Autoregulation of Endogenous Glucose Production During Hyperglucagonemia

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Increased endogenous glucose production (EGP) contributes to fasting hyperglycemia in type II diabetes. In nondiabetic subjects, increased gluconeogenesis from lactate does not increase EGP. Type 2 diabetes is associated with hyperglucagonemia. The present study was undertaken to examine whether physiologic elevation of plasma glucagon overrides autoregulation of EGP. Eight healthy volunteers were studied on 2 occasions, once during a 3-hour infusion of 30 μmol/kg/min Na-lactate and once during a control infusion of Na-bicarbonate. Plasma glucagon, insulin, and growth hormone were clamped at identical levels in both experiments. Rates of appearance of glucose, lactate, and gluconeogenesis from lactate were measured by tracer techniques. Glucagon infusion rate was elevated when the lactate or bicarbonate infusions were started to induce physiologic hyperglucagonemia. Plasma glucagon increased from baseline levels (234 ± 21 ng/L and 211 ± 23 ng/L) to 313 ± 47 ng/L (bicarbonate experiments) and 329 ± 43 ng/L (lactate experiments, means ± SE, $P > .3$). Lactate infusion increased plasma lactate concentrations from 1.1 ± 0.9 to 4.6 ± 0.5 mmol/L ($P = .0003$). Lactate conversion to glucose increased from 1.5±0.3 to 2.8±0.8 μmol/kg/min ($P = .03$) and from 1.7 ± 0.3 to 8.1 ± 0.8 μmol/kg/min ($P = .0003$) in the bicarbonate and lactate experiments, respectively. The increments in lactate conversion to glucose differed significantly ($P = .0008$). Nevertheless, plasma glucose and EGP were not different in the bicarbonate and lactate experiments: 5.4 ± 0.5 versus 6.6 ± 0.7 mmol/L ($P = .21$), and 10.5 ± 0.6 versus 11.6 ± 0.6 μmol/kg/min ($P = .19$). We conclude that in normal volunteers, neither hyperglucagonemia nor the combination of hyperglucagonemia and increased substrate availability alters the autoregulation of EGP.

RECENT EVIDENCE indicates that gluconeogenesis and glycogenolysis are responsible for approximately equal proportions of endogenous glucose production (EGP) in normal postabsorptive humans.[1] Among the factors regulating gluconeogenesis, substrate availability[2,3] and hormones (eg, insulin, glucagon, and catecholamines)[4,5] are considered to be of prime importance. With respect to substrate availability, however, increases in gluconeogenesis induced by infusions of lactate,[6] glycerol,[3,7] and alanine[8] do not result in increases in EGP. This phenomena has been referred to as hepatic autoregulation[6,9] and has been proposed to be the result of reciprocal decreases in glycogenolysis.[2,10,11]

Type 2 diabetes is a condition characterized by increased circulating glucagon and substrate concentrations.[12,13] Elevation of plasma glucagon in healthy persons leads to a waning increase in EGP, mainly accounted for by glycogen breakdown.[14] When plasma glucose and insulin concentrations rise, EGP falls again. If glucagon levels remain elevated for more than 90 minutes, and insulin levels are unchanged, the rate of gluconeogenesis increases and contribute by about 67% to EGP.[14]

Patients with type 2 diabetes have increased glucose production[15-17] and increased rate of gluconeogenesis[18] despite of elevated levels of plasma glucose and insulin.[19] Thus, autoregulation of EGP appears to be inoperative in this disorder, possibly due to the combination of increased circulating glucagon and increased substrate availability.

The present studies were therefore undertaken to test the hypothesis that hyperglucagonemia inactivates autoregulation of EGP during increased gluconeogenesis induced by infusions of lactate.

MATERIALS AND METHODS

Subjects

Informed, written consent was obtained from 8 nondiabetic, nonobese, healthy volunteers (5 women, 3 men) aged 34 to 57 years, (mean ± SEM, 45 ± 3 years) with a body mass index of 24.5 ± 1 kg/m^2. None had a family history of diabetes mellitus or took any medication. All subjects had undergone a physical examination and routine laboratory screening to exclude illnesses. For 3 days before the experiments, all subjects consumed a weight-maintaining diet containing at least 200 g of carbohydrates and abstained from alcohol.

Experiments

After obtaining approval from the local institutional review board, the subjects were admitted to the Clinical Research Unit at 7 PM the day before the experiments on 2 occasions. A standard meal was given at 7.30 PM (10 kcal/kg; 50% carbohydrate, 35% fat, 15% protein). After an overnight fast, a primed (30 μCi), continuous (0.30 μCi/min) infusions of [6-^{3}H]glucose and [U-^{14}C] lactate (New England Nuclear, Boston, MA) were started in a left antecubital vein at 6 AM. A left dorsal hand vein was cannulated and placed in a thermobox maintaining 65°C for sampling of arterialized venous blood.[20] Endogenous secretion of insulin, glucagon, and growth hormone was suppressed by infusing somatostatin (250 μg/h), and replaced by exogenous glucagon (0.7 ng/kg/min), growth hormone (3 ng/kg/min), and insulin infusions to reproduce basal levels of these glucoregulatory hormones.[21] The hormone infusions were given in the left antecubital vein and started at 7 AM. Plasma glucose concentrations were kept between 6 and 6.5 mmol/L by adjustment of the insulin infusion rate until stable glucose concentrations were obtained with a fixed insulin infusion rate that was maintained throughout the rest of the experiments. Six hours after the

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Submitted August 13, 2001; accepted March 4, 2002.

