Effect of Dexfenfluramine on Fat Mass Distribution in a High-Fat Rat Model

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It has been shown that in contrast to peripheral fat, visceral fat is an important risk factor for cardiovascular diseases and diabetes. In this study, we investigated whether dexfenfluramine (dF), a compound known to decrease body fat, affects fat mass differentially in various regions of the body. We used a moderately obese rat model fed a high-fat diet (40% fat). After 35 days on the diet, rats were divided into three groups: a dF-treated group ([D] 2.5 mg/kg intraperitoneally twice daily), a pair-fed group (Cp), and a control group (C) fed ad libitum. C and Cp rats were injected with saline. After 4 weeks of treatment, body fat, fat cell morphology, and metabolism were determined in subcutaneous (inguinal [ING]) and visceral (retroperitoneal [RET] and mesenteric [MES]) fat tissues. Food intake in D and Cp rats was similar, and was lower than in the C group. In comparison to Cp and C rats, D rats had lower body weight and body fat, smaller ING and RET fat pad weights, and smaller fat cell size in all depots. No significant differences were observed in fat mobilization between groups; however, fat accumulation tended to be lower in D rats. These data suggest that dF has an effect on adipose tissue independent of its effect on food intake. However, this effect seems to occur without regional specificity.

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THE IMPORTANCE of regional fat distribution as a risk factor for a number of prevalent diseases has been recently well documented.[1-8] It was shown that increased central fat mass, particularly in visceral regions, was positively associated with hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and insulin resistance, which are the main risk factors for cardiovascular diseases and diabetes. Furthermore, in comparison to subcutaneous depots, visceral fat depots, particularly those that drain their free fatty acids directly into the portal vein (mesenteric [MES] and omental fat), have a high metabolic turnover, which suggests that fat is easily accumulated and released from these tissues.[9] It has been hypothesized that the high catecholamine-stimulated lipolysis or fat mobilization observed in these depots might be one contributor to the pathophysiology of abdominal obesity.[10]

Several studies in rats and humans have shown that long-term administration of dexfenfluramine (dF) decreases food intake, body weight, and body fat.[11-14] At the adipose tissue level, a decrease in body fat can be explained by at least two major mechanisms: dF could stimulate basal or catecholamine-stimulated lipolysis (fat mobilization) or inhibit lipoprotein lipase (LPL) activity (fat accumulation). A stimulation of lipolysis both in vitro and in vivo has been suggested in previous studies.[11,15-18] In addition, Chaouloff et al[19] have suggested that in conscious rats, short-term administration of dF promoted norepinephrine release from sympathetic nerve terminals. This in turn might stimulate adipose tissue lipolysis. A decrease in fat accumulation after dF administration was shown in female rats by Carlton and Rowland.[20]

The available literature concerning a potential regional specificity of action of dF on fat mobilization or fat accumulation, particularly in fat regions associated with risk factors for diseases such as visceral fat, is scarce. However, regional differences observed, in response to catecholamine-stimulated lipolysis,[21-22] in the catecholamine content[23] of various adipose tissues, and in fat accumulation,[24] particularly between visceral and subcutaneous fat depots, suggest that dF might preferentially reduce visceral fat through catecholamine stimulation of lipolysis or a decrease in fat accumulation.

Rats fed a high-fat diet were used in this study to investigate whether dF affects adipose tissue distribution and metabolism differentially in various regions of the body (visceral *v* subcutaneous).

MATERIALS AND METHODS

Animals

Fifty-one male Sprague-Dawley rats (Charles River Laboratories, Cambridge, MA) weighing 210 to 230 g were housed in individual wire-mesh cages in a room maintained at 20° ± 2°C with a 12-hour light cycle (7 AM to 7 PM). Rats were fed ad libitum a high-fat diet (40% fat, 38% corn starch, 20% protein, plus vitamins, minerals, and fiber; Research Diets, New Brunswick, NJ). Food intake was recorded daily and body weight biweekly.

