Regulation of Gluconeogenesis in Hepatocytes from Fasted Alloxan-diabetic Rats

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SUMMARY

Hepatocytes from fasted, alloxan-diabetic rats were incubated in the absence of gluconeogenic substrates to deplete residual glycogen stores. Glucose production from lactate and pyruvate was enhanced in cells from diabetic rats relative to similarly treated hepatocytes from fasted, nondiabetic control rats. Gluconeogenesis from dihydroxyacetone, fructose, or glycerol was not increased but the formation of lactate plus pyruvate from dihydroxyacetone was decreased.

The stimulation of gluconeogenesis by exogenous fatty acids was decreased by diabetes. The rates of gluconeogenesis in the presence of lactate plus pyruvate plus oleate were equal in hepatocytes from diabetic and control rats and indicate that the maximal rate of gluconeogenesis was not increased.

With lactate plus pyruvate as substrates, stimulation of gluconeogenesis by norepinephrine or dibutyryl-cAMP was not altered by diabetes. The catecholamine stimulation of gluconeogenesis from glycerol also was unaffected. In contrast, diabetes decreased the maximal stimulation of gluconeogenesis from dihydroxyacetone by dibutyryl-cAMP, glucagon, or norepinephrine and this decrease was proportional to the decreased production of lactate plus pyruvate. The concentrations of glucagon or norepinephrine required for half-maximal stimulation were not altered by diabetes. Thus, the hormonal stimulation of gluconeogenesis from dihydroxyacetone is decreased by diabetes, probably because of decreased pyruvate kinase activity, but the interaction of glucagon and norepinephrine with hepatocytes and the subsequent stimulation of gluconeogenesis from physiologic substrates is not impaired. DIABETES 1985; 34:767–73.

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Diabetes alters the total concentrations of several enzymes of glucose metabolism in liver; increases of P-enolpyruvate carboxykinase, pyruvate carboxylase, glucose-6-phosphatase, and possibly fructose-1,6-bisphosphatase,[1–5] along with decreases of glucokinase, phosphofructokinase, pyruvate kinase, and Fru-6-P 2-kinase have been reported.[5–10]* These changes correspond to an increased gluconeogenic capacity and a potentially decreased sensitivity of gluconeogenesis to the feedback inhibition of high glucose concentrations via the actions of Fru-2,6-P_2 on phosphofructokinase and fructose-1,6-bisphosphatase. The rates of gluconeogenesis from lactate are greater in perfused livers or isolated hepatocytes from fed diabetic rats than from fasted or fed control rats.[11–15]

Glucagon stimulation of gluconeogenesis is decreased in livers or hepatocytes from fed diabetic rats relative to fed control rats.[12,13] A decreased generation of cAMP in response to glucagon has been suggested to account for at least part of the decreased stimulation of gluconeogenesis.[12–14] Although several studies have attempted to correlate the decreased gluconeogenic effect in diabetes with changes in the glucagon receptor, decreases in receptor number or affinity have not been observed consistently.[16–21] In contrast, fasting also decreases the cAMP-dependent stimulation of hepatic gluconeogenesis,[22,23] but this effect has been explained by the greater amounts of pyruvate kinase activity in fed rat livers and the correspondingly greater importance of pyruvate kinase inhibition for the stimulation of gluconeogenesis. Fasting did not affect the apparent affinity of hepatocytes for glucagon.[22]

This study reexamines the effects of alloxan diabetes on gluconeogenesis and its regulation. The stimulation of glu-

*The abbreviations used are: Fru-6-P, fructose-6-phosphate; Fru-2,6-P_2, fructose-2,6-bisphosphate; and dibutyryl-cAMP, N^6, $O^{2'}$-dibutyryladenosine-3′,5′-cyclic monophosphate.

