Regulation of Net Hepatic Glycogenolysis and Gluconeogenesis

by Epinephrine in Humans

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Running Head: Epinephrine Regulation of Hepatic Glucose Metabolism in Humans

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ABSTRACT

The relative contributions of net hepatic glycogenolysis (NHG) and gluconeogenesis to rates of glucose production during a physiological increment in plasma epinephrine concentrations, independent of changes in plasma insulin concentrations, were determined in seven fasting, healthy young subjects. Plasma insulin concentrations were kept constant by infusing somatostatin (0.1 µg/kg/min), and replacing basal insulin (24 pmol/m²/min). Epinephrine (1.2 µg/m²/min) was infused for 90 min while NHG was assessed directly by \(^{13}\)C Magnetic Resonance Spectroscopy (MRS). The rate of glucose production was assessed using [6,6-\(^2\)H\(_2\)]glucose, and gluconeogenesis was calculated as the difference between the rate of glucose production and NHG. Plasma epinephrine concentrations increased rapidly from \(\sim 100\) to \(\sim 2000\) pmol/L \((P<0.00001)\) accompanied by an increase in plasma glucose concentrations from 4.3±0.2 to 13.3±0.3 mmol/L at 90 min \((P=0.00001)\). This increase in plasma epinephrine concentration resulted in a 2.5-fold increase in glucose production (from 14.4±1.0 µmol/kg/min to 35.7±2.0 µmol/kg/min, \(P<0.0001\)), which lasted for approximately 60 min (Phase 1), after which glucose production decreased to 31.2±1.9 µmol/kg/min \((P<0.0001\) vs. Basal) during the last 30 min of the epinephrine infusion (Phase 2). Hepatic glycogen concentrations decreased almost linearly during Phase 1 and rates of NHG were 19.9±3.0 µmol/kg/min \((P=0.005\) vs. Basal), which could account for \(~\)60% of glucose production. During Phase 2, NHG decreased to 7.3±2.8 µmol/kg/min \((P=0.02\) vs. Peak), accounting for only \(~20\)% of glucose production.

In conclusion, in the presence of basal plasma insulin and glucagon concentrations, a physiological increase in plasma epinephrine concentrations stimulates glucose production with an initial, 60 min transient phase caused by stimulation of NHG and a second phase, which can mostly be attributed to a two-fold increase in rates of gluconeogenesis.

Key words: Net hepatic glycogenolysis, gluconeogenesis, epinephrine, humans
It is well established that hepatic glucose production increases in response to a physiological increase in plasma epinephrine concentration but the role of epinephrine in the regulation of the relative contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in humans is unknown (14, 19). Surprisingly only a few studies have examined the effects of epinephrine on glucose production in humans and none of these studies have directly assessed the relative contributions of net hepatic glycogenolysis and gluconeogenesis to this process due to the difficulty of measuring these processes in vivo.

In this regard, $^{13}$C Magnetic Resonance Spectroscopy (MRS) provides a non-invasive method to directly measure changes in liver glycogen content in humans in real time allowing for calculation of rates of net hepatic glycogenolysis (10, 15). Rates of gluconeogenesis can be calculated by subtracting rates of net hepatic glycogenolysis from rates of whole body glucose production as determined with $[6,6-^{2}\text{H}_2]$ glucose. The purpose of the present study was to use $^{13}$C MRS in combination with $[6,6-^{2}\text{H}_2]$ glucose to directly estimate the relative contributions of net hepatic glycogenolysis and gluconeogenesis to overall glucose production in humans during a physiological increase in plasma epinephrine concentration.

Since epinephrine not only stimulates hepatic glucose production but also directly and indirectly alters pancreatic beta cell function, we infused epinephrine in combination with somatostatin to inhibit endogenous insulin and glucagon secretion and infused insulin to maintain basal peripheral insulin concentration. Using this approach, we were able to examine the effects of epinephrine per se on the relative contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in man.
METHODS

Subjects

We studied seven healthy, nonsmoking volunteers (5 males, 2 females, 22±2 years, body weight 68±4 kg, Body Mass Index: 21.7±0.7 kg/m²). None of the subjects were taking any medication or had a family history of diabetes. Informed consent was obtained from each subject after the purpose, nature, and potential risks of the study had been explained. The protocol was reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine.

