Sustained decreases in weight and serum insulin, glucose, triacylglycerol and cholesterol in JCR:LA-corpulent rats treated with D-fenfluramine

¹†David N. Brindley, †Paul Hales, †Abdulbasit I.I. Al-Sieni & *James C. Russell

†Department of Biochemistry, *Department of Surgery and †Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, T6G 2S2, Canada

1 The effects of D-fenfluramine were studied in the JCR:LA-corpulent rat that is grossly obese, hyperphagic, hyperlipidaemic, hyperinsulinaemic and atherosclerosis-prone.

2 Daily doses of 1, 2.5 and 5 mg kg⁻¹ of D-fenfluramine produced sustained decreases in body weight and food intake over a period of 30 days in 6 month old female rats fed *ad libitum*. This was accompanied by decreases in the circulating concentrations of glucose, triacylglycerol, free cholesterol and insulin.

3 Food restriction imposed by meal feeding also decreased circulating glucose, triacylglycerols, cholesterol and insulin and diminished the effect of D-fenfluramine on these parameters in male and female rats.

4 Addition of D-fenfluramine to drinking water to give a dose of about 0.25 mg kg⁻¹ daily produced a sustained decrease in body weight and food intake of male and female rats over a nine week period.

5 The results show that the JCR:LA-corpulent rat is very sensitive to the pharmacological effects of D-fenfluramine. These rats should provide an appropriate animal model for determining the mechanisms of action of this anti-obesity agent and whether apparently beneficial changes in metabolism translate into long-term protection against premature atherosclerosis.

Keywords: Adipose tissue; anorectics; atherosclerosis; diabetes; glucocorticoids; hypercholesterolaemia; hypertriglyceridaemia; insulin; lipogenesis; obesity

Introduction

The JCR:LA-corpulent rat, if homozygous for the cp gene (cp/cp), is grossly obese, hyperphagic, insulin-insensitive and hyperlipidaemic (Russell *et al.*, 1987; Dolphin *et al.*, 1987; 1990). The hyperlipidaemia is mainly caused by high concentrations of very low density lipoprotein (VLDL) caused by the oversecretion of this lipoprotein which is more pronounced in the females (Russell *et al.*, 1989a; Vance & Russell, 1990). The corpulent male rats spontaneously develop both atherosclerotic and myocardial lesions. By contrast the female cp/cp rats do not develop cardiovascular lesions even though they are more hypertriglycerdemic (Russell & Amy, 1986a,b; Amy *et al.*, 1988; Russell *et al.*, 1990a; Dolphin *et al.*, 1990). These characteristics make the JCR:LA-cp rat an ideal experimental model to investigate the relationship between sex, obesity, diabetes, hyperlipidaemias and the progression to cardiovascular disease.

In the present work, the corpulent rats were treated chronically with D-fenfluramine which is used clinically to manage obesity. This compound has also been reported to improve insulin sensitivity and to have hypoglycaemic and hypolipidaemic effects (Dannenberg, 1983; Brindley, 1983; Arnaud & Nathan, 1990). The main purpose of the work was therefore to determine whether D-fenfluramine would correct the metabolic derangements of the JCR:LA-corpulent rat. These animals were treated in three different ways and also with different concentrations of D-fenfluramine to establish dose-response relationships. This was determined in terms of body weight changes, adipose tissue mass, food intake, the concentrations of circulating hormones and metabolites, and the capacity of the adipose tissue to synthesize lipids. The results demonstrate that relatively low concentrations of D-fenfluramine were effective in partially reversing the metabolic abnormalities present in the JCR:LA-corpulent rat. A preliminary

nary abstract of some of these results has been presented (Brindley *et al.*, 1989).

Methods

Male and female JCR:LA rats that were cp/cp were raised in the established colony at the University of Alberta as previously described (Russell & Amy, 1986a,b). Rats were killed by guillotine and blood was collected from the necks and the serum obtained after centrifugation. The entire fat depots from the subcutaneous, inguinal, mesenteric and perirenal regions were dissected and weighed.

Series 1

In the first series of experiments, 5–6 month old rats were transferred to a room that was lit from 08 h 00 min to 20 h 00 min and which was maintained at 22°C. Rats were housed in groups of two or three in grid-bottomed cages. They were fed *ad libitum*, on Wayne Lab Blox (Continental Grain Co., Chicago, IL, U.S.A.) which had an energy content of 1240 kJ g⁻¹. The D-fenfluramine was injected i.p. in 0.16 M NaCl at 1, 2.5 or 5 mg kg⁻¹ between 08 h 00 min and 10 h 00 min each day. Control rats were injected with the equivalent volume of 0.16 M NaCl. The weights of the rats and the food intake were recorded daily.

Series 2

In the second series of experiments, which were concerned with pair-feeding, 6 month old rats were put on a reversed light cycle so that the room was lit from 22 h 00 min to 10 h 00 min. There were two rats per grid-bottomed cage. They were then allowed 5 days to become accustomed to this change before restricting the period of feeding to 6 h from 10 h

¹ Author for correspondence at: Lipid & Lipoprotein Research Group, 328 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, T6G 2S2, Canada.

Figure 1 Effect of different doses of D-fenfluramine on the body weights and food intakes of female JCR:LA-corpulent rats. Female corpulent rats were fed *ad libitum* and injected daily for 30 days with 0.16 M NaCl, alone (controls, ■), or with 0.16 M NaCl containing a dose of 1 mg kg⁻¹ (□), 2.5 mg kg⁻¹ (●), or 5 mg kg⁻¹ (○) of D-fenfluramine (Series 1). The initial body weights of these rats were 464 ± 17 g (9), 462 ± 30 g (9), 456 ± 31 g (8), and 474 ± 18 g (9) respectively. The mean weight change relative to the weight of each rat at the beginning of the treatment is given in (a). The mean daily food consumption for three cages of rats is given in (b). The s.d. values have been omitted from the figure for the sake of clarity.

