The Reciprocal Regulation of Lipoprotein Lipase Activity and Hormone-sensitive Lipase Activity in Rat Adipocytes*

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SUMMARY

The regulation of lipoprotein lipase activity was studied in rat adipocytes. Incubation of fat cells for 60 min in buffer without glucose and insulin resulted in a 50% decrease in lipoprotein lipase activity. This decrease was prevented by glucose and insulin. Inhibition of fat cell protein synthesis by cycloheximide abolished the effect of glucose and insulin and caused a rapid decay of lipoprotein lipase activity (T1/2 = 24 min). Lipolytic concentrations of N6,O2'-dibutyryl cyclic adenosine 3',5'-monophosphate (dibutyryl cAMP) in the presence of glucose and insulin decreased rat cell lipoprotein lipase activity and also decreased the rate of 1-leucine-1-14C incorporation into fat cell protein. A close correlation (r = .95) was found between changes in the rate of protein synthesis and changes in lipoprotein lipase activity caused by lipolytic agents, cycloheximide, and by the omission of glucose and insulin.

Incubation with dibutyryl cAMP decreased fat cell ATP content. In the presence of glucose and insulin this occurred without an intracellular accumulation of fatty acid, and it is suggested that the decrease was due to consumption of ATP by the process of re-esterification of fatty acids. Thus the decrease in lipoprotein lipase activity caused by lipolytic hormones could be the last step in a series of events. Activation of lipolysis with re-esterification of some of the fatty acids results in an increased consumption of ATP, and therefore the ATP available for protein synthesis is limited. This leads to a decrease in the rate of protein synthesis and then, because of the rapid decay of this enzyme, to a decrease in lipoprotein lipase activity.

In adipose tissue the processes of uptake of circulating triacylglyceride and of release of intracellular triacylglyceride both require hydrolysis of the triglyceride molecule, and these hydrolytic reactions are mediated by two distinct lipase systems (1-3). The mobilization of adipose tissue triacylglyceride as glycerol and fatty acids requires the action of a lipase that is activated by catecholamines and a variety of other hormones, and there is evidence that this activation follows the intracellular accumulation of cyclic 3',5'-AMP (4, 5). The activity of this enzyme, hormone-sensitive lipase, as manifested by glycerol release, is greater in adipose tissue from fasted than from fed animals (6). The second adipose tissue lipase, lipoprotein lipase, regulates the rate of uptake of plasma triglyceride by adipose tissue because of its action in catalyzing the hydrolysis of circulating chylomicron and lipoprotein triglyceride that must occur prior to the uptake of the fatty acid components (7-10). This enzyme is more active in adipose tissue from fed than fasted animals (8, 9, 11, 12), and the activity in tissue from fasted animals can be increased by in vitro incubation of tissue slices with glucose and insulin (8), a process that requires protein synthesis (13).

Since fasting and feeding produce opposite changes in the activities of these two adipose tissue lipase systems, it is possible that a mechanism exists within the fat cell that produces coordinated reciprocal changes in the activities of the two enzymes. That such a mechanism may be operative is further suggested by data indicating that hormonal activation of lipolysis is accompanied by a simultaneous reduction in the activity of lipoprotein lipase (13-16). It has also been shown that the in vitro induction of lipoprotein lipase can be inhibited by exposure of the tissue to N6,O2'-dibutyryl cyclic adenosine 3',5'-monophosphate, an agent which also produces an increase in the rate of lipolysis (17).

The present study was designed to explore in detail the relationship between activation of the hormone-sensitive lipase and reduction in the activity of lipoprotein lipase. Since cyclic 3',5'-AMP influences the activity of both enzymes, our attention was focused on the action of this nucleotide.

MATERIALS AND METHODS

Male Wistar rats were fed ad libitum and were used when they weighed 180 to 210 g. Bacterial collagenase (crude) and Glucostat reagent kits were obtained from Worthington. Crystalline bovine serum albumin, Cohn Fraction V, was from Brickman and Company, Montreal, Quebec, Canada. Epinephrine bitartrate, theophylline, cyclic 3',5'-AMP, and dibutyryl cAMP were obtained from Schwarz BioResearch; cycloheximide was from Sigma. Triethanolamine, ATP, NAD+, NADH, and trisodium 5-phosphosuccinate, glycyrrhizinate (85% solution)

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units per mg), glycerol-1-phosphate dehydrogenase (36 units per mg), glyceraldehyde-3-phosphate dehydrogenase (36 units per mg), and 3-phosphoglycerate kinase (180 units per mg) were from Boehringer-Mannheim. l-Leucine-1-\textsuperscript{14}C (20 mCi per mmole) was from New England Nuclear, and crystalline zinc insulin was obtained from Connaught Laboratories. All other chemicals were from Fisher and were of American Chemical Society grade or higher.

