Exercise-Induced Enhancement of Lipid Peroxide Metabolism in Tissues and Their Transference into the Brain in Rat

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Summary This study was conducted to investigate whether a moderate running exercise would enhance or prevent the lipid peroxidation in animal body and also stimulate or depress the degradation-excretion of lipid peroxides (LIPOX, thiobarbituric acid reactive substances determined as malon dialdehyde) in young female rats. Compared with sedentary rats, voluntary wheel-running exercised rats did not show any significant difference in total LIPOX contents in plasma and several tissues including brain, and whole body during 4 weeks of experiment with a vitamin E-free low LIPOX diet. On the contrary, when rats were previously fed a high LIPOX diet and then allowed voluntary exercise with a vitamin E-added low LIPOX diet, total LIPOX contents per whole body reduced significantly faster in the exercised rats than in the sedentary controls during 2 weeks of exercise. At that period, LIPOX were progressively increased in the brain in both groups of animals, but was significantly greater in the exercised group. Interestingly, more than 80% of total LIPOX contents in whole body were found to be stored in carcass portions regardless of greater or lesser amounts of LIPOX contents in rats. These data suggest that a moderate exercise of several weeks might enhance the degradation-excretion of LIPOX but not the formation-accumulation of LIPOX in rats. Exercise also seems to modulate LIPOX transference among tissues.

Key Words voluntary running exercise, lipid peroxide, brain, carcass, whole body, rat

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The elevation of lipid peroxide (LIPOX) levels in the blood and tissues of animals has generally been considered as one of the causes that enhance the process of ageing and increase the prevalence of cerebral and cardiovascular diseases. Several papers have recently reported increased levels of LIPOX (defined as thiobarbituric acid reactive substances, TBARS) in erythrocytes of horses (1) and in skeletal muscle and liver of rats (2) after an exhaustive exercise. In addition, expired pentane, which is thought as a peroxidation product from ω -6 series of fatty acids, was reported to be increased in man during bicycle ergometer exercise (3) and in rats after a bout of exhaustive swimming exercise (4). On the other hand, a moderate swimming exercise training for several weeks has been reported to reduce plasma and liver LIPOX contents in rats (5).

These findings, however, are not clear as to whether exercise enhance or lower lipid peroxidation in animal body, since exercise may have the effects on the LIPOX distribution among organs and tissues without changing the total content of LIPOX in the whole animal body. Additionally, the elevated pentane output in the expired gas may result from hyperventilation *per se* during exercise.

The present study, therefore, was carried out in an effort to elucidate whether exercise would enhance or prevent the lipid peroxidation and stimulate or depress the degradation-excretion of LIPOX in rats. For these purposes, the estimation of total content and distribution of LIPOX in the whole animal body was conducted in so designed experimental animals.

EXPERIMENTAL

Two sets of experiments were conducted using female JCL®-Sprague-Dawley rats (CLEA Japan Inc., Tokyo). Compositions of experimental diets and feeding schedules were shown in Table 1 and Fig. 1, respectively. Animals were cared in a temperature controlled room ($22 \pm 1^\circ\text{C}$) with the lights on between 07.00–19.00 hr with free access to water.

Experiment 1. To study the effects of voluntary running on the formation-accumulation of LIPOX, twenty six rats of 21 days old were used. They were *ad libitum* fed a vitamin (V.) E-added basal diet for 5 days. Then the feeding pattern was shifted to meal-feeding twice a day (08.00–09.00 hr and 20.00–21.00 hr). Rats were meal-fed a V.E-free basal diet for 5 days and a V.E-free low LIPOX diet for the successive 28 days of an exercise period. This V.E-free low LIPOX diet was prepared to minimize dietary supply of LIPOX but allow lipid peroxidation in animal body during the exercise period. On the previous day of switching diet from the basal diet to the low LIPOX diet, 6 rats were killed at 13.00 hr, and the remaining rats were divided into sedentary (10 rats) and exercised (10 rats) groups. Rats of the sedentary group were individually housed in ordinary cages, and those of the exercised group in cages with revolving wheels (6). The exercised group was allowed voluntary running in wheels during 24 hr daily.

