# Effect of aging and cellularity on lipolysis in isolated mouse fat cells

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**Abstract** The effects of age and cellularity on lipolysis have been investigated in isolated epididymal fat cells from both Swiss albino mice and Sprague-Dawley rats. No significant lipolytic response to glucagon could be demonstrated with adipocytes from either young or old mice, while glycerol output was increased by this hormone with fat cells from young rats. Larger adipocytes from older mice showed significantly greater isoproterenol-stimulated lipolysis than those from younger animals if the glycerol output was expressed on a per cell basis. However, the lipolytic response per cell appeared to be equivalent in young and old rat adipocytes with either isoproterenol or ACTH-(1-24). In a complete aging study, relationships between body weight, epididymal fat pad weight and cellularity were examined covering the life span of the mouse. ACTH-(1-24)- and dibutyryl cyclic AMP-stimulated lipolysis increased with age and cell size but fell at senescence when adipocyte size diminished. Although an effect of aging per se cannot be ruled out with the experimental techniques used in the present study, a dominant influence of adipocyte size on the lipolytic process was demonstrated.—**Jolly, S. R., Y. B. Lombardo, J. J. Lech, and L. A. Menahan.** Effect of aging and cellularity on lipolysis in isolated mouse fat cells. *J. Lipid Res.* 1980. **21:** 44–52.

**Supplementary key words** glycerol output · isoproterenol · glucagon · ACTH · dibutyryl cyclic AMP · adipocyte number · fat cell diameter · cellular triacylglycerol content

There has been recent interest in studying obesity in the mouse since there are well defined genetic models within the species. Isolated mouse adipocytes have been utilized to investigate the factors which influence the lipolytic process (1). Herberg, Gries, and Hesse-Wörtmann (2) have examined basal- and stimulated-lipolysis in New Zealand obese mice and obese hyperglycemic mice of different ages and body weights using minced adipose tissue, and have observed increased lipolytic capacity with age. Lipolysis in isolated fat cells from the genetically obese hyperglycemic (ob/ob) mouse has also been investigated, using adipocytes from obese mice and lean controls of the same age (3–5). Previous work in our laboratory (5) has shown a selective depression of the lipolytic response to isoproterenol in adipocytes from obese mice, while lipolytic stimulation by ACTH-(1-24) was equivalent in fat cells from both obese and lean mice. Adipocytes isolated from obese mice had a mean cell diameter that was twice as great as those from lean controls. This appeared to be analogous to results with large and small epididymal fat cells from rats of different ages (6, 7). However, the effects of aging and cellularity on the lipolytic process in the mouse have not been extensively investigated.

In the present study, the effects of age and cell size on hormone-stimulated glycerol release of isolated epididymal fat cells prepared from Swiss albino mice have been studied in comparison with those from Sprague-Dawley rats. The comparative lipolytic response to glucagon of adipocytes from both young and old mice or rats was investigated in depth since conflicting results have been reported regarding its efficacy as a lipolytic hormone in the mouse (1, 4, 5). The consequences of aging and cell size on the lipolytic process in the adipose tissue of the mouse were examined by developing a total aging profile in which adipose tissue cellularity and in vitro lipolytic responses were examined over the lifespan of the mouse.

## MATERIALS AND METHODS

### Materials

Bovine Fraction V albumin was purchased from Miles Laboratories, Inc., Elkhart, IN (Lot #271) and crude collagenase, Type CLS (150 units/mg) was obtained from Worthington Corporation, Freehold, NJ (Lot #46K285). ACTH-(1-24) was a commercial preparation from Organon, Inc., West Orange, NJ (Lot #27234). Glucagon was donated by the Eli Lilly Research Laboratories, Indianapolis, IN (Lot #258

Abbreviations: ACTH-(1-24), corticotrophin-(1-24)-tetracosadecapeptide; dibutyryl cyclic AMP, $N^6$,2′-O-dibutyryl adenosine 3′,5′-monophosphate.

VO-16-36) through the courtesy of Dr. Mary Root. Isoproterenol was purchased as the racemic mixture from the Aldrich Chemical Company, Milwaukee, WI, and dibutyryl cyclic AMP (sodium salt) from Boehringer Mannheim, Indianapolis, IN. All other enzymes and nucleotides were obtained commercially either from Boehringer Mannheim, PL Biochemicals, Milwaukee, WI or Sigma Chemical Company, St. Louis, MO.

