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d-Fenfluramine in a Rat Model of Dietary Fat-Induced Obesity

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FISLER, J. S., S. J. UNDERBERGER, D. A. YORK AND G. A. BRAY. d-*Fenfluramine in a rat model of dietary fat-induced obesity.* PHARMACOL BIOCHEM BEHAV 45(2) 487–493, 1993.—*d*-Fenfluramine is an appetite suppressant drug that acts by releasing serotonin from axon terminals and inhibiting its reuptake. S 5B/Pl rats, which are resistant to dietary-fat induced obesity, and Osborne-Mendel rats, which are sensitive, were adapted to ad lib feeding of either a low- or high-fat diet. *d*-Fenfluramine (10 mg/kg, IP) was injected daily for 12 days. Other than a slightly greater suppression of food intake in Osborne-Mendel rats, there was little difference in response to *d*-fenfluramine between S 5B/Pl and Osborne-Mendel rats eating the low-fat diet. However, in Osborne-Mendel rats *d*-fenfluramine completely abolished the excess food intake and weight gain associated with the high-fat diet. Purine nucleotide (GDP) binding on day 13 was higher in S 5B/Pl rats than in Osborne-Mendel rats and was increased by *d*-fenfluramine in animals of both strains eating the low-fat diet. The high-fat diet increased GDP binding only in S 5B/Pl rats and blocked the fenfluramine-induced increase in GDP binding in both strains. We speculate that *d*-fenfluramine blocks a feeding reward system stimulated by the high-fat diet.

High-fat diet S 5B/Pl rats Osborne-Mendel rats Food intake GDP binding Serotonin

WHEN rats eat a high-fat diet, some strains become obese whereas others do not. At one extreme is the Osborne-Mendel strain, which is sensitive to dietary fat-induced obesity, and at the other extreme is the S 5B/Pl strain, which is resistant to obesity induced by a high-fat diet. The resistant and sensitive strains differ in a number of physiological characteristics that could promote the sensitivity to dietary fat; Osborne-Mendel rats have lower sympathetic nervous system activity than S 5B/Pl rats (6,8,27), lower levels of 3-hydroxybutyrate peripherally (7,27) and in the brain (3,7), and lower brain serotonin and norepinephrine levels than S 5B/Pl rats (H. Shimizu, J. S. Fisler, and G. A. Bray, unpublished) or Sprague-Dawley rats (13).

Because brain serotonin levels are lower in the sensitive Osborne-Mendel rats than in other strains, we questioned whether Osborne-Mendel rats might respond to enhanced serotonin release with increased resistance to dietary fat-induced obesity. The indirect agonist, *d*-fenfluramine, was chosen as the experimental agent. Fenfluramine is a racemate with l-fenfluramine having dopaminergic activity whereas *d*-fenfluramine is more specific for the serotonergic system [reviewed in (10)]. *d*-Fenfluramine is a powerful and selective inhibitor of serotonin uptake with somewhat lesser activity on release of granular serotonin stores (10). Fenfluramine both suppresses appetite and enhances thermogenesis (1,15,16).

We examined the effects of *d*-fenfluramine on food intake, body weight, and GDP binding, an index of sympathetic nervous system activity, in S 5B/Pl and Osborne-Mendel rats eating either low- or high-fat diets. Other than a slightly greater suppression of food intake in Osborne-Mendel rats, there was little difference in response to *d*-fenfluramine between S 5B/Pl and Osborne-Mendel rats eating the low-fat diet. However, in Osborne-Mendel rats *d*-fenfluramine completely abolished the excess food intake and weight gain associated with the high-fat diet.

METHOD

Animals

S 5B/Pl rats were obtained from the colony of Dr. Rachel Schemmel (Michigan State University, East Lansing, MI) and Osborne-Mendel rats were obtained from Rockland (Gilbertsville, PA). All rats were housed individually in wire-bottomed hanging metabolic cages in a temperature-controlled room (26 ± 2°C) with lights on from 0600–1800 h daily.

