Prevention of Dietary Hypercholesterolemia in Rats Using Sunflower-Oil-Fried Sardines. Effects on Cholesterol and Serum Enzymes

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The acceptability, hypocholesterolemic effect, and possible harmfulness of cholesterol-enriched diets containing different kinds of sunflower-oil-fried sardines were studied in growing Wistar rats. Group 1 was fed a diet containing casein and sunflower oil. For group 2 the only source of protein and fat was a mixture of fried sardines from the first and second fryings in sunflower oil. Group 3 received a mixture of sardines from the 8th to 10th fryings in sunflower oil. Weight gain and food acceptability were lower in group 3. The hypercholesterolemic effect of diet 1 was markedly checked in fried sardine diet groups by reducing the cholesterol content in the lower density lipoproteins (VLDL + LDL). The hepatosomatic index of group 3 appears to be significantly increased. Lactate dehydrogenase, α-hydroxybutyrate dehydrogenase, and aspartate aminotransferase appear to be increased in group 1. These levels were markedly decreased when diets containing sunflower-oil-fried sardines were consumed. Alanine aminotransferase indicates some increase in membrane permeability of liver cells in sardine groups. γ-Glutamyltransferase suggests possible liver damage in rats of group 3.

INTRODUCTION

Some dietary components such as fat have been implicated in many major public health diseases, particularly as risk factors in coronary heart disease (CHD), atherosclerosis, brain stroke, cancer, and allergies (Kinsella et al., 1990; Lands, 1986; Simopoulos et al., 1991).

Several models to induce experimental hypercholesterolemia have been employed; they are nutritional methods widely used (Dolphin, 1981; Durand et al., 1985; Wissler and Vesselinovich, 1987). High amounts of cholesterol in diets are known to increase both serum and liver cholesterol levels and to produce hepatomegalia and steatosis (Dolphin, 1981; Durand et al., 1985; Wissler and Vesselinovitch, 1987).

Recent evidence suggests that polyunsaturated fatty acids (PUFA) of the n-3 (ω-3) configuration, by competing with n-6 (ω-6) fatty acids, may modify the effects of n-6 fatty acids, thereby diminishing the undesirable effects of excessive eicosanoid production (Kinsella et al., 1990; Lands, 1986; Simopoulos et al., 1991). Moreover, n-3 fatty acid supplements have been suggested to reduce myocardial cell injury or liver disease by inhibiting both prostanoid and leukotriene pathways and growth factors (Visser and Meijer, 1990; Weber and Leaf, 1991).

Most of the PUFA n-3 studies have been carried out on fish oil concentrates and on fatty fish (Kinsella et al., 1990; Schouten and Beynen, 1986); however, there is scarce information about the effect of the consumption of fried fish on the prevention of dietary hypercholesterolemia. Recently our group (Sánchez-Muniz et al., 1991) has studied the usefulness of diets containing olive-oil-fried sardines in the prevention of dietary hypercholesterolemia in rats.

Nevertheless, information is scarce on the possible deleterious effect of diets containing oily fish fried in different oils which had been used several times. During successive fryings, and mainly due to the action of temperature, atmospheric oxygen, and water on the foodstuff, hydrolysis and thermoxidative degradation take place in the fat (Blumenthal, 1991; Dobarganes et al., 1988).

Nutritionists are now suggesting increased consumption of unsaturated fats in the diet, particularly rich in n-3 fatty acids. Taking into account the vulnerability to oxidation of these fatty acids, toxicologists, on the other hand, suggest that consumption of oxidized lipids may have severe consequences.

Recognizing the increasing consumption of oily-fish, fried food stuff and thereby of fried oily-fish the aims of this study are (1) to compare the acceptability of diets containing cholesterol and different kinds of sunflower-oil-fried sardines, (2) to investigate the preventive effect of such diets on the alimentary induction of hypercholesterolemia, and (3) to study the possible harmfulness of those diets.

MATERIALS AND METHODS

Materials. Refined sunflower oil and sardines (*Sardina pilchardus* WALB.) were purchased at a local store.

Methods. *Performance of Frying.* The fried sardines included in the diets given to groups 2 and 3 (see Dietary Treatments) were prepared as follows: sardines (600–700 g), head, scales, guts, and backbone removed, were opened into a fan shape, floured in wheat flour (~8%), and fried in sunflower oil for 4 min at 180 °C as normally is done in Spanish homes and bars. Domestic fryers with a capacity of 3 L were used as cooking receptacles. The same oil was used 10 times in succession without any turnover during 3 days to fry the sardines following the experimental scheme set out in previous papers (Sánchez-Muniz et al., 1990; Sánchez-Muniz et al., 1992). Once fried, the sardines were freeze-dried and kept at −20 °C under nitrogen atmosphere until both analyses and diet preparation were made.

