Effect of diet and exercise on norepinephrine-induced thermogenesis in male and female rats

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ABSTRACT

Leblanc, Jacques, Jean Dussault, Diane Lupien, and Denis Richard. Effect of diet and exercise on norepinephrine-induced thermogenesis in male and female rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 52(3): 556-561, 1982.-Male and female rats were fed standard laboratory chow or a highly palatable diet (cafeteria diet) for 10 wk. The cafeteria diet caused an increase in caloric intake and in body weight, and it induced thermogenesis that was associated with elevated plasma triiodothyronine (T3) levels, increased brown adipose tissue size, and enhanced metabolic response to norepinephrine. For a comparable caloric intake, body-weight gain was significantly greater in female than in male rats possibly because of difference in thermogenesis as suggested by the response to norepinephrine. Exercise training (swimming 2 h/day for 10 wk) reduced food intake and body-weight gain and failed to increase norepinephrine-induced thermogenesis in rats fed laboratory chow. In animals fed the cafeteria diet, food intake and body-weight gain were also reduced by exercise training, which at the same time diminished the diet-induced thermogenesis (as evidenced by the diminution of 1) brown fat hypertrophy, 2) the elevation of plasma T3, and 3) the hyperthermic response to injected norepinephrine. It is suggested that the thyroid hormone and catecholamines through their actions on the brown adipose tissue are the important regulators of thermogenesis. Exercise training would reduce the diet-induced thermogenesis by preventing increased T3 production. Enhanced thermogenesis may be considered an adaptive reaction as it serves to reduce fat deposition in animals fed cafeteria diet and to promote nonshivering heat production in the cold. On the other hand, exercise training reduces thermogenesis and thus prevents energy wasting.

Thermogenesis was shown to be an important factor in the control of energy balance (1, 22). Cold-induced thermogenesis has been known for some time (11), and the participation of brown adipose tissue in the development of nonshivering thermogenesis has also been shown by various investigators (10, 13, 20, 33). Excessive food intake was also shown to produce what has been called "dietary-induced thermogenesis" (22, 32). Exercise produces an increased caloric utilization and is sometimes associated with diminution in food intake. However, no studies have been reported on the role of exercise-training in thermogenesis. Thermogenesis has been associated with brown fat hypertrophy (20, 29, 30, 33), increased triiodothyronine secretion (6, 16), and enhanced response to the metabolic effect of catecholamines (14, 20, 29). An experiment was designed to study the effect of prolonged exercise training on thermogenesis of male and female rats fed either standard laboratory chow or very palatable food called "cafeteria diet," which has been shown to induce a significant thermogenesis (29, 30). The thermogenesis measured in our study was that produced by standard norepinephrine injections. It seemed also interesting to examine the effect of a sexual difference in thermogenesis because female rats did not lose as much body fat as males when submitted to prolonged training periods (26, 27), and they gained more weight than the male rats when fed a cafeteria diet (31). This raises the possibility of a sexual difference in thermogenesis that was also estimated by the response to norepinephrine.

Protocol

Effects of exercise and diet on hormonal and growth changes. Sedentary and exercise-trained Wistar rats were fed either normal laboratory stock diet (Purina chow) or a special regimen called cafeteria diet (29). A total of four groups of male and four groups of female rats were used and were kept at 27°C throughout the experiment. The average initial body weight of the male rats was 213 g and that of the female rats was 197 g. All groups initially included a total of 15 animals. For two male and two female groups, the exercise training program was the same as the one described previously (27). The rats swam for 2 h, 6 days/wk in an agitated water tank kept at 36°C. During the swimming session the rats carried small loads on their backs corresponding to 2% of their body weight. This period of training lasted 10 wk. When not swimming, these rats were kept in individual cages, along with the other four sedentary groups in a room maintained at 27°C. The four groups of rats on stock diet were fed Purina chow ad libitum. The following procedure was adopted for the groups of rats on the cafeteria diet. These animals were given various food items in excess of predicted requirements. Twice a week all food items consumed by the animals were measured, and this allowed transformation of grams of food into calories consumed. For the other 5 days of the week food consumed per animal was weighted in bulk, and the transformation of grams of food into calories was made on the basis of results obtained for the other 2 days. Consequently the caloric intake was measured for 2 days and estimated for the other 5 days. The cafeteria diet included two meals that were fed on alternate days.
meal consisted of bologna, fruitcake, popcorn, and laboratory chow and the other of pâté, chocolate chip cookies, sunflower seeds, and laboratory chow. The pâté is a mixture of ground pork meat, bread crumbs, and seasonings. The cafeteria diet correspond to an average caloric content of 19.8 kJ/g of wet weight derived from fat (60%), carbohydrate (30%), and protein (10%). The corresponding values for the Purina chow were 14.4 kJ/g derived from fat (12%), carbohydrate (60%), and proteins (28%). The various food items that were very palatable proved to be most efficient in promoting food intake and gain in body weight as was previously described (29, 31). Two days before the end of the experiment, all animals were given a subcutaneous injection of norepinephrine (300 μg/kg) in saline, and colonic temperature was measured before and 1 h after the injection. This allowed a qualitative estimate of the thermogenic effect of norepinephrine (22). At the end of the experiment, the animals were decapitated and plasma was kept at −40°C for later thyroxine and triiodothyronine determinations. At autopsy, epididymal fat pads and interscapular brown adipose tissue were dissected and weighed.

