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Changes in Energy Metabolism and Metabolite Patterns of Obese Rats After Application of Dexfenfluramine

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BOSCHMANN, M., U. FRENZ, C. M. MURPHY AND R. NOACK. *Changes in energy metabolism and metabolite patterns of obese rats after application of dexfenfluramine.* PHARMACOL BIOCHEM BEHAV 53(3) 549–558, 1996.— Serotonergic neuronal networks are important for food intake and body weight regulation. Dexfenfluramine (dF), a serotonin releaser and reuptake inhibitor, was used to investigate changes in food intake, body weight development, energy expenditure, respiratory quotient, and substrate oxidation rates for 12 days. Rats, which had been made obese by early postnatal overfeeding, received an energy-controlled mash diet and water ad lib and were intraperitoneally injected daily with either saline, 5 or 10 mg dF/kg. Compared to controls, food intake, body weight development, and energy expenditure were decreased in a dose-dependent manner, especially during the first 6 days. Lipid oxidation was increased while oxidation of carbohydrates was decreased. Pair-feeding experiments over 2 days revealed that this was not solely a result of diminished food intake but also an additional metabolic effect of dF, different from its anorectic effect. At the end of these experiments, plasma glucose and liver glycogen were unchanged after dF, but plasma free fatty acids were significantly decreased. Insulin-sensitivity was probably improved, indicated by decreased insulin levels and increases in muscle glycogen contents and activities of muscle pyruvate kinase. Liver-glutamine and contents of valine, leucine, and isoleucine in the muscle were significantly decreased after dF-treatment, the latter indicating a diminished proteolysis. The plasma tryptophan/large neutral amino acids ratio of the dF-rats was unchanged but that of the paired-fed rats was changed, despite similar changes in food intake. It is concluded that both increased oxidation of endogenous fat and reduced food intake could mediate the body weight reducing effect of dF.

Obesity Dexfenfluramine Energy metabolism Calorimetry Serotonin Amino acids Free fatty acids Glucose Glycogen Insulin Pyruvate kinase

A MULTITUDE of different and integrated mechanisms exist for the regulation of food intake and energy metabolism. These consist of central and peripheral neuronal networks as well as hormonal and metabolic signals derived from various organs (1–4). Recently, our knowledge has been increased about some central neuronal processes, especially those located within the hypothalamus for the regulation of eating behavior (5). Above all, there is increasing interest in hypothalamic neurones with serotonergic (5-HT) transmission. These neurons can be stimulated effectively by dexfenfluramine (dF), a 5-HT releaser and reuptake inhibitor (6). Application of this drug has been shown to reduce food intake, to diminish craving for carbohydrate- or fat-enriched meals, and to spare protein consumption (7–9). Because the application of this drug produced no significant side effects, it was introduced in clinical treatment of obesity (10).

Various animal models have been developed to study central or peripheral processes leading to hunger or satiety (for an overview see Refs. 11 and 12). However, the metabolic processes leading to body weight reduction after dF treatment are rather unclear. Levitsky et al. (13) reported an enhanced thermic effect of feeding after dF-treatment. Lupien et al. (14) and Rothwell and LeFeuvre (15) found a significant increase in the thermogenic activity of brown adipose tissue indicated by an increased GDP-binding to mitochondria from this tissue after acute dF treatment, while McCormack et al. (16) have demonstrated that dF maintained the thermogenic activity of brown adipose tissue that was decreased in their pair-fed ani-

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mals. With regard to substrate utilization, Geelen (17) reported an insulin-like effect of dF on isolated hepatocytes, consisting of an activation of glycogenesis and inhibition of gluconeogenesis and fatty acid oxidation. In isolated muscle cells, an insulin-permissive effect was found (18). Even et al. (19, 20) found an enhanced release and utilization of free fatty acids and/or an enhancement of metabolic rate during spontaneous locomotor activity after application of dF to normal rats, and postulated the so-called "lipostatic, ischymetric mechanism" for body weight reduction.

The aim of this study was to assess metabolic processes and metabolite patterns that may be changed under dF treatment and to clarify whether these changes are simply a consequence of reduced food intake after dF application or whether they are primary metabolic effects of dF-initiating satiety. For this purpose, we used male rats that had been made obese by postnatal overnutrition (PNO-rat) after reduction of nest size. According to Widdowson (21) the reduced nest size induces overnutrition in the preweaning period and leads to long-lasting obesity. The metabolic processes studied were the oxidation rates of carbohydrate, fat, and protein measured by indirect calorimetry. The metabolites included liver and muscle glycogen as well as plasma glucose and free fatty acids. Because amino acid profiles are a good indicator of metabolic changes (22–24) and are important neurotransmitter precursors, their concentrations in plasma, liver, and muscle were also investigated.

In addition, food intake, body weight development, and energy expenditure of obese rats receiving an energy-controlled mash diet and water ad lib were investigated. These experiments were compared with pair-feeding studies to assess the differences, if any, between the pharmacological effects of dF and the consequences of reduced food intake only.

MATERIALS AND METHODS

Animals

For all experiments male, obese Wistar rats (250 g) were used. Rats had been made obese by postnatal overfeeding (PNO). To this end, litters of 8–12 pups were used only. On the day of delivery the pups were randomly distributed in such a way that one group of mother animals got 12, and the other group only 2 male pups per nest (large and small sizes). After weaning at 28 days, the young animals from the large and small nests were maintained singly in cages. Then they had free access to food and water. Whereas usually our normal rats achieved the desired body weight of about 250 g during the 10th week after delivery, our PNO-rats had already achieved this during their 7th week after delivery. Beginning with our experiments, the animals were housed in single cages at 25°C on a 12 h light/dark cycle beginning with the light phase at 6.00 a.m. The rats obtained an energy-controlled (800 $kJ/kg^{0.75}/d$) mash diet (Altromin, Lage/Westf., Germany), which was equivalent to 1.2 times their maintenance energy requirement (composition of the commercial diet (weight%): carbohydrate 50.5, fat 4.0, protein 19.0, fiber 6.0, ash 7.0, and water 13.5; brutto energy: 21.75 kJ/g; metabolizable energy: 12.3 kJ/g). This allowed growth but prevented great differences in body weight development. The corresponding food amount was presented to each rat after these had been injected with either saline or dexfenfluramine. The rats had free access to drinking water.

