Regulation of glucose production by NEFA and gluconeogenic precursors during chronic glucagon infusion

Owen P. McGuinness, Joseph Ejiofor, Laurent P. Audoly, and Nancy Schrom. Regulation of glucose production by NEFA and gluconeogenic precursors during chronic glucagon infusion. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E432–E439, 1998.—We previously reported that simulation of the chronic hyperglucagonemia seen during infection was unable to recreate the infection-induced increase in hepatic glucose production. However, chronic hyperglucagonemia was accompanied by a fall in the arterial levels of gluconeogenic precursors as opposed to a rise as is seen during infection. Thus our aim was to determine whether an infusion of gluconeogenic precursors could increase hepatic glucose production in a setting of hyperglucagonemia. Studies were done in 11 conscious chronically catheterized dogs in which sampling (artery and portal and hepatic veins) and infusion catheters (splenic vein) were implanted 17 days before study. Forty-eight hours before infusion of gluconeogenic (GNG) precursors, a sterile fibrinogen clot was placed into the peritoneal cavity. Glucagon was infused over the subsequent 48-h period to simulate the increased glucagon levels (~500 pg/ml) seen during infection. On the day of the experiment, somatostatin was infused peripherally, and basal insulin and simulated glucagon were infused intraportally. After a basal period, a two-step increase in lactate and alanine was initiated (120 min/step; n = 5). Lactate (1479 ± 25 and ∆1,780 ± 85 µM; expressed as change from basal in periods I and II, respectively) and alanine (∆94 ± 13 and ∆287 ± 44 µM) levels were increased. Despite increases in net hepatic GNG precursor uptake (∆0.7 ± 0.3 and ∆1.1 ± 0.4 mg glucose·kg⁻¹·min⁻¹), net hepatic glucose output did not increase. Because nonesterified fatty acid (NEFA) levels fell, in a second series of studies, the fall in NEFA was eliminated. Intralipid and heparin were infused during the two-step substrate infusion to maintain the NEFA levels constant in period I and increase NEFA availability in period II (∆29 ± 29 and ∆689 ± 186 µM; n = 6). In the presence of similar increases in net hepatic GNG precursor uptake and despite increases in arterial glucose levels (∆17 ± 5 and ∆38 ± 12 mg/dl), net hepatic glucose output increased (∆0.6 ± 0.1 and ∆0.7 ± 0.2 mg·kg⁻¹·min⁻¹). In summary, a chronic increase in glucagon, when combined with an acute increase in gluconeogenic precursor and maintenance of NEFA supply, increases hepatic glucose output as is seen during infection.

alanine; lactate; lipolysis; inflammation; gluconeogenesis

However, recent work in our laboratory suggests that hyperglucagonemia alone cannot explain the increase in glucose production during infection. A noninfected animal exposed to chronic simulation of the increase in glucagon seen during infection does not increase hepatic glucose production (20). Hepatic glucose production cannot increase because glucagon, although having potent effects in enhancing the ability of the liver to extract gluconeogenic precursors, does not increase gluconeogenic precursor supply. Consequently, the circulating levels of gluconeogenic substrates decrease markedly during chronic hyperglucagonemia, offsetting the glucagon-mediated rise in the fractional extraction of gluconeogenic precursors by the liver (20).

Simply increasing the supply of gluconeogenic precursors also does not increase hepatic glucose production in vivo. Although in vitro increases in gluconeogenic precursor supply increase hepatic glucose production (7), this has yet to be established in vivo. In vivo, increases in lactate, alanine, or glycerol levels in a setting of fixed insulin and glucagon levels do not increase hepatic glucose production in overnight-fasted dogs and humans (4, 6, 13). Because hepatic glycolysis contributes a large portion of hepatic glucose production after an overnight fast, a rise in gluconeogenesis could be offset by a fall in hepatic glycogenolysis. Yet, even in prolonged fasted states in which gluconeogenesis accounts for the majority of the glucose released by the liver, infusion of gluconeogenic substrates does not increase hepatic glucose production (6, 13). Suppression of gluconeogenic precursor availability using dichloroacetate in prolonged fasted humans also does not decrease hepatic glucose production; however, the accompanying suppression of lipolysis complicates the interpretation of the results (13).

