Glucose production during strenuous exercise in humans: role of epinephrine

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Howlett, Kirsten, Mark Febbraio, and Mark Hargreaves. Glucose production during strenuous exercise in humans: role of epinephrine. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E1130–E1135, 1999.—The increase in hepatic glucose production (HGP) that occurs during intense exercise is accompanied by a simultaneous increase in epinephrine, which suggests that epinephrine may be important in regulating HGP. To further investigate this, six trained men were studied twice. The first trial (control [Con]) consisted of 20 min of cycling at 40 ± 1% peak oxygen uptake (VO2peak) followed by 20 min at 80 ± 2% VO2peak. During the second trial (epinephrine [Epi]), subjects exercised for 40 min at 41 ± 2% VO2peak. Epinephrine was infused during the latter 20 min of exercise and resulted in plasma levels similar to those measured during intense exercise in Con. Glucose kinetics were measured using a primed, continuous infusion of [3-3H]glucose. HGP was similar at rest (Con, 11.0 ± 0.5 and Epi, 11.1 ± 0.5 µmol·kg−1·min−1). In Con, HGP increased (P < 0.05) during exercise to 41.0 ± 5.2 µmol·kg−1·min−1 at 40 min. In Epi, HGP was similar to Con during the first 20 min of exercise. Epinephrine infusion increased (P < 0.05) HGP to 24.0 ± 2.5 µmol·kg−1·min−1 at 40 min, although this was less (P < 0.05) than the value in Con. The results suggest that epinephrine can increase HGP during exercise in trained men; however, epinephrine during intense exercise cannot fully account for the rise in HGP. Other glucoregulatory factors must contribute to the increase in HGP during intense exercise.

liver; catecholamines; glucose kinetics

HEPATIC GLUCOSE PRODUCTION (HGP) during exercise is regulated by a complex interaction between both hormonal and neural mechanisms. Previous research examining the regulation of hepatic glucose output during exercise has generally focused on mechanisms that operate at moderate intensities (for review, see Ref. 5). Studies in humans have demonstrated that hepatic glucose output during moderate-intensity exercise is mediated mainly by alterations in glucagon and insulin (12, 32), although catecholamines, in particular epinephrine, are important when such alterations do not occur (22). In contrast, during high-intensity exercise, the changes in glucagon and insulin are insufficient to account for the marked increase in hepatic glucose output (6, 30). However, such exercise is associated with an augmented increase in plasma epinephrine, which has been shown to occur simultaneously with that in hepatic glucose output (4, 20, 23, 26, 28, 30).

These findings suggest, but do not establish, a causal relationship between plasma epinephrine and hepatic glucose output. Only one study has previously infused epinephrine and measured hepatic glucose output in exercising humans (18). During intense exercise, infusion of a high physiological dose of epinephrine increased hepatic glucose output; however, interpretation of the results is complicated since the subjects had undergone anesthetic blockade of the celiac ganglion, which impairs sympathetic activity to the liver, pancreas, and adrenal medulla (18). The aim of the present study was to determine whether epinephrine was responsible for the increase in hepatic glucose output during intense exercise in trained men, by infusing epinephrine during low- to moderate-intensity exercise to obtain plasma levels similar to those observed during a previous high-intensity exercise bout.

METHODS

Subjects. Six endurance-trained males (20.5 ± 0.9 yr, 71.0 ± 2.8 kg, mean ± SE) volunteered to serve as subjects for the experiment. The experimental procedures and possible risks of the study were explained to each subject verbally and in writing. All subjects gave their informed, written consent, and the experiment was approved by the Human Research Ethics Committee of The University of Melbourne.

Preexperimental protocol. All subjects performed an incremental workload test to exhaustion on an electromagnetically braked cycle ergometer (LODE Instrument, Groningen, The Netherlands) to determine their peak pulmonary oxygen uptake (VO2peak). Mean VO2peak was 4.34 ± 0.14 l/min. For the day preceding each trial, the subjects consumed a food package (~14,000 kJ, 80% carbohydrate) and abstained from strenuous exercise, tobacco, caffeine, and alcohol. In addition, they were instructed to consume 5 ml tap water/kg body wt upon waking to ensure euhydration. The subjects reported to the laboratory in the morning after a 10- to 12-h overnight fast.

Experimental protocol. Each subject performed two experimental trials, separated by at least 7 days. To determine whether epinephrine was an important regulator of hepatic glucose output during intense exercise, epinephrine was infused during low- to moderate-intensity exercise (Epi) at a rate that was estimated to elevate plasma epinephrine to a level similar to that measured during high-intensity exercise in a control trial (Con). The exercise trials were performed on the same stationary cycle ergometer used in the VO2peak determination. Subjects performed all exercise tests in a laboratory at a temperature of 20–22°C, and an electric fan circulated air to minimize thermal stress.

