PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 513-516 (1977)

Protein Kinase Activity of Isolated Rat Adipocytes as Related to Cell Size[1] (39706)

M. DIGIROLAMO,[2] J. L. OWENS, J. G. PATRICK, AND J. F. KUO

Departments of Medicine and Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30303

Introduction. Enlarged adipocytes from older fatter rats present a number of metabolic alterations when compared to smaller fat cells from leaner younger rats (1–5). Among the most significant findings, large adipocytes (mean volume, 400–600 pl) present a rate of basal lipolysis which is 3- to 4-fold greater than that of smaller fat cells (40–60 pl) (2–4); in addition, large adipocytes present a rate of glyceride synthesis from glucose which is three to four times greater than that of the smaller cells (3–5). These findings have indicated an accelerated intracellular turnover of glyceride in the enlarged cells (5), the purpose and mechanism(s) of which are not known at present.

Activation of the hormone-sensitive lipase (6, 7) in adipocytes follows stimulation of adenyl cyclase, formation of cyclic AMP, and subsequent activation of cyclic AMP-dependent protein kinase. Hartman *et al.* (2) have reported that norepinephrine-activated adenyl cyclase activity in adipocytes, expressed on a per-cell basis, was unaffected by cell size. Cooper and Gregerman (8) found that both basal and epinephrine-activated adenyl cyclase activities in isolated adipocytes declined progressively and significantly in rats from 2 to 24 months of age. Forn *et al.* (9) have shown that, in intact fat cells, the norepinephrine-stimulated adenyl cyclase activity was similar in cells from 5- to 6-week-old and 10- to 12-week-old rats, but was markedly decreased in cells from 18- to 24-week-old rats.

These authors also found an increase in phosphodiesterase activity with aging and/or enlarging cell size in the rat. No values could be found in the literature for unstimulated cyclic AMP content of fat cells as related to cell size.

Since these reported changes could not readily explain the increased basal rate of lipolysis in enlarged fat cells, the possibility was explored that the cyclic AMP content and the adipocyte protein kinase activity may be altered with enlarging fat cell size in the rat, in a manner more closely related to the changing lipolytic rate. In the present report we have compared basal, cyclic AMP-dependent, and cyclic GMP-dependent protein kinase activities in adipocytes differing in mean cell volume, both on the basis of protein content and also on a per cell basis. The cellular content of cyclic AMP was also compared.

Materials and methods. Animals. Male Wistar rats (Royalhart Laboratory, New Hampton, N.Y.), fed Purina laboratory chow ad libitum since weaning, were housed in a temperature-controlled (23°) room and exposed to 12-hr intervals of light and darkness. Water was available at all times. The animals were sacrificed by cervical dislocation at 9–10 A M in the fed state.

Conditions of incubation and analytical techniques. At sacrifice, the epididymal fat pads were removed and weighed; after mincing, they were incubated at 37° in Krebs–Ringer bicarbonate medium (KRB), at pH 7.4, with collagenase from *Cl. Histolyticum* (Worthington, 5–10 mg/g adipose tissue), as described by Rodbell (10). The fat cells were then washed four times and resuspended in fresh medium by techniques previously employed (4). The resuspended fat cells were incubated for 15 min at 37° in a Dubnoff metabolic shaker at 80 strokes/min. Three-milliliter aliquots of the cell suspension were transferred to a plastic centrifuge tube, kept at 0°, and sonicated for 10 sec. Additional aliquots of the cell suspensions were removed for the determination of lipid content and for optical sizing of the cell

[1] This work was supported in part by Grants AM-13940, AM-17403, and HL-15696 from the United States Public Health Service.

[2] Address all correspondence to Mario DiGirolamo, M. D., Department of Medicine, Emory University, School of Medicine, 69 Butler Street, Atlanta, Georgia 30303.

ISSN 0037-9727

diameters; from these, the fat cell number was calculated (11).

