Effect of epinephrine on muscle glycogenolysis during exercise in trained men

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Febbraio, M. A., D. L. Lambert, R. L. Starkie, J. Proietto, and M. Hargreaves. Effect of epinephrine on muscle glycogenolysis during exercise in trained men. J. Appl. Physiol. 84(2): 465–470, 1998.—To test the hypothesis that an elevation in circulating epinephrine increases intramuscular glycogen utilization, six endurance-trained men performed two 40-min cycling trials at 71 ± 2% of peak oxygen uptake in 20–22°C conditions. On the first occasion, subjects were infused with saline throughout exercise (Con). One week later, after determination of plasma epinephrine levels, subjects performed the second trial (Epi) with an epinephrine infusion, which resulted in a twofold higher plasma epinephrine concentration in Epi compared with Con. Although oxygen uptake was not different when the two trials were compared, respiratory exchange ratio was higher throughout exercise in Epi compared with Con (0.93 ± 0.01 vs. 0.89 ± 0.01; P < 0.05). Muscle glycogen concentration was not different when the trials were compared preexercise, but the postexercise value was lower (P < 0.01) in Epi compared with Con. Thus net muscle glycogen utilization was greater during exercise with epinephrine infusion (224 ± 37 vs. 303 ± 30 mmol/kg for Con and Epi, respectively; P < 0.01). In addition, both muscle and plasma lactate and plasma glucose concentrations were higher (P < 0.05) in Epi compared with Con. These data indicate that intramuscular glycogen utilization, glycolysis, and carbohydrate oxidation are augmented by elevated epinephrine during submaximal exercise in trained men.

catecholamines; carbohydrate metabolism; training

It is well established that endogenous epinephrine secretion is increased by exercise alone (13), but release of this hormone during exercise may be augmented by many factors, including hypoxia (23), caffeine ingestion (14), heat stress (9, 16), and dehydration (17). It has been suggested that an increase in endogenous epinephrine secretion results in a concomitant increase in intramuscular glycogen utilization (13) because glycogen phosphorylase activity is enhanced by β-adrenergic stimulation (34). We have demonstrated on many occasions that intramuscular glycogen utilization closely matched the plasma epinephrine response during exercise and hyperthermia in endurance-trained men (9, 11, 17). These previous studies do not, however, establish a causal link between epinephrine and muscle glycogen utilization. The effect of epinephrine on intramuscular carbohydrate metabolism is a well-studied, but nonetheless complex, phenomenon. Studies conducted in which animals were used have demonstrated that epinephrine infusion increases glycogen utilization, during either voluntary submaximal exercise or electrical stimulation, in both rats (34, 35) and dogs (21). In addition, removal of the adrenal medulla (19, 33) or β-adrenergic blockade (20) reduces glycogen use in these animals. Studies in humans, however, that have manipulated plasma epinephrine concentration via exogenous infusion have produced conflicting results. Those that have demonstrated that epinephrine infusion enhances muscle glycogenolysis have infused doses that are high and often “pharmacological” (22, 39). In contrast, two studies have recently demonstrated that glycogenolysis is not increased during intense dynamic (6) or prolonged (44) exercise when epinephrine is increased to physiological concentrations. It has been suggested that the effect of epinephrine on glycogen phosphorylase is diminished by cellular regulatory mechanisms such as Ca2+ release, substrate availability, and posttransformation allosteric modulators such as free AMP and free IMP (6, 32, 44). We have, however, observed an increase in glycogen utilization during exercise and heat stress in trained men in circumstances in which there has been little, if any, disruption to the intracellular milieu (9, 10), suggesting, therefore, that epinephrine may play a role in the regulation of carbohydrate metabolism during prolonged exercise.

The purpose of the present study, therefore, was to infuse epinephrine during exercise to levels similar to those that we have observed in subjects during exercise in the heat (9, 16) and examine intramuscular carbohydrate metabolism. We hypothesized that such an infusion would augment intramuscular glycogen utilization.

METHODS

Subjects. Six endurance-trained triathletes [age 24.2 ± 1.9 (SE) yr; weight 75.2 ± 2.3 kg] took part in this study after being informed of all the risks and stresses and giving their informed consent. The study was approved by The University of Melbourne Human Research Ethics Committee. Peak oxygen uptake (V̇O2peak) was measured during incremental cycling to fatigue by using an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) at 20–22°C, and the value averaged 4.81 ± 0.16 l/min.

