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Dietary Modulation of the Anorectic Potency of Amphetamine

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MARKS-KAUFMAN, R. AND R. B. KANAREK. *Dietary modulation of the anorectic potency of amphetamine*. PHARMACOL BIOCHEM BEHAV **35**(2) 301–306, 1990.—The effects of intake of an amphetamine solution on food and fluid intakes, body weight (b.wt.) and feed efficiency (FE) were examined in rats fed either a high-carbohydrate (HC) (65% of total calories) or high-fat (HF) (65% of total calories) diet. During a 17-day predrug period, neither caloric intake, fluid intake, b.wt. nor feed efficiency (FE) differed as a function of diet. When given a 0.1 mg/ml amphetamine sulfate (AMPH) solution as their sole source of fluid, rats in both diet groups decreased fluid intake by an equivalent amount. While diet did not influence AMPH intake, it did alter the drug's effects on caloric intake, b.wt. and FE. In both diet groups, rats drinking AMPH decreased caloric intake, b.wt. gain and FE relative to rats which drank water. However, rats fed the HC diet decreased caloric intake less, but lost more weight than rats fed the HF diet. Further, rats fed the HC diet displayed a rapid tolerance to the anorectic effects of AMPH, with no tolerance to the drug's effect on b.wt. In contrast, rats fed the HF diet suppressed caloric intake throughout the drug period, but weighed more than rats fed the HC diet. Thus, when drinking the AMPH solution, FE of rats fed the HC diet was significantly lower than that of rats fed the HF diet. When the drug was removed, rats eating the HC diet failed to alter caloric intake and b.wt. In contrast, rats fed the HF diet increased caloric intake and gained weight. These data indicate that dietary factors must be considered when evaluating the anorectic actions of psychopharmacological agents.

Amphetamine High-fat diet High-carbohydrate diet Caloric intake Body weight Feed efficiency Anorectic drugs

MOST research examining the interaction of pharmacological agents and feeding behavior and energy regulation has concentrated on the effects of the drug of choice on food intake and body weight gain [for reviews see: (1, 5, 28)]. However, recent work has demonstrated that the other side of this interaction, that is the effects of alterations in feeding behavior on drug actions must also be considered [e.g., (2–4, 13, 14)]. Dietary variables can modify the intake of a variety of psychoactive drugs (2–4, 13, 14, 23). One dietary variable which can influence drug administration is the availability of food. For example, food deprivation results in increased intake of a wide variety of drugs commonly self-administered by animals including cocaine, phencyclidine, morphine and amphetamine (3, 4, 14). In contrast, giving animals access to a palatable sweet tasting food or solution (2, 13, 23) can reduce intake of psychoactive drugs. For example, we recently observed that rats consumed significantly less of an oral amphetamine solution when given access to two food cups, one containing granulated sucrose and the other a standard laboratory diet, than when given the standard diet alone (13,23). To further explore the role of dietary variables on drug self-administration, the present study examined the effects of modifying the macronutrient content of the diet on the intake of the classic anorectic agent, amphetamine. In addition, this study allowed for the examination of the effects of amphetamine on food intake and body weight gain in animals maintained on diets varying in macronutrient content.

METHOD

Animals

Thirty-two male virus and antibody free Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 160 to 185 g at the beginning of the experiment, were used. Animals were housed individually in standard stainless-steel cages in a temperature- and humidity-controlled room maintained on a 12-12-hr light/dark cycle (lights on: 0800–2000).

Diets and Drugs

The high-carbohydrate diet contained, on a per 1000 kilocalorie basis, 62.5 g (250 kcal) vitamin-free casein (ICN Pharmaceuticals, Cleveland, OH), 11.25 g (100 kcal) hydrogenated fat (MFB Shortening, Wesson), 162.5 g (650 kcal) corn starch (Teklad Test Diets, Madison, WI), 5.0 g vitamin diet fortification mix (ICN