On arrival at the laboratory, all subjects rested quietly on a couch, and indwelling Teflon catheters were inserted in an antecubital vein of one arm for blood sampling and in the contralateral arm for infusion. The catheter for blood sampling was kept patent by flushing with 0.5 ml of 0.9% saline containing 5 units of heparin every 30 min. After a priming
dose of 40 µCi, D-[3-3H]glucose (Du Pont, Biotechnology Systems, Wilmington, DE) was infused continuously at a rate of 0.40 ± 0.01 µCi/min for the duration of the 2-h rest period and 40 min of exercise. Upon completion of the rest period, the subject moved to the cycle ergometer, and exercised for 20 min at a workload requiring 40 ± 1% VO₂peak, immediately followed by a 20-min exercise bout at 80 ± 2% VO₂peak. Venous blood samples were obtained at 5-min intervals for the last 15 min of the rest period and throughout exercise for later analysis of plasma glucose and [³H]glucose specific activity. Samples obtained immediately before the commencement of exercise, at 20, 25, and 30 min, and at the completion of exercise were analyzed for catecholamines. Additional samples were taken at 0, 20, and 40 min for analysis of plasma lactate, insulin, glucagon, cortisol, and free fatty acids (FFA). Blood for glucose, lactate, and cortisol was placed in fluoride heparin tubes, catecholamines and FFA were placed in plain tubes containing EGTA and reduced glutathione, insulin was placed in lithium heparin tubes, and glucagon was placed in lithium heparin tubes containing 200 µl of a protease inhibitor (10% Trasylol). Upon completion of exercise, the blood samples were spun, and the plasma was removed and stored at −20°C for later analysis. Plasma for catecholamine analysis was stored at −80°C. In preparation for the lactate assay, 250 µl of plasma were deproteinized in 500 µl of 8% perchloric acid and spun again, and the supernatant was removed and stored at −20°C. Expired gases were collected in Douglas bags at 10-min intervals during exercise for measurement of oxygen uptake and respiratory exchange ratio (RER). Heart rate was measured continuously via telemetry (Polar sports tester; Polar Electro Finland) and was recorded every 10 min during exercise. Subjects were permitted to drink water ad libitum during the trials.

For the Epi trial, all subjects undertook the same protocol as described above. However, the exercise protocol required the subjects to cycle for 40 min at 41 ± 2% VO₂peak. During the latter 20 min of exercise, an epinephrine solution was delivered via a three-way stopcock to allow simultaneous infusion of tracer and epinephrine. Epinephrine was infused in a stepwise manner by a peristaltic pump. Based on the plasma epinephrine concentrations measured in the Con trial and assuming a clearance of 2.4 l/min (9), an epinephrine solution (1 µg/ml) was infused at a rate that averaged 0.19 ± 0.04, 0.46 ± 0.09, and 0.76 ± 0.17 µg/min for the exercise intervals of 20–25, 25–30, and 30–40 min, respectively.

In addition, five endurance-trained males (30.8 ± 2.1 yr, 74.4 ± 2.9 kg, VO₂peak = 4.52 ± 0.20 l/min, mean ± SE) were studied during a single exercise bout. The subjects underwent an identical experimental protocol as described above except the subjects exercised for 40 min at 40 ± 2% VO₂peak without epinephrine infusion (WEI).

Analytic techniques. Oxygen and carbon dioxide contents of dried expirate were analyzed using Applied Electrochemistry S-3A/I1 and CD-3A analyzers (Ametek, Pittsburgh, PA), whereas volume was measured using a Parkinson Cowan gas meter. Plasma glucose was measured using an automated glucose oxidase method (YSI 2300; Yellow Springs, OH), and lactate was determined using an enzymatic spectrophotometric method (21). Plasma insulin (Incstar, Stillwater, MN), glucagon (2), and cortisol (Orion, Espoo, Finland) were measured by RIA. Plasma catecholamines were determined using a single-isotope, radioenzymatic method (TRK 995; Amersham, Amersham, UK). FFA were measured by an enzymatic colorimetric method (Wako NEFA C test kit; Wako Chemicals). Plasma [³H]glucose specific activity was measured as previously described (14). Rates of plasma glucose appearance (= HGP) and disappearance (glucose R₁) at rest and during exercise were calculated using a modified one-pool non-steady-state model (31) with the assumption of a pool fraction of 0.65 and estimation of the apparent glucose space as 25% of body weight. The metabolic clearance rate (MCR) of glucose was calculated by dividing glucose R₁ by the corresponding plasma glucose concentration. Data from two trials (Con and Epi) were compared by a two-way ANOVA for repeated measures. A one-way ANOVA was employed to compare differences over time for WEI. An unpaired t-test was utilized to compare the change in HGP during the final 20 min (20–40 min) of low- to moderate-intensity exercise either with (Epi) or without (WEI) epinephrine infusion. The level of significance was set at P < 0.05. Specific differences were determined using the Student-Newman-Keuls post hoc test. All data are reported as means ± SE.