### Animals

Sprague-Dawley rats were obtained either from ARS/Sprague-Dawley, Madison, WI or King Animal Laboratories, Oregon, WI and Swiss albino (ARS HA (ICR) f) mice from ARS/Sprague-Dawley, Madison, WI.

In the experiments comparing mice or rats, weanling mice and rats were raised in our colony to body weights of 19–30 g (1–1.5 mo) and 140–230 g (~1.5 mo) to constitute the respective young groups. Retired breeder mice, 40–60 g (6–8 mo) and rats 400–550 g (~10 mo), were obtained respectively as older animals for comparison; there was an acclimatization period of at least two weeks. Animals in the complete aging profile of mice varied in age from 1–24 months. The profile was developed by obtaining groups of at least ten weanling mice at 2–3-month intervals for approximately a 2-year period.

All animals were maintained on a 12-hour light–dark cycle with ad libitum access to laboratory mouse or rat chow and water until they were killed.

### Preparation of isolated adipocytes

In the experiment comparing young and old rats or mice, all animals were killed by cervical dislocation. Epididymal fat pads were obtained from three to eight animals to obtain sufficient tissue, and more animals of the younger groups were used per experiment. In the complete aging profile, the mice were killed by exsanguination under pentobarbital anesthesia (110 mg/kg, intraperitoneal). Each group contained five animals. Several mice, 22–24 months in age, were not used in preparing adipocytes due to overt pathology at the time of the experiment. Epididymal fat pads were weighed prior to use.

All epididymal fat pads were washed in warm buffer (37°C) after dissection and minced for cell dissociation. Isolated cells were prepared according to the method of Rodbell (8). Cell separation, washings, and incubations were carried out at 37°C in Krebs-Henseleit phosphate buffer (pH 7.4) with 1.25 mM $Ca^{2+}$. The buffer contained 5.5 mM glucose and 40 mg/ml of dialyzed bovine serum albumin. Chang, Huang, and Cuatrecasas (9) reported difficulties in preparing adipocytes from obese mice. In our experiments, modifications were made in the cell dissociation procedure for both mice and rats in order to minimize cell breakage. Only 1 mg/ml of crude collagenase was used in the incubation to dissociate the adipose tissue. Also, the incubations were limited to 30 min. These conditions are similar to those of Shepherd et al. (3) in preparing isolated adipocytes from obese mice. After 30 min of incubation with the dissociating medium, cells were strained through nylon mesh to remove stroma and blood vessels. Cells were then washed three times in fresh, warm (37°C) buffer. The isolated adipocytes were incubated in a total volume of 1 ml containing the indicated effector for 45 min at 37°C in a metabolic shaker (60 cycles/min) under 95% $O_2$–5% $CO_2$. Glycerol release was used as an index of lipolysis in the present study. In time–dependency studies, appearance of glycerol in the incubation medium was followed at 15 min intervals between 0 and 90 min, inclusive. The best fit of these points was determined by linear regression analysis. In all other cases, glycerol release has been expressed as nmoles measured after 45 min with duplicate incubations for each experimental condition.

### Lipolysis and cellularity determinations

Glycerol release was measured enzymatically as described by Wieland (10). Aliquots of adipocyte suspensions were taken for determination of triacylglycerol by the method of Laurell (11). Fifty cells per group were measured microscopically by the method of Di Girolamo, Mendlinger, and Fertig (12) and all such measurements were conducted by the same person throughout the present study to improve precision as suggested by Khan et al. (13). Cell volume was calculated according to Goldrick (14). The observed mean cell diameter, the content of triacylglycerol and the density of triolein (0.915) in aliquots of fat cell suspensions were used to calculate the number of adipocytes per incubation (14, 15). Cell surface was calculated by the formula: Area $= \pi(\sigma^2 + \bar{x}^2)$ where $\bar{x}$ is the mean cell diameter and $\sigma^2$ is the variance, as given by Zinder and Shapiro (16). Total adipocyte surface was used to evaluate cellularity and is the product of mean cell surface and average number of adipocytes per incubation.

Evaluation of lipolytic responses to various effectors by cells pooled from the same species was accomplished by analysis of variance and Duncan's multiple range test (17). Comparison between old and young animals (rats or mice) was made using Student's *t* test (17).

