

Antilipolytic actions of insulin on basal and hormone-induced lipolysis in rat adipocytes

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Abstract The levels of insulin, free fatty acids (FFA), and triglycerides in rat sera increase with age. The increase in serum FFA levels accompanied the stimulation of basal lipolysis (i.e., lipolysis in the absence of lipolytic agents) in fat cells and enlargement of the diameter of the cells. An overnight fast resulted in a significant increase in basal lipolysis in fat cells from 6- and 8-week-old rats. Although insulin inhibited lipolysis induced by norepinephrine and ACTH at a concentration of 10^{-10} M, it failed to inhibit basal lipolysis even at a concentration of 10^{-6} M. Propranolol, another antilipolytic agent like insulin, also did not affect basal lipolysis. Insulin did not inhibit the accelerated basal lipolysis in enlarged fat cells, fasted fat cells, and sonicated cells. These results indicate that insulin inhibits only the lipolysis induced by lipolytic agents such as norepinephrine and ACTH but not the basal lipolysis found in the absence of lipolytic agents. The possibility that free fatty acids produced by enlarged fat cells initiate insulin resistance and diabetes mellitus, is discussed. —Morimoto, C., T. Tsujita, and H. Okuda. Antilipolytic actions of insulin on basal and hormone-induced lipolysis in rat adipocytes. *J. Lipid Res.* 1998. 38: 957–962.

Supplementary key words insulin • basal lipolysis • fat cells • norepinephrine • propranolol

Plasma FFA levels are affected by fat cell lipolysis which is regulated by hormone-dependent and hormone-independent lipolytic activities. The latter is termed basal lipolysis.

Our previous study demonstrated that basal lipolysis is elevated in the enlarged fat cells of obese rats as a result of a reduction in the surface phosphatidylcholine concentration of endogenous lipid droplets (1, 2). An active hormone-sensitive lipase (HSL) is present in fat cells even in the absence of lipolytic hormones, and phosphatidylcholine on the surface of endogenous lipid droplets causes inhibition of the lipolytic action of HSL. The decrease in surface phosphatidylcholine concentrations in endogenous lipid droplets thus causes the increase in basal lipolysis.

One of the most important metabolic actions of insulin is the inhibition of lipolytic activity in fat cells (3). Activation of the adipocyte cGMP-inhibited cAMP phosphodiesterase by insulin is believed to be the major mechanism whereby insulin reduces cellular cAMP which then leads to inactivation of the cAMP-dependent protein kinase, net

dephosphorylation of HSL and antilipolysis (3–6). This mechanism of insulin-mediated antilipolysis presupposes that HSL activity is reduced.

However, there is no direct evidence as to whether insulin-induced antilipolysis accompanies reduction in HSL activity. Further experiments are therefore needed to clarify the precise mechanism of the antilipolytic action of insulin. Although insulin is known to inhibit hormone-induced lipolytic activity in fat cells, there have been no studies that have examined the effect of insulin on hormone-independent or basal lipolysis. The present investigation was designed to clarify whether insulin inhibits basal lipolysis in rat fat cells.

MATERIALS AND METHODS

Animals

Young male Wistar-King rats, 6, 8 and 10 weeks of age, were given a standard laboratory diet (Oriental Yeast Co. Ltd) and water ad libitum. They were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) and venous blood samples were obtained at 10:00 am; then their epididymal adipose tissues were quickly removed.

Materials

Collagenase (type IV) was purchased from Worthington Biochemical Corp. (Freehold, NJ). Norepinephrine was obtained from Sankyo Co. Ltd. ACTH was from Daiichi Seiyaku Co. Ltd. (Tokyo, Japan). Bovine serum albumin, TES, Glucose B-test wako, NEFA C-test wako, triglyceride E-test wako, cholesterol E-test wako and GLAZYME Insulin-EIA TEST were purchased from Wako Pure Chemical Industries (Osaka, Japan). Bovine serum albumin was extracted by the method of Chen (7) to remove free fatty acids.

Analytical procedure

The levels of serum insulin, glucose, FFA, triglyceride and cholesterol in rats were estimated using commercial kits from Wako Pure Chemical Industries.

Abbreviations: FFA, free fatty acids; HSL, hormone-sensitive lipase; ACTH, adrenocorticotrophic hormone; TES, N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid.