Experimental Protocol

After 35 days on the diet, rats were divided into three groups: control (C), dF-treated (D), and a group pair-fed to the treated rats (Cp). D rats were injected intraperitoneally twice daily with dF 2.5 mg · kg^{-1} in .16 mol/L NaCl (Laboratories Servier, Neuilly, France), whereas C and Cp rats were injected with the same volume of .16 mmol/L NaCl only. The last injection was given in the evening before rats were killed. C and D rats were fed ad libitum with the high-fat diet, and Cp rats were given the mean amount of food consumed the previous day by D rats.

After 28 days of treatment, all rats were killed. Blood was collected from the trunk for further determination of glucose, insulin, and triglyceride levels. Adipose tissues were carefully removed from one subcutaneous (inguinal [ING]) and two visceral (retroperitoneal [RET] and MES) regions. Tissues were weighed and sampled for determination of LPL activity, fat cell size, and lipolysis.

Methods

LPL activity was determined after elution with heparin, using a stable radioactive triolein emulsion according to the method

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reported by Nilsson-Ehle and Schotz.[25] Fat cell size and lipolysis were measured on adipocytes isolated by collagenase. Lipolysis was measured as glycerol release in the basal condition and in the presence of various concentrations of norepinephrine (10^{-7} to 10^{-4} mol/L) as previously described.[26] Fat cell size was measured using a microscope[27] and was expressed as fat cell weight (micrograms of lipid per cell). Both enzyme activities were expressed per number of cells or per cell surface area to take into account differences in fat cell size. Glucose and triglycerides were determined enzymatically (Sigma Diagnostics Kit, Sigma, St Louis, MO). Plasma insulin level was measured by radioimmunoassay using a double-antibody method.[28]

Statistical Analysis

One-way ANOVA was used to analyze the data (Super Anova program for MacIntosh, Abacus Concepts, Berkeley, CA). Comparisons were considered significant at P less than .05.

RESULTS

As shown in Fig 1, treatment with dF decreased food intake dramatically for 2 days in D and Cp rats. However, this difference tended to diminish with time and was not significantly different between C and D groups during the last 10 days of treatment. Body weights also decreased for 2 to 3 days in D and Cp animals, and thereafter weight gain in both groups paralleled that of C rats (not shown). When body weights were expressed as percentage gain from the first day of treatment, not only was a significant difference observed between C and D rats, but D rats also had lower body weights than Cp rats (Fig 2). Similar differences ($P < .05$) between D and Cp rats were also observed when the sum of the three fat pads studied (ING + RET + MES) was determined (not shown).

Figure 3 shows the effect of dF treatment on fat pad weights, fat cell weights, and LPL activities for subcutaneous (ING) and visceral (RET and MES) fat pads. In comparison to Cp and C rats, D rats had smaller fat pads in both ING and RET ($P < .05$) but not in the MES region. Similarly, fat cell weights were significantly decreased in all three tissues in D rats in comparison to Cp and C rats ($P < .05$). LPL activity showed the same pattern, with lower ING and MES activities in D rats in comparison to Cp and C rats; however, these differences did not reach full significance ($P < .08$). Similar data were observed when LPL activities were expressed per cell surface area (not shown). In contrast, whether results were expressed per cell (Fig 4) or per cell surface area (not shown), no significant differences were observed in basal or norepinephrine-stimulated lipolysis between D, Cp, and C rats in any of the tissues studied. No differences in sensitivity, as measured by ED_{50} values, were observed between groups (not shown).

Treatment with dF did not affect glucose, insulin, and triglyceride concentrations (Table 1).