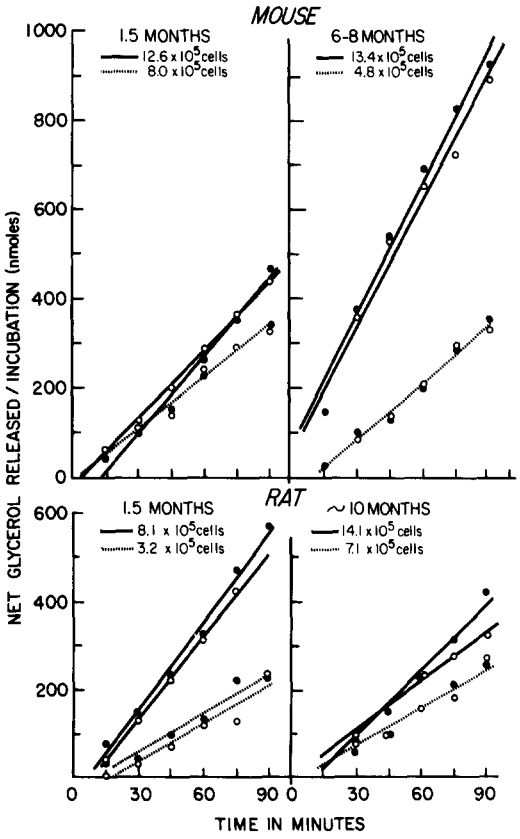


**Fig. 1.** Time dependency and influence of cell number on hormone-stimulated glycerol release in adipocytes of mice or rats differing in age. Net glycerol release to (●) $10^{-6}$ M isoproterenol and (○) $10^{-7}$ M ACTH-(1-24) is given. Experimental and incubation conditions are described in detail in Materials and Methods.

## RESULTS

### Comparison of young and old rats or mice

In order to relate the lipolysis in adipocytes from rats and mice and examine the effects of aging in both species, it was necessary to look at effects of aging in both species on the time–dependency of glycerol release. **Fig. 1** summarizes our consistent finding that the rate of glycerol release to either $10^{-6}$ M isoproterenol or $10^{-7}$ M ACTH-(1-24), both maximally stimulating concentrations of the respective hormones, was linear over 90 min with adipocytes obtained from mice or rats at both ages examined, 1.5 and 6–10 months. Basal, unstimulated, lipolysis was not linearly related to time of incubation and was not studied per se in the experiments described below. When measurable, basal release has been subtracted from the values presented. Cell number per incubate has been shown to influence lipolysis in isolated rat adipocytes (18). Therefore, time–dependency of hormone-stimulated lipolysis was studied at widely differing cell concentrations. The data given in Fig. 1 indicate that the lipolytic response to either $10^{-6}$ M isoproterenol or $10^{-7}$ M ACTH-(1-24) was linear with cell concentrations that differed approximately two- to three-fold with mice and rats of both ages.

In these studies we examined the combined effects of aging and cell size on lipolysis in two rodent species: the Sprague-Dawley rat and the Swiss albino mouse. Factors defining the isolated epididymal adipocytes from mice and rats are given in **Table 1.** Body weight, fat cell diameter and adipocyte triacylglycerol content were significantly greater in older, larger mice or rats. Fat cells from old and young mice or rats were examined in parallel experiments on a given day under identical incubation conditions to minimize variations.

The lipolytic response of rat adipocytes to ACTH-(1-24), glucagon and isoproterenol is given in **Fig. 2** and is expressed in terms of net glycerol output on a per cell basis. In young rats, the lipolytic response to both isoproterenol and ACTH-(1-24) was significant at all concentrations tested ($P < 0.05$); and glucagon, at concentrations of $10^{-7}$ M and $10^{-6}$ M, also stimulated glycerol output from young rat adipocytes. In older rats, isoproterenol and ACTH-(1-24) in concentrations above $10^{-8}$ M stimulated glycerol release. However, glucagon was ineffective as a lipolytic agent with adipocytes from older rats at a concentration as high as $2 \times 10^{-6}$ M. The lipolytic response, under maximally stimulating concentra-