Supported by grants from the Norwegian Diabetes Association, Nordic Research Funding, Odd Fellow Research Funding, Odd Berg Gruppen Medical Research Funding, and Hoechst Norway Research Funding.

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0026-0495/02/5109-0008$35.00/0

doi:10.1053/meta.2002.34702

tracer infusions were started, a 3-hour continuous infusion of Na-L-lactate (30 μmol/kg/min) or equimolar amounts of Na-bicarbonate was started, and the glucagon infusion rate was raised to 1.68 ng/kg/ min in order to simulate the hyperglucagonemia seen in type 2 diabetes.[12]

Na-L-lactate was prepared by diluting the acid form of L-lactate (Sigma Chemical Co, St Louis, MO) in sterile water, titrating it with 10 N NaOH to pH 5.10 and filtering the solution through a 0.22-μmol/L Millipore Filter (Millipore Corp, Bedford, MA). Lactate infusate concentration was measured in each study and included in the calculations. Blood samples were drawn at -30, 0, 60, 120, 150, and 180 minutes for the determination of plasma [^{14}C] and [^{3}H] glucose specific activities, [^{14}C] lactate specific activity, and plasma glucose, lactate, alanine, insulin, C-peptide, growth hormone, and glucagon concentrations, blood pH, and bicarbonate concentrations.

Analytical Procedures

Plasma glucose concentrations were determined by a Yellow Springs Instruments glucose analyzer (2300 STAT PLUS, Yellow Springs, OH). Plasma lactate and alanine were determined by standard fluorometric methods.[22,23] Ion-exchange chromatography was used for isolation of glucose and lactate before determining plasma glucose and lactate specific activities.[24] Recovery was corrected for by use of external standards. Blood pH and bicarbonate concentrations were measured on a co-oximeter (Ciba Corning, Medfield, MA). Plasma insulin,[25] C-peptide,[26] glucagon,[27] and growth hormone[28] were measured by radioimmunoassay methods. Serum nonesterified fatty acids (NEFA) were analyzed by an acyl-coenzyme A oxydase-based colometric kit (Wako Nefa C Kit, Osaka, Japan).

Calculations

Rates of glucose and lactate appearance (Ra) and disappearance (Rd) were calculated by means of the non–steady-state equation of DeBodo et al.[29] The pool fraction used was 0.65. Distribution volumes of glucose and lactate were defined as 200 mL/kg[29] and 500 mL/kg,[30] respectively. The rate of lactate conversion to glucose was determined using the non–steady-state equation of Chiasson et al[31]:

$$\frac{(\text{Glucose Rd} \times [^{14}\text{C}]\text{Glucose SA}) + \text{PV}(\Delta[^{14}\text{C}]\text{Glucose})(\Delta t)^{-1}}{[^{14}\text{C}]\text{Lactate SA}}$$

where glucose Rd, [^{14}C]glucose SA, and [^{14}C]lactate represent the mean values between 2 consecutive time points. Δ[^{14}C]-glucose radioactivity and Δt represent the change in values for plasma glucose counts and time expressed in disintegrations per milliliter and per minute, respectively. P is the fractional constant and V is the distribution volume for glucose. The percentage of glucose Ra derived from lactate was calculated with the following equation:

$$\frac{(\text{Lactate Conversion to Glucose} \times 0.5)}{\text{Glucose Ra}} \times 100$$

Changes during experiments were calculated by the value obtained at the end of the experiments minus the value obtained before bicarbonate or lactate infusions. These data were analyzed by 1-sample *t* test. Differences between the 2 experiments were analyzed both by analysis of variance corrected for repeated measurements and by 2-sample *t* tests. *P* values were identical. *P* values obtained by the *t* tests are reported, because values obtained during minutes 150 to 180 of the experiments were normally distributed, highly correlated ($r > 0.80$), and had similar variances. *P* values less than .05 were considered statistically significant. Data are given as means ± SE. All data were analyzed using the SAS software package (SAS Institute, Cary, NC).

