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FAILURE OF FENFLURAMINE TO AFFECT BASAL AND INSULIN-STIMULATED HEXOSE TRANSPORT IN RAT SKELETAL MUSCLE

SHLOMO SASSON, BARUCH KUNIEVSKY, CHRISTINE NATHAN* and EROL CERASI†‡
The Departments of Pharmacology and †Endocrinology and Metabolism, Hebrew University Hadassah Medical Center, Jerusalem, Israel, and *Institut de Recherches Internationales Servier, Paris, France

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Abstract—Fenfluramine is an effective appetite supressant that mediates its action via serotoninergic neurons. We studied the effect of pure *d*- and *l*-fenfluramine on *in vitro* hexose transport in isolated rat soleus muscles and skeletal muscle cells in culture. We found no evidence to suggest that the fenfluramine enantiomers affect the basal transport activity. Furthermore, the drugs did not interfere with the ability of glucose to regulate its own transport. Muscle responsiveness to insulin was not altered by the enantiomers, nor did insulin unmask any effect of fenfluramine on muscle hexose transport. These conclusions are based on experiments performed with a wide concentration range of drug and insulin, from the therapeutic to suprapharmacological levels. We discuss our results in view of published data on the effects of fenfluramine on peripheral glucose metabolism.

Fenfluramine is a clinically effective serotoninergic appetite suppressant used for two decades. Recent studies have shown that *d*-fenfluramine is stereospecific in causing anorexia [1] and that 5-HT_1§ receptors are preferentially involved in this action [2, 3]. Interest in metabolic effects unrelated to the anorectic effect of fenfluramine was generated in the early 1970s, when it was shown in animal experiments that the drug may lower blood glucose [4, 5].

In 1972 Bliss *et al.* [6] reported that fenfluramine significantly reduced the blood glucose level of non-obese patients with peripheral vascular complications. Other studies on obese type II diabetics indicated that the drug may improve glucose tolerance and insulin secretion [7]. Muscle is the major peripheral tissue involved in glucose homeostasis. Several studies suggested that the metabolic effect of fenfluramine exerts itself at the level of skeletal muscle. Thus, studies on isolated human gluteus muscle or rat hemidiaphragm showed that fenfluramine, in the presence of physiological concentrations of insulin, increased the glucose uptake of the tissue [8–11]. No significant effect was demonstrated by fenfluramine in the absence of insulin when the drug was present in therapeutic concentrations (50–100 ng/ml; 0.19–0.37 μM); however, at higher concentrations (1–10 μg/ml; 3.7–37.0 μM) the drug was effective in the absence of insulin [10, 12].

With the recent availability of *d*-fenfluramine for therapeutic purposes [2, 3], interest has been renewed on the possible metabolic effects of the drug. We therefore undertook to study in detail the action of *d*- and *l*-fenfluramine in a sensitive *in vitro* model of skeletal muscle [13, 14]. Furthermore, since recent studies from our laboratory indicate that the reduced peripheral glucose utilization in type II diabetes may be the consequence of a physiological regulatory mechanism involving autoregulation of hexose transport by glucose [13, 14], we also investigated the effect of fenfluramine on this phenomenon.

MATERIALS AND METHODS

Chemicals. The 2-[1-2-^{3}H]deoxy-glucose (dGlc), 30.2 Ci/mmol, was purchased from New England Nuclear (Boston, MA). [U-^{14}C]Sucrose, 556 mCi/mmol, was from the Radiochemical Centre (Amersham, U.K.). Crystalline porcine insulin (26.8 U/mg) was a gift from Eli Lilly & Co. (Indianapolis, IN). Stock solutions (1 mg/ml) in 10^{-3} N HCl, pH 3.0, were kept at 4°; for further use, the solution was diluted in buffer containing 0.25% (w/v) bovine serum albumin (BSA). HEPES, BSA (fraction V), dGlc and cytochalasin B were purchased from Sigma Chemical Co. (St Louis, MO). *d*(+)Fenfluramine and *l*(−)fenfluramine were a gift from Technologie Servier (Orléans, France). Waymouth medium and Dulbecco's modified Eagle's medium (DMEM) were from Gibco Laboratories (Grand Island, NY). Fetal calf serum (FCS), L-glutamine, penicillin G and streptomycin sulphate were obtained from Biological Industries (Kibbutz Beth-Haemek, Israel). All other chemicals were reagent grade.

