RELEASE OF FATTY ACIDS FROM ADIPOSE TISSUE IN GENETICALLY OBESE (ob/ob) MICE

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1. Introduction

Conditions likely to promote the release of fatty acids from depot fat in obese (ob/ob) mice have produced conflicting results, and it is not clear to what extent fat mobilisation occurs in these animals. Ob/ob mice survive starvation and this is accompanied by a rise in plasma fatty acids similar to that in normal mice [1] but cold exposure for about 5 h results in death, whereas lean mice adapt to cold [2]. Injection of noradrenaline increases the level of plasma fatty acids to the same extent in ob/ob and lean mice [1]. However, in vitro studies with isolated adipose tissue preparations indicate that hormone stimulated lipolysis is lower in ob/ob mice than in normal controls [3–7].

2. Experimental

Male obese mice (ob/ob) and their lean littermates (ob/ob$^+$) aged 6–8 weeks were obtained from Biochemistry Department, Imperial College, London and were 2–3 months old when used in experiments. They were fed Oxoid 41B diet ad libitum.

The saline medium used in the preparation and incubation of isolated adipocytes was Krebs-Henseleit saline [8], containing half the Ca^{2+} concentration of the original saline medium and with the addition of 4% (w/v) bovine serum albumin (fraction V, Armour). Glassware was siliconized with Repelcote (Hopkin and Williams).

2.1. *Preparation of isolated adipocytes*

Isolated adipocytes were prepared by a modification of the method in [9]. The modification appears to give a cell preparation which contains few broken cells from adipose tissue of obese mice, judged by the release of oil and lactate dehydrogenase into the medium. Paired, chopped whole epididymal fat pads were incubated for 1 h at 37°C with shaking, in 3 ml saline medium containing 10 mg collagenase, (type 1 from *Clostridium histolyticum*, Sigma) and 2 mg/ml glucose. Saline medium, 10 ml, was then added and the suspension centrifuged at 250 × *g* for 1 min. Surface oil and cell free medium were discarded, 10 ml fresh medium were added to the adipocytes and after gentle dispersion any cell clumps were removed. The suspension was allowed to separate from the medium under gravity for 3 min at 37°C. The bottom aqueous layer was removed and the isolated adipocytes made to 5 ml and 7 ml with saline medium, for cells prepared from lean and obese mice, respectively. Preparations contained approx. 2×10^6 cells/ml.

2.2. *Counting of adipocytes*

Isolated adipocytes were routinely counted on a 0.2 mm haemocytometer. Adipocytes from obese mice are much larger than those from lean mice due to increased triglyceride content, and it was necessary to independently assess the method for counting cells. For this purpose DNA in isolated cell preparations was estimated by the method in [10]. Cell suspension, 1 ml, was sonicated with an MSE Mullard 60 W ultrasonic drill and centrifuged at 12 000 × *g* for 15 min at 0°C. The supernatant, after removal of fat, was extracted once with cold 10% (w/v) perchloric acid and twice with hot 10% (w/v) perchloric acid. The combined extracts were used for DNA estimation and gave values of (mean ± SEM) 4.2 ± 0.9 (5) pg/cell and 4.0 ± 0.9 (5) pg/cell for adipocytes from lean and

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obese mice, respectively. The close similarity of DNA contents per cell indicates that the method used for counting adipocytes appeared to be valid.

Blood samples were obtained from mice after anaesthetisation with diethyl ether/air and decapitation. Serum was obtained from clotted blood after centrifugation (12 000 × *g*; 15 min).

2.3. *Incubation of adipocytes*

The effect of noradrenaline on fatty acid release from adipocytes was determined as follows. Isolated cell suspension, 400 μl, saline medium, 600 μl (with or without glucose; final conc. 10 mM) and noradrenaline, 100 μl, in distilled water (at concentrations indicated in fig.1) were added to 25 ml flasks, gassed with O_2/CO_2 (95%/5%) and incubated with shaking at 37°C for 45 min. In control flasks 100 μl distilled water replaced noradrenaline. After incubation the contents of the flasks were centrifuged at 250 × *g* for 1 min and the bottom aqueous layer used for fatty acid determination. The release of fatty acids from adipocytes in the presence of noradrenaline was almost linear for 1 h indicating a low degree of cell damage during incubation.

Fatty acids from incubated cells and serum were estimated by the method in [11].

