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Lipostatic and Ischymetric Mechanisms Originate Dexfenfluramine-Induced Anorexia[1]

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EVEN, P., H. COULAUD AND S. NICOLAÏDIS. *Lipostatic and ischymetric mechanisms originate dexfenfluramine-induced anorexia.* PHARMACOL BIOCHEM BEHAV **30**(1) 89–99, 1988.—Rats treated with physiological saline or dexfenfluramine (Isoméride, Laboratoires SERVIER, Neuilly, France) (dF), 1.75, 3.50, and 7.00 mg/kg, were studied in a computer-controlled open-circuit metabolic chamber, in which temperature was regulated at 24°C. Total metabolic rate (TMR), respiratory quotient (RQ), locomotor activity (LA), energy cost of LA, and thus, locomotor free metabolic rate, were scanned at 10-second intervals throughout 22-hour uninterrupted recording sessions. The feeding pattern was also measured in relation to the dF-induced changes in the above parameters. It was found that dF induced a sustained decrease in TMR and RQ, while periodically enhancing the increase in TMR and RQ produced by the periods of LA. All phenomena occurred in a dose-dependent fashion. Anorexia was also increased as a function of the dF treatments and metabolic changes. It is concluded that dF-induced anorexia may be related to the enhanced release and utilisation of free fatty acids (lipostatic mechanism), and/or to the enhancement of metabolic rate during locomotor activity (ischymetric mechanism). In addition. it appeared that the increased prandial thermogenesis, previously reported from the measurement of changes in TMR, may be due to the dF-induced increase in the energy cost of LA, rather than to an effect of feeding per se. Indeed, in our experimental conditions, it was measured that the energetic cost of LA accounted for more than 60% of the peri-prandial increase in TMR.

Rat Dexfenfluramine Lipostatic Ischymetric Metabolic Food intake Anorexia
Thermic effect of feeding Activity Energetic cost

DEXFENFLURAMINE (dF) is extensively used in both pharmacotherapy and basic research in order to produce reduction of feeding and of body weight (b.wt). The latter is currently considered to be the consequence of the former. However, b.wt. can decrease as a consequence of both decrease in feeding and increase in energy expenditure. In effect, besides the evidence of its central serotonergic anorexigenic action ([1, 10, 12], for a review see [6]), dF has also been shown to affect various processes of metabolites' utilization (reviews in [7] and [47]). In theory, dF could affect energy expenditure via one of the following mechanisms: (a) primary inhibition of the central mechanism of feeding which will bring about changes in metabolism and b.wt. or, (b) primary change of metabolism which will bring about changes in feeding and b.wt. or (c) parallel changes of both the central mechanism of satiety and of peripheral metabolism so that b.wt. loss occurs. To be complete, another mechanism, more difficult to assess, could be the primary decrease of the level of a hypothetical body weight set point [20]. This would have resulted in a decrease in feeding as well as an increase in energy loss until the new (lower) b.wt. has been reached. As far as energy expenditure is concerned, dF may increase it via the enhancement of its various components, i.e., basal metabolism and extra expenditures associated with feeding, thermoregulation, muscular contraction, and mobilization and processing of macronutrients (glucides, lipids, and amino acids).

The effect of dF on total metabolism [32,45] and various components of metabolism [16,17] is being better documented. In particular, dF was shown to enhance the release and utilization of endogenous fat stores [11, 16, 40].

In order to get a better insight on the causal relationship between the metabolic and behavioral changes induced by dF treatments, we measured the total energy expenditure (TEE), respiratory quotient (RQ), locomotor activity (LA), extra energy expenditure associated with LA and feeding, and further investigated correlations between these parameters on free-feeding, free-moving rats housed in an open-circuit calorimetric device monitored by a computer.

METHOD

Animals and Housing

The experiment was carried out on 18 adult female Wistar

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rats initially weighing 246 g (±7 SEM). Females were preferred to males because of a slower rate of b.wt. gain. In calorimetric studies, this avoids large changes in the magnitude of the energy expenditure throughout the experiments, and thus avoids resorting to Kleiber coefficient [28] for comparing energy expenditure of subjects of different b.wt.

Before the experimental procedure, the animals were adapted to the laboratory conditions housed in individual wired cages in a room maintained at 24°C. Lights were on from 08:00 to 20:00 hr. Standard chow (Extralabo M25, 13.4 kJ/g, carbohydrate 62.2%, lipid 6.5%, protein 31.3%) and water were available ad lib throughout the experiment.

Experimental Procedure

The rats received a saline (0.5 ml) (6 rats) or a dF injection (1.75, 3.50 and 7.00 mg/kg in 0.5 ml of saline) (4 rats for each dose). One injection was made each day at 18:00 on a rat sampled at random. The treated rat was housed immediately after the injection in an open-circuit calorimeter for measurement of metabolic and behavioral parameters during a 16-hour recording session.

Description of the Calorimetric Device

An open-circuit calorimeter was used in this experiment [14,16]. The cage (Fig. 1) was equipped with piezo-resistive strain gauges so that the locomotor activity of the animals could be quantitatively recorded. A food cup on a 0.1 g sensitive scale allowed monitoring of the feeding pattern. Data acquisition was performed at 10-second intervals on the 7 following apparatus:

(1) A mass sensitive flow-meter (measurement and monitoring of air flow throughout the cage according to the open-circuit principle) (Precision flow-device, 2SLM).

(2) An oxygen analyser (measurement of changes in O_2 content of air passed through the cage) (S-3A, Applied Electro Chemistry).

(3) A carbon dioxide analyser (measurement of changes in CO_2 content of air passed through the cage) (Infralyt 4, Veb Junkalor Dessau).

(4) Three piezo-resistive strain gauges (locomotor activity) (Kistler, gauges 9203, Amplifier 5001).

(5) A microscale (food intake) (Schlumberger, gauge CD0272, Amplifier CH9036).

