Activation of Central Lactate Metabolism Lowers Glucose Production in Uncontrolled Diabetes and Diet-Induced Insulin Resistance

Madhu Chari,[1,2] Carol K.L. Lam,[1,2] Penny Y.T. Wang,[2] and Tony K.T. Lam[1,2,3]

OBJECTIVE—Hypothalamic lactate metabolism lowers hepatic glucose production and plasma glucose levels in normal rodents. However, it remains unknown whether activation of hypothalamic lactate metabolism lowers glucose production and plasma glucose levels in rodents with diabetes and obesity.

RESEARCH DESIGN AND METHODS—We performed intracerebroventricular (ICV) administration of lactate to enhance central lactate metabolism in *1*) early-onset streptozotocin-induced uncontrolled diabetic rodents, *2*) experimentally induced hypoinsulinemic normal rodents, and *3*) early-onset diet-induced insulin-resistant rodents. Tracer-dilution methodology was used to assess the impact of ICV lactate on the rate of glucose production in all three models.

RESULTS—We first report that in the absence of insulin treatment, ICV lactate administration lowered glucose production and glucose levels in rodents with uncontrolled diabetes. Second, ICV lactate administration lowered glucose production and glucose levels in normal rodents with experimentally induced hypoinsulinemia. Third, and finally, ICV lactate administration lowered glucose production in normal rodents with diet-induced insulin resistance.

CONCLUSIONS—Central lactate metabolism lowered glucose production in uncontrolled diabetic and normal rodents with hypoinsulinemia and in rodents with diet-induced insulin resistance. These data suggest that insulin signaling is not required for central lactate to lower glucose production and that the activation of hypothalamic lactate metabolism could consequently bypass insulin resistance and lower glucose levels in early-onset diabetes and obesity. ***Diabetes*** **57:836–840, 2008**

Recent studies indicate that the hypothalamus detects a rise in nutrients and hormones in order to regulate peripheral metabolic processes in normal rodents (1–9). Specifically, hypothalamic administration of glucose and its subsequent conversion to lactate lowers glucose production and plasma glucose levels in the presence of basal insulin levels (10). No studies to date, to our knowledge, have examined whether activation of hypothalamic lactate metabolism lowers glucose production and plasma glucose levels in models with diabetes or obesity. We hypothesized that hypothalamic lactate metabolism regulates glucose homeostasis independent of insulin signaling and could consequently bypass insulin resistance to lower glucose production and glucose levels in diabetes and obesity.

Here, we first tested whether an enhancement of central lactate metabolism regulates glucose homeostasis in uncontrolled diabetic rodents. Since uncontrolled diabetes is associated with metabolic disturbances and hypoinsulinemia, we next alternatively and selectively evaluated whether central lactate metabolism regulates glucose homeostasis in normal rodents with experimentally induced hypoinsulinemia. Finally, we tested whether central lactate metabolism bypasses insulin resistance to regulate glucose homeostasis in high-fat diet–induced insulin-resistant rodents. These studies could reveal novel therapeutic targets in the hypothalamus to bypass insulin resistance and lower glucose levels in diabetes and obesity.

RESEARCH DESIGN AND METHODS

We studied 8-week-old male Sprague-Dawley rats (Charles River Laboratories, Montreal, Canada). Rats were housed in individual cages and subjected to a standard light-dark cycle. We implanted a single catheter into the third cerebral ventricle, for intracerebroventricular (ICV) infusions, by stereotaxic surgery. After ~1 week of recovery, additional catheters were placed in the internal jugular vein and carotid artery for infusion and sampling, respectively, during the eventual in vivo infusion procedure. Recovery from surgery was monitored by measuring daily food intake and body weight gain in days preceding the infusion procedure. All study protocols were reviewed and approved by the institutional animal care and use committee of the Toronto General Hospital Research Institute, University Health Network.

