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THE EFFECTS OF CALORIC DENSITY OF THE FOOD ON RUNNING ENDURANCE AND GENERAL CONDITION OF RATS AND HAMSTERS RESTRICTED IN FOOD INTAKE OR FED AD LIBITUM

by

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NATICK, MASSACHUSETTS 01760

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Young adult male rats and hamsters, after a treadmill training period, were divided into 6 groups, receiving 20, 40 or 70% dietary fat calories and fed ad libitum or 40% by weight of average ad libitum intake. (For the hamsters under tropical climatic conditions the latter figure was about 80%.) All animals were run to exhaustion once a week. Increased dietary fat level was not correlated with superior running performance at either food intake level, although during restricted feeding the high fat groups received more calories.

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Increased running endurance and kgm performance were correlated with lower body weight at both food intake levels. The rate of performance increase with decreasing body weight during food restriction was significantly higher for the low than for the high fat rat group. Water consumption was considerably lower during restricted than during ad libitum feeding. Plasma glucose in the rats was lower for the 70% than for the other fat levels at both levels of food intake, lower from day 8 on during restricted than during ad libitum food intake and lower on day 15 than on day 8 of the restricted intake. In the hamsters, average plasma triglyceride levels were 76% and plasma total cholesterol levels 22% (significant) higher in non-exercised than in regularly exercised animals right after running to exhaustion. In the hamsters kept at tropical climatic conditions, plasma total cholesterol at both food intake levels was significantly (an average of 92 and 118%) higher on the 70 than on the 20% dietary fat level. The implications of ti· findings have been discussed.

SUMMARY

Young adult male rats and hamsters, after a treadmill training period, were divided into 6 groups, receiving 20, 40 or 70% dietary fat calories and fed ad libitum or 40% by weight of average ad libitum intake. (For the hamsters under tropical climatic conditions the latter figure was about 80%.) All animals were run to exhaustion once a week. Increased dietary fat level was not correlated with superior running performance at either food intake level, although during restricted feeding the high fat groups received more calories. Increased running endurance and kgm performance were correlated with lower body weight at both food intake levels. The rate of performance increase with decreasing body weight during food restriction was significantly higher for the low than for the high fat rat group. Water consumption was considerably lower during restricted than during ad libitum feeding. Plasma glucose in the rats was lower for the 70% than for the other fat levels at both levels of food intake, lower from day 8 on during restricted than during ad libitum food intake, and lower on day 15 than on day 8 of the restricted intake. In the hamsters, average plasma triglyceride levels were 76% and plasma total cholesterol levels 22% (significant) higher in non-exercised than in regularly exercised animals right after running to exhaustion. In the hamsters kept at tropical climatic conditions, plasma total cholesterol at both food intake levels was significantly (an average of 92 and 118%) higher on the 70 than on the 20% dietary fat level. The implications of these findings have been discussed.

PREFACE

The experiments reported on here were conducted to document the degree to which physical performance and — condition of rats and hamsters are affected by caloric density of the food when fed ad libitum or restricted. This information is necessary to determine during a food intake restricted to a uniform, relatively low weight, which dietary fat level will result in the best running performance, among other factors, and to compare these parameters with those obtained at similar dietary fat levels during ad libitum feeding.

The author is grateful to Dr. William K. Calhoun for his suggestions and to Julian W. Ratteree, Bonita M. Atwood and Lawrence M. Breslouf for their technical assistance.

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INTRODUCTION

An answer was desired on the question how caloric density or fat content of the food would affect walking or running endurance and some related physical parameters of young adult humans. This information was and is primarily of interest under conditions of restricted and equal weight of food intake for periods of up to ten days, and different climatic conditions.

Recognizing the limitations of using small animals as models in a first approximation of the objective to be attained, such an initial approximation still has its advantages in terms of numbers available, important in a statistical evaluation, possibilities in experimental design, and ease and cost of execution.

Where weight of food to be carried is an important consideration, it becomes desirable to know how an increased caloric density of the food, meaning higher fat content, affects physical performance such as walking or running endurance.

Most information regarding the effect of diet on exercise endurance has been obtained in human subjects and, generally, under ad libitum-fed conditions.

Many authors have reported a reduced ability of human subjects to work on high-fat diets, as compared to "normal" or high-carbohydrate diets (1-9). On a high-carbohydrate diet there was no progressive change in efficiency, but on a high-fat diet the efficiency progressively declined to at least the 7th day, according to Marsh and Murlin (10). Hollmann and coworkers (8) have reported an average increase of 61% in ergometer cycling duration on an all-carbohydrate as compared to a "normal" diet. These differences in performance with different dietary fat and carbohydrate levels were not observed, however, during brief periods of strenuous exercise (11). Takagi (12) observed that treadmill running endurance of dogs decreased as the fat content of their diet increased. Ershoff (13) found that, at a water temperature of 20°C., the swimming performance of mice and two strains of rats on a low-fat ration was significantly longer than that of animals fed a similar diet supplemented with fat. Deuel, Scheer and coworkers (14, 15), on the other hand, found that young rats swam longer on a 20% than on a 5% fat diet. No further improvement occurred on a 40% fat diet. However, after an initial adjustment by means of weights for different specific gravities by these authors, the same additional weights were attached during swimming, regardless of body size or specific gravity of the rats. Since the rats on the higher fat diets generally were heavier, and since a positive correlation existed between body weight and swimming duration, this methodology favored the rats on the higher fat diets. Swimming times here averaged only 13 to 20 minutes. Voluntary activity of rats on an unlimited intake has been reported (16) to be significantly higher on a high-starch diet than on either of two high-fat diets. On food intake limited to the unlimited intake of the least active group, voluntary activity for the high-starch group was about twice that of the high-soybean-oil group and about four times that of the high-coconut-oil group. All experiments quoted so far, unless otherwise mentioned above, were under conditions of ad libitum feeding and of up to six hours or more [such as in (2)] of exercise per test per day.

Physical fitness on a high-fat, low-carbohydrate diet deteriorated so fast as to render human subjects completely useless operationally within three days (2). Chief complaints were nausea, weakness and excessive fatigue. The same experience on high-fat diets, including extreme fatigue, not only localized in the exercising limbs but also generally, as well as dizziness, headache, irritability, and a greater need for sleep has also been reported by others (5, 10, 17-19). These effects of the high-fat diets lasted for several days after terminations(10). Apparently, more than 10% of the calories from carbohydrate (more than 85 g per day for the human (10)) is required to prevent these symptoms during ad libitum feeding.

In addition to the factors mentioned above, on the average 10 to 11% less of the combustion energy is available for muscular work from fat than from carbohydrate (1, 3, 10, 17, 18). Per kgm of work output, less oxygen is used up during the oxidation of carbohydrate. This increases the maximal work output possible on carbohydrate. White muscle, and to some extent red muscle, appears potentially capable of deriving more usable energy by oxidizing carbohydrate than fat (20).

The relative contribution of carbohydrate and fatty acids as energy source for the working muscle depends on both the work intensity (4, 7, 21-24) and previous diet (1, 3, 21, 25-27). The higher the work intensity the higher the relative contribution from carbohydrate. At moderate or high work intensities, the carbohydrate contribution to total energy output cannot be replaced by any other substrate (24). Over a 6-hour period after intake of a standard meal, the percentage of energy during exercise derived by dogs from carbohydrate oxidation was much higher than in the postabsorptive state (28).

A close, positive correlation has been found to exist between initial glycogen content of the working muscles and exercise endurance time (other than for brief, highly intensive exercise bouts) (4, 5, 25, 27). This has been found true for performance times up to about 6 hours and moderate to fairly heavy exercise levels (40 to 77% of maximal oxygen uptake). The glycogen content of the working muscle is one of the main determinants of the capacity for prolonged continuous exercise. When the glycogen stores are exhausted, work cannot be continued at the same rate possible when glycogen is available (4, 8, 23, 29-31). Liver glycogen, also, is metabolized during exercise (32-34). In mice, liver glycogen has been reported to be closer correlated with swimming time than is muscle glycogen (32).

Furthermore, a strong, positive correlation has been observed between initial muscle and liver glycogen content, and carbohydrate content of the previous diet (4, 5, 26, 35).