The purity of the *d*- and *l*-enantiomers of fenfluramine was checked by several methods including specific optic rotation, mass spectroscopy, infra-red spectra and chemical analysis. Based upon these measurements we concluded that the preparations were >99% pure.

Cells and culture conditions. Myogenic cells of the

‡ To whom correspondence should be sent at: Department of Endocrinology and Metabolism, Hadassah University Hospital, P.O. Box 12000, Jerusalem 91120, Israel.

§ Abbreviations used: BSA: bovine serum albumin; dGlc: 2-deoxy-D-glucose; FCS: fetal calf serum; 5-HT: 5-hydroxytryptamine; HEPES: 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; KRB; Krebs–Ringer bicarbonate buffer.

L_8 cell line, originally established by Dr D. Yaffe (Weizmann Institute of Science, Rehovot, Israel) were obtained through his courtesy. The cells were grown as previously described [15]. In brief, mononucleated myogenic cells were plated in 35-mm gelatin-coated tissue culture plates (10^5 cells/plate) in Waymouth medium supplemented with 15% (v/v) FCS. At confluency, the medium was changed 18–20 hr prior to the experiment to glucose-free DMEM containing 15% (v/v) FCS supplemented with glucose at the desired concentration.

Animals. Young male albino rats (40–60 g) of the Sabra strain (Hebrew University, Jerusalem, Israel) received standard laboratory chow and water *ad lib.* Their serum glucose concentration at sacrifice ranged between 8–10 mM.

Measurement of dGlc uptake in myocytes. The myocytes in culture were rinsed 8 times with 2 ml of KRB (supplemented with 10 mM HEPES, pH 7.4). After rinsing, 1 ml of KRB buffer containing 0.5 μCi [^{3}H]dGlc, 0.1 mM unlabeled dGlc, and 0.02 μCi [^{14}C]sucrose was added to the plates (in triplicates). Incubation was carried out for 5 min at room temperature and terminated by aspirating the medium and rinsing the cells 5 times with 2 ml ice-cold KRB. After digestion of the cells with 1 ml 1 N NaOH (60 min at 37°) and neutralization with concentrated HCl, aliquots of 800 μl were taken for liquid scintillation counting. Extracellular space was determined by calculating the [^{14}C]sucrose space. Cytochalasin B (5 μM) effectively inhibited the uptake of dGlc when added prior to the hexose. Usually the noninhibitable uptake of dGlc was similar to the dGlc content of the extracellular space as determined by [^{14}C]sucrose.

Based upon measurements of the intracellular concentrations of free and phosphorylated dGlc we have shown that the [^{3}H]dGlc uptake assay measures the rate of the hexose transport and not its intracellular phosphorylation [13]. It was also shown that the cell washing procedure eliminates the extracellular glucose and that no free glucose accumulates in the cells [13].

Measurement of dGlc uptake in isolated soleus muscles. Non-fasted rats were killed by cervical dislocation, the soleus muscles quickly removed and incubated in 20 ml glass vials sealed with a rubber stopper, continuously gassed with O_2/CO_2 (95:5, v/v) in a metabolic shaker (40 cycles/min) at 37°. To measure dGlc uptake, 4–6 soleus muscles, weighing 10–25 mg, were incubated for various times in DMEM containing glucose concentrations and other agents as indicated. At the end of the first incubation period, the muscles were washed three times in 20 ml KRB buffer and transferred to vials with 1.5 ml KRB containing 0.5 mM dGlc, 10.0 μCi [^{3}H]dGlc, and 0.5 μCi [^{14}C]sucrose. After a 5 min incubation, the uptake was terminated by 3 rapid washes in 20 ml ice-cold KRB. The muscles were then frozen in liquid nitrogen and weighed. After thawing, the individual muscles were dissolved in 1 ml 1 N NaOH (10 min at 80–90°), cooled and neutralized with concentrated HCl. The radioactivity was counted in 10 ml Lumax scintillation mixture. The data were corrected for the extracellular space. In experiments where insulin effect was studied, KRB with 0.25% (w/v) BSA was used for the first incubation.