(6) A thermometer for measurement and regulation of the ambient temperature in the cage (24°C) (TRI-R Instruments).

(7) A hygrometer (air is dried at the output of the cage before being driven to the gas analysers and the absence of humidity in the air is controlled) [Humidity Indicator HMI 11 (sensitivity 0–100%), Vaisala].

Parameters Provided by the Metabolic Device

(1) O_2 consumption calculated from changes in O_2 content (%) and air flow (l/mn).

(2) CO_2 production calculated from changes in CO_2 content (%) and air flow (l/mn).

(3) Respiratory quotient (RQ) calculated as the ratio of carbon dioxide production to oxygen consumption. In long-lasting measurements, RQ is a faithful index of the proportion of carbohydrate and lipid actually used for energy production [49].

(4) Total metabolic rate (TMR) calculated from O_2 consumption and RQ according to Lusk Formula [33]. TMR is the instantaneous expression of EE. It is expressed in watts, i.e., joules/second. The integration over a period of time of TMR provides the energy produced during this period (in joules). This was used to study the effects of dF treatments on total energy expenditure, energy cost of locomotor activity and thermic effect of feeding.

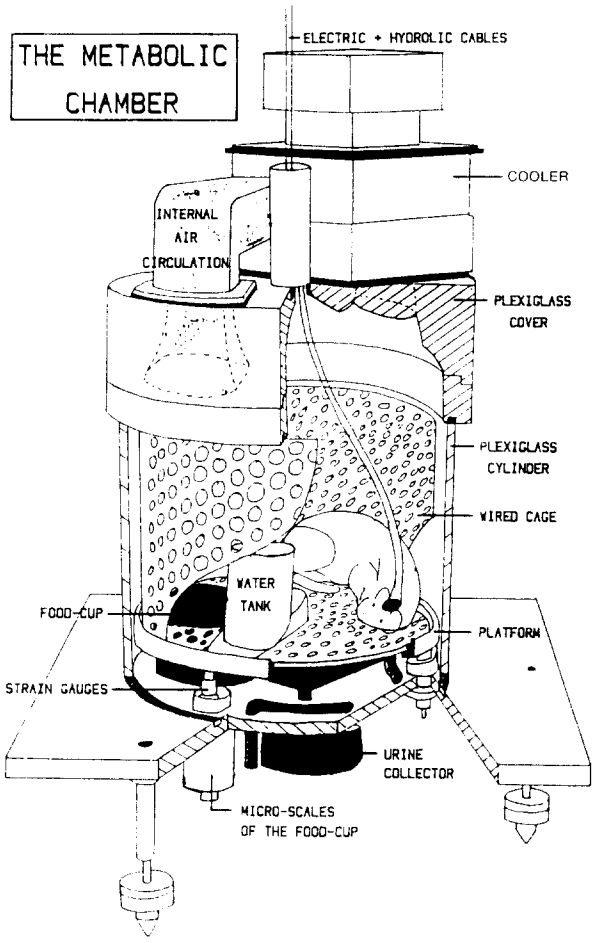

FIG. 1. The metabolic chamber.

(5) Meal pattern (meal size, duration and intermeal interval) was recorded from the continuous measurement of the weight of the food cup (sensitivity 0.1 g).

(6) Quantitative intensity of locomotor activity (LA) was obtained from the continuous measurement and integration, over the 10 second time unit, of the electrical signal (volt) produced by the 3 strain gauges (sensitivity 1 gf). In this paper, we defined a unit of locomotor activity (ULA) as the amount of activity producing a signal of 1 volt during the 10-second time unit.

(7) Energy cost of LA was computed according to the method described in Fig. 5. For each rat, all the periods of LA free from ingestive events were extracted, then the cost of LA was calculated by integrating the increase in TMR above the base line resting value throughout the period of LA (ΣdTMR) and by reporting this value to the cumulated LA (ΣdLA): The energy cost of LA is expressed as δTMR per unit of LA = δTMR/ULA.

(8) RQ changes during LA were computed by using the same method that was used for TMR changes (Fig. 5) and are expressed as δRQ/ULA.

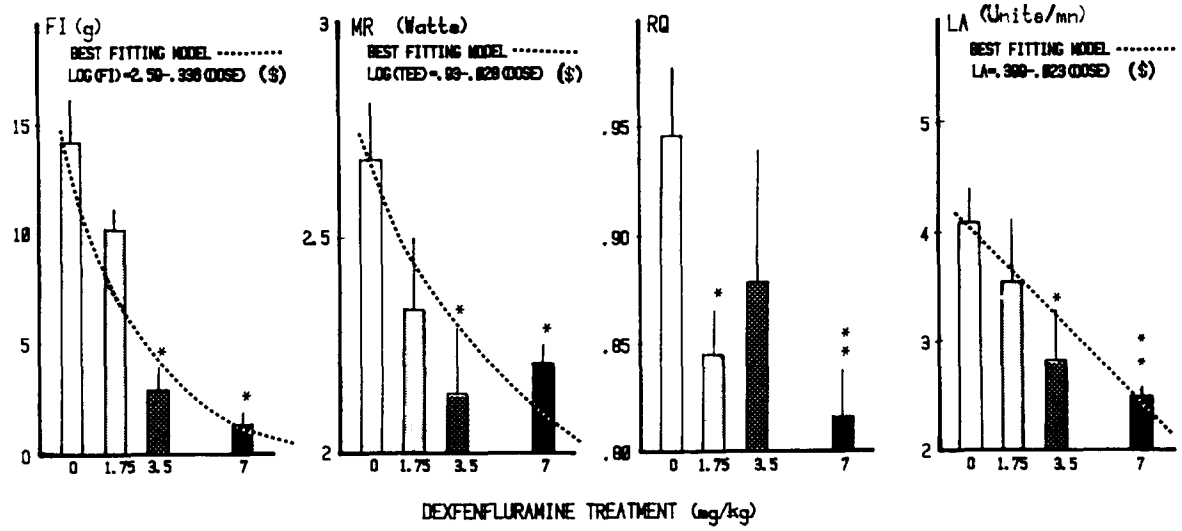

FIG. 2. Effects of dexfenfluramine treatment on food intake (FI), total metabolic rate (TMR), respiratory quotient (RQ), and locomotor activity [LA (unit of LA=volt)]. *$p<0.05$ (Mann-Whitney U-test); **$p<0.01$ (Mann-Whitney U-test); $$p<0.01$ (ANOVA).

