Dependence of rates of lipolysis, esterification, and free fatty acid release in isolated fat cells on age, cell size, and nutritional state

Michael F. Reardon, R. Brian Goldrick, and Noel H. Fidge

Department of Clinical Science, The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T. Australia

Abstract Rates of lipolysis, esterification, and free fatty acid release were estimated in isolated epididymal fat cells prepared from rats fed either ad lib. or with a restricted caloric intake. Basal and epinephrine- or theophylline-stimulated rates of lipolysis correlated positively with cell size in the ad lib.-fed group only. Rates of esterification, both basal and epinephrine-stimulated, correlated positively with cell size in the ad lib.-fed group but negatively in the caloric-restricted group. These findings indicate that nutritional factors can modify any possible influence of adipose cell size on lipolysis and esterification. On the other hand, in both groups of rats, epinephrine- and theophylline-stimulated rates of lipolysis correlated positively with the basal rates of lipolysis. Also, rates of esterification in the presence of epinephrine correlated positively with the basal rates of esterification, suggesting that stimulated rates of lipolysis and esterification are at least partly determined by the basal rates regardless of nutritional status. The activity of glycerokinase measured in homogenates of isolated fat cells, if applicable to intact fat cells, was sufficient to cause considerable underestimations of the basal rates of lipolysis (using glycerol production as an index). When lipolysis was stimulated, the potential errors of estimating lipolysis by glycerol production alone were negligible.

Supplementary key words epinephrine · theophylline · epididymal fat pad · glycerokinase

A PRECISE EVALUATION of the influence of the size of the adipose cell on its metabolic function is difficult to obtain because of other influencing factors such as hormonal environment, age, and nutritional state. Positive correlations of rates of basal lipolysis with cell size have been demonstrated in mice (1), rats (2, 3), and humans (4). However, there is conflicting evidence in the literature concerning the relationship of stimulated rates of lipolysis to cell size (2–4). The rate of conversion of glucose to glyceride-glycerol has been shown to be a direct function of cell size in isolated fat cells from rats, guinea pigs, hamsters (5), and humans (4). Few studies have considered the simultaneous effects of other factors such as age and nutrition on the cell size relationships. Salans and Dougherty (6) have described the dependence of insulin sensitivity of adipose cells of similar sizes on the nutritional state. Increased rates of basal and epinephrine-stimulated lipolysis (2) and conversion of glucose to glyceride-glycerol (7) have been demonstrated in larger fat cells prepared from a given adipose depot. These latter two studies have demonstrated the causal effect of cell size on adipose tissue metabolism.

The following study was designed to examine the dependence of rates of lipolysis, esterification, and FFA release on age, nutritional state, and adipose cell size. The relationships between rates of lipolysis, esterification, and FFA release measured in the basal state and in the presence of epinephrine and theophylline were also investigated. Simultaneous estimations were made of rates of lipolysis, esterification, and FFA release in isolated fat cells prepared from two groups of rats growing at different rates and having different adipose cell sizes and nutritional states.

We have also attempted to determine the relevance of glycerokinase activity (8, 9) to estimations of rates of lipolysis and esterification when using the "balance" method of Vaughan and Steinberg (10).

MATERIALS

$^{63}NiCl_2$ and [2-^{3}H]glycerol were obtained from the Radiochemical Centre, Amersham, England. α-[2-^{3}H]-

Abbreviations: FFA, free fatty acids.

Glycerophosphate was prepared by incubating purified glycerokinase (Boehringer, Mannheim, Germany) with [2-^{3}H]glycerol using the incubation conditions described by Robinson and Newsholme (11). The incubation mixture was applied to an AG-3 (H^+) column (1.5 × 5 cm) and the labeled glycerol was eluted with water; the bound α-glycerophosphate was eluted with 1 M NH_4OH and the solvent was removed by lyophilization.