Table 1. dGlc uptake by myocytes in culture incubated with *d*-fenfluramine: dose dependency

d-Fenfluramine (μM)	dGlc uptake pmol dGlc/10^6 cells/min 1.7 mM glucose	17.0 mM glucose
None	67.2 ± 1.5	33.5 ± 1.5
0.037	67.9 ± 2.6	33.1 ± 2.9
0.19	67.0 ± 3.0	36.3 ± 2.0
0.37	72.1 ± 1.0	35.9 ± 2.7
0.93	67.7 ± 2.1	35.7 ± 1.9
1.90	70.3 ± 1.0	34.2 ± 2.5
2.80	68.1 ± 1.5	35.3 ± 2.0
3.70	72.2 ± 2.0	35.2 ± 1.5

L_8 myocytes were grown to confluency. After preincubation (20 hr) with DMEM containing 3.0 or 20.0 mM glucose, the hexose levels fell to 1.7 and 17.0 mM, respectively. The media were then changed to DMEM containing the same glucose levels, FCS and increasing *d*-fenfluramine concentrations, and incubated in triplicates for 4 hr at 37°. After rapid washings with KRB the standard [^{3}H]dGlc uptake assay was performed. Mean ± SE (N = 4).

We [13, 14] and others [16] showed that an intracellular pool of free glucose in soleus muscle incubated at various glucose concentrations is unlikely to dilute the free intracelluar [^{3}H]dGlc in the uptake assay. We have previously established that the standard washing procedure effectively eliminates free glucose from the extracellular space [13].

Glucose determination. Glucose concentration in plasma samples from rats and in aliquots from incubation media was determined by the glucose oxidase method using a glucose analyzer.

Statistical analysis. Results are expressed as mean ± SE. Non-parametric tests were used for the statistical analysis: the Mann–Whitney test with ties ranks for simple comparison between two experimental groups, and the Kruskal–Wallis test for multiple comparisons [17].

RESULTS

Lack of effect of fenfluramine on hexose transport in L_8 myocytes

The effect of *d*- and *l*-fenfluramine on the hexose transport system was studied in L_8 myocytes maintained at different glucose concentrations. Table 1 demonstrates the lack of effect of *d*-fenfluramine: myocytes conditioned at 1.7 or 17.0 mM glucose maintained their basal dGlc transport rates unchanged in the presence of 0.037–3.7 μM *d*-fenfluramine. Similar results were obtained with *l*-fenfluramine (data not shown). In additional experiments (data not shown) neither *d*-fenfluramine nor *l*-fenfluramine when present at 3.7 μM (1 μg/ml) for up to 4 hr had any significant effect on the basal transport rates of myocytes conditioned at 2.7, 10.0 or 19.5 mM glucose, the corresponding dGlc uptake rates being 64.9 ± 0.9, 49.5 ± 1.5 and 22.1 ± 0.5 pmol/10^6 cells/min.

Fenfluramine had no effect on the autoregulatory mechanism of the hexose transport system. Figure

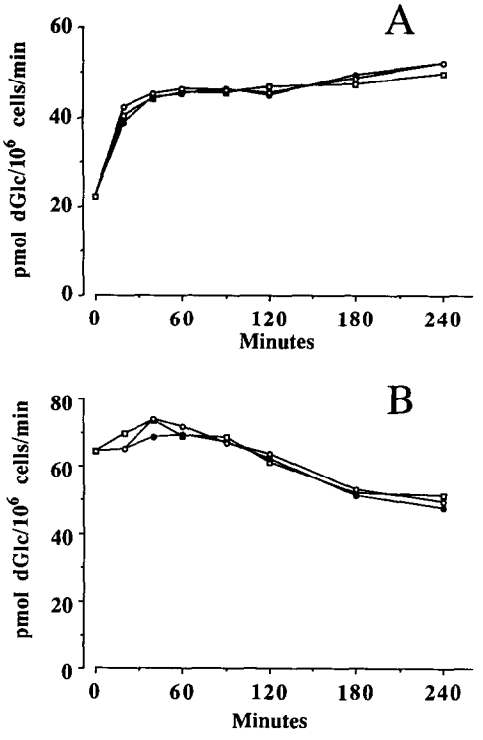

Fig. 1. The autoregulation of dGlc uptake in L_8 myocytes in the presence of *d*- and *l*-fenfluramine. L_8 myocytes were grown to confluency. After preincubation (20 hr) with DMEM containing FCS and 19.7 (A) or 2.1 mM (B) glucose, the medium glucose fell to 17.0 and 1.1 mM, respectively. The media were then changed to DMEM containing FCS and 2.6 (A) or 10.5 mM (B) glucose without (□) or with 3.7 μM *d*-fenfluramine (●) or *l*-fenfluramine (○), and triplicate cultures incubated for the indicated times at 37°. After rapid washing with KRB the standard [^{3}H]dGlc uptake assay was performed. Means of four experiments. The SE bars were smaller than the symbols.