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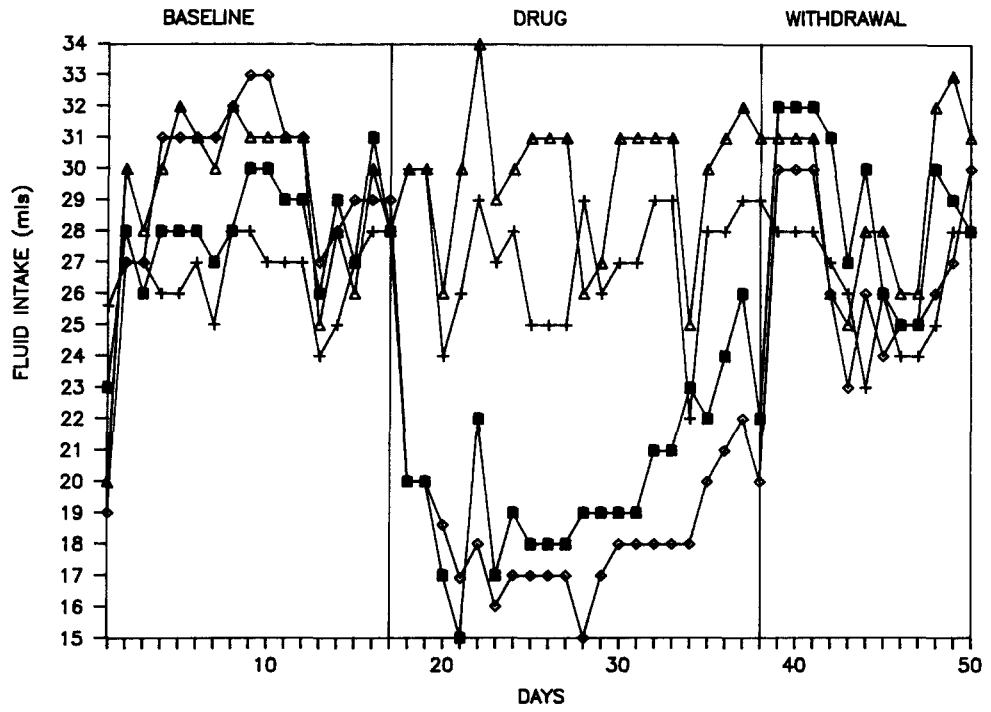

FIG. 1. Mean daily fluid intake for rats fed a high-fat diet and drinking either an amphetamine solution (■) or water (+), and for rats fed a high-carbohydrate diet and drinking either an amphetamine solution (◇) or water (△). During the 17-day predrug baseline period, all rats drank water. Rats in the amphetamine groups then were given the drug solution as their sole source of fluid for 21 days, at the end of which time the drug solution was removed and all rats were given water for a 12-day withdrawal period.

Pharmaceuticals) and 10 g AIN-76 mineral mix (ICN Pharmaceuticals). On a percentage basis, the high-carbohydrate diet contained 25% of its calories as protein, 65%, as carbohydrate and 10%, as fat. The caloric density of the high-carbohydrate diet was 3.9 kcal/g. On a per 1000 kcal basis, the high-fat diet contained 62.5 g (250 kcal) vitamin-free casein, 72.2 g (650 kcal) hydrogenated vegetable fat, 25.0 g (100 kcal) corn starch, 5.0 g vitamin diet fortification mix, and 10 g AIN-76 mineral mix. On a percentage basis, the high-fat diet contained 25% of its calories as protein, 10%, as carbohydrate and 65%, as fat. The caloric density of the diet was 5.7 kcal/g. The diets were presented to the rats in spill-proof Wahmann LC-306A stainless-steel food cups.

D-Amphetamine sulfate (Smith, Kline and French, Philadelphia, PA) was dissolved in water at a concentration of 0.1 mg of the salt/ml. The amphetamine solution was provided to the animals in 250 ml glass bottles with nonspill stainless-steel drinking spouts.

Procedure

Animals initially were divided into two groups matched on the basis of body weight. Animals in the first group (N = 16) were given ad lib access to the high-carbohydrate diet and those in the second group (N = 16), access to the high-fat diet. For the first 17 days of the experiment, all animals were given water to drink. From day 18 to day 38, ten animals in each of the diet conditions were given the 0.1 mg/ml amphetamine sulfate solution as their sole source of fluid. The remaining six rats in each diet condition continued to receive water. From day 39 to day 50, all animals again were given water to drink. Food and fluid intakes and body weights were measured on a daily basis throughout the experiment.

Statistical Analyses

Data were analyzed using analysis of variance followed by post hoc multiple comparisons (Bonferroni *t*-test). All data reported as significant have a p value of 0.05 or less.