Collagenase, type B, was obtained from Worthington Biochemical Corp., Freehold, N.J. Theophylline and epinephrine hydrogen lactate were obtained from British Drug Houses Ltd., Poole, England. Bovine serum albumin (fraction V) was supplied by the Armour Pharmaceutical Co., Eastbourne, England. Rat chow was supplied by Drug Houses of Australia, Sydney, Australia.

METHODS

Source of adipose tissue

In order to obtain two groups of rats with different adipose cell sizes at the same age, 64 male Wistar rats of the John Curtin School strain were randomly divided into two equal groups at the age of 4 wk. The rats in one group were fed regular laboratory chow ad lib. and were observed to feed from approximately 6 p.m. to 6 a.m. The second group was placed on a restricted caloric intake (7 g chow/100 g body wt/day) which was just sufficient to maintain a state of positive caloric balance. These animals were observed to consume all their daily ration within 3.5 hr and could thus be classified as "meal feeders." Eight rats from each group were randomly selected at the ages of 10, 12, 19, and 27 wk, fasted for 16 hr in order to induce measurable rates of basal lipolysis, and killed by cervical dislocation. The fat pads were immediately excised, washed in warm (37°C) isotonic saline, and weighed. It is not known if the 16-hr fasting period affects the metabolism of both groups of rats similarly.

Incubation conditions

Krebs-Henseleit buffer (12) containing half the suggested concentration of Ca^{2+}, 5.55 mM glucose, and 40 mg/ml albumin was employed as the incubation medium. The pH was adjusted to 7.4 under an atmosphere of 95% O_2–5% CO_2 with saturated sodium bicarbonate. Incubations were performed in rubber-capped siliconized flasks at 37°C in a shaking water bath set at 90 cycles/min. The gas phase was 95% O_2–5% CO_2. At no time during incubations did the concentration of FFA rise sufficiently to saturate the available binding sites on the albumin.

Preparation of isolated fat cells

2 g of fat pad from each rat was incubated for 1 hr in 8 ml of buffer containing 16 mg of collagenase. After incubation, the cells were washed three times with buffer by flotation, concentrated, and then dispersed into 10 ml of the buffer.

Determination of rates of lipolysis, esterification, and FFA release (or uptake)

1-ml aliquots of cell suspension were dispersed into incubation flasks containing 7 ml of buffer. Where appropriate, the incubation medium contained epinephrine hydrogen tartrate (0.4 μM) or theophylline (5 mM). These concentrations were selected to produce maximal glycerol production (13). Aliquots of the incubation mixture were taken at zero time and 2 hr later for (*a*) deproteinization using 15% $HClO_4$ and subsequent enzymatic determination of glycerol concentration (14) and (*b*) extraction of FFA with isopropanol–heptane–1 N H_2SO_4 40:10:1 (v/v/v) using the double wash procedure described by Dole and Meinertz (15). A double wash extraction was found necessary to eliminate factors that interfered with the determination of FFA concentration. These factors were possibly pyruvate, lactate, or short-chain fatty acids. FFA was estimated as the ^{63}Ni complex.[1] An aliquot of the upper phase of the extraction system containing the FFA was evaporated to dryness under a stream of nitrogen. The FFA was redissolved in chloroform and mixed with an aqueous solution of $^{63}NiCl_2$ (sp act 10 μCi/mg in 1 ml) in 1 M triethanolamine. During the mixing period, the FFA complexed with nickel to form nickel soaps of fatty acid. The chloroform phase (containing the soaps) was separated from the aqueous phase (containing noncomplexed nickel). The chloroform was evaporated and the nickel soaps were counted in a toluene scintillator, 0.4% 2,5-diphenyloxazole and 0.05% 1,4-bis[2-(4-methyl-5-phenoxazolyl)]benzene. The radioactivity was proportional to the molar concentration of long-chain fatty acids, as shown by the routine inclusion of internal standards.[1]