On the 14-day and 28-day of the exercise period, 5 rats of each group were

Fig. 1. Feeding schedules for experiments 1 and 2. V.E means vitamin E, and + and - represent added and deprived, respectively. Exercise means voluntary wheel-running which was allowed only in the exercised group of rats for 24 hr.

killed by decapitation at 13.00 hr with exercise restriction for the exercised rats from 21.00 hr of the previous day of killing. Plasma, liver, heart, lungs, kidneys, perirenal and inguinal adipose tissues, and brain were taken from each rat. Carcass was also obtained from the remaining portion after removing digestive tract, mesenteric adipose tissue and other organs. Obtained organs and tissues were freeze-dried, homogenized in 0.9% NaCl solution, and then determined for TBARS contents (7). Plasma TBARS contents were determined by the fluorescence method (8). TBARS levels were expressed as nmol malon dialdehyde (MDA)/tissue or plasma/100 g body weight of rat. Total TBARS contents per whole body were calculated by summing up TBARS contents in the examined tissues and organs per whole rat. Percentage distribution of TBARS in plasma and tissues was also shown. Plasma volume per rat was calculated from the body weight (9).

Experiment 2. To see the effects of voluntary running on the degradation-excretion of LIPOX, another twenty six rats of 21 days old were used. These animals were fed a high LIPOX diet (51.1 μmol MDA/100 g, Table 1) *ad libitum* for the first 5 days and then meal-fed twice a day (08.00–09.00 hr and 20.00–21.00 hr) for another 5 days. Then the V.E-added low LIPOX diet was fed to minimize both dietary supply of LIPOX and lipid peroxidation in animal body during 14 days of the exercise period. On the previous day of switching diet to the V.E-added low LIPOX diet, 6 rats were killed at 13.00 hr and the remaining rats were divided into a

Table 1. Composition of experimental diets.

	Diets		
	Basal	Low LIPOX	High LIPOX
		(g/100 g diet)	
Wheat flour (hard)	84.6	84.6	
Sucrose	2.0	6.0	
Soybean oil	4.0		
(oxidized) ^a			3.0
Cellulose powder	4.0	4.0	
L-Lysine HCl	0.4	0.4	
Salt mixture ^b	4.0	4.0	
Vitamin mixture ^b	0.85	0.85	
Choline chloride	0.15	0.15	
Commercial diet ^c			97.0
		(IU/100 g diet)	
Vitamin A	2,000	2,000	
Vitamin D	200	200	
D,L- α -Tocopherol	0/10	0/80	
		(μ mol MDA/100 g diet)	
Lipid peroxide	3.7/4.1	1.6/1.8	51.5

^a Soybean oil was oxidized by heating at 110°C for 8 hr with aeration. Peroxide value was 197 meq/kg (10). ^b CLEA Japan Inc., Tokyo; according to Harper (11). ^c CE-7 (powder); CLEA Japan Inc., Tokyo.

sedentary (10 rats) and an exercised (10 rats) group. Rats of both groups were housed individually and rats of exercised group were allowed voluntary running in wheels as those of experiment 1.

On the 7-day and 14-day of the exercise period, 5 rats of each group were killed at 13.00 hr with exercise restriction for the exercised group from 21.00 hr of the previous day of killing. Plasma, several organs, adipose tissues and carcasses obtained were prepared and determined for TBARS in the same manner as described for experiment 1.

In both experiments 1 and 2, exercised rats were pair-fed with sedentary rats during the exercise periods. Data were analyzed for significance by the paired *t*-test.

RESULTS

Effects of voluntary exercise on the formation-accumulation of LIPOX in rats (experiment 1)

Since exercised rats were pair-fed with sedentary rats, weight gain and LIPOX intake over 28 days of the exercise period were almost the same between the groups

Table 2. Body weight changes and total consumption of food and lipid peroxides during 4 weeks (experiment 1; vitamin E-free low LIPOX diet) and 2 weeks (experiment 2; vitamin E-added low LIPOX diet) of the exercise periods in female rats.

