Lipoic Acid Acutely Induces Hypoglycemia in Fasting Nondiabetic and Diabetic Rats

Mogher Khamaisi, Assaf Rudich, Ruth Potashnik, Hans J. Tritschler, Alisa Gutman, and Nava Bashan

Lipoic acid (LA) is a unique antioxidant that increases peripheral glucose utilization in diabetic patients. This study was conducted to investigate whether the inhibition of glucose production could be an additional mechanism for the action of LA. Intravenous (IV) LA injection (100 or 60 mg/kg body weight) to fasting nondiabetic or streptozotocin (STZ)-induced diabetic rats caused a rapid reduction in blood glucose with no effect on circulating insulin levels. In vivo conversion of fructose to glucose was not inhibited by LA, whereas the gluconeogenesis flux from alanine was completely prevented. Reduced liver pyruvate carboxylase (PC) activity in vivo is suggested by the finding that LA induced a decrease in liver coenzyme A (CoA) content (44% and 28% reduction in nondiabetic and diabetic rats, respectively, compared with vehicle-treated animals) and liver acetyl CoA content (80% and 67% reduction in nondiabetic and diabetic rats, respectively). A reduction in plasma free carnitine (42% and 22% in nondiabetic and diabetic rats, respectively) was observed in LA-treated animals, and acylcarnitine levels were increased twofold. This could be attributed to elevated levels of C16 and C18 acylcarnitine, without a detectable accumulation of lipoylcarnitine. Under such conditions, a significant increase in the plasma free fatty acid (FFA) concentration (204% in nondiabetic and 151% in diabetic animals) with no elevation in β-hydroxybutyrate levels was noted. In conclusion, this study suggests that short-term administration of LA at high dosage to normal and diabetic rats causes an inhibition of gluconeogenesis secondary to an interference with hepatic fatty acid oxidation. This may render LA an antihyperglycemic agent for the treatment of diabetic subjects, who display glucose overproduction as a major metabolic abnormality.

UNDER FASTING CONDITIONS, glucose homeostasis is maintained by the balance between systemic glucose production and total-body glucose utilization. In both non–insulin-dependent (type 2) and insulin-dependent (type 1) diabetes mellitus, glucose overproduction is a major contributor to hyperglycemia.[1,2] The increase in glucose production can be largely attributed to an elevation in the gluconeogenesis rate and appears as the main cause of fasting hyperglycemia, which is particularly evident in a certain percentage of diabetic patients.[1,3] Although mechanisms leading to the enhanced gluconeogenesis have not been fully characterized, they are believed to represent a combined effect of a reduced hepatic insulin sensitivity and an elevation in free fatty acid (FFA) oxidation by the liver.[3,4]

Fatty acid oxidation provides the primary stimulus for the activation of gluconeogenesis.[5] This is due to an elevated NADH to NAD^+ ratio and an increased acetyl coenzyme A (CoA) production. NADH provides reducing equivalents for the gluconeogenesis process, and acetyl CoA stimulates gluconeogenesis via both allosteric activation of pyruvate carboxylase (PC) and an increase in the citrate concentration, which in turn results in an increase in the relative activity of the gluconeogenetic enzyme fructose-1,6-bisphosphatase to the glycolytic enzyme phosphofructokinase.[5] In addition, fatty acid oxidation can also provide the energy (adenosine triphosphate) required for the gluconeogenesis pathway.[3,6] The increased release of lactate, alanine, and glycerol from skeletal muscle and adipose tissue provides the substrate for the enhanced gluconeogenic flux.[6]

The inhibition of glucose production may be a therapeutic target for optimizing glycemic control, especially in patients in whom fasting hyperglycemia represents the major metabolic abnormality. Several antidiabetic agents have been developed to reduce hepatic glucose production by the ability to inhibit either hepatic FFA oxidation or FFA release from adipose tissue. Inhibition of lipolysis is the primary mode of action of the adenosine A1 agonist SDZ WAG 994 and the nicotinic acid derivative acipimox, whereas the carnitine palmitoyltransferase-1 (CPT-1) inhibitor aminocamitine, etomoxir, and SDZ 51-641 have an inhibitory effect on hepatic FFA oxidation.[7] Furthermore, inhibition of hepatic glucose production has been demonstrated as a complementary mode of action of both metformin and thiazolidinediones.[8-10]

The antioxidant α-(+)-lipoic acid ([LA] thioctic acid) has been shown to relieve the symptoms of diabetic polyneuropathy in a cohort of diabetic patients. Lately, it has been shown that LA enhances glucose utilization by skeletal muscle in a cell culture system[11] and in animal models of diabetes.[12-14] In non–insulin-dependent diabetic patients, short- and long-term administration of LA resulted in an approximately 30% increase in peripheral glucose disposal.[15] These data suggest that increased glucose utilization by peripheral tissues may represent a mechanism for the hypoglycemic effect of LA. Another potential mechanism for its hypoglycemic effect is suggested in previous studies by Blumenthal[16] using isolated hepatocytes, in which LA was demonstrated to inhibit gluconeogenesis by sequestration of the biochemical expression of coenzyme A (HS CoA), resulting in reduced acetyl CoA, the essential activator of the key gluconeogenetic enzyme PC.

