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# Effects of Prolonged Fenfluramine Administration in Obese and Nonobese Mice (39616)

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The amphetamine derivative, fenfluramine (*N*-ethyl-α-methyl-3-trifluoromethyl-β-phenylethylamine), produces anorexia in man and other animals without the central nervous system stimulation produced by the parent amphetamine (1). While the majority of studies in humans has involved obese patients, most of the animal experiments have been performed in normal weight animals, although a few studies were done in rodents made obese by electrical (2) or chemical (3) lesioning of the hypothalamus. Although these investigations have been principally concerned with the anorectic action of fenfluramine, a number of metabolic effects has also been reported, including increased glucose uptake by muscle (4), decreased plasma glucose and insulin (5), decreased depletion of liver glycogen (6), increased plasma free fatty acids, free glycerol, and ketones (7), and decreased plasma triglycerides (8). Because fenfluramine exhibits concomitant anorectic and hypoglycemic effects, it has been suggested for possible use in the treatment of the obese, maturity onset, diabetic patient (9). Since the genetically obese mouse (ob/ob) is characterized by obesity associated with hyperglycemia, hyperinsulinemia, and insulin resistance similar to that observed in maturity onset diabetes, we investigated the effects of prolonged fenfluramine administration in this animal in comparison to the effects in mice made obese by gold thioglucose injections (GTG) and in nonobese mice.

*Materials and methods.* Animals used in the study were female, genetically obese mice of the C57BL/6J-ob strain (genotype, ob/ob), female mice of the C57BL/6J strain made hyperphagic and obese by a single ip injection of 600 mg/kg body weight GTG in distilled water at 9 weeks of age, and female nonobese C57BL/6J-ob (genotypes, +/+ and ob/+) mice (Jackson Laboratories, Bar Harbor, Maine). Animals were housed singly in wire-bottom cages in a light- (12-hr light/dark cycle) and temperature- (20 ± 2°) controlled room and were fed ground laboratory chow (Ralston Purina Company, St. Louis, Mo.) and water *ad libitum*. Food intake, water consumption, and body weight were recorded three times a week. At 15 weeks of age, animals were divided into groups of seven mice and given either tap water or a 60 mg/100 ml solution of fenfluramine · HCl (A. H. Robins Company, Richmond, Va.) in place of drinking water. After 4 weeks, the concentration of the fenfluramine solution was increased to 90 mg/100 ml. Both the fenfluramine solutions and the tap water were flavored with orange extract to minimize any differences in water intake due to taste.

At 2, 4, and 6 weeks after treatment was begun, nonfasted mice were anesthetized with 50% $CO_2$:50% $O_2$, and blood samples were collected from the retroorbital sinus in heparinized capillary tubes. Plasma glucose concentrations were determined by the method of Fales (10), plasma insulin concentrations by the method of Herbert *et al.* (11), and plasma triglyceride concentrations by an enzymatic method using a diagnostic reagent kit (Dow Diagnostics, Indianapolis, Ind.). After 8 weeks of treatment, all animals were fasted for 18 hr, killed by decapitation, and their blood was collected in heparinized tubes for final determinations of insulin, glucose, and triglycerides. A 500 mg sample of liver was quickly excised and digested in boiling 1 *N* NaOH, and the glycogen content was determined with anthrone reagent (12). Body composition was determined using the procedure of Leshner *et al.* (13). The results were analyzed by Student's *t* test for independent samples comparing fenfluramine-treated animals to

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untreated animals, and expressed as mean ± SE.