Nonesterified free fatty acids (NEFAs) may play an important role in modulating hepatic glucose production (27). NEFAs can inhibit insulin suppression of glucose production. Thus they may interact with gluconeogenic substrate availability to support an increased rate of gluconeogenesis and glucose production. NEFA levels have generally not been measured in studies in which gluconeogenic substrates were infused. Infusion of gluconeogenic precursors, such as lactate, can alter the availability of NEFA (4). NEFAs are the major fuel oxidized by the liver (15). In fact, inhibition of fat oxidation using a fatty acid oxidation inhibitor suppresses hepatic glucose production (22). Thus sustaining an increase in gluconeogenesis may necessitate adequate fat oxidation by the liver.

We hypothesized that to recreate the increase in glucose production and gluconeogenesis seen in our model of infection, both the appropriate hormonal...
environment and the supply of gluconeogenic precursors and NEFA must be adequate to support an increase in gluconeogenesis. Thus the aim of this study was to determine in a chronically hyperglucagonemic state whether acute increases in gluconeogenic precursor supply can increase hepatic glucose production and whether this interaction is dependent on NEFA availability. Studies were done in chronically catheterized conscious dogs in which gluconeogenic precursor and NEFA supply were modulated in livers primed to support an increase in gluconeogenesis by chronic glucagon infusion.

MATERIALS AND METHODS

Animal preparation. Experiments were carried out on 11 conscious mongrel dogs (21 ± 1 kg) of either gender. Before being studied, they received a diet consisting of KalKan meat (Vernon, CA) and Purina dog chow (St. Louis, MO) once daily. The composition of the diet was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber based on dry weight. The dogs were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the experimental protocols were approved by the Vanderbilt University Medical Center Animal Care Subcommittee.

Experimental protocol. Fourteen to seventeen days before study, a laparotomy was performed under general anesthesia (isoflurane). As previously described (20), infusion catheters were placed into the splenic vein and a jejunal vein for intraportal infusion of insulin and glucagon. Sampling catheters (0.04 inch ID) were also inserted into the portal vein and the left common hepatic vein for blood sampling. In addition, a catheter (0.04 inch ID) for blood sampling was inserted into the femoral artery after an incision in the left inguinal region. The catheters were then filled with saline containing heparin (200 U/ml). Doppler flow probes were placed about the portal vein and hepatic artery after the gastroduodenal vein was ligated. The venous catheters and the Doppler flow probe leads were exteriorized and placed in a subcutaneous pocket in the abdominal region. The free end of the splenic venous infusion catheter was exteriorized, tunneled subcutaneously, and placed under the skin between the scapulae. The femoral arterial catheter was placed under the skin in the inguinal region (19).

Two weeks after catheter implantation, all animals had 1) a good appetite (consuming the entire daily ration), 2) normal stools, 3) a hematocrit above 35%, and 4) a leukocyte count below 18,000 mm⁻³ before the study. To simulate the control protocol used in our infection model, dogs were anesthetized with a short-acting anesthetic (sodium thiopental; 15 mg/kg), and a sterile fibrin clot (described below) was placed into the peritoneal cavity with the use of sterile technique. The implantation of a sterile fibrin clot was not required to answer the primary question of this study. However, it aided in comparing this response to our infection model, in which we implanted a fibrin clot containing Escherichia coli. Twelve hours after clot implantation, animals received 300–500 ml sterile saline for volume resuscitation. Dogs were not fed after receiving the clot. All dogs received an exogenous infusion of glucose into the portal vein via the splenic venous infusion catheter. The rate of glucagon infusion was varied to simulate the time course of the rise in glucagon seen after septic clot implantation. Specifically, immediately after clot implantation, glucagon was infused at rates of 20 ng·kg⁻¹·min⁻¹ for 18 h, 15 ng·kg⁻¹·min⁻¹ for 12 h, and 10 ng·kg⁻¹·min⁻¹ for the remaining duration of the study. Because of the tendency for glucagon to aggregate at high concentrations, glucagon was prepared in a dilute solution containing the dog’s own plasma and infused at 4 ml/h. The glucagon solution was filtered (0.2 µm) under sterile conditions and was prepared fresh every 12 h. The dog was placed in a jacket (Alice King Chatham, Los Angeles, CA) containing a pocket into which a portable infusion pump was placed (Infused 200; Medfusion Systems, Norcross, GA).