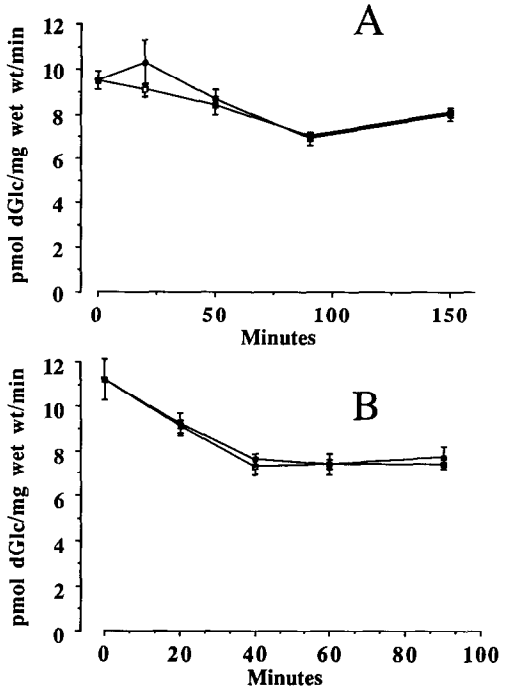

Fig. 2. The dGlc uptake of soleus muscles exposed to 3.7 μM fenfluramine: time dependency. Soleus muscles were incubated in DMEM containing 10.0 mM glucose in the absence (□) or the presence (■) of (A) 3.7 μM *d*-fenfluramine or (B) 3.7 μM *l*-fenfluramine for the indicated time periods. After washing, the muscles were taken for the standard [^{3}H]dGlc uptake assay. Mean ± SE (N = 4–10).

1A describes the upregulation of hexose transport in L_8 myocytes conditioned at 17.0 mM. When the glucose concentration of the medium was reduced to 2.6 mM the transport rate increased from 22.5 ± 0.5 to 51.0 ± 2.0 pmol dGlc/10^6 cells/min. Neither *d*- nor *l*-fenfluramine altered the magnitude or kinetics of this process. A similar ineffectiveness of the fenfluramine enantiomers was observed in the down-regulation of the transport (Fig. 1B). When the myocytes received fresh medium containing 10.5 mM glucose after the conditioning period with 2.1 mM glucose, the transport rate was reduced gradually from 64.4 ± 1.5 to 50.0 ± 2.0 pmol dGlc/10^6 cells/min within 4 hr whether fenfluramine was absent or present.

To test the hypothesis that fenfluramine may potentiate the action of insulin, L_8 myocytes were conditioned at 1.3 and 20.0 mM glucose and exposed to the hormone without or with the drug. As previously reported [13], muscle does not respond to a short-term insulin stimulus when hexose transport is upregulated by glucose deprivation. Indeed, the upregulated dGlc uptake of myocytes following conditioning at 1.3 mM glucose was not further stimulated by insulin (54.0 ± 1.2 and 56.0 ± 2.0 pmol/10^6 cells/min, respectively). In contrast, following conditioning at 20.0 mM glucose, insulin (85.0 nM, 30 min exposure) stimulated significantly the hexose transport rate (30.8 ± 0.6 vs 40.0 ± 0.4 pmol/10^6 cells/min, $P < 0.01$). The addition of 3.7 μM *d*-fenfluramine had no effect on either basal (57.4 ± 0.6 and 32.2 ± 1.0 pmol/10^6 cells/min in up- and down regulated myocytes) or insulin-stimulated dGlc uptake (57.0 ± 4.4 and 41.2 ± 1.2 pmol/10^6 cells/min, respectively).

Lack of effect of fenfluramine on hexose transport in isolated rat soleus muscles

The rat soleus is a sensitive physiological muscle preparation commonly used for *in vitro* studies [13, 14, 16]. Figure 2A shows that *d*-fenfluramine (3.7 μM) had no significant effect on the basal dGlc uptake rate of muscles incubated in the presence of 10.0 mM glucose for up to 150 min. Similarly, addition of 3.7 μM *l*-fenfluramine failed to alter the hexose transport rates (Fig. 2B). Also, in muscles maintained at 16.7 mM glucose under similar experimental conditions, fenfluramine was without effect (data not shown).