Early-onset uncontrolled diabetes model. Uncontrolled diabetes was induced with a single injection of streptozotocin (STZ) (60 mg/kg i.v.; Sigma, St. Louis, MO), a potent diabetogenic agent that is cytolytic against pancreatic β-cells, dissolved in sterile saline, at the end of the vascular surgery. The glycemic status of diabetic rats was monitored daily using a hand-held glucometer (Accu-Chek Compact Plus; Roche Diagnostics, Laval, Canada); only rats that were sufficiently hyperglycemic (blood glucose >15 mmol/l) were included in the diabetes studies. Fifty-four to sixty hours post-STZ injection, the in vivo ICV infusion experiments were carried out, and lasted a total of 3 h, in rats that were limited to 20 g of standard chow the previous night. We infused ICV vehicle (0.9% wt/vol NaCl) or ICV 5 mmol/l L-lactate throughout the experiments. A primed-continuous infusion of [3-^{3}H]glucose (40 μCi bolus, 0.4 μCi/min thereafter; Perkin Elmer) was initiated at 90 min and maintained throughout the study to assess glucose kinetics. Plasma samples were collected in the final 0.5 h (150–180 min) for determination of [^{3}H]glucose specific activity as well as hormone levels.

Experimentally induced hypoinsulinemic model. These in vivo infusion experiments lasted a total of 270 min and were carried out in normal rats that were limited to 20 g of standard chow the previous night. A primed-continuous infusion of [3-^{3}H]glucose (40 μCi bolus, 0.4 μCi/min thereafter; Perkin Elmer) was initiated at the start of the protocol along with somatostatin (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Sigma). An infusion of ICV saline or 5 mmol/l lactate was initiated at 90 min and maintained until the end of the experiments (270 min). Plasma glucose level was monitored every 10 min throughout the protocol. Plasma samples were collected throughout the protocol for the determination of

From the [1]Department of Physiology, University of Toronto, Toronto, Ontario, Canada; the [2]Toronto General Hospital Research Institute, University Health Network, Toronto, Ontario, Canada; and the [3]Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

Address correspondence and reprint requests to Dr. Tony Lam, MaRS Centre, Toronto Medical Discovery Tower, Room 10-706, 101 College St., Toronto, Ontario M5G 1L7, Canada. E-mail: tony.lam@uhnres.utoronto.ca.

Received for publication 12 October 2007 and accepted in revised form 5 January 2008.

Published ahead of print at http://diabetes.diabetesjournals.org on 9 January 2008. DOI: 10.2337/db07-1464.

CNS, central nervous system; ICV, intracerebroventricular.

