Regulation of Lipoprotein Lipase by Glucose in Primary Cultures of Isolated Human Adipocytes

Relevance to Hypertriglyceridemia of Diabetes

PHILIP A. KERN, ANGELA MANDIC, AND ROBERT H. ECKEL

SUMMARY

Human adipose tissue lipoprotein lipase (LPL) is stimulated in vivo by an insulin-glucose infusion. However, previous work by us showed no effect of physiologic insulin concentrations on LPL in isolated human adipocytes. To pursue further the regulation of LPL in vitro, primary cultures of isolated human adipocytes were prepared and exposed to glucose concentrations of 0–4.5 mg/ml. LPL activity was measured as activity secreted into the culture medium (CM), released from cells by heparin (HR), and extracted from cell digests (EXT). After 5 h in culture, a stimulatory effect of glucose on HR was observed. After 24 h there was a gradual increase in CM, HR, and EXT in parallel with increasing glucose concentrations of 0–1.0 mg/ml. At glucose concentrations >1.0 mg/ml, however, there was a decrease in CM. At a glucose concentration of 4.5 mg/ml, CM was only 51 ± 14% ($P < .02$) of its value at glucose concentrations of 1.0 mg/ml. Cellular LPL (HR and EXT) was not affected by high glucose concentrations. Response of cellular LPL to the hormonal regulator insulin-like growth factor I (IGF-I) was modulated by medium glucose. HR in cultures treated with 50 ng/ml IGF-I was 166 ± 40 and 147 ± 23% of HR in control cultures at glucose concentrations of 1.0 and 2.5 mg/ml, respectively ($P \leq .05$). However, IGF-I failed to stimulate HR at glucose concentrations >2.5 mg/ml or <1.0 mg/ml. Adipocyte protein synthetic rate was not inhibited by medium containing 4.5 mg/ml glucose, suggesting that these high glucose concentrations were not generally toxic to cells. The hexoses galactose, L-glucose, and 3-O-methylglucose had no effect on LPL, but pyruvate and D-glucose stimulated HR. Cellular ATP was relatively constant regardless of medium conditions and bore no relationship to LPL. Thus, glucose plays an important role in the regulation of LPL in isolated human adipocytes. In addition, the decrease in CM and the blunted response of HR to IGF-I in high glucose suggest that hyperglycemia per se may contribute to the decreased LPL and consequent hypertriglyceridemia of poorly controlled diabetes.

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Adipose tissue lipoprotein lipase (LPL) hydrolyzes the core of triglyceride-rich lipoproteins into free fatty acids and monoacylglycerol (1). These free fatty acids are the major source of substrate for adipocyte triglyceride synthesis because adipocytes synthesize very little free fatty acid de novo (2). LPL is synthesized in the adipocyte, secreted, and transported to the capillary endothelium where triglyceride hydrolysis takes place (1). Because LPL is localized to several sites in adipose tissue, studies on LPL regulation can provide especially useful information when carried out in isolated cells.

LPL is decreased in adipose biopsies and in postheparin plasma in most patients with poorly controlled diabetes mellitus complicated by hypertriglyceridemia (3–5). In addition, LPL increases when these patients are placed on appropriate therapy. When insulin and glucose are infused into normal subjects, adipose LPL also increases (6). Together with data from other studies (reviewed in refs. 7 and 8), these observations in diabetic patients and normal subjects suggest that insulin is an important regulator of LPL. Recent studies have confirmed the important role of insulin in rat adipocytes by demonstrating an increase in LPL after insulin is added in vitro (9,10). When similar experiments were carried out in human adipocytes (11), however, insulin stimulated LPL only at a very high concentration (400 ng/ml). Thus, rat adipocytes respond to insulin differently from human cells, and human adipose cells respond differently depending on whether insulin is administered in vivo or in

From the Department of Medicine, Cedars-Sinai Medical Center (P.A.K., A.M.), Los Angeles, California, and the Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado.

Address correspondence and reprint requests to Philip A. Kern, MD, Division of Endocrinology, Room 1735, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

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vitro (6). Because of the close links between insulin levels and glucose metabolism, we wondered whether glucose independently regulates adipocyte LPL.

To approach this question, we studied LPL activity in primary cultures of isolated human adipocytes that were exposed to a spectrum of glucose concentrations under a variety of conditions. Together, these data complement observations in other systems and provide new insights into the mechanism of decreased LPL in poorly controlled diabetes.

MATERIALS AND METHODS

Preparation of adipocytes. Cells were prepared from omental adipose tissue from 38 patients undergoing elective surgery for benign conditions. Patients gave informed consent, were aged 21–57 yr (mean 37 yr), and were <132% (12) of ideal body weight (mean ± SE 100 ± 2.5%). Mean body mass index was 22.5 ± 0.52 kg/m^2. No patient was taking any drug known to affect lipid metabolism, had diabetes, or was chronically ill.