This lack of drug effect was not a result of the concentration chosen. In experiments where a wide range of concentrations were used, encompassing both the therapeutic and suprapharmacological levels (0.037–3.7 μM; 10–1000 ng/ml), the basal dGlc

Table 2. The dGlc uptake of soleus muscles exposed to fenfluramine: dose dependency

Fenfluramine (μM)	dGlc uptake pmol dGlc/mg wet wt/min	
	l-Fenfluramine	*d*-Fenfluramine
None	7.8 ± 0.2	7.2 ± 0.2
0.037	8.5 ± 0.3	7.6 ± 0.2
0.37	7.3 ± 0.5	8.1 ± 0.5
1.90	8.2 ± 0.4	8.0 ± 0.3
3.70	8.3 ± 0.3	8.5 ± 0.3

Soleus muscles were incubated in DMEM containing 10.9 mM glucose and the indicated concentrations of *l*- or *d*-fenfluramine for 90 min. After washing, the muscles were taken for the standard [^{3}H]dGlc uptake assay. Mean ± SE (N = 6).

uptake of soleus muscles remained unresponsive to the enantiomers of fenfluramine (Table 2).

The possible effect of fenfluramine on the responsiveness of soleus muscle to insulin was also investigated. As shown in Fig. 3 the freshly isolated muscles respond to 1.7 nM (0.25 mU/ml) insulin by almost doubling the rate of [^{3}H]dGlc uptake. When insulin was added together with *d*- or *l*-fenfluramine to the incubate, the drug failed to alter the effect of insulin on soleus dGlc uptake (Fig. 3). In experiments not shown, similar results were obtained with lower *d*- or *l*-fenfluramine concentrations (0.037–1.9 μM). With prolonged incubation in DMEM containing 10.0 mM glucose the responsiveness of the muscle to insulin was reduced. This deterioration is specific for the insulin effect since the basal dGlc uptake (8.5 ± 0.6 pmol/mg wet wt/min) was not significantly altered after 150 min incubation without or with *d*-fenfluramine (3.7 μM) treatment during the last 20 min (7.6 ± 0.4 and 7.9 ± 0.3 pmol/mg wet wet/min, respectively). *d*-Fenfluramine had no effect on the response to insulin, nor did it prevent the deterioration of the response. In another series of experiments soleus muscles were preconditioned by exposure to 1.7 nM insulin for 130 min, then 3.7 μM *d*-fenfluramine was added for 20 min. As shown in Table 3, also under these conditions the drug failed to modify the muscle dGlc uptake.

Since a synergistic interaction with fenfluramine could be operative at lower insulin levels, soleus muscles were incubated for 90 min with 0.7–85 nM (0.1–12.5 mU/ml) insulin and fenfluramine. As shown in Fig. 4, already 0.7 nM insulin was stimulatory on muscle dGlc uptake; fenfluramine did not modify the insulin effect at the physiological as well as pharmacological hormone level (up to 85 nM, data not shown).

The inability of both fenfluramine enantiomers to affect the basal and insulin stimulated dGlc uptake was also evident when the muscles were incubated in high glucose medium (16.7 mM). Table 4 shows that the basal dGlc uptake rate of muscles incubated at 16.7 mM glucose was lower than in muscles incubated at 10.0 mM glucose due to the downregulatory process. Neither *d*- nor *l*-fenfluramine improved the response to insulin which amounted to 50% stimulation of dGlc uptake under these conditions.

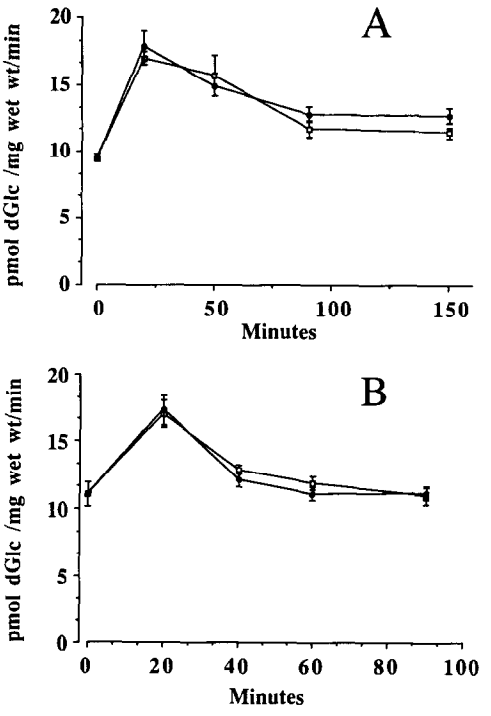

Fig. 3. Lack of effect of fenfluramine on basal and insulin-stimulated dGlc uptake by soleus muscle. Soleus muscles were incubated in DMEM containing 10.0 mM glucose and 1.7 nM (0.25 mU/ml) insulin in the absence (□) or the presence (●) of 3.7 μM *d*-fenfluramine (A) or 3.7 μM *l*-fenfluramine (B) for the indicated times. After washing, the muscles were taken for the standard [^{3}H]dGlc uptake assay. Mean ± SE (N = 4–15).