Diets

All rats were fed a pelleted chow diet ad lib (Wayne Lab-Blox, Chicago, IL) prior to the onset of the experiments. Diet

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composition by weight, according to the manufacturer's specifications, was 24.5% protein, 4.4% fat, and 46.9% carbohydrates. Rats were then adapted to either a low-fat diet (10% of kcal as fat) or to a high-fat diet (56% of kcal as fat). The composition of these diets is given in Table 1.

Drugs

In ad lib-fed rats, the 50% inhibitory dose (DI_{50}) for *d*-fenfluramine given as a single injection during the first 24 h is 10 mg/kg (4). A preliminary experiment in a separate set of rats showed that the DI_{50} dose given once daily to high-fat fed rats resulted in a differential response in food intake in S 5B/Pl and Osborne-Mendel rats over a 9-day period (data not shown). Therefore, *d*-fenfluramine (gift of Les Laboratoires Servier, Gidy, France) was injected IP at 10 mg/kg body weight (5 mg/ml saline).

Purine Nucleotide Binding (GDP binding)

The interscapular brown adipose tissue (IBAT) was dissected from surrounding muscle and white adipose tissue and weighed, and mitochondria were prepared as previously described (6). The binding of guanosine 5'-diphosphate to IBAT mitochondria was measured immediately by a modification (5) of the method of Nicholls (17). Protein was estimated by a modification of the Lowry method (21).

Food Intake and Body Weight

Food intake, corrected for the minimal spills that occurred, was recorded daily, as was body weight. Lee Index, defined as $[\text{body weight (g)}]^{1/3}/\text{naso-anal length (cm)}$, was measured in completely anesthetized (Metofane, Pitman-Moore, Inc., Denver, CO) animals 1 day prior to sacrifice.

Experimental Protocol

Male 3.5-month-old S 5B/Pl and Osborne-Mendel rats were adapted to the low-fat, semisynthetic diet for 7 days. One half of the rats were transferred to the high-fat diet just 2 days prior to beginning drug treatment to prevent excess weight gain before beginning the study. Four groups were studied: a) low-fat diet and saline injections (S 5B/Pl, $n = 5$; Osborne-Mendel, $n = 6$); b) low-fat diet and *d*-fenfluramine (10 mg/kg) injections (S 5B/Pl, $n = 6$; Osborne-Mendel, $n = 7$); c) high-fat diet and saline injections (S 5B/Pl, $n = 5$; Osborne-Mendel, $n = 6$); d) high-fat diet and *d*-fenfluramine (10 mg/kg) injections (S 5B/Pl, $n = 5$; Osborne-Mendel, $n = 6$). Injections were given daily for 12 days at 0900 h, at which time food intake and body weight were also recorded. On day 12, naso-anal length was measured. On day 13, rats were killed by decapitation for measurement of GDP binding in IBAT.

Analysis of Data

All data are presented as means ± SE. Comparisons were made by analysis of variance (ANOVA) and ANOVA with measures repeated over time (26). If there was a significant interaction, simple main effects were computed (26). When the ANOVA indicated a significant effect of *d*-fenfluramine, the comparison of individual means was done by *t*-test using the within-cell mean square, corrected for sample size differences in the groups, from the appropriate ANOVA table (26).

RESULTS

Prior to *d*-fenfluramine treatment, energy intake for all groups combined was greater in Osborne-Mendel rats than in S 5B/Pl rats ($p < 0.005$) and was higher in both strains eating the high-fat diet ($p < 0.005$) (Table 2). The 12-day cumulative energy intake was also slightly greater in Osborne-Mendel rats ($p < 0.05$) and in animals eating the high-fat diet ($p < 0.05$). *d*-Fenfluramine decreased energy intake, expressed either as kcal or as percent of baseline, in both strains and across both diets ($p < 0.0005$) (Table 2 and Fig. 1). Energy intake in saline-treated S 5B/Pl rats eating either diet was fairly constant throughout the experiment (see Fig. 1). With *d*-fenfluramine, energy intake of S 5B/Pl rats decreased to 40% of baseline but by day 3 did not differ from that of saline-treated animals. The energy intake of saline-treated Osborne-Mendel rats eating the low-fat diet was also constant throughout the experiment. Saline-treated Osborne-Mendel rats eating the high-fat diet increased their food intake to 145% of baseline on the second day, followed by a gradual return to baseline. In Osborne-Mendel rats, energy intake of

