Role of endogenous lipid droplets in lipolysis in rat adipocytes

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Abstract Sonication of rat fat cells caused an increase in lipolysis in the absence of lipolytic stimulants (basal lipolysis) and loss of epinephrine responsiveness. Sonication of endogenous lipid droplets from fat cells also induced an increase in lipolysis in the presence of hormone-sensitive lipase (HSL) and loss of epinephrine responsiveness in a cell-free system consisting of lipid droplets and HSL. This increase in lipolysis was found not to be due to an increase in the surface area resulting from sonication, but seemed to be due to a decrease in the phosphatidylcholine concentration on the surface of the lipid droplets. Addition of phosphatidylcholine to the sonicated lipid droplets reduced the hydrolysis of triglyceride by HSL in the cell-free system, whereas addition of phospholipase C to a cell-free system consisting of HSL and intact lipid droplets or a lipid emulsion containing phosphatidylcholine increased lipolysis. These results suggest that phosphatidylcholine on the surface of the lipid droplets may be a regulatory factor for lipolysis in fat cells.—**Okuda, H., C. Morimoto, and T. Tsujita.** Role of endogenous lipid droplets in lipolysis in rat adipocytes. *J. Lipid Res.* 1994. **35:** 36–44.

Supplementary key words fat cells • intact lipid droplets • sonicated lipid droplets • surface area

The rate-limiting step in lipolysis in fat cells, hydrolysis of triacylglycerol, is catalyzed by hormone-sensitive lipase (HSL) (1). Belfrage et al. (1) and Holm, Fredrikson, and Belfrage (2) purified HSL from rat adipose tissue and demonstrated that it exhibits the same amphiphilicity as typical intrinsic membrane proteins. Huttunen, Aquino, and Steinberg (3) suggested that the pure HSL is associated with a high-molecular weight lipoprotein particle. A cDNA from a rat library encoding the HSL polypeptide has recently been cloned and sequenced, and the primary structure of HSL of 757 amino acids has been deduced (4). The activity of rat HSL is believed to be controlled by cyclic AMP-dependent phosphorylation of a single serine residue (Ser-563) in the regulatory phosphorylation site (4, 5). HSL is activated by its phosphorylation by cyclic AMP-dependent protein kinase, and inactivated by its dephosphorylation by protein phosphatases (5). There have been many studies on the characteristics of HSL, but no attention has been paid to the role of endogenous lipid droplets of fat cells in the HSL-mediated lipolytic reaction except for a few reports (6–8).

In most studies on HSL, artificial lipid emulsions, such as triolein emulsified with gum arabic, have been used as substrates, although the physiological substrate of HSL is endogenous lipid droplets in fat cells. In general, lipases show differences in specificities that are related to the physico-chemical natures, rather than the chemical configurations of their substrates.

Tsujita, Muderkwa, and Brockman (9) examined the hydrolysis of 1,3-dioleoylglycerol in mixed lipid films of the substrate and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine by human pancreatic carboxylester lipase, porcine pancreatic carboxylester lipase, and human milk bile salt-stimulated lipase. They found that the percentage of 1,3-dioleoyl-glycerol hydrolyzed increased abruptly from almost zero to nearly 100% with an increase in the proportion of substrate in the films. In their experiments, the lipase concentration exposed to the interface of the films remained constant, and the phospholipid in the films was not hydrolyzed.

Previously, we isolated endogenous lipid droplets from rat fat cells and analyzed their constituents (8, 10). We found that the main component was triglyceride, with phospholipid, cholesterol, carbohydrate, and protein as minor constituents, suggesting that these minor constituents might be localized on the surface of the lipid droplets. Therefore, it seems likely that the physicochemical nature of the surface of endogenous lipid droplets is quite different from that in artificial lipid emulsions.

In the present study, we have focused on the phospholipid contents of the lipid droplets and have discussed

Abbreviations: HSL, hormone-sensitive lipase; TG, triglyceride; TES, N-Tris(hydroxymethyl)methyl-1-2-aminoethanesulfonic acid; BES, N,N,-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; FFA, free fatty acids.

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the role of endogenous lipid droplets in the HSL-mediated lipolytic reaction.

MATERIALS AND METHODS

Animals

Young male Wistar-King rats, weighing 150 to 200 g, were given a standard laboratory diet and water ad libitum. They were killed by cervical dislocation to minimize endogenous catecholamine secretion, and their epididymal adipose tissues were quickly removed.

Materials

Collagenase (type IV) was purchased from Worthington Biochemical Corp. (Freehold, NJ). Epinephrine was obtained from Daiichi Seiyaku Co. (Tokyo, Japan). TES was from Wako Pure Chemical Industries, (Osaka, Japan). Egg phosphatidylcholine was from Serdary Research Laboratories, (London, Canada). Heparin-Sepharose was from Pharmacia, LKB, Biotechnology (Uppsala, Sweden). Phospholipase C (type 1, from *Clostridium perfringens*) was from Sigma and was purified further by affinity chromatography on agarose-linked egg yolk lipoprotein, followed by gel filtration on Sephadex G-100 (11) before use. The purified enzyme appeared homogeneous on polyacrylamide gel electrophoresis and its specific activity was 210 U/mg protein per min. A crude commercial preparation of phospholipase D (from *Streptomyces chromofuscus;* Boehringer-Mannhein GmbH, Germany) was used. One enzyme unit (U) was defined as the amount required to hydrolyze 1 μmol of lecithin/min. Bovine serum albumin was from Wako Pure Chemical Industries and was extracted by the method of Chen (12) to remove free fatty acid.

