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RESPONSE OF ANTIOXIDANT SYSTEM IN RATS TO DIETARY FAT AND PHYSICAL ACTIVITY

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Abstract : The influence of feeding hydrogenated fat (HF) or refined peanut oil (PO) diet and regular swimming exercise on hepatic and skeletal muscle antioxidant enzymes i.e., catalase and glutathione peroxidase (GPX) as well as tissue lipid peroxidation was investigated in male Wistar rats. Two groups of rats were fed diet with HF or PO as the only fat source. Both the groups were further divided into 4 subgroups each according to physical activity : Two each for sedentary (HFS3, POS3) and two for swimming, HFE3 and POE3 [30 minutes a day, 6 days a week, for 3 months or HFS6, POS6, HFE6 and POE6 for 6 months. A mild increase in lipid peroxidation was observed in both liver and muscle tissues of PO-diet fed rats of E1. Swimming augmented further the lipid peroxidation in liver. GSH level was decreased in the liver of exercising rats, in contrast, it was increased in skeletal muscle by 70% in POE6 and 26% in HFE6. Compared to POS3 swimming elevated GPX activity of about 70% in liver from POE3 as well as about 60% in skeletal muscle from POE3 and POE6. The catalase activity was enhanced in muscle of HFE3 and POE3 by 250% while it remained unaltered in rats of 6 months. These data indicate an adaptive-response of antioxidant enzymes in liver and skeletal muscle to reduce oxidative stress induced by unsaturated fat (PO) and exercise.

Key words : antioxidants catalase glutathione glutathione peroxidase lipid peroxidation

INTRODUCTION

The use of vegetable oils rich in polyunsaturated fatty acids (PUFA) has been recommended to lessen the deleterious effects of saturated fatty acids by lowering of serum cholesterol and prevention of development of atherosclerosis. Nevertheless, because PUFA are substrates for free radical reactions, a high intake of PUFA might overwhelm the normal antioxidant defences due to overproduction of reactive oxygen species (ROS) leading to peroxidative damage of biological membranes (1–3), more so in the presence of induced oxidative stress/insults. Diet and physical exercise are the two important factors linked to free radical generation and the antioxidant

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defence system. Diets rich in PUFA and strenuous exercise were demonstrated to augment production of ROS (2–4). On the other hand, diet rich in either monounsaturated fatty acid (MUFA) or saturated fatty acid (SFA) did not result in any enhancement of lipid peroxidation (1). This has been attributed to the resistant nature of oleic acid, predominant in MUFA diet, to oxidation and SFA being a non-substrate for peroxidation (1). However, little information is available in the literature regarding the influence of peanut oil-diet on the tissue lipid peroxidation and antioxidant enzymes. It is also reported that regular exercise of moderate intensity may be beneficial to an individual's health due to probable enhanced antioxidant defence system to offset the barrage of ROS generated during exercise (5). However, much has not been documented in the literature on the interactions between physical exercise and the type of dietary fat on the tissue antioxidant system. Hence, the present study was undertaken to assess the influence of the type of dietary fat (vegetable hydrogenated fat for SFA and peanut oil for MUFA rich) and regular exercise on non-enzymatic antioxidants such as glutathione (GSH) and enzymatic antioxidants such as catalase (CAT) and glutathione peroxidase (GPX) in liver and skeletal muscle.

METHODS

The Meals-Ready-to-Eat (MRE) ration developed at Defence Food Research Laboratory, Mysore for Indian Armed forces was used for the study (6). Each ration pack consisted of sooji halwa, vegetable pulav, chapati, potato peas curry, soft bar (made up of sugar, liquid glucose, vanaspati, milk powder, emulsifier and flavor having total fat about 1% dry wt.), tealeaves, skimmed milk powder and sugar. The ration was either prepared with vegetable hydrogenated fat, vanaspati (HF) as source of SFA or with refined peanut oil (PO) as MUFA-rich source. Carbohydrates, fat and proteins contributed 58, 27 and 15% respectively, of the total energy in this diet. The fatty acid profile of the Indian vanaspati (Dalda) and peanut oil was analyzed by gas chromatography and is given in Table I. The general chemicals used were of AR grade obtained from either E. Merck or SRL (India).

