Impact of Acute Epinephrine Removal on Hepatic Glucose Metabolism During Stress Hormone Infusion

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We examined the effect of acute discontinuation of an epinephrine (EPI) infusion on hepatic glucose metabolism during stress hormone infusion (SHI). Glucose metabolism was assessed in 11 conscious, 20-hour fasted dogs using tracer and arteriovenous techniques after a 3-day exposure to SHI. SHI increased EPI, norepinephrine, cortisol, and glucagon levels (~sixfold to 10-fold), which led to marked hyperglycemia, hyperinsulinemia, and accelerated glucose metabolism. On day 3, EPI infusion was acutely discontinued for 180 minutes in five dogs while infusion of the other hormones was continued (SHI − EPI). In the remaining six dogs, all hormones were continued for the duration of the study (SHI + EPI). In SHI − EPI, EPI levels decreased from 1,678 ± 191 to 161 ± 47 pg/mL. Isoglycemia (183 ± 10 to 185 ± 15 mg/dL) was maintained with an exogenous glucose infusion. Arterial insulin levels increased from 41 ± 8 to 64 ± 8 μU/mL. Whole-body glucose utilization increased from 3.5 ± 0.5 to 9.4 ± 1.9 mg/kg/min. Nonesterified fatty acids ([NEFAs] 763 ± 292 to 147 ± 32 μmol/L) decreased. Net hepatic glucose output decreased (2.6 ± 0.6 to 0.1 ± 0.3 mg/kg/min). In SHI + EPI, hepatic glucose metabolism remained unaltered. In summary, EPI plays a pivotal role during SHI by stimulating glucose production and inhibiting glucose utilization. In part, these effects are mediated by restraining pancreatic insulin secretion.

THE METABOLIC RESPONSE to stress is accompanied by marked increases in counterregulatory hormone levels. We previously reported that chronic stress hormone infusion ([SHI] combined infusion of glucagon, epinephrine [EPI], norepinephrine, and cortisol for 70 hours) in the dog caused marked hyperglycemia and accelerated glucose production (rate of appearance [R_a]).[1] The increase in the glucose R_a was due to a combined increase in glycogenolysis and gluconeogenesis. The latter resulted from increases in net hepatic gluconeogenic precursor uptake and renal gluconeogenesis.

We previously completed a series of studies examining the long-term interaction of glucagon, cortisol, EPI, and norepinephrine in regulating carbohydrate metabolism in the conscious dog.[1-4] We observed that when EPI was not included in the SHI, the SHI-induced increase in hepatic glycogenolysis was markedly reduced. While EPI can enhance gluconeogenesis by augmenting the gluconeogenic precursor supply when administered acutely,[5] this action plays a relatively minor role when it is infused long-term with other counterregulatory hormones. In fact, glucagon and cortisol were the primary determinants of the SHI-induced increase in gluconeogenesis.

While it is clear that the absence of EPI during SHI limits the increase in hepatic glycogenolysis, the role of EPI is uncertain when all of the stress hormones are also present. Since many of the actions of these hormones overlap, the actions of one hormone may compensate for the absence of another hormone in supporting a metabolic process (eg, gluconeogenesis). The consequence is that the role of a given hormone in the overall response could be underestimated. In addition, the chronic effects of a hormone can differ from its acute effects. This difference was most readily apparent when we contrasted the acute and chronic roles of glucagon during SHI. During SHI, acute removal of glucagon for 180 minutes decreased hepatic glycogenolysis and diverted gluconeogenic carbon to glycogen,[6] while the chronic absence of an increase in glucagon limited the increase in gluconeogenesis observed during SHI.

It is not known if the acute and chronic roles of EPI differ as well during SHI. During a chronic stress such as infection, in which multiple stress hormones are elevated, adrenergic blockade has been used to assess the role of catecholamines in the metabolic response.[7] While adrenergic blockade had multiple effects (augmentation of lipolysis and glucose production and inhibition of insulin secretion), the study could not discriminate between the effects of circulating EPI and neurally derived catecholamines.[8] The aim of the present study was to examine the impact of acute EPI removal during chronic SHI on hepatic and whole-body glucose metabolism in the dog.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 11 fasted (20 hours) conscious mongrel dogs (22 ± 2 kg) of either gender receiving a diet of Kal-Kan 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 meets American Association for Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

