Effect of epinephrine on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans

DIDIER LAURENT,1 KITT FALK PETERSEN,1 RAYMOND R. RUSSELL,1 GARY W. CLINE,1 AND GERALD I. SHULMAN2

1Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520; and 2Howard Hughes Medical Institute, Bethesda, Maryland 20814

Laurent, Didier, Kitt Falk Petersen, Raymond R. Russell, Gary W. Cline, and Gerald I. Shulman. Effect of epinephrine on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans. Am. J. Physiol. 374 (Endocrinol. Metab. 37): E130–E138, 1998.—To examine the effects of a physiological increase in plasma epinephrine concentration (−800 pg/ml) on muscle glycogenolysis and insulin-stimulated glycogenesis, we infused epinephrine (1.2 µg·m−2·body surface−1·min−1) for 2 h and monitored muscle glycogen and glucose 6-phosphate (G-6-P) concentrations with 13C/31P nuclear magnetic resonance (NMR) spectroscopy. Epinephrine caused an increase in plasma glucose (Δ−50 mg/dl), lactate (Δ−1.4 mM), free fatty acids (Δ−1.200 µM at peak), and whole body glucose oxidation (Δ−0.85 mg·kg−1·min−1) compared with levels in a group of control subjects (n = 4) in the presence of slight hyperinsulinemia (−13 µU/ml, n = 8) or basal insulin (−7 µU/ml, n = 7). However, epinephrine did not induce any detectable changes in glycogen or G-6-P concentrations, whereas muscle inorganic phosphate (P) decreased by 35%. Epinephrine infusion during a euglycemic-hyperinsulinemic clamp (n = 8) caused a 45% decrease in the glucose infusion rate that could be mostly attributed to a 73% decrease in muscle glycogen synthesis rate. After an initial increase to ~160% of basal values, G-6-P levels decreased by ~30% with initiation of the epinephrine infusion. We conclude that a physiological increase in plasma epinephrine concentration 1) has a negligible effect on muscle glycogenolysis at rest, 2) decreases muscle P, which may maintain phosphorylase activity at a low level, and 3) causes a major impairment in insulin-stimulated muscle glycogen synthesis, possibly due to inhibition of glucose transport-phosphorylation activity.

nuclear magnetic resonance spectroscopy; glucose oxidation; lipid oxidation; energy expenditure

IN THEIR PIONEERING STUDIES, Cori and Cori (11) demonstrated that epinephrine injection into rats caused muscle glycogenolysis and formation of lactate, which entered the circulation and was then deposited as liver glycogen, thus establishing the cycle that bears their name. This occurs through epinephrine-induced transformation of the inactive muscle phosphorylase b to the active form a by a sequence of reactions mediated by an increase in intracellular adenosine 3',5'-cyclic monophosphate or Ca2+ (16, 39). However, this study and most subsequent studies used pharmacological doses of epinephrine (0.1–0.5 mg/kg) to examine its effects on muscle glycogen metabolism in vivo (21, 27). Therefore the effect of a more physiological increase (~800 pg/ml) in plasma epinephrine concentration on muscle glycogen metabolism, which occurs under most typical stress conditions such as hypoglycemia or strenuous exercise, remains to be determined. Additionally, the in vivo effects of epinephrine on muscle glycogenolysis, independent of changes in plasma insulin concentrations, have not been conclusively documented. Epinephrine is known to inhibit insulin secretion (28) but will also cause hyperglycemia, which can secondarily increase insulin secretion (15).

Finally, it is well known that an increase in plasma epinephrine concentration causes insulin resistance (13, 37), although the mechanism for this effect remains unclear. Muscle biopsy studies have provided evidence that epinephrine inhibits glycogen synthase activity (31). However, the biopsy procedure has many limitations, including poor time resolution due to the small number of tissue samples typically obtained and stimulation of endogenous catecholamine release due to pain. Furthermore, the measurement of enzyme activity by itself does not necessarily reflect in vivo substrate flux. Thus the postulated mechanism that epinephrine causes diminution of insulin-mediated glucose disposal by direct inhibition of muscle glycogen synthesis remains to be determined.

In this study, we examined the effects of a physiological increase (~600–1,000 pg/ml) in the plasma epinephrine concentration on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans. This was accomplished by using 13C/31P nuclear magnetic resonance (NMR) spectroscopy to simultaneously monitor changes in muscle glycogen and glucose 6-phosphate (G-6-P) concentrations noninvasively (29, 36).

