Potentiation by Glucose of Lipolytic Responsiveness of Human Adipocytes

COLETTE MOUSSALLI, ROBERT W. DOWNS, AND JAMES M. MAY

SUMMARY

The effect of glucose on lipolytic regulation was studied in isolated human adipocytes. Glucose enhanced adipocyte glycerol release in the presence and absence of the β-adrenergic agent ritodrine by 150–200% of control rates. The glucose effect was maximal at just >1 mM glucose and could not be attributed to prevention of a time-dependent decline in lipolysis. Glucose not only increased lipolytic stimulation at each of several concentrations of ritodrine but also enhanced the sensitivity to stimulation at low concentrations of the agent. Ritodrine-stimulated lipolysis was inhibited by insulin by 50–60%; although glucose increased absolute rates of lipolysis, it did not affect the relative inhibition of lipolysis by insulin or the sensitivity to the hormone. In investigating a possible cause of the glucose effect on lipolysis, it was found that the addition of adenosine deaminase increased lipolytic rates in the absence of glucose and blunted the relative stimulation of lipolysis by glucose, the latter implicating extracellular adenosine in the mechanism of the glucose effect. DIABETES 1986; 35:759–63.

Glucose paradoxically increases both the synthesis and breakdown of triglyceride in adipocytes prepared from rat adipose tissue.[1-7] This acceleration of triglyceride turnover or cycling results from the ultimate conversion of glucose carbon to fatty acid and the glycerol backbone of triglyceride on one hand and from the stimulation of lipolysis on the other. The mechanism for the lipolytic effect of glucose is unknown but has been attributed to removal of inhibitory intracellular free fatty acids by reesterification with glucose-derived glycerol 3-phosphate[1,2] or to repletion of ATP stores consumed during lipolysis.[4] Whether glucose actually stimulates lipolysis or merely prevents a time-dependent decrease in lipolytic rates is uncertain.[2,6] Early studies with fragments of human fat also showed that glucose enhanced basal and isoprenaline-stimulated lipolysis.[8] However, more recent results in isolated fat cells from the same laboratory failed to confirm the lipolytic effect of glucose.[9]

Our studies were performed to determine whether glucose plays a role in the regulation of lipolysis in human subcutaneous adipocytes. We found that low concentrations of glucose did in fact enhance basal and stimulated lipolytic rates in human cells and that extracellular adenosine may be involved in the effect.

From the Division of Endocrinology and Metabolism, Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Address reprint requests to Dr. James M. May, Box 111, MCV Station, Richmond, VA 23298.

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MATERIALS AND METHODS

Materials. Collagenase (type III) was obtained from Worthington (Freehold, NJ), ritodrine hydrochloride was a gift from Phillips-Duphar (Weesp, Holland), and bovine serum albumin (fraction V) was purchased from Sigma (St. Louis, MO). The albumin was extensively dialyzed as previously described[10] and was treated by the method of Chen[11] to remove bound fatty acid. The buffer for all studies was Krebs-Henseleit Hepes, pH 7.4.[12] Enzymes were obtained from Boehringer-Mannheim (FRG).

Preparation of isolated human adipocytes. Subcutaneous abdominal adipose tissue was obtained from healthy women either at the time of elective surgery or by suction lipectomy. All subjects were under 45 yr of age, were within 20% of ideal body weight as defined by the Metropolitan Life Insurance Tables, had no known disorder of carbohydrate or lipid metabolism, and were taking no interfering medications. Surgical procedures included hysterectomies, exploratory laparotomies, and ovarian cystectomies. For surgical procedures, anesthesia was general, and tissue was obtained at initiation of surgery. Local skin anesthesia was used for lipectomies; no deep infiltration with epinephrine or hypotonic saline was employed before removal of adequate tissue for the studies. There was no difference in cell size, rates of