TABLE 1

COMPOSITION OF EXPERIMENTAL DIETS

	Low-Fat Diet		High-Fat Diet	
	g/100 kcal	% of kcal	g/100 kcal	% of kcal
Vitamin-free casein*	6.00	24	6.00	24
Cornstarch†	16.50	66	5.00	20
Corn oil†	0.67	6	0.67	6
Vegetable shortening‡	0.44	4	5.56	50
Mineral mix*	0.88	–	0.88	–
Vitamin mix*	0.22	–	0.22	–
Choline bitartrate§	0.05	–	0.05	–
D,L-Methionine*	0.03	–	0.03	–
Alphacel nonnutritive bulk*	2.50	–	2.50	–

Low-fat diet, 3.62 kcal/g; high-fat diet, 4.72 kcal/g.
*ICN Nutritional Biochemicals (Cleveland, OH).
†Best Foods, CPC International (Englewood Cliffs, NJ).
‡Procter and Gamble (Cincinnati, OH).
§Sigma Chemical Co. (St. Louis, MO).

TABLE 2

FOOD INTAKE, BODY WEIGHT, AND LEE INDEX OF S 5B/Pl AND OSBORNE-MENDEL RATS

	Low-Fat Diet				High-Fat Diet			
			Saline vs. d-Fenfluramine				Saline vs. d-Fenfluramine	
	Saline	*d*-Fenfluramine	*t*	*p*	Saline	*d*-Fenfluramine	*t*	*p*
S 5B/Pl rats								
Number	5	6			4	5		
Initial food intake (kcal/d)	72 ± 3	67 ± 7	–	–	76 ± 9	84 ± 12	–	–
Cumulative intake (kcal)	877 ± 50	752 ± 42	2.02	0.05	951 ± 63	751 ± 78	2.91	0.01
Initial body weight (g)	263 ± 16	262 ± 12	–	–	276 ± 15	261 ± 23	–	–
Final body weight (g)	290 ± 18	275 ± 16	0.77	NS	309 ± 19	279 ± 23	1.38	NS
Lee Index ($g^{1/3}$/cm)	0.295 ± 0.008	0.286 ± 0.004	1.56	NS	0.303 ± 0.006	0.288 ± 0.003	2.14	0.05
Osborne-Mendel rats								
Number	6	7			6	6		
Initial food intake (kcal/d)	85 ± 5	82 ± 5	–	–	94 ± 10	95 ± 5	–	–
Cumulative intake (kcal)	952 ± 23	762 ± 19	3.33	0.01	1066 ± 42	814 ± 28	4.26	0.001
Initial body weight (g)	359 ± 7	364 ± 8	–	–	362 ± 7	364 ± 5	–	–
Final body weight (g)	396 ± 8	380 ± 10	0.89	NS	421 ± 7	385 ± 7	1.93	NS
Lee Index ($g^{1/3}$/cm)	0.295 ± 0.003	0.298 ± 0.003	0.52	NS	0.302 ± 0.003	0.298 ± 0.004	0.67	NS

Data are means ± SE. NS, not statistically significant. Three-way ANOVA: Initial food intake, average of days −2 and −1— Osborne-Mendel rats ate more than S 5B/Pl rats, $F(1, 36) = 10.21$, $p < 0.005$; intake of the high-fat diet was greater than intake of the low-fat diet, $F(1, 36) = 9.67$, $p < 0.005$. Cummulative food intake, days 1–12: Osborne-Mendel rats ate more than S 5B/Pl rats, $F(1, 36) = 4.88$, $p < 0.05$; intake of the high-fat diet was greater than intake of the low-fat diet, $F(1, 36) = 4.13$, $p < 0.05$; fenfluramine-treated rats ate less than saline controls, $F(1, 36) = 42.1$, $p < 0.0005$. Initial body weight: Initially, Osborne-Mendel rats weighed more than S 5B/Pl rats, $F(1, 36) = 138$, $p < 0.0005$. Final body weight: On day 13, Osborne-Mendel rats weighed more than S 5B/Pl rats, $F(1, 36) = 130$, $p < 0.0005$; fenfluramine-treated rats weighed less than saline-treated rats, $F(1, 36) = 7.03$, $p < 0.025$. Lee Index: Lee Index was less in fenfluramine-treated rats than in saline-treated rats, $F(1, 36) = 5.08$, $p < 0.05$. All other main effects and all interactions were not significant. When ANOVA indicated a significant effect of fenfluramine, the comparison of saline vs. fenfluramine within each group was done by *t*-test using the within-cell mean square, corrected for differences in sample sizes, from the appropriate ANOVA table.