TABLE 1. Effect of aging on body weight and the characteristics of isolated adipocytes from rats and mice

| Animals | Approximate Age | No. of Experiments | Body Weight | Cells per Incubation $\times 10^5$ | Triacylglycerol | Cell Diameter |
|---|---|---|---|---|---|---|
| | *mo* | | *g* | | *nmol/cell* | *μm* |
| Rats | 1.5 | 3[a] | 168 ± 2[b] | 14.3 ± 2.0 | 0.042 ± 0.002[b] | 40.4 ± 0.8[b] |
| | 10.0 | 5 | 464 ± 10 | 13.7 ± 0.6 | 0.26 ± 0.02 | 73.8 ± 1.8 |
| Mice | 1–1.5 | 6 | 24.8 ± 1.2[b] | 13.5 ± 2.0[b] | 0.036 ± 0.006[b] | 38.0 ± 2.5[b] |
| | 6–8.0 | 6 | 44.3 ± 1.2 | 4.1 ± 0.5 | 0.74 ± 0.01 | 102.1 ± 5.9 |

[a] Values are means ± SEM of duplicate determinations for the number of experiments shown for each group.
[b] Indicates a difference at least significant at $P < 0.05$ between young and old rats or mice by Students' $t$ test.

tions of either ACTH-(1-24) or isoproterenol, was similar in cells from old and young rats, despite a two-fold difference in cell diameter (Fig. 2, Table 1).

Adipocytes from both young and old Swiss albino mice were tested with similar effective concentrations of isoproterenol or ACTH-(1-24) and these results have been summarized in **Table 2** and **Fig. 3.** The response of isolated mouse fat cells to isoproterenol is presented in Fig. 3. The three isoproterenol concentrations used produced significant lipolysis ($P < 0.05$) compared to unstimulated release in both young and old mice. Since the mean cell diameter of adipocytes from older mice was approximately twice that of younger mice, as with young and old rats (Table 1), cell size has been evaluated as a parameter for expressing lipolytic response. In Fig. 3, glycerol output is expressed in terms of cell number, surface area, and triacylglycerol content. In terms of cell number, fat cells from older mice were more active with the difference being significant at least at the $P < 0.05$ level for each concentration of isoproterenol tested. When expressed in terms of either surface area or triacylglycerol content, however the converse was observed. However, this dampened isoproterenol responsiveness observed with cells from older mice was only significant when expressed on a triacylglycerol basis. A difference in lipolysis between old and young rat adipocytes on a per cell basis was not observed (Fig. 2) under similar conditions in which lipolysis in mouse adipocytes was shown to be related to age and cell size (Fig. 3). In Table 2, net glycerol output in terms of cell number in response to isoproterenol ($10^{-6}$ M) is given as a relative index of lipolytic activity. Glucagon was ineffective as a lipolytic agent in a concentration as high as $2 \times 10^{-6}$ M in either young or old mouse adipocytes. In a second experiment (Table 2, Exp. 2), the lipolytic response to ACTH-(1-24) was demonstrated and the lack of glucagon response was again observed (data not shown).

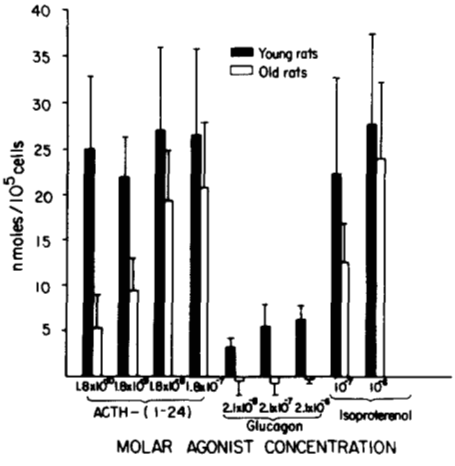


**Fig. 2.** Effect of lipolytic agents on glycerol release in adipocytes of young (1.5 mo) or old (10 mo) rats. Basal glycerol response has been subtracted. Values represent mean ± SEM with $N = 3$ for young and $N = 5$ for old rats. In each experiment, duplicate determinations were made. Incubation conditions are described in detail in the Materials and Methods.

TABLE 2. Comparative effects of lipolytic agents on lipolysis in adipocytes from young and old mice

| Addition | Mice: Young (1–1.5 mo) | Mice: Old (6–8 mo) |
|---|---|---|
| | *nmol glycerol/$10^5$ cells*[a] | |
| Experiment 1[b] | | |
| Isoproterenol $10^{-6}$ M | 31.2 ± 3.2 | 128 ± 37 |
| Glucagon | | |
| $2 \times 10^{-9}$ M | 0.1 ± 1.6 | −3.0 ± 11.7 |
| $2 \times 10^{-8}$ M | 1.2 ± 2.0 | −2.0 ± 13.2 |
| $2 \times 10^{-7}$ M | 0.9 ± 1.5 | −2.9 ± 13.8 |
| $2 \times 10^{-6}$ M | 2.3 ± 1.7 | 4.3 ± 11.9 |
| Experiment 2[c] | | |
| Isoproterenol $10^{-6}$ M | 46.4 | 276.0 |
| ACTH-(1-24) | | |
| $2 \times 10^{-9}$ M | 22.0 | 78.5 |
| $2 \times 10^{-8}$ M | 46.9 | 207.0 |
| $2 \times 10^{-7}$ M | 44.0 | 220.0 |