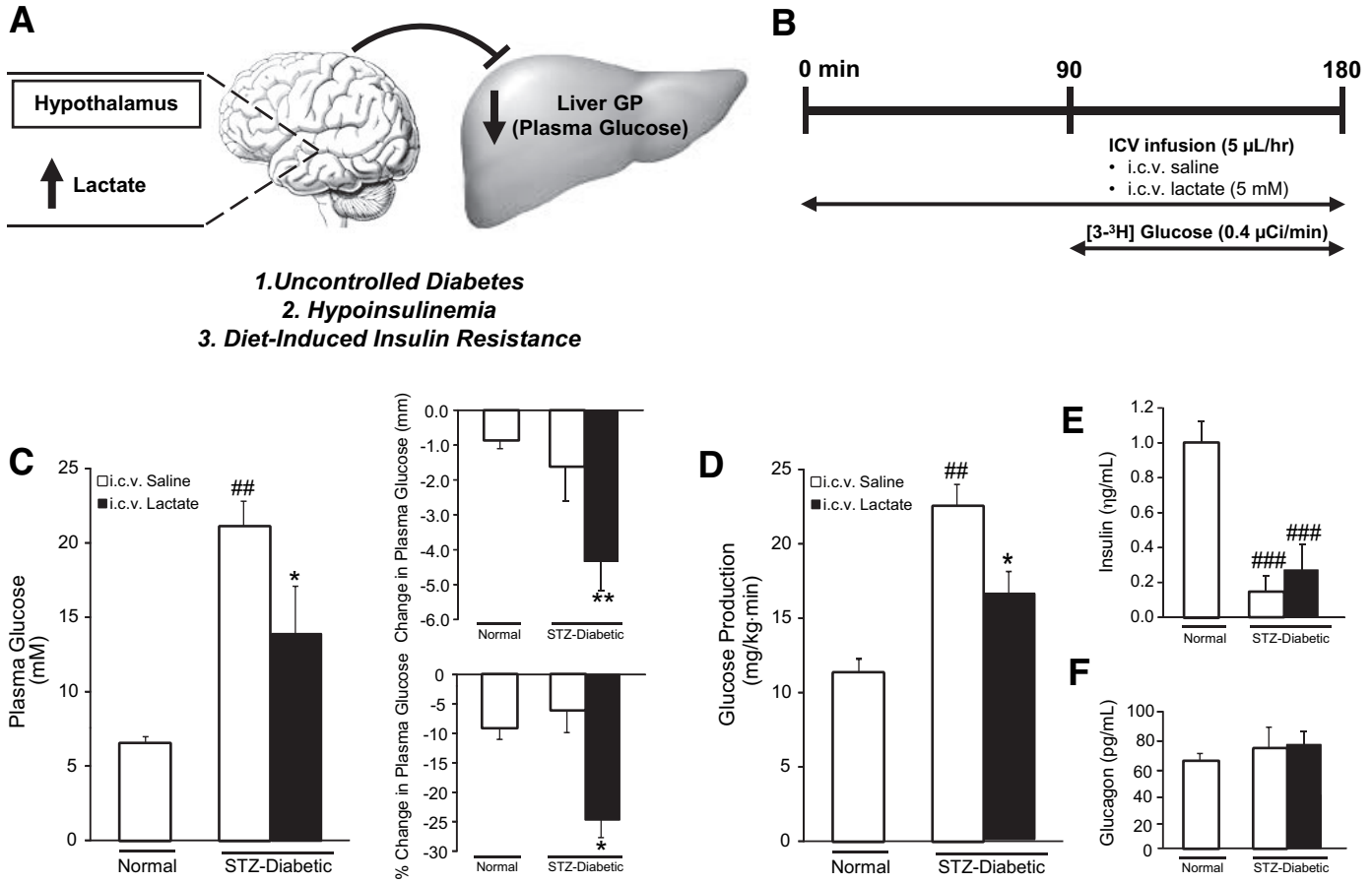

FIG. 1. Activation of central lactate metabolism lowers glucose production and plasma glucose levels in uncontrolled diabetes. *A*: Schematic representation of the working hypothesis. Activation of hypothalamic lactate metabolism lowers glucose production and plasma glucose in the early onset of uncontrolled diabetes, selective hypoinsulinemia, and diet-induced insulin resistance. *B*: Experimental protocol of ICV infusion in normal and STZ-induced diabetic rats. *C*: STZ injections increased plasma glucose level to ~18–20 mmol/l in ICV saline ($n = 6$) ($^{\#\#}P < 0.01$ vs. ICV saline normal, $n = 7$). Administration of ICV lactate ($n = 6$) lowered plasma glucose levels compared with ICV saline in STZ rats ($^{*}P < 0.05$). Lactate ICV lowered plasma glucose levels by 4 mmol/l or 25% from pre-ICV (or basal) ($^{**}P < 0.01$ or $^{*}P < 0.05$ vs. ICV saline STZ). *D*: STZ injection increased glucose production to ~22 mg · kg^{-1} · min^{-1} in ICV saline ($^{\#\#}P < 0.01$ vs. normal). Administration of ICV lactate lowered glucose production ($^{*}P < 0.05$ vs. ICV saline STZ). *E*: STZ injection lowered plasma insulin levels in ICV saline-administered or lactate-treated STZ rats ($^{\#\#\#}P < 0.001$ vs. normal). *F*: Glucagon levels were comparable in all groups. The plasma glucose levels for pre-ICV (or basal) were 7.2 ± 0.2 (ICV saline normal), 22.5 ± 2.6 (ICV saline STZ), and 18.3 ± 2.7 mmol/l (ICV lactate STZ) (STZ ICV saline or lactate vs. ICV saline normal, $P < 0.01$).

[^{3}H]glucose specific activity as well as hormone levels. This experimental approach decreased plasma insulin levels by 50% in both ICV saline- or lactate-infused rats. In ICV saline-infused rats, plasma glucose level was elevated by 180–200 min, and this elevation was sustained until the end of the experiments. Thus, this is an acute model of hypoinsulinemia/hyperglycemia that does not have long-term metabolic disturbances, as seen in uncontrolled diabetes.

