

Long term dietary restriction ameliorates swimming exercise-induced oxidative stress in brain and lung of middle-aged rat

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Exhaustive exercise may generate oxidative stress in brain and reported findings are conflicting. Long term dietary restriction (DR) may be useful in the inhibiting of free oxygen radicals generated during exhaustive exercise in the brain of rat. Hence, in this study we evaluated beneficial effects of long term DR on the oxidative stress and antioxidant enzyme systems in brain cortex and lung in rats after different intensities of swimming exercise. Sprague-Dawley rats (60) were assigned as DR and *ad libitum* (AL) groups, and each group was further subdivided into three groups namely control (sedentary), submaximal exercise (endurance exercise) and maximal exercise (exhaustive swimming exercise) groups. Animals in the endurance exercise group swam 5 days/week for 8 weeks while exhaustive swimming group was subjected to an acute bout of exercise. With the increase in intensity of exercise, degree of lipid peroxidation (LP) and protein oxidation (PO) were also increased in DR and AL groups; however rate of increase was lower in DR group than AL group. Glutathione (GSH) and glutathione peroxidase (GSH-Px) activity were lower but glutathione reductase (GR) activity was higher in DR group compared to AL group in endurance and exhaustive swimming exercise. With increase in exercise intensity, GSH and GR enzyme activity decreased, whereas an increase was observed in GSH-Px enzyme activity. There was no difference in LP, PO, GSH and GR activity between DR and AL groups. GSH-Px activity in brain cortex was significantly lower in DR group than in AL group and sedentary rats. Results indicate that long term dietary restriction may protect against endurance and exhaustive swimming exercise-induced oxidative stress in rats by inhibiting oxidative stress.

Keywords: Antioxidants, Brain, Dietary restriction, Exercise, Oxidative stress

Long-term diet restriction (DR) has been reported to lengthen the lifespan in rodents and underfed rats lived longer than rats fed *ad libitum*. DR delays onset of various age-related neurodegenerative diseases such as Alzheimer's disease and insulin resistance¹. DR was also reported to ameliorate oxidative stress-related impairment in mitochondria² although the mechanism whereby DR is beneficial in brain of exercised animals is not yet understood.

It is accepted that both superoxide anion and hydroxyl radicals correlate to oxidative stress and cause an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential. Reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide and hydroxyl radical act as subcellular messengers in mitogenic signal transduction, gene expression, and regulation of cell proliferation. ROS are generated excessively when enzymatic and non-enzymatic defence systems

are impaired³. In response to acute or single bout exercise, the body can not adapt to oxidative challenge due to the short duration. Physical exercise generates increased levels of ROS, and results in oxidative damage to macromolecules⁴. Regular exercise through its continuous radical generating effect, can also significantly contribute to the oxidative status. The most marked effect of regular exercise is that it causes adaptation to the exercise-induced oxidative stress⁵.

Although most of the oxygen used in brain tissue and lung is converted to CO₂ and water, small amounts of oxygen forms ROS. Presence of polyunsaturated fatty acids, targets of ROS, in brain makes it more sensitive to oxidative damage⁶. There are various antioxidant mechanisms in brain that neutralize the harmful effects of ROS. However, increased production of free radicals, loss of efficiency of antioxidant mechanisms and alterations in mitochondrial systems results in increase in free radical formation due to mitochondrial overproduction of fats, amino acids and carbohydrates³.

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Many neurotransmitters are autoxidizable molecules. In the brain, DR protects neurons against oxidative and metabolic insults by increasing the production of neurotrophic factors⁶. Studies have also shown that DR protects against acute pulmonary oxidant challenge in rats⁷ and alleviates lipid peroxidation (LP) and promotes activity of enzymatic antioxidants in chickens⁸.

Exhaustive exercise may increase mitochondrial ROS production in the brain cortex⁶ although DR may decrease the ROS production. However, the effect of DR and swimming exercise to modify the alterations in antioxidant system and oxidative stress in brain has not been reported. Suppression of mitochondrial ROS production by means of DR may cause decrease in ROS production of in brain cortex and lung. Hence, the objective of this study was to investigate effect of long-term dietary restriction on protection from oxidative stress caused by increasing intensities of swimming exercise.

Materials and Methods

Chemicals—All chemicals were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and all organic solvents from Merck KGaA (Darmstadt, Germany). All reagents were of analytical grade and phosphate buffers were prepared each day and stored in refrigerator at +4°C. Reagents were equilibrated at room temperature for 30 min before use.

