Pathological Changes in Rats Associated with Feeding Free Fatty Acids and Fatty Acid Methyl Esters.* (29695)

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The effects of feeding different fatty acids as measured by a number of parameters have been investigated. Biological values of these compounds have been determined when they were supplied in the form of free fatty acids, methyl or ethyl esters, or as triglyceride precursors. It has been shown that free fatty acids are less well utilized than the corresponding triglycerides by the rat(1) and the chick(2). Thomasson and Gottenbos(3) have reported that triglycerides were more efficiently utilized than their methyl or ethyl esters when administered simultaneously with linoleic acid to essential fatty acid-deficient rats. In the present investigation, triglycerides, free fatty acids, and methyl esters of fatty acids were fed to rats and compared for their effects on growth, feed conversion ratios, fat absorbability, carcass composition, organ weights, serum polyunsaturated fatty acids and cholesterol levels, and liver and kidney histology.

Experimental. Prime steam lard was used as the source of triglycerides and as the starting material from which the free fatty acids and methyl esters of fatty acids were prepared. Free fatty acids were obtained by saponifying the lard with potassium hydroxide, acidifying the soaps, washing and drying over anhydrous sodium sulfate. Methyl esters were obtained by interesterification with methanol using sodium methylate as a catalyst. Nitrogen gas was bubbled through the preparation throughout the procedure. All 3 materials were checked for impurities by official methods of the American Oil Chemist's Society(4). Results of the analyses are shown in Table I.

All test materials were incorporated into a synthetic diet at a concentration of 10%. The composition of the diet was as follows: isolated soybean protein, 20.00%; glucose, 30.56%; dextrin, 30.00%; mineral mix (Jones-Foster), 4.50%; cellulose, 3.00%; dl-methionine, 0.75% vitamin mix, 1.19%; and test material, 10.00%. The vitamin mixture was that described by Rice *et al*(5) with the following additions per 100 g of diet: 500 USP units of vit. A, 150 IU units of vit. D, and 0.04 g dl-alpha-tocopherol.

White, male, weanling rats of the Holtzman strain were individually caged and fed a control diet containing 10% refined corn oil for a preliminary period of 6 days, whereupon they were fasted overnight, weighed and grouped according to both weight and gain during the preliminary period. Twelve rats were randomly assigned to each treatment. Fresh feed was weighed daily and supplied according to appetite. Water was supplied *ad libitum* throughout the feeding trial. Weight gains were recorded weekly. After 6 weeks of feeding the animals were fasted overnight, blood samples taken from the tail artery, and then the animals were killed. The livers and kidneys from 6 animals in each

TABLE I. Characteristics of Fat Materials Used in the Feeding Trial.

	Lard	Free fatty acids	Methyl esters
Moisture and volatile matter (%)	.01	.83	.10
Impurities (%)	.00	.00	.02
Unsaponifiable matter (%)	.06	.24	.80
Free fatty acids (as oleic) (%)	.23	101.2	—
Peroxide value (me/kg)	7.0	—	1.5
Iodine value (Wijs)	64.4	67.8	64.5

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treatment group were removed, blotted, weighed and fixed in 10% neutral formalin. The entire carcass of each remaining animal was frozen, lyophilized, ground and analyzed for proximate composition by standard methods. Histological sections taken from the left lateral lobe of the livers and longitudinal sections of the kidneys were stained with hemotoxylin and eosin and examined. Additional sections from the livers were stained with Oil Red O and examined for fat content.

Absorbabilities of the different fat materials were estimated from total fat intake and excretion during the sixth week of the feeding trial. Feces from 6 animals in each treatment group were collected and were analyzed separately using the method described by Hopkins *et al*(6). The blood sera were analyzed for polyunsaturated fatty acids by the lipoxidase method described by MacGee(7) and for cholesterol levels by the method of Bowman and Wolf(8).

A second experiment was performed in which the following materials were tested at the concentration indicated: triglycerides (lard), 10.0%; free fatty acids, 9.7%; free fatty acids, 9.7% + glycerol, .5%; methyl esters of fatty acids, 9.7%; and methyl esters of fatty acids, 9.7% + glycerol, 0.5%. The concentrations of the test materials were calculated to provide the same level of fatty acids in all diets. Twenty rats were started on each treatment. Three were sacrificed at the end of the third week of the feeding period, 14 at the end of the sixth week, and the remaining 3 were fed for a total of 144 days. Sections of the livers and kidneys from those animals killed at the end of the third week were examined histologically as in Exp. 1. All other conditions were the same as for Exp. 1.