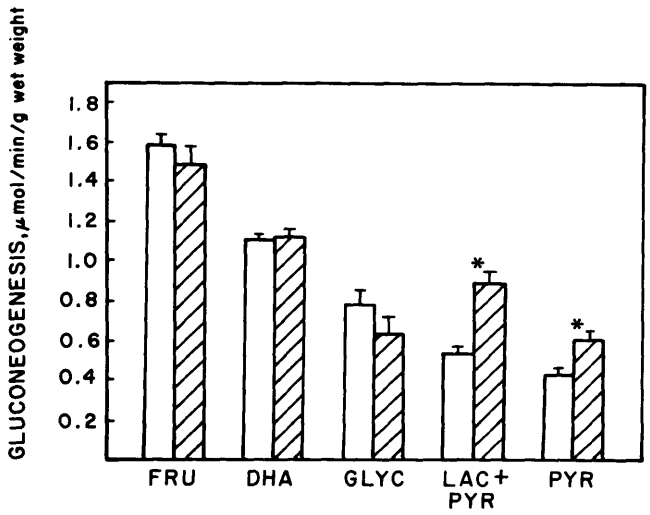

FIGURE 1. Rates of gluconeogenesis in hepatocytes from fasted nondiabetic and diabetic rats. Hepatocytes from fasted nondiabetic (□) or diabetic (▨) rats were incubated in the presence of 2.4 mM Ca^{2+} with 5 mM fructose (FRU), 5 mM dihydroxyacetone (DHA), 2.5 mM glycerol (GLYC), 10 mM lactate plus pyruvate (LAC + PYR), or 10 mM pyruvate (PYR). Each value is the mean ± SEM of 5–10 experiments. *P < 0.05 versus nondiabetic control.

coneogenesis by glucagon, norepinephrine, or exogenous fatty acids was compared for hepatocytes from fasted control and diabetic rats. Since fasting for 24 h does not completely deplete hepatic glycogen stores in the diabetic rat as it does in normal rats,[24–26] isolated hepatocytes were incubated in the absence of substrates before studies of gluconeogenesis to deplete glycogen further and to minimize possible interactions between glucose synthesis and glycogen degradation. The data presented in this report indicate that the hormonal regulation of lactate and pyruvate gluconeogenesis was not diminished, despite the increased rates of gluconeogenesis and decreased stimulation by fatty acids, but that hormonal stimulation of dihydroxyacetone gluconeogenesis is decreased proportionately to decreased lactate formation from this substrate.

MATERIALS AND METHODS

Materials. Alloxan monohydrate, bovine serum albumin, and dibutyryl-cAMP were obtained from Sigma (St. Louis, Missouri). The sources of other materials are given in ref. 27. Bovine serum albumin was treated with charcoal by the method of Chen[28] and dialyzed against Krebs-Henseleit bicarbonate buffer before use.

Animals. Male Sprague-Dawley rats (200–225 g) were obtained from King Laboratories (Madison, Wisconsin). Diabetes was induced in rats fasted for 24 h by intravenous (i.v.) injection of alloxan (38 mg in saline/kg body wt) and was verified by blood glucose concentrations >300 mg/dl after 1 wk. Urinalysis indicated that the diabetic rats were not ketotic. Generally no further weight gain was observed in the alloxan-injected animals, and thus the age-matched, nondiabetic control rats had a slightly greater average body weight at the time of the experiments.

Preparation and incubation of hepatocytes. Hepatocytes were isolated as previously described[27] from normal rats fasted 24 h or from diabetic rats fasted 26–30 h, except that Krebs-Henseleit bicarbonate medium without added $CaCl_2$ was used throughout the perfusion and isolation. Isolated hepatocytes were incubated at 37°C in Krebs-Henseleit medium containing 1.3% bovine serum albumin and 2.4 mM $CaCl_2$ with oxygenation for 45 min to deplete residual glycogen stores, then washed and incubated for 40 min as in ref. 27, at approximately 20 mg wet wt/ml, in Krebs-Henseleit bicarbonate medium containing 1.5% bovine serum albumin and various gluconeogenic substrates and effectors as described in the legend to each figure.