Early-onset, high-fat diet–induced, insulin-resistant model. Animals were fed a high-fat (lard oil enriched) diet (catalog no. 9389; Purina Mills) that was generated by supplementing the standard chow with 10% lard for 3 days. The caloric content of the high-fat diet was 45% carbohydrate, 22% protein, and 33% fat and 44, 24, and 6.19%, respectively, for the standard chow. The total calories provided by digestible nutrients was 5.14 kcal/g for the high-fat diet versus 3.3 kcal/g for the standard chow. The in vivo infusion experiments lasted a total of 4.5 h. A primed-continuous infusion of [3-^{3}H]glucose (40 µCi bolus, 0.4 µCi/min thereafter; Perkin Elmer) was initiated at 0 min. We infused ICV vehicle (0.9% wt/vol NaCl) or ICV 5 mmol/l L-lactate from time 90 min and onward until 270 min. A basal pancreatic insulin clamp was performed in the final 2 h (150–270 min) of the study: a continuous coinfusion of insulin (0.8 mU · kg^{-1} · min^{-1}) and somatostatin (3 µg · kg^{-1} · min^{-1}) was administered, and a variable infusion of 25% glucose solution was administered as needed to clamp and maintain the plasma glucose concentration at levels similar to those of the basal state. Plasma samples were collected in the final 0.5 h (240–270 min) for determination of [^{3}H]glucose specific activity as well as hormone levels. Plasma insulin (Fig. 3*C*) and glucagon (Fig. 3*D*) levels were maintained at ~0.9 ng/ml and ~37 pg/ml, respectively. A separate set of hyperinsulinemic (insulin dosage: 3 mU · kg^{-1} · min^{-1})-euglycemic clamp experiments was performed in this high-fat–fed model to evaluate whether the high-fat diet induces insulin resistance.

Biochemical analysis. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer GM9; Analox Instruments, Lunenberg, MA). Plasma insulin, glucagons, and leptin concentrations were measured by RIA (Linco Research, St. Charles, MO).

Calculation and statistical analysis. One-way ANOVA was performed to compare differences between treatments (ICV saline versus ICV lactate) in specific individual groups. Two-way ANOVA was performed to compare differences between the effects of ICV lactate in STZ, experimentally induced hypoinsulinemia, and high-fat–fed rodents using treatment (ICV saline vs. ICV lactate) and experimental (STZ vs. experimentally induced hypoinsulinemia vs. high-fat fed) groups as independent variables. Statistical calculations were performed using SAS software (SAS, Cary, NC). Analysis revealed no interaction between the treatment and the experimental groups, indicating that ICV lactate similarly lowered glucose production in all three models. Data are presented as means ± SEM. The final 30 min of the experiments were averaged for the "ICV" time period and used for the calculation of plasma glucose, insulin, and glucagon levels and glucose production rates. The start of the experiment ($t = 0$ min) was used for the "pre-ICV" time period and for the calculation of starting plasma glucose levels.

RESULTS AND DISCUSSION

To examine whether the activation of central nervous system (CNS) lactate sensing mechanisms lower glucose