00 min to 16 h 00 min. After a further 18 days the rats were divided into three groups: control, D-fenfluramine-treated and a group pair fed to the treated group. The treated rats were injected i.p. with D-fenfluramine 2.5 mg kg⁻¹, whereas other rats were injected with the equivalent volume of 0.16 M NaCl. The pair-fed animals were fed the equivalent weight of food consumed the day before by the paired rat which most closely matched their weight on day 18. An additional quantity of food equivalent to that wasted by the pair-fed rats on the previous day was added to the food allowance. The treatment of six rats from the D-fenfluramine group was discontinued at day 33 and these rats were then allowed free access to food during the 6 h period. All rats were killed on day 47, 2 h after the last injection. Access to food was not allowed during this

2 h period. The reversal of the light cycle facilitated the injection of the D-fenfluramine just before the feeding period.

Series 3

In the third series of experiments male and female corpulent rats were given D-fenfluramine in drinking water at 6 weeks of age. The concentration was adjusted so that the rats consumed 0.25 mg kg⁻¹ body weight and this had no effect on water consumption. Treatment continued up to 15 weeks of age; the rats were caged individually on wood chip bedding in polycarbonate cages. Control rats were provided with tap water and food was available *ad libitum*.

Analytical methods

Most of the methods and the suppliers of reagents have been described previously (Brindley *et al.*, 1988; Al-Sieni *et al.*, 1989). The rates of lipid synthesis were measured in samples of subcutaneous, or inguinal adipose tissue by determining the incorporation of [¹⁴C]-glucose into the fatty acid and glycerol moieties of triacylglycerol (Al Sieni *et al.*, 1989). Phospholipids, triacylglycerol and fatty acids in blood serum were determined enzymically with kits purchased from Wako Chemicals Inc. Richmond, VA, U.S.A. The assays followed the manufacturer's instructions, but they were miniaturized and absorbancies were measured with a microtitration plate reader. Triacylglycerol concentrations were corrected by subtracting the concentration of free glycerol from the total glycerol concentration determined with the Wako kit. Glycerol concentrations were determined on 10 µl of serum in a reaction mixture which had a final volume of 250 µl. This contained: triethanolamine 200 mM buffered to pH 7.5 with HCl, diaphorase 0.18 units ml⁻¹; glycerol 3-phosphate dehydrogenase 1.69 units ml⁻¹; 1-(4,5-dimethylthiazole-2-yl)-3,5-diphenyltetrazolium bromide 0.4 mg ml⁻¹, ATP 1.23 mM, NAD 1.6 mM, MgCl₂ 12.4 mM and Triton X-100 2 µl ml⁻¹. The reaction was started with glycerol kinase 0.63 units ml⁻¹. The NADH-dependent formation of formazan was determined at the completion of the coupled reaction with a microtitration plate reader at 600 nm. Results were calculated by use of a standard curve with glycerol concentrations from 0.1–1.0 mM prepared in dialysed rat serum.

Statistical evaluation

Results are expressed as means ± s.d. Statistical differences between the groups were established by ANOVA and differences between groups were determined with an unpaired *t* test, *P* < 0.05 in a two-tailed test being considered significant.

Table 1 Effects of D-fenfluramine on adipose tissue mass

Adipose site	Control (9)	Dose of D-fenfluramine		
		1 mg kg ⁻¹ (9)	2.5 mg kg ⁻¹ (8)	5 mg kg ⁻¹ (9)
Subcutaneous (g)	80.3 ± 11.6	67.8 ± 12.1 <i>P</i> < 0.025	58.6 ± 7.1 <i>P</i> < 0.0005	60.6 ± 8.4 <i>P</i> < 0.005
Inguinal (g)	14.3 ± 1.3	13.5 ± 2.1	13.9 ± 1.9	12.2 ± 1.3 <i>P</i> < 0.005
Mesenteric (g)	13.3 ± 1.5	11.4 ± 1.6 <i>P</i> < 0.025	11.4 ± 1.6 <i>P</i> < 0.025	10.4 ± 2.1 <i>P</i> < 0.005
Peri-renal (g)	25.5 ± 3.9	20.4 ± 3.9 <i>P</i> < 0.01	20.4 ± 2.3 <i>P</i> < 0.005	19.8 ± 3.5 <i>P</i> < 0.005

JCR:LA-corpulent female rats (Series 1) were fed *ad libitum* and treated daily for 30 days with the dose of D-fenfluramine indicated in Figure 1. Adipose tissue from the various sites was then obtained by dissection. The weights are expressed as means ± s.d. for the number of rats indicated in parentheses. The *P* values indicate the significance of the difference between the treated group and the untreated control rats.

Table 2 Effects of D-fenfluramine treatment of JCR:LA-corpulent female rats on the rate of fatty acid synthesis from glucose by subcutaneous adipose tissue

Insulin (ng ml ⁻¹)	Control n = 9	Glucose (mmol incorporated per depot) Dose of D-fenfluramine		
		1 mg kg ⁻¹ n = 9	2.5 mg kg ⁻¹ n = 8	5 mg kg ⁻¹ n = 9
0	3.62 ± 1.55	2.28 ± 0.59 <i>P</i> < 0.025	2.03 ± 0.83 <i>P</i> < 0.025	2.42 ± 1.35
0.1	3.63 ± 1.41	2.04 ± 0.49 <i>P</i> < 0.005	1.91 ± 0.74 <i>P</i> < 0.01	2.62 ± 1.40
1	3.80 ± 1.30	2.65 ± 0.60 <i>P</i> < 0.025	2.18 ± 0.78 <i>P</i> < 0.025	2.64 ± 1.67
10	5.01 ± 0.94	3.27 ± 0.92 <i>P</i> < 0.005	2.52 ± 0.56 <i>P</i> < 0.005	3.07 ± 1.52 <i>P</i> < 0.01
100	6.88 ± 2.77	4.00 ± 1.09 <i>P</i> < 0.01	2.96 ± 0.70 <i>P</i> < 0.005	3.64 ± 2.37 <i>P</i> < 0.025