The present study was conducted to evaluate whether in vivo inhibition of gluconeogenesis in normal and streptozotocin

From the Department of Clinical Biochemistry, Faculty of Health Sciences, Soroka Medical Center and Ben-Gurion University of the Negev, Beer-Sheva, Israel; Asta Medica, Frankfurt, Germany; and Department of Clinical Biochemistry, Hadassah Medical Center, Jerusalem, Israel.

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Address reprint requests to Nava Bashan, PhD, Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

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(STZ)-diabetic rats is a complementary mechanism for the hypoglycemic effect of LA.

MATERIALS AND METHODS

LA (racemic mixture) was provided by Asta Medica (Frankfurt, Germany). Glucagon was purchased from Novo Nordisk (Bagsvaerd, Denmark). [U-^{14}C]glucose 1-phosphate, [1-^{14}C]pyruvic acid, [1-^{14}C]acetyl CoA, and sodium [^{14}C]bicarbonate were purchased from Amersham International (Buckingham, UK). All other chemicals were purchased from Sigma (St Louis, MO) unless otherwise stated.

Animals

Male Sprague-Dawley rats were purchased from Harlan Laboratories (Jerusalem, Israel). They were kept on a 12-hour light-dark cycle at 23°C and housed in groups of four per cage. Standard rat chow and tap water were provided ad libitum. Diabetes was induced at the age of 6 to 8 weeks by a single intraperitoneal (IP) injection of a freshly prepared solution of STZ (65 mg/kg body weight) in 100 mmol/L citrate buffer (pH 4.5). The experiments were performed 7 days after the induction of diabetes in animals with fed tail-blood glucose levels above 400 mg/dL on two separate occasions (Glucometer Elite; Bayer Diagnostic, Tarrytown, NY). Fasting conditions were induced by 12 hours of food deprivation. All experimental procedures were authorized by the Institutional Animal Care Committee.

LA Treatment and Specimen Collection

Rats were injected intravenously (IV) with either 120 mmol/L Tris buffer (pH 7.4, 1 mL) or LA (at the concentrations indicated) dissolved in the same buffer. At the time points indicated in each experiment, blood samples were obtained by clipping the tail and glucose levels were determined. Alternatively, the animals were anesthetized (phenobarbital 80 mg/kg IP), the liver was surgically removed and rapidly frozen in liquid nitrogen, and 2 mL blood was drawn by cardiac puncture into EDTA-containing tubes, followed by centrifugation (3,000 × *g* for 10 minutes) at 4°C. Liver or plasma samples were kept at −70°C until analyzed.

Metabolites and Enzymes in Plasma

Pyruvate and β-hydroxybutyrate concentrations were analyzed as previously described.[17,18] Insulin levels were measured using a rat insulin radioimmunoassay kit (Linco Research, St Louis, MO). Plasma FFA and carnitine levels were determined using conventional methods.[19-21] Plasma acylcarnitine was determined by tandem mass spectrometry with electrospray ionization as described by Rashed et al[22] on a VG Quattro II ESI-MS-MS instrument (England). Derivatization of the samples was performed essentially as previously described.[23] Plasma levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, and creatinine were measured using an optimized enzymatic calorimetric assay kit (Boehringer, Mannheim, Germany).

Liver Enzyme Activity and Metabolite Concentration

PC activity was determined by measuring ^{14}C incorporation into oxaloacetate as described by Fowden et al.[24] Phosphoenolpyruvate carboxykinase (PEPCK) activity was determined by measuring ^{14}C incorporation into malate as described previously.[25] Liver glycogen was determined enzymatically after digestion in 33% potassium hydroxide as previously described.[26] CoA was determined by a spectrophotometric method as previously described.[27] Briefly, denatured samples were incubated in a final volume of 1 mL containing 50 mmol/L arsenate (pH 7.2), 0.1 mmol/L NAD, 2 mmol/L 2-oxoglutarate, and 0.4 mg EDTA. The absorbance at 340 nm was evaluated before and after the addition of 5 μg/mL oxoglutarate dehydrogenase. The assay method for acetyl CoA is based on the synthesis of citrate from oxaloacetate in the presence of citrate synthase as previously described.[28] Briefly, denatured samples were incubated in a final volume of 1 mL containing 100 mmol/L Tris buffer, pH 8.1, 5 mmol/L DL-malate, and 1.5 mmol/L NAD. The absorbance at 340 nm was evaluated before and after addition of 1 U/mL malate dehydrogenase and 100 mU/mL citrate synthase. The protein concentration was measured using the Bio-Rad (Munich, Germany) protein assay.[29]

Statistical Analysis

All data are reported as the mean ± SE. The significance of differences between variables was calculated by Student's *t* test for nonpaired groups.

