A PRELIMINARY STUDY OF THE EFFECT OF NORFENFLURAMINE ON LIPOGENESIS BY HUMAN ADIPOSE TISSUE *In vitro*

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1 Norfenfluramine is thought to be the major active metabolite of fenfluramine in man. Therefore its *in vitro* effect on lipogenesis in human adipose tissue from twelve patients was investigated.

2 Although inhibition of lipogenesis was demonstrated, the concentrations of norfenfluramine necessary (>1 mM) were much higher than normal blood levels of the drug.

3 There was no correlation between the degree of inhibition by norfenfluramine *in vitro* and fat cell size, age or relative weight of the subject. Greater degrees of inhibition were however associated with samples of tissue from patients with higher blood glycerol concentrations and lower blood triglyceride concentrations.

Introduction

The role of fenfluramine (Ponderax, Servier Laboratories Ltd) in aiding weight loss has been ascribed to both anorectic (Anand, 1971) and peripheral metabolic effects (Butterfield & Whichelow, 1968; Pawan, 1969). The metabolic effects observed *in vivo* have suggested that lipid synthesis is reduced in adipose tissue and that glucose is diverted into muscle. However, it is very difficult to distinguish the metabolic effects attributable to the drug from those attributable to weight reduction alone, and Garrow, Belton & Daniels (1972) have questioned the 'glycolytic' action of fenfluramine. More convincing evidence for the direct action of fenfluramine on adipose tissue is needed and yet very few groups so far have demonstrated the effect of fenfluramine and its derivatives *in vitro*.

Dannenburg & Kardian (1970) have reported metabolic effects of fenfluramine (1 mM) *in vitro* using rat fat cells, namely the inhibition of hormone-stimulated lipolysis and glyceride-fatty acid and free fatty acid synthesis. Wilson & Galton (1971) showed that fenfluramine at concentrations greater than 1 mM could also inhibit lipogenesis in pieces or homogenates of human adipose tissue.

Since these concentrations of fenfluramine necessary to produce an *in vitro* effect are about 10,000 times more than the concentration of plasma fenfluramine on the recommended dosage (Campbell, 1970), it was thought that fenfluramine itself might not be the active metabolite. Le Douarec (1971) has suggested that norfen-

fluramine (NFF) is the major active metabolite in animals and man and for this reason, it was decided to study the effects of norfenfluramine on *in vitro* lipogenesis by human adipose tissue.

Methods

Adipose tissue was obtained from subcutaneous and extraperitoneal sites of twelve patients undergoing routine abdominal surgery under general anaesthesia.

Norfenfluramine chlorhydrate (NFF) was kindly supplied by Servier Laboratories Ltd, London. [14 C]-U-glucose was obtained from the Radiochemical Centre, Amersham, Bucks. Scintillation solution grade 1,4-di[(2-(5-phenyl-oxazolyl))] -benzene (POPOP) and 2,5-diphenyl-oxazole (PPO) were purchased from Nuclear Enterprises Ltd, Edinburgh, Scotland.

Incubations were performed at 37° for 2 h either using small pieces of tissue (about 60 mg/tube) or isolated fat cells (about 20 mg/tube) prepared according to Rodbell (1964). Krebs Ringer bicarbonate buffer (2 ml) (pH 7.4) plus [14 C]-U-glucose (0.05 μ Ci/ml) (specific activity 300 mCi/mmol) containing 4% BSA and glucose (1 mg/ml) was used and all tubes were gassed initially with O₂ : CO₂ (95 : 5). Solutions of NFF were also prepared in appropriate concentrations in this buffer. At the end of the incubation, neutral lipids were extracted from the fat pieces or fat cells

using the technique of Folch, Lees & Sloane Stanley (1957). Radioactivity appearing in total lipids was counted in scintillation solution (10 ml) (0.4% PPO, 0.05% POPOP in toluene by a liquid scintillation counter (LKB Wallac 81000).

Relative weight of patients was calculated as a ratio of their actual weight divided by their ideal weight for height as given by the Metropolitan Life Insurance Company (1959).

Fat cell size of all human fat samples was determined microscopically using isolated cell suspensions (Ashwell, Priest & Bondoux, in preparation).

Blood samples were obtained during the operation for the analysis of glucose (Trinder, 1969), glycerol and triglycerides (Boehringer Corporation Ltd, Test Combination No. 15989), and lipoproteins (Stone & Thorpe, 1966).

All subjects except four (JW, BB, ER and JL) were within the normal weight range. In spite of this there was a considerable variation in fat cell diameter thus reflecting a wide variation in total fat cell number in these subjects. Three subjects (SB, MH and JW) had triglyceride values higher than the normal range.

Results

Table 1 shows the general characteristics of the patients from whom fat was obtained. Table 2 shows that lipogenesis in fat samples from all eleven patients was inhibited to a certain extent by NFF, particularly by concentrations exceeding 1 mM. The range of NFF effect at 5 mM was quite large (13.8% of basal to 77.3% of basal) and therefore the results were analysed to see whether the degree of inhibition by NFF (5 mM) could be correlated with any of the measurements made on the fat or the blood.

