Alpha-adrenergic responses in rabbit white fat cells: the influence of obesity and food restriction[1]

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Abstract The present work was carried out to separate the influence of age from that of fat cell size in rabbit white fat cells, in order to assess the importance of changing cell size to the age-related decrease of epinephrine responsiveness. Epinephrine action and adrenergic receptor site activities were explored in two main groups of rabbits. One group, 5–6 months of age, was divided into three subgroups: the control, group I, was fed usual laboratory chow; group II was subjected to dietary restriction (3 months); group III was fed the usual diet with chronic administration of insulin (1.5 UI/kg per day for 3 months) known to induce fat cell size increment. The other main group, composed of 15- to 16-month-old rabbits, was also divided into three subgroups: group IV, control; group V, subjected to dietary restriction (1½–2 months) after previous development of adipose mass; and group VI, diet-restricted for 4 months. The loss of epinephrine-responsiveness of rabbit fat cells can be either prevented by restricting food intake (groups III, V) or promoted by chronic insulin administration (group II). Isoproterenol-induced lipolysis was maintained whatever the fat-cell size, while the changes in the ability of epinephrine to promote lipolysis were linked to a variable alpha-adrenergic activity (increase of epinephrine-induced lipolysis promoted by the alpha-antagonist drug, phentolamine). Alpha-adrenergic responsiveness is increased in large fat cells (groups III and IV) while a reduced alpha-adrenergic activity is observed in small fat cells of underfed rabbits (group II). After dietary restriction, large fat cells (with an increased alpha-adrenergic responsiveness) were reduced in size and a significant restoration of the lipolytic effect of epinephrine was shown (group V). In conclusion, these results indicate that cell size, in addition to age, is an important factor affecting epinephrine-responsiveness in rabbit adipocytes. The loss or the recovery of the lipolytic effect of epinephrine could be explained by a modification of the alpha-receptor activity; the beta-receptor activity was less modified.—**Lafontan, M.** Alpha-adrenergic responses in rabbit white fat cells: the influence of obesity and food restriction. *J. Lipid Res.* 1981. **22:** 1084–1093.

Supplementary key words perirenal adipocytes · glycerol · isoproterenol · epinephrine · beta-receptor sites · alpha-receptor sites · fat cell size · aging

In a previous report it was shown that an increased alpha-adrenergic responsiveness caused the decrease and the loss of epinephrine-induced lipolysis in isolated white adipocytes of aging rabbits (1). These results did not allow a clear dissociation to be made between aging and increased body weight or fat cell size.

The mechanisms regulating the development of the adipose mass are virtually unknown. However, previous investigators have described the time course of the growth of the adipose organ in the rabbit (2, 3). Stable cellularity of the perirenal fat pad is not reached until 10 months of age (3) and the main changes in the composition and cellularity occur during the first year of life (2). It is well known that some aspects of adipose tissue metabolism such as the rate of lipolysis or lipogenesis and hormone responsiveness are closely associated with fat cell size (4–12). In humans (4–6) and rats (7–12), some investigators have proposed that a decreased responsiveness to epinephrine is associated with some critical degree of fat cell enlargement as in the rabbit (1). However, since aging in experimental animals is generally associated with larger adipose mass and cell size, more research is needed to find the determinant of the modifications of hormonal responsiveness.

The present investigation in the rabbit was undertaken in order to expand our preliminary results (1). We have attempted to separate the influence of age from that of fat cell size and to establish whether fat cell size is the major determinant in the lipolytic response of rabbit fat cells to epinephrine. Epinephrine-responsiveness with regard to alpha- and beta-receptor site activity was investigated on isolated perirenal white adipocytes from a group of rabbits during the growth of the adipose tissue and on a group of

Abbreviation: ACTH, adrenocorticotropic hormone.

[1] A preliminary report of a portion of these data has been published in abstract form at the 7th International Congress of Pharmacology held in Paris, July 1978, Abstract 1491 and at the INSERM-EMBO workshop "Cellular and Molecular Aspects of Obesity" held in Nice (France), August 1979, Abstract 16.

rabbits that had reached stable cellularity of the perirenal fat pad.