Dexfenfluramine Treatment

Twelve animals were divided into three groups, each with four rats, and received IP injections of either 5, 10 mg dF/kg/d or saline for 12 days, 2 h before the dark phase began. dF was kindly donated by Servier (Courbevoie, France). At the end of the 12 experimental periods, after caloric studies had been finished, rats were killed by decapitation after they had been anesthesized with hexobarbital (dissolved in saline). There were no visible signs of postinjection excitement. Blood was collected and liver and thigh muscle were removed. Before removing the liver any remaining blood was washed out via the vena cava with 10 ml saline.

Assays

Plasma-glucose, liver and muscle glycogen, and activities of liver and muscle pyruvate kinase (at 37°C) were measured by standard procedures (25). Free amino acids were determined by RP-HPLC (chromatography system by Waters Chromatography, Div. of Millipore, Milford, MA) using precolumn derivatization with *o*-phthalaldehyde and fluorescence detection as described by Algermissen et al. (26). The "Maxima 820" software (Waters Chromatography, Div. of Millipore, Milford, MA) was used for the estimation of amino acid concentrations. Insulin concentration was determined using an radioimmunoassay (Diagnostic Product Corp., Los Angeles, CA, USA).

Indirect Calorimetry

Apparatus. To estimate the respiratory quotient (RQ) and energy expenditure (EE), four rats from each group were placed in an open circuit calorimeter for 23 h at Experimental Days 1, 2, 5, 6, 11, and 12. Within the calorimeter rats had access to food and water. The oxygen concentration in the outflowing air was measured paramagnetically with a "Permolyt 2" analyser and the CO_2 concentration by infrared absorption with an "Infralyt 4" analyser (both analysers from Junkalor, Dessau, Germany). Air and catalytically burned alcohol were used for calibration purposes. All electrical signals from the six different channels (four for animals, two for calibration) were monitored and received by a "unit 575/AMM 2" (Keithley Instruments GmbH, München, Germany) and a computer (IBM.PC-AT 386 SX). To calculate RQ, EE, resting metabolic rate (RMR), and locomotor activity (LA), the spreadsheet program "Quattro Pro 4.0" (Borland Inc., Langen, Germany) was used.

Calculation of LA. Assuming that the animals rest at least once every 2 h and that such a rest is long enough to be recorded in the EE values, a value of locomotor activity (LA) can be calculated by defining a curve between the EE minima in periods of 2 h. The difference between the EE curve and the minimum curve is taken as the LA (for detailed information cf. Ref. 27).

Statistics. For each animal EE, LA, and oxidation rates of fat, carbohydrate, and protein were summarized over 24 h and only the means and standard deviations of these values of each experimental group are presented in the figures and result tables. In contrast to that, all data points of the RQ values obtained for one animal over 24 h were averaged and only the means of the various animals of one group, including standard deviations, are expressed in the figures and result tables. Generally, statistical analyses of all data (including data of the assays) were carried out using the *U*-test (Mann–Whitney–Wilcoxon) or (if indicated) Wilcoxon's matched-pairs signed rank tests.

RESULTS

Controlled Feeding

Food intake and body weight development. It is generally accepted that dF exerts its effect on body weight mainly

through the reduction of food intake and by changing food preferences (12). We used food intake and body weight development to control the effectiveness of dF in relation to the doses applied.

After injection of dF on the first day (Fig. 1, upper panel) the treated rats reduced their food intake dose-dependently from 795 ± 3 kJ/kg$^{0.75}$ (saline-injected controls) to 435 ± 191 kJ/kg$^{0.75}$ ($p < 0.005$) and 329 ± 101 kJ/kg$^{0.75}$ ($p < 0.005$) for 5 and 10 mg dF/kg, respectively. Even after 2 days, both dF-treated groups showed an increase in their food intake reaching nearly control values on the fourth day. But, compared to control animals, rats treated with 10 mg dF/kg showed a significantly lower food intake after a 12 day treatment. dF-Treatment led also to a body weight reduction (−10 g) during the first 3 days for both dF doses used. Then there was an increase in body weight with the same rate for both dF-treated groups. After 7 days this process seemed to be faster for rats treated with 10 mg/kg$^{0.75}$ compared to rats injected with the lower dose, but the difference was not statistically significant. Nevertheless, body weights of dF-treated rats were always lower compared to control animals.

Energy metabolism. Changes in food intake and body weight development should be accompanied by changes in energy expenditure and nutrient oxidation rates. We hoped to elucidate whether or not dF initially effected nutrient oxidation rates that could in turn lead to the above-mentioned changes in food preferences. To study this, calorimetric estimation was carried out.

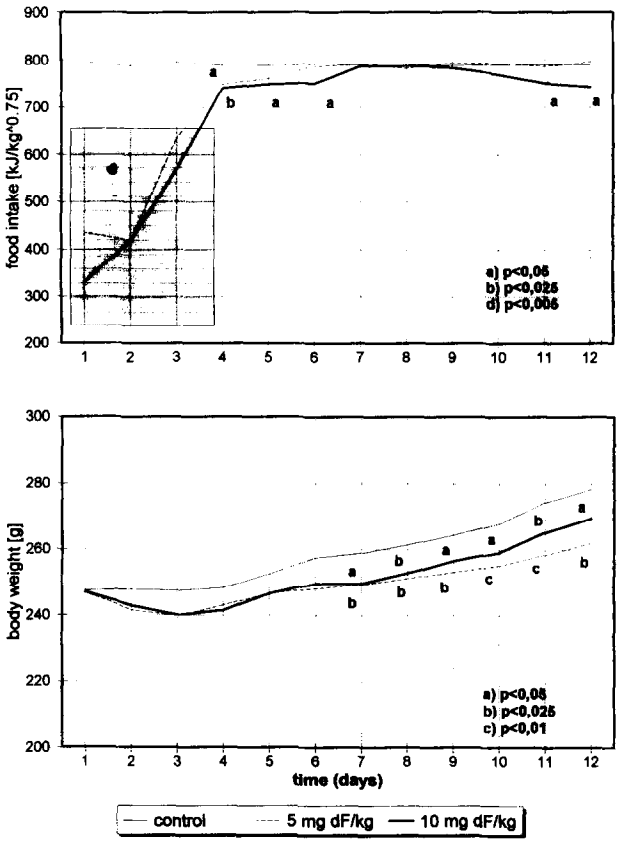

FIG. 1. Effect of dexfenfluramine on food intake and body weight development of male PNO-rats. Animals received an energy-controlled mash diet and were injected with either various doses of dexfenfluramine or saline (control) for 12 days as described in the methods section. Data are expressed as mean values of six animals. Values of the dF-treated rats indicated by a letter are significantly different compared to control values. All data within the shaded areas have the same significance value.