The rate of FFA release was computed from the differences in FFA concentration at zero and 2-hr incubation times. A positive value indicated a net release of FFA into the incubation medium and, conversely, a negative value indicated a net uptake of FFA from the incubation medium. The rate of lipolysis was derived from the differences in glycerol concentration at the zero and 2-hr incubation times. The rate of esterification of FFA was computed from the rates of glycerol and FFA release (10). With this technique it is assumed that 1 mole of glycerol produced represents the production of 3 moles of FFA and that the difference between this value (glycerol × 3) and the observed release of FFA represents the reesterification of FFA (10). Glycerol production (lipolysis) has therefore been expressed as its equivalent in FFA, i.e., moles of glycerol × 3.

[1] Simpson-Morgan, M. Personal communications.

Determination of adipose cell size and number

Adipose cell diameter was measured using the technique described by McLeod, Goldrick, and Whyte (16). Briefly, isolated fat cells were photographed in the plane of maximal diameter using a Zeiss photomicroscopy system. The negative obtained was examined on a Zeiss particle size analyzer TGZ 3 to obtain a frequency distribution of cell diameters. Metabolic data were expressed per unit number of fat cells. The number of fat cells per incubation flask was determined by dividing the total lipid content of the flask by the average lipid content per cell. Total lipid content was assayed on the upper phase of the FFA extraction system (17). Average lipid content per cell was derived from the mean and variance of the diameter (18) and was expressed as μmoles of triolein.

Statistical analyses

The frequency distribution of rates of lipolysis, esterification, and FFA release were positively skewed and so were normalized by logarithmic transformation. To simplify the analysis, all values, prior to logarithmic transformation, were made greater than 1. For values of lipolysis and esterification the addition of 1 was necessary, but for values of FFA release where many values were negative (FFA uptake) the addition of 30 was found necessary. It should be noted that for zero values of lipolysis, esterification, and FFA release, after logarithmic transformation, the values would be 0, 0, and 1.477, respectively.

The significance of differences was determined using Student's t test. Correlations, partial correlations, and comparison of regression line slopes were performed as described by Bailey (19). Bars representing ± SEM are illustrated in Fig. 1 where they exceed the size of the symbol or where purposes of illustration allow.

Glycerokinase assay

Glycerokinase activity was assayed in homogenates of adipose cells from ad lib.-fed rats, aged 6 and 34 wk. Prior to homogenization, the isolated fat cells were incubated as described for estimations of rates of lipolysis, esterification, and FFA release, but for 1 hr only. Isolated fat cells from the same preparations were also incubated for 2 hr for estimations of lipolysis and esterification. After the 1-hr preincubation the cells were concentrated by flotation, washed with buffer, and homogenized at 2°C in an equal volume of 1 mM EDTA in 1% KCl using a Teflon–glass homogenizer. Assays were performed using the incubation conditions described by Robinson and Newsholme (11), with [2-^{3}H]glycerol as the substrate. Control assays were routinely included; in these assays all components except the adipose homogenate were included. The method was modified by separating glycerol from α-glycerophosphate by thin-layer chromatography on silica gel G, using n-propanol–ethyl acetate–water 23:1:2 (v/v/v) as the solvent system. With this system, glycerol had an R_F of 0.7 while α-glycerophosphate remained at the origin. 20-μl aliquots of the glycerokinase assay medium were spotted on the plates. After development of the chromatogram, the silica gel at the origin was scraped into a counting vial and counted in 15 ml of toluene–Triton (20) scintillator plus 1 ml of water. The counting efficiency was 17%. Control assays gave zero activities at all times.

Validation of glycerol/α-glycerophosphate separation

[2-^{3}H]Glycerol and [2-^{3}H]glycerophosphate were chromatographed as described above. Radioautographs were used to locate the position of glycerol and α-glycerophosphate on the chromatograms. Excellent reproducibility of separation was achieved using amounts similar to those of the experimental assay system. This separation system, when used for large numbers of estimations, was found to be faster and more reproducible than the separation system described by Robinson and Newsholme (11). Total lipid content of the cell homogenate was determined and the glycerokinase activity was expressed per unit number of adipose cells.