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TABLE 1. Characteristics of non-fasted rats

Age	Body weight	Insulin	Glucose	Free fatty acids	Triglyceride	Cholesterol
weeks	g	mU/l	mg/l	$\mu\text{Eq/l}$	mg/l	mg/l
6	178 \pm 1	31.0 \pm 3.1	2110 \pm 63	131 \pm 16	581 \pm 39	712 \pm 15
8	289 \pm 5 ^a	62.4 \pm 6.5 ^a	1980 \pm 16	209 \pm 14 ^b	1250 \pm 85 ^a	728 \pm 16
10	360 \pm 13 ^a	49.1 \pm 2.1 ^b	2370 \pm 40 ^a	324 \pm 17 ^a	1290 \pm 130 ^a	697 \pm 50

Values are means \pm SE of four separate experiments.
^a $P < 0.01$ and ^b $P < 0.05$ vs. value in rats at 6 weeks.

Measurement of lipolytic activity in fat cells

Isolated fat cells were obtained from rat epididymal adipose tissue by the method of Rodbell (8). The fat cells (50 μl packed volume) were incubated for 1 h at 37°C in 250 μl of buffer A (25 mm TES, pH 7.4, containing 135 mm NaCl, 5 mM KCl and 1 mM MgCl_2) supplemented with 2.5% bovine serum albumin and the test samples. Then 3 ml of chloroform–heptane 1:1 (v/v) containing 2% (v/v) methanol was added and any FFA released were measured with copper reagent and bathocuproine as described by Okuda, Tsujita, and Kinutani (9).

Estimation of the diameter of fat cells

The isolated fat cells were mixed with buffer A containing 2% osmium chloride. The mixture stood for 3 h at 4°C and was then subjected to scanning electron microscopy (Hitachi H-500).

The diameters of the fat cells were estimated from the electron micrographs with an image analyzer.

Analysis of data

Statistical analysis was performed by one-way ANOVA and multiple comparisons were tested with Scheffe's method.

RESULTS

The levels of insulin, FFA, and triglyceride in the non-fasted rat sera increased with age as shown in **Table 1**. It is likely that the increase in FFA caused a rise in triglyceride levels through an elevation in VLDL formation in the