Experimental period (age, days)	Group	Body weight		Total consumption	
		Initial	Final (g)	Food (g/rat)	Lipid peroxides (μ mol MDA/rat)
Experiment 1					
32–45	Sedentary	60 $\pm$ 1	81 $\pm$ 4	113 $\pm$ 8	1.8 $\pm$ 0.1
	Exercised	60 $\pm$ 2	80 $\pm$ 3	112 $\pm$ 8	1.8 $\pm$ 0.1
32–59	Sedentary	60 $\pm$ 2	107 $\pm$ 7	238 $\pm$ 25	3.8 $\pm$ 0.4
	Exercised	58 $\pm$ 1	100 $\pm$ 6	243 $\pm$ 7	3.9 $\pm$ 0.1
Experiment 2					
32–38	Sedentary	86 $\pm$ 2	91 $\pm$ 3	74 $\pm$ 5	1.3 $\pm$ 0.1
	Exercised	88 $\pm$ 1	93 $\pm$ 3	72 $\pm$ 5	1.3 $\pm$ 0.1
32–45	Sedentary	85 $\pm$ 2	97 $\pm$ 4	121 $\pm$ 7	2.2 $\pm$ 0.1
	Exercised	84 $\pm$ 3	96 $\pm$ 4	126 $\pm$ 6	2.3 $\pm$ 0.1

Values are shown as mean $\pm$ SEM for 5 rats of each group.

(Table 2). Exercised rats had a voluntary running activity of 4-8 km/day.

Changes in plasma and tissue LIPOX contents per rat were illustrated in Fig. 2, and their LIPOX distribution expressed on the basis of 100 g body weight and as percentages were shown in Table 3. Although rats were fed a relatively low LIPOX containing diet for the first 10 days in this experiment, their whole body LIPOX contents were unexpectedly as high as $16.5 \pm 4.8 \mu\text{mol MDA/rat}$ at the age of 31 days (Fig. 2). During the successive 14-day feeding of the low LIPOX diet with voluntary exercise, body LIPOX stores per rat markedly decreased to 40% of the level at the age of 31 days in exercised rats and to 35% in sedentary rats. However, in both groups body LIPOX stores per rat increased during the remaining 14 days of the exercise period and reached almost equal levels. The increasing rates were much lower in the exercised group (13%) than in the sedentary group (27%).

Carcasses accumulated significantly large portions of body total LIPOX stores as 90% in 31 day old rats and about 80% in 45 and 59 day old rats in both groups (Table 3). Carcass LIPOX contents per rat considerably decreased through 31 to 45 days of age in both groups of rats, but afterwards increased by age of 59 days. Thus, changes in carcass LIPOX contents were largely responsible for those of whole animal body. The observed increase in carcass LIPOX contents per rat during the final 2 weeks of experiment, as whole body LIPOX stores, were much lower in the exercised group (13%) than in the sedentary group (37%).

Liver, next to carcass, contained LIPOX highly at about 6% of body total LIPOX contents per 100 g body weight in 31 day old rats and 9-13% in 45 and 59

Fig. 2. Effects of voluntary running exercise on plasma, tissue and whole body lipid peroxide contents per rat in female rats (experiments 1 and 2). Each point represents mean for 5 to 6 rats of each group. —, sedentary group; ----, exercised group. —●—, ---●---, experiment 1; —○—, ---○---, experiment 2. ^aSignificantly different ($p < 0.05$) from the corresponding sedentary group.

day old rats in both groups. During the exercise period, liver LIPOX contents per rat increased at 45 days of age and then decreased at 59 days of age in sedentary rats. In exercised rats, on the other hand, its changing pattern was the reverse of

sedentary rats. But there was no significant difference in the liver LIPOX contents per rat between the two groups of animals.

Brain stored slightly more LIPOX than kidneys and lungs, and LIPOX storage levels of these three organs were about 2% each of body total LIPOX stores per 100 g body weight. Brain LIPOX contents per rat slightly increased and then decreased during the exercise period, and there was no difference between exercised and sedentary rats.

LIPOX contents in kidneys and lungs per rat slightly increased by the end of the exercise period, although a marked decrease occurred in lung LIPOX contents in exercised rats at the age of 45 days. Heart and combined perirenal and inguinal adipose tissues stored LIPOX in less than 1% each of the body total LIPOX stores per 100 g body weight. Their storage levels per rat increased over the exercise period in both groups, however, in adipose tissues the accumulation of LIPOX seemed to be lowered during the last 14 days of voluntary running exercise.

