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Effects of starvation, refeeding, and fat feeding on adipocyte ghost adenyl cyclase activity

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Abstract Basal adenyl cyclase activity and its response to epinephrine and glucagon were studied in isolated adipocyte ghosts obtained from fed, starved, refed, and fat-diet-adapted rats. Epinephrine stimulation of adenyl cyclase was significantly increased in fasted rats, but the glucagon response did not change. Rats fasted for 48 hr and refed a high carbohydrate, low fat diet for 48 or 96 hr showed no differences from chow-fed animals in either basal or hormone-stimulated adenyl cyclase activity. Rats adapted to a high fat, low carbohydrate diet showed an initial and transitory increase in basal activity but a progressive loss of epinephrine- and glucagon-stimulated enzyme activities. The loss in hormone responsiveness correlated well with a decrease in hormone-stimulated lipolysis of fat pads and was associated with a significant increase in fat cell diameter.

Supplementary key words fat diet · isolated fat cells · adipocyte ghosts · adenosine-3′-5′-monophosphate · cell size · hormone-stimulated lipolysis

Adenyl cyclase is a plasma membrane-associated enzyme which plays a central role in the stimulation of lipolysis by a variety of hormones (1, 2). There is some evidence that the activity of this enzyme and its sensitivity to hormonal stimulation may be induced to change by nutritional (3) and hormonal (4) manipulation of the animals. Therefore, we elected to study the adaptability of basal and hormone-stimulated adenyl cyclase activity in plasma membrane sacs (ghosts) of isolated rat fat cells in chow-fed, fasted, fasted–refed, and fat-diet-adapted rats.

MATERIALS AND METHODS

Male Holtzman rats initially weighing 150–160 g were used. Basal and hormone-stimulated adenyl cyclase activities were measured in *1*) animals fasted for 48 hr, *2*) animals fasted 48 hr and refed a high carbohydrate diet for 48 or 96 hr, and *3*) animals fed a high fat diet for 1, 3, 5, and 7 days. The compositions of the experimental diets and the control chow diet are shown in Table 1. Since porcine lard has a fatty acid composition comparable to rat epididymal fat pads (5), it was chosen as the primary constituent of the high fat diet. All diets contained calorically similar amounts of protein.

Adenyl cyclase measurement

The rats were decapitated and the fat pads were rapidly removed and placed into 1-oz plastic bottles that contained 3 ml of Krebs-Ringer Tris buffer (pH 7.4) with 4% bovine albumin (Armour Pharmaceutical Co.) and 3 mg/ml collagenase (Worthington, 130–180 units/mg); fat cells and plasma membrane sacs (ghosts) were then prepared according to Rodbell (6). By this method, epididymal fat pads are subjected to collagenase digestion for 1 hr at 37°C in a shaking water bath. After passage through a silk screen, and several washings to remove stromal and vascular tissue, the fat cells are osmotically ruptured in a hypotonic solution. The broken cells are then centrifuged at 900 *g* for 15 min, and the plasma membrane sacs are resuspended in 3 vol of 1.0 mM $KHCO_3$. Adenyl cyclase in ghosts was assayed according to Rodbell (6), with modifications as reported by Pohl, Birnbaumer, and Rodbell (7), from the rate of formation of cyclic 3′-5′-AMP from [^{32}P]ATP. The reac-

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TABLE 1. Diet compositions as percentages of total weight and calories

Diet		Protein[a]	Fat[b]	Carbohydrate		Vitamins[c]	Salts[d]	Ash and Fiber
				Sucrose	Starch			
					%			
Chow	Weight	24.0	5.0		51.9	4.0	4.0	11.1
	Calories	28.0	12.0		60.0			
High carbohydrate	Weight	27.0		60.0		3.8	4.4	4.8
	Calories	32.0		68.0				
High fat	Weight	42.1	43.9			2.8	4.3	6.9
	Calories	30.0	70.0					

[a] Vitamin-free casein (Nutritional Biochemicals).
[b] Porcine lard.
[c] Vitamin supplement (Nutritional Biochemicals).
[d] USP XIV (Nutritional Biochemicals).