Two weeks before each experiment, a laparotomy was performed under general anesthesia (acepromazine 0.55 mg/kg and sodium pentobarbital 25 mg/kg). Silastic catheters (0.03-in ID; Dow Corning, Midland, MI) were placed into the inferior vena cava for long-term infusion of hydrocortisone, EPI, and norepinephrine and into a splenic vein for long-term infusion of glucagon as described elsewhere.[1] Catheters for blood sampling (0.04-in ID) were inserted into the portal vein, right renal vein, and left hepatic vein. In addition, a catheter (0.04-in ID) for blood sampling was inserted into the femoral artery following an incision in the left inguinal area. The catheters were then filled with saline containing heparin (200 IU/mL). Doppler flow probes

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were placed about the portal vein and hepatic artery after the gastroduodenal vein was ligated. The infusion catheters were exteriorized and passed through a subcutaneous tunnel to the back between the scapulas, where they were placed in a subcutaneous pocket. The portal, renal, and hepatic blood sampling catheters and leads from the Doppler flow probes were exteriorized and placed in a subcutaneous pocket in the abdominal area. The arterial blood sampling catheter was placed under the skin in the inguinal region.

All animals studied had (1) a good appetite (consuming all of the daily ration), (2) normal stools, (3) a hematocrit greater than 35%, and (4) a leukocyte count less than 18,000/μL on days 0 and 3.

Experimental Protocol

Hormone infusion catheters located in the back were removed from their respective pockets under local anesthesia (2% lidocaine; Abbott Laboratories, North Chicago, IL). The dog was then placed in a jacket (Alice King Chatham, Los Angeles, CA) containing two pockets, into each of which was placed a portable infusion pump (Auto Syringe; Travenol Laboratories, Hooksett, NH). Hydrocortisone was dissolved in saline and infused with one pump at a rate of 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (240 μL/h) into the inferior vena cava. EPI and norepinephrine were dissolved in saline containing ascorbic acid (0.7 mg/mL), and both were infused at a rate of 0.08 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (240 μL/h) into the inferior vena cava using the other pump. Glucagon, because of its tendency to aggregate at high concentrations, was prepared in a dilute solution containing each dog's own plasma (3% by vol). A swivel-tether system (Alice King Chatham) was used to infuse this hormone. The swivel was attached to the cage and the silastic catheter was passed through the tether connecting the dog jacket to the swivel. This system allowed the dog freedom of movement while maintaining a constant infusion of glucagon (5 $ng \cdot kg^{-1} \cdot min^{-1}$, 70 μL/min; Harvard Apparatus, Millis, MA) into the portal vein. All solutions were filtered (0.2 μm) under sterile conditions before infusion. Fresh solutions were prepared every 12 hours on each of 3 infusion days. On the third day following a 20-hour fast, metabolism was assessed.

The catheters were removed from their respective pockets under local anesthesia on the day of the study. An Angiocath (18-gauge; Deseret Medical, Sandy, UT) was inserted percutaneously into a cephalic vein. A primed (50-μCi)-constant infusion of high-performance liquid chromatography (HPLC)-purified 3-^{3}H-glucose (0.4 μCi/min) and constant infusions of U-^{14}C-alanine (0.4 μCi/min), *p*-aminohippuric acid (0.3 $mg \cdot kg^{-1} \cdot min^{-1}$), and indocyanine green (0.1 $mg \cdot m^{-2} \cdot min^{-1}$) were started via the right cephalic vein and continued throughout the entire experiment. The experiment consisted of three periods, an equilibration period (−180 to −60 minutes), a basal period (−60 to 0 minutes), and an experimental period (0 to 180 minutes). Arterial, portal vein, and hepatic vein blood samples were taken every 15 minutes during the basal period and every 30 minutes during the experimental period. In six of 11 dogs, chronic EPI infusion was continued throughout the 180-minute experimental period (SHI + EPI). In the remaining five dogs, chronic EPI infusion was discontinued at the end of the basal period and a variable exogenous glucose infusion was administered via a cephalic vein to maintain a constant glucose level (SHI − EPI). The effect of acute EPI removal on renal substrate metabolism could not be estimated accurately, since the renal vein catheter failed in four dogs in which EPI was acutely removed. At the end of the experimental period, the dog was killed with an overdose of Phenobarbital (Sigma, St Louis, MO).

Tracer Methods and Calculations

The rates of total (ie, whole-body) glucose production (R_a) and utilization (rate of disappearance [R_d]) were calculated according to the method of Wall et al[9] as simplified by DeBodo et al.[10] A pool fraction of 0.65 and a volume of distribution of 220 mL/kg were used. When glucose was infused, endogenous glucose production was calculated as the difference between the whole-body R_a and the exogenous glucose infusion rate.