As can be seen in Table 1, all dF-rats showed decreased energy expenditure (EE). This effect was marked for both dF-treated groups and was clearly dose-dependent during the first few days. At the end of the experiment the differences between the doses used became smaller, but EE-values of rats treated with 10 mg dF/kg remained significantly lower than controls.

In addition, all dF-rats showed a marked reduction of their RQ-values in a dose-dependent manner during the first two days of treatment (Table 1), with the greatest reduction being displayed by the highest dF-concentration (from about 0.92 for controls to 0.78 for 10 mg dF/kg, $p < 0.001$). Derived from these data an increase of fatty acid oxidation rates from 3.92 to about 12.95 g/kg$^{0.75}$/d ($p < 0.001$) was found corresponding to a decrease of carbohydrate oxidation from about 34.00 to 8.60 g/kg$^{0.75}$/d ($p < 0.001$). This effect became smaller in the following days, but was detectable even after 12 days. The protein oxidation in all groups, estimated by the measurement of *N*-excretion in the urine during 24 h, was significantly reduced during the first 2 days (from about 4.8 g/kg$^{0.75}$/d for controls to about 2.8 g/kg$^{0.75}$/d for 10 mg dF/kg, $p < 0.001$), but was practically unchanged from the 5th day on.

Finally, it should be mentioned that during the experimental time saline-injected control rats showed a decrease of both energy expenditure and fat oxidation rate and for this trend degrees of determination of 68.9% and 69.0% were found for EE and fat oxidation rate, respectively.

Pair-Feeding Experiments

The changes in EE, RQ, and substrate oxidation after application of dexfenfluramine could be interpreted in different ways: they could be simply the consequence of the reduced food intake of the animals due to the satieting properties of dF, or they could be the expression of metabolic effects of dF per se, in addition to, or combined with the anorectic effect of the drug.

To evaluate these alternatives, in a second study, pair-feeding experiments combined with indirect calorimetric measurements were performed. To this end, PNO-rats (245 ± 5 g) were again housed singly in metabolic cages and were treated as outlined in Table 2.

Energy metabolism. Table 3 summarizes the daily changes of the measured variables during the pair-feeding experiment. The dF-treatment reduced the energy intake by about 45% compared to the day preceeding the experimental period. This reduction in food intake was therefore in the same range as that which was achieved in the aforementioned 12 day study. There was also a reduction in energy expenditure but these changes didn't reach any value of significance.

Within the P-dF group a notable reduction in 24 h RQ and daily carbohydrate oxidation, and an increase in fat oxidation were found, especially on the first experimental day. Within the P-P group a similar trend was observed. Contrary to the P-dF group the reduction in the oxidation of amino acids reached statistical significance in the P-P group, possibly due to the significantly higher amino acid oxidation rate of this group on the day preceeding the experimental period compared to that of the P-dF group on the same day.

Between both groups it is evident that on the first experi-

TABLE 1

ENERGY EXPENDITURE, RQ AND NUTRIENT OXIDATION LEVELS OVER 24 H OF OBESE RATS TREATED WITH VARYING DOSES OF DEXFENFLURAMINE OR SALINE OVER 12 DAYS

dF-Treatment Group [mg/kg]	Day 1	Day 2	Day 5	Day 6	Day 11	Day 12
Energy expenditure [kJ/kg$^{0.75}$]						
Saline	849.2 ± 3.11	857.6 ± 28.4	834.8 ± 46.4	867.2 ± 13.0	766.6 ± 16.2	792.9 ± 2.5
5	754.1 ± 58.3[a]	730.8 ± 63.9[a]	789.5 ± 28.6	823.3 ± 48.2	769.7 ± 1.2	782.7 ± 18.1
10	723.8 ± 35.2[a]	697.8 ± 35.1[a]	754.4 ± 10.3[a]	757.8 ± 33.9[a]	702.7 ± 19.7[a]	704.8 ± 16.0[a]
RQ-values						
Saline	0.923 ± 0.001	0.934 ± 0.009	0.92 ± 0.007	0.936 ± 0.016	0.934 ± 0.028	0.958 ± 0.006
5	0.798 ± 0.046[a]	0.811 ± 0.061[a]	0.902 ± 0.007[a]	0.923 ± 0.013	0.922 ± 0.018	0.933 ± 0.017[a]
10	0.775 ± 0.028[a]	0.792 ± 0.016[a]	0.886 ± 0.008[a]	0.895 ± 0.018[a]	0.898 ± 0.019	0.905 ± 0.023[a]
Protein oxidation [g/kg$^{0.75}$]						
Saline	4.76 ± 0.22	5.26 ± 1.03	5.25 ± 0.35	4.74 ± 0.31	5.99 ± 0.64	6.56 ± 0.45
5	2.82 ± 0.65[a]	3.69 ± 1.59	4.42 ± 0.26[a]	5.37 ± 0.95	6.01 ± 0.70	6.26 ± 0.93
10	2.75 ± 0.08[a]	3.55 ± 0.42[a]	4.57 ± 0.51	5.35 ± 0.88	5.06 ± 1.01	5.48 ± 1.18
Fat oxidation [g/kg$^{0.75}$]						
Saline	3.92 ± 0.01	2.97 ± 0.48	3.85 ± 0.88	3.10 ± 1.36	2.27 ± 2.04	0.67 ± 0.53
5	11.91 ± 2.14[a]	10.30 ± 3.27[a]	5.00 ± 0.48[a]	3.49 ± 0.80	3.08 ± 1.41	2.32 ± 1.30
10	12.95 ± 1.37[a]	11.18 ± 0.78[a]	5.70 ± 0.63[a]	4.83 ± 1.14[a]	4.34 ± 1.18[a]	3.78 ± 1.62[a]
CHO oxidation [g/kg$^{0.75}$]						
Saline	34.0 ± 0.1	36.0 ± 0.6	32.8 ± 1.0	36.9 ± 2.0	31.7 ± 3.0	36.2 ± 0.9
5	12.6 ± 7.8[a]	14.0 ± 9.5[a]	28.5 ± 1.4[a]	32.8 ± 3.7	30.0 ± 2.5	32.2 ± 3.0
10	8.6 ± 4.6[a]	10.3 ± 2.6[a]	24.8 ± 0.9[a]	26.1 ± 3.5[a]	24.4 ± 3.1[a]	25.3 ± 3.3[a]