DISCUSSION

This study has shown that, as observed previously in humans and in other rat models,[11-14] long-term administration of dF decreased food intake in this animal model of rats rendered moderately obese with a high-fat (40%) diet. This was accompanied by a decrease in body weight in both D and Cp rats. Furthermore, this decrease was significantly

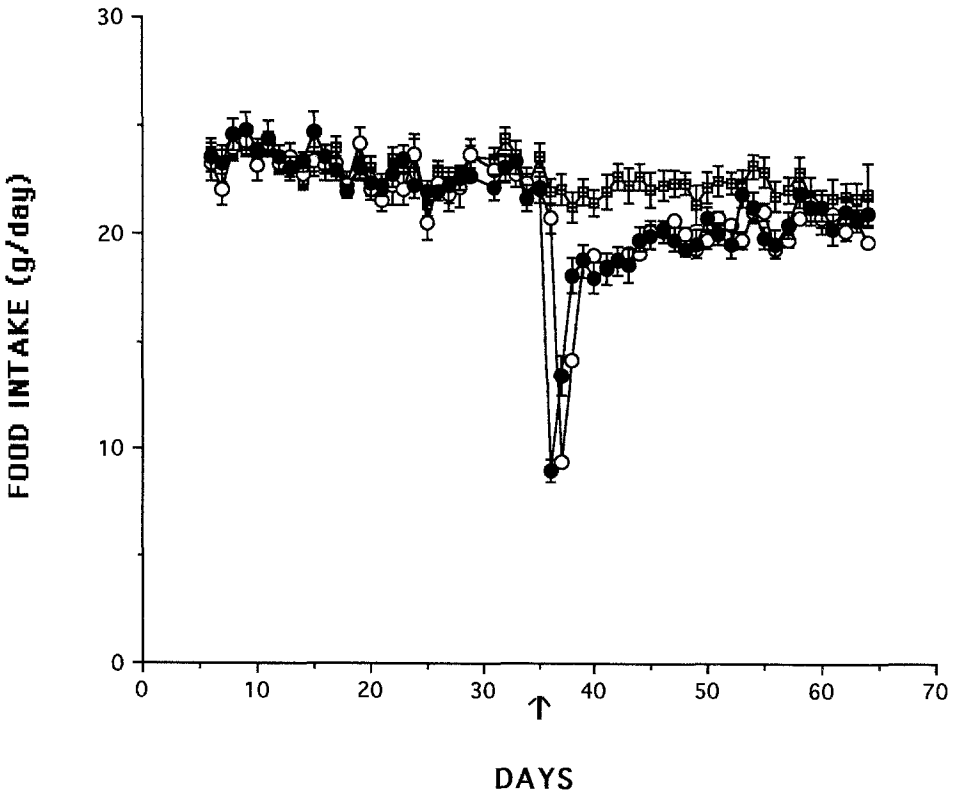

Fig 1. Food intake before and after (↑) treatment with dF (2.5 mg · kg^{-1} twice daily) in C (⊞), Cp (○), and D (●) rats. Mean ± SE.