TABLE 1

EFFECTS (±SE) OF dF TREATMENTS ON FOOD INTAKE

Treatment	Meal Number	Meal Size (g)	Intake (g)	n
Control	11.7 (1.97)	1.27 (0.500)	14.4 (4.82)	6
FF 1.75 mg/kg	12.3 (2.52)	0.875 (0.256)	10.4 (1.60)	4
FF 3.50 mg/kg	4.75* (2.22)	0.583† (0.118)	2.96* (2.07)	4
FF 7.00 mg/kg	3.00* (1.41)	0.465† (0.283)	1.36* (0.950)	4

*$p<0.05$ vs. Control (M&W U-test).
†$p<0.01$ vs. Control (M&W U-test).

Statistical Analysis

All data are reported as mean±SE. Means were compared using Mann-Whitney U-test. Relationships between factors were studied by regression analysis.

RESULTS

Effect on Feeding

Food intake was reduced in a dose-dependent semi-logarithmic fashion (Fig. 2). In the 3.50 and 7.0 mg/kg-treated groups, both size and number of meals were reduced (Table 1). In the 1.75 mg/kg-treated rats, only meal size tended to be reduced ($p=0.08$ Mann-Whitney U-test).

The feeding pattern (Fig. 3) showed that in the 1.75 mg/kg-treated subjects, food intake (FI) was inhibited during 4 to 5 hours only. During this period, meal size but not meal number was affected. Then, the rats recovered their normal feeding rate but did not compensate for their ingestive deficit. Indeed, the 4 g difference in total FI finally observed between the untreated and 1.75 mg/kg-treated subjects was already attained 5 hours after the administration of the treatment. In contrast, in the 3.5 and 7 mg/kg-treated subjects, FI remained depressed throughout the 16 hours of the recording sessions (Fig. 3).

Effect on Locomotor Activity (LA)

The spontaneous locomotor activity was reduced in a dose-dependent manner by the dF treatment (Table 2, Fig. 2). Examination of the pattern of activity showed that this reduction was due to the attenuation of intensity rather than the number of the periods of LA (see an example on Fig. 4). LA remained reduced during the entire period of recording in the 7 mg/kg-treated subjects. This was different in the other treated groups where LA was variable and related to feeding and sleep episodes as it was in the control group (Figs. 3 and 4).

Effects on Metabolic Parameters

Total metabolic rate (TMR). Mean TMR was reduced in a dose-dependent semi-logarithmic fashion (Table 2, Fig. 2). This was mostly related to the pattern of FI, as testified by the parallelism between TMR and feeding profiles (Fig. 3). However, a specific effect of dF on energy expenditure may be also suspected from the fact that the resting metabolic rate was less reduced in both the 3.5 and 7.0 mg/kg-treated rats than in the 1.75 mg/kg-treated ones, despite the continuous reduction of feeding (Table 3). In other words, when FI and LA were sufficiently reduced to allow for a comparison, dF appears to enhance energy expenditure.

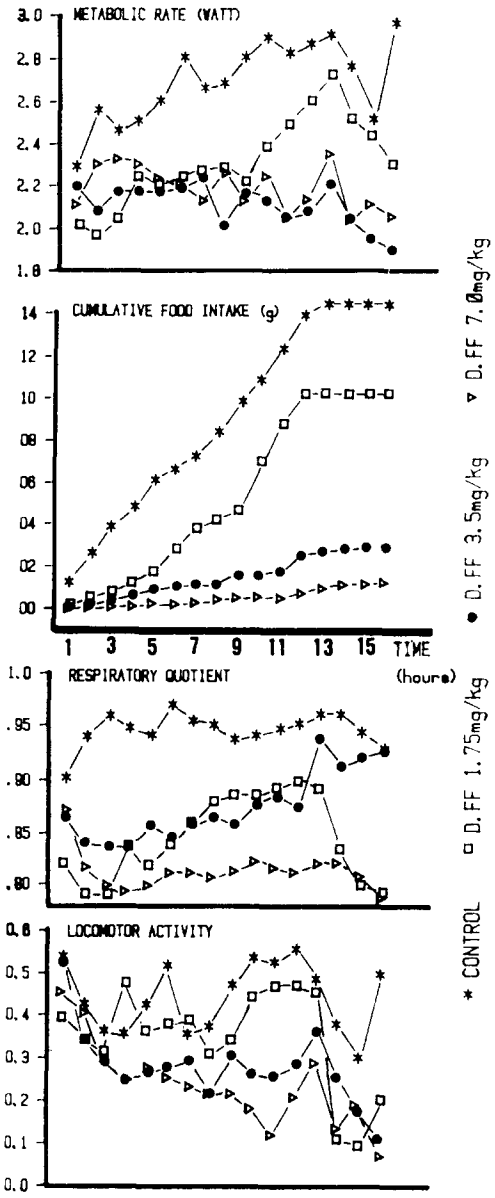

FIG. 3. Hourly evolution of the metabolic and behavioral parameters in the different groups. Dexfenfluramine or saline treatment was applied immediately before the animals were housed in the calorimeter. Unit of LA=volt.

Respiratory quotient (RQ). RQ was decreased by the dF treatment but not in a dose-dependent manner (Table 2, Fig. 2). The RQ decrease was significant in both the 1.75 and 7 mg/kg-treated groups, but not so in the 3.5 mg/kg-treated group due to large individual variations.