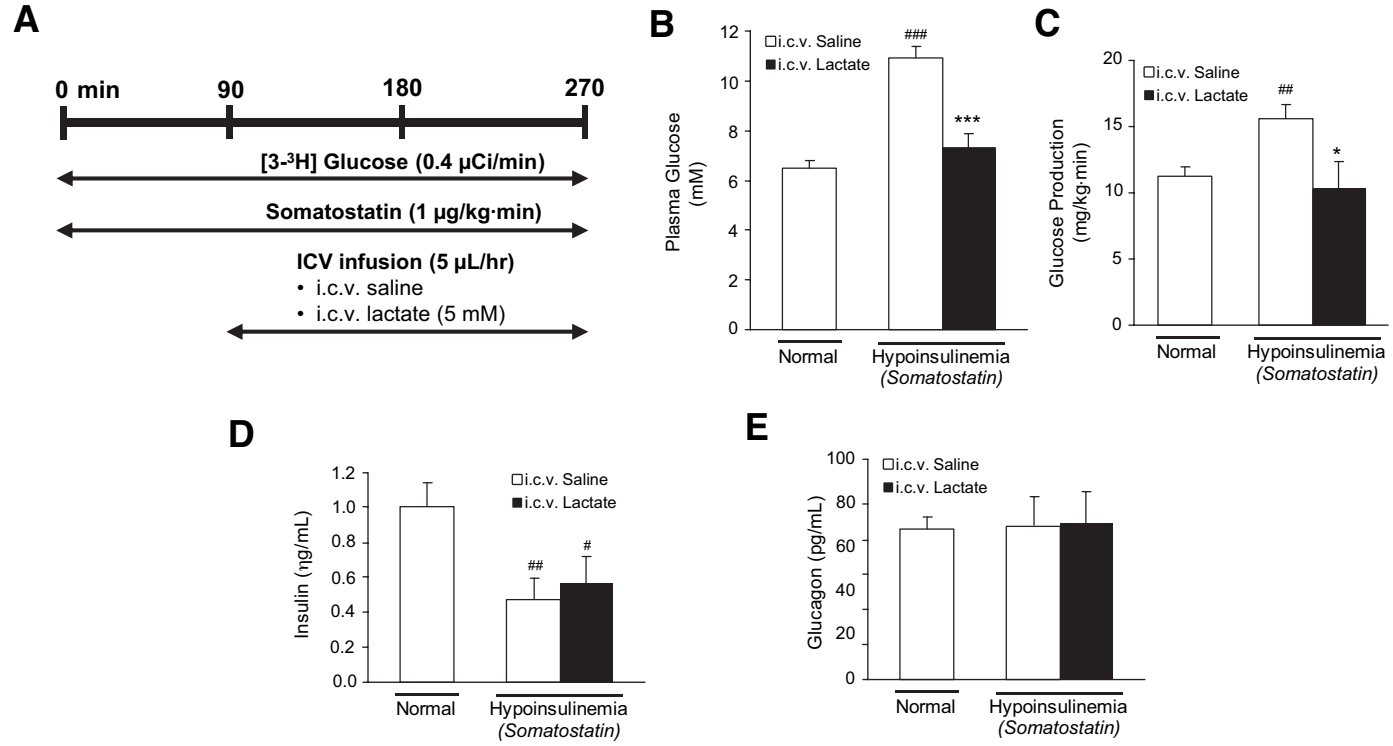

FIG. 2. Activation of central lactate metabolism lowers glucose production and plasma glucose level in experimentally induced hypoinsulinemia. *A*: Experimental protocol of ICV infusion in normal or hypoinsulinemic rats. Administration of intravenous somatostatin with ICV saline ($n = 6$) increased plasma glucose levels ($^{###}P < 0.001$ vs. ICV saline normal, $n = 6$) (*B*) and glucose production ($^{##}P < 0.01$ vs. ICV saline normal) (*C*) and lowered insulin levels ($^{##}P < 0.01$ vs. ICV saline normal rats) (*D*). *E*: Administration of intravenous somatostatin with ICV lactate ($n = 6$) prevented the rise in plasma glucose levels ($^{***}P < 0.001$ vs. ICV saline hypoinsulinemic rats, $n = 6$) (*B*) and glucose production ($^{*}P < 0.05$ vs. ICV saline hypoinsulinemic rats) (*C*) in the presence of low insulin levels ($^{#}P < 0.05$ vs. ICV saline normal rats) (*D*). *E:* Glucagon levels were comparable in all groups.

production and glucose levels in diabetes (Fig. 1*A*), we first established an early-onset uncontrolled diabetic rat model with an intravenous STZ injection (11). Within 30 h of the STZ injection (60 mg/kg), plasma glucose levels were elevated to at least 15 mmol/l. The metabolic effects of CNS lactate sensing were tested in the STZ rats whose plasma glucose levels were elevated to ~20 mmol/l for ~24–30 h (or 54–60 h post-STZ injections) (Fig. 1*C*). We first examined the peripheral glucose metabolism in these STZ rats using tracer-dilution methodology (Fig. 1*B*) (10). Hepatic glucose production in ICV saline–administered STZ rats was elevated to ~22 from 11.2 ± 0.7 mg · kg^{-1} · min^{-1} (as seen in normal rats) (Fig. 1*D*). This elevation of glucose production increased plasma glucose levels to ~20 mmol/l (Fig. 1*C*) in the presence of very low plasma insulin (Fig. 1*E*) but normal glucagon levels (Fig. 1*F*).

