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Dextrofenfluramine[1] Increases Energy Cost of Muscular Effort

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EVEN, P AND S NICOLAIDIS *Dextrofenfluramine increases energy cost of muscular effort* PHARMACOL BIOCHEM BEHAV **24**(3) 647–655, 1986 —A peripheral action of dextrofenfluramine (d FF) was investigated The effects of d FF on total energy expenditure (TEE), locomotor activity (LA), and respiratory quotient (RQ) were quantitatively monitored for 22 hours by a computerized metabolic device The precise temporal evolution of RQ allowed calculation of glucidic versus lipidic substrates used in all instances It appeared that in d FF-treated rats, TEE and resting energy expenditure (REE) were not significantly changed, RQ and LA were significantly decreased Moreover, in d FF-treated rats, LA induced a two to five fold increase energy expenditure over vehicle-treated control subjects and it was observed that there was an LA related increase in RQ which was not observed in control subjects Therefore, d FF causes LA to be a highly inefficient process by inducing what seems to be an exaggerated catabolism of glucides These may be only partially used for muscular contraction because it was calculated from relative changes in RQ and TEE during LA that 70% of the catabolized glucides seems to be diverted toward lipogenesis This process probably represents the way futile cycles are triggered by d FF in order to exacerbate LA associated energy cost

Dextrofenfluramine Total energy expenditure Locomotor activity Respiratory quotient Energy cost of locomotor activity

TREATMENT with fenfluramine (FF) is well known to result in a reduction in body weight (BW) [1, 12, 23, 32, 44] and it is generally considered that this negative energy balance is achieved by means of a relative reduction of food intake since FF has a strong anorexigenic effect [1, 3, 4, 9, 44] However, various studies have raised the possibility that, in addition to its anorexigenic effect, FF may affect the rate of thermogenesis as its administration is accompanied by a rise in core temperature [2, 35, 36, 38], and an increase in the thermogenesis associated with feeding [35,38] These observations raise the possibility that FF might induce BW reduction, at least in part, through its effect on thermogenesis It might also be that the anorexigenic effect of FF could be (at least partially) the consequence of the increased energy expenditure [7, 23, 26, 32, 46] We have proposed that the physiological signal of satiety is generated from the rate of basal power production itself, that is the ischymetric hypothesis (IM) [29] According to this hypothesis, FF will increase IM and thus augment satiation (or decrease total FI)

In addition to the hypothesis that FF may reduce BW by increasing energy expenditure, there is the subsidiary question of how FF is able to affect the rate of energy expenditure In particular, does FF affect the resting metabolism or the meal associated extra losses only, or could it be possible that this compound modifies the energy efficiency of various biochemical processes [17, 20, 21, 22, 23, 25, 31, 37, 39, 40, 43, 45] and consequently those of muscular contraction

In order to investigate these questions, we used an original computerized metabolic device which, in addition to measuring O_2 and CO_2 exchanges, was designed to precisely quantify the locomotor activity (LA) and to compute the metabolic surplus corresponding to moment to moment level of LA By examining the relation between rest, movement and power production, we were able to show that dextrofenfluramine affects energy expenditure by increasing the energy cost of LA Some additional observations on the nature of metabolites used in resting and in locomotor conditions were also made by monitoring the animal's respiratory quotient (RQ) RQ is the CO_2 production/O_2 consumption ratio of the total animal It is well established that this ratio depends on the chemical composition of the oxidized molecule, and particularly that it decreases toward 0 7 when lipids are used and increases up to 1 0 when carbohydrates are used

METHOD

Animals and Housing

Experiments were carried out on 13 adult male Wistar rats with an initial weight of 313±14 g Before the experimental procedure, the animals were housed in individual wired cages in a room maintained at 24°C Lights were on from 08 00 hr to 20 00 hr Standard chow (extralabo M25) and water were available ad lib throughout the experiment

Data Acquisition

The experiment consisted of 22 hour periods during which behavioral and respiratory parameters were monitored

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TABLE 1

FOOD INTAKE AND BODY WEIGHT GAIN, (±SD) DURING THE 22 HOURS OF RECORDING SESSIONS

	Body Weight gain (g)	Food Intake (g)
Control group	+8 4 (3 4)	24 8 (5 1)
d FF Treated Group	−10 8 (4 0)	3 04 (3 1)
Value of p (M and W U Test)	0 001	0 001

Temperature in the cage was maintained at 29°C Details of structure and function of the metabolic devices, and methods of calculations have been published elsewhere [14,15] The calorimetric chamber monitored by an on-line computer (Hewlett-Packard 9835A) provided a semi-continuous (1 sample every 10 seconds) numerical record of the following parameters

