# Hormone-stimulated lipolysis in isolated fat cells from "young" and "old" rats

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**Abstract** The biphasic nature of the lipolytic dose-response curve of epinephrine in fat cells from "young" rats (40–45 days) was confirmed. The first phase (Lipolysis I) occurred at concentrations of from $10^{-7}$ M to $3 \times 10^{-6}$ M. The second phase (Lipolysis II) occurred at concentrations of from $10^{-5}$ M to $3 \times 10^{-4}$ M. Insulin (0.1 mU/ml) abolished Lipolysis I and slightly augmented Lipolysis II. Higher concentrations of insulin (1.0 mU/ml) augmented Lipolysis II even further. These results may help to explain some of the conflicting reports in the literature concerning the effects of insulin on lipolysis. The dose-response curve of epinephrine using fat cells from "old" rats (14–16 months) was monophasic. Based on results with propranolol, $K^+$-free media, and insulin, it was concluded that the lipolytic response in tissue from older animals corresponds to Lipolysis II in tissue from younger rats. The lipolytic response to ACTH was greatly reduced in the cells from the older rats, but the response to theophylline was unaltered.

**Supplementary key words** epinephrine · ACTH · theophylline · propranolol · insulin · aging

Increasing body weight and senescence in the rat are accompanied by alterations in adipose tissue metabolism. Lipoprotein lipase activity and chylomicron uptake are diminished (1) while the conversion of glucose to glyceride-glycerol is increased (2). Benjamin et al. (3) demonstrated extensive changes in fat pad metabolism in rats 647 days old as compared with rats 38 days old. In fat pads from the older animals, there was decreased lipid synthesis from acetate, a reduction in the activity of the pentose shunt, and a decreased rate of lipolysis. There is now considerable evidence that the sensitivity of hormone-stimulated lipolysis decreases with aging (4–6), although the exact biochemical mechanisms involved are unknown.

It is generally accepted (7) that the lipolytic response to hormones is the result of activation of adenylate cyclase at the membrane with a subsequent increase in the intracellular levels of cyclic 3′, 5′-adenosine monophosphate (cyclic AMP). The cyclic nucleotide then activates a protein kinase which in turn activates a triglyceride lipase (8). Any age- or weight-related change in hormone-stimulated lipolysis could occur through alterations in any or all of these processes or at some event unrelated to cyclic AMP such as an alteration in the cell membrane.

Previous studies in this laboratory have demonstrated the existence of a biphasic increase in lipolysis with increasing concentrations of catecholamines (9). Both phases of lipolysis appear to be cyclic AMP-mediated but differ in several other aspects (10, 11). The present study was undertaken to determine what effect age, with the accompanying increased body weight, has on this and other lipolytic responses.

## METHODS

Two groups of fed, male Cox-Holtzman rats were used. The rats in the first group were 40–45 days of age and weighed 180–220 g. Those in the second group were 14–16 months old and weighed 500 g or more. All animals were allowed free access to water and standard laboratory chow. Rats were stunned by a blow to the head and exsanguinated. The epididymal fat pads were removed, and fat cells were isolated by the method of Lech and Calvert (12). Aliquots of the fat cells were placed in polyethylene bottles containing Krebs-Ringer bicarbonate buffer (pH 7.4) with bovine serum albumin (4% w/v) and appropriate drugs and hormones. The final volume was 3.0 ml. Unless otherwise indicated, all incubation and wash media contained 5.0 mM $K^+$. In the series of experiments in which $K^+$ was omitted from the media, the $K^+$ was replaced with an appropriate amount of $Na^+$.

