Glucagon chronically impairs hepatic and muscle glucose disposal

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Chen SS, Zhang Y, Santomango TS, Williams PE, Lacy DB, McGuinness OP. Glucagon chronically impairs hepatic and muscle glucose disposal. Am J Physiol Endocrinol Metab 292: E928–E935, 2007. First published November 28, 2006; doi:10.1152/ajpendo.00063.2006.—Defects in insulin secretion and/or action contribute to the hyperglycemia of stressed and diabetic patients, and we hypothesize that failure to suppress glucagon also plays a role. We examined the chronic impact of glucagon on glucose uptake in chronically catheterized conscious depancreatized dogs placed on 5 days of nutritional support (NS). For 3 days of NS, a variable intraportal infusion of insulin was given to maintain isoglycemia (~120 mg/dl). On day 3 of NS, animals received a constant low infusion of insulin (0.4 mU·kg⁻¹·min⁻¹) and either no glucagon (CONT), basal glucagon (0.7 ng·kg⁻¹·min⁻¹; BasG), or elevated glucagon (2.4 ng·kg⁻¹·min⁻¹; HiG) for the remaining 2 days. Glucose in NS was varied to maintain isoglycemia. An additional group (HiG + I) received elevated insulin (1 mU·kg⁻¹·min⁻¹) to maintain glucose requirements in the presence of elevated glucagon. On day 5 of NS, hepatic substrate balance was assessed. Insulin and glucagon levels were 10 ± 2, 9 ± 1, 7 ± 1, and 24 ± 4 μU/mL, and 24 ± 5, 39 ± 3, 80 ± 11, and 79 ± 5 pg/mL, CONT, BasG, HiG, and HiG + I, respectively. Glucagon infusion decreased the glucose requirements (9.3 ± 0.1, 4.6 ± 1.2, 0.9 ± 0.4, and 11.3 ± 1.0 mg·kg⁻¹·min⁻¹). Glucose uptake by both hepatic (5.1 ± 0.4, 1.7 ± 0.9, −1.0 ± 0.4, and 1.2 ± 0.4 mg·kg⁻¹·min⁻¹) and nonhepatic (4.2 ± 0.3, 2.9 ± 0.7, 1.9 ± 0.3, and 10.2 ± 1.0 mg·kg⁻¹·min⁻¹) tissues decreased. Additional insulin augmented nonhepatic glucose uptake and only partially improved hepatic glucose uptake. Thus, glucagon impaired glucose uptake by hepatic and nonhepatic tissues. Compensatory hyperinsulinemia restored nonhepatic glucose uptake and partially corrected hepatic metabolism. Thus, persistent inappropriate secretion of glucagon likely contributes to the insulin resistance and glucose intolerance observed in obese and diabetic individuals.

glycogen; glucokinase; muscle

NORMALIZATION OF HYPERGLYCEMIA is the goal of therapy for individuals with diabetes or those with glucose intolerance who, as a consequence of an accompanying stress, have persistent hyperglycemia (25). Defects in both insulin action and insulin secretion contribute to the hyperglycemia and are therapeutic targets. An appropriate response to hyperglycemia is an increase in insulin secretion and a fall in glucagon secretion. Yet, in individuals with non-insulin-dependent diabetes and in stressed patients, glucagon secretion is not suppressed and may increase (3). Inappropriate glucagon secretion in the face of hyperglycemia and hyperinsulinemia may exacerbate the glucose intolerance.

Defects in α-cell function have the potential to modulate whole body glucose metabolism. Acute increases in glucagon can antagonize net hepatic glucose uptake (NHGU) by stimulating hepatic glucose production and inhibiting hepatic glyco-gen synthesis (9). Yet, isolated acute increases in glucagon alone have limited effects on the glucose concentration if compensatory hyperinsulinemia is allowed to occur. In the fasted setting, elevated glucagon concentrations explain, in part, the increase in hepatic glucose production during infection (23) and diabetes (2, 3). Very high concentrations of glucagon, when given chronically, can amplify gluconeogenesis (22, 24) or impair NHGU in a setting of elevated catecholamine levels (14). Neutralization of glucagon or genetic loss of hepatic glucagon receptor or pancreatic α-cell glucagon secretion (PC2⁻/⁻) lower glucose levels in diabetic and non-diabetic models (4, 15, 19, 35). An alteration in glucose concentration is not always observed, presumably because compensatory decreases in insulin can mask the effect. However, the tissues responsible for the alterations in glucose levels and the long-term impact of sustained elevations in glucagon observed in chronic stress and diabetes have not been examined, especially when compensatory changes in insulin are not allowed to occur. Moreover, the extent to which pancreatic insulin secretion can compensate for defects initiated by inappropriate glucagon secretion is unclear.

In this study, we tested the hypothesis that whole body glucose disposal is sensitive to chronic increases in glucagon concentration and that compensatory increases in insulin cannot completely reverse the metabolic effects of glucagon. We used the chronically catheterized, conscious, well-controlled, depancreatized dogs so as to both control the glucagon and insulin environment and to simultaneously measure hepatic and nonhepatic glucose metabolism. Animals were placed on continuous nutritional support (NS) to augment NHGU (9) to allow detection of hepatic and extrahepatic effects of glucagon.