both low- and high-fat diets was decreased by *d*-fenfluramine treatment to 30% of baseline and remained below that of control animals to day 7.

Saline-treated S 5B/Pl rats eating either diet showed a steady growth pattern, reaching 110–112% of initial body weight by day 12 (Fig. 1). In S 5B/Pl rats treated with *d*-fenfluramine, body weight decreased to 98% of baseline by day 2, after which the rate of growth was the same as for saline-treated rats. Saline-treated Osborne-Mendel rats eating the low-fat diet had similar growth curves as the S 5B/Pl rats, reaching 110% of initial body weight by day 12. Saline-treated Osborne-Mendel rats eating the high-fat diet, on the other hand, increased body weight to 117% of initial body weight. The differential effect of diet on body weight was eliminated by *d*-fenfluramine treatment, with the rate of growth of both low- and high-fat diet groups being similar to that of saline-treated low-fat fed Osborne-Mendel rats. Lee Index was modestly reduced ($p < 0.05$) by *d*-fenfluramine, with the primary effect seen in S 5B/Pl rats eating the high-fat diet (Table 2).

Interscapular brown adipose tissue (IBAT) weight was greater in S 5B/Pl rats than in Osborne-Mendel rats ($p < 0.0005$) and was reduced in animals treated with fenfluramine ($p < 0.0005$), with this reduction being primarily in S 5B/Pl rats (Table 3). IBAT total protein ($p < 0.0005$) and mitochondrial protein ($p < 0.0005$) were also higher in S 5B/Pl rats than in Osborne-Mendel rats. *d*-Fenfluramine reduced mitochondrial protein ($p < 0.025$), primarily in high-fat fed S 5B/Pl rats, but did not significantly affect total IBAT protein. The specific binding of purine nucleotide (GDP) to mitochondria was greater in all groups of S 5B/Pl rats than in Osborne-Mendel rats ($p < 0.0005$) (Fig. 2). There was a small increase in specific GDP binding with chronic *d*-fenfluramine in both strains eating the low-fat diet (ANOVA, $p < 0.05$); however, comparison of individual means, a fairly conservative estimate of probability, did not show this difference. Perhaps a larger sample size would compensate for the relatively large variation inherent in this measure. In saline-treated S 5B/Pl rats, the high-fat diet increased the specific GDP binding by 50%, whereas there was only a 13% increase in specific GDP binding in Osborne-Mendel rats under the same conditions. In rats fed the low-fat diet, *d*-fenfluramine increased specific GDP binding in both strains. The high-fat diet, on the other hand, blocked the *d*-fenfluramine-induced increase in GDP binding in both strains. Analysis of total GDP binding in IBAT gave virtually the same statistical results (data not shown).

DISCUSSION

The hypothesis upon which this experiment was based was that raising hypothalamic serotonin levels would prevent dietary fat-induced obesity in the Osborne-Mendel rat. This hypothesis was suggested by several observations; first, that serotonin in the brainstem is lower in the Osborne-Mendel rat than in the Sprague-Dawley rat (13); second, that basal release of serotonin in the hypothalamus, as measured by microdialysis, is lower in Osborne-Mendel rats than in S 5B/Pl rats (H. Shimizu, J. S. Fisler, and G. A. Bray, unpublished); third, that *d*-fenfluramine, a drug that releases serotonin and prevents its reuptake, lowers food intake and increases thermo-