Animals and Maintenance. Male Wistar rats [Instituto de Nutrición y Bromatología (CSIC), Facultad de Farmacia, Universidad Complutense de Madrid] weighing approximately 65 g at the outset were randomly divided into groups of six rats each. The animals were housed individually in metabolic cells and were kept in a room under controlled temperature (22.3 ± 1.8 °C) and with a 12-h light/dark cycle.

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Table I. Composition of Semisynthetic Diets Fed to Experimental Groups

ingredient	amount, g/100 g of dry matter food, in diet 1	2	3
protein	14.50[a]	14.31	15.51
fat	14.92[b]	14.37	13.88
wheat starch	34.69	35.43	34.72

major fatty acid	amount, % of total fatty acids, in diet 1	2	3
myristic (C14:0)		3.39	0.68
palmitic (C16:0)	7.80	14.41	9.64
palmitoleic (C16:1, *n*–7)	0.11	3.26	0.59
stearic (C18:0)	4.90	4.68	4.80
oleic (C18:1, *n*–9)	29.92	24.31	28.07
linoleic (C18:2, *n*–6)	54.63	33.10	48.91
eicosapentaenoic (C20:5, *n*–3)		5.41	1.46
docosahexaenoic (C22:6, *n*–3)		4.71	1.91
total saturated fatty acids	11.92	23.22	15.47
PUFA *n*–3[c]		10.76	3.50

[a] Casein plus 0.2% DL-methionine. [b] Refined sunflower oil. Sunflower-oil-fried sardines from the first and second fryings for diet 2 and sunflower-oil-fried sardines from the 8th–10th fryings for diet 3. All other constituents were the same for each diet (see Materials and Methods). [c] PUFA *n*–3: eicosapentaenoic + docosapentaenoic + docosahexaenoic acids.

Dietary Treatments. The rats were fed commercial rat pellets (Panlab SL., Barcelona) after weaning and then switched, without any adaptive period, to experimental diets. Water and food were provided ad libitum over a 4-week experimental period. Group 1 was fed casein supplemented with 20 g kg^{-1} DL-methionine and sunflower oil. Group 2 was fed a mixture of fried sardines from the first and second fryings in sunflower oil as a combined protein/fat source. Group 3 was fed sardines from the 8th to the 10th fryings in sunflower oil as a combined protein/fat source. Diets contained roughly 15% protein, 17% lipid (fat plus cholesterol), and 34% wheat starch. Cholesterol (2%) plus 0.5% bovine bile was used as a serum cholesterol-raising agent (Table I).

Other basic dietary components were as follows: 5% crude fiber (microcrystalline cellulose); 3.17% mineral and 0.12% vitamin supplement [in accordance with the guidelines of the National Research Council (1978)], 22% sucrose, 0.25% BHT, and 0.25% BHA. Caloric densities of diets, estimated according to their protein, fat, and carbohydrates contents and multiplied by 4, 9, and 3.75, respectively (corresponding to 1 kcal to 4.184 kJ), were 1694 kJ/100 g of dry matter for group 1, 1646 kJ/100 g dry of matter for group 2, and 1674 kJ/100 g of dry matter for group 3.

General Procedures. Food intake was checked every day; body weight variations were measured every 2 days. Group 1 was pair-fed with respect to group 2. Blood samples were collected in fasting conditions from the rat's tail vein at the start of the experiment (initial value) and by carotidic puncture 4 weeks later. Serum was separated and stored at −20 °C until analysis. When the experiment ended, the animals were killed and their livers were removed and weighed.

Fatty Acid Analyses. Fish fat was extracted according to the method of Bligh and Dyer (1959), saponificated with 0.5 N of sodium hydroxide, and then methylated following the method of Metcalfe et al. (1966).

The fatty acid methyl esters of olive and sunflower oil and the fish fats were analyzed by gas chromatography. A Hewlett-Packard 5710 chromatograph with a steel column packed with 10% Supelcoport 2330 on 100/120 Chromosorb W AW, 2 m × $^1/_8$ in., was used. The temperature of the column was held for 8 min at 170 °C and then increased to 240 °C at 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 Perkin-Elmer Minigrator M-2 integrator. The fatty acids were identified by comparing their relative and absolute retention times with those of commercial standards. The major fatty acid compositions of diets are presented in Table I.