Measurement of lipolytic activity in fat cells

Isolated fat cells were obtained from rat epididymal adipose tissues by the method of Rodbell (13). The resultant fat cell fraction was divided into two portions. One portion (50 μl packed volume) was incubated for 1 h at 37°C in 225 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 25 μl of epinephrine solution in buffer A. The other portion was suspended in 125 μl of buffer A per 50 μl 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, 100 μl of buffer A containing 2.5% albumin was added. The mixture was incubated for 1 h at 37°C with 25 μl of epinephrine solution in buffer A, and then the free fatty acids released were measured as described previously (14). Briefly, the incubation mixture (250 μl) was mixed with 3 ml of a 1:1 (v/v) mixture of chloroform and heptane containing 2% (v/v) methanol and extracted by shaking the tube horizontally for 10 min in a shaker. The mixture was centrifuged (2,000 *g*, 5 min), the upper aqueous phase was removed by suction, and 1 ml of copper reagent was added to the lower organic phase. The tube was shaken for 10 min, the mixture was centrifuged (2,000 *g*, 10 min), and 0.5 ml of the upper organic phase, which contained the copper salts of the extracted fatty acids, was treated with 0.5 ml 0.1% (w/v) bathocuproin in chloroform containing 0.05% (w/v) 3-tert-butyl-4-hydroxyanisole. Then its absorbance at 480 nm was measured. Lipolysis was expressed as microequivalents (μEq) of free fatty acids (FFA) released per ml of packed fat cells per h.

Preparation of endogenous lipid droplets

Isolated fat cells were obtained by the method of Rodbell (13), and 1 ml packed volume of cells was suspended in 4 ml of 5 mM Tris-HCl buffer (pH 7.4). The suspension was mixed by slowly inverting the centrifuge tube three times and then centrifuged at 200 *g* for 3 min at room temperature. The fat layer was mixed with 4 ml of 5 mM Tris-HCl buffer (pH 7.4) containing 0.025% Triton X-100 by slowly swinging the tube three times and the mixture was centrifuged at 200 *g* for 3 min at room temperature. The fat layer was washed once with buffer A, incubated with buffer A at 37°C for 10 min, and centrifuged at 200 *g* for 3 min. Then, it was washed with buffer A. Approximately 95% of protein was removed from the fat layer by these procedures. Addition of epinephrine to the fat layer did not stimulate lipolysis in the absence of added lipase (10). The fat layer consisted of (per gram dry weight) 870 μmol triglyceride, 0.71 μmol phospholipid, 0.52 μmol cholesterol, 342 μg carbohydrate, and 63 μg protein. Although the fat layer was a crude preparation, it was used as endogenous lipid droplets in experiments.

Preparation of lipoprotein lipase-free HSL solution

Rat epididymal adipose tissue (0.7 g) was cut into small pieces with scissors and homogenized in 1 ml of buffer A (25 mM TES buffer, pH 7.4, containing 135 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$) in a Potter-Elvehjem homogenizer by five strokes by hand of a Teflon pestle. The homogenate was centrifuged (2,500 *g*, 15 min) at 10°C, and the resultant supernatant was applied to a heparin-Sepharose column (5 × 20 mm), equilibrated with buffer A, to remove lipoprotein lipase. The unadsorbed fraction was used as the HSL solution. HSL activity in this fraction was not reduced by 1 M NaCl or antiserum to bovine lipoprotein lipase, indicating that lipolytic activity due to lipoprotein lipase was minimal in the preparation.

Estimation of diameter of endogenous lipid droplets

Fat cells, sonicated fat cells, lipid droplets, and sonicated lipid droplets were mixed with buffer A containing

2% osmium chloride. The mixtures stood for 3 h at 4°C and were then subjected to scanning electron micrography (Hitachi H-500). The diameters of lipid droplets were estimated from the electron micrographs with an image analyzer.

Lipolysis in a cell-free system consisting of HSL and endogenous lipid droplets

A sample of 25 μl of packed endogenous lipid droplets was incubated at 37°C for 1 h with 100 μl HSL solution, 25 μl epinephrine solution, 100 μl buffer A containing 2.5% bovine serum albumin, and 25 μl buffer A. Another sample of 375 μl of packed endogenous lipid droplets was mixed with 750 μl buffer A containing 2.5% bovine serum albumin and 16.95 mg gum arabic. The mixture was sonicated for 5 min, and the sonicate (75 μl) was incubated with 100 μl HSL solution and 100 μl buffer A containing 2.5% bovine serum albumin for 1 h at 37°C. Lipolysis was expressed as μEq FFA per mg protein of HSL solution.

Analysis of data

Student's *t*-test was used to determine the significance of differences.