Effect of exercise on catecholamines of trained and untrained animals. In a previous unpublished experiment, sedentary and exercise-trained male rats fed stock diet were cannulated in order to draw blood for catecholamine determination. For that purpose, a polyethylene tube was placed permanently into the jugular vein. This tube was exteriorized through a neck incision that allowed blood sampling at rest and during exercise bouts without unduly disturbing the animal. Blood samples (0.4 ml) for determination of epinephrine and norepinephrine were obtained while the animals rested quietly in their cage and while they were swimming at 1.5 h after the beginning of exercise. The catecholamines were determined by the radioenzymatic method (8), the thyroxine by the method of Murphy and Jachan (24), and the triiodothyronine by the radioimmunological method of Mitsuma et al. (23). The statistical analysis was performed by using the Student’s t test to compare two sets of results and the Duncan multiple-range test for more than two groups (9).

RESULTS

Daily exercise was shown to reduce food intake in male rats fed standard chow or cafeteria diet especially between the 2nd and 8th wks of the experiment (Fig. 1). In the female rats this effect was observed only with the cafeteria diet (Fig. 2). The average body weight of exercised male rats was 75 g less than that of the sedentary animals (Fig. 3). The equivalent difference in the female rats was only 25 g (Fig. 4). In an attempt to verify these differences in body weight in terms of changes at the level of the white adipose tissue, weights of epididymal and parametrial fat pads were measured. On that basis, the difference in body weight between male and female rats could not be related to these changes in the white adipose tissue because a comparable reduction of approximately 50% in epididymal or parametrial fat pads was found in the exercised animals in spite of a marked difference due to sex with regard to the total body weight (Fig. 5). Body weight increase was greatly enhanced by the cafeteria diet in both male and female rats, and this was accompanied by a threefold increase in epididymal and parametrial pads. When exercise was combined with the cafeteria diet, the body weight increase was reduced as were the body fat pads in both male and female rats (Figs. 3–5). Food intake was significantly increased by the cafeteria diet, but this effect was reduced in exercise-trained animals. The difference in weight gain among rats fed the cafeteria and the stock diet was larger in the male rats fed standard laboratory chow (S) and cafeteria diet (C) with and without exercise (E). For daily food intake, C groups show greater values than S groups (P < 0.01). For mean food intake C group values are also greater than S groups (P < 0.01) while S > (S + E) and C > (C + E) both with P < 0.01.

FIG. 1. Daily food intake and mean food intake over 10-wk period in male rats fed standard laboratory chow (S) and cafeteria diet (C) with and without exercise (E). For daily food intake, C groups show greater values than S groups (P < 0.01). For mean food intake C group values are also greater than S groups (P < 0.01) while S > (S + E) and C > (C + E) both with P < 0.01.

FIG. 2. Daily food intake and mean food intake over a 10-wk period in female rats fed standard laboratory chow (S) and cafeteria diet (C) with and without exercise (E). For daily food intake, C groups show greater values than S groups (P < 0.01). For mean food intake C group values are also greater than S groups while C > (C + E) with P < 0.01.
female (92 g) than in the male rats (51 g), although the equivalent difference in caloric intake, expressed in total kJ for the 10-wk period, was not significantly different between the female (14,700 kJ) and the male rats (13,850 kJ) (Fig. 6).

The size of the brown adipose tissue was significantly increased in both male and female rats fed the cafeteria diet ($P < 0.01$), but this increase was reduced in exercised animals ($P < 0.01$). In addition, exercise had no effect on the brown adipose tissue of either male or female animals fed the stock diet (Fig. 5). The injection of norepinephrine failed to modify body temperature significantly in male or female rats fed the stock diet whether they were sedentary or on the exercise program. All animals fed the cafeteria diet showed an increase in body temperature after norepinephrine injection. This increase was larger in male than in female rats, and it was somewhat reduced by exercise training in male rats only ($P < 0.05$) (Fig. 5).