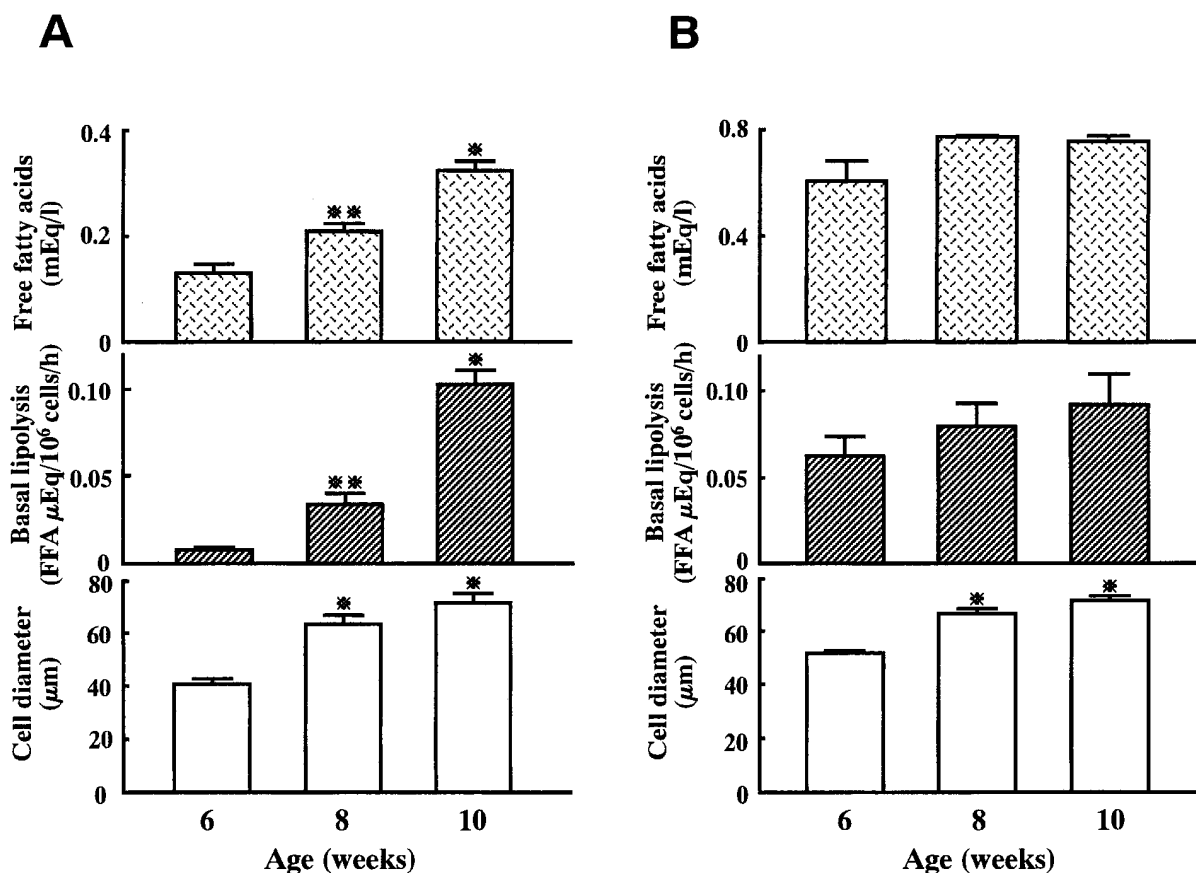


Fig. 1. Basal lipolysis and serum free fatty acids in the non-fasted (A) and the overnight fasted (B) rats. Basal lipolysis was estimated by measuring FFA released from fat cells in the absence of lipolytic hormones. * $P < 0.01$, ** $P < 0.05$ vs. value in rats at 6 weeks. Each column represents the means \pm SE of four separate assays.

TABLE 2. Characteristics of overnight fasted rats

Age weeks	Body weight g	Insulin mU/l	Glucose mg/l	Free		
				Fatty Acids $\mu\text{Eq/l}$	Triglyceride mg/l	Cholesterol mg/l
6	173 \pm 2	9.7 \pm 1.1	742 \pm 79	606 \pm 78	764 \pm 136	746 \pm 35
8	273 \pm 6 ^a	17.0 \pm 3.9	773 \pm 68	775 \pm 4	594 \pm 60	668 \pm 45
10	349 \pm 3 ^a	17.1 \pm 3.1	941 \pm 16	758 \pm 20	654 \pm 45	680 \pm 33

Values are means \pm SE of four separate experiments.
^a $P < 0.01$ vs. value in rats at 6 weeks.

liver. The increase in serum FFA levels accompanied stimulation of basal lipolysis (lipolysis in the absence of lipolytic agents) in fat cells and enlargement of the diameter of the cells (Fig. 1A). The levels of serum FFA increased and the levels of serum glucose and insulin decreased with overnight fasting as compared with non-fasted rats (Table 2). The increase in serum FFA levels accompanied stimulation of basal lipolysis in fat cells by overnight fasting; for rats 6 weeks of age, fasting resulted in a marked rise of serum FFA levels (4.6-fold) and basal lipolysis (7.8-fold) (Fig. 1B). Taken together, it seems likely that the rise in serum FFA levels is due to stimulation of basal lipolysis in fat cells.

Figure 2 shows antilipolytic actions of insulin on lipolysis in fat cells from overnight fasted rats. Although insulin inhibited lipolysis elicited by norepinephrine and ACTH at a concentration of 10^{-10} M, it failed to inhibit basal li-

