

# Effects of age and cell size on rat adipose tissue metabolism

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**Abstract** In order to analyze separately the effects of cell size and age on the metabolism of rat adipose tissue, fat cells of different sizes were obtained from the same animals. The rats were 4 or 15 wk old. The results show that age as well as cell size influences the metabolic rates. At a given cell size, the basal lipolysis, the lipolytic effects of glucagon and noradrenaline, the rate of glucose incorporation into the triglycerides, and the effect of insulin on glucose metabolism were considerably increased in the young animals. Furthermore, irrespective of fat cell size the lipolytic action of glucagon was reduced in old animals. The data thus show that experiments with large fat cells from old rats and with small cells from young animals cannot be directly compared because both variables may influence metabolic reactions.

**Supplementary key words** lipolysis · glucose metabolism · insulin · glucagon · catecholamines

It seems quite clear that adipose tissue metabolism is influenced by the size of the constituent cells in both rat (1–4) and man (5–8). However, the possible influence of the age of the donor is not clear. In previous work in which the effect of aging on the metabolism of rat adipose tissue was studied, results were expressed in terms of the tissue weight (9). Because adipocyte size increases with age in the rat (10), those results may merely reflect differences in fat cell size between young and old animals. Conversely, in several metabolic studies on the effect of fat cell size, large fat cells were obtained from old animals and small fat cells from young (2, 11–13). The results of such studies may be influenced by the age of the animals.

In order to circumvent these problems, the present study was carried out in which fat cells of different sizes were obtained from the same donors. The results of these experiments clearly show that age as well as fat cell size influences the metabolic rates and the responsiveness of cells to hormones.

## MATERIALS AND METHODS

Male Sprague-Dawley rats of different weights (about 80 g and 400 g) and ages (about 4 wk and 15 wk) were obtained from Anticimex (Stockholm, Sweden). The rats were kept in cages in the laboratory for about 1 wk prior to being killed. They had unlimited access to tap water and were fed ad lib. a laboratory chow (EWOS, Södertälje, Sweden) containing, by weight, 5% fat, 55% carbohydrate, and 22.5% protein, plus minerals and vitamins.

The rats were killed by a sudden blow on the head. In each experiment the epididymal fat pads were excised from 2 old and 15 young animals. The fat pads from each group were pooled, and the cells were isolated from the stroma by incubating smaller specimens with collagenase (14). The fat cells from each group of animals were then separated into different size classes as previously described (15). This technique utilizes the fact that fat cells of different sizes have different rates of flotation in dialysis tubing. Cell fractions were collected as previously described (15), and the mean cell size of each fraction was determined (16). The cell fractions were collected in Krebs-Ringer bicarbonate buffer containing 40 mg/ml albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.) and 5.6 mM glucose. Aliquots of each cell fraction were then incubated in a total volume of 2 ml of buffer with 0.15  $\mu$ Ci of [ $^{14}$ C]glucose (New England Nuclear, Frankfurt/Main, W. Germany), recrystallized glucagon-poor insulin (Vitrum AB, Stockholm, Sweden), and noradrenaline (Astra AB, Södertälje, Sweden) or glucagon (Novo AB, Copenhagen, Denmark) for 2 hr as indicated. The gas phase was 5% CO<sub>2</sub>–95% O<sub>2</sub>. The reaction was stopped by adding 5 ml of Dole's extraction mixture (17). Samples of the incubation media were also taken for the determination of glycerol (18).

After separating and washing the phases, triglycerides were determined according to Carlson (19). An aliquot of the heptane phase was taken for the determination of ra-

dioactivity in the total lipids in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., La Grange, Ill.). Quenching was corrected for by internal standardization. The number of fat cells was calculated from the mean fat cell size of the fraction and the triglyceride content. Mean cell volume was calculated as described by Goldrick (20) and mean cell weight as described by Hirsch and Gallian (21), assuming that the density of fat cells is equal to that of triolein. The metabolic rates are expressed in terms of the number of fat cells.

## RESULTS

When the data from the different experiments were pooled, fat cell size was found to influence the metabolic rates in both young and old animals. Thus, with the exception of the lipolytic effect of glucagon, correlations between fat cell size and the basal rate of glucose incorporation into the lipids, basal lipolysis, and the lipolytic effect of noradrenaline were found in the old group (Figs. 1 and 2). In the young animals, all metabolic parameters studied were increased in the large cells except basal lipolysis, where the correlation coefficient did not reach statistical significance due to interindividual variations. In the separate experiments an effect of cell size was also found on this parameter.

However, all the metabolic rates differed considerably between the young and the old animals, as shown in Figs. 1 and 2. The most pronounced differences were found for the basal rate of glucose incorporation into the triglycerides, the effect of insulin on glucose metabolism, and the lipolytic effect of glucagon. A resistance to glucagon was found in the old animals irrespective of fat cell size. The young group responded to this hormone, and the larger the fat cells, the greater the lipolytic effect (Fig. 2). These data are further shown in Table I, where the metabolic rates of fat cells of the same size from young and old animals are compared.