Microscopic examination indicated 90–95% viability for hepatocytes from fasted normal rats and 85–95% viability for hepatocytes from fasted diabetic rats. The average size of hepatocytes from fasted diabetic rats was consistently smaller than those obtained from fasted control rats and the cell count/g wet wt was correspondingly increased for the cells from diabetic rats. Data are calculated on the basis of wet weight of cells, assuming a conversion factor of 3.7 mg wet wt/mg dry wt.[29] Rates of gluconeogenesis have been corrected for endogenous glucose production, which was usually <0.2 μmol/min/g wet wt for hepatocytes from diabetic rats and was not substantially increased by norepinephrine, glucagon, or dibutyryl-cAMP.

Measurement of metabolites. Glucose was determined in perchloric acid extracts by the glucose-oxidase method.[30] Lactate, pyruvate, β-hydroxybutyrate, acetoacetate, and dihydroxyacetone were assayed spectrophotometrically in the

TABLE 1
Dihydroxyacetone metabolism in hepatocytes from fasted normal and diabetic rats

	Control	Diabetic
Glucose synthesis (μmol/min/g wet wt)	1.12 ± 0.06 (10)	1.08 ± 0.04 (16)
Lactate plus pyruvate formed (μmol/min/g wet wt)	1.32 ± 0.05 (10)	0.61 ± 0.04 (16)*
β-Hydroxybutyrate plus acetoacetate formed (μmol/min/g)	0.151 ± 0.015 (5)	0.141 ± 0.009 (7)
Lactate/pyruvate	3.05 ± 0.12 (10)	4.18 ± 0.15 (16)*
β-Hydroxybutyrate/acetoacetate	0.166 ± 0.017 (5)	0.120 ± 0.021 (7)

Hepatocytes were incubated 40 min in the presence of 10 mM dihydroxyacetone and 2.4 mM $CaCl_2$. Each value is the mean ± SEM of the number of observations given within the parentheses.
*P < 0.001 versus control.

TABLE 2
Dihydroxyacetone utilization by hepatocytes from fasted normal and diabetic rats

	DHA consumed (μmol/min/g)	Glucose formed (% of DHA)	Lac + Pyr formed (% of DHA)	Net C_3 units recovered*
Normal	3.53 ± 0.38	59 ± 2	38 ± 3	3.38 ± 0.25
Diabetic	2.83 ± 0.23	78 ± 5	20 ± 2	2.88 ± 0.23

Hepatocytes were incubated 40 min in the presence of 10 mM dihydroxyacetone and 2.4 mM $CaCl_2$. Each value is the mean ± SEM of four experiments.
*Net C_3 units recovered in glucose, lactate, and pyruvate was calculated as (2 × Glc) + (Lac) + (Pyr).

neutralized perchloric acid extracts by established enzymatic methods.[30]

RESULTS

Rates of gluconeogenesis in hepatocytes from fasted diabetic rats. Diabetes caused a >50% increase in the rate of gluconeogenesis from lactate plus pyruvate or from pyruvate alone (Figure 1), substrates that enter the pathway before pyruvate carboxylase. The rates of gluconeogenesis from fructose, dihydroxyacetone, or glycerol, substrates that enter as triose phosphates, were not increased by diabetes (Figure 1).

Metabolism of dihydroxyacetone. The production of lactate plus pyruvate from dihydroxyacetone was decreased in hepatocytes from diabetic rats and the ratio of lactate/pyruvate was slightly increased (Table 1). A similar decrease in lactate plus pyruvate production, without an accompanying increase in gluconeogenesis, was observed when hepatocytes from diabetic rats were incubated with 2.5 mM fructose (data not shown). Neither the formation of β-hydroxybutyrate and acetoacetate nor the ratio β-hydroxybutyrate/acetoacetate was affected by diabetes (Table 1).