RESULTS

Fluid Intake

No difference in fluid intake was observed during the first 17 days of the study when all animals had water to drink (Fig. 1). During this period, mean daily fluid intake of rats fed the high-fat diet was 27.8 ml and intake of rats fed the high-carbohydrate diet 29.5 ml.

Substituting the amphetamine solution for water led to a reduction in fluid intake. During the 21-day drug period, animals drinking the amphetamine sulfate solution consumed significantly ($p<0.05$) less fluid than rats drinking water. On the high-fat diet, daily fluid intake averaged 20.5 ml for rats drinking the amphetamine solution, and 28.0 ml for rats drinking water. On the high-carbohydrate diet, daily fluid intake averaged 19.0 ml for rats drinking the amphetamine solution, and 30.8 ml for those drinking water. Thus, average daily amphetamine intake was 2.05 mg for rats fed the high-fat diet and 1.90 mg for rats fed the high-carbohydrate diet. Amphetamine intake did not differ as a function of diet.

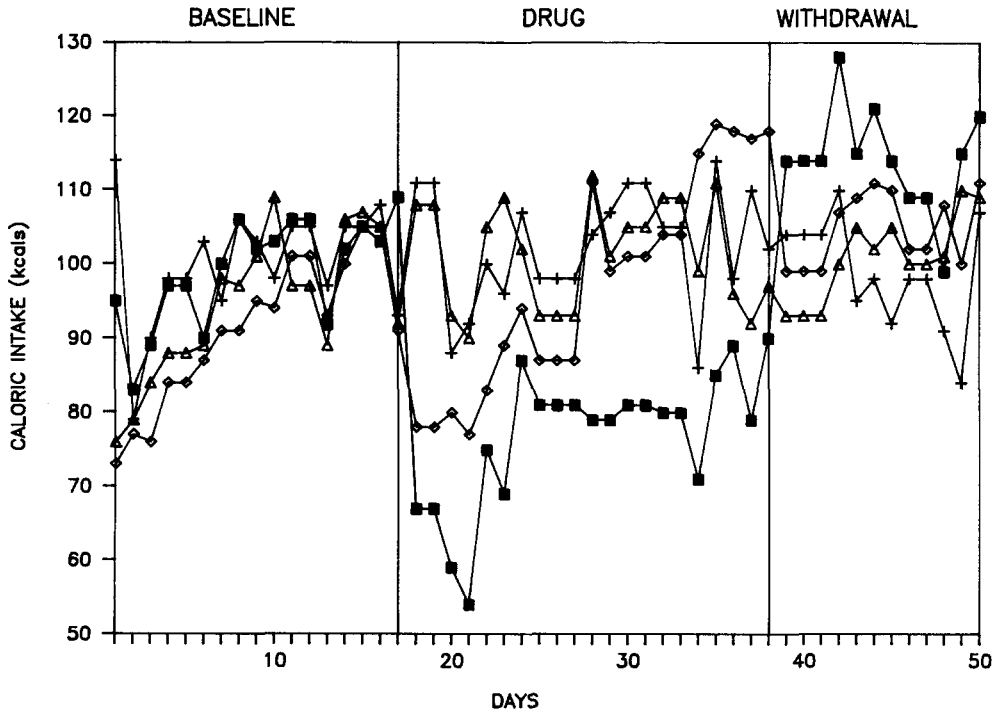

FIG. 2. Mean daily caloric intake for rats fed a high-fat diet and drinking either an amphetamine solution (■) or water (+), and for rats fed a high-carbohydrate diet and drinking either an amphetamine solution (◇) or water (△). During the 17-day predrug baseline period, all rats drank water. Rats in the amphetamine groups then were given the drug solution as their sole source of fluid for 21 days, at the end of which time the drug solution was removed and all rats were given water for a 12-day withdrawal period.

Fluid intake returned to predrug levels when amphetamine was replaced with water. For rats fed the high-fat diet, mean daily water intake during withdrawal was 29.1 ml for rats which had previously received amphetamine and 26.9 ml for rats which had drunk water throughout the experiment. For rats fed the high-carbohydrate diet, mean daily water intake was 27.1 ml for rats which had previously received amphetamine, and 29 ml for rats which had drunk water throughout the experiment.