RESULTS

The epididymal fat pads were obtained from rats in the growing phase. Weights of the fat pads were direct functions of the body weights (ad lib., $r = 0.774$, $P < 0.001$; restricted, $r = 0.854$, $P < 0.001$).

In the ad lib.-fed group, the weights of the epididymal fat pads (Fig. 1*A*) increased between the ages of 10 and 12 wk ($P < 0.001$), after which there was no further enlargement of this fat depot. In rats fed a restricted amount of chow, the rate of growth of the epididymal fat pads (Fig. 1*A*) was retarded such that at 10, 12, 19, and 27 wk all values were significantly lower ($P < 0.001$) than those of the ad lib.-fed group. Nevertheless, the most rapid rate of growth of the epididymal fat pads occurred between the ages of 10 and 12 wk ($P < 0.01$), with a slower but significant increase in weight between 12 and 19 wk ($P < 0.01$). There was no evidence of growth of the epididymal fat pads in the restricted group after the age of 19 wk. In both groups of rats, the enlargement of the epididymal fat cells paralleled the changes in weight of the epididymal fat pads, and thus the diameters of the fat cells were closely related to the weight of the fat pads in individual rats (ad lib., $r = 0.809$, $P < 0.001$; restricted, $r = 0.811$, $P < 0.001$).

The rates of lipolysis, esterification, and FFA release at different ages are illustrated in Fig. 1, *B*, *C*, and *D*, respectively, for both groups of rats. The correlation coefficients for the basal rates of lipolysis, esterification,