**Preparation and Incubation of Cells—**Rats were anesthetized by intraperitoneal injection of sodium pentobarbital, and the lumbar and epidymal adipose tissue were excised. Free fat cells were prepared by the method of Rodbell (18). Adipose tissue was incubated in Krebs-Ringer bicarbonate buffer containing half the recommended concentration of calcium (19) with bovine serum albumin, 50 mg per ml, collagenase, 1.5 mg per ml, glucose, 2 mg per ml, and insulin, 10 mg per ml. Prior to use the Krebs-Ringer bicarbonate buffer with albumin was dialyzed for 24 hours against 10 volumes of Krebs-Ringer bicarbonate buffer, then filtered through a 0.45-μm membrane filter. The fat cells were washed 3 times with 5 volumes of Krebs-Ringer bicarbonate buffer, then incubated in Krebs-Ringer bicarbonate buffer with albumin, and dispensed in 4-m1 aliquots into 1-ounce plastic botules. Any additions were made in small volumes, then the suspensions were brought to 5 ml with Krebs-Ringer bicarbonate buffer with albumin, and incubated at 37° with shaking in a Dubnoff metabolic incubator with the gas phase, 96% O\textsubscript{2} to 5% CO\textsubscript{2}. Approximately 500 to 800 mg of fat cells were incubated in each botule. Following incubation, the fat cells were separated from the medium and frozen in dry ice methanol for subsequent extraction of lipoprotein lipase activity. When fat cell ATP concentration was determined, the incubation medium was removed, and the cells were homogenized immediately in 2 ml of 0.5 M perchloric acid at 4°.

**Analytical Procedures—**Lipoprotein lipase was extracted from acetone-ether dried powders of fat cells with 25 mM NH\textsubscript{4}Cl-N\textsubscript{2}H\textsubscript{4}OH at pH 8.2 and assayed in a system containing bovine serum albumin defatted by the method of Chen (20), 60 mg per ml, Tris-HCl, 15 mM, heparin, 5 μg per ml, dog chylomicon triglyceride, 2.95 mg per ml, and NaCl, 7 mM at pH 8.2. The details of preparation of acetoacetate-ether powders, extraction and assay of lipoprotein lipase activity, and preparation of dog chylomicon tracylcerides have been previously described (21). Lipoprotein lipase activity was expressed as microequivalent of free fatty acid produced per hour per g of fat cell acetone-ether extractable lipid.

Glycerol was determined by a modification of the method of Wieland (22). To 10-ml aliquots of incubation medium 0.1 ml of 30% perchloric acid was added. The resulting precipitate was washed twice with 0.5 ml of 3% perchloric acid, all the supernatant solutions were combined, and the pH was adjusted to 9.0 with 2.0 M K\textsubscript{2}PO\textsubscript{4}. The volume was adjusted to 2.5 ml with distilled water, the mixtures were allowed to stand overnight at 4°, and then were centrifuged at 4° for 20 min at 1200 x g. The supernatant solution (0.5 ml) was added to a mixture consisting of 0.2 ml glycine, 1 ml hydrazine hydrate, 2 mM MgCl\textsubscript{2}, 1.65 mM ATP, 0.75 mM NAD\textsuperscript{+}, 0.1 mg per ml of glycerol-1-phosphate dehydrogenase, and 0.015 mg per ml of glycerokinase, pH 9.8. Blanks and standards prepared in Krebs-Ringer bicarbonate buffer with 5% bovine serum albumin and deproteinized as above were assayed simultaneously. The mixtures were incubated at 37° for 1 hour, then were cooled in an ice water bath, and the optical density at 366 μm determined. The change in optical density was proportional to glycerol concentration over a range of 0.01 to 0.6 μmole.

For determination of ATP content, fat cells were homogenized in 2.0 ml of 0.6 M perchloric acid at 4°. The homogenate was centrifuged at 4° for 15 min at 1200 x g, and the clear fluid between the upper fat layer and the protein precipitate was then removed. This infranatant (0.9 ml) was adjusted to pH 7.0 with 2.0 M K\textsubscript{2}HPO\textsubscript{4}; the mixtures were allowed to stand for 20 min at 4° and were then centrifuged at 4° for 10 min at 1200 x g. The supernantant solution (0.2 to 0.4 ml) was assayed for ATP by a fluorometric micromethod using 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, in which the disappearance of NADH was measured (23).