Caloric Intake

Caloric intake did not differ between the dietary groups during the first 17 days of the study when all rats were drinking water (Fig. 2). Rats fed the high-fat diet consumed an average of 100.0 kcal/day, and those fed the high-carbohydrate diet, an average of 93.6 kcal/day. During the remainder of the experiment, caloric intake did not vary as a function of diet for animals drinking water. During the drug period, when water was available, daily caloric intake averaged 104.1 kcal for rats fed the high-fat diet, and 100.5 kcal for rats fed the high-carbohydrate diet. During withdrawal, daily caloric intake averaged 100.0 kcal for rats fed the high-fat diet, and 101.0 kcal for rats fed the high-carbohydrate diet.

Caloric intake differed significantly as a function of drug availability. When initially given the amphetamine solution to drink, animals in both dietary conditions significantly decreased caloric intake. However, diet differentially influenced caloric intake of rats drinking the amphetamine solution. By the third day of access to the drug, caloric intake was no longer significantly suppressed in animals maintained on the high carbohydrate diet. Rats consuming the high-carbohydrate diet increased caloric intake throughout the drug period and actually were consuming more calories than their controls drinking water by the end of the drug period. In comparison, caloric intake of rats fed the high-fat diet did not increase across the 21-day drug period and by day 15 of the study was significantly less than that of rats consuming the high-carbohydrate diet and drinking the AMPH solution. Across the drug period, mean daily caloric intake for rats fed the high-fat diet was 78.0 kcal and for rats fed the high-carbohydrate diet was 98.1 kcal.

When the amphetamine solution was replaced with water, rats in the high-carbohydrate group did not significantly alter caloric intake. In contrast, during drug withdrawal, rats in the high-fat group increased caloric intake and consumed more calories than their diet controls which had drunk water throughout the study. For rats which had drunk the amphetamine solution, mean daily caloric intake during withdrawal was significantly greater in the high-fat diet group (114.9 kcal) than in the high-carbohydrate group (104.6 kcal).

Body Weight

No differences in body weight were observed during the first 17 days of the study when all animals were drinking water (Fig. 3). Body weight did not differ as a function of diet for animals drinking water for the remainder of the experiment.

Amphetamine availability influenced body weight with rats drinking the drug solution gaining less weight than animals drinking water. Rats in the amphetamine groups weighed signifi-

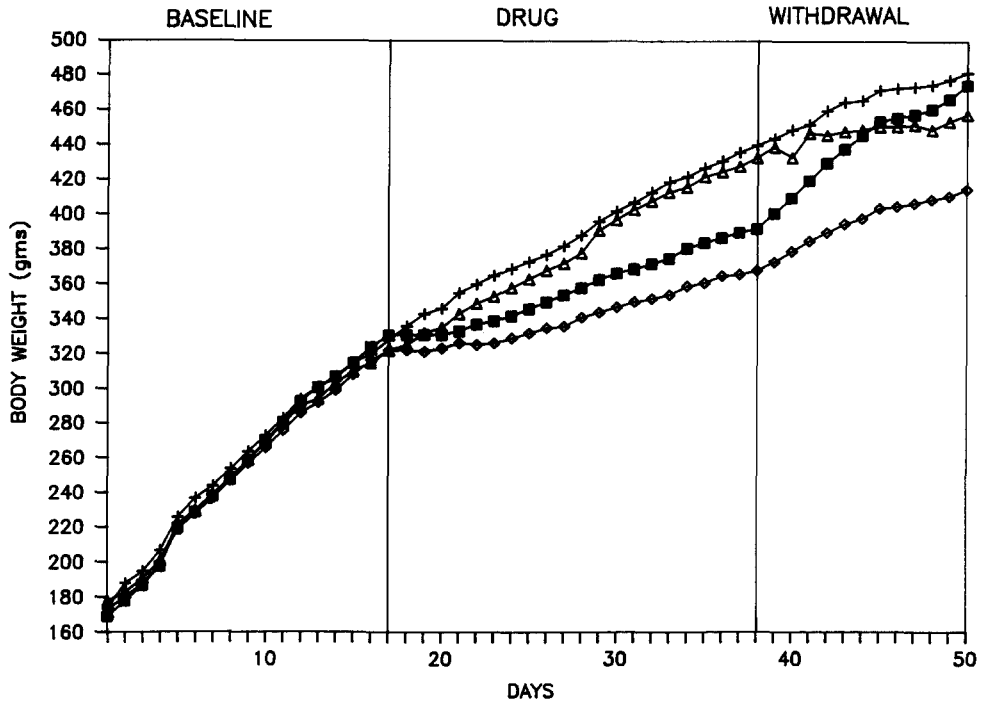

FIG. 3. Mean daily body weights for rats fed a high-fat diet and drinking either an amphetamine solution (■) or water (+), and for rats fed a high-carbohydrate diet and drinking either an amphetamine solution (◇) or water (△). During the 17-day predrug baseline period, all rats drank water. Rats in the amphetamine groups then were given the drug solution as their sole source of fluid for 21 days, at the end of which time the drug solution was removed and all rats were given water for a 12-day withdrawal period.