Experimental Protocols

For 3 days before the study, subjects were given a weight-maintaining diet (30-40 Kcal/kg body weight, 60% carbohydrate, 20% protein, and 20% fat) prepared by the metabolic kitchen of the Yale University-Center for Clinical Investigation Hospital Research Unit (HRU). The diet was given as breakfast, lunch and dinner each meal providing 1/3 of the daily caloric intake. On the evening before the study, participants were admitted to the HRU and at 10 p.m. they were given an extra meal in liquid form (1,000 Kcal, 60% carbohydrate with 80% as sucrose, 20% protein, and 20% fat). After this meal the subject fasted overnight until the end of the study the following day.

At 6 a.m. a Teflon catheter was inserted into an antecubital vein in each arm for blood drawing and infusions and the subjects were brought to the MRRC. Liver volume was measured using Magnetic Resonance Imaging (MRI) with three-dimensional reconstruction performed in a 1.5-T magnet (Signa III, General Electric, Milwaukee, WI), as previously described (15). At 7:30 a.m. the subjects were placed in the supine position on the MRS bed (Bruker I Biospec Spectrometer, Bruker Instruments, Billerica, MA; 2.1T, 1m bore), and the receiver coil was placed on the abdomen over the liver. After an initial image to confirm the position of the coil over the liver, the 2 hour Baseline Period was begun with continuous measurements of liver glycogen concentrations (10) along with infusions of [6,6 $^2$H$_2$] glucose (primed: 5
mg/kg – continuous: 0.05 mg/kg/min), somatostatin (0.1 µg/kg/min - to inhibit endogenous insulin and
glucagon release), and insulin (24 pmol/kg/min; to replace basal peripheral insulin concentrations). At the
end of the Baseline Period blood was collected for determination of plasma $[6,6^{-2}\text{H}_2]$ glucose enrichment
(Atom Percent Enrichment APE) and concentrations of glucose, epinephrine, insulin, C-peptide,
glucagon, and lactate.

At time zero min, while the patients remained inside the MR Spectrometer, the epinephrine
infusion was started (1.2 µg/m$^2$/min) and continued for 90 min. The $^{13}$C MRS measurements of liver
glycogen concentration measurements were continued throughout the epinephrine infusion. Every 5
minutes, blood samples were drawn for determination of plasma glucose and lactate concentrations, and
every 15 minutes for determination of plasma $[6,6^{-2}\text{H}_2]$ glucose enrichment, epinephrine, insulin, C-
peptide, and glucagon concentrations.

**Analytical Procedures**

Every 5 min plasma glucose and lactate concentrations were measured using the glucose oxidase
method (YSI 2700A, Yellow Springs Instruments, Yellow Springs, CA). Plasma free fatty acid (FFA)
concentrations were measured using a microfluorimetric method (8) and plasma glycerol concentrations
were measured as previously described (7). Plasma immunoreactive insulin, C-peptide and glucagon
concentrations were measured using double antibody radio-immuno assay kits (Diagnostic Systems
Laboratories, Webster, TX). Plasma epinephrine concentrations were measured using a radio-enzymatic
assay (Amersham, Arlington Heights, IL). Plasma atom percent enrichment (APE) of $[6,6^{-2}\text{H}]$ glucose
was determined every 15 min by gas chromatography-mass spectrometry (GC-MS) of the penta-acetate
derivative of plasma glucose after deproteinization and deionization as previously described (10).
Hepatic glycogen concentrations were monitored throughout the 120 minutes of the Baseline Period and the 90 minutes of the epinephrine infusion (Epinephrine Period) using $^{13}$C MRS, as previously described (15). Briefly, a 9-cm $^{13}$C observation coil and a 12- x 14-cm coplanar butterfly $^1$H-decoupling coil placed rigidly over the lateral aspect of the abdomen of the supine subject, the position of the coil over the liver was confirmed with an image, and the C1 glycogen resonance (100.6 ppm) was quantified by integration and comparison with a glycogen solution.