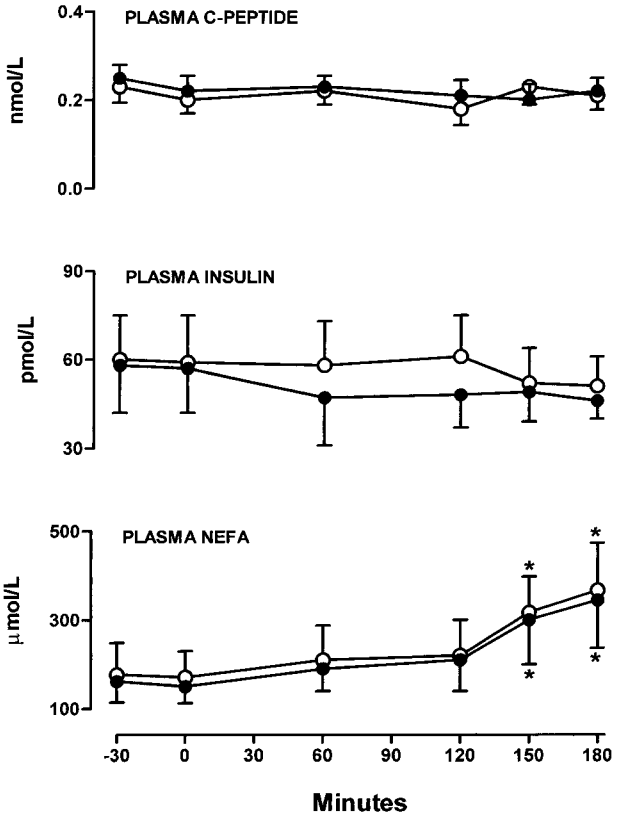

Fig 1. Levels of plasma C-peptide, insulin, and nonesterified fatty acids (NEFA) before and during a 3-hour infusion of 30 μmol/kg/min Na-lactate (●) or equimolar amounts of Na-bicarbonate (○) in 8 healthy subjects who underwent a pancreatic-adrenocortical-pituitary clamp where glucagon levels were raised to mimic the hyperglucagonemia seen in type 2 diabetes mellitus. Data are means ± SE. *$P < .05$ for differences between baseline values and values obtained at the end of the study (1-sample *t* tests).

RESULTS

Plasma C-Peptide, Insulin, NEFA, Growth Hormone, Glucagon, and Alanine

Basal plasma C-peptide levels were 0.220 ± 0.002 nmol/L in the bicarbonate experiment and 0.230 ± 0.001 nmol/L in the lactate experiment ($P > .3$). The corresponding basal insulin levels were 59 ± 19 and 57 ± 16 pmol/L ($P > .3$); basal NEFA levels were 177 ± 72 and 157 ± 41 μmol/L ($P > .3$); basal growth hormone levels were 1.40 ± 0.30 and 1.00 ± 0.20 μg/L ($P > .3$); and basal glucagon levels were 234 ± 21 and 211 ± 23 ng/L ($P > .3$). At 0 minutes, the infusion rate of glucagon was raised, and at the end of the study plasma glucagon was 329 ± 43 ng/L in the bicarbonate and 313 ± 47 ng/L in the lactate experiments ($P > .3$). Plasma C-peptide and growth hormone concentrations remained constant throughout experiments in both lactate and bicarbonate studies (Figs 1 and 2). In lactate experiments, plasma insulin tended to decline slightly from 57 ± 16 to 50 ± 9 pmol/L at the end of the experiments ($P = .25$). In the bicarbonate experiments plasma insulin was 59 ± 19 at baseline and 53 ± 11 pmol/L at the end of the experiments ($P = .31$). Plasma NEFA levels increased simi-

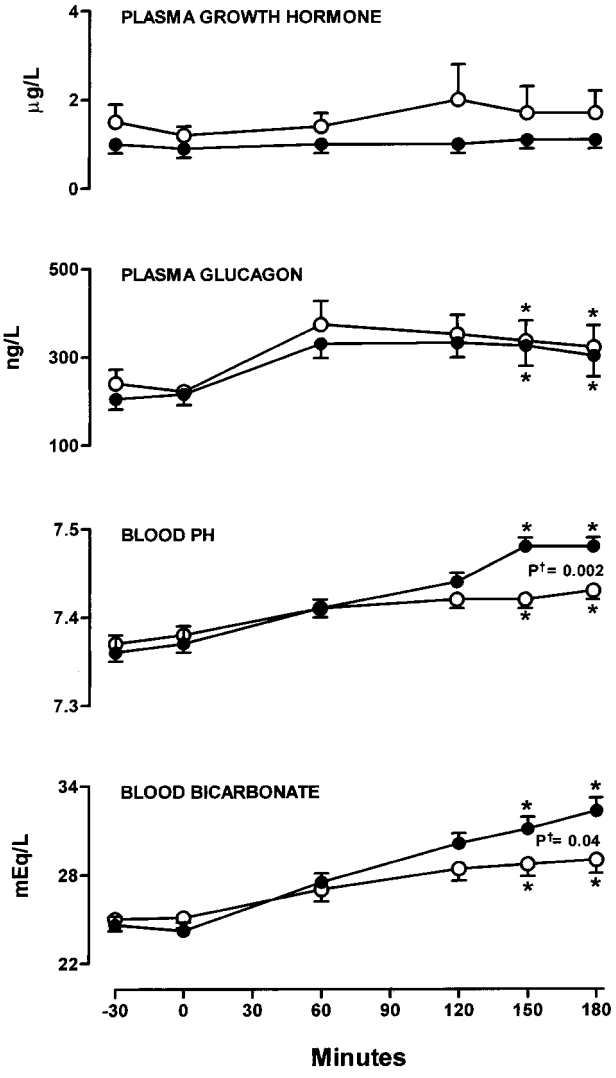

Fig 2. Levels of plasma growth hormone, glucagon, blood pH, and bicarbonate before and during a 3 -hour infusion of 30 μmol/kg/min Na-lactate (●) or equimolar amounts of Na-bicarbonate (○) in 8 healthy subjects who underwent a pancreatic-adrenocortical-pituitary clamp where glucagon levels were raised to mimic the hyperglucagonemia seen in type 2 diabetes mellitus. Data are means ± SE.*$P < .05$ for differences between baseline values and values obtained at the end of the study (1-sample *t* tests) †P values for differences between experiments (2-sample *t* tests).

larly at the end of bicarbonate (333 ± 101 μmol/L, $P = .05$ *v* baseline) and lactate (302 ± 78 μmol/L, $P = .04$ *v* baseline) infusions (lactate *v* bicarbonate infusion: $P > .3$). In bicarbonate studies, plasma alanine concentration remained constant (471 ± 19 *v* 450 ± 16 μmol/L, $P > .3$). In lactate infusion experiments, plasma alanine increased slightly from 476 ± 28 μmol/L in the basal state to 535 ± 44 μmol/L ($P = .03$) at the end of the studies.