A decrease in blood glucose concentration has been reported (1, 4, 5, 7, 25) in human subjects, fed ad libitum, during and after moderate intensity exercise to exhaustion and in most cases of fairly heavy exercise (about 50% to about 70%, respectively, of maximal oxygen uptake). This drop in blood glucose can be one of the factors limiting endurance during fairly heavy work of several hours duration (4, 7). Under such conditions, central nervous system symptoms typical of hypoglycemia often occur at the point of exhaustion (5, 7). According to Hultman and coworkers (30, 36), the fall in blood glucose concentration is less pronounced during more severe work, leading to more rapid exhaustion. However, even here somewhat more of a fall in blood glucose concentration occurred close to exhaustion after a high fat diet than after a high carbohydrate diet. Bergström et al. (5) report that the blood glucose concentration of human subjects during and after fairly heavy exercise (75% of maximal oxygen uptake) fell to extremely low levels only after a high-fat diet. In this situation the subjects experienced fatigue, not only localized in the exercised limbs but also generally.

Henschel et al. (37), in a five-day starvation study, during which weight losses of human subjects averaged 7.8%, observed a considerable decrease in exercise performance towards the end of the test period. On the other hand, on 500 to 580 kcal per day, mostly or entirely from carbohydrate, no significant decrease in exercise efficiency and stamina of human subjects occurred during a ten- to twelve-day period (38, 39). On an average intake of 1800 kcal per day from a high-fat, low-carbohydrate diet, coupled with fairly heavy, long-term exercise of human subjects, frank incapacitation with extreme general tiredness and nausea was observed within three days (2). Others (40, 41), likewise, have observed a lack of energy and excessive tiredness within days when human subjects were placed on low-carbohydrate, low-caloric (1500, respectively 1800 kcal per day) diets. These symptoms were lessened but not entirely eliminated even with the inclusion of 104 g of carbohydrate per day substituted for fat in the diet (19). Swimming endurance of rats has been found to decrease with decreasing caloric intake (15). On a caloric intake of about one-third of ad libitum, no significant improvement in swimming time with increasing percentage of fat in the diet was found for these rats. On a caloric intake of about two-thirds of ad libitum, a higher average swimming endurance time was observed on a diet containing 40% fat than on a lower fat level. However, results tended to be biased towards the heavier rats, being in the higher fat level groups, because of a lower percentage weight attachment to the heavier rats during the course of the swimming period. Furthermore, swimming times were of relatively short duration (about 5 to 20 minutes).

When the preceding diet is of average composition, human beings as well as most animals, including rats, show an initial decrease in blood sugar level during the first few days of fasting and semi-starvation, followed by a gradual return to normal (4 -45). The blood glucose levels in a group of human subjects on a 1000 kcal per day intake for 5 days fell most for the group receiving a very-low-carbohydrate diet (pemmican), least for the group receiving the most sugar (80 g/day) (44). Muscle and liver glycogen also decrease during the first few days of starvation in both man and rats (42, 46).

The previous diet also has been shown to affect both blood glucose levels of man and rats (43, 47, 48) and exercise endurance of rats (48) during subsequent fasting.

Swank and Cullen have observed an increase in blood viscosity and slowing, even cessation in certain areas, of the circulation, accompanied by increased aggregation and adhesiveness of red blood cells, in hamsters after high fat meals (49-51). The blood viscosity began to increase about 3 hours after cream feeding and reached a peak in 6 to 9 hours after the fat meal. An observed increase of 50 to 100% in the blood viscosity was not unusual. The hamsters with elevated blood viscosity exhibited much more than the usual degree of cyanosis of the venous blood (51). These changes were reversible. In rabbits (52), but not in dogs (53), circulation times were also prolonged after fat feeding. Intravenous infusions of fat emulsions have been observed to lower arterial blood oxygen content in dogs (54, 55) and in human subjects (54), and to decrease the circulation rate of the blood in peripheral vessels. Control of lipemia in patients with clinical atherosclerosis by the use of low fat diets resulted in a rise in arterial oxygen saturation, and in an increase in exercise tolerance (54).

It hardly requires mentioning that changes in blood viscosity and, thereby, circulation rate, affect the supply of nutrients, including oxygen, to the working muscles, as well as the work required by the heart and, thus, affect exercise performance.

In the physiological range of blood flow, blood behaves as if it were a Newtonian fluid (56). Blood viscosity under these conditions has been found to increase exponentially with an increase in hematocrit (56, 57). Aggregation of red blood cells in humans and dogs has been observed 4 to 9 hours after a large fat meal, accompanied by a decrease in hematocrit (49). In the hamster, during the first 9 hours after a fat meal only minor variations occurred in the hematocrit. The blood viscosity returned to normal by the end of 24 hours (51). This was accompanied by a significant drop in the hematocrit. The latter then returned to normal by the end of 72 hours.

MATERIALS AND METHODS

I. Rats. Temperate climatic conditions. Male, young adult Long-Evans rats[1] were housed individually in stainless steel cages at 21 ± 1°

[1] Obtained from Simonsen Labs., CA.

(mean ± SD) C. and 50 ± 4% relative humidity. When received, they were 60 days old and weighed 245 ± 14 g. For the first 4 months after arrival they were fed commercial rat chow[2] pellets, after that for 3 weeks a semipurified diet containing 40% of the total calories from fat, as described below. Following this, starting the actual experimental phase, they were divided into six groups, each receiving its own diet, namely, ad libitum: 20%, 40%, and 70% of the total calories from fat; restricted food intake (40% of the average ad libitum intake): 20%, 40%, and 70% of the calories from fat. These diets will be described below in further detail. A treadmill training program was started one week after the rats were received. This treadmill was a modification of a design used by Kimeldorf (59), and employed an endless, motor-driven belt with a number of ventilated cages suspended above it. The 7 weeks preceding the actual experimental phase the rats were run to exhaustion once a week. One day after being put on the different experimental diets and with one week intervals from there on they were, likewise, run to exhaustion. These runs to exhaustion took place at a speed of 22.3 mpm and an incline of 12 1/2°. The food was taken away 1 hour prior to the running. Of the 72 animals that had been received, 48 were selected for the experimental phase. These remaining animals were randomly divided in equal numbers between the six groups.

The composition, in %, of the diet containing 40% fat calories was as follows: vitamin-free casein[3], 20.0; vegetable shortening[4], 10.45; lard[5], 10.45; corn starch, 30.3; dextrin, 10.1; dextrose, 10.1; cellulose, 4.0; mineral mixture, 4.2; vitamin mixture, 0.4. The diets containing 20% and 70% fat calories contained, respectively, 4.55 and 23.3% of, each, vegetable shortening and lard. The difference, compared to the 40% fat diet, was made up by adjustments in the amount of corn starch. The mineral mixture was composed as follows (%): $CaCO_3$, 15.8; $CaHPO_4$, 35.8; KCl, 16.5; Na_2HPO_4, 17.2; $MgSO_4.7H_2O$, 12.4; $MnSO_4.H_2O$, 0.44; $ZnCO_3$, 0.11; $CuSO_4.5H_2O$, 0.19; Fe-citrate, 1.46; KIO_3, 0.005; Cr(III)-acetate (59-61), 0.05. The vitamin mixture, per kg of diet, consisted of (mg, unless stated otherwise): vitamin A, 6000 I.U.; vitamin D, 3000 I.U.; alpha-tocopherol, 80; menadione, 0.30; thiamine.HCl, 6.0; riboflavin, 5.0; pyridoxine.HCl, 3.0; niacin, 30; Ca-pantothenate, 16; choline (as dihydrogen citrate), 1.23 g; vitamin B_{12}, 0.056; ascorbic acid, 60; inositol, 17; p-aminobenzoic acid, 17; folic acid, 2.0. The diets were kept refrigerated.

For logistic reasons, as explained in the introduction, the animals on restricted intake all received the same weight of food.

Heparinized blood samples were collected, either from the tail

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2 Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO.

3 All ingredients, unless otherwise stated, were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

4 Commercial, general purpose, high stability (100 AOM) shortening.

5 Armour Co., Chicago.

or during exsanguination, within 2 minutes after exhaustion of the rats run on the treadmill, as well as from rats not exercised. Deproteinization was effected by using 6% $ZnSO_4.7H_2O$ and approximately 0.3 N NaOH (titrated against the $ZnSO_4$ solution), as a modification of the method of Somogyi (62).