MATERIALS AND METHODS

Animal housing and dietary conditions

Male Fauve de Bourgogne rabbits were housed at 20 ± 2°C under a natural light-dark cycle and fed a standard rabbit chow (UAR-Paris) with water available at all times. The composition of the laboratory chow was described previously (1). Control rabbits were fed ad libitum. The experimental schedule is shown in **Table 1** (see Results). Two main groups of rabbits were used. One of these groups was composed of 5 to 6-month-old adult rabbits during the phase of development of the adipose tissue. These rabbits were divided into three groups: a control group fed ad libitum (group I); a group of diet-restricted rabbits (group II) which, for 3 months were maintained on approximately one-half of their normal dietary intake (60–70 g/day instead of 130–150 g/day); and a group of insulin-treated rabbits (group III) given insulin (1.5 UI/kg/day) for 3 months twice a day (except the day before being killed) and fed ad libitum. Another main group was composed of older rabbits, 15 to 16 months old, which, according to previous investigations (2–3), had reached stable cellularity of the perirenal fat pad. These rabbits were also divided into three groups: a control group fed ad libitum (group IV), a diet-restricted group (group V) maintained on one-half of their normal daily intake for 1½–2 months and a diet-restricted group (group VI) subject to 4 months of caloric restriction. Rabbits in groups V and VI were slightly older than the controls in group IV. Diet-restricted animals received their chow at 9 AM and they ate their daily ration faster than the controls. Thus, when necessary, before they were killed, they were given food later to prevent prolonged fasting periods.

Experimental procedures: Preparation and incubation of isolated fat cells

The experiments took place from September to April for 2 years. All the studies were done in the morning. The animals were killed by cervical dislocation after an 18-hr fast and the perirenal adipose tissue was taken immediately after. Fat cells were isolated and incubated as previously described (1) with only minor modifications for the management of large adipocytes. Large fat cells of aged controls and insulin-treated rabbits are fragile and an increased cell breakage occurred in some fat-cell preparations. Cell breakage is the largest obstacle in the study of large fat cells. The choice of a suitable collagenase batch associated to a limited enzyme exposure, the use of siliconized glassware and polythene tubing and vials, the omission of the centrifugation procedure originally described by Rodbell (13) (the fat-cells were allowed to float to the surface, the infranatant being removed by suction), and the prevention of thermic shocks allowed us to obtain preparations of large fat cells with reduced disruption bearing sufficient functional cells for the investigations.

The washed adipocytes were resuspended in the incubation medium. Appropriate dilutions of the fat cells were carried out to obtain approximately the same cell concentration in each preparation (whatever the size of the fat cell). In this investigation the number of fat cells was not directly counted. Incubations of 90 min were done in duplicate or triplicate. The adipokinetic substances and other agents were added (10 μl) just before starting the incubation. The lipolytic response of each incubation set was checked with a strong lipolytic agent, adrenocorticotrophic hormone (0.1 or 1 μg/ml incubation medium).

Analytical technique

The fat cells of one vial were stained with Giemsa stain and the diameters of 400 fat cells were determined by optical sizing as previously described (1); the mean fat cell diameter and volume were calculated according to the method of Hirsch and Gallian (14). The number of fat cells in the cell suspension was calculated by dividing the total lipid content of the isolated adipocytes by the mean fat cell triglyceride content (corrected mean cell volume × 0.915). Glycerol released in the incubation medium was analyzed according to the enzymatic method of Wieland (15). Total lipids were extracted according to the method of Dole and Meinertz (16) and the mass was found by gravimetric determination. The lipolytic activity was expressed per 100 mg of total lipid or per 10^6 adipose cells. The results of some experiments are expressed on a percentage basis according to the formula: (stimulated lipolysis minus basal lipolysis/basal lipolysis)×100.

The mean values are given with standard error. The significance of the difference among the sets within an experiment was estimated with Student's paired *t*-test.

The pharmacological agents used in the incubations were: l-epinephrine hydrochloride (Aguettant Labs., France) or l-epinephrine bitartrate (Sigma), isoproterenol (Winthrop), phentolamine (Ciba-Geigy), propranolol (I.C.I. Pharma), theophylline (Bruneau Labs. or Sigma), porcine ACTH (Choay Labs.). Pork-beef slow acting insulin was purchased from Novo-Copenhagen. Fatty acid-poor bovine serum albumin

TABLE 1. Basal information relative to the experimental groups: epinephrine, isoproterenol, and ACTH-responsiveness in perirenal adipocytes of rabbits