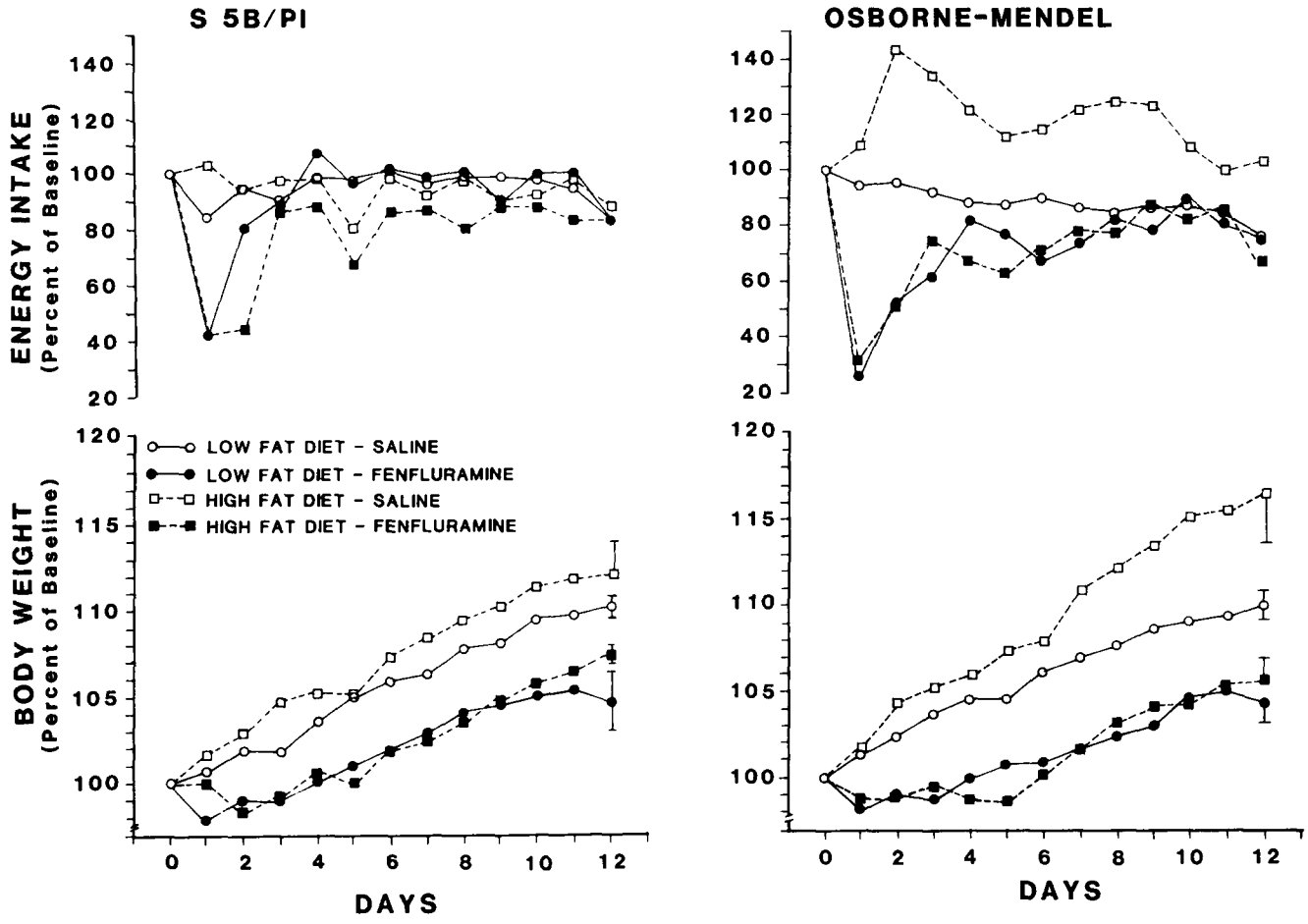

FIG. 1. (A) Energy intake expressed as percent of baseline values of S 5B/Pl rats eating either the low-fat diet or the high-fat diet and receiving either saline or fenfluramine injections. Three-way analysis of variance (ANOVA) with measures repeated over time: effect of time, $F(5, 80) = 6.00$, $p < 0.0005$; the reduction in energy intake occurred only in fenfluramine-treated S 5B/Pl rats, $F(5, 80) = 3.18$, $p < 0.025$. All other effects and interactions, not significant. (B) Body weights expressed as percent of baseline values of S 5B/Pl rats. Three-way ANOVA with measures repeated over time: *d*-Fenfluramine reduced body weight in S 5B/Pl rats eating either the low- or high-fat diet, $F(1, 16) = 71.3$, $p < 0.0005$; effect of time, $F(5, 80) = 99.1$, $p < 0.0005$. (C) Energy intake of Osborne-Mendel rats. Three-way ANOVA with measures repeated over time: Fenfluramine abolished the different pattern of food intake seen in Osborne-Mendel rats eating the low- or high-fat diet, $F(5, 100) = 4.38$, $p < 0.005$. (D) Body weights of Osborne-Mendel rats. Three-way ANOVA with measures repeated over time: *d*-Fenfluramine reduced body weight in Osborne-Mendel rats eating either the low- or high-fat diet, $F(1, 20) = 38.2$, $p < 0.0005$; effect of time, $F(5, 100) = 105$, $p < 0.0005$; saline-treated Osborne-Mendel rats eating the high-fat diet gained more weight over time than those eating the low-fat diet, $F(5, 100) = 5.80$, $p < 0.0005$; drug × time interaction, $F(5, 100) = 4.93$, $p < 0.0005$.

genesis (1,16). In the present study, when rats were treated with *d*-fenfluramine their food intake was acutely depressed, whether or not they were likely to develop obesity when eating a high-fat diet. Chronic treatment with *d*-fenfluramine reduced food intake to a slightly greater extent in Osborne-Mendel rats and the excess weight gain due to the high-fat diet was completely abolished. These data are, thus, consistent with our hypothesis.