FFA were extracted and titrated by the method of Dole and Meinertz (24). The rate of esterification of FFA was calculated according to the method of Vaughan (6). The uptake of glucose by fat cells was determined by measuring the medium glucose concentration before and after incubation. Glucose was measured by a glucose oxidase method commercially available in kits (Glucostat). Protein was determined by the method of Lowry et al. (25), using crystalline bovine serum albumin as standard.

The incorporation of l-leucine-1-\textsuperscript{14}C into fat cell protein was determined by applying 0.1-ml aliquots of the fat cell suspension to filter paper discs and treating the discs according to the method of Mans and Novelli (26). The uptake of l-leucine-1-\textsuperscript{14}C by fat cells was determined by measuring the radioactivity of the medium before and after incubation. In these experiments, 0.1-ml aliquots of the medium were digested at 50° for 60 min with 0.5 ml of a commercial mixture of quaternary ammonium compounds (27) prior to counting. Radioactivity was counted in a Nuclear-Chicago 720 series scintillation counter using a dioxane scintillator (28).

**RESULTS**

**Maintenance of Fat Cell Lipoprotein Lipase Activity—**It is well known that incubation of adipose tissue slices in the absence of glucose and insulin results in a rapid decline of lipoprotein lipase activity and that glucose and insulin can prevent this decline (8). In the first experiments the effect of glucose and insulin on the lipoprotein lipase activity of isolated fat cells was studied (Table I). When fat cells were incubated for 60 min in the absence of glucose and insulin, lipoprotein lipase activity fell to 50% of zero time control values, but when glucose and insulin were present, fat cell activity was maintained almost at control values. Other experiments showed that the fall in lipoprotein lipase activity was not prevented by insulin alone but was largely prevented by glucose alone. However, the presence of both glucose and insulin was necessary for optimum maintenance of lipoprotein lipase activity.

It has also been shown in intact adipose tissue that inhibition of protein synthesis can prevent the increase in lipoprotein lipase activity that occurs when tissue from fasted animals is incubated with glucose and insulin (13). In the next experiments, with the use of fat cells from fed animals, a study was made of the effect of cycloheximide on lipoprotein lipase activity and on l-leucine-1-\textsuperscript{14}C incorporation into fat cell protein (Fig. 1). When fat cells were incubated for 50 min with glucose and insulin only, lipoprotein lipase activity remained constant, and l-leucine-1-\textsuperscript{14}C incorporation into protein was not significantly different from control values. In contrast, when cycloheximide was added to the incubation medium containing glucose and insulin, lipoprotein lipase activity declined to 30% of control values, and l-leucine-1-\textsuperscript{14}C incorporation into protein was also markedly reduced. These results indicate that lipoprotein lipase activity in isolated fat cells is regulated by glucose and insulin, and that the fall in lipoprotein lipase activity is prevented by cycloheximide.
incorporation into cell protein was linear. The addition of cycloheximide (10 μg per ml) caused complete inhibition of L-leucine-1-14C incorporation into protein and also produced a rapid decline in lipoprotein lipase activity, which decayed with a half-life of 25 min.

In other experiments omission of glucose and insulin from the incubation medium resulted in a 50% decrease in both lipoprotein lipase activity and L-leucine-1-14C incorporation into protein. In this instance cycloheximide (10 μg per ml) also produced a further decrease in lipoprotein lipase activity and totally abolished L-leucine-1-14C incorporation into fat cell protein.

These results established that the lipoprotein lipase activity of fat cells is unstable to incubation, that glucose and insulin are necessary for the maintenance of this activity during incubation, and that this effect of glucose and insulin can be prevented by inhibition of protein synthesis. It is not known whether glucose and insulin maintain enzyme activity by influencing the synthesis of enzyme protein or of another protein necessary for enzyme activity. The rapid decay of lipoprotein lipase activity that occurs when protein synthesis is inhibited indicates that the activity of this enzyme can be rapidly altered by changes in the rate of fat cell protein synthesis.