Experimental Groups	Fat Cell Volume	Basal Lipolysis[b]	Epinephrine × 10^{-6} M				Isoproterenol[f] 5×10^{-6} M	ACTH[f] (1 μg/ml incubation medium)
			0.05	0.5	5	50		
	pl							
5–6 Months old								
Group I (10)[a]								
Controls 5–6 months old	258 ± 21	0.254 ± 0.029	3.5 ± 3.1	−7.1 ± 2.6	17.7 ± 4.3	92.5 ± 13.2[d,**]	185.1 ± 24**	1274 ± 203**
Group II (12) Restricted 3 months	138 ± 14	0.233 ± 0.018	6.8 ± 2.1[c]	33.9 ± 8.2*	172.5 ± 35.2**	323.2 ± 56.1**	246.0 ± 38**	1570.1 ± 286**
Group III (10) Insulin-treated 3 months	730 ± 60	0.595 ± 0.050	−18.3 ± 2.1[e,○]	−50.7 ± 8.3[○○]	−17.1 ± 7.1[○]	+35.8 ± 12.2*	147.6 ± 20.2**	420.8 ± 75.2**
15–18 Months old								
Group IV (11) Aged controls	880 ± 90	0.540 ± 0.080	−23.2 ± 4.1[○]	−52.5 ± 5.1[○○]	−20.1 ± 3.1[○]	+8.9 ± 10.8	150.3 ± 19.2**	395.2 ± 80.6**
Group V (12) Restricted 1½–2 months	303 ± 31	0.270 ± 0.031	−4.3 ± 4.7	−7.8 ± 7.9	116.8 ± 38.1**	298.7 ± 35.4**	362.2 ± 45.4**	795 ± 225**
Group VI (11) Restricted 4 months	92 ± 18	0.216 ± 0.041	—	6.2 ± 3.1	50.1 ± 21.3*	184.8 ± 40.4**	212.5 ± 50.4**	1135 ± 234**

[a] Number of animals in each group in parentheses.

[b] Basal lipolysis is expressed as μmol of glycerol released/100 mg total lipids over 90 min.

[c] Positive results indicate a stimulation of lipolysis and negative results indicate an inhibition of basal lipolysis.

[d] *, $P < 0.05$; **, $P < 0.01$: positive results significantly different from control values (by Student's paired t test).

[e] ○, $P < 0.05$; ○○, $P < 0.01$: negative results significantly different from control values (by Student's paired t test).

[f] The lipolytic response of larger cells to ACTH as well as to isoproterenol is reduced on expressing the adipocyte responsiveness on a percentage basis when the basal lipolytic rate is increased. The glycerol release induced by these agents does in fact reach those obtained in group I rabbits.

Adipocytes responsiveness to lipolytic agents is expressed according to the formula: (stimulated lipolysis minus basal lipolysis/basal lipolysis) × 100. Results are expressed as means ± SEM. Experimental conditions are described in detail in Materials and Methods.

was obtained from Calbiochem; crude collagenase from Worthington Biochemical Corp. Enzymes came from Boehringer Mannheim.

RESULTS

Evaluation of the cell size and definition of the experimental groups

Isolated fat cells from the different groups of rabbits were studied. Group I was composed of 10 control normal adult rabbits, 5 to 6-months old; their mean body weight was 2.940 ± 0.160 kg and the fat cell volume was 258 ± 21 pl.

Group II was composed of 12 calorie-restricted rabbits, 5 to 6 months in age; the mean body weight was 2.520 ± 0.090 kg and the mean fat cell volume was 138 ± 14 pl.

Group III consisted of 10 insulin-treated rabbits, 6 months in age; the mean body weight was 3.380 ± 0.170 kg and the mean fat cell volume was 730 ± 60 pl.

The three other rabbit groups totaled 40 older rabbits, 15 to 16-months old; their mean body weight was 3.950 ± 0.170 kg.

Group IV consisted of 17 older controls, the mean fat cell volume was 880 ± 90 pl.

Group V was composed of 12 calorie-restricted animals; the mean weight loss was 303 ± 31 g after 1½–2 months of caloric restriction. The mean fat cell volume was 245 ± 35 pl.

Group VI consisted of 11 rabbits submitted to prolonged caloric restriction (4 months). The mean weight loss was 664 ± 59 g; the mean fat cell volume 92 ± 18 pl.

Our investigation did not involve the study of the development of adipose tissue cellularity. However, on investigation of fat cell size, we observed that, for animals of comparable age, rabbits on a restricted diet for 3 months showed a lower increase ($P < 0.01$) in fat cell size than the control rabbits (group I). In contrast, the average fat cell size increased more rapidly in insulin-treated rabbits (group III). In the older rabbits a moderate dietary restriction decreased the fat cell size to a level reaching approximately that of 5 to 6-month-old rabbits (group I). After a longer period of caloric restriction, the mean cell size of the rabbits, which were then approximately 20 months old, was reduced to a level that was equivalent to that of the 50 to 70-day-old rabbits previously studied (1).

