The response of rats to long-term feeding with diets containing oxidized fat. 2. Biochemical indicators in the serum, liver, and bone mineralization*

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ABSTRACT

Selected biochemical indicators in blood and liver and indicators of femur mineralization were determined in rats fed for eight weeks on diets containing 10% fat with a peroxide value below 5, 40, 80, 120, 160, or 200 meq O_2/kg. The average body weight of the rats was 259.9±10.5 g, the experimental groups contained 12 animals. Fat with a high peroxide value (160 and 200 meq O_2/kg) significantly increased the concentration of malondialdehyde in the serum and slightly in the liver. The most sensitive indicators of the reaction of rats to oxidation of dietary fat were the activity of glutathione peroxidase in erythrocytes, which increased with the rising degree of dietary fat oxidation, i.e. at 40 meq O_2/kg, the activity of serum aspartate aminotransferase and the vitamin A content in the liver, which fell at a peroxide value of 80 meq O_2/kg. A less sensitive indicator was erythrocyte peroxide dismutase activity, which did not increase until fat with a peroxide value of 160 meq O_2/kg was fed. The degree of fat oxidation did not significantly affect the activity of serum alanine aminotransferase, liver enzymes (aspartate aminotransferase, alanine aminotransferase, acid phosphatase, and lactate dehydrogenase), serum and liver triglycerides levels, total cholesterol and HDL cholesterol, or femur mineralization indicators.

KEY WORDS: rats, lipid peroxidation, serum, liver lipids, enzymes, bone mineralization

INTRODUCTION

The results of studies implicating free radicals in the pathogenesis of numerous diseases (Baynes, 1991; Kehrer, 1993) have led to increased interest in the quality of dietary fat in human and animal nutrition. Numerous studies have shown that the composition and degree of oxidation of consumed fat has an adverse effect on liver cell function (Lambert et al., 1998) and lipid metabolism (Sanchez-Muniz et al., 1998). Oxidized dietary fat can disturb many metabolic processes (Corcos Benedetti et al., 1987), among others, by inducing oxidation of membrane lipids (Hayam et al., 1987). If the oxidation of fats is extensive, leading to formation of polymerized and cyclic derivatives, a very distinct adverse impact of this kind of fat on feed intake, growth, and liver secretory function becomes apparent (Lopez-Varela et al., 1995). The harmful consequences of feeding fat with lesser degrees of oxidation without polymers are still little recognized. Many studies have indicated that the detrimental effect of such fat on feed intake and animal growth may be small (Hochgraf et al., 1997; Zduńczyk et al., 2000).

The objective of this study was to investigate the response of rats to long-term (8 weeks) feeding with diets containing fat oxidized to various degrees. Selected biochemical indicators in blood and liver and mineralization indicators in the femur were analyzed as the indicators of metabolic changes caused by oxidized dietary fat.

MATERIAL AND METHODS

Animals and diets

The study was conducted on six groups of rats fed for eight weeks with diets containing 10% fat with peroxide values of under 5, 40, 80, 120, 160, 200 meq O_2/kg. The average body weight of the rats was 259.9 ± 10.5 g, the experimental group numbered 12 animals. Details of the diet composition, conditions of oxidizing fat, feed consumption and utilisation, and growth of rats are given in the preceding paper (Zduńczyk et al., 2000).

Preparation of samples

The rats were anaesthetized with a 20% solution of urethane (Sigma), then blood was sampled and the liver and femur were isolated. The activities of superoxide dismutase (SOD) and glutathione peroxidase (PGx) were assayed immediately in erythrocytes of blood sampled into heparinized tubes. Unheparinized blood was left at room temperature to coagulate and the serum was removed by

centrifugation at 2500 g/min for 15 min. Serum and isolated liver (weighed and frozen in liquid nitrogen) were stored at - 40°C until analysis.

Analytical methods

Superoxide dismutase (SOD) and glutathione peroxidase (PGx) activities were assayed using kits from Randox Laboratories Ltd. (Cat. No. SD125 and No. RS505, respectively). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as the serum triglycerides (TAG), total cholesterol (TC) and HDL fractions were determined using kits from Alpha Diagnostics. The content of malondialdehyde (MDA) in serum was determined spectrophotometrically using a Beckman (model DV 7500) spectrophotometer, by analyzing the reaction products with tiobarbituric acid (TBA) by the Yaga method (1980). In order to assay TAG, total and HDL cholesterol fractions in the liver, preliminary extraction of lipids with a 2:1 mixture of chloroform-methanol was carried out according to Folch et al. (1957), then as described by Prasad and Saraswathy (1996). The MDA content in liver tissue was determined spectrophotometrically using the method of Uchiyama and Mihara (1978).

