The Effects of Various Physical Activity Levels and Dietary Carbohydrate Source on Rat Cholesterol Levels[1] (34940)

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(Introduced by M. Womack)

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Because of the association between higher levels of cholesterol in the blood serum and atherosclerosis, much interest has been manifested in the effects of exercise and physical training on the serum lipids (1). Mayer states that "very hard, prolonged physical work at a level difficult to achieve in urbanized Western societies does lower serum cholesterol levels even in subjects consuming a high-fat diet" (2). In the continuing study of the relationship between diet and atherosclerosis, emphasis in the past decade has shifted to include a study of the role of dietary carbohydrate as well as of dietary fat (3). Ahrens *et al.* (4) reported in 1968 that young adult male rats fed cornstarch demonstrated a lower level of carcass cholesterol than rats fed sucrose, lactose, or a mixture of carbohydrates representative of an average "market basket" diet in the United States. The rats fed the mixture of carbohydrates increased in cholesterol at a more rapid rate as body weight increased than did those fed the single carbohydrate sources. This finding has recently been confirmed in young growing male rats (5). We felt the need to examine the effect of intensity of exercise on serum and tissue cholesterol levels in young adult male rats fed high-fat diets differing only in their source of dietary carbohydrate.

Methods and Materials. Ninety 85-day old, male Sprague-Dawley rats[3] with a mean body weight of 260 g were housed individually in wire-bottomed cages and were fed a stock diet[4] for a week during which they were trained to exercise by swimming with weights attached to their tails. At the end of the week, 10 rats were selected randomly and killed for initial carcass and serum cholesterol analyses. The remaining rats were randomly divided into two groups of 40 each which received either a diet in which cornstarch was the only carbohydrate or in which the carbohydrate was a mixture representative of that found in U.S. "market basket" diets (6). These were both nutritionally adequate diets[5] with 38% of the calories from protein, 50% from fat, and 12% from one of the two carbohydrate sources (4, 5) as indicated in Table I. We reported previously that the effect of the carbohydrate source on cholesterol accumulation is just as evident at 12% of calories as at 54% (4).

The rats in each dietary group were randomly subdivided into 4 forced-exercise treatment groups of 10 rats each: (1) immersed in water up to their necks while in restraining cages; (2) swimming with a weight equivalent to 2% of initial mean body weight attached to the tail (moderate exercise); (3) swimming with a weight equivalent to 4% of body weight (heavy exercise); (4) swimming with a weight equivalent to 6% of body weight (exhausting exercise). The water temperature was maintained at 27°, and

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[3] From Rockland Farms, Gilbertsville, Pennsylvania.

[4] Rockland Rat/Mouse Diet. Chesapeake Feed Company, Inc., Beltsville, Md.

[5] Prepared by General Biochemicals, Chagrin Falls, Ohio.

TABLE I. Composition of the Diets Fed to Rats Subjected to Exercise Treatments of Different Degrees of Intensity.

Ingredients	Cornstarch diet (g/kg)	Mixture diet (g/kg)
Lactalbumin	480.7	480.7
Cornstarch	155.0	62.0
Lactose	—	31.0
Dextrin, white, technical	—	15.5
Sucrose	—	15.5
Glucose	—	15.5
Fructose (levulose)	—	15.5
Safflower oil	67.0	67.0
Beef tallow, edible	214.0	214.0
Salt mix, Bernhardt–Tomarelli (7)	54.0	54.0
Non-nutritive fiber	28.1	28.1
Vitamin mix (4)	1.2	1.2
Total	1000.0	1000.0
Gross energy (kcal/g)	5.07	5.07

the daily exercise period was limited to 10 min, since this was the maximum endurance time of the rats swimming with a 6% load.

At the end of 60 days each rat was anesthetized with sodium amytal and blood removed by heart puncture for serum cholesterol analysis. The aorta was removed, fixed in formalin, and stained with Sudan IV in a 1:1 mixture of acetone and ethanol. The stained aorta, cut open and fixed in glycerol, was then graded three times by three independent graders on a scale from 1 to 5 reflecting the intensity of staining at the intimal surface. The gastrointestinal tract was removed, flushed of its contents, and returned to the animal carcass. After weighing, the ingesta-free carcasses were diluted with added water and ice at a ratio of 2:1 and homogenized in a Waring Blendor. Cholesterol analysis of carcass homogenates and blood serum samples was done by the method of Koval (8). Changes in cholesterol content over the 60-day experimental period were determined by comparing these levels with those found in the 10 initial control rats. This was a 2 × 4 factorial design conducted as a randomized complete block experiment with 10 replications.