Epinephrine-responsiveness of adipocytes of restricted and insulin-treated rabbits: comparison with isoproterenol and ACTH responsiveness

As shown in Table 1, in the control group (group I), epinephrine was without any noticeable lipolytic effect on rabbit fat cells; a slight but significant stimulation ($P < 0.01$) occurred only at 50×10^{-6}M.

In the group submitted to a restricted diet (group II), a marked increase in epinephrine-responsiveness was clearly shown. Epinephrine elicited a strong adipokinetic effect: a significant ($P < 0.05$) lipolytic response occurred at 0.5×10^{-6}M epinephrine. Higher doses induced a 2- to 3-fold stimulation of the basal lipolytic rate.

In the insulin-treated group (group III), the basal rate of lipolysis was higher than that observed in the two other groups (Table 1). Expressed per 10^6 cells, the difference was highly significant ($P < 0.001$). Epinephrine did not increase lipolysis in the insulin-treated animals except for the highest dose used (50×10^{-6}M epinephrine). A significant decrease of the basal rate of lipolysis occurred at low concentrations of epinephrine (0.05, 0.5, and 5×10^{-6}M).

The glycerol production induced by the beta-adrenomimetic drug (isoproterenol) was several times higher than that induced by epinephrine in the control group and in the insulin-treated group. In the restricted rabbits, the effects of 5×10^{-6}M epinephrine or isoproterenol were almost the same. ACTH (1 μg/ml in the incubation medium) exerted a marked lipolytic action on all three groups of rabbits.

Epinephrine and isoproterenol responsiveness of older rabbit adipocytes after a dietary restriction (2 months)

After 2 months of dietary restriction, group V showed an important decrease of perirenal fat cell size (from 880 ± 90 pl in the control group to 245 ± 35 pl). The mean cell size was equivalent to that of 5 to 6-month-old rabbits. Lipolytic responses of adipocytes are shown in **Fig. 1** before and after dietary restriction. The control group (group IV) of older obese rabbits showed an increased basal level of lipolysis and the absence of any lipolytic effect of epinephrine which even inhibited lipolysis at 0.05, 0.5, and 5×10^{-6}M, while isoproterenol exerted a lipolytic effect at 0.5, 5, and 50×10^{-6}M. The anti-lipolytic effect of epinephrine was suppressed by the alpha-adrenoblocking drug (phentolamine) which unmasked, at 20×10^{-6}M, a significant stimulation of lipolysis (not shown) as reported in a previous paper (1).

In the diet-restricted group (group V), the basal rate of lipolysis was lower than that of the control group (group IV). The most striking fact was the occurrence of clear epinephrine-induced lipolysis in the adipocytes of the restricted group at 5 and 50 $\times 10^{-6}$M, while isoproterenol responsiveness was not

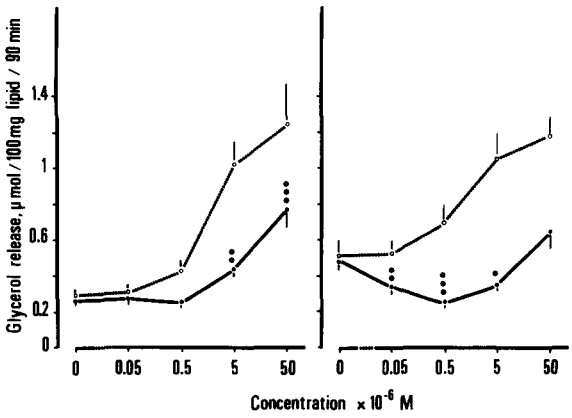

Fig. 1. Epinephrine (●) and isoproterenol (○) responsiveness of older obese rabbit adipocytes (group IV—right) and older rabbit adipocytes after 2 months of dietary restriction (group V—left). Drugs were added at zero time; values are means ± S.E.M.; n = 11 (group IV) or n = 12 (group V). Vertical lines represent standard errors. *, $P < 0.02$; **, $P < 0.01$; ***, $P < 0.001$; results of epinephrine-induced lipolysis significantly different from basal values (by Student's paired t test). The responses to 0.5, 5, and 50 × 10^{-6}M isoproterenol are significantly different from basal values. Incubation conditions are described in detail in Materials and Methods.

