Fenfluramine Increases Insulin Action in Patients With NIDDM

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These studies examined the effect of fenfluramine on insulin action and insulin secretion in healthy subjects and patients with non-insulin-dependent diabetes mellitus (NIDDM). In the first study, a double-blind crossover design was used in healthy subjects to compare the effect of short-term fenfluramine therapy (60 mg orally for 3 days) with placebo. Insulin secretion and whole-body insulin sensitivity (determined by frequently sampled intravenous glucose tolerance tests with analysis by the minimal-model method) were unchanged by fenfluramine. In the second study, involving patients with NIDDM inadequately controlled on submaximal to maximal doses of oral hypoglycemic agents, a double-blind crossover strategy was used to compare baseline studies (conducted after a run-in period) with fenfluramine (60 mg orally) or placebo for 4 wk. There was a signficant fall in fasting blood glucose after therapy with fenfluramine compared with the baseline study period (13.0 ± 1.2 vs. 8.4 ± 0.89 mM, mean ± SE, $P < .01$) with no significant fall in fasting serum insulin (20 ± 2 vs. 24 ± 3 μU/ml) or C-peptide (1.3 ± 0.2 vs. 1.3 ± 0.1 nM). During euglycemic-hyperinsulinemic (1 mU · kg^{-1} · min^{-1}) clamp studies there was a significant increase in insulin action from 12.7 ± 2.3 to 17.3 ± 1.8 min^{-1} · 10^3 μU · ml^{-1} ($P < .05$), although clamp insulin levels were lower after fenfluramine treatment (136 ± 14 vs. 96 ± 9 μU/ml, $P < .02$), reflecting an enhanced metabolic clearance rate for insulin (12.7 ± 1.5 vs. 20.1 ± 2.1 ml · kg^{-1} · min^{-1}, $P < .025$). When insulin action was normalized for the prevailing insulin level during the clamp, the increase of insulin action/insulin concentration was more marked (0.11 ± 0.02 to 0.22 ± 0.04 min^{-1} · 10^3 μU · ml^{-1}, $P < .005$). The insulin secretory response to arginine was unchanged from 21 ± 4 to 22 ± 6 μU/ml at similar levels of glycemia. Chronic fenfluramine therapy can lower fasting plasma glucose and increase insulin sensitivity without affecting insulin secretion in patients with NIDDM. Acute fenfluramine treatment in healthy individuals has no effect on glucose metabolism. We conclude that in patients with inadequately controlled NIDDM, fenfluramine may serve as a useful adjunct to sulfonylurea therapy. *Diabetes Care* 12:252–58, 1989

Glucose 1 mM = 18 mg/dl

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Reduced insulin secretion and resistance to insulin are both believed to contribute to the glucose intolerance of non-insulin-dependent diabetes mellitus (NIDDM; 1–6). Sulfonylurea agents are known to improve insulin secretion and action and are used widely in the treatment of this condition (7–9). However, sulfonylurea therapy may be associated with weight gain, and secondary failure is well described (10). For these reasons, other oral hypoglycemic agents such as metformin have been used instead of or in conjunction with sulfonylureas. Another potentially useful adjunct to sulfonylurea therapy is the phenylethylamine derivative fenfluramine, which has been shown previously to improve glucose tolerance independent of its effect on weight loss (11–13). Although the mechanism for this improvement has not been fully determined, it has been attributed to an increase in insulin sensitivity rather than an increase in insulin secretion (11–13).

The conclusion from earlier studies of increased whole-body insulin sensitivity during fenfluramine therapy in patients with NIDDM is based on the indirect evidence of lower glucose levels in the presence of

lower insulin levels after an oral glucose tolerance test (OGTT) (11,14). However, this method of assessment does not give a quantitative measure of insulin sensitivity. The one study that directly examined the effect of fenfluramine on insulin action did so in healthy subjects and showed only a short-term increase in forearm glucose uptake after 90 min but not after 165 min of intra-arterial fenfluramine infusion (11). In addition, conflicting data exist on the effect of fenfluramine on insulin secretion with evidence for increased (15), unchanged (11,13), or reduced (14,16) insulin secretion.

The aim of our study was to examine the effect of fenfluramine administration on glycemic control, insulin secretion, and insulin action in groups of subjects with and without NIDDM with a double-blind crossover design. Given the possibility that plasma levels of insulin may be altered either directly by drug-induced changes in pancreatic insulin secretion or indirectly by changes in cellular binding of insulin and hence clearance of insulin, we also measured the metabolic clearance rate of insulin during these studies (17).