The major products of dihydroxyacetone metabolism in perfused livers or isolated hepatocytes from fasted rats are glucose, lactate, and pyruvate.[31,23] As shown in Table 2, the production of glucose, lactate, and pyruvate accounted for >95% of dihydroxyacetone consumption in hepatocytes from fasted control and diabetic rats. Thus, there was no significant utilization of dihydroxyacetone for glycogen synthesis or pyruvate oxidation. The decreased metabolism of dihydroxyacetone presumably reflects a decreased rate of phosphorylation of dihydroxyacetone by the cells from diabetic rats.

The addition of 3-mercaptopicolinate, an inhibitor of P-enolpyruvate carboxykinase,[31] did not affect the rates of glucose or lactate plus pyruvate production from dihydroxyacetone even at concentrations sufficient to inhibit the rate of glucose synthesis from lactate plus pyruvate by >90% (data not shown). Thus, the decreased accumulation of lactate plus pyruvate was not due to a recycling of these substrates to glucose.

Effects of oleate and lysine on gluconeogenesis. Previous studies have shown that lysine or oleate stimulates gluconeogenesis from lactate plus pyruvate and that the combination of both lysine and oleate gives the maximal rate of glucose formation from this substrate pair.[33] Gluconeogenesis in hepatocytes from diabetic rats was stimulated to a lesser extent by lysine or by oleate (Table 3). In the presence of oleate, there was no difference between the rates of gluconeogenesis from lactate plus pyruvate in diabetic or nondiabetic hepatocytes (Table 3).*

Effects of decanoyl-D-carnitine on gluconeogenesis. The presence of high levels of endogenous fatty acids in the diabetic rat hepatocytes could account for the relative insensitivity to further addition of exogenous fatty acids and for the increased rates of gluconeogenesis and decreased rates of glycolysis that were observed in these cells. Decanoyl-D-carnitine, an inhibitor of long-chain fatty acid utilization at the carnitine palmitoyl transferase step,[34] was added to diabetic and nondiabetic rat hepatocytes to assess this possibility. In both cases, 2 mM decanoyl-D-carnitine blocked the stimulation by oleate of lactate plus pyruvate gluconeogenesis but did not affect gluconeogenesis in the presence of octanoate, which bypasses the site of inhibition (Table 4). In agreement with earlier studies,[35–37] decanoyl-D-carnitine inhibited gluconeogenesis from lactate plus pyruvate even in the absence of added fatty acids (Table 4). The degree of inhibition of gluconeogenesis in the control cells of this study, approximately 50%, was comparable to that observed previously with perfused rat livers and with isolated rat hepatocytes.[35–37] In hepatocytes from diabetic rats, this inhibition was only approximately 20% (Table 4). Furthermore, decanoyl-D-carnitine did not affect the metabolism of dihydroxyacetone in diabetic rat hepatocytes (data not shown). These data indicate that endogenous extramitochondrial fatty acids are less important in supporting gluconeogenesis in the hepatocytes from diabetic rats than from fasted control rats and cannot account for the alterations of lactate plus pyruvate gluconeogenesis or of dihydroxyacetone metabolism, but possible contributions of intramitochondrial fatty acids are not excluded.

Attempts to demonstrate a requirement for endogenous lipid mobilization were unsuccessful with both normal and diabetic hepatocytes. No specific inhibition of lactate plus pyruvate gluconeogenesis was observed with glycodiazine, an inhibitor of triacylglyceride hydrolysis.[38] Concentrations of glycodiazine sufficient to inhibit gluconeogenesis from lactate plus pyruvate in the absence of added fatty acids were also inhibitory in the presence of exogenous oleate (data not shown).

*The addition of oleate had little effect on dihydroxyacetone metabolism in hepatocytes from diabetic rats in contrast to the 35% stimulation of gluconeogenesis by oleate in hepatocytes of normal fasted rats.

