Effects of Dexfenfluramine on Free Fatty Acid Turnover and Oxidation in Obese Patients With Type 2 Diabetes Mellitus

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To test the potential effects of dexfenfluramine (dF) on enhancing free fatty acid (FFA) turnover and oxidation rates, 11 obese female non–insulin-dependent diabetes mellitus (NIDDM) outpatients (age, 52.5 ± 1.5 years; weight, 81.3 ± 3.2 kg; height, 158 ± 3.04 cm; body mass index, 32.4 ± 0.7 kg/m^2) received a primed-constant infusion of 1-^{14}C-palmitate. The waist to hip ratio (WHR) was 0.91 ± 0.04. Fat body mass and lean body mass, assessed by dual-energy x-ray densitometry, were 32.0 ± 1.5 and 49.30 ± 2.67 kg, respectively. All patients had an average hemoglobin A_1 of 6.3% ± 0.3% in the month preceding the study and had not received oral hypoglycemic agents. Gas exchange was measured both basally and during a ventilated-hood system, indirect-calorimetry session. The protocol was a randomized, placebo-controlled, single-blind design. Subjects received dF 30 mg acutely (n = 6) or a placebo (n = 5). A dose of dF 15 mg twice daily or placebo was then administered over 15 days (chronic). To obtain serum peak level of the drug, dF was administered 2 hours before starting palmitate infusion. A free diet was allowed throughout the study, and the group treated with dF lost approximately 0.5 kg body weight. Acute and chronic dF administration resulted in a significant increase in FFA oxidation, expressed as a percentage of the dose of radiocarbon (respectively, 11.47% ± 0.46% *v* 9.50% ± 0.46% [*P* < .01] and 12.06% ± 0.71% *v* 9.88% ± 0.62% [*P* < .01]). FFA turnover rate was higher after both acute and chronic dF administration (respectively, 10.71 ± 2.18 *v* 7.79 ± 1.48 μmol/kg/min [*P* < .05] and 11.92 ± 2.74 *v* 8.43 ± 1.86 μmol/kg/min [*P* < .05]). Serum FFA concentration during both acute and chronic dF administration increased, but not significantly. Mean serum glucose level decreased after acute dF from 114.3 ± 8.6 to 86.5 ± 5.1 mg/dL (*P* < .001) and after chronic dF from 120.3 ± 7.3 to 89.8 ± 5.8 mg/dL (*P* < .001). Serum insulin was not affected by dF administration. In conclusion, oral acute and chronic dF administration increase FFA turnover and oxidation rates in NIDDM obese patients. This may play an important role in weight reduction. In addition, dF shows a weight-independent effect on glucose metabolism, reducing serum glucose levels without acting on insulin secretion.

DEXFENFLURAMINE (dF), the dextro-optical isomer of fenfluramine, is a serotoninergic drug that appears to act not merely by reducing food intake but also by modifying food selection. In fact, clinical studies[1-4] have indicated that in obese patients, dF selectively reduces the consumption of foods with a high carbohydrate content that is maintained by carbohydrate craving. It has been suggested that the increased levels of serotonin in the brain mimic the effect of a carbohydrate-rich meal.[1] In animals[5] and humans,[6-10] it has been demonstrated that dF improves insulin sensitivity and glucose disposal regardless of the degree of weight loss. In a double-blind study[8] on 34 overweight patients with type 2 diabetes mellitus in which dF or a placebo was added for 12 weeks to metformin with or without a sulfonylurea, it was shown that dF was a valuable adjunct to the treatment of these patients.

A direct effect of dF on lipid metabolism has also been recently demonstrated, both in rats[11,12] and in man.[13] dF administration caused a sustained decrease in both the total metabolic rate and the respiratory quotient (RQ) in rats.[11,12] This phenomenon appeared to be dose-dependent like the dF-induced anorexia. Due to the decrease in RQ, the investigators proposed that dF could induce anorexia not only through a central mechanism but also by enhancing the release and utilization of free fatty acids ([FFA] lipostatic mechanism), and/or by increasing the total metabolic rate during locomotor activity (ischymetric mechanism).[11,12]

In obese patients of both sexes, similar to what was observed in animal studies, dF induced a significant increase of FFA turnover and oxidation.[13]

Few data are available in the literature concerning the effect of dF on lipid metabolism in non–insulin-dependent diabetes mellitus (NIDDM) obese patients. The present randomized, single-blind, placebo-controlled study was designed to test whether and to what extent dF increases FFA mobilization and oxidation in diabetic obese patients.

