RELEASE OF LIPOPROTEIN LIPASE FROM RAT ADIPOSE TISSUE CELLS GROWN IN CULTURE

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SUMMARY: Fibroblast-like stromal cells obtained from rat epididymal fat pads were grown in culture. It has been shown that these cells release lipoprotein lipase into the culture medium for approximately 10 hr upon exposure of the cells to heparin. Continued incubation of such heparin treated cells in the absence of heparin results in the replenishment of releasable lipase pools over a three day period. The released enzyme is inhibited by NaCl and protamine sulfate.

Lipoprotein lipase* (EC 3.1.1.3) has been shown to be a key enzyme in the metabolism of serum lipoproteins, initiating the conversion of triglyceride-rich chylomicrons and VLDL* to "intermediate" or "remnant" particles (1,2). Adipose tissue is a major site of LPL synthesis, and the synthesis, release and purification of this enzyme from adipose cells has been extensively studied (3,4,5). Attempts to cultivate adipose cells in vitro have met with only limited success; however, recent investigations have demonstrated that fibroblast-like cells having the biochemical characteristics of adipose cells can be obtained from adipose tissue and grown in culture (6,7). Such cells may represent a population of "pre-adipocytes", and the ability of such cells, growing in culture, to synthesize and release LPL is the subject of this report.

METHODS

Cells and Culture Conditions

Epididymal fat pads from three month old Fischer 344 rats (approximately 200 gms) were removed and 2 gm of epididymal tissue incubated for 120 min in 15 ml Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1.3% bovine albumin (Sigma) and 3.33 mg/ml collagenase

*Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoproteins; FBS, fetal bovine serum.

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(Worthington, Type I). The digest was filtered through a 250 micron nylon mesh into a 250 ml separatory funnel. After 10 min the lower infranatent fraction containing the stromal cells was drawn off and diluted with 5 ml of Williams medium E (Flow Labs) supplemented with 10% FBS* (Microbiological Assoc.). The upper adipocyte fraction was washed 5 times with 5 ml of Williams medium E and resuspended in 5 ml. Aliquots of each fraction were placed in 75 cm^2 plastic tissue culture flasks (Lux) at a density of approximately 5 x 10^5 cells/flask and diluted so that each flask contained 25 ml Williams medium E supplemented with 10% FBS. Flasks were incubated at 37° in an atmosphere of 6% CO_2-94% air. Attachment and spreading of stromal cells was rapid, whereas only a limited number of fat-filled, adipose cells were observed to attach to the flasks, and there was no evidence of continued multiplication of these cells. Within 1 to 2 weeks the stromal cells in both cell fractions formed confluent monolayers. These primary cultures were detached with 0.25% trypsin and inoculated into new flasks using a 1:2 split ratio. These cells are designated first passage level. Subsequent 1:2 splits of confluent monolayers are designated by increasing passage level number. Monolayers were either split when confluent or maintained without subculturing by weekly refeeding with fresh medium. Confluent monolayers containing approximately 3 x 10^6 cells/flask which was equivalent to approximately 2 mg cell protein. Stromal cells, which had the morphological appearance of fibroblasts are referred to as preadipocytes.

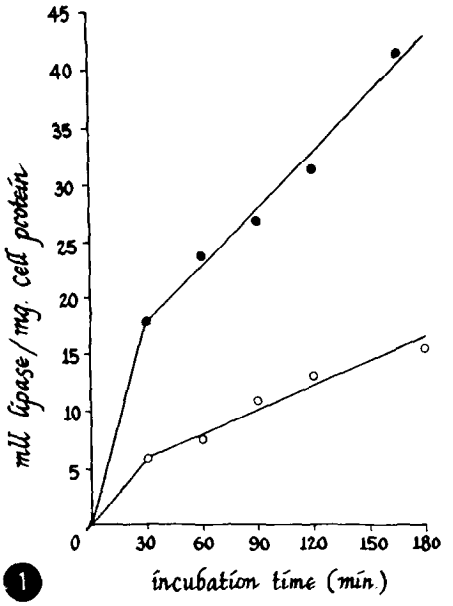
LPL Release and Enzyme Assay

One day prior to use flasks were refed with fresh medium. At the beginning of the release period growth medium was removed, monolayers were washed 4 times with serum-free medium and 6 ml to 12 ml of release medium added. Unless otherwise indicated this medium consisted of Williams medium E buffered to pH 7.5 with 25 mm HEPES (Sigma) supplemented with 4% fasted human serum (heated 60°/30 min), 10 mg/ml crystalline bovine albumin (Sigma) and 10 units/ml heparin (Calbiochem). Flasks were incubated at 37°C and at timed intervals 0.2 ml aliquots of medium were removed and assayed for LPL activity. If sterility was maintained throughout these operations the monolayers could be washed with serum-free medium at the end of the release period, refed with fresh medium and reused in subsequent experiments.