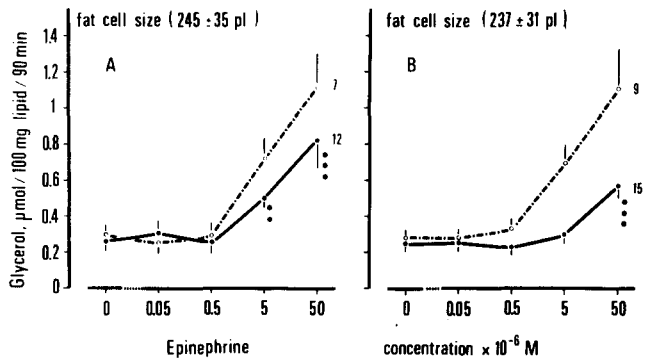

Fig. 2. Comparison of dose-reponse curves for epinephrine alone (●) and in the presence of (20 × 10^{-6}M) phentolamine (○) in adult rabbit adipocytes (group I—B) and older rabbit adipocytes after a 2-month dietary restriction (group V—A). Mean fat cell size is equivalent in the two groups. Drugs were added at zero time. **, $P < 0.01$; ***, $P < 0.001$; results significantly different from basal values (by Student's paired t test). Vertical lines represent standard errors and number is the number of animals. Fat cell volume ± S.E.M. is given in picoliters.

modified. Control experiments were performed on seven rabbits (group IV) to evaluate the changes occurring in the fat cell responsiveness after several days of caloric restriction (10 days) before the occurrence of any significant reduction in fat cell size. A weak increase in the basal rate of lipolysis was observed (0.670 ± 0.040 in this group versus 0.540 ± 0.080 µmol/100 mg lipid per 90 min in the controls). The antilipolytic effect of 0.5 and 5 × 10^{-6}M epinephrine was practically the same in the two groups (60 ± 7.1 per cent inhibition versus 52.5 ± 5.1 in the control group for 0.5 × 10^{-6}M epinephrine).

Furthermore, we compared (**Fig. 2**) the lipolytic responses of the adipocytes of the restricted group with those of younger control rabbits (group I) since the mean fat cell size was equivalent (245 ± 35 pl for group V rabbits and 237 ± 31 pl for group I rabbits).

The adipocytes of the restricted group had a higher epinephrine responsiveness than those of younger animals (group I). With 5 × 10^{-6}M epinephrine, lipolysis was stimulated in restricted animals, but showed no noticeable modification in younger controls. This difference was also maintained at the higher dose (50 × 10^{-6}M epinephrine). The alpha-adrenoblocking drug phentolamine potentiated the epinephrine-induced lipolysis which approximately reached isoproterenol-stimulated levels. It was observed that the effect of epinephrine on lipolysis was potentiated in the presence of low concentrations of phentolamine (from 2 × 10^{-6}M to 20 × 10^{-6}M) while higher concentrations (10^{-4} and 10^{-3} M) were found to inhibit epinephrine-induced lipolysis or even ACTH-stimulated lipolysis. This biphasic effect of phentolamine on hormone-induced lipolysis has been previously reported in rat (17) and hamster fat cells (18). Nevertheless, the difference between the lipolytic response obtained with epinephrine alone or associated with phentolamine could be considered as a reflection of the alpha-adrenergic activity. The response (after the adjunction of a suitable concentration of the alpha-adrenoblocking drug) nearly reached that elicited by isoproterenol alone.

Hormonal responsiveness after prolonged dietary restriction

Food restriction (4 months) resulted in reduction of body weight ranging from 15 to 18% and had a strong effect on cell size. The cells of the diet-restricted rabbits (group VI) were as small as those observed in very young rabbits (50–60 days). The average size was 92 ± 18 pl after food restriction and 108 ± 18 pl for young rabbits.

Isoproterenol and epinephrine (Table 1) stimulated lipolysis less in group VI rabbit fat cells than in young rabbit adipocytes as previously reported (1) and shown in **Fig. 3** (left). No significant difference was found between the effect of epinephrine and that of isoproterenol in the small fat cells of diet-restricted rabbits (Fig. 3). Moreover, phentolamine (20 × 10^{-6}M) was without any effect on epinephrine-induced lipolysis in small fat cells of diet-restricted rabbits, suggesting a lack of any alpha-adrenergic activity.

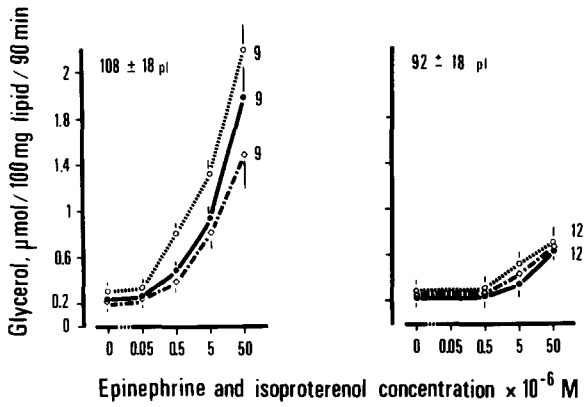

Fig. 3. Comparison of dose-response curves for isoproterenol (○) and epinephrine alone (●) and in the presence of (20×10^{-6}M) phentolamine (◇) in older rabbit adipocytes after a 4-month dietary restriction (group VI, right) and young rabbit adipocytes (left panel). The young rabbits were 50–60 days old; the mean body weight was 1.100 ± 0.020 kg. The addition of phentolamine failed to modify significantly the response to epinephrine. Drugs were added at zero time. Vertical lines represent standard errors and number is the number of animals. Fat cell volume ± S.E.M. is given in picoliters.