Results. Weight gains, feed conversion ratios, absorbability values and serum polyunsaturated fatty acid and cholesterol levels obtained in the 2 experiments are presented in Tables II and III. Feed conversion ratios and fat absorbability were significantly ($P < .01$) poorer for both the free fatty acid and methyl esters of fatty acids preparations than for the intact triglycerides in Exp. 1. Serum polyunsaturated fatty acids levels were significantly ($P < .01$) higher in animals fed corn oil than in the other groups. No differences were found in the proximate composition of the carcasses or in the fresh weights of livers and kidneys.

Large areas of cellular degeneration and necrosis were found in the histological sections of the free fatty acid treated group. There was a general disruption of tissue architecture and many cells were without either cell boundaries or nuclei. All sections of the liver from the animals which had received the methyl esters of fatty acids preparation showed general cellular degeneration and necrosis. Liver sections from animals in the lard and the corn oil groups appeared normal.

The Oil Red O stained liver sections from animals fed free fatty acids contained large fat globules around the portal and central veins. Small globules of fat were found within the cells in the portal areas of the other groups.

Kidneys from the group fed methyl esters of fatty acids showed numerous calculi and areas of fibrosis. The other groups showed some inflammation around the larger arteries and some leucocyte invasion, but no calculi.

In the second experiment, the weight gains and feed conversion ratios were better than in the first experiment but still followed the same pattern. Organ weights, carcass composition, serum polyunsaturated fatty acids and serum cholesterol levels did not show any effect that could be attributed to the treatments. The livers and kidneys of rats killed at 3 weeks showed some indications of similar changes that were observed in the first experiment. Fat globules were accumulated in the areas of the central veins in the livers of the free fatty acid treated group, and the kidneys of the methyl esters of fatty acids treated group showed the first signs of calculi formation.

Discussion. The histological changes observed in the livers and kidneys of rats fed methyl esters of fatty acids indicate that preparations of methyl esters of fatty acids may be toxic when fed at high levels. Reiser *et al*(9) have reported that methyl ester preparations of tallow fatty acids were not

TABLE II. Effects of Triglycerides, Free Fatty Acids and Methyl Esters of Fatty Acids on Weight Gains, Feed Conversion Ratios, Absorbabilities and Serum Polyunsaturated Fatty Acid and Cholesterol Levels in Rats of Experiment 1.*

Test material	6 wk wt gain (g)	Feed : gain	Fat absorbability (%)	Serum PUFA (mg %)	Serum cholesterol (mg %)
Corn oil	220 ± 26	2.92 ± .11	89 ± 2.2	113 ± 15	102 ± 14
Lard	224 ± 25	2.95 ± .48	85 ± 2.6	81 ± 17	103 ± 14
Free fatty acids	214 ± 16	3.13 ± .12	69 ± 1.7	75 ± 18	95 ± 12
Methyl esters of fatty acids	214 ± 32	3.13 ± .22	74 ± 7.2	76 ± 13	99 ± 10

* Values shown are means ± sample standard deviation.

TABLE III. Effects of Triglycerides, Free Fatty Acids and Methyl Esters of Fatty Acids on Weight Gains, Feed Conversion Ratios, and Serum Polyunsaturated Fatty Acid and Cholesterol Levels in Rats of Experiment 2.*

Test material	6 wk wt gain (g)	Feed : gain	Serum PUFA (mg %)	Serum cholesterol (mg %)
Lard	250 ± 16	2.64 ± .19	75 ± 16	103 ± 10
Free fatty acids	241 ± 7	2.73 ± .06	69 ± 17	95 ± 10
Idem + glycerol	233 ± 14	2.81 ± .19	66 ± 13	94 ± 8
Methyl esters of fatty acids	216 ± 20	2.86 ± .25	68 ± 21	96 ± 11
Idem + glycerol	235 ± 12	2.76 ± .10	78 ± 10	97 ± 11

* Values shown are means ± sample standard deviation.

toxic to chickens, but they did not report histological observations. Raulin *et al*(10) found that free fatty acids in a diet containing only 0.05% choline were toxic to rats and that high levels of choline protected the animals against the toxic effects. The diet used in the present investigation contained 0.16% choline, which is more than twice the requirement for rats as stated by the National Research Council. Further investigations are needed to evaluate the findings with both the free fatty acid and methyl esters of fatty acids preparations.