Preparation of human adipocytes generally followed the method of Rodbell (13) and has been described previously (11). Briefly, the fat was minced and digested in Krebs-Ringer bicarbonate buffer containing 2 mg/ml collagenase II (Worthington, Freehold, NJ) at 37°C until the digestion was almost complete (~30–60 min). After filtering out the undigested pieces, adipocytes were separated from stromal cells by flotation. Cells were washed twice in medium 199 containing 10% fetal bovine serum (Irvine, Santa Ana, CA) and twice in serum-free medium 199. A final wash was performed with glucose-free medium 199 before placing a 0.075 ml aliquot of cells into polypropylene tubes containing 0.75 ml of the desired medium. Cells were then incubated in a tissue culture incubator at 37°C and 5% CO_2. Each experiment was carried out with cells from each patient.

Measurement of LPL. The LPL activity against an emulsified [1-^{14}C]triolein substrate was measured as described previously (11). LPL activity that was spontaneously secreted into the culture medium (CM) was first assayed. Cells were then washed, and LPL was released from the cells (HR) with 13 μg/ml heparin (Fisher, Springfield, NJ) in Krebs-Ringer phosphate buffer. Cells were then transferred to a microhomogenizer, washed, and disrupted in buffer containing 0.5% deoxycholate, 0.02% Nonidet P 40, and 125 μg/ml heparin as described previously (10). After spinning up the lipid cake, the extracted LPL (EXT) was assayed. LPL activity was expressed as nanoequivalents of free fatty acid released per minute per 10^6 cells. Cell number was determined by fixing cells in osmium tetroxide and counting them in a Coulter counter (14) or by staining with methylene blue and counting in a hemacytometer.

Incorporation of [^{35}S]methionine into protein. Isolated adipocytes were prepared and incubated overnight in medium 199 containing 10% fetal bovine serum. The next day, cells were washed and placed in 2 ml medium 199 containing 10% dialyzed fetal bovine serum, 30 μCi [^{35}S]methionine, and glucose concentrations of 1.0 or 4.5 mg/ml. Methionine concentration of medium 199 was 0.2 mM. At 30 min and 1, 2, and 4 h an aliquot of cells was removed, washed, and homogenized in 1 ml of cold 10% trichloroacetic acid (TCA). After 4 h at 4°C the precipitated proteins were pelleted, washed twice with cold acetone, dissolved by heating in 2% sodium dodecyl sulfate, and counted in a liquid scintillation counter.

ATP assay. The ATP was measured with firefly luciferase-luciferin (Sigma, St. Louis, MO) as described previously (15). Adipocytes that had been cultured overnight were disrupted and delipidated as described above for EXT activity; 0.8 mg luciferase-luciferin reagent was added to a 0.1-ml sample of adipocyte extract, and maximum fluorescence was recorded in an Aminco luminescence photometer. The ATP standards were diluted in the same buffer and assayed in parallel. Data are expressed as nanomoles ATP per 10^6 cells.

Statistics. All data are expressed as means ± SE, with the number of experiments shown or stated. Data were analyzed nonparametrically with the Wilcoxon matched-pairs signed-ranks test. Although some data are expressed as a percent of control, statistical analysis was performed on paired experiments with the raw data.

RESULTS

As described previously (11), LPL production by isolated cultured human adipocytes increased shortly after the cells were placed in culture medium containing glucose (1 mg/ml). Thus, to study the effects of other glucose concentrations on LPL, primary cultures were exposed to different glucose concentrations during this initial 24-h period.

Time course and dose response. To determine how much time is required for glucose to affect adipocyte LPL, cells from three patients were cultured in medium containing glucose concentrations of 0–4.5 mg/ml, and HR was measured 3, 5, and 24 h after preparation (Fig. 1). Soon after preparation (3 h), there was no discernible effect of glucose on HR. However, 3–5 h after preparation, cells in medium containing glucose demonstrated a more pronounced rise in HR

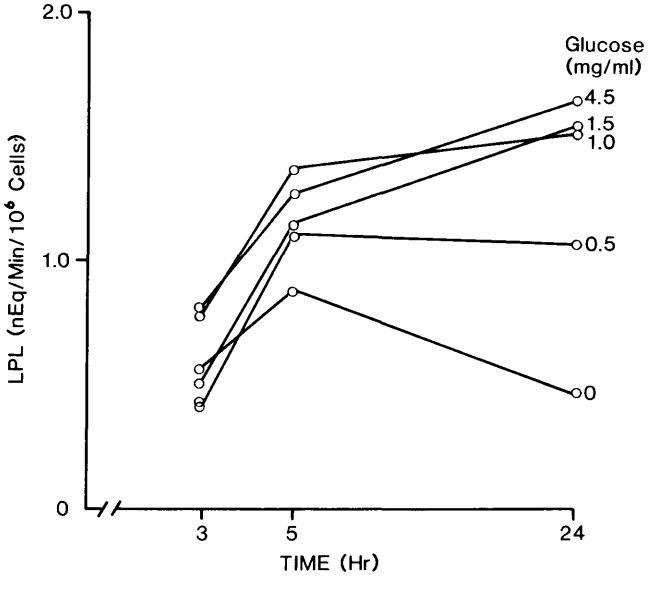


FIG. 1. Time course of glucose effect on heparin-releasable activity (HR). Adipocytes were prepared as described in MATERIALS AND METHODS and cultured in medium 199 containing 10% dialyzed fetal bovine serum and indicated glucose concentrations. HR was assayed 3, 5, and 24 h after preparation. Data are means of 3 experiments. LPL, lipoprotein lipase.