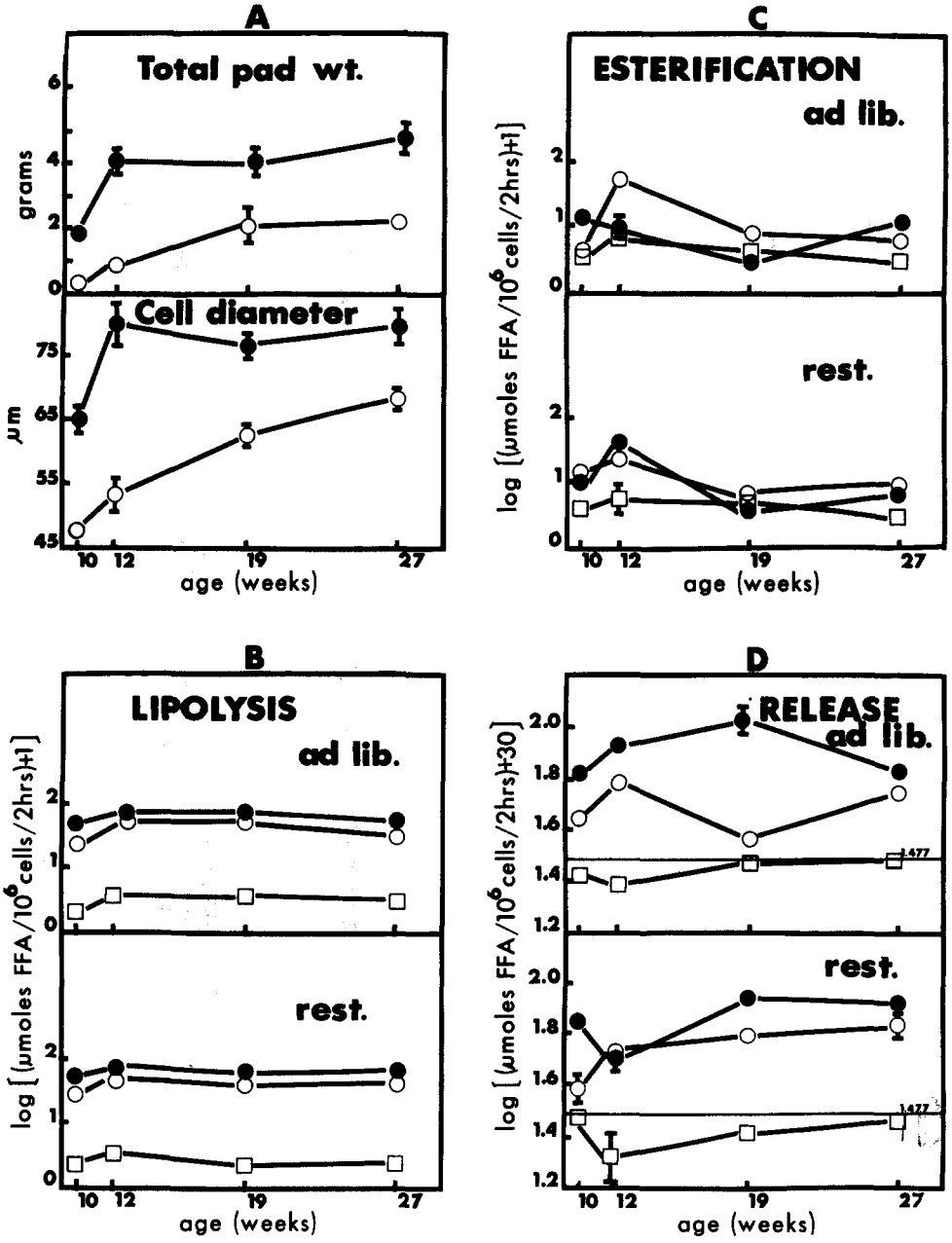

FIG. 1. *A*, morphological data obtained on the epididymal fat pads of the ad lib.-fed (●) and calorie-restricted (○) rats during growth. Each point represents the mean ± SE of eight observations. *B*, *C*, and *D*, rates of lipolysis, esterification, and FFA release measured in isolated epididymal fat cells incubated in the absence (□) or presence of epinephrine (○) or theophylline (●). Each point represents the mean ± SE of values obtained from eight rats fed ad lib. or fed a restricted (rest.) amount of chow. Although originally derived as μmoles glycerol, rates of lipolysis (*B*) are expressed as μmoles FFA for sake of comparison with rates of esterification and FFA release. In *D*, the line 1.477 ($\log_{10}$ 30) refers to zero FFA uptake or release. Figures greater than 1.477 refer to FFA release and, conversely, figures less than 1.477 refer to FFA uptake.

and FFA release with the values recorded in the presence of epinephrine or theophylline are listed in Table 1. For the ad lib.-fed rats, the rate of basal lipolysis rose between 10 and 12 wk of age ($P < 0.001$) and was unchanged thereafter. The rates of lipolysis in the presence of epinephrine, though 10 to 20 times the basal values, followed closely the basal values. The absolute increments above basal values were unaffected by the age of the rats. Hence, as shown in Table 1, there was a close correlation between the basal and epinephrine-stimulated rates of lipolysis. The rates of lipolysis in the presence of theophylline were similar to those in the pres-

TABLE 1. Correlations between basal metabolic parameters and parameters measured in the presence of epinephrine or theophylline

		Correlation Coefficient	
		Ad Lib.	Restricted
Lipolysis	Basal vs. epinephrine	0.617[a]	0.467[b]
	Basal vs. theophylline	0.348[c]	0.341[c]
Esterification	Basal vs. epinephrine	0.558[a]	0.570[a]
	Basal vs. theophylline	−0.001	0.249
Release	Basal vs. epinephrine	−0.134	0.309
	Basal vs. theophylline	0.171	0.233

32 pairs were used for calculating each correlation coefficient.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

TABLE 2. Correlations of cell size with rates of lipolysis, esterification, and FFA release