All data given as means ± SD, $n = 4$. [a]$p \leq 0.05$ (relative to control values of the same day).

mental day the RQ and the carbohydrate oxidation were much more reduced and the fat oxidation was much more increased in the P-dF group than in the P-P group. Compared to the P-P group, the oxidation of amino acids was also lower in the P-dF group but this difference didn't reach any significance. It was obvious that these changes in energy metabolism were not only a consequence of the central anorectic effect of dF but also an additional metabolic effect of dF.

TABLE 2

DESIGN OF THE PAIR-FEEDING EXPERIMENTS

Day	P-C	P-dF	P-P
0	calorimetry (saline-injected)	calorimetry (saline-injected)	calorimetry (saline-injected)
1	calorimetry (saline-injected)	calorimetry (dF-injected)	calorimetry (saline-injected)
2	sacrificed	calorimetry (dF-injected)	calorimetry (saline-injected)
3		sacrificed	sacrificed

P-C: saline-injected control rats, not pair-fed; P-dF: dF-treated rats, 10 mg dF/kg; P-P: pair-fed parallels to P-dF rats, saline injected. For each group, $n = 4$.

Rats of all groups were acclimatized to experimental conditions by being placed in the calorimeter 24 h before starting the experiment (Day 0) and receiving an energy controlled mash diet (800 kJ/kg$^{0.75}$) and injections of saline. The same amount of energy was given to P-C and P-dF rats on the experimental days. P-P rats were only fed the amount of food consumed by P-dF rats (54 and 59% of the amount presented to P-dF rats on Days 1 and 2, respectively). Experiments (P-C, P-dF, and P-P) were performed in consecutive experimental periods. Food presentation, injections, and sampling of organs after the rats had been decapitated were performed as described in the method section.

All the calorimetric data mentioned above are mean daily figures. Taking into consideration that food intake, RQ-values, and energy expenditure are dependent on circadian rhythm as well as the halflife of dF, Fig. 2 displays the time-dependence of energy expenditure, EE (upper panel), and RQ (lower panel) with 10 mg dF/kg during this pair-feeding experiment on the first experimental day.

The P-dF group showed a prompt decrease in EE immediately after injection of dF and rats nearly remained at this lower value until the end of this experimental day. This effect was accompanied by a decreased locomotor activity, possibly due to the reduced food intake.

A contrary picture was obtained with the P-P group (Fig. 2). During the early night time, these rats showed the same increasing values of EE and locomotor activity compared to those of the P-C animals, but after they had eaten the restricted diet these animals reduced their EE and locomotor activity to values lower than that of P-C rats and, especially, that of P-dF animals.

Looking at the RQ-values, injection of 10 mg dF/kg led to a prompt decrease of RQ-values of the P-dF group from about 0.95 to 0.75, and remained at this level for nearly 12 h. In the morning an increase of RQ-values to control-levels was observed. P-P animals showed contrary results: as long as the restricted energy supply was available the RQ-values were the same as for controls but, after the restricted diet had been eaten the RQ-values decreased to 0.75 indicating the beginning of a fasting period and enhanced fatty acid oxidation. These daily and dF-induced changes have to be taken into account

TABLE 3

ENERGY INTAKE (EI), ENERGY EXPENDITURE (EE), EE FOR LOCOMOTION (LA), RQ AND SUBSTRATE OXIDATION OF dF-INJECTED RATS (P-dF) AND THEIR SALINE INJECTED PAIR-FED PARALLELS (P-P)

P-dF, 10 mg dF/kg		Untreated Day Preceeding Exp. Period (0)	Treated 1st Exp. Day (1)	2nd Exp. Day (2)	Wilcoxon-test: p (0)/(1)	p (0)/(2)
EI	($kJ/kg^{0.75} \times d$)	763 ± 27	428 ± 115	473 ± 94	< 0.05	< 0.05
EE	($kJ/kg^{0.75} \times d$)	834 ± 43	798 ± 56	792 ± 68	NS	NS
LA	($kJ/kg^{0.75} \times d$)	129 ± 22	97 ± 15	133 ± 16	<0.05	NS
RQ		0.889 ± 0.034	0.807 ± 0.006[b]	0.838 ± 0.030	<0.05	<0.05
CHO-Oxid.	($g/kg^{0.75} \times d$)	28.48 ± 4.69	14.15 ± 1.53[c]	19.12 ± 5.23	<0.05	<0.05
Fat-Oxid.	($g/kg^{0.75} \times d$)	5.89 ± 2.75	11.36 ± 0.52[d]	9.26 ± 2.01	<0.05	<0.05
AA-Oxid.	($g/kg^{0.75} \times d$)	4.90 ± 0.81[a]	3.83 ± 0.37	4.08 ± 0.52	NS	NS
P-P, saline		Day Preceeding Exp. Period (0)	1st Exp. Day (1)	2nd Exp. Day (2)	p (0)/(1)	p (0)/(2)
EI	($kJ/kg^{0.75} \times d$)	738 ± 38	424 ± 113	473 ± 94	<0.05	<0.05
EE	($kJ/kg^{0.75} \times d$)	848 ± 34	779 ± 26	759 ± 33	<0.05	<0.05
LA	($kJ/kg^{0.75} \times d$)	126 ± 13	113 ± 13	115 ± 13	NS	NS
RQ		0.907 ± 0.063	0.854 ± 0.024[b]	0.832 ± 0.021	<0.05	<0.05
CHO-Oxid.	($g/kg^{0.75} \times d$)	31.45 ± 1.20	22.55 ± 3.99[c]	18.62 ± 4.23	<0.05	<0.05
Fat-Oxid.	($g/kg^{0.75} \times d$)	4.43 ± 0.58	7.42 ± 1.76[d]	8.47 ± 1.15	<0.05	<0.05
AA-Oxid.	($g/kg^{0.75} \times d$)	5.90 ± 0.37[a]	4.32 ± 1.08	4.72 ± 0.72	<0.05	<0.05