The activities of aspartate (AST) and alanine (ALT) aminotransferase, alkaline phosphatase (AP) and lactate dehydrogenase (LDH) in the liver were determined using Alpha Diagnostics kits, after preparation of a liver homogenate according to the method of Rivetz and Bogin (1982), Farnson et al. (1985), and Webb et al. (1991). The vitamin A content of liver was determined by HPLC according to Cuesta Sanz and Castro Santa-Gruz (1986).

Femur mineralization was evaluated using two indicators, Ca and P contents and breaking resistance. The breaking force was determined using an INSTRON (model 1011) apparatus. After ashing the bone at a temperature of 450°C, the calcium content of the ash was determined using a diagnostic kit from Alpha Diagnostics, while phosphorus was determined by the method of Fiske and Subbarow (Tomaszewski, 1970).

The results were subjected to statistical analysis using one-way analysis of variance and the Duncan multiple range test.

RESULTS AND DISCUSSION

The blood triglyceride levels (TAG) and total serum cholesterol (TC) were similar in all groups (Table 1). A significant reduction of the serum HDL fraction from 57.8 mg/dl in the control group (I) to 51.1-51.4 mg/dl in the groups receiving diets containing fats with peroxide value of 160 and 200 meq O_2/kg (groups V and VI) was found. The TAG content (from 64.1 to 65.9 mg/dl) and serum cholesterol

(from 73.0 to 78.1 mg/dl) were similar to that in rats fed diets with a similar amount of fat (9%) from various sources (Kirchgessner et al., 1993). In the experiment of Sanchez-Muniz et al. (1998) a 15% addition of oxidized sunflower oil to diets caused a significant rise in total serum cholesterol in comparison with a group fed non-oxidized oil. Also in the experiment of Hochgraf et al. (1997) highly oxidized dietary fat (1300 meq O_2/kg fat) elevated serum cholesterol levels in rats.

Rats fed diets containing fat with peroxide value of 160 and 200 meq O_2/kg (groups V and VI) had significantly higher serum malondialdehyde (MDA) concentrations (Table 1), most likely as the result of absorption of an increased amount of peroxide degradation products.

TABLE 1

Triglycerides (TAG), total cholesterol (TC), HDL-cholesterol (HDL-C) and malondialdehyde (MDA) content in serum of rats[1]

	Group[2]						SEM
	I	II	III	IV	V	VI	
TAG, mg/dl	68.5	64.1	65.9	65.5	65.9	65.4	1.256
TC, mg/dl	78.1	73.0	74.2	75.0	75.7	74.8	1.202
HDL-C, mg/dl	57.8	52.7	53.6	55.7	51.1	51.4	0.759
MDA, µg/ml	0.401[ABbc]	0.361[Bc]	0.405[ABbc]	0.395[ABbc]	0.494[ABab]	0.506[Aa]	0.014

[1] values represent means and standard error of the mean (SEM), n = 12
[2] peroxide value of fat introduced into diets of particular groups was: below 5, 40, 80, 120 160 and 200 meq O_2/kg, respectively
[a, b] – P<0.05
[A, B] – P<0.01

Table 2 presents data on the activity of selected enzymes in serum and erythrocytes. In rats fed diets containing fat oxidized to 80-200 meq O_2/kg (groups III-VI), a significant reduction of serum aspartate aminotransferase (AST) activity from 204.9 U/dl in the control group to 164.6-176.1 U/dl was found. The activity of alanine aminotransferase (ALT) did not differ among groups and ranged from 44.5 to 50.3 U/dl. The activities of AST and ALT in the serum were close to the values obtained by Nalbone et al. (1989), who fed rats diets with a high (17%) fat content.

Greater fat oxidation caused a distinct rise in the activity of superoxide dismutase (SOD) and glutathione peroxidase (PGx) in erythrocytes. In group VI, in which rats were fed a diet containing fat with a peroxide value of 200 meq O_2/kg, PGx in erythrocytes demonstrated an activity of 1021 U/dl, SOD of 429 U/ml, while in the control group, the respective activities of these enzymes were 609 U/dl and 311 U/ml. Hayam et al. (1995) also found higher SOD and PGx activities in erythrocytes of rats fed for 7 weeks soyabean oil oxidized to a pero-

TABLE 2

The activity of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (PGx)[1]