Sixty-four male Wistar rats about 2 months old, bred in the animal house of Defence Food Research Laboratory, Mysore, weighing 140±10 g were used throughout the experiments. They were fed on commercially prepared pellet diet before the commencement of the experiment. The Institutional Animal Ethics Committee had approved this animal experimentation. The rats were housed individually in stainless steel cages under a 12-hr photoperiod and fed on diet prepared by mincing and thoroughly mixing the contents of the MRE ration. The diet was prepared on every 3rd day and stored in polyester/aluminium foil/cast-polypropylene (PEP) pouches at 4°C. It was fed ad libitum to the rats after warming to room temperature. The rats were also provided with water ad libitum.

Experiment protocol

After being on the MRE ration diet for 2 weeks the rats were grouped randomly. Swimming was administered 30 min a day,

6 days per week for either 3 months or 6 months to the groups subjected to exercise. To familiarize them to water immersion, the rats were made to swim, in the first week, for 10 min initially and gradually increased to 30 min. In experiment 1, 32 rats were divided into four groups of 8 each. Group 1 and 3 were fed on HF diet while group 2 and 4 on PO diet. Rats in group 3 (HFE3) and 4 (POE3) were subjected to swimming for 3 months. Group 1 (HFS3) and 2 (POS3) served as sedentary controls for exercising groups. In experiment 2 another 32 rats were similarly divided into 4 groups however, group 7 and 8 (HFE6, POE6) rats were allowed to swim for 6 months and group 5 and 6 (HFS6, POS6) were respective controls. Parallel controls were run throughout the experiment period to eliminate the influence of age. The body weight of all rats was recorded weekly.

At the end of the experimental period, the animals were allowed to rest for about 20 hours after the last bout of exercise and killed by cervical dislocation between 9–10 am. The liver and muscle tissue of left hind limb were excised, rinsed in ice-cold physiological saline and stored at –20°C pending analysis.

Biochemical analysis

All biochemical analyses were carried out at 0–4°C. A 50% homogenate of liver and muscle, separately, was prepared in 5 ml of cold physiological saline and centrifuged at 100 xg. The supernatant was used for determining the activities of CAT, GPX, level of glutathione (GSH) and malondialdehyde (MDA). The activity of CAT was determined by spectrophotometry (Shimadzu UV/Visible spectrophotometer) while the activity of GPX was estimated by spectrofluorimetry (Elico spectrofluorimeter). CAT activity was measured by monitoring the decomposition of H_2O_2 at 240 nm as described by Cohen at al (7). GPX was determined according to the method of Weiss et al (8) using NADPH and H_2O_2 as substrates. Tissue GSH content was determined by the method of Ellman (9) using 5, 5'-dithiobis-2-nitrobenzoic acid reagent. Lipid peroxidation in tissues was estimated by measuring MDA as described by Girotti et al (10).

Statistical analysis

The data shown are mean ± SE. A two-way ANOVA was performed for the effects of dietary fat and physical exercise and significant ($P<0.05$) interaction terms were evaluated by Duncan's multiple range tests while Student's t-test was employed to compare respective groups between 3 months and 6 months and level of significance was set at $P<0.05$.

RESULTS

Rats from all groups freely consumed the mixed diet of MRE ration items. There was no appreciable difference in food consumption among the rats of different groups. The body weight of rats of various groups at the conclusion of the experiment did not alter significantly (data not shown).

Malondialdehyde

The level of MDA was higher in the liver

by about 10% in rats of POS3 compared to those of HFS3. In exercising rats it was increased by 50% in HFE3 and 55% in POE3 and 5–10% in HFE6 and POE6 compared to respective sedentary rats. Similarly, in muscle an increase of about 16% was observed only in POS3 rats compared to HFS3. However, exercise found to increase MDA level by 16% in HFE3 and 12% in POE3 and 7% in HFE6 and 20% in POE6 rats (Fig. 1).

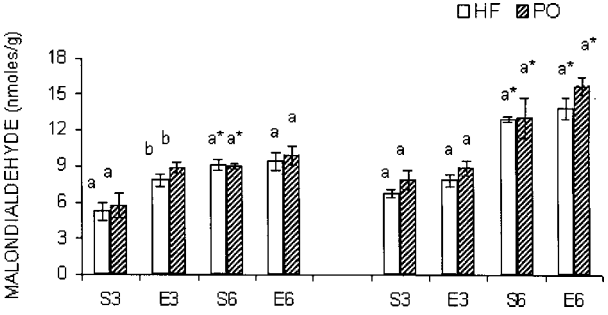

Fig. 1: Malondialdehyde in liver (left panel) and skeletal muscle (right panel) in nmoles/g.