RESULTS

As shown in **Fig. 1,** epinephrine induced lipolysis in intact fat cells at concentrations of over 10^{-7} M, but not at 10^{-8} M. No lipolysis in intact fat cells occurred in the absence of epinephrine, but sonication of the intact fat cells

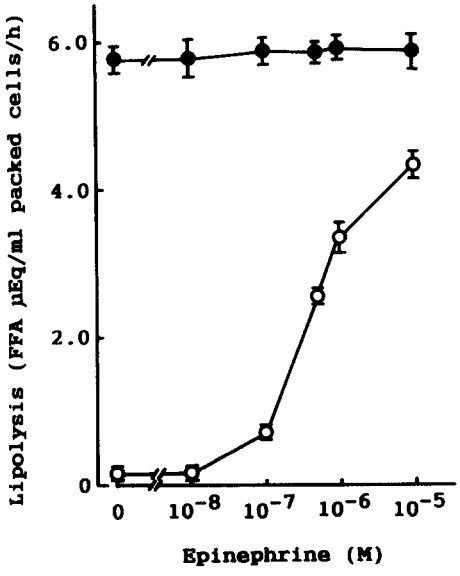


Fig. 1. Lipolysis of intact and sonicated fat cells in the presence and absence of epinephrine. The procedures are as described in Materials and Methods; (○), intact fat cells; (●), sonicated fat cells. Each point represents the mean ± SE of four separate assays.

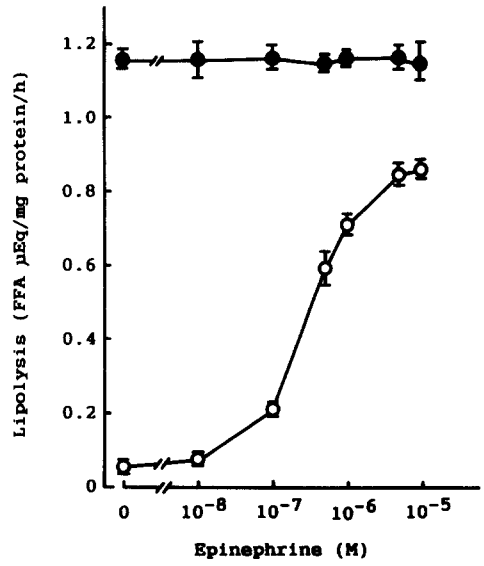


Fig. 2. Lipolysis of intact and sonicated lipid droplets in the presence of HSL. The procedures are described in Materials and Methods; (○), intact lipid droplets; (●), sonicated lipid droplets. Each point represents the mean ± SE of four separate assays.

elicited marked lipolysis, which was not affected by the addition of epinephrine. These results raise two questions. Why did sonication of intact fat cells result in greater lipolysis than that induced by epinephrine? Why did epinephrine not induce lipolysis in sonicated fat cells? Experiments were carried out to answer these questions.

First, we examined lipolysis in a cell-free system consisting of lipoprotein lipase-free HSL and endogenous lipid droplets as substrate. With intact lipid droplets as substrate, epinephrine induced lipolysis at concentrations of over 10^{-7} M in this system (**Fig. 2**). Sonication of the lipid droplets induced a much higher rate of lipolysis in the absence of epinephrine than that induced maximally by the hormone, and this rate of lipolysis was not enhanced further by addition of epinephrine (Fig. 2). Scanning electron micrographs of the intact and sonicated lipid droplets are shown in **Fig. 3.** These results suggested that the increase in basal lipolysis and loss of responsiveness to epinephrine after sonication of fat cells might be mainly due to destruction of the structure of intact lipid droplets in the cells.

However, the HSL concentration also affected lipolysis in the cell-free system. Therefore, before examining the effect of sonication of the intact lipid droplets we examined the effect of HSL concentration on lipolysis and the epinephrine responsiveness (**Fig. 4**). Addition of HSL did not affect lipolysis in intact lipid droplets in the absence of epinephrine, the rate being nearly zero even on addition of 88 μg protein of HSL per reaction mixture, which was the enzyme concentration used in other experiments with the cell-free system. On the other hand, with