RESULTS

LA Treatment Reduces Blood Glucose in Fasting Nondiabetic and STZ-Induced Diabetic Rats

A single IV injection of 100 mg/kg LA to nondiabetic rats after 12 hours of fasting resulted in a dramatic reduction in blood glucose levels (Fig 1A). At 1 hour following LA, blood glucose was reduced from 76 ± 3 to 38 ± 3 mg/dL ($P < .01$). Hypoglycemia persisted for 4 hours, and glucose returned to pretreatment concentrations by 8 hours. A shorter duration of the hypoglycemic effect was observed with a dose of 60 mg/kg body weight (Fig 1A), and 30 mg/kg failed to reduce blood glucose (data not shown). In diabetic rats, LA administration led to a decrease in blood glucose, which was progressive for up to 8 hours postinjection (Fig 1B). In contrast to the hypoglycemic effect observed under fasting conditions, administration of 100 mg/kg LA in the fed state had no effect on blood glucose in both nondiabetic and diabetic rats (Fig 1A and B). To assess whether LA exerts the hypoglycemic effect by stimulating insulin secretion, circulating insulin levels were measured following 100 mg/kg LA. Insulin levels in diabetic rats were 50% of the levels in nondiabetic animals. In both nondiabetic and diabetic animals insulin was not significantly elevated following LA administration (Fig 1C and D).

Systemic (hepatic and renal) glucose production is essential for maintaining glucose homeostasis under fasting conditions. To eliminate the possibility of hepatotoxic or nephrotoxic effects of LA as the underlying cause of hypoglycemia, plasma levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, and creatinine were measured at 0, 1, 4, 12, and 24 hours following LA administration. There was no elevation in any of these markers of hepatic or renal damage in either nondiabetic or diabetic rats (data not shown).

LA Treatment Prevents the Hyperglycemic Response to Glucagon in Fasted Nondiabetic and Diabetic Animals

Glucagon (20 μg per rat) was injected IP to fasted rats 30 minutes after administration of vehicle or 100 mg/kg LA. Blood glucose in nondiabetic and diabetic rats increased 30 minutes after glucagon injection. In LA pretreated animals, no such hyperglycemic response was observed. Furthermore, the fact that the reduction in blood glucose was not prevented by administration of glucagon suggests an interference of LA in gluconeogenesis and/or glycogenolysis (Fig 2).