Energy cost of locomotor activity. The increase in TMR in relation to LA (δTMR/ULA) increased as a function of the dF treatment:

$$\delta TMR/ULA = Cost = 0.753(\pm 0.081)\,LA + 0.097(\pm 0.021\ dF\ dose),\ F(1,17)=21,\ p<10^{-4}.$$

According to this relationship, the energy cost of LA was increased from 0.8 watt/ULA in untreated subjects, to 1.38 watt/ULA in the 7 mg/kg-treated subjects (Table 3).

We investigated the metabolic parameters underlying this phenomenon by studying in the different groups the value of the energy cost of locomotor activity in a multiple regression. The dependent variables used in the analysis were the values of TMR and RQ prevailing during the resting period that immediately preceded the period of LA and the amplitude of the increase in RQ during the time-course of locomotion (dRQ/ULA) (Fig. 5).

The increase in TMR during LA, i.e., the energy cost of muscular effort, was best expressed as a function of both the intensity of the LA and δRQ/ULA (Table 4, Fig. 6). In all the groups the increase in TMR specifically devoted to LA appeared to be a constant close to 0.6 watts/ULA. On the other hand, the extra cost induced by the increase in RQ during LA (δRQ/ULA) appeared slightly greater in the 3.5 and 7.0 mg/kg dF-treated rats than in untreated and 1.75 mg/kg-treated ones. However, the relationship was the best fitted when the data from the different groups were pooled (see F values, Table 4). Thus:

$$dTMR/ULA = 0.6(LA\ intensity) + 9.4(dRQ/ULA)\ \text{(Table 4)}.$$

On the other hand, the lower was the RQ value during the resting periods that preceded the periods of LA, the greater was the increase in RQ during LA ($p<0.01$). Since dF generally decreased the resting RQ, the increase in RQ during LA was greater in the dF-treated subjects. Therefore, the increase in TMR during LA in these subjects was more specifically related to the greater RQ increase during LA (Tables 3 and 4).

Relationship Between Anorexia and the Energy Cost of Locomotor Activity

Since FI as well as δRQ/ULA and δTMR/ULA were affected by dF treatment in a significant and dose-dependent way, we investigated if FI could be expressed as a function of these metabolic parameters.

The study of FI in relation to both δRQ/ULA and δTMR/ULA showed that dF-induced anorexia was correlated with both parameters (Table 5). It was, however, shown, that δTMR/ULA, i.e., the energy cost of LA was the parameter best correlated with feeding (Table 5). This indicated the possibility of a close relationship between the metabolic effect of dF on peripheral metabolism and on anorexia.

Prandial Thermogenesis

Total thermogenesis in relation to feeding. We initially studied the relation between feeding and TMR changes, i.e., did not discriminate, within the total peri-prandial increase in energy expenditure, the part specifically devoted to locomotion. In other words, we studied the feeding-induced increase in TMR in the way that the previous investigators have studied it on the rat, i.e., δTMR versus food intake.

In both control and dF-treated ad lib subjects, the peri-prandial increase in TMR was integrated from the onset of the meal to the end of the post-prandial metabolic rebound [9,15]. This post-prandial rebound lasted 20 to 40 minutes depending on meal size (Fig. 7). Contrary to previous reports, no difference between control and dF-treated subjects was found (Table 6). The peri-prandial extra energy expenditure was 720 joules per gram of food ingested, i.e., 5.2% of the ingested calories.

TABLE 2

EFFECTS (±SE) OF dF TREATMENTS ON TOTAL METABOLIC RATE (TMR), RESPIRATORY QUOTIENT (RQ) AND LOCOMOTOR ACTIVITY (LA)

Treatment	TEE	RQ	LA	n
Control	2.68 (0.13)	0.946 (0.03)	4.10 (2.8)	6
FF 1.75 mg/kg	2.33 (0.23)	0.845* (0.02)	3.58 (0.60)	4
FF 3.50 mg/kg	2.14* (0.16)	0.878 (0.06)	2.85* (0.50)	4
FF 7.00 mg/kg	2.21* (0.04)	0.816† (0.02)	2.51† (0.13)	4

*$p<0.05$ vs. Control (M&W U-test).
†$p<0.01$ vs. Control (M&W U-test).