All data are given as mean ± SD, $n = 4$.
Within each column, values with same superscripts differ significantly at $p < 0.05$ level (U-test).

when states of metabolism and metabolites are studied at defined time points.

Plasma glucose and free fatty acids, and glycogen contents of liver and muscle. The changes in nutrient oxidation rates derived from the calorimetric data should also be followed by changes in some metabolite patterns. To do this, rats of all groups (P-C, P-dF and P-P) were killed immediately after calorimetric estimation was completed (15:00) and blood and livers were removed for metabolite analysis.

As shown in Table 4, the plasma glucose levels after a 2-day treatment with dF were not different from P-C rats. P-P animals showed a decrease in their plasma glucose levels from 9.93 (P-C) to 9.05 mmol/l (not significant), possibly in response to their fasting situation. The changes in liver glycogen contents were more pronounced. Whereas P-dF groups didn't show changes in their glycogen contents, P-P animals displayed drastically reduced liver glycogen (6.72 mg/g wet weight for P-P and 38.67 mg/g wet weight for P-C). Muscle glycogen contents were rather increased after dF-treatment, whereas P-P animals didn't show any changes. Plasma-free fatty acids were significantly decreased after dF-treatment, but were unchanged in the P-P group. Additionally, the insulin levels were reduced in both P-dF and P-P groups. Whereas the activity of pyruvate kinase (PK), a main glycolytic enzyme, was significantly decreased in livers of these groups, PK-activities in muscles were highly increased.

Free amino acids in plasma, liver, and muscle. The different effects relating to carbohydrate and lipid metabolism displayed by our control- and paired-feeding experiments could have some influences on protein turnover. A main link between carbohydrate and protein turnover is given by the free amino acid pool in the various organs. On the other hand, according to the concept of Fernström and Wurtmann (26, 28), a carbohydrate-rich meal results in an insulin-stimulated uptake of large neutral amino acids (LNAAs) into the muscle leading to an increase of plasma TRP/LNAA-ratio. This is discussed as a condition of enhanced TRP-uptake into the brain and therefore activated 5-HT synthesis. At least this increase in 5-HT concentration results in a reduction in food intake and carbohydrate consumption. We analyzed the free amino acid profile changes in plasma, liver, and muscle of the same groups of animals as tested above.

Compared to the P-C animals, the P-dF rats didn't show any changes in plasma-free amino acids, and therefore the TRP/LNAA ratio was also unchanged (Table 5). On the other hand, all free amino acid concentrations were significantly increased in the plasma of P-P rats, and this increase was most considerable for the non-essential amino acids. Because the increase in the concentrations of LNAA was greater than for TRP, the TRP/LNAA-ratio showed a slight but not significant decrease in P-P animals.

With regard to the changes of free amino acids in the liver (Table 6) the picture was different and less drastic: compared to P-C, both P-dF and P-P groups showed decreases of SER, GLY, THR, VAL, PHE, ILE, and LYS. In addition, levels of ALA and LEU were diminished for P-P rats. GLN-levels of untreated PNO-rats (P-C rats) were higher compared with levels found in normal, non-obese rats (data not shown), but these higher levels were normalized after dF-treatment. In contrast to that, there was a further, significant increase of GLN-content in P-P rats compared to P-C animals.

In skeletal muscle (Table 7) ILE and LEU levels were decreased after dF-treatment (from 88 ± 16 and 160 ± 62 nmol/mg w.w. for P-C rats to 59 ± 12 and 105 ± 14 nmol/mg w.w. ($p < 0.05$) for P-dF rats). P-P rats didn't show changes of ILE (76 + 7 nmol/mg w.w.) but LEU (333 + 67 nmol/g w.w.) was highly increased. All the other muscle free amino acids levels were unchanged.

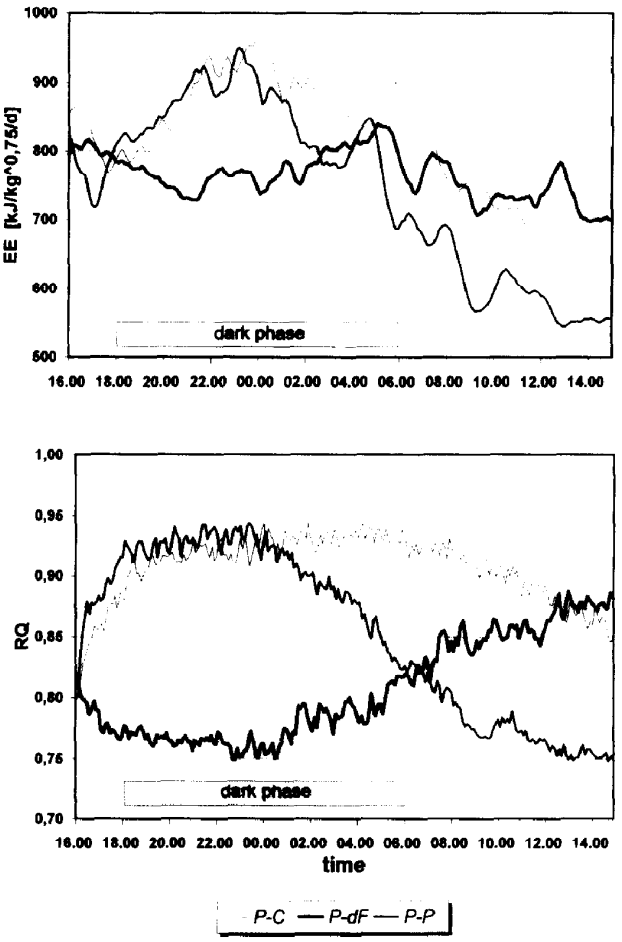

FIG. 2. Energy expenditure (EE) and respiratory quotient (RQ) of male rats treated as outlined in Table 2. P-dF animals were injected with 10 dF mg/kg. All animals were injected at 16.00. Data are expressed as mean values of four animals over a 24 h period on the first day of treatment. Energy expenditure of the P-dF rats was significantly different relative to P-P rats between 22.00 and 2.00 and between 12.00 and 14.00 ($p < 0.05$). The difference of RQ values between these two groups was significant between 20.00 and 5.00 and between 12.00 and 14.00 ($p < 0.05$).