Animals—Male, 1-year-old Sprague-Dawley rats (60) were used in this study. Experiments were conducted as for the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For the first one year the animals had been reared in the Experimental Animals Breeding and Research Centre, Uludag University, Bursa, Turkey at 23° ± 2°C, 60 ± 5% RH and 12 hr artificial light-12 hr dark cycle with lights on between 06:00 hr to 18:00 hr daily. Rats (3-4) were housed in polycarbonate cages with wire lids and had free access to tap water, and fed with a laboratory chow diet (MBD Laboratory Animal Food Company, Gebze, Kocaeli, Turkey) at 08:00 hr daily. Composition of diet was as follows - protein 18% (min), lipid 2.5% (min), fiber 4% (max), ash 5.5% (max), nitrogen free extract 57.0% (max), metabolic energy 2650 kcal kg⁻¹ (min), water 13% (max) and amino acids, minerals, and vitamins (data obtained from the supplier).

Study design—Two main groups - dietary restricted (DR) and *ad libitum* (AL) 30 rats each - were assigned. In DR group, animals were fed only on Monday, Wednesday, and Friday mornings at 08:00 hr. Previous studies have shown that rats and mice maintained on intermittent feeding will consume fewer calories over time and live longer than animals fed AL².

In the AL group, rats were given more food than they consumed daily, so that food was available *ad libitum*. Amount of food given was gradually increased weekly to ensure that rats maintained their healthy and steady growth. This feeding schedule was maintained on for 6 months. After 6 months of dietary restriction, animals of the DR and AL groups were divided into three subgroups of 10 each for sedentary, endurance and exhaustive swimming exercise. Increase in body weights of sedentary animals was monitored bi-weekly. The study was performed after approval of the Animal Care and Use Committee of Uludag University.

Exercise—Swimming was performed in a cylindrical tank with 120 cm diameter and 50 cm depth, containing water at 30°-32°C. Exercises were performed between 18:30-20:30 hours, when the animals were more active, by the same person (SK). As rats in groups promote more vigorous exercise than rats allowed swimming alone, group swimming was preferred. An electric hair-dryer was used to dry animals immediately after swimming.

During the first week, rats in all groups were made to swim 15 min for familiarization. The swimming training program used in the study was as follows: In the endurance group, exercise endurance capacity was estimated for each animal. A mass of 2% of the body weight of each animal was tied to tail part and then they were forced to swim in that condition. Time point at which rats remained below water surface for 10 seconds was recorded as exercise endurance capacity. Sixty percent of this value was considered as endurance exercise training time. Animals were exercised 5 working days a week for a period of 8 weeks. Taking gradual increase in exercise capacity in time into consideration, an updated exercise endurance capacity was determined at the first exercise day of each week, and the new value was used during that week. Average endurance capacities at first swimming exercise day were as 48 min for DR and 50 min for AL groups, while the values reached 75 min and 82 min for the two groups after 8 weeks, respectively.

During the 8 weeks period, animals in exhausted exercise group and sedentary groups were not exercised. At the end of 8th week, rats in exhausted exercise group were forced to swim until exhaustion and were then immediately sacrificed, while sedentary rats were sacrificed at rest.

Tissue sampling—All animals were sacrificed by decapitation while under diethyl ether anaesthesia at the end of 8th week of swimming period after fasting. In endurance exercise training group, rats were rested for 24 hr before decapitation. All animals were fasted at least 12 hr prior to decapitation. Body cavities were opened and right lung was quickly excised.

Brain cortex was dissected out after brain was split in mid-sagittal. Cortex of brain and lung tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freezer (−20°C) until processing (maximum 10 hr). After weighing, cortex of the brain and lung were placed on ice, cut into small pieces using scissors, and homogenized (2 min at 5000 rpm) in a five volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), by using a glass Teflon homogenizer (Caliskan Cam Teknik, Ankara, Turkey). All preparation procedures were performed at 4°C.

Lipid peroxidation (LP) determination—LP levels in tissue homogenate were measured with the thiobarbituric acid reaction by the method of Ohkawa *et al.*⁹. Quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing absorption to standard curve of malondialdehyde (MDA) equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3-tetramethoxypropane. Values of MDA were expressed as nmol per g of tissue.