Blood pH and Bicarbonate Concentrations

Basal blood pH and bicarbonate concentrations were not different in bicarbonate and lactate experiments (pH 7.37 ± 0.02 *v* 7.37 ± 0.01; bicarbonate 25.0 ± 0.7 *v* 24.4 ± 0.6 mEq/L, respectively, $P > .3$ for both). Both blood pH and bicarbonate increased significantly during the studies. For the last 30 minutes of the experiments, blood pH was 7.43 ± 0.01 during bicarbonate infusion and 7.48 ± 0.01 during lactate infusion ($P = .002$, lactate *v* bicarbonate experiments). Corresponding values for blood bicarbonate were 29.0 ± 0.5 mEq/L and 31.6 ± 0.9 mEq/L ($P = .04$, lactate *v* bicarbonate experiments; Fig 2).

Plasma Lactate Concentrations, Ra, Rd, Conversion to Glucose, and Percentage of Lactate Rd Converted to Glucose

Basal plasma lactate concentrations in bicarbonate and lactate experiments were 1.1 ± 0.1 and 1.2 ± 0.2 mmol/L ($P > .3$). Corresponding baseline values for lactate Ra (14.9 ± 0.2 *v* 14.5 ± 0.6 μmol/kg/min), lactate Rd (14.5 ± 0.5 *v* 14.5 ± 0.6 μmol/kg/min), and lactate conversion to glucose (1.5 ± 0.3 *v* 1.7 ± 0.3 μmol/kg/min) were also similar in the 2 experiments ($P > .3$ for all). During bicarbonate experiments, lactate Ra increased to 17.2 ± 1.5 μmol/kg/min over the last 30 minutes of the experiments ($P = .03$ *v* basal level). Lactate Rd increased nonsignificantly (from 14.5 ± 0.5 to 15.8 ± 1.8 μmol/kg/min, $P = .15$), and no change in plasma lactate could be detected at the end of experiment (1.1 ± 0.9 mmol/L, $P = .23$ *v* baseline). During lactate infusion, significant increments occurred in plasma lactate (4.6 ± 0.5 mmol/L, $P = .0003$ *v* baseline), lactate Ra (46.0 ± 1.4 μmol/kg/min, $P = .0001$ *v* baseline), and lactate Rd (46.0 ± 1.6 μmol/kg/min, $P = .0001$ *v* baseline). These values were also significantly different from the corresponding values in the bicarbonate experiments ($P < .001$ for all). Rates of lactate conversion to glucose for the final 30 minutes increased significantly both in the bicarbonate (2.8 ± 0.8 μmol/kg/ min, $P = .03$) and the lactate experiments (8.1 ± 0.8 μmol/kg/min, $P = .0005$). These rates were also significantly different from one another ($P = .0003$) (Fig 3). Baseline percentage of lactate Rd diverted into glucose conversion were similar in the bicarbonate (10.2% ± 1.9%) and lactate experiments (11.9% ± 1.9%, $P > .3$). The percentage of lactate Rd diverted into glucose conversion increased similarly during the 2 experiments to 17.0% ± 3.1% ($P = .003$ *v* basal level) during bicarbonate experiments, and to 18.1% ± 3.6% ($P = .03$ *v* basal level) during lactate experiments (Fig 4).

Plasma Glucose Concentration, Glucose Ra, and Percent Glucose From Lactate

Basal plasma glucose levels (6.2 ± 0.3 *v* 6.4 ± 0.2 mmol/L) were comparable in the bicarbonate and lactate experiments ($P > .3$). Corresponding values for glucose Ra (12.0 ± 0.3 *v* 11.7 ± 0.2 μmol/kg/min) and percent glucose derived from lactate (6.2% ± 0.3% *v* 7.1% ± 1.3%) were not significantly different ($P > .3$ for both). During bicarbonate infusions, a transient rise occurred at 60 minutes for plasma glucose (7.0 ± 0.3 mmol/L, $P = .03$ *v* baseline) and glucose Ra (14.5 ± 0.8 μmol/kg/min, $P = .04$ *v* baseline) was observed. The percent of glucose coming from lactate increased to 11.6% ± 2.9% ($P = .008$ *v* baseline) at the end of the bicarbonate experiments. During lactate infusion, the amount of glucose coming from