Plasma glucose levels were determined employing an automated microtechnique[6] which involved a modification of the ferri-ferrocyanide oxidation-reduction method according to Hoffman (63) and a 1:10 sample dilution with 0.9% NaCl solution. Since heparin has been found to raise recorded glucose values (64), standards were run only after the saline diluent was heparinized.

Lactic acid determinations were performed on deproteinized blood samples, diluted to different extents with distilled water, depending on the anticipated range of lactic acid values. The method adapted for automation according to Hochella and Weinhouse (65) was employed, except that the heating bath was kept at 37°C.

II. Hamsters. Temperate climatic conditions

A. This experiment served as a preliminary study for the next one under similar conditions.

Male, young adult Syrian hamsters[7] were housed individually in galvanized steel cages at 22 ± 1°C. (mean ± SD) and 50 ± 4% relative humidity. When received, they were 12 weeks old and weighed 114 ± 4 g. For the first 3 weeks after arrival they were fed commercial chow[2] pellets, after that for 7 weeks a semipurified diet containing 40% of the calories from fat, as described below. Following this, starting the actual experimental phase, they were divided into six groups, each receiving its own diet, similar to those used for the rat experiment described above. A treadmill training program was started 2 weeks after the hamsters had been received. Two days after having been put on the different experimental diets and one week hereafter they were run to exhaustion. These runs to exhaustion took place at a speed of 27.4 mpm and an incline of 14°. The food was, again, taken away 1 hour prior to the running. Of the 90 animals that had been received, 68 were selected for the test phase and randomly divided between the six groups.

The composition of the diets was the same as for the rat experiment described above, except for the mineral and vitamin mixtures. The mineral mixture used was one according to Rogers-Harper[8]. The vitamin mixture, per kg of diet, consisted of the following (in mg, unless stated otherwise): vitamin A, 12000 I.U.;

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6 Technicon Auto Analyzer methodology N-9, Technicon Instrument Corp., Chauncey, N.Y.

7 Obtained from Lakeview Hamster Colony, Newfield, N.J.

8 Obtained from General Biochemicals, Chagrin Falls, Ohio.

vitamin D, 3000 I.U.; alpha-tocopherol, 100; menadione, 0.30; thiamine.HCl, 10.0; riboflavin, 6.0; pyridoxine.HCl, 5.0; niacin, 40; Ca-pantothenate, 40; choline, 705; vitamin B_{12}, 0.050; ascorbic acid, 60; inositol, 200; p-aminobenzoic acid, 50; folic acid, 2.0; biotin, 0.10. The diets were, again, kept refrigerated.

The animals on restricted intake, again, all received the same weight of food.

Heparinized blood was collected from the abdominal aorta as described by Manning and Giannina (66), except that no hemostat was used in order to reduce pressure. The collection from animals run on the treadmill, again, occurred within 2 minutes after exhaustion. Deproteinization of the blood was effected by adding 0.3 ml to 1.7 ml distilled water, then adding 0.5 ml 0.3 N $Ba(OH)_2 \cdot 8H_2O$, 0.65 ml 5% $ZnSO_4 \cdot 7H_2O$ and centrifuging.

Plasma triglyceride levels were determined according to the micro method of Van Handel (67), plasma cholesterol levels according to the method of Abell and coworkers (68). Both determinations were done on animals fed ad libitum only.

B) Male, young adult Syrian hamsters[7] were housed as in the previous experiment, mentioned above, at 24 $\pm$ 1°C. and 57 $\pm$ 3% relative humidity. They were transferred to stainless steel cages with urine and feces collection equipment during the last 3 weeks of the experiment. When received, they were 10 weeks old and weighed 112 $\pm$ 4 g. For the first 4 weeks after arrival they were fed commercial chow[2] pellets, after that for 12 weeks a diet containing 40% of the calories from fat, identical to the one used for hamsters in the experiment mentioned above, except that it contained (%): corn starch, 36.5; sucrose, 12.4; dextrose, 1.6; corn oil, 3.7; vegetable shortening[4], 8.6; and lard[5], 8.6. Following this, starting the actual experimental phase, they, again, were divided into six dietary treatment groups. In this case, these all had a restricted food intake and were equally divided into groups receiving (calories from fat, and daily food intake, respectively): 20%: 3.5 g, 4.1 g; 40%: 3.5 g, 3.5 g; 70%: 3.5 g, 2.7 g. The 3.5 g represented an average of about 40% by weight of the ad libitum consumption on the 40% fat calories diet. The other intakes represented an equal amount of calories from the different diets. Moisture contents for the diets containing 20, 40 and 70% fat calories were, respectively: 9.6, 8.2, and 4.6%. Treadmill training, again, was started 2 weeks after the hamsters had been received. They were run to exhaustion twice while still all fed ad libitum on the diet with 40% fat calories; and twice on the actual experimental regimen with one week interval, the first time 2 days after the start of this regimen. These runs to exhaustion took place at 31.4 mpm and 6° incline. The food was, again, taken away 1 hour prior to the running. Of the 90 animals that had been received, 66 were, again, selected for the test phase and randomly divided between the six groups.

Caloric density of the diets and feces was determined in an oxygen bomb calorimeter[9], after drying of the samples for 18 hours at 70°C. and 1 cm Hg.

In order to determine transit time of the food through the gastro-intestinal tract, 9 hamsters of the same age and source and treated the same way as in the main experiment, but not part of this experiment, were used. Diets thoroughly mixed with 0.3% carmine[10] were fed at 16:15 hrs., 7 hours after removal of the food. All animals ate the marked diets nearly immediately.

Total urine ketone bodies were determined according to the method of Chernick (69), removing impurities by means of using $CuSO_4$ and $Ca(OH)_2$, as described.

Total lipid content of the feces was determined by employing the method of Amenta (70), adapted for small samples.

III. <u>Hamsters. Tropical climatic conditions</u>. Male, young adult Syrian hamsters[7] were housed in stainless steel cages, initially at 23 ± 1°C. and 51 ± 4% relative humidity. When received, they were 10 weeks old and weighed 102 ± 5 g. For the first 3 months after arrival they were fed commercial chow[2] pellets, and after that for 7 weeks a diet containing 40% of the calories from fat, identical to the one used for hamsters, as described above under (IIB). Following this, starting the actual experimental phase, they were divided into six dietary treatment groups, similar to those in experiment I and IIA, except that the groups on a restricted, equal amount of food intake received about 80% of the amount of the ad libitum fed animals. The room temperature was gradually increased, starting at 26.5°C. one week before being put on the 40% fat calories diet, to 34 ± 1°C. 5 weeks later. The humidity was kept about the same and during the main experimental phase averaged 55 ± 2%. Treadmill training, again, was started 2 weeks after the hamsters had been received. They were run to exhaustion once while still all fed ad libitum on the diet with 40% fat calories and twice on the actual experimental regimen, as in the previous experiments with hamsters, recorded above. These runs to exhaustion took place at 24.4 mpm and 9° incline. The food was, again, taken away 1 hour prior to the running. The number of animals started with and selected for the test phase was identical to those in the previous experiment, recorded above. The same is true for the method of caloric density determination of food and feces used.

Blood flow rates as an indication of viscosity were determined using a water-jacketed, glass capillary tube with an inner diameter

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[9] Model 1221 equipped with model 2611-1525 adiabatic control system, Parr Instrument Co., Moline, Ill. 61265.