A relatively high daily dose of *d*-fenfluramine (10 mg/kg, IP) was chosen for this study because that is the 50% inhibitory dose in ad lib-fed rats during the first 24 h following a single injection (4). However, it is clear that the 10-mg/kg dose affects other catecholamines than just serotonin and its metabolites. As measured by in vivo microdialysis, a single injection of 10 mg/kg *d*-fenfluramine increases serotonin in the ventromedial hypothalamus and lateral hypothalamus (LH) (23). That dose also increases norepinephrine in the ventromedial hypothalamus and dihydroxyphenylacetic acid (DOPAC) in all three brain regions (23). Because the dose of *d*-fenfluramine used can affect several of the catecholamines, and because hypothalamic serotonin was not measured in the present study, we cannot conclude that the effects of *d*-fenfluramine observed are specifically attributable to increased neuronal release of serotonin. To test whether the effect observed in this study was due to serotonin release, the experiment would need to be repeated replacing the *d*-fenfluramine injections with microinfusion of serotonin into the hypothalamus.

The prevention of dietary obesity could result from a decrease in energy intake, an increase in energy expenditure, or

TABLE 3

IBAT WEIGHT AND CONTENT OF TOTAL AND MITOCHONDRIAL PROTEIN IN S 5B/Pl AND OSBORNE-MENDEL RATS

	Low-Fat Diet				High-Fat Diet			
			Saline vs. d-Fenfluramine				Saline vs. d-Fenfluramine	
	Saline	*d*-Fenfluramine	*t*	*p*	Saline	*d*-Fenfluramine	*t*	*p*
S 5B/Pl rats								
Number	5	6			4	5		
IBAT weight (mg)	581 ± 76	400 ± 50	2.69	0.05	696 ± 71	459 ± 68	3.18	0.01
Total protein (mg)	7.1 ± 1.1	6.9 ± 0.7	–	–	9.7 ± 1.8	7.2 ± 0.9	–	–
Mitochondrial protein (mg)	4.6 ± 0.4	4.5 ± 0.4	0.22	NS	5.5 ± 0.4	4.1 ± 0.3	2.82	0.01
Osborne-Mendel rats								
Number	6	7			6	6		
IBAT weight (mg)	315 ± 23	266 ± 16	0.79	NS	325 ± 26	271 ± 41	0.84	NS
Total protein (mg)	4.8 ± 0.3	4.5 ± 0.3	–	–	4.3 ± 0.3	4.0 ± 0.5	–	–
Mitochondrial protein (mg)	2.2 ± 0.1	2.2 ± 0.3	0	NS	2.6 ± 0.2	2.2 ± 0.3	0.94	NS