METHODS

Animal preparation. Twenty-one male and female nonpregnant mongrel dogs were fed standard Kal-Kan meat (Vernon, CA) and Purina Lab Canine Diet No. 5006 (Purina Mills, St. Louis, MO) once daily and had free access to water. The composition of the diet, based on dry weight, was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Pancreas was added to the diet to facilitate digestion after the pancreas was removed. Dogs were housed in a facility that met Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. All dogs were depancreatized and were treated with subcutaneous injections of regular (10.8 ± 0.5 U; Eli Lilly, Indianapolis, IN) and NPH (17.8 ± 0.7 U) insulin daily until the continuous NS was initiated. The protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. The health of the animals was determined before surgery and before NS administration as having: a good appetite (i.e., consumed at least 75% of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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of the daily ration), normal stools, hematocrit >35%, and leukocyte count <18,000 mm⁻³.

**Experimental preparation.** A laparotomy was performed using sterile techniques with general anesthesia (15 mg/kg thiopental sodium iv for induction and 1.0% isoflurane as an inhalant during surgery) on healthy dogs. During the laparotomy, the dogs were depancreatized. Blood sampling catheters were placed in the portal and left common hepatic veins. Infusion catheters were placed in the splenic vein for insulin and/or glucagon infusion to mimic the physiological route of insulin and glucagon delivery and in the inferior vena cava (IVC) for infusion of nutritional support. Flow probes (Transonic Systems, Ithaca, NY) were positioned about the portal vein, hepatic artery, and right external iliac artery. Sampling catheters were also placed in the left common iliac vein with the tip positioned distal to the anastomosis with the IVC and in the abdominal aorta via the right external iliac artery.

All catheters were filled with 0.9% NaCl (saline) containing heparin (200 U/ml). The free ends of the catheters and flow probes were exteriorized and placed in subcutaneous pockets. The dogs received penicillin G (500,000 U iv) in 1 liter of saline to minimize the possibility of infection. Fluimixamine (0.1 mg/kg; Fort Dodge Laboratory, Fort Dodge, IA) was injected intramuscularly immediately after wound closure for acute pain relief. Dogs also received penicillin G (600,000 U im) for 3 days after surgery.

**NS.** After allowing ≥14 days for recovery from surgery, the free catheter ends were exteriorized from the subcutaneous pocket behind the left clavicle under local anesthesia (2% lidocaine; Abbott, North Chicago, IL). NS was infused into one of IVC catheters with an ambulatory infusion pump (Dakmed, Buffalo, NY). Insulin or glucagon was infused into the catheter in the splenic vein by means of an infusion pump (Walkmed-350; Mckinley, Lakewood, CO). Dogs wore a jacket (Alice King Chatham, Los Angeles, CA) with two large pockets to hold the NS bag and pumps.

The dogs received NS as the sole exogenous caloric source for 5 days. The NS was designed to be isocaloric, based on predicted resting energy expenditure (26). The composition of the NS included glucose, lipids, amino acids, saline (2.9 ml·kg⁻¹·min⁻¹), potassium phosphates (90 mg·kg⁻¹·day⁻¹), and a multivitamin supplement (MV1-12; Astra USA, Westborough, MA). Glucose (50% dextrose, Abbott) comprised 75% of the nonprotein calories, and a fat emulsion (20% Intralipid; Baxter Healthcare, Deerfield, IL) constituted the remaining 25% of the energy requirements. Travasol (Baxter) was infused to supply basal nitrogen requirements (~12 g protein/day), calculated with the formula 1.5 × body wt⁰.⁶⁷ (in kg). NS was prepared under sterile conditions.

**Experimental design.** Chronically catheterized dogs received NS continuously for 5 days. NS was changed once daily. For the first 48 h of NS infusion, a variable infusion of insulin was given into the portal vein by a catheter in the splenic vein to maintain isoglycemia (~120 mg/dl). The insulin requirements were highest during the first 24 h (peak requirements ~1.5 mU·kg⁻¹·min⁻¹) and gradually diminished to very low rates (~0.4 mU·kg⁻¹·min⁻¹) by 48 h. After 48 h of NS, animals were assigned to one of four groups. Three groups received a constant low infusion of insulin (0.4 mU·kg⁻¹·min⁻¹) with either no replacement of glucagon (CONT; n = 6), a basal infusion of glucagon (0.7 ng·kg⁻¹·min⁻¹; BasG; n = 5), or 3.4 times basal infusion of glucagon (2.4 ng·kg⁻¹·min⁻¹; HiG; n = 5) for the next 2 days. The high-dose glucagon stimulated the glucagon response that we had observed in our infection model (27). Since glucagon infusion decreased whole body glucose disposal [i.e., glucose infusion rate (GIR)], we wished to see whether normalizing whole body glucose disposal with insulin reversed the glucagon effect. Thus, another group received elevated glucagon (2.4 ng·kg⁻¹·min⁻¹) and a higher insulin infusion rate (HiG + I; 1 mU·kg⁻¹·min⁻¹; n = 5) to normalize whole body glucose disposal. On the basis of pilot studies, this dose of insulin normalized whole body glucose disposal in the presence of normoglycemia (120 mg/dl) when glucagon was infused.

In all groups during the remaining 2 days of nutritional support, the GIR was adjusted to maintain isoglycemia at 120 mg/dl. The glucose level was monitored using the other IVC catheter four times a day, and NS was changed twice daily. On the basis of the average plasma glucagon concentration, the glucose content of the NS was varied; by altering the composition of the NS, the amount of glucose administered could be adjusted so as to not alter the infusion rate of the other NS components.