Plasma LIPOX contents per rat slightly increased during the exercise period, but there was no significant difference between the two groups.

Effects of voluntary exercise on the degradation-excretion of LIPOX in rats (experiment 2)

During 14 days of the exercise period, weight gain and LIPOX intake were almost the same between sedentary and exercised groups, since the two groups of animals were pair-fed as those in experiment 1 (Table 2). Voluntary running activity of exercised rats was 4–6 km/day during the 2-week exercise period.

Owing to the 10-day feeding of the high LIPOX diet prior to the exercise period, body LIPOX stores per rat at the age of 31 days were significantly as high as about 50 μ mol MDA/rat (Fig. 2), which were about 3 times higher than those in experiment 1. Eighty percent of the initial body total LIPOX stores per rat disappeared during the first 7 days of the exercise period in two experimental groups. In sedentary rats, about 19% of the body total LIPOX stores per rat at the age of 38 days old further decreased during the last 7 days of experiment. In exercised rats, the reduction of LIPOX stores per rat through 38 to 45 days old was 28% of the body total LIPOX stores per rat at 38 days old, and the LIPOX contents per rat at the age of 45 days were significantly lower than those in the sedentary rats ($p < 0.05$).

As observed in experiment 1, considerably large portions of total LIPOX in whole body were accumulated in the carcass which stored 97% or 80–82% of the body total LIPOX stores per 100 g body weight before the exercise period or after the 7-day and 14-day voluntary running exercise, respectively (Table 3). In exercised rats, the reduction of carcass LIPOX stores per rat during the exercise period was larger than that in sedentary rats, resulting in significantly lower LIPOX contents in the carcass of exercised rats than those of sedentary rats after 14 days of voluntary exercise. Carcass LIPOX contents per rat at the age of 45 days in both groups were almost similar to those at the ages of 45 and 59 days in experiment 1.

LIPOX contents in liver, kidneys and plasma per rat decreased during the

Table 3. Effects of voluntary running exercise on plasma, tissues and body lipid (experiments 1 and 2).

Age of rats		Experiment 1					
		31 days		45 days		59 days	
		nmol MDA/ 100 g b.w.	%	nmol MDA/ 100 g b.w.	%	nmol MDA/ 100 g b.w.	%
Plasma	Sed ¹	17 ± 1 ^a	0.1 ^b	16 ± 1 ^a	0.2 ^b	14 ± 1 ^a	0.2 ^b
	Ex ²			15 ± 1	0.2	13 ± 1	0.2
Carcass	Sed	26,650 ± 7,956	90.4	5,562 ± 394	77.8	5,698 ± 356	83.5
	Ex			6,645 ± 690	82.1	6,041 ± 291	82.5
Brain	Sed	271 ± 14	1.3	203 ± 32	2.8	110 ± 10	1.6
	Ex			212 ± 70	2.7	127 ± 16	1.7
Liver	Sed	1,235 ± 100	5.6	908 ± 113	12.9	627 ± 38	9.3
	Ex			804 ± 47	10.3	777 ± 116	10.5
Heart	Sed	82 ± 8	0.4	67 ± 5	1.0	63 ± 9	1.0
	Ex			67 ± 8	0.9	69 ± 8	0.9
Lungs	Sed	198 ± 28	1.0	168 ± 21	2.4	114 ± 12	1.7
	Ex			103 ± 11*	1.3	139 ± 26	2.0
Kidneys	Sed	195 ± 14	1.0	137 ± 8	1.9	114 ± 4	1.7
	Ex			150 ± 10	1.9	118 ± 8	1.6
Adipose tissue ³	Sed	61 ± 16	0.2	68 ± 20	1.0	66 ± 18	1.0
	Ex			47 ± 22	0.6	39 ± 15	0.6
Whole body	Sed	28,707 ± 8,043	100	7,129 ± 363	100	6,805 ± 335	100
	Ex			8,043 ± 655	100	7,323 ± 327	100

¹ Sedentary group. ² Exercised group. ³ Perirenal and inguinal adipose tissues. different ($p < 0.05$) from sedentary group.

exercise period, but there was no significant difference between the two groups.