In the same group of rabbits, the lipolysis of small fat cells was examined in the presence of ACTH and theophylline. There was no effect of 0.3×10^{-3}M theophylline on the lipolysis of the small fat cells of restricted rabbits while the same dose elicited a clear lipolytic response in control rabbits (2- to 3-fold). Theophylline was found to have a lipolytic effect only at higher doses (2 or 5×10^{-3}M). ACTH (0.1 µg/ml incubation medium) was without any lipolytic effect while 10-fold stimulation occurred in control rabbits (group I); a higher dose (1 µg/ml incubation medium) was clearly lipolytic (Table 1).

Effects of diet and fat cell size on basal lipolysis

There was a positive and significant correlation between basal glycerol release and fat cell size during the experiments of caloric restriction (**Fig. 4**). When the data for the obese rabbits are observed alone, the correlation is only slightly significant ($r = 0.47$; $P < 0.05$). Food restriction led to a strong reduction in fat cell size in group V and group VI rabbits and the basal lipolytic rate and fat cell size decreased concomitantly. The basal glycerol release of group VI rabbits was equivalent to that observed in younger animals. Investigation of the basal lipolytic rates demonstrated the existence of a significant relationship between the level of basal lipolysis and the extent of the antilipolytic effect of epinephrine ($r = 0.89$; $P < 0.01$); the higher the basal rate of lipolysis, the stronger the antilipolytic effect; such a result fits our recent findings in human fat cells (19).

DISCUSSION

The results clearly demonstrate that the loss of epinephrine-responsiveness of rabbit fat cells can either be prevented by restricting food intake or promoted by chronic insulin administration in 6-month-old rabbits. Moreover, after the restriction of food intake in older rabbits, the animals showed a weight loss associated to a decrease of the adipose mass; the lipolytic sensitivity of the adipocytes to epinephrine was partly recovered.

The results pinpoint the importance in the rabbit of fat cell size in the modulation of the epinephrine-responsiveness; the response to the other lipolytic agents being less affected.

Preliminary studies (1), were focused on the physiological variation of the expression of the alpha-adrenergic responsiveness, i.e., the increment of epinephrine-induced lipolysis promoted by the antagonist drug (phentolamine) and the difference between epinephrine and isoproterenol induced lipolysis.

The same indirect methods were used for the investigation of alpha-adrenoceptor activity since a more appropriate protocol had not been established when the experiments were carried out. The validity of the indirect approach using phentolamine is questionable since this drug is known to exert direct nonspecific inhibitory effects on lipolysis (17, 18). At the concentrations used (2 to 20×10^{-6}M) we did not find inhibiting effects of phentolamine on basal, ACTH- or isoproterenol-induced lipolysis. Higher doses (10^{-4} or 10^{-3}M) did exert antilipolytic effects and did not permit a complete exploration of the alpha-adrenergic sensitivity (enhancement of epi-

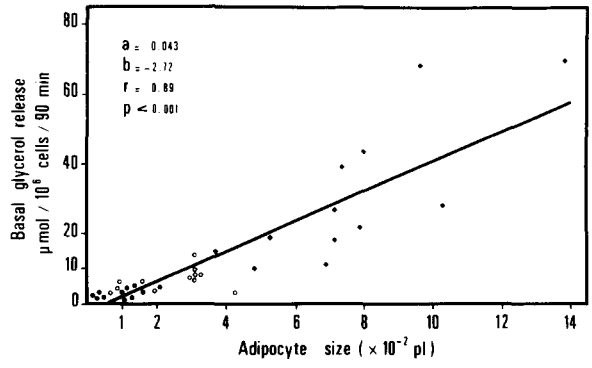

Fig. 4. Relationship between mean fat cell volume and basal rate of lipolysis in the adipose tissue of obese controls (◆) and rabbits subjected to a 2-month (○) or 4-month (●) dietary restriction. a, slope of the regression line; b, y intercept from the equation $y = ax + b$; r = correlation coefficient. The rate of basal glycerol release is a linear function of the mean fat cell volume (picoliters).