METHODS

Subjects

Twenty-six healthy nonsmoking volunteers (10 males, 16 females, mean age 26 ± 1 yr, mean body wt 66 ± 2 kg, mean body mass index 22 ± 1 kg/m2) without a family history of diabetes mellitus, hypertension, or any major diseases were studied, and none of the subjects was taking medications. Subjects were instructed not to participate in strenuous physical activity for 3 days before the study. Experimental procedures were carried out in compliance with the guidelines of the Human Investigation Committee of Yale University School of Medicine. Each subject gave informed consent after the purpose, nature, and potential risks of the study were explained.

Experimental Protocols

For 3 days before the study, a weight-maintaining diet of 30–40 kcal/kg body wt, consisting of 60% carbohydrate, 20% protein, and 20% fat, prepared by the metabolic kitchen of the General Clinical Research Center (GCRC) was given. On the evening before the study, participants were admitted to the GCRC/New Haven Hospital and fasted overnight (10–12 h) until the beginning of the study at 8 AM. A Teflon catheter
was inserted into an antecubital vein in each arm for blood drawing and infusions. For each protocol, epinephrine (Parke-Davis, Detroit, MI) was infused at a rate of 1.2 µg·m⁻²·body surface⁻¹·min⁻¹. The epinephrine infusate was freshly prepared in sterile saline containing ascorbic acid (1 mg/ml) to protect against oxidation. Infusions were started after 40 min of ¹³C/³¹P NMR spectroscopy for determination of basal metabolite concentrations. During the infusion period, the time course for the change in muscle glycogen concentration and utilization of high-energy phosphate compounds was examined by NMR spectroscopy in 11- and 22-min blocks, respectively. Blood samples were obtained at 5- to 30-min intervals until completion of the study.

Effect of Epinephrine on Muscle Glycogen Breakdown

The effects of epinephrine on muscle glycogenolysis were assessed over a 2-h infusion period. In the first set of experiments, epinephrine (epinephrine, 8 subj) or 0.9% saline (control, 4 subj) was administered. Physiological elevations in the plasma epinephrine concentration usually produces a rapid rise in hepatic glucose output (14). Because a slight increase in plasma insulin concentration might in turn affect the rate of muscle glycogenolysis, a second set of experiments (epinephrine-insulin-somatostatin) was performed on seven subjects to examine the effects of a physiological increase in plasma epinephrine per se on muscle glycogen breakdown. To inhibit endogenous insulin secretion, an infusion of somatostatin (Bachem, Torrance, CA; 0.1 µg·kg body wt⁻¹·min⁻¹) was initiated 5 min before the start of the epinephrine infusion. An infusion of insulin (Humulin, Eli Lilly, Indianapolis, IN) was started at the same time as the epinephrine infusion at a rate of 0.6 pmol·kg body wt⁻¹·min⁻¹ to maintain basal circulating concentrations of insulin.

Effect of Epinephrine on Muscle Glycogen Synthesis

In this series of experiments (8 subj), euglycemia-hyperinsulinemia was achieved with the glucose-insulin-clamp technique (12). At time 0, insulin was administered as a prime and continuous infusion of 6 pmol·kg⁻¹·min⁻¹ to raise plasma insulin concentration to ~450 mU·ml⁻¹ and maintain that concentration for the duration of the study. At the same time, a variable infusion of glucose was begun to maintain plasma glucose levels at ~5.5 mM for 240 min. Throughout the study, the plasma glucose concentration was measured every 5 min, and the infusion rate of a [¹³C₅]-glucose solution (1.11 M, 40% ¹³C enrichment) was periodically adjusted to maintain euglycemia. After 2 h, an infusion of epinephrine (1.2 µg·m⁻²·body surface⁻¹·min⁻¹) was started and maintained for 2 h.

Indirect Calorimetry

Continuous indirect calorimetry was performed to determine rates of total body glucose and lipid oxidation at baseline and at 100–120 and 220–240 min into the study as previously described (38). Power deposition, assessed by magnetic vector potential specific absorption rate calculation, was ~4 W/kg. The total scan time for each interleaved spectrum was 5.5 min.