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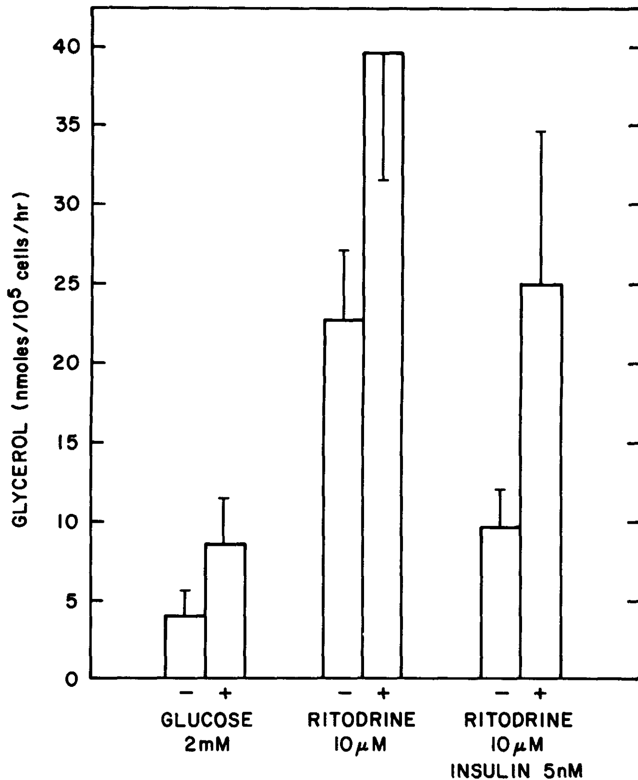

FIGURE 1. Stimulation of lipolysis by glucose. Adipocytes were incubated for 1 h in presence (+) or absence (−) of 2 mM glucose and agents as indicated. Rates of lipolysis were measured as described in MATERIALS AND METHODS. Combined data from 6 experiments are shown. Glucose effect was significant across the 3 different conditions with $P < .01$ (two-way ANOVA with replication) as well as within each condition with $P < .05$ (paired Student's t test).

basal or ritodrine-stimulated lipolysis, or the antilipolytic effect of insulin between cells obtained by the two methods (not shown). All subjects gave informed consent under the protocol approved by the Medical College of Virginia Human Subjects Review Committee.

Approximately 5–10 g of fat tissue was placed immediately in normal saline and processed within 20 min. The tissue was washed in saline and minced in 4 ml of Hepes buffer containing 40 mg/ml bovine serum albumin and 1 mg/ml collagenase. Digestion was performed at 37°C for 60–90 min in a shaking water bath (140 cycles/min). The digest was filtered through 400-μM-aperture nylon mesh (Tetko, Elmsford, NY) and washed with 10 ml of 37°C albumin buffer. The cells were allowed to float to the surface, the infranatant was carefully removed with a plastic catheter, and the washing was repeated twice more with 10 ml of buffer. Cells were counted by the hemocytometer method of Gliemann,[13] and the final cell suspension was adjusted to 50,000 cells/ml. As noted by Kashiwagi et al.,[14] it was important in preparing the cells to use only polyethylene or polypropylene containers, not to centrifuge the cells, and to continuously agitate them gently at 37°C to prevent aggregation.

Assays. Lipolysis was estimated by glycerol release from 10^5 cells incubated in 2 ml of 40 mg/ml albumin buffer at 37°C with incubation times and additives as noted. Glycerol was measured by the method of Chernick[15] in triplicate aliquots of 0.5 ml of cell-free buffer.

RESULTS

The effect of glucose on adipocyte glycerol release is shown in Figure 1. Basal rates of lipolysis were more than doubled by the addition of 2 mM glucose. Whereas ritodrine increased lipolytic rates severalfold in the absence of glucose, ritodrine-stimulated lipolysis was almost doubled by 2 mM glucose. A maximal amount (5 nM) of insulin inhibited ritodrine-stimulated lipolysis by 40–50% regardless of whether glucose was present. Nevertheless, glucose addition increased absolute rates of lipolysis even in the presence of insulin. In experiments not shown, there was no effect of glucose on the sensitivity of antilipolysis to increasing doses of insulin, because the half-maximal inhibition of lipolysis occurred at 0.2 nM insulin in the presence or absence of 2 mM glucose. In two experiments, also not shown, the time course of lipolysis was linear for at least 1 h, and at least a doubling of lipolysis was evident in cells incubated with glucose from the earliest time point (20 min). This confirms that glucose had actually stimulated lipolysis and not just maintained it at later time points.

The lipolytic response to increasing amounts of glucose is illustrated in Figure 2. Enhancement of ritodrine-stimulated lipolysis (expressed relative to lipolytic rates with ritodrine alone) increased progressively up to a glucose concentration of ~1 mM, then plateaued. The glucose effect was therefore maximal at subphysiologic glucose concentrations.