The NFF effect of 5 mM was not significantly correlated with age of the subject or the relative weight of the subject. Nor was there any correlation with fat cell size. However, there was a significant ($P < 0.05$) association between NFF inhibition and blood glycerol and triglyceride levels (Table 3). High degrees of NFF inhibition of lipogenesis in fat samples were associated with high blood glycerol and low triglyceride levels.

In some experiments, the effect of NFF on basal lipolysis (i.e. release of glycerol) and on [^{14}C]-glucose uptake into adipose tissue was measured, but no significant change was noted from controls in the range of concentrations of NFF which affected lipogenesis.

Table 1 General characteristics of patients

Patient and sex	Age (years)	Relative weight*	Mean fat cell	Serum glycerol (mg%)	Serum triglycerides (mg%)	Lipoproteins			Plasma glucose (mmol/l)
			diameter (μM)			Large (g/l)	Medium (g/l)	Small (g/l)	
EM ♀	70	1.17	57.6	0.85	92	0.04	1.65	3.49	4.3
ER ♀	61	1.29	88.8	1.17	103	0.04	1.65	4.31	5.0
JH ♀	71	1.04	64.4	1.32	177	0.04	1.65	4.53	4.1
ED ♀	49	1.15	87.5	1.40	177	0.04	1.65	3.65	4.7
SB ♂	57	1.12	88.2	0.92	276	0.04	1.65	5.93	6.0
JL ♂	58	1.26	96.5	0.83	111	0.04	2.75	2.88	3.8
GW ♀	54	1.15	83.0	+	+	+	+	+	+
MH ♀	63	1.11	79.3	0.81	254	0.04	1.65	1.95	5.2
JW ♂	43	1.30	96.9	0.61	216	0.04	1.65	3.74	4.4
EC ♀	40	0.97	+	1.13	106	+	+	+	4.4
BB ♀	18	1.74	92.4	1.18	95	0.04	4.71	3.9	3.9
RG ♀	75	1.13	83.9	0.94	120	0.04	1.65	4.79	8.4
Mean	54.9	1.20	83.5	1.01	157	0.04	2.07	3.92	4.93
S.e. mean	4.6	0.05	3.8	0.07	20.2	0	0.31	0.34	0.40
n	12	12	11	11	11	10	10	10	11

+ Results not available.

* Normal range 0.8-1.2.

Table 2 The effect of norfenfluramine on lipogenesis in human adipose tissue

Norfenfluramine concentration (mM)	% basal lipogenesis	
	Mean $\pm$ s.e. mean	Range
1	95.4 $\pm$ 4.2 (n = 11)	74.8-121
3	74.4 $\pm$ 3.7 (n = 11)	54.4-90.3
5	47.2 $\pm$ 6.8 (n = 12)	13.8-77.3

Human adipose tissue was incubated as described in the text. The values obtained for lipogenesis in the presence of NFF were compared with the basal value of lipogenesis for the same fat sample. (Average value for basal lipogenesis was 541 p mol [14 C]-glucose incorporated into lipid/2 hours).

Duplicate samples were incubated for each drug concentration with each fat sample and the results averaged.

Discussion

The results presented in this paper show that norfenfluramine, like fenfluramine, will inhibit lipogenesis from [14 C]-glucose in human adipose tissue but only in concentrations which are very much higher than the normal circulating concentrations of the drug (Campbell, 1970).

Wilson & Galton (1971), working with human fat, suggested that fenfluramine may act at the acyl-transfer reaction in the pathway of triglyceride synthesis since it reduced radioactivity in the neutral lipid from labelled palmitate but did not reduce radioactivity in the long chain acyl-CoA fraction. Dannenburg & Kadian (1970) showed that fenfluramine (1 mM) inhibited lipogenesis from [14 C]-glucose by reducing fatty acid synthesis from glucose. In my studies, I did not pursue the site of action of norfenfluramine but since glucose uptake is unaffected the effect must be intracellular. Preliminary experiments

Table 3 Correlation of norfenfluramine (5 mM) inhibition of lipogenesis *in vitro* with *in vivo* characteristics of patients

Regression equation	r	P
Serum glycerol $y = 107 - 62x$	-0.64	<0.05
Serum triglycerides $y = 12 + 21x$	0.61	<0.05

y = % basal value for lipogenesis.

x = concentration of serum glycerol (mg%) or serum triglycerides (mg%).

There was no significant correlation between NFF (5 mM) effect and age, relative weight, cell diameter, blood glucose or blood lipoproteins.

with rat fat cells (Ashwell, unpublished observations) showed that norfenfluramine, like fenfluramine, inhibited [14 C]-glucose oxidation to [14 CO $_2$] at similar concentrations to those needed for inhibition of lipid formation. Unfortunately, a smaller proportion of [14 C]-glucose is oxidized to [14 CO $_2$] by human adipose tissue and it was not possible to measure the effect of norfenfluramine.

The correlation of norfenfluramine inhibition *in vitro* with the blood levels of glycerol and triglycerides is interesting. Gilbert, Kaye & Galton (1974) have recently shown a correlation between plasma levels of free fatty acids and the release of fatty acids from adipose tissue incubated *in vitro*. This suggests that the metabolic activity of adipose tissue *in vitro* may continue to reflect the immediate physiological situation from which it was removed. If norfenfluramine inhibition at physiological concentrations could be shown to correlate with these physiological variables, then there would be some indication that its effect on adipose tissue from some subjects would be greater than others.

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