# Effects of Dietary Fat and Oils on Body Compositions of the Exercised Rats

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#### Abstract

Nutritional effects of dietary lipids on body compositions of the exercised rats were determined. Body weights of the exercise groups significantly decreased as compared with those of the sedentary groups. Crude lipids, especially triacylglycerol, in the liver and carcass decreased significantly due to loading of endurance exercise. Glycogen reserves in the liver tissues were promoted by feeding of plant-or marine fish-oil diets. Protein contents in the carcass had a tendency to increase in corn oil and marine fish oil diet groups.

From above results of reductions of crude lipids and triacylglycerol in the animal body, it is suggested that body fat can be apparently consumed as an energy source of the exercise.

Key words: exercise, corn oil, beef tallow, marine fish oil, triacylglycerol, cholesterol.

Recently many investigators have focussed their interests on polyunsaturated fatty acids from view point of protections of hypercholesterolemia and ischemic heart disease. Since the evidences of the Green land Eskimo have been reported<sup>1,2)</sup>, their interests on marine fish oils containing much of polyunsaturated fatty acids are expanded quickly.

The effects of sardine and squid-liver oils on lipid metabolism in rats fed a low-protein diet were investigated in our laboratory<sup>3)</sup>. From our results, serum protein, albumin and monoamin oxidase activity significantly increased in sardine oil diet group as compared to the corn oil diet group, although the serum iron contents decreased in all of marine fish oil diet groups<sup>3)</sup>.

In general, it is well known that carbohydrate and fat in the body were equally consumed (50% each) in the case of endurance exercise, especially low grade exercise (<50% Vo<sub>2</sub> max)<sup>4</sup>). It is interesting how much fatty acids released from adipose tissues where dietary fat have been deposited, are utilized as an energy source of exercise.

In the presence we conducte to study the influences of dietary oils and fat on body compositions of the exercised rats. Using diets containing pollack-liver oil (*Theragra chalocogramma*) as a marine fish oil, changes of body compositions of the exercised rats were investigated in the present study.

# Materials and Methods

Diets

Three kinds of diets were prepared for the experiment. Composition of these diets is shown in Table 1. These three diets consisted of 15% dietary lipids [corn oil (C-group), beef tallow (B-group) and marine

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Diet groups	C-group	B-group	T-group
Protein(casein)a	18	18	18
Fat and oil	corn oil <sup>c</sup> 15	beef tallow 15	marine fish oil <sup>ь</sup> 15
salt mixture <sup>d</sup>	5	5	5
vitamin mixture <sup>e</sup>	1	1	1
Choline chloride <sup>f</sup>	0.2	0.2	0.2
Corn starch <sup>g</sup>	60.8	60.8	60.8
Total	100.0	100.0	100.0

Table 1. Composition of diets (%)

a: vitamin free casein, Oriental Yeast Co. Ltd., b: Riken Vitamin Co. Ltd., c: Corn salad oil, Ajinomoto Co. Inc., d: OrientalYeast Co. Ltd. (composition: CaHPO<sub>4</sub> • 2H<sub>2</sub>O, 0.43g, KH<sub>2</sub>PO<sub>4</sub>, 34.31g, NaCl, 25.06g, Fe-citrate, 0.623g, MgSO<sub>4</sub> • 7H<sub>2</sub>O, 9.98g, ZnCl<sub>2</sub>, 0.02g, MnSO<sub>4</sub> 4-5H<sub>2</sub>O, 0.121g, CuSO<sub>4</sub> • 5H<sub>2</sub>O, 0.156g, KI, 0.0005g, CaCO<sub>3</sub>, 29.29g, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>4H<sub>2</sub>O, 0.0025g, Total 99.993g) e: Oriental Yeast Co. Ltd., (composition: Vitamin A acetate  $5 \times 10^5$ IU, 93.2mg(46,600IU), vitamin D  $4 \times 10^7$ IU, 0.5825mg(23,300IU), vitamin E acetate, 1,200.0mg, vitamin K<sub>3</sub>, 6.0mg, vitamin B<sub>1</sub> hydrochloride, 59.0mg, vitamin B<sub>2</sub>, 59.0mg, vitamin B<sub>6</sub> hydrochloride, 29.0mg, vitamin B<sub>12</sub>, 0.2mg, vitamin C, 588.0mg, D-biotin, 1.0mg, folic acid, 2.0mg, calcium pantothenate, 235.0mg, nicotinic acid, 294.0mg, inositol, 1,176.0mg, filled up to 100g with lactose), f: Sigma Chemical Co. (St. Louis, U. S. A.) 100g of choline chloride was dissolved into 50% ethanol to a final volume of 200ml, g: Amicol C, Nichiden Chemical Co. (Osaka).