tion mixture contained final concentrations of 3.2 mM (Tris)–[^{32}P]ATP (International Chemical and Nuclear Co., specific activity 15–30 cpm/pmole), 5 mM $MgCl_2$, 25 mM Tris–HCl (pH 7.4), 10 mM theophylline, 0.1% serum albumin, and an ATP-regenerating system consisting of 20 mM creatine phosphate and creatine kinase, 1 mg/ml (Sigma, 50 units/mg). Final concentrations of both epinephrine (Parke, Davis) and natural glucagon (Eli Lilly Co.) were 10 μg/ml. Reactions were initiated by the addition of 40–60 μg of membrane protein. The total reaction volume of 0.05 ml was incubated for 10 min, and the reaction was terminated by the addition of 0.10 ml of a solution containing 34 mM sodium dodecyl sulfate, 40 mM ATP, 0.15 μCi of cyclic [^{3}H]3′-5′-AMP (Schwarz/Mann), plus sufficient cyclic 3′-5′-AMP to give a final concentration of 12.5 mM. After boiling for 3.5 min, the reaction volumes were diluted with 0.4 ml of water, and the cyclic 3′-5′-AMP was purified by passage through a Dowex AG 50W-8X column (Calbiochem) and a $BaSO_4$ precipitation step (6). The eluate from the $BaSO_4$ step was suspended in 15 ml of scintillation fluid and counted in a Nuclear-Chicago scintillation system no. 720. Appropriate reaction blanks (without enzyme) were used with all assays. The [^{3}H]3′-5′-AMP was used to calculate the recovery (about 35%) of [^{32}P]3′-5′-AMP from the columns. Cyclase activity was calculated as nmoles of [^{32}P]3′-5′-AMP/10 min/mg of protein, determined by the method of Lowry et al. (8).

Lipolysis was estimated in fat pads according to Bizzi and Carlson (9) by measuring glycerol release into a medium containing 2 ml of Krebs-Ringer bicarbonate buffer with 3% bovine serum albumin and 0.1% glucose, adjusted to pH 7.4. Hormones were added at concentrations of 0.5 μg/ml for epinephrine and 5 μg/ml for glucagon. Glycerol was determined by the method of Lambert and Neish (10), as modified by Korn (11).

Adipocyte diameters were measured by using a micrometer eyepiece (American Optical, no. 1407A). 150 fat cells were measured for each animal, and the mean values from four animals per group are reported.

RESULTS

Diet and adenyl cyclase hormone responsiveness

Rats fasted for 48 hr show a significant increase in epinephrine-stimulated adenyl cyclase activity (Table 2). The basal and glucagon-stimulated specific activities do not appear to be different from those of chow-fed animals (Table 2).

TABLE 2. Adenyl cyclase activity under different nutritional states

Diet	Hormone[a] Treatment	Adenyl[b] Cyclase Activity	*P* Value Compared with Corresponding Response in Chow-fed Controls
Chow	None	0.44 ± 0.07 (7)[c]	
	Epinephrine	1.35 ± 0.02 (7)	
	Glucagon	0.69 ± 0.02 (7)	
48-hr Fast	None	0.42 ± 0.07 (5)	N.S.[d]
	Epinephrine	1.99 ± 0.14 (5)	<0.05
	Glucagon	0.72 ± 0.07 (5)	N.S.
48-hr Fast–48-hr refeed, high carbohydrate	None	0.44 ± 0.08 (3)	N.S.
	Epinephrine	1.26 ± 0.17 (3)	N.S.
	Glucagon	0.66 ± 0.07 (3)	N.S.
48-hr Fast–96-hr refeed, high carbohydrate	None	0.56 ± 0.08 (5)	N.S.
	Epinephrine	1.58 ± 0.13 (5)	N.S.
	Glucagon	0.79 ± 0.05 (5)	N.S.
1-day High fat	None	1.07 ± 0.23 (3)	<0.05
	Epinephrine	1.46 ± 0.12 (3)	N.S.
	Glucagon	0.86 ± 0.16 (3)	N.S.
3-day High fat	None	0.34 ± 0.07 (3)	N.S.
	Epinephrine	0.74 ± 0.05 (3)	<0.05
	Glucagon	0.27 ± 0.04 (3)	<0.05
5-day High fat	None	0.34 ± 0.07 (3)	N.S.
	Epinephrine	0.46 ± 0.06 (3)	<0.05
	Glucagon	0.31 ± 0.08 (3)	<0.05
7-day High fat	None	0.46 ± 0.03 (6)	N.S.
	Epinephrine	0.58 ± 0.04 (6)	<0.05
	Glucagon	0.38 ± 0.03 (6)	<0.05