SUBJECTS AND METHODS

Subjects

Eleven white obese women with NIDDM, which had been diagnosed 2 to 3 years before enrollment, participated in the study. Body mass index was 32.4 ± 0.7 kg/m^2, and the mean age was 52.5 ± 1.5 years. None of the patients were treated with hypoglycemic agents. The baseline value for hemoglobin A_1 was 6.3% ± 0.3%. The waist to hip ratio (WHR) was 0.91 ± 0.04. Clinical characteristics of patients are listed in Table 1.

Lean body mass and fat body mass were measured by dual-energy x-ray densitometry.[14] Basal values for lean body mass and fat body mass were 49.3 ± 2.7 and 32.0 ± 1.5 kg, respectively.

All subjects were in good health as assessed by medical history, physical examination, and routine hematologic, biochemical, and urine analysis. The study took place after a 15-day run-in period, with the aim being to ascertain body weight stability of subjects included in the protocol; a change in body weight of ±1 kg was one of the exclusion criteria. During the trial, patients were maintained on a free diet. Before beginning the experimental procedure, the following analyses were performed to allow definite inclusion into the study: serum creatinine, aspartate aminotransferase, alanine

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Table 1. Clinical Characteristics of Obese NIDDM Patients

Patient No.	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/cm²)	FBM (kg)	LBM (kg)
1	59	80	152	34.7	27	39.5
2	59	75.4	148	34.5	25.3	40
3	51	86	161	33.2	35.5	50.5
4	49	74	152	32.1	32	42
5	47	101.3	175	33.1	36.9	64.1
6	49	95.7	171	32.7	35.2	62.3
7	47	87.3	171	29.9	28.3	58.7
8	50	64	148	29.2	33.6	48.4
9	58	74	153	31.7	26.5	46.2
10	58	74.5	149	29.1	20.6	41.4
11	50	82	158	36.8	31.5	
Mean	52.45	81.3	158	32.1	30.2	49.3
± SD	4.9	10.7	10.1	2.8	5.1	8.9

Abbreviations: BMI, body mass index; FBM, free body mass; LBM, lean body mass.

aminotransferase, fasting blood glucose, hemoglobin A_1, triglycerides, and cholesterol.

The protocol was approved by the Ethics Committee of the Catholic University School of Medicine in Rome. Written informed consent was obtained in all cases.

Experimental Protocol

On days 0 and 15, patients were admitted to the Internal Medicine Institute, Department of Metabolic Diseases, Catholic University, Rome, Italy. Six patients were assigned to the dF group and five patients to the control group (placebo) in a randomized, single-blind manner. In the acute phase (day 0), patients received dF 30 mg orally or an equivalent formulation of placebo. From days 1 to 15, patients assigned to the first group continued to be treated with dF 30 mg (15 mg twice daily) taken orally before meals, and the second group of patients received one capsule of placebo twice a day.

On days 0 and 15, after an overnight fast, subjects were placed in the study room after voiding at 8 AM. A catheter was inserted into an antecubital vein for tracer infusion; a dorsal hand vein was cannulated contralaterally in a retrograde fashion and placed in a heating device to facilitate sampling of arterialized venous blood. After 30 minutes of stabilization, a blood sample was taken for basal metabolite estimation. Solid palmitic acid (1-^{14}C) from Amersham (Amersham, England; specific activity, 54 μCi/mmol) was prepared according to the method reported by Nordenstrom et al.[15] The albumin-bound 1-^{14}C-palmitate was administered using a syringe infusion pump (Harvard Apparatus, Southnatick, MA) at a constant rate of 0.5 $\mu Ci \cdot min^{-1}$ for 70 minutes after a priming dose of 8 μCi. Blood samples were obtained every 10 minutes from the start of infusion for 180 minutes to analyze both labeled and cold FFA.