fish oil (*Theragra chalcogramma*, T-group)] and the other ingredients at the same level (casein 18%, corn starch 60.8%, adequate amounts of vitamins and minerals).

Animals, dietary treatments and exercise loading.

Male rats of Sprague-Dawley Crj-CD strain (Charles River Japan Inc., Atsugi) were housed individually in stainless steel cages in an air-conditioned room ( $22\pm2^{\circ}$ C,  $64\pm12\%$  of relative humidity) with 12-h cycle of light and dark (lights on at 8:00). The rats were given a commercial diet (MF, produced by Oriental Yeast Co. Ltd., Tokyo) for 20 days for pre-training of exercise prior to the experiment, and were divided into 6 groups of 4 to 5 rats. The initial body weight of the rats was  $213\pm34g$ . The rats were given free access to the diet of each group and tap water for 4 weeks. In the case of T-diets, residual diet was discarded when intake of diet was measured every day, and diet bowl was filled up with a new T-diet. The body weight and food intake for each animal were usually measured at 10:00 to 12:00 AM every day.

After pre-training the exercise, these rats were exercised in running (30 m/min, 60 min/day) for 26 days (each E-group) except for the sedentary groups (C-C, B-C and T-C groups). After finishing the final exercise and subsequently being fasted for 9 hours, all rats were killed by bleeding under light anesthesia of diethyl ether and some organs were removed.

Blood was drawn from the abdominal aorta of the rats with a heparin. The blood obtained from each animal was separated plasma by centrifugation (3000 r.p.m. for 20 min) at 4°C. Plasma was stored in a refrigerator below 4°C prior to the biochemical assays.

The various organs except for adipose tissues were removed from the carcass and skin was stripped off from the carcass and finally a head was rejected on the neck. And then carcass was comminuted and minced twice by a meat grinder (National MKG-1, Matsushita Elect. Co. Ltd.). The paste ground down carcass was mixed well and stored in a freezer at  $-30^{\circ}$ C prior to biochemical assay.

# Biochemical assays.

Total lipids were extracted with minimum of 8 ml of chloroform-methanol (2:1, v/v) from liver and carcass samples (1 and 0.5 g, respectively) essentially according to the method of Folch et al<sup>5</sup>). These procedures of lipid extraction were repeated three times, and total volumes of the extract were made up to 30 ml. On crude lipids in the liver and carcass, adequate amounts of the extracts above described were measured weights after desiccating.

Triacylglycerol (TG), total cholesterol and free cholesterol in the plasma, liver and carcass.

Plasma, liver and carcass TGs were measured using a triacylglycerol assay kit from the Wako Pure Chemicals Ind. Ltd. (Triglyceride Test Wako) and total cholesterols in the plasma, liver and carcass were estimated using cholesterol assay kit from the Wako Pure Chemicals Ind. Ltd. (Cholesterol C-test). On the other hand, free cholesterol was measured using free-cholesterol C-test (Wako Pure Chemicals Ind. Ltd., Osaka).

Glycogen in the liver.