Effect of Lipolytic Agents on Fat Cell Lipoprotein Lipase Activity—It is known that the lipoprotein lipase activity of intact epididymal fat pads from fed rats is decreased when the pads are incubated with lipolytic hormones (15, 16) and that these hormones can block the increase in lipoprotein lipase activity that occurs when fat pads from fasted rats are incubated with glucose and insulin (13, 17). In the following experiments the effect of the lipolytic agent dibutyryl cAMP on lipolysis and lipoprotein lipase activity was investigated using isolated fat cells. In the first experiments, a study was made of the effect of dibutyryl cAMP on fat cells incubated in the presence and absence of glucose and insulin (Table II). As shown previously, fat cell lipoprotein lipase activity was maintained by glucose and insulin, and this activity fell to 50% of zero time values when cells were incubated without glucose or insulin. The addition of dibutyryl cAMP (1 mM) to fat cell suspensions incubated with glucose and insulin was associated with a 50% reduction in lipoprotein lipase activity. When the same concentration of nucleotide was added to cells incubated without glucose or insulin, there was no effect on the lipoprotein lipase activity of these cells. In the presence of 1 mM dibutyryl cAMP, the release of fatty acids from cells incubated without glucose or insulin was twice that of cells incubated with glucose and in sulin, but the concentrations of medium fatty acids that were reached were not sufficient to saturate the estimated primary binding sites for FFA of the medium albumin (29). In other experiments it was found that dibutyryl cAMP in concentrations greater than 1.3 mM decreased the lipoprotein lipase activity of

### Table I

<table>
<thead>
<tr>
<th>Additions</th>
<th>Incubation time</th>
<th>Lipoprotein lipase activity</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>0 min</td>
<td>μmoles FFA/hr/g lipid</td>
</tr>
<tr>
<td>None</td>
<td>60 min</td>
<td>43.4 ± 4.0</td>
</tr>
<tr>
<td>Glucose (2 mg per ml) + insulin (10 μg per ml)</td>
<td>60 min</td>
<td>37.6 ± 4.0</td>
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*p < 0.001, (t test), where p is the difference from cells incubated 0 min.

*p < 0.01, where p is the difference from cells incubated without glucose and insulin.

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**Additions**

- None
- Glucose (2 mg per ml) + insulin (10 μg per ml)

**Incubation Time (min)**

- 0 min
- 60 min

**Lipoprotein Lipase Activity**

- μmoles FFA/hr/g lipid

### Table II

<table>
<thead>
<tr>
<th>System</th>
<th>Lipoprotein lipase activity</th>
<th>Fatty acid release</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>μmoles FFA/hr/g lipid</td>
</tr>
<tr>
<td>Complete system</td>
<td>58.0 ± 7.8</td>
<td>−0.9 ± 0.3</td>
</tr>
<tr>
<td>Plus dibutyryl cAMP</td>
<td>49.5 ± 9.0</td>
<td>10.3 ± 3.2</td>
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<tr>
<td>Minus glucose and insulin</td>
<td>54.0 ± 5.8</td>
<td>0.5 ± 0.1</td>
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<tr>
<td>Minus glucose and insulin, plus dibutyryl cAMP</td>
<td>50.0 ± 5.2</td>
<td>20.4 ± 3.0</td>
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*Percentage of lipoprotein lipase activity of nominunibated control fat cells.
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**INCUBATION TIME (min)**

**FIG. 2.** The relationship between the onset of lipolysis and the decrease in lipoprotein lipase activity. Fat cells were incubated for the times shown at 37°C in Krebs-Ringer bicarbonate buffer containing dialyzed bovine serum albumin, 50 mg per ml, glucose, 2 mg per ml, insulin, 10 μg per ml, and dibutyryl cAMP, 1.5 mM. Control fat cells were incubated with glucose and insulin but with no dibutyryl cAMP. Following incubation the medium was removed and assayed for glycerol content. Acetone-ether powders of the fat cells were prepared and assayed for lipoprotein lipase activity. Lipoprotein lipase activity was expressed as a percentage of the activity recovered from control fat cell suspensions incubated with glucose and insulin alone. A----A, lipoprotein lipase activity; O----O, glycerol release.