cantly less than their respective diet control group drinking water by day 10 of the study for rats eating the high-carbohydrate diet, and by day 13 for rats eating the high-fat diet. As can be seen in Fig. 3, rats given the high-carbohydrate diet weighed less than those fed the high-fat diet throughout the drug period.

When the amphetamine solution was replaced with water, different patterns of weight gain were observed as a function of dietary conditions. Rats fed the high-fat diet rapidly increased their weight gain. By day 2 of drug withdrawal, these animals no longer weighed less than rats drinking water throughout the study. In contrast, animals fed the high-carbohydrate diet only slowly increased body weight during the withdrawal period and by the end of the experiment continued to weigh significantly less than rats on the high-fat diet that had been withdrawn from amphetamine.

Feed Efficiency

The preceding data indicate that when given amphetamine, animals fed the high-carbohydrate diet consumed more calories but gained less weight than rats fed the high-fat diet. To quantify this observation, feed efficiency (weight gained per 100 kcal consumed) was calculated. No differences in feed efficiency were observed during the first 17 days of the study (Fig. 4).

During the drug period, feed efficiency did not differ as a function of diet for animals drinking water. However, diet did have an effect on feed efficiency for animals drinking the amphetamine solution. In both dietary conditions, animals consuming amphetamine gained less weight per 100 kcal consumed than their respective controls, however, this effect was more pronounced for rats given the high-carbohydrate diet. Rats drinking amphetamine and fed the high-carbohydrate diet were significantly (0.05) less efficient at using calories for weight gain than animals fed the high-fat diet.

When the amphetamine solution was replaced with water, animals in both diet conditions increased feed efficiency. However, this measure was again influenced by dietary conditions.

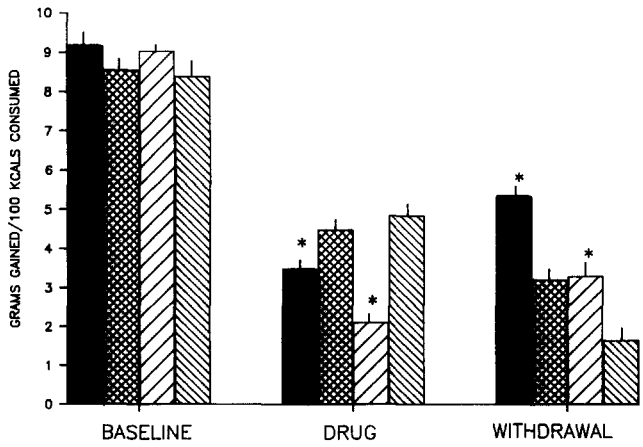

FIG. 4. Mean food utilization (weight gained/100 kcal consumed) across a 17-day predrug baseline period, 21-day drug period, and 12-day withdrawal period for rats fed a high-fat diet and drinking either an amphetamine solution (solid bars) or water (cross-hatched bars), and rats fed a high-carbohydrate diet and drinking either an amphetamine solution (lightly hatched bars) or water (heavily hatched bars). *$p<0.05$, significantly different from respective water control group.

Rats eating the high-carbohydrate diet continued to gain significantly less weight per 100 kcal consumed than rats given the high-fat diet.

DISCUSSION

In the present study giving animals access to a single diet that varied in macronutrient content did not differentially modify oral self-administration of amphetamine. These data can be contrasted with the results of our previous experiment in which rats consumed less of an amphetamine solution when given a choice of a standard laboratory diet and granulated sucrose than when they were given the laboratory diet alone (13). Several variables including the strain of the rats, and the design of the experiment (i.e., within subjects versus between subjects design), may help to explain the difference in results between the two studies. However, the most important difference between these two studies may be that in the first study, rats were given a choice of two dietary components, while in the present study only one diet was presented. We have found that providing alternatives, be they palatable foods or access to running wheels [(14), Kanarek and Marks-Kaufman, unpublished results], can have significant effects on drug intake.