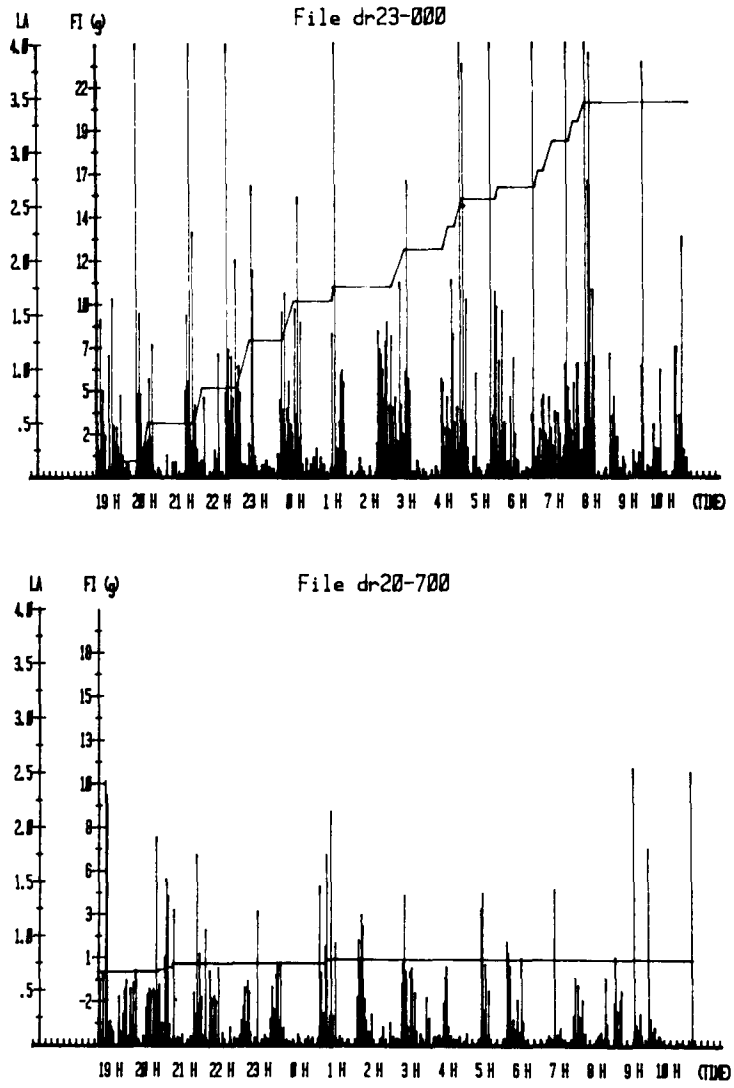

FIG. 4. Patterns of locomotor activity [LA (in volt)] and feeding (FI) of a saline (top) and a dexfenfluramine- (7 mg/kg) (bottom) treated rat. In dF-treated rats LA is reduced by means of a reduction of the intensity of the periods of LA rather than by means of a reduction of the number of periods of LA. In the untreated rats LA is mostly due to the occurrence of feeding events.

TABLE 3

EFFECTS (±SE) OF dF TREATMENTS ON THE PARAMETERS RELATED TO PERIODS OF LOCOMOTOR ACTIVITY FREE FROM MEAL

Treatment	RMR	RRQ	dRQ/ULA	dTMR/ULA	n
Control	2.14 (0.31)	0.955 (0.017)	0.0277 (0.005)	0.800 (0.23)	32
FF 1.75 mg/kg	1.75† (0.23)	0.823‡ (0.057)	0.0606‡ (0.057)	1.02† (0.24)	12
FF 3.50 mg/kg	1.88* (0.26)	0.811‡ (0.097)	0.0323* (0.097)	1.10‡ (0.32)	11
FF 7.00 mg/kg	1.93† (0.15)	0.792‡ (0.038)	0.0624‡ (0.038)	1.38‡ (0.52)	22

RMR: Pre-activity resting metabolic rate.
RRQ: Pre-activity resting RQ level.
dRQ/ULA: Changes in RQ per Unit of LA.
dTMR/ULA: Changes in TMR per unit of LA, i.e., energy cost of locomotion (watt/volt).
n: Number of periods of LA studied.
*$p<0.05$ vs. Control (M&W U-test).
†$p<0.01$ vs. Control (M&W U-test).
‡$p<0.001$ vs. Control (M&W U-test).

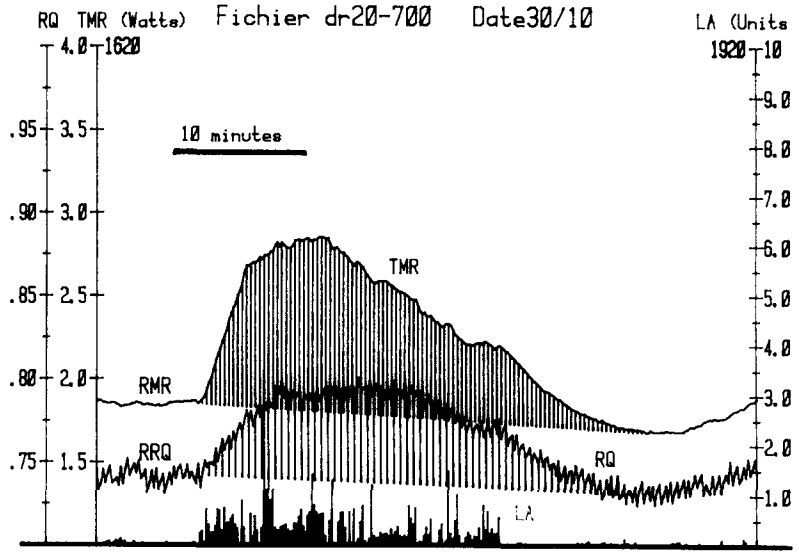

FIG. 5. A typical example of changes in total metabolic rate (TMR) and respiratory quotient (RQ) induced by a period of activity (unit of LA=volt) in a dexfenfluramine-treated rat. RMR: pre-activity resting metabolic rate; RRQ: pre-activity resting respiratory quotient. Other legends same as in Fig. 1. Top tracing narrow vertical shading: δTMR induced by LA. Center tracing wide vertical shading: δRQ induced by LA. δTMR/ULA was studied in relation to δRQ/ULA, RMR and RRQ. The intensity of δTMR/ULA is dependent on δRQ/ULA.

This universally used method of computation includes also the energy produced by the LA associated with feeding. We observed that LA increased in relation to meal size. The relationship between LA and meal size was different in untreated and dF-treated subjects (Table 7), but, in all groups, LA could be suspected to be responsible for more than 50% of the post-prandial increase in TMR. Therefore, it appeared important to discriminate the respective roles of feeding and LA in the post-prandial increase in TMR.

Respective role of feeding and LA in the prandial and post-prandial increase in TMR. In a previous section we showed that the increase of TMR in relation to LA was also a function of changes in RQ. This held for periods of LA outside from meals, where it was possible to assume that the RQ changes were directly related to the concomitant periods of LA. Such an assumption cannot be made when another specific metabolic event susceptible to modify RQ occurs. That was the case around feeding.

Since it was impossible to know a priori which was the influence of feeding on RQ, and thus, the influence of RQ on the energy cost of activity during feeding, we could not apply the formula used above to extract the energy cost of LA around feeding. Consequently, we applied a method of "general non-linear model fitting" on the following model:

$$\delta(TMR) = a(MS) + b(LA)$$

with: a(MS) = δ(TMR) due to feeding
b(LA) = δ(TMR) due to locomotor activity.