Assay for LPL was conducted as described by Nilsson-Ehle and Schotz (8) using 0.1 ml of a stable emulsion of tri-[9,10-^{3}H] oleoylglycerol (Amersham-Searle) in glycerol and an incubation time of 60 min at 37°C. Liberated ^{3}H-oleic acid was recovered by solvent partition (8) and counted in Aquasol (New England Nuclear) using a Packard scintillation spectrometer. The data are expressed as milli units (mU) of LPL which were calculated as described by Nilsson-Ehle and Schotz (8). Preliminary experiments demonstrated linearity of the assay for at least 90 min and that addition of double or one-half of the substrate had no effect on the observed activity. The amount of LPL in the total volume of medium was calculated from the value obtained from 0.2 ml aliquot and is expressed as mU/flask or mU/mg cell protein. All values represent the average of at least 2 replicate aliquots obtained from duplicate flasks. Assays for enzyme sensitivity to NaCl or protamine sulfate (Sigma) were performed using a final concentration of 1 M NaCl and 0.15 mg protamine sulfate/0.2 ml of released enzyme.

RESULTS

The addition of heparin to the incubation medium resulted in the stimulation of release of LPL from cultured preadipocytes when compared to controls. In the presence of heparin rapid release occurs within the first 30 min of exposure followed by a decreased rate of release (Fig. 1). Stimulation of release by heparin was dose dependent with maximum re-



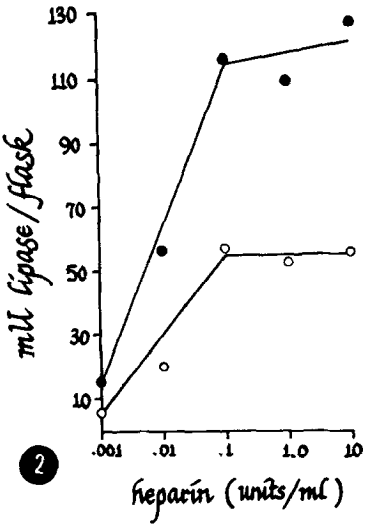
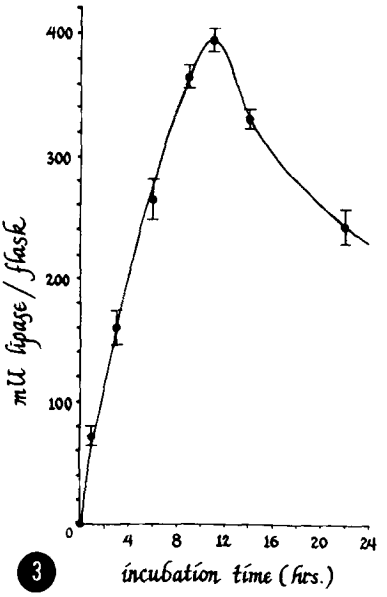


Figure 1. Release of lipoprotein lipase from rat epididymal preadipocytes. Average of duplicate determinations obtained from two flasks for each curve. O—O no heparin, ●—● 10 units/ml heparin.

Figure 2. Effect of heparin concentration on release of lipoprotein lipase. Average of duplicate determinations obtained from two flasks for each concentration. O—O lipase released after 30 min incubation; ●—● release after 3 hr incubation.

lease obtained at 0.1 units/ml and similar dose response patterns were obtained after 30 min and 3 hr of incubation (Fig. 2). When the released LPL was assayed in the presence of 1 M NaCl or protamine enzyme activity was reduced by 96% and 79% respectively.