	Correlation Coefficient	
	Ad Lib.	Restricted
Lipolysis		
Basal	0.683[a]	−0.289
Epinephrine	0.544[a]	0.033
Theophylline	0.383[b]	0.224
Esterification		
Basal	0.380[b]	−0.364[b]
Epinephrine	0.418[b]	−0.356[b]
Theophylline	0.045	−0.236
Release		
Basal	−0.124	−0.142
Epinephrine	0.155	0.146
Theophylline	0.067	0.229

32 pairs were used for calculating each coefficient.
[a] $P < 0.001$.
[b] $P < 0.05$.

ence of epinephrine. However, because the values obtained from theophylline-stimulated cells were similar at all ages, only a low-order relationship was demonstrable with the rates of basal lipolysis. In the restricted group, the basal and stimulated rates of lipolysis showed no significant change during the period of observation. Epinephrine and theophylline stimulated lipolysis 10–20-fold, as in the ad lib.-fed rats. Likewise, the rates of lipolysis recorded in the presence of epinephrine and theophylline were positively correlated with the basal values. The only difference in rates of lipolysis between the two groups was at 19 wk, when higher rates of basal lipolysis were recorded in the ad lib. group ($P < 0.05$).

In both groups of rats, there were no significant variations in basal rates of esterification with age (Fig. 1*C*). The effects of epinephrine and theophylline upon esterification were not consistent. Thus, the rates of esterification in the presence of epinephrine were elevated above the basal levels, in the ad lib.-fed group, at 12 and 27 wk ($P < 0.001$ and $P < 0.05$, respectively) but not at 10 and 19 wk. In the restricted group, epinephrine stimulated the rate of esterification at 10, 12, and 27 wk (for all values $P < 0.05$) but not at 19 wk. With theophylline, significant stimulation of the rates of esterification in the ad lib.-fed group occurred only at 10 and 27 wk ($P < 0.001$ and $P < 0.001$, respectively) and in the restricted group only at 10 and 12 wk ($P < 0.05$ and $P < 0.01$, respectively). These inconsistent findings may well be partly a reflection of the fact that rates of esterification reflected the combined errors of estimating glycerol and FFA levels. In both groups of rats, the rates of esterification in the presence of epinephrine were directly proportional to the basal rates. However, no such relationship was demonstrable with theophylline in either group of rats.

In the basal state, the rate of esterification approached or exceeded the rate of lipolysis in both groups of rats so that there was a zero or net uptake of FFA from the incubation medium at all ages. In the presence of epinephrine or theophylline, the rate of lipolysis exceeded the rate of esterification, resulting in a net release of FFA. No significant variations with age in basal rates of FFA uptake were demonstrable in either group of rats (Fig. 1*D*). Similar rates of FFA uptake were recorded in both groups except at the age of 19 wk, when higher values ($P < 0.05$) were recorded in the calorie-restricted rats. In this connection, it should be noted that at 19 wk the adipose cells were still enlarging in the calorie-restricted group only. Hence, it is possibly significant that the differences in rates of FFA uptake at 19 wk appeared to be due to differing rates of lipolysis rather than to differences in the rates of esterification.

Correlations of cell size with rates of lipolysis, esterification, and FFA release are shown in Table 2. In the ad lib.-fed rats, but not in the calorie-restricted rats, the rates of basal and stimulated lipolysis were direct functions of adipose cell diameter. Rates of esterification in the presence or absence of epinephrine were directly proportional to the diameter of the adipose cells in the ad lib.-fed rats but were inverse functions of adipose cell diameter in the calorie-restricted group. In neither group of animals was there any relationship between the diameter of the fat cell and the rates of FFA release in the presence or absence of epinephrine or theophylline. Partial correlations of basal and stimulated rates of lipolysis while holding cell size constant illustrated the independence of the relationship of basal with epinephrine-stimulated lipolysis to cell size (ad lib., $r = 0.400$, $P < 0.05$; restricted, $r = 0.498$, $P < 0.01$). No similar result could be demonstrated with theophylline-stimulated lipolysis (ad lib., $r = 0.142$, NS; restricted, $r = 0.312$, NS). Although there was no significant relationship between basal rates of lipolysis and cell size in the calorie-restricted group, it would appear from Fig. 2 that a relationship between cell size and basal rates of