TABLE 3
Effects of lysine and oleate on gluconeogenesis in hepatocytes from fasted control and diabetic rats

Substrates	Rate of gluconeogenesis (μmol/min/g wet wt)	
	Control	Diabetic
10 mM Lactate + 1 mM pyruvate	0.54 ± 0.04	0.89 ± 0.07*
+ 10 mM lysine	0.73 ± 0.06 (135%)	1.03 ± 0.05 (116%)*
+ 1 mM oleate	1.57 ± 0.12 (291%)	1.58 ± 0.18 (178%)
+ lysine + oleate	1.68 ± 0.09 (311%)	1.71 ± 0.15 (192%)

Hepatocytes were incubated 40 min with 2.4 mM $CaCl_2$ and substrates as indicated. Each value is the mean ± SEM of seven experiments.
*$P < 0.05$ versus control.

Stimulation of gluconeogenesis by hormones. Despite the accelerated rates of gluconeogenesis from lactate plus pyruvate, the stimulation of gluconeogenesis from this substrate mixture by dibutyryl-cAMP or norepinephrine was not significantly affected by diabetes (Figure 2). The Ca^{2+}-dependent stimulation by norepinephrine of gluconeogenesis from glycerol was unaffected by diabetes. Therefore, neither the responses to cAMP nor the effects of norepinephrine were inhibited directly in diabetic rat hepatocytes.

In contrast, diabetes decreased the stimulation by dibutyryl-cAMP of gluconeogenesis from dihydroxyacetone (Figure 2). Similar results were obtained when fructose was substituted for dihydroxyacetone as the substrate (data not shown) or when glucagon was substituted for dibutyryl-cAMP (Figure 3B).

Calcium addition decreased the catecholamine stimulation of gluconeogenesis from dihydroxyacetone in hepatocytes from fasted control rats, as previously described.[27] In the absence of Ca^{2+}, the stimulation by norepinephrine was similar to that by dibutyryl-cAMP and was decreased by diabetes (Figure 2). Norepinephrine stimulation of dihydroxyacetone gluconeogenesis in the presence of Ca^{2+} was approximately 30% and was unchanged by diabetes (data not shown). Thus, no net inhibition of norepinephrine stimulation by Ca^{2+} was observed in diabetic rat hepatocytes.

With dihydroxyacetone as a substrate and in the absence of added $CaCl_2$, the stimulation of gluconeogenesis by maximally effective concentrations of norepinephrine was decreased in hepatocytes from diabetic rats but the concentration of norepinephrine required for half-maximal stimulation was not altered significantly (Figure 3A). The maximal activation of dihydroxyacetone gluconeogenesis by glucagon was also decreased, from 63% to 36%, without affecting the concentration of glucagon required for half-maximal stimulation (Figure 3B).

Effects of norepinephrine or dibutyryl-cAMP on dihydroxyacetone metabolism. Norepinephrine or dibutyryl-cAMP decreased the production of lactate plus pyruvate from dihydroxyacetone in hepatocytes from diabetic rats (Table 5). The rates of lactate plus pyruvate formation in the presence of dibutyryl-cAMP were comparable for hepatocytes from diabetic or nondiabetic fasted rats but the net decrease brought about by dibutyryl-cAMP or norepinephrine was less in hepatocytes from diabetic rats as a result of the already low rates of lactate plus pyruvate formation in the absence of hormones (Table 5). In diabetic cells, the decrease in glycolytic flux from dihydroxyacetone correlated with the magnitude of the stimulation of gluconeogenesis. These data suggest that the apparently low stimulation of gluconeogenesis from dihydroxyacetone is a consequence of the already low rates of glycolysis and the subsequently diminished capacity for further diversion toward glucose, rather than a defect in the coupling mechanisms or second messenger generation during diabetes.