	Group[2]						SEM
	I	II	III	IV	V	VI	
AST, U/dl	204.9[a]	180.6[ab]	176.1[b]	170.9[b]	164.9[b]	164.6[b]	3.74
ALT, U/dl	44.5	45.5	46.3	50.3	49.8	50.1	1.62
PGx, U/dl	609.1[Aa]	835.3[ABb]	851.2[ABb]	817.0[ABb]	905.3[Bb]	1020.9[Bb]	30.39
SOD, U/ml	311.3[Aa]	345.1[ABa]	382.5[ABab]	370.0[ABab]	426.0[Bb]	428.7[Bb]	10.91

[1] values represent means and standard error of the mean (SEM), n = 12

[2] peroxide value of fat introduced into diets of particular groups was: below 5, 40, 80, 120 160 and 200 meq O_2/kg, respectively

[a, b] – P<0.05; [A, B] – P<0.01

xide value of 420 meq O_2/kg, in comparison with a group fed non-oxidized oil. These authors also observed a concomitant increase in the activities of serum alanine (ALT) and aspartate (AST) aminotransferases.

The results given in Table 2 indicate that the most sensitive indicators of a biological reaction of rats to oxidation of dietary fat was glutathione peroxidase, whose activity increased when the peroxide value of dietary fat was only 40 meq O_2/kg, and aspartate aminotransferase (activity lowered at a peroxide value of 80 meq O_2/kg), while the least sensitive indicator was superoxide dismutase, whose activity did not decline until the peroxide value of dietary fat reached 160 meq O_2/kg. The degree of dietary fat oxidation did not affect the activity of serum alanine aminotransferase.

Increasing the oxidation of dietary fat did not cause changes in the weight of the liver or its fat, triglyceride, or cholesterol contents (Table 3). The triglyceride content ranged from 12.2 to 14.7 mg/g, total cholesterol, 3.1-3.2 mg/g liver, and was similar to the content of these compounds in the liver of rats that were fed 10% non-oxidized animal fat or sunflower seed oil (Nagata et al., 1980; Wachnik et al., 1989). The content of HDL-cholesterol in the liver of animals fed diets containing the most highly oxidized fat (200 meq O_2/kg) was 1.55 mg/g and was lower than in the remaining groups (1.80-1.96 mg/g). The MDA content of the liver was highest in rats fed the diets with the highest level of fat oxidation (groups V and VI, difference nonsignificant). In the experiments of Sanchez-Muniz et al. (1998) and Hochgraf et al. (1997) feeding rats diets containing highly oxidized fat caused a significant increase in the MDA content of the liver. In the studies of Hochgraf et al. (1997) a concomitant significant fall in the cholesterol content of the liver was found as compared with animals fed unoxidized fat, whereas in the experiment of Corcos Benedetti et al. (1987), replacing part of fresh fat with oxi-

Liver weight, triglycerides (TAG), total cholesterol, (TC), HDL-cholesterol (HDL-C) and malondialdehyde (MDA) content in the liver[1]

	Group[2]						SEM
	I	II	III	IV	V	VI	
Liver weight, g/100 BW	3.43	3.46	3.36	3.50	3.45	3.53	0.026
Fat content, %	4.68	4.37	4.61	4.50	4.73	4.55	0.035
TAG, mg/g	13.77	12.17	14.72	14.22	13.32	13.94	0.339
TC, mg/g	3.12	3.21	3.18	3.05	3.11	3.07	0.042
HDL-C, mg/g	1.89	1.96	1.83	1.80	1.80	1.55	0.054
MDA, µg/g	6.39	6.19	6.21	6.52	6.56	6.76	0.075

[1] values represent means and standard error of the mean (SEM), n = 12

[2] peroxide value of fat introduced into diets of particular groups was: below 5, 40, 80, 120 160 and 200 meq O_2/kg, respectively

dized and polymerized soyabean oil did not cause significant changes in serum and liver MDA contents.

Table 4 presents results pertaining to the activity of selected liver enzymes. No differences were found in the activity of alanine and aspartate aminotransferases, but there was a tendency towards a declining activity of alkaline phosphatase, from 3.97 U/g in group I to 3.05-3.11 U/g in groups IV-VI, i.e. as the dietary fat became more oxidized. In the case of LDH, a reverse relationship was found, i.e. the activity of this enzyme rose as the diet contained more oxidized fat, but the differences among groups did not reach statistical significance. Feeding rats oxidized fat caused a significant reduction in the vitamin A content of the liver from

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH) and vitamin A content in liver[1]

	Group[2]						SEM
	I	II	III	IV	V	VI	
AST, U/g	108.6	114.4	103.9	111.1	111.6	110.8	4.395
ALT, U/g	73.6	70.5	76.0	70.7	75.6	72.4	2.193
AP, U/g	3.97	3.61	3.18	3.05	3.11	3.07	0.042
LDH, U/g	439.66	434.25	453.78	457.51	475.86	461.51	10.112
Vitamin A, mg/100 g	85.07[Aa]	83.67[ABa]	67.91[BCb]	61.92[Cbc]	63.56[Cbc]	52.94[Cc]	2.129