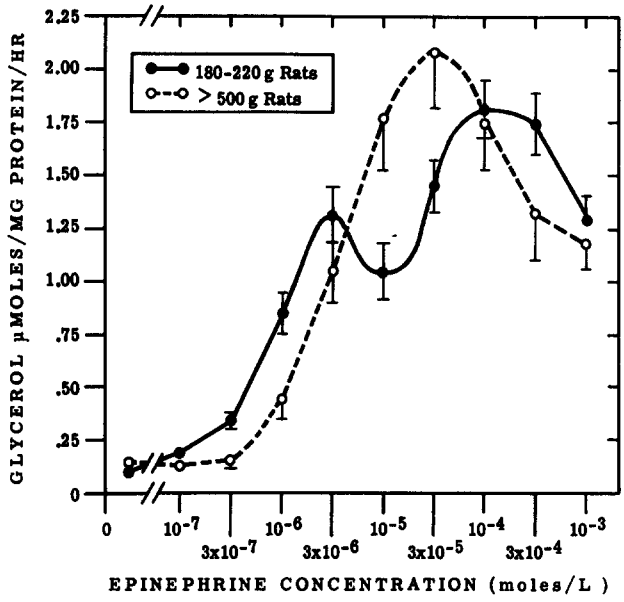


FIG. 1. Lipolytic effect of epinephrine on isolated fat cells from rats weighing 180–220 g and over 500 g. Results are means ± SEM of six experiments.

Incubations were carried out in a shaking water bath for 60 min at 37°C under an atmosphere of 95% $O_2$–5% $CO_2$. Rates of lipolysis are linear over this period of time. The incubations were terminated by adding an aliquot of cells and medium to trichloroacetic acid (5%), and the rate of lipolysis was determined by measuring the production of glycerol (13).

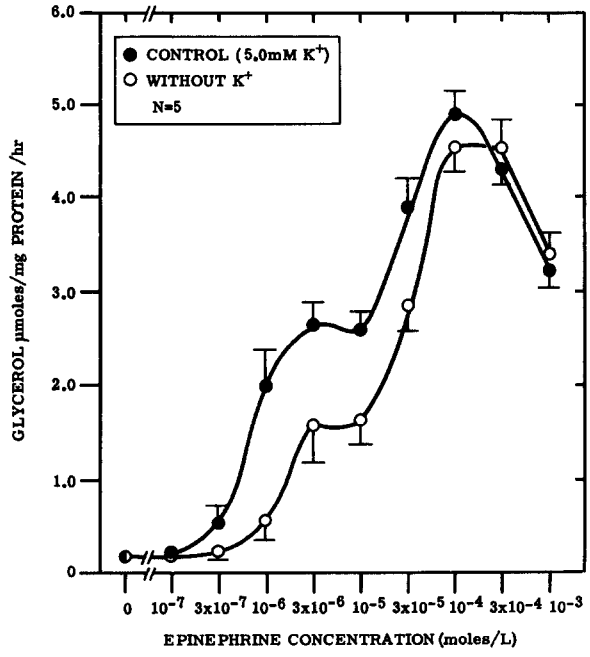


FIG. 2. Effect of lack of $K^+$ on the lipolytic effect of epinephrine on isolated fat cells from rats weighing 180–220 g. Results are means ± SEM of five experiments.

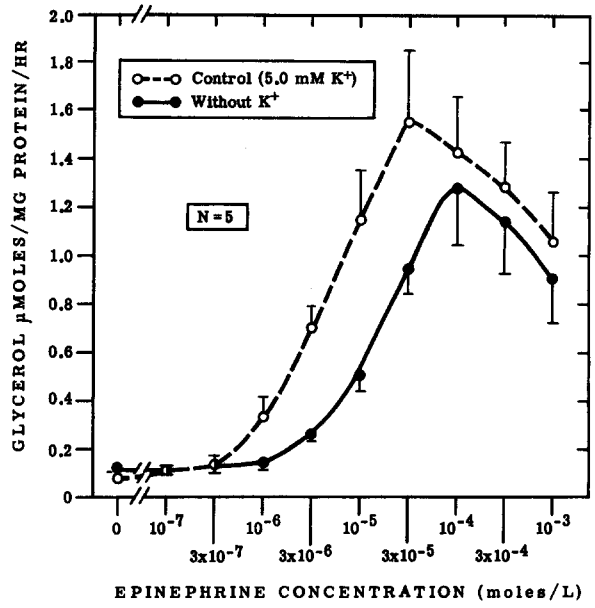


FIG. 3. Effect of lack of $K^+$ on the lipolytic effect of epinephrine on isolated fat cells from rats weighing over 500 g. Results are means ± SEM of five experiments.