Protein kinase activity was determined in the infranatant obtained after centrifuging the sonicated cells for 10 min at 30,000*g*. The standard assay system for cyclic AMP-dependent and cyclic GMP-dependent protein kinases was essentially the same as described elsewhere (12), except that the isolated stimulatory modulator (13) of cyclic GMP-dependent protein kinase replaced the crude protein kinase modulator. The incubation mixture contained, in a final volume of 0.2 ml: potassium phosphate buffer, 10 μmole, pH 7.0; arginine-rich histone (HA, Worthington), 40 μg; $MgCl_2$, 2 μmole; [γ-^{32}P]ATP (New England Nuclear), 1 nmole, containing about 1.2×10^6 cpm; stimulatory modulator from bovine heart (45 μg); with or without cyclic AMP or cyclic GMP, 80 pmole as indicated. The reaction was carried out for 10 min at 30°. One unit of the enzyme is defined as that amount of enzyme that transferred 1 pmole of ^{32}P from [γ-^{32}P]ATP to recovered histone under the assay conditions. The protein content of the infranatant was determined by the method of Lowry (14). Protein kinase activity was expressed as units of activity per milligram of cell protein and also per fat cell. Cyclic AMP was determined by the method of Kuo and Greengard (15) and expressed as pmole/10^6 fat cells.

Results. The determinations were carried out in two groups of rats: group A, lean rats (6–7 weeks in age) weighing between 150 and 170 g; and group B, older fatter rats (5–6 months in age) weighing between 450 and 550 g. The epididymal fat pads of five to six animals of group A were pooled to obtain sufficient adipocytes in a single experiment, whereas in group B the pads of only one animal were used. The mean fat cell volume of group A rats was 58 pl, that of group B rats was 371 pl. Table I shows that the larger adipocytes from group B rats contained 2.3 times more protein per fat cell than the smaller adipocytes from group A rats. It also shows that basal protein kinase activity was significantly greater for group B rats than group A rats, whether the activity was expressed as units/milligram of protein (230 vs 168, $p < 0.02$), or as units/10^6 fat cells (422 vs 131, $p < 0.001$). Similarly, the cyclic AMP-dependent protein kinase activity was also greater in the large adipocytes of group B rats than the small adipocytes of group A ($p < 0.005$), independently of the unit of expression. No significant protein kinase activity specific for cyclic GMP was found in extracts of fat cells from either group. This enzyme activity, however, has been shown to become evident upon further purification (12). The cyclic AMP-stimulated protein kinase activity was markedly and significantly greater ($p < 0.001$) than basal kinase activity in both types of cells. When expressed as relative increase over the basal levels, the enzyme activity was 252% of basal values in group A, and 270% of basal in group B. Thus, even though cyclic AMP-dependent protein kinase activity was markedly greater in the large fat cells (46% higher on protein basis, or 246% higher on per cell basis) when compared to small cells, the relative stimulation by cyclic AMP was similar in adipocytes from both groups. Table II shows that the basal cyclic AMP levels, expressed on a per-cell basis, were significantly greater ($p < 0.025$) in the larger adipocytes from group B than in the smaller adipocytes from group A. It is of

TABLE I. PROTEIN KINASE ACTIVITY OF ADIPOCYTES OF DIFFERENT SIZE FROM TWO GROUPS OF RATS.

			Protein Kinase Activity					
	Mean fat cell Volume	Protein/fat cell	Basal	+Cyclic GMP	+Cyclic AMP	Basal	+Cyclic GMP	+Cyclic AMP
Group[a]	(pl)	(ng)	(U/mg of protein)			(U/10^6 fat cells)		
A (n = 6)	58 ± 3	0.78 ± 0.05	168 ± 11	172 ± 9	424 ± 16	131 ± 13	135 ± 12	331 ± 22
B (n = 7)	371 ± 23	1.82 ± 0.12	230 ± 18	243 ± 18	621 ± 48	422 ± 55	445 ± 53	1144 ± 150
p[b]	<0.001	<0.001	<0.02	<0.01	<0.005	<0.001	<0.001	<0.001

[a] Group A rats were 6–7 weeks old, body weight 150–170 g; Group B rats were 5–6 months old, body weight 450–550 g; n is the number of experiments in each group of animals. Figures are given as means ± SE.