Indirect calorimetry was continuously performed by a Deltatrac (Datex Instrumentarium, Oslo, Norway) starting 45 minutes before beginning the experimental session. O_2 consumption ($mL/min \cdot m^2$), CO_2 production ($mL/min \cdot m^2$), and RQ were measured directly by a computer (Deltatrac). Expired air was collected over 2-minute periods at 10, 20, 30, 40, 70, 80, 90, 100, 110, 130, 180, 190, 260, 310, 320, 380, and 390 minutes after the start of ^{14}C-palmitate infusion. A solution of methylbenzethonium hydroxide 1 mol/L in methanol was prepared as previously described[16] and transferred into a bubbling device to trap $^{14}CO_2$ from the expired air.

All subjects underwent a 24-hour urine collection so that protein metabolism could be estimated through measurement of urea, creatinine, and uric acid excretion.[17]

Analytic Procedures

Blood samples were immediately centrifuged to separate the serum, which was then frozen and stored at −20°C until analysis. Fifty micrograms of erucic acid was added to 1 mL of each serum sample as an internal standard. Proteins were precipitated with trichloroacetic acid, lipids were extracted three times with 0.6 mL chloroform-methanol (2:1 vol/vol), and supernatants were collected together. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under a nitrogen stream. Single fractions of serum lipid extracted in this way were separated by thin-layer chromatography.

The plates were first developed in benzene-hexane (70:30 vol/vol), with the solvent front arriving 1 cm below the upper limit of the plates. Plates were then placed into a tank containing hexane-diethyl ether-acetic acid (70:30:1.8 vol/vol/vol) as a second solvent system. Elution was stopped once the solvent front had reached approximately 10 cm from the line of sample deposition. The different lipid fractions were evidenced by iodine vapors. Spots corresponding to the FFA standard were scraped off the plates and extracted with 3 vol chloroform-methanol (2:1 vol/vol). After evaporation of the solvent under a nitrogen stream, extracted solutes from serum FFA were dissolved in 1 mL acetonitrile-methanol (4:1 vol/vol) and added to 6 mg *p*-bromo-phenacylbromide dissolved in CH_3CN and 14 mL *N,N*-diisopropylethylamine as a catalyst. The mixture was heated to 60°C for 15 minutes. Aliquots from 20 to 40 μL were automatically injected into a liquid chromatograph (Hewlett Packard 1050, Palo Alto, CA) with an integrator and a scanning spectrophotometer operating in the 190- to 600-nm wavelength range (deuterium lamp) and equipped with an 8-μL flow cell.

FFA derivatives were separated on a reverse-phase column (25 cm × 4 mm ID; RP-18, 5-μm Supelcosil LC 18, Bellafonte, PA) according to the method reported by Passi et al.[18]

After a 5-minute isocratic elution with 60% CH_3CN in water adjusted to pH 3.10 with H_3PO_4, a gradient was performed at 100% CH_3CN for 60 minutes. The conditions were as follows: flow rate, 1 mL/min; absorbance, −0.3 to 1.000 absorbance units; absorbance noise, 2.5×10^{-5} absorbance units at 245 to 255 nm; chart speed, 0.25 cm/min; and UV detector operation wavelength, 254 nm.

A mixture of derivatized fatty acids (myristic, palmitoleic, palmitic, oleic, linoleic, steraic, and erucic acids) was used as a reference standard.

Fatty acids as *p*-bromophenacyl esters were fractionated by reverse-phase high-performance liquid chromatography, individually collected, and placed in counting vials in the presence of a standard scintillation solution.

Radioactivity of excreted $^{14}CO_2$ and serum high-performance liquid chromatography eluates were detected by a β-scintillation counter (Packard Tricarb 4691). Quenching was checked by the internal standard method.

Serum glucose concentration was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Fullerton, CA). Serum insulin concentrations were determined by the standard radioimmunoassay method.