Subcutaneous adipose tissue was taken from the rats described in Table 1 and the rate of incorporation of [U-¹⁴C]-glucose into fatty acids was measured over a 2 h period in the presence of various concentrations of insulin. Results are means ± s.d. for the number of rats indicated. The *P* values indicate the significance of the difference between tissue from untreated (control) rats and the D-fenfluramine-treated rats at the equivalent concentration of insulin.

Results

Effects of D-fenfluramine treatment on rats fed ad libitum

Treatment of the 6 month old cp/cp female rats with D-fenfluramine (Series 1) produced a sustained and dose-dependent loss in relative body weight over a 28 day period compared to the control rats (injected with NaCl above) that slowly increased their body weights (Figure 1a). There was a decrease in the absolute body weight relative to the controls over this period of 32 g (*P* < 0.025), 52 g (*P* < 0.0005) and 67 g (*P* < 0.0005) respectively at the 1, 2.5 and 5 mg kg⁻¹ doses. These decreases were largely accounted for by decreases in the size of adipose tissue depots (Table 1). The subcutaneous depot was the largest and it showed the greatest decrease.

D-Fenfluramine treatment also produced a sustained decrease in food intake. This was more pronounced at the

beginning, but persisted (*P* < 0.0005) through the treatment period (Figure 1b). During the last 7 days of treatment the average daily food intake for the control rats was 17.6 ± 2.4 g which was higher (*P* < 0.005) than that for the rats treated with 1, 2.5 and 5.0 mg kg⁻¹ of D-fenfluramine which consumed 14.8 ± 1.9 g, 12.9 ± 2.3 g and 12.6 ± 1.5 g of food per day respectively.

Samples of subcutaneous and inguinal adipose tissue were incubated with 5.5 mM glucose to determine whether metabolism was altered by D-fenfluramine treatment. Subcutaneous adipose tissue from the rats treated with D-fenfluramine at all doses had lower rates of fatty acid synthesis from [U-¹⁴C]-glucose when this was calculated for the whole adipose tissue depot (Table 2) by using the weights given in Table 1. These values also remained statistically significant when the results were calculated per g of tissue for the group treated with 1 mg kg⁻¹ of D-fenfluramine at all insulin concentrations, and for the 2.5 mg kg⁻¹ group at 10 and 100 ng ml⁻¹ of insulin. The greater variability in the group treated with

Table 3 Effects of D-fenfluramine treatment of JCR:LA-corpulent female rats, on the concentrations and some metabolites and hormones in the blood serum

	Control n = 9	Dose of D-fenfluramine		
		1.0 mg kg ⁻¹ n = 9	2.5 mg kg ⁻¹ n = 8	5.0 mg kg ⁻¹ n = 9
Glucose (mM)	6.90 ± 0.53	6.40 ± 0.53 <i>P</i> < 0.05	6.27 ± 0.48 <i>P</i> < 0.025	6.56 ± 0.77
Lactic acid (mM)	3.25 ± 0.88	3.36 ± 0.94	2.63 ± 0.42 <i>P</i> < 0.05	3.23 ± 1.18
Glycerol (mM)	0.647 ± 0.263	0.793 ± 0.212	0.623 ± 0.194	0.718 ± 0.299
Fatty acid (mM)	0.743 ± 0.414	0.991 ± 0.331	0.705 ± 0.249	0.642 ± 0.383
Triacylglycerol (mM)	8.28 ± 3.36	6.60 ± 2.82	4.99 ± 2.72 <i>P</i> < 0.025	5.10 ± 2.84 <i>P</i> < 0.025
Total cholesterol (mM)	3.20 ± 0.99	2.75 ± 0.52	2.52 ± 0.70	2.86 ± 0.49
Free cholesterol (mM)	1.88 ± 0.81	1.60 ± 0.64	1.07 ± 0.39 <i>P</i> < 0.025	1.28 ± 0.50
Esterified cholesterol (mM)	1.37 ± 0.50	1.15 ± 0.31	1.45 ± 0.45	1.59 ± 0.54
Phospholipids (mM)	4.40 ± 1.061	3.94 ± 0.919	3.16 ± 0.66 <i>P</i> < 0.01	3.56 ± 0.636 <i>P</i> < 0.05
Insulin (μU l ⁻¹)	260 ± 161	139 ± 90 <i>P</i> < 0.05	131 ± 57 <i>P</i> < 0.025	104 ± 50 <i>P</i> < 0.01
Corticosterone (nM)	502 ± 473	611 ± 754	946 ± 388 <i>P</i> < 0.025	1273 ± 1493

The rats were fed *ad libitum* (Series 1) and treated with the daily dose of D-fenfluramine indicated in Figure 1. The results are expressed as means ± s.d. for the number of animals indicated. The *P* values show the significance of the difference between the treated group and the untreated (control) rats.

Figure 2 Effects of food restriction and D-fenfluramine on food intake and body weights in male and female corpulent rats. Female (a and b) and male (c and d) corpulent rats were restricted to a feeding period of 6 h/day (controls, ■) as described for Series 2. After 18 days 12 female and 6 male rats were injected daily with 2.5 mg kg^{-1} of D-fenfluramine (●). Six female and 4 male rats were pair-fed (△) with the treated female and male rats respectively. At day 33, six of the female rats were withdrawn from D-fenfluramine treatment (▲) and all rats were killed on day 48. Results for relative weights and food intakes are the means for the groups; s.d. are not shown for the sake of clarity.