*Results.* During the first 4 weeks of treatment, the ob/ob mice received a significantly ($P < 0.05$) smaller dose of fenfluramine when expressed on a body weight basis, 52 ± 3 mg/kg/day compared to 71 ± 7 mg/kg/day for GTG mice and 72 ± 6 mg/kg/day for nonobese mice. This difference was attributable to the greater body weight of the ob/ob mice since they ingested a total of 2.1 ± 0.2 mg of fenfluramine per day, while the GTG and nonobese mice ingested 2.1 ± 0.2 and 1.6 ± 0.1 mg, respectively. During this period there were no significant differences in food consumption, water intake, and body weight between treated and untreated ob/ob mice. Both GTG and nonobese mice exhibited a significant ($P < 0.01$) and sustained reduction in water intake with a significant ($P < 0.05$), but transitory, reduction in food intake which paralleled the transitory reduction in body weight (Fig. 1). When the concentration of fenfluramine in the drinking water was increased to 90 mg/100 ml, all animals received an average daily dose of 104 ± 8 mg/kg. At this dose, all animals showed significant ($P < 0.05$) reductions in food and water intake. Body weights were also significantly ($P < 0.05$) reduced for all treated mice during the final 4 weeks when compared to untreated mice (Fig. 1). Fenfluramine treatment reduced body weight and prevented weight gain more effectively in the GTG mice than in the ob/ob mice and was least effective in nonobese mice.

The results of the biochemical determinations made during the final four weeks of treatment, when all animals received the same dose of fenfluramine, are presented in Table I. A striking effect of fenfluramine treatment was the reduction in plasma glucose concentrations in the ob/ob mice at both the sixth and eighth weeks of treatment under fed and fasted conditions, respectively. Fenfluramine significantly reduced plasma glucose concentrations in fed, but not in fasted, GTG mice. In contrast, there was no effect in the nonobese mice. Plasma insulin concentrations were reduced by fenfluramine treatment in the normally hyperinsulinemic ob/ob mice, but not to any significant degree in the GTG and nonobese mice. Fenfluramine treatment lowered plasma triglyceride concentrations in all treated animals, but only significantly in the GTG mice. The glucose and triglyceride values for fed animals (sixth week) presented in Table I are representative of the values obtained after 2 and 4 weeks of treatment at the lower dose, when the effects on food intake and body weight were much less. However, the significant reduction in plasma insulin concentrations in the ob/ob mice occurred only during the last weeks of

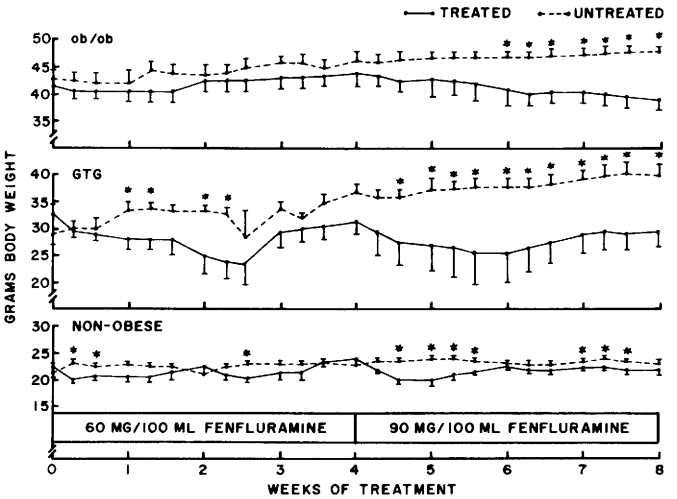


FIG. 1. Body weight changes in ob/ob, GTG, and nonobese mice treated with fenfluramine in drinking water. Bars represent SE and asterisks indicate significant ($P < 0.05$) differences between treated and untreated mice.