Calculations

The time–plasma palmitate curves corresponding to the entire tracer infusion-postinfusion period were interpreted using a simple one-compartment model (Fig 1). The model (Fig 2) used is similar to the one on which Steele's equation[19] is based, and it directly allows the use of all measured serum radioactivity points. No enrichment steady state is assumed. The model parameters are the rate of appearance of palmitate (micromoles per kilogram per

$$\frac{dC}{dt} = \frac{\frac{R_i - (R_{as} W\, C)}{N}}{V_{ds}\, W}$$

$$C(0) = \frac{P}{V_{ds}\, W}$$

Fig 1. C = tracer concentration [μCi/L]; V_{ds} = specific volume of distribution [L/kg BW]; W = body weight (BW) [kg]; R_i = infusion rate of tracer [μCi/min]; R_{as} = specific turnover rate [μmol/min/kg BW]; N = NEFA concentration [μmol/L]; P = priming dose of tracer [μCi].

minute) and the volume of distribution of palmitate (liters per kilogram body weight).

The model assumes that for the duration of the experiment, the rate of appearance of palmitate equals its rate of disappearance, and therefore equals the turnover rate.

The estimate of the volume of distribution depends on the time allowed, in this case a few hours, for the equilibration of the substance with slow peripheral compartments. Such an estimate may be much larger than that obtained from the extrapolated initial concentration in a well-stirred single compartment.

Even though a previously published sampling schedule (points at 55, 60, 65, and 70 minutes) was used,[15] this model was used because an apparent isotopic steady state is obtained. More frequent sampling, like that used in the present study, indicates that a plateau indeed is not reached in the considered time period. An example of fitting the experimental data of serum labeled palmitate concentrations, obtained after placebo or short-term administration of dF, is reported in Fig 3.

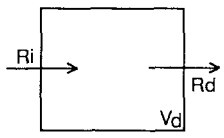
Statistical Methods

The model was fitted by a standard unweighted least-squares two-stage procedure. Parameter values are expressed as the mean ± SD. Groups were compared by Student's *t* test.

RESULTS

A slight (0.50 ± 0.12 kg) but significant ($P < .05$) body weight reduction occurred during the 15-day treatment with dF. After short-term administration of dF, fasting blood glucose levels were significantly lower than after placebo (86.5 ± 5.1 *v* 114.3 ± 8.6 mg/dL, $P < .001$). Long-term administration of dF resulted in a significant decrease of serum blood glucose levels (89.8 ± 2.4 *v* 120.3 ± 3.0 mg/dL, $P < .001$). Serum insulin concentrations did not change significantly with short- or long-term treatment.

FFA turnover rate was significantly ($P < .05$) greater in



$$Vd = Vds \cdot W$$

where $Ra = Ras \cdot W$

$Rd = Ra \cdot \frac{C}{N}$ is the rate of disappearance of the tracer

Fig 2. Block diagram of the single-compartment model used for 1-^{14}C-palmitate kinetic analysis. R_i, rate of infusion; R_d, rate of disappearance; V_d, volume of distribution; R_a, rate of appearance. For further details, see Fig 1.

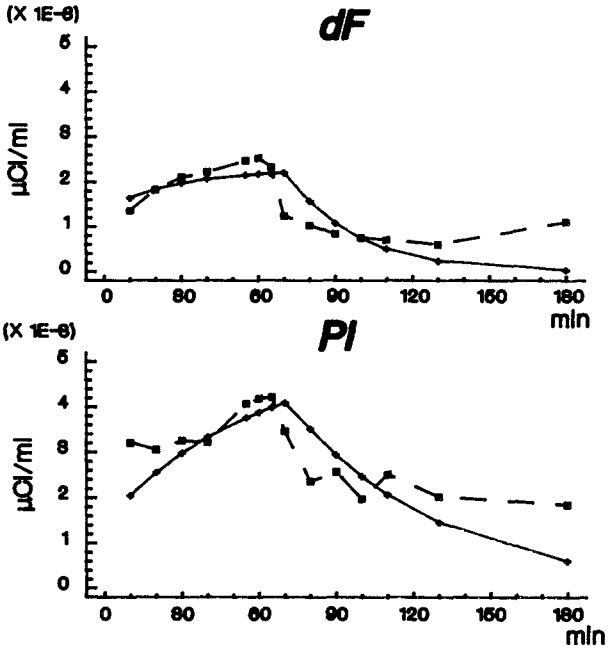


Fig 3. Model prediction of labeled palmitate concentration versus time (——) and experimental data curve (-----) in two different subjects treated with dF or placebo (PI).