5 mg kg^{-1} prevented the results expressed per g of adipose tissue being significantly different from the control group. The incorporation of glucose into glyceride-glycerol was about 3.5 fold higher than that for fatty acid synthesis. This incorporation was not significantly changed by D-fenfluramine treatment when expressed as per depot or per g of tissue. There

were also no consistent changes in the total uptake of glucose, or output of lactate by the subcutaneous adipose tissue (results not shown).

Similar measurements were made on the inguinal adipose tissue. D-Fenfluramine treatment produced no consistent changes in glucose uptake, lactate output, or in the synthesis of glyceride-glycerol or fatty acids independently of whether this was expressed as per depot, or per g of tissue.

The concentrations of insulin, corticosterone and some metabolites were also determined in blood samples from the female rats. Treatment of the female rats with 1 mg kg^{-1} of D-fenfluramine decreased insulin concentrations by about 47% and the effect appeared to increase at the higher doses (Table 3). The concentration of corticosterone was only increased significantly at the 2.5 mg kg^{-1} dose. However, this was not accompanied by an increased stress reaction as indicated by the unaltered concentrations of fatty acids and glycerol.

Glucose concentrations were decreased significantly at the 1 and 2.5 mg kg^{-1} doses whereas circulating triacylglycerol was only decreased significantly at the 2.5 and 5 mg kg^{-1} doses. Total circulating phospholipids were also decreased at these two higher doses. Cholesterol concentrations showed little change except for a significant decrease in unesterified cholesterol at the 2.5 mg kg^{-1} dose.

Effect of food restriction on the actions of D-fenfluramine (Series 2)

A major pharmacological property of D-fenfluramine is its anorectic effect. Experiments were therefore performed to investigate the extent to which the metabolic changes produced in Table 3 by D-fenfluramine might be simply a reflection of diminished food intake. Pair-fed rats, with decreased food intake, consume their food rapidly. It was therefore considered more appropriate to limit the time during which both treated and pair-fed control rats consumed their food rather than to permit feeding throughout the day. Restriction of the female corpulent rats to a feeding schedule of 6 h per day caused decrease of about 3% in body weight on the first day (Figure 2a), and this was accompanied by a substantial decrease in food intake (Figure 2b). The female rats were able to increase food intake during the first 18 days (Figure 2b), but not sufficiently to maintain a normal increase in body weight (Figure 2a). On day 18, some of the rats were then treated with 2.5 mg kg^{-1} of D-fenfluramine. An anorectic effect of D-fenfluramine was seen during the next 7 days of treatment ($P < 0.0005$) such that the mean daily food intake for the treated female rats was $8.2 \pm 1.2 \text{ g}$ per rat compared with $11.1 \pm 1.9 \text{ g}$ for the control rats. From day 30 to day 47, there was no significant difference between the control and D-fenfluramine-treated rats and this is reflected in their weights (Figure 2a and b). After 16 days of administering D-fenfluramine to some of the female rats the treatment was discontinued. During the last 7 days of the experimental period $16.9 \pm 2.2 \text{ g}$ of food per day per rat was consumed by these animals which was higher ($P < 0.0005$) than the $14.5 \pm 1.4 \text{ g}$ eaten by the controls. The relative body weights of the four groups of rats at the end of the treatment period were similar (Figure 2a).

The results of the analysis of metabolites and hormones are given in Table 4. No significant differences were detected among the female groups with respect to the concentrations of glucose, lactate, fatty acids, triacylglycerol and corticosterone. Glycerol concentrations were decreased by D-fenfluramine treatment. The concentration of free cholesterol was raised in groups that were treated with D-fenfluramine for the full period, those that were only treated for 16 days and in those that were pair-fed compared to the controls. Insulin concentrations were decreased in the D-fenfluramine-treated rats compared to untreated rats that were allowed free access to food.

Table 4 Effects of food restriction on the concentrations of some metabolites, insulin and corticosterone in male and female corpulent rats

	Females				Males		
	Controls (6)	Pair-fed (6)	Fenfluramine treated (6)	Fenfluramine treatment withdrawn (6)	Controls (4)	Pair-fed (4)	Fenfluramine treated (6)
Glucose (mM)	6.56 ± 0.34	6.37 ± 0.37	6.55 ± 0.44	6.61 ± 0.36	5.78 ± 0.52	6.30 ± 0.5	6.22 ± 0.89
Lactate (mM)	3.46 ± 0.73	3.20 ± 0.78	3.59 ± 0.69	3.75 ± 0.63	4.38 ± 0.94	4.04 ± 0.89	4.45 ± 0.77
Glycerol (mM)	1.01 ± 0.14	0.98 ± 0.15	0.66 ± 0.21	0.78 ± 0.35	1.16 ± 0.16	0.66 ± 0.27	1.07 ± 0.10
			$P < 0.005$			$P < 0.01$	
Fatty acid (mM)	1.07 ± 0.29	1.09 ± 0.11	1.03 ± 0.22	1.18 ± 0.35	0.99 ± 0.03	1.12 ± 0.12	1.03 ± 0.16
						$P < 0.05$	
Triacylglycerol (mM)	2.31 ± 1.22	2.18 ± 0.77	3.18 ± 0.61	3.03 ± 1.95	1.27 ± 0.33	1.21 ± 0.55	0.63 ± 0.57
Total cholesterol (mM)	2.58 ± 0.42	3.55 ± 0.64	3.05 ± 0.69	3.09 ± 0.43	3.23 ± 0.44	2.77 ± 0.13	2.76 ± 0.36
		$P < 0.01$		$P < 0.05$		$P < 0.05$	
Free cholesterol (mM)	1.08 ± 0.11	1.43 ± 0.24	1.32 ± 0.26	1.29 ± 0.21	1.22 ± 0.14	1.09 ± 0.07	1.01 ± 0.07
		$P < 0.005$	$P < 0.05$	$P < 0.05$			$P < 0.025$
Esterified cholesterol (mM)	1.5 ± 0.33	2.12 ± 0.43	1.72 ± 0.45	1.80 ± 0.31	2.01 ± 0.33	1.69 ± 0.07	1.76 ± 0.3
		$P < 0.01$					
Phospholipids (mM)	3.12 ± 0.35	3.67 ± 0.42	3.54 ± 0.55	3.66 ± 0.47	3.33 ± 0.38	2.94 ± 0.19	2.81 ± 0.24
		$P < 0.025$		$P < 0.05$			$P < 0.05$
Insulin ($\mu\text{M l}^{-1}$)	78 ± 31	55 ± 22	45 ± 24	68 ± 40	205 ± 90	120 ± 85	108 ± 46
			$P < 0.05$				
Corticosterone (nM)	1155 ± 537	816 ± 282	1167 ± 744	1042 ± 425	649 ± 156	562 ± 155	533 ± 180

