RELEASE OF LIPOPROTEIN LIPASE FROM FAT CELLS

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When isolated fat cells, prepared from rat epididymal pads, were incubated, increasing amounts of lipase activity were found in the cell-free medium. Most of this lipase activity had characteristics of lipoprotein lipase. Increasing the temperature that fat cells were incubated from 23° to 37° markedly increased the appearance of lipase activity in the medium. In contrast, the appearance of this enzyme activity was markedly decreased when cells were incubated in the presence of cyanide. The data presented suggest that the release of lipoprotein lipase activity on incubation of fat cells is an energy-dependent process.

Lipoprotein lipase is one of several lipases found in adipose tissue (1). This enzyme specifically hydrolyzes triglyceride of chylomicron and lipoprotein complexes. Several investigators have suggested that hydrolysis of these triglycerides by lipoprotein lipase occurs on or near the capillary endothelium (2,3) and that this hydrolysis regulates the uptake of triglyceride into adipose tissue (4,5). However, on examination of the distribution of lipoprotein lipase in adipose tissue it has been shown that most of the enzyme activity is associated with fat cells and not the encompassing capillary network (6,7). This dichotomy of data suggesting hydrolysis of lipoprotein triglyceride occurring in the capillary and the finding that most of the lipoprotein lipase is in the fat cell led Rodbell and Scow (8) to suggest that fat cells could control incorporation of plasma lipoprotein triglyceride by synthesizing and secreting lipoprotein lipase. The secreted enzyme could become associated with the endothelial cells of capillaries where hydrolysis of circulating triglyceride is thought to take place.

Recently, data obtained from a study of incorporation of very low density lipoprotein triglyceride into isolated fat cells suggested that hydrolysis occurred prior to incorporation of the triglyceride into the cell lipids (9).

These findings led us to test one possible interpretation, that is, that fat cells were releasing lipoprotein lipase into the medium under these *in vitro* conditions. The present report is concerned with determining whether lipoprotein lipase is released by incubated fat cells and if this release is energy-dependent.

The epididymal fat pads of *ad libitum* fed male Sprague Dawley rats weighing 180-220 g were used for preparation of isolated fat cells by modification of Rodbell's procedure (10). Fat cells were isolated and incubated in Krebs-Ringer bicarbonate buffer containing 0.1 mg glucose per ml and fatty acid-free albumin (3%) (11). To liberate the fat cells the flasks were shaken at 160 cycles per min for 20-25 min at 37°. These cells were washed 4 times at 23° and resuspended in the previously mentioned buffer, dispensed into vials, gassed with 95% O_2-5% CO_2 and incubated for varying intervals.

Throughout all procedures involved in preparation and incubation, plasticware was used. Fat cells were counted essentially as described by Gliemann (12). Medium was separated from fat cells by filtering through Millipore (Millipore Filter Corp., Bedford, Mass.) filters. The cell-free medium was incubated either in the presence or in the absence of 1 M NaCl for 30 min at 0° prior to lipase assay. Lipase activity was assayed at 37° for 1 hr by adding 2 ml of medium to a mixture of 0.1 ml serum-activated triglyceride (4% Ediol (Schenlabs Pharmaceuticals, Inc., New York) incubated 30 min at 37° (1:1) with dog serum) and 0.2 ml of 1 M Tris, pH 8.5. The reaction was stopped by the addition of 4 ml of isopropyl alcohol: 3 N H_2SO_4 (39:1). Hydrolysis of triglyceride was determined by titrating the extracted free fatty acid (13). The difference between 1 M NaCl inhibitable and non-inhibitable lipase activity was designated lipoprotein lipase activity. Lipoprotein lipase activity is expressed as μmoles of fatty acid produced in the medium from 10^6 cells per hr.

Malic dehydrogenase activity was assayed fluorometrically in a 1 ml system at 37° (14). Malic dehydrogenase activity is expressed as μmoles NAD converted to NADH per min in the medium from 10^6 cells.