[a] Hormone concentrations were 10 μg/ml for epinephrine and glucagon.
[b] Means ± SEM of enzyme activity, reported as nmoles of [^{32}P]3′-5′-AMP/10 min/mg of protein.
[c] (n) = number of experiments. Each experiment represents the pooled adipocyte ghosts from three or more rats.
[d] N.S. = not significant ($P > 0.05$).

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When animals are fasted for 48 hr and refed for 48 hr on a high carbohydrate diet, they show no changes in either the basal activity or the epinephrine- or glucagon-stimulated cyclase activities when compared with chow-fed animals (Table 2). If the high carbohydrate diet is fed for 96 hr after a 48-hr fast, the basal activity and the epinephrine- and glucagon-stimulated specific activities are not significantly elevated when compared with chow-fed animals (Table 2).

Maintenance of rats on a high fat diet results in changes in the basal and epinephrine-stimulated, as well as the glucagon-stimulated, adenyl cyclase activities (Table 2). After 24 hr of fat feeding, the basal adenyl cyclase activity is significantly elevated (240% of chow-fed levels), while the glucagon- and epinephrine-stimulated activities parallel the chow-fed controls. After 72 hr of fat feeding, the basal activity has returned to control levels, the glucagon response is completely lost, while the epinephrine response at this time is slightly depressed. By the 5th day, the basal activity remains normal, the glucagon response is still absent, and the epinephrine response has fallen further. Finally, after 7 days, the basal activity remains unchanged, addition of glucagon is without effect, and the epinephrine response remains low (Table 2).

Fat feeding and lipolysis

To relate the loss in hormone responsiveness of adipocyte ghost adenyl cyclase seen in fat-fed animals to whole tissue lipid metabolism, lipolysis was measured in epididymal fat pads from rats after 3 and 7 days on a high fat diet. Decreases in the rate of lipolysis, as measured by glycerol release, in response to epinephrine and glucagon were found in both sets of fat-fed animals, while the basal lipolytic rate was unchanged (Table 3). In fat-fed animals, the decrease in the rate of fat pad lipolysis in response to hormones correlates strongly ($r = 0.98$) with the time course associated with the loss of hormone responsiveness in the adenyl cyclase system of adipocyte ghosts (Fig. 1).

TABLE 3. Epididymal fat pad lipolysis in response to hormones

Diet	Hormone[a] Additions	μmoles of Glycerol/hr/g of Tissue[b]	P Value Compared with Corresponding Response in Chow-fed Controls
Chow	None	2.78 ± 0.44	
	Epinephrine	7.45 ± 1.15	
	Glucagon	4.72 ± 0.30	
3-day High fat	None	2.23 ± 0.22	N.S.[c]
	Epinephrine	4.54 ± 0.30	<0.05
	Glucagon	2.67 ± 0.33	<0.05
7-day High fat	None	2.55 ± 0.22	N.S.
	Epinephrine	3.68 ± 0.24	<0.05
	Glucagon	2.80 ± 0.29	<0.05

[a] Hormone concentrations were 0.5 μg/ml for epinephrine and 5.0 μg/ml for glucagon.
[b] Values are the means ± SEM for six animals.
[c] N.S. = not significant ($P > 0.05$).