Serum Analyses. Serum total cholesterol (TC) in both serum and high-density lipoproteins (HDL), after precipitation of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) with phosphotungstic acid and magnesium chloride (Burstein et al., 1970), was analyzed according to the enzymatic cholesterol esterase–cholesterol oxidase method proposed by Boehringer Mannheim, FRG. Creatine kinase (CPK) (EC 2.7.3.2) was determined according to the method of Gerhardt (1987), lactate dehydrogenase (LDH) (EC 1.1.1.27) according to the Wahlefeld (1987) method, α-hydroxybutyrate dehydrogenase (α-HBDH) (EC 1.1.1.27) following the method of Henderson (1987), aspartate aminotransferase (AST or GOT) (EC 2.6.1.1) according to the method of Rej and Hørder (1987), alanine aminotransferase (ALT or GPT) (EC 2.6.1.2) according to the method of Rej et al. (1987), and γ-glutamyltransferase (γ-GT) (EC 2.3.2.2) according to the method of Wahlefeld and Bergmeyer (1987).

A chow group, matched by sex and age and fed commercial pellets, was used as reference for enzymatic determinations. In one rat of both group 3 and the chow group quite a small volume of blood was obtained, thereby not allowing any serum enzyme determinations.

Statistical Analyses. The various groups were compared using the ANOVA one-way and Duncan test. Differences were accepted as significant when $p \leq 0.05$ (Domenech, 1982).

RESULTS AND DISCUSSION

Fatty Acid Diet Composition. The fatty acid content of fried-sardine diets (Table I) clearly indicates that the fat composition of the fried sardines tends to be similar to that of the frying oil. This fact concurs with data of other studies (May et al., 1975; Nawar et al., 1990; Sánchez-Muniz et al., 1992). However, differences in linoleic acid content of fried-sardine diets must be assigned to the higher fat content of the raw sardines used in diet 2 than those used in diet 3, because a higher fat content of the raw fish produces fewer lipid changes during frying (May et al., 1975). Fried sardines used in diets 2 and 3 contained modest amounts of EPA and DHA.

Food Intake. Table II shows the daily food and cholesterol intakes of the experimental groups during 4 weeks. Group 3 showed a 20% lower diet acceptability than group 2. The food intake observed in group 2 was rather low in comparison with the results obtained in other experiments in which olive-oil-fried sardines were used (Sánchez-Muniz et al., 1991; Viejo, 1992).

Moreover, Sánchez-Muniz et al. (1991), using an analogous experimental design, found a 36% lower intake in rats fed fried sardines from the 8th–10th fryings in olive oil than in rats fed fried sardines from the first and second fryings.

Naim et al. (1977) suggested that factors such as taste, smell, and texture primarily influence diet intake. Oxidation and hydrolysis of the fat affect its palatability, spoiling the taste of food even when the food contains only very small quantities of fat.

One of the most obvious impacts of lipid oxidation is on flavor. Undersirable flavors and odors develop in fish oils at very low peroxide values at an early stage of oxidation, even during the induction period. The rate of fish oil oxidation is significantly different from that of other oils. The break in the induction curve is less sharp, and the beginning of the increase in peroxide number occurs earlier (Liston et al., 1963; Stansby, 1967).

The peroxide values of the different kinds of sardines used in the current study (data not shown) were rather low and are not apparently related with the number of occasions the oils were used for frying. As is well-known, hydroperoxides are very unstable and break down to produce many types of secondary products (Frankel, 1991).

Table II. Food and Cholesterol Intakes, Body Weight Gains, Liver Weights, Hepatosomatic Index, and Serum Cholesterol Levels of Rats Fed Experimental Diets

measurement	values for rats fed diet[a]		
	1[b]	2	3
food intake, g/day	9.60	9.60 ± 0.45[a]	7.70 ± 0.33[b]
cholesterol intake, mg/day	192	192 ± 9.03[a]	154 ± 6.53[b]
weight gain, g	90.4 ± 9.03[a]	92.6 ± 8.14[a]	50.2 ± 3.76[b]
liver weight, g	9.70 ± 0.46[a]	9.60 ± 0.40[a]	8.60 ± 0.67[a]
hepatosomatic index[c]	6.33 ± 0.09[a]	6.05 ± 0.09[a]	7.43 ± 0.38[b]
initial serum cholesterol, mg/dL	93.2 ± 9.2[a]	88.2 ± 12.3[a]	102.5 ± 13.6[a]
final serum cholesterol, mg/dL	798.0 ± 113.7[a]	104.8 ± 7.4[b]	118.7 ± 15.2[b]
final HDL cholesterol, mg/dL	40.0 ± 3.9[a]	55.4 ± 8.1[a]	75.5 ± 17.2[a]
final VLDL + LDL cholesterol, mg/dL	758.0 ± 90.3[a]	49.4 ± 7.6[b]	43.2 ± 16.0[b]

