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Influence of Cell Size on the Effects of Insulin and Noradrenaline on Human Adipose Tissue

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Summary. In the present study dose-response relationships of the effects of noradrenaline and insulin on fat cells of different sizes were performed. Adipose cells larger than 100 μm were more responsive (expressed as absolute effects) to the lipolytic action of noradrenaline as well as to the antilipolytic effect of insulin. This suggests that in the larger cells the *capacity*, i.e. the sum of factors contributing to the ability to stimulate or inhibit the metabolic rates, was greater than in the smaller ones. In contrast the *sensitivity* to these agents, i.e. the readiness to respond, was not different between small and large cells.

It is shown that the concentrations of insulin needed to obtain an antilipolytic effect is far below that needed to stimulate glucose incorporation. This discrepancy in insulin concentrations required may be due to binding of insulin to receptors with different affinity.

Key words: Human adipose tissue, lipolysis, noradrenaline, insulin.

It is now well established that fat cell size is of importance for the cellular responsiveness to certain hormones. In human adipose tissue it has been shown that the lipolytic effect of catecholamines is enhanced in large fat cells [1–4]. Furthermore, data have been presented indicating that the antilipolytic action of insulin is more pronounced in large cells as well [2, 5]. However, fairly high concentrations of catecholamine and insulin have generally been used for the studies. Thus the results show that the metabolic *capacity*, i.e. the sum of factors contributing to the ability to stimulate or inhibit the metabolic rates, is increased in large fat cells. In order to analyse the cellular *sensitivity* to these hormones, which well may be a physiologically more relevant parameter, dose-response relationships have to be analysed.

In the present study different concentrations of insulin and noradrenaline were used and the sensitivity for these agents was analysed in fat cells below or above 100 μm.

Materials and Methods

Specimens of subcutaneous adipose tissue were obtained from patients operated upon for an isolated abdominal disorder, generally cholelithiasis. Patients with jaundice, malignant or endocrine disorders were excluded. In the studies of the effect of catecholamine tissue biopsies were obtained in local field block anesthesia with 2% lidocain (Xylocain, ASTRA, Södertälje, Sweden) by incision in the lower lateral quadrant of the abdominal wall. Care was taken not to infiltrate the tissue biopsy with the anesthetic agent, since such agents inhibit lipolysis [6, 7]. The biopsies were immediately placed in medium 199 and smaller specimens, weighing about 25–50 mg each, were then prepared.

The incubation procedure has been described in detail previously [2]. Briefly, after preincubation for 30 min [8] the tissue specimens were incubated for 2 hours at pH 7.4 in 2.0 ml medium 199 (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) modified to a glucose concentration of 1.0 mM and containing 40 mg/ml albumin (Fraction V, Sigma Chemical Co., St. Louis, Mo.). Noradrenaline (ASTRA AB, Södertälje, Sweden) and glucagon-poor insulin (Vitrum AB, Stockholm, Sweden) were added as indicated. The release of glycerol was taken as an index of the lipolysis and determined enzymatically [9]. For reasons previously discussed [10] the

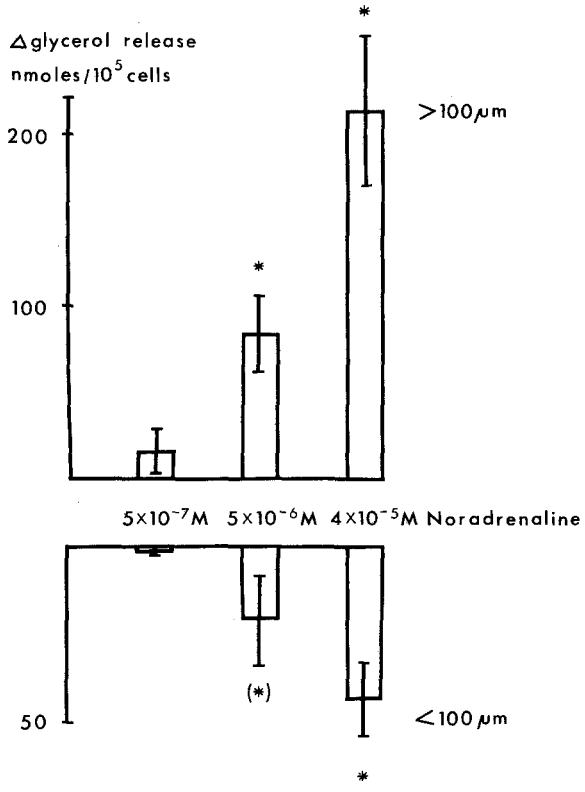

Fig. 1. The lipolytic effect of different concentrations of noradrenaline on fat cells above (n = 9) or below (n = 8) 100 μm. N = number of donors. * indicates significant lipolytic effect of noradrenaline ($p < 0.05$), (*) indicates $0.05 < p < 0.1$. The basal lipolysis has been subtracted from both groups. Results ± SEM