Rats were treated as described in the text (Series 2) and Figure 2 and the results are expressed as means ± s.d. for the number of rats indicated. The P values show the significance of the difference between the appropriate group and the untreated (control) rats.

The same experimental protocol was applied to male corpulent rats (Figure 2c and d). There was again a decrease in body weight and food intake on the first day while the rats became accustomed to the restricted period of feeding. Body weights then remained relatively constant from days 1 to 18. The rats treated with D-fenfluramine at day 18 showed an anorectic effect which prevented the further increase in food intake (Figure 2d) and weight gain (Figure 2c) that was seen with the control rats fed *ad libitum* for 6 h. The treated rats lost weight as did the rats that were pair-fed with them (Figure 2c).

The differences in the metabolite and hormone concentrations for the male rats were not marked. Treatment with D-fenfluramine appears to decrease the concentrations of blood lipids as seen for phospholipids, free cholesterol and tri-

acylglycerol, although the latter value did not reach statistical significance. Insulin concentrations appeared to decrease by 42 and 48% with pair-feeding and D-fenfluramine-treatment, but the results were not statistically significant with the number of animals used. This was because of the large inter-animal variations which are normal with this rat model. However, other work has shown that food restriction is effective in decreasing circulating insulin in the corpulent rat (Russell *et al.*, 1990b). There were no statistically significant differences in metabolites or hormones on day 42 (when the rats were killed) between the rats treated with D-fenfluramine and those that were pair-fed.

Effects of prolonged treatment of young JCR:LA corpulent rats with D-fenfluramine (Series 3)

The third experimental protocol was to treat 6 week old corpulent rats with D-fenfluramine in drinking water. Both male and female rats gained substantially less weight ($P < 0.001$ for males and $P < 0.01$ for females) when treated with 0.25 mg of D-fenfluramine kg^{-1} daily (Figure 3a). The mean decreases in body weight were equivalent to 54 g and 80 g respectively for the male and female rats after the 9 weeks of treatment. The decreased weight was associated with a sustained decrease in food intake (Figure 3b).

Discussion

The response curve with D-fenfluramine demonstrates that daily injections of 1 mg kg^{-1} of D-fenfluramine produced significant decreases in body weight, adipose tissue mass and food intake that were maintained throughout the 30 day treatment period of the female rats (Figure 1). This was accompanied by a decreased ability of the subcutaneous adipose tissue to synthesize fatty acids from glucose (Table 2). Decreases in fatty acid synthesis after D-fenfluramine-treatment have also been observed in lean male Wistar rats (Al-Sieni *et al.*, 1989) and in old female Wistar rats (Brindley *et al.*, 1989). However, we did not observe a diversion of glucose to lactate in the corpulent rats as was observed with male Wistar rats (Al-Sieni *et al.*, 1989).

JCR:LA-corpulent rats and old female Wistar rats (Brindley *et al.*, 1990) were much more sensitive to the weight reducing effects of D-fenfluramine than were lean male Wistar rats that were actively growing (Brindley *et al.*, 1985; Al-Sieni

Figure 3 Effects of adding D-fenfluramine to the drinking water of male and female JCR:LA corpulent rats. D-Fenfluramine was added to the drinking water of 6 male (○) and 6 female (●) rats (Series 3) and the concentration was continually adjusted to produce a daily dose of about 0.25 mg kg^{-1} of D-fenfluramine, 6 male (□) and 6 female (■) rats received untreated water (controls). The results are means ± s.d. Food intake is given as the mean daily intake per rat and the rats were housed individually.

et al., 1989). Treatment of male and female corpulent rats from 6 weeks of age with 0.25 mg kg^{-1} daily of D-fenfluramine substantially decreased body weight (Figure 3). To our knowledge this is the lowest dosage that has been reported to have such a profound effect (Arnaud & Nathan, 1990). However, this treatment did not completely prevent the appearance of obesity; neither did restricting the food intake of male rats to 12 g day^{-1} (Russell *et al.*, 1990b) or making the rats run for $6\text{--}8 \text{ km day}^{-1}$ (Russell *et al.*, 1989b).