Glycogen contents (mg/g) in the livers were estimated by the method of Lo, Rusell and Taylor<sup>6)</sup>. An aqueous sample of alcoholic precipitate from the liver glycogen was spectrophotometorically measured at 490 nm by adding 5% phenol reagent (1 ml) and concentrated  $H_2SO_4$  (5 ml) using a bovine liver glycogen as a standard.

Protein in the carcass.

Protein content of the carcass was estimated by the method of Lowry et al.71

Water content in the carcass.

For water content in the carcass, adequate amounts of the paste were measured weight differences before and after desiccating.

Statistical analysis.

In the tables each value represents mean ± half range of the confidence interval (confidence limit) at 95% level. The symbol >> shows significant difference between both sides of the symbol. According to the procedure of Pollard<sup>8)</sup> and Masuyama<sup>9)</sup>, statistical analysis was performed for the differences between means.

## Results and Discussion

Body weight gains and food intakes.

The growth curves are presented in Fig. 1. Body weight gain of the C-C group during the later experimental period was significantly larger than those of the other two groups (B-C and T-C groups). While body weight gains of the exercise groups had a tendency to be lower than those of each sedentary group. Especially reductions of body weights in the C-E and T-E groups were significant as compared with value of each sedentary group. The cumulative consumptions of foods are shown in Fig. 2. Food intakes in each of the sedentary and exercise groups were no differences among variously dietary treatments. In comparison of the sedentary and exercise groups, food intakes of the exercise groups were significantly less than those of the sedentary groups after the 2nd experimental week, regardless of dietary treatments.

Plasma concentrations of triacylglycerol (TG).

Plasma concentrations of TG in the sedentary groups were in the order of C-C group>B-C group>T-C group. On the other hand, plasma TG concentrations in the exercise groups decreased below half levels of the sedentary groups, although that of the T-E group which was at the lowest level in the sedentary status, was at almost similar level of the T-C group (Fig. 3).

Total cholesterol in the plasma.

Total cholesterols in the plasma of the sedentary groups were in the order of C-C group>B-C group> T-C group with significant difference between C-C and T-C groups as shown in Fig. 4. Total cholesterols in the exercise groups had a tendency to decrease, showing significant differences in the C-E group as compared to the C-C and B-E groups.

Crude lipid contents in the liver tissues. (Fig. 5)

Crude lipid contents in the liver tissues of the exercise groups decreased slightly, particularly showing significant decrease in T-E group rats as compared with the sedentary group (T-C group). For C-E and B-E groups, however, there were no differences between the sedentary and exercise groups.

Triacylglycerol (TG) contents in the liver. (Fig. 6)

In the C-E and T-E groups, TG contents in the livers decreased slightly, especially showing significant reduction in the T-E group, but TG content in the B-E group did not differ from the level of the sedentary group.

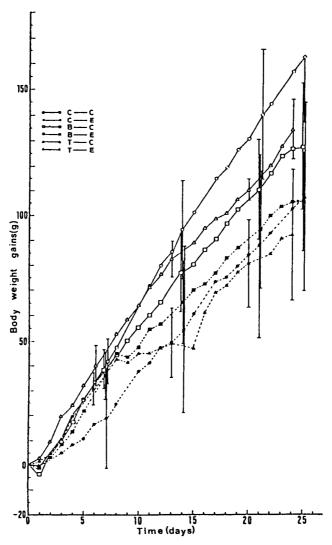


Fig 1. Body weight gains (g).

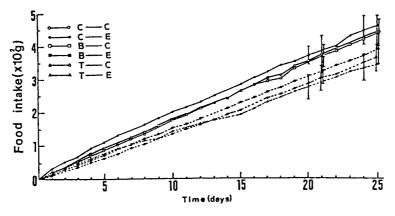


Fig 2. Food intake ( $\times 10^2$ g).

Total cholesterol in the livers. (Fig. 7)

Total cholesterol contents in the livers of the sedentary rats were in the order of T-C group > B-C group > C-C group. In the exercise groups, contents of total cholesterol in the livers decreased as compared with each of the sedentary groups, particularly indicating significant reduction of the T-E group.