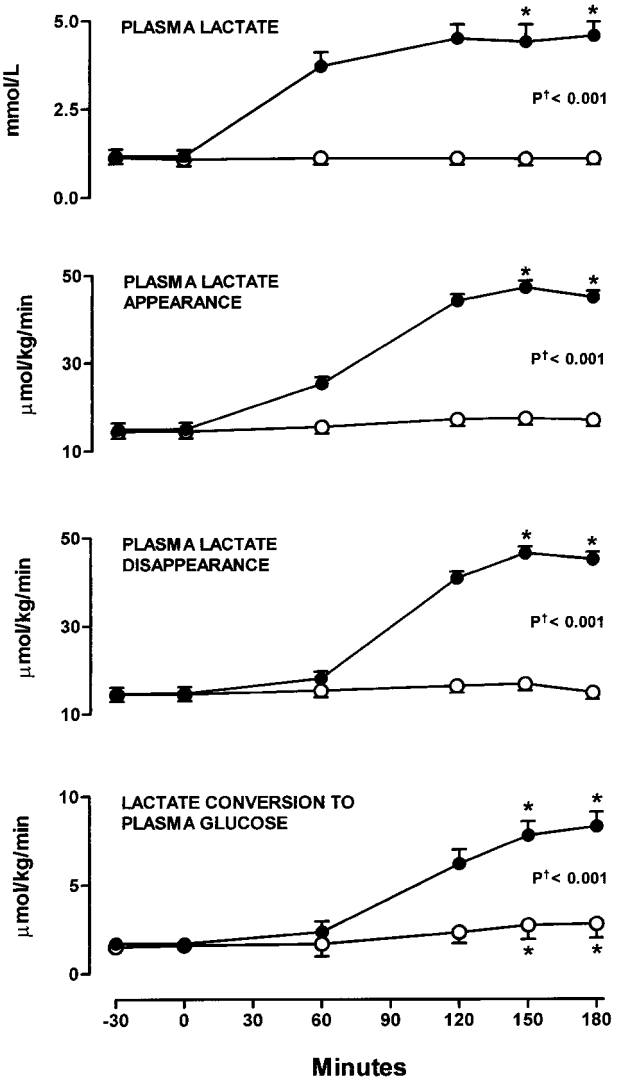

Fig 3. Plasma lactate, rate of appearance of lactate, rate of disappearance of lactate, and rate of lactate conversion to glucose (expressed in lactate equivalents) before and during a 3-hourr infusion of 30 μmol/kg/min Na-lactate (●) or equimolar amounts of Na-bicarbonate (○) in 8 healthy subjects who underwent a pancreatic-adrenocortical-pituitary clamp where glucagon levels were raised to mimic the hyperglucagonemia seen in type 2 diabetes mellitus. Data are means ± SE. *$P < .05$ for differences between baseline values and values obtained at the end of the study (1-sample *t* tests) †P values for differences between experiments (2-sample *t* tests).

lactate increased to 34.3% ± 8.4% ($P = .0005$ compared to bicarbonate experiments). Plasma glucose and glucose Ra rose transiently at 60 minutes also during lactate infusions: to 7.4 ± 0.3 mmol/L ($P = .01$ *v* baseline) and 13.5 ± 0.5 μmol/kg/min ($P = .04$ *v* baseline), respectively. This increase was not different from that in the bicarbonate experiments ($P = .25$). Mean plasma glucose levels during the third hour of the clamp did not differ significantly in the bicarbonate (5.4 ± 0.5 mmol/L) and lactate (6.6 ± 0.7 mmol/L) experiments ($P = .21$). Corresponding values for glucose Ra were 10.5 ± 0.5 μmol/kg/min in the bicarbonate and 11.6 ± 0.5 μmol/kg/min in the lactate experiments ($P = .20$, bicarbonate *v* lactate experiments). During both bicarbonate and lactate infusions, plasma glucose and glucose Ra were similar to baseline levels by the end of the experiments (Fig 4).

DISCUSSION

The present studies were undertaken to test the hypothesis that hyperglucagonemia, such as found in type 2 diabetes, might override the autoregulation of EGP that occurs during substrate induced increases in gluconeogenesis. Comparisons of changes in EGP rates are difficult due to interindividual

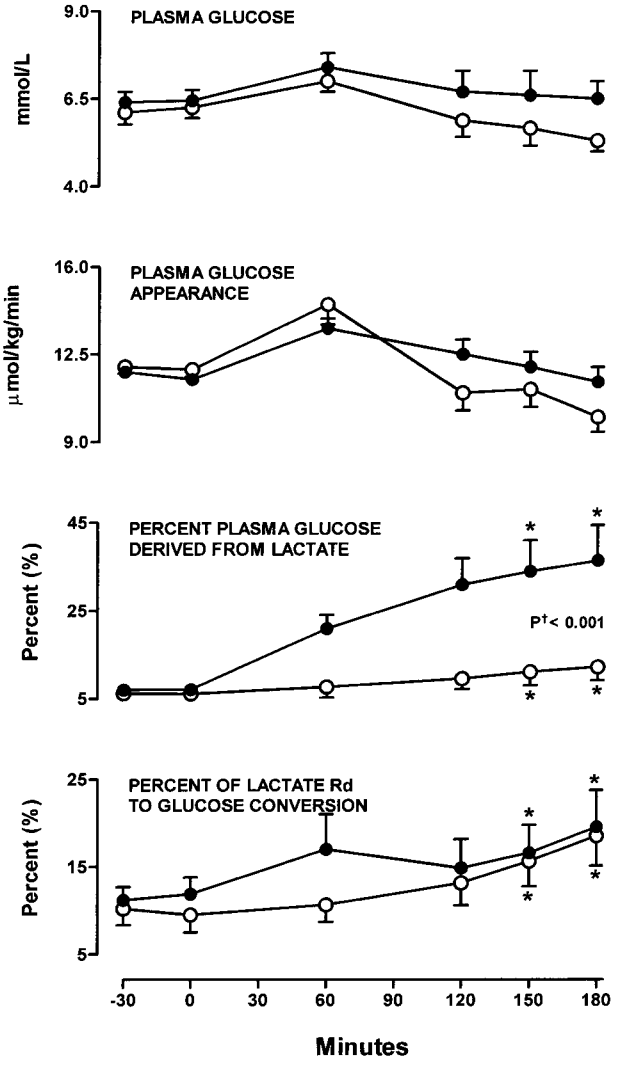

Fig 4. Plasma glucose, rate of appearance of glucose, percent of plasma glucose that was derived from lactate, and percent of lactate disappearance diverted into glucose conversion before and during a 3-hour infusion of 30 μmol/kg/min Na-lactate (●) or equimolar amounts of Na-bicarbonate (○) in 8 healthy subjects who underwent a pancreatic-adrenocortical-pituitary clamp where glucagon levels were raised to mimic the hyperglucagonemia seen in type 2 diabetes mellitus. Data are means ± SE. *$P < .05$ for differences between baseline values and values obtained at the end of the study (1-sample *t* tests) †P values for differences between experiments (2-sample *t* tests).