TABLE I. EFFECT OF FENFLURAMINE ON PLASMA CONCENTRATION OF GLUCOSE, INSULIN, AND TRIGLYCERIDES, AND ON LIVER GLYCOGEN IN MICE.

| Parameter measured | Treatment week[a] | ob/ob | | GTG | | Nonobese | |
|---|---|---|---|---|---|---|---|
| | | Treated | Untreated | Treated | Untreated | Treated | Untreated |
| Plasma glucose (mg/100 ml) | 6 | 128 ±4 (6)[b] | 285 ±27 (5) | 122 ±17 (3) | 171 ±3 (4) | 110 ±6 (4) | 110 ±8 (6) |
| | | $P < 0.001$ | | $P < 0.05$ | | NS | |
| | 8 | 90 ±3 (5) | 195 ±5 (6) | 97 ±21 (3) | 137 ±8 (6) | 82 ±14 (4) | 89 ±11 (7) |
| | | $P < 0.05$ | | NS | | NS | |
| Plasma insulin ($\mu$U/ml) | 6 | 36.7 ±1.4 (3) | 63.5 ±6.6 (5) | 11.5 ±1.4 (3) | 18.8 ±3.3 (4) | 11.9 ±3.9 (4) | 27.2 ±6.6 (5) |
| | | $P < 0.05$ | | NS | | NS | |
| | 8 | 66.5 ±7.8 (5) | 90.0 ±5.1 (6) | 34.8 ±2.6 (3) | 26.2 ±3.9 (6) | 32.3 ±3.7 (4) | 44.9 ±1.8 (7) |
| | | $P < 0.05$ | | NS | | NS | |
| Plasma triglycerides (mg/100 ml) | 6 | 219 ±39 (3) | 251 ±38 (5) | 78 ±5 (3) | 154 ±4 (4) | 80 ±10 (4) | 125 ±36 (5) |
| | | NS | | $P < 0.05$ | | NS | |
| | 8 | 80 ±14 (5) | 99 ±22 (6) | 72 ±7 (3) | 114 ±14 (4) | 62 ±2 (4) | 99 ±26 (6) |
| | | NS | | $P < 0.05$ | | NS | |
| Liver glycogen (mg/g) | 8 | 6.5 ±0.3 (5) | 8.4 ±0.9 (6) | 4.4 ±1.3 (3) | 4.9 ±0.9 (6) | 3.2 ±0.1 (5) | 4.7 ±0.7 (7) |
| | | NS | | NS | | NS | |

[a] Six and eight treatment weeks were the second and fourth weeks after fenfluramine was increased to 90 mg/100 ml of drinking water.

[b] Values are mean ± SE; numbers in parentheses indicate number of determinations.

TABLE II. PERCENTAGE BODY COMPOSITION OF FENFLURAMINE-TREATED AND UNTREATED MICE.

| | ob/ob | | GTG | | Nonobese | |
|---|---|---|---|---|---|---|
| | Treated | Untreated | Treated | Untreated | Treated | Untreated |
| Water | 39.1 ± 1.2 (5)[a] | 38.8 ± 2.1 (6) | 48.0 ± 1.0 (3) | 46.1 ± 4.2 (6) | 61.6 ± 1.9 (5) | 68.0 ± 1.5 (7) |
| | NS | | NS | | $P < 0.05$ | |
| Protein | 10.0 ± 1.1 (5) | 9.5 ± 0.3 (4) | 14.7 ± 3.8 (3) | 11.2 ± 1.1 (6) | 8.8 ± 1.7 (5) | 6.3 ± 1.0 (7) |
| | NS | | NS | | NS | |
| Fat | 50.0 ± 8.3 (5) | 43.7 ± 2.6 (6) | 23.7 ± 1.9 (3) | 27.8 ± 3.6 (6) | 13.6 ± 1.2 (5) | 10.1 ± 1.0 (7) |
| | NS | | NS | | $P < 0.05$ | |

[a] Values are mean ± SE; numbers in parentheses indicate number of determinations.

treatment when body weight was also significantly reduced. Liver glycogen concentrations were not significantly affected by drug treatment in any of the mice. The body composition data are given in Table II. Despite the significant reduction in body weight, the percentage of body fat was not significantly reduced by fenfluramine treatment.

