metabolism of consumers, however this culinary procedure has to be gently done to avoid a high level of potential toxic compounds.

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