Although the body weights of the adult corpulent rats tended to stabilize at a lower level after about 15 days of treatment, D-fenfluramine did prevent the normal increase in weight that occurred in the untreated rats (Figure 1a). This pattern of response is reminiscent of the clinical experience in using D-fenfluramine to treat obesity (Rowland & Carlton, 1986). The failure to continue to lose weight should not be taken as an indication of pharmacological tolerance. It has been proposed that fenfluramine persistently lowers body weight set-point, and that anorexia is secondary to the weight suppressing effects (Levitsky *et al.*, 1981; Stunkard, 1982). This conclusion is supported by the observation that fenfluramine does not have an anorectic effect in rats that had previously had their weights decreased by food deprivation (Levitsky *et al.*, 1987; Stunkard, 1982). Similarly, the effects of D-fenfluramine in the female rats were not so prolonged when they were allowed to feed for only 6 h per day (Figure 2b). These female corpulent rats were unable to consume enough food to maintain and increase their body weights as they would have done had they been fed *ad libitum* (Figure 1). This finding was unexpected since in previous work we used a less extreme form of obesity, the aged adult female Wistar rat. These rats were able to maintain their body weights when fed during the same 6 h feeding period, and D-fenfluramine at 1, 2.5, 5 and 10 mg kg^{-1} decreased their body weight by 2, 4, 7 and 8% respectively in 8 days (Brindley *et al.*, 1989). The male corpulent rats were also able to gain weight in the 6 h feeding period and D-fenfluramine produced a marked reduction in body weight (Figure 2c).

The stabilization of body weight after treatment for about 15 days with D-fenfluramine (Figure 1b) does not mean that there were no further beneficial metabolic effects. The decrease in circulating insulin reached 60% after 29 days of treatment at the 5 mg kg^{-1} dose in female corpulent rats (Table 3). This indicates a marked improvement in insulin sensitivity and was reflected in the decrease in circulating glucose. D-Fenfluramine did not increase lactate concentrations in the corpulent rats as do some biguanides.

D-Fenfluramine treatment also produces decreases of up to 40% in circulating triacylglycerol with smaller effects on cholesterol and phospholipids. These changes are compatible with a suppression of the high rates of VLDL secretion in the corpulent rats (Russell *et al.*, 1991). Insulin resistance is often associated with hypertriglyceridaemia, which could be caused by the high insulin concentrations. Alternatively, the relatively low effectiveness of insulin together with increased glucocorticoid action could be responsible (Brindley & Rolland, 1989). Glucocorticoids stimulate the secretion of lipids (Mangiapan & Brindley, 1986; Bartlett & Gibbons, 1988) and apolipoproteins (Martin-Sanz *et al.*, 1990) in VLDL. Furthermore excessive glucocorticoid action may be an important factor in the development of obesity (Bray & York, 1979; Berdanier, 1989; Devenport *et al.*, 1989). Treatments that decrease glucocorticoid secretion (Bray & York, 1979), or effectiveness (Baulieu 1989; Langley & York, 1990) can partially reverse the obesity; and D-fenfluramine may produce some of its metabolic effects by preventing the excessive secretion of glucocorticoids (Brindley, 1983; Brindley *et al.*, 1988). However, decreases in circulating triacylglycerols, or the weight of the corpulent rats were not associated with decreased corticosterone concentrations (Tables 3 and 4).

The anorectic effect of D-fenfluramine could in itself have the effect of raising corticosterone concentrations, which in this condition could promote catabolism rather than anab-

olism (Dallman *et al.*, 1989). This catabolic phase is also observed in the first few days when lean rats were treated with fenfluramine and it is associated with anorexia and a marked stimulation of lipolysis (Dannenberg, 1983; Brindley, 1983). Furthermore, fenfluramine when administered acutely can cause the release of corticosterone (Schettini *et al.*, 1979; Fuller *et al.*, 1981). Glycerol and fatty acid concentrations were raised in the food-restricted rats as expected, but there was no evidence for a specific effect of D-fenfluramine in promoting lipolysis in this chronic treatment (Table 4), or in the rats fed *ad libitum* (Table 3). This conclusion was also made in other work where lean Wistar rats were treated chronically with D-fenfluramine (Brindley *et al.*, 1985).

Food restriction also played a major part in decreasing the hypertriglyceridaemia of the female rats from 8.3 mm to 2.3 mm (Tables 3 and 4). In our male corpulent rats, serum triacylglycerol concentrations are normally about 2 mm when the rats are fed *ad libitum*. This was decreased to 1.27 mm when the rats were fed for only 6 h per day (Table 4). There was little evidence for a pronounced hypolipidaemic action of D-fenfluramine after food restriction. However, D-fenfluramine was still able to decrease insulin concentrations in the females and possibly in the males, although the latter effect was not statistically significant (Table 4). Other work with D-fenfluramine has demonstrated improvements in insulin sensitivity and hypolipidaemic effects in animals and human beings that are unrelated to anorexia and weight loss (Brindley *et al.*, 1985; Arnaud & Nathan, 1990). However, it is concluded that the anorectic action of D-fenfluramine is an important component in producing the hypolipidaemic effects and some of the improvement in insulin sensitivity in the JCR:LA-corpulent rat.

Similar metabolic effects to those observed with D-fenfluramine were obtained by treatment of the corpulent rat with a related drug, benfluorex at 25 mg kg^{-1} . This produced decreases in food intake and body weight similar to that seen with 5 mg kg^{-1} of D-fenfluramine in the female rats (Figure 1). However, benfluorex appeared to have a greater effect in decreasing the concentration of circulating lipids (Brindley *et al.*, 1991). Benfluorex and D-fenfluramine depress feeding through their central 5-hydroxytryptaminergic actions (Garratini, 1983; Samanin, 1983) which could also account for the beneficial changes in hormonal balance resulting in hypoglycaemic and hypolipidaemic effects (Brindley *et al.*, 1985; 1988).