**Calculations**

During the baseline period rates of glucose production were calculated for steady state conditions as the ratio of the tracer/tracee atom percent enrichment multiplied by the rates of [6,6-$^2$H$_2$] glucose infusion, corrected for the glucose infusion rate (10). During the Epinephrine Period rates of glucose production were calculated using a modification of the two-compartment model of Radziuk et al. for non-steady state conditions (13).

Rates of net hepatic glycogenolysis were calculated from the slope of the best linear fit of glycogen concentrations over time. Rates of net hepatic glycogenolysis (mmol/kg/min) were calculated by multiplication with the liver volume and division by the body weight. Rates of gluconeogenesis were calculated as the difference between rates of whole body glucose production and rates of net hepatic glycogenolysis (10, 15).

**Statistical Tests**

Data are given as means ± SE. Statistical comparisons within and between groups were performed by the use of the paired Student’s test.
RESULTS

Plasma metabolite concentrations

The time courses of plasma concentrations of glucose, and lactate are shown in Figure 1. During the 2-hour Baseline Period after start of the somatostatin infusion plasma glucose concentrations remained constant at 4.3±0.2 mmol/L and lactate at 1.5±0.2 mmol/L. At time zero min when the epinephrine infusion was begun plasma glucose concentrations immediately increased and reached a peak of 13.3±0.3 mmol/L by the end of the epinephrine infusion ($P=0.00001$ vs. Baseline) (Figure 1). After 20 minutes of epinephrine infusion plasma lactate concentrations began to increase and by the end of the epinephrine infusion reached a peak of 4.6±0.7 mmol/L ($P<0.00001$ vs. Baseline). During the epinephrine infusion plasma concentrations of FFA increased ~5-fold from 265±41 to 1093±136 μmol/L ($P<0.0002$), and plasma glycerol concentrations increased ~4-fold (Basal Period: 42±5 μmol/L vs. Epinephrine Period: 135±18 μmol/L; $P=0.02$).

Hormone concentrations

The plasma concentrations of epinephrine, insulin, C-peptide and glucagon are shown in Figure 2. There were no changes in plasma epinephrine concentrations from Fasting to the Baseline Period. In contrast once the somatostatin infusion was started ($t = -120\text{min}$), peripheral plasma concentrations of insulin decreased from 115±11 to 24±3 pmol/L ($P<0.0001$), C-peptide concentrations decreased from 293±24 to 42±8 pmol/L ($P<0.0001$), and plasma glucagon concentrations decreased from 60±3 to 29±3 ng/L ($P<0.0001$). At time zero min, when the epinephrine infusion was begun, plasma epinephrine concentrations immediately increased from 125±130 to 2058±250 pmol/L ($P<0.0001$), while peripheral plasma insulin, C-peptide and glucagon concentrations remained suppressed, despite the epinephrine-
induced hyperglycemia. The average heart rate increased from 64±2 beats/min during the Basal Period to 82±3 beats/min during the epinephrine infusion ($P<0.0001$).

### Rates of glucose production, net hepatic glycogenolysis and gluconeogenesis

**Baseline Period.** During the Baseline Period whole body glucose production was 14.4±1.0 µmol/kg/min with net hepatic glycogenolysis accounting for 36±11% (4.8±1.5 µmol/kg/min) and gluconeogenesis for 64±11% (9.6±2.0 µmol/kg/min) (Figure 3).

**Epinephrine Period.** Upon starting the epinephrine infusion whole body glucose production immediately increased and reached a maximum of 35.7±2.0 µmol/kg/min ($P=0.0002$ vs. Basal) at 60 min. During this Phase 1 of the epinephrine infusion, plasma glucose concentrations increased to a peak of 11.6±0.3 mmol/L ($P<0.001$ vs. Basal) (Figure 1). During Phase 2 of the epinephrine infusion (60 to 90 min), glucose production slowly decreased but remained 2-fold higher than during the Baseline Period (31.2±1.9 µmol/kg/min; $P=0.02$ vs. Peak and $P<0.0001$ vs. Basal) (Figure 3a).