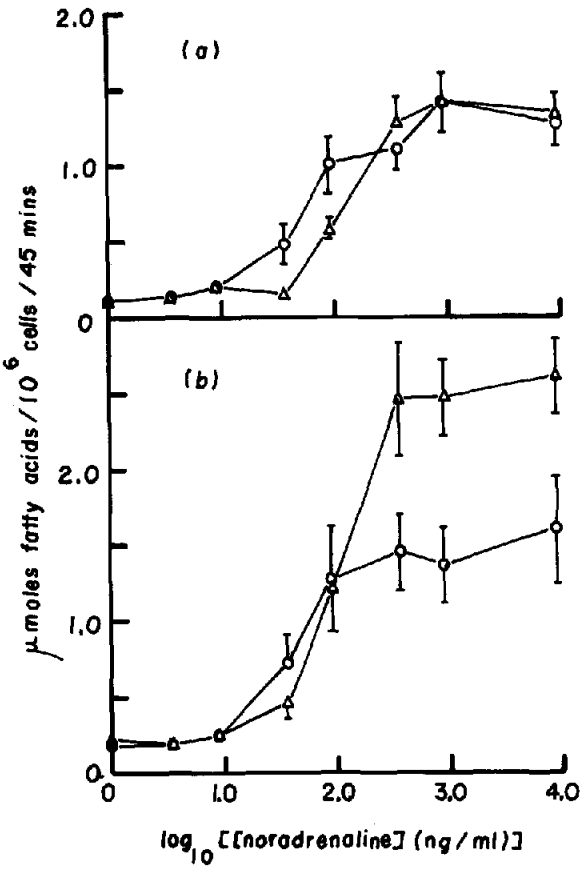

Fig.1. The effect of noradrenaline concentration on fatty acid release from isolated adipocytes. Isolated adipocytes, prepared from lean (○), or obese (△) mice were incubated in saline medium containing various concentrations of noradrenaline. The fatty acid concentration in the medium was measured after 45 min. In (a) the incubation medium was glucose-free and in (b) the incubation medium was 10 mM with respect to glucose. Results are means of 7 incubations and bars indicate the SEM.

3. Results and discussion

The concentrations of serum fatty acids increased 4-fold in both fed lean and obese mice after cold exposure at 4°C for 1 h. In lean mice, fatty acids expressed as μmol/ml serum were raised from the control value of 0.25 ± 0.04 to 1.00 ± 0.10; in obese mice the corresponding values were 0.36 ± 0.06 and 1.47 ± 0.13, showing that fatty acids were rapidly mobilised on cold exposure. Means ± SEM for 6 observations are given. Failure of obese mice to survive cold exposure [2] may be due to an inability to utilize rapidly mobilised fatty acids. This defective response to cold in obese mice has been found in preweanling ob/ob mice [12] and is probably not a secondary effect of their obesity.

The effect of noradrenaline on fatty acid release from adipocytes of lean and obese mice, in the absence of glucose, is shown in fig.1a. The release on a cell basis is similar for lean and obese mice at all noradrenaline concentrations tested. This result is in agreement with recent work [13] which showed that, although cyclic AMP accumulation by isolated fat cells in response to noradrenaline is much lower in obese mice, lipolysis was essentially unaltered. However it is at variance with results in [3–7]. Fatty acid release calculated on the basis of tissue weight, gave lower results for obese mice compared with lean mice [5,6], presumably because the no. adipocytes/unit weight is much lower in obese mice due to their higher

triglyceride content. Fatty acid release based on whole tissue protein gave lower results for obese mice [3], presumably because this includes protein from mast cells which are increased 15-fold in adipose tissue from obese mice compared with lean mice [14]. Complications of mast cells and cell mass are eliminated by using cell numbers as the basis for the expression of results.

Lower levels of fatty acid release from obese mice have been claimed, based on experiments measuring fatty acid release from pieces of adipose tissue, expressing results on the basis of cell numbers [4,7]. Cell numbers were gauged by estimating their lipid content and diameter. The use of tissue pieces involves questions of the extent of penetration of noradrenaline into the preparation, and release of fatty acids into the medium (especially with obese mice). These difficulties do not occur with isolated adipocytes. We have obtained maximal effects of noradrenaline at concentrations of 0.5% of those necessary when tissue pieces are used [4].

In the presence of glucose, maximal stimulation of fatty acid release by noradrenaline was greatly increased by cells from obese but not lean mice, strongly supporting the view that there is no impairment of lipolysis in obese mice (fig.1b). The effect of glucose may be due to a stimulation of triglyceride turnover or to increased energy available for cyclic AMP biosynthesis. Results suggest the former, since in adipocytes from obese mice the ratio of fatty acids released : glycerol released is reduced from 3:1 to 2:1 in the presence of glucose.

The in vitro results described here with isolated adipocytes and the in vivo results obtained on cold exposure are compatible with previous in vivo work. Plasma fatty acid concentrations increase equally on starvation and after injection of noradrenaline in lean and obese mice [1]. Also plasma fatty acid levels are similar in fed lean and obese mice, although the rate of removal of fatty acids from the bloodstream is greater in the obese mouse [15]. We conclude that noradrenaline stimulated lipolysis is not defective in obese mice, as suggested by other in vitro work [3–7], and that impaired lipolysis is not a contributory factor to their obesity or failure to survive cold stress. This failure may be due to an impaired utilization of mobilised fatty acids.

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