Forty-two hours after clot implantation, the blood-sampling catheters and Doppler flow probe leads were removed from subcutaneous pockets under local anesthesia (2% lidocaine). The dog was then placed into a Pavlov harness. Intravenous catheters (Angiocath, 20 gauge; Becton Dickinson Vascular Access, Sandy, UT) were inserted percutaneously into right and left cephalic veins and left saphenous vein. Experiments consisted of an equilibration period (−120 to −40 min), a sampling period (−40 to 0 min), and an experimental period (0 to 240 min). At −120 min, a primed (50 µCi), constant infusion of [3-3H]glucose (0.4 µCi/min) and a constant infusion of indocyanine green dye (0.1 mg·kg⁻¹·min⁻¹) were begun through the right cephalic vein and continued throughout the entire experiment. Somatostatin was infused into the left saphenous vein (0.8 µg·kg⁻¹·min⁻¹). Insulin (300 ± 43 µU·kg⁻¹·min⁻¹) and glucagon (10 ng·kg⁻¹·min⁻¹) were infused into the portal vein via the splenic and jejunal veins. The exogenous chronic intraportal glucagon infusion was maintained constant, whereas the insulin infusion rate was adjusted to maintain euglycemia. Once glucose levels had stabilized at the desired value and 30 min after the last adjustment in the insulin infusion rate, the basal sampling period (−30 to 0 min) was initiated. After the basal period, a two-step (120 min/step) combined infusion of lactate and alanine was initiated into the left cephalic vein. Alanine and lactate were infused at 2 and 12.5 µmol·kg⁻¹·min⁻¹, respectively, during the first step (0 to 120 min) and at 4 and 25 µmol·kg⁻¹·min⁻¹ during the second step (120 to 240 min). The pH of the lactate and alanine solution was adjusted to pH 3.8 to eliminate the alkalizing effects of lactate infused at pH 7.4 (10). Two different protocols were instituted at 0 min (Fig. 1). In protocol 1 (−NEFAs; n = 5), saline was also infused. Because we observed a fall in NEFAs in this protocol, a second group was designed to prevent the fall in NEFAs seen in protocol 1. In protocol 2 (−NEFAs; n = 6), a primed (500 U), continuous infusion of heparin (0.5 U·kg⁻¹·min⁻¹) was also initiated at 0 min and continued for the duration of the experimental period. During the second period, 20% intralipid (0.0075–0.01 ml·kg⁻¹·min⁻¹; Abbott, Evanstan, IN) was also infused into a peripheral vein. Femoral arterial, portal venous, and hepatic venous blood samples were taken every 10 min during the basal sampling period and every 30 min during the experimental period. At the end of the study, each dog was euthanized with an overdose of pentobarbital sodium.

Fibrinogen clot preparation. A 1% bovine fibrinogen solution (10 ml/kg) was prepared in sterile saline and filtered through a sterile 0.45 µm filter (Corning, Corning, NY). Thrombin was then added (1,000 units), and 30 min were allowed for clot formation (18).

Processing of blood samples. Blood samples were drawn into heparinized syringes and transferred to chilled tubes containing potassium EDTA (15 mg). The collection and immediate processing of blood samples have been previously described (2). Radioactivity in plasma glucose was measured using established methods (2). Blood lactate, glycerol, and alanine were analyzed using the method of Lloyd et al. (16) on
a Monarch 2000 centrifugal analyzer (Lexington, MA). Plasma glucose was assayed immediately with the use of a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma NEFAs were determined spectrophotometrically (Wako Chemicals, Richmond, VA). Immunoreactive insulin (29) was assayed using a double-antibody technique (Pharmacia Diagnostics, Piscataway, NJ; intra-assay coefficient of variation (CV) of 11%). Plasma treated with 500 KIU of Trasylol (Miles, Kankakee, IL) was assayed for immunoreactive glucagon (1) using a procedure similar to the one for insulin (intra-assay CV of 8%). Plasma cortisol (8) was assayed with Clinical Assays Gamma Coat radioimmunoassay kit (intra-assay CV of 6%). Hepatic arterial and portal venous blood flows were assessed using Transonic flow probes (Transonic Systems, Ithaca, NY). Blood flow was converted to plasma flow by multiplying by 1 hematocrit ratio. Indocyanine green dye was used to verify the placement of hepatic venous catheter and to provide an additional estimate of hepatic plasma flow. The labeled concentrations of plasma alanine and lactate and the unlabeled concentration of alanine were determined using a short column ion exchange chromatographic system (3).