The present work establishes that the JCR:LA-corpulent rat is very sensitive to the actions of D-fenfluramine. Very low doses of this compound produce a sustained depression of food intake, weight loss, together with marked decreases in the concentrations of circulating insulin and triacylglycerol. These effects are all in the direction that should decrease the risk of developing premature atherosclerosis. The corpulent rats appear to provide an ideal model for the combined obese-diabetic-hyperlipidaemic syndrome that is associated with an increased incidence of coronary heart disease in human beings. The animals not only develop mature cardiovascular diseases spontaneously, but this disease expression can be modified by metabolic manipulation or pharmacological treatment (Russell *et al.*, 1989b,c; 1990c). The present work provides the foundation for future studies on the mode of action of D-fenfluramine in this experimental model. Most significantly this model can be used to investigate if prolonged treatment with D-fenfluramine can decrease the incidence of cardiovascular lesions. Such knowledge will be extremely important in deciding whether to continue treating patients with drugs such as D-fenfluramine even after the initial loss of body weight has been achieved.

This work was supported by the Alberta Heritage Foundation for Medical Research, the Alberta Heart and Stroke Foundation, the Juvenile Diabetes Foundation and the Institut de Recherches Internationales Servier. Dorothy Koeslag, Sandra Graham and Cindy Labine provided valuable technical assistance.