For each parameter the metabolic rates were significantly increased in the young animals. The effect of insulin on glucose metabolism was several times higher in the young animals. However, even at the same cell size the effect of insulin varied considerably in the young group. In the old group no significant effect of insulin on glucose metabolism was found. It should be noted that, in spite of a consistent effect of insulin on glucose metabolism in the young group, no consistent antilipolytic action was found (Table I).

## DISCUSSION

In order to analyze separately the contributions of age and cell size to the metabolic rates, it is necessary to sepa-

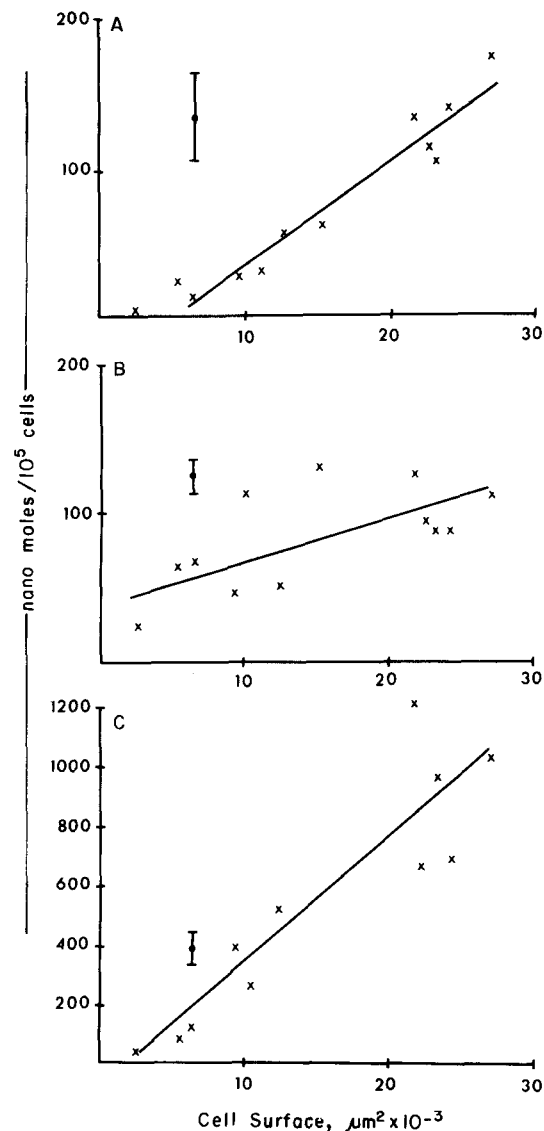


Fig. 1. Correlation between cell surface area and metabolism of fat cells from old rats (X). The average metabolic rate of fat cells from young animals ( $\pm$  SEM; 12 experiments) is also shown. (a) Basal rate of glucose incorporation into the triglycerides ( $n = 12$ ;  $y = 7.0x - 36.1$ ;  $r = 0.97$ ;  $P < 0.001$ ); (b) basal lipolysis ( $n = 12$ ;  $y = 2.5x + 44.7$ ;  $r = 0.63$ ;  $P < 0.05$ ); (c) lipolytic effect of  $5 \times 10^{-5}$  M noradrenaline ( $n = 11$ ;  $y = 42.1x - 88.6$ ;  $r = 0.91$ ;  $P < 0.001$ ).

rate fat cells of different sizes from the same donors. Such techniques are now available (4, 7, 15) and were utilized in the present study. The results show that both parameters influence the metabolic rates. Thus, for both young and old animals, the larger the fat cells, the greater the metabolic capacity, as has repeatedly been demonstrated for both rat (1-4) and human (5-8) adipose tissue.

Basal lipolysis was increased in the young animals. Novak, Melichar, and Hahn (22) have shown that lipolysis is enhanced in newborn infants and that the lipolytic rates then rapidly decline. However, the data were expressed per unit tissue weight, which makes the interpretation less

TABLE 1. Comparison of metabolism in fat cells of the same size from rats of different ages

Parameter	Young Animals (n = 12)	Old Animals (n = 5)	P Level
Cell surface, $\mu\text{m}^2 \times 10^{-2}$	6.3 $\pm$ 0.9	6.7 $\pm$ 1.4	>0.1
Glucose incorporation <sup>a</sup>	135 $\pm$ 35	9 $\pm$ 3	<0.005
Glucose incorporation + insulin	461 $\pm$ 162	12 $\pm$ 3	<0.01
% Increase	241	17	
Basal lipolysis	125 $\pm$ 17	62 $\pm$ 15	<0.05
Basal lipolysis + insulin	126 $\pm$ 17	60 $\pm$ 15	<0.05
Effect of noradrenaline	387 $\pm$ 45	176 $\pm$ 65	<0.05
Effect of glucagon <sup>b</sup>	289 $\pm$ 45	48 $\pm$ 25	<0.005

Fat cells of different sizes were separately obtained from young and old animals with the flotation method described in the text. In this table the results obtained with fat cells of the same size from young and old animals are compared. Incubations were performed for 2 hr with 0.15  $\mu\text{Ci}$  of [ $1\text{-}^{14}\text{C}$ ]glucose and  $10^3 \mu\text{U/ml}$  insulin,  $5 \times 10^{-5}$  M noradrenaline, and  $2.9 \times 10^{-7}$  M glucagon as indicated. Values are means  $\pm$  SEM of the number of experiments shown in parentheses.