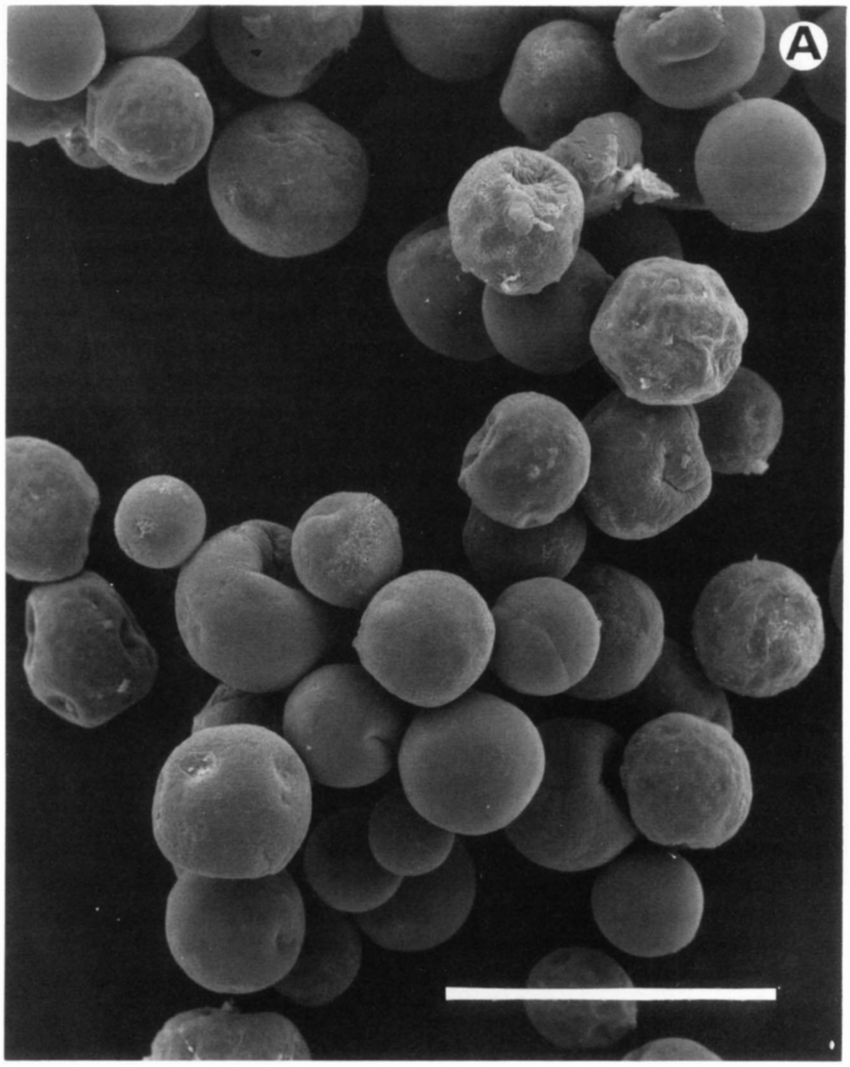
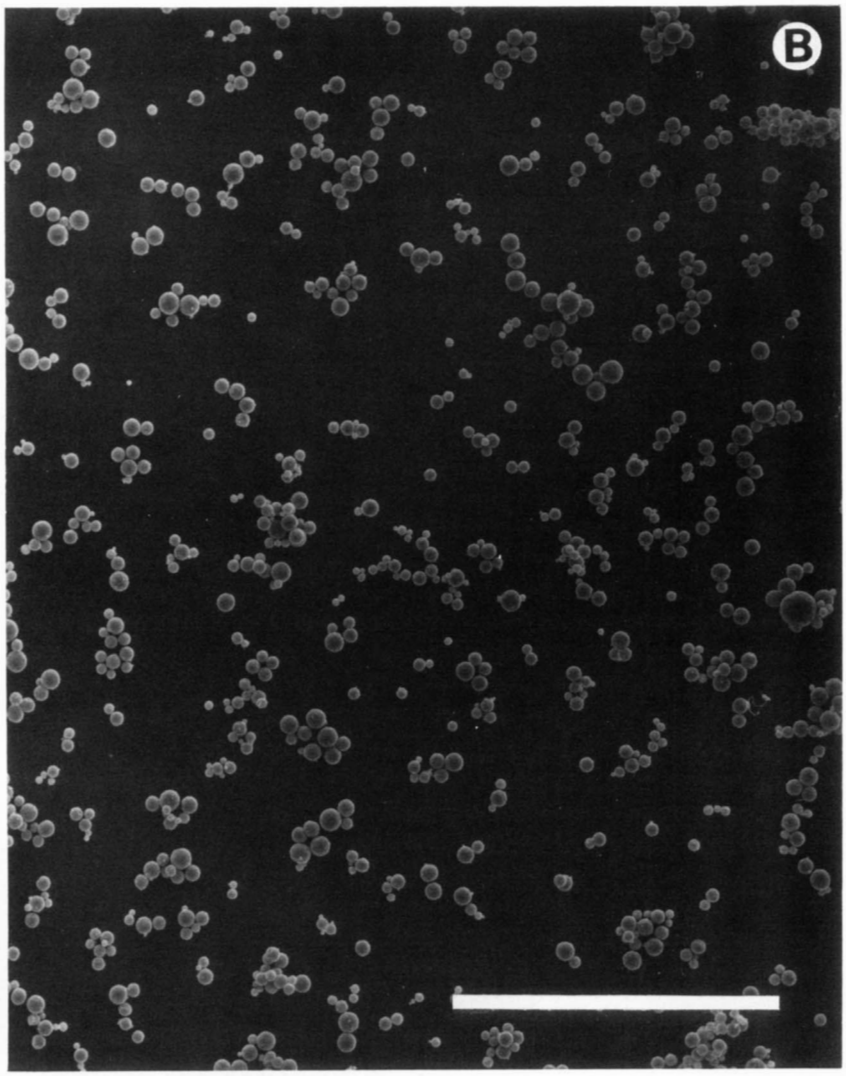


Fig. 3. Scanning electron micrographs of (A) intact and (B) sonicated lipid droplets from rat fat cells. The methods for preparation of intact lipid droplets and their sonication are described under Materials and Methods. Bar = 150 μm.

sonicated lipid droplets, the rate of lipolysis increased linearly with an increase in the amount of HSL added, reaching 178 ± 3.5 FFA μEq/reaction mixture per h at 88 μg protein of HSL. These results suggest that the HSL concentration does not affect the basal lipolysis with intact lipid droplets. Furthermore, they suggest that some mechanism may inhibit HSL in intact lipid droplets and that sonication of the intact droplets may destroy this mechanism, resulting in an elevation of the rate of basal lipolysis.