[1] values represent means and standard error of the mean (SEM), n = 12

[2] peroxide value of fat introduced into diets of particular groups was: below 5, 40, 80, 120 160 and 200 meq O_2/kg, respectively

[a,b] – P<0.05; [ABC] – P<0.01

85.1 mg/100 g in group I to 52.9 mg/100 g in group VI; a significant reduction was already noted after feeding the diet containing fat oxidized to a peroxide value of 80 meq O_2/kg. A similar decline in vitamin A content in the liver of rats was found in a study by Corcos Benedetti et al. (1987), in which part of fresh fat was replaced with a oxidized fraction of soyabean oil.

No unequivocal dependence between the degree of oxidation of dietary fat and the calcium and phosphorous contents of the femur in rats was found (Table 5). The breaking strength of the femur was similar in groups I-V, the lowest in group VI, but these differences were not statistically significant. The lowest breaking strength of the femur in group VI was in line with the lowest calcium and phosphorous content in bone tissue. In this group fat digestibility was also significantly worse (Zduńczyk et al., 2000), which could have caused the increased excretion of calcium soaps in faeces (Matyka and Korol, 1991).

TABLE 5

Calcium and phosphorus content (% of bone ash) and breaking force of the rat femur[1]

	Degree of fat oxidation, meq O_2/kg						SEM
	5	40	80	120	160	200	
Ca	38.34[ab]	38.14[ab]	38.83[a]	37.24[b]	38.00[ab]	36.94[b]	0.203
P	16.41	16.30	16.13	16.51	16.65	16.11	0.070
Femur breaking force (Newton)	84.00	84.49	85.87	86.50	85.85	81.87	1.229

[1] values represent means and standard error of the mean (SEM), n = 12

[2] peroxide value of fat introduced into diets of particular groups was: below 5, 40, 80, 120 160 and 200 meq O_2/kg, respectively

[a, b] – P<0.05

CONCLUSIONS

The results of this study show that the most sensitive indicators of a response of rats to oxidation of dietary fat are erythrocyte glutathione peroxidase activity, which increased with rising degree of dietary fat oxidation, i.e. when fat with a peroxide value of 40 meq O_2/kg was fed, and the activity of serum aspartate aminotransferase and vitamin A content of the liver (reduced when the peroxide value of dietary fat was 80 meq O_2/kg). A less sensitive indicator was erythrocyte superoxide dismutase activity, which did not increase until the peroxide value of dietary fat reached 160 meq O_2/kg. The degree of dietary fat oxidation did not have a significant effect on the activity of serum alanine aminotransferase, nor on the activities of AST, ALT, AP, LDH in the liver, serum and liver lipid profiles, or bone mineralization indicators.

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STRESZCZENIE

Reakcja szczurów na długotrwałe karmienie dietami zawierającymi utleniony tłuszcz. 2. Wskaźniki biochemiczne surowicy i wątroby oraz mineralizacja kości

Oznaczono wybrane wskaźniki biochemiczne w krwi i wątrobie oraz mineralizację kości u szczurów żywionych przez 8 tygodni dietami zawierającymi 10% tłuszczu o liczbie nadtlenkowej 5, 40, 80, 120, 160 i 200 meq O_2/kg. Średnia masa ciała szczurów wynosiła 259,9 g; grupy doświadczalne składały się z 12 zwierząt. Tłuszcz o wysokiej liczbie nadtlenkowej (160 i 200 meq O_2/kg) powodował istotny wzrost koncentracji dialdehydu malonowego w surowicy krwi i niewielki wzrost w wątrobie. Najbardziej czułym wskaźnikiem reakcji szczurów na utlenienie tłuszczu diety była aktywność peroksydazy glutationowej w erytrocytach, która zwiększała się już przy najmniejszym stopniu utlenienia tłuszczu, tj. przy 40 meq O_2/kg, a także aktywność aminotransferazy asparaginianowej w surowicy i zawartość witaminy A w wątrobie, które obniżały się przy liczbie nadtlenkowej 80 meq O_2/kg. Stopień utlenienia tłuszczu nie miał istotnego wpływu na aktywność aminotransferazy alaninowej w surowicy, enzymy wątrobowe (transferaza asparaginianowa, aminotransferaza alaninowa, kwaśna fosfataza i dehydrogenaza kwasu mlekowego), poziom trójglicerydów w surowicy krwi i wątrobie, ogólną zawartość choresterolu i frakcję HDL, oraz na wskaźniki mineralizacji kości.