Analytical Procedures

Plasma glucose was measured every 5 min by the glucose oxidase method (Beckman glucose analyzer, Fullerton, CA). Plasma immunoreactive insulin, glucagon, and C peptide were measured every 15–30 min using commercially available double-antibody radioimmunoassay kits (insulin: Diagnostic Systems Laboratories, Webster, TX; glucagon: Linco Research, St. Charles, MO; C peptide: Diagnostic Products, Los Angeles, CA). Plasma lactate concentrations were measured by the lactate dehydrogenase method. Catecholamines were determined every 30–60 min using a ³H radioenzymatic assay (Amersham, Buckinghamshire, UK). Plasma free fatty acid was measured every 30–60 min by use of a microfluorometric assay. Plasma amino acid concentrations were determined with an automated amino acid analyzer (Dionex, Sunnyvale, CA). During the clamp study, the ¹³C enrichment of plasma glucose was determined every 15 min by gas chromatography-mass spectrometry of the pentaacetate derivatives of plasma glucose after deproteinization and deionization as previously described (43).

In Vivo NMR Spectroscopy

Interleaved natural-abundance ¹³C/³¹P NMR spectroscopy was performed at 4.7 T on a Bruker Biospec spectrometer (Billerica, MA) with a 30-cm-diameter magnet bore as previously described (29). During the measurements, subjects remained supine with the right leg positioned within the homogenous volume of the magnet and with the lower portion of that leg resting on the stage of a radio frequency (RF) probe. The spectrometer was equipped with a modified RF relay that allowed the hardware to switch the RF power between the ¹³C (50.4 MHz) and ³¹P (81.1 MHz) channels with a 10-µs switching time. A 5.1-cm-diameter circular ¹³C/³¹P double-tuned surface coil RF probe was used for interleaved acquisitions. The double-tuned circuit was optimized for the ³¹P channel so that the NMR sensitivity was enhanced to detect G-6-P. Shimming, imaging, and ¹H decoupling at 200.4 MHz were performed with a 9 × 9 cm series butterfly coil. Proton water-line widths were shimmed to ~50 Hz. A microsphere containing ¹³C and ³¹P reference standards was fixed at the center of the double-tuned RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that used a longitudinal relaxation time (T₁)-weighted gradient-echo image (repetition time (Tₑ) = 82 ms, echo time (Tₑ) = 21 ms). The subject’s lower leg was typically positioned so that the isocenter of the magnetic field was ~1 cm into the medial head of the gastrocnemius muscle. By determination of the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This maximized suppression of the lipid signal that arises from the subcutaneous fat layer and optimized signal from the muscle.

The interleaved ¹H-decoupled ¹³C/³¹P RF pulse sequence was designed so that 72 ³¹P transients were acquired during the same period that 2,736 ¹³C were obtained (38 ¹³C scans/³¹P relaxation period), and free induction decays were saved separately in two blocks. The repetition time for ³¹P acquisition was 4.6 s to allow for the long T₁ of ³¹P resonances. Because the acquisition times of both channels had to be identical due to a spectrometer limitation, the optimized acquisition time was 87 ms. ¹H continuous-wave decoupling could not be turned on during the entire acquisition time because ³¹P power deposition would have been excessive. Power deposition, assessed by magnetic vector potential specific absorption rate calculation, was <4 W/kg. The total scan time for each interleaved spectrum was 5.5 min.

Intramuscular glycogen concentrations were determined by comparison with an external standard solution (150 mM glycogen + 50 mM KCl) in a cast of a leg that electrically loaded the RF coil the same as the subject’s leg (29, 35). ¹³C spectra were processed by methods that have previously been described (29, 35). Briefly, Gaussian broadened spectra (30 Hz) were baseline corrected ±500 Hz on either side of the [¹⁻¹³C]glycogen resonance of both subject spectra and standard spectra. Peak areas were then assessed ±200 Hz about the resonance. The ¹³C NMR technique for assessing intramus-
cular glycogen concentrations has been validated in situ in frozen rabbit muscle (22) and by comparison with human gastrocnemius muscle biopsies (41).

Concentrations of phosphorylated compounds were calculated from $^{31}$P NMR spectra as described previously (36). The area of the β-ATP resonance peak was used as an internal concentration standard, assuming a constant concentration of 5.5 mM for resting muscle (24). Chemical shifts are referenced to phosphocreatine (PCr) at 0.00 ppm. The resonance for G-6-P is in a region of the $^{31}$P NMR spectrum in close proximity to other phosphomonoester resonances. Any potential contribution from other phosphomonoester resonances, which have chemical shifts upfield (lower ppm) to G-6-P, was minimized by integrating over the chemical shift range of the downfield half of the G-6-P resonance (7.43–7.13 ppm) and multiplying by two, as described previously (35, 36). The G-6-P measurement has been validated in an animal model (3).