DISCUSSION

Based on these results the following conclusions have been drawn: (a) dose-dependent reduction in food intake, body weight, energy expenditure, and RQ occur; (b) dF-mediated metabolic effects on nutrient oxidation rates are independent of its anorectic effects; (c) the decline in energy expenditure with increased fat oxidation rate could promote body weight reduction; (d) insulin sensitivity remains improved, especially in skeletal muscle; and (e) there is further evidence for the protein-sparing effect of dF during body weight reduction, because plasma, liver, and muscle amino acids were nearly unchanged after dF-treatment.

The reduction in food intake after application of dF was clearly dose dependent and biphasic with respect to the duration of dF-treatment. At both doses, 5 and 10 mg dF/kg, there was a strong decrease in food intake during the first 2 days of treatment. After 3 days the food intake of both dF-groups returned to higher values. Nevertheless, rats treated with 10 mg dF/kg displayed a significantly reduced food intake (about −10%) after a 12-day treatment. The tolerance developed to the anorectic effect of dF has been described by others (30–34). It could be a consequence of either reduced central 5-HT receptor numbers (6), reduced hypothalamic 5-HT release, especially during long-term treatment with high dF-doses (33), or an induction of contraregulatory processes in different brain areas (34). The reduced food intake led to a decrease in body weight in both dF-groups. After 4 days this was followed by a resumption of weight gain and this development seemed to be faster for rats treated with 10 mg dF/kg, but the difference to the group treated with the lower dF-dose was not statistically significant. Nevertheless, both groups reflected lower body weight at the end compared to controls (e.g., −20 and −10 g for 5 and 10 mg dF/kg, respectively).

Our calorimetric data did not indicate any enhancement of a thermogenic response after dF-treatment as reported by others (13–16). On the contrary, and corresponding with our results after application of dF to normal, nonobese rats (35), energy expenditure was almost consistently reduced for groups treated with 5 or 10 mg dF/kg (Table 1). On the other hand daily energy expenditure did not reflect the distinct initial reduction of energy intake of these rats, indicating that endogenous reserves had to be used as fuel. This is confirmed by the fat oxidations rates of the first two experimental days calculated from the RQ-values of the indirect calorimetric measurements. At both doses used (5 and 10 mg dF/kg) fat oxidation rates were significantly different from the saline-injected control group. As usual the increased fat oxidation was combined with a reduced oxidation of carbohydrates. Protein oxidation was significantly decreased during the first days. One explanation for the reduced energy expenditure of dF-treated rats could be the high rate of fatty acid oxidation induced by dF. Increased intracellular levels of free fatty acids can lead to a partial inactivation of the mitochondrial ATP/ADP translocase, especially in the liver (36,37). The following diminished cytosolic ATP-supply could result in a lowered rate of some synthetic processes, for example triglyceride synthesis (30). The reduced energy expenditure could also be a consequence of a slight sedative effect of dF (38). On the other hand, the different development of feeding bevavior and metabolism with increasing dF doses (the least food intake and body weight with the lower dF dose but highest lipid oxidation rate with the highest dose used) indicate that different 5-HT pools (central and peripheral) could be affected by dF.

To prove whether such dF-induced effects were the consequence of reduced food intake or were due to metabolic changes, pair-feeding experiments were performed. These experiments clearly demonstrated that under similar reduced food-intake conditions additional metabolic effects of dF could be measured (Table 3). When compared with the P-P group the P-dF group revealed greater reductions in RQ and in the rate of carbohydrate oxidation whereas fat oxidation was more stimulated over 24 h. At the first pair-feeding day, the differences in these values between both groups were always significant ($p < 0.05$). These findings are similar to what was reported by Even et al. (19,20), thus clarifying that the dF-induced effects on fat and carbohydrate oxidation are not simply a consequence of the reduced food intake but are additionally of metabolic origin.

Further analysis of the paired feeding data (Fig. 2) revealed that P-dF and P-P rats displayed contrary courses of 24 h energy expenditure and RQ.

TABLE 4

PLASMA-GLUCOSE, LIVER AND MUSCLE GLYCOGEN, FREE FATTY ACIDS, INSULIN AND ACTIVITIES OF LIVER AND MUSCLE PYRUVATE KINASE OF PNO-RATS FED AN ENERGY CONTROLLED DIET* AND RECEIVING INJECTIONS OF SALINE (P-C) AND OF PAIR-FED† RATS INJECTED WITH EITHER 10 mg dF/kg (P-dF) OR SALINE (P-P)

	P-C	P-dF	P-P
Glucose	9.93 ± 1.10	9.70 ± 1.06	9.05 ± 0.50
Glycogen-Liver	38.67 ± 9.08	34.57 ± 13.01	6.72 ± 3.85[a]
Glycogen-Muscle	4.89 ± 0.81	5.82 ± 0.75	4.46 ± 0.20
FFA	0.178 ± 0.039	0.093 ± 0.012[a]	0.182 ± 0.036
Insulin	173.12 ± 35.11	77.19 ± 3.61[a]	80.15 ± 8.87[a]
PK-Liver	11.56 ± 0.86	7.60 ± 0.80[a]	6.40 ± 1.70[a]
PK-Muscle	74.60 ± 10.54	112.50 ± 21.80[a]	147.20 ± 32.40[a]

*controlled diet: 800 kJ/kg$^{0.75}$/day.
**pair-fed: 430 kJ/kg$^{0.75}$/day.
Glucose: plasma-glucose in mmol/l as $x \pm SD$, $n = 4$; glycogen: liver and muscle glycogen in mg/g wet weight as $x \pm SD$, $n = 4$; FFA: free fatty acids in mmol/l as $x \pm SD$, $n = 4$; insulin: plasma-insulin in mU/l as $x \pm SD$, $n = 4$; PK: pyruvate kinase in U/g w.w. as $x \pm SD$, $n = 4$.
[a] $p < 0.05$ (relative to P-C).