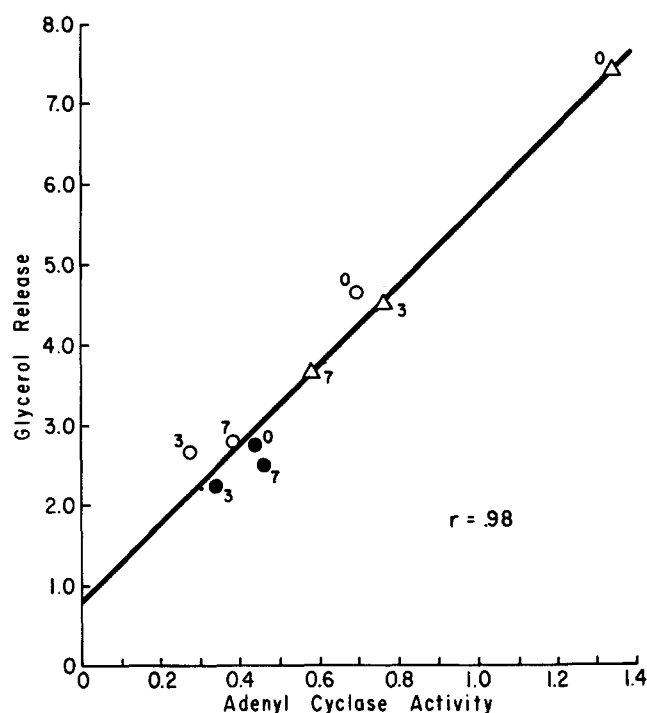

FIG. 1. Regression plot of hormone-stimulated lipolytic activity in epididymal fat pads and hormone-responsive adenyl cyclase activity in fat-fed animals. Glycerol release is expressed as μmoles of glycerol/hr/g of adipose tissue. Adenyl cyclase activity is reported as nmoles of [^{32}P]3′-5′-AMP/10 min/mg of protein. ●, basal activity; ○, glucagon-stimulated; △, epinephrine-stimulated. Numerals represent the duration in days of fat feeding at each point.

Adipose tissue hormone responsiveness and adipocyte diameters

The mechanism responsible for the loss of hormone responsiveness in fat-fed animals is not known. However, large fat cells are less responsive to epinephrine- and ACTH-induced lipolysis (12), and recent evidence indicates that when fat cell lipid exceeds 80% of fat pad wet weight, norepinephrine-induced lipolysis is inhibited (13). Since our 7-day fat-fed animals had fat pads that contained 84% lipid[1] by wet weight, and since increases in lipid parallel increases in cell size (14), the mean cell size in these animals was measured. Mean adipocyte diameter of rats fed a high fat diet for 3 or 7 days was significantly increased when compared with that of chow-fed control animals, but there was no significant increase after 1 day on the high fat diet (Table 4). Fat feeding results in a progressive increase in mean cell size, a 21% increase after 3 days, and a 43% enlargement after 7 days. As one would expect, the mean cell diameter after

[1] Gorman, R. R. Unpublished experiments.

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TABLE 4. Mean rat adipocyte diameter

Diet	Mean Body Wt at Time of Killing	Mean Cell Diameter	*P* Value Compared with Chow-fed Controls
	g	*μm*	
Chow	172.3 ± 4.43	58 ± 0.72	
48-hr Fast	125.5 ± 5.85	42 ± 1.32	<0.05
48-hr Fast–96-hr refeed	175.8 ± 6.63	61 ± 1.11	N.S.[a]
1-day High fat	174.6 ± 3.33	62 ± 0.44	N.S.
3-day High fat	179.4 ± 4.34	70 ± 0.55	<0.05
7-day High fat	176.2 ± 4.84	83 ± 1.20	<0.05

Values are means ± SEM of four rats per group. 150 cells per rat were measured.
[a] N.S. = not significant ($P > 0.05$).

fasting was significantly reduced. Cell size after refeeding of a high carbohydrate diet, where adenyl cyclase response to hormones was unchanged, was not statistically different from that found in chow-fed animals (Table 4).

DISCUSSION

The increase observed in this study in the specific activity of adenyl cyclase in response to epinephrine after fasting is in agreement with the earlier work of Brodie, Krishna, and Hynie (4). This group reported an increase in the amount of activable adenyl cyclase in response to norepinephrine without a change in phosphodiesterase activity. Additionally, these authors indicate that the adenyl cyclase system in vivo, after fasting, is stimulated by a nonadrenergic system, and that the norepinephrine stimulation is actually an expression of an increased catalytic capacity of the adenyl cyclase system. Since glucagon, after fasting, did not activate adenyl cyclase above levels of glucagon stimulation found in chow-fed controls, it appears that glucagon, unlike the catecholamines, is incapable under these conditions of effecting activation of adenyl cyclase to its fullest potential.