The protein content of the fat cells was assayed as described by Lech and Calvert (12). The amount of protein in fat cells does not radically change between the ages of 3 and 16 months in the rat (14) and is a useful parameter on which to base results.

All data are expressed as micromoles of glycerol/milligram of protein/hour (mean ± SEM). Unless otherwise stated, $P$ values were calculated using Student's $t$ test. A $P$ value of $<0.05$ was considered to be significant.

ACTH was purchased from Parke, Davis & Co. (Detroit, Mich.). The DL-propranolol (1-isopropylamino-3-naphthyloxy-2-propanol) was generously supplied by Ayerst Laboratories (New York). Bovine serum albumin (fraction V) was obtained from Sigma Chemical Co. (St. Louis, Mo.). The insulin was a gift of Eli Lilly and Co. (Indianapolis, Ind.).

## RESULTS

In Fig. 1, the lipolytic response to increasing concentrations ($10^{-7}$ to $10^{-3}$ M) of epinephrine in fat cells from 180–220-g rats is compared with the response in fat cells from rats weighing over 500 g. The response in the cells from younger animals was biphasic in nature. The first lipolytic response occurred at epinephrine concentrations of $10^{-7}$ to $10^{-5}$ M and has been previously designated Lipolysis I (9). The second, Lipolysis II, occurred at $10^{-5}$ to $10^{-3}$ M. In the fat cells from old animals, the dose-response curve was monophasic, occurring at $10^{-6}$ to $10^{-4}$ M epinephrine. The maximum response in older rats, which occurred at $3 \times 10^{-5}$ M, did not differ signif-

icantly in magnitude from the maximum response seen in Lipolysis II at $10^{-4}$ M epinephrine in the younger animals.

On the basis of the results shown in Fig. 1, it was impossible to identify the monophasic response as being similar to Lipolysis I or to Lipolysis II. Therefore, experiments were carried out to better identify the monophasic response. Fig. 2 shows the effects of a lack of $K^+$ on Lipolysis I and II in the younger rats. The lack of $K^+$ in the medium caused a significant inhibition of Lipolysis I and a slight shift to the right of Lipolysis II. If $K^+$ was present, a biphasic dose-response curve was observed. The effect of the lack of $K^+$ on fat cells from old animals is shown in Fig. 3. A monophasic response was seen in both the presence and absence of $K^+$ but was shifted to the right when $K^+$ was absent. The maximum response in older animals in the presence of $K^+$ occurred at an epinephrine concentration of $3 \times 10^{-5}$ M, but in the absence of $K^+$, the maximum response to epinephrine occurred at $10^{-4}$ M.

The effects of $10^{-5}$ M propranolol on the lipolytic response to epinephrine in fat cells from the two different groups of rats are summarized in Figs. 4 and 5. $\beta$-Adrenergic blockade abolished Lipolysis I in fat cells obtained from younger animals (Fig. 4) and produced a reduction in the maximum response and a shift to the right of Lipolysis II. The effect of propranolol in fat cells from old rats (Fig. 5) was to reduce the maximum response and shift the curve to the right so that the effective concentration of epinephrine needed to achieve the maximum response was increased from $3 \times 10^{-5}$ to $10^{-4}$ M. The effect of propranolol on the Lipolysis II response and on the monophasic response was essentially the same in fat cells from young and old rats, respectively.