TABLE 1
Cell number and lipoprotein lipase (LPL) production in presence and absence of glucose

	No glucose			1 mg/ml glucose		
		Total cell LPL (neq/min per 10^6 cells)			Total cell LPL (neq/min per 10^6 cells)	
Experiment	Cell no. ($\times 10^{-5}$)	3 h	24 h	Cell no. ($\times 10^{-5}$)	3 h	24 h
1	0.70	0.71	0.96	0.62	0.54	2.20
2	1.02	1.00	0.80	0.95	1.06	2.35
3	1.06	0.21	1.18	1.25	0.58	1.95

Cell number determined after 24 h of culture; total cell LPL is sum of cellular and secreted LPL (11) measured 3 and 24 h after cell preparation.

than those in the glucose-free cultures. At 24 h the effect of glucose was most apparent and maximally separated HR according to medium glucose concentration (Fig. 1). Thus, adipocytes from these three patients required a prolonged exposure to glucose to affect LPL. In further experiments, therefore, a 24-h exposure to different glucose concentrations was used.

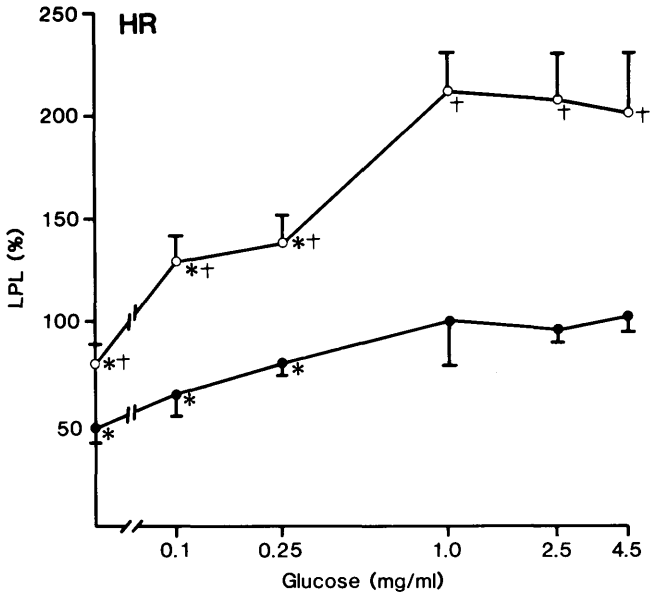
LPL synthesis. To demonstrate that this increase in HR was due to an increase in cell LPL production, total cell LPL (cellular and secreted activity) was measured in freshly prepared cells and again after 24 h of culture (Table 1). Shortly after cell preparation, glucose had little effect on total cell LPL. After 24 h of culture, however, there was a two- to threefold increase in total cell LPL in the glucose-containing cultures and only small, inconsistent changes in the glucose-free cultures. This was not related to cell breakage, because there was no difference in cell number in the presence or absence of glucose.

Glucose-serum interactions and cell LPL. The effect of glucose on cellular LPL at 24 h in the presence and absence of fetal bovine serum is illustrated in Fig. 2. Glucose had similar effects on HR both in the absence and presence of serum. These effects included an increase in HR with increasing glucose up to a concentration of 1.0 mg/ml. Thus, in the presence of 10% dialyzed fetal bovine serum, HR in glucose-free medium was 42 ± 6% of HR in medium containing 1 mg/ml glucose. Similarly, in the absence of serum, HR in glucose-free medium was 48 ± 7% of HR in medium containing 1.0 mg/ml glucose. Increases in glucose concentration to >1.0 mg/ml did not increase HR activity further, and the response of LPL to glucose was not affected by fat cell size or the subjects' body mass index. Serum stimulated HR at all concentrations, even in the absence of glucose. As with HR, EXT varied with the glucose concentration, although the stimulatory effect of glucose was less pronounced (Fig. 2). In both the absence and presence of serum, EXT in glucose-free medium was ~72% of EXT in medium containing 1.0 mg/ml glucose. In addition, serum stimulated EXT in both the absence and presence of glucose. Glucose at 4.5 mg/ml did not significantly affect EXT compared with glucose concentration of 1.0 mg/ml. Thus, human adipocytes incubated in medium 199 containing a wide range of glucose concentrations increased cellular LPL, and this increase was most pronounced after 24 h in culture. In addition, LPL was still measureable after 24 h in the absence of glucose and could be stimulated by serum.

In the above experiments, LPL was assayed in cells that were in the same medium since the time of preparation. To determine how HR would respond to acute changes in medium glucose, cells were cultured overnight in serum-free medium containing glucose concentrations of either 0 or 1.0 mg/ml, and then medium was changed to either the same or the opposite medium. The HR was then measured 4 h after the medium change (Fig. 3). Cells that were initially incubated in a glucose concentration of 1.0 mg/ml and changed to glucose-free medium showed a prompt fall in HR. In contrast, cells incubated in glucose-free medium demonstrated an increase in HR when changed to medium containing glucose. A slight but statistically insignificant increase in HR was seen when cells cultured without glucose were changed into fresh medium that also contained no glucose (Fig. 3). Thus, adipocytes that had been in culture for 24 h retained the ability to respond to acute changes in medium glucose. In addition, the responsiveness of the cells cultured initially in 0 mg/ml glucose suggests that adipocytes can survive and produce LPL in culture without the availability of glucose.