To test the metabolic impact of CNS lactate sensing in STZ rats, lactate was administered ICV at a concentration (10) that rapidly lowers glucose production and plasma glucose levels in normal rodents (Fig. 1*B*). Lactate ICV administration lowered glucose production by ~6 mg · kg^{-1} · min^{-1} (Fig. 1*D*), which led to a drop in plasma glucose levels of 4.3 ± 0.8 mmol/l or a suppression of 25 ± 4% from pre-ICV lactate (Fig. 1*C*) in STZ rats. Importantly, this occurred in the presence of very low insulin (Fig. 1*E*) but normal glucagon levels (Fig. 1*F*). Given the fact that the activation of hypothalamic insulin signaling via direct insulin injection lowers glucose levels in uncontrolled diabetes (11), activation of hypothalamic lactate metabolism could serve as a complementary approach that targets the brain to lower glucose levels in diabetes. An activation of central lactate metabolism lowers glucose levels in uncontrolled diabetes in the absence of insulin injections.

Because uncontrolled diabetes is associated with metabolic disturbances and hypoinsulinemia, we alternatively and selectively examined whether central lactate metabolism regulates glucose homeostasis in the presence of hypoinsulinemia. We first established an experimentally induced hypoinsulinemic model in normal rodents with a method similar to that used previously in dogs (12) (Fig. 1*A* and Fig. 2*A*). Somatostatin administered at a dosage of 1 μg · kg^{-1} · min^{-1} in normal rats decreased plasma insulin levels by 50% (Fig. 2*D*), but plasma glucagon levels were not affected (Fig. 2*E*, as seen in STZ rats). This experimental approach resulted in an elevation of glucose production (15.8 ± 1.4 mg · kg^{-1} · min^{-1}) and plasma glucose levels (10.8 ± 0.3 mmol/l) by 180–200 min. Glucose production was elevated to 15.6 ± 1.1 mg · kg^{-1} · min^{-1} (Fig. 2*C*) and plasma glucose levels to 10.9 ± 0.5 mmol/l (Fig. 2*B*) in normal rodents with ICV saline administration by the end of the infusion studies (240–270 min). In contrast, in rats that received ICV lactate administration starting at 90 min, the rise in glucose production (10.2 + 2.1 mg · kg^{-1} · min^{-1}) (Fig. 2*C*) and plasma glucose levels (7.3 + 0.6 mmol/l) was prevented (Fig. 2*B*) throughout the infusion protocol. Together with the findings on STZ rats, these data indicate that insulin signaling is not required for central lactate metabolism to lower glucose production and glucose levels and suggest that the activation of hypothalamic lactate metabolism could bypass insulin