Results. Table II shows the comparison of means for kilocalories consumed per day, body weight gain, serum cholesterol change, and scores given for affinity of the aorta for lipophilic stain (Sudan IV). An analysis of variance indicated that the mean food intake of the rats fed the diet containing the mixture of carbohydrates (70.6 kcal/day) was higher ($p<.05$) than that of the rats consuming the starch diet (67.6 kcal/day). The rats exposed to the 2% (moderate exercise) swimming treatment gained the least weight ($p<.05$). This was evident on both experimental diets.

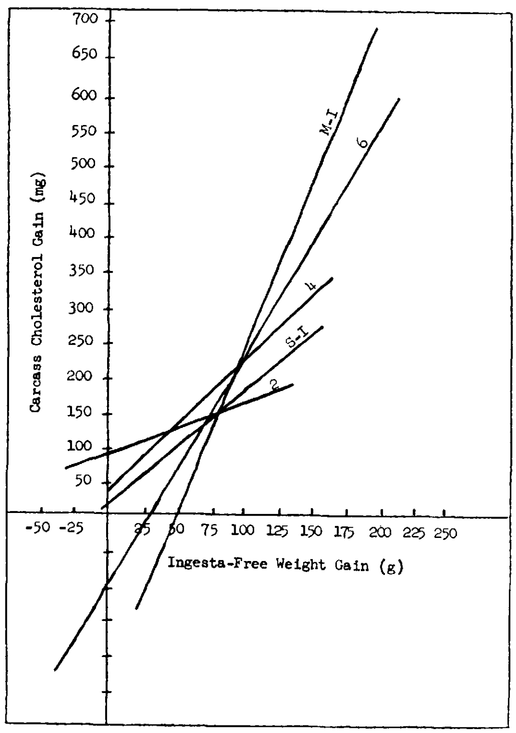

FIG. 1. Regression lines of body cholesterol gain vs ingesta-free weight gain. The diet fed significantly affected these regressions only in the sedentary (immersed) animals. Therefore, the S-I and M-I lines represent 10 observations each while the 2, 4, and 6% lines represent 20 observations each. S = starch diet; M = mixture of carbohydrates; I = immersed; 2, 4, and 6% are the fractions of initial body weight attached to the tail while swimming. The regression coefficients were as follows: M-I, 4.953 mg/g; S-I, 1.792; 2%, 0.756; 4%, 1.941; and 6%, 3.707.

TABLE II. Comparison of Means for Kilocalories Consumed per Day, Body Weight Gain, Serum Cholesterol Change, and Intensity of Lipophilic Staining of the Aorta.

Treatment group	No. of rats	*Ad libitum* intake (kcal/day)	Body wt gain (ingesta-free, g)	Serum cholesterol (mg/100 ml change)	Aorta stain Sudan IV (mean score)[a]
Starch—Immersed	10	68.8 ± 1.7[b]	83.7 ± 15.1	3.9 ± 7.7	2.57 ± 0.37
Starch—2%[c]	10	64.5 ± 1.6	43.4 ± 19.3	7.0 ± 2.6	1.93 ± 0.25
Starch—4%	10	68.9 ± 1.5	93.9 ± 11.0	−0.5 ± 4.7	2.56 ± 0.19
Starch—6%	10	68.1 ± 1.2	89.5 ± 16.7	13.7 ± 4.1	3.13 ± 0.21
Mixture[d]—Immersed	10	70.4 ± 2.0	96.5 ± 17.0	12.5 ± 4.4	2.75 ± 0.15
Mixture—2%	10	70.5 ± 1.5	78.0 ± 10.7	14.0 ± 4.7	2.40 ± 0.15
Mixture—4%	10	72.5 ± 1.4	93.7 ± 14.3	−5.9 ± 4.9	4.21 ± 0.22
Mixture—6%	10	69.0 ± 1.1	93.4 ± 15.0	−10.9 ± 5.5	2.53 ± 0.32

[a] Mean score represents the average of three scorings apiece by three independent scorers (nine scorings) of each aorta on a scale of 1 to 5 reflecting intensity of staining with a lipophilic stain (Sudan IV).