Glucose and LPL secretion. Within several hours after the initiation of primary culture in serum-containing medium (11), human adipocytes spontaneously secrete LPL activity into the medium. In the absence of serum there is no detectable activity in the medium. Because the secreted LPL activity has a finite half-life (11), CM probably represents a steady state of new enzyme secretion and enzyme degradation or denaturation. To determine whether glucose regulates CM, cells were cultured for 24 h in a spectrum of glucose concentrations and CM was measured. Between 0 and 1.0 mg/ml, CM increased with increasing glucose concentrations (Fig. 4). In glucose-free medium, CM was 30 ± 7% of CM in medium containing 1.0 mg/ml glucose. Hence, the rise in CM paralleled the rise in cellular LPL between glucose concentrations of 0 and 1.0 mg/ml. At higher glucose concentrations, however, CM did not remain stable but fell by ~50% (Fig. 4).

To determine whether this effect of high glucose levels would occur after an acute medium change, cells were cultured overnight in medium 199 containing 1.0 mg/ml glucose and 10% fetal bovine serum. Medium was then changed to either the same medium or to medium containing glucose concentrations of 1.5, 2.5, or 4.5 mg/ml, and CM was assayed 4 h later (Fig. 5). As with the results from the 24-h cultures, CM at 4 h after the medium change was lower in



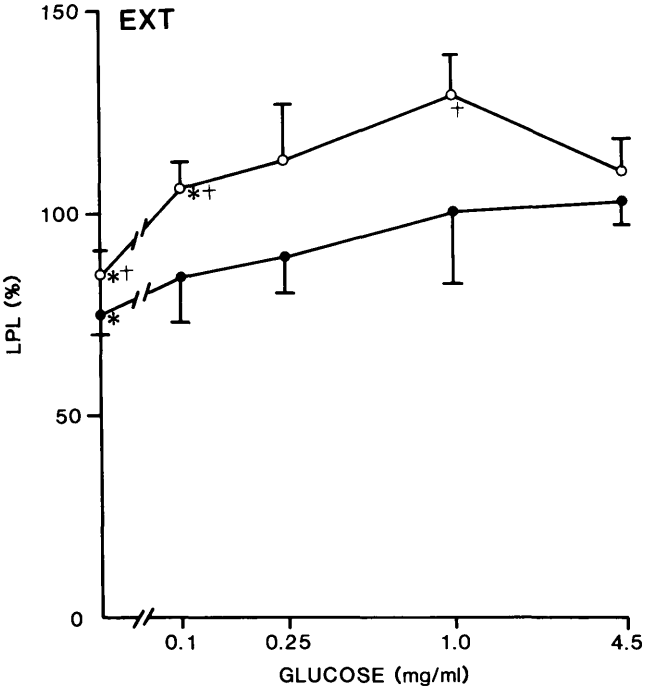


FIG. 2. Effect of glucose concentration on cellular lipoprotein lipase (LPL) at 24 h. Adipocytes were cultured for 24 h in medium 199 containing different glucose concentrations in presence (○) or absence (●) of 10% dialyzed fetal bovine serum. Each *point* represents $n \geq 5$ experiments and is expressed as percent of activity at glucose concentration of 1.0 mg/ml without serum [1.29 ± 0.28 and 1.25 ± 0.22 neq/min per 10^6 cells for heparin-releasable activity (HR) and extractable activity (EXT), respectively]. *$P < .05$ vs. glucose concentration of 1.0 mg/ml with same serum concentration. †$P < .05$ vs. no serum at same glucose concentration.

cells changed to medium containing 2.5 or 4.5 mg/ml glucose. In contrast, the medium change had no effect on HR or EXT. Thus, medium glucose concentration had a biphasic effect on CM. At low to physiologic concentrations, CM reflected cellular LPL activity and increased with increasing glucose concentrations. At high glucose concentrations, however, CM fell despite a maintenance of cellular activity.

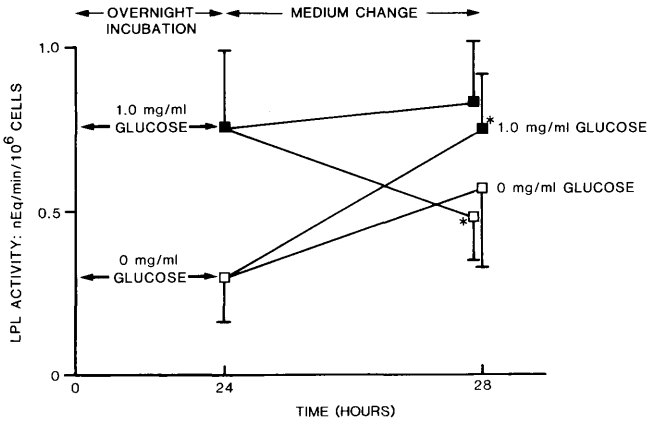


FIG. 3. Effect of medium change on heparin-releasable activity (HR). Cells were cultured overnight in serum-free medium containing glucose concentrations of either 0 or 1.0 mg/ml. HR was measured and then medium was changed to either glucose-containing (1.0 mg/ml) or glucose-free medium; HR was then assayed 4 h later. Data are means ± SE of 9 experiments. LPL, lipoprotein lipase. *$P < .05$ vs. HR before medium change.