DISCUSSION

This study demonstrates unequivocally that fenfluramine has no direct effect on the hexose transport of skeletal muscle *in vitro*. Neither *d*-fenfluramine nor *l*-fenfluramine at therapeutic or suprapharmacological concentrations changed the basal dGlc uptake in L_8 myocytes and in isolated soleus muscles maintained at low, normal or high levels of glucose. The drug had no influence on the autoregulatory process of the hexose transport system operated by extracellular glucose, the up- or downregulatory mechanisms of hexose uptake remaining unchanged in the presence of the two enantiomers. Furthermore, we could not demonstrate any interaction between insulin and fenfluramine in isolated soleus muscles. Preincubation of the muscles with insulin, at near physiological and pharmacological levels, did not reverse the lack of responsiveness to fenfluramine. Likewise, preincubation with *d*- or *l*-fenfluramine did not alter the stimulation of hexose transport by insulin.

These observations were made with similar concentrations of drug and for similar incubation periods as previously reported [8–11]. Yet, the present data do not agree with the early reports. Kirby [10], for instance, showed that 1.9 μM (500 ng/ml) fenfluramine racemic mixture increased the glucose uptake in isolated human gluteus muscle; at lower

Table 3. Lack of effect of *d*-fenfluramine on long-term insulin stimulation of dGlc uptake in soleus muscles

Addition	dGlc uptake pmol/mg wet wt/min	% change
None	9.8 ± 0.4	
d-Fenfluramine	10.0 ± 0.4	+2
Insulin	13.0 ± 0.6	+33*
Insulin + *d*-fenfluramine	13.2 ± 0.6	+35*

Soleus muscles were incubated in DMEM containing 9.5 mM glucose in the absence or the presence of 1.7 nM insulin for 150 min. Twenty minutes before the end of the incubation 3.7 μM *d*-fenfluramine was added to the appropriate groups. Thereafter the muscles were washed and taken for the standard dGlc uptake assay. Mean ± SE (N = 4).

* $P < 0.01$, Kruskal–Wallis test.

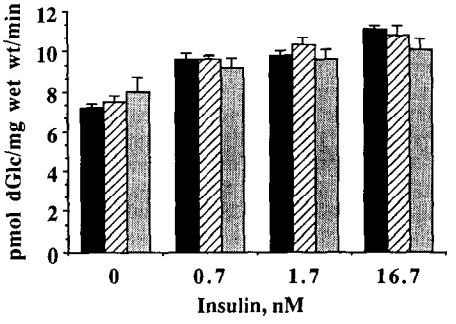

Fig. 4. Lack of effect of fenfluramine on dGlc uptake by soleus muscles in the presence of insulin. Soleus muscles were incubated in DMEM containing 8.7 mM glucose (black columns), and 3.7 μM *d*- (hatched columns) or *l*-fenfluramine (shaded columns) together with insulin at the indicated concentrations for 90 min. After washing, the muscles were taken for the standard [^{3}H]dGlc uptake assay. All insulin concentrations stimulated significantly the uptake (Kruskal–Wallis test). Mean ± SE (N = 6–10).

concentrations (0.037–0.19 μM) the drug had no effect whether it was added alone or together with insulin. In another report, Kirby and Turner [9] showed that *l*-fenfluramine stereospecifically increased the glucose disappearance from the incubation medium of rat hemidiaphragms in the presence of insulin (0.7 nM) while *d*-fenfluramine had no effect. Bajaj and Vallance-Owen [12] found that 37.0 μM (10 μg/ml) fenfluramine increased the glucose uptake of rat diaphragms to the same extent as 3.3 nM (0.5 mU/ml) insulin. These studies led Dannenburg [18] to suggest that *l*-fenfluramine is the metabolically active enantiomer and that insulin may be required for the activity of the drug on glucose uptake. The differences among the various studies were reconciled on the basis of the large differences in the fenfluramine and insulin concentrations used.