DISCUSSION

Glycogen-depleted hepatocytes from fasted diabetic rats had greater rates of gluconeogenesis from substrates entering the pathway before pyruvate carboxylase and lesser rates of glycolysis from substrates entering as triose phosphates than cells from fasted control rats (Figure 1 and Table 1). The increased rates of gluconeogenesis from lactate plus pyruvate are consistent with previous observations of perfused livers and isolated hepatocytes and most likely result

TABLE 4
Decanoyl-D-carnitine effects on gluconeogenesis in hepatocytes from fasted normal and diabetic rats

Added fatty acid	Control		Diabetic	
	−DDC*	+DDC	−DDC	+DDC
	(Gluconeogenesis, μmol/min/g wet wt)			
None	0.51 ± 0.09	0.27 ± 0.04	0.79 ± 0.05†	0.64 ± 0.03†
Oleate	1.52 ± 0.34	0.45 ± 0.08	1.79 ± 0.09	0.76 ± 0.07†
Octanoate	1.10 ± 0.19	1.09 ± 0.20	1.33 ± 0.06	1.38 ± 0.07

Hepatocytes were incubated for 40 min with 2.4 mM $CaCl_2$, 10 mM lactate, and 1 mM pyruvate. Decanoyl-D-carnitine and exogenous fatty acid, where indicated, were added to final concentrations of 2 mM and 1 mM, respectively. Each value is the mean ± SEM of three experiments.
*DDC, decanoyl-D-carnitine.
†$P < 0.05$ versus nondiabetic control.

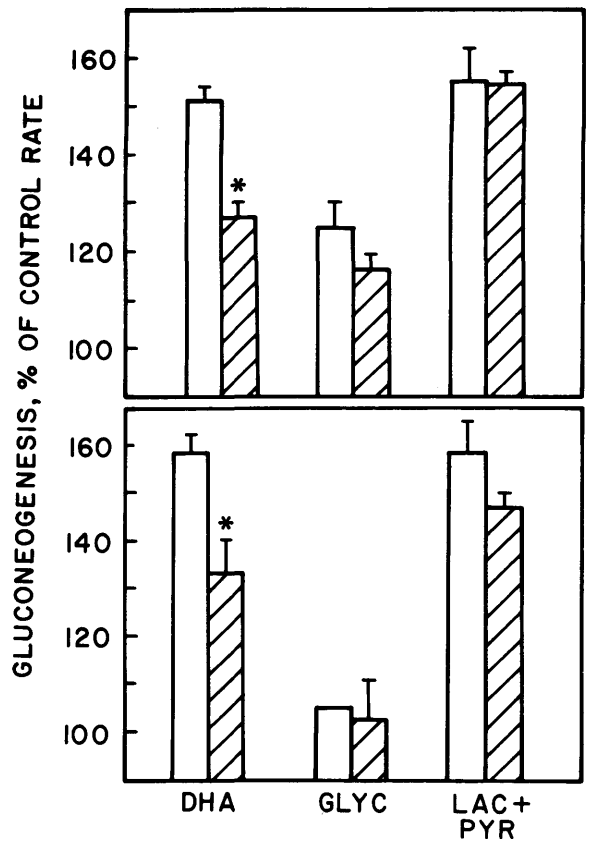

FIGURE 2. Stimulation of gluconeogenesis by norepinephrine and dibutyryl-cAMP in hepatocytes from fasted nondiabetic and diabetic rats. Hepatocytes from fasted nondiabetic (□) or diabetic (▨) rats were incubated with 5 mM dihydroxyacetone (DHA), 2.5 mM glycerol (GLYC), or 10 mM lactate plus pyruvate (LAC + PYR). Upper panel, stimulation of gluconeogenesis by 10 μM norepinephrine with 2.4 mM $CaCl_2$ present for glycerol or lactate-pyruvate but omitted for dihydroxyacetone. Lower panel, stimulation of gluconeogenesis by 50 μM dibutyryl-cAMP with Ca^{2+} present for all substrates. Each value is the mean ± SEM of 3–10 experiments, with the control rates of gluconeogenesis given in Figure 1. *P < 0.05 versus nondiabetic control.