Glucose and insulin-like growth factor I (IGF-I) effects. In addition to independently regulating LPL, glucose may modulate the effect of other substances on LPL. One substance that has previously been shown to stimulate LPL in cultured human adipocytes is IGF-I/somatomedin C, which produced a dose-dependent increase in HR (11). To assess the effect of glucose on the IGF-I–mediated increase in HR, cells were cultured in serum-free medium containing glucose concentrations of 0–4.5 mg/ml in the presence of 50 ng/ml IGF-I (AMGen, Thousand Oaks, CA). Control cultures were

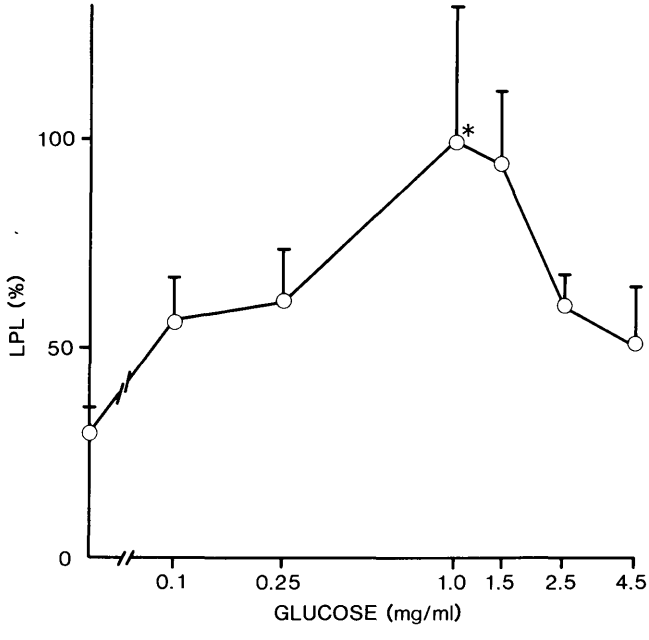


FIG. 4. Effect of glucose concentration on culture medium activity. Cells were cultured for 24 h in medium 199 containing 10% dialyzed fetal bovine serum plus a spectrum of glucose concentrations. Lipoprotein lipase (LPL) was then assayed from medium. Each *point* represents mean ± SE of at least 4 experiments and is expressed as percent of value at glucose concentration of 1.0 mg/ml (1.50 ± 0.50 neq/min per 10^6 cells). *$P < .05$ vs. values at glucose concentrations of 0, 0.1, 0.25, and 4.5 mg/ml.

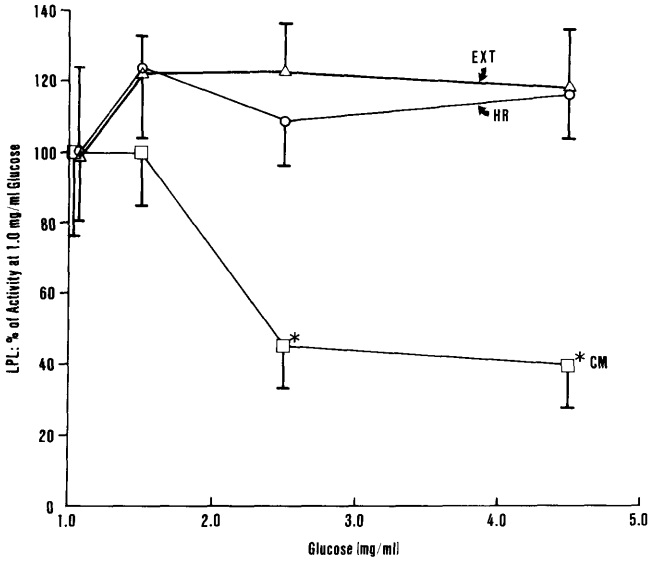


FIG. 5. Acute effect of high-glucose medium on culture medium activity (CM). Adipocytes were cultured overnight in medium 199 containing 10% serum and 1.0 mg/ml glucose. Medium was then changed to medium 199 containing serum and glucose concentrations of 1.0, 1.5, 2.5, and 4.5 mg/ml. CM, heparin-releasable activity (HR), and extractable activity (EXT) were assayed after 4 h in these new media. Data are expressed as percent of activity in control culture, in which medium was changed to 1.0 mg/ml glucose (0.96 ± 0.26, 0.60 ± 0.15, and 0.31 ± 0.08 neq/min per 10^6 cells for EXT, HR, and CM, respectively). Each *point* represents at least 6 experiments. LPL, lipoprotein lipase. *$P < .05$ vs. value at glucose concentration of 1.0 mg/ml.