Energy expenditure of the P-P rats was high during the first 12 h and this was accompanied by high RQ-values, reflecting high carbohydrate oxidation and indicating that food had been consumed. Having eaten their restricted diet, energy expenditure and RQ declined, reflecting the beginning of a fasting period. Fluctuations in energy expenditure were also reduced—a sign of reduced locomotor activity.

P-dF rats displayed practically the opposite time course to the P-P rats with the exception that energy expenditure at the end of the experimental day did not reach control value. The main contrast with the P-P rats is the prompt decrease of the RQ values and practically no greater fluctuations in energy expenditure after the application of dF. It seems that the activation of lipid oxidation is a pure effect of dF, mediated through the activation of some sympathetic neurons (4). It indicates an activation of short-term regulation processes and it can be inferred that this prompt stimulation of lipid oxidation may be itself responsible for, or at least contribute to, the initiation of the reduction in food intake.

Some metabolite patterns were studied at the end of the experiments in P-C, P-dF, and P-P rats. This allowed us to compare rats after acute treatment with dF (P-dF) to rats that were caloric restricted (P-P) or received an energy-controlled diet (P-C).

TABLE 5

PLASMA AMINO ACID VALUES AND TRP/LNAA-RATIO OF RATS TREATED AS IN TABLE 4

AA	P-C	P-dF	P-P
GLU	107.7 ± 15.8	122.9 ± 14.5	240.0 ± 87.6[a]
SER	142.7 ± 25.1	118.9 ± 12.7	266.6 ± 73.7[a]
GLN	505.2 ± 47.9	565.7 ± 161.3	1270.1 ± 181.7[a]
HIS	33.0 ± 6.1	46.0 ± 11.6	90.5 ± 23.1[a]
THR	124.4 ± 52.6	116.1 ± 16.9	206.7 ± 57.4[a]
ARG	119.3 ± 42.1	144.6 ± 14.9	229.6 ± 58.1[a]
ALA	304.5 ± 50.2	292.2 ± 71.2	533.8 ± 129.0[a]
TYR	42.6 ± 11.4	79.5 ± 31.2	153.2 ± 28.0[a]
TRP	60.7 ± 13.3	69.9 ± 10.1	83.3 ± 21.4[a]
MET	30.3 ± 4.7	37.8 ± 4.9	54.1 ± 9.2[a]
VAL	128.6 ± 33.0	140.4 ± 22.2	190.7 ± 41.6[a]
PHE	35.3 ± 7.5	36.9 ± 6.2	62.6 ± 13.8[a]
ILE	61.0 ± 14.7	68.7 ± 16.6	103.7 ± 17.2[a]
LEU	97.6 ± 20.9	117.6 ± 12.9	163.1 ± 30.0[a]
ORN	37.7 ± 8.4	46.1 ± 2.7	59.1 ± 16.7[a]
LYS	273.4 ± 73.8	228.3 ± 61.5	353.3 ± 53.1[a]
TRP/LNAA	0.167 ± 0.023	0.159 ± 0.017	0.123 ± 0.021

Concentrations are given in nmol/ml as $x \pm SD$, $n = 4$.
[a] $p < 0.05$ (relative to P-C).

TABLE 6

AMINO ACID PATTERNS OF LIVERS OF RATS TREATED AS IN TABLE 4

AA	P-C	P-dF	P-P
GLU	923 ± 84	890 ± 251	935 ± 99[a]
SER	235 ± 66	171 ± 24	129 ± 32[a]
GLN	3340 ± 259	2251 ± 355[a]	4445 ± 965[a]
GLY	1518 ± 186	1231 ± 177	1141 ± 116[a]
THR	142 ± 41	91 ± 10[a]	87 ± 18[a]
ARG	1424 ± 362	1039 ± 204	1350 ± 431
ALA	1927 ± 185	1791 ± 318	1228 ± 261[a]
VAL	165 ± 38	115 ± 11[a]	98 ± 24[a]
PHE	52 ± 14	34 ± 7[a]	30 ± 6[a]
ILE	92 ± 22	71 ± 9	58 ± 13[a]
LEU	149 ± 40	145 ± 16	103 ± 12[a]
LYS	331 ± 40	167 ± 47[a]	149 ± 30[a]

Concentrations are given in nmol/mg w.w. as $x \pm SD$, $n = 4$.
[a] $p < 0.05$ (relative to P-C).

TABLE 7

AMINO ACID PATTERNS OF MUSCLE OF RATS TREATED AS IN TABLE 4

AA	P-C	P-dF	P-P
ASP	132 ± 34	112 ± 38	107 ± 32
GLU	901 ± 274	752 ± 129	770 ± 151
ASN	155 ± 25	143 ± 25	180 ± 25
SER	354 ± 69	381 ± 45	482 ± 77
GLN	2517 ± 608	2644 ± 166	3070 ± 255
HIS	181 ± 45	206 ± 16	209 ± 46
GLY	2407 ± 1060	2853 ± 761	2185 ± 84
CIT	204 ± 35	193 ± 16	227 ± 25
THR	331 ± 120	356 ± 67	339 ± 59
ARG	192 ± 43	217 ± 50	205 ± 35
ALA	1464 ± 346	1147 ± 195	1316 ± 81
TYR	238 ± 38	274 ± 69	299 ± 69
TRP	24 ± 4	27 ± 1	25 ± 3
MET	50 ± 8	45 ± 8	56 ± 8
VAL	194 ± 37	156 ± 23	159 ± 8[a]
PHE	70 ± 20	41 ± 6[a]	55 ± 18
ILE	88 ± 16	59 ± 12[a]	76 ± 7
LEU	160 ± 62	105 ± 14	333 ± 67[a]
LYS	322 ± 136	346 ± 105	298 ± 69

Concentrations are given in nmol/g w.w. in $x \pm$ SD, $n = 4$.