RESULTS

Plasma epinephrine was not different between Con and Epi at rest and after 20 min of low- to moderate-intensity exercise. In Con, when the exercise intensity was increased, there was a marked rise (P < 0.05) in the plasma epinephrine concentration. Infusion of epinephrine during the last 20 min of exercise in Epi resulted in plasma levels that were similar to those measured during high-intensity exercise in Con (Fig. 1). There were no differences between Con and Epi for oxygen uptake, RER, and heart rate during the first 20 min of exercise. When the workload was increased during the final 20 min of exercise in Con, oxygen uptake, RER, and heart rate increased significantly and were higher (P < 0.05) than Epi. During the final 20 min of exercise, in Epi there was no change in oxygen uptake, but there was a significant increase in RER and heart rate (Table 1).

Plasma glucose was similar at rest and increased (P < 0.05) during exercise in both Con and Epi. During the final 15 min of exercise, plasma glucose was significantly higher in Con compared with Epi (Fig. 2). HGP was similar at rest between trials (Con, 11.0 ± 0.5 and
In Con, HGP increased (P, 0.05) to 17.7 ± 1.0 µmol·kg⁻¹·min⁻¹ after 20 min of low- to moderate-intensity exercise. When the exercise intensity was increased, there was a marked rise (P < 0.05) in HGP to a peak of 41.0 ± 5.2 µmol·kg⁻¹·min⁻¹ at 40 min. In Epi, HGP was not different from Con during the first 20 min of exercise (16.6 ± 1.2 µmol·kg⁻¹·min⁻¹ at 20 min). During the final 20 min of exercise, infusion of epinephrine resulted in an increase (P < 0.05) in HGP to 24.0 ± 2.5 µmol·kg⁻¹·min⁻¹ at 40 min of exercise, although this was less (P < 0.05) than Con (Fig. 2). Glucose R_d and MCR were not different between Con and Epi at rest. In Con, glucose R_d and MCR increased (P < 0.05) and were greater (P < 0.05) than Epi during the latter stages of exercise. Glucose R_d and MCR were not affected by epinephrine infusion during exercise (Fig. 3).

Plasma insulin, glucagon, and the glucagon-to-insulin molar ratio were not different between Con and Epi at rest and during exercise (Table 2). During the first 20 min of exercise, plasma norepinephrine, cortisol, and lactate were not different between trials. In Con, plasma norepinephrine, cortisol, and lactate increased (P < 0.05) during the final 20 min of exercise and were greater (P < 0.05) than Epi.

**Table 1.** Oxygen uptake, RER, and heart rate during 40 min of exercise without and with epinephrine infusion commencing at 20 min.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Oxygen uptake, l/min</th>
<th>RER</th>
<th>Heart rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.74 ± 0.08 1.74 ± 0.08 3.40 ± 0.12† 3.48 ± 0.09†</td>
<td>0.92 ± 0.02 0.89 ± 0.03 0.99 ± 0.02† 0.97 ± 0.02†</td>
<td>116 ± 3 120 ± 3 171 ± 3† 174 ± 3†</td>
</tr>
<tr>
<td>20</td>
<td>1.79 ± 0.13 1.78 ± 0.10 1.71 ± 0.04 1.75 ± 0.11</td>
<td>0.89 ± 0.01 0.88 ± 0.02 0.92 ± 0.02† 0.91 ± 0.02†</td>
<td>113 ± 3 116 ± 2 123 ± 3† 129 ± 3†</td>
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<tr>
<td>30</td>
<td>3.40 ± 0.12† 3.48 ± 0.09†</td>
<td>0.92 ± 0.02 0.89 ± 0.03 0.99 ± 0.02† 0.97 ± 0.02†</td>
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<tr>
<td>40</td>
<td>3.48 ± 0.09†</td>
<td>0.97 ± 0.02†</td>
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</table>

Values are means ± SE; n = 6 subjects. RER, respiratory exchange ratio; Con, control group; Epi, group that received epinephrine infusion. P < 0.05, significant difference from Epi (*) and 20 min (†).
epinephrine did not alter the plasma concentrations of norepinephrine, cortisol, and lactate (Table 2). Plasma FFA were significantly different between Con and Epi at rest and during exercise. In Con, plasma FFA decreased (P < 0.05) during the high-intensity exercise bout, whereas infusion of epinephrine in Epi resulted in an increase (P < 0.05) in plasma FFA levels (Table 2).