Experiments were undertaken to determine the effect of insulin on epinephrine-stimulated lipolysis in both types of fat cells. Fig. 6 summarizes the effects of two concentrations of insulin on fat cells from 180–220-g rats. Insulin at 0.1 mU/ml completely abolished Lipolysis I but had no effect on Lipolysis II except to augment the maximum response at $3 \times 10^{-5}$ M epinephrine. A higher concentration of insulin (1.0 mU/ml) augmented the lipolytic response to concentrations of epinephrine at $10^{-5}$ M and above. Insulin (0.1 or 1.0 mU/ml) increased the maximum response of Lipolysis II and caused the dose-response curve to be shifted to the left. In the fat cells from old rats (Fig. 7), 0.1 mU/ml insulin slightly reduced the response to $3 \times 10^{-5}$ M epinephrine but had no effect on the response to any other concentration of epinephrine.

There are other drugs and hormones that are potent stimulators of lipolysis. The lipolytic response to ACTH (Fig. 8) was monophasic with fat cells from both young and old animals, yet the magnitude of the response in the cells from the older rats was markedly decreased. In both cases the maximum response to ACTH was obtained with a concentration of 0.3 U/ml of ACTH in the incubation medium.

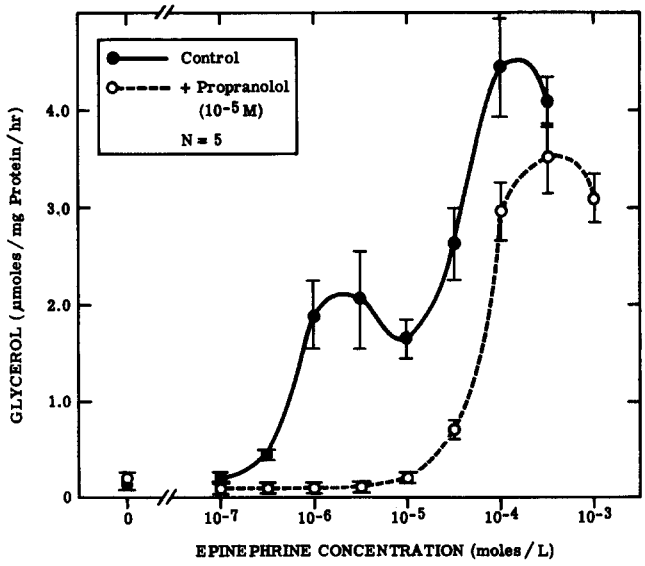


FIG. 4. Effect of propranolol ($10^{-5}$ M) on the lipolytic effect of epinephrine on isolated fat cells from rats weighing 180–220 g. Results are means ± SEM of five experiments.

Dose-response curves of increasing concentrations of theophylline ($10^{-5}$ to $10^{-2}$ M) were also done (Fig. 9).

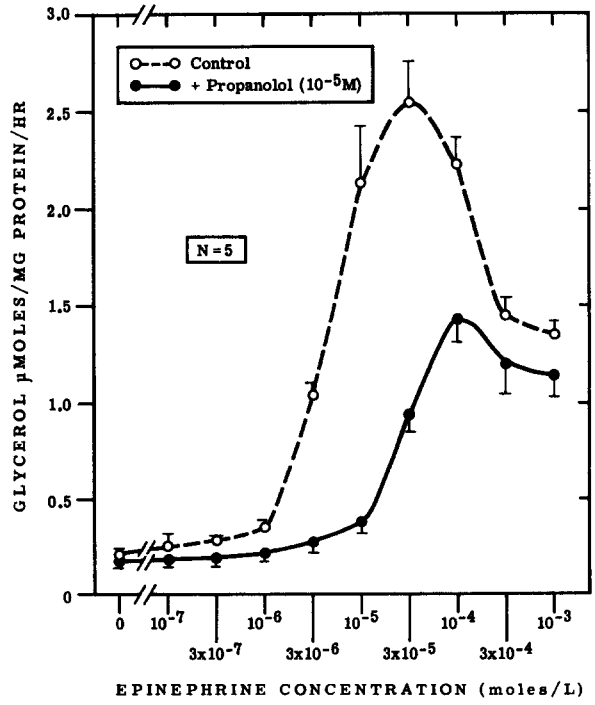


FIG. 5. Effect of propranolol ($10^{-5}$ M) on the lipolytic effect of epinephrine on isolated fat cells from rats weighing over 500 g. Results are means ± SEM of five experiments.