[b] Standard error of the mean.

[c] Intensity of swimming determined by percentage of body weight attached to tail.

[d] Dietary carbohydrate source was a mixture of carbohydrates approximating that found in U. S. "market basket" diets (6).

Serum cholesterol change followed a more consistent pattern among the rats fed the mixture of carbohydrates than among those fed only starch. On the mixture diet the rats that swam with a 6% load (exhausting exercise) showed the greatest decrease in serum cholesterol concentration, and this decrease was larger ($p < .01$) than the change noted in the rats that were moderately exercised (2% load).

The intensity of Sudan IV staining in the excised aortas was not a direct reflection of the serum cholestrol change. Among the rats on both diets the aortas from those that were moderately exercised (2% load) had the least affinity for the lipophilic stain. An analysis of variance indicated that the mean Sudan IV staining score for the rats that swam with a 2% load was less than that found in those that swam with a 4% load ($p < .01$) or a 6% load ($p < .05$). This agrees quite well with the cholesterol gain in the ingesta-free carcass (Fig. 1). The dietary carbohydrate source, even at only 12% of calories, significantly affected the rate of cholesterol accumulation in the carcass, but only among the sedentary (immersed) animals. The regression coefficient of 4.953 (mg cholesterol gain/g body weight gain) for the 10 immersed-mixture fed rats was larger ($p < .05$) than the regression coefficient of 1.792 found in the 10 immersed starch-fed rats. The effect of exercise on this regression was similar on both diets and the regression coefficient of 3.707 found in the 20 rats swimming with a 6% load (exhausting exercise) was larger than the 1.941 found in the 20 rats swimming with 4% load (heavy exercise) ($p < .05$) or the 0.756 found in the 20 rats swimming with a 2% load (moderate exercise) ($p < .01$).

Discussion. First of all, we did confirm the observation of Mayer that "very hard, prolonged physical work . . . does lower serum cholesterol levels. . . ." (2). The rats consuming a high fat diet with 12% of the calories from a mixture of carbohydrates similar to that found in U.S. "market basket" diets (6) did show the greatest reduction of serum cholesterol at the more severe levels of exercise (4% and 6% loading). The source of that 12% of the calories was critical, however, as a similar pattern was not discernible when starch was the only carbohydrate source in the diet.

The amount of body weight gained, the rate of carcass cholesterol accumulation, and the intensity of Sudan IV staining in the aorta were all lowest at the more moderate level of exercise (2% loading). This indicates quite clearly that serum cholesterol level alone is not a suitable criterion for making far-reaching statements about conditions which affect the health of the individual. The moderately exercised rats had the lowest body weight, the lowest rate of increase in tissue cholesterol as they gained in weight, and the lowest accumulation of material in the aortic tissue that responded to lipophilic staining. All of this evidence indicates that, under the conditions of this experiment, a program of moderate daily exercise was more desirable than programs of heavy or exhausting activity.

The rate of accumulation of cholesterol in the carcass as the rats gained in weight was significantly dependent on the dietary carbohydrate source as we have reported before (4, 5), but only in the sedentary (immersed) rats. The effect of daily exercise was to override this dietary effect. Results noted in sedentary subjects can be safely applied only to other sedentary subjects. Exercise changes many patterns of dietary utilization.

Summary. Three levels of forced swimming (2%, 4%, and 6% of body weight attached to the tail) were compared to the sedentary state (immersion) in young adult male rats fed a high-fat diet containing 12% of the calories as either cornstarch or a mixture of carbohydrates similar to that found in U.S. "market basket" diets. The more strenuous levels of exercise gave the greatest reduction of serum cholesterol in the rats fed the carbohydrate mixture. However, the most moderate level of exercise (2% loading) led to the lowest body weight, the lowest rate of increase in tissue cholesterol as the rats gained in weight, and the lowest accumulation of material in the aortic tissue that responded to lipophilic staining. Although the dietary carbohydrate source did have an effect on the rate of tissue cholesterol accumulation, this was true only in the sedentary rats.

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