Net hepatic glucose output was calculated using the formula $[H - ([F_a \times A] + [F_p \times P])] \times HBF$, where H, A, and P are 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.[11] Net hepatic lactate (alanine or glycerol) uptake was calculated using the formula $[H - ([F_a \times A] + [F_p \times P])] \times HBF$, where H, A, and P are blood lactate (alanine or glycerol) concentrations in the hepatic vein, femoral artery, and portal vein, respectively. In the present study, Doppler flow probes were functional in seven of 11 dogs that received the SHI. Based on our previous study,[1] neither the fractional contribution of hepatic arterial blood flow to total HBF nor the HBF were altered by SHI. The contribution of hepatic artery blood flow for the entire group was assumed to equal the mean distribution found in the subgroup with functional Doppler flow probes.

Processing of Blood Samples

The collection and immediate processing of blood samples has been previously described.[12] Blood lactate, glycerol, and alanine were analyzed using the method of Lloyd et al.[13] Plasma glucose was assayed immediately using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma treated with 500 Kallikrien inhibitory units (KIU) of Trasylol (FBA Pharmaceuticals, New York, NY) was assayed for immunoreactive glucagon using the 30K antiserum of Aguilar-Parada et al[14] (coefficient of variation [CV], 8%). Immunoreactive insulin[15] was assayed using a Sephadex-bound antibody technique (Pharmacia Diagnostics, Piscataway, NJ; CV, 11%). Plasma cortisol was assayed with the Clinical Assays Gamma Coat radioimmunoassay kit[16] (CV, 6%). Plasma collected from blood samples that were immediately treated with EGTA and glutathione was assayed for EPI and norepinephrine using HPLC techniques[17] (CV, 14%). The indocyanine green dye content was measured spectrophotometrically (810 nm) to estimate total HBF and to verify placement of hepatic vein catheters.[18] Doppler-determined blood flow was obtained using an ultrasonic range-gated, pulsed-Doppler flow meter.[19, 20]

Materials

Glucagon was purchased from Eli Lilly and Co (Indianapolis, IN). EPI, norepinephrine, and *p*-aminohippuric acid were obtained from Sigma Chemical (St Louis, MO). Hydrocortisone was purchased from Abbott Laboratories. Glucagon 30K antiserum was obtained from the University of Texas Southwestern Medical School (Dallas, TX). Purified glucagon and ^{125}I-glucagon were obtained from Novo Research Institute (Copenhagen, Denmark). Cortisol assay kits were obtained from Upjohn Diagnostics (Kalamazoo, MI). 3-^{3}H-glucose (HPLC-purified) was obtained from New England Nuclear Research Products (Wilmington, DE).

Data Analysis

Statistical comparisons were made using two-way ANOVA (Systat, Cambridge, MA). A univariate post hoc F test was used when a significant F ratio was found. Statistical significance was accepted at a P level .05. Data from the control group (SHI + EPI) have been previously reported.[6]

RESULTS

Hormone Levels and Glucose Metabolism

SHI increased arterial plasma glucagon, EPI, norepinephrine, and cortisol to approximately fivefold to 10-fold the values

found in a normal dog.[1] Acute discontinuation of the EPI infusion (SHI − EPI) decreased plasma EPI from 1,678 ± 191 to 161 ± 47 pg/mL by 180 minutes. Despite maintenance of isoglycemia, arterial plasma insulin increased from 42 ± 7 to 64 ± 7 μU/mL following EPI withdrawal, but remained unchanged in SHI + EPI (Fig 1). Arterial plasma glucagon (Fig 1), norepinephrine (1,301 ± 111 to 1,209 ± 242 and 1,685 ± 305 to 1,678 ± 120 pg/ml for SHI + EPI and SHI − EPI, respectively), and cortisol (13 ± 1 to 13 ± 1 and 12 ± 1 to 14 ± 1 μg/dL) levels remained unaltered regardless of whether EPI was acutely removed. The arterial plasma glucose concentration did not change when EPI was acutely removed, because glucose was infused to maintain euglycemia (8.7 ± 2.0 mg · kg^{-1} · min^{-1}). The whole-body R_a increased from 3.4 ± 0.4 to 9.7 ± 2.0 mg · kg^{-1} · min^{-1}. However, endogenous glucose production (whole-body R_a- exogenous glucose infusion) and net hepatic glucose output decreased 2.5 ± 0.4 and 2.2 ± 0.4 mg · kg^{-1} · min^{-1} ($P < .05$), respectively (Fig 2). The whole-body R_d increased from 3.5 ± 0.5 to 9.4 ± 1.9 mg · kg^{-1} · min^{-1} and whole-body glucose clearance increased from 1.9 ± 0.2 to 5.1 ± 1.0 mL · kg^{-1} · min^{-1} (Fig 3). When EPI infusion was continued, endogenous glucose production, net hepatic glucose output, and glucose R_d were not altered.