[10] Fisher, Certified Biological Stain, Fisher Scientific Co., Pittsburgh, Pa. 15219.

of 0.446 mm (determined by filling with mercury), to which a suction of 7.5 cm H_2O was applied. This tube was bent 90° at a distance of 6.4 cm from the tip. The tip was vertically immersed in the blood so that the horizontal section was 5.9 cm above the surface of the blood. The horizontal section had two markings, 10.0 cm apart. From 5.0 cm above the tip and 5.0 cm before the first mark to 1.5 cm beyond the last mark the tube was water-jacketed, the water being kept at 37 $\pm$ 0.5°C. Heparinized, 0.5 ml aliquots of blood were used for the determinations, pre-warmed for 3 minutes at 37° and mixed by inversion immediately prior to the readings. The shear rates produced in this manner were in the range of 7-10 cm^{-1}, compatible with blood flow conditions in medium-sized arterioles (71). In a comparison of EDTA[11] (1.5 mg/ml) and heparin[12] (0.4 mg/ml) as anticlotting agents, blood flow rate in the presence of heparin was found to change somewhat less with variations in time that the blood was kept after drawing. Blood obtained 1 hour and 2 hours, respectively, after drawing, and kept in the refrigerator until used for the determinations, was compared for this purpose. Little, if any, effect on the blood flow rate was observed from a change in heparin concentration of 0.40 to 0.51 mg/ml of blood in a test for this purpose. The blood flow rate 1 hour after drawing with the two anticlotting agents mentioned was comparable at the concentrations used.

Plasma cholesterol was determined employing the method of McDougal and Farmer (72).

RESULTS

I. Rats. Temperate climatic conditions

Running performance. If only the last 4 times of running to exhaustion are used before the experimental phase with different diets, the preliminary, unweighted average times for what were to become the groups ad libitum, 20%, 40% and 70% fat calories, and restricted, 20%, 40% and 70% fat calories are, respectively, 44.8, 45.7, 52.2, 42.0, 38.0, and 44.1 minutes. If, proceeding in time towards the actual experimental phase, for each prospective diet group the following weights are attached to the 4 preliminary running times: 2, 2, 3, and 4, the average preliminary times as given in table 1 are obtained. These times were equalized, by using appropriate multiplication factors, in order to facilitate comparison of the average times obtained, subsequently, on the different diet regimen. This equalizing of the preliminary times introduced a relatively insignificant amount of error, as will be discussed later.

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11 Disodium-EDTA, Sigma Chemical Co., St. Louis, Mo. 63118.
12 Panheprin, Abbott Laboratories, No. Chicago, Ill.

TABLE 1

TREADMILL RUNNING TIMES, MIN., OF LONG-EVANS MALE, YOUNG ADULT RATS ON DIETS WITH DIFFERENT FAT LEVELS, FED AD LIBITUM AND AT A CALORICALLY RESTRICTED INTAKE. TEMPERATE CLIMATIC CONDITIONS

Preliminary Average[a]		Diet Groups					
Not equalized	(a)	(41.8)	(42.7)	(48.7)	(39.9)	(35.8)	(42.0)
Equalized	(b)	41.8	41.8	41.8	41.8	41.8	41.8

Days on diet regimen		Ad libitum			Restricted[b]		
		Calories from fat					
		20%	40%	70%	20%	40%	70%
1	(a)	(41.1)	(39.6)	(51.1)	(41.1)	(39.6)	(44.0)
	(b)	41.1	38.7	43.9	43.1	46.3	43.8
8	(a)	(39.4)	(39.5)	(54.2)	(49.7)	(44.0)	(52.2)
	(b)	39.4	38.7	46.6	52.1	51.4	52.0
15	(a)	(36.3)	(39.6)	(47.9)	(56.6)	(54.0)	(48.3)
	(b)	36.3	38.7	41.2	59.3	63.1	48.0
22	(a)	(40.1)	(39.7)	(47.1)			
	(b)	40.1	38.7	40.5			
36	(a)	(43.0)	(39.6)	(47.9)			
	(b)	43.0	38.7	41.1			
43	(a)	(40.3)	(39.6)	(43.4)			
	(b)	40.3	38.7	37.3			

[a] On a preliminary (pre-experimental phase) diet with 40% of the calories from fat, weighted (see text) average of 4 runs.

[b] On about 40% of the ad libitum intake.

None of the average running times as affected by fat level, both on the ad libitum and on the restricted intake, were significantly different (73), using the equalized preliminary times in this comparison. However, the differences due to caloric intake level were significant, according to Student's t test, for all fat levels ($P < 0.02$) on both the 8th and 15th day after the institution of the different diet regimen, and highly significant ($P < 0.01$) if only the 20% and 40% fat levels were used in the comparison. The increases (%) for the restricted, as compared to the ad libitum intake groups, on, respectively, the 1st, 8th, and 15th day were for the 20% fat level: 5, 32, and 63; for the 40% fat level: 20, 33, and 63; and for the 70% fat level: 0, 12, and 17. These increases were significantly higher during all three times for the 20% and 40% than for the 70% fat level, according to a nonparametric ranking test such as the Mann-Whitney U test (74). Average body weights (g) on the diet with 40% calories from fat for the 2 weeks before the start of the experimental period with different diet regimen were for what were to become the groups ad libitum, 20%, 40%, and 70% fat calories, and restricted, 20%, 40%, and 70% fat calories, respectively: 494, 502, 478, 524, 555, and 514. Average weights (changes, %) on the 15th day of the experimental period were, respectively: 491 (-0.8), 505 (+ 0.7), 470 (-1.5), 455 (-13.2), 467 (-15.9), and 457 (-11.1). Weight changes (%), from the weights mentioned during the pre-experimental period, for the three ad libitum fed groups on the 43rd experimental day were, respectively: +0.6, +4.0, and -1.2. Treadmill running performance in kgm for the different groups is given in table 2. (These values were obtained by taking the weight, in kg, of each animal during a particular run to exhaustion, and multiplying this by the distance, in meters, that the animal had lifted itself on the inclined belt during the run.) Again, none of the average performance values, expressed in kgm, as affected by fat level, both on the ad libitum and on the restricted intake, were significantly different. But the differences due to caloric intake were significant, according to the Mann-Whitney U test, for all fat levels on both the 8th and 15th day after the institution of the different diet regimen, and highly significant for all test days if only the 20% and 40% fat levels are considered. The increases (%) for the restricted, as compared to the ad libitum intake groups, were on, respectively, the 1st, 8th, and 15th day for the 20% fat level: 5, 22, and 43; for the 40% fat level: 19, 18, and 37; and for the 70% fat level: -1, 2, and 4.

If, for the 4 preliminary running times mentioned on the diet with 40% of the calories from fat, the performance, in kgm, of all animals is compared with their weight, a first order regression between these values is found to exist: $Y = -.2216X + 216.5$ (within the range of 95 to 123 kgm performance and 454 to 559g body

TABLE 2

TREADMILL RUNNING PERFORMANCE, KGM, OF LONG-EVANS MALE, YOUNG ADULT RATS ON DIETS WITH DIFFERENT FAT LEVELS, FED AD LIBITUM AND AT A CALORICALLY RESTRICTED INTAKE. TEMPERATE CLIMATIC CONDITIONS

Preliminary Average[a]		Diet Groups					
Not equalized	(a)	(100.0)	(104.0)	(111.4)	(101.3)	(96.0)	(104.4)
Equalized	(b)	102.8	102.8	102.8	102.8	102.8	102.8
Days on		Ad libitum			Restricted[b]		
		Calories from fat					
diet regimen		20%	40%	70%	20%	40%	70%
1	(a)	(96.8)	(96.1)	(116.7)	(103.3)	(105.4)	(107.8)
	(b)	99.5	95.0	107.7	104.8	112.9	106.1
8	(a)	(93.3)	(95.9)	(122.3)	(115.5)	(104.7)	(117.1)
	(b)	95.9	94.8	112.9	117.2	112.1	115.3
15	(a)	(85.9)	(96.5)	(108.7)	(124.2)	(121.7)	(106.4)
	(b)	88.3	95.4	100.3	126.0	130.3	104.8
43	(a)	(96.8)	(99.7)	(98.8)			
	(b)	99.5	98.5	91.2			

[a] On a diet with 40% of the calories from fat, weighted average (see text) of 4 runs.
[b] On about 40% of the ad libitum intake.

weight) with an F ratio = 35.4 (highly significant), in which Y = performance, in kgm, and X = weight, in g. The fit was better for a linear than for an exponential relationship. For the same running times, a correlation coefficient, r = -.922 was found to exist between weight of the animals and running times to exhaustion. If a comparison is made between performance (kgm) - body weight relationships on ad libitum and restricted food intake for the 20% and 70% fat calorie level, on days 8 and 15 of the different diet regimen, using the average values, the results as shown in fig. 1 are obtained. Equalized average values for the preliminary period, shortly before the start of the different diet regimen, were used here. The validity of a comparison on this basis will be discussed later. In order to better compare the slopes for the lines depicted at the 20% and at the 70% fat calorieslevel, appropriate performance multiplication factors were used, one for each of all 20% fat, 8th day values, similarly one for the 20% fat, 15th day, and so on, thus obtaining one average line instead of the separate 8th and 15th day lines for each fat level. Using the individual adjusted values, thus obtained, the regression equation for the diets with 20% of the calories from fat is: $Y = -.439X + 309.9$, that for the diets with 70% of the calories from fat: $Y = -.051X + 126.6$, both equations covering a minimum range of 426 to 534 g body weight, in which Y and X denote the same parameters as above. The fits in each of both cases, again, were better for a linear than for an exponential relationship. According to an analysis of covariance, using the individual, adjusted values, the slope for the 70% fat values is highly significantly ($P < 0.005$) different from that for the 20% fat values. The slopes for the 40% fat values were in between those for the 20% and 70% fat values.