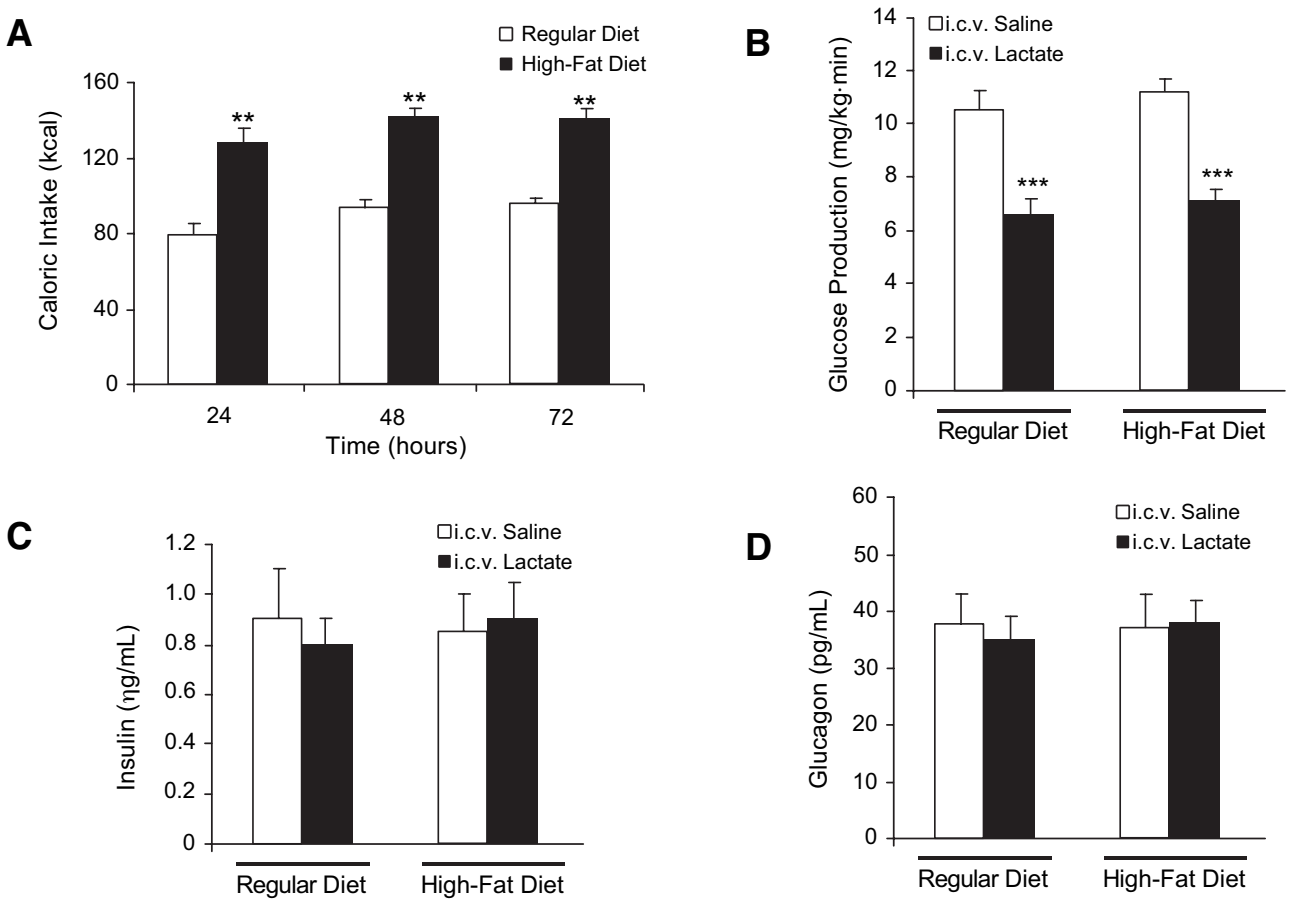

FIG. 3. Activation of central lactate metabolism lowers glucose production in the high-fat diet–induced insulin-resistant model. *A*: Normal male Sprague-Dawley rats (weight ~240 g, $n = 5$) had a caloric intake of ~85 kcal/day (standard chow at Toronto General Hospital) (□). Once the rats were switched to a lard oil–enriched diet ($n = 5$; ■), the rats increased their caloric intake to ~130 kcal within 1 day, and the elevation of caloric intake was maintained for 3 days ($P < 0.01$ vs. regular diet). *B*: Administration of ICV lactate lowered glucose production in the high-fat–fed rats (***$P < 0.001$ vs. ICV saline high-fat diet) to the same extent as in the regular chow–fed rats (***$P < 0.001$ vs. ICV saline regular diet) during the clamp. *C*: Insulin levels were comparable in all groups during the clamp. *D*: Glucagon levels were comparable in all groups during the clamp.**

resistance and lower glucose production in diet-induced insulin-resistant models.

To directly test this hypothesis, the metabolic effects of lactate ICV were tested in an early-onset high-fat diet–induced insulin-resistant model (13,14) (Fig. 1*A*). Here, we confirm that rats fed a lard oil–enriched diet rapidly increased their food intake (Fig. 3*A*), developed hepatic insulin resistance (% glucose production suppression from basal obtained by hyperinsulinemic-euglycemic clamps: 75 ± 5% [regular chow, $n = 4$] vs. 23 ± 3% [high-fat diet, $n = 6$], $P < 0.001$), and had elevated plasma insulin (0.8 ± 0.2 ng/ml [regular chow] vs. 1.9 ± 0.3 ng/ml [high-fat diet], $P < 0.01$) and leptin (1.1 ± 0.3 nmol/l [regular chow] vs. 2.1 ± 0.2 nmol/l [high-fat diet], $P < 0.01$) levels. In this high fat–fed model, ICV lactate administration lowered glucose production during the pancreatic basal insulin clamps (Fig. 3*B*). Importantly, this metabolic effect of lactate ICV was comparable with that in regular chow–fed rats (Fig. 3*B*). These data indicate that central lactate metabolism bypassed insulin resistance to lower glucose production in a diet-induced insulin-resistant model.