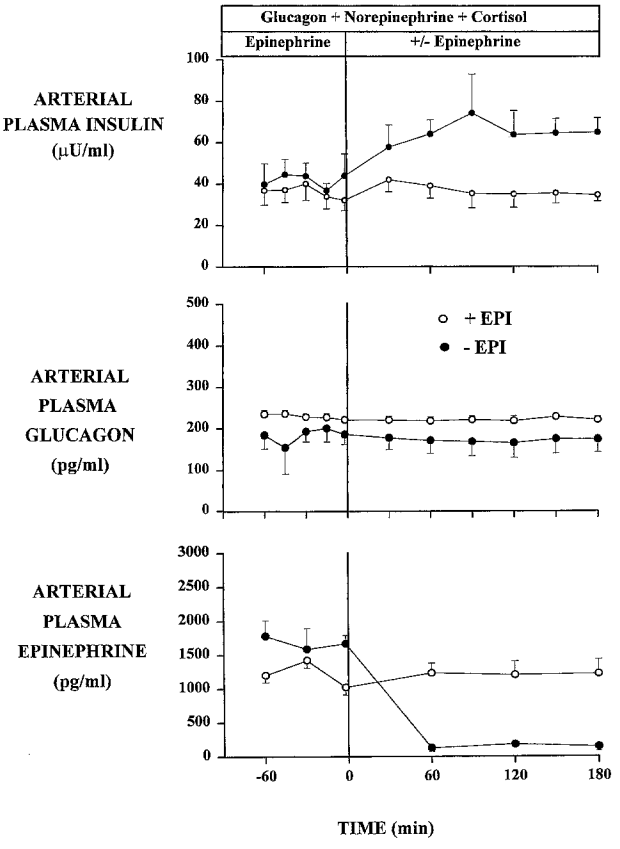

Fig 1. Effect of acute epinephrine removal following a 70-hour chronic SHI on arterial plasma insulin, glucagon, and epinephrine concentrations in conscious, 20-hour fasted dogs. Data are the mean ± SEM.

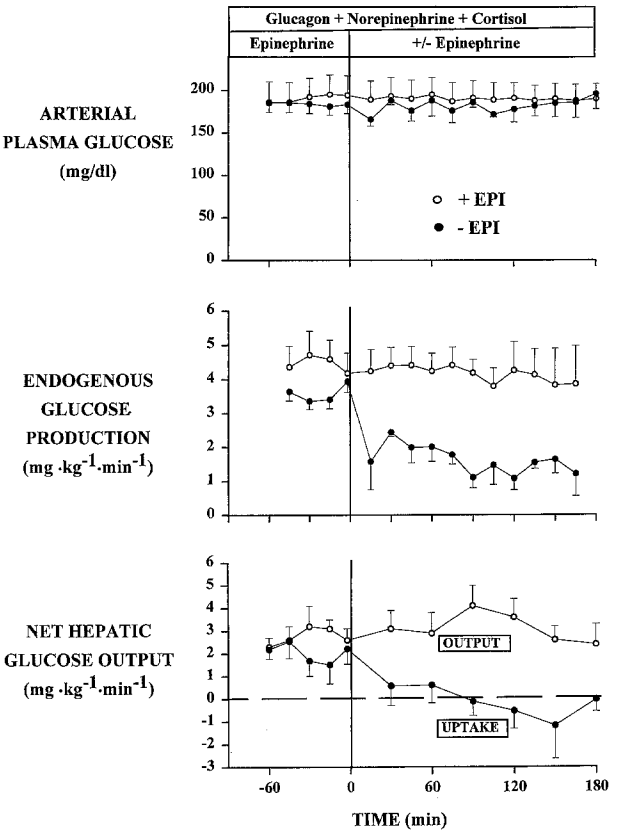

Fig 2. Effect of acute epinephrine removal following a 70-hour chronic SHI on arterial plasma glucose, endogenous glucose production, and net hepatic glucose output in conscious, 20-hour fasted dogs. Data are the mean ± SEM.