<sup>a</sup> Metabolic rates are expressed as nmoles/ $10^5$  cells.

<sup>b</sup> n = 9 and n = 3 for young and old animals, respectively.

clear. Recently, we found that basal lipolysis is increased in children under 1 yr of age compared with older children when the data are analyzed in terms of cellularity.<sup>1</sup>

The effect of lipolytic hormones was increased in the young animals. Irrespective of fat cell size, old animals were resistant to glucagon whereas the young animals responded. Furthermore, the larger the cell size, the greater the effect of glucagon in this latter group. Recently, Manganiello and Vaughan (23) reported that large fat cells from old animals were less responsive to glucagon than small cells from younger animals. The adenylyl cyclase activity in response to glucagon was diminished in the resistant cells. Other studies have verified that large fat cells from old rats are resistant to glucagon (4, 13). The mechanism for this resistance has been suggested to be a diminished binding of glucagon to the cells (13) as well as an increased phosphodiesterase activity (24) in large fat cells. It was suggested in these latter studies that the metabolic differences noted were due to the different fat cell sizes. However, because the large cells were obtained from old rats this conclusion was unwarranted. The present results show quite clearly that the glucagon responsiveness is diminished in old rats irrespective of fat cell size. In the younger animals, however lipolysis in response to glucagon was increased, and the larger the fat cells, the greater this effect.

The reasons for the diminished lipolytic effects of glucagon and noradrenaline in fat cells from old rats are unclear but may be associated with diminished binding of the hormones to the cells (13). Furthermore, diminished adenylyl cyclase activity and increased phosphodiesterase activity may also be of importance (24, 25). However, further studies with the present techniques are required to elucidate this problem.

The basal rate of glucose metabolism and the insulin responsiveness were considerably increased in the young ani-

mals. Only total lipids were measured in the present work, but it may well be that the decrease in lipid synthesis in the old rats is mainly due to a decreased rate of fatty acid synthesis, as previously suggested (11, 12).

Insulin responsiveness at the same cell size was several times greater in the young animals. This, however, was true only for the effect of insulin on glucose metabolism and not for the antilipolytic action. The effect of insulin on basal lipolysis was variable under the present experimental conditions, as was also found by other investigators (26, 27). The discrepancy between the effect of insulin on glucose metabolism and the effect on lipolysis would seem to argue against differences in the binding of the hormone to the cells, as discussed above for the lipolytic hormones. However, it is possible that the insulin receptors mediating

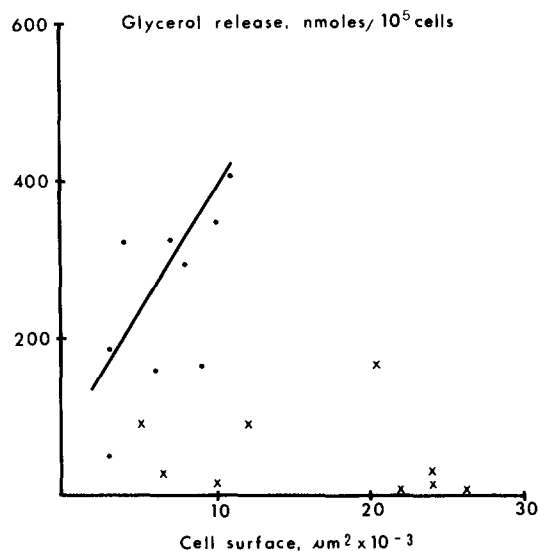



Fig. 2. Correlation between cell surface area and lipolytic effect of  $2.9 \times 10^{-7}$  M glucagon on fat cells from old rats ( $\times$ ) (n = 9;  $r = 0.20$ ;  $P > 0.1$ ) and young rats ( $\bullet$ ) (n = 9;  $y = 33.4x + 55.4$ ;  $r = 0.76$ ;  $P < 0.05$ ).

<sup>1</sup> Nyberg, G., G. Mellgren, and U. Smith. Unpublished results.

the effect on glucose metabolism differ from those mediating the antilipolytic action (28).

Irrespective of the mechanisms involved, the present results show quite clearly that fat cell size as well as age influences rat adipose tissue metabolism. Thus, studies in which large fat cells from old rats are compared with small cells from young rats are difficult to interpret because both variables may contribute to the results. 

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