incubated in medium containing the same glucose concentrations but without IGF-I. As shown in Fig. 6, IGF-I failed to stimulate HR in the presence of glucose concentrations of 0, 0.25, and 0.50 mg/ml. At a glucose concentration of 1.0 mg/ml, IGF-I stimulated a 66% increase in HR. With higher glucose concentrations, however, the IGF-I effect diminished. At glucose concentrations of 2.5 and 4.5 mg/ml, IGF-I stimulated HR by only 47 and 18%, respectively. The effect of IGF-I at a glucose concentration of 4.5 mg/ml was not statistically different from that on the control cells. Thus, glucose had a biphasic effect on the response of HR to IGF-I. The presence of a physiologic concentration of glucose was essential for the stimulation of LPL by IGF-I, yet both lower and higher concentrations of glucose blunted the effect of IGF-I.

Glycemia and cytotoxicity. To determine whether high glucose was generally cytotoxic to adipocytes, [^{35}S]methionine incorporation into TCA-precipitable protein was measured in cells cultured in medium containing glucose concentrations of 1.0 and 4.5 mg/ml (Fig. 7). Cells were incubated overnight in medium containing a glucose concentration of 1.0 mg/ml and 10% fetal bovine serum and then changed to medium containing [^{35}S]methionine, 10% serum, and glucose concentrations of either 1.0 or 4.5 mg/ml. [^{35}S]methionine incorporation was higher in cells changed to the high-glucose medium than in the cells in normal-glucose (1.0 mg/ml) medium. Thus, high-glucose medium did not decrease protein synthesis under these conditions, suggesting that the decrease in LPL with high-glucose medium is not due to a generalized toxicity to the adipocyte.

Effects of other substrates on LPL and cellular ATP. To approach the mechanism by which glucose may influence LPL, the effects of other substrates on HR were tested. Cells were incubated overnight in the presence and absence of glucose as well as with the following compounds substituted for glucose: galactose, L-glucose, 3-*O*-methylglucose, and pyruvate. In this way, substrates were chosen that were metabolized but not actively transported (galactose and pyruvate), actively transported but not metabolized (3-*O*-methylglucose), and neither actively transported nor metabolized (L-glucose). As shown in Fig. 8, HR in cells cultured in galactose, L-glucose, or 3-*O*-methylglucose was indistinguishable from HR in cells cultured without glucose. In contrast, pyruvate (2.0 mg/ml) stimulated HR as well as glucose (1.0 mg/ml).

To determine whether adipocyte ATP content was affected by these different medium conditions, cellular ATP levels were measured. There were no statistically significant differences in ATP levels in the presence or absence of glucose, galactose, pyruvate, or serum. Thus, the cellular ATP level of adipocytes appears to remain constant in the presence and absence of various potential substrates for energy production.

DISCUSSION

Except for our previous study in human adipocytes (11), most data on the regulation of human LPL by hormones and substrates are derived from studies in vivo. Whole adipose tissue LPL increases after a meal (4,16), after treatment of an insulin-deficient (3) or insulin-resistant (4) state, and after a glucose-insulin infusion (6). Thus, the association of LPL with insulin levels suggests that insulin plays some role in regulating the enzyme. However, recent studies suggest an important role of glucose in in vivo LPL responsiveness. When subjects were given an insulin infusion and maintained at mild hyperglycemia (140 mg/dl), a rise in LPL was noted earlier than when glucose was maintained at euglycemia and

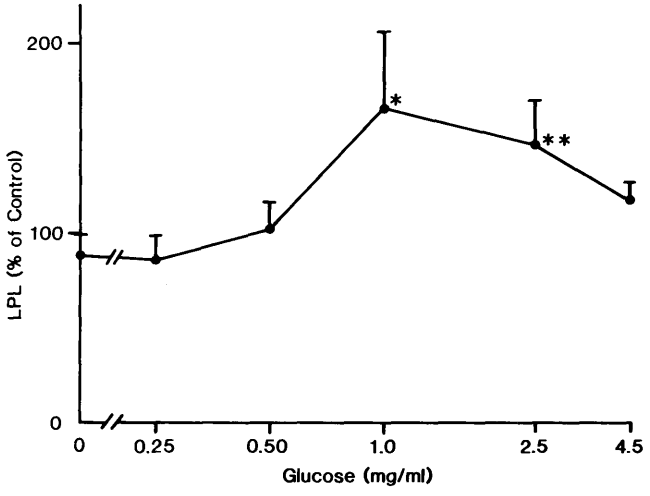


FIG. 6. Modulation of effect of insulin-like growth factor I (IGF-I) by glucose. Adipocytes were cultured overnight in serum-free medium containing various glucose concentrations in presence and absence of IGF-I at concentration of 50 ng/ml. Each *point* represents 4–8 paired experiments and is expressed as heparin-releasable activity (HR) in presence of IGF-I as percent of HR in absence of IGF-I. LPL, lipoprotein lipase. *$P = .02$ and **$P = .05$ vs. control.