Measurement of protein carbonyl levels—Tissues were homogenized with NaCl (137 mM), KCl (4.6 mM), KH₂PO₄ (1.1 mM), MgSO₄ (0.6 mM), leupeptin (0.5 µg/ml), pepstatin (0.7 µg/ml), phenylmethyl-sulfonyl fluoride (40 µg/ml), aprotinin (0.5 µg/ml), EDTA (1.1 mM). Protein oxidation (PO) levels were expressed as protein carbonyl amount and it was measured by using method of Levine *et al.*¹⁰. Absorbance at 595 nm was measured in blanks, samples and bovine serum albumin standards, and was recorded as nmol carbonyl per mg protein.

GSH assay—Tissues were homogenized with 0.15 M KCl, 0.125 M NaH₂PO₄, 20 mM Tris (pH 7.4) and GSH levels were determined according to the method of Beutler¹¹. The absorbance of the reduced chromogen 5, 5'-dithiobis (2-nitrobenzoic acid) was

measured at 412 nm and directly proportioned to the GSH concentration. The level of GSH was expressed as µmol/g of tissue.

Measurement of GSH-Px activity—GSH-Px activity was measured by the method of Paglia and Valentine¹². Enzyme reaction in the tube, which contains NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase, was initiated by addition of H₂O₂, and change in absorbance at 340 nm was monitored by a spectrophotometer. Milimolar extinction coefficient of 6.22 mM/cm was used to determine the activity of GSH-Px and was expressed as µmol NADPH min/g of tissue.

Determination of glutathione reductase (GR) and protein levels—GR enzyme activity was analyzed according to the method of Beutler¹¹. Fifty µl of NADPH (2 mM) in 10 mM Tris-HCl buffer (pH 7.0) was added in a cuvette containing 50 µl of GSSG (20 mM) in phosphate buffer (0.5 M, pH 7.0) and 850 µl of phosphate buffer was incubated 37°C for 10 min. Tissue sample (10 µl) was added to the NADPH-GSSG buffered solution and measured at 340 nm for 30 min. The milimolar extinction coefficient of 6.22 mM/cm was used to determine the activity of GR. Activity of GR was expressed as µmol NADPH min/g of tissue.

Protein content in the tissues was measured by method of Lowry *et al.*¹³ with bovine serum albumin as standard.

Statistical analysis—Statistical analysis was performed using SPSS version 15.0 for Windows. (SPSS Inc., Illinois, USA). Comparisons of body weight and statistical analysis of difference in DR and AL animals in each exercise subgroup were done with independent samples 't' test. Kolmogorov Smirnov Lilliefors test was performed for evaluation of nonparametric variables. Descriptive statistics of variables were computed. Mean ± standard errors (SE) were computed. Kruskal-Wallis test was used for comparison of the nonparametric variables and ANOVA was used for parametric variables for more than two groups. Mann-Whitney U test was used for comparison of the nonparametric variables and 't' test was used for parametric variables between two groups. Comparison of LP, PO, GSH, GSH-Px and GR between DR group and AL group, in sedentary, endurance swimming exercise training and exhaustive swimming exercise groups was done using two-way ANOVA. All tests were two-tailed and P<0.05 was considered statistically significant.

Results

Monthly changes in body weights of the DR and AL fed rats are presented in Fig. 1. Body weight was significantly lower in DR rats compared to AL counterparts. Mean body weight of AL sedentary rats at the end of 8 months was 40% higher than body weight of their DR counterparts.

LP and PO levels in brain and lung of rats fed dietary restricted and *ad libitum* are presented in Table 1. LP and PO levels in DR groups were lower than AL group except level of PO in brain cortex. Feeding the DR diet lowered LP levels in both organs regardless of exercise intensity. In DR and AL endurance swimming exercise groups, LP levels were lower in cortex brain ($P<0.001$) and lung ($P<0.01$) in DR animals. In exhaustive exercise groups, only LP level in lung of DR rats was significantly lower ($P<0.01$) than in lung of AL animals. Significant differences were observed for LP levels by two-way ANOVA either between the DR and AL groups or among the exercise groups. When exercise intensity was disregarded and groups were compared, LP levels in DR group were significantly lower in brain cortex and lung ($P<0.001$). With increase in the intensity of exercise, LP levels in all organs showed an increase ($P<0.001$), however, the increase in DR group was at a lower rate.

When the effect of diet restriction on PO levels was compared (Table 1), lower ($P<0.001$) PO levels were

seen in brain cortex in exhaustive exercise group of DR animals than AL. In the investigation of the effect of groups and exercise on PO level, the PO levels in brain cortex of the DR group was lower ($P<0.05$) than AL group, and PO levels in lung increased ($P<0.01$) with the increase in severity of exercise.