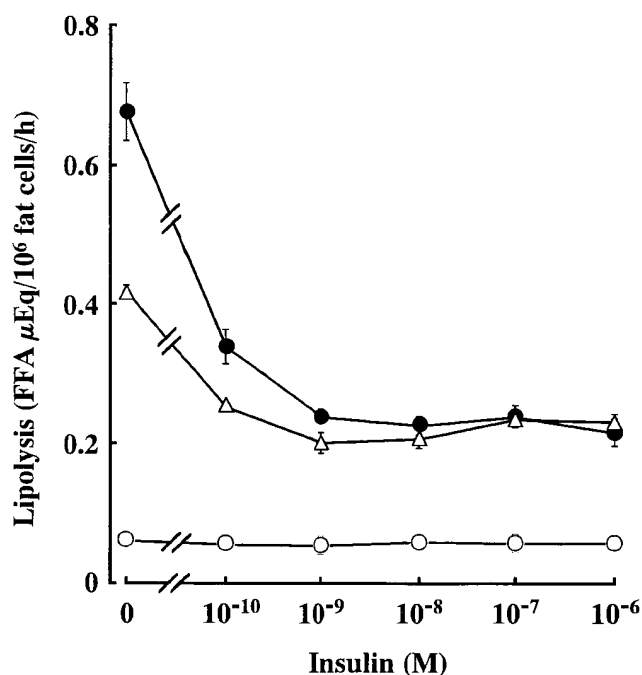


Fig. 2. Antilipolytic actions of insulin on norepinephrine- and ACTH-induced lipolysis in fat cells. After an overnight fast, fat cells isolated from rats 6 weeks of age were incubated at 37°C for 1 h with norepinephrine (0.1 $\mu\text{g/ml}$ (●)) and ACTH (0.1 $\mu\text{g/ml}$ (Δ)) or without lipolytic hormone (○) in the presence of the indicated amount of insulin. Each point represents the means \pm SE of four separate assays.

polysis in fat cells isolated from rats who were 6 weeks old even at a concentration of 10^{-6} M. Similar results were observed in fat cells isolated from 6-week-old non-fasted rats (data not shown). Figure 3 shows the effect of time on basal lipolysis in fat cells from overnight fasted rats. The accumulation of released FFA increased time-dependently for at least 1 h. Insulin did not inhibit the accelerated basal lipolysis in enlarged fat cells isolated from non-fasted rats who were 8 and 10 weeks of age (Fig. 4A). Insulin also did not inhibit the accelerated basal lipolysis in fat cells isolated from overnight fasted rats (Fig. 4B).

In 1994, we first reported that sonication of fat cells elicited marked lipolysis in the absence of epinephrine (2). In other words, the sonication of fat cells caused a marked increase in basal lipolysis. As shown in Fig. 5, insulin also failed to inhibit the accelerated basal lipolysis in sonicated cells. These results clearly indicate that insulin only inhibited the lipolysis induced by lipolytic agents such as norepinephrine and ACTH but did not affect the basal lipolysis found in the absence of lipolytic agents.

It is well known that the β -blocker, propranolol, inhibits lipolysis induced by norepinephrine, whereas the α -blocker phenoxybenzamine does not inhibit lipolysis. Figure 6 shows the effect of β -blocker on lipolysis in fat cells isolated from overnight fasted rats. Propranolol completely inhibited the lipolysis elicited by norepinephrine at a concentration of 10^{-5} M whereas it failed to inhibit basal lipolysis at a concentration of 10^{-4} M. Similar results were observed in fat cells isolated from 6-week-old non-fasted rats (data not shown). Propranolol also failed to inhibit the basal lipolysis accelerated by sonication of the cells (data not shown).

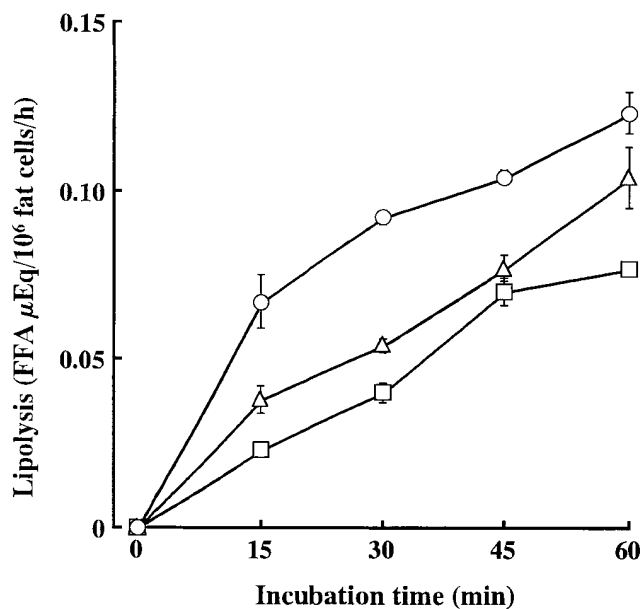


Fig. 3. Effect of time on basal lipolysis in fat cells isolated from the overnight fasted rats. Basal lipolysis (lipolysis in the absence of lipolytic agents) was examined using fat cells isolated from rats of 6 (□), 8 (Δ) and 10 weeks (○) of age. Each point represents the means \pm SE of four separate assays.