Water consumption on the diet with 70% of the calories from fat, fed ad libitum, was significantly ($P < 0.05$) lower, namely on the average 7%, than on the diet with 20% of the calories from fat, fed ad libitum, add highly significantly ($P < 0.01$) lower, on the average 11%, than on the diet with 40% of the calories from fat, fed ad libitum, over the period of 2 to 23 days from the start of the different diet regimen. Water consumption on the restricted food intakes at all fat levels was considerably, and for the 4th through 11th day on the diet regimen (not measured beyond this time) highly significantly ($P < 0.01$) lower than on the ad libitum food intakes at the same fat levels. The reductions (%) in water consumption for the restricted intake regimen on day 2, 4, 9, and 11 from the start of the different diet regimen were, respectively, for the diet with 20% of the dalories from fat: 18, 38, 43, and 42; for the diet with 40% of the calories from fat: 23, 41, 40, and 38; and for the diet with 70% of the calories from fat: 12, 23, 29, and 36. The difference in reduction between the diet with 70%, and those with 20% and 40% of the calories from fat was

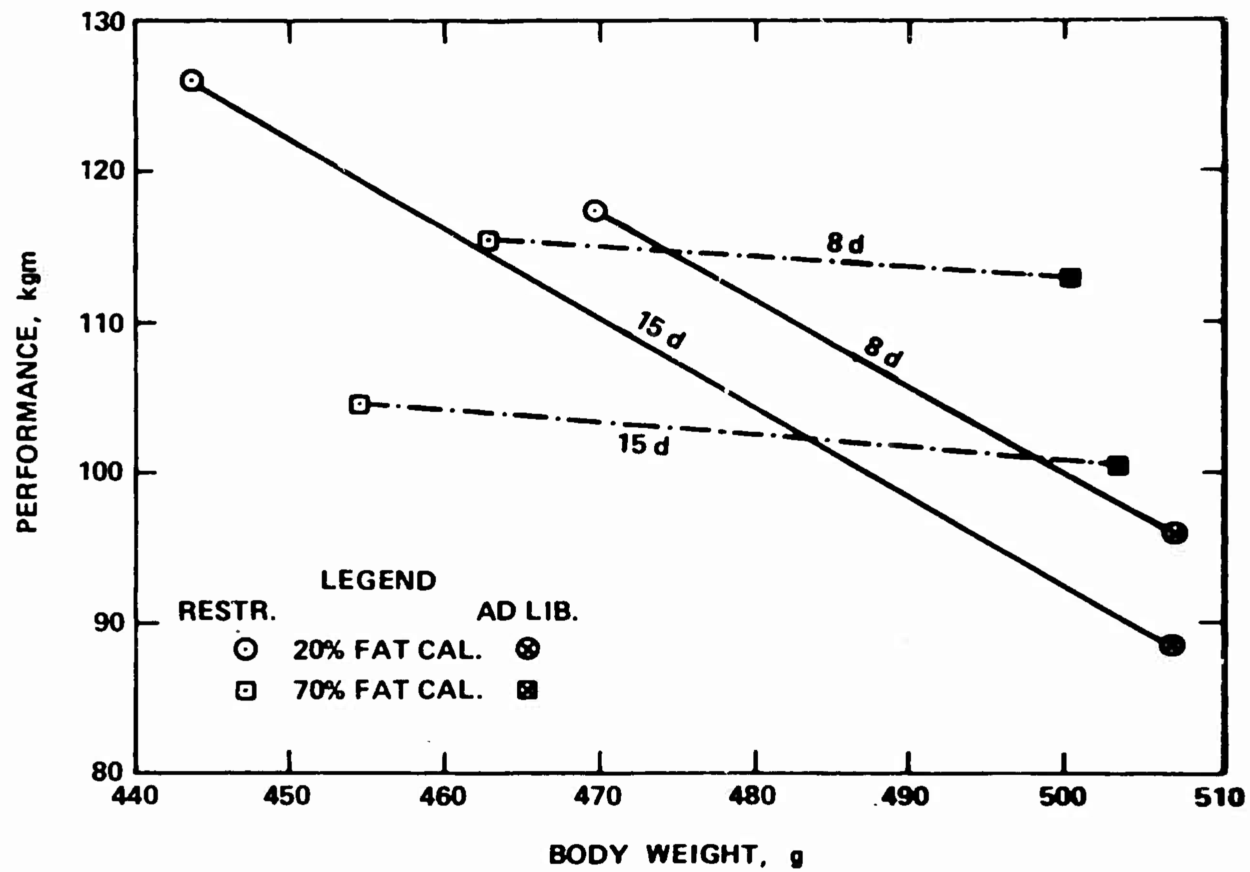

Fig. 1. Performance (kgm) - body weight relationships on restricted and ad libitum fed diets during the 8th and 15th day on different diet regimen. Long-Evans rats, temperate climatic conditions.

significant ($P < 0.05$). No significant differences in water consumption occurred between the different restricted-fed groups.

The plasma lactic acid values between the different fat levels were not significantly different at each determination time, neither for the ad libitum nor for the restricted intake groups. Therefore, the values for the different fat levels at each determination time were averaged. This yielded the following values (mg/100 ml) on, respectively, day 1, 8, 15, and 22 after the start of the different diet regimen for the ad libitum fed rats: 107, 126, 130, and 150; and for the animals on restricted food intake: 115, 127, and 195 (no determinations on day 22). These compare with an average value for 6 non-exercised animals of 23 mg/100 ml. The differences between the values for the animals run to exhaustion and those not run were highly significant ($P < 0.001$) at all times. If the data for day 1, 8 and 15 are combined, the average values for the 20%, 40%, and 70% fat calorie groups, fed ad libitum, are, respectively (mg/100 ml): 117, 131, and 109; for the restricted-fed groups these are, respectively: 147, 143, and 155. According to Duncan's multiple range test, the plasma lactic acid values during the restricted food intake were significantly ($P < 0.05$) higher than on the ad libitum intake for all fat levels, right after running to exhaustion.

Adjusted to the same average preliminary plasma glucose value (of 102.9 mg/100 ml on the diet with 40% fat calories, shortly before the different diet regimen), the values found on the different diet regimen, within 2 minutes after the running to exhaustion, are given in table 3. The plasma glucose levels on day 8aand 15 on the different diet regimen were significantly ($P < 0.02$) lower on the restricted than on the ad libitum intake for all fat levels. The plasma glucose levels were, further, significantly ($P < 0.01$) lower on the 70% than on the 20% and 40% fat calorie diets on the ad libitum food intake during the whole period measured. In addition, on the restricted food intake, for all days measured, the diet with 70% fat calories yielded significantly ($P < 0.05$) lower plasma glucose levels than the 40% fat calories diet, and the latter, in turn, significantly ($P < 0.05$) lower glucose values than the diet with 20% of the calories from fat. Finally, plasma glucose levels on the restricted food intake on day 15 were significantly ($P < 0.05$) lower than on day 8.