The AL diet was associated with decreased brain cortex ($P<0.001$) and lung ($P<0.01$) GSH level in endurance exercise group, whereas reduced lung GSH levels ($P<0.01$) was observed in exhaustive exercise groups. The effect of groups and exercise on GSH levels were also investigated. Though exercise

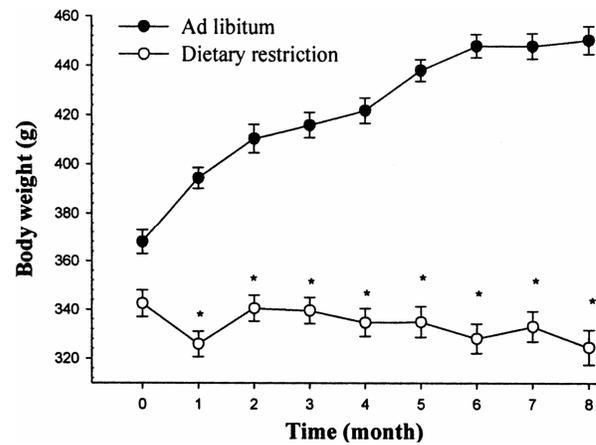


Fig. 1—Body weights of *ad libitum* sedentary and dietary restricted sedentary rats. Values are mean \pm SE of 20 animals *Significantly different from *ad libitum* levels ($P<0.05$)

Table 1—Effects of swimming exercise on the level of lipid peroxidation and protein oxidation in different organs of dietary restricted and *ad libitum* fed rats

[Values are mean \pm SE from 8-10 animals assayed in duplicate]

Tissue	Group	Sedentary	Endurance swimming exercise training	Exhaustive swimming exercise	Two-way ANOVA factors	
					Group	Exercise
Lipid peroxidation (mol / g/ tissue)						
Brain	DR	3.45 \pm 0.21 ^a	3.16 \pm 0.17 ^a	5.96 \pm 0.25 ^b	***	***
	AL	4.07 \pm 0.30 ^I	5.68 \pm 0.51 ^{I,II,###}	6.59 \pm 0.42 ^{II}		
Lung	DR	2.85 \pm 0.40 ^a	3.44 \pm 0.29 ^a	4.15 \pm 0.32 ^a	***	***
	AL	3.52 \pm 0.47 ^I	4.72 \pm 0.23 ^{I,##}	6.01 \pm 0.25 ^{II,##}		
Protein oxidation (mol carbonyl / mg/ protein)						
Brain	DR	2.14 \pm 0.26 ^a	2.69 \pm 0.15 ^a	3.20 \pm 0.30 ^a	*	NS
	AL	1.76 \pm 0.20 ^I	2.45 \pm 0.24 ^I	7.80 \pm 0.41 ^{II,###}		
Lung	DR	2.11 \pm 0.21 ^a	2.71 \pm 0.30 ^{a,b}	3.20 \pm 0.30 ^a	NS	**
	AL	2.70 \pm 0.26 ^I	3.00 \pm 0.18 ^I	3.69 \pm 0.33 ^I		

The level of significance between DR and AL groups in the same column ; P : # <0.05 ; ## <0.01 ; ### <0.001 .

a, b, c; and I, II, III : For each exercise group, different letters in the same row show statistical differences. NS - non significant; P : * <0.05 ; ** <0.01 ; *** <0.001 .

significantly lowered the lung ($P<0.001$) GSH levels, the group parameter did not have any effect (Table 2).

DR feeding was associated with lower GSH-Px enzyme activity in brain and spleen ($P<0.01$) in the sedentary animals. GSH-Px enzyme activity was lower in endurance exercise group in lung ($P<0.001$) in DR animals, and brain ($P<0.001$) and lung ($P<0.01$) in the exhaustive exercise group. An increase was observed in GSH-Px enzyme activity with the increasing exercise intensity regardless of diet. Significant differences in GSH-Px activity were observed in brain and lung of DR and AL groups and among different exercise groups.

Feeding DR reduced GR enzyme activity in the brain ($P<0.001$) in exhaustive exercise swimming groups. A decrease was observed in GR enzyme activity with the increasing exercise intensity in both DR and AL groups. GR enzyme activity in DR group was high in the brain, whereas low in the lung and spleen. GR enzyme activity in all organs decreased with the increase in exercise intensity. Brain may be more significantly affected than lung by oxidative stress of exhaustive exercise and AL diet.