**Experimental protocol.** Hepatic and nonhepatic metabolism was assessed 48 h after infusing glucagon and/or additional insulin, which was the 5th day of the 5-day NS infusion. On the morning of the study, the free ends of all catheters were exteriorized under local anesthesia, and their contents were aspirated and flushed with saline. The free ends of the flow probes were also exteriorized and connected to a flowmeter (Transonic Systems). The dog was placed in a Pavlov harness for the duration of the study. The study consisted of two periods: a tracer equilibration (0–120 min) and a sampling period (120–240 min). The NS and hormones infused for the prior 48 h were continued for the duration of the study. Radioactive tracers were infused into the inferior vena cava. Primed (44 and 27 μCi) constant infusions (0.4 and 0.3 μCi/min) of [³H]- and [U-¹⁴C]glucose (New England Nuclear, Wilmington, DE), respectively, were begun at least 120 min before sampling. Blood pressure and heart rate (Micro-Med, Louisville, KY) were assessed. Blood samples in the artery, portal vein, hepatic vein, and iliac vein were taken every 30 min during the 120-min experimental period. At the end of the study, the animals were killed with an overdose of pentobarbital sodium (Veterinary Lab, Lenexa, KS). Tissue samples from each of the seven liver lobes and a muscle (caductor magnus at brevis) were freeze-clamped with Wallenberg clamps precooled in liquid nitrogen and were stored at −70°C until analysis. The entire liver was removed rapidly and weighed.

**Sample processing.** The collection and immediate processing of blood samples have been described previously (20). Blood ¹⁴C0₂ was assessed in triplicate on arterial, portal vein, and hepatic vein samples, as described by Chan and Dehaye (8). For the glucagon assay, 1 ml of plasma was added to 30 μl of Trasylol (500 kallikrein inhibitor units; Miles, Kankakee, IL). Plasma samples (0.5 ml) were deproteinized with Ba(OH)₂ and ZnSO₄ (30), and resin was added to remove charged intermediates (22), dried, and counted to assess plasma [³H]- and [¹⁴C]glucose specific activity (SA). The remaining plasma was stored at −70°C for later analyses.

**Analysis.** Insulin, glucagon, and cortisol were assayed as previously described (10). Analysis of lactate, alanine, β-hydroxybutyrate (BOHB), and glycerol in blood was performed using a modification of the method of Lloyd et al. (20). The concentration of nonesterified fatty acids (NEFA) was determined spectrophotometrically (Wako Chemicals, Richmond, VA).

**Calculations.** The hepatic substrate load (Loadₘₐₓ) was calculated as Aₜ × HABF + PVᵣ × PVBF, where Aₜ and PVᵣ represent the blood or plasma substrate concentrations in the iliac artery and portal vein, and HABF and PVBF represent blood flow in the hepatic artery and portal vein, respectively. Similarly, the substrate load leaving the liver (Loadₘᵢ₞) was the product of HV × HBF, in which HV, and HBF represent the hepatic vein substrate concentration and total hepatic blood (HABF + PVBF) or plasma flow [blood flow × (1 – hematocrit)]. Net hepatic substrate uptake was calculated as the difference between Loadₘᵢₚ and Loadₘₐₓ. Net hepatic substrate fractional extraction was calculated as the ratio of net hepatic substrate uptake and Loadₘᵢₚ. These equations were used to calculate net hepatic glucose, ¹⁴C0₂, lactate, alanine, BOHB, glycerol, and NEFA balances. In cases where the liver was a producer of substrate (i.e., negative uptake), these data were presented as positive values and denoted as net output. Plasma glucose was converted to blood glucose by a correction factor of 0.73.

NHGU is the net balance of two processes, hepatic glucose production and unidirectional uptake. Unidirectional hepatic glucose
GLUCAGON AND GLUCOSE UPTAKE

uptake (HGU) was calculated as the ratio of hepatic [1H]glucose uptake and the corresponding [1H]glucose inflowing glucose SA. Hepatic glucose production (HGP) was calculated as the difference between unidirectional HGU and NGHU. Net hindlimb glucose uptake was calculated with the formula (A_e - V_e) × ABF, where A_e and V_e represent glucose concentrations in the iliac artery and iliac vein and ABF represents blood flow in the iliac artery. Plasma flow was calculated by multiplying blood flow by (1 – hematocrit).

Net nonhepatic glucose uptake was calculated as the difference between exogenous GIR and NGHU. Non-net hepatic carbohydrate uptake was equal to the sum of net nonhepatic glucose uptake and net hepatic lactate output. Given the very high fractional turnover rate of glucose in the NS animals, the 120-min tracer equilibration period allowed the plasma specific activity to achieve a steady state. Despite this, non-steady-state analysis was used, and the rate of whole body glucose appearance (Ra) and disappearance were calculated with a two-compartmental model, as described by Mari et al. (21). Endogenous glucose production (EGP) was the difference between Ra and exogenous GIR.

Hepatic conversion of [14C]glucose to CO2 (hepatic glucose oxidation) was calculated as net hepatic [14C]glucose production divided by the hepatic [14C]glucose precursor SA. The hepatic [14C]glucose precursor SA was considered to be the weighted average of [14C]glucose SA in portal vein and hepatic artery.