Strangely, with sonicated lipid droplets, basal lipolysis was significantly higher than that with intact lipid droplets, even in the absence of HSL. Probably a small amount of HSL was still present in the intact lipid droplets fraction and exerted its lipolytic action after sonication of the droplets. With intact lipid droplets, epinephrine-induced lipolysis increased with an increase in the amount of HSL added, but with sonicated lipid droplets, epinephrine did not stimulate lipolysis at any concentration of HSL. These results suggest that the loss of epinephrine responsiveness of sonicated lipid droplets may be due to factors other than the HSL concentration. Therefore, we examined the effect of sonication of intact lipid droplets on an increase in basal lipolysis and loss of epinephrine responsiveness.

In the present experiments, the average diameter of the endogenous lipid droplets was found to be 56.6 ± 0.7 μm. Sonication decreased the average diameter of the lipid droplets to 4.6 ± 0.2 μm, with an increase in the droplet number. Thus, the increase in the surface area of the droplets was proportional to the ratio of the average diameter of the intact lipid droplets to that of the sonicated ones. The average surface area of sonicated lipid droplets was calculated to be about 12 times that of intact lipid



droplets. It seemed possible that this increase in the surface area might result in an increase in basal lipolysis and loss of epinephrine responsiveness, so we next examined these possibilities.

Fig. 5 shows results on lipolysis examined at fixed surface areas of intact and sonicated lipid droplets. In the absence of epinephrine, lipolysis (basal lipolysis) at 400 cm^2 per ml of incubation mixture was found to be 0.06 ± 0.002 FFA μEq/mg per h with intact lipid droplets and 2.93 ± 0.02 FFA μEq/mg per h with sonicated lipid droplets, suggesting that the increase of basal lipolysis with sonicated lipid droplets was not due to an increase in the surface area. At 400 cm^2 per ml of incubation mixture, the rates of lipolysis in the presence and absence of epinephrine, respectively, were found to be 2.79 ± 0.11 and 0.06 ± 0.002 FFA μEq/mg per h with intact lipid droplets, and 2.97 ± 0.03 and 2.93 ± 0.02 FFA μEq/mg per h with sonicated lipid droplets. Thus, there was marked induction of lipolysis by epinephrine with intact lipid droplets but not with sonicated lipid droplets of the same surface area. These results clearly indicate that the increase of basal lipolysis and loss of epinephrine responsiveness on sonication of intact lipid droplets were caused by some factor other than an increase in the surface area of the lipid droplets.

Previously we reported that triglyceride is the main component of intact lipid droplets, with phospholipid, cholesterol, carbohydrate, and protein as minor constituents. Of the phospholipid fraction, 75% was found to be phosphatidylcholine and 25% to be phosphatidylethanolamine (10). The minor constituents may be localized at the surface of the droplets and affect their physico-chemical nature. As mentioned above, in this study the average diameter of intact lipid droplets was 56.6 ± 0.7 μm and that

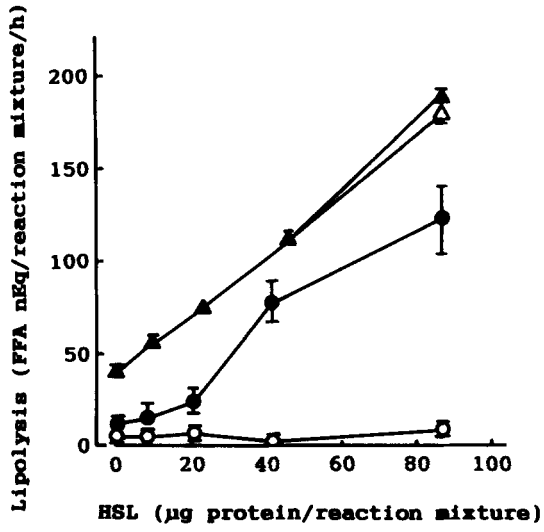


Fig. 4. Effect of HSL concentration on lipolysis in the cell-free system. Reaction mixtures consisting of lipid droplets and various amounts of HSL were incubated in the presence (●, ▲) and absence (○, △) of epinephrine (5.5 μM) as described in Materials and Methods; (●, ○), intact lipid droplets; (▲, △), sonicated lipid droplets. Each point represents the mean ± SE of four separate assays.

of sonicated droplets was 4.6 ± 0.2 μm. Therefore, the average surface area of sonicated droplets was about 12 times that of intact droplets. Therefore, if they are located at the surface of the droplets, the concentrations of the minor constituents such as phosphatidylcholine at the surface might be reduced about 12-fold by sonication.