[a] Net glycerol release during a 45 min incubation after subtraction of basal lipolysis. (See Table 1 for absolute values).
[b] Means ± SEM of six separate preparations performed in duplicate are shown.
[c] The mean of two separate preparations performed in duplicate is given. In all cases, both young and old mice were compared on the same day.

## Complete aging profile of lipolytic response in mice

Interrelationships between aging, epididymal fat pad growth and lipolytic responses of isolated fat cells were examined in Swiss albino mice in this series of experiments. Influence of aging on body and epididymal fat pad weight is shown in **Fig. 4** A–C. Maximal body weight was reached several months before maximal epididymal fat pad weight (Fig. 4B, C). Fat pad weight declined with advancing age (Fig. 4B) in spite of a relatively stable total body weight (Fig. 4A). Mean adipocyte diameter (**Fig. 5**A) and triacylglycerol content per fat cell (Fig. 5B) increased rapidly in the first 2 months of age. Epididymal fat pad weight (Fig. 4A) and mean adipocyte size (Fig. 5A) declined at extreme age (>20 mo).

It was of interest to follow hormonal responsiveness with aging. ACTH-(1-24), dibutyryl cyclic AMP and

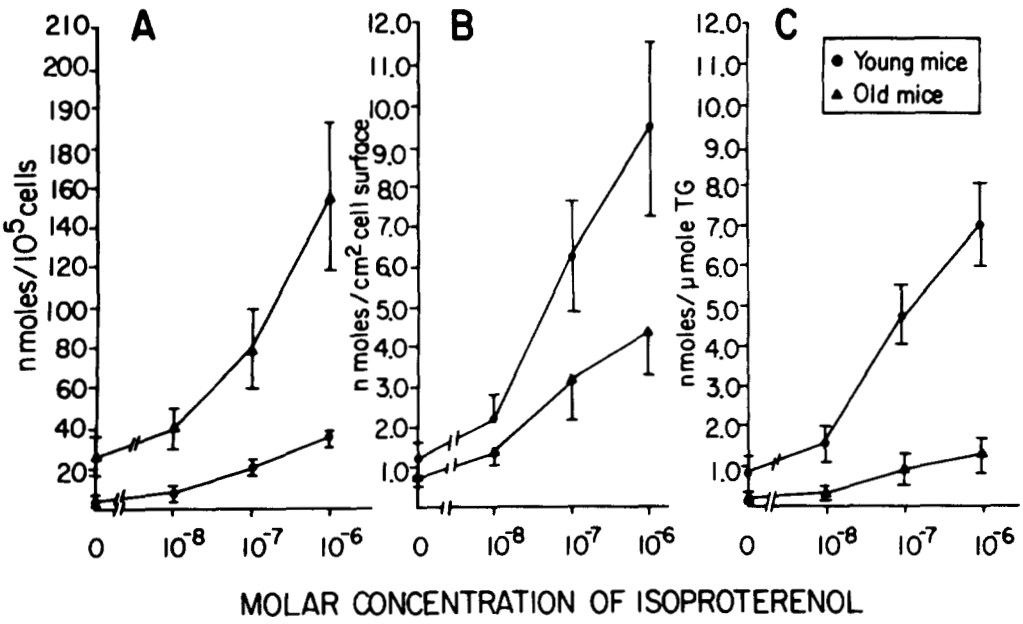


**Fig. 3.** Lipolytic effect of isoproterenol on isolated mouse adipocytes expressed on the basis of number of adipocytes (A), cell surface area (B) and cellular triacylglycerol content (C). Values are given as mean ± SEM ($N = 6$) for both young (1–1.5 mo) and old (6–8 mo) groups. Each experimental incubation was performed in duplicate. Differences between glycerol output for young and old mice were significant in terms of adipocyte number or cellular triacylglycerol content ($P < 0.05$).