II. Hamsters. Temperate climatic conditions. A.

Running Performance. Running times to exhaustion for the different diet groups are given in table 4. On the restricted intake, the running times for the diets with 20 and 40% of the calories from fat on day 9 of the different diet regimen were

TABLE 3
PLASMA GLUCOSE VALUES IN LONG-EVANS MALE, YOUNG ADULT RATS ON DIFFERENT DIET REGIMEN, WITHIN 2 MIN. AFTER RUNNING TO EXHAUSTION. TEMPERATE CLIMATIC CONDITIONS.

Days on diet regimen	Ad libitum fed			Restricted fed[a]		
	Dietary calories from fat					
	20%	40%	70%	20%	40%	70%
	mg/100 ml[b]					
1	109.4	111.5	63.5	119.3	113.1	75.1
8	102.8	101.6	74.2	93.4	89.6	72.0
15	101.0	97.0	80.9	84.0	77.5	51.9
22	95.7	99.1	68.1			

[a]40% of the average ad libitum intake by weight.
[b]Mean values. N = 7-8.

TABLE 4

TREADMILL RUNNING TIMES, MIN., OF SYRIAN MALE, YOUNG ADULT HAMSTERS ON DIETS WITH DIFFERENT FAT LEVELS, FED AD LIBITUM AND AT A CALORICALLY RESTRICTED INTAKE. TEMPERATE CLIMATIC CONDITIONS. EXPERIMENT A

Days on diet regimen	Diet groups					
	Ad libitum			Restricted[a]		
	Calories from fat					
	20%	40%	70%	20%	40%	70%
2	94.1	97.1	98.4	113.6	93.0	99.7
9	94.4	98.8	96.4	125.9	102.6	101.2

[a] On about 40% of the ad libitum intake.

significantly longer ($P < 0.02$) than on day 2. Percentage changes in body weight, running times, and performance (in kgm) for day 9, as compared to day 2, for the restricted-fed animals are given in table 5. For the case of a linear relationship between body weight and performance, the values for an equal percentage weight loss within a similar absolute range are given between brackets. The change in performance with a given change in weight was not significantly different for the diets with 20% and 70% of calories from fat during the restricted intake.

For unknown reasons, these hamsters had failed to gain weight during the four weeks on Purina chow, but slowly gained weight after this on the semi-purified diet with 40% of calories from fat. Just before the period with the different diet regimen their average weight was still considerably lower (142 _versus_ 186 g) than that of the hamsters in the next experiment. At autopsy, at the termination of the experiment, no gross symptoms were observed that could serve to adequately explain the growth retardation earlier.

Average _plasma triglyceride_ levels right after running to exhaustion for the ad libitum fed animals, including all fat levels, were 241 ± 128 (mean ± SD) mg/100 ml. For the otherwise comparable but not exercised animals this value was 425 ± 129 mg/100 ml. This difference was significant ($P < 0.05$).

Average _plasma total cholesterol_ levels for the ad libitum fed animals right after running to exhaustion, including all fat levels, were 125 ± 14 mg/100 ml, for the otherwise comparably treated but non-exercised animals: 153 ± 28 mg/100 ml. This difference was also significant ($P < 0.05$). Neither for the triglyceride nor for the cholesterol levels were the differences between fat levels significant for the animals fed ad libitum.

II. _Hamsters. Temperate climatic conditions. B._

Running performance. Percentage changes in body weight, running time, and performance in kgm from day 2 to day 9 on restricted food intake during the period on different diet regimen are given in table 6. For the case of a linear relationship between body weight and performance, the values for an equal percentage weight loss within a similar absolute range are given between brackets. All values for running time and performance in kgm in this table are those after multiplication by a factor of 0.9421 to compensate for a possible increase due to a training effect as seen in a one-week time interval during the running to exhaustion occasions before the period of different diet regimen. The difference between fat levels in performance, and that of change in rate of performance with a given change in weight, again, was not significant. Assuming that the increases in running time and performance in kgm from day 2 to day 9 of the different diet regimen are, indeed, a relatively close approximation of

TABLE 5

PERCENTAGE CHANGES IN BODY WEIGHT, TREADMILL RUNNING TIME, AND PERFORMANCE (IN KGM) FOR DAY 9, AS COMPARED TO DAY 2, ON DIFFERENT, RESTRICTED-FED[a] DIET REGIMEN FOR SYRIAN, MALE, YOUNG ADULT HAMSTERS. TEMPERATE CLIMATIC CONDITIONS. EXPERIMENT A[b]

Parameters	Diet groups		
	20%[c]	40%	70%
Body weight	- 16.7 (- 12.2)	19.3 (- 12.2)	- 12.2 (- 12.2)
Running time	+ 10.8 (+ 7.9)	+ 10.3 (+ 6.5)	+ 1.5 (+ 1.5)
Performance, kgm	- 7.7 (- 5.6)	- 11.0 (- 7.0)	- 10.9 (- 10.9)

[a] At about 40% of the ad libitum intake.

[b] For the case of a linear relationship between body weight and performance, the values accompanying equal percentage weight loss are given between brackets.

[c] Percent of calories from fat in the diet.

TABLE 6

PERCENTAGE CHANGES IN BODY WEIGHT, TREADMILL RUNNING TIME, AND KGM PERFORMANCE FOR DAY 9, AS COMPARED TO DAY 2, ON DIFFERENT RESTRICTED-FED[a] DIET REGIMEN FOR SYRIAN, MALE, YOUNG ADULT HAMSTERS. TEMPERATE CLIMATIC CONDITIONS. EXPERIMENT B[b]

Parameters	Diet groups				
	20%, 3.5g[c]	20%, 4.1g	40%, 3.5g	70%, 3.5g	70%, 2.7g
Body weight	- 9.7 (- 8.2)	- 8.8 (- 8.2)	- 8.2 (- 8.2)	- 8.8 (- 8.2)	- 11.0 (- 8.2)
Running time[d]	+ 88.1 (+ 74.5)	+ 76.2 (+ 70.8)	+ 62.2 (+ 62.2)	+ 76.2 (+ 71.3)	+ 88.9 (+ 66.4)
Performance, kgm[d]	+ 69.9 (+ 60.2)	+ 60.7 (+ 56.8)	+ 48.9 (+ 48.9)	+ 60.8 (+ 57.3)	+ 68.2 (+ 52.8)

[a] At about 40% of the ad libitum weight intake with 3.5 g/day. The other levels are equicaloric to the intake at 40% dietary fat calories.

[b] For the case of a linear relationship between body weight and performance, the values accompanying equal percentage weight loss are given between brackets.

[c] Percent of calories from fat in the diet, and daily food intake, respectively.

[d] Adjusted (through multiplication of all values by 0.9221) for a possible training effect.

the true differences (controls were not used here because this was beyond the original purpose of the experiment), these differences were highly significant ($P < 0.001$). The average running time on day 2 was 74 minutes; on day 9 the average, adjusted time was 132 minutes. The regression equation for the average change in performance (in kgm) with change in weight between days 2 and 9 is given by: $Y = -1.607 X + 315.2$, in which Y = performance (kgm), and X = body weight (g) within the range of 155 and 170 g. An 8% lower weight, under these conditions, on the average, thus, corresponds with a 53.9% increase in performance, in kgm (or 67.4% increase in running time). During the period when the animals were fed ad libitum, before the period with different diet regimen, on the diet with 40% of the calories from fat, the relationship between body weight (g) and performance (kgm) is expressed by the equation: $Y = -.330 X + 87.8$ ($P < 0.005$), valid for the body weight range of 140 to 197 g. An 8% lower weight, under these conditions, on the average, coincided with a 15.4% higher performance in kgm.

Transit time through the GI tract: the feces was examined 15 hours after the administration of the carmine with their food of the hamsters who had received this at the 3 different dietary fat levels used. One-third to one-quarter of the feces collected over this period was colored red.

The caloric density, in cal./g, of feces and food from the different diets is shown in table 7. Total fecal lipid, in %, is given in table 8.

Urine total ketone levels for day 7 through 9 of the period with different diet regimen, for the following diets (calories from fat, and daily food intake, respectively): 20%, 3.5 g; 20%, 4.1g; 40%, 3.5g; 70%, 3.5g; and 70%, 2.7g were respectively, 0.54, 0.59, 0.58, 0.87, and 0.70 mg/day. The differences between the values for the 20%, 3.5g and 20%, 4.1 g diets on the one hand and that for the 70%, 3.5g diet on the other was significant ($P < 0.02$), and that for the 20%, 3.5g diet and for the 70%, 2.7g diet was significant ($P < 0.05$).