In WEI, there was no change in oxygen uptake (1.83 ± 0.11 l/min, average value), RER (0.87 ± 0.02), and heart rate (107 ± 5 beats/min) during 40 min of low- to moderate-intensity exercise. There was no significant change in plasma glucose during exercise (Table 3), and the increases in HGP, MCR, and glucose R_d were not statistically significant (Table 3). Plasma epinephrine did not rise during exercise, unlike plasma norepinephrine, which was elevated (P < 0.05) after 40 min of exercise (Table 3). Plasma levels of glucagon, insulin, FFA, and lactate did not change during exercise (Table 3). The change in HGP during the final 20 min of low- to moderate-intensity exercise in Epi (7.4 ± 2.1 µmol·kg⁻¹·min⁻¹) was greater (P < 0.05) than that in WEI (2.3 ± 0.9 µmol·kg⁻¹·min⁻¹).

### DISCUSSION

The results from the present study suggest that in trained men the marked increase in plasma epinephrine observed during exercise at 80% V̇O₂peak cannot fully account for the rise in hepatic glucose output. Other glucoregulatory factors must contribute to the increase in glucose production during intense exercise.

Previous studies have proposed that catecholamines, specifically epinephrine, are the main mediators of the increment in hepatic glucose output during intense (≥80% V̇O₂peak) exercise (20, 23, 26, 30). In contrast, the results from the present study argue against epinephrine as the major humoral mediator of the increase in hepatic glucose output. Infusion of epinephrine during low- to moderate-intensity exercise that elevated plasma epinephrine to a level similar to that measured during intense exercise in Con (Fig. 1) could only account for ~30% of the rise in hepatic glucose output (Fig. 2). It is possible that, during intense exercise, changes in the internal milieu may influence the effect of the exercise-induced rise in epinephrine on HGP such that, in the present study, the increase in the epinephrine concentration, per se, during low- to moderate-intensity exercise may have underestimated the contribution of epinephrine to glucose production during intense exercise. However, a recent study in dogs showed that infused phentolamine and propranolol in the portal vein to selectively block hepatic α- and β-adrenergceptors, respectively, were unable to attenuate the rise in hepatic glucose output during heavy (85% of maximum heart rate) exercise (7). Taken together, these findings suggest that epinephrine may not play the major role in mediating the increase in hepatic glucose output during intense exercise.

Given that the augmented increase in plasma epinephrine observed during intense exercise cannot fully account for the rise in hepatic glucose output (Fig. 2), other glucoregulatory factors must contribute to the increase in glucose production. Sympathetic neural innervation of the liver has been proposed to play an important role in the regulation of hepatic glucose output during exercise in humans (6, 13, 23, 26, 30). In contrast, HGP was not reduced during intense exercise in normal subjects when sympathetic activity to the liver was impaired by anesthetic blockade of the celiac ganglion (18) and in liver transplant patients (19). These findings suggest that sympathetic neural activity does not play a role in hepatic glucoregulation during exercise in these circumstances. However, as indicated by Sigal et al. (30), it is difficult to extrapolate the findings from liver transplant patients to normal healthy individuals. Furthermore, hepatic neural activation may be more important during exercise at

### Table 2. Plasma insulin, glucagon, glucagon-insulin molar ratio, norepinephrine, cortisol, free fatty acids, and lactate during 40 min of exercise without and with epinephrine infusion commencing at 20 min