Hepatic Substrate Uptake

Acute removal of EPI did not significantly alter arterial blood lactate levels; however, net hepatic fractional lactate extraction and net hepatic lactate uptake tended to decrease (Table 1). Arterial alanine levels and hepatic alanine uptake and fractional extraction were unaltered. Arterial glycerol levels decreased during acute EPI removal; however, net hepatic fractional glycerol extraction and glycerol uptake were not significantly altered. Arterial nonesterified fatty acid (NEFA) levels de-

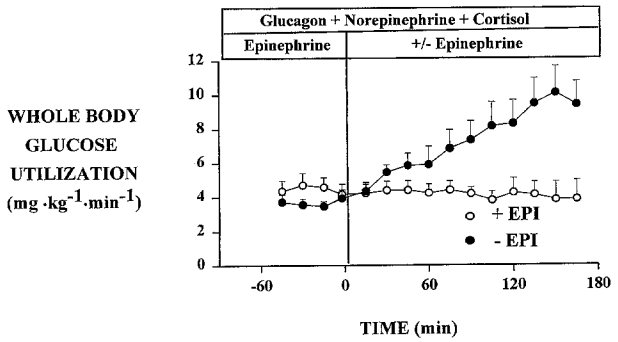

Fig 3. Effect of acute epinephrine removal following a 70-hour chronic SHI on whole-body glucose utilization in conscious, 20-hour fasted dogs. Data are the mean ± SEM.

Table 1. Effect of Acute Epi Removal Following a 70-Hour Chronic SHI on the Arterial Concentration, Net Hepatic Uptake, and Net Fractional Hepatic Extraction of Lactate, Alanine, and Glycerol in Chronically Catheterized Conscious Dogs (n = 5)

Parameter	SHI + EPI		SHI − EPI	
	Basal Period	Experimental Period	Basal Period	Experimental Period
Lactate				
Arterial level (μmol/L)	864 ± 263	872 ± 257	415 ± 86	586 ± 66
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	7.3 ± 2.9	6.5 ± 3.0	8.7 ± 1.1	5.5 ± 1.7
Net fractional hepatic extraction	0.38 ± 0.16	0.33 ± 0.09	0.67 ± 0.05	0.37 ± 0.09*
Alanine				
Arterial level (μmol/L)	78 ± 11	85 ± 22	84 ± 19	116 ± 44
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	2.2 ± 0.3	2.0 ± 0.4	1.6 ± 0.1	1.7 ± 0.4
Net fractional hepatic extraction	0.59 ± 0.04	0.56 ± 0.02	0.60 ± 0.02	0.60 ± 0.06
Glycerol				
Arterial level (μmol/L)	139 ± 33	119 ± 35*	170 ± 17	115 ± 33*
Net hepatic uptake (μmol · kg^{-1} · min^{-1})	3.1 ± 0.7	3.0 ± 1.1	2.4 ± 0.4	1.9 ± 0.9
Net fractional hepatic extraction	0.67 ± 0.04	0.67 ± 0.02	0.58 ± 0.04	0.66 ± 0.05

NOTE. Data are the mean ± SEM.
*Significantly different *v* basal.

creased (763 ± 292 to 147 ± 32 μmol/L) and net hepatic NEFA uptake decreased (1.86 ± 0.67 to 0.3 ± 0.3 μmol · kg^{-1} · min^{-1}) following EPI removal.

DISCUSSION

Based on these studies, acute removal of EPI during SHI has profound effects on whole-body glucose metabolism. Whole-body glucose utilization increased nearly threefold and was associated with a decrease in NEFA levels. Net hepatic glucose output and endogenous hepatic glucose production were also suppressed. The observed response may have been mediated in part by the reflex increase in insulin secretion following discontinuation of EPI infusion. Regardless of whether the effects of EPI are direct and/or indirect (via suppression of insulin secretion), it is clear that the hormone exerts profound effects in sustaining the hyperglycemia observed during SHI.

Withdrawal of EPI led to a marked increase in arterial insulin, indicating that a high circulating EPI level exerts a tonic inhibitory effect on pancreatic insulin secretion likely mediated by the α-adrenergic receptor.[21] This impact of EPI on pancreatic insulin secretion was not detected in our previous study when EPI was not included in the SHI.[4] The most likely reason is that circulating glucose levels were lower, which obscured the insulin-suppressing effects of EPI. By preventing the decrease in glucose levels in the present study, the inhibitory effect of EPI on the pancreatic β cell became apparent.