(a) Mass sensitive air flow through animal's cage (Precision Flow device, 2SLM),

(b) Oxygen (O_2) consumption (oxygen S-3A analyser, Applied Electro Chemistry Inc),

(c) Carbon dioxide (CO_2) production (CO_2 analyser Infralyt 4, Veb Junkalor Dessau),

(d) Ambiant humidity (humidity indicator HMI 11, Vaissala),

(e) Variations of pressure produced on the platform by the rat's movements (integration of 3 piezo-resistive strain-gauges Kiag-Swiss arranged as a triangle under the rat's living platform) (Resolution=0 1 gf),

(f) Weight of the food cup (±0 01 g micro-scales Schlumberger),

(g) Cage ambient temperature (electric thermometer, laboratory made thermo-couple)

Data Computation

Using these measurements, the following parameters were calculated on-line by the computer

(1) Respiratory quotient (RQ), expressed as the ratio of CO_2 release over O_2 consumption,

(2) Total energy expenditure (TEE) expressed in watts from oxygen consumption and RQ's value using Lusk formula [5,24],

(3) Meal pattern, expressed as the variations in grams of the weight of the food cup,

(4) Quantitative intensity of locomotor activity (LA), expressed in arbitrary units as the integration over time unit of the electric signal produced by the strain gauge (N B if required, these units can be converted into watts following calibration) Because of the extreme sensitivity of the strain gauges, the signal recorded is filtered from environmental vibrations (pumps, motors, steps) (Low pass filter 25 Hertz),

(5) Energy cost of LA, expressed in watt/unit from simultaneous relative variations in intensity of TEE and LA

Analysis of Respiratory Quotient

The RQ of any individual foodstuff can be determined from the equation representing its oxidative breakdown to the final products which are H_2O and CO_2, as shown in the following examples taken from Lusk [5,24]

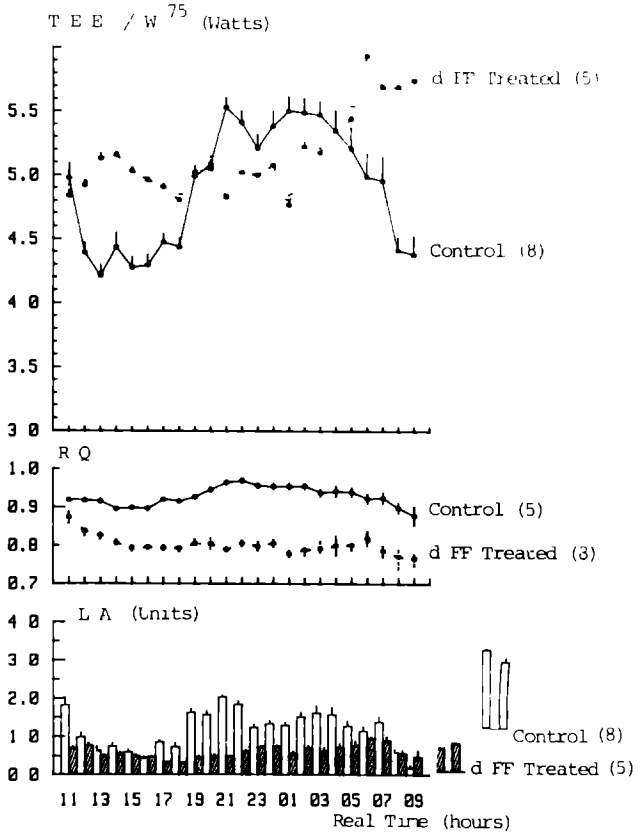

FIG 1 Hourly evolution (±SEM) of total energy expenditure (TEE), respiratory quotient (RQ) and locomotor activity (LA) throughout the 22 hours of the recording sessions

Carbohydrates

General formula $(CH_2O)n$ Example for glucose

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$

$$RQ = 6 \text{ vol } CO_2/6 \text{ vol } O_2 = 1\ 00$$

Fat

In the oxidation of this substance, extramolecular oxygen is required not only for the oxidation of carbon but also for the oxidation of hydrogen For this reason RQ is less than 1 00 and usually reaches 0 70 Example for Tripalmitin

$$2(C_{51}H_{98}O_6) + 145\ O_2 = 102\ CO_2 + 98\ H_2O$$

$$RQ = 102 \text{ vol } CO_2/145 \text{ vol } O_2 = 0\ 703$$

Protein

Calculation of the RQ of protein oxidation is more complex, but it is close to 0 80