Calculations

During the insulin-glucose-clamp study, increments in muscle glycogen concentration ($\Delta$[Gly]) during each 11-min intervals were calculated from the increment in C1 glycogen peak intensity ($\Delta$[Gly]) divided by the intensity of the basal glycogen peak ([Gly]₀). This ratio was multiplied by the basal glycogen concentration ([Gly]₀), the natural $^{13}$C enrichment (1.1%), and divided by the plasma [1-13C]glucose enrichment (atom% excess, APE), measured during the specific 11-min interval as previously described (39)

$$\Delta$[Gly] = $\frac{\Delta$[Gly]}{[Gly]₀} \times \frac{[Gly]₀ \times 1.1}{(APE) + 1.1}$

Rates of glycogen synthesis were then calculated from the slope of the least-squares linear fit to the glycogen concentration curve during the 2nd h of both periods with and without epinephrine infusion.

Hepatic glucose production (HGP) was estimated at the end of both periods (insulin period: 100–120 min; insulin + epinephrine period: 220–240 min) of the clamp experiment from the glucose infusion rate (GIR) and the [1-13C]glucose enrichment in the plasma (APEₚ) and the infusate (APEᵢᵣ) as follows

$$HGP = GIR \times \left( \frac{APEᵢᵣ}{APEₚ} - 1 \right)$$

Whole body glucose uptake was then calculated by adding hepatic glucose production to the mean rate of glucose infusion during the same periods.

All values are expressed as means ± SE. Statistical comparisons between groups were performed by use of the paired Student’s t-test and analysis of variance (ANOVA) for repeated measurements when appropriate.

RESULTS

Effects of Epinephrine on Muscle Glycogen Breakdown

Plasma catecholamines, glucose, insulin, glucagon, and C peptide. Plasma epinephrine concentrations were maintained at ~600 and ~1,100 pg/ml in epinephrine- and epinephrine-insulin-somatostatin-treated subjects, respectively (Fig. 1A). Plasma norepinephrine concentrations remained essentially the same throughout each experiment (~320 pg/ml in all groups). During
infusion of epinephrine alone, plasma glucose concentrations quickly rose and within 1 h reached a plateau value of \( \sim 140 \text{ mg/dl} \). When infusions of epinephrine, insulin, and somatostatin were combined, plasma glucose concentration increased to \( \sim 170 \text{ mg/dl} \) (\( P < 0.0001 \) vs. epinephrine) during the 2nd h (Fig. 1B). In contrast, the mean plasma concentration of glucose remained unchanged in control subjects. The mean plasma insulin concentration increased by \( \sim 80\% \) at the end of the epinephrine infusion (\( P < 0.0001 \), \( t = 60–120 \text{ min} \); \( 13 \pm 1 \text{ \mu M/ml vs. baseline} \)), whereas it remained at basal levels during both the saline- and insulin-replacement studies (Fig. 1C). Plasma glucagon concentration remained at baseline values in epinephrine and control subjects but decreased in the epinephrine-insulin-somatostatin-treated subjects (\( P < 0.0001 \), \( t = 30–120 \text{ min} \); \( 25 \pm 3 \text{ pg/ml vs. baseline} \)). Plasma C peptide concentration increased and reached steady-state values of 677 \( \pm 62 \text{ pM} \) in the epinephrine study (\( t = 60–120 \text{ min} \), \( P < 0.0001 \) vs. baseline), whereas it steadily declined (\( P < 0.0001 \)) in the insulin-replacement study and did not change in the control study (Fig. 1D).

Plasma lactate, free fatty acids, and amino acids. Mean plasma lactate concentration increased during the epinephrine infusion until it reached a plateau value (\( t = 90–120 \text{ min} \)) in both the epinephrine-treated subjects (1.64 \( \pm 0.18 \text{ mM} \), \( P < 0.0001 \) vs. baseline) and epinephrine-insulin-somatostatin-treated subjects (2.26 \( \pm 0.30 \text{ mM} \), \( P < 0.0001 \) vs. baseline) but did not change during saline infusion (Fig. 2A). Plasma values of free fatty acids increased promptly to peak levels (epinephrine: 1.838 \( \pm 224 \text{ \mu M} \), epinephrine-insulin-somatostatin: 1.629 \( \pm 210 \text{ \mu M} \) vs. baseline, \( P < 0.0001 \)) and thereafter steadily decreased (\( P < 0.0001 \) to basal concentration, despite ongoing epinephrine infusion (Fig. 2B). Similarly, plasma concentrations of branch-chained amino acids (valine + isoleucine + leucine) decreased both during epinephrine and epinephrine-insulin-somatostatin protocols (\( t = 120 \text{ min} \); 286 \( \pm 26 \text{ and 356 \pm 42 \text{ \mu M vs. baseline: 443 \pm 31 and 487 \pm 61 \text{ \mu M, P < 0.005, respectively}} \)), whereas plasma gluconeogenic amino acids (threonine + glutamate + glutamine + glycine + alanine + valine + cysteine + methionine + isoleucine + histidine + arginine) remained unchanged (\( \sim 1,800 \text{ \mu M} \)).