MATERIALS AND METHODS

The first study used a randomized double-blind crossover technique to compare the effects of fenfluramine with those of placebo on insulin secretion and whole-body insulin sensitivity in healthy subjects. Informed consent to a protocol approved by the St. Vincent's Hospital research ethics committee was obtained from eight healthy volunteers aged 33–51 yr in good health and on no medications. Dietary content was kept constant among subjects with at least 180 g of carbohydrate consumed each day of treatment. Fenfluramine (60 mg) or placebo capsules were taken each morning for 3 days before the study and on the morning of the study 30 min before the test, which was performed after a 12-h overnight fast. Allocation to initial treatment with fenfluramine or placebo was randomized, and a 2-wk washout period was allowed before crossover.

Insulin secretion and insulin sensitivity were measured by the dynamic interaction between glucose and insulin during a frequently sampled intravenous glucose tolerance test (FSIGT) with analysis based on the minimal-model method described by Bergman et al. (18). FSIGTs were performed as previously described with a glucose dose of 300 mg/kg given over 1 min (19). Data analyses were performed by modification of the method of Bergman et al. (18) with the simulation and modeling program SAAM (20). We have modified the method of analysis so that the information base is maximized and analysis enhanced by simultaneously fitting individual data sets from individuals on fenfluramine and placebo and determining parameter values common to both sets and those that are unique.

Patients with NIDDM. The second study involved nine patients (4 men, 5 women) aged 44–74 yr with inadequately controlled NIDDM (mean HbA_{1c} 9.6%, normal <6.6%) on moderate to maximal doses of the sulfonylurea agent glyburide (Table 1). The patients did not have renal or hepatic disease or advanced diabetic complications. Informed consent to a protocol approved by the St. Vincent's Hospital research ethics committee was obtained from all patients before the study. After an initial baseline period, studies of insulin action and secretion were performed before randomization to fenfluramine (60 mg) or placebo, each given for 4 wk in a double-blind crossover design with an intervening 2-wk washout period. A constant dietary intake with at least 180 g of carbohydrate daily was maintained with the assistance of a dietitian, and the dose of sulfonylurea remained constant throughout the study. Patients fasted for 12 h and took their medications 30 min before the studies.

Because the FSIGT method requires endogenous insulin secretion that is abnormal in NIDDM (5), the euglycemic clamp method described by Pacini et al. (21) was used in this part of the study to examine the effects of fenfluramine treatment on insulin sensitivity in patients with NIDDM. The insulin infusion (1 $mU \cdot min^{-1} \cdot kg^{-1}$ body wt) was prepared from neutral porcine insulin (Actrapid MC CSL, Novo, Melbourne, Australia) in 0.9% saline containing 25 ml of 5% haemaccel

TABLE 1
Clinical details of patients with non-insulin-dependent diabetes mellitus

Patients	Sex	Age (yr)	Weight (kg)	Height (cm)	Duration of diabetes (yr)	HbA_{1c} (%)*	Dose of glyburide (mg/day)
1	M	72	70.5	160	8	7.4	10
2	M	59	75.0	170	3	8.7	10
3	M	44	95.0	180	7	7.6	10
4	M	66	94.0	173	4	14.8	10
5	F	59	84.5	168	4	9.1	7.5
6	F	74	75.0	150	5	8.7	10
7	F	63	67.0	157	4	11.4	15
8	F	55	70.5	158	4	10.8	5
9	F	64	76.0	157	10	7.7	10

*Normal range <6.6%.

(1/20 saline dilution of degraded gelatin polypeptide, Hoechst, Melbourne, Australia) and infused with a Gilson pump (Villiers Le Bel, France). Before the insulin infusion, blood samples were drawn for measurement of baseline glucose, insulin, C-peptide, glucagon, nonesterified fatty acids (NEFA), cholesterol, triglyceride, fenfluramine and norfenfluramine levels, and monocyte insulin-receptor binding.