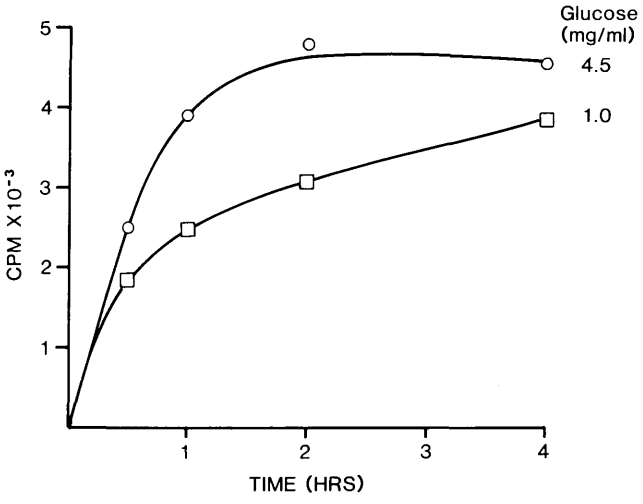


FIG. 7. Effect of glucose on [^{35}S]methionine incorporation into protein. Adipocytes were cultured overnight in medium containing 10% fetal bovine serum and 1 mg/ml glucose. Cells were then placed in 2 ml medium containing 10% serum, 30 μCi [^{35}S]methionine, and glucose concentrations of either 1.0 or 4.5 mg/ml. At indicated times, cells were removed, washed, and cell proteins were precipitated with 10% trichloroacetic acid and counted. Data are from 1 representative experiment out of 2.

glucose disposal rate correlated with rise in LPL (17). Other studies have also suggested an important role for glucose disposal rate in the regulation of adipose LPL (18). However, these studies cannot separate the independent effects of glucose and insulin nor provide any information on the mechanism of their effects on adipose tissue. Because we recently failed to demonstrate an effect of physiologic insulin concentrations on adipocyte LPL (11), we turned our attention to glucose.

The heparin-releasable fraction of adipose tissue appears to be most representative of functional in vivo LPL (3,9,11,19). Hence, the effect of glucose on HR was carefully studied. Cells were incubated in medium 199, which contained all 20 amino acids as well as a metabolizable pentose (ribose). Glucose stimulated total cellular LPL synthesis, especially HR, although this increase in HR was most apparent after 24 h in culture. A physiologic glucose concentration was necessary for maximum LPL activity in HR, and further increases in glucose did not increase HR further. Glucose was especially important for the development of full activity in the presence of serum. In addition, the effect of glucose could be reversed in vitro by changing the medium to one with a different glucose concentration.

Previous studies on the regulation of adipose tissue LPL have focused on glucose and other factors present in the postprandial state. Early experiments studied LPL in whole fat pads from fed and fasted rats and consistently measured more LPL in the fat from fed rats (19–23). To determine what component of the postprandial state was responsible for LPL stimulation, subsequent studies incubated freshly prepared rat fat pads with various combinations of glucose, insulin, and cycloheximide. The addition of glucose to fat pads that were being incubated in buffer containing cycloheximide produced brief increases in whole fat pad LPL (24,25), and the addition of insulin in vitro potentiated this effect of glucose.

Several studies have examined the effect of glucose in isolated cell systems. In 3T3-L1 cells, glucose produced an increase in cellular LPL (26). In adipocytes, Stewart and Schotz (27) showed glucose-mediated stimulation of LPL release, whereas Kornhauser and Vaughan (28) showed no such effect. In this study the effect of glucose became apparent only after 5 h in a culture medium environment. Thus, studies that examined the effect of glucose after shorter periods or under different incubation conditions may not have been able to show any effect.

LPL activity that is spontaneously secreted from human adipocytes is only measurable when cells are cultured in medium containing serum (11). Because CM correlates strongly with HR (11), HR probably represents cellular LPL that is an immediate precursor to the secreted enzyme. At glucose concentrations of 0–1.0 mg/ml, this relationship between HR and CM was maintained, because CM increased in parallel with HR in response to glucose. However, at glucose concentrations >1.0 mg/ml, HR remained stable, whereas CM fell. This effect of high glucose was even demonstrable over a shorter period after cells had incubated overnight. Because incorporation of [^{35}S]methionine into protein was not diminished by high glucose concentrations and cellular LPL (HR and EXT) and cellular ATP remained stable, the effect of high glucose was probably not due to a general decrease in adipocyte viability.