nephrine-induced lipolysis). So, the indirect means based on the inhibition of the alpha-adrenergic activity by an antagonist is not the best experimental design to investigate alpha-adrenergic responses. However, the fact that clear-cut differences exist in epinephrine effects in rabbit fat cells certainly permits easier investigation through indirect means. Since the time when these investigations were made, we have improved the characterization of the alpha-receptors of the fat cell by a direct means (inhibition of basal or theophylline-induced lipolysis by alpha$_2$ agonists (20, 21). To assess the results obtained by the indirect approach, we have studied the inhibition of theophylline-induced lipolysis by clonidine (alpha$_2$-agonist) on young and aged rabbit fat cells (**Table 2**). The results confirm our previous investigations (1) and permit a validation of the results obtained with phentolamine for the investigation of alpha-adrenergic responses.

The data obtained from the experiments on adipocytes of underfed (group II) or insulin-treated (Group III) rabbits support the view that alpha-receptors seem more effective in large fat cells than in small fat cells.

The findings obtained with older rabbits confirmed those reported in the first step of the experiments. By restricting calorie intake, older animals with reduced perirenal adipocytes (group V) could be observed. Clearly, food restrictions lead to a change in epinephrine responsiveness which was recovered and even enhanced if we compare it to that of younger ones (group I) with an equivalent cell size (Fig. 2). Isoproterenol responsiveness was unchanged. These data, showing that epinephrine responsiveness is restored as cells shrink in old adult rabbits, suggest that the previously reported age-related loss of hormone responsiveness is associated to adipocyte enlargement rather than to aging. The differences between the lipolytic potencies of epinephrine, alone or associated to phentolamine, and those of isoproterenol (Fig. 2) bring additional evidence for variation of alpha-adrenergic activity according to size in rabbit fat cells. This result is assessed by the comparison between the responsiveness of the adipocytes of group V rabbits and that of rabbits submitted to shorter periods of calorie restriction (before the occurrence of any changes in fat cell size). It was seen that no significant changes in the antilipolytic effect of epinephrine existed while the basal rate of lipolysis was only slightly increased. So, in the rabbit, the alpha-adrenergic responses of the adipocytes are not increased by calorie restriction when fat cell size is not modified. These findings are different from those reported for human fat cells (22, 23). An accelerated basal rate of lipolysis was observed during fasting (22) or calorie restriction (23) in obese subjects; it was associated to the occurrence of an inhibitory effect on basal glycerol release of norepinephrine or epinephrine, while these agents are clearly lipolytic in control patients. These results lead to the conclusion that the alpha-adrenergic response is increased in human subcutaneous adipose tissue during short periods of fasting or calorie restriction. The conflicting results in the rabbit may be due to interspecies differences (mainly the occurrence of caecotrophy in the rabbit)

TABLE 2. Percent inhibition by an alpha$_2$-agonist (clonidine) of the theophylline-induced lipolysis in isolated perirenal white fat cells of young and old rabbits.

	Clonidine			
	0.05	0.5	5	50
			(μM)	
Young rabbits (5)[a]	1.6 ± 2.0[b] NS[c]	3.7 ± 4.0 NS	5.1 ± 6.2 NS	11.8 ± 9.3 NS
Old rabbits (10)	44.2 ± 7.1[d]	73.8 ± 9.1[d]	78.6 ± 8.5[d]	87.2 ± 6.2[d]

[a] Number of animals in each group of rabbits in parentheses.

[b] Values are means ± SEM. The results are expressed as the percentage of inhibition of theophylline-induced glycerol release/100 mg lipid per 90 min.

[c] NS, not statistically significant.

[d] Statistically significant difference between control values and stimulated values obtained from Student's paired t test. ($P < 0.001$).

Isolated fat cells were incubated in the presence of 1 mM theophylline and the indicated concentration of clonidine. Theophylline-stimulated lipolysis reached the same level in young and old rabbit fat cells. Clonidine strongly inhibited theophylline-induced lipolysis in large fat cells (860 ± 50 pl) of older rabbits and was without any effect on theophylline-induced lipolysis in small fat cells (110 ± 11 pl) of young rabbits. Alpha$_2$-mediated responses (antilipolytic effects) only occur in large fat cells of older rabbits. The result confirms the results obtained by the indirect method (with the alpha-adrenoblocking agent, phentolamine).

and to the fact that, under our experimental conditions, the basal rate of lipolysis is spontaneously increased in the large fat cells of the rabbit (Fig. 4) and is less responsive to short term calorie restriction.