Glycogen contents in the livers (Fig. 8)

Glycogen contents in the livers of the sedentary and exercise rats after 9 hour-fast were in the order of

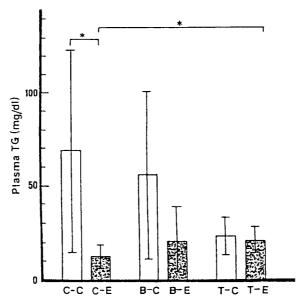


Fig 3. Plasma TG (mg/dl).

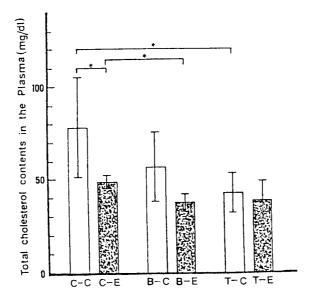


Fig 4. Total cholesterol contents in the Plasma (mg/dl).

C-C group>T-C group>> B-C group. Those of B-C and B-E groups were at the lowest level of hepatic glycogen as compared with the other two dietary groups. In the exercise groups, hepatic glycogen contents tended to increase as compared to each of the sedentary groups, although no elevation due to exercise loading in the rats fed beef tallow diet was observed. The T-E group showed significant elevation of glycogen in the liver.

Crude lipid contents in the carcass.

Figure 9 shows comparison of crude lipid contents in the carcass. For the crude lipid in the carcass, the order of B-C group>T-C group>C-C group was observed in the sedentary groups. In the exercise groups, those of the carcass significantly decreased except for the C-C group. In the exercise groups, TG contents in the carcass also significantly decreased as compared with the sedentary groups except for the C-E group. (not shown in figure)

Protein contents in the carcass.

Protein contents in the carcass are shown in Fig. 10. The protein contents in the carcass were in the order of T-C group>C-C group>B-C group in the sedentary groups. The difference between T-C and B-C groups

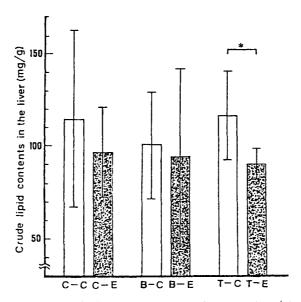


Fig 5. Crude lipid contents in the liver (mg/g).

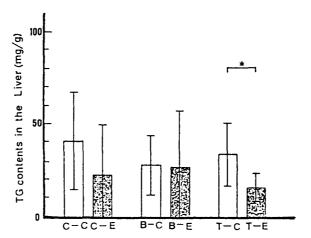


Fig 6. TG contents in the Liver (mg/g).

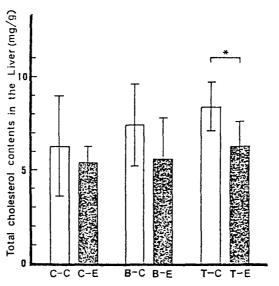


Fig 7. Total cholesterol contents in the Liver (mg/g).

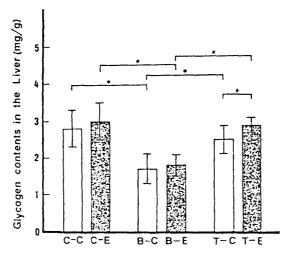


Fig 8. Glycogen contents in the Liver (mg/g).

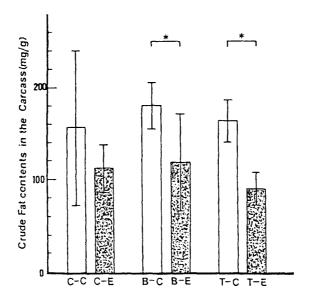


Fig 9. Crude Fat contents in the Carcass (mg/g).