Indirect calorimetry. Changes for the mean respiratory quotient (RQ), mean rates of glucose and lipid oxidation, and energy expenditure rate are summarized in Table 1. In contrast to a progressive decrease of RQ as observed in the control group, the epinephrine infusion in both epinephrine- and epinephrine-insulin-somatostatin-treated subjects blunted this usual effect of fasting (\( P < 0.05 \)), reflecting increased glucose oxidation (epinephrine and epinephrine-insulin-somatostatin vs. control, \( P < 0.05 \)) and unchanged lipid oxidation (epinephrine and epinephrine-insulin-somatostatin vs. control, \( P < 0.05 \)). In addition, the energy expenditure increased by \( \sim 20\% \) in both cases (vs. baseline, \( P < 0.0005 \)) as a result of the epinephrine infusion, whereas no significant change was measured in the control study.

Glycogen content. Mean basal muscle glycogen concentrations were similar before each study (epinephrine: 70 \( \pm 6 \text{ mmol glucosyl U/kg muscle} \); control: 77 \( \pm 7 \text{ mmol glucosyl U/kg muscle} \); Fig. 3). Two-hour infusion of epinephrine did not cause any detectable glycogen degradation either in presence of a slight hyperinsulinemia (epinephrine protocol) or with maintenance of basal insulin concentrations (epinephrine-insulin-somatostatin protocol). By 2 h, glycogen content had decreased by 10.2 \( \pm 0.33 \text{ mmol glucosyl U/kg muscle} \) in the epinephrine study and did not change in the control study, whereas it increased by 8.2 \( \pm 0.44 \text{ mmol glucosyl U/kg muscle} \) in the epinephrine-insulin-somatostatin study.

### Table 1. Effects of epinephrine on whole body metabolism as measured by indirect calorimetry

<table>
<thead>
<tr>
<th></th>
<th>Saline Infusion</th>
<th>Epinephrine Infusion</th>
<th>Epinephrine Infusion + Somatostatin Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta RQ )</td>
<td>-0.33 ( \pm 0.28 )</td>
<td>0.04 ( \pm 0.01^* )</td>
<td>0.03 ( \pm 0.01^{\dagger} )</td>
</tr>
<tr>
<td>( \Delta \text{Rate of glucose oxidation, mg·kg}^{-1}·\text{min}^{-1} )</td>
<td>-0.33 ( \pm 0.49 )</td>
<td>0.86 ( \pm 0.28^* )</td>
<td>0.85 ( \pm 0.20^{\dagger} )</td>
</tr>
<tr>
<td>( \Delta \text{Rate of lipid oxidation, mg·kg}^{-1}·\text{min}^{-1} )</td>
<td>0.26 ( \pm 0.12 )</td>
<td>-0.04 ( \pm 0.10 )</td>
<td>0.08 ( \pm 0.05 )</td>
</tr>
<tr>
<td>( \Delta \text{Energy expenditure rate, kcal/24 h} )</td>
<td>87 ( \pm 23 )</td>
<td>326 ( \pm 64^* )</td>
<td>370 ( \pm 86^* )</td>
</tr>
</tbody>
</table>

Data are means \( \pm \text{SE} \). RQ, respiratory quotient. Significance vs. saline: *\( P < 0.05 \); †\( P < 0.01 \).
rine-insulin-somatostatin protocol). During the entire study, deviation from the baseline glycogen concentration was <5% in each subject.

Phosphorylated metabolites. Neither epinephrine nor saline infusion significantly affected the absolute concentrations of G-6-P over the entire period of each study (basal, epinephrine: 0.146 ± 0.023; epinephrine-insulin-somatostatin: 0.145 ± 0.013; control: 0.173 ± 0.018 mM). In addition, muscle concentrations of PCR did not change significantly. However, absolute concentrations of inorganic phosphate (P; basal, epinephrine: 2.19 ± 0.59, epinephrine-insulin-somatostatin: 2.10 ± 0.21, control: 2.00 ± 0.05 mM) decreased by ~35% (P < 0.01) during the epinephrine infusion in both epinephrine and epinephrine-insulin-somatostatin experiments. A continuous decrease (P < 0.0001) up to 0.03 pH unit by the end of the epinephrine infusion period was measured in muscle of both epinephrine- and epinephrine-insulin-somatostatin-treated subjects.