Experimental procedures. At least 7 days after V̇O2peak determination, subjects reported to the laboratory in the morning after an overnight fast, after having abstained from alcohol, caffeine, and strenuous exercise for 24 h. On arrival, they voided and were weighed, and a rectal thermometer (Monatherm Mallinkrodt Medical, St. Louis, MO) was positioned 10–15 cm beyond the anal sphincter. Catheters (20 gauge; Terumo, Tokyo, Japan) were then inserted into an antecubital vein of each arm for saline infusion and blood
sampling. After an initial blood sample was drawn, a muscle sample was obtained from the vastus lateralis by using the percutaneous needle-biopsy technique modified to include suction and was quickly frozen in liquid nitrogen. Muscle temperature was measured immediately after the biopsy by using a needle thermistor (YSI 525, Yellow Springs Instruments, Yellow Springs, OH) inserted to a depth of 4 cm through the biopsy incision. Subjects then moved to the cycle ergometer where a heart rate monitor (Sports Tester, Polar, Finland) was positioned. Immediately before exercise, the infusion of saline commenced and was maintained throughout exercise at a rate of 1 ml/min (Con). Subjects cycled for 40 min at a workload corresponding to 71% of VO$_2$peak in the laboratory, which was maintained at 20–22°C. Blood samples (5 ml) were obtained at 10-min intervals during exercise and together with the resting sample were stored for analyses of plasma epinephrine, norepinephrine, glucose, lactate, and free fatty acids (FFA). After being drawn, ~3-ml samples of blood were placed in a tube containing fluoride heparin and were spun. Some plasma was decanted and stored for glucose determination. A further 250 µl of plasma were placed in a tube containing 3 M perchloric acid and spun, and the supernatant stored at −20°C for lactate determination. A 1.5-ml sample of whole blood was placed in tubes containing 30 µl of a preservative consisting of ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and reduced glutathione and was spun. The plasma was stored at −80°C until analysis for epinephrine, norepinephrine, and FFA. Expired gas samples were collected into Douglas bags at 5, 15, 25, and 35 min during exercise for measurement of oxygen uptake (VO$_2$) and respiratory exchange ratio (RER). Immediately on cessation of exercise, a second muscle sample was obtained from a separate incision in the same leg and was quickly frozen in liquid nitrogen. The time between cessation of exercise and freezing was <20 s. This sample and the one obtained at rest were analyzed for glycogen and lactate. Muscle temperature was measured immediately after the biopsy. Rectal temperature was monitored at 10-min intervals throughout exercise.

After this trial, plasma epinephrine was determined, and 7 days later subjects performed a second trial (Epi) that was identical to the first except that the saline infusion contained epinephrine. The infusion was calculated to obtain a difference in plasma epinephrine similar to those we had previously observed when exercise at 40°C was compared with that at 20°C (9, 16). Briefly, a calculated amount of 1 µg/ml stock solution of epinephrine (adrenaline chloride injection, Parke-Davis) was added to 500 ml of sterile saline and infused at the same rate as in Con. The average epinephrine infusion rate was 0.009 ± 0.002 µg·kg$^{-1}$·min$^{-1}$.

Analytic techniques. Expired gases were analyzed for oxygen and carbon dioxide with Applied Electrochemistry S-3AII and CD-3A analyzers, respectively (Ametek, Pittsburgh, PA), which were calibrated with commercial gases of known composition. Volumes were measured by using a gas meter (Parkinson-Cowan, Manchester, UK) calibrated against a Tissot spirometer. Plasma glucose was analyzed by using an automated glucose oxidase method (YSI 23AM, Yellow Springs Instruments, OH), and plasma lactate was determined by using an enzymatic spectrophotometric analysis (25). Plasma epinephrine and norepinephrine were analyzed using a single-isotope ($^3$H) radioenzymatic assay system (TRK 995, Amer sham). Plasma FFA were analyzed by using an enzymatic colorimetric method (NEFA C code 279-75409, Wako, Tokyo, Japan). Muscle samples were weighed and subsequently freeze-dried. After being freeze-dried, the samples were reweighed, dissected free of any blood and connective tissue, powdered, and placed into two separate aliquots. One was extracted according to the procedure of Harris et al. (18) and was analyzed for lactate by using standard enzymatic, fluorometric techniques (25). Muscle glycogen concentrations were determined on the second freeze-dried aliquot, which was extracted, neutralized, and analyzed according to the procedure of Passonneau and Lauderdale (29).