When animals are fasted then refed a high carbohydrate diet, the metabolic balance of the adipocyte is tipped in the direction of triglyceride synthesis and storage and away from lipolysis, a condition termed "adaptive hyperlipogenesis" (15). In our hands, fasting followed by refeeding for 48 or 96 hr a high carbohydrate diet had no significant effect on either basal or epinephrine- or glucagon-stimulated adenyl cyclase activities. Preliminary work has been reported that indicates an increase in both basal and epinephrine-stimulated activity of the enzyme under refeeding conditions (3). It is difficult to evaluate the possible physiological significance of increased adenyl cyclase activity upon refeeding. Froesch et al. (16) recently reported that although glycerol release is high in fasted–refed rats, the increased triglyceride lipase activity is dependent on insulin secretion, and is occurring at a time when intracellular cyclic AMP concentrations are supposedly low. Therefore, under these conditions, an increase in adenyl cyclase activity would not seem necessary. In any case, the reason for the discrepancy between our results and those previously reported are not apparent to us.

Our data showing decreased adenyl cyclase activity in response to epinephrine and glucagon are compatible with the reduction in hormone-stimulated lipolysis in epididymal fat pads of fat-fed animals that we describe in this report. These data confirm previously reported diminished lipolytic response to epinephrine in adipose tissue of fat-fed animals (17) and show for the first time that glucagon responsiveness is also reduced.

It is puzzling that 1 day of fat feeding increases basal adenyl cyclase activity but does not change the hormone-stimulated levels. This was the only instance in which basal activity significantly changed from levels in chow-fed animals, and it occurred in three separate experiments. The mechanism responsible for the loss in hormone responsiveness of the adenyl cyclase system after 3 days of fat feeding is not clear, but it is tempting to postulate that the gradual loss of hormone responsiveness of the enzyme is the result of adipocyte enlargement, and not due to the fat diet directly. Although there is disagreement in the literature (18), old rats (400–500 g) appear to lose hormone responsiveness in their adenyl cyclase systems (19) and have very large fat cells (14). It now appears that this loss of hormone responsiveness in these "old rats" is really the result of cellular enlargement, not age (13). Our fat-fed animals, although not old in terms of body weight (180 g), are comparable to old animals when the mean adipocyte diameters are considered. Goldrick and McLoughlin (20) postulated that there could be some critical degree of cellular enlargement associated with a loss in hormone responsiveness in fat tissue, but our data indicate it is probably a gradual loss of activity paralleling the increase in cell size.

If cellular enlargement results in decreased adenyl cyclase activity in response to hormones while the basal enzymatic activity is unchanged, as our data indicate, then it is plausible that the mechanism responsible for the loss in hormone sensitivity is related to either a loss of hormone receptors or to alterations in the relationships between the hormone receptors and the adenyl cyclase macromolecule, rather than to a loss of active enzyme. Further support of this concept is found in preliminary experiments from our laboratory which indicate that the total amount of activable adenyl cyclase, as measured by NaF stimulation, is unchanged in rats regardless of diet.[1]

Although the cell size hypothesis is consistent with the data available, metabolic effects induced by the high fat diet itself could be the cause of decreased hormonal

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responsiveness. Lipids appear to play a critical role in the effective coupling of the glucagon receptor to the catalytic component of the adenyl cyclase system (21), but little is known about the synthesis or control of this union. The high circulating concentrations of fatty acids that occur in fat-fed animals could, in some manner, inhibit or impair normal coupling between the hormone receptor and the catalytic unit of adenyl cyclase, resulting in reduced hormone responsiveness. Recently, palmitate has been reported to inhibit elevations in cyclic AMP in fat cells stimulated by epinephrine, suggesting that fatty acids may have direct effects on the ability of adenyl cyclase to respond to hormones (22). Experiments now in progress were designed to test these alternative hypotheses.

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