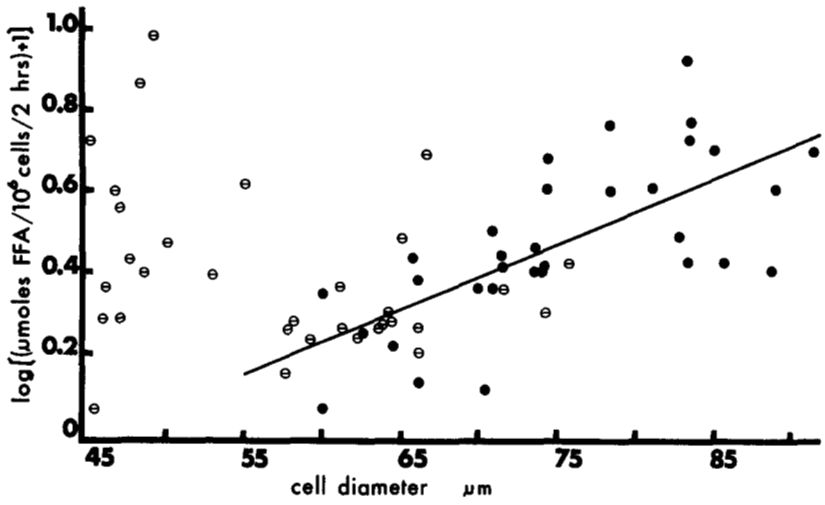

FIG. 2. Plot of individual values obtained for basal rates of lipolysis against cell diameter. Each point represents the value obtained for each animal in the ad lib.-fed group (●) and the calorie-restricted group (⊖). The line defined by the equation for the significant correlation between cell diameter and basal rates of lipolysis in the ad lib.-fed group is shown.

lipolysis, similar to that found for the ad lib.-fed group, could exist for cells having diameters greater than 55 μm. Hartman et al. (3), who also found a relationship between adipose cell size and basal lipolysis, reported that in cells having diameters less than 55 μm there appeared to be little correlation between cell size and basal glycerol release. No similar phenomenon was obvious for the other metabolic parameters recorded.

The activity of glycerokinase measured in homogenates of fat cells prepared from ad lib.-fed rats (Table 3) was similar at the ages of 6 and 34 wk and was apparently unaltered by preincubations of the fat cells with epinephrine or theophylline. On the assumption that the observed activities were representative of those in the intact fat cells, we have corrected the basal and stimulated rates of glycerol production measured in aliquots of parent fat cell suspensions. The data in Table 3 show that in the basal state a significant amount of glycerol released by lipolysis could have been phosphorylated and so would not be estimated in the glycerol assay. On the other hand, when the rate of lipolysis was enhanced with epinephrine or theophylline, the amount of glycerol being phosphorylated was negligible in relation to the rate of glycerol production.

DISCUSSION

Koschinsky, Gries, and Herberg (9) have shown that glycerokinase activity in isolated fat cells from normal mice did not vary with age. The same findings for rats are apparent from our comparisons of glycerokinase activities. The validity of extrapolating values obtained for glycerokinase activity in a homogenate of isolated fat cells to intact fat cells is unknown. However, the values obtained for glycerol phosphorylation in the fat cell homogenates indicated a potentially significant underestimation of rates of lipolysis (based on glycerol production) in isolated fat cells. When glycerol production was stimulated by epinephrine or theophylline, the errors appeared to be negligible. Glycerokinase activity did not appear to be affected by preincubation with epinephrine or theophylline although glycerokinase appears to be inducible by insulin (9). The absence of any significant effect of epinephrine or theophylline on glycerokinase activity could be the result of the small number of observations or of the preincubation period being too short. Although we are reluctant to use the glycerokinase data to determine the true rate of basal lipolysis, the errors involved appeared to be constant for all ages. Thus, relationships established from comparisons of rates of lipolysis and esterification should be valid. The finding that the rates of basal glycerol production were directly related to rates of glycerol production when stimulated with epinephrine or theophylline further suggests that any errors in estimating rates of lipolysis were constant.