diabetic obese patients who received a short-term oral dose (30 mg) of dF than in the control group (10.71 ± 2.18 *v* 7.79 ± 1.48 μmol/kg/min). Long-term administration of dF also resulted in a significant ($P < .05$) increase of FFA turnover rate (11.92 ± 2.74 *v* 8.43 ± 1.86 μmol/kg/min).

FFA oxidation, expressed as the percentage of administered radioactivity recovered in expired CO_2 (% of administered μCi), was significantly greater ($P < .001$) after both short- and long-term dF administration than after placebo (short-term dF *v* placebo, 11.46% ± 0.46% and 9.50% ± 0.46%, respectively; long-term dF *v* placebo, 12.06% ± 0.71% and 9.88% ± 0.62%, respectively). The time course of expired radioactivity (microcuries per minute) plotted against time (minutes) is shown in Fig 4.

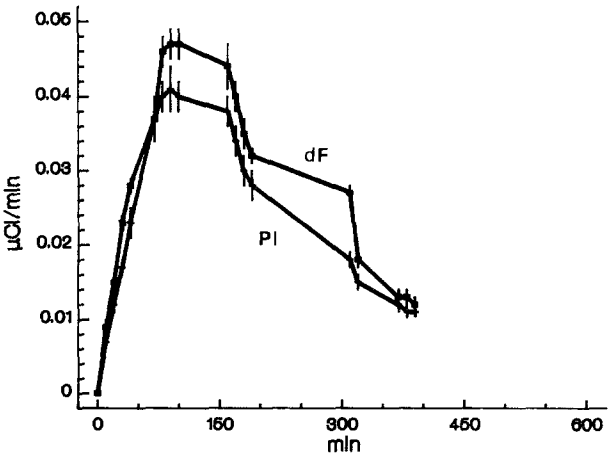


Fig 4. Rate of $^{14}CO_2$ excretion (μCi/min, mean ± SD) *v* time after short-term administration of dF or PI.

Whole-body net fat oxidation measured by indirect calorimetry increased nonsignificantly, after short-term dF treatment, which is shown by the lower values for nonprotein RQ observed in patients who received both short- and long-term dF (Fig 5). No significant changes in the mean values for resting energy expenditure were observed in the two groups of patients at days 0 and 15.

DISCUSSION

Our data for basal FFA turnover rates in obese women with a high WHR agree with those reported by Jensen et al,[20] which showed that women with upper-body obesity have increased adipose tissue FFA release relative to lean body mass. In this study, palmitate turnover was measured under overnight postabsorptive (basal), epinephrine-stimulated, and insulin-suppressed (euglycemic hyperinsulinemic clamp) conditions, but no data concerning substrate oxidation were reported, since labeled CO_2 was not measured and indirect calorimetry was not performed. In addition, the obese patients studied by Jensen et al[20] were not affected by diabetes mellitus. In our series, FFA oxidation appears to be greater than that reported in healthy people with an ideal body weight.[20] Therefore, subjects with a predominance of central fat not only have an increased FFA turnover but also an increased FFA oxidation.

In subjects with a high WHR, as compared to patients with the same mass but a peripheral distribution of adipose tissue, a higher degree of insulin resistance, affecting both nonoxidative and oxidative pathways of glucose metabolism, has been demonstrated.[21,22] It has been suggested that the accelerated lipolysis in these patients could contribute to reducing glucose uptake via the glucose–fatty acid cycle.[23,24]

In contrast, we observed that control of glycemia improved during both short- and long-term treatment with dF despite the simultaneous increase of FFA turnover and oxidation rates. Since this phenomenon has already been observed after short-term oral dF administration and since there was only a slight weight loss after 15 days of dF treatment, it seems likely that this improvement of blood glucose control is related to a primary effect of the drug, and is not due to the weight loss.