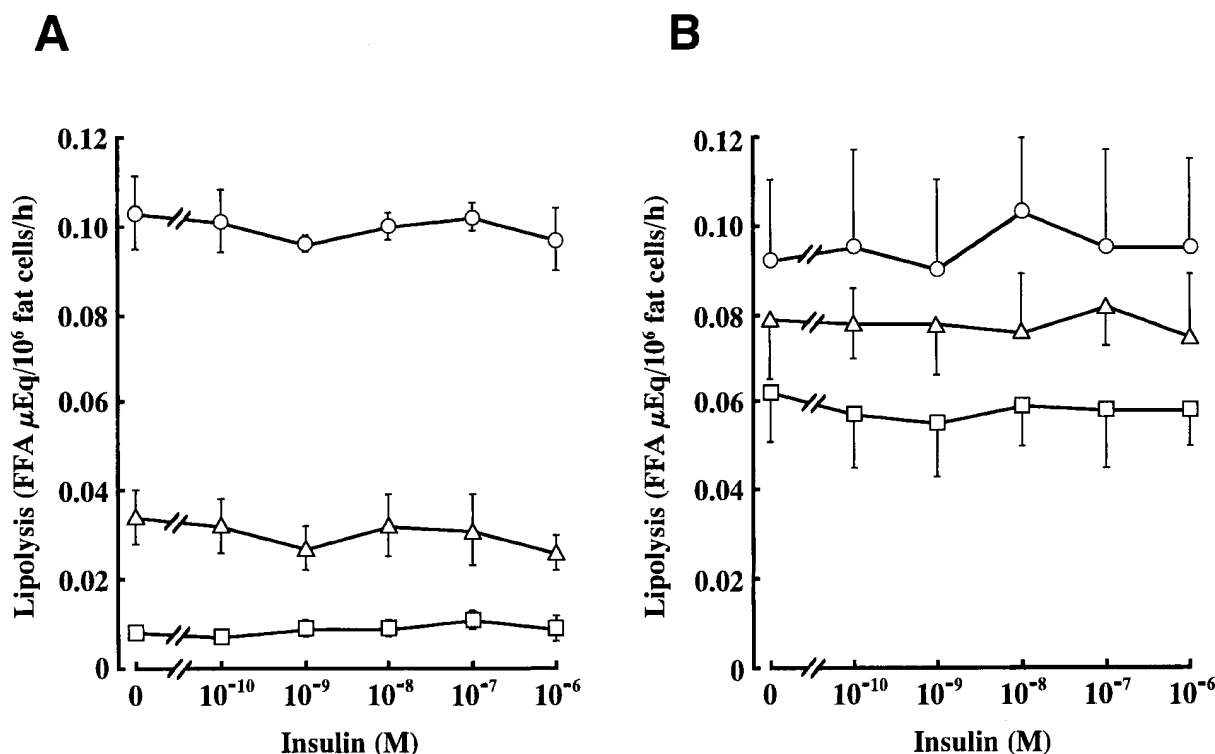


Fig. 4. Effect of insulin on basal lipolysis in fat cells isolated from the non-fasted (A) and the overnight fasted (B) rats. Basal lipolysis (lipolysis in the absence of lipolytic agents) was examined using fat cells isolated from rats of 6 (\square), 8 (\triangle), and 10 weeks (\circ) of age, respectively, in the presence and absence of insulin. Each point represents the means \pm SE of four separate assays.

DISCUSSION

Although insulin inhibited lipolysis induced by norepinephrine or ACTH in fat cells, it did not affect basal lipolysis in these cells (Figs. 2 and 4). Propranolol, another antilipolytic agent, also failed to inhibit basal lipolysis (Fig. 6). It has been reported that an enlargement in fat cell size causes an increase in basal lipolysis (1). Aging caused an enlargement in fat cell size and an increase in basal lipolysis, which possibly induced elevation in serum FFA levels (Table 1 and Fig. 1). It has been suggested that elevation in basal lipolysis in enlarged fat cells is induced by the reduction in the surface phosphatidylcholine concentration of the enlarged lipid droplets in these cells (1). Surface phosphatidylcholine is known to inhibit the lipolytic reaction between lipid droplets and HSL (1, 2).