Here we report that a selective central activation of lactate metabolism similarly lowered glucose production in the early-onset models of uncontrolled diabetes, hypoinsulinemia, and diet-induced insulin resistance. It still remains unknown whether hypothalamic lactate sensing regulates glucose homeostasis in chronic models of diabetes and obesity. Furthermore, metabolic effects of lactate sensing in other regions of the brain (i.e., hindbrain) warrant future investigations, since inhibition of neuronal lactate transport (the reverse of direct lactate administration, as done in the current study) in the hindbrain increases plasma glucose levels (15). Finally, although selective activation of central lactate metabolism lowered glucose production and plasma glucose levels in uncontrolled diabetes in the absence of insulin injections, glucose production and glucose levels were not fully normalized. Taken together, we postulate that therapeutic strategy designed to activate hypothalamic lactate metabolism could bypass insulin resistance and serve as one of the complementary approaches to insulin injections to lower glucose levels in diabetes and obesity.

ACKNOWLEDGMENTS

This work was supported by a research grant to T.K.T.L. from the J.P. Bickell Foundation. M.C. was supported by a Banting and Best Diabetes Centre Charles Hollenberg summer studentship. T.K.T.L. holds the John Kitson McIvor Endowed Chair in Diabetes Research at the University of Health Network and University of Toronto.

REFERENCES

1. Obici S, Zhang BB, Karkanias G, Rossetti L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 8:1376–1382, 2002
2. Flier JS: Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 116:337–350, 2004
3. Schwartz MW, Porte D Jr: Diabetes, obesity, and the brain. *Science* 307:375–379, 2005
4. Lam TK, Schwartz GJ, Rossetti L: Hypothalamic sensing of fatty acids. *Nat Neurosci* 8:579–584, 2005
5. Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, Teshigawara K, Matsuki Y, Watanabe E, Hiramatsu R, Notohara K, Katayose K, Okamura H, Kahn CR, Noda T, Takeda K, Akira S, Inui A, Kasuga M: Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* 3:267–275, 2006
6. Caspi L, Wang PY, Lam TK: A balance of lipid-sensing mechanisms in the brain and liver. *Cell Metab* 6:99–104, 2007
7. Cota D, Proulx K, Seeley RJ: The role of CNS fuel sensing in energy and glucose regulation. *Gastroenterology* 132:2158–2168, 2007
8. Konner AC, Janoschek R, Plum L, Jordan SD, Rother E, Ma X, Xu C, Enriori P, Hampel B, Barsh GS, Kahn CR, Cowley MA, Ashcroft FM, Bruning JC: Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. *Cell Metab* 5:438–449, 2007
9. Lam TK, Gutierrez-Juarez R, Pocai A, Bhanot S, Tso P, Schwartz GJ, Rossetti L: Brain glucose metabolism controls the hepatic secretion of triglyceride-rich lipoproteins. *Nat Med* 13:171–180, 2007
10. Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L: Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science* 309:943–947, 2005
11. Gelling RW, Morton GJ, Morrison CD, Niswender KD, Myers MG Jr, Rhodes CJ, Schwartz MW: Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. *Cell Metab* 3:67–73, 2006
12. Cherrington AD, Lacy WW, Chiasson JL: Effect of glucagon on glucose production during insulin deficiency in the dog. *J Clin Invest* 62:664–677, 1978
13. Morgan K, Obici S, Rossetti L: Hypothalamic responses to long-chain fatty acids are nutritionally regulated. *J Biol.Chem.* 279:31139–31148, 2004
14. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L: Overfeeding rapidly induces leptin and insulin resistance. *Diabetes* 50:2786–2791, 2001
15. Patil GD, Briski KP: Lactate is a critical "sensed" variable in caudal hindbrain monitoring of CNS metabolic stasis. *Am J Physiol Regul Integr Comp Physiol* 289:R1777–R1786, 2005