We next examined the effects of these minor constituents on lipolysis in the cell-free system consisting of HSL and sonicated lipid droplets. For this, intact lipid droplets were sonicated with gum arabic and each of the minor constituents, and then added as substrates in the cell-free system. Lipolysis, expressed as a percentage of the value without addition of the constituents to the sonicated droplets was 0% at a ratio of phosphatidylcholine to triglyceride of 0.05, 210% at a ratio of phosphatidylethanolamine to triglyceride of 0.031, and 38% at a ratio of cholesterol to triglyceride of 0.025. These levels of phosphatidylcholine, phosphatidylethanolamine, and cholesterol correspond to 6.3-, 7.6-, and 7.8-times those in intact lipid droplets. Other constituents such as carbohydrate and protein, isolated as residual delipidated materials after treatment of the lipid droplets with ether, did not affect lipolysis (data not shown).

Of these minor constituents, phosphatidylcholine and/or cholesterol in the sonicated lipid droplets inhibited lipolysis, while phosphatidylethanolamine stimulated it. Addition of phosphatidylcholine to the sonicated lipid droplets markedly inhibited lipolysis in the cell-free system, but did not restore epinephrine responsiveness, as shown in **Fig. 6.** The size of the sonicated lipid droplets was not changed by addition of phosphatidylcholine (data not shown).

The inhibitory effect of cholesterol on lipolysis with sonicated lipid droplets was limited: the rates of lipolysis at ratios of 0, 0.0125, 0.025, 0.0375, and 0.05 of cholesterol to triglyceride were 506 ± 19, 249 ± 5,

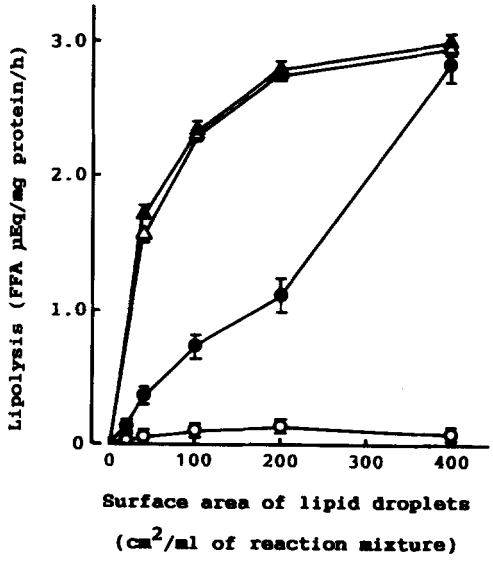


Fig. 5. Effect of surface area of lipid droplets on lipolysis in the presence of HSL. A mixture of lipid droplets and HSL solution was incubated at 37°C for 1 h in the presence (●, ▲) and absence (○, △) of epinephrine (5.5 μM) as described in Materials and Methods; (○, ●), intact lipid droplets; (△, ▲), sonicated lipid droplets. Each point represents the mean ± SE of four separate assays.

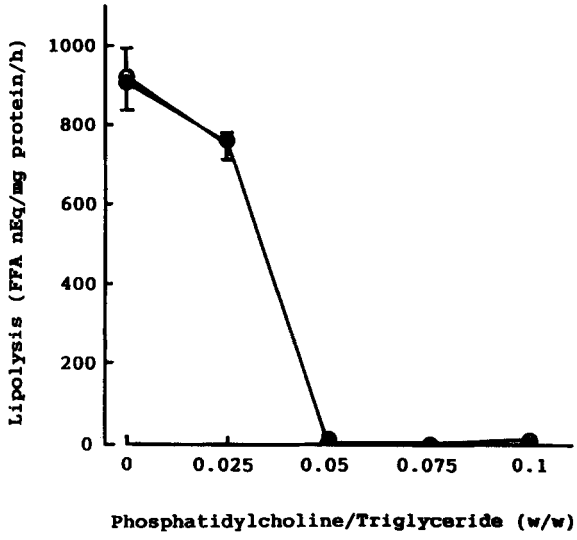


Fig. 6. Effect of phosphatidylcholine on hydrolysis of sonicated lipid droplets in the presence of HSL. Intact lipid droplets were sonicated with gum arabic and various amounts of phosphatidylcholine. Phosphatidylcholine was added at ratios of 0, 0.025, 0.05, 0.075, and 0.1 to triglyceride (w/w). The resultant sonicated lipid droplets were incubated with HSL solution as described in Materials and Methods in the presence (●) and absence (○) of epinephrine (5.5 μM). Each point represents the mean ± SE of four separate assays.

192 ± 11, 202 ± 16, and 200 ± 13 FFA μEq/mg per h, respectively. Thus cholesterol in the sonicated lipid droplets did not inhibit lipolysis completely. Lipolysis in the cell-free system consisting of intact lipid droplets and HSL was markedly enhanced by phospholipase C, but not by phospholipase D, even in the absence of epinephrine (**Fig. 7**). These results suggested that, of the minor constituents in the intact lipid droplets, phosphatidylcholine is the most likely to inhibit lipolysis in the droplets.