Net rate of incorporation of [14C]glucose into liver glycogen was calculated as the ratio of hepatic [14C]glucose accumulation (dpm.g-liver-1.min-1) into glycogen and the average inflowing [14C]glucose SA. This is an underestimate to the extent plasma glucose SA does not reflect liver glucose 6-phosphate (G-6-P) SA.

Tissue analysis. Hepatic glycogen content was determined using the enzymatic method of Chan and Exton (8). Tissue glucokinase (GK) and glucose-6-phosphatase (G-6-Pase) activities were analyzed on the quadrate lobe with the methods described by Barzilai and Rossetti (1). Total GK activity was calculated as the difference between activities at 100 and 0.5 mM glucose. G-6-Pase was measured at 10 mM G-6-P. Protein content was assessed with the Biuret method. Pyruvate kinase activities were analyzed on the quadrate lobe with the methods described by Tanaka et al. (18).

Statistics. All mean and SE values reported are the means of the 120-min period using five time points. Statistical comparisons were made with two-way ANOVA followed by an F-test (SYSTAT, Evanston, IL). P ≤ 0.05 was regarded as significant.

RESULTS

Prestudy data. During the first 48 h of NS infusion, the exogenous insulin infusion rate (InsIR) was varied to maintain the IVC plasma glucose concentration near 120 mg/dl. In all four groups, the insulin requirements were high during the first 12 h of NS infusion (peak 1.5 mU·kg⁻¹·min⁻¹), after which the insulin requirements gradually diminished to be ~0.4 mU·kg⁻¹·min⁻¹ and were held constant for the duration of the study in all four groups. The GIR was similar in the four groups (9.4 ± 0.05, 9.4 ± 0.02, 9.4 ± 0.04, and 9.5 ± 0.04 mg·kg⁻¹·min⁻¹; CONT, BasG, HiG, and HiG + I) on the first 2 days. In CONT, the GIR remained unchanged for the duration of the 5-day study (9.28 ± 0.14 mg·kg⁻¹·min⁻¹), whereas it had to be markedly and rapidly decreased when the glucagon infusion was initiated in both BasG and HiG. On day 4 (48 h after initiation of glucagon infusion), GIR was decreased (4.6 ± 1.2 and 0.93 ± 0.35 mg·kg⁻¹·min⁻¹, BasG and HiG, respectively, P < 0.05 vs. CONT). The compensatory hyperinsulinemia in HiG + I group was able to sustain the glucose infusion rate (11.3 ± 1.0 mg·kg⁻¹·min⁻¹) despite concomitant hyperglucagonemia.

Hemodynamics. Blood pressure and heart rate were not different among the four groups during the study period. Blood flow (hepatic and iliac arteries) was similar in CONT, BasG, HiG, and HiG + I groups (Table 1). Portal vein blood flow was elevated in HiG (P < 0.05 vs. BasG) but not in BasG and HiG + I.

Hormone concentrations. In CONT, BasG, and HiG groups, insulin concentrations were similar and were elevated in the HiG + I (P < 0.05 vs. all groups). As expected, the arterial and portal vein plasma glucagon concentration increased as the glucagon infusion rate increased (Table 1). Cortisol did not significantly change over time (Table 1).

Hepatic glucose metabolism. The arterial plasma glucose concentration (Fig. 1) was clamped at similar concentrations in the four groups (~120 mg/dl). In the absence of glucagon infusion, NGHU was 5.6 ± 0.6 mg·kg⁻¹·min⁻¹ (CONT). NGHU decreased as the dose of glucagon was increased (P < 0.05 vs. CONT). At the highest dose of glucagon, the liver switched to a net producer of glucose. Interestingly, the compensatory hyperinsulinemia, while able to restore the GIR, was unable to completely reverse the glucagon-mediated decrease in NGHU (P < 0.05, HiG vs. HiG + I). Net fractional hepatic glucose extraction (NHFGE) was dramatically reduced after infusion of glucagon (P < 0.05 vs. CONT, all groups). Al-

Table 1. Body and liver weight, basal hemodynamic parameters, and hormone in CONT, BasG, HiG, and HiG + I groups receiving NS for 5 days