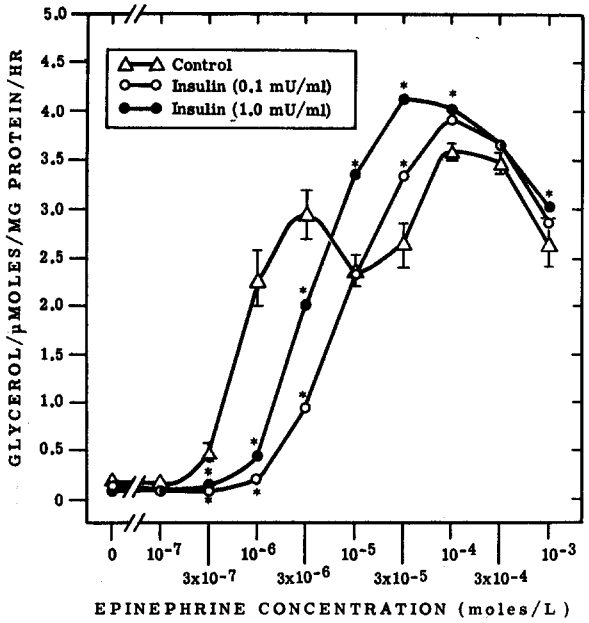


FIG. 6. Effect of insulin (0.1 and 1.0 mU/ml) on the lipolytic effect of epinephrine on isolated fat cells from rats weighing 180–220 g. Results are means ± SEM of five experiments. Asterisk indicates a significant difference ($< 0.05$) from the control.

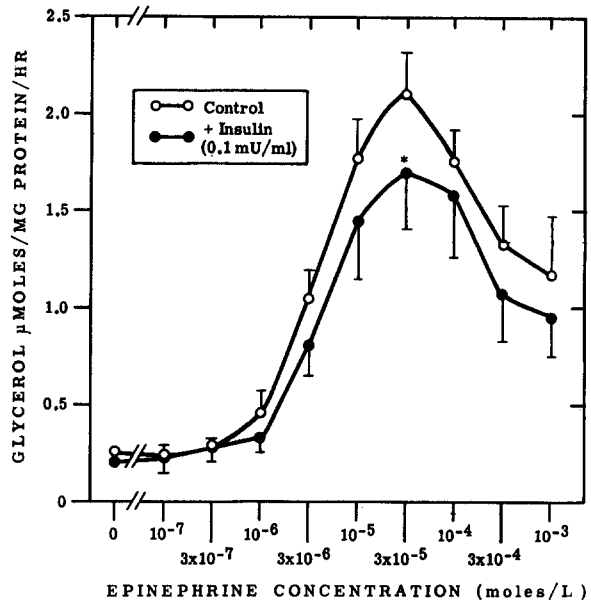


FIG. 7. Effect of insulin (0.1 mU/ml) on the lipolytic effect of epinephrine on isolated fat cells from rats weighing over 500 g. Results are means ± SEM of five experiments. Asterisk indicates a significant difference ($< 0.05$) from the control.

In these experiments the response to theophylline was monophasic and identical in fat cells from both young and old rats. The maximum response was obtained at $3 \times 10^{-3}$ M. The response to theophylline was independent of age.

## DISCUSSION

By definition an old rat is an obese rat (15). A rat allowed free access to food with age becomes fatter by increasing the deposition of neutral fat in its fat cells. In the present studies, no differentiation has been made between an old rat and an obese rat. All rats, both young and old, were allowed free access to food, and although the older rats were obese, this is their normal physiological state.