There was a possibility that phospholipase C-mediated lipolysis was not due to hydrolysis of triglyceride in the droplets, but to that of diglyceride formed by the action of phospholipase C on the phosphatidylcholine in the droplets. For examination of this possibility, [^{3}H]triolein was emulsified with gum arabic and various amounts of phosphatidylcholine and the mixtures were sonicated for 5 min. The resulting radioactive triolein emulsions were incubated for 1 h as described in the legend for **Fig. 8.** The rates of lipolysis at ratios of phosphatidylcholine to triglyceride of 0, 0.025, 0.05, 0.1, 0.2, and 0.3 were found to be 355, 367, 314, 91, 69, and 55 nmol/mg protein per h, respectively. Thus phosphatidylcholine in the triolein emulsion also inhibited lipolysis catalyzed by HSL. Therefore, we examined the effect of phospholipase C on hydrolysis of [^{3}H]triolein using emulsion in which the ratio of phosphatidylcholine to triolein was 0.3. As shown in Fig. 8, phospholipase C markedly increased lipolysis of [^{3}H]triolein emulsified with phosphatidylcholine. On the other hand, it did not stimulate lipolysis of [^{3}H]triolein emulsion in the absence of added phosphatidylcholine (data not shown).

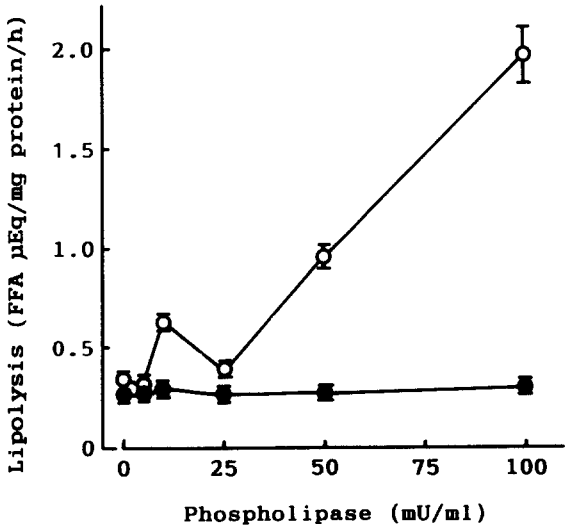


Fig. 7. Effects of phospholipases on lipolysis of intact lipid droplets in the presence of HSL. Various amount of phospholipase C or D was added to the reaction mixture as described in Materials and Methods except that epinephrine was omitted; (○), phospholipase C; (●), phospholipase D. Each point represents the mean ± SE of four separate assays.

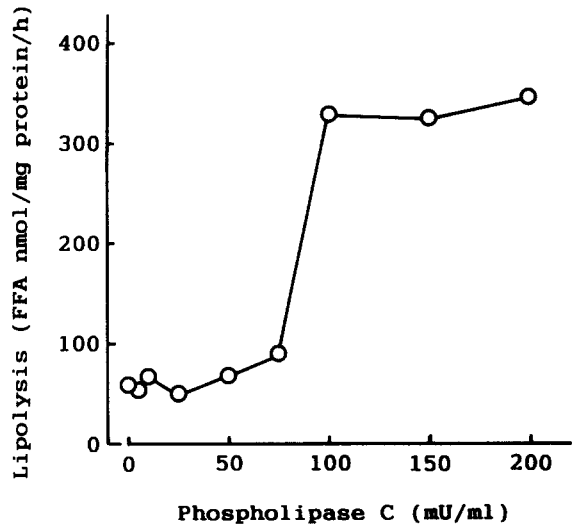


Fig. 8. Effect of phospholipase C on lipolysis of [^{3}H]triolein emulsified with gum arabic and phosphatidylcholine. A mixture of 50 mg triolein, 8.14 MBq [^{3}H]triolein, and 15 mg phosphatidylcholine in 3.75 ml 5% (w/v) gum arabic solution was sonicated for 5 min. The assay system contained the following components in a total volume 100 μl: 50 μl HSL solution, 0.14 μmol triolein, 47 nmol phosphatidylcholine, 0.45 mg gum arabic, 1.43 mg bovine serum albumin, 8 μmol KCl, 2 μmol NaCl, 0.1 μmol $CaCl_2$, 4.5 μmol BES, and various amounts of phospholipase C as indicated. Incubations were carried out for 1 h at 37°C at pH 6.8 and released [^{3}H]oleic acid was measured as reported previously (5).

In contrast to phospholipase C, phospholipase D did not affect the lipolysis of [^{3}H]triolein in emulsion containing phosphatidylcholine: the rates of lipolysis of [^{3}H]triolein emulsion at phospholipase D concentrations of 0, 50, 100, 500, and 1000 mU/ml were 56, 68, 66, 66, and 71 nmol/mg protein per h, respectively. These results suggested that phosphatidylcholine, possibly its phosphate group, in artificial lipid emulsion and intact lipid droplets inhibited lipolysis of triglyceride catalyzed by HSL.