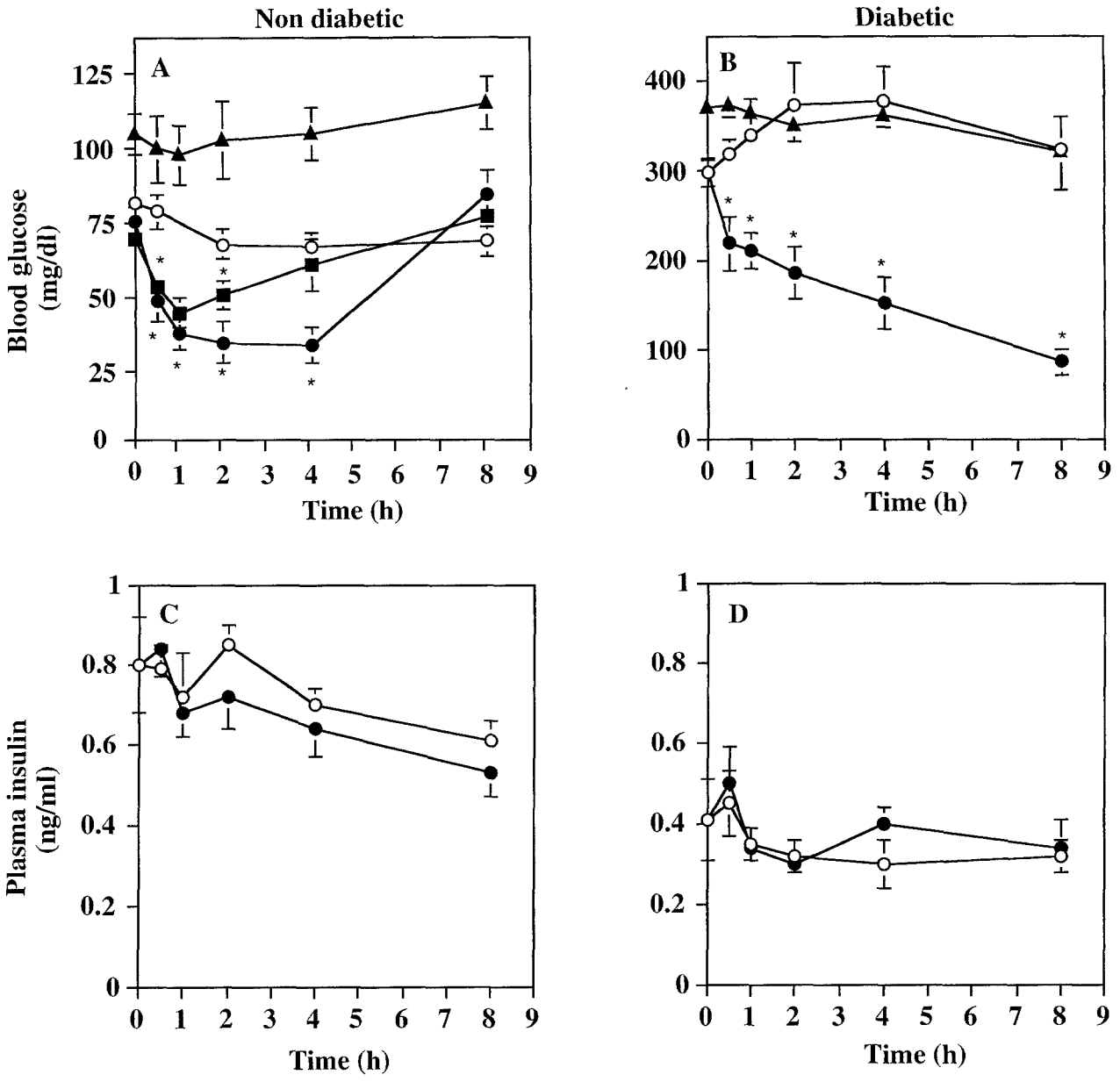

Fig 1. Effect of LA on blood glucose and insulin concentrations in nondiabetic and STZ-induced diabetic rats. (A) Nondiabetic rats in the fed state were injected IV with LA 100 mg/kg body weight (▲) or fasted for 12 hours and injected IV with either vehicle (120-mmol/L Tris buffer, pH 7.4, 1 mL, ○) or LA 60 mg/kg (■) or 100 mg/kg (●). Tail vein blood was obtained at the indicated times, and the glucose level was measured using a glucometer. (B) Seven days after induction of diabetes, rats in the fed state were injected IV with LA 100 mg/kg body weight or fasted for 12 hours and injected IV with either vehicle (120-mmol/L Tris buffer, pH 7.4, 1 mL) or LA 100 mg/kg body weight. Glucose was measured as in A. (C) Nondiabetic animals were fasted for 12 hours and injected IV with either vehicle or LA 100 mg/kg body weight. Blood samples were obtained from the tail vein at the indicated time points and centrifuged for 10 minutes, and plasma insulin was measured using a rat insulin radioimmunoassay kit. (D) Seven days after induction of diabetes, animals were fasted for 12 hours and injected IV with either vehicle or LA 100 mg/kg body weight. Insulin was measured as in C. Values are the mean ± SE for 10 different animals. *$P < .01$ *v* vehicle-treated rats at each time point.

To evaluate whether LA inhibited liver glycogen degradation, resulting in fasting hypoglycemia, the glycogen content was determined in nondiabetic and diabetic rats following vehicle or LA treatment. Fasting liver glycogen content was 110 ± 30 and 60 ± 10 mg/g protein in vehicle-treated nondiabetic and diabetic animals, respectively ($P < .01$). Following LA administration, liver glycogen content was 23 ± 5 and 30 ± 3 mg/g protein, respectively, suggesting that glycogenolysis was not inhibited by LA treatment.

LA Treatment Inhibits the Gluconeogenesis Pathway

To further determine whether LA may impair glucose production by interference with the gluconeogenetic flux and to dissect the potential location within this metabolic pathway, in vivo