Tracer methods and calculations. The rates of total glucose production and utilization were calculated according to the method of Wall et al. (28), as simplified by DeBodo et al. (5). Net hepatic glucose output was calculated using the formula 

\[ \text{H} = (F_a + F_p - P) \] 

where H, A, and P are the blood glucose concentrations in the hepatic vein, femoral artery, and portal vein, respectively, and F_a and F_p represent the fractional contribution of the hepatic artery and portal vein, respectively, to total hepatic blood flow (HBF). Plasma glucose concentrations were converted to whole blood concentrations using a correction factor of 0.73 (21). The above equation was used to calculate net hepatic lactate, alanine, and glycerol output. However, because the liver generally was a net consumer of these substrates (i.e., negative output), the data are presented as positive values and denoted as net uptake. Gluconeogenic substrate uptake was obtained by summing net hepatic lactate, alanine, and glycerol uptake.

Statistics. Statistical comparisons were made using ANOVA (Systat for Windows; Systat, Evanston, IL). A univariate post hoc F test was used when a significant F ratio was found. Statistical significance was accepted at P < 0.05. Because previous studies in a similarly fasted dog (26) indicated that hepatic glucose metabolism is constant over the time course of the study, the data are expressed as changes from basal and are averaged over the last 30 min of each period and are indicated by Δ.

RESULTS

Hormone levels, NEFA, and glucose kinetics. Chronic glucagon infusion increased arterial plasma glucagon levels in both groups (574 ± 99 and 499 ± 86 pg/ml; -NEFA and +NEFA, respectively) during the basal period (normal range 40–80 pg/ml), and levels remained elevated during the experimental period (Fig. 2). Arterial plasma insulin levels were 9 ± 2 and 9 ± 2 µU/ml (-NEFA and +NEFA, respectively) in the basal period.
period and did not change during the experimental period. Arterial plasma cortisol levels were 1.5 ± 0.6 and 2.3 ± 0.8 µg/dl (−NEFA and +NEFA, respectively) in the basal period and did not change during the experimental period.

In −NEFA, arterial NEFA levels were 940 ± 143 µM in the basal period, tended to fall in period I (Δ −156 ± 93 µM), and fell further in period II (Δ −435 ± 55 µM; P < 0.05; Fig. 3). Basal net hepatic NEFA uptake (4.3 ± 0.7 µmol·kg⁻¹·min⁻¹) decreased by 1.4 ± 0.5 and 2.2 ± 0.3 µmol·kg⁻¹·min⁻¹ in periods I and II, respectively. Associated with this fall, net hepatic β-hydroxybutyrate (β-OHB) production fell from a basal rate of 2.1 ± 0.9 µmol·kg⁻¹·min⁻¹ by 1.3 ± 0.4 and by 1.9 ± 0.8 µmol·kg⁻¹·min⁻¹.

In +NEFA, arterial plasma NEFA levels were 1,005 ± 177 µM in the basal period, did not fall during period I (Δ −27 ± 38 µM), and rose in the second period by 654 ± 243 µM (P < 0.05; Fig. 3). Basal net hepatic NEFA uptake (3.6 ± 0.7 µmol·kg⁻¹·min⁻¹) was unaltered (Δ0.5 ± 0.4 µmol·kg⁻¹·min⁻¹) in period I. Net hepatic NEFA balance could not be accurately determined during period II because of the intralipid infusion. Despite maintenance of NEFA levels and net hepatic NEFA uptake, net hepatic β-OHB production decreased from a basal rate of 2.7 ± 0.7 µmol·kg⁻¹·min⁻¹ by 1.6 ± 0.4 and by 2.4 ± 0.8 µmol·kg⁻¹·min⁻¹ during periods I and II, respectively.