TABLE 4

EXPRESSION (±SE) OF THE INCREASE IN TEE DURING LOCOMOTOR ACTIVITY AS A FUNCTION OF INTENSITY OF LA (LA) AND CHANGES IN RQ RELATIVE TO THE INTENSITY OF LA (dRQ/ULA)

	n	Intercept	Slope	p	F
Control	29	0.61 (0.04)	05.90 (0.97)	<0.001	F(1–27)=36.5
dF 1.75 mg/kg	12	0.69 (0.11)	05.45 (1.64)	<0.01	F(1–10)=11.0
CT & dF 1.75 mg/kg	41	0.62 (0.04)	06.09 (0.74)	<0.001	F(1–39)=68.2
dF 3.50 mg/kg	10	0.49 (0.25)	16.56 (6.57)	<0.05	F(1–08)=06.4
dF 7.00 mg/kg	21	0.50 (0.22)	12.90 (3.22)	<0.01	F(1–19)=16.0
dF 3.5 & dF 7 mg/kg	31	0.59 (0.15)	12.04 (2.36)	<0.001	F(1–29)=26.0
dF pooled	43	0.66 (0.12)	09.35* (1.88)	0.001	F(1–41)=24.7
All rats	72	0.60 (0.06)	09.39* (1.12)	0.001	F(1–70)=70.2

p lev.: Probability level of the multiple regression according to analysis of variance.
*Value significantly different from control value ($p<0.05$, t-test).
n: Number of periods of locomotor activity studied.

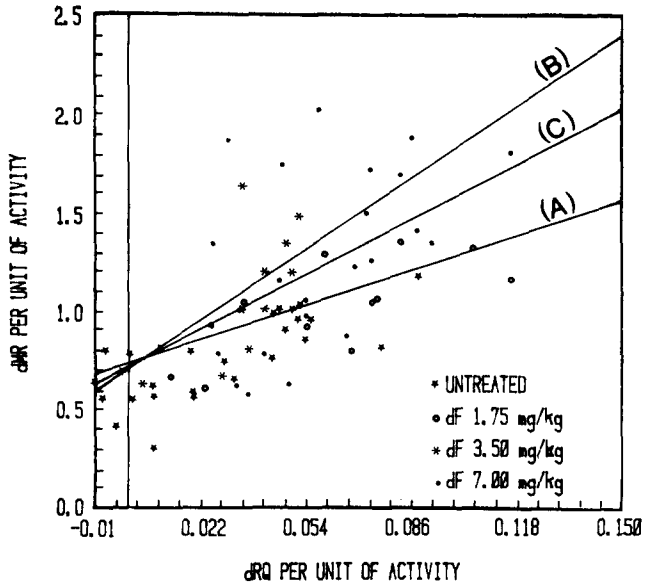

FIG. 6. Relationship between energy cost of locomotor activity (δMR per unit of activity) and changes in respiratory quotient during LA (δRQ per unit of activity). (A) Relationship fitting for saline and 1.75 mg/kg dF-treated rats; (B) relationship fitting for 3.5 and 7.0 mg/kg dF-treated rats; (C) relationship fitting for all the rats. A,B,C: see also Table 4.

i.e., δTMR during and after feeding is expected to be the result of the intensity of LA and the size of the meal simultaneously, but the respective roles of these 2 parameters, introduced as the undefined a and b coefficient is unknown. This method performs the same kind of treatment as a multiple regression, but avoids the possible bias due to the fact that LA and RQ changes were probably correlated.

It happened that the a and b coefficients could be precisely fitted for both untreated and dF-treated rats, and did not significantly vary between the groups (Table 8). This agreed with the fact that if the cost of LA was previously shown to be increased in dF-treated rats, LA intensity during feeding was reduced in these same rats. With each phenomenon compensating for the other, it resulted in a fairly constant increase in energy expenditure in relation to LA in all groups. As a result, the increase in energy expenditure specifically related to feeding was only 245 of the 700 J/g, i.e., 35% of the total increase of energy expenditure.

The cost of peri-prandial LA simultaneously estimated did not account for the entire peri-prandial RQ increases. Therefore, as suspected above, only a fraction of the RQ increases were related to the muscular effort while the remaining part was due to a feeding-associated response.

DISCUSSION

This experiment shows that dF treatment induces a number of metabolic phenomena that parallel and perhaps originate its anorectic effect. dF affects catabolic processes in a typical way that starts with increased release and utilization of endogenous lipid stores and brings about increased energy expenditure during muscular effort.

Most of the observations reported here were made possible only recently by using a computerized metabolic device that allows minute to minute measurement of total metabolic rate, respiratory quotient, intensity and cost of locomotor activity, and thus, continuous computation of the part of metabolism devoted to the resting metabolism of a freely-behaving animal. These distinct measurements enabled us to evaluate the energy cost of muscular effort under various doses of dF treatment and then to study the thermic effect of feeding free from artefactual variations due to changes in intensity and cost of locomotor activity.

The prime effect of dF is the sustained diminution of RQ. This observation confirms previous reports [16, 17, 36, 40] and demonstrates that, in the intact animal, dF simultaneously brings about a strong lipolysis and subsequently an increased utilization of the released FFA. The mechanism of lipolysis is not known. It is not due to a direct action of dF on the adipocytes, since dF was shown not to increase lipolysis in vitro [21], nor to some inhibition of insulin release since dF was rather shown to possess insulin-like properties [29, 43, 49]. As a result, one can assume that, in vivo, some central action of dF activates either the sympathetic and adrenomedullary system [30], or a hypothetical lipolytic agent [40] by means of which lipolysis and lipo-utilization may be enhanced.