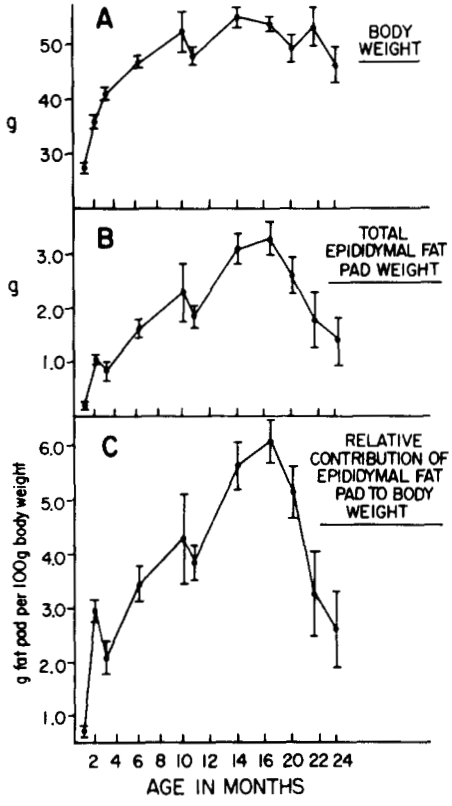


**Fig. 4.** Body weight (A) and total epididymal fat pad weight (B) for a complete aging profile in the mouse are given. The relative contribution of fat pad weight to total body weight has been calculated (C). Each point represents the mean ± SEM for three to ten mice per group.

isoproterenol were added to incubations of adipocytes in concentrations which would most likely reveal age effects on lipolysis. The choice of the concentrations of ACTH-(1-24) and isoproterenol, $5 \times 10^{-8}$ M and $5 \times 10^{-7}$ M, respectively, was based on our first experimental series (Table 2, Fig. 3). Due to the negative findings in the first series, glucagon was not tested. The response to isoproterenol in this series was comparable to the data given in Fig. 3, and qualitatively similar to either dibutyryl cyclic AMP or ACTH-(1-24) so these data are not shown. The lipolytic response of epididymal adipocytes from mice to dibutyryl cyclic AMP and ACTH-(1-24) is summarized in **Fig. 6** A–C. Cell size in relation to lipolytic response has been evaluated throughout the life span of the mouse from weaning to senescence. When expressed in terms of number of cells, the larger adipocytes from the older mice showed greater glycerol release. When expressed on cell triacylglycerol content, the age groups with smaller cells appeared to show more activity. In this series, no difference between age groups was apparent when the results were expressed in terms of cell surface area.

## DISCUSSION

This study examines the effect of age and cell size on the lipolytic response of mice of different ages.

Two series of experiments were performed. In the first series, mouse and rat adipocytes were compared with both young and older animals which had reached a plateau in body weight. In a second series, mice of differing ages were examined.

### Lipolytic response of adipocytes in rats and mice in relation to cell size and aging

Catecholamine-stimulated glycerol release was significantly augmented in larger fat cells from older, heavier mice. Our observations using rat adipocytes revealed no difference between isoproterenol-stimulated lipolysis in the two age groups despite a two-fold difference in mean cell diameter. Since both mouse and rat adipocytes were treated similarly, a species difference may account for these results.

Effects of age and cell size on catecholamine-augmented lipolysis in the Swiss albino mouse have not been extensively studied. In the rat, the dependence of stimulated lipolysis on cell size is less than clear, since age may depress lipolytic responsiveness. Björntorp and Sjöström (19) have demonstrated that larger epididymal rat fat cells from the same pool have greater lipolytic response to stimuli than smaller cells. A similar relationship has been reported by others (16, 20). Holm et al. (20) have shown that fat cell populations from young Sprague-Dawley rats exhibited lipolytic capacity greater than fat cells of similar mean diameter from older animals. This represents the best evidence for an age-related deficit in lipolysis in the rat. However, if larger adipocytes are more fragile, the dialysis tubing flotation technique used by Holm et al. (20) to separate subpopulations of rat adipocytes differing in mean diameter may differentially affect the recovery and performance of large and small cells. When age and fat cell size have been examined simultaneously in the rat, as in the present study, observations concerning catecholamine-stimulated lipolysis have varied. Elevation in lipolytic response with increasing age and cell size has been reported (16, 21). Hartman and coworkers (6, 7) have found similar glycerol release in adipocytes of Sprague-Dawley rats of differing age. But Hubbard and Matthew (22) have reported decreased lipolysis from fat cells obtained from older rats compared to those from younger animals. Possibly, this was related more to the obesity of the older rats than to age itself since such a reduction was not observed in older rats receiving a restricted diet in their experiments (22). Miller and Allen (23) have demonstrated qualitative differences in the dose response of catecholamine-stimulated lipolysis in older rats when compared to younger animals. The