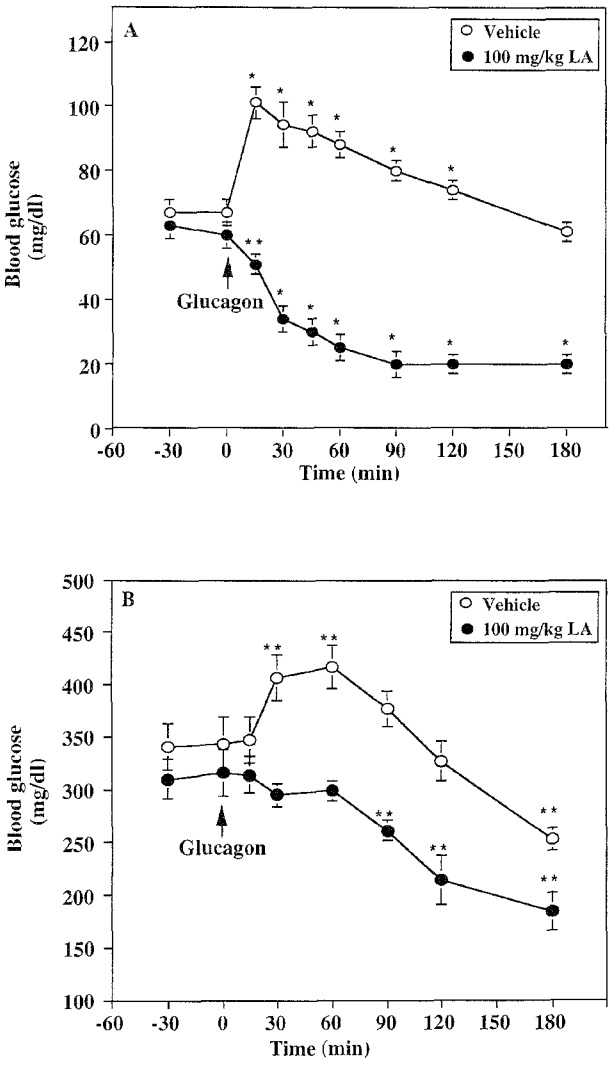

Fig 2. LA prevents the hyperglycemic response to glucagon. (A) Nondiabetic or (B) diabetic rats were injected IV with either vehicle (120-mm Tris buffer, pH 7.4, 1 mL) or LA 100 mg/kg body weight after a 12-hour fast; 30 minutes later (time 0), the animals were injected IP with glucagon (20 μg per rat). Blood samples were obtained from the tail vein at the indicated times, and glucose was measured using a glucometer. Values are the mean ± SE for 7 different animals. *$P <$ .001 *v* time 0. **$P <$ .01 *v* time 0.

loading tests with various gluconeogenetic substrates were performed. Fructose (2 g/kg IP) injected to fasted nondiabetic rats resulted in an elevation of blood glucose both in vehicle-treated and in LA-treated rats (Fig 3A), indicating that the flux from glyceraldehyde-3-phosphate to glucose is intact following LA administration. In turn, this eliminates the possibility that LA inhibits the activity of fructose-1,6-bisphosphatase and glucose-6-phosphatase. In contrast to the results obtained with fructose, alanine loading (2 g/kg IP) to nondiabetic or diabetic animals (Fig 3B and C, respectively) increased blood glucose in vehicle-treated but not LA-treated animals, indicating inhibition of the gluconeogenetic pathway with alanine as the substrate.

To define the mechanism for the reduction in the gluconeogenesis flux from alanine following LA treatment, the activities of PC and PEPCK were measured in the liver. One hour and 2 hours after LA treatment, no inhibition of either PC or PEPCK activity was found (Table 1). These results indicate that inhibition of hepatic gluconeogenesis cannot be attributed to a reduction in the maximal velocity (Vmax) of PC or PEPCK. However, substrate (pyruvate) depletion or an allosteric modulation of PC and/or PEPCK activity cannot be excluded. Plasma pyruvate concentrations were measured 1 hour after administration of either vehicle or 100 mg/kg LA, and were found to be significantly increased in both nondiabetic and diabetic LA-treated animals compared with vehicle-treated controls