Effects of Epinephrine on Muscle Glycogen Synthesis

Plasma glucose, insulin, glucagon, C peptide, and catecholamines. Euglycemic conditions were maintained within a range of 90–110 mg/dl throughout the clamp period. Likewise, plasma insulin concentrations rapidly increased and reached steady-state values within <20 min (t = 30–240 min: 70 ± 6 μU/ml). Plasma glucagon concentrations remained at baseline values, whereas plasma C peptide concentrations started to decrease when the epinephrine infusion was initiated (~60% at 4 vs. 2 h, P < 0.0001). During that latter period, plasma epinephrine concentrations reached steady-state values of 556 ± 47 pg/ml and plasma norepinephrine concentrations remained unchanged.

Plasma lactate, free fatty acids, and amino acids. Plasma lactate concentrations reached a plateau value during the first period of the clamp (t = 60–120 min: 1.12 ± 0.08 mM vs. baseline, P < 0.0001), and increased further when the epinephrine infusion was started (t = 180–240 min: 2.16 ± 0.14 mM vs. baseline, P < 0.0001). Plasma free fatty acid levels decreased to 140 ± 10 μM (P < 0.0001) before the infusion of epinephrine and remained at this level until completion of the study (t = 180–240 min: 145 ± 15 μM). In addition, plasma concentrations of both branch-chained and gluconeogenic amino acids steadily declined throughout the clamp period to reach concentrations of 153 ± 8 μM (vs. baseline, P < 0.0001) and 1,176 ± 71 μM (vs. baseline, P < 0.0001), respectively.

Indirect caloriometry, glucose infusion rate, plasma [1-13C]glucose enrichment, hepatic glucose production, and whole body glucose uptake. Values determined by indirect calorimetry are given in Table 2. Under euglycemia-hyperinsulinemia, glucose oxidation rate increased by approximately twofold (vs. baseline, P < 0.001) and lipid oxidation rate decreased by ~27% (vs. baseline, P < 0.001). After 2 h of epinephrine infusion, glucose oxidation remained elevated (vs. baseline, P < 0.05), whereas lipid oxidation returned to the baseline value. As in the previous protocols described above, the energy expenditure increased by ~19% (vs. baseline, P < 0.05) after the epinephrine infusion. Meanwhile, the mean rate of glucose infusion decreased by ~45% (t = 60–130 min: 7.57 ± 0.68 vs. t = 200–220 min: 4.19 ± 0.81 mg·kg⁻¹·min⁻¹, P < 0.01). The [1-13C]glucose enrichment (APE) reached a steady-state value at 13.9 ± 0.5% during the first period of the clamp (90–120 min) and remained the same (14.3 ± 0.4%) during the epinephrine infusion period (210–260 min). Likewise, epinephrine infusion did not induce any significant change in hepatic glucose production (100–120 min: 0.83 ± 0.56 mg·kg⁻¹·min⁻¹, 200–220 min: 0.49 ± 0.29 mg·kg⁻¹·min⁻¹), whereas whole body glucose uptake decreased by 55% (P < 0.01) during the same period (100–120 min: 8.4 ± 0.7 mg·kg⁻¹·min⁻¹, 200–220 min: 4.6 ± 1.2 mg·kg⁻¹·min⁻¹).

Glycogen synthesis rate. The effects of epinephrine on muscle glycogen synthesis rate are shown in Fig. 4. The increase in muscle glycogen content (basal concentration: 68 ± 4 mM) over the course of the study was measured from peak area changes of the C1 glycogen peak. The initial rate of glycogen synthesis in the 2nd h of the clamp period was 105 ± 19 μmol glucosyl U·l⁻¹·muscle⁻¹·min⁻¹. One hour after initiation of the epinephrine infusion, glycogen synthesis had decreased by