III. Hamsters. Tropical climatic conditions.

Running performance. No significant difference was observed for running times, or performance in kgm between the different fat levels, both for the restricted and for the ad libitum fed animals, when the changes from day 2 to day 9 on the different diet regimen were compared. However, although there was no significant differences due to fat level, a significant ($P < 0.05$) difference occurred due to difference in level of food intake: The animals on the restricted food intake, on the average, ran 10.2% longer on day 9 than on day 2. Percentage weight losses on day 9, as compared to day 2, during the restricted intake on the diets with 20, 40, and 70% fat calories were, respectively: 4.3, 3.5, and 2.6. On the ad libitum fed diets no significant

TABLE 7

CALORIC DENSITY, IN CAL./G DRY WEIGHT, OF THE DIETS USED, AND OF FECES COLLECTED AT DIFFERENT PERIODS, WHEN DIETS WITH DIFFERENT FAT LEVELS AND FED AT DIFFERENT LEVELS OF INTAKE WERE CONSUMED BY EXERCISED SYRIAN, MALE, YOUNG ADULT HAMSTERS, AT TEMPERATE CLIMATIC CONDITIONS.
EXPERIMENT B

Material	Day	Diet groups				
		20%, 411g[a]	20%, 3.5g	40%, 3.5g	70%, 2.7g	70%,3.5g
Feces	2	3786[b]	3802	3911	4087	4140
	7-9	3630[b]	3652	3830	4205	4254

Material	Diets		
	20%[a]	40%	70%
Food	4691[b]	5427	6385

[a] Percent of dietary calories from fat, and daily food intake, respectively.

[b] All values not connected below by the same line are either significantly ($P < 0.05$) or highly significantly ($P < 0.01$) different.

TABLE 8

TOTAL FECAL LIPID, IN % OF DRY WEIGHT, OF FECES COLLECTED AT DIFFERENT PERIODS, WHEN DIETS WITH DIFFERENT FAT LEVELS AND FED AT DIFFERENT LEVELS OF INTAKE WERE CONSUMED BY EXERCISED, MALE, YOUNG ADULT SYRIAN HAMSTERS, UNDER TEMPERATE CLIMATIC CONDITIONS. EXPERIMENT B

Day	Diet groups				
	20%, 4.1g[a]	20%, 3.5g	40%, 3.5g	70%, 2.7g	70%, 3.5g
2	7.2[b]	9.2	9.9	13.9	16.3
7-9	6.5[b]	6.6	10.3	17.5	16.0

[a] Percent of dietary calories from fat, and daily food intake, respectively.

[b] All values not connected below by the same line are either significantly ($P < 0.05$) or highly significantly ($P < 0.01$) different.

TABLE 9

CALORIC DENSITY IN CAL./G DRY WEIGHT, OF THE DIETS USED, AND OF FECES COLLECTED ON DAY 8 AND 9 OF DIFFERENT DIET REGIMEN FOR EXERCISED, MALE, YOUNG, ADULT SYRIAN HAMSTERS, UNDER TROPICAL CLIMATIC CONDITIONS

Material	Diet groups					
	20%[a]		40%		70%	
	Restr.[b]	Ad lib.	Restr.	Ad Lib.	Restr.	Ad lib.
Feces	4093[c]	4125	4349	4577	4601	5107

Material	Diets		
	20%[a]	40%	70%
Food	4683	5393	6473

[a] Percent of dietary calories from fat.

[b] Food intake level. The restricted intake represents an average of about 80% of the ad libitum intake.

[c] All values not connected below by the same line are either significantly ($P < 0.05$) or highly significantly ($P < 0.01$) different.

weight changes occurred during this period.

Water donsumption. On the average, water consumption of the restricted-fed groups was 13% and significantly ($P < 0.02$) lower than that of the ad libitum fed groups. No signficant difference in this decrease occurred due to difference in fat level.

Caloric density values of the different diets and of the feces from days 8 and 9 on the different diet regimen are given in table 9.

Relative blood viscosity values (51, 71) were not significantly different between the different individual diet groups and, under the measurement conditions, averaged 10.8 seconds/cm. However, the difference between the average for all restricted-fed groups (10.17 seconds/cm) and that for the group on 70% fat calories, fed ad libitum (12.72 seconds/cm) was significant ($P < 0.05$). Further, the ratio of relative blood viscosity to hematocrit was significantly ($P < 0.05$) lower for the average of all restricted-fed animals (0.221 seconds/cm.%). than for that of the group on 70% fat calories, fed ad libitum (0.283 seconds/cm.%).

The plasma cholesterol values for the groups on 20, 40, and 70% of calories from fat, fed ad libitum were, respectively: 107.1, 147.5, and 205.8 mg/100 ml. For the restricted-fed groups these values were, respectively: 93.4, 113.5, and 204.0 mg/100 ml. The difference between the 20 and 70% fat group, fed ad libitum was significant ($P < 0.02$). Further, among the restricted-fed animals the difference between the 20 and 40% fat groups, and between the 40 and 70% fat group was significant ($P < 0.05$).

DISCUSSION

The most outstanding feature in the results of the four experiments described above may well be the increased running times and performance in kgm during the course of the first two weeks of reduced caloric intake, under the conditions as described. For the rats, the restricted-fed 20% fat calorie group on the 15th day of the diet regimen, on the average, ran 63% longer, with a 43% higher performance in kgm, for a 12.4% weight loss, as compared to the ad libitum fed group on the same dietary fat level. For the restricted-fed 70% fat calorie group these figures were, respectively, 17, 4, and 9.6%, for the restricted-fed 40% fat calorie group 63, 37, and 16.6%, respectively. Thus, a 9.6% weight loss, assuming a linear relationship, for the restricted-fed 20, 40, and 70% fat calorie groups corresponded with, respectively, a 49, 36, and 17% increase in running time, and a 33, 21, and 4% increase, respectively, in performance in kgm. This difference in rate of increase in kgm performance between the 20% and 70% fat calorie groups was significant ($P < 0.005$). During the preliminary period, when the rats were fed ad libitum on a 40% fat calorie diet that in all other respects had the same composition as the diets during the period with different fat levels, rats with a 9.6% lower weight, on the average, had an 18.5% longer running time and a 15.1% higher kgm performance. Both, during this ad libitum feeding period and during the period of restricted feeding, the relationship between weight change and kgm performance could better be expressed by a linear regression equation than by an exponential one within the range of determinations used.

In order to better allow a comparison of running time and kgm performance between the different experimental groups, results for the different groups were multiplied by different factors at all times, such that the average preliminary values were equalized. However, body weight has been shown to be negatively correlated with kgm performance and, thus, also running time ($t = p/X-q$, in which: t = running time, X = body weight, p and q are constants under the conditions described). Distribution of average preliminary body weights indicates that the use of multiplication factors providing an equalization of average preliminary running times and kgm performance introduced an average error into the experimental results of not more than about 1.4%, the largest being about 3.3% of the result (for the 20% fat calorie, ad libitum fed group). This does not affect the relative significance of the results.