In heart and adipose tissues, LIPOX contents per rat markedly increased during the first 7 days of the exercise period, and the increases were smaller in exercised rats than in sedentary rats. After 7 days of voluntary running, exercised rats showed significantly lower adipose tissue LIPOX contents per rat ($p < 0.05$) as compared with sedentary rats. In heart and adipose tissues of both groups, LIPOX contents per rat decreased during the last 7 days of the exercise period.

Lung LIPOX contents per rat continuously increased in sedentary rats, while in exercised rats, they increased during the first 7 days and then decreased during the last 7 days of the exercise period.

Brain LIPOX contents per rat progressively elevated throughout the exercise period in both groups of animals, and the increasing rates were considerably larger in exercised rats than in sedentary rats. This resulted in a significantly higher brain LIPOX contents per either 100 g body weight of rat ($p < 0.05$) in exercised rats at the end of the exercise period as compared with sedentary rats.

peroxide contents per 100 g body weight and their percentage distribution in female rats

Age of rats		Experiment 2					
		31 days		38 days		45 days	
		nmol MDA/ 100 g b.w.	%	nmol MDA/ 100 g b.w.	%	nmol MDA/ 100 g b.w.	%
Plasma	Sed ¹	17 ± 1 ^a	0.1 ^b	19 ± 1 ^a	0.2 ^b	16 ± 1 ^a	0.2 ^b
	Ex ²			17 ± 1	0.2	15 ± 1	0.2
Carcass	Sed	52,392 ± 9,736	96.6	8,672 ± 467	79.7	6,971 ± 405	81.4
	Ex			8,532 ± 1,453	81.7	5,765 ± 175*	80.5
Brain	Sed	244 ± 32	0.4	302 ± 22	2.8	320 ± 37	3.9
	Ex			357 ± 83	3.6	451 ± 73*	6.2
Liver	Sed	1,198 ± 53	2.1	747 ± 46	6.9	641 ± 25	7.8
	Ex			817 ± 42	8.5	637 ± 37	8.9
Heart	Sed	60 ± 3	0.1	85 ± 7	0.8	61 ± 5	0.7
	Ex			71 ± 7	0.7	52 ± 4	0.7
Lungs	Sed	131 ± 17	0.2	128 ± 16	1.2	152 ± 22	1.9
	Ex			166 ± 37	1.9	108 ± 13	1.5
Kidneys	Sed	181 ± 13	0.3	163 ± 3	1.5	100 ± 6	1.2
	Ex			146 ± 11	1.5	118 ± 9	1.7
Adipose tissue ³	Sed	91 ± 20	0.2	537 ± 96	6.9	14 ± 2	0.2
	Ex			214 ± 189*	1.9	24 ± 7	0.3
Whole body	Sed	61,822 ± 7,629	100	10,854 ± 423	100	8,276 ± 418	100
	Ex			10,318 ± 1,473	100	7,169 ± 247*	100

^a Mean ± SEM for 6 (31 days) or 5 (38, 45 and 59 days) rats. ^b Mean. * Significantly

DISCUSSION

This study presented interesting findings that the amounts of more than 80% of the total LIPOX in the rat whole body were distributed in the carcass portions regardless of the amounts of whole body LIPOX stores per rat. Carcass obtained here was mainly composed of subcutaneous adipose tissue, skeletal muscle and skin. As abdominal adipose tissues were found to contain only minor parts of body LIPOX stores in the present experiments, LIPOX stored in carcass might be largely accumulated in muscle and skin portions.

Whole body LIPOX stores of 31 day old rats which were previously fed the low LIPOX diets after weaning were significantly higher than those of 45 and 59 days old (experiment 1). A possible transference of LIPOX from mothers to babies through placenta during pregnancy and/or through milk after delivery may at least in part account for such findings.

In experiment 2, adipose tissues appeared to actively uptake and accumulate LIPOX while carcass was putting out large amounts of LIPOX, suggesting that adipose tissues may have a role of transient stores for LIPOX. However, the LIPOX accumulation in adipose tissues was lowered by voluntary running exercise. This indicates that either an increased lipolytic activity of adipose tissues by exercise training or a smaller uptake of LIPOX released from carcass portions by adipose tissues than by the other tissues might be partly responsible for the lowered LIPOX accumulation in the adipose tissues of exercised rats.