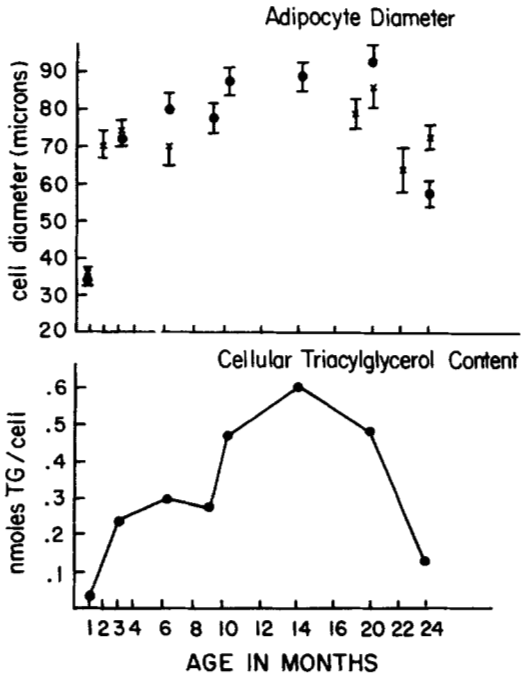


**Fig. 5.** Mean adipocyte diameter (A) and cellular triacylglycerol content (B) are summarized from mice of 1–24 months of age. Mean cell diameter ± SEM as an average of 50 observations and cell triacylglycerol (duplicate determinations) were made as described in the Materials and Methods.

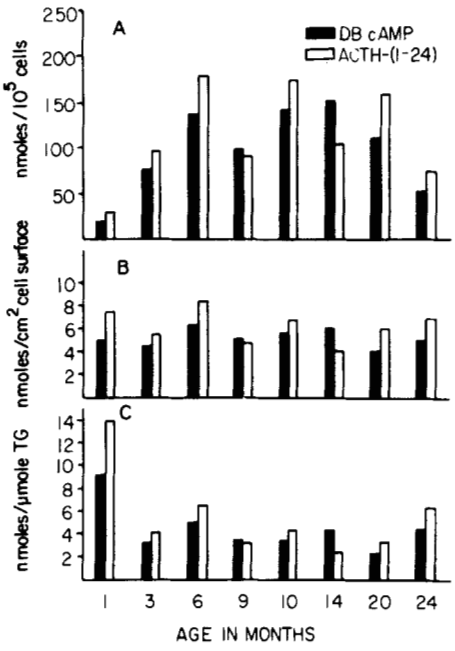


**Fig. 6.** Lipolytic response of isolated adipocytes from mice to effectors during aging. Net glycerol release to $5 \times 10^{-8}$ M ACTH-(1-24) and $5 \times 10^{-4}$ M dibutyryl cyclic AMP is given in terms of number of adipocytes (A) or cell surface area (B) or cellular triacylglycerol content (C). Each value is the mean of duplicate determinations from pooled adipocytes from a different age group (three to five mice) with basal glycerol release subtracted. With all age groups examined, the number of fat cells per incubate was within the range of $1.5–5.0 \times 10^5$ cells. Experimental and incubation conditions are described in detail in Materials and Methods.

results reported in this study are generally in agreement with those of Hartman and colleagues (6, 7) that hormone-stimulated lipolysis was similar in rats of differing age despite the larger adipocytes of rats which had plateaued in body weight.

In the present study, no selective loss of lipolytic response to ACTH-(1-24) was found in older mice or rats. A similar response of mouse adipocytes to ACTH has been described by Heindel, Cushman, and Jeanrenaud (1). The present study extends these results to an older age group. A selective loss of response to ACTH with age in the rat has been reported (23). Others (16, 24) have not observed any such loss of responsiveness to ACTH with aging or increased cell size. Experiments with isolated rat adipocytes in which cell dissociation takes place in the absence of albumin (25) have indicated that the lipolytic response to ACTH-(1-24) and glucagon[1] may suffer in comparison to fat cells prepared according to Rodbell (8). However, a decrease in ACTH-stimulated adenylate cyclase activity with age has been reported in the rat (26) which might also explain the loss of ACTH lipolytic responsiveness with adipocytes from older rats (23).