References

- AL-SIENI, A.I.I., PLESTED, P., ROLLAND, Y. & BRINDLEY, D.N. (1989). Decreased incorporation of glucose into lipids and increased lactate production by adipose tissue after long-term treatment of rats with D-fenfluramine. *Biochem. Pharmacol.*, **38**, 3661–3667.
- AMY, R.M., DOLPHIN, P.J., PEDERSON, R.A. & RUSSELL, J.C. (1988). Atherogenesis in two strains of obese rats. The fatty Zucker and LA/N-corpulent. *Atherosclerosis*, **69**, 199–209.
- ARNAUD, O. & NATHAN, C. (1990). Antiobesity and lipid lowering agents with antidiabetic activity. In *New Anti-Diabetic Drugs* ed. Bailey, C.J. & Flatt, R.P. pp. 133–142. Nishima: Smith Gordon Co. Ltd.
- BARTLETT, S.M. & GIBBONS, G.F. (1988). Short- and longer-term regulation of very-low-density lipoprotein secretion by insulin, dexamethasone, and lipogenic substrates in cultured rat hepatocytes. A biphasic effect of insulin. *Biochem. J.*, **249**, 37–43.
- BAULIEU, E.E. (1989). Contragestion and other clinical applications of RU486, an antiprogesterone at the receptor. *Science*, **245**, 1351–1357.
- BERDANIER, C.D. (1989). Role of glucocorticoids in the regulation of lipogenesis. *FASEB J.*, **3**, 2179–2183.
- BRAY, G.A. & YORK, D.A. (1979). Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol. Rev.*, **59**, 719–809.
- BRINDLEY, D.N. (1983). Phenylethylamines and their effects on the synthesis of fatty acids, triacylglycerols and phospholipids. In *Biochemical Pharmacology of Obesity* ed. Curtis-Prior, P.B. pp. 285–308. Amsterdam: Elsevier.
- BRINDLEY, D.N., AKESTER, H., DERRICK, G.P., IRVINE, D.D., PATMORE, R.D., SPENCER, H., YULE-SMITH, A., FINNERTY, C., SAXTON, J., MACDONALD, I.A. & ROLLAND, Y. (1988). Effects of chronic administration of benfluorex to rats, on the metabolism of corticosterone, glucose, triacylglycerols, glycerol and fatty acids. *Biochem. Pharmacol.*, **37**, 695–705.
- BRINDLEY, D.N., AL-SIENI, A.I.I., HALES, P., HOPKINS, L., INMAN, R. & RUSSELL, J.C. (1989). JCR:LA-corpulent rats are very sensitive to the weight reducing effects of D-fenfluramine. *Int. J. Obesity*, **13**, 547.
- BRINDLEY, D.N., HALES, P., AL-SIENI, A.I.I. & RUSSELL, J.C. (1991). Decreased serum lipids, serum insulin and triacylglycerol synthesis in adipose tissue of JCR:LA-corpulent rats treated with benfluorex. *Biochim. Biophys. Acta*, **1085**, 119–125.
- BRINDLEY, D.N. & ROLLAND, Y. (1989). Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin. Sci.*, **77**, 453–461.
- BRINDLEY, D.N., SAXTON, J., SHAHIDULLAH, A. & ARMSTRONG, M. (1985). Possible relationships between changes in body weight set-point and stress metabolism after treating rats chronically with D-fenfluramine. *Biochem. Pharmacol.*, **34**, 1265–1271.
- DALLMAN, M.F., DARLINGTON, D.N., SUEMARU, S., CASCIO, C.S. & LEVIN, N. (1989). Corticosteroids in homeostasis. *Acta Physiol. Scand.*, **136**, Suppl. 583, 27–34.
- DANNENBERG, W.N. (1983). Metabolic effects of phenylethylamine drugs on glucose and fatty acids in various tissues. In *Biochemical Pharmacology of Obesity* ed. Curtis-Prior, P.B. pp. 263–283. Amsterdam: Elsevier.
- DEVENPORT, L., KNEHENS, A., SUNDSTROM, A. & THOMAS, T. (1989). Corticosterone's dual metabolic actions. *Life Sciences*, **45**, 1389–1396.
- DOLPHIN, P.J., AMY, R.M. & RUSSELL, J.C. (1990). Effect of age on serum lipids and lipoproteins of male and female JCR:LA corpulent rats. *Biochim. Biophys. Acta*, **1042**, 99–106.
- DOLPHIN, P.J., STEWART, B., AMY, R.M. & RUSSELL, J.C. (1987). Serum lipids and lipoproteins in the atherosclerosis prone LA/N-corpulent rat. *Biochim. Biophys. Acta*, **919**, 140–148.
- FULLER, R.W., SNODDY, H.D. & CLEMENS, J.A. (1981). Elevation by fenfluramine of 3,4-dihydroxyphenylacetic acid in brain and of corticosterone and prolactin in serum of fenfluramine-pretreated rats. *Pharmacol. Res. Commun.*, **13**, 275–280.
- GARRATINI, S. (1983). Central anorectic effects of phenylethylamines. In *Biochemical Pharmacology of Obesity* ed. Curtis-Prior, P.B. pp. 243–262. Amsterdam: Elsevier.
- LANGLEY, S.C. & YORK, D.A. (1990). Effects of antigluco-corticoid RU486 on development of obesity in obese fa/fa Zucker rats. *Am. J. Physiol.*, **259**, R539–R544.
- LEVITSKY, D.A., STRUPP, B.J. & LUPOLI, J. (1981). Tolerance to anorectic drugs: pharmacological or artifactual. *Pharmacol. Biochem. Behav.*, **14**, 661–667.
- MANGIAPANE, E.H. & BRINDLEY, D.N. (1986). Effects of dexamethasone and insulin on the synthesis of triacylglycerols and phosphatidylcholine and the secretion of very-low-density lipoproteins and lysophosphatidylcholine by monolayer cultures by rat hepatocytes. *Biochem. J.*, **233**, 151–160.
- MARTIN-SANZ, P., VANCE, J.E. & BRINDLEY, D.N. (1990). Stimulation of apolipoprotein secretion in very-low-density and high-density lipoproteins from cultured rat hepatocytes by dexamethasone. *Biochem. J.*, **271**, 575–583.
- ROWLAND, N.E. & CARLTON, J. (1986). Neurobiology of an anorectic drug: fenfluramine. *Prog. Neurobiol.*, **27**, 13–62.
- RUSSELL, J.C., AHUJA, S.K., MANICKAVEL, V., RAJOTTE, R.V. & AMY, R.M. (1987). Insulin resistance and impaired glucose tolerance in the atherosclerosis prone LA/N-corpulent rat. *Arteriosclerosis*, **7**, 620–626.
- RUSSELL, J.C. & AMY, R.M. (1986a). Early atherosclerotic lesions in a susceptible rat model. *Atherosclerosis*, **60**, 119–129.
- RUSSELL, J.C. & AMY, R.M. (1986b). Myocardial and vascular lesions in the LA/N-corpulent rat. *Can. J. Physiol. Pharmacol.*, **64**, 1272–1280.
- RUSSELL, J.C., AMY, R.M., MANICKAVEL, V., DOLPHIN, P.J., EPLING, W.F., PIERCE, D. & BOER, D. (1989b). Prevention of myocardial disease in the JCR:LA-corpulent rat by running. *J. Appl. Physiol.*, **66**, 1649–1655.
- RUSSELL, J.C., AMY, R.M., MANICKAVEL, V. & DOLPHIN, P.J. (1989c). Effects of chronic ethanol consumption in atherosclerosis-prone JCR:LA-corpulent rats. *Arteriosclerosis*, **9**, 122–128.
- RUSSELL, J.C., AMY, R.M., MICHAELIS, O.E., MCCUNE, S.M. & ABRAHAM, A.A. (1990a). Myocardial disease in the corpulent strains of rats. In *Frontiers in Diabetes Research: Lessons from Animal Diabetes*, III, ed. Shafrir, E. pp. 402–407. London: Smith Gordon Co. Ltd.
- RUSSELL, J.C., DOLPHIN, P.J., HAMEED, M., STEWART, B., KOESLAG, D.G., ROSE-KAHN, G. & BAR-TANA, J. (1991). Hypolipidemic effect of β - β' -tetra-methyl hexadecanedioc acid (MEDICA 16) in hyperlipidemic JCR:LA-corpulent rats. *Arteriosclerosis Thromb.*, **11**, 602–609.
- RUSSELL, J.C., KOESLAG, D.G., AMY, R.M. & DOLPHIN, P.J. (1989a). Plasma lipid secretion and clearance in hyperlipidemic JCR:LA-corpulent rats. *Arteriosclerosis*, **9**, 869–876.
- RUSSELL, J.C., KOESLAG, D.G., DOLPHIN, P.J. & AMY, R.M. (1990c). Prevention of myocardial lesions in JCR:LA-corpulent rats by nifedipine. *Arteriosclerosis*, **10**, 658–664.
- RUSSELL, J.C., KOESLAG, D.G., MANICKAVEL, V., AMY, R.G. & DOLPHIN, P.J. (1990b). The effects of advancing age and severe food restriction on pathological processes in the insulin resistant JCR:LA-corpulent rat. *Diabetes Res.*, **9**, 53–62.
- SAMANIN, R. (1983). Drugs affecting serotonin and feeding. In *Biochemical Pharmacology of Obesity* ed. Curtis-Prior, P.B. pp. 339–356. Amsterdam: Elsevier.
- SCHETTINI, G., QUATTRONE, M., GIANFRANCO, D.R. & PREZIOSI, P. (1979). Effect of selective degeneration of brain serotonin-containing neurons on plasma corticosterone levels: studies with D-fenfluramine. *Pharmacol. Res. Commun.*, **11**, 545–553.
- STUNKARD, A.J. (1982). Anorectic agents lower a body weight set point. *Life Sci.*, **30**, 2043–2055.
- VANCE, J.E. & RUSSELL, J.C. (1990). Hypersecretion of VLDL, but not HDL by hepatocytes from the JCR:LA-corpulent rat. *J. Lipid Res.*, **31**, 1491–1501.

(Received May 20, 1991
Revised October 28, 1991
Accepted November 1, 1991)