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Con</th>
<th>Epi</th>
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<tr>
<td>20</td>
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<tr>
<td>40</td>
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**Table 3. HGP, glucose R_d, MCR, plasma hormones and metabolites during 40 min of exercise at 40 ± 2% V̇O₂peak Without epinephrine infusion**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Rest</th>
<th>20</th>
<th>40</th>
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<tbody>
<tr>
<td>HGP, µmol·kg⁻¹·min⁻¹</td>
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<tr>
<td>Glucose R_d, µmol·kg⁻¹·min⁻¹</td>
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<tr>
<td>MCR, ml·kg⁻¹·min⁻¹</td>
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<tr>
<td>Glucose, mmol/l</td>
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<td>Lactate, mmol/l</td>
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<tr>
<td>Free fatty acids, mmol/l</td>
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<tr>
<td>Epinephrine, nmol/l</td>
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<tr>
<td>Norepinephrine, nmol/l</td>
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<tr>
<td>Insulin, pmol/l</td>
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<tr>
<td>Glucagon, ng/l</td>
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Values are means ± SE; n = 5 subjects. HGP, hepatic glucose production; R_d, rate of disappearance; MCR, metabolic clearance rate; V̇O₂peak, peak oxygen uptake. *P < 0.05, significant difference from rest.
higher absolute or relative intensities (6) than at those used in these studies. Given the evidence outlined above, the role of sympathetic liver nerves in the regulation of glucose production during exercise in humans remains equivocal. In the present study, plasma norepinephrine correlated significantly with the increase in HGP in Con (r = 0.70, P < 0.05). Thus it is possible that activation of sympathetic liver nerves may account, at least in part, for the rise in hepatic glucose output during intense exercise.

In agreement with previous studies (6, 23, 26, 30), the findings from this study suggest that pancreatic hormones are unlikely to account for the rise in hepatic glucose output during intense exercise, as plasma insulin, glucagon, and the molar ratio were not different between trials (Table 2). However, it is possible that peripheral levels of insulin and glucagon may not reflect significant changes in the portal vein concentration of these hormones. A small increment in the portal vein concentration of glucagon and/or a decrement in insulin could account for the marked increase in hepatic glucose output in Con (Fig. 2).

Factors other than catecholamines and pancreatic hormones could be responsible for the increase in hepatic glucose output during intense exercise (Fig. 2). Elevated plasma cortisol levels in Con (Table 2) may contribute to the rise in hepatic glucose output. The effect of this hormone on glucose production during exercise has not been directly established. However, cortisol is likely to play only a minor role (for review, see Ref. 8), as this hormone acts by increasing gluconeogenesis, which does not contribute significantly to hepatic glucose output at high intensities. Changes in the plasma glucose concentration may also contribute to the increase in glucose output, although in the present study this is unlikely as elevated plasma glucose levels (Fig. 2) have been shown to attenuate the rise in hepatic glucose output during strenuous exercise (11, 14, 24). Hepatic blood flow was not measured, but it is possible that reduced splanchnic blood flow during high-intensity exercise could alter glucose production (17). Decreased blood flow, which is thought to reflect an increase in local vascular resistance mediated by increased sympathetic nervous activity (for review, see Ref. 29), could affect hepatic glucose output either directly or indirectly by altering the delivery of hormones and substrates to the liver. However, this remains to be determined. Furthermore, it cannot be excluded that the possibility of other, as yet unidentified, factors may contribute to the increase in hepatic glucose output observed during high-intensity exercise.

The results from the present study also suggest that, in trained men during low- to moderate-intensity exercise, infusion of epinephrine that results in plasma concentrations that were higher than those normally observed, increased hepatic glucose output (Fig. 2). It is unlikely that the rise in hepatic glucose output in Epi was simply a time-dependent increase, since in an additional group of five trained males who exercised for 40 min at 40 ± 2% VO2peak without epinephrine infusion, hepatic glucose output did not increase significantly from 20 to 40 min of exercise (Table 3). The change in HGP during the final 20 min of low- to moderate-intensity exercise with epinephrine infusion was significantly greater than that in those subjects who did not receive the epinephrine infusion (WEI). The effect of elevated plasma epinephrine levels on HGP during exercise is in accordance with previous findings (18) and is likely a result of epinephrine directly stimulating liver glycogenolysis. Epinephrine could also increase the supply of gluconeogenic precursors by stimulating muscle glycogen breakdown (1, 25, 27).

It has been suggested that an increase in the plasma epinephrine concentration could reduce muscle glucose uptake during exercise (15) as a result of direct effects on muscle glucose transport (3) and/or indirect effects on glucose metabolism via changes in muscle glycogenolysis (16) and FFA availability (10). In contrast, results from the present study suggest that epinephrine infusion does not significantly influence muscle glucose uptake and glucose clearance during exercise (Fig. 3). Similarly, muscle glucose uptake was not altered during exercise in celiac ganglion-blocked humans either with or without infusion of epinephrine (18). However, in the present study, epinephrine infusion resulted in an increase in carbohydrate oxidation (Table 1), which, in the absence of changes in glucose Rg, suggests that epinephrine may have stimulated muscle glycogen degradation.

In summary, the results from the present study suggest that, in trained men, the augmented increase in plasma epinephrine observed during intense exercise cannot fully account for the magnitude of the rise in hepatic glucose output. Other glucoregulatory factors must also contribute to the increase in glucose production during intense exercise.

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