### Lipolytic response to glucagon in adipocytes from mice and rats

In adipocytes isolated from the epididymal fat pad of the mouse, no stimulation of the lipolytic response to glucagon, even in extremely high concentration, was observed in the present study. This is in contrast with a previous report (1) in which a significant lipolytic effect was observed with glucagon in isolated epididymal adipocytes from mice. Dehaye, Winand, and Christophe (4) have failed to demonstrate a significant lipolytic effect of glucagon using minced adipose tissue from mice. This would suggest that our present finding and that reported previously from our laboratory (5), were not due to loss of receptors during preparation of adipocytes in the presence of collagenase. Furthermore Dehaye et al. (4) obtained a uniformly negative lipolytic response with a series of glucagon polypeptides. In addition, a glucagon-sensitive adenylate cyclase could not be demonstrated in either preadipocytes or adipocytes during differentiation of murine 3T3-L1 fibroblasts (27). We would conclude from results of the present study and those just discussed, that a significant lipolytic response to glucagon is absent in the mouse.

Under similar experimental conditions, in which no glucagon responsiveness could be shown with mouse adipocytes, significant stimulation of lipolysis by glucagon was observed in adipocytes from young rats. This response was attenuated in older rats. Our results are consistent with observations by others (20, 24, 26, 28, 29). This loss does not appear to be due to cell size per se since larger cells from young rats respond to a greater degree than smaller cells from the same pool of adipocytes (20). Furthermore, it has been demonstrated that older rats on a restricted diet retain their responsiveness to glucagon (29), suggesting that nutritional factors, obesity, and age itself may have interrelated effects. Glucagon-stimulated adenylate cyclase activity also falls with age (24, 26). A loss of specific glucagon binding has also been reported in fat cell membranes from older rats (28).

### Lipolysis in mice over the lifespan

A complete profile of aging in mice extended the examination of lipolysis and its interrelationship to growth of the epididymal fat pad and the adipocyte. The results of this profile, which covered the expected lifespan, support those initially observed in our limited age group comparison with this species. Lipolytic response in the presence of either ACTH-(1-24), isoproterenol or dibutyryl cyclic AMP was greater with the larger adipocytes. Again, no differential loss of response to these agents was observed even in adipocytes of older mice approximately 2 years of age, approaching their normal life expectancy.

This extended investigation of aging effects on lipolysis in mice primarily addressed itself to the question: would lipolysis change beyond the plateau of body weight? To the best of our knowledge, this has not been previously examined in the mouse. Decrease in catecholamine-stimulated lipolysis during both maturation (8–10 mo) and senescence (12–30 mo) in the rat has been reported (30, 31). At older ages decreased lipolysis was associated with a decline in catecholamine binding and stimulation of adenylate cyclase (30). Other complete aging studies in the rat have focused on changes in lipoprotein lipase (32) and cellularity (33). Hirsch and Han (34) and Johnson and Hirsch (35) have reported that adipocyte hypertrophy was the primary cause of increased adipose tissue mass in the epididymal fat pad beyond early development in rats and mice. However, these experiments did not follow changes in adipose tissue cellularity beyond the plateau of body weight. More recently, significant hyperplasia in the perirenal fat depot has been observed in older rats of the Fischer 344 strain (36) and changes in subpopulations of epididymal adipocytes during aging have also been observed in this same strain of rats (33). An overall decrease in mean adipocyte diameter with senescence was also observed

[1] Jolly, S. R., J. J. Lech, and L. A. Menahan. Unpublished observations.

by Stiles, Francendese, and Masoro (33). A fall in adipocyte size was also shown in senescent mice in the present study. Even with mice of an advanced age, ACTH-(1-24), dibutyryl cyclic AMP, and isoproterenol markedly stimulated glycerol release in our experiments. Since no loss of hormonal responsiveness was observed relative to the dibutyryl cyclic AMP response, which bypasses membrane–receptor interaction, we have demonstrated that both discriminator and catalytic components of the lipolytic response remain functional with increasing age in the mouse.

Adipocytes from mice approaching senescence did show decreased lipolysis per cell. This was associated with decreased cell size and a concurrent drop in epididymal fat pad weight. Expressing hormone-stimulated lipolysis in terms of cell surface equalized the glycerol output of very young, intermediate aged, and senescent mice. This would suggest that changes in lipolysis during aging may be controlled by factors which influence adipocyte size in the epididymal fat pad of the mouse.

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