Lipolytic responses to increasing amounts of ritodrine in the presence and absence of glucose were evaluated as shown in Figure 3. Not only were lipolytic rates greater in the presence of glucose at all ritodrine concentrations, but the relative effect of glucose was much greater at low ritodrine concentrations, indicating a leftward shift in the dose-response curve. Glucose, therefore, not only enhanced lipolytic responsiveness but also its sensitivity to β-adrenergic stimulation in human adipocytes.

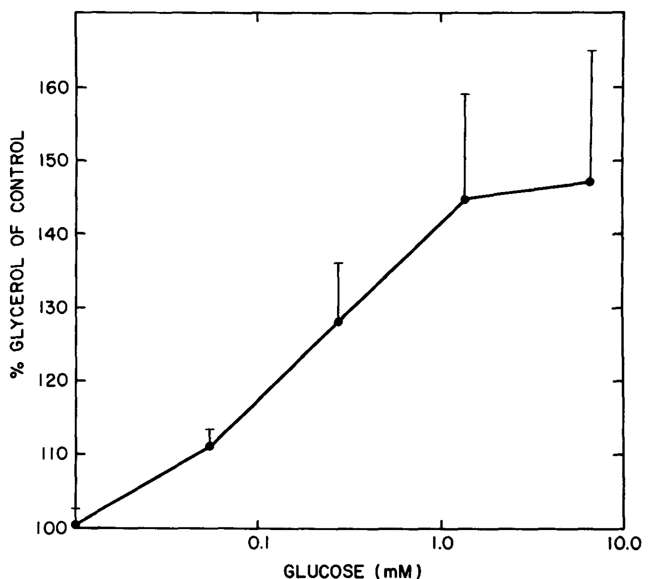

FIGURE 2. Effect of glucose concentration on stimulated lipolysis. Adipocytes were incubated for 1 h at 37°C with 10 μM ritodrine and indicated concentration of glucose. Values at each glucose concentration are expressed as percent of rate obtained with ritodrine alone (86 ± 25 nmol · 10^5 cells^{-1} · h^{-1}) ± SE for 5 experiments. Glucose was significant with $P < .05$ by one-way ANOVA with replication.

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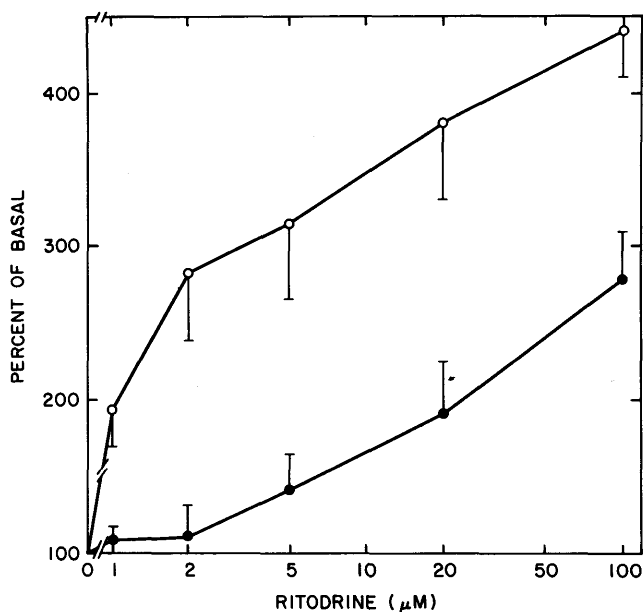

FIGURE 3. Effect of glucose on lipolysis stimulated by increasing concentrations of ritodrine. Adipocytes were incubated for 1 h with indicated concentration of ritodrine in presence (○) or absence (●) of 2 mM glucose. Values from 4 experiments are expressed as percent of basal rates of lipolysis ±SE measured in presence or absence of glucose, respectively. Glucose effect was significant with $P < .01$ by two-way ANOVA with replication.