For experiment A with hamsters it is also of interest to compare performance of the restricted-fed animals to that of the ad libitum fed ones on the same dietary fat level. Thus compared, on the 9th day of the dietary regimen, measured from the 2nd day, the restricted-fed 20% fat calorie group, on the average, ran 10.5% longer for a 12.6% weight loss. These values for the restricted-fed 40% fat calorie group were, respectively, 8.4 and 18.2% and for the restricted-fed 70% fat calorie group 3.6 and 11.6%. Thus, an 11.6% weight loss, assuming a linear relationship, for the restricted-fed 20, 40, and 70% fat calorie groups corresponded with, respectively, a 9.7, 5.4, and 3.6% increase in running time. It is possible that the effect of the cause of the lack of growth during the 4 weeks on commercial chow made itself still felt in terms of exercise performance in later stages of the experiment, and that this explains the relatively small increases in running time. On the other hand, in experiment B with hamsters very large percentage increases in running time and kgm performance were obtained for the restricted-fed animals. These percentage increases, per unit of weight loss, were larger than those observed with the rats and amounted, on the average, to increases of about 69% in running time and 55% in kgm performance for an 8.2% weight loss. The correction for a possible training effect, based on performance differences for the same time interval observed in earlier runs to exhaustion, should be a reasonably close estimate. It is unlikely, based on this experience, that the real increases in performance during the weight loss described would have been much smaller. During ad libitum feeding, on a 40% fat calorie diet, an 8.2% lower weight corresponded with a 26% longer running time and 16% higher kgm performance. For the hamsters under tropical climatic conditions, a 3.5% weight loss during restricted food intake on the 40% fat calories diet corresponded with a 10.1% increase in running time and a 6.2% increase in kgm performance. Assuming that this relationship can to this extent be extrapolated, an 8.2% weight loss under these conditions would correspond with increases of 24% in running time and 14% in kgm performance.

Both, in the experiments with rats and with hamsters at room temperature, where enough data are available to make such a comparison, the indications are that these animals under the conditions described and on restricted food intake experience a higher increase in running time and kgm performance for a given percentage weight loss, within a comparable absolute range, than the animals on ad libitum food intake. It is quite possible that the lighter weight animals during ad libitum feeding had a lower percentage body fat than the heavier animals and, thus, could be more efficient in their running performance. This was not investigated because it was not part of the original purpose of these experiments. A possible explanation for the higher rate of increase in running performance with a given weight loss for the restricted-fed animals might be an increased adrenalin secretion (75) as a result of the stress imposed by the restricted food intake. The lower rate of gain in running performance with weight loss in the first experiment with hamsters and in the one under tropical climatic conditions might be due to a stress in addition to the one imposed by the restricted food intake, resulting in a decreased efficiency effect in the organism. In the first experiment with hamsters, the coincidence of start of body weight gain with the change-over from the commercial chow to the semi-purified diet would tempt one to postulate the occurrence of an incorrect nutrient mixture (too little or too much of one or more ingredients) in the commercial chow, the effects of which were to some extent still noticeable during later stages of the experiment. In the experiment under tropical climatic conditions, the minor degree of food restriction, relative to the ad libitum intake, could have been a contributing factor in the relatively smaller rate of performance increase with weight loss. In connection with the observations, reported above, of running performance increase during food restriction, it should be of interest to report the findings of Samuels and coworkers (48) that exercise (running) ability of adult male rats increased from the 9th day of fasting on, as compared to the 2nd through 4th day. No information on the magnitude of a training effect is provided by these authors, however, exhaustion tests having been initiated only after the start of fasting. Tollenaar, previously, has reported a significant increase in running endurance of rats on the 7th, as compared to the 3rd day of a restricted food intake, as well as compared to preliminary running times while still fed ad libitum (76). The results reported in the present paper regarding running performance during food restriction are not in agreement with those found by Scheer and coworkers (15) during swimming of rats.

No significant difference in change in running times and kgm performance was observed between the different dietary fat level groups in the experiments with rats and hamsters, conducted by the author and described here. This was the case both during the ad libitum and during the restricted food intake. However, if instead of making only a comparison within the restricted — or within the ad libitum fed groups, the restricted-fed groups at each fat level were compared with the ad libitum fed ones at the same fat level, then for the rats differences could be noted. A comparison in this manner showed that on the diet with 70% fat calories a given percentage change in body weight corresponded with a significantly smaller percentage change in kgm performance than on the diet with 20% fat calories, both on the 8th and on the 15th day of the different diet regimen (fig. 1). Tollenaar, previously, reported a significantly longer running endurance of rats on a diet with 14% fat calories from corn oil than on a similar diet but with 41% fat calories from butter (76). Since on the restricted food intake the weight of food offered was the same at all fat levels, the amount of calories provided decreased from the diet with 70% to that with 20% fat calories. However, the higher amount of calories offered provided no advantage in terms of running time or kgm performance. On the ad libitum fed regimen, a tendency existed for the rats on the diet with 70% fat calories to initially show a better running performance than on the other diets. This difference diminished more and more with time, however.

Average caloric density of the feces of the restricted-fed hamsters was higher on the diets with 70% fat calories than on the lower dietary fat levels, both at temperature and at tropical climatic conditions. Yet, from measurements of food intake and amount of feces produced it became apparent that, notwithstanding the higher caloric density and higher total lipid content of the feces at the higher dietary fat levels, on balance more calories were assimilated at the 70% than at the 20% dietary fat level. This, also, is expressed in the smaller body weight losses at the 70% dietary fat level in most experiments.

Water consumption was significantly lower on the restricted than or the ad libitum food intake for both rats and hamsters, where measured. This was to be expected from the lower amount of solutes presented in the former case. With the rats, the water consumption was also significantly lower on the diet with 70% fat calories than on the other dietary fat levels during the ad libitum feeding. This, again, was to be expected since in the combustion of the same weight of fat more water is formed than in the combustion of carbohydrate. However, during the restricted feeding of the rats, and in the tests with hamsters, no significant differences between dietary fat levels were found. The differences in water consumption found with the rats are in agreement with those found by Tollenaar with rats earlier (77).

The lower plasma glucose levels of rats on the high fat diet, compared with the lower fat levels, both during ad libitum and during restricted feeding, as reported above, are in agreement with results reported earlier for humans (5, 30, 36, 44). The plasma sugar levels, observed in the rats right after exhaustion, not only were lower on the restricted than on the ad libitum feedi g regimen but, also, were lower on the 15th than on the 8th day of restricted feeding. Only on this 15th day, on the high-fat diet, were levels observed suggestive of serious hypoglycemia. Results observed earlier in rats support the present findings regarding lower blood glucose levels during restricted feeding or fasting (48, 77).

Exercise significantly lowered plasma triglyceride and total cholesterol levels in the rats, fed ad libitum. Hamsters seemed to be affected more by dietary fat level in their plasma cholesterol levels than rats, based on the data available.

Although a higher relative blood viscosity was observed for the hamster group on 70% fat calories, fed ad libitum, than for the restricted-fed groups, the ability to measure differences in blood flow rate may have been somewhat impaired by the use of an anti-coagulant, such as heparin (51). The ratio of blood viscosity to hematocrit has been reported (reference lost) to be positively correlated with both incidence and severity of atherosclerosis. This ratio was 28% higher for the high-fat goup than for the average of all restricted-fed groups. No significant difference in hematocrit values occurred between the different dietary groups.

In exercise to exhaustion, blood lactate values, after about the first 4 minutes, have been found to decrease with increase in time from the start of the exercise, both in man and in rats (78-82). Peak blodd lactic acid values of about 95 mg/100 ml, 3 minutes after the start of exercise, have been reported in the rat (81). In man, peak blood lactic acid values mostly in the range of 100-140 mg/100 ml have been observed, occurring the first 2-7 minutes of very high relative work rates and during the first 3 minutes after such work (79, 83-86). Individual peak values as high as 220 mg/100 ml, obtained during very high intensity, intermittent work have been reported (87). A slight increase in blood lactate level close to the point of exhaustion has been observed (1, 75). Plasma lactate values correspond with about 1.15 times blood lactate values (81). In trained animals, lower blood lactic acid values are seen at the same relative work rate than in untrained ones (88, 89).

Intravenous infusion of adrenaline has been shown to increase the blood lactate concentration (90). This may explain the higher plasma lactic acid value on day 15 of the diet regimen in the restricted — as compared to the ad libitum fed rats, the semi-starvation likely functioning as an additional stress factor.

According to Carlson, Pentecost and coworkers (91, 92) during work the blood lactate concentration is a good indicator of the adequacy of oxygen supply to the tissues. If, in the experiment with rats, described above by the author, the heart was the limiting factor in the exercise endurance, thus limiting the blood and oxygen flow to the exercising muscles at the time of and shortly before exhaustion, then we should have been about the same high plasma lactic acid values for all measurements, both in the ad libitum — and in the restricted fed animals. However, this does not exclude the possibility that in the runs with the highest plasma lactic acid values the heart function at the time of exhaustion was the limiting factor, considering the level of the lactic acid values observed.

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