<table>
<thead>
<tr>
<th></th>
<th>CONT n = 6</th>
<th>BasG n = 5</th>
<th>HiG n = 5</th>
<th>HiG + I n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
<td>21 ± 1</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Liver weight, g/kg</td>
<td>50 ± 3</td>
<td>39 ± 6</td>
<td>30 ± 1*</td>
<td>30 ± 3*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>103 ± 6</td>
<td>109 ± 32</td>
<td>129 ± 20</td>
<td>97 ± 18</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>105 ± 15</td>
<td>106 ± 7</td>
<td>113 ± 4</td>
<td>125 ± 24</td>
</tr>
<tr>
<td>Hepatic arterial blood flow, ml·kg⁻¹·min⁻¹</td>
<td>4.5 ± 0.7</td>
<td>5.5 ± 1.4</td>
<td>6.9 ± 0.9</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Portal vein blood flow, ml·kg⁻¹·min⁻¹</td>
<td>24.3 ± 4.1</td>
<td>18.6 ± 2.2*</td>
<td>28.5 ± 1.9</td>
<td>19 ± 2.8</td>
</tr>
<tr>
<td>Lactic arterial blood flow, ml·kg⁻¹·min⁻¹</td>
<td>6.5 ± 0.6</td>
<td>7.2 ± 1.2</td>
<td>7.6 ± 1.2</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>Arterial plasma insulin, μU/ml</td>
<td>10.2 ± 2.3</td>
<td>9.4 ± 1.4</td>
<td>7.1 ± 0.9</td>
<td>24.1 ± 3.6*</td>
</tr>
<tr>
<td>Glucagon (artery), pg/ml</td>
<td>24 ± 5.6</td>
<td>39 ± 3*</td>
<td>80 ± 10*</td>
<td>79 ± 5*</td>
</tr>
<tr>
<td>Glucagon (portal vein), pg/ml</td>
<td>25 ± 5.6</td>
<td>55 ± 6*</td>
<td>128 ± 12*</td>
<td>155 ± 14*</td>
</tr>
<tr>
<td>Cortisol, μg/dl</td>
<td>3.4 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>3.7 ± 0.8</td>
<td>4.2 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. CONT, control with only basal insulin dose; BasG, basal glucagon dose; HiG, higher glucagon dose; HiG + I, high glucagon plus higher insulin dose; NS, nutritional support. *P < 0.05 compared with CONT; **P < 0.05 vs. HiG.
though improved ($P < 0.05$, HiG vs. HiG+I), NFHGE was not completely reversed with a high dose of insulin. The glucagon-mediated decrease in NHGU was due to a decrease in unidirectional HGU, as glucagon did not increase HGP (Table 3). Concomitant hyperinsulinemia partially corrected the effect of glucagon on HGU.

Whole body $R_g$ was $11.5 \pm 0.7$ mg·kg$^{-1}$·min$^{-1}$ in the CONT group. In parallel with the decrease in GIR, whole body $R_g$ was decreased by glucagon infusion ($6.0 \pm 0.7$ and $2.8 \pm 0.3$ mg·kg$^{-1}$·min$^{-1}$, BasG and HiG, respectively, $P < 0.05$). Whole body $R_g$ was restored to CONT rates by the compensatory hyperinsulinemia ($12.3 \pm 0.5$ mg·kg$^{-1}$·min$^{-1}$).

**Metabolic substrate kinetics.** The arterial blood lactate concentration was similar in CONT and BasG groups (Fig. 2). After an infusion of 3.4-fold basal concentration of glucagon, it was significantly decreased in the HiG group ($P < 0.05$ vs. CONT) and remained suppressed in the presence of hyperinsulinemia (HiG+I, $P < 0.05$ vs. CONT). The liver was a net producer of lactate in the CONT group ($3.6 \pm 0.4$ mg·kg$^{-1}$·min$^{-1}$), and net hepatic lactate release decreased progressively as the glucagon infusion increased. At the high dose of glucagon, the liver was no longer a net producer of lactate. Because elevated insulin partially improved NHGU, the liver remained a net producer of lactate ($0.9 \pm 0.4$ mg·kg$^{-1}$·min$^{-1}$), but it was significantly decreased compared with CONT ($P < 0.05$).

The arterial blood alanine concentration progressively fell as the glucagon infusion was increased ($P < 0.05$ vs. CONT, all groups). It decreased further when insulin was increased ($P < 0.05$, HiG vs. HiG+I). As expected, net hepatic alanine uptake (NHAU) and net hepatic fractional extraction were increased by glucagon ($P < 0.05$ vs. CONT, all groups). Both were diminished by concomitant hyperinsulinemia ($P < 0.05$, HiG vs. HiG+I; Table 2).

The arterial blood glycerol concentration was slightly but not significantly higher in HiG group (Table 2). However, net hepatic glycerol uptake was markedly elevated in the HiG group ($P < 0.05$ vs. CONT). Net fractional hepatic glycerol extraction was not altered. The arterial plasma NEFA concentration progressively increased as the dose of glucagon was increased. Hyperinsulinemia decreased the arterial NEFA concentration in the HiG+I group ($P < 0.05$, HiG vs. HiG+I). The arterial blood BOHB concentration was slightly higher in the HiG group. However, BOHB concentration was significantly decreased in the HiG+I group ($P < 0.05$ vs. CONT and HiG). Net hepatic BOHB output (i.e., negative uptake) was increased by glucagon infusion; the increase was glucagon dose dependent (Table 2). Hepatic glucose oxidation rate (Table 2) was decreased by glucagon infusion, and this was not reversed by compensatory hyperinsulinemia ($P < 0.05$, HiG vs. CONT and HiG+I).
Glucagon decreased tracer-determined glycogen synthesis rate and glycogen content (Table 3), and hyperinsulinemia did not reverse this decrease. The large decrease in liver mass due to glucagon infusion was due to a fall in glycogen content, as total protein content was unaffected (10.0 ± 0.6, 9.7 ± 1.7, 7.7 ± 1.0, and 6.9 ± 1.3 g/kg body wt; CONT, BasG, HiG, and HiG + I, respectively). Hepatic G-6-Pase, but not GK, activity was altered by glucagon infusion (Table 3). Surprisingly, hepatic pyruvate kinase activities were elevated in the HiG group in 5.9 mM phosphoenolpyruvate (288 ± 26 vs. 572 ± 56 mM/g liver, CONT vs. HiG, P < 0.05). However, the pyruvate kinase activity ratio, representing the ratio of activity measured with 0.1 mM phosphoenolpyruvate to that measured with 5.9 mM phosphoenolpyruvate, was similar in the two groups (0.06 ± 0.01 vs. 0.01, CONT vs. HiG).