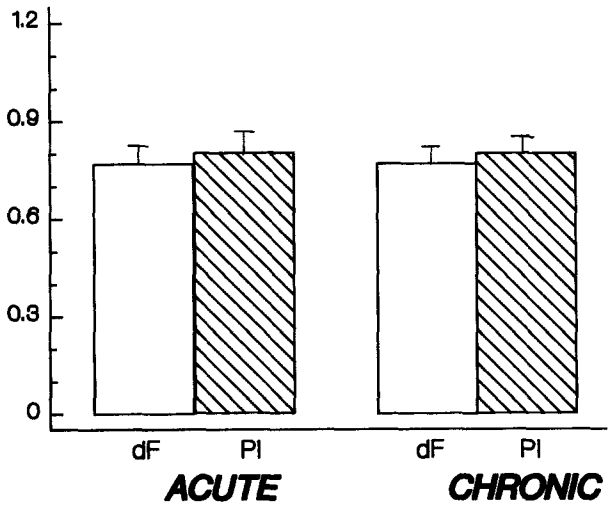


Fig 5. Mean nonprotein RQ (± SD) after acute and chronic administration of dF or PI.

In the study reported by Scheen et al[7] on the short-term effects of dF on insulin-induced glucose disposal in obese NIDDM patients, a significant increase of tissue glucose uptake was observed after dF administration, whereas no significant changes in the insulin metabolic clearance rate or endogenous (hepatic) glucose production were observed. This and other studies[6,8,9] suggest that dF improves insulin sensitivity in NIDDM patients.

Recently, Brindley et al[25] showed that long-term treatment of JCR:LA-corpulent rats with dF induces a decrease in circulating concentrations of glucose, triacylglycerols, free cholesterol, and insulin. They[25] suggest that by reducing the release of glucocorticoids, dF improves insulin sensitivity. In our series, we found a simultaneous increase in both FFA turnover and oxidation rates, which resulted in an equilibrium of circulating FFA concentrations.

Upon normalizing FFA levels in NIDDM patients with upper-body obesity, a simultaneous improvement of insulin sensitivity with a converse decrease of blood glucose levels becomes evident. In fact, since no significant difference in insulin levels was observed in the patients between short- and long-term dF administration periods, we suppose that this drug exerts a weight-independent effect on glucose cell metabolism without increasing insulin delivery.

The combined syndrome of android obesity, diabetes mellitus, hyperlipidemia, and hypertension is related to insulin resistance and to an increased control of metabolism by cortisol. The antagonism of the two hormones has been invoked as the factor responsible for hyperglycemia, hypertriglyceridemia, and hypercholesterolemia. As suggested by Brindley,[26] dF might act through the serotoninergic system in the hypothalamus both by decreasing food consumption and by normalizing hormonal balance through the hypothalamic-pituitary-adrenal axis. However, more detailed studies, using euglycemic hyperinsulinemic clamps combined with FFA turnover assay and fuel substrate oxidation measurements by indirect calorimetry, might improve our understanding of the role of dF in both glucose and lipid metabolism.

The findings of this study, ie, improvement of glucose metabolism and increase in FFA turnover and oxidation rates, mimic what happens during body weight reduction, in which an improvement of insulin resistance is associated with increased FFA turnover and oxidation rates. Therefore, at equilibrium the increase in glucose oxidation might be associated with a corresponding decrease in the rate of FFA oxidation, with no changes occurring in overall adenosine triphosphate production, as stated by Randle et al.[23] In contrast, in a dynamic situation such as during weight loss, there is no compensatory shift between glucose and FFA oxidation; instead, both of them are increased, thus inducing weight loss.