Thus, monitoring the RQ of whole animals allows the detection of preferential use of lipids (low RQ) or of glucides (high RQ)

Experimental Protocol

The 13 rats were studied in random order At 10 00, eight

TABLE 2

MEAN VALUES (±SD) OF TOTAL ENERGY EXPENDITURE, RESPIRATORY QUOTIENT, AND LOCOMOTOR ACTIVITY, AVERAGED THROUGHOUT THE 22 HOURS OF RECORDING SESSIONS

	En Exp /$W^{0.75}$ (Watts)	Resp Quo	Loct Act /10 sec/100 g (Units)
Control (total)	4 94 (0 302)	0 930 (0 044)	3 37 (0 87)
d FF Treated (total)	5 18 (0 906)	0 802 (0 054)	2 26 (0 23)
Value of p (M and W U Test)	0 222	0 004	0 004

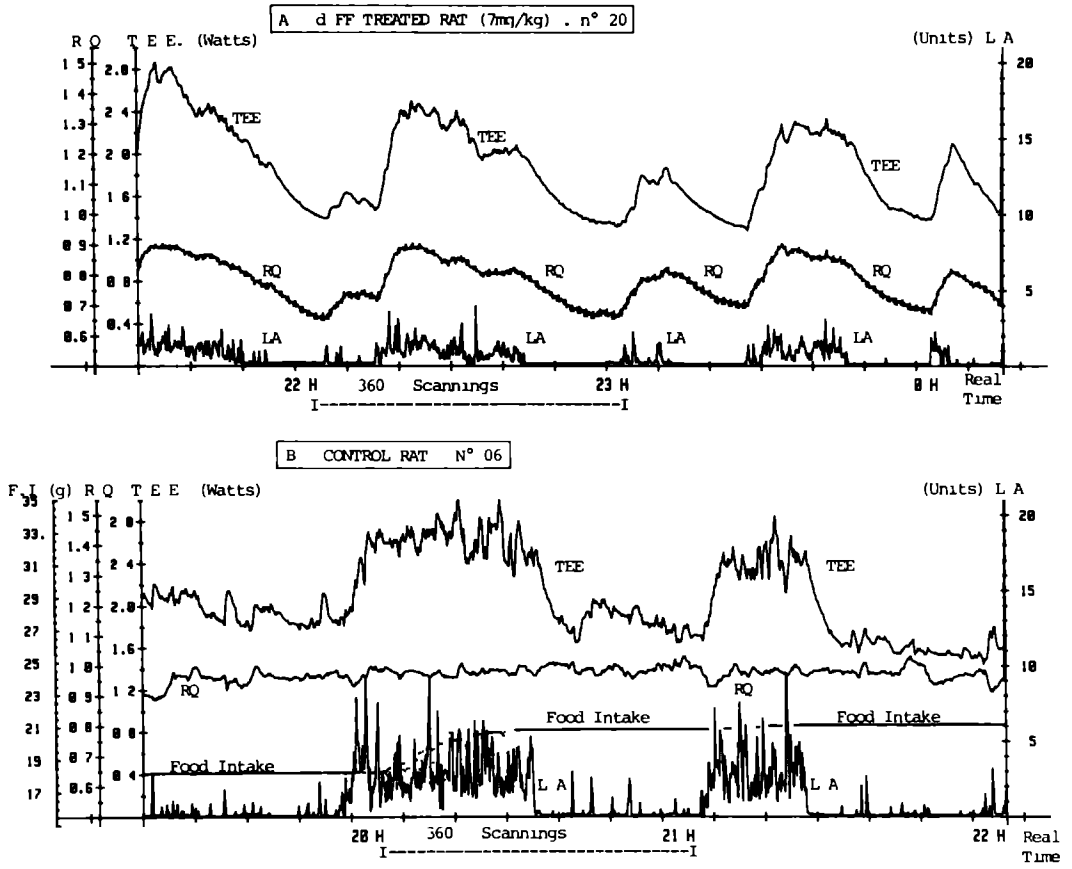

FIG 2 Two individual examples (control vs d FF-treated) of total energy expenditure (TEE) and respiratory quotient (RQ) changes in relation to locomotor activity (LA) as they can be observed between 4 and 20 hours after the injections

of them received a control IP injection of 0 5 ml of saline and five of them an IP injection of d FF (7 mg/kg) diluted in 0 5 ml of saline Injections were made just before the rats were housed in the calorimeter and measurements were started 30 min later A dose of 7 mg/kg was chosen as preliminary experiments had demonstrated that this dose evoked a clear anorexia reducing the 24 hours consumption by 60% the first day of treatment