*Discussion.* Body weight loss in man and other animals is correlated with the steady-state plasma concentration of fenfluramine and its active metabolite norfenfluramine, which represents a dynamic equilibrium between fenfluramine absorbed and that accumulated in body tissues (9). Le Douarec and Neveu (1) suggest that fenfluramine should be given at the time when food is eaten to be effective. In either case, administration of fenfluramine in the drinking water should be expected to promote the most effective reduction in food intake and body weight. Maximal effects of this compound were probably observed during the final 4 weeks of treatment when the animals were receiving 104 ± 8 mg/kg/day, which is five times the $ED_{50}$ for ip fenfluramine in mice and 43% of the $LD_{50}$ for orally administered

fenfluramine (1). However, it is possible that if treatment had continued, the effectiveness of this higher dose would have diminished as it did for the lower dose during the first 4 weeks of treatment (Fig. 1). On the other hand, the tolerance observed during the initial 4-week period could represent failure of this lower dose to produce an effective plasma fenfluramine concentration. Our results fail to confirm reports (1, 7, 14) that tolerance to the anorectic action of fenfluramine does not develop readily.

Reports of increased lipolysis (7) and decreased lipogenesis (15) in fenfluramine-treated animals have led to the hypothesis that fenfluramine may contribute to weight loss via direct effects on lipid metabolism as well as by reducing caloric intake. While a decrease in plasma triglyceride concentration and the commonly associated increases in free fatty acids and ketones are often used as an indication of lipolysis in fenfluramine-treated animals, Garattini *et al.* (8) have shown that fenfluramine may reduce plasma triglycerides by reducing the absorption of lipids from the gut. Furthermore, Dannenburg *et al.* (16) have shown that in the rat, fenfluramine reduces available energy by reducing fat absorption, but that fenfluramine does not affect body composition. While the reduction in plasma triglyceride concentration observed in the GTG mice (Table I) may or may not be indicative of lipolysis, the failure of 8 weeks of fenfluramine administration to significantly reduce the percentage of body fat in any of the treated mice (Table II) leads us to the conclusion that fenfluramine does not enhance weight loss by enhancing lipolysis in adipose tissue. Our results also fail to confirm the observation of Duhault (6) that fenfluramine prevents the depletion of liver glycogen during fasting (Table I).

The observation *in vivo* (5, 17), that fenfluramine reduced blood glucose and improved glucose tolerance, has been explained on the basis of *in vitro* observations that fenfluramine increased the uptake of glucose by muscle (4), that this effect was dose dependent and significantly increased by the presence of insulin (18), and that the mechanism of fenfluramine-induced glucose uptake was different from that of insulin (19). Under the conditions described in our experiment, fenfluramine reduced blood glucose concentrations only when some degree of hyperglycemia was seen (Table I). The reduction in blood glucose observed in the ob/ob mice when the dose of fenfluramine was not sufficient to produce the other effects of this drug proves the direct hypoglycemic action of fenfluramine. Since the elevated insulin concentrations in the ob/ob mice were reduced in the fenfluramine-treated animals concomitantly with body weight, it was not possible to determine whether this reduction was a consequence of the reduction in body weight or the reduction in blood glucose levels. The report by Weisweiler and Schwandt (5) that fenfluramine improved glucose tolerance and lowered basal plasma insulin and glucose concentrations without any weight reduction suggests that fenfluramine may reverse the normal sequence of an initial weight loss leading to decreased insulin resistance and improved glucose tolerance. Further investigations in the ob/ob might clarify the means by which fenfluramine improves glucose tolerance.

*Summary.* Oral administration of fenfluramine for 8 weeks reduced food intake and body weight in ob/ob, GTG, and nonobese mice. The hyperglycemia and hyperinsulinemia present in the ob/ob mice were significantly reduced by this drug. Plasma glucose, but not insulin, was significantly reduced in GTG mice; neither was significantly reduced in nonobese mice. Fenfluramine reduced plasma triglycerides, but had no effect on liver glycogen concentration and did not reduce the percentage of body fat.

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