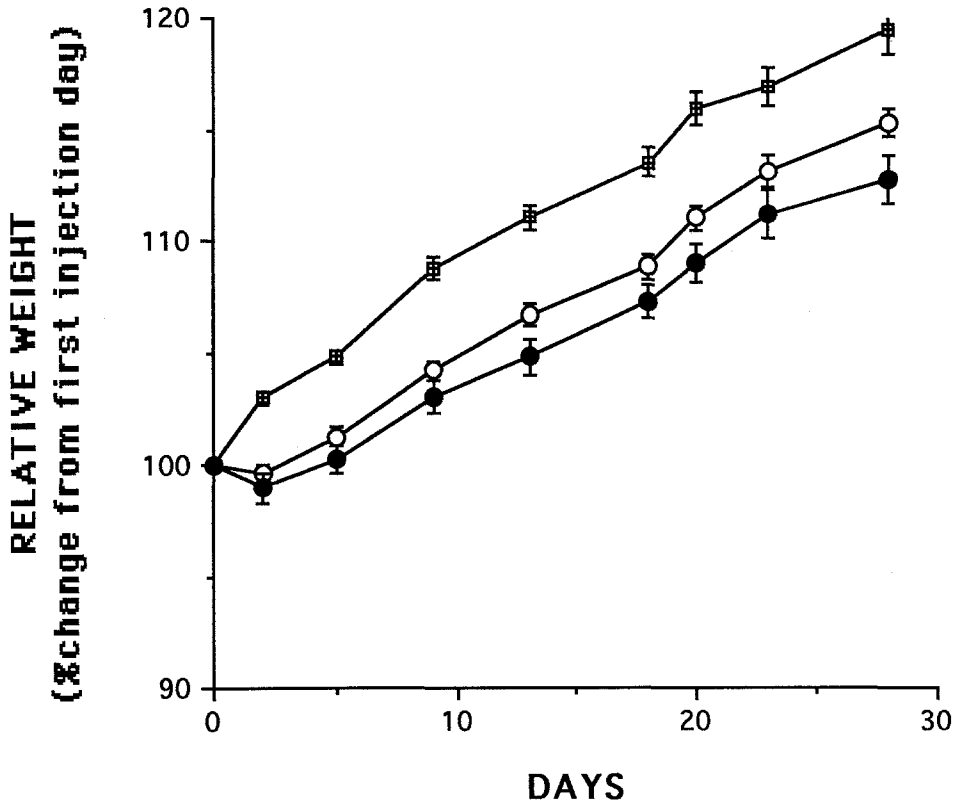

Fig 2. Relative body weight during 28 days of dF treatment, expressed as % increase from first day of treatment, in C (⊞), Cp (○), and D (●) rats. Mean ± SE.

more pronounced in D rats in comparison to Cp rats fed the same amount of the high-fat diet. Comparisons between D and Cp rats also showed that D rats had smaller fat pads and fat cells in both subcutaneous and visceral tissues. This suggests that the effect of dF on adipose tissue morphology is not only the result of changes in food intake. Studies performed in other rat models such as genetically obese female rats[13] or female rats fed a high-carbohydrate diet[20] have also noted an effect of dF on adipose tissue independent of its anorectic action.

To understand the mechanisms by which fat cell weights were decreased in D rats, both fat accumulation and fat mobilization were determined. It was found that in comparison to Cp rats, LPL activity in D rats tended to decrease in ING and MES fat tissues, whereas no differences in basal or norepinephrine-stimulated lipolysis were observed. It therefore seems that the decreased fat cell weight observed after dF administration might be due to a decrease in fat accumulation rather than to a stimulation of fat mobilization. Stimulation of lipolysis with dF had in fact only been suggested[15,16,29] or was demonstrated in an in vitro study.[18] In the present study, the maximum responsiveness and the sensitivity to norepinephrine of adipocytes obtained from the three groups of rats were not significantly different between groups and within tissues. However, as shown previously,[21,22] visceral fat tissues indeed had a higher lipolytic activity than subcutaneous fat in all three groups. It is also important to take into account that, as suggested by Scheurink et al,[30] dF might have different effects on lipolysis depending on dose and length of treatment. This might also explain the variability between results obtained from these different studies.

Recently, it was suggested[31] that the decrease in fat mass observed after dF treatment might be due to a decrease in fat accumulation. Furthermore, Carlton and Rowland[20] indeed measured LPL activity after long-term dF treatment and found a decrease in LPL activity in RET fat, whereas no changes were observed in the ING region. The trend for a decrease in both subcutaneous (ING) and visceral (MES) LPL activity observed in our rat model strengthens the hypothesis that dF decreases fat accumulation, but it is not known if dF can affect adipose tissue in a direct manner. However, it is known that fat accumulation is regulated by a number of factors such as genetic[32] and neuroendocrine, particularly by steroid hormones.[9] Further investigations on the potential role of and/or interactions between dF and both sex steroid and glucocorticoid hormones might therefore be helpful for understanding the mechanisms by which dF reduces body fat mass.