DISCUSSION

With intact fat cells, basal lipolysis was extremely low and epinephrine induced lipolysis at concentrations of over 10^{-7} M. When the fat cells were sonicated with gum arabic, basal lipolysis increased markedly with a loss of epinephrine responsiveness.

Fat cells are known to contain two major lipase systems, HSL and lipoprotein lipase, which catalyze the hydrolysis of triglyceride to FFA and glycerol in about neutral and alkaline pH ranges (1, 15, 16). It seemed probable that the increase in the basal lipolysis in sonicated fat cells might be due to the lipolytic activity of lipoprotein lipase.

Basal lipolysis in the cell-free system consisting of lipid droplets and HSL solution was markedly enhanced by sonication of the intact lipid droplets with gum arabic. In lipolytic reaction with HSL solution, the effect of contamination with lipoprotein lipase was minimal as

described in Materials and Methods. These facts suggested that the increase in lipolysis after sonication of intact fat cells was due to destruction of the intact lipid droplets, and not to the lipolytic action of lipoprotein lipase. As shown in Fig. 4, it was demonstrated that a small amount of HSL was still present in the intact lipid droplets fraction and exerted its lipolytic action after sonication of the droplets, although the degree of the lipolysis was extremely low. It was difficult to estimate the HSL content associated with the lipid droplets, because endogenous triglyceride in the droplets interfered with the measurement of HSL activity. However, correct determination of HSL content in the droplets is needed for progress in this study. Sonication of the intact lipid droplets reduced their diameter from 56.6 ± 0.7 μm to 4.6 ± 0.2 μm, and resulted in an estimated 12-fold increase in the surface area of the droplets. In 1958, Sarda and Desnuelle (17) proposed that the surface area of the substrate is one of the factors regulating lipase activity. Therefore, it seemed possible that the increase in basal lipolysis on sonication of the intact lipid droplets might be due to their increased surface area. However, we found that basal lipolysis of sonicated lipid droplets was considerably higher than that of intact droplets of the same surface area (Fig. 5). Thus the increase in basal lipolysis on sonication of the intact lipid droplets was due to factors other than increases in the surface area of the droplets.

Of the minor constituents of intact lipid droplets, phosphatidylcholine and cholesterol were found to inhibit lipolysis in the cell-free system consisting of the sonicated lipid droplets and HSL, whereas phosphatidylethanolamine increased it. Cholesterol inhibited lipolysis of the sonicated lipid droplets only partially, whereas phosphatidylcholine inhibited it completely (Fig. 6). The size of the sonicated lipid droplets was not changed by addition of phosphatidylcholine which was localized as a monolayer on the surface of the droplets by examination with transmission electron micrographs (data not shown). Thus we concluded that phosphatidylcholine on the surface of intact droplets inhibits lipolysis. This conclusion was supported by the finding that lipolysis of intact lipid droplets in the cell-free system was considerably activated by phospholipase C (Fig. 7).

Addition of phosphatidylcholine to [^{3}H]triolein emulsified with gum arabic inhibited lipolysis in the presence of HSL, and this inhibition of hydrolysis by HSL was reversed by phospholipase C. These results suggested that phosphatidylcholine on the surface of intact lipid droplets or the artificial lipid emulsion inhibited lipolysis by HSL and that an increase in the rate of basal lipolysis in the cell-free system on sonication of the intact lipid droplets might be due to a reduction of the phosphatidylcholine concentration resulting from an increase in the surface area.

Generally, interfacial reactions such as lipolysis was known to be subject to regulation by the physical properties of the surface containing the substrate (18). Tsujita et al. (9) prepared films of mixtures of 1,3-dioleoylglycerol and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine at an argon–buffer interface and exposed these lipid films to various lipases, such as pancreatic carboxyester lipase and milk carboxyester lipase. They found that the extent of hydrolysis of 1,3-dioleoylglycerol was less than 5% up to a molar fraction of 0.5 and increased abruptly to 95% at a mole fraction of 0.6. They showed that this "switching" was not related to the surface pressure, reaction time, or enzyme concentration, but was due to the ratio of 1,3-dioleoylglycerol to phosphatidylcholine in the mixed lipid films. Their results are also consistent with our hypothesis mentioned above.

Sonication of fat cells and/or intact lipid droplets in the cell-free system induced not only an increase in the rate of basal lipolysis but also a loss of epinephrine responsiveness (Figs. 1 and 2). Moreover, although addition of phosphatidylcholine to the sonicated lipid droplets resulted in reduction of basal lipolysis, it did not restore epinephrine responsiveness (Fig. 6).

Previously, we have suggested that phosphatidylcholine in intact lipid droplets might be involved in the mechanism of the stimulatory effect of epinephrine (10, 19). However, the present experiments suggest that some other factors in addition to phosphatidylcholine may participate in epinephrine-induced lipolysis. Addition of other minor constituents such as cholesterol, phosphatidylethanolamine, carbohydrate, and protein to the sonicated lipid droplets did not restore the epinephrine responsiveness in the cell-free system (data not shown). However, perilipin reported by Greenberg et al. (20) may be included in the protein fraction. They speculate that perilipin plays a role in the specialized lipid storage function of adipocytes. Further work must be done to characterize the proteins present in the droplets.

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