Statistical Significance Test

Comparison between control and experimental values of

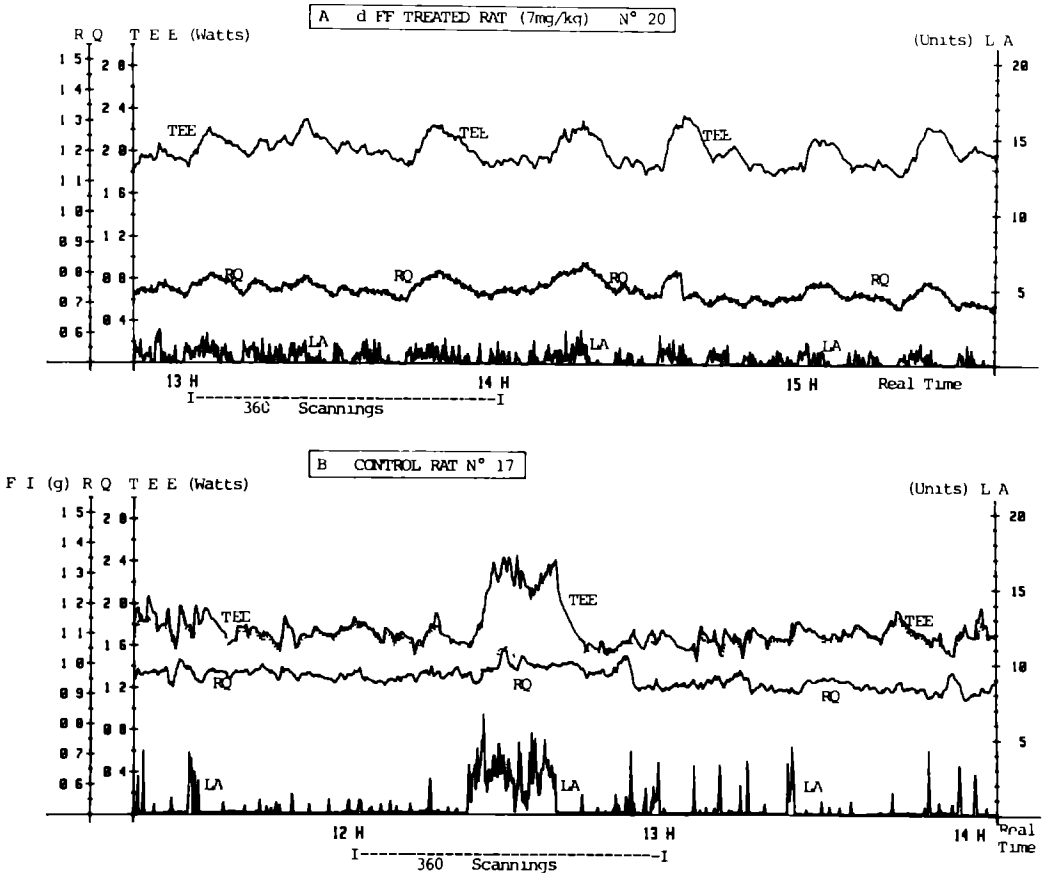

FIG 3 Two individual examples (control vs d FF-treated) of total energy expenditure (TEE), respiratory quotient (RQ) and locomotor activity (LA) as they can be observed between 0 and 4 hours after the injections

FI, TEE, REE, RQ, and LA were done using Mann and Whitney U test (two-tailed)

RESULTS

Feeding and BW

The dose of 7 mg/kg d FF reduced the mean 22 hour intake from 24 8 g in control to 3 04 g in treated rats (Table 1) None of the d FF-treated rats ate during the first 19 hours after treatment The BW of the d FF-treated group was reduced by 10 8 g compared with an 8 4 g of increase of BW of the C group (Table 1)

Locomotor Activity

The C subjects showed the usual circadian nocturnal hyperactivity and diurnal hypoactivity (Fig 1) Locomotion occurred as discrete episodes and alternated with periods of rest, including sleep Meals were usually preceded and followed by periods of locomotion (Fig 2B) In contrast, the d FF-treated animals showed an overall reduction in activity in both the dark and light phases Furthermore, in these animals locomotion was more continuous and of low intensity (2 26 units/10 sec/kg (0 23 SEM) as compared with 3 37 units/10 sec/kg (0 87 SEM) in C subjects) (Table 2) The effect of d FF on LA was maximal during the first three hours post-injection (Fig 3) During the following period (4–22) locomotion partially recovered its normal uneven pattern (Fig 2) However, the activity periods remained shorter and weaker and were usually not associated with feeding (Figs 2, 4)