Another interesting finding observed in the present study (experiment 2) was that the brain also appeared to have an active uptake of LIPOX during the period in which the large amounts of stored LIPOX were removed from the animal body. Furthermore, the LIPOX accumulation into the brain was significantly enhanced by daily voluntary running exercise. In experiment 1, however, such increases in the brain LIPOX levels were not observed in either sedentary or exercised rats. Therefore, the increased LIPOX levels in the rat brain in experiment 2 may not be due an increased LIPOX formation in the brain tissue *per se* by exercise but due to an increased transference of LIPOX from carcass and other tissues into the brain. A transient increase of LIPOX in lungs of exercised rats (experiment 2) may also reflect the exercise-induced increase in LIPOX transference into lungs.

The marked increase of LIPOX observed in the brain of rats with voluntary running exercise seemed to be important with regard to the development and prevalence of cerebrovascular diseases.

There has been reported that bouts of exhaustive exercise increased LIPOX levels in liver and muscle (2) and on the contrary, exercise training decreased LIPOX levels in liver and plasma (5) in rats. Our present study, however, has evaluated all the LIPOX distribution in whole animal body and clearly demonstrated that moderate exercise such as voluntary running enhanced the degradation-excretion of LIPOX in the animal body (experiment 2), but did not increase the formation-accumulation of LIPOX (experiment 1). It was also shown that voluntary running exercise may modulate the LIPOX transference between organs and tissues of rats. These findings, therefore, suggest that the exercise-induced increase or decrease of LIPOX levels in certain organs and tissues may not necessarily indicate the net elevation or reduction of lipid peroxidation in those organs and tissues.

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REFERENCES

- 1) Brady, P. S., Ku, P. K., and Ullrey, D. E. (1978): Lack of effect of selenium supplementation on the response of equin erythrocyte glutathione system and plasma enzymes to exercise. *J. Anim. Sci.*, **47**, 492–496.

- 2) Brady, P. S., Brady, L. J., and Ullrey, D. E. (1979): Selenium, vitamin E and the response to swimming stress in the rat. *J. Nutr.*, **109**, 1103–1109.
- 3) Dillard, C. J., Litov, R. E., Savin, W. M., Dumelin, E. E., and Tappel, Al. L. (1978): Effects of exercise, vitamin E, and ozone on pulmonary function on lipid peroxidation. *J. Appl. Physiol.: Respir. Environ. Exercise Physiol.*, **45**, 927–932.
- 4) Gee, D. L., and Tappel, Al. L. (1981): The effect of exhaustive exercise on expired pentane as a measure of *in vivo* lipid peroxidation in the rat. *Life Sci.*, **8**, 2425–2429.
- 5) Tani, Y., and Aoki, M. (1981): Effects of dietary protein levels and exercise on liver and serum lipids composition of rats. *Eiyo To Shokuryo (J. Jpn. Soc. Food Nutr. Sci.)*, **34**, 59–64.
- 6) Suzuki, M., Hashiba, N., and Kajuu, T. (1982): Influence of timing of sucrose meal feeding and physical activity on plasma triacylglycerol levels in rat. *J. Nutr. Sci. Vitaminol.*, **28**, 295–310.
- 7) Ohkawa, H., Ohishi, N., and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351–358.
- 8) Yagi, K. (1975): Micro-determination of lipoperoxide in blood plasma or serum. *Vitamins* (in Japanese), **49**, 403–405.
- 9) Bagdade, J. D., Yee, E., Albers, J., and Pykalisto, O. J. (1976): Glucocorticoids and triglyceride transport: effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat. *Metabolism*, **25**, 533–542.
- 10) International Chemical Union (1947): The modified Lea's method, in *Experiments in Agricultural Chemistry* (1967) (revised edition with supplement), Vol. 3, ed. by Department of Agricultural Chemistry of Kyoto Univ., Sangyo Tosho Pub. Co., Ltd., Tokyo, p. 1125.
- 11) Harper, A. E. (1959): Amino acid balance and imbalance. 1. Dietary protein level of protein and amino acid imbalance. *J. Nutr.*, **68**, 405–418.