Incubation of fat cells at 37° for 25 min resulted in demonstrable lipase activity released into the medium. As shown in Table 1 the lipase activity was markedly reduced by previous incubation with 1 M NaCl. A similar reduction of lipase activity was noticed when serum was absent from the assay system. Both of these findings are characteristics which distinguish lipoprotein lipase from other lipases and suggest approximately 70% of the lipase activity released from fat cells is lipoprotein lipase (15).

A rapid decline of lipoprotein lipase activity was obtained when cell-free medium was incubated at 37°. Approximately 50% of this activity was lost in 30 min. The rate of loss of activity was 0.033 μmoles free fatty acid per min and appeared to follow zero order kinetics during 30 min incubation. When the

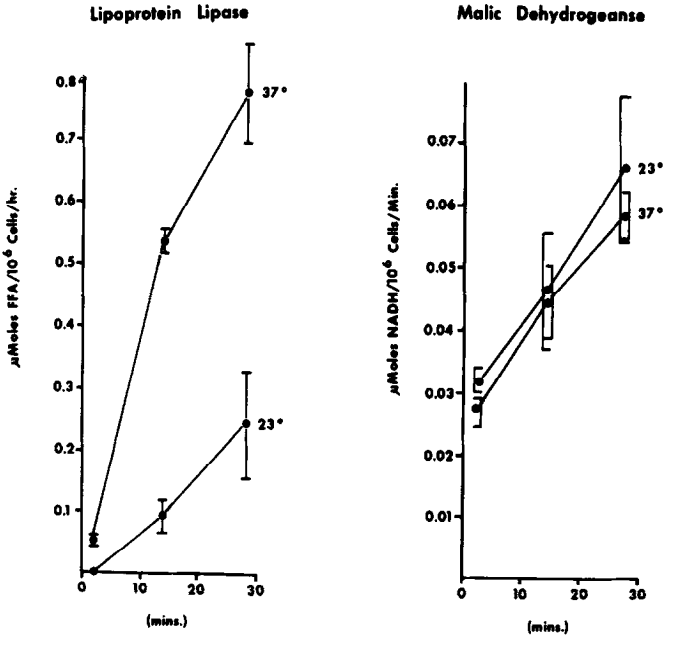

Fig. 1. The effect of temperature on release of lipoprotein lipase and malic dehydrogenase activities from free fat cells. Isolated fat-cell suspensions were incubated at 23° or 37°. Aliquots of cell suspensions were removed from the incubation flasks at 3, 14, and 24 min. The medium was separated from the fat cells and placed at 0° until assays for lipoprotein lipase or malic dehydrogenase activities were performed. The data points represent the mean of 3 experiments (duplicate analyses), with vertical bars indicating standard deviation of the means.

medium was incubated at 23° for 30 min the loss of lipoprotein lipase activity was not significant. In those experiments which were carried out at 37° the true rate of release was estimated by correcting the observed rate of release of lipoprotein lipase activity by the amount of enzyme destroyed.

The effect of temperature on the appearance of lipoprotein lipase activity is shown in Fig. 1. During the 30 min of incubation the amount of enzyme activity released at 37° and 23° increased with time. In addition, the amount of lipoprotein lipase activity released at 37° was 3.5 times greater than at 23°. The three-fold difference in the enzyme activity released into the medium on incubation by fat cells is the order expected for a 14° temperature change if an energy-requiring process is involved in its release (16).

Another possible explanation for greater amounts of lipoprotein lipase activity in the incubation medium at 37° is that higher temperatures break fat cells or alter their stability. To rule out this possibility we examined the release of malic dehydrogenase activity from incubated fat cells under the same experimental condition. This stable enzyme has been shown to be a sensitive indicator of cell instability (14). As shown in Fig. 1, the amount of malic dehydrogenase released into the medium by fat cells increased with time but in contrast the release of malic dehydrogenase activity is not significantly altered by a change in temperature. This finding suggests there has been no change in cell stability at these temperatures.