Additional support of previous conclusions is offered by the observations made on the small fat cells of aged rabbits submitted to sustained calorie restriction (group VI). Although no investigation has been made into the metabolic state and endocrine gland activity to define the biochemical environment of the cells, the results obtained with the adipocytes of group VI rabbits fit our previously described observations concerning catecholamine responsiveness. After a prolonged restriction of calorie intake, the cell size was equivalent to that observed in younger rabbits (60–70 days). Compared to the sensitivity to lipolytic agents of the fat cells of younger rabbits (Fig. 3), the lipolytic activity of group VI rabbit adipocytes was profoundly decreased. However, although food restriction reduced the sensitivity of the adipocytes to the various lipolytic agents used, adrenergic-induced lipolysis is interesting to consider since there was no difference between the lipolytic effects of isoproterenol and those of epinephrine. Moreover, phentolamine was without any effect upon epinephrine-induced lipolysis (Fig. 3). Since the lipolytic response to isoproterenol and epinephrine alone, or in the presence of phentolamine, is reduced, a desensitization of the beta-receptors (24) or a diminished number of available catecholamine receptors cannot be excluded (25). Concerning the alpha-receptor activity, no noticeable adrenergic responsiveness could be seen in small adipocytes, whatever the age of the rabbit.

From these findings we can say that: *1*) the modifications of epinephrine-responsiveness in rabbit fat cells involve alpha-adrenoceptors stimulated by epinephrine and *2*) the alpha-adrenergic activity may represent a regulatory mechanism characterized, in the rabbit, by fat-cell size-dependence.

Alpha-adrenoceptors have been described in hamster (26–28), human (29, 30), and dog adipose tissue (31). At present, the mechanism of the alpha-adrenergic effect and the importance of the interplay in the beta- and alpha-adrenergic responsiveness are not elucidated. Although the role of alpha-receptors in the physiological control of the lipolytic activity of the adipocytes is not immediately apparent, an increment of alpha-adrenergic responsivesss during prolonged starvation was reported in human fat cells (32). Moreover, pathologically increased alpha-adrenergic responsiveness was reported in the adipose tissue of hypothyroid subjects (33, 34). Recently, local differences in the lipolytic responsiveness of human adipose tissue to epinephrine or norepinephrine were considered by us (19) and two other groups (35, 36) to be linked to a variable alpha-receptor activity; the adrenaline responsiveness of the femoral adipocytes was associated to a prominent alpha-adrenergic effect (19, 35). More recently, reports about alpha-adrenergic responsiveness and cell size or age modulation have appeared in the literature for rabbit and dog fat cells. The alpha-adrenergic responsiveness increases with age and/or fat cell size in dog (31) and in rabbit adipocytes (1). Recently, Pecquery and Giudicelli (37) have shown that the presence of alpha-receptors is only detectable by a binding method ([^{3}H]dihydroergocryptine binding) after the first month of life in hamster fat cells; the number of alpha-receptors increased with age, with a parallel variation of the alpha-adrenergic responsiveness. The present data fit our previous observations in rabbit fat cells (1) and strongly support the view, in another species, that the alpha-adrenergic responsiveness increases with age and /or fat cell size. These results do not agree with those of Arner, Engfeldt and Ostman (22) who reported that the alpha effect was stronger in small human adipocytes.

In conclusion, the results reported here allow aging, increased body weight, and fat cell size to be dissociated. Over the life span considered in the experiments, fat cell size changes seem more important than aging. These experiments strongly suggest that the alpha-adrenergic responsiveness is a variable parameter in the adrenergic responsiveness of rabbit adipose tissue. They support the physiological significance of the alpha-adrenergic function in the regulation of lipolysis in vitro, since the interplay with beta-adrenergic activity influences the cellular sensitivity to catecholamines. Fat cell size, in addition to age, is an important factor explaining epinephrine resistance in rabbit adipocytes. The molecular basis of the modifications is unknown and could presumably involve various steps such as altered binding, altered number of alpha- and beta-receptors, or even modifications of the coupling between the receptors and the effector systems.

Moreover, if these results are extended to other species, an increased alpha-receptor activity could represent a pathological state (explaining epinephrine unresponsiveness) particularly serious in adipose tissue unresponsive to peptidic hormones, such as human adipose tissue. In the rabbit, the low efficiency of catecholamines in the induction of lipid mobilization, did not limit the occurrence of the phenomenon, since in obese rabbits, ACTH or a related peptide is of

greater physiological importance than catecholamines in the control of lipid mobilization (38, 39).

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