differences in glucoregulatory hormones. Accordingly, we clamped the gluconeogenic hormones at baseline levels and infused glucagon along with lactate or bicarbonate to determine whether EGP would increase during infusion of lactate. At the end of the lactate experiments, EGP did not increase above baseline or above that observed when glucagon was infused without lactate (bicarbonate experiments), despite a 5-fold increase in gluconeogenesis from lactate, resulting in a comparable increase in the proportion of plasma glucose derived from lactate. When using tracer technique for measurement of EGP one must consider some methodologic problems. Incorporation of lactate into glucose measured with the [U-^{14}C] lactate tracer may be underestimated by as much as 55%[13] due to exchange of labeled carbons with unlabeled carbons in the Krebs cycle,[32-34] and elevation of the lactate supply during experiments probably also influenced tracer dilution. Calculation of the rate of lactate incorporation into glucose based on measurement of specific activity of lactate in peripheral blood will also underestimate the lactate conversion rate due to tracer dilution when tracer mixes with lactate formed in the splanchnic bed. Thus, the lactate conversion rates measured with [U-^{14}C] lactate tracer must be regarded as minimum estimates of lactate gluconeogenesis.

The clamping of hormones may also influence the results in the present study. Overinsulinization would have inhibited EGP and also indirectly inhibited gluconeogenesis through suppression of lipolysis. Oxidative NEFA turnover parallels plasma NEFA concentrations[35] and facilitate gluconeogenesis by the activation of key enzymes for gluconeogenesis.[36] Plasma insulin concentrations ranged between 60 and 50 pmol/L during experiments, which corresponds with fasting levels. Overinsulinization is unlikely since no decreases in NEFA levels were observed. Insulin levels tended to fall slightly during the experiments and a corresponding increase in NEFA levels was seen at the end of experiments. These changes may lead to increased lactate conversion to oxalacetate and thereby increased lactate gluconeogenesis, and may thus have reduced the underestimation of gluconeogenesis from lactate calculated with tracer technique.

Even if measurements of lactate conversion to glucose are minimum estimates, this does not modify the finding that hyperglucagonemia does not modulate autoregulation of EGP in healthy volunteers during augmented gluconeogenesis from lactate. Hyperglucagonemia does not provide an explanation for the apparent inoperation of this phenomena in patients with type 2 diabetes,[13,16] unless, of course, glucagon has unique effects in diabetic patients, or a more prolonged period of hyperglucagonemia is necessary to alter autoregulation of EGP.

Glycogenolysis was not measured in the present study. However, one may speculate that the stimulatory effect of glucagon on glycogenolysis may have been counteracted by the fact that increased gluconeogenesis from lactate probably increased the glucose-6-phosphate pool, which is known to inhibit net glycagon breakdown.[37] It is beyond the scope of this study to determine the net effects of these processes on glycogenolysis. Increased glucose-6-phosphatase activity is found in obese type 2 diabetes.[38] This could be secondary to increased intracellular glucose-6-phosphate accumulation, or a primary mechanism for the increased EGP seen in this condition. Assuming that hyperglucagonemia in the present study stimulated both gluconeogenesis and glycogenolysis, intracellular glucose-6-phosphate formation probably rose. However, if this was the case, glucose-6-phosphatase activity must have been correspondingly downregulated since EGP did not rise.

Other mechanisms could be involved. It has been proposed that such a mechanism might be the inhibition of gluconeogenesis from other substrates.[3,39] This is unlikely because in patients with type 2 diabetes, gluconeogenesis from lactate,[13,17] alanine,[16,17] and glycerol[40] are simultaneously increased in concert with increased EGP. Jenssen et al[6] showed that alanine conversion to glucose actually increased during infusions of lactate. In the present study, alanine levels increased slightly during lactate infusion. This may either reflect increased alanine production from lactate or reduced alanine utilization, possibly explained by inhibited gluconeogenesis. The rate of glucose production at the end of the lactate infusion was 12 μmol/kg/min. Gluconeogenesis accounts for approximately 50% of the glucose production,[41,42] ie, 6 μmol/kg/min. Gluconeogenesis from lactacte increased to approximately 8 μmol/kg/min, so even if the contribution from all other gluconeogenic substrates were suppressed to zero, glucose Ra should still have increased if the autoregulation of EGP was inoperative.

Although plasma NEFA increased to a comparable extent in lactate and control experiments in the present study, the levels were generally lower than those observed in type 2 diabetes,[40] and the metabolism of NEFA was not assessed (eg, NEFA oxidation). It is possible that the abnormalities in NEFA metabolism found in type 2 diabetes might be a possible explanation for the altered autoregulation of EGP during the increased gluconeogenesis seen in this condition. Experiments on catheterized dogs support this view, as infusions of glucagon and lactate did not alter EGP, but the addition of Intralipid infusion (Abbott, Evansville, IN) in order to keep plasma NEFA slightly elevated during the clamp experiments resulted in an 1.5-fold increase in EGP.[43] Alternatively, some defect intrinsic to type 2 diabetes might be involved in the abnormal autoregulation of EGP.