Measurements of plasma thyroid hormones gave the following results (Fig. 7). Cafeteria diet increased the plasma levels of triiodothyronine ($T_3$) in both male and female rats ($P < 0.01$), whereas exercise reduced these levels in both males ($P < 0.05$) and females ($P < 0.01$) fed either the stock or the cafeteria diet. On the contrary, cafeteria diet had no effect on thyroxine ($T_4$) concentrations. The only significant change observed in $T_4$ concentrations was a decrease in exercising male rats ($P < 0.01$) fed the standard chow diet.

Plasma catecholamine determinations gave the following results. There was a sixfold increase in norepinephrine when the rats swam for the first time, and this increase was not diminished after rats had been swimming for 10 wk. A fivefold increase in plasma epinephrine was observed in rats after swimming for the first time, but this effect was significantly reduced after 10 wk of swimming (Fig. 8).

![Figure 3](image3.png)
**FIG. 3.** Variations of body weight of male rats over 10-wk period for 4 groups.

![Figure 4](image4.png)
**FIG. 4.** Variations of body weight of female rats over 10-wk period for 4 groups.

![Figure 5](image5.png)
**FIG. 5.** Variations in epididymal and parametrial fat pads in male and female rats as affected by various diets. C diets increased size of fat deposit ($P < 0.01$) and exercise caused drop in both C and S groups ($P < 0.01$). C diets increased size of interscapular brown fat ($P < 0.01$), which was reduced in male and female exercise groups ($P < 0.01$). C diets increased response to norepinephrine (0.3 μg/100 g body wt), but this effect is more pronounced in male than in female rats ($P < 0.01$). Exercise training reduced effect observed with C diet in male rats.
DIET, EXERCISE, AND THERMOGENESIS

DISCUSSION

The marked influence of palatable food, termed cafeteria diet by Rothwell and Stock (29, 30), on body growth has been confirmed in both male and female rats. In the males the cafeteria diet increased caloric intake by 13,850 kJ; the animals gained 51 g more than the Purina-fed animals during the 10-wk period, and epididymal fat pads were three times larger. In the female rats caloric intake increased by 14,770 kJ, the extra weight gain amounted to 92 g, and the parametrial fat pads were also three times larger. Thus although the effect of cafeteria diet on caloric intake is somewhat comparable for both sexes, the body-growth increase was significantly larger in the female than in the male rats. The extra weight gain observed in the females is due to either a facilitation of body-weight increase or a reduced thermogenic action of cafeteria diet in female compared with male rats. The results obtained when norepinephrine was injected tend to support a reduced thermogenesis in female rats. Indeed, the increase in body temperature in response to norepinephrine was greater in males (2.8 ± 0.21°C) than in females (1.2 ± 0.15°C) (P < 0.01). Thus reduced thermogenesis could explain why female rats gained more weight than male rats while the food intake was comparable for both sexes. It should be mentioned however that the size of the brown adipose tissue (BAT), which was markedly increased by the cafeteria diet, was not

![Graph showing difference in caloric intake and body weight gain between cafeteria (C) and standard chow (S) diets for males and females.](image1)

![Graph showing difference in caloric intake and body weight gain between exercise-trained (E) and non-exercise-trained (control) animals for males and females.](image2)

![Graph showing plasma levels of triiodothyronine and thyroxine for males and females fed standard chow or cafeteria diet with or without exercise training.](image3)

![Graph showing plasma levels of epinephrine and norepinephrine for males and females during rest and exercise.](image4)
different when male and female rats were compared. However, this finding does not exclude the possibility for a role of the BAT in explaining the difference in thermogenesis between male and female rats. Indeed, it is possible that the metabolically active tissue present in the BAT is not related exclusively to its size. For example, growth of BAT accompanied by a marked increase in the total number of β-adrenergic receptor sites can be considered an important factor in thermogenesis (3).