Total and Resting Energy Expenditure

The 22 hour averaged values of TEE and REE of both groups were not significantly different (Tables 2, 3) However it must be noted that four of the five d FF-treated rats showed increased TEE and REE On the other hand, the L/D profile of TEE of the two groups showed remarkable differences (Fig 1) One hour after the injections, the total LA of both groups were similar and this lasted until 17 00 hr During this period of similarly low LA in both groups there was a striking increase in TEE in the treated subjects, thus d FF dramatically increased the overall energy expenditure during the first 7 to 8 hours From 17 00 onwards, the differences in the profiles of TEE in the d FF and C groups were due essentially to increases in LA and feeding of the C subjects However, short bursts of LA were also observed in the d FF-treated group and they allowed us to study the energy cost of these locomotor events in comparison to equivalent locomotor events of the C group

Energy Cost of Locomotor Activity

Analysis of the data showed that the cost of LA in d FF-treated subjects was dramatically increased It reached 2 258

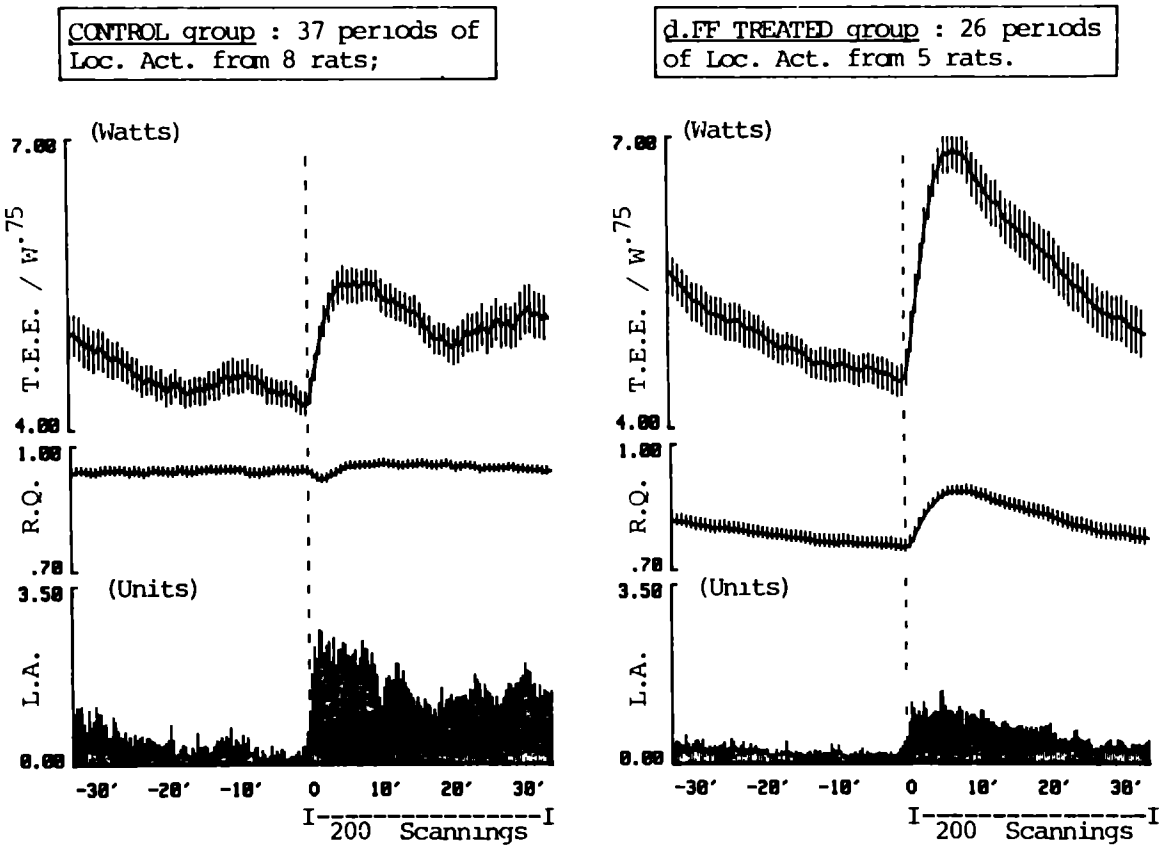

FIG 4 Mean evolutions of total energy expenditure (TEE ±SEM), and respiratory quotient (RQ ±SEM) in relation to locomotor activity (LA) in the control and the d FF-treated groups