The sensitivity and accuracy of the [^{3}H]dGlc uptake assay used here is much higher than when the disappearance of glucose from the incubation medium is measured as was done by others [8–12, 19, 20]. dGlc is commonly used for this purpose since it is a nonmetabolizable glucose analogue that enters the cell via the glucose transporter and is

Table 4. Lack of effect of fenfluramine on basal and insulin stimulated dGlc uptake by soleus muscles maintained at high glucose concentration

Glucose (mM)	Addition	dGlc uptake pmol dGlc/mg wet wt/min
10.0	None	8.1 ± 0.2*[a,b]
16.7	None	6.0 ± 0.4[b,c]
16.7	*d*-Fenfluramine	5.6 ± 0.4
16.7	*l*-Fenfluramine	6.0 ± 0.2
16.7	Insulin	8.4 ± 0.4[c]
16.7	*d*-Fenfluramine + insulin	8.6 ± 0.4
16.7	*l*-Fenfluramine + insulin	8.8 ± 0.6
Fresh muscles	None	13.2 ± 0.4[a]

Soleus muscles were incubated in DMEM containing the indicated glucose concentrations in the absence or the presence of 1.7 nM (0.25 mU/ml) insulin, 3.7 μM *d*- or *l*-fenfluramine. After 90 min incubation the muscles were washed and taken for the standard [^{3}H]dGlc uptake assay. Mean ± SE (N = 4).

* Statistical comparisons relate to differences between groups indicated by the same letter, i.e. a vs a, b vs b, etc. All were $P < 0.01$, Kruskal–Wallis test.

phosphorylated by the hexokinase reaction. We and others [13, 16] have shown that under the experimental conditions used here dGlc uptake represents the transporter activity and not the hexokinase reaction. Moreover, the rate of hexose transport determines its rate of utilization in isolated soleus muscle and skeletal cells in culture under similar incubation conditions [14, 16]. Studies on lactate production in human skeletal muscle *in vitro* suggested that fenfluramine increases the transmembrane transport of glucose and thereby the glycolytic flux [21]. However, this observation was not in agreement with other *in vivo* measurements of lactate production [22, 23]. The effect of fenfluramine on glycogenesis was studied by Jorgensen [22] and others [8] who found no significant effect of the drug on glycogen content in the rat hemidiaphragm. These inconsistent results of studies performed in the 1970s suggest to us that the methodology used was inadequate. Our present extensive *in vitro* studies, using two muscle systems in which a variety of physiologic modulations of hexose uptake have been well-documented [13, 14], lead us to conclude that fenfluramine lacks direct effects on glucose transport following short-term exposure.

Several reports [24–26] on the effects of fenfluramine and related compounds on hepatic intermediary metabolism suggest that these drugs alter gluconeogenesis and fatty acids synthesis as well as the activities of several microsomal enzymes. It is therefore likely that some of the effects of fenfluramine on glucose homeostasis are mediated through its hepatic interactions. The effects of fenfluramine in the central nervous system are multiple: it modifies the release of prolactin, ACTH, and growth hormone [27–29], and it releases 5-HT from serotoninergic neurons and inhibits its re-uptake [1]. It is therefore possible that the verified effects of *d*-fenfluramine on the *in vivo* glucose metabolism [6, 7] are mediated indirectly by these or similar mechanisms. However, we cannot exclude that exposure of several days' duration to the drug may unmask direct effects on muscle cells not detected in our study.