TABLE 5

RELATIONSHIP (±SE) BETWEEN LOG (FI) AND THE CHANGES IN RESPIRATORY QUOTIENT ASSOCIATED WITH ACTIVITY (dRQ/ULA) AND THE ENERGY COST OF LOCOMOTOR ACTIVITY (dTMR/ULA)

	n	Intercept	Slope	p
LOG(FI) vs. dTMR/ULA	18	3.45 (0.24)	−1.61 (0.26)	
& vs. dRQ/ULA	18		−2.16 (3.44)	<0.001
LOG(FI) vs. dTMR/ULA	18	3.45 (0.24)	−1.70 (0.21)	<0.001

Result shows that FI decreases as a function of the increase in the energy cost of activity.

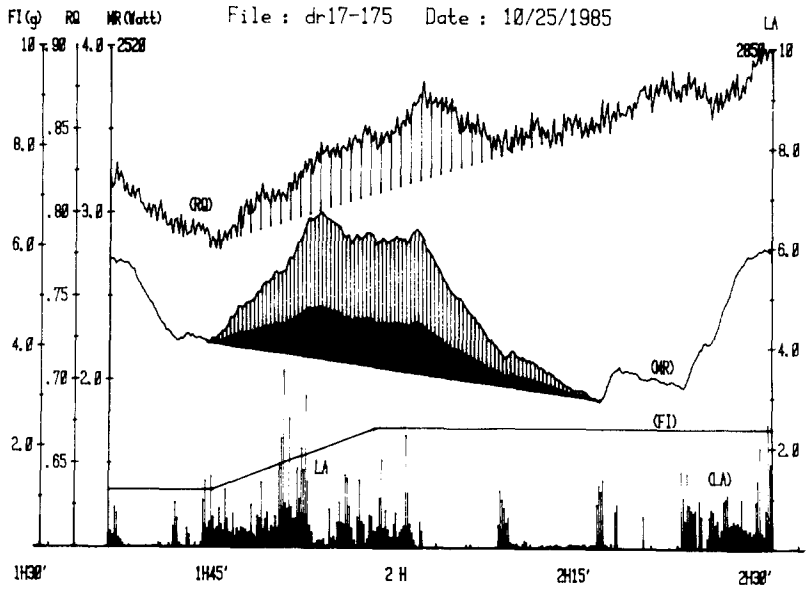

FIG. 7. An example of measurement of the metabolic increase observed in relation to a 1.23 g meal in a 1.75 mg/kg-treated rat. Discrimination between the contribution of feeding and LA in the increase of TMR was calculated using the energy cost of LA reported in Table 8, i.e., δTMR/ULA was introduced as equal to 0.76 watt/ULA. It resulted that of the total 864 kJ expended in relation to feeding, 521 kJ were due to LA and 343 kJ were due to the 1.23 g meal, i.e., 279 kJ/g. Legends same as in Fig. 5. Top tracing, vertical shading: δRQ induced by LA and feeding. Center tracing: total metabolic increase (864 kJ). Vertical shading: part devoted to LA (521 kJ); solid area: part devoted to feeding (343 kJ).

This experiment also confirms the reduction of LA under dF treatment [16, 17, 48, 50, 51] and brings further information on the possible causes and consequences of the increased energy cost of LA under dF treatment. It was shown that increased energy cost of LA was proportional to the dose of dF injected and mostly related to dF-induced RQ increases during LA. Furthermore, it appeared that the anorexia was correlated with these phenomena.

Although cellular and enzymatic processes are insufficiently known, it is possible to speculate on the biochemical events that may support the simultaneous increase of both thermogenesis and RQ during muscular effort. The following observations in the intact animal as well as in isolated muscle preparation may suggest that this phenomenon is due to the initiation by dF of glucide-lipid recycling during muscular effort. Under dF treatment, it was reported that the increased glucose uptake by the muscle [5,25] was neither accompanied by storage under glycogen form [26], nor by increase in CO_2 production in the muscle [24]. Only lactate content was increased within the muscle [27]. From these data, it seems that the glucose taken up by the muscle is not stored, but degraded in the glycolytic pathway. The accumulation of lactate may suggest that the citric acid cycle does not catabolize completely all the pyruvate provided by glycolysis because more glucose than is required for the muscular effort enters the myocytes. Therefore, lactate has to be recycled (via the hepatocytes?) towards either glucose (gluconeogenesis) or lipids (lipogenesis) since dF was shown to increase insulin secretion during exercise [46]. Such a biochemical scheme could account for the simultaneous increase of both RQ and energy expenditure in relation to LA. If so, the dF-induced exaggerated increase in both RQ and metabolic rate should occur after some delay since it is not the energy cost of muscular effort by itself that is increased,

TABLE 6

RESULT OF THE REGRESSION THROUGH THE ORIGIN ON THE PERIPRANDIAL INCREASE IN TEE (JOULES) BY MEAL SIZE

	Slope*	Cor. Coef	F	p lev.
Control	721.3 (4.7)	0.75	F(1–37)=235	$<10^{-6}$
dF Treated	690.9 (6.3)	0.74	F(1–20)=119	$<10^{-6}$

*Slope ⟷ Extra energy produced per gram of food ingested.

TABLE 7

RESULT OF THE REGRESSION THROUGH THE ORIGIN OF MEAL SIZE ON THE PERIPRANDIAL LA INTENSITY

	Slope*	Cor. Coef	F	p lev.
Control	64.9 (4.8)	0.70	F(1–37)=184	$<10^{-5}$
dF Treated	47.4 (6.1)	0.58	F(1–20)=061	$<10^{-3}$

*Slope ⟷ Units of locomotor activity developed per gram of food ingested.