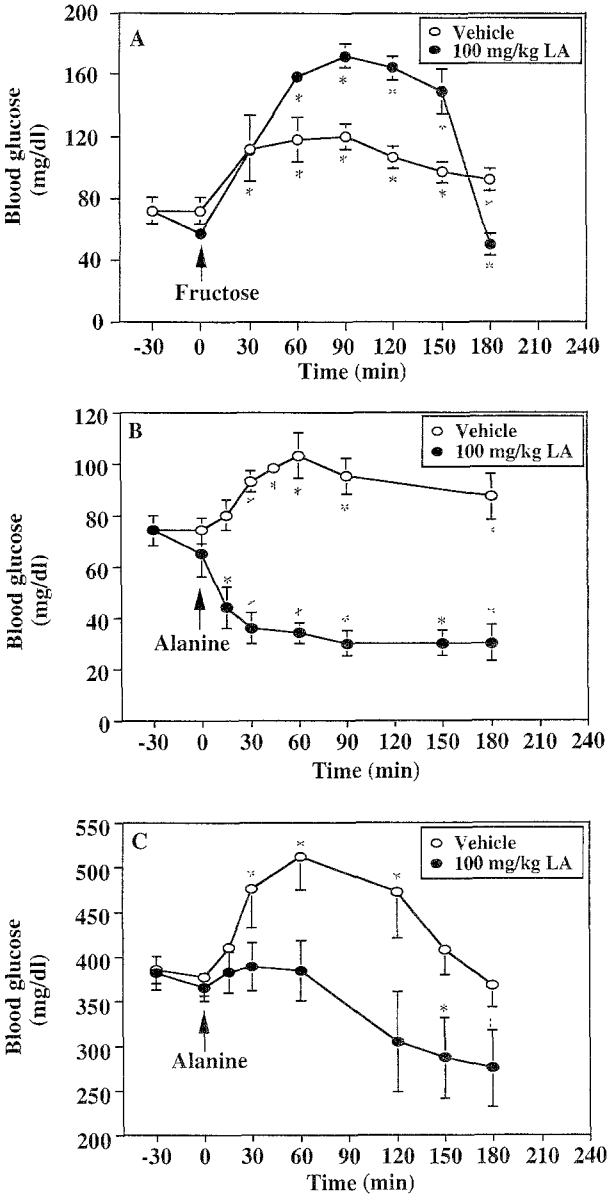

Fig 3. LA prevents glucose production from alanine but not from fructose. Following a 12-hour fast, rats were injected with either vehicle or LA as described in Fig 2; 30 minutes later (time 0), nondiabetic animals were injected IP with either fructose (A) or alanine (B) 2 g/kg body weight, and diabetic rats were injected with alanine (C). Blood samples were obtained from the tail vein at the indicated time points, and glucose was measured using a glucometer. Values are the mean ± SE for 7 different animals. *$P <$ .05 *v* time 0.

Table 1. Effect of LA on Liver Enzymes and Metabolites

Treatment	PC Activity (nmol/mg protein/min)	PEPCK Activity (nmol/mg protein/min)	CoA (nmol/g wet tissue)	Acetyl CoA (nmol/g wet tissue)
Nondiabetic				
Vehicle	44 ± 4	235 ± 14	112 ± 5	30.45 ± 9.78
LA	47 ± 2	343 ± 27*	63 ± 7*	6.24 ± 0.96†
Diabetic				
Vehicle	42 ± 3	209 ± 15	187 ± 6*	53.55 ± 11.22
LA	39 ± 4	227 ± 23	135 ± 10‡	18.46 ± 4.81§

NOTE. Nondiabetic and diabetic rats fasted for 12 hours were injected via the tail vein with 1 mL of either vehicle (120-mmol/L Tris buffer, pH 7.4) or LA 100 mg/kg dissolved in the same buffer. After injection (1 hour for nondiabetic and 2 hours for diabetic rats), animals were killed by phenobarbital overdose injection (80 mg/kg IP) and the liver was immediately removed, frozen in liquid nitrogen, and kept at −70°C until used. Liver PC and PEPCK activity and CoA and acetyl CoA concentrations were determined. Values are the mean ± SE for 5 determinations from different rats, each performed in duplicate.

*$P < .001$ *v* vehicle-treated nondiabetic.

†$P = .008$ *v* vehicle-treated nondiabetic.

‡$P = .004$ *v* vehicle-treated diabetic.

§$P = .023$ *v* vehicle-treated diabetic.

(0.20 ± 0.03 and 0.29 ± 0.02 mmol/L, in nondiabetic rats, $P < .05$; 0.25 ± 0.03 and 0.33 ± 0.02 mmol/L in diabetic rats. $P < .05$). These results exclude the possibility that substrate depletion was the cause of reduced gluconeogenetic flux following LA administration.

To address the possibility that in vivo PC activity is reduced due to depletion of its essential modulator acetyl CoA, liver acetyl CoA and free CoA concentrations were determined. Table 1 demonstrates that LA treatment caused a significant reduction in intrahepatic free CoA and acetyl CoA concentrations in both nondiabetic and diabetic rats.

Effect of LA on Plasma Fatty Acids, β-Hydroxybutyrate, and Acylcarnitine Levels

In the fasting state, β-oxidation of FFA provides the major source of liver acetyl CoA. To assess the possibility that LA causes a reduction in fatty acid β-oxidation, plasma levels of FFA, β-hydroxybutyrate, and total and free carnitine were determined. In both nondiabetic and diabetic rats, LA administration resulted in a significant increase in FFA (Table 2). This was associated with a reduced free carnitine level, while total carnitine levels remained unchanged. No elevation in blood β-hydroxybutyrate was noted in nondiabetic animals.

To exclude the possibility that LA as a short-chain fatty acid sequestered carnitine, plasma acylcarnitine methyl esters were analyzed by ESI MS/MS. Quantitatively, the major acylcarnitine species in plasma are acetylcarnitine and propionylcarnitine. No specific peak that could correspond to lipoylcarnitine was observed in the plasma of LA-treated animals (not shown). In both nondiabetic and diabetic rats, palmitoyl (C16), stearyl (C18:0), oleyl (C18:1), and linoleyl (C18:2) carnitines were all increased 2- to 2.5-fold 1 hour following LA injection compared with vehicle treatment (Table 3). Taken together, the data presented herein are consistent with the proposition that high concentrations of LA inhibit liver β-oxidation, leading to increased formation of long-chain fatty acylcarnitine.