Data are means ± SE. NS, not statistically significant. Three-way ANOVA: IBAT weight— S 5B/Pl rats had heavier IBAT than Osborne-Mendel rats, $F(1, 36) = 55.7, p < 0.0005$; fenfluramine reduced IBAT weight, $F(1, 36) = 16.4, p < 0.0005$; the effect of fenfluramine on IBAT weight occurred primarily in S 5B/Pl rats, $F(1, 36) = 6.13, p < 0.025$; the difference between strains was greater in saline-treated animals ($p < 0.0005$) than in fenfluramine-treated animals ($p < 0.005$). Total protein: IBAT total protein was higher in S 5B/Pl rats than in Osborne-Mendel rats, $F(1, 36) = 39.2, p < 0.0005$. Mitochondrial protein: Mitochondrial protein was higher in S 5B/Pl rats than in Osborne-Mendel rats, $F(1, 36) = 126, p < 0.0005$; fenfluramine reduced mitochondrial protein, $F(1, 36) = 5.48, p < 0.025$. All other main effects and interactions, not significant. When ANOVA indicated a significant effect of fenfluramine, the comparison of saline vs. fenfluramine within each group was done by *t*-test using the within-cell mean square, corrected for differences in sample sizes, from the appropriate ANOVA table.

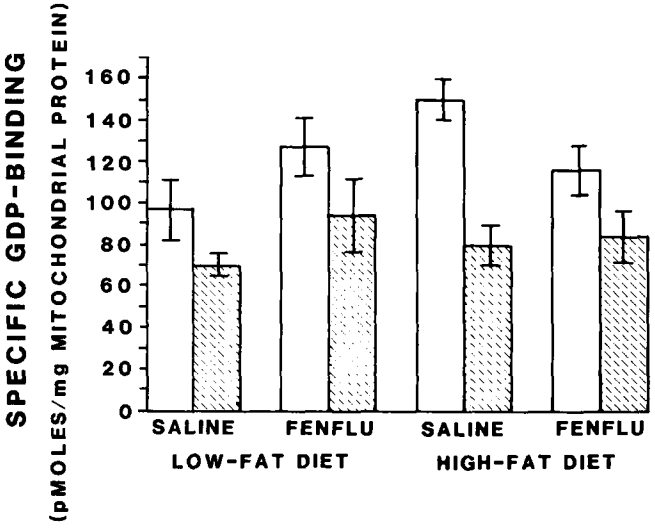

FIG. 2. Effect of dietary fat and fenfluramine on the binding of purine nucleotide to mitochondria of interscapular brown adipose tissue in S 5B/Pl (open bars) and Osborne-Mendel (cross-hatched bars) rats. Three-way analysis of variance (ANOVA): Across all groups, S 5B/Pl rats had higher specific binding than did Osborne-Mendel rats, $F(1, 36) = 18.3, p < 0.0005$; diet × fenfluramine interaction, $F(1, 36) = 4.72, p < 0.05$. GDP binding increased with the high-fat diet in saline-treated ($p < 0.05$), but not fenfluramine-treated, animals.

some combination of the two. It is clear that *d*-fenfluramine reduced energy intake in both strains of rats. This reduction occurred after a single dose, confirming a large body of scientific data (18). Chronic treatment also reduced food intake but the effects differed between strains and was modified by diet. In the leaner S 5B/Pl rats, the reduction in food intake was of relatively short duration, with subsequent recovery to control levels. This response was unaffected by the fat content of the diet, consistent with previous reports (19). In the dietary fat-sensitive Osborne-Mendel rats, the depression in food intake during *d*-fenfluramine treatment lasted longer (8 vs. 3 days, Fig. 1) and was proportionally greater in rats eating the high-fat diet. Even so, the reduction in energy intake was probably not sufficient to account for the lower maintained body weight in both strains and the prevention of dietary fat-induced obesity in Osborne-Mendel rats.