A wide variety of drugs and hormones are capable of stimulating lipolysis in isolated fat cells of the rat. Previous studies in this laboratory with fat cells from rats weighing from 120 to 200 g have demonstrated the existence of the biphasic lipolytic dose-response curve with catecholamines (9–11). The response at low concentrations has been termed Lipolysis I and that at high concentrations Lipolysis II (9). Although the two phases of the lipolytic response differ in their sensitivity to $K^+$, $\beta$ blockers, and insulin, they both appear to be mediated by cyclic AMP (10). If the rats used were older and heavier (more than 500 g), it was found that the dose-response curve to epinephrine was monophasic in character. The results of the present study with propranolol, insulin, and $K^+$-free medium demonstrated that this monophasic dose-response curve strongly resembled Lipolysis II.

Insulin showed three different effects on lipolysis depending upon the age of the rats. Jungas and Ball (16) and Mahler et al. (17) were among the first to report that if a high concentration of epinephrine was used to stimulate lipolysis in younger animals, insulin was a less effective inhibitor of lipolysis than if lower concentrations of epinephrine were used. Later, Hales et al. (18) showed that in the presence of 0.1 μg/ml epinephrine, increasing concentrations of insulin first inhibited and then augmented the release of glycerol. These data can be explained by the fact that insulin was antilipolytic to Lipolysis I but augmented Lipolysis II. Kono (19) demonstrated that concentrations of insulin that augment lipolysis also increase levels of cyclic AMP. This effect on lipolysis was independent of glucose transport (20) and seems to involve the insulin receptor on the cell.

Fat cells from 180–220-g rats are different from fat cells obtained from rats weighing more than 500 g. Hirsch and Han (21) and others (22, 23) have shown that the epididymal fat pad increases in size by hyperplasia until the rat weighs about 600 g. Fat pads from 180–220-g rats are capable of increasing in size by hyperplasia until the rat is 9–15 wk of age or weighs up to 300 g. However, hypertrophy continues until the rat weighs about 600 g. Fat pads from 180–220-g rats are capable of increasing in size by hyperplasia and/or hypertrophy. These fat cells have a lower triglyceride

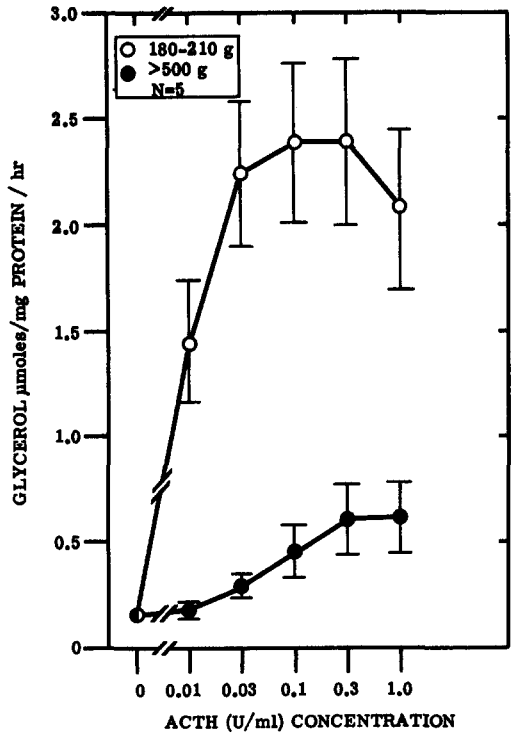


FIG. 8. Lipolytic effect of ACTH on isolated fat cells from rats weighing 180–210 g or over 500 g. Results are means ± SEM of five experiments.

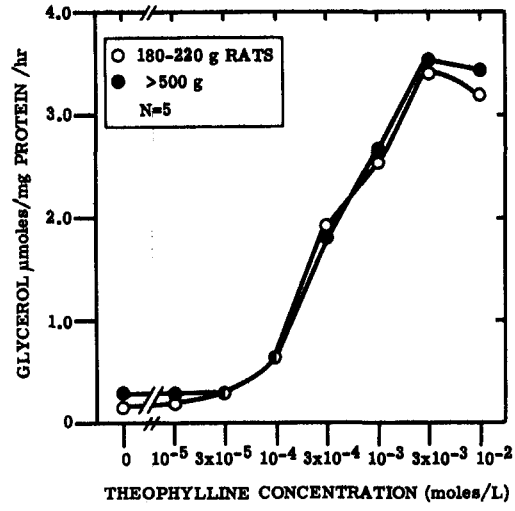


FIG. 9. Lipolytic effect of theophylline on isolated fat cells from rats weighing 180–220 g or over 500 g. Results are means ± SEM of five experiments.