Rates of net hepatic glycogenolysis are shown in Figure 3b. During Phase 1 of the epinephrine infusion rates of net hepatic glycogenolysis increased from 4.8±1.5 µmol/kg/min to a peak at 19.9±3.0 µmol/kg/min ($P=0.005$ vs. Basal), which could account for 58±10% of rates of glucose production.

During Phase 2 of the epinephrine infusion rates of net hepatic glycogenolysis decreased to 7.3±2.8 µmol/kg/min ($P=0.017$ vs. Peak, $P=NS$ vs. Basal) to account for 21±9% of glucose production.

During Phase 1 of the epinephrine infusion rates of gluconeogenesis remained relatively constant ($P=0.12$ vs. Basel) and could account for 42±10% of rates of glucose production. During Phase 2 of the epinephrine infusion (60-90 min) rates of gluconeogenesis increased dramatically and peaked at 23.9±2.2 µmol/kg/min ($P=0.02$ vs. Baseline) to account for 79±9% of glucose production.
DISCUSSION

The effects of a physiological increase in plasma epinephrine concentration, on rates of whole body glucose production were examined in combination with $^{13}$C MRS measurements of rates of net hepatic glycogenolysis to assess the relative contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production. Infusing epinephrine, at a rate of 1.2 µg/m²/min, increased plasma epinephrine concentrations to ~2,000 pmol/L, which is equivalent to the levels observed during exercise at 60-80% of VO$_2$max (11), and increased the average heart rate ~30% from resting. This increment in plasma epinephrine concentration increased glucose production in a biphasic manner with an initial ~2.5-fold increase during the first 60 minutes of the infusion (Phase 1), which could be accounted for by a 4-fold increase in rates of net hepatic glycogenolysis and no appreciable changes in rates of gluconeogenesis.

This was followed by a Second Phase of 30 min (60-90min), during which rates of glucose production rates decreased by ~15% compared to the peak, but still were slightly more than 2-fold higher than basal rates. During this Second Phase, net hepatic glycogenolysis decreased to ~20% of glucose production and gluconeogenesis increased ~2.5-fold from basal to account for ~80% of whole body glucose production.

Previous studies in humans have examined the effects of epinephrine on glucose production, but no study has yet quantified the individual contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production. Rizza et al., using somatostatin to prevent increases in plasma insulin and glucagon levels during the epinephrine administration, found a transient but sustained increase in glucose production, similar to our results, but did not estimate the relative contribution of hepatic glycogenolysis and gluconeogenesis to glucose production (14). Using arterial-hepatic venous difference, Sacca et al. estimated net splanchnic glucose output during an epinephrine infusion (16). Plasma epinephrine levels were not measured in that study and the authors did not control the endogenous production of insulin and glucagon with somatostatin. In response to this epinephrine infusion, plasma
glucose concentration rose slowly to a peak of ~6.7 mmol/L after 60 min and then remained stable for the last 30 minutes. At the same time, net splanchnic glucose output increased rapidly during the first 30 minutes to a peak of ~22 μmol/kg/min, and then decreased gradually to basal rates during the last 60 minutes of the epinephrine infusion. This early decrease in glucose production contrasts with the sustained increase in rate of glucose production, observed in our study, which peaks at ~36μmol/kg/min, and is most likely due to the significant compensatory rise in plasma insulin concentrations, in response to the hyperglycemia. These authors also examined the incorporation of [U-14C] alanine into 14C glucose during these studies and found that it was delayed, leading them to conclude that the initial increase in glucose production by epinephrine was due to glycogenolysis and that gluconeogenesis was activated at a later time. However they were not able to quantify the relative contributions of these processes to hepatic glucose production using this approach (16).