Arterial plasma glucose levels were unaltered during the experimental period in −NEFA (Fig. 4). In addition, both tracer-determined glucose appearance (Δ0.1 ± 0.2 and Δ0.1 ± 0.2 mg·kg⁻¹·min⁻¹ in periods I and II, respectively; expressed as change from basal) and net hepatic glucose output (Δ0.1 ± 0.3 and Δ0.1 ± 0.2 mg·kg⁻¹·min⁻¹) were not altered. However, with combined intralipid and gluconeogenic substrate infusion (+NEFA), arterial glucose levels increased by 17 ± 5 and 38 ± 12 mg/dl during periods I and II, respectively. Despite this increase, tracer-determined glucose appearance did not fall in period I and increased in period II. In addition, net hepatic glucose output increased by 0.6 ± 0.1 and by 0.7 ± 0.2 mg·kg⁻¹·min⁻¹ in periods I and II, respectively.

Gluconeogenic precursor kinetics. In −NEFA, the infusion of lactate increased arterial lactate levels in a stepwise manner (Δ479 ± 25 and Δ1,780 ± 85 µM; Fig 5). Despite this increase, net hepatic lactate uptake only
The infusion of alanine increased arterial alanine levels in a stepwise manner ($\Delta 94 \pm 13$ and $\Delta 287 \pm 44 \mu M$) in $-\text{NEFA}$ (Fig 6). Net fractional hepatic alanine extraction was unaltered ($\Delta 0.03 \pm 0.03$ and $\Delta -0.06 \pm 0.03$), and net hepatic alanine uptake increased ($\Delta 2.1 \pm 0.6$ and $\Delta 44.4 \pm 0.6 \mu mol \cdot kg^{-1} \cdot min^{-1}$). In $+\text{NEFA}$, a similar response was observed. The infusion of alanine increased arterial alanine levels in a stepwise manner ($\Delta 62 \pm 4$ and $\Delta 211 \pm 16 \mu M$). Net fractional hepatic alanine extraction was unaltered ($\Delta 0.03 \pm 0.05$ and $\Delta -0.06 \pm 0.03$), and net hepatic alanine uptake increased ($\Delta 2.1 \pm 0.6$ and $\Delta 44.4 \pm 0.6 \mu mol \cdot kg^{-1} \cdot min^{-1}$).
creased arterial glycerol levels in a step-wise manner ($\Delta -5 \pm 4$ and $\Delta -27 \pm 8 \mu M$; Fig. 7), whereas net fractional hepatic glycerol extraction was unaltered ($\Delta 0.03 \pm 0.02$ and $\Delta 0.02 \pm 0.03$) and net hepatic glycerol uptake decreased in period II ($\Delta -0.1 \pm 0.4$ and $\Delta -0.6 \pm 0.3 \mu mol \cdot kg^{-1} \cdot min^{-1}$). In +NEFA, arterial glycerol levels did not fall ($\Delta 9 \pm 5$ and $\Delta 10 \pm 17 \mu M$).

In -NEFA, the infusion of lactate and alanine decreased arterial glycerol levels in a step-wise manner ($\Delta -5 \pm 4$ and $\Delta -27 \pm 8 \mu M$; Fig. 7), whereas net fractional hepatic glycerol extraction was unaltered ($\Delta 0.03 \pm 0.02$ and $\Delta 0.02 \pm 0.03$) and net hepatic glycerol uptake decreased in period II ($\Delta -0.1 \pm 0.4$ and $\Delta -0.6 \pm 0.3 \mu mol \cdot kg^{-1} \cdot min^{-1}$). In +NEFA, arterial glycerol levels did not fall ($\Delta 9 \pm 5$ and $\Delta 10 \pm 17 \mu M$).

Fig. 7. Change in arterial blood glycerol level, net fractional hepatic glycerol extraction, and net hepatic glycerol uptake in hyperglucagonemic dogs receiving an acute 2-step increase in lactate and alanine with (+NEFA) or without (-NEFA) a combined heparin-intralipid infusion. In basal period, arterial blood glycerol levels, net fractional hepatic glycerol extraction, and net hepatic glycerol uptake were 101.69 $\pm$ 0.70 $\mu M$, 0.70 $\pm$ 0.05, and 1.7 $\pm$ 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, in -NEFA. In +NEFA, during basal period, arterial blood glycerol levels, net fractional hepatic glycerol extraction, and net hepatic glycerol uptake were 101.69 $\pm$ 0.70 $\mu M$, 0.70 $\pm$ 0.05, and 1.7 $\pm$ 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively. Data are presented as means $\pm$ SE. In -NEFA, arterial blood glycerol levels and net hepatic glycerol uptake were decreased in period II ($P < 0.05$).