TABLE 3

MEAN VALUES (±SD) OF APPROACHED RESTING ENERGY EXPENDITURE AND RESPIRATORY QUOTIENT, AVERAGED THROUGHOUT THE 22 HOURS OF RECORDING SESSIONS, FROM PERIODS IN WHICH NO BURSTS OF LOCOMOTOR ACTIVITY WERE MEASURED

	En Exp /$W^{0.75}$ (Watts)	Resp Quo	Loct Act /10 sec/100 g (Units)
Control (resting)	4 29 (0 262)	0 914 (0 042)	0 976 (0 47)
d FF Treated (resting)	4 57 (0 751)	0 771 (0 060)	0 992 (0 53)
Value of p (M and W U Test)	0 170	0 004	—

Residual values of locomotor activity are due to permanent small movements, heart beating, and breathing recorded by the strain gauges

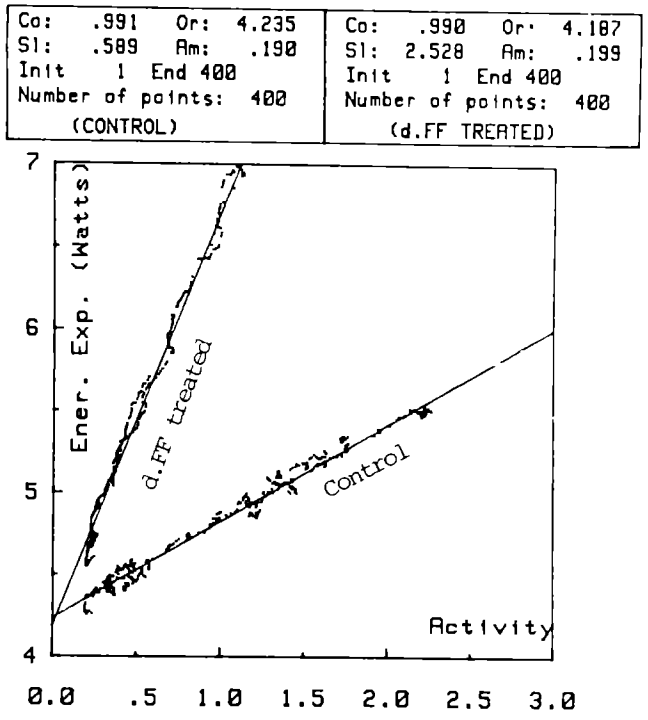

FIG 5 Total energy expenditure/locomotor activity linear regressions computed from data Fig 4 The slopes of the regressions express the energy cost of locomotor activity

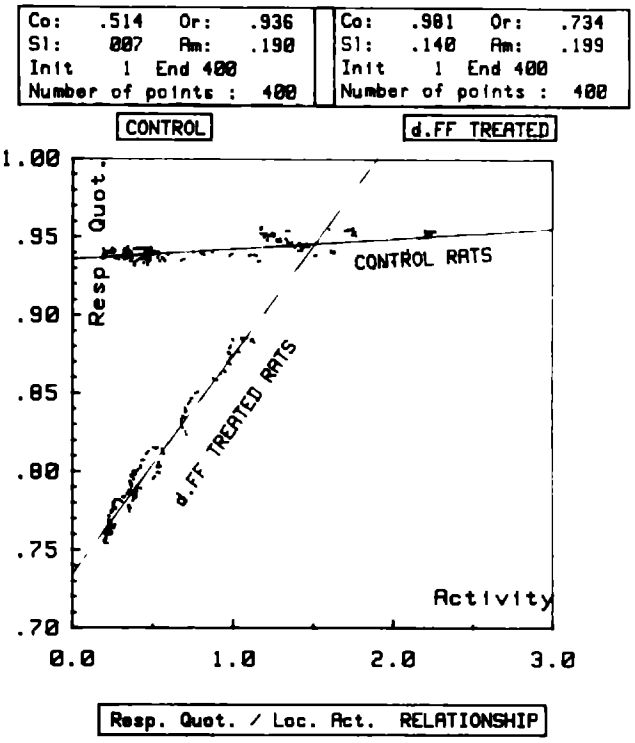

FIG 6 Respiratory quotient/locomotor activity linear regressions computed from data of Fig 4 The slopes of the regressions express the amplitude of the increase in RQ associated with locomotor activity

watts/weight$^{0.75}$/unit of LA in the treated subjects vs 0 589 watts/weight$^{0.75}$/unit of LA in C subjects (Figs 4, 5) An individual record of the short term profile of LA bursts and the corresponding supplement of energy expenditure in C and treated individuals (Fig 2) clearly illustrates this phenomenon In this figure, the LA-elicited increases in TEE were chosen to be equivalent in the treated and C individuals in order to show that the corresponding bursts of LA were at least three times less intense in the d FF-treated subjects than in the C subjects This phenomenon of increased energy cost of LA of treated subjects could be seen even when their bursts were compared with a burst of the C subject which occurred during a 2 61 meal, despite the fact that meals are known to bring about an additional increase of energy expenditure [14, 28, 30]