The poor performance of the free fatty acids as indicated by both absorbability and feed conversion ratios was similar to the results of Carroll and Richards(1). The high concentrations of serum polyunsaturated fatty acids in the control group reflect the high concentrations of linoleic acid in corn oil. Differences between the remaining 3 groups were not significant. Serum cholesterol levels in the corn oil group do not appear to be reduced below those observed in the lard group. The higher serum polyunsaturated fatty acids may account for this apparent ineffectiveness of corn oil in reducing serum cholesterol. Other workers‖ in our laboratories have since found that corn oil, linoleic and linolenic acids interfere with analyses for cholesterol when ferric chloride is used as the color reagent. Since there was no pre-treatment of the serum to separate the cholesterol from the fatty acids before the color reaction was initiated, the cholesterol values obtained may be erroneously high.

Summary. Triglycerides, free fatty acids and methyl esters of fatty acids were compared for their effects on growth, feed conversion ratios, absorbability, serum polyunsaturated fatty acid and cholesterol levels, organ weights, carcass composition and liver and kidney histology when fed to young male rats. The livers from the animals fed free fatty acid and methyl esters of fatty acids showed evidence of cellular degeneration. Animals fed free fatty acids also showed some fatty infiltration. Kidneys from the animals fed methyl esters of fatty acids had numerous calculi. Neither the free fatty acids nor the preparations containing methyl esters of fatty acids were absorbed as well as the intact triglycerides. Rats fed the free fatty acids or the methyl esters of fatty acids required more feed per unit gain than did the animals fed the intact triglycerides. No dif-

‖ Kirk, M. C., Siedler, A. J., unpublished results.

ferences were found in weight gains, organ weights, carcass composition or serum cholesterol levels. Serum polyunsaturated fatty acid levels were not changed by feeding fatty acids in the different forms. Corn oil did elevate the polyunsaturated acid levels in the serum above those observed in the lard-fed group of rats.

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Effects of Exercise on Coxsackie A9 Myocarditis in Adult Mice.* (29696)

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A previous study of experimental viral myocarditis in adult mice suggested that spontaneous exercise of a mild degree and infection with Strain 13 of Coxsackie A9 virus each increases the relative weight of the heart and that these effects are additive(1). The present study was designed to determine the effects of forced, severe exercise under otherwise comparable conditions.

Materials and methods. A total of 108 male C_3H mice, 10 months old and weighing 23.9 to 41.6 g were housed 6 to a cage and fed standard Purina Rat Chow. They were divided into 4 groups: A. (36 mice) inoculated with virus and exercised; B. (36 mice) inoculated with virus, not exercised; C. (18 mice) not inoculated with virus, but exercised; and D. (18 mice) not inoculated with virus and not exercised. The exercise consisted of swimming in aquaria filled with water at 33°C. The mice in groups A and C were exercised for increasing periods,—once daily for 5, 15, 30 and 60 minutes on days 7, 6, 5 and 4, respectively, and twice daily for 60 minutes on the last 3 days *before* they were inoculated with virus. Following the inoculations, the swim period was 45 minutes twice a day for the first 3 days. After that, because the animals experienced difficulty toward the end of the swim periods on the third day, the period of exercise was shortened to 40 minutes twice daily until the day of sacrifice. All animals were weighed to the nearest 0.1 g before the training was begun, on the day of inoculation and immediately before they were sacrificed; surviving animals were also weighed on days 3, 6 and 9 after inoculation.

Viral infection. Mice of groups A and B were given an intraperitoneal injection of 10^6 $TCID_{50}$† of the third rhesus kidney passage of strain 13, Coxsackie A9 virus(2) in a volume of 0.03-0.05 ml. Mice in groups C and D were given an intraperitoneal injection of uninfected tissue culture media containing

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† $TCID_{50}$ is the dose of virus which infects 50% of inoculated tissue culture tubes.