To avoid differences in glucose utilization that might occur due to different periods of insulinization, comparable periods of insulin infusion before the clamp were used in each individual for the three studies (22). The time taken to lower plasma glucose to 6.5 mM before the clamp during the baseline study was matched for the placebo and fenfluramine studies, with exogenous glucose being infused when necessary to prevent blood glucose falling below 6.5 mM. During the clamp, the venous blood glucose level was maintained at 6.5 mM for 90 min by adjusting the rate of glucose infused according to an algorithm based on 10-min bedside measurements of blood glucose by the glucose oxidase method with a glucose analyzer (YSI, Yellow Springs, OH; 21). Thirty minutes after conclusion of the clamp experiment, 5 g i.v. arginine monohydrochloride was given over 15 s to assess the insulin secretory response. The acute insulin response to arginine was calculated as the mean elevation above the prestimulus level of the insulin values at 2, 3, 4, and 5 min after the arginine injection (23). The plasma glucose levels before administration of arginine were similar for the baseline, fenfluramine, and placebo periods being 5.0 ± 0.3, 5.0 ± 0.3, and 5.5 ± 0.4 mM, respectively.

The minimal-model–based glucose clamp allows calculation of the fractional disappearance rate of glucose, known as *x*, which is a measure of insulin action (21). Values for insulin action during the baseline, fenfluramine, and placebo periods were derived from data over the last 60 min of the euglycemic clamp. The mean of the coefficients of variation for steady-state plasma insulin and glucose during this part of the clamp were 9 and 4.5%, respectively. The metabolic clearance rate of insulin during the clamp studies was calculated as previously described with plasma insulin and C-peptide levels (24).

Analytic methods and statistical analyses. Plasma glucose was measured with an automatic analyzer (YSI) by a glucose oxidase method. Plasma insulin and glucagon were estimated by radioimmunoassay with dextran-charcoal separation of bound and free fractions (25,26). C-peptide was assayed by the Novo C-peptide radioimmunoassay kit (Copenhagen) with synthetic human C-peptide and guinea pig anti-human C-peptide antiserum (27). NEFAs were measured with a modification of the method of Carruthers and Young (28). Binding of ^{125}I-labeled insulin to human monocytes was measured by the Ficoll-Hypaque method of Boyum (29) for monocyte separation. Fenfluramine and its metabolite norfenfluramine were measured by gas chromatography (30). Statistical analyses were conducted with the nonparametric Wilcoxon's signed-rank test. However, all data are expressed as means ± SE.

RESULTS

Healthy subjects. In healthy subjects, no significant differences in fasting glucose, insulin, C-peptide, NEFA, glucose tolerance, insulin sensitivity, insulin secretion, or glucose-mediated glucose disposal were found between fenfluramine and placebo treatments (Table 2).

Patients with NIDDM. No significant weight loss occurred in the fenfluramine-treated group compared with the baseline (78.8 ± 3.4 vs. 78.3 ± 3.4 kg). The only side effect was self-limited loose stools reported by three patients at the start of the fenfluramine-treatment period and not during placebo (Table 1).

During therapy with fenfluramine when compared with the baseline study, there was a significant fall in the fasting glucose level (13.0 ± 1.2 vs. 8.4 ± 0.9 mM, $P < .01$) with no significant change in fasting serum insulin (20 ± 2 vs. 24 ± 3 μU/ml) or C-peptide (1.3 ± 0.1 vs. 1.3 ± 0.2 nM) (Fig. 1; Table 3). A significant decrease in mean serum insulin concentration during insulin infusion at the time of plateau in the euglycemic clamp occurred in fenfluramine treatment compared with baseline study, from 136 ± 14 to 96 ± 9 μU/ml, $P < .02$. Despite the lower insulin level there was a significant increase in glucose infusion rate during the clamp from 2.5 ± 0.5 to 3.8 ± 0.5 mg · kg^{-1} · min^{-1}, $P < .005$. Insulin action also increased from 12.7 ± 2.3 to 17.3 ± 1.8 min^{-1} · 10^3 μU · ml^{-1}, $P < .05$, (Fig. 2). When insulin action was normalized with respect to the prevailing plateau insulin level (insulin action/insulin concentration) the change of insulin action was more marked, increasing from 0.11 ± 0.02 to 0.22 ± 0.04 min^{-1} · 10^3 μU · ml^{-1}, $P < .005$ (31). With calculations based on the serum insulin and C-peptide levels during

TABLE 2
Minimal-model analysis of intravenous glucose tolerance test data for healthy individuals

	Fenfluramine	Placebo
Fasting glucose (mM)	4.6 ± 0.2	4.6 ± 0.1
Fasting insulin (μU/ml)	7.0 ± 0.8	8.0 ± 1.0
Fasting C-peptide (nM)	0.38 ± 0.05	0.39 ± 0.06
Fasting nonesterified fatty acids (mM)	0.62 ± 0.08	0.54 ± 0.04
Glucose tolerance (kg/min)	1.7 ± 0.1	1.8 ± 0.1
Glucose-mediated glucose disposal (min^{-1} × 10^{-2})	2.6 ± 0.4	2.5 ± 0.3
Insulin sensitivity (min^{-1}per μU/L × 10^{-4})	3.1 ± 0.7	3.6 ± 0.8
First-phase insulin secretion (μU · ml^{-1} · min per mg/dl)	4.4 ± 0.6	4.9 ± 1.0
Second-phase insulin secretion (μU · ml^{-1} · min^2 per mg/dl)	5.8 ± 0.9	7.0 ± 1.5

Values are means ± SE.