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REFERENCES

1. Garattini S, Mennini T, Bendotti C and Invernizzi R, Neurochemical mechanism of action of drugs which modify feeding via the serotoninergic system. *Appetite* **7**(suppl.): 15–38, 1986.
2. Garattini S, Mennini T and Samanin R, From fenfluramine racemate to *d*-fenfluramine. *Ann NY Acad Sci* **499**: 156–166, 1987.
3. Goodall E and Silverstone T, The effect of the 5-HT releasing drug *d*-fenfluramine and the 5-HT receptor blocker, metergoline, on food intake in human subjects. *Ann NY Acad Sci* **499**: 321–323, 1987.
4. Chandler PT, Dannenburg WN, Polan CE and Thompson NR, Effects of fenfluramine on appetite and lipid metabolism of the young ruminant. *J Dairy Sci* **53**: 1747–1756, 1970.
5. Kneebone GM, The non-anorectic effects of fenfluramine hydrochloride at an experimental level. *S Afr Med J* **45**(suppl.): 20–23, 1971.
6. Bliss BP, Kirk CJC and Newall RG, The effect of fenfluramine on glucose tolerance, insulin, lipid and lipoprotein levels in patients with peripheral arterial disease. *Postgrad Med J* **48**: 409–413, 1972.
7. Dykes JW, The effect of a low-calorie diet with or without fenfluramine on the glucose tolerance and insulin secretion of obese maturity onset diabetics. *Postgrad Med J* **49**: 318–324, 1973.
8. Frayn KN, Hedges A and Kirby MJ, Stimulation by fenfluramine of glucose uptake into skeletal muscle *in vitro*. *Horm Metab Res* **6**: 86, 1974.
9. Kirby MJ and Turner P, Effect of the (−) and (+) isomer of fenfluramine and norfenfluramine on glucose uptake by the isolated rat hemidiaphragm. *Br J Pharmacol* **51**: 120P–121P, 1974.
10. Kirby MJ, Dose-related effect of fenfluramine and norfenfluramine on glucose uptake into human isolated skeletal muscle. *Br J Clin Pharmacol* **1**: 511–512, 1974.
11. Kirby MJ and Turner P, Effect of fenfluramine and norfenfluramine on glucose uptake into skeletal muscle. *Postgrad Med J* **51**(suppl). 1): 73–76, 1975.
12. Bajaj JS and Vallance-Owen J, Fenfluramine and glucose uptake by muscle. *Horm Metab Res* **6**: 85, 1974.
13. Sasson S and Cerasi E, Substrate regulation of the glucose transport system in rat skeletal muscle. *J Biol Chem* **261**: 16827–16833, 1986.
14. Sasson S, Edelson D and Cerasi E, *In vitro* autoregulation of glucose utilization in rat soleus muscle. *Diabetes* **36**: 1041–1046, 1987.
15. Yaffe D, Cellular aspects of muscle differentiation *in vitro*. *Curr Top Develop Biol* **4**: 37–77, 1969.
16. Chaudry IH and Gould MK, Kinetics of glucose uptake in isolated soleus muscle. *Biochim Biophys Acta* **177**: 527–536, 1969.
17. Zar JH, *Biostatistical Analysis*, (2nd Edn), pp. 138–145 and 199–201, Prentice-Hall, Englewood Cliff, New Jersey, 1984.
18. Dannenburg WN, Metabolic effects of phenylethylamine drugs on glucose and fatty acids in various tissues, In: *Biochemical Pharmacology of Obesity* (Ed. Curtis-Prior PB), pp. 263–283, Elsevier Science Publishers, Amsterdam, 1983.
19. Kirby MJ and Turner P, The effect of methysergide and other receptor antagonists on fenfluramine-induced glucose uptake into the isolated rat hemidiaphragm. *Arch Int Pharmacodyn Ther* **225**: 25–28, 1977.
20. Bichi LA, Franklin CS and Turner P, Dose 5-hydroxytryptamine influence the facilitating effect of fenfluramine on glucose uptake into rat isolated hemidiaphragm? *Br J Pharmacol* **68**: 169P–170P, 1980.
21. Kirby MJ and Turner P, Increases in human skeletal muscle lactate production by fenfluramine. *Nature* **262**: 617, 1976.
22. Jorgensen KC, Action of fenfluramine on glucose uptake *in vitro* and *in vivo*. *Acta Pharmacol Toxicol* **40**: 401–417, 1977.
23. Asmal AC, Leary WP and Lockett CJ, The effect of fenfluramine on carbohydrate metabolism in obesity. *Res Commun Chem Pathol Pharmacol* **18**: 95–103, 1977.
24. Geelen MJ, Mechanisms responsible for the inhibitory effects of benfluorex on hepatic intermediary metabolism. *Biochem Pharmacol* **32**: 1765–1772, 1983.

25. Geelen MJ, The effects of *d*-fenfluramine on the metabolism of isolated hepatocytes in the rat. *Biochem Pharmacol* **32**: 3321–3324, 1983.
26. Mounie J, Goudonnet H, Escousse A and Truchot RC, Effets de divers hypolipoprotéinémiants sur l'activité des enzymes microsomales hépatiques. *J Pharmacol* **17**: 308–315, 1986.
27. Van de Kar DL, Urban HJ, Richardson DK and Bethea LD, Pharmacological studies on the serotoninergic and nonserotonin-mediated stimulation of prolactin and corticosterone secretion by fenfluramine. *Neuroendocrinology* **41**: 283–288, 1985.
28. Fuller RW, Serotoninergic stimulation of pituitary adrenocortical functions in rats. *Neuroendocrinology* **32**: 118–127, 1981.
29. Smythe GA and Lazarus L, Growth hormone regulation by melatonin and serotonin. *Nature (Lond)* **244**: 230–231, 1973.