Discussion

Several factors, including the diet and exercise, are known to influence free radical production and efficiency of antioxidant defense system. Although, an effective antioxidant defense system exists in the body, it may not be sufficient to protect against exercise-induced LP and other conditions that produce an excess of ROS². The present study investigated effect of DR on oxidative damage and antioxidant enzyme systems at different intensities of swimming exercise in adult male rats. Results indicate that DR may have beneficial effects on oxidative stress and antioxidant enzyme systems.

Body weight increases with increase in age from weaning to senility in Sprague-Dawley rats. In this study body weight of AL sedentary rats were significantly higher than their DR counterparts throughout the experiment (Fig. 1). Body weight of DR sedentary rats decreased for the first month, increased to beginning level by second month and kept a level under beginning weight with no significant decreases afterwards, reflecting an adaptive mechanism to dietary restriction in adult rats. Rats fed *ad libitum* ingest more energy than needed

Table 2—Effects of swimming exercise on levels of GSH, GSH-Px and GR activity in different organs of rat dietary restricted and *ad libitum* fed rats

[Values are mean \pm SE from 8-10 animals assayed in duplicate]

Tissue	Group	Sedentary	Endurance swimming exercise training	Exhaustive swimming exercise	Two-way ANOVA factors	
					Group	Exercise
GSH level ($\mu\text{mol} / \text{g tissue}$)						
Brain	DR	9.00 \pm 0.36 ^a	8.75 \pm 0.32 ^a	6.44 \pm 0.42 ^b	NS	NS
	AL	12.35 \pm 1.06 ^I	8.57 \pm 0.52 ^{II,###}	2.14 \pm 0.26 ^{III}		
Lung	DR	8.83 \pm 0.56 ^a	7.75 \pm 0.32 ^{a,b}	6.29 \pm 0.52 ^b	NS	***
	AL	10.56 \pm 0.93 ^I	6.31 \pm 0.48 ^{II,##}	4.29 \pm 0.38 ^{III,##}		
GSH-Px level ($\mu\text{mol NADPH} / \text{min} / \text{g tissue}$)						
Brain	DR	9.81 \pm 0.39 ^a	9.09 \pm 0.32 ^a	4.20 \pm 0.23 ^b	***	***
	AL	14.18 \pm 1.10 ^I	10.13 \pm 0.55 ^{II}	15.73 \pm 0.85 ^I		
Lung	DR	2.58 \pm 0.24 ^a	4.00 \pm 0.18 ^a	5.02 \pm 0.54 ^b	***	**
	AL	3.50 \pm 0.48 ^I	5.46 \pm 0.16 ^{II,###}	7.53 \pm 0.55 ^{III,##}		
GR level ($\mu\text{mol NADPH} / \text{min} / \text{g tissue}$)						
Brain	DR	1.09 \pm 0.19 ^{a,b}	1.09 \pm 0.11 ^a	0.67 \pm 0.07 ^b	**	***
	AL	1.93 \pm 0.20 ^I	1.08 \pm 0.12 ^{II}	0.89 \pm 0.10 ^{II,###}		
Lung	DR	2.59 \pm 0.21 ^a	2.13 \pm 0.13 ^{a,b}	1.63 \pm 0.26 ^b	*	***
	AL	2.44 \pm 0.21 ^I	1.79 \pm 0.21 ^{I,II}	1.08 \pm 0.14 ^{II}		

The level of significance between DR and AL groups in the same column ; P : # <0.05 ; ## <0.01 ; ### <0.001

a, b, c; and I, II, III : For each exercise group, different letters in the same row show statistical differences. NS - non significant; P : * <0.05 ; ** <0.01 ; *** <0.001

which leads to increased body weight primarily as a result of fat deposition².

In sedentary animals LPO levels were not different between AL and DR animals. However, feeding DR had a positive effect on oxidative stress after exercise: LPO values in DR animals were decreased in lungs of exhaustive swimming group and in lung and brain of the endurance exercise group. Increase in LPO levels after exercise may reflect increased ROS formation and insufficient antioxidant defense¹⁴.

Some researchers have suggested that the whole body metabolic rate, and hence rate of free radical generation from cellular metabolism, would be similar in DR and AL rats¹⁵. Protective role may be due to increased production of antioxidants¹⁶. Findings of this study however, show both LPO and PO levels, as well antioxidant enzyme levels, were lower in DR rats. DR may increase efficiency of mitochondria, resulting in production of same energy levels with less free radical production¹⁷. Other reports indicated that feeding DR decreased oxidative damage to lipids by altering the rate of free radical production in endurance exercise training group^{16,18} possibly via an increase in efficiency of mitochondrial function¹⁷.