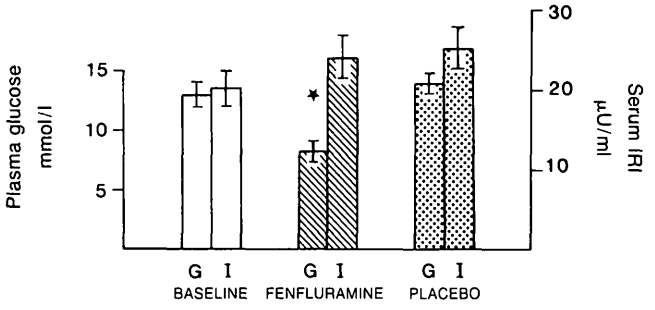

FIG. 1. Fasting plasma glucose (G) and insulin (I) expressed as means ± SE for 9 patients with non-insulin-dependent diabetes mellitus during baseline (run-in), fenfluramine, and placebo phases. *$P < .01$, fenfluramine vs. placebo or baseline.

the euglycemic clamp, the metabolic clearance rate of insulin increased in the fenfluramine-treated group from 12.7 ± 1.5 to 20.1 ± 2.1 mg · kg^{-1} · min^{-1}, $P < .025$ (24). However, basal monocyte insulin binding was unchanged, being 3.57 ± 1.0% during the baseline period compared with 3.42 ± 0.32% during fenfluramine. The insulin secretory response to arginine was not altered by fenfluramine treatment compared with the baseline study (Fig. 3).

When fenfluramine treatment was compared with placebo, fasting plasma glucose was significantly lower (8.4 ± 0.9 vs. 13.8 ± 0.9 mM, $P < .01$) with no difference in fasting insulin (24 ± 3 vs. 25 ± 3 μU/ml) or C-peptide (1.3 ± 0.2 vs. 1.4 ± 0.2 nM). During the euglycemic clamp, glucose infusion rate was significantly greater (3.8 ± 0.5 vs. 2.9 ± 0.5 mg · kg^{-1} · min^{-1}, $P < .025$). Insulin action also tended to be greater (17.3 ± 1.8 vs. 15.2 ± 2.4 min^{-1} · 10^3 μU · ml^{-1}) and insulin action/insulin concentration was significantly greater (0.22 ± 0.04 vs. 0.15 ± 0.04 min^{-1} · 10^3 μU · ml^{-1}, $P < .01$). The insulin secretory response to arginine was unchanged (Fig. 3). There were no significant differences between baseline and placebo for any other parameters examined (Figs. 1–3).

TABLE 3
Metabolic data in patients with non-insulin-dependent diabetes mellitus after run-in or baseline period, and after 4-wk treatment with fenfluramine or placebo

	Baseline	Fenfluramine	Placebo
Basal C-peptide (nM)	1.3 ± 0.1	1.3 ± 0.2	1.4 ± 0.2
Basal nonesterified fatty acids (mM)	0.73 ± 0.1	0.69 ± 0.07	0.77 ± 0.08
Basal glucagon (pg/ml)	68 ± 9	106 ± 22	122 ± 22
Clamp C-peptide (nM)	0.77 ± 0.09	1.01 ± 0.2*	0.74 ± 0.09
Clamp nonesterified fatty acids (mM)	0.18 ± 0.05	0.22 ± 0.06	0.20 ± 0.07
Clamp glucagon (pg/ml)	45 ± 9	48 ± 15	69 ± 14
Basal cholesterol (mM)	5.8 ± 0.6	4.5 ± 0.4	5.1 ± 0.5
Basal triglyceride (mM)	3.3 ± 0.9	1.9 ± 0.3	2.7 ± 0.6
Fenfluramine (ng/ml)		98.0 ± 16.4	ND
Norfenfluramine (ng/ml)		32.3 ± 5.11	ND

Values are means ± SE. ND, not detected.
*$P < .05$ vs. baseline.