Fig. 8. Change in net hepatic gluconeogenic precursor uptake in hyperglucagonemic dogs receiving an acute 2-step increase in lactate and alanine with (+NEFA) or without (-NEFA) a combined heparin-intralipid infusion. During basal period, net hepatic gluconeogenic precursor uptake was 1.1 $\pm$ 0.3 and 1.4 $\pm$ 0.2 $mg$ glucose $\cdot kg^{-1} \cdot min^{-1}$ in -NEFA and +NEFA, respectively. Data are presented as means $\pm$ SE. In both +NEFA and -NEFA, net hepatic gluconeogenic precursor uptake increased from basal ($P < 0.05$).

Net fractional hepatic glycerol extraction ($\Delta 0.03 \pm 0.02$ and $\Delta 0.00 \pm 0.02$) and net hepatic glycerol uptake were unaltered ($\Delta -0.3 \pm 0.1$ and $\Delta 0.1 \pm 0.3 \mu mol \cdot kg^{-1} \cdot min^{-1}$). In both periods, the increase in net hepatic gluconeogenic precursor uptake was similar in the two protocols ($\Delta 0.7 \pm 0.3$ and $\Delta 1.1 \pm 0.4 mg$ glucose equivalents $\cdot kg^{-1} \cdot min^{-1}$ in -NEFA; $\Delta 0.6 \pm 0.1$ and $\Delta 1.1 \pm 0.1 mg$ glucose equivalents $\cdot kg^{-1} \cdot min^{-1}$ in +NEFA; Fig. 8).

**DISCUSSION**

These data demonstrate that chronic hyperglucagonemia, when accompanied by increases in gluconeogenic precursor availability and adequate circulating concentrations of NEFA, can contribute to the infection-induced increase in glucose production and gluconeogenesis. These data confirm that even in an animal reliant predominantly on gluconeogenesis, combined increases in lactate and alanine uptake by the liver are unable to increase hepatic glucose output. If the substrate-induced suppression of NEFAs is prevented and/or NEFAs are increased, increases in gluconeogenic precursor supply can support an increase in hepatic glucose production.

Chronic hyperglucagonemia markedly enhanced the importance of the liver in disposal of the exogenous alanine. As expected, the uptake of alanine by the liver increased in parallel with the rise in alanine levels. This is reflected in the constancy of net fractional hepatic alanine extraction in the face of increases in blood alanine concentration (6). Remarkably, all of the exogenous alanine infused was disposed of by the liver. In periods I and II, net hepatic alanine uptake increased by $\sim 2$ and 4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which was equal to the rate of alanine infusion in each period. In the 42-h fasted dog, 50% of the exogenous alanine infused was consumed by the liver and 50% was...
removed by peripheral tissues (6). In the present study, the glucagon-mediated twofold increase in net fractional hepatic extraction of alanine (20) is the likely explanation for the enhancement in net hepatic alanine uptake. The lack of a contribution of the peripheral tissues may be due to the lower alanine levels in this study or to a decrease in peripheral alanine clearance. Peak alanine levels in period II were ~350 µM, whereas, in the prior study (6), they were ~500 µM.

In the case of lactate, the coupling between the increase in the arterial lactate level and the increase in the hepatic uptake of lactate is nonlinear. Net hepatic lactate uptake increased only modestly from period I to period II, despite a fourfold increase in the arterial lactate level. Net fractional hepatic lactate extraction decreased progressively from the basal period to periods I and II. In addition, although the liver still removed ~40% of the infused lactate in each period, net hepatic lactate uptake increased only modestly in period II, despite the much larger increase in the arterial lactate levels. Interestingly, in the overnight-fasted dog (4), when lactate levels were increased to similar levels (~3,000 µM), the liver increased its uptake by 9 µmol·kg⁻¹·min⁻¹. This increase is equal to the increase from basal seen in the second period (~9 µmol·kg⁻¹·min⁻¹). This suggests that chronic hyperglucagonemia does not facilitate concentration-dependent lactate entry.