Statistical analyses. The data from the two trials were compared by using two-factor (time and treatment) analysis of variance (ANOVA) with repeated measures. Simple main-effects analyses and Newman-Keuls post hoc tests were used to locate differences when ANOVA revealed a significant interaction. Where appropriate, paired comparisons were made by t-test. A biomedical data-processing computer software program was used to compute these statistics. All comparative data are reported as means ± SE.

RESULTS

On average, plasma epinephrine was twofold higher (P < 0.01) in Epi compared with Con. The difference in plasma epinephrine when Epi is compared with Con was similar to that observed when 40°C is compared with 20°C in our previous studies (9, 16) at both 10 min (0.88 ± 0.15 vs. 0.70 ± 0.23 nmol/l; P > 0.05) and 40 min (1.00 ± 0.20 vs. 1.31 ± 0.22 nmol/l; P > 0.05) (Fig. 1). Muscle and rectal temperatures were not different either before or after exercise, and VO$_2$ was not different throughout exercise in Epi compared with Con (Table 1). In contrast, RER (Fig. 2) and heart rate (Table 1) were higher (P < 0.05) throughout exercise in Epi compared with Con. Plasma glucose concentrations tended to be higher throughout exercise in Epi compared with Con, with the difference being significant (P < 0.05) at 10 min, and plasma lactate was higher (P < 0.05) at 20 and 40 min during exercise in Epi (Table 2). No differences were observed in plasma FFA or norepinephrine in Epi compared with Con (Table 2). The dry weight-to-wet weight ratio averaged 0.245 ± 0.005 and was not affected (P > 0.05) by either treatment or exercise. Muscle glycogen concentration was not different when the trials were compared at rest, but the postexercise value was lower (P < 0.01) in Epi compared with Con (Fig. 3). Thus net muscle glycogen utilization was greater during exercise with epinephrine infusion (224 ± 37 vs. 303 ± 30 mmol/kg for Con and Epi, respectively; P < 0.01). In addition, muscle lactate was higher (P < 0.05) in Epi compared with Con (Fig. 3).

DISCUSSION

The data from the present study demonstrate that net intramuscular glycogen use, glycolysis, and carbohydrate oxidation are augmented by a twofold increase in circulating plasma epinephrine. In addition, because the magnitude of the increase in epinephrine in this study was similar to that observed in our previous studies that have examined carbohydrate utilization during exercise and heat stress (9, 16), these data suggest a role for epinephrine as a mechanism for increased glycogenolysis during exercise in the heat.

An increase in epinephrine concentration has been demonstrated to augment contracting skeletal muscle
glycogenolysis in both animals (20, 21, 34, 35) and humans (15, 22, 39). In addition, glycogen utilization has also been observed in noncontracting skeletal muscle with epinephrine infusion (5, 12, 27, 45). In contrast, two recent studies (6, 44) have demonstrated that epinephrine infusion had no effect on the rate of muscle glycogenolysis during voluntary exercise. In these studies (6, 44) the epinephrine was infused at physiological concentrations, leading the authors to speculate that it plays a minor role in glycogenolytic processes during exercise. The data from the present study clearly demonstrate that, in trained humans, infusion of epinephrine to mimic physiological concentrations increases net muscle glycogen use, as well as muscle lactate accumulation and carbohydrate oxidation. The differences between the present and previous studies are likely to be related to a number of significant methodological differences. In the study by Chesley et al. (6), exercise was conducted at 85% maximal oxygen uptake ($\dot{V}O_2$max), which was likely to have fully activated glycogenolysis irrespective of epinephrine concentration via local factors such as Ca$^{2+}$ release from the sarcoplasmic reticulum and posttransformational factors such as Pi, free ADP, and free AMP concentrations (32). Accordingly, calculated concentrations of Pi, free ADP, and free AMP were markedly elevated and not different when the two trials in this previous experiment are compared (6). Although the exercise intensity used in the study by Wendling et al. (44) was similar to that of the present study, those authors hypothesized that the effect of the elevated epinephrine was rendered unimportant because of the marked changes in local, regulatory factors. We have, however, demonstrated that, during 40 min of exercise at 70% $\dot{V}O_2$max in endurance-trained men in 40°C conditions, net muscle glycogen use and circulating epinephrine were elevated, relative to 20°C conditions, without any difference in muscle energy metabolism (9, 10). Indeed, in our previous study (9), such exercise had little effect on the intracellular milieu because there was no exercise-induced change in IMP, the total adenine nucleotide pool, or the calculated energy charge potential, irrespective of the environmental temperature. Hence, the data from this present study, along with our previous findings (9, 10), suggest that epinephrine is a major regulator of muscle glycogenolysis during exercise in trained humans.