[a] Protein and fat sources are identivied in Table I; all other constituents were the same for each diet (see Materials and Methods). Results (mean ± SEM of six rats) bearing different superscripts are significantly different. [b] Pair-fed to group 2. [c] Hepatosomatic index: liver weight/body weight × 100.

Fat oxidation must affect the palatability of fried-sardine diets, causing the different diet acceptabilities that in turn condition the intake of cholesterol and fatty acids (e.g., PUFA *n*–3).

Body Weight Gains. Body weight gains were similar in groups 1 and 2 but lower in group 3 (Table II).

According to Kahl and Hildebrandt (1986), the presence of products of lipid peroxidation in food is undesirable because the nutritional value of food decreases with the destruction of unsaturated fatty acids and other essential food constituents possessing an unsaturated lipid structure, most notably vitamin A. Nielsen et al. (1985) reported that almost all amino acids react with primary and secondary products of oxidized lipids, thereby decreasing the digestive utilization of both protein amino acids and fats, which may affect weight gains.

In 1985 Billeck presented results of a sunflower oil that was used for the industrial production of fish-fingers and that was studied before discarding. This oil was fractionated by column chromatography into unpolar and polar fractions and included in the diet at 20% by weight over a period of 1.5 years; the group fed with the polar fraction exhibited growth retardation.

Rats fed a diet containing 5% highly oxidized cuttle fish oil (peroxide value = 240) showed abrupt decreases in body weight and died within a week (Matsuo, 1954). The consumption of less oxidized oil (peroxide value = 30) was not followed by any noticeable toxic effects. Rats fed a diet containing 5% of this oil gained weight similarly to those on a control diet (Matsuo, 1954).

Sánchez-Muniz et al. (1991) have found significantly lower body weight gain in rats fed with sardines fried in an olive oil used several times for frying sardines than in rats fed with a mixture of fried sardines from the first and second frying uses of the olive oil.

Liver Weight and Hepatosomatic Index. Hepatomegalia and steatosis were found in all livers studied, liver weights being similar in all groups (Table II). Dolphin (1981) showed that high content of cholesterol in thrombogenic diets produced a liver size increase in male rats.

To prevent fish oil oxidation 0.25% BHT and 0.25% BHA were used in diets. According to Powell et al. (1986), hepatomegalia was found at autopsy of rats dosed for 28 days with BHT and was related to the amount of antioxidant administered.

Liver weight of group 3 (~10% lower, but not significantly, than of group 2) does not seem to be related to the final body weight of this group (~27% significantly lower than of group 2); the hepatosomatic index significantly increased in group 3 (Table II). Due to the lower food intake, group 3 ate less cholesterol and antioxidants than the other groups; thus, the differences in the hepatosomatic index might be mainly attributed to the possible alterations in sardines consumed by group 3.

Sánchez-Muniz et al. (1991) have described higher hepatosomatic index in rats fed with sardines that had been fried in an olive oil used several times for frying sardines than in rats fed with sardines from the first and second fryings.

Serum Lipoprotein Cholesterol Levels. TC increased in all groups. However, the cholesterol-raising effect of the diet in group 1 was much more noticeable than in the sardine groups (Table II). The hypercholesterolemic effect of diet consumed by group 1 concurs with findings by Durand et al. (1985) and Sautier et al. (1990), who pointed out that cholesterol–casein diets produced a significant cholesterol increase.

Similar final TC values were obtained by the consumption of fried sardines in all other groups, independent of the cholesterol intake or the content of saturated fatty acids, linoleic acid, or PUFA *n*–3 of the different kinds of fried-sardine diets (Table I).