TABLE 8

RESULT (±SE) OF NON-LINEAR MULTIPLE REGRESSION OF THE INCREASE IN TEE IN RELATION TO THE PERI-PRANDIAL ACTIVITY AND MEAL SIZE

	J/ULA	J/(g of food)	Cor. Coef	F	p lev.
Control	7.8 (1.0)	212.2 (70.8)	0.91	F(37–2)=348	$<10^{-6}$
dF Treated	8.8 (1.3)	275.2 (72.4)	0.92	F(20–2)=213	$<10^{-6}$
All rats	7.6 (0.8)	246.9 (53.8)	0.90	F(57–2)=525	$<10^{-6}$

None of the regressions differ at the 0.05 level.
J/ULA: Joules expended per unit of locomotor activity.
J/(g of food): Joules expended per gram of food ingested.

but rather the extramuscular metabolic rate during and after locomotion (energy cost of the recycling of lactate). We tested this hypothesis by running on the original data, TMR and LA, a very recently developed computer software of numerical filtering [22, 35, 41]. This software performs a continuous comparison between the results provided according to a modelization of the system and the results experimentally observed and extracts the energy expenditure specifically devoted to LA. An example of this method in dF-treated rats is shown in Fig. 8. In agreement with the above hypothesis, this method of computation showed that the energy cost of LA was not modified by dF treatment, but rather, that resting energy expenditure was increased with a delay comparable to that actually observed for RQ. Such an observation may further support the hypothesis that non-locomotor organs such as the liver may be involved in the apparent increase of energy cost of muscular effort.

The present data on the thermic effect of feeding result from a large improvement on the method of computation which allowed a precise discrimination between the respective roles of LA and feeding in the production of the post-prandial increase in energy expenditure. They contradict the previous data in the literature [44,45] including our own reports [18] since they show that the increase in energy expenditure in relation to feeding was (a) identical in saline- and dF-treated subjects when total energy expenditure was taken into account and (b) 30% higher in dF-treated subjects if energy cost of LA was taken into account, but this increase did not reach significance. However, as the present data were collected from freely-feeding rats, the occurrence of repeated meals did not allow the study of long-delayed responses (more than 40 minutes post-meal). Thus, these results do not allow us to conclude on a possible influence of dF on the production of extra-energy expenditure related to feeding. But, the fact that more than 60% of the post-prandial total increase in energy expenditure was specifically due to LA points out that it is very important to take LA and energy cost of LA into account when measuring energy expenditure in relation to feeding. The fact that dF treatment influences both the cost and the amount of LA makes measurement of this parameter even more important.

Finally, the present data also evoke some speculation on the mechanism of dF-induced anorexia other than its central serotonergic action.

The 1.75 mg/kg-treated subjects show the previously reported effect on meal size only [2,42]. According to the classic theories, decreased feeding may be due to increased rate of utilization either of lipids (lipostatic hypothesis [7, 23, 31]), of carbohydrates (glucostatic hypothesis [3, 13, 34]) or of both lipids and carbohydrates (ischymetric hypothesis [8, 15, 37–39]). The present data confirm that dF enhances the release of inner fat stores and prompt their cellular utilization during the entire period of anorexia. This allows us to suspect that the satiating effect of dF is mediated by a lipostatic mechanism. On the other hand, as pointed out above, the LA associated with feeding results in a supplement of energy production and so in an increase of metabolic rate. This prandial thermogenic supplement is not due to ulilization of lipids since it is accompanied by a clearcut elevation of RQ, i.e., more carbohydrates are being used when satiety occurs. This double increase in the rate of utilization of both lipids (resting periods) and carbohydrates (active periods) was proposed by the ischymetric hypothesis to be one of the factors of satiety [19]. Therefore, the present data also support the idea that dF enhances satiety via an ischymetric mechanism, i.e., via hypermetabolic state at the expense of both glucose and lipids.

In conclusion, the metabolic correlates of dF treatments have been studied and provided results which aid in the understanding of the way dF acts to induce anorexia and body

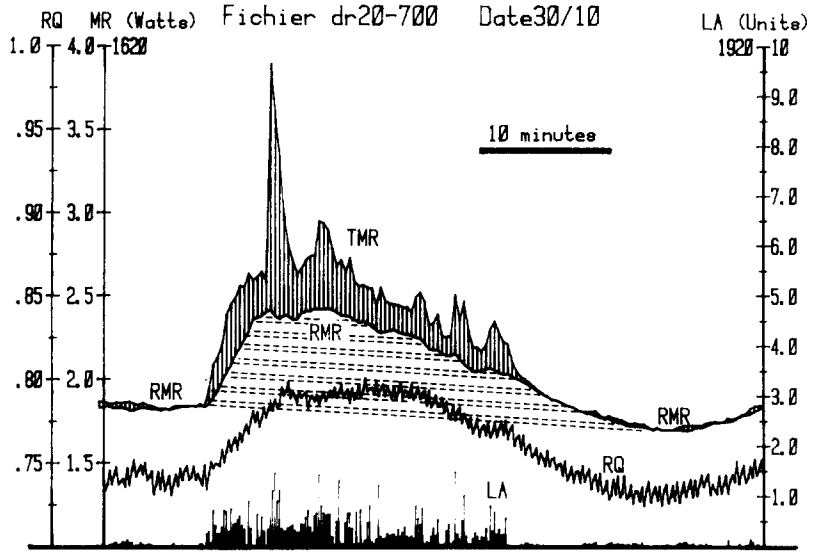

FIG. 8. Extraction of the energy cost of the locomotor activity after filtering of the data according to the method of Kalman. The period is the same as in Fig. 5. This resulted in a metabolic signal in which energy cost of LA remained low while the "resting" energy expenditure during LA increased in parallel with the RQ increase. Legends same as in Fig. 5. Shaded area: total metabolic increase. Vertical shading: part devoted to LA; horizontal shading: part devoted to increase in RMR.

weight loss. It was clear that some of the peripheral effects of dF are closely related with the anorexia and may be responsible for it in the framework of the lipostatic and the ischymetric hypothesis. Furthermore, the increase in energy expenditure during ordinary activity (and may be also during rest) improve the effect of anorexia on body weight loss. This dual effect (decreased energy intake plus increased energy expenditure) is the most efficient body weight loss or leptogenic mechanism.

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