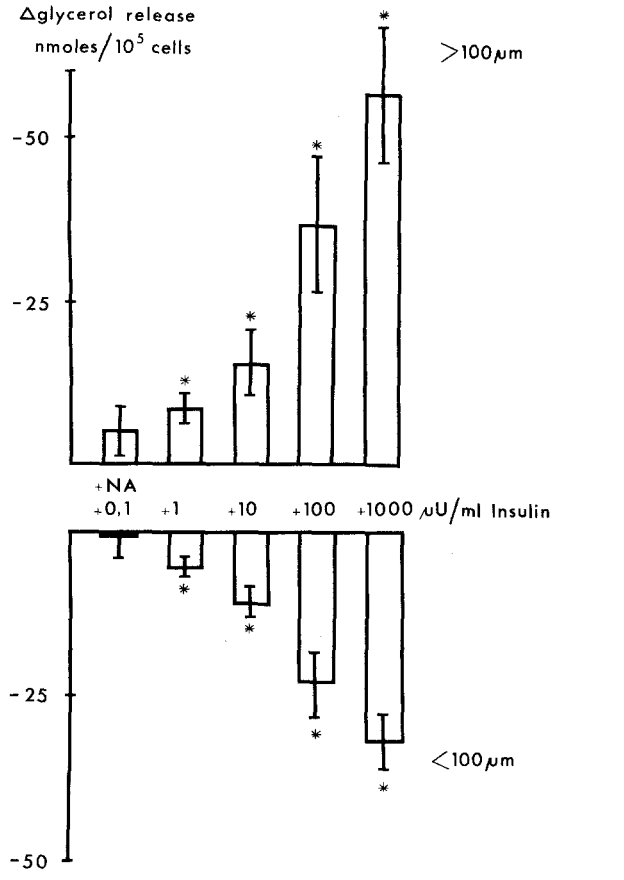

Fig. 2. The antilipolytic effect of different concentrations of insulin on the noradrenaline-stimulated lipolysis (5×10^{-5}M) in fat cells above or below 100 μm. * indicates significant antilipolytic effect of insulin ($p < 0.05$). The number of donors, n = 10 in both groups. Results ± SEM

glycerol release was expressed in terms of the cellularity of the tissue specimens.

Mean cell diameter was determined on cells isolated with collagenase as previously described [11]. Mean cellular volume was calculated as suggested by Goldrick [12]. Mean cell weight was calculated according to Hirsch & Gallian [13]. The tissue triglyceride content was analysed in the chloroform phase [14], as described by Carlson [15]. When the triglyceride content and mean cellular weight are known the number of fat cells of the specimens can be determined.

The results were analysed in relation to cell sizes larger and smaller than 100 μm. The mean ± SEM for cells larger than 100 μm was 105.35 ± 1.70 and for cells smaller than 100 μm 90.0 ± 2.5. The difference was statistically significant ($p < 0.001$).

The ages of the donors were similar and the average for the group with the larger cell size was 52.4 years and for the group with the smaller cells 49.3 years.

Results

Noradrenaline-Stimulated Lipolysis

The lipolytic effect of noradrenaline was enhanced in large fat cells. However, in both groups a consistent effect was found at 5×10^{-6}M (Fig. 1) and the concentration required for half-maximal effect was similar in both groups (about 10^{-5}M).

Antilipolytic Effect of Insulin

Previous experiments have shown that in these intact specimens a maximal lipolytic effect of noradrenaline was obtained with 4×10^{-5}M and for the antilipolytic action of insulin 1000 μU/ml was required [2].

The antilipolytic effect of insulin on the noradrenaline-stimulated lipolysis was analysed in two groups, i.e. specimens with a mean cell size above or below 100 μm. As shown in Fig. 2 the absolute effect of insulin on the lipolysis was, at all tested concentra-

tions, more pronounced in the large cells. On a percentage basis, however, no difference was found between the two groups (Fig. 3). Furthermore, irrespective of fat cell size, a significant antilipolytic effect was found in these intact tissue specimens at 1.0 μU/ml and half-maximal effect was exerted in both groups at about 30 μU/ml. These findings were not influenced by an effect of insulin on the basal lipolysis since in the presence of glucose it was inconsistent and variable (see below).

In Table 1 the effect of insulin at different concentrations on glucose metabolism is shown. The results indicate that the insulin effect on glucose disposal is obtained at higher concentrations than the effects exerted on lipolysis.