The energy cost of LA was rather constant in the C subjects [15] but was quite variable in d FF-treated subjects, ranging from 2 to 5 fold greater than control value

Respiratory Quotient (RQ)

In contrast to the RQ of C subjects, d FF dramatically and persistently decreased the RQ of treated subjects, indicating a massive mobilization and utilization of animal's lipid reserves (Fig 1) (Table 2) It is also instructive to examine the short term fluctuations of RQ associated with bursts of LA Figures 2, 4 and 6 show that in C subjects the usual bursts of LA had either no effect or a very weak effect on the RQ In contrast to this stability, the RQ of d FF-treated rats showed dramatic LA-associated fluctuations As soon as LA episodes started, the initially low RQ of close to 0 70 increased toward 0 9 (Figs 4, 6) This phenomenon is also obvious when Table 2 is compared with Table 3 It appears that the substraction of a large part of the periods exhibiting LA produces a simultaneous decrease of the 22 hours averaged value of the RQ in the d FF-treated group When the RQs of all the treated subjects were pooled together, the low figures were 0 755 (0 049 SD), vs 0 930 (0 022 SD) in C subjects In treated subjects during episodes of LA, RQ rapidly reached an average of 0 885 This RQ increase was proportional to the increase of LA and of TEE (Figs 5, 6) The RQ increase, specifically due to LA associated energy expenditure (RQ'), can be calculated by the following equation,

$$(RQ' \times 2\ 32) = (0\ 885 \times 6\ 81) - (0\ 755 \times 4\ 49) = 2\ 637$$

thus

$$RQ' = (2\ 637/2\ 320) = 1\ 137$$

with 2 32=mean change of TEE from rest to LA, 0 885=mean RQ value during LA, 0 755=mean RQ value during rest, 6 81=mean TEE value during LA, 4 49=mean TEE value during rest

The fact that the value of RQ is 1 136 is certainly due to the fact that, during LA episodes, glucose oxidation by the muscle (RQ=1) occurs simultaneously with a metabolic process of lipid synthesis (RQ=1 2) [5,34] The part of glucose

directly oxidized by the muscle and the part used for a lipogenetic process can be calculated as if the observed value of RQ' (1 136) was the result of these two simultaneous processes corrected by the percentage of glucose respectively used for each of them Therefore,

$$1\ 14 = (X \times 1\ 0) + ((100-X) \times 1\ 2)$$

where X=% glucose used for energy production and providing an RQ value of 1 0,
and 100−X=% glucose used for lipogeneses and providing an RQ value of 1 2
As a result, X=30% and (100−X)=70%

From the above, it appears that, during LA episodes, 30% of glucose is taken up into the muscle and 70% is diverted toward lipid synthesis This diversion of a large part of the glucose towards lipogenesis is compatible with the increased energy cost of LA in the d FF-treated rats

DISCUSSION

This research has revealed a previously unreported property of dextrofenfluramine, that is, an increase in the cost of muscular contraction On the other hand, these data confirmed previous reports of decreased LA under FF treatment [40, 41, 47, 48], and are in agreement with a recent study by Schuster and Levitsky who reported that FF does not produce extra energy losses, at least in the absence of FI [35] The data obtained in these experiments did not confirm however previous reports of increased energy expenditure due to d FF treatment [23, 32, 38] However, it seems that most of the parameters involved in the total energy production (REE, FI, intensity of diet induced thermogenesis, LA, energy cost of LA, thermogenesis) are modified by FF, and it might be that the magnitude of the modifications depends on the dose of FF injected as well as on the duration of the treatment The fact that none of the experiments performed hitherto has fully controlled all of these parameters may certainly account for the discrepancies in the previously published results

In addition to the LA-associated extra energy losses, an augmented TEE can be observed even during the resting period since the metabolic rate was still higher in the treated group (Fig 1) between 2–8 hours post-treatment when the LA of both C and treated group was minimal Despite this difference in TEE it cannot be definitely stated whether d FF increases either basal or resting metabolism because even during the period of minimal activity, small but almost continuous movements were always recorded in the treated subjects This residual LA may well account for the overall augmented metabolic rate since the energy cost of muscular contraction is dramatically exaggerated by d FF