Values (mean±SE) with different alphaphets are significantly different (P<0.05).

*Statistically significant compared to respective group of 3 months (P<0.05).

Glutathione

The levels of GSH in liver and skeletal muscle are shown in Fig. 2. There was an increase of 5–10% in GSH level in livers of POS3 and POS6 rats. But it was decreased in exercising rats by 13–15% in POE3 and 17–20% in POE6. In skeletal muscle, it was decreased by 5% in POS3 rats compared to HFS3 (except in muscle of sedentary group of 6 months). However, the level of GSH was increased significantly (about 70%) in muscle of exercising rats especially in POE6 rats.

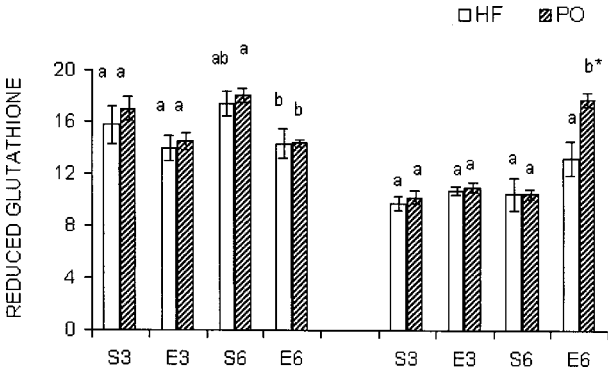

Fig. 2: Reduced glutathione in liver (left panel, umole/g) and skeletal muscle (right panel, x4 nmoles/g).

Values (mean±SE) with different alphaphets are significantly different (P<0.05).

*Statistically significant compared to respective group of 3 months (P<0.05).

Glutathione peroxidase

The GPX activity was significantly increased in the livers of HFE3 (45%) and POE3 (about 70%) while it remained unaltered in livers of HFE6 and POE6 rats (Fig. 3). On the other hand in case of muscle tissue a significant increase in GPX activity

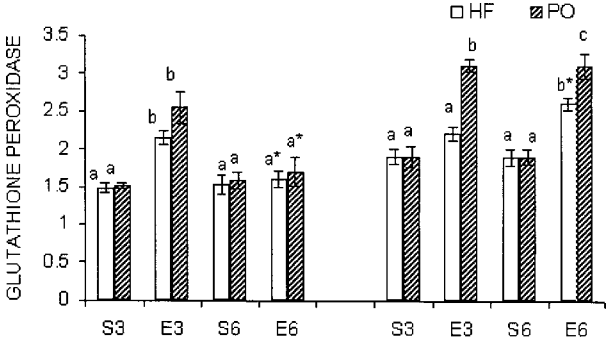

Fig. 3: Activity of Glutathione in liver (left panel, umole/g) and skeletal muscle (right panel, x10 nmoles/g).

Values (mean±SE) with different alphaphets are significantly different (P<0.05).

*Statistically significant compared to respective group of 3 months (P<0.05).

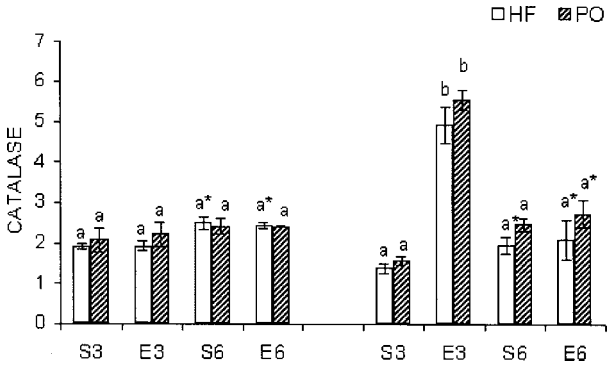

Fig. 4: Catalase activity in liver (left panel, x10^3 units/g) and skeletal muscle (right panel, x10 units/g).
Values (mean±SE) with different alphaphets are significantly different (P<0.05).
*Statistically significant compared to respective group of 3 months (P<0.05).

was observed (Fig. 4) in POE3 (40%), HFE6 (40%) and POE6 (60%).