Durand et al. (1985) found that replacing half of the olive oil in the diet by sardine oil checked and reversed the hypercholesterolemic effect of the diet. Sánchez-Muniz et al. (1991) found that olive-oil-fried sardines markedly decrease the cholesterol-raising effect of cholesterol in the diet. According to Nestel (1990), the usefulness of fish oils in treating hypercholesterolemia is doubtful except when the excess of cholesterol is in VLDL. In rats, dietary cholesterol has been shown to stimulate production of VLDL rich in esterified cholesterol, changing the rat's normal cholesterolemic profile (Durand et al., 1985; Dolphin, 1981). With regard to TC, HDL cholesterol, and VLDL + LDL cholesterol values, fried-sardine diets seem to decrease the cholesterol enrichment of the lower density lipoproteins (VLDL + LDL) seen in rats fed the casein plus sunflower oil diet, probably by reducing outflow of VLDL from the liver.

The tendency observed in animals fed diet 3 to have higher HDL cholesterol levels seems to be related to the 20% lower cholesterol intake of group 3.

The hypocholesterolemic effect of fried-sardine diets could mainly be attributed to its PUFA *n*–3 content, but the protein composition would also change cholesterol levels (Jacques, 1990; Zang and Beynen, 1990). Furthermore, Nestel (1990), in a recent review of *n*–3 fatty acids, wonders whether it is the fish or the fish oil that is protective.

Enzyme Activity Changes. Some enzymes were selected and tested as markers of heart and liver functionalities on the basis of the hypothesis that cholesterol-enriched diets would (1) affect myocardial functionality in the presence of high levels of linoleic acid (sunflower

Table III. Serum Creatine Kinase (CPK), Lactate Dehydrogenase (LDH), α-Hydroxybutyrate Dehydrogenase (α-HBDH), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and γ-Glutamyltransferase (γ-GT) Activities of Rats Fed Experimental Diets

measurement	values for rats fed diet[a] (serum/group)			
	1 (6)	2 (6)	3 (5)	chow (5)
CPK,[b] IU/L	888.5 ± 112.6[a]	572.0 ± 115.0[a]	524.0 ± 135.3[a]	827.4 ± 111.9[a]
LDH,[b] IU/L	3165.2 ± 586.8[a]	561.5 ± 100.5[b]	535.0 ± 75.7[b]	268.4 ± 59.6[c]
α-HBDH,[b] IU/L	1153.8 ± 138.7[a]	113.7 ± 20.6[b]	109.7 ± 16.7[b]	89.7 ± 12.2[b]
AST,[b] IU/L	214.8 ± 24.7[a]	95.5 ± 13.1[b]	99.3 ± 6.7[b]	95.3 ± 13.9[b]
ALT,[b] IU/L	ND[c]	25.8 ± 3.3[a]	29.3 ± 0.9[a]	12.3 ± 3.4[b]
γ-GT,[b] IU/L	0.2 ± 0.2[a]	4.8 ± 0.8[b]	25.3 ± 1.7[c]	1.5 ± 1.2[a]

[a] Protein and fat sources are identified in Table I; all other constituents were the same for each diet (see Materials and Methods). Chow group: group fed with commercial pellets. [b] Calculated at 30 °C. Results (mean ± SEM, for the indicated number of serum samples analyzed) bearing different superscripts are significantly different. [c] ND, not determined.

oil) due to the β-VLDL production and the possible platelet membrane saturation by arachidonic acid that would increase tromboxane A2 production and (2) change the liver and biliary tract functionalities as a consequence of the hepatomegalia produced. On the other hand, such marker enzymes would be useful to study the possible effects (positive or negative) of the consumption of cholesterol-enriched sunflower-oil-fried sardine diets.

CPK values of the different groups are shown in Table III. CPK appears to be elevated in some muscular dystrophies and some myopathies but does not increase in liver diseases (Moss et al., 1986). Results of the current study suggest the lack of a relationship between both cholesterol and PUFA *n*–3 intakes and CPK values. The kind of sardines consumed did not affect these CPK values either.

LDH and α-HBDH activities of the different dietary groups are shown in Table III. Similar activities were found in all groups for LDH and α-HBDH activities, but these activities were significantly higher than those in the chow group. The group consuming casein plus sunflower oil shows 6-fold LDH and 10-fold α-HBDH levels compared to those of the sardine groups. LDH evaluation has been defined as a useful test of malignity on illness, and this enzyme is particularly high in heart, muscle, liver, and kidney tissues. Faster electrophoretic LDH isoenzymes reduce the α-hydroxybutyrate more quickly than lower electrophoretic ones. This α-hydroxybutyrate reduction has been named the α-HBDH activity. The α-HBDH/LDH ratio increases in serum due to the LDH isoenzymes of cardiac origin. In acute hepatitis the α-HBDH increase is lower than the increase of LDH (Cava, 1990).