In this study, dF seemed to affect adipose tissue mass similarly in visceral and subcutaneous tissues. In contrast, Brindley et al[31] reported a decrease in fat pad weights and Carlton and Rowland[20] reported a decrease in LPL activity in visceral fat, but not in subcutaneous fat. Differences in gender might account for this divergence. It should also be kept in mind that this effect is small, is additional to the effect produced by the decrease in food intake, and might therefore be difficult to demonstrate. Compositions of the diets were different among the studies (high-fat *v* high-carbohydrate) and might have affected the results differently. However, more importantly, both in the study reported by Carlton and Rowland[20] and in ours, it was shown that dF decreases fat accumulation in visceral (RET and MES) adipose tissues. This effect is particularly interesting

in light of recent findings that visceral fat is strongly correlated with increased risk factors for metabolic diseases.[5-8] Furthermore, it is suggested that before any conclusions can be drawn on the specificity of action of dF on adipose tissue, it would be important to perform further studies that take into account the sex and age of the animals, composition of the diet, and doses and modes of administration of the drug.

Gender differences might also explain why, in contrast to a previous report,[33] the high-fat diet–induced hyperinsu-

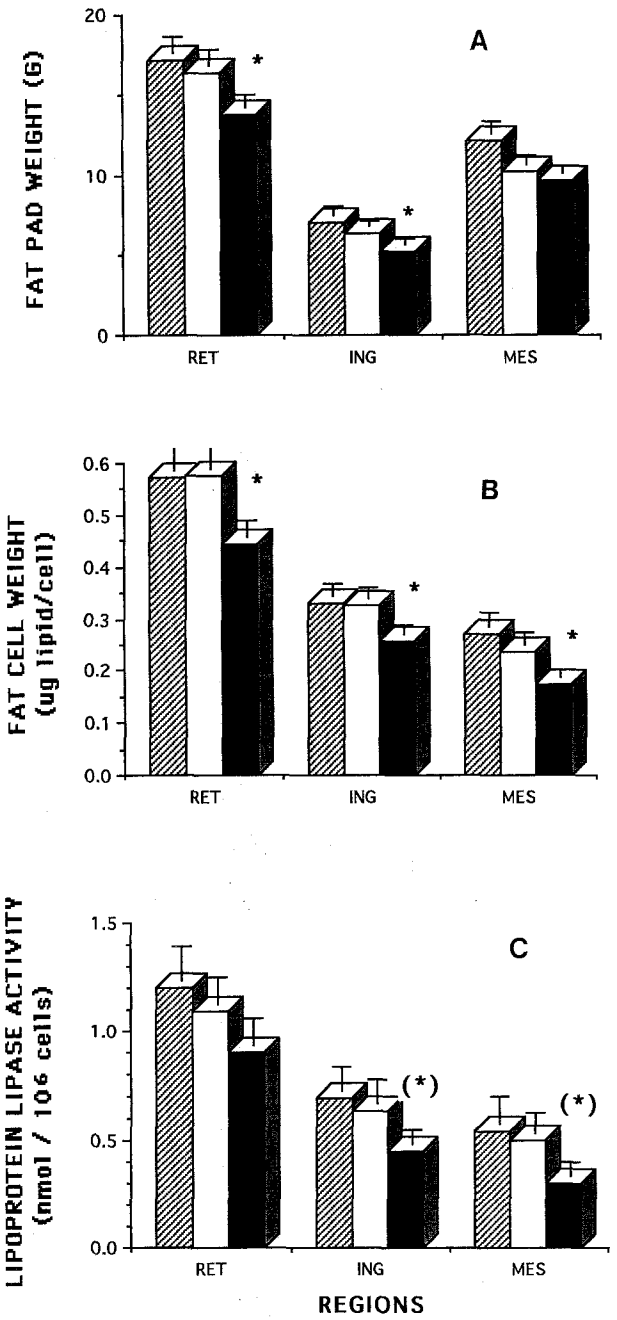

Fig 3. Fat pad weight (A), fat cell weight (B), and LPL activity (C) in RET, ING, and MES fat tissues from C (▨), Cp (□), and D (■) rats. Mean ± SE. (*).05 < *P* < .1; **P* < .05. Comparisons are between groups.