The increase of TEE of the C group during the dark period, in relation to increased LA and FI, nearly compensates for the increased TEE of the d FF-treated group during the light period, thus resulting in an almost equivalent energy expenditure of the two groups along the 22 hour recording sessions This is also reported for REE in Table 3 It is, however, impossible to conclude that REE is also the same for the two groups because it appears that "resting" periods nevertheless provide non negligible values for LA If theoretical REE's were computed from the approximative REE's values of Table 3 by using the slope of the activity-energy expenditure relationship computed in Fig 5, the REE of the d FF-treated group would be 3 912 watts and REE of the C group would be 4 090 watts The REE value thus appears to be reversed, but the difference between the groups remains very weak and tends to confirm that energy expenditure measured at the level of the TEE and REE seems to be similar in both groups Furthermore, it is not certain that the relationship remains linear at this very low level of activity

From the present data of augmented energy expenditure during LA, it can be suggested that d FF causes a reduction in energy efficiency of metabolites used in muscular contraction Although this interesting observation requires further biochemical investigations, data from the present study shed some light on the underlying mechanism Firstly, d FF rapidly results in a permanently lipolytic metabolism (fall of RQ to 0 75) indicating massive utilization of endogenous fat reserves This permanently low RQ is promptly switched upwards during LA, tending to reach values close to 0 9 which indicates a switch towards the utilization of a carbohydrate substrate for muscular contraction Such changes of RQ in association with LA are not observed in untreated subjects ([19] and P Even, unpublished observations) Moreover the rise of RQ in treated subjects expresses an exaggerated consumption of glucidic substrates, and it is calculated that only 30% of these glucides are utilized as fuel for power production The remaining 70% of the catabolized glucides can be accounted for by lipid synthesis This LA-induced lipogenesis is proportional to the increase of TEE produced by LA It appears that the primary event is the initiation of an exaggerated glycolytic tendency which, above a threshold, results in the shifting of the excessive glucidic catabolites towards a lipid "sink" Exaggerated glucose catabolism in vivo in muscle perfused with FF, and the concomitant exaggerated amount of insulin [3, 4, 5, 6, 9, 10, 17, 32, 37] argue in favor of the idea that d FF engages futile cycles that ultimately result in unnecessary glycolysis and lipogenesis Our data are also consistent with previous reports of increased glucose uptake, complete aerobic metabolism, and absence of glycogen storage in treated tissue [8, 17, 20, 21, 22, 23, 37, 40, 45]

The question remains whether the effects related to muscular contraction are directly related to the treatment or a consequence of the d FF-induced food deprivation and/or drop of RQ Previously reported data by Heusner [18,19] have established that various RQ levels due to circadian metabolic events or to starvation do not affect the cost of LA That low levels of RQ are not themselves affected by the energy cost of LA has been repeately confirmed in our laboratory (Even, unpublished data) and also appears in the data presented in this article, i e , in C subjects the LA-associated energy production remains unchanged during the early post-injection period (4 hours), when feeding does not occur and RQ approaches low lipolytic values Dealing specifically with RQ, since the initial value of RQ was low (0 75) in the d FF-treated group, one could suspect its increase during LA to originate from some LA-associated glucose combustion This does not seem to be the case according to the calculations that resulted in a LA-associated RQ greater than 1 0 [1,14] In addition, Heusner has shown that in rats which had no access to food for 24 hours (which resulted in a RQ of approximately 0 75), LA did not result in any changes of RQ [19] Moreover, we have observed in ad lib fed rats (P Even, unpublished observations) that when initial resting RQ value is around 0 75 (low values of RQ can be transitorily observed during the light period), locomotor events did not seem to produce any changes in RQ despite its initial low

value (A study on 12 periods of locomotor activity showed a 0 02 increase of RQ per each unit of LA)

Whatever the biochemical mechanisms of the increased cost of LA, this property should be used in the d FF treatment of obesity Thus, dextrofenfluramine may be particularly efficient treatment if used in conjunction with a substantial amount of LA through which an extra amount of energy might be dissipated It may even be possible that those patients who do not lose weight under FF treatment are those who have a particularly low LA

This mechanism does not exclude the possibility that d FF also affects the basal metabolism the meal associated thermogenesis [35,38] and, of course, a direct and/or indirect central anorectic action [2, 3, 4, 7, 8, 11, 13 16] According to the ischymetric hypothesis which proposes that satiety is enhanced by high metabolic rates [30], the marked thermogenesis which accompanies the meal-associated LA may induce a premature offset of feeding Futile metabolic cycles engaged under the action of dextrofenfluramine might therefore be responsible for its well described action in reducing more specifically the size rather than the frequency of meal taking [3,4]

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