While diet did not influence drug intake in this study, diet did play a major role in determining the effects of drugs on energy intake and body weight gain. When given an amphetamine solution as their sole source of fluid, rats eating a high-fat diet consumed less calories, but gained more weight than rats eating a high-carbohydrate diet. Further, when the amphetamine solution was replaced with water, rats given the high-fat diet displayed an immediate increase in caloric intake and rapidly regained weight, while those given the high-carbohydrate diet failed to alter caloric intake and to regain weight.

There have only been a limited number of studies that have investigated the role of the macronutrient composition of the diet on the effects of potential anorectic agents on feeding behavior and body weight (22,23). Maggio *et al.* (22) recently reported that cholecystokinin was more effective in reducing food intake in both lean and obese Zucker rats maintained on a high-fat diet than in animals maintained on either an isocaloric fat diet or chow. Additionally, we recently observed that the anorectic actions of fenfluramine also are altered by dietary conditions. Using the same high-carbohydrate and high-fat diets as in the present experiment, both acute and chronic fenfluramine administration led to a significantly greater reduction in caloric intake and body weight in rats fed the high-fat diet than in animals fed the high-carbohydrate diet (Kanarek and Marks-Kaufman, unpublished data). Finally, we recently reported that rats drinking an AMPH solution lost more weight and had significantly lower FE when given a simultaneous choice of a chow diet and granulated sucrose than when given the chow diet alone (13,23). Taken together, the preceding data indicate that the macronutrient content of the diet can significantly alter the effects of a number of anorectic agents. In studies comparing the effects of anorectic agents in rats consuming high-carbohydrate or high-fat diets, the drugs invariably led to greater reductions in intake of high-fat diets than of high-carbohydrate diets.

The present study also illustrates that one must not only look at food intake, but must also examine body weight gain and feed efficiency to understand the interaction between diet composition and the pharmacological action of drugs. While animals maintained on the high-carbohydrate diet actually consumed more food than animals on the high-fat diet, they gained less weight. Therefore, animals on the high-carbohydrate diet were less efficient in utilizing calories than animals maintained on the high-fat diet. As mentioned above, this is similar to findings in our laboratory in which animals drinking an amphetamine solution decreased their feed efficiency more when given access to sucrose and chow then when given the chow diet alone. Thus, the above studies suggest that there is an interaction between amphetamine and the macronutrient composition of the diet which can alter not only the anorectic potential, but also the leptogenic characteristics of the drug (25).

One possible explanation for these findings relates to the effects of both high-carbohydrate diets and amphetamine on the noradrenergic sympathetic nervous system and brown adipose tissue (BAT). BAT is a specialized organ located primarily in the interscapular and paraspinal regions that gets its characteristic brown color from its many mitochondria containing high concentrations of cytochrome pigments (6,24). Heat production or thermogenesis in BAT is mediated by a proton-conductance pathway which is under the control of beta-adrenergic receptors. Evidence is accumulating that both diet (8, 9, 12, 17, 26, 29) and a number of anorectic compounds (7, 19–21, 27) can moderate thermogenic activity in BAT. With respect to diet, for example, it has been found that sucrose can increase BAT mass and stimulate norepinephrine turnover and the metabolic potential for thermogenesis in the tissue (10–12, 15, 16). Additionally, Glick (8) has reported that a high-carbohydrate meal stimulates thermogenic activity in BAT to a greater degree than a high-fat meal. With respect to drugs, it has been demonstrated that a number of anorectic agents, including both amphetamine and fenfluramine, can activate BAT metabolism (7, 19–21, 27). Recent work by Levitsky and colleagues (18) provides support for the idea that diet and drugs may interact in stimulating thermogenesis. These investigators observed that fenfluramine had a significant thermogenic effect when injected in conjunction with a high-carbohydrate meal, but not with a high-fat meal. Thus, it could be hypothesized that the greater weight loss observed in rats fed the high-carbohydrate diet in this experiment, is the result of a synergistic effect of the diet and amphetamine on thermogenesis.

Understanding the role of diet in the actions of anorectic agents could provide important insights into methods of combining therapeutic strategies in the clinical treatment of obesity. Diets which produce greater weight loss, less tolerance and less rebound weight gain when used in conjunction with anorectic drugs would obviously be of benefit and a simple adjunct to pharmacological treatment of obesity.

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