It is well known that fasting results in a decrease in serum concentration of insulin and glucose, and a marked rise in FFA. In our experiment, the serum glucose and insulin levels decreased and the serum FFA levels increased with overnight fasting (Tables 1 and 2). The increased FFA levels were accompanied by the accelerated basal lipolysis (Fig. 1). However, in rats at 10 weeks of age, the levels of basal lipolysis did not increase with overnight fasting (Fig. 1). Insulin may play a pivotal role in the hormonal control of metabolic adaptation of fasting. The known ability of insulin to inhibit the lipolysis suggests that it should have an important role in controlling the serum FFA levels: fasting causes a decrease in serum insulin lev-

els, which induces lipolysis, and results in an increase in serum FFA levels. However, the accelerated basal lipolysis induced by fasting was not affected by insulin (Fig. 4). Further experiments are needed to clarify the mechanism of acceleration of basal lipolysis by fasting.

Sonication of fat cells is known to increase basal lipolysis (1, 2). Sonication of fat cells decreased the average diameter ($56.6 \pm 0.7 \mu\text{m}$) of the lipid droplets to $4.6 \pm 0.2 \mu\text{m}$, with an increase in the droplet number. The increase in the surface area of the droplets was directly proportional to the ratio of the average diameter of the intact to the sonicated lipid droplets. The average surface area of the sonicated lipid droplets was calculated to be about 12 times that of intact lipid droplets. Thus, the concentration of phosphatidylcholine on the surface of the lipid droplets was reduced by about 12-fold by sonication. The reduction in phosphatidylcholine concentration accelerated the association between the sonicated lipid droplets and HSL, resulting in elevated basal lipolysis (2).

The present study clearly shows that insulin fails to inhibit the elevated basal lipolysis found in sonicated fat cells (Fig. 5). Taken together, the results show that insulin inhibits lipolysis in the presence of lipolytic agents such as norepinephrine and ACTH, but does not affect lipolysis in the absence of these agents (basal lipolysis). Norepinephrine- and ACTH-induced lipolysis was not completely inhibited by insulin, whereas it was completely inhibited by β -blocker (Figs. 2 and 6). Some proportion of hormone-stimulated lipolysis was "insulin-

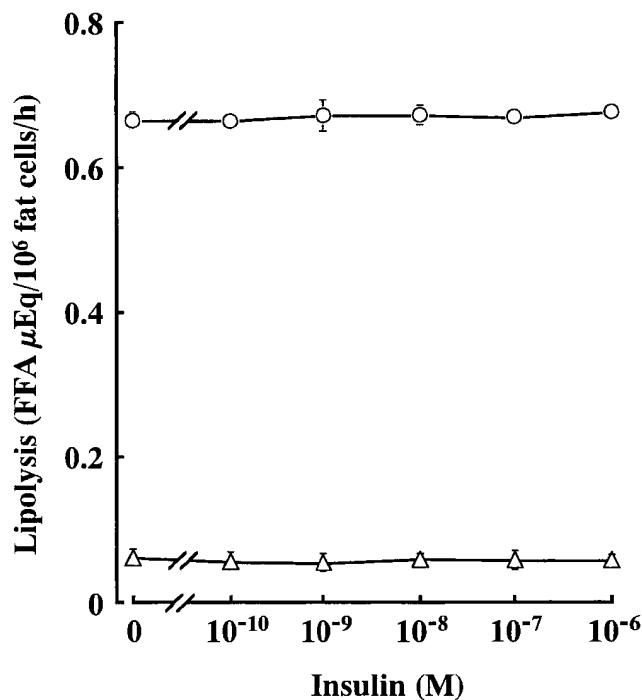


Fig. 5. Effect of insulin on basal lipolysis in sonicated fat cells. After an overnight fast, fat cells were isolated from rats 6 weeks of age. The resulting fat cell fraction was divided into two portions. One portion (50 μ l packed volume) (Δ) was incubated for 1 h at 37°C in 225 μ l of buffer A supplemented with 2.5% bovine serum albumin and 25 μ l of insulin solution in buffer A. The other portion (\circ) was suspended in 100 μ l of buffer A per 50 μ l packed volume of the cells supplemented with 2.5% bovine serum albumin and 2.26 mg gum arabic. After sonication of the mixture for 5 min, 125 μ l of buffer A containing 2.5% albumin was added. The mixture was incubated for 1 h at 37°C with 25 μ l of insulin solution in buffer A, and then the free fatty acids released were measured. Each point represents the means \pm SE of four separate assays.

resistant." Further experiments are needed to clarify this lipolysis.