Because cell concentrations were relatively high in these studies (50,000/ml), the glucose effect may have been related to some factor produced by the cells, such as adenosine. If glucose prevented the breakdown of ATP to adenosine, extracellular concentrations of the nucleoside would be lowered and lipolysis removed from tonic inhibition. In fact, as shown in Figure 4, the addition of adenosine deaminase enhanced both glucose- and ritodrine-stimulated lipolysis, suggesting that tonic inhibition of lipolysis was indeed present under these conditions. Thus, any effect of glucose on adenosine concentrations must have been small compared with that of adenosine deaminase. The relative effect of adenosine deaminase in the presence of various concentrations of glucose is shown in Figure 5. Lipolysis in the presence of glucose and ritodrine was expressed as a percent of rates in cells treated with ritodrine alone (*open circles*). A similar percentage calculation derived from lipolytic rates in cells treated with glucose, adenosine deaminase, and ritodrine versus rates in cells treated with adenosine deaminase and ritodrine is also shown (*closed circles*). The relative glucose stimulation was less at all glucose concentrations when adenosine deaminase was present. Such a decrease would be expected if glucose enhanced lipolysis by lowering adenosine release; i.e., removal of most if not all of the adenosine by adenosine deaminase would obscure any glucose-induced fall. On the other hand, because glucose was still capable of stimulating lipolysis to some extent when adenosine deaminase was present, the glucose effect was not due entirely to decreased cellular release of adenosine. It is also possible that the relative glucose effect was lessened in the presence of both ritodrine and adenosine deaminase because maximal rates of lipolysis had been reached.

DISCUSSION

The major finding of our work was that low concentrations of glucose enhanced lipolysis in isolated human adipocytes. Although rates of lipolysis were variable between subjects, the stimulation by glucose was consistently 150–200% of rates in cells incubated without glucose. This effect of glucose persisted even when lipolysis was stimulated with the β-adrenergic agent ritodrine or when ritodrine-stimulated lipolysis was inhibited with insulin (Figure 1). In at least two studies in rat adipocytes, glucose prevented a late decline in stimulated lipolytic rates, thus making the "stimulation" of

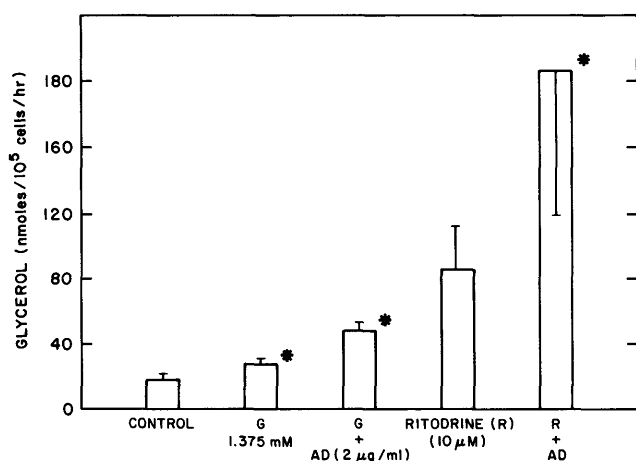

FIGURE 4. Stimulation of lipolysis by adenosine deaminase. Adipocytes were incubated for 1 h with indicated concentrations of glucose (G), ritodrine (R), and adenosine deaminase (AD), with glycerol release measured as described in MATERIALS AND METHODS. Results shown are from 5 experiments. *Asterisk* indicates $P < .05$ by the paired Student's *t* test compared with adjacent control.

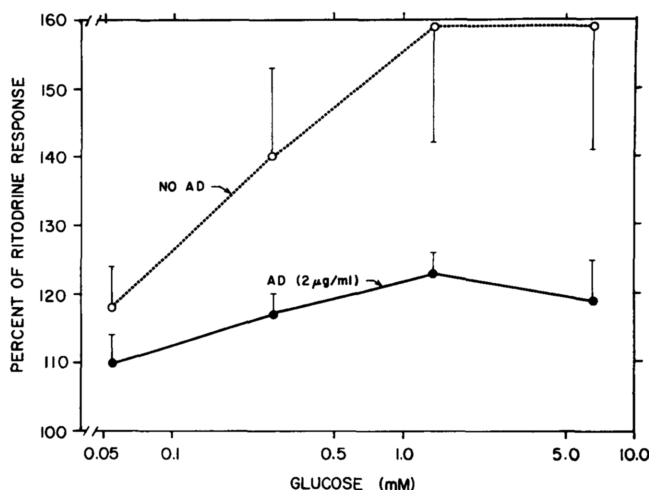

FIGURE 5. Relative effect of adenosine deaminase on ritodrine-stimulated lipolysis in presence or absence of glucose. Adipocytes were incubated for 1 h with 10 μM ritodrine, indicated concentration of glucose, and with or without adenosine deaminase (AD) as shown. Data from 6 experiments are expressed as percent of rates in cells treated with ritodrine and adenosine deaminase alone (●) or with ritodrine alone (○) for each glucose concentration, with SE indicated. Effect of adenosine deaminase was significant at $P < .01$ across glucose concentrations by two-way ANOVA with replication.