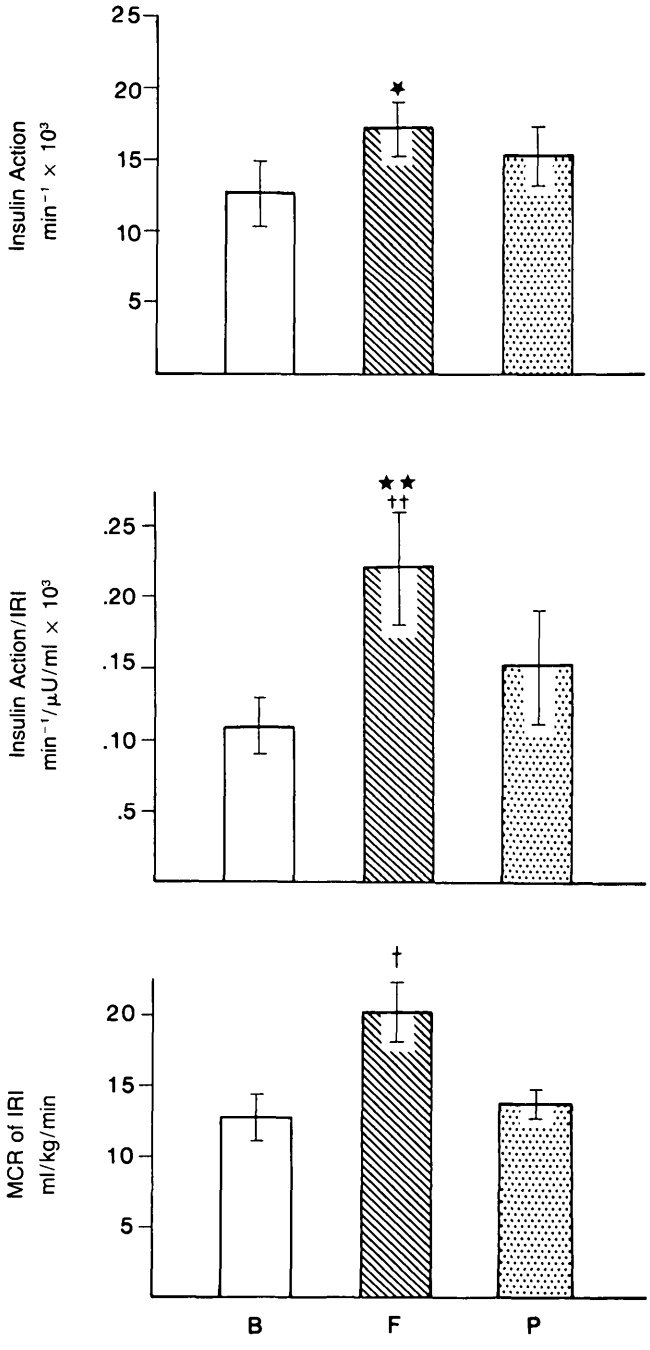

FIG. 2. Insulin action, insulin action and plasma insulin level (IRI), and metabolic clearance rate of insulin (MCR of IRI) expressed as means ± SE for 9 patients with non-insulin-dependent diabetes mellitus during run-in or baseline (B), fenfluramine (F), and placebo (P) phases. *$P < .05$, **$P < .005$, and †$P < .025$ vs. baseline. ‡$P < .01$ vs. placebo.

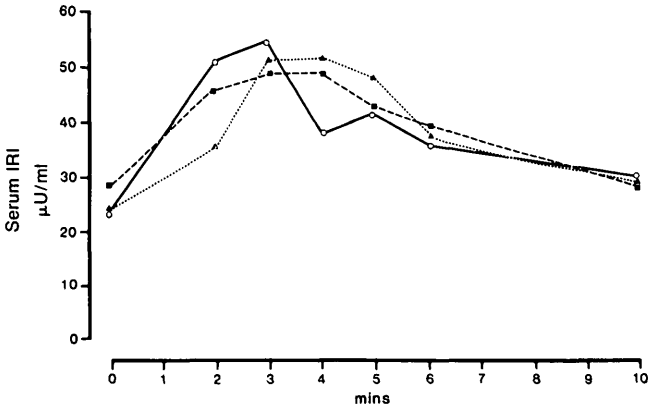

FIG. 3. Mean insulin secretory response to 5 g i.v. arginine monohydrochloride at matched glucose levels for 9 patients with non-insulin-dependent diabetes mellitus during baseline (run-in; ○), fenfluramine (■), and placebo (▲) phases.