to DNA content, and wet mounts show they are smaller in size than fat cells from rats greater than 500 g. The fat pads from the older animal can increase in size only through hypertrophy.

With respect to the effect of epinephrine on lipolysis, there is an increased sensitivity of the fat cell to the Lipolysis II component with age. The shift of the Lipolysis II response curve to the left in old fat cells can explain some of the discrepancies reported in the literature on the effect of age on adipose tissue (24, 25). Depending on the concentration of epinephrine used, the response in old fat cells can be significantly decreased ($10^{-6}$ M), increased ($10^{-5}$ M), or identical ($10^{-4}$ M) compared with the response observed in younger fat cells.

Lipolysis I is sensitive to changes in the physiological environment such as those occurring in aging or after adrenalectomy (11). The lack of Lipolysis I in old animals may explain why higher levels of epinephrine are needed to stimulate lipolysis. It is unknown whether the catecholamines are the major physiological stimulators of lipolysis in the aged rat, but any mobilization of free fatty acids in response to epinephrine in these animals must be via the Lipolysis II pathway.

ACTH interacts with the fat cell membrane at a site distinct from the epinephrine receptor (26) and produces a monophasic increase in lipolysis (9). Age or increased weight has a different effect on the lipolytic response to ACTH than it does on the epinephrine lipolytic response. Not only is the magnitude of the dose response decreased but a higher concentration of ACTH is needed to elicit a response in old fat cells than in young fat cells. This is similar to the effect of aging on the glucagon response as reported by Manganiello and Vaughan (27). The increased cell size may cause changes in the ACTH receptor or in the membrane, which in some way alters the interaction of ACTH and its receptor. The lipolytic response to theophylline was the same in fat cells from the two different age groups.

Aging is a complex phenomenon that is poorly understood. In the rat it is complicated by obesity. However, it is known that specific processes within the cell are altered. The changes leading to these alterations are unknown. The results reported here could be secondary to increased body weight or fat cell size as well as aging. The answer must await further study.

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## REFERENCES

1. Nestel, P. J., W. Austin, and C. Foxman. 1969. Lipoprotein lipase content and triglyceride fatty acid uptake in adipose tissue of rats of differing body weights. *J. Lipid Res.* **10:** 383–387.
2. Di Girolamo, M., and D. Rudman. 1968. Variations in glucose metabolism and sensitivity to insulin of the rat's adipose tissue, in relation to age and body weight. *Endocrinology.* **82:** 1133–1141.
3. Benjamin, W., A. Gellhorn, M. Wagner, and H. Kundel.