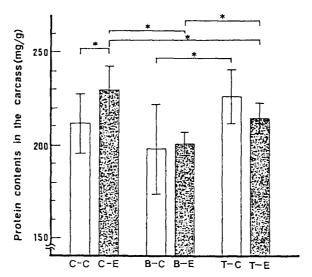


Fig 10. Protein contents in the carcass (mg/g).

was significant. On the other hand, the protein contents in the carcass of the exercise groups were in the order of C-E group >> T-E group >> B-E group. In comparison of protein contents in the carcass among the all groups, C-E group was significantly greater than those of the C-C, B-E and T-E groups. Between the B-C and B-E groups, there was no difference. The protein content of the B-E group was at the lowest level as compared to those of the other two exercise groups.

Water contents in the carcass.

As shown in Fig. 11, there were no differences among dietary treatments of the sedentary groups. For exercise groups, however, every group showed significant increases in water contents of the carcass as

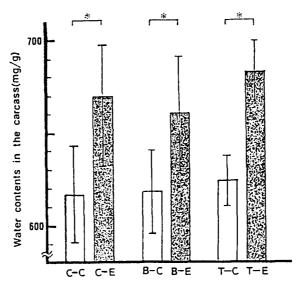


Fig 11. Water contents in the carcass (mg/g).

compared to each of the sedentary groups.

Free cholesterol in the carcass.

Free cholesterol content in the carcass was in the order of C-C group>B-C group>>T-C group. On the other hand, the C-E and B-E groups had a tendency to decrease free cholesterol in the carcass, although that in the T-E group apparently increased as compared to the T-C group. (Fig. 12)

In general food intakes of the exercise groups were less than those of the sedentary groups. On the other hand, the body weights of the exercise groups markedly decreased as compared to the sedentary rats except

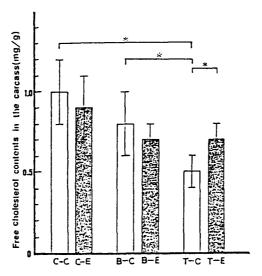


Fig 12. Free cholesterol contents in the carcass (mg/g).

for the beef tallow diet group. Thus reduction of body weight due to exercise loading might be influenced by differences of dietary treatments.

In the sedentary rats, plasma TG and total cholesterol levels were in the order of C-C group > B-C group > T-C group and were at the lowest in the marine fish oil-diet group, but those of the exercised rats fed marine fish oil were not reduced, although rats given the other two oils showed reduction of TG and total cholesterol in plasma. Namely as described by Dyerberg et al.<sup>10)</sup> Lands<sup>11)</sup>, and Tamura et al.<sup>12)</sup>, marine fish oil itself may have a lowering effect for both TG and cholesterol in plasma. However multiplication of dietary and exercise effects was not obtained. In comparison of plasma concentration of TG between C-C and T-C groups, plasma TG of the T-C group was lower than that of the C-C group regardless of almost similar level of hepatic TG. As pointed out by Wong et al.<sup>13)</sup>, secretion of TG with VLDL might be inhibited by eicosapentaenoic acid (EPA) of marine fish oil.

In comparison of both liver and plasma in the sedentary groups, total cholesterol contents were in the order of T-C group>B-C group>C-C group and C-C group>B-C group>T-C group, respectively. That is, the order in the liver tissue contents was inversely related to the order of it's concentration in plasma. While three dietary treatments did not differ in total cholesterol levels of plasma and livers for the exercised rats. However cholesterol lowering effects due to exercose loading as compared to the sedentary groups were observed.

From evidence of glycogen reserve in the liver, dietary treatment of oil containing polyunsaturated fatty acids, especially marine fish oil, might be more effective for the spare action of glycogen in the liver tissues as described by Suzuki<sup>14)</sup>.

Water contents in the carcass increased significantly in the exercised rats maintained on every diet. Namely water contents in the carcass were apparently elevated according to reduction of body fat.

From results of reduction of crude lipids and TG in the carcass of the exercise groups, it might be confirmed that body fat could be consumed significantly as an energy source of exercise.

# Acknowledgment

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