DISCUSSION

In patients with inadequately controlled NIDDM already on moderate to maximal doses of sulfonylurea, addition of fenfluramine was associated with a significant fall in fasting blood glucose levels over 4 wk of treatment. These data support earlier studies that showed an improvement in fasting glucose during fenfluramine treatment (12,13). Other studies found that fenfluramine improved oral glucose tolerance in NIDDM but did not report fasting glucose level results (11,14,32). These improvements occurred independent of weight loss as noted in our study. In contrast with the effect in NIDDM, this study failed to demonstrate any effect of short-term fenfluramine treatment on glucose tolerance in healthy individuals. Although other studies have shown improved glucose tolerance in healthy subjects after fenfluramine, this effect occurred either transiently after acute injection of fenfluramine or after significant weight loss with longer-term therapy (11,33). Our study suggests that the direct hypoglycemic effect of fenfluramine therapy may be confined to patients with NIDDM, although healthy individuals were only studied acutely and with a different protocol.

Previously, the mechanism for improved glucose tolerance in patients with NIDDM following fenfluramine treatment has not been fully evaluated. In earlier studies, changes in whole-body insulin sensitivity were assessed indirectly from the glucose and insulin levels following an OGTT. However, in addition to insulin sensitivity, other factors determine oral glucose tolerance and a quantitative assessment of insulin sensitivity cannot be derived from OGTT data (34). Direct evidence for an effect of fenfluramine to improve insulin sensitivity in patients with NIDDM was obtained in this study with a version of the euglycemic-hyperinsulinemic clamp that was designed to compare total-body glucose disposal at matched insulin and glucose levels (21).

Improved insulin action in the clamp study during fenfluramine treatment may be due to an effect on peripheral glucose disposal or to suppression of hepatic glucose output, because the latter was not estimated directly. Previous studies in our laboratory have shown 90% suppression of glucose output in patients with NIDDM given insulin <1 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} (35), but other studies have shown considerably less suppression at these levels (36). On the other hand, the fall in fasting plasma glucose during fenfluramine is more likely to reflect reduced hepatic glucose output than improved peripheral glucose disposal. Several studies have shown a strong correlation between fasting plasma glucose and hepatic glucose output in NIDDM (35,37,38), and there is evidence from animal studies that fenfluramine can reduce hepatic glucose output (39).

The finding of lower insulin levels despite the same rate of insulin infusion during the euglycemic-hyperinsulinemic clamp while on fenfluramine therapy reflects increased insulin clearance because C-peptide levels showed that endogenous insulin secretion was not suppressed more by the infused insulin. Previous studies have shown reduced insulin clearance in insulin resistant states and the increased insulin clearance during fenfluramine therapy is therefore consistent with the increased insulin sensitivity (40,41). Increased insulin sensitivity and clearance could be due to increased insulin-receptor binding at either hepatic or peripheral sites. Although monocyte insulin-receptor binding characteristics were not altered by fenfluramine in this study, increased insulin-receptor binding to adipose tissue has been observed previously (17). Alternatively, improved insulin action may be due to a postbinding step, as has been observed during sulfonylurea therapy (8,9).

Some studies have suggested that insulin secretion is inhibited by fenfluramine, both in vivo (14,16) and in vitro (16). However, like the previous assessments of insulin sensitivity, conclusions from in vivo studies were based on OGTT data in which glucose levels differed between fenfluramine-treated and control groups, and changes in insulin clearance were not considered. In this study, basal and dynamic measures of β-cell function in healthy subjects and patients with NIDDM showed that insulin secretion was not altered by fenfluramine treatment. Previous conclusions about reduced insulin secretion during OGTT probably did not take sufficient account of the lower glucose levels or increased insulin clearance caused by fenfluramine therapy.

In summary, fenfluramine therapy markedly lowered fasting plasma glucose in a group of patients with sulfonylurea-treated NIDDM, independent of weight loss and without any significant change of insulin levels. Insulin action, measured with the hyperinsulinemic-euglycemic clamp, was improved by fenfluramine, and insulin clearance was enhanced, effects that may be causally linked. However, insulin secretion was unaltered by fenfluramine. The effects of fenfluramine on glucose metabolism were not seen with short-term administra-

tion in healthy subjects. We conclude that fenfluramine may be a useful adjunct to sulfonylurea therapy in patients with inadequately controlled NIDDM.

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