**INCUBATION TIME (min)**

**FIG. 3.** The effect of dibutyryl cAMP on lipolysis, lipoprotein lipase activity, and protein synthesis. Fat cells were incubated for 60 min at 37°C in Krebs-Ringer bicarbonate buffer containing dialyzed bovine serum albumin, 50 mg per ml, glucose, 2 mg per ml, insulin, 10 μg per ml, L-leucine-1-14C, 0.5 μM (0.4 μCi per mole), and the concentrations of dibutyryl cAMP shown. After incubation 0.1 ml of the fat cell suspension was applied to filter paper discs for determination of radioactivity in protein. Incubation medium was then removed from the remaining cells and assayed for glycerol and FFA. Acetone-ether powders of the cells were prepared and assayed for lipoprotein lipase activity. O----O, control; A----A, cycloheximide; □□□, cycloheximide and dibutyryl cAMP.

**FIG. 4.** The effect of cycloheximide plus dibutyryl cAMP on fat cell lipoprotein lipase activity. Fat cells were incubated for the times shown at 37°C in Krebs-Ringer bicarbonate buffer containing dialyzed bovine serum albumin, 50 mg per ml, glucose, 2 mg per ml, insulin, 10 μg per ml, and, when used, cycloheximide, 10 μg per ml and dibutyryl cAMP, 1.5 mM. After incubation 0.1 ml of the fat cell suspensions was applied to filter paper discs for determination of radioactivity in protein. Incubation medium was removed from the remaining cells and assayed for glycerol and FFA. Acetone-ether powders of the cells were assayed for lipoprotein lipase activity. O----O, control; ▲▲▲, cycloheximide; □□□, cycloheximide and dibutyryl cAMP.

**Relationship between Protein Synthesis and Lipoprotein Lipase Activity**—Since the maintenance of adipocyte lipoprotein lipase activity requires protein synthesis, and since lipolytic agents such as epinephrine reduce the rate of protein synthesis in fat tissue (30), it was considered possible that the reduction in lipoprotein lipase activity produced by dibutyryl cAMP was secondary to an effect of the nucleotide on fat cell protein synthesis. To investigate this possibility, fat cells were incubated with varying concentrations of dibutyryl cAMP, and determinations were made of lipolytic rate, lipoprotein lipase activity, and L-leucine-1-14C incorporation into fat cell protein. As in other experiments, glycerol release occurred at lower concentrations of dibutyryl cAMP than were required to affect lipoprotein lipase activity. It is evident that dibutyryl cAMP decreased the rate of incorporation of L-leucine-1-14C into fat cell protein and that this effect and the percentage of decrease in lipoprotein lipase activity were of similar magnitude and occurred at similar concentrations of the nucleotide.

Fat cells incubated without glucose or insulin, but this occurred only after the medium albumin had become saturated with FFA.

In the next experiments the relationship in time between activation of hormone-sensitive lipase and the decrease in lipoprotein lipase activity was studied (Fig 2). Glucose and insulin were present during the incubations. When dibutyryl cAMP (1.5 mM) was added, stimulation of hormone-sensitive lipase activity, as evidenced by accumulation of glycerol in the incubation medium, occurred well before a reduction in lipoprotein lipase activity was evident. However, because of active re-esterification of FFA, in most experiments the fall in lipoprotein lipase activity occurred before the release of fatty acid into the medium.

In other experiments it was found that the minimal concentration of dibutyryl cAMP required to stimulate glycerol release was consistently slightly less than was required to decrease lipoprotein lipase activity. However, because of re-esterification of fatty acids in the presence of glucose and insulin, in most experiments the concentration of dibutyryl cAMP required to cause the release of FFA into the medium was greater than that necessary to influence lipoprotein lipase activity.
Our previous studies on the effects of glucagon, insulin, cyclic 3',5'-AMP and other cyclic nucleotides on lipase activity in liver, kidney and adipose tissue and on protein synthesis in liver and adipose tissue, suggested that cyclic 3',5'-AMP and cyclic nucleotides in general, could mediate changes in lipase activity and protein synthesis by affecting transport processes. In the study presented here the effect of dibutyryl cyclic 3',5'-AMP on lipase activity and protein synthesis in adipose tissue was examined. The results shown in Figs. 1-4, compared the effects of dibutyryl cyclic 3',5'-AMP on both lipase activity and protein synthesis of fat cell homogenates and intact fat cells. In the homogenates dibutyryl cyclic 3',5'-AMP caused a concentration dependent decrease in lipoprotein lipase activity and an increase in protein synthesis. In intact fat cells dibutyryl cyclic 3',5'-AMP caused a similar decrease in lipoprotein lipase activity, but had no effect on protein synthesis.