In the present study, the effects of epinephrine to stimulate rates of net hepatic glycogenolysis were transient but could not be accounted for by depletion of hepatic glycogen stores. After 60 min of epinephrine infusion (Phase 2), rates of net hepatic glycogenolysis declined despite liver glycogen concentrations of 248±17 mmol/L, which are similar to the levels of hepatic glycogen after an overnight fast but ~6-fold higher than after a prolonged fast of 64 hours (42±9 mmol/L) (15). This observation of an evanescent effect of a continuous hormone stimulation on rates of net hepatic glycogenolysis is similar to what has previously been observed for glucagon (6). This reduction in hepatic glucose production is likely due to the ability of hyperglycemia per se to inhibit hepatic glucose production (18), through inhibition of glycogen phosphorylase activity (9, 17, 19), as well as the intrinsic post-G coupled receptor down-regulation of signaling (4, 5). Epinephrine, at this physiologic concentration (~2,000 pmol/L), appears to be as potent as glucagon (at physiological levels of ~300 ng/L) to stimulate net hepatic glycogenolysis, in humans, as reflected by similar maximal rates of net hepatic glycogenolysis in response to epinephrine and to glucagon [19.9±3.0 μmol/kg/min and 21.7 μmol/kg/min, respectively (6)]. It is possible that the contribution of epinephrine on net hepatic glycogenolysis may represent an underestimate the effects of
epinephrine on net hepatic glycogenolysis due to portal vein hypo-glucagonemia during the epinephrine infusion.

In the Second Phase of epinephrine infusion (60-90min), when net hepatic glycogenolysis had waned, rates of gluconeogenesis increased by 150% and accounted for most of the increased glucose production (~80%). This likely reflects epinephrine’s ability to stimulate gluconeogenesis through up-regulation of transcription factors and co-factors involved in the regulation of hepatic gluconeogenesis (e.g. CREB, PGC1-α)(12), resulting in an increased expression of key gluconeogenic enzymes (phosphoenol-pyruvate carboxy kinase [PEPCK], glucose-6-phosphatase [G-6-Pase]) (1). In addition, the increase in plasma lactate concentrations, due to epinephrine-induced muscle glycogenolysis (3), and the increase in plasma glycerol concentrations were more pronounced during the second phase of epinephrine administration, and provided increased gluconeogenic substrate supply to the liver during this time period. The significant increase in plasma FFA concentrations during the epinephrine infusion is consistent with epinephrine-induced lipolysis and as previously shown in man, this acute increase in plasma FFA may also have contributed to a direct stimulation of gluconeogenesis (2). Thus, the increase in gluconeogenic substrates, combined with an increased gluconeogenic enzymes expression/activation, might explain the later increase rate of gluconeogenesis observed in this study.

In summary, our studies show that, under conditions of basal peripheral insulin and glucagon concentrations, a physiological increase in epinephrine concentration in humans induces a sustained and biphasic increase in glucose production. Using natural abundance $^{13}$C MRS, we demonstrate that during the first 60 minutes of epinephrine infusion, activation of hepatic glycogenolysis is responsible for the increase in glucose production. We also demonstrate that the activation of net hepatic glycogenolysis is only transient and that during the later phase of epinephrine infusion (60-90 min), rates of net hepatic glycogenolysis return to basal. As that point, increased rates of glucose production are sustained due to a 2.5 fold increase in gluconeogenesis.
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**FIGURE LEGENDS**

*Figure 1:* Effect of epinephrine on plasma glucose and lactate concentrations. Values are expressed as means ± sem. $ P<0.01, * P<0.001.$

*Figure 2:* Effect of epinephrine on plasma epinephrine, insulin, C-peptide and glucagon concentrations. Values are expressed as means ± sem.

*Figure 3:* a) Time course for the change in glucose production under basal conditions and during an epinephrine infusion. b) Effect of epinephrine on rates of glucose production, net hepatic glycogenolysis and gluconeogenesis. Values are expressed as means ± sem. Significance is indicated by paired student t-test result.
References


Rates of Glucose Production (µmol/kg/min)

Epinephrine Infusion

Time (minutes)

Net Hepatic Glycogenolysis
Gluconeogenesis

Basal Phase 1 (0-60min) Phase 2 (60-90min)

4.8 9.6 19.9 23.9

P<0.001 P=0.02 P=0.017 P=0.06

P=0.12

P=0.05