[a] $p < 0.05$ (relative to P-C).

Generally all metabolite data from paired feeding experiments with dF should be carefully considered because dF treatment resulted in the animals changing their feeding pattern: P-dF rats seemed to be in the middle of their feeding cycle when sacrificed, in comparison to the P-P rats who were in a postabsorptive state. But, despite these considerations there are some interesting differences in metabolite patterns between the P-dF and P-P groups.

Metabolite data showed that the P-P group was nearly depleted of liver glycogen, whereas, for the P-dF and P-C groups, liver glycogen was in the normal range. In accordance with this, P-P rats had the lowest blood glucose level of the three groups. This could be expected because, about 14 h after dF application, the energy expenditure and the RQ of the dF rats began to increase, indicating locomotor activity and food intake, whereas the P-P rats were inactive because food consumption had already taken place (Fig. 2). But, it seems also probable that the conservation of glycogen stores by dF treatment could be another contributing factor. Additional experiments in which rats had been treated in the same way (pair-feeding experiment) but were killed in the early morning (6.00) did not show any changes of liver glycogen of P-dF rats despite strongly reduced values of RQ and food intake, but the plasma free fatty acids were reduced to the same extent as has been observed at the end of the pair-feeding experiment (liver glycogen: P-C rats, 42.93 ± 9.99 mg/g.w.w., P-dF rats 34.66 ± 13.60 mg/g.w.w., n.s.; free fatty acids: P-C rats, 0.194 ± 0.05 mmol/l, P-dF rats 0.085 ± 0.006 mmol/l, $p < 0.05$, $n = 4$).

Surprisingly, insulin levels in both Groups P-dF and P-P were decreased to nearly the same level after 2 days despite the different nutritional state of the rats at the end of the experiment. Therefore, this development could be rather a consequence of reduced overall food intake than of pharmacological origin. Additionally there should be an improvement of insulin sensitivity in these rats indicated by decreased insulin levels and increased muscle glycogen contents which has been reported by others (30,39,40). The decreased PK-activity in livers but increased activity of this important regulating glycoltic enzyme in muscles of both P-dF and P-P rats reflects a possible shift in glycolytic activity from liver to muscle. On the other hand, this could also be a consequence of enhanced insulin sensitivity (muscle) or activated lipid oxidation (liver).

In this connection it must be explicitly mentioned that any influence of the hexobarbital anesthesia on metabolite concentrations (e.g., those of glucose and free fatty acids in plasma), can be excluded, because hexobarbital-injected and unanesthesized control rats showed no differences for these parameters.

During periods of spontaneous physical activity both P-P and P-dF rats behaved similarly with respect to energy expenditure and glucose and fatty acid oxidation rates. Even et al. (19,20) reported that dF increased the energy expenditure and the rate of glucose oxidation during periods of enhanced locomotor activity compared to pair-fed rats. We cannot confirm these findings. Probably, this contributed to the fact that during our calorimetric estimation body weight of rats (250 g) and room temperature (25°C) were different from that used by Even et al. (19,20) with 350 g rats and 29°C, respectively.

It has been postulated that under appropriate experimental conditions there is a moderate increase in protein intake without activation of proteolysis after application of dF (the so-called protein sparing effect, [41]). The altered free amino acid patterns of plasma, liver, and muscle found in our experiments could confirm this hypothesis to some extent. Whereas plasma amino acids did not show any changes in the P-dF group, all amino acids were highly increased in the P-P group, indicating enhanced proteolysis (42). The TRP/LNAA ratio was unchanged after dF-treatment and comparable to that of untreated control rats (P-C group). In contrast, this ratio was rather decreased in the P-P group, giving the possibility of diminished TRP-uptake into the brain, and hence diminished serotonin synthesis in some critical brain areas (ventromedial hypothalamus) that could in turn lead to hunger signalling (concept of Fernström and Wurtman [28,29]).

The situation in the liver is more complex. Interestingly, untreated control rats (P-C) showed higher GLN levels than normal, non-obese rats (35) but these levels were normalized after dF, whereas these were further increased in the P-P group. This could be an indication of a shift in ammonia metabolism between urea synthesis or GLN production. On the other hand, GLN has an anabolic effect within liver metabolism (43), and therefore this effect could be diminished after dF.

Finally, the sum of branched chain amino acids within the muscle was significantly decreased after dF-treatment, a sign of diminished proteolysis. This ties in well with the fact of diminished *N*-excretion of dF-rats during our calorimetric experiments. On the other hand, branched chain amino acids were highly increased in the P-P group, especially for LEU, indicating proteolysis, possibly due to the beginning hunger situation. Again, measurements of free amino acids of plasma, liver, and muscle of dF-treated rats in the early morning (6.00) and therefore in a metabolic state comparable to P-P rats at the end of experiment, also did not show any changes compared to P-C rats (data not shown).

All these data indicate that the metabolic changes during reduction of RQ and energy expenditure after dF treatment

are somewhat different compared to metabolic changes observed at rats during development of a hunger situation (P-P rats). During the feeding of a carbohydrate-rich diet as in our experiments, dF-treatment exerts not only a protein-sparing but also a carbohydrate-sparing effect (reduction of both carbohydrate uptake and its oxidative metabolism).

In conclusion, the results of this study confirm that dF (and hence serotonin) activates lipid oxidation in addition to its anorectic effect. Serotonergic stimulation decreased carbohydrate oxidation without effecting plasma glucose and liver glycogen levels but there are some indications of improved insulin-sensitivity in the muscle. Protein oxidation was diminished. The prompt increase in fat oxidation rates coupled with a decline in energy expenditure could mediate the body weight reducing effect of dF. Due to enhanced lipid oxidation after dF-treatment, an activation or modulation of some adrenergic neurons, especially in liver and white adipose tissue, should be included into the mechanism of dF-action.

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