The present design permits some observations on the effects of glucagon on lactate metabolism and lactate gluconeogenesis. The bicarbonate control experiments indicated that hyperglucagonemia within the physiologic range caused alterations of the lactate metabolism. Although plasma lactate concentrations did not change, lactate production increased from 14.9 to 17.2 μmol/kg/min and was accompanied by an increase in lactate utilization (from 14.5 to 15.8 μmol/kg/min). The rate of lactate conversion to glucose nearly doubled, and the percentage of lactate utilization diverted towards gluconeogenesis increased from 10% to 18%, without altering EGP. For comparison, no increase in lactate conversion to glucose during a fixed glucagon infusion at rates mimicking the glucagon levels observed in healthy people after an over nights fast ($\sim$ 230 ng/L), was seen in a previous study[6] under identical experimental conditions. In that study,[6] additional lactate infusion at rates increasing lactate appearance to 38 μmol/kg/min doubled lactate gluconeogenesis (from 1.5 to 3.5 μmol/kg/min). In the present study, a 5-fold increase in gluconeogenesis from lactate (from 1.7 to 8.1 μmol/kg/min) was seen when lactate of similar rates was infused

along with physiologic elevated glucagon levels. These observations are in accordance with the view that prolonged hyperglucagonemia stimulates gluconeogenesis.[14]

In both experiments, an early transient rise in plasma glucose occurred during hyperglucagonemia, reflecting a waning increase in EGP caused by increased glycogenolysis as previously seen during glucagon stimulation.[13,14,44] The prevailing hyperglycemia probably contributed to the subsequent downregulation of EGP.[44] Increased glucagon associated stimulation of glycogenolysis during simultaneously enhancement of gluconeogenesis from lactate did not increase EGP above baseline at the end of the experiments. Glucose levels at the end of the experiments were not significantly different, but the slightly higher glucose concentration observed at the end of the lactate experiments may have contributed to suppress EGP. In diabetes, however, EGP is increased despite increased glucose and insulin levels.[19] If the defect autoregulation of EGP seen in this condition was due to the combination of hyperglucagonemia and increased substrate supply, one would expect EGP to increase during lactate and glucagon infusion, although perhaps not as much as if glucose concentration had remained totally unchanged.

It is tempting to speculate that the newly formed glucose emerging from simultanously increased glycogenolysis and gluconeogenesis may be converted through other pathways than the release into circulation, ie, towards the pentose pathway, towards glycolysis, or towards de novo lipogenesis through the acetyl coenzyme A pool.

In summary, the present study indicates that in normal volunteers physiologic hyperglucagonemia increases total body lactate production, the rate of lactate conversion to glucose, and the proportion of lactate metabolized through gluconeogenesis. In spite of this, hyperglucagonemia does not alter the autoregulation of EGP. The combination of physiologic hyperglucagonemia and increased supply of lactate leads to a 5-fold increase in the rate of lactate conversion to glucose and a comparable increase in the percentage of plasma glucose derived from lactate, without increasing EGP.

We conclude that in normal volunteers, neither hyperglucagonemia nor the combination of hyperglucagonemia and increased substrate availability alters the autoregulation of EGP.

ACKNOWLEDGMENT

We appreciate the superb technical assistance of Jorunn Eikrem and Åse Lund Bendiksen.

REFERENCES

1. Landau BR, Wahren J, Chaandramouli V, et al: Contributions of gluconeogenesis to glucose production in the fasted state. J Clin Invest 98:378-385, 1996

2. Jenssen T, Nurjhan N, Consoli A, et al: Dose-response effects of lactate infusions on gluconeogenesis from lactate in normal man. Eur J Clin Invest 23:448-454, 1993

3. Jahoor FE, Peters J, Wolfe RR: The relationship between gluconeogenic substrate supply and glucose production in humans. Am J Physiol 258:E288-E296, 1990

4. Hue L: Gluconeogenesis and its regulation. Diabetes Metab Rev 3:111-126, 1987

5. Gerich JE: Control of glycaemia. Baillieres Clin Endocrinol Metab 7:551-586, 1993

6. Jenssen T, Nurjahan N, Consoli A, et al: Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal man: Demonstration of hepatic autoregulation without a change in plasma glucose concentration. J Clin Invest 86:489-497, 1990

7. Winkler B, Rathgeb I, Steele R, et al: Conversion of glycerol to glucose in the normal dog. Am J Physiol 219:497-502, 1990

8. Diamond M, Rollings R, Steiner K, et al: Effect of alanine concentration independent of changes in insulin and glucagon on alanine and glucose homeostasis in the conscious dog. Metabolism 37:28-33, 1988

9. Tappy L: Regulation of hepatic glucose production in healthy subjects and patients with non-insulin-dependent diabetes mellitus. Diabetes Metab 21:233-240, 1995

10. Paquot N, Schneiter Ph, Jèquier E, et al: Effects of ingested fructose and infused glucagon on endogenous glucose production in obese NIDDM patients, obese non-diabetic subjects, and healthy subjects. Diabetologia 39:580-586, 1996

11. Müller C, Assimacopoulos-Jeannet F, Mosimann F, et al: Endogenous glucose production, gluconeogenesis and liver glycogen concentration in obese non-diabetic patients. Diabetologia 40:463-468, 1997