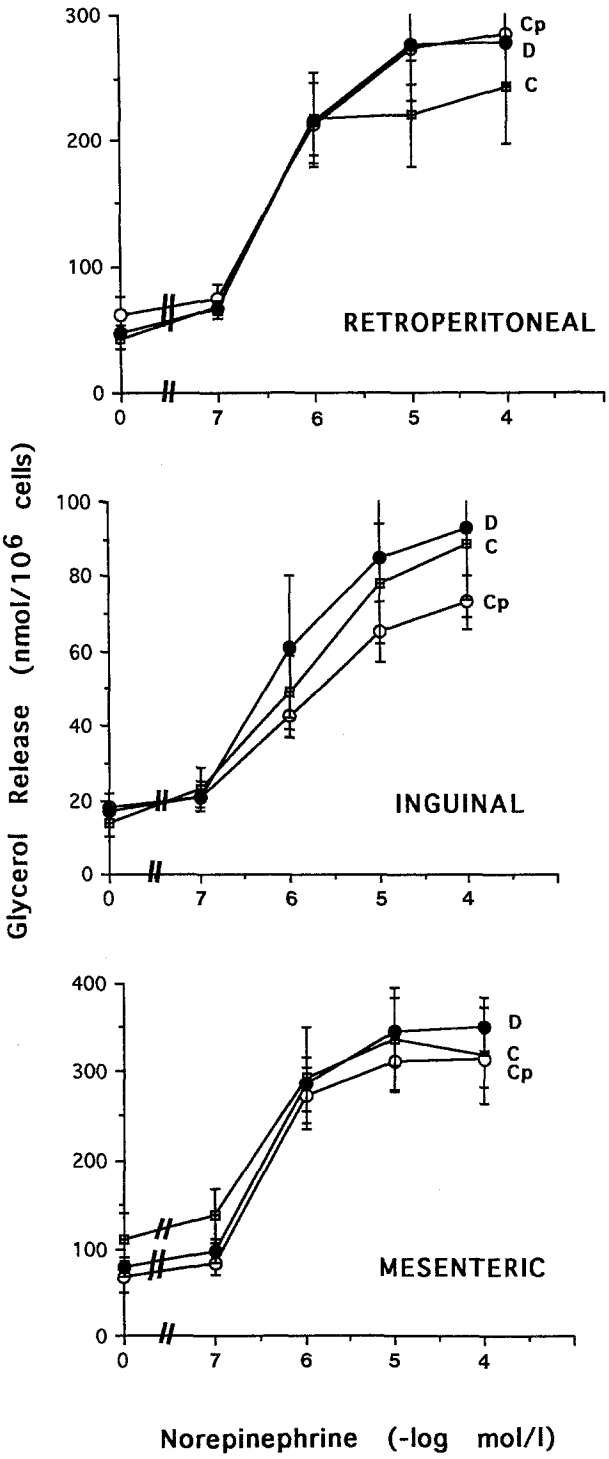

Fig 4. Basal and norepinephrine-stimulated glycerol release from adipocytes obtained from RET, ING, and MES fat in C, Cp, and D rats.

Table 1. Metabolic Variables of C, Cp, and D Rats

	C	Cp	D
Glucose (mg/dL)	116 ± 5	126 ± 6	132 ± 5
Insulin (μU/mL)	144 ± 11	148 ± 16	143 ± 12
Triglycerides (mg/dL)	154 ± 16	179 ± 25	173 ± 18

NOTE. Results are the means ± SE.

linemia found in all three groups was not decreased by dF treatment in this study of young male rats. Brindley et al[31] reported recently that in the obese and hyperinsulinemic JCR:LA-corpulent rat, dF decreased insulin concentrations in females but not in males.

In conclusion, this study has shown that in a moderately obese male rat model fed a high-fat diet, long-term administration of dF decreases adipose tissue weights, fat cell weights, and fat accumulation. This effect on adipose tissue mass seems to occur independently of the action of the drug on food intake and appears to affect fat mass without regional specificity.

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