It was also established that dibutyryl cyclic 3',5'-AMP increased the rate of degradation of lipoprotein lipase activity by inhibiting protein synthesis, whereas cycloheximide increased lipoprotein lipase activity by inhibiting protein synthesis. However, the data did not exclude the possibility that dibutyryl cyclic 3',5'-AMP increased the rate of degradation of lipoprotein lipase activity by inhibiting protein synthesis.

The data shown in Figs. 2 and 3 supported the thesis that both hormone-sensitive lipase activity and lipoprotein lipase activity can be controlled in a reciprocal manner by cyclic 3',5'-AMP and suggested that the effect of this nucleotide on lipoprotein lipase activity was secondary to an effect on protein synthesis. However, the data did not exclude the possibility that dibutyryl cyclic 3',5'-AMP increased the rate of degradation of lipoprotein lipase activity. This possibility was investigated by determining whether dibutyryl cyclic 3',5'-AMP accelerated the decline of lipoprotein lipase activity observed when protein synthesis in fat cells was totally inhibited by incubation with cycloheximide (Fig. 4). Since the rate of decline of fat cell lipoprotein lipase activity in the presence of cycloheximide was not influenced by the presence of dibutyryl cyclic 3',5'-AMP, it is unlikely that the nucleotide influenced the rate of enzyme degradation.

To explore further the possibility that dibutyryl cyclic 3',5'-AMP decreased lipoprotein lipase activity by inhibiting protein synthesis, a comparison was made between the effects of this nucleotide and those of cycloheximide on both lipoprotein lipase activity and Leucine-1-14C incorporation into protein. A concentration of cycloheximide was chosen that would give approximately the same degree of inhibition of protein synthesis as the usual concentrations of dibutyryl cyclic 3',5'-AMP. As shown in Fig. 5, when a similar degree of inhibition of protein synthesis was achieved by these agents, there was also a similar decrease in lipoprotein lipase activity. Additional evidence that lipolytic agents decrease lipoprotein lipase activity by inhibiting protein synthesis was obtained by pooling the data of many experiments in which both lipoprotein lipase activity and protein synthesis were altered by incubation of fat cells with glucose and insulin, and those in which dibutyryl cyclic 3',5'-AMP, cycloheximide, epinephrine, or theophylline were used, or those where incubation was without glucose or insulin. The results shown in Fig. 6 are expressed as percentage of decrease from values obtained when fat cells were incubated with glucose and insulin only. The data show that a close relationship exists between changes in the rate of protein synthesis and changes in lipoprotein lipase activity and that there were no differences irrespective of whether these changes were caused by the various lipolytic agents, by cycloheximide, or by incubation without glucose or insulin.

In additional experiments it was shown that neither dibutyryl cyclic 3',5'-AMP nor cycloheximide influenced the uptake of glucose or leucine by fat cells; this finding excluded the possibility that the effect of these agents was secondary to an effect on transport. It was also established that dibutyryl cyclic 3',5'-AMP and cycloheximide had no effect on the assay of extracted lipoprotein lipase. In other experiments the lipoprotein lipase activity released by fat cells into the incubation medium was determined. It was found that when fat cells were incubated with dibutyryl cyclic 3',5'-AMP or cycloheximide, the lipoprotein lipase activity of the incubation medium decreased in parallel with the decrease in fat cell enzyme activity, indicating that the effect of these agents was not secondary to increased release of lipoprotein lipase from the cells. Also, there was no increase in trichloracetic acid insoluble radioactivity in the incubation medium following incubation of fat cells with these agents; this indicates that protein was not lost from the cells.

The data of all studies in which the relationship between protein synthesis and lipoprotein lipase activity was explored provide very suggestive evidence that rapid alterations in lipoprotein lipase activity can result from inhibition of synthesis of a protein of short half-time that is essential for enzyme activity. As pure lipoprotein lipase has not been studied, it is not known whether this protein is the enzyme itself.

**Effect of Activation of Lipolysis on Fat Cell ATP**—Although cyclic 3',5'-AMP can alter protein synthesis by causing the release of polypeptides from liver ribosomes (31), the data presented above suggested an alternate explanation of the effect of dibutyryl cyclic 3',5'-AMP on protein synthesis in intact fat cells. Small concentrations of dibutyryl cyclic 3',5'-AMP inhibited protein synthesis and decreased lipoprotein lipase activity. The results of 64 experiments in which fat cells were incubated under different conditions for 60 min and in which both protein synthesis and lipoprotein lipase activity were determined have been pooled. The results are expressed as percentage of decrease from cells incubated with glucose, 2 mg per ml and insulin, 10 mg per ml alone. - dibutyryl cyclic 3',5'-AMP, 0.3 to 1.5 mM; H, cycloheximide, 0.05 to 0.5 µg per ml; □, epinephrine, 0.1 to 5.0 µg/; O, theophylline, 1 mM; △, incubation without glucose or insulin. Probability, 0.99 ≤ P ≤ 0.97 = 0.01.