Table 2. Effect of LA on Plasma FFA, β-Hydroxybutyrate, and Carnitine Levels

Treatment	FFA (nmol/mL)	β-Hydroxy-butyrate (mmol/L)	Total Carnitine (nmol/mL)	Free Carnitine (nmol/mL)
Nondiabetic				
Vehicle	497 ± 34	1.25 ± 0.21	19.80 ± 0.61	13.02 ± 0.18
LA	1,018 ± 81*	1.40 ± 0.05	17.30 ± 0.17	7.61 ± 0.31*
Diabetic				
Vehicle	289 ± 31*	ND	17.64 ± 1.23	12.36 ± 0.45
LA	437 ± 47†	ND	17.66 ± 1.69	9.66 ± 0.39†

NOTE. Rats were treated as described in Table 1. After death, the blood was sampled via cardiac puncture and collected into EDTA-containing tubes. Plasma was separated by centrifugation for 10 minutes at 2,000 × *g*. FFA, β-hydroxybutyrate, and free and total carnitine were determined. Values are the mean ± SE for 5 determinations from different rats, each performed in duplicate.

Abbreviation: ND, not determined.

*$P < .001$ *v* vehicle-treated nondiabetic.

$P < .05$ *v* vehicle-treated diabetic.

DISCUSSION

This study demonstrates a blood glucose–lowering effect of LA in both nondiabetic and diabetic rats via inhibition of systemic glucose production. LA, which was found to be beneficial in the treatment of diabetic polyneuropathy,[30,31] may exert its effects by one or more well-documented biological properties: (1) it is a potent antioxidant, as observed in a wide variety of experimental systems[32,33]; (2) it is a cofactor of mitochondrial ketoacid dehydrogenases[32]; and (3) it increases insulin-stimulated glucose transport into skeletal muscle.[14] In accordance with this, we previously reported that 10 days of treatment of STZ-diabetic rats with 30 mg/kg LA improved glycemia by enhancing skeletal muscle insulin-stimulated and GLUT4-dependent glucose transport,[12,13] a phenomenon similarly observed in both the skeletal muscle of fatty Zucker rats[14] and muscle cells in culture.[11] In this study, the reduction in blood glucose observed after a single high-dose (100 mg/kg) treatment of LA to fasted rats (Fig 1A and B) could not be attributed to a similar mode of action. The effect of LA under

Table 3. Effect of LA on Plasma Acylcarnitine Levels (nmol/mL)

	Carnitine			
Treatment	C16	C18	C18:1	C18:2
Nondiabetic				
Vehicle	0.16 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
LA	0.41 ± 0.05*	0.17 ± 0.03*	0.21 ± 0.04*	0.15 ± 0.02*
Diabetic				
Vehicle	0.17 ± 0.02	0.13 ± 0.01	0.10 ± 0.02	0.07 ± 0.01
LA	0.41 ± 0.01†	0.24 ± 0.03†	0.21 ± 0.03†	0.13 ± 0.03†

NOTE. Rats were treated as described in Table 1. After death, the blood was sampled via cardiac puncture and the plasma was separated. Acylcarnitine levels were determined by tandem mass spectrometry with electrospray ionization. Values are the mean ± SE for 5 determinations from different rats, each performed in duplicate.

*$P < .05$ *v* vehicle-treated nondiabetic.

†$P < .02$ *v* vehicle-treated diabetic.

these conditions was restricted to the fasting state, and direct measurements of glucose uptake activity in isolated soleus muscle failed to demonstrate increased skeletal muscle glucose transport activity.[12] Hence, an inhibition of systemic glucose production, which is activated during fasting to maintain euglycemia, was suggested by these findings as the mechanism for LA action under these conditions.

The hypoglycemic response to LA occurred although liver glycogenolysis was intact. This could be explained by the fact that the contribution of glycogenolysis to glucose production is low after 12 hours' fasting. The return of blood glucose to pretreatment levels in nondiabetic animals (Fig 1A) may represent the termination of the LA effect on systemic glucose production, since the half-life of LA is approximately 25 minutes.[34] Alternatively, it may result from the activation of counterregulatory systems in response to hypoglycemia. The different kinetics in the hypoglycemic response to LA between nondiabetic and diabetic rats (Fig 1A and B, respectively) may reflect either differences in the pharmacokinetics of LA and/or a different degree of activation of the counterregulatory response.