1961. Effect of aging on lipid composition and metabolism in the adipose tissues of the rat. *Amer. J. Physiol.* **201:** 540–546.
4. Zinder, O., and B. Shapiro. 1971. Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells. *J. Lipid Res.* **12:** 91–95.
5. Nakano, J., A. C. Gin, and T. Ishii. 1971. Effect of age on norepinephrine-, ACTH-, theophylline- and dibutyryl cyclic AMP-induced lipolysis in isolated rat fat cells. *J. Gerontol.* **26:** 8–12.
6. Jelínková, M., and E. Stuchlíková. 1968. Age changes in the metabolism of the adipose tissue. *Exp. Gerontol.* **3:** 193–195.
7. Robinson, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Lipolysis in adipose tissue. *In* Cyclic AMP. Academic Press, New York. 286–316.
8. Huttunen, J. K., and D. Steinberg. 1971. Activation and phosphorylation of purified adipose tissue hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *Biochim. Biophys. Acta.* **239:** 411–427.
9. Allen, D. O., C. C. Hillman, and J. Ashmore. 1969. Studies on a biphasic lipolytic response to catecholamines in isolated fat cells. *Biochem. Pharmacol.* **18:** 2233–2240.
10. Allen, D. O., and P. J. MacLaren. 1970. Effect of potassium ion, theophylline and propranolol on the biphasic lipolytic response to some catecholamines. *Biochem. Pharmacol.* **19:** 2569–2578.
11. Allen, D. O., and R. Beck. 1972. Alterations in lipolysis, adenylate cyclase, and adenosine 3′,5′-monophosphate levels in isolated fat cells following adrenalectomy. *Endocrinology.* **91:** 504–510.
12. Lech, J. J., and D. N. Calvert. 1966. Protein content and osmotic behavior of isolated fat cells. *J. Lipid Res.* **7:** 561–564.
13. Korn, E. D. 1959. The assay of lipoprotein lipase *in vivo* and *in vitro*. *Methods Biochem. Anal.* **7:** 145–192.
14. Salans, L. B., and J. W. Dougherty. 1971. The effect of insulin upon glucose metabolism by adipose cells of different size. Influence of cell lipid and protein content, age and nutritional state. *J. Clin. Invest.* **50:** 1399–1410.
15. Williams, H. H., N. Galbraith, M. Kaucher, E. Z. Moyer, A. J. Richards, and I. G. Macy. 1945. The effect of growth on the lipid composition of rat tissues. *J. Biol. Chem.* **161:** 475–484.
16. Jungas, R. L., and E. G. Ball. 1963. Studies on the metabolism of adipose tissue. XII. Effects of insulin and epinephrine on free fatty acid and glycerol production in the presence and absence of glucose. *Biochemistry.* **2:** 383–388.
17. Mahler, R., W. S. Stafford, M. E. Tarrant, and J. Ashmore. 1964. The effect of insulin on lipolysis. *Diabetes.* **13:** 297–302.
18. Hales, C. N., J. M. Chalmers, M. C. Perry, and D. R. Wade. 1969. Investigations on the hormonal control of lipolysis. *In* Protein and Polypeptide Hormones. M. Margoulis, editor. Excerpta Medica, Amsterdam. 432–443.
19. Kono, T. 1972. A paradoxical effect of insulin on lipolysis in isolated rat epididymal fat cells. *Federation Proc.* **31:** 243. (Abstr.)
20. Desai, K. S., and A. Angel. 1971. Effects of insulin and glucose on initial rates of lipolysis and intracellular (IC) FFA levels in isolated adipocytes. *Can. Fed. Biol. Soc.* **14:** 92.
21. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10:** 77–82.
22. Goldrick, R. B. 1967. Morphological changes in the adipocyte during fat deposition and mobilization. *Amer. J. Physiol.* **212:** 777–782.
23. Hartman, A. D., A. J. Cohen, C. J. Richane, and T. Hsu. 1971. Lipolytic response and adenyl cyclase activity of rat adipocytes as related to cell size. *J. Lipid Res.* **12:** 498–505.
24. Hubbard, R. W., and W. T. Matthew. 1971. Growth and lipolysis of rat adipose tissue: effect of age, body weight, and food intake. *J. Lipid Res.* **12:** 286–293.
25. Altschuler, H., M. Lieberson, and J. J. Spitzer. 1962. Effect of body weight on free fatty acid release by adipose tissue *in vitro*. *Experientia.* **18:** 91–92.
26. Bär, H. P., and O. Hechter. 1969. Adenyl cyclase and hormone action. I. Effects of adrenocorticotropic hormone, glucagon and epinephrine on the plasma membrane of rat fat cells. *Proc. Nat. Acad. Sci. USA.* **63:** 350–356.
27. Manganiello, V., and M. Vaughan. 1972. Selective loss of adipose cell responsiveness to glucagon with growth in the rat. *J. Lipid Res.* **13:** 12–16.