Although LPO levels in all tissues after exhaustive exercise were lower in DR group, no significant differences were observed between LPO levels in DR and AL groups except in lung. This may be due to excessive level of free radical damage in the brain after exhaustive training which can not be compensated by DR.

Gul *et al.*¹⁹ had observed that exercise to exhaustion resulted in much higher levels of oxygen consumption in trained animals than in untrained animals and suggested that trained animals were most likely exposed to much higher levels of ROS than untrained group. The present study reports similar results in DR animals. Increases in both DR and AL animals after exercise in this study may be due to use of untrained animals.

Oxidative damage to proteins is accompanied by an increased number of carbonyl residues¹⁰. In this study it is observed that protein carbonyl amounts were significantly increased only in brains of exhaustively exercised DR animals. Brain is a high level of oxygen consuming organ containing areas that are rich in pro-oxidant iron²⁰ and polyunsaturated fatty acids which all make it very susceptible to oxidative damage²¹. Mechanism underlying protective effect of dietary restriction however is unclear. It may be that the

DR may decrease accumulation of damaged proteins by increasing rate of their proteolytic degradation²².

GSH, a biologically versatile antioxidant system, is known to affect cellular functions under altered redox state as observed in the case of low GSH contents in several pathological conditions³. DR animals had lower GSH and GSH-Px activity which presumably reflects lower protein expression levels and can be attributed to lower rates of oxidative stress. Oxidation of GSH in various tissues has been reported as reliable index of exercise-induced oxidative stress²³. DR inhibits generation of oxidative molecules, whereas it does not directly increase antioxidant enzyme activity^{2,17}. Aydin *et al.*² reported that increase in antioxidant levels in heart, liver and kidney in response to carbohydrate-energy restriction may be due to decrease in substrate oxidative molecules.

After endurance exercise training, the GSH level decreased in brain and lung in DR animals, but after exhaustive exercise it decreased in the lung in DR animals compared to AL. Decreases in GSH levels with increase in exercise severity had already been reported²³ and attributed to diminishing GSH pool, regulation of redox balance and may be due to a possible increase in formation of GSSG out of cell²⁴.

In this study an increase in LP levels with the decrease in GSH levels suggest a strong negative correlation between them. Existence of such a correlation is supported by studies on long duration treadmill run performance²⁵.

Antioxidants alleviate oxidant load before they damage cellular components by catalyzing the decomposition of oxidants and free radicals. GSH-Px enzyme activities were consistently lower in DR animals than in AL animals and may be due to low levels of oxidative injury in DR animals. Beneficial effects of restricted diets may be due to increased activity or efficiency of cellular antioxidant defenses²⁶. Some studies have supported this hypothesis^{16,27}, while others have failed to demonstrate consistent enhancement of antioxidant defenses associated with the restricted diets^{23,28}. In this study antioxidant activity increased with increase in exercise intensity, whereas the increase in DR group was lower than in AL group. DR may reduce free radical damage at various steps, e.g. by reducing the generation of ROS²⁹ affecting sensitivity of cellular components, including DNA, to free radical oxidation; and increasing expression of genes

encoding antioxidant enzymes³⁰. Further DR may increase the endogenous levels of NADP⁺, glucose-6-phosphate dehydrogenase (G6PDH) and the efficiency of GSH-Px enzyme system²⁹.

GSH-Px levels were significantly increased after 8 weeks of training (endurance swimming exercise vs. sedentary) in AL animals, but not in DR animals. This may be because DR induces a metabolic reprogramming characterized by a transcriptional shift towards energy metabolism, increased biosynthesis and protein turnover³¹.

Effect of exercise on GR activity showed a decrease with increase in exercise intensity and this decrease may be due to disturbance of the balance between GSSG/GSH and NADPH/NADP. Conversion of GSSG to GSH also affects NADPH production by changing activity of hexose monophosphate pathway³². In this case demand for energy is provided by the alternative creatine kinase pathway. Indeed, the increase in the creatine kinase levels in parallel with the increase in exercise intensity (unpublished data) supports this hypothesis.

It has been reported earlier that DR has a positive effect on aging and that regular and mild exercise minimizes oxidative injury and adapts body for a maximum bout of exercise. Findings of this study indicate that DR has a beneficial effect against tissue damage caused by free radicals generated excessively during various intensities of swimming exercise.

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