In an effort to support this inference that release of lipoprotein lipase activity is energy-dependent, the effect of a metabolic inhibitor on incubating fat cells was investigated. Fig. 2 demonstrates the effect of 1×10^{-3} M sodium cyanide in the incubation medium on the release of lipoprotein lipase and malic dehydrogenase activities at 23°. In this experiment cyanide completely inhibited the release of lipoprotein lipase activity at 22 min and caused a 78% inhibition of lipoprotein lipase activity released at 45 min. In a separate experiment the insulin-stimulated conversion of glucose-^{14}C to $^{14}CO_2$ at 23° for 45 min was depressed 75% in the presence of 1×10^{-3} M cyanide.

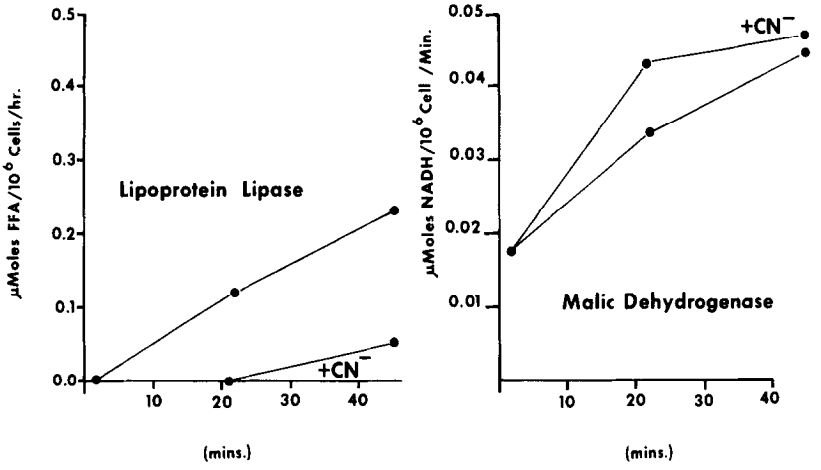

Fig. 2. The effect of 1 x 10^{-3} M sodium cyanide on release of lipoprotein lipase and malic dehydrogenase activities from isolated fat cells. Isolated fat cells were incubated in the presence or in the absence of 1.0 x 10^{-3} M sodium cyanide at 23°. At 22 and 45 min aliquots of cell suspensions were removed from incubation flasks, medium separated from fat cells and assayed for lipoprotein lipase and malic dehydrogenase activities. There was a slight inhibition of lipoprotein lipase by cyanide in the lipase assay, therefore all samples were assayed in the presence of 1 x 10^{-3} M NaCN. The results are the mean of duplicate analysis.

Table 1.

Pre-assay incubation	Assay incubation	µ moles FFA/10^6 cells/hr
-	serum-activated triglyceride	1.88
-	non-activated triglyceride	0.70
-	serum-activated triglyceride	0.67

The effect of inhibitors and activators on lipase activity released from free fat cells. Fat cells were incubated at 37° for 15 min. Aliquots of cell-free medium, incubated in the presence or in the absence of 1 M NaCl for 30 min at 0°, were assayed with activated (see text) or non-activated triglyceride substrate. Non-activated triglyceride substrate was prepared by incubation of equal volumes of Ediol and 0.85% NaCl at 37° for 30 min. Results are the mean of duplicate analysis.

These data suggest that cyanide has altered the release of lipoprotein lipase activity from fat cells by altering key metabolic pathways. The amount of malic dehydrogenase activity released into the medium by cells incubated in the presence and absence of cyanide increases with time; however, in contrast to the lipoprotein lipase data consistently more malic dehydrogenase activity was found in the presence of cyanide. The reason for this slight increase is not clear, but could suggest some impairment of cell integrity.

The effects of temperature changes and of cyanide upon the release of lipoprotein lipase activity offer strong evidence in support of the hypothesis that the release of lipoprotein lipase from isolated fat cells is an energy-dependent process.

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