[b] p represents the statistical significance of the difference between means of respective measurements in group A and group B rats.

TABLE II. CYCLIC AMP CONTENT OF ADIPOCYTES OF DIFFERENT SIZE FROM TWO GROUPS OF RATS.

Group[a]	Mean fat cell volume (pl)	Cyclic AMP (pmole/10^6 fat cells)
A	64 ± 3	10.36 ± 1.13
B	439 ± 29	27.25 ± 4.98
p[b]	<0.001	<0.025

[a] Group A rats were 6-7 weeks old, body weight 150-170 g; Group B rats were 5-6 months old, body weight 450-550 g. Figures given are means ± SE.

[b] p represents the statistical significance of the difference between means of respective measurements in group A and group B rats.

note that the magnitude of the difference in cyclic AMP between mean values of large and small fat cells (2.63 ×) per cell was approximately similar to the difference in basal protein kinase activity per cell (3.22 ×) in the two groups.

Discussion. Large adipocytes from old fat rats differ in several morphological and metabolic parameters when compared to smaller adipocytes from young lean rats (1-5, 11). The present study provides an additional instance of a marked difference in an important regulatory system between small and large adipocytes.

Protein kinase in fat cells is known to mediate the effect of cyclic AMP on lipase activation (6, 7). Subsequent to this enzyme activation, triglycerides are hydrolyzed and both glycerol release and free fatty acid mobilization ensue. The present findings of a 3-fold greater cyclic AMP content and a 3- to 4-fold higher basal protein kinase activity in large adipocytes are consonant with the corresponding greater rate of basal lipolysis in these cells as compared to that of smaller adipocytes from younger leaner rats (2-4).

The observed differences between small and large fat cells in basal and cyclic AMP-stimulated protein kinase activity are more pronounced when the results are expressed on the basis of fat cell number than when they are expressed on the basis of cellular protein. This is probably due to a greater protein content in the enlarged adipocytes; in the present study, approximately 2.3 times more cellular protein is found in enlarged fat cells than in small fat cells. Thus, an enlargement of fat cell size leads to an increase not only in surface area and volume but also in the amounts of cellular protein.

It is of interest that other investigators have reported, with aging and/or enlarging cell size in the rat, no change or a decrease in basal and epinephrine-stimulated adenylate cyclase (2, 8, 9) and also an increase in phosphodiesterase activity (8) of the adipocytes. Neither of these enzymatic changes could be readily reconciled with the greater rate of basal lipolysis in the large fat cells. The present study, by demonstrating that larger adipocytes contain greater amounts of cyclic AMP and present a higher basal protein kinase activity, suggest that these two cellular parameters may be interrelated and possibly responsible for the greater baseline lipolytic activity observed in the large as compared to the smaller fat cells. Addition of cyclic AMP to the cellular extract in this study stimulated protein kinase activity to a similar extent in both small and large fat cells. These findings may also be consonant with a similar degree of lipolytic stimulation exerted by lipolytic hormones on fat cells of different size (2, 15, 16), although one must be cautious in attempting to correlate enzyme activation with overall metabolic responses such as lipolysis (9).

Summary. We have studied cellular cyclic AMP content and protein kinase activity in extracts of small and large adipocytes from two groups of rats differing in age and body weight. The data, expressed on a per cell basis, show that large adipocytes from larger rats present a significantly greater ($p < 0.02$) content of cyclic AMP and a 3-fold greater ($p < 0.001$) level of basal protein kinase activity than small adipocytes. Cyclic AMP (0.4 μM) stimulated the cyclic AMP-dependent protein kinase activity to a similar degree (about 3-fold) in both types of cells. No significant amount of cyclic GMP-dependent protein kinase activity was detected in extracts of adipocytes from either group of rats.

The increased cellular content of cyclic AMP and the elevated protein kinase activity in large adipocytes are probably responsible for the greater basal rate of lipolysis observed in these cells.

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Received October 11, 1976. P.S.E.B.M. 1977, Vol. 154.