In general, obesity is often associated with insulin resistance; indeed, circulating FFA levels are chronically elevated roughly in proportion to the degree of obesity (10, 11). Randle et al. (12) initially reported that FFA inhibit glucose utilization in cardiac and diaphragmatic muscles and Garland, Newsholme, and Randle (13) showed that FFA inhibit muscle glucose utilization by decreasing glucose transport and inhibiting glycolysis and pyruvate oxidation. FFA also stimulate gluconeogenesis from lactate, alanine and pyruvate (14). Lee et al. (15) examined the effect of a lipid-heparin infusion on glucose metabolism in ten normal subjects by the euglycemic glucose clamp technique and isotopic determination of glucose turnover to test the hypothesis that an increase in lipolysis is related to insulin resistance. Their conclusion was that artificial induction of intravascular lipolysis by a lipid-heparin infusion led to a state of insulin resistance in humans (15). Based on these results, it seems likely that insulin resistance associated with obesity may be caused by an increase in basal lipolysis. Although the elevation in basal lipolysis

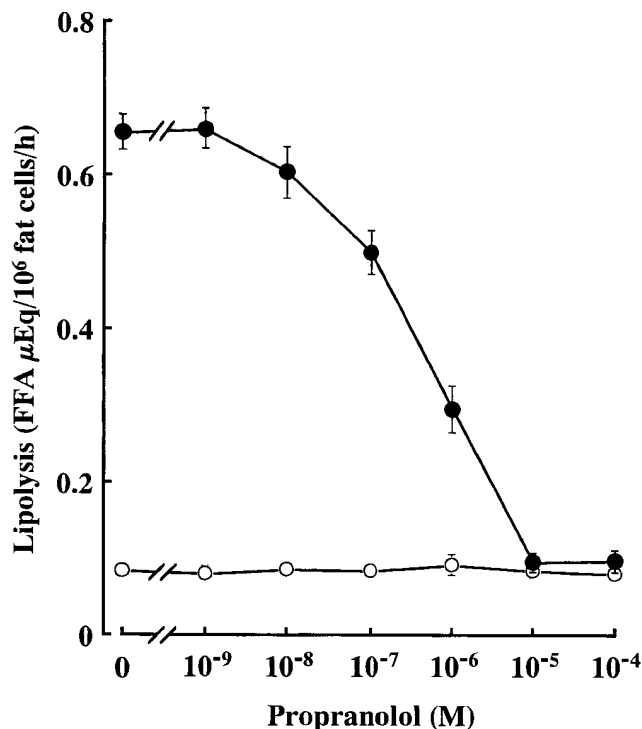


Fig. 6. Effect of propranolol on norepinephrine-induced lipolysis in fat cells. After an overnight fast, fat cells were isolated from rats 6 weeks of age and incubated at 37°C for 1 h with (\bullet) or without (\circ) norepinephrine in the presence of the indicated amount of propranolol. Each point represents the means \pm SE of four separate assays.

induces hyperinsulinemia in humans and rodents (16–18), insulin does not inhibit the lipolysis (Figs. 2 and 4). Milburn et al. (19) indicated that FFA produced by the increase in basal lipolysis induced β -cell hyperplasia and enhanced low K_m glucose metabolism in the islet cells.

Thus, FFA levels elevated by the increase in basal lipolysis cause insulin resistance resulting in compensatory hyperinsulinemia. However, the hyperinsulinemia fails to reduce FFA levels produced by the increase in basal lipolysis. In summary, therefore, it seems probable that the increase in basal lipolysis induced by the enlargement of lipid droplets in fat cells may initiate insulin resistance and result in diabetes mellitus. Further experiments are needed to prove this hypothesis. \square

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