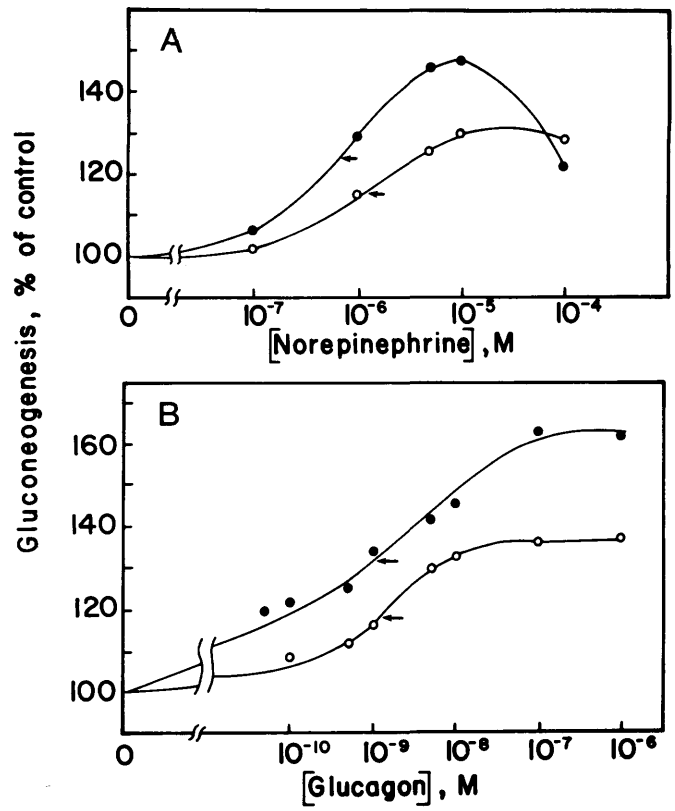

FIGURE 3. Hormonal stimulation of gluconeogenesis from dihydroxyacetone in hepatocytes from fasted normal and diabetic rats. Hepatocytes from control (●) and diabetic (○) rats were incubated for 40 min with 5 mM dihydroxyacetone in the absence of added Ca^{2+} and in the presence of various concentrations of norepinephrine (upper panel) or glucagon (lower panel). The rates of gluconeogenesis in the absence of hormones were 0.92 ± 0.16 and 0.95 ± 0.08 μmol/min/g wet wt, respectively, for hepatocytes from control and diabetic rats. Each point represents the mean of 3–4 experiments.

from the increased concentrations of P-enolpyruvate carboxykinase and of pyruvate carboxylase and the decreased concentration of pyruvate kinase in livers of fasted, diabetic rats relative to fasted control rats.[1,4,9]

The decreased rates of lactate plus pyruvate production from dihydroxyacetone indicate a decrease in the relative proportion of DHAP metabolized via glyceraldehyde phosphate dehydrogenase versus via fructose-1,6-bisphosphatase that probably results from the decreases of pyruvate kinase and fructose-6-phosphate 2-kinase activities during diabetes.[9,10] A decreased accumulation of Fru-2,6-P_2 during dihydroxyacetone metabolism could favor gluconeogenesis at the fructose-1,6-bisphosphate/phosphofructokinase site as well as the decreased glycolytic capacity.[39,40]

Previous studies of alloxan-diabetic rats used fructose as

TABLE 5
Effects of norepinephrine and of dibutyryl-cAMP on dihydroxyacetone metabolism

Conditions	Lac + pyr formation (μmol/min/g wet wt)	
	Normal	Diabetic
2.4 mM $CaCl_2$	1.38 ± 0.07	0.67 ± 0.06
2.4 mM $CaCl_2$ + 50 μM Bt_2-cAMP	0.25 ± 0.02*	0.25 ± 0.01*
$CaCl_2$ omitted	1.33 ± 0.07	0.68 ± 0.06
$CaCl_2$ omitted + 10 μM norepinephrine	0.65 ± 0.07†	0.43 ± 0.04†