Consistent with other studies done in vivo, increases in gluconeogenic precursor supply did not increase net hepatic glucose output even in a setting of chronic glucagon excess. In the present study, net hepatic gluconeogenic precursor uptake increased by 0.7 and 1.0 mg glucose·kg⁻¹·min⁻¹ in periods I and II, respectively, whereas net hepatic glucose output was unaltered. This is consistent with previous reports that, even in prolonged fasted states, increases in gluconeogenic substrates alone do not increase glucose production (4, 6, 13). Presumably, the additional carbon taken up was either oxidized or used to synthesize lipid or glycogen.

Maintenance of NEFA availability allows increases in gluconeogenic precursor uptake to increase hepatic glucose output. During lactate and alanine infusion, NEFA levels decreased. When the fall in NEFA concentration was prevented (period I) or when levels were allowed to rise (period II), net hepatic glucose output increased. Previous investigators either did not measure NEFA levels when they infused gluconeogenic substrates, or, if NEFAs were assessed, they were not prevented from falling (4). In vitro studies clearly indicate that NEFA can stimulate gluconeogenesis. In vivo it has been difficult to demonstrate that increases in NEFA enhance gluconeogenesis (7, 15). However, NEFA oxidation is essential in maintaining hepatic glucose production in the fasted and infected states (15). NEFAs are important inhibitors of insulin-mediated suppression of hepatic glucose production (9, 27). They restrain glycolysis and subsequent lactate release by the liver of the overnight-fasted dog (27). However, in a prolonged fasted state in which glycolysis is already suppressed, decreases in NEFAs did not alter hepatic glucose production (11). Because NEFAs inhibit pyruvate oxidation, it is possible that they limit the oxidative disposal of the gluconeogenic precursors in the liver and thereby enhance gluconeogenesis (23).

The potency of the NEFA effect is even more evident given that net hepatic glucose output increased despite increases in arterial glucose levels that are suppressive to hepatic glucose output. This mild hyperglycemia should suppress net hepatic glucose output by ~0.5–1 mg·kg⁻¹·min⁻¹ (26). In period I, glucose levels increased only modestly (17 mg/dl), and net hepatic glucose output increased by 0.6 mg·kg⁻¹·min⁻¹. However, in period II, the arterial plasma glucose levels increased by 38 mg/dl, yet net hepatic glucose output remained elevated. Thus, if the suppressive effects of hyperglycemia are taken into account, net hepatic glucose output increased by 1.15 mg·kg⁻¹·min⁻¹. The mechanism whereby NEFAs interact with this suppressive effect of hyperglycemia is unclear.

Despite maintenance of NEFA levels, the anti-ketogenic effects of lactate and alanine persisted. Hepatic β-OHB production decreased irrespective of whether NEFA levels were clamped. This is seen in the perfused rat liver (17), and it is likely due to substrate-induced increase in malonyl-CoA. Yet, in the overnight-fasted dog, lactate infusion did not alter ketone body synthesis (4). One possibility is that the addition of alanine may have combined with lactate to exert an anti-ketogenic effect. An additional possibility is that the higher rate of ketogenesis in the longer fasted dog makes it more susceptible to the anti-ketogenic effects of gluconeogenic substrates.

In summary, in a setting of chronic hyperglucagonemia, combined increases in lactate and alanine levels can increase hepatic glucose production if the antilipolytic effect of the substrate infusion is eliminated. Thus these results suggest that the infection-induced increase in glucagon, when combined with increases in gluconeogenic precursor and adequate fatty acid availability, can contribute to the infection-induced increase in glucose production.

We are grateful for the technical assistance of D. Brooks Lacy, Pat Donahue, Pamela Venson, and Eric Allen.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-43748 (O. P. McGuinness, Principal Investigator) and Diabetes Research and Training Center Grant P60-DK-20593.

Address for reprint requests: O. P. McGuinness, 702 Light Hall, Dept. of Molecular Physiology and Biophysics, Vanderbilt Univ., Nashville, TN 37232-0615.

Received 26 November 1997; accepted in final form 6 May 1998.

REFERENCES