Table 2. Effects of epinephrine on whole body metabolism as measured by indirect calorimetry under euglycemic-hyperinsulinemic conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose Oxidation Rate, mg·kg⁻¹·min⁻¹</th>
<th>Lipid Oxidation Rate, mg·kg⁻¹·min⁻¹</th>
<th>Energy Expenditure Rate, kcal/24 h</th>
</tr>
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<tbody>
<tr>
<td>Basal</td>
<td>0.97 ± 0.24</td>
<td>1.51 ± 0.09</td>
<td>1.76 ± 0.63</td>
</tr>
<tr>
<td>Insulin clamp (100–120 min)</td>
<td>2.20 ± 0.18*</td>
<td>1.10 ± 0.07*</td>
<td>1.826 ± 73</td>
</tr>
<tr>
<td>Insulin clamp + epinephrine</td>
<td>2.32 ± 0.27*</td>
<td>1.29 ± 0.03*</td>
<td>2.090 ± 164†</td>
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</table>

Data are means ± SE. Significance vs. basal: *P < 0.001; †P < 0.05.
73% (28 ± 12 μmol glucosyl U·muscle−1·min−1, P < 0.005). In addition, this change in the glycogen synthesis rate correlated strongly with the decrease in the glucose infusion rate (r = 0.72, P < 0.0001).

Phosphorylated metabolites. The time course for changes in the G-6-P concentration during the clamp study is shown in Fig. 5: after an initial increase to 156% (P < 0.005) of the basal value (0.164 ± 0.024 mM), G-6-P levels started to decrease (P < 0.05, repeated-measures ANOVA) to resting concentrations (t = 200–260 min: 0.186 ± 0.031 mM) with the initiation of the epinephrine infusion. This change in the G-6-P concentration correlated strongly with the decrease in whole body glucose uptake (r = 0.71, P < 0.0001). A slight muscle acidosis was noted during the first period of the clamp and persisted during epinephrine infusion (on average −0.03 pH units vs. baseline, P < 0.0001).

Effects of Epinephrine on Muscle Glycogenolysis

The fact that no significant change in muscle glycogen concentration occurred during epinephrine infusion is surprising given the number of studies that predict or assume glycogen depletion under stress conditions. There is, in fact, very little in vivo data that has directly measured muscle glycogen concentration before and after an epinephrine challenge. Chasiotis et al. (8) reported ~15% decrease in the glycogen content of human muscle biopsy specimens after 15 min of an epinephrine infusion, with the almost total transformation of glycogen phosphorylase into the active form. However, in this study, plasma epinephrine concentrations were likely to be in the high physiological range, since the infusion rate was approximately threefold higher than that used in the present study. More recent studies (5, 18) have reported net lactate release from the human forearm together with a decrease in glucose uptake, suggesting a glycogenolytic effect of epinephrine on skeletal muscle. However, because epinephrine was infused locally into the brachial artery, it is likely that high local concentrations of epinephrine may have contributed to this observation. Therefore the glycogenolytic effect of epinephrine observed in earlier studies might have been due to supraphysiological (>1,000 pg/ml) concentrations of epinephrine.

The NMR spectroscopic technique used in this study is capable of detecting small changes in muscle glycogen concentration, with an intraobserver variability of <5% (25). The major contributory factor to this variation is the signal-to-noise ratio, which is usually ~20:1. By use of differential spectral analysis, greater precision can be gained by measuring small sequential changes in muscle glycogen concentration. The detection of glycogenosis is surprising given the number of studies that predict or assume glycogen depletion under stress conditions. There is, in fact, very little in vivo data that has directly measured muscle glycogen concentration before and after an epinephrine challenge. Chasiotis et al. (8) reported ~15% decrease in the glycogen content of human muscle biopsy specimens after 15 min of an epinephrine infusion, with the almost total transformation of glycogen phosphorylase into the active form. However, in this study, plasma epinephrine concentrations were likely to be in the high physiological range, since the infusion rate was approximately threefold higher than that used in the present study. More recent studies (5, 18) have reported net lactate release from the human forearm together with a decrease in glucose uptake, suggesting a glycogenolytic effect of epinephrine on skeletal muscle. However, because epinephrine was infused locally into the brachial artery, it is likely that high local concentrations of epinephrine may have contributed to this observation. Therefore the glycogenolytic effect of epinephrine observed in earlier studies might have been due to supraphysiological (>1,000 pg/ml) concentrations of epinephrine.

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tion threshold for minimal change is ~5 mmol glycogen/l muscle, which is ~7% of the baseline concentration. This is almost 30% less than the variation reported by Chasiotis et al. (8) using biopsies, suggesting that the NMR method has sufficient sensitivity to measure variations at least as low as the smallest decreases that have been reported for the biopsy method. Also, needle-biopsy techniques are painful, causing endogenous catecholamine release, and are prone to sampling errors due to the small amount of tissue that is observed. Therefore natural-abundance 13C NMR estimates of muscle glycogen concentration are likely to be more precise than those obtained from biopsies.