**Fig. 5.** Comparison of the effects of cycloheximide and dibutyryl cyclic 3',5'-AMP on fat cell lipoprotein lipase activity and protein synthesis. Fat cells were incubated for 60 min at 37° in Krebs-Ringer bicarbonate buffer containing dialyzed bovine serum albumin, 50 mg per ml, glucose, 2 mg per ml, insulin, 10 mg per ml, and when added, dibutyryl cyclic 3',5'-AMP, 1 mM or cycloheximide, 0.05 µg per ml. After incubation 0.1 ml of the fat cell suspension was applied to filter paper discs for determination of radioactivity in protein. The incubation medium was removed from the remaining cells, and acetone-ether powders of the cells were prepared and assayed for lipoprotein lipase activity. The results are the means ± S.E. of five experiments.

**Fig. 6.** The relationship between changes in protein synthesis and changes in lipoprotein lipase activity. The results of 64 experiments in which fat cells were incubated under different conditions for 60 min and in which both protein synthesis and lipoprotein lipase activity were determined have been pooled. The results are expressed as percentage of decrease from cells incubated with glucose, 2 mg per ml and insulin, 10 mg per ml alone. - dibutyryl cyclic 3',5'-AMP, 0.3 to 1.5 mM; H, cycloheximide, 0.05 to 0.5 µg per ml; □, epinephrine, 0.1 to 5.0 µg/ml; O, theophylline, 1 mM; △, incubation without glucose or insulin. Probability, 0.99 ≤ P ≤ 0.97 = 0.01.
synthesis only in the presence of glucose and insulin, and under these conditions there is extensive re-esterification of the fatty acid products of lipolysis. This process requires ATP (32), and it is possible that high rates of esterification might lead to the consumption of sufficient ATP to limit other ATP-requiring processes, such as protein synthesis. It is also known that in the absence of glucose and insulin, lipolytic agents reduce fat cell ATP content, an effect possibly due to accumulation of FFA or their derivatives (33). An investigation was therefore undertaken of the effects of dibutyryl cAMP on fat cell ATP content.

In the initial study, fat cells were incubated with dibutyryl cAMP in the presence and absence of glucose and insulin. Preliminary experiments showed that there was no change in fat cell ATP content during 60-min incubation whether or not glucose and insulin were present. It was also established that dibutyryl cAMP had no effect when added to the ATP assay system. However, as shown in Fig. 7, when fat cells were incubated with increasing concentrations of dibutyryl cAMP in the presence of glucose and insulin there was a marked reduction in fat cell ATP content. While dibutyryl cAMP also decreased ATP content in the absence of glucose and insulin, the latter effect occurred at much higher concentrations of the nucleotide than were required to influence ATP content when glucose and insulin were present.

It therefore seemed likely that following activation of lipolysis, both the process of esterification of fatty acids in the presence of glucose and insulin and the accumulation of fatty acids occurring in the absence of glucose and insulin could be operative in reducing fat cell ATP levels. To investigate these possibilities, experiments were done in which both the esterification rate and the intracellular FFA concentration were varied by incubation of fat cells for 60 min with dibutyryl cAMP in the presence and absence of glucose and insulin, with and without albumin in the incubation medium. The effect of incubating fat cells on intracellular FFA concentration under these various conditions is shown in Fig. 8a. When fat cells were incubated with dibutyryl cAMP in the presence of albumin a small rise in intracellular FFA concentration occurred which was not influenced by the presence or absence of glucose and insulin. However, when albumin was omitted from the incubation medium, dibutyryl cAMP caused a marked increase in the intracellular FFA concentration, and in this instance the effect of the nucleotide was more pronounced in cells incubated in the absence of glucose and insulin. Fig. 8b shows the rate of esterification of FFA that occurred in these experiments. There was no esterification of FFA by fat cells at any concentration of dibutyryl cAMP when glucose and insulin were omitted, whether or not albumin was present in the incubation medium. However, when glucose and insulin were present there was extensive esterification of the fatty acid products of lipolysis, and this occurred both in cells incubated with and without albumin. At low concentrations of dibutyryl cAMP (0.4 mM) the quantity of FFA esterified was less in cells incubated in albumin-free medium, as under these conditions the lipolytic rate was slower than when albumin was present. Fig. 8c shows the effect of these manipulations on fat cell ATP. Dibutyryl cAMP had no effect on the ATP content of fat cells incubated in albumin-containing medium without glucose and insulin.
glucose or insulin; under these conditions there was little accumulation of intracellular FFA and no esterification of FFA. When glucose and insulin were added, the progressive increase in the quantity of FFA esterified caused by increasing concentrations of dibutyryl cAMP was associated with a progressive fall in fat cell ATP content. However, when high levels of intracellular FFA were achieved by incubation with dibutyryl cAMP in the absence of glucose, insulin, or albumin there was also a profound fall in fat cell ATP, although esterification of FFA under these conditions was negligible. In this instance, at low concentrations of dibutyryl cAMP (0.4 mM), the addition of glucose and insulin had a protective effect on fat cell ATP by promoting esterification and thus preventing the accumulation of intracellular FFA.