The loading and stimulation tests (Figs 2 and 3) clearly indicate that although hepatic glucose production was not measured directly, inhibition of systemic gluconeogenesis occurred following LA administration. Since the conversion of alanine, but not fructose, to glucose was inhibited by LA (Fig 3A to C), the gluconeogenesis defect could be mapped to steps between pyruvate and glyceraldehyde-3-phosphate (GA3P), in which PC and PEPCK are the two regulatory enzymes. The data presented in this study are consistent with a possible impairment in the in vivo activity of PC after LA treatment, due to a reduced availability of its activator acetyl CoA.

The main source of acetyl CoA production during starvation is β-oxidation of FFA. Elevated plasma FFA concentrations following LA treatment in both nondiabetic and diabetic rats (Table 2) suggest that adipocyte lipolysis is not inhibited by LA. The increased production of long-chain fatty acylcarnitines (Table 3) associated with a reduction of free carnitine (Table 2) suggests that the cytosolic steps in FFA catabolism (acyl CoA synthetase and CPT-1 activity) are intact following LA administration (Fig 4). Therefore, although a reduction in the total liver CoA concentration was observed following LA treatment (Table 1), it does not seem to be rate-limiting for cytosolic acyl CoA synthetase activity. However, the reduced liver acetyl CoA concentrations and lack of elevation in plasma ketone bodies are consistent with inhibition of the intramitochondrial steps of fatty acid oxidation. These may include an inhibition of CPT-2 or acyl CoA dehydrogenase(s) or a reduction of intramitochondrial CoA (Fig 4).[3] The latter possibility of a direct sequestration of intramitochondrial CoA by LA has been previously suggested in hepatocytes,[16] and if it correctly reflects the in vivo situation, it provides a plausible explanation for the gluconeogenesis-inhibitory effect of LA.

Recent studies suggest that the kidney is a significant contributor to systemic glucose production under fasting conditions.[35,36] The lack of a hyperglycemic response following both glucagon stimulation and alanine loading indicates an inhibition of systemic glucose production (Figs 2A and B and 3B and C, respectively). Thus, it is plausible that an inhibition of FFA oxidation by LA resulting in an inhibition of the gluconeogenetic flux represents a common mechanism for both the liver and the kidney.

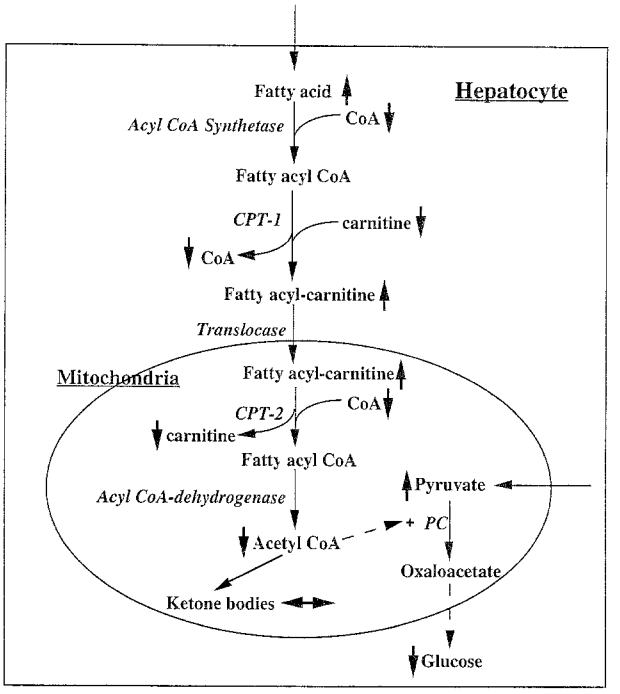

Fig 4. Proposed mode for the inhibitory effect of LA on fatty acid oxidation. Bold arrows indicate changes measured in the liver or plasma following LA treatment. Enzymes are italicized.

In the search for an antihyperglycemic agent for selective inhibition of liver glucose production but without an effect on skeletal muscle β-oxidation, the tissue specificity of CPT-1 rendered it a potential therapeutic target.[37-39] Although LA does not seem to inhibit FFA oxidation by interfering with CPT-1 activity, influencing its relative accumulation in the liver and kidney versus skeletal muscle by the administration route and dosage may provide tissue-specific effects. However, one of the basic drawbacks of agents that inhibit FFA oxidation is the accompanying elevation in circulating FFA, which may have deleterious effects such as insulin resistance and enhanced atherogenesis.[40] These should be weighed against the expected benefit in the treatment of the individual patient.

In conclusion, this study suggests that short-term administration of LA at high dosage to normal and diabetic rats causes inhibition of systemic glucose production secondary to interference with hepatic fatty acid oxidation. This mode of action for the hypoglycemic effect of LA raises its potential therapeutic value, particularly for diabetic patients in whom fasting hyperglycemia due to glucose overproduction is a major metabolic abnormality. This therapeutic potential should be further confirmed in clinical trials in both type 1 and type 2 diabetic subjects.

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