Rates of lipolysis were positive functions of adipose cell size in the ad lib.-fed group. As both the stimulated and basal rates correlated with cell size in this group of rats, correlations of basal rates of lipolysis with stimulated rates would be expected. However, in the restricted group, where there was no relationship between rates of lipolysis and cell size, the rates of lipolysis in the presence of epinephrine or theophylline were still direct functions of the basal rates. As the concentrations of epinephrine or theophylline were sufficient to maximally

TABLE 3. Glycerokinase activities in isolated fat cell homogenates and their potential effects on the estimations of rates of lipolysis and esterification in rats 6 and 34 wk old

	Glycerokinase	Lipolysis		Esterification	
		Before Correction	After Correction	Before Correction	After Correction
	μmoles glycerol phosphorylated	*μmoles glycerol released*		*μmoles FFA esterified*	
6 wk[a]	0.139 ± 0.032	0.309 ± 0.087	0.478 ± 0.119	0.416 ± 0.513	0.833 ± 0.609
34 wk[a]	0.158 ± 0.017	0.213 ± 0.057	0.371 ± 0.074	0.666 ± 0.094	1.140 ± 0.145
34 wk[b] (+ lipolytic stimulants)	0.149 ± 0.052	12.414 ± 0.918	12.513 ± 0.970	2.093 ± 0.964	2.540 ± 1.120

The fat cells were preincubated for 1 hr in the presence or absence of epinephrine or theophylline prior to homogenization. Rates of lipolysis and esterification were determined on intact fat cells. Values are means ± SE. All values are expressed per 2 hr per 10^6 cells.

[a] Six observations.

[b] Four observations.

stimulate the rate of lipolysis, the rates of lipolysis in the presence of these agents should reflect a complete activation of the hormone-sensitive lipase. Basal rates of lipolysis should reflect a low level of active lipase which, by equilibrium, would always be a constant fraction of the total (active plus inactive) lipase concentration. Therefore, both stimulated and basal rates of lipolysis would be dependent on the total concentration of hormone-sensitive lipase, and so both parameters would be functions of the same factor. The significant partial correlations of basal lipolysis with epinephrine-stimulated lipolysis while holding cell size constant is further evidence to support this conclusion. The failure to observe similar results with theophylline-stimulated lipolysis was possibly due to the low-order correlations observed in the presence of theophylline.

Although it cannot be demonstrated statistically, it appears probable that adipose cells of the calorie-restricted group having diameters greater than 55 μm correlate positively with basal rates of lipolysis, thus following the same relationship as was observed for cells of the ad lib.-fed group. The mean cell diameters for the ad lib.-fed group were all greater than 55 μm. It is thus possible that if the cells of the calorie-restricted group were in a similar range of diameters to those of the ad lib.-fed group, a relationship between cell size and basal rates of lipolysis, similar to that found for the ad lib.-fed group, would have been observed. The relationships we have found between cell size and basal, epinephrine-stimulated, or theophylline-stimulated rates of lipolysis in the ad lib. group confirm previous reports (1, 2, 4). In these studies, homogeneous groups of animals were used. However, in the present investigation, where two age-matched groups of animals having different feeding patterns and stages of development were studied, it was apparent that the relationship between cell size and rates of lipolysis tended to break down, indicating that cell size is not the sole, nor an over-riding, factor in influencing rates of lipolysis and esterification. On the other hand, it is of interest that the relationship between basal and stimulated rates of lipolysis and esterification were not as severely affected, indicating the close dependence of these parameters on each other.

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