Fig. 3 shows the LPL release pattern throughout a 22 hr exposure period in the presence of heparin. Near linear release was obtained for approximately 10 hrs after which the level of enzyme activity in the medium steadily declined. To establish if the failure to observe continued LPL release beyond 10 hrs resulted from an inability of cells to synthesize LPL, replicate flasks were incubated with heparin for 24 hrs at which time releasable stores of LPL appeared to be depleted. These flasks were refed with growth medium (no heparin) and



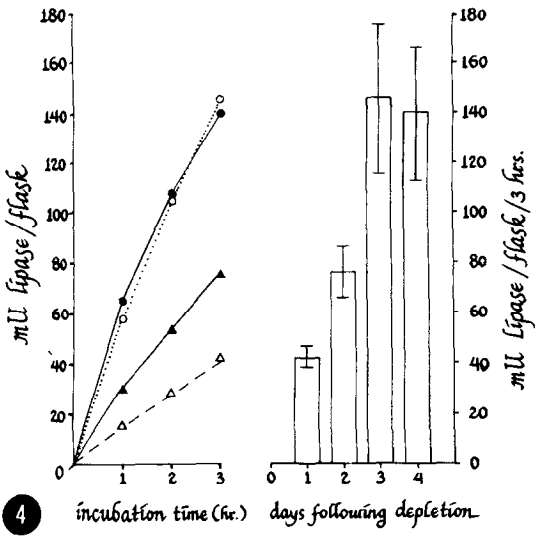


Figure 3. Release of lipoprotein lipase in the presence of 10 units/ml heparin. average and S.E.M. N = 8.

Figure 4. Release of lipoprotein lipase from preadipose cultures preincubated 24 hr in medium containing 10 units/ml heparin (depletion phase) and re-incubated in absence of heparin. Left panel: release during a 3 hr incubation period upon re-exposure to heparin. Days following depletion; Δ–Δ 1 day, 2 days, O···O 3 days, 4 days. Right panel: lipase released in 3 hrs from depleted cells after re-incubation. Average and S.E.M. of values obtained from 3 independent experiments (N = 8).

incubated at 37°C. At daily intervals following refeeding, replicate flasks were tested for their ability to release LPL during a 3 hr incubation in the presence of heparin. Fig. 4 demonstrates that the releasable pool of LPL increased for 3 to 4 days following depletion. Continued incubation beyond 4 days resulted in no further increase in the amount of enzyme released. The ability of cells to release LPL appeared to steadily decrease as the passage level of the cells was increased. Cells at passage level 6 exhibited only 20% to 40% of the activity of early populations and only trace activity was obtained from cells at passage level 10. Rat preputial fibroblasts (population doubling 1–5) had no significant lipase activity when tested in parallel with preadipocytes.

The release of LPL from adipose tissue has been the subject of intensive investigation. Many such studies have been limited by the inability to maintain functional fat cells *in vitro* for more than a few hours. The present investigation demonstrates that a population of cells can be obtained from rat epididymal tissue which can grow *in vitro* and which has the ability to synthesize and release LPL. The enzyme has the properties of adipose LPL in that it is released by heparin and inhibited by NaCl and protamine sulfate. The concentration of heparin required for maximum release is similar to that previously shown to be required for adipose tissue (9).

Although this investigation did not attempt to correlate the level of intracellular LPL to released LPL, the data shown in Fig. 3 suggest that these cells contain a pool of LPL which continues to be released for approximately 10 hrs following exposure to heparin. The replenishment of this pool is relatively slow, requiring 3 to 4 days before maximum release is achieved in previously depleted cells (Fig. 4). The decrease in enzyme activity in the medium after 10 hr exposure to heparin (Fig. 3) may be explained by a rate of thermal inactivation of enzyme exceeding the cellular rate of synthesis and release.

In vivo studies have suggested the presence of a population of precursor cells which accumulate triglyceride and assume the morphological appearance of fat cells (10, 11). It has been suggested that adipose tissue development occurs primarily between birth and weaning and that adipose tissue in mature animals has only a very limited proliferative capacity (12,13). Recent studies have demonstrated the ability to grow cells from adult humans which have the morphological appearance of fibroblasts but the enzyme patterns of mature adipocytes (6). The present study demonstrates that such cells from adult rats are capable of synthesizing and releasing adipose LPL. The presence of such preadipocytes, with a continuing proliferative capacity, in mature animals must be reconciled with the current concepts of adipose tissue cellularity. The ability to grow LPL containing cells in culture will provide a powerful tool for the study of the regulation of synthesis and release of LPL.

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