Nonhepatic and hindlimb glucose metabolism. Basal non-NHGU was 3.7 ± 0.6 mg·kg⁻¹·min⁻¹ in the CONT group (Fig. 3). It was decreased by glucagon in a dose-dependent manner (P < 0.05, HiG vs. CONT). Not surprisingly, non-NHGU was significantly increased in the HiG + I group (P < 0.05, HiG + I vs. CONT and HiG). Non-net hepatic carbohydrate uptake (glucose + lactate uptake) was also decreased by glucagon (P < 0.05, HiG and HiG + I vs. CONT, HiG + I vs. HiG; Fig. 3). Consistent with these net changes, new hindlimb glucose uptake (0.24 ± 0.04, 0.14 ± 0.05, 0.10 ± 0.05, and 0.49 ± 0.13 mg·kg⁻¹·min⁻¹, CONT, BasG, HiG, and HiG + I, respectively; P < 0.05 HiG vs. CONT) and fractional extraction (0.05 ± 0.01, 0.03 ± 0.01, 0.02 ± 0.01, and 0.11 ± 0.03, P < 0.05, HiG vs. CONT) decreased as glucagon was increased and were increased by compensatory hyperinsulinemia (P < 0.05, HiG + I vs. CONT).

**DISCUSSION**

This study demonstrates that both the liver and muscle are very sensitive to the chronic effects of glucagon. Net hepatic glucose uptake was markedly inhibited by concentrations of glucagon normally seen in the basal state. Moreover, increases in glucagon to levels as can be seen in diabetes and inflammatory stress further diminished net hepatic glucose uptake. Even more strikingly, glucagon impaired glucose uptake by nonhepatic tissues (i.e., muscle). The combined effect of glucagon on hepatic and nonhepatic tissues impaired whole body glucose uptake. Although compensatory hyperinsulinemia was able to augment muscle glucose uptake and reverse the glucagon-mediated fall in whole body glucose uptake, the glucagon-mediated impairment in net hepatic glucose uptake persisted.

Glucagon markedly impairs net hepatic glucose uptake during nutritional delivery. Using the insulin-treated pancreatectomized canine model we isolated the chronic effects of glucagon in the absence of changes in glucose and insulin concentrations. Placing animals on continuous nutritional support augmented net hepatic glucose uptake (9), allowing us to detect glucagon-mediated effects on net hepatic glucose uptake in the absence of hyperglycemia. Restoration of glucagon levels to normal postprandial levels in glucagon levels to basal decreased net hepatic glucose uptake by ~70%. Prior work examining the impact of basal glucagon on net hepatic glucose uptake was unable to detect an effect on an acute basis.

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**Table 2. Arterial metabolite concentrations, hepatic uptake, FE, and hepatic glucose oxidation rate (CO₂)**

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>BasG</th>
<th>HiG</th>
<th>HiG + I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine Conc.</td>
<td>1.026±1.53</td>
<td>545±101*</td>
<td>139±19*</td>
<td>84±10*</td>
</tr>
<tr>
<td>Uptake</td>
<td>0.88±0.67</td>
<td>2.21±0.25</td>
<td>2.42±0.42</td>
<td>0.59±0.12*</td>
</tr>
<tr>
<td>Glycero Conc.</td>
<td>0.03±0.02</td>
<td>0.20±0.07*</td>
<td>0.44±0.04*</td>
<td>0.25±0.05**</td>
</tr>
<tr>
<td>Uptake</td>
<td>1.02±0.10</td>
<td>1.01±0.24</td>
<td>2.14±0.32*</td>
<td>0.87±0.11</td>
</tr>
<tr>
<td>FE</td>
<td>0.61±0.05</td>
<td>0.70±0.04</td>
<td>0.71±0.04</td>
<td>0.71±0.07</td>
</tr>
<tr>
<td>BOHB Conc.</td>
<td>25±2</td>
<td>26±3</td>
<td>37±7</td>
<td>15±2*</td>
</tr>
<tr>
<td>Uptake</td>
<td>−0.27±0.08</td>
<td>−0.51±0.05*</td>
<td>−0.81±0.45*</td>
<td>−0.16±0.11</td>
</tr>
<tr>
<td>NEFA Conc.</td>
<td>220±34</td>
<td>396±35</td>
<td>533±55*</td>
<td>122±25*</td>
</tr>
<tr>
<td>Uptake</td>
<td>0.14±0.19</td>
<td>1.39±0.56</td>
<td>0.29±0.82</td>
<td>0.40±0.17</td>
</tr>
<tr>
<td>FE</td>
<td>0.03±0.06</td>
<td>0.20±0.12</td>
<td>0.01±0.06</td>
<td>0.28±0.12**</td>
</tr>
<tr>
<td>Hepatic glucose oxidation</td>
<td>0.49±0.06</td>
<td>0.13±0.18*</td>
<td>0.20±0.08*</td>
<td>0.15±0.16*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. FE, fractional hepatic extraction; BOHB, β-hydroxybutyrate; NEFA, nonesterified fatty acids. Concentrations in μM; uptake rates in μmol·kg⁻¹·min⁻¹; glucose oxidation rates are in mg·kg⁻¹·min⁻¹. *P < 0.05 vs. CONT; **P < 0.05 vs. HiG.