12. Baron A, Schaeffer D, Shragg L, et al: Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes 36:274-283, 1987

13. Consoli A, Nurjhan N, Capani F, et al: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes 38:550-561, 1990

14. Magnusson I, Rothman DL, Gerard DP, et al: Contribution of hepatic glycogenolysis to glucose production in response to a physiological increase in plasma glucagon. Diabetes 44:185-189, 1995

15. Jeng CY, Sheu WHH, Fuh MMT, et al: Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. Diabetes 43:1440-1444, 1994

16. Perriello G, Pampanelli S, DelSindaco P, et al: Evidence of increased systemic glucose production and gluconeogenesis in an early stage of NIDDM. Diabetes 46:1010-1016, 1997

17. Tappy L, Acheson K, Curcod B, et al: Overnight glucose metabolism in obese non-insulin-dependent diabetic patient and in healthy lean individuals. Clin Physiol 14:251-265, 1994

18. Magnusson I, Rothman DL, Katz LD, et al: Increased rate of gluconeogenesis in type II diabetes mellitus. A ^{13}C nuclear magnetic resonance study. J Clin Invest 90:1323-1327, 1992

19. Mevorach M, Giacca A, Aharon Y, et al: Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. J Clin Invest 102:744-753, 1998

20. McGuire E, Halderman JJ, Tobin R, et al: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 41:565-573, 1976

21. DeFeo PG, Perriello MM, Ventura P, et al: The pancreatic-adrenocortical-pituitary clamp technique for study of counter-regulation in humans. Am J Physiol 252:E565-E570, 1987

22. Lowry OH, Passonneau JV: A Flexible System of Enzymatic Analysis. Orlando, FL, Academic Press, 1972, p 200

23. Karl I, Pagliaris A, Kipnis D: A microfluorometric enzymatic assay for determination of alanine and pyruvate in plasma and tissue. J Lab Clin Med 80:434-441, 1972

24. Kreisberg R, Siegal A, Crawford-Owen D: Glucose-lactate interrelationship effect of ethanol. J Clin Invest 50:175-185, 1971

25. Herbert V, Gottlieb C, Bleicher S: Coated charcoal immunoassay of insulin. J Clin Endocrinol 25:1375-1384, 1971

26. Faber OK, Binder C: C-peptide response to glucagon: A test for

the residual beta-cell function in diabetes mellitus. Diabetes 26:605-610, 1977

27. Faloona G, Unger R: Glucagon, in Jaffe B, Behrman H (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic Press, 1974, pp 317-330

28. Peake G: Growth hormone, in Jaffe B, Behrman H (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic Press, 1974, pp 103-121

29. DeBodo RR, Steele N, Altszuler A, et al: On the hormonal regulation of carbohydrate metabolism: Studies with ^{14}C glucose. Recent Prog Horm Res 19:445-448, 1963

30. Searle G, Cavalieu R: Determination of lactate kinetics in human analysis of data from single injection vs continuous infusion methods. Proc Soc Exp Biol Med 139:1002-1006, 1972

31. Chiasson JJ, Liljenquist W, Lacy A, et al: Gluconeogenesis: Methodological approaches in vivo. Fed Proc 36:229-235, 1977

32. Tayek JA, Katz J: Glucose production, recycling, and gluconeogenesis in normals and diabetics: A mass isotopomer [U^{13}C]glucose study. Am J Physiol 270:E709-E717, 1996

33. Hetenyi G Jr: Correction for the metabolic exchange of ^{14}C for ^{12}C in the pathway of gluconeogenesis in vivo. Fed Proc 41:104-109, 1985

34. Katz J: Determination of gluconeogenesis in vivo with ^{14}C-labelled substrates. Am J Physiol 248:R391-R399, 1986

35. Groop LC, Saloranta C, Shank M, et al: The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 72: 96-107, 1991

36. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

37. Hems D, Whitton P: Control of hepatic glycogenolysis. Physiol Rev 60:1-50, 1980

38. Clore JN, Stillman J, Sugerman H: Glucose-6-phosphatase flux in vitro is increased in type 2 Diabetes. Diabetes 49:969-974, 2000

39. Steele R, Winkler B, Altszuler N: Inhibition by infused glycerol of gluconeogenesis from other precursors. Am J Physiol 221:883-888, 1971

40. Puhakainen I, Koivisto VA, Yki-Järvinen H: Lipolysis and gluconeogenesis from glycerol are increased in patients with non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 75:789-794, 1992

41. Diraison F, Large V, Brunengraber H, et al: Non-invasive tracing of liver intermediary metabolism in normal subjects and in moderately hyperglycemic NIDDM subjects. Evidence against increased gluconeogenesis and hepatic fatty acid oxidation in NIDDM. Diabetologia 41:212-220, 1998

42. Pimenta W, Nurjhan N, Jansson PA, et al: Glycogen: Its mode of formation and contribution to hepatic glucose output in postabsorbtive humans. Diabetologica 37:697-702, 1994

43. McGuinness OP, Ejiofor J, Audoly LP, et al: Regulation of glucose production by NEFA and gluconeogenic precursors during chronic glucagon infusion. Am J Physiol 275:E432-E439, 1998

44. Gerich JE: Hormonal and substrate determinants of hepatic glucose production in man, in Veneziale CM (ed): The Regulation of Carbohydrate Formation and Utilization in Mammals. Baltimore, MD, University Park Press, 1981, pp 419-457