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lipolysis a function of the time of sampling.[2,6] In our studies, however, the potentiating effect of glucose on lipolysis was not an artifact of the time of sampling, because relatively linear rates of lipolysis were enhanced at every time point up to 60 min. The maximal effect was attained at ~1 mM glucose (Figure 2).

Although the effect of glucose on lipolysis was evident in the presence or absence of insulin (Figure 1), and glucose enhanced both the sensitivity and maximal responsiveness to stimulation by ritodrine (Figure 3), neither sensitivity nor responsiveness to the antilipolytic effects of insulin was modified. These results are logical from the viewpoint that under our conditions, the general effect of glucose is to promote lipolysis and lipolytic sensitivity. In contrast, at the low cell concentrations used by Arner et al.,[9] glucose did not enhance basal or stimulated lipolysis but did improve the sensitivity to the antilipolytic effects of insulin. Although the results of both studies are internally consistent, the markedly different findings emphasize the need to understand the mechanism of the glucose effect on lipolysis as modulated by different incubation conditions.

Although several hypotheses have been suggested regarding the cause of the potentiation of lipolysis by glucose in rat adipocytes, none has been proven. The effect has been attributed to glucose-induced reesterification of intracellular free fatty acids,[1–3] thus decreasing their concentrations and preventing their well-known inhibition of lipolytic activity.[2,16–18] On the other hand, two other studies failed to show that glucose actually does decrease total intracellular free fatty acids in adipocytes.[7,19] Another possibility is that the metabolism of glucose causes a change in the concentration of a factor that in turn potentiates lipolysis.[5] Glucose has been shown to maintain intracellular ATP concentrations during rapid lipolysis,[4,6] which in turn could prevent an energy-related decline in lipolysis. However, in perifused adipocytes, glucose did not prevent the decline in intracellular ATP concentrations induced by an uncoupler of oxidative phosphorylation when present during epinephrine-stimulated lipolysis, whereas it did allow the inhibited rates of lipolysis to return to control levels with removal of the uncoupler.[20] This makes a direct role for ATP unlikely under such conditions.

Glucose could also modify the concentration of adenosine, which accumulates when ATP is degraded and, in rat adipocytes, is well known to inhibit basal and stimulated lipolysis at cell concentrations similar to those used in some studies.[21,22] Moreover, adipocytes appear to be very sensitive to adenosine, because addition of adenosine deaminase to a perifused adipocyte system (in which extracellular buffer is constantly removed) caused an 80% increase in epinephrine-stimulated lipolysis.[23] In this regard it is important to compare the experimental conditions used in studies of the effects of glucose on basal and stimulated rates of lipolysis in human adipocytes. Although the early studies from Östman's laboratory used pieces of omental fat rather than adipocytes prepared from subcutaneous fat, a stimulatory effect of glucose on lipolysis was evident.[8] In the more recent studies from the Swedish group[9] a sensitive bioluminescent assay for glycerol was used, allowing measurement of glycerol in one-third the number of cells as in the current work and certainly fewer than in their earlier studies.[8] Under such conditions there was little accumulation and, thus, effect of adenosine.[24] An adenosine effect did in fact appear to influence our results, because adenosine deaminase, which converts adenosine to the less active inosine, was capable of increasing both basal and stimulated rates of lipolysis (Figure 4). Glucose may have lowered adenosine release from cells by preventing the breakdown of ATP to adenosine. A fall in extracellular adenosine would then enhance lipolysis by decreasing the tonic inhibition of lipolysis mediated via the adenosine receptor.[21,22] We were able to show that adenosine deaminase did decrease the relative enhancement of ritodrine-stimulated lipolysis by glucose, thus supporting a role for adenosine. On the other hand, the effect of glucose was not entirely abolished in the absence of adenosine (Figure 5). Moreover, a decreased relative effect of glucose could also be due to the fact that lipolytic rates in the presence of ritodrine and absence of adenosine were already near maximal. Clearly, although we have presented indirect evidence that the glucose effect could be due to extracellular adenosine, further studies are necessary to determine the extent to which adenosine contributes to the effect.

In conclusion, it is possible to show that low concentrations of glucose enhance both basal and stimulated rates of lipolysis in human subcutaneous adipocytes and that the effect may be due at least in part to inhibition of adenosine accumulation by glucose. Whatever its cause, this effect of glucose may be an important factor in the regulation of adipocyte lipolysis.

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