Table 1. Physiological responses during 40 min of exercise at 70% $\dot{V}O_2$peak with or without epinephrine infusion

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Epi</th>
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<tbody>
<tr>
<td>Oxygen uptake, l/min</td>
<td>3.42 ± 0.16</td>
<td>3.38 ± 0.10</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>161 ± 2</td>
<td>167 ± 3*</td>
</tr>
<tr>
<td>Rectal temperature, ºC</td>
<td>37.2 ± 0.2</td>
<td>37.1 ± 0.2</td>
</tr>
<tr>
<td>Muscle temperature, ºC</td>
<td>39.8 ± 0.2</td>
<td>39.9 ± 0.2</td>
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Fig. 1. Plasma epinephrine concentration of subjects during 40 min of cycling exercise at 70% peak oxygen uptake at 40 and 20°C from previous studies (data taken from Refs. 9 and 16; A) and with (Epi) or without (Con) epinephrine infusion (B). Values are means ± SE; n = 20 men for 40 vs. 20°C and 6 men for Epi vs. Con. *Significant difference, P < 0.05.

Fig. 2. Respiratory exchange ratio (RER) during 40 min of cycling exercise at 70% peak oxygen uptake with (Epi) or without (Con) epinephrine infusion. Values are means ± SE; n = 6 men. *Main effect for treatment, P < 0.05.
with our previous findings (9–11, 16, 17), suggest a role for epinephrine in the control of intramuscular glycogenolysis in circumstances in which the energy turnover is relatively low. In contrast, in circumstances in which exercise results in marked changes in local metabolic factors, epinephrine may not affect fuel selection. In addition, exercise duration may play an important role when previous results (44) are compared with those from the present study. During the study by Wendling et al. (44), muscle biopsies were taken before and after 90 min of exercise compared with 40 min in the present study, and this difference may be important. The role of epinephrine in glycogenolytic processes diminishes over time (1), probably because the conversion of phospho-
ylase from the inactive \( b \) to the active \( a \) form occurs early during contractions before there is a progressive reversion back to phospho-
ylase \( b \) (31). Indeed, in the present study, the magnitude of difference in RER was greatest at the initial measurement point and then progressively diminished (Fig. 2). Finally, in the present study, and in our previous studies (9–11, 16, 17), subjects were fasted overnight. In contrast, in the previous studies in which epinephrine was infused into humans to mimic physiological concentrations (6, 44), subjects were fed on the morning of the experiments. Although speculative, this may have predisposed the subjects in the present study to use lipid in Con and, therefore, made it more likely to switch to greater carbohydrate utilization when epinephrine was elevated.

The observed twofold increase in muscle lactate concentration (Fig. 3) suggests that production of lactic acid is increased during exercise with epinephrine infusion. Epi infusion has been demonstrated to increase lactic acid production in both contracting and noncontracting dog muscle in situ (41, 42). Furthermore, blood lactate accumulation increases during exercise in humans with epinephrine infusion (43, 44). We did not measure lactate flux across the contracting muscle, and, therefore, we cannot totally discount the possibility that the higher postexercise muscle lactate concentration in Epi was due to increased uptake of circulating lactate late in exercise, because lactic acid output is marked early in exercise and is transient in

Table 2. Plasma lactate, glucose, free fatty acids, and norepinephrine concentrations during 40 min of exercise at 70% \( \dot{V}_\text{O}_{2\text{peak}} \) with or without epinephrine infusion