This effect of high glucose concentrations on adipocyte LPL secretion suggests a possible mechanism for the LPL disorder in diabetes. The decreased LPL in poorly controlled diabetes has usually been ascribed to insulin deficiency or insulin resistance (29). Although insulin administered in vivo certainly results in increased adipose LPL, insulin does not have the same effect in vitro. Therefore, insulin may act in-

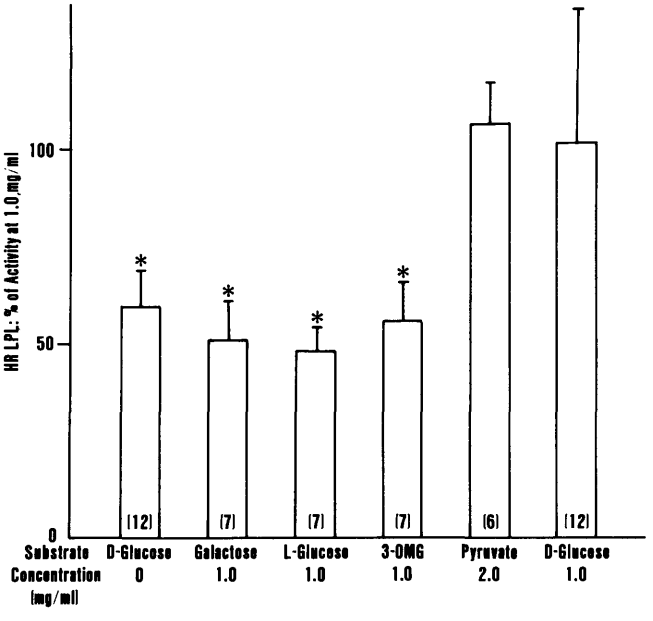


FIG. 8. Effects of other substrates on heparin-releasable activity (HR). Cells were cultured overnight in serum-free medium containing substrates listed on *abscissa*. HR was then measured and expressed as percent of activity in cultures containing 1.0 mg/ml glucose (1.08 ± 0.35 neq/min per 10^6 cells). Experimental *n* indicated in parentheses. LPL, lipoprotein lipase. 3-OMG, 3-*O*-methylglucose. *$P < .02$ vs. pyruvate and D-glucose.

directly in vivo to stimulate LPL. However, if hyperglycemia in patients affects adipocyte LPL in a manner analogous to the data herein, then adipocyte LPL would remain stable but secretion would fall. Decreased secretion would eventually lead to less extra-adipocyte LPL (interstitial space, endothelium bound) and hence less total LPL in adipose tissue biopsies and in postheparin plasma, as has been demonstrated previously (3–5). In addition, a decrease in endothelium-bound LPL would result in decreased triglyceride-rich lipoprotein catabolism and consequent hypertriglyceridemia. Thus, our data in isolated adipocytes are consistent with previous studies in adipose tissue.

In addition to its effects on cellular and secreted LPL, glucose also modulated the response of adipocytes to IGF-I. These data can be compared with the response of LPL in 3T3-L1 cells to glucose and insulin. Spooner et al. (26) examined the effect of glucose on the increase in cell LPL in response to a very high concentration of insulin (560 ng/ml). In both human adipocytes and 3T3-L1 cells, low glucose concentrations prevented the full expression of the hormonal effect on LPL. In human adipocytes, however, high glucose concentrations also inhibited the effect of IGF-I on LPL, whereas similar glucose concentrations did not affect LPL response to high insulin concentrations in 3T3-L1 cells. These differences may be the result of using cells from different species or the differences inherent in comparing a primary culture to a cell line.

The exact physiologic role of IGF-I as a regulator of LPL is not yet clear. Because plasma IGF-I levels in humans remain relatively constant (30), IGF-I may tonically maintain adipocyte LPL—an effect that hyperglycemia would perhaps abate. On the other hand, IGF-I may be a locally produced regulator of LPL (31). Thus, the interference with IGF-I action by elevated glucose concentrations may be crucial to LPL pathophysiology. In addition, if hyperglycemia also diminishes the response of LPL to other hormonal regulators, then LPL in diabetic adipose tissue may not adequately respond to the postprandial state and thus not properly clear triglyceride-rich plasma lipoproteins.

To gain some insight into the mechanism of action of glucose on adipocyte LPL, other hexoses and pyruvate were substituted for glucose. Only pyruvate stimulated HR and glucose. Galactose, which is metabolizable to glucose in many cells (32), had no effect on LPL and 3-*O*-methylglucose, and L-glucose was also unable to substitute for D-glucose. In contrast, LPL in 3T3-L1 cells did not respond to either galactose or pyruvate (26). One explanation for the above data would be an inability of adipocytes to metabolize the amino acids in medium 199 into energy and an inability to metabolize galactose. As such, these cells may require glucose or pyruvate for the generation of metabolic energy. To probe this possibility, we measured ATP levels in adipocytes cultured for 24 h under various conditions. No significant difference was found in ATP levels regardless of substrate or presence of serum, although ATP levels may not reflect ATP production because generation and utilization of ATP may be in equilibrium. Alternatively, adipocytes may be capable of utilizing amino acids for energy production in the absence of glucose and hence maintain ATP production. Thus, whether glucose affects LPL by modulating cellular energy production cannot be answered by these data.

In conclusion, the glucose concentration in the medium of cultured human adipocytes plays an important role in the regulation of LPL. A physiologic concentration of glucose is necessary for full expression of cellular and secreted LPL, but glucose concentrations above the physiologic range decrease CM and blunt the increase in HR in response to IGF-I. Thus, human adipocyte LPL is not responsive to physiologic concentrations of insulin, as is the rat enzyme, but does respond to glucose. There are probably other cell-cell interactions in adipose tissue that will help reconcile in vivo data with those reported herein.

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