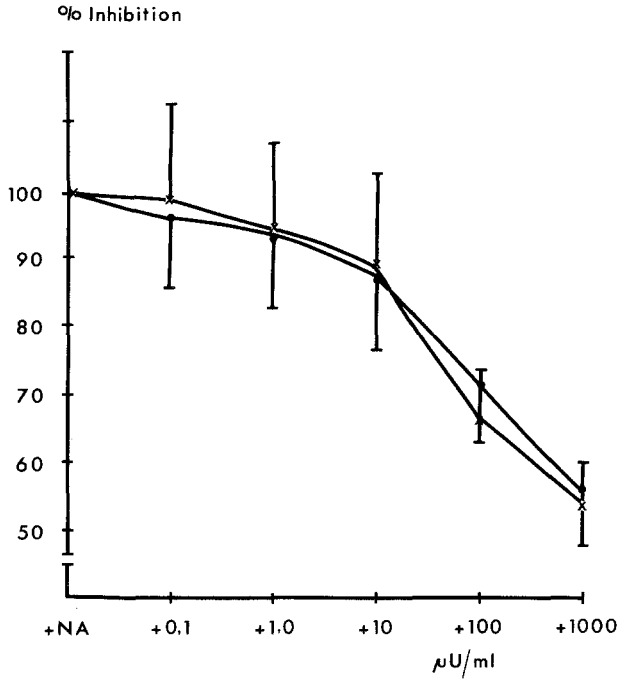

Fig. 3. The antilipolytic effect of different concentrations of insulin on the noradrenaline-stimulated lipolysis (5×10^{-5}M). The results are expressed as percent of the lipolysis obtained in the absence of insulin (= 100%). N = 10 in both groups. Results ± SEM

Table 1. The effect of different concentrations of insulin on glucose metabolism in human fat cells

Insulin concentration μU/ml	Incorporation of glucose nmoles/10^5 cells
0	6.8 1.0
0.1	6.0 1.1
1.0	6.3 1.3
10	7.0 1.0
100	8.7 1.5[a]
1000	9.4 1.4[a]

Explants were incubated for 2 hrs with 0.15 μCi [1–^{14}C] glucose. The incorporation into the total lipids was determined. n = 5. All specimens had a mean cell size larger than 95 μm.
[a] indicates significant increase over basal values ($p < 0.05$).

Discussion

It is now quite clear that the effect of certain hormones is more pronounced in large fat cells. However, since high hormone concentrations generally have been used it is not clear whether the sensitivity to hormones may differ between small and large cells.

The present data show that the lipolytic effect of noradrenaline as well as the antilipolytic action of insulin on catecholamine-stimulated lipolysis were more pronounced in large fat cells at all concentrations tested. This implies that the cellular metabolic capacity is increased in large fat cells. However, the cellular *sensitivity*, determined from the dose-response relationships, was similar in large and small cells. The findings of similar sensitivity to noradrenaline is in agreement with a report by Gries et al. [16] where fat cells from obese and non obese individuals were compared.

Since glucose was used in the incubation medium the effect of insulin on the basal lipolysis was inconsistent, as also previously reported by several investigators [2, 17, 18]. In contrast to the present and other studies [2, 3, 5] Gries [19] reported that large fat cells were less responsive than small cells to the antilipolytic effect of insulin. The reason for this discrepancy is unclear but may be due to methodological differences. For instance 'in Gries' [19] study no glucose was present in the incubation medium and a lower catecholamine concentration was used.

The fact that large fat cells are as sensitive as small cells to the antilipolytic effect of insulin seems at variance with the effect of the hormone on glucose metabolism. It has been shown that there is a negative correlation between adipocyte size and the stimulatory effect of insulin on glucose metabolism [20, 21]. Since this tissue resistance has by some investigators [22], but not all [23], been attributed to a diminished number of insulin receptors the present results may at first seem difficult to reconcile with this concept. However, several explanations are possible. It has, for instance, been suggested that there are different types of insulin receptors [24] in the cell membrane. Some support for this concept is offered by the findings that there are two populations of insulin receptors in the cell membrane; high affinity and low affinity receptors [26, 27]. Furthermore, it may well be that a smaller portion of the total number of insulin receptors have to be occupied to elicit an antilipolytic response as compared to that required for the effect on glucose

metabolism. The antilipolytic action requires 10–100 times less insulin than that needed for the effect on glucose metabolism both in rat adipocytes [25] and in human fat cells (Table 1), which may support such a concept. It may well be then, that binding of insulin to the high affinity receptors initiates the antilipolytic effect while the low affinity receptors are mainly concerned with glucose metabolism.

The reason for the increased metabolic *capacity* in the large fat cells is unclear at present. It has been suggested that the number of receptors for catecholamines increases as the cells enlarge [28]. However, in analogy with the discussion above it seems likely that the cellular sensitivity should have been increased rather than only the metabolic capacity. Although several other possibilities may be considered the observation that large fat cells are more responsive to dibutyryl cAMP [4, 16] indicates that the triglyceride lipase may be increased in these cells.

Irrespective of the mechanism involved the present data clearly show the importance of performing dose-response curves, since an increased metabolic capacity cannot be extrapolated to imply an increased cellular sensitivity.

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