In the next experiments, the effect on fat cell ATP of esterification of exogenous fatty acids was studied by adding sodium oleate to the incubation medium (Fig. 9). When fat cells were incubated with increasing concentrations of sodium oleate in the absence of glucose or insulin; O—O, glucose, 2 mg per ml and insulin, 10 μg per ml.

These experiments demonstrate that activation of lipolysis can result in the depletion of fat cell ATP. Under different conditions this depletion can be a result of either esterification of fatty acids or intracellular accumulation of fatty acids.

Relationship between ATP Depletion, Protein Synthesis, and Lipoprotein Lipase Activity—In the final experiments the relationship between the effects of dibutyryl cAMP on fat cell ATP and the effects of this nucleotide on protein synthesis and lipoprotein lipase activity was investigated. Fat cells were incubated for 60 min in the presence of albumin, glucose and insulin, and varying concentrations of dibutyryl cAMP (Fig. 10). As shown previously, lipoprotein lipase activity and L-leucine-1-14C incorporation into fat cell protein were decreased by approximately the same concentrations of dibutyryl cAMP. However, fat cell ATP content was reduced by lower concentrations of dibutyryl cAMP. Other experiments demonstrated that the reduction in ATP usually preceded in time, but on occasion was synchronous with, the decrease in the rate of protein synthesis.
activity requires continual synthesis of new enzyme. The fact that the relationship between the effects of dibutyryl cAMP on protein synthesis and lipoprotein lipase activity was entirely analogous to the relationship observed when cycloheximide was used strongly suggests that the effect of the cyclic nucleotide on lipoprotein lipase activity was mediated through changes in synthesis either of enzyme itself or of another protein essential for enzyme action.

However, the mechanism by which cyclic 3',5'-AMP inhibits protein synthesis in fat cells remains a matter of speculation. It is clear that dibutyryl cAMP decreases fat cell ATP and that this can occur either by consumption of ATP in the process of re-esterification of FFA or, when no glucose or insulin are present, by an effect of high intracellular concentrations of FFA as suggested by Hepp, Challoner, and Williams (33). It has been established that an increased consumption of ATP caused by the rapid phosphorylation of fructose or by the formation of S-adenosyl derivatives of ethionine can deplete the ATP content of liver, and this phenomenon is associated with inhibition of protein synthesis in this tissue (34, 35). Hence it remains possible, although unproven, that the effect of cyclic 3',5'-AMP on protein synthesis in fat cells is indirect and secondary to depletion of fat cell ATP.

In conclusion, the effect of lipolytic hormones on lipoprotein lipase activity could be the last step in a series of events beginning with activation of adenyl cyclase and the accumulation of cyclic 3',5'-AMP. This nucleotide activates hormone-sensitive lipoprotein lipase resulting in the production of FFA. In the presence of glucose and insulin some of the FFA are re-esterified to triacylglycerol, leading to an increased consumption of ATP, hence to a decrease in the rate of protein synthesis and to a consequent reduction in lipoprotein lipase activity.

Such a regulatory mechanism would be physiologically meaningful as it would lead to a reduction in triglyceride uptake by adipose tissue during periods of fat mobilization, increasing the triglyceride available to other tissues. Conversely it could permit an increase in triglyceride uptake by adipose tissue during periods when the process of lipolysis was inactive.

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