Hepatocytes were incubated for 40 min with 10 mM dihydroxyacetone and other additions as indicated. Each value is the mean ± SEM of eight experiments with diabetic hepatocytes and five with controls.
*P < 0.05 versus control with $CaCl_2$.
†P < 0.05 versus control without $CaCl_2$.

a substrate entering the gluconeogenic pathway as triose phosphates, and gave two different results. No change in glucose or lactate production from fructose was seen on comparison of perfused livers from fed or diabetic rats,[12] but the net rate of glucose synthesis in isolated hepatocytes from fed diabetic rats was greater than the rates in hepatocytes from fed or fasted control rats.[14,15] However, these experiments used high concentrations of fructose (10 mM or greater), which can cause a depletion of cellular adenine nucleotide pools[41] and thus may have influenced glycogen metabolism and pyruvate kinase activity. More recently, Zaleski et al.[42,43] have reported effects of alloxan diabetes on gluconeogenesis in rabbit hepatocytes that are analogous to the effects reported here for rat hepatocytes.

The similar rates of gluconeogenesis in normal or diabetic cells incubated with lactate plus pyruvate plus oleate indicate that a common point in the pathway is limiting in the presence of oleate and that the capacity for this reaction is not increased by diabetes. Two possible sites of oleate action are an activation of pyruvate carboxylase by acetyl-CoA and an inhibition of phosphofructokinase by citrate.[35] In the presence of oleate, the rate of gluconeogenesis from lactate plus pyruvate equals or exceeds that attained with fructose. This indicates that the process is probably limited by conversion of triose phosphates to glucose rather than by the formation of PEP from pyruvate. One possibility is that the activity of fructose-1,6-bisphosphatase becomes limiting under this condition. The concentration of fructose-1,6-bisphosphatase is increased to a similar extent by fasting or diabetes and only small and variable differences have been reported.[3-5]

Several lines of evidence suggest that diabetes does not interfere directly with hormone-receptor interactions or second messenger production induced by glucagon or catecholamines: hormonal stimulation of lactate plus pyruvate gluconeogenesis and catecholamine stimulation of glycerol gluconeogenesis are not affected by diabetes; the maximal activation of gluconeogenesis by glucagon is equivalent to that by dibutyryl-cAMP for each substrate; and the concentrations of each hormone required for half-maximal stimulation of gluconeogenesis are similar in hepatocytes from diabetic or fasted rats. There was no indication of the decreased sensitivity to glucagon that was previously observed by comparison of diabetic rats to fed control rats.[12,13] Because the ability of each hormone to stimulate gluconeogenesis from dihydroxyacetone is diminished to the same extent as the production of lactate plus pyruvate is diminished, the decreased stimulation of gluconeogenesis by norepinephrine, glucagon, or dibutyryl-cAMP is most likely secondary to the inhibition of glycolysis in hepatocytes from diabetic rats.

The hormonal stimulation of gluconeogenesis from lactate plus pyruvate was not diminished by diabetes despite the increased rates of gluconeogenesis from these substrates (Figure 2) and the apparent decrease of flux through pyruvate kinase as indicated by decreased lactate production from dihydroxyacetone. These observations indicate that the further diminution of pyruvate kinase by diabetes does not influence the hormonal regulation of gluconeogenesis from lactate plus pyruvate and that the inhibition of pyruvate kinase is apparently less important for the hormonal activation of gluconeogenesis from this substrate pair than from dihydroxyacetone or fructose. Several additional sites of hormone action have been proposed to play a role in the stimulation of gluconeogenesis from lactate or pyruvate, including the possible stimulation of P-enolpyruvate carboxykinase, pyruvate carboxylase, and pyruvate transport into the mitochondria (for review see ref. 44). One or more of these additional sites probably are the predominant mediators of the stimulation of gluconeogenesis in diabetes.

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