Acute removal of EPI led to a marked inhibition of hepatic glucose production (~2.2 mg · kg^{-1} · min^{-1}). This decrease was primarily due to a decrease in hepatic glycogenolysis. Net hepatic gluconeogenic precursor uptake (sum of net hepatic uptake of lactate, alanine, and glycerol) only decreased about 0.5 mg glucose · kg^{-1} · min^{-1} and thus could account for no more than 25% of the decrease in hepatic glucose production. These data are consistent with the known potent effects of EPI on hepatic glycogenolysis.[22] They are also consistent with our previous observation that the absence of EPI in the SHI markedly attenuated the increase in hepatic glycogenolysis.[4] Since insulin also has potent effects on hepatic glucose production,[23] it is difficult to determine if the decrease in hepatic glucose production was directly due to the effects of the decrease in EPI and/or the increase in insulin. Renal glucose production contributes to the SHI-induced increase in glucose flux. Since renal glucose production is responsive to EPI,[24] it is possible that a small portion of the decrease in tracer-determined glucose production was renal in origin. However, the reduction in endogenous glucose production following EPI withdrawal can be explained quantitatively by a decrease in net glucose output by the liver. Thus, the contribution of the kidney to the reduction in endogenous glucose production is likely minimal.

Acute EPI infusion may have inhibited net hepatic lactate uptake by decreasing net hepatic fractional lactate extraction. The extent of the decrease is difficult to quantify, since net hepatic fractional extraction of lactate was different in the basal period in the two groups. However, irrespective of the extent of the decrease, the effect is likely modest. While EPI acutely augments gluconeogenesis by increasing lactate supply,[5] in the context of elevations in other counterregulatory hormones, this effect clearly does not persist to the same extent chronically.

NEFA levels decreased markedly (80%) following acute withdrawal of EPI. Interestingly, arterial glycerol levels decreased modestly (15%) in both groups, indicating that lipolysis was not altered by EPI removal. Since the decrease in NEFAs was substantially greater than the decrease in lipolysis, this suggests that acute withdrawal of EPI enhanced reesterification of the NEFAs. In the acute setting in the absence of changes in insulin, EPI has a greater effect on glycerol levels than on NEFA levels.[25] Thus, EPI withdrawal alone would not be expected to have a potent effect on NEFA levels. Since insulin is a potent stimulator of reesterification, the specific effect of EPI withdrawal on reesterification likely reflects the actions of the ensuing hyperinsulinemia.[26]

The potent ability of EPI to restrain peripheral glucose utilization contributed markedly to the hyperglycemia during SHI. Following acute removal of EPI, whole-body glucose utilization (R_d) and clearance increased 2.5-fold. In the presence of hyperglycemia (220 mg/dL) and hyperinsulinemia (40 μU/mL), whole-body glucose utilization should be approximately 10 mg · kg^{-1} · min^{-1}.[11] Following SHI, it was only 2.6 mg · kg^{-1} · min^{-1}, clearly indicating that SHI led to severe

insulin resistance. EPI can directly inhibit skeletal muscle glucose uptake.[27] Thus, this effect combined with the suppression of insulin secretion to limit peripheral glucose.

The hormonal environment created by SHI was not designed to mimic a specific stress. Since the endocrine environment in any given stress will differ from that found in SHI, the relative importance of EPI versus other counterregulatory hormones in modulating whole-body glucose metabolism will change. In the present study, EPI levels were increased to about 1,600 pg/mL. These are similar to the levels found in severe stress such as cardiogenic shock and sepsis but higher than the levels found in moderate stress. Thus, it is clear that EPI at these levels can have potent effects on β cells, as well as other organs, which can contribute to the accompanying hyperglycemia. However, in a stress in which EPI levels are lower, the importance of EPI will also depend on the levels of the other counterregulatory hormones. A direct comparison of this study to studies in which adrenergic blockade was used is difficult, since combined α- and β-adrenergic blockade would block the effects of both circulating EPI and norepinephrine and neurally derived norepinephrine.

In summary, EPI exerts potent effects on carbohydrate and fat metabolism during SHI. Moreover, many of its effects are dependent, in part, on its ability to restrain pancreatic insulin secretion and thus sustain the hyperglycemia found during chronic stress.

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