**Table 3. Enzyme and substrate analysis of the liver in CONT, BasG, HiG, and HiG + I groups**

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>BasG</th>
<th>HiG</th>
<th>HiG + I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidirectional HGU, mg·kg⁻¹·min⁻¹</td>
<td>5.5±0.5</td>
<td>2.7±0.9**</td>
<td>0.5±0.4</td>
<td>1.8±0.5**</td>
</tr>
<tr>
<td>Hepatic glucose production, mg·kg⁻¹·min⁻¹</td>
<td>0.4±0.4</td>
<td>0.5±0.4</td>
<td>−0.3±0.3</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>¹³C-determined glycogen synthesis, mg·kg⁻¹·min⁻¹</td>
<td>0.94±0.19</td>
<td>0.08±0.03*</td>
<td>0.10±0.02*</td>
<td>0.19±0.07*</td>
</tr>
<tr>
<td>Tracer-determined glycogen synthesis/HGU, %</td>
<td>16.1±4.1</td>
<td>2.6±0.7*</td>
<td>5.2±5.7</td>
<td>19.2±9.0</td>
</tr>
<tr>
<td>Glycogen, mg/g liver</td>
<td>106.9±12.4</td>
<td>61.9±12.6*</td>
<td>18.7±4.3*</td>
<td>33.4±9.9*</td>
</tr>
<tr>
<td>Glycogen, g/kg body wt</td>
<td>5±0.7</td>
<td>2.6±0.8*</td>
<td>0.5±0.1*</td>
<td>1.0±0.4*</td>
</tr>
<tr>
<td>G-6-Pase, uU/mg protein</td>
<td>127±44</td>
<td>148±24</td>
<td>217±35*</td>
<td>158±45</td>
</tr>
<tr>
<td>GK, μU/mg protein</td>
<td>11.5±1.5</td>
<td>9.7±1.1</td>
<td>12.2±3.8</td>
<td>17.8±3.3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. HGU, hepatic glucose uptake; G-6-Pase, glucose-6-phosphatase; GK, glucokinase. *P < 0.05 vs. CONT; **P < 0.05 vs. HiG.
means expected to persist over 2 days (11). However, when other production wanes over a few hours and thus would not be prevented. Prior work suggests that glucagon-stimulated hepatic glucose uptake. The difference may in part be due to the glucagon-mediated decrease in hepatic glycogen content and tracer incorporation into glycogen (3). The glucagon-infusion glycogenolysis and hepatic glucose production were activated, and this waned as hepatic glucose utilization was attenuated. As the glucose utilization rate abruptly decreased and remained decreased for the 48 h, it is likely the transition between activation of glucogenolysis and inhibition of hepatic glucose uptake was gradual.

The mechanism for the glucagon-mediated fall in liver glucose uptake is unclear. Hepatic lactate release, a major metabolic fate of the glucose during nutritional support, decreased in proportion to the fall in net hepatic glucose uptake. Thus, the metabolic control points are likely prior to pyruvate. One possible mechanism by which glucagon could limit liver glucose uptake is inhibition of glucokinase (29, 30). However, chronic activation of glucokinase (i.e., chronic fructose infusion) alone cannot correct the infection-induced impairment in net hepatic glucose uptake (13). In addition, glucokinase and glucose 6-phosphatase activities were unaffected by basal glucagon despite having pronounced effects on net hepatic glucose uptake. High glucagon concentrations did enhance glucose 6-phosphatase activity. Glucokinase activity was unaffected. This is surprising, because glucagon is a negative regulator of glucokinase (12, 34). It is possible that intracellular localization of glucokinase may have been altered by glucagon (28), which would not be detected with the enzymatic assay.

Compensatory hyperinsulinemia, although able to restore whole body glucose uptake (i.e., glucose infusion rate), was unable to completely correct the glucagon-mediated decrease in net hepatic glucose uptake. Whole body glucose uptake was decreased by over 90% in HiG, and this was reversed by the additional insulin infusion (0.4 vs. 1.0 mU·kg\(^{-1}\)·min\(^{-1}\)). Yet, despite this restoration by additional insulin, net hepatic glucose uptake was still decreased by 80%. Moreover, the glucagon-mediated decrease in hepatic glycogen content and tracer incorporation into hepatic glycogen were not reversed. The additional insulin primarily diverted the exogenous glucose to peripheral tissues. Acutely, insulin is a potent inhibitor of glucagon action (31); these studies suggest that in the chronic setting glucagon is a very powerful hormone as well. In stressed patients receiving nutritional support, accompanying hyperglycemia is managed by administering exogenous insulin (25). In insulin-resistant individuals, insulin secretion increases except when glucagon was increased above basal concentrations (17). In normal animals, acute modest changes in insulin, glucagon, and glucose levels seen during nutritional support would not shift the liver to a glucose consumer, suggesting that a chronic sustained fall in glucagon likely played an essential role in allowing the liver to sustain net hepatic glucose uptake. In individuals with diabetes, glucagon concentration is normal or elevated (2, 19), which is inappropriate given the prevailing insulin and glucose concentration. These data would suggest that the very subtle chronic alterations in glucagon secretion observed in these individuals likely contribute to the observed defects in hepatic metabolism.