Catalase activity

CAT activity in liver and skeletal muscle is shown in Fig. 4. The hepatic CAT activity was increased non-significantly only in PO fed rats of 3 months while exercise as such has not influenced this parameter in liver. On the other hand, a highly significant increase of about 250% was observed in muscle of HFE3 and POE3 rats compared to the respective sedentary rats. However, in skeletal muscle of HFE6 and POE6 it was increased by only 8–10%.

DISCUSSION

The present study deals with the response of antioxidant defence system in liver and skeletal muscle to different dietary fat and exercise in rats. The diets in this study consisted of MRE ration items prepared using isocaloric fat (about 27% total energy) so that the results could be attributed to the effects of different proportions of saturates, MUFA and PUFA (Table I). MRE ration is usually prepared using hydrogenated fat (vanaspati) to avoid rancidity and obtain longer shelf life. Long-term intake of such diets may contribute to obesity and coronary heart disease. Earlier, we have shown that the deleterious effects of HF-diet consumption for a prolonged period could be reduced by regular exercise (unpublished observation). The purpose of the present study was to delineate the effects of induced oxidative stress on account of exercise with the MRE ration prepared either with HF or PO.

TABLE I: Fatty acid profile (g/100 g) hydrogenated fat (HF) peanut oil (PO) used for the preparation of diets.

	HF	*PO*
12:0	–	1.11
14:0	1.87	
16:0	40.60	25.81
18:0	3.80	6.50
18:1	23.80	50.27
18:2	–	13.03
SFA	46.27	33.46
MUFA	23.80	50.27
PUFA	–	13.03

Lipid peroxidation due to free radical attack on the membrane is used as an indicator of tissue oxidative stress (4). MDA in liver of exercised groups in both diets (HFE3 and POE3) was significantly higher than that of respective sedentary rats and it was also significantly higher in livers of HFS6 and POS6 compared to HFS3 and POS3 rats (Fig. 1). However, the lipid peroxidation in skeletal muscle was not augmented significantly in sedentary rats fed on PO-diet but there was a non-significant increase in the PO fed exercising

rats (Fig. 1). These results are in agreement with the earlier reports wherein the olive oil (MUFA rich) based diet did not result in any appreciable increase in lipid peroxidation (1, 12). On the other hand, irrespective of the type of fat-diet a significant increase in MDA level was observed in muscle of both sedentary and exercising rats of 6 months compared to 3 months attributable to the accumulation with age (11). However, it has also been reported that liver and muscle MDA levels are not affected by regular exercise for longer duration and found to be more during acute exercise (13).

The level of GSH in tissues forms a consistent index of exercise-induced oxidative stress (14). GSH level was significantly increased in muscle of POE6 rats while it was depleted in liver (Fig. 2). The decreased level of GSH in liver in these exercising rats could be due to differential activity of the enzyme, γ-glutamyltransferase (GGT) in liver and muscle to ensure the availability of precursors of GSH synthesis in the active peripheral tissues having acute needs. The GSH dependent enzyme, GPX activity was significantly increased with exercise in livers of HFE3 and POE3 rats while the dietary lipid composition as such did not affect GPX activity in liver. On the other hand, dietary lipid composition has an influence in GPX activity of muscle tissue; it was increased significantly in muscle of POE3 compared to HFE3, and in HFE6 rats compared to respective sedentary rats (Fig. 3). Our above results are mostly in agreement with an earlier report (15). Even the fish oil-diet, rich in PUFA was earlier found not to affect this enzyme activity. However, treadmill training decreased the activity of the enzyme in liver (16).

In this study, a significant increase by about 250% in CAT activity was observed only in muscle of exercising rats, HFE3 and POE3 (Fig. 4). In an earlier report the CAT activity was found to be increased by 300–400% in all tissues, wherein the sampling was done immediately after a bout of exercise (17). However, in the present study the samples were taken for analysis 20 hours after the last bout of exercise. In contrast, unaltered CAT activity in liver and decreased activity in muscle has been reported (13).

In summary, the findings from the present study revealed that the intake of diet containing unsaturated fat induced a mild lipid peroxidation, which was further augmented significantly by swimming exercise in liver only. In the muscle of exercising rats it was less, probably due to increased GSH level. The response of antioxidant system to regular swimming exercise induced oxidative stress was much more evident in shorter duration (3 months) while it got stabilized over a longer period (6 months). Changes due to exercise suggest an oxidative stress and existence of active scavenging mechanism in the tissues. They were capable of adapting to exercise to minimize oxidative injury produced by free radicals.

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