An increase in the activity of the sympathetic nervous system and an associated increase in thermogenesis was also anticipated with *d*-fenfluramine treatment. Fenfluramine increases GDP binding (16) and the firing rate of sympathetic nerves to IBAT (1). Chronic treatment with fenfluramine produces a lower body weight even after food intake returns to normal (15,16), suggesting that energy expenditure is elevated by fenfluramine. In animals eating the low-fat diet, *d*-fenfluramine increased GDP binding to mitochondria from IBAT, consistent with increased sympathetic nervous system activity and thermogenesis. Data on the effect of a high-fat diet on sympathetic nervous system activity are contradictory. Acutely, a high-fat diet increases sympathetic nervous system outflow to IBAT whether measured by norepinephrine turnover (22,27) or by electrical firing rate (20). Chronic feeding of a high-fat diet, however, may result in a loss of sympathetic activity (6,8,20). In the present experiment, feeding the high-

fat diet for 14 days increased GDP binding to mitochondria from interscapular brown fat, with the effect being greater in rats resistant to dietary fat-induced obesity. *d*-Fenfluramine, however, did not further increase GDP binding in rats eating the high-fat diet.

The differential response between diets to *d*-fenfluramine in Osborne-Mendel rats could be explained if *d*-fenfluramine selectively inhibited fat intake in that strain. However, *d*-fenfluramine is reported to suppress carbohydrate intake early in the dark period and has no effect on fat intake at any time (24). Thus, the effect of *d*-fenfluramine on macronutrient selection should, if anything, cause greater reduction in food intake in rats eating the low-fat, high-carbohydrate diet. Nutrient selection in response to *d*-fenfluramine has not been measured in S 5B/Pl or Osborne-Mendel rats.

The interaction of dietary fat and *d*-fenfluramine in Osborne-Mendel rats could also be due to a difference in biological activity of *d*-fenfluramine as a function of diet. Obese Zucker rats have a slightly slower clearance of high-dose (6.25 mg/kg) *d*-fenfluramine from the plasma than do lean Zucker controls (9). In the present study, however, body weight did not differ between Osborne-Mendel rats eating the low- and high-fat diets, so differences in the anorexic effect of *d*-fenfluramine could not be due to differences in obesity between the two groups. The plasma half-life of *d*-fenfluramine in rats is 2 h and that of its biologically active metabolite, norfenfluramine, is 12 h (18). Thus, biological activity following *d*-fenfluramine should be present for greater than 24 h. To be certain that the differential response to diet in Osborne-Mendel rats was not due to giving injections 12 h prior to the onset of the primary feeding period, a second experiment, giving *d*-fenfluramine just prior to the dark period, was conducted. The results of that experiment (data not shown) were essentially the same as the present study.

An attractive mechanism to explain the present observations is that a feeding reward system responds to the high-fat diet in Osborne-Mendel rats, but not in S 5B/Pl rats, and that *d*-fenfluramine inhibits some aspect of that system. Hoebel et al. (11) described a feeding reward system that projects through the midbrain ventral tegmental area and the taste center in the nucleus tractus solitarius, with the LH being an important juncture. Electrical stimulation of the LH can connect to this system, which is excited by tastes and modulated by local metabolic factors and insulin, as well as by monoamine systems. If an animal is below weight, it will overeat and over-self-stimulate until the "appropriate" weight is achieved. Conversely, if an animal is overweight it will undereat and under-self-stimulate until body weight decreases to "normal" (11). Although it is clear that an intact LH region is not necessary for the anorexic effect of fenfluramine (2), there is some evidence that *d*-fenfluramine could be acting through serotonin release to inhibit some aspect of the reward system that involves the LH (12). *d*-Fenfluramine (10 mg/kg) increases serotonin release in the LH (14,23) and direct injection of serotonin into the LH suppresses food intake (25). *d*-Fenfluramine, in doses of 1.5–4.5 mg/kg, selectively decreases self-stimulation, reflecting inhibition of the feeding-reward system (11). Thus, we speculate that *d*-fenfluramine blocks a feeding reward system stimulated by the high-fat diet in Osborne-Mendel rats.

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