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>10 Min</th>
<th>20 Min</th>
<th>30 Min</th>
<th>40 Min</th>
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</thead>
<tbody>
<tr>
<td><strong>Lactate, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.0</td>
<td>3.6</td>
<td>3.5</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Epi</td>
<td>1.2</td>
<td>4.9</td>
<td>4.8*</td>
<td>4.2</td>
<td>4.6*</td>
</tr>
<tr>
<td><strong>Glucose, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>5.0</td>
<td>5.1</td>
<td>5.4</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Epi</td>
<td>4.9</td>
<td>5.5*</td>
<td>5.9</td>
<td>6.1</td>
<td>6.2</td>
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<tr>
<td><strong>FFA, mmol/l</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>0.25</td>
<td>0.24</td>
<td>0.25</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>Epi</td>
<td>0.19</td>
<td>0.20</td>
<td>0.26</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Norepinephrine, nmol/l</strong></td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>1.92</td>
<td>7.18</td>
<td>9.50</td>
<td>11.31</td>
<td>10.89</td>
</tr>
<tr>
<td>Epi</td>
<td>1.24</td>
<td>6.49</td>
<td>8.19</td>
<td>9.06</td>
<td>9.19</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 men. FFA, free fatty acids. *Difference compared with Con, \( P < 0.05 \).
nature (40). This explanation is unlikely, however, because net muscle lactate release and muscle glycogenolysis is maintained throughout 60 min of submaximal knee-extension exercise (36). It is likely, therefore, that the augmented postexercise muscle lactate concentration in Epi was due to an increase in glycolysis within the contracting muscle. Although it has been suggested that an increase in glycolysis may result from an increased uptake and subsequent oxidation of blood-borne glucose (44), this is unlikely because glucose uptake in untrained men is either decreased at rest (12) or unaffected during exercise (24) by epinephrine infusion.

Apart from an increase in glycolysis and glycolysis, the higher RER in Epi compared with Con, in the presence of a similar VO₂, suggests an increased rate of carbohydrate oxidation, supporting previous observations (43). The mechanism by which epinephrine may increase carbohydrate oxidation is unclear, but may be related to activation of the pyruvate dehydrogenase (PDH) complex (PDHC). The activity of PDHC is dependent on the balance between the activation of PDH phosphatase and inhibition of PDH kinase. PDH phosphatase is activated by increased Ca²⁺ concentration, whereas PDH kinase is inhibited by increased pyruvate and ADP concentrations (30). Although pyruvate was not measured directly in the present study, the increased glycogen utilization, lactate accumulation, and carbohydrate oxidation suggest an increased formation of pyruvate, which was metabolized both aerobically and anaerobically. The higher RER in Epi compared with Con reflects a substrate shift toward reduced lipid catabolism, which may be a consequence of increased carbohydrate utilization and inhibition of mitochondrial FFA oxidation (38). Because epinephrine is a potent stimulus for lipolysis (26), but the reliance on lipid by the contracting skeletal muscle was reduced during Epi, it was possible that plasma FFA concentration would be elevated in Epi. This was not the case. However, the relationship between lipolysis and epinephrine during exercise is complex because epinephrine results in an α-receptor-mediated reduction in adipose tissue blood flow during exercise (3), whereas elevated circulating lactate (2) and glucose (4) inhibit lipolysis during exercise. We did not measure plasma FFA kinetics in the present study.

The magnitude of the difference in epinephrine concentration between Epi and Con was similar to our previous studies that have compared exercise at 40°C with that at 20°C (9, 16). Although muscle glycogen utilization during exercise and heat stress is influenced by other factors, such as muscle temperature (7, 8), the observation of similar core and muscle temperatures when Epi is compared with Con (Table 1) suggests that the elevated epinephrine also influences glycogenolytic processes during exercise and heat stress (9, 10, 17). During this study we were not able to measure leg arterial epinephrine concentrations. Because heat stress causes cardiovascular adaptations during exercise, we are not certain that the epinephrine directly perfusing the contracting limbs was the same concentration in this study compared with our previous experiments (9, 16). It is likely, however, that the delivery of epinephrine to the contracting muscles during this and our previous experiments was similar because blood flow to contracting limbs during exercise in the heat is not reduced in humans (28, 37).

In summary, our data demonstrate that a physiological elevation in plasma epinephrine during submaximal exercise in trained subjects increases intramuscular glycogen utilization, lactate accumulation, and carbohydrate oxidation. The data also suggest that the increase in muscle carbohydrate utilization that occurs during exercise in the heat is mediated, in part, by increased circulating epinephrine levels.

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