REFERENCES

1. Wurtman JJ, Wurtman RJ, Mark S, et al: D-Fenfluramine selectively suppresses carbohydrate snacking by obese subjects. Int J Eating Disord 4:89-99, 1985

2. Hill AJ, Blundell JE: Model system for investigating the actions of anorectic drugs: Effects of dexfenfluramine, in Ferrari E, Brambilla F (eds): Disorders of Eating Behaviour: A Psychoneuronendocrine Approach. New York, NY, Pergamon, 1986, pp 377-389

3. Blundel JE, Hill AJ: Serotoninergic modulation of the pattern of eating and the profile of hunger-satiety in humans. Int J Obes 11:141-155, 1987

4. Blundell JE: Serotonin manipulations and structure of feeding behaviour. Appetite 7:39-56, 1986

5. Storlien AH, Thorburn AW, Smythe GA, et al: Effect of D-fenfluramine on basal glucose turnover and fat-feeding–induced insulin resistance in rats. Diabetes 38:499-503, 1989

6. Glaser B, Assad Y, Norynberg C, et al: Acute and chronic effects of dexfenfluramine on glucose and insulin response to intravenous glucose in diabetic and non-diabetic obese subjects. Diabetes Res 19:165-176, 1992

7. Scheen AJ, Paolisso G, Salvatore T, et al: Improvement of insulin-induced glucose disposal in obese patients with NIDDM after 1-wk treatment with D-fenfluramine. Diabetes Care 14:325-332, 1991

8. Willey KA, Molyneaux LM, Overland JE, et al: The effects of dexfenfluramine on blood glucose control in patients with type 2 diabetes. Diabetic Med 9:341-343, 1992

9. Pestell RG, Crock PA, Ward GM, et al: Fenfluramine increases insulin action in patients with NIDDM. Diabetes Care 12:252-258, 1989

10. Hammet P, Verdy I, Chiasson JL, et al: Hypoglycemic effect of fenfluramine in insulin-treated diabetics. Clin Invest Med 9:12-14, 1986

11. Even P, Coulaud H, Nicolaidis S: Lipostatic and ischymetric mechanisms originate dexfenfluramine-induced anorexia. Pharmacol Biochem Behav 30:89-99, 1988

12. Even P, Coulaud H, Aucouturier JL, et al: Correlation between metabolic and behavioral effects of dexfenfluramine treatment. Clin Neuropharmacol 11:S93-S96, 1988 (suppl)

13. Greco AV, Mingrone G, Tataranni PA, et al: Turnover and oxidation rates of plasma free fatty acids in obese patients treated with dexfenfluramine. Ann Nutr Metab 37:237-244, 1993

14. Heymsfield SB, Wang J, Aulet M, et al: Dual photon absorptiometry: Validation of mineral and fat measurements. Basic Life Sci 55:327-337, 1990

15. Nordenstrom J, Carpentier YA, Askanazi J: Free fatty acid mobilization and oxidation during total parenteral nutrition in trauma and infection. Ann Surg 198:725-735, 1983

16. Wolfe RR: Collection of CO_2, in Liss AR (ed): Tracers in Metabolic Research. New York, NY, Raven, 1984

17. Long JM, Wilmore DW, Mason AD: Effect of carbohydrate and fat intake on nitrogen excretion during total intravenous feeding. Am Surg 185:417-422, 1977

18. Passi S, Rothschild-Boros MC, Fasella P: An application of high performance liquid chromatography to analysis of lipids in archeological samples. J Lipid Res 22:778-784, 1981

19. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

20. Jensen MD, Morey WH, Rizza RA: Influence of body fat distribution on free fatty acid metabolism in obesity. J Clin Invest 83:1168-1173, 1989

21. Evans DJ, Hoffman RG, Kalkhoff RK, et al: Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. Metab Clin Exp 33:68-75, 1984

22. Evans DJ, Murray R, Kissebah AH: Relationship between skeletal muscle, insulin resistance, insulin-mediated glucose disposal, and insulin binding: Effects of obesity and body topography. J Clin Invest 74:1515-1525, 1984

23. Randle PJ, Garland PB, Hales CN: The glucose–fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

24. Ferrannini E, Barrett EJ, Bevilacqua S: Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72:1737-1747, 1983

25. Brindley DN, Hales P, Al-Sieni AII, et al: Sustained decreases in weight and serum insulin, glucose, triacylglycerol and cholesterol in JCR: LA-corpulent rats treated with D-fenfluramine. Br J Pharmacol 105:579-685, 1992

26. Brindley DN: Neuroendocrine regulation and obesity. Int J Obes 16:S73-S79, 1992 (suppl)