The reduction of adiposity due to exercise training in rats fed standard laboratory food, which was reported by other investigators (5, 26, 27), was confirmed in the present study. Exercise training was also found to significantly modify the effects of the cafeteria diet in both male and female rats. Caloric intake in relation to energy expenditure was reduced by exercise, and this explained the reduced body-weight gain especially in cafeteria-fed animals. This reduction in body weight parallels a significant decrease in white adipose tissue size of exercise-trained animals compared with their sedentary controls. In these same animals it was found that exercise prevented some of the BAT hypertrophy and reduced the hyperthermic effect of norepinephrine in cafeteria-fed male and female rats. In other words, exercise training reduced the thermogenesis produced by the cafeteria diet. This finding is somewhat unexpected. Indeed, we have shown that the level of exercise used in the present study produced a marked activation of the sympathetic nervous system as evidenced by the large increase in plasma epinephrine and norepinephrine. Previous studies have demonstrated that such an increase in sympathetic nervous system activity as produced by cold exposure or repeated daily injections of catecholamines results in BAT enlargement and enhanced response to the metabolic effect of norepinephrine, both phenomena being associated with an increased thermogenesis (19). At this point, the role of the thyroid hormones in the genesis of thermogenesis would seem to be relevant in the present discussion. In cold-induced thermogenesis the plasma level of $T_3$ is increased as shown previously (10). In diet-induced thermogenesis the levels of $T_3$ are also increased in humans (6) and in rats (30), and these results are confirmed by the present study. In exercise-trained male or female rats, the levels of $T_3$ are reduced compared with the levels in sedentary animals; this difference is observed whether the animals are fed stock diet or cafeteria diet. Moderate exercise in humans has also been shown to decrease plasma $T_3$ levels (25). These results are in agreement with previous findings showing that thyroid stimulating hormone response is reduced by many stressful situations (17) but not by cold exposure (4). Kinetic techniques have shown that the increase in plasma $T_3$ observed with overeating in humans is associated with a greater production rate of $T_3$ (23). Similar findings were reported during prolonged cold exposure in the rats (16). We have shown that repeated thyroid hormone injections increase the size of the BAT and the response to norepinephrine (20). Thus cold exposure and overeating increase the level of $T_3$ in the blood, indicating that this metabolic hormone is concerned with the induction of enhanced thermogenesis. Following the same reasoning it can be argued that the reduction of diet-induced thermogenesis by exercise could be related to a concomitant reduction of $T_3$ production.

The BAT is accepted as the important site of thermogenesis of nonmuscular origin (10, 13, 30, 33). What are the factors involved in the transformation of BAT into a highly metabolic tissue? The fact that daily injections of norepinephrine (18, 19) or isoproterenol produce a BAT hypertrophy and promote thermogenesis may indicate the importance of these substances in cold- and diet-induced thermogenesis, because these two conditions are characterized by an enhanced catecholamine secretion (21, 35). However exercise training, as described in the present experiment, also causes a marked increase in plasma catecholamines, and yet no evidence of thermogenesis was found in rats fed a standard laboratory diet, whereas in rats fed the cafeteria diet the induced thermogenesis was partly reduced by training. Another substance that might be suggested as a factor in thermogenesis induction and in BAT metabolic activation is the thyroid hormone. Indeed $T_4$ and $T_3$ have been shown to stimulate mitochondrial protein synthesis in vitro and to induce an increased respiratory activity in the mitochondria (9, 34). Yet we have shown that repeated injections of $T_4$ failed to increase respiratory enzyme activity in the BAT (12, 28). However, cold exposure and isoproterenol injections produced significant increases in the activity of these enzymes. Just the same $T_3$ injections resulted in an increased thermogenesis comparable with that observed after repeated injections of isoproterenol. Obviously different mechanisms are involved in $T_3$- and catecholamine-induced thermogenesis, for catecholamines the important factor may be the increased activity of mitochondrial enzymes, whereas the effect of $T_4$ could be explained by an increase in the Na⁺-K⁺-ATPase activity (15). In cold-adapted rats the activity of respiratory enzymes in BAT is increased but no more than in isoproterenol-injected rats, and yet cold-adapted animals are much more resistant to cold. Because not only catecholamine but also thyroid hormones are secreted in larger quantities in cold-adapted animals, it is possible that the marked thermogenesis observed is due to an increase of both Na⁺-K⁺-ATPase and respiratory enzyme activity in the BAT. For the same reasons the same situation may prevail in the diet-induced thermogenesis. Thus the diminution, but not the complete abolition, of thermogenesis by exercise training could be explained by the suppression of the increase in plasma $T_3$ levels normally observed in diet-induced thermogenesis.

Feeding palatable food stimulates caloric intake and increases body-weight gain. At the same time plasma $T_3$ is increased, the BAT hypertrophies and the animals become more sensitive to the thermogenic effect of norepinephrine. This enhanced heat production would have a corrective action on this excessive food intake and serve a useful purpose in helping to prevent fat deposition (1, 22, 32). In that sense thermogenesis is an adaptive reaction. Exercise training has no such effect per se. When associated with palatable food, the habit of exercise diminished excessive food intake and reduced the increase in plasma $T_3$ and the hypertrophy of the BAT. This results in a diminution of the norepinephrine-induced thermogenesis. It is also suggested that the sexual
difference in norepinephrine thermogenesis observed in animals fed palatable food contributes to the caloric-sparing effect observed in female rats, which by consuming quantities of food similar to male rats were found to gain significantly more weight during the experimental period.

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REFERENCES