The reason for the lack of effect of epinephrine on muscle glycogenolysis despite the abundant in vitro data supporting this effect is unclear but may be due to the higher concentrations of epinephrine used in the in vitro studies. It is also possible that Ca2+ release is essential for activation of the phosphorylase reaction (7), which occurs mainly with muscle contraction. Thus, in the presence of high amounts of epinephrine, an α-receptor-mediated increase in Ca2+ influx into the cell (30) can activate glycogen phosphorylase. Another alternative is that low concentrations of Pi at the active site of the phosphorylase maintain in vivo activity at a low level (6, 32). Our data support this latter possibility, since a decrease in free Pi was detected during prolonged epinephrine infusion. It is also possible that epinephrine-stimulated lipolysis as reflected by an increase in plasma free fatty acid concentration contributed to the sparing of muscle glycogen. Finally, glucose itself may inhibit glycogenolysis and counterbalance any effect of epinephrine on this parameter. However, this effect would be expected to be mediated through an increase in G-6-P concentration, which we did not observe.

Significant increases in plasma lactate concentration and whole body glucose oxidation were measured in the presence of both basal insulin and mild hyperinsulinemia. This is probably due to the mass effect of hyperglycemia to promote its own uptake in muscle and other tissues, since similar increases in plasma lactate concentration have been observed under conditions of hyperglycemia while basal insulinemia and hypoglycagoneemia were maintained (33, 44). However, one cannot totally exclude the possibility that very low rates of glycogenolysis (<0.04 glucosyl U·muscle−1·min−1) in the vastus lateralis muscle or other muscle groups might have contributed to this increase in plasma lactate concentration. Although hepatic glycogenolysis might also have contributed to this increase in plasma lactate concentration, this is unlikely in view of recent studies which have found that similar increases in plasma epinephrine concentration cause an increase in net hepatic uptake of lactate (40). Together these data suggest that a physiological increase in plasma epinephrine concentration stimulates glucose cycling between liver and periphery without significantly altering muscle glycogen content.

Effects of Epinephrine on Muscle Insulin-Stimulated Glycogen Synthesis

Previous studies have demonstrated an inhibitory effect of epinephrine on insulin-mediated glucose uptake (1, 2), which occurs mostly in muscle tissue (5, 13). The present study is the first direct demonstration that inhibition of muscle glycogen synthesis is a major component of the decrease in glucose disposal during a physiological increase in the plasma epinephrine concentration. Additionally, the finding that the G-6-P concentrations decreased during the epinephrine infusion, as opposed to other clamp studies during which G-6-P remained high in absence of epinephrine for >4 h (34), suggests that either reduced activity of muscle glycogen transporter or hexokinase activity is responsible for the impaired muscle glycogen synthesis. These data are consistent with previous data showing that glucose transport and/or phosphorylation controls the rate of the synthase reaction through the concentration of G-6-P (4). This is, however, in apparent contradiction to a recent study by Raz et al. (31), who reported a marked increase in G-6-P concentration while in the presence of similar plasma epinephrine and insulin concentrations. The low sensitivity of measuring G-6-P with muscle biopsy may be one of the potential explanations for the discrepancy with our results. Also, Raz et al. (31) have suggested that epinephrine stimulation would result in a net decrease of glucose uptake because of the inhibitory effect of G-6-P on hexokinase activity. However, such a hypothesis is based on the assumption that glycogenolysis is the primary source of G-6-P, which our results do not support. The possibility of enhanced muscle glycolysis resulting in a net decrease in G-6-P concentrations is also unlikely, since the increase in the whole body glucose oxidation rate was <10% of the decrease in muscle glycogen synthesis. Meanwhile, the changes in G-6-P correlated strongly with the changes in the total body glucose uptake. A decrease in G-6-P concentration therefore most likely reflects an epinephrine-induced impairment of insulin-stimulated glucose transport and/or phosphorylation.

In summary, we found that a physiological increase in plasma epinephrine concentration 1) had a negligible effect on muscle glycogenolysis, 2) decreased muscle Pi, which may maintain phosphorylase activity at a low level, and 3) caused significant impairment in insulin-stimulated muscle glycogen synthesis, possibly due to inhibition of glucose transport-phosphorylation activity.

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