High intakes of PUFA *n*–6 would produce arachidonic acid saturation in many cell membranes, which in turn will increase the eicosanoid production in response to several stimuli, increasing the incidence and severity of some diseases such as CHD (Kinsella et al., 1990; Lands, 1986; Simopoulos et al., 1991). These results would explain the high activities of LDH and α-HBDH in response to both cholesterol and PUFA *n*–6 intakes in group 1.

However, PUFA *n*–3 replace comparable PUFA *n*–6 in membrane phospholipids and, thereby, change the profile of eicosanoids produced by a tissue upon its activation, resulting in formation of eicosapentaenoic acid-derived eicosanoids, the majority of which have lower biological activities than their arachidonic acid-derived counterparts (Kinsella et al., 1990; Hornstra and Heemskerk, 1991; Simopoulos et al., 1991). This observation would explain lower changes in LDH and α-HBDH in the sunflower-oil-fried sardine group than in group 1. Also, the highest α-HBDH/LDH ratio observed in group 1 (date not shown) suggests a possible heart injury.

AST and ALT activities appear in Table III. As is well-known, both enzymes show an important role in the amino acid metabolism. High levels of AST are found in heart, liver, skeletal muscle, and kidney, whereas ALT is rather abundant in liver. When a myocardial injury is produced, AST activities go up, while ALT increases slightly. Liver alterations produce considerable increases of both enzymes, predominantly on ALT (Balistreri and Shaw, 1986). In the current study AST levels in group 1 appear to be increased in respect to all groups. A similar consideration to the above-described LDH and α-HBDH could be done for AST activities.

However, the ALT values were higher in all sardine groups than in the chow one. According to Hohnadel (1989) high serum enzymes such as ALT are caused by increased membrane permeability of the hepatocyte and the release of enzymes from liver cells into the serum. ALT values seem to be independent of the cholesterol ingested since group 3 ate 20% less cholesterol and had ALT activities similar to those of group 2. However, results in sardine groups suggest the hypothesis that the possible antagonistic effect of dietary cholesterol and dietary PUFA *n*–3 would affect ALT activities by changing the membrane permeability of liver cells. Oxidized fatty acid of sardine diets would also affect the hepatocyte membrane permeability and thereby the presence of higher serum ALT in groups 2 and 3.

Billeck (1985) has pointed out that ALT values were significantly higher after feeding the polar fraction of the heated sunflower oil used for frying fish-fingers than after feeding the unheated oil, the whole heated oil, or the unpolar fraction.

Activities of γ-GT are also shown in Table III. γ-GT is defined as an enzyme of the biliary tract, which is released as a consequence of treatments with drugs, medicaments, and toxic agents (Hohnadel, 1989; Moss et al., 1986). γ-GT is of great utility in the diagnosis of cholecystitis and hepatic necrosis but mainly in the diagnosis of cholestasis (Balistreri and Shaw, 1986; Moss et al., 1986). Leonard et al. (1984) found that serum γ-GT activity is very low in rats but can be a good indictor of liver injuries.

The data of the current study clearly indicate a different induction of this enzyme in the various group diets studied. Group 2 showed a γ-GT activity 3.2 times higher than that of the chow group. Moreover, group 3, consuming sardines fried in oil used several times, shows the highest γ-GT levels. These data indicated a possible hepatotoxic effect of diets containing fried sardines from the 8th–10th fryings. This effect seems to be related with the fat alteration of the sardine diets, the diet of group 3 being very rich in linoleic and having a modest content of PUFA *n*–3 (Table I).

Following suggestions of Powell et al. (1986) and considering that fats from diets of group 3 were more altered than those from the other groups, it could be possible that the normal detoxifying pathway of BHT in

diet 3 was overwhelmed. This would explain the higher hepatosomatic index and the γ-GT induction in this dietary group.

Conclusions. In comparison with a casein–sunflower oil diet, sunflower-oil-fried sardine diets showed a powerful check effect on the cholesterol-raising effect induced by dietary cholesterol. Fried sardine diet acceptability decreases when frying is carried out in oils used several times, resulting in both lower intake and weight gain, leading to an increase of the hepatosomatic index. The theoretical antagonism of dietary cholesterol–dietary PUFA *n*–3 possibly produces changes in ALT activities. The levels of some heart and/or liver cell injury markers (LDH, α-HBDH, and AST) appear to be decreased in cholesterol-enriched sardine diets with respect to the cholesterol-enriched casein plus sunflower oil diet. However, findings in γ-GT values suggest possible biliary tract damage in rats fed with cholesterol and sardines fried in sunflower oil used several times.

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