Because the liver can both produce and consume glucose, decreases in net hepatic glucose uptake could be due to either a decrease in unidirectional hepatic glucose uptake and/or to an increase in hepatic glucose production. Basal and high-dose replacement of glucagon decreased unidirectional hepatic glucose uptake and did not alter hepatic glucose production. Glucagon also decreased hepatic glycogen levels and tracer incorporation into glycogen. This contrasts with prior studies where acute increases in glucagon enhanced hepatic glucose production (17) and had limited effects on unidirectional hepatic glucose uptake. The difference may in part be due to the duration of exposure to the change in glucagon (3 vs. 48 h). Prior work suggests that glucagon-stimulated hepatic glucose production wanes over a few hours and thus would not be expected to persist over 2 days (11). However, when other stress hormones (catecholamines and cortisol) are also elevated, glucagon has marked stimulatory effects on gluconeogenesis (22). Interestingly, chronic elevations in glucagon help restrain the chronic effects of cortisol to augment hepatic glycogen synthesis (14). Our results are novel, as the effects of glucagon persisted in the absence of accompanying increases in stress hormones. On the basis of our prior work, glucogen stores increase during the first 3 days of nutritional support and then stabilize for the duration of nutritional support. Glucogen content was decreased by chronic glucagon infusion. Because hepatic glucose production was very low during nutritional support, inhibition of unidirectional hepatic glucose uptake could not explain the extent of the fall in glycogen. Net glucogenolysis must have been activated for a brief period of time during the 48 h of glucagon infusion to explain a decrease of 5 g/kg body wt. Glucogenolysis would have to have increased by \(~ 1.5\ mg·kg\(^{-1}\)·min\(^{-1}\) for the entire 48 h. Because hepatic glucose production was not different from zero at 48 h in the glucagon-infused group, it is likely that at the onset of the glucagon infusion glucogenolysis and hepatic glucose production were activated, and this waned as hepatic glucose utilization was attenuated. As the glucose infusion rate abruptly decreased and remained decreased for the 48 h, it is likely the transition between activation of glucogenolysis and inhibition of hepatic glucose uptake was gradual.

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to control glucose levels. If diabetes develops, drugs are given to augment insulin secretion and/or insulin action. Insulin-resistant individuals and individuals with type 2 diabetes secrete inappropriate amounts of glucagon and/or have abnormal high glucagon-to-insulin ratios (2, 5, 33); our data suggest that compensatory hyperinsulinemia can correct the hyperglycemia associated with a relative excess of glucagon. Yet, although insulin can correct whole body glucose disposal, it does not restore the underlying disturbances in hepatic metabolism mediated by inappropriate glucagon secretion.

Consistent with previous work, net hepatic alanine fractional extraction and consequently hepatic disposal of alanine were very sensitive to glucagon. In a dose-dependent manner, net hepatic alanine fractional extraction increased 40-fold as glucagon levels were increased. These responses are consistent with previous work using an acute infusion of glucagon (32). Glucagon infusion also enhanced net hepatic alanine uptake by $\sim 2 \mu$mol·kg$^{-1}$·min$^{-1}$. Because exogenous alanine given in the nutritional support ($\sim 0.9 \mu$mol·kg$^{-1}$·min$^{-1}$) could not account for the increase in hepatic alanine uptake, it suggests that glucagon diverts both exogenous and endogenous alanine carbon to the liver. Insulin infusion decreased arterial alanine levels and net hepatic alanine uptake. Since the nutritional support-derived exogenous alanine infusion was not altered, it is consistent with known effects of insulin to diminish nonhepatic proteolysis. The insulin-mediated fall in net hepatic alanine fractional extraction is likely due to the very low alanine levels that limited the extraction process; since in the setting of adequate alanine availability insulin does not attenuate glucagon-stimulated net hepatic alanine fractional extraction (31).

Surprisingly, glucagon inhibited glucose uptake by peripheral tissues. Infusion of basal glucagon decreased nonhepatic glucose uptake by 22%, and this was further decreased to 50% by HiG. The liver releases a large fraction of net hepatic glucose uptake as lactate, which in turn is used by peripheral tissues. Total nonhepatic carbohydrate uptake (glucose + lactate) was decreased to an even greater extent, 45 and 79%, when basal and high glucagon were infused. Glucagon receptors are expressed in cardiac muscle and possibly to a lesser extent in skeletal muscle (6, 16). There is little evidence that glucagon directly impairs muscle glucose uptake in the acute setting. Interestingly, nonhepatic glucose uptake decreased with the high dose of glucagon to $1.9 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which is the rate seen in the basal state in an overnight-fasted dog where insulin levels are lower than that seen during nutritional support. Because insulin levels were elevated, it suggests that high doses of glucagon may impair insulin action.

In part, this could be mediated by the rise in NEFA, which can impair insulin-stimulated glucose uptake. The effects of physiological glucagon concentrations on lipolysis are consistent with work in humans where low doses of glucagon in the acute setting augment lipolysis (7). The present studies suggest that these effects are not transient and can persist for days.

In summary, both hepatic and peripheral tissues are exquisitely sensitive to the chronic action of glucagon. The effects of glucagon on the liver are not as readily reversed by exogenous insulin as the effects on nonhepatic tissues. Thus, inappropriate secretion of glucagon for the prevailing glucose and insulin environment, as is seen in stressed patients and in individuals with type 2 diabetes (3), can exert profound long-term effects on glucose homeostasis.

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