Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia

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Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. Am J Physiol Endocrinol Metab 280: E908–E917, 2001. —In the present study the hypothesis tested was that prior exercise may blunt counterregulatory responses to subsequent hypoglycemia. Healthy subjects (15 females (15 males (m), age 27 ± 1 yr, body mass index 22 ± 1 kg/m², hemoglobin A1c 5.6 ± 0.5%) were studied during 2-day experiments. Day 1 consisted of a 2-h hypoglycemic clamp in all subjects. Endogenous glucose production (EGP) was measured using [3-3H]glucose. Muscle sympathetic nerve activity (MSNA) was measured using microneurography. Day 2 insulin (87 ± 6 μU/ml) and plasma glucose levels (54 ± 2 mg/dl) were equivalent after priorEXE and priorREST. Significant blunting (P < 0.01) of day 2 norepinephrine (−30 ± 4%), epinephrine (−37 ± 6%), glucagon (−60 ± 4%), growth hormone (−61 ± 5%), pancreatic polypeptide (−47 ± 4%), and MSNA (−90 ± 8%) responses to hypoglycemia occurred after priorEXE vs. priorREST. EGP during day 2 hypoglycemia was also suppressed significantly (P < 0.01) after priorEXE compared with priorREST. In summary, two bouts of exercise (90 min at 50% VO2 max) significantly reduced glucagon, catecholamines, growth hormone, pancreatic polypeptide, and EGP responses to subsequent hypoglycemia. We conclude that, in normal humans, antecedent prolonged moderate exercise blunts neuroendocrine and metabolic counterregulatory responses to subsequent hypoglycemia.

EXERCISE-ASSOCIATED HYPGLYCEMIA frequently occurs in type I diabetes mellitus (20). Although the in vivo mechanisms responsible for this phenomenon have not been completely elucidated, causal hypotheses include the presence of relative or absolute hyperinsulinemia, possibly associated with increased insulin sensitivity (39) and blunting of glucose counterregulatory responses (6, 37). The latter is supported by the recent findings of a reduction in the exercise-induced epinephrine response in type I diabetics with autonomic neuropathy (6) and of reduced catecholamine responses to exercise during hypoglycemia in intensively treated type I diabetics (37).

Glucocorticoids are elevated during both hypoglycemia and physical exercise. Recently, prior elevations of cortisol have been demonstrated to produce blunted counterregulatory responses to subsequent hypoglycemia (18). Because both hypoglycemia and exercise trigger similar neuroendocrine and autonomic nervous system (ANS) responses, we have previously hypothesized that these two kinds of stress may reciprocally blunt their respective counterregulatory responses. Support for this hypothesis comes from several studies in which prior elevations of glucocorticoids resulted in blunted counterregulatory responses to a variety of other stresses (8, 18, 27, 29, 34, 36). Furthermore, a recent study from our laboratory (14) demonstrated that two prior 120-min episodes of hypoglycemia (50 mg/dl) significantly blunted counterregulatory responses to next-day exercise (14). Two studies have addressed the reciprocal question of whether antecedent exercise blunts counterregulatory responses to subsequent hypoglycemia, and they produced conflicting results (28, 35). In one study (28), dogs displayed a decreased epinephrine response to 2-deoxyglucose-induced neuroglycopenia after exhaustive exercise. A subsequent study performed in humans reported that prior exercise did not blunt counterregulatory responses to subsequent hypoglycemia, but intensity and duration of exercise may not have been sufficient to affect hypoglycemic responses (35).

The present study was therefore designed to establish whether prolonged exercise may blunt neuroendocrine and ANS responses to subsequent hypoglycemia. Thirty healthy young subjects were studied over 2 days; during the 1st day, two 90-min episodes of static cycling exercise at 50% VO2 max were performed, and on the 2nd day, the metabolic, neuroendocrine, and ANS responses to 120 min of moderate (54 mg/dl) hypoglycemia were recorded.
RESEARCH DESIGN AND METHODS

Subjects

We studied 30 healthy volunteers (15 male/15 female) aged 27 ± 1 yr with a body mass index of 22 ± 1 kg/m² and glycosylated hemoglobin A₁c of 5.6 ± 0.5% (normal range 4.0–6.5%). No subject was taking medications or had a family history of diabetes. Each subject had normal blood count, plasma electrolytes, and liver and renal function. All subjects gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board. The subjects were asked to avoid any exercise and to consume their usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt Clinical Research Center (CRC) at 5:00 PM on the evening before an experiment. All subjects were studied after an overnight 10-h fast.

Experimental Design

Experimental procedures were performed over two consecutive days. On day 1, subjects were assigned to one of two groups: one group (priorEXE, n = 16, 8 m/8 f) performed two 90-min exercise bouts at ~50% maximal O₂ uptake (VO₂ max); the other group (priorREST, n = 14, 7 m/7 f) was maintained in resting conditions for an equivalent period of time. Some of the data from the priorREST group have been previously included in related publications from our laboratory (17, 19). Day 2 procedures were identical in both priorEXE and prior-REST.

Preliminary Exercise Testing

At least 2 wk before the initial study, subjects assigned to the priorEXE group performed an incremental work test on a stationary cycle ergometer to determine VO₂ max and anaerobic threshold (AT). Airflow and O₂ and CO₂ concentrations in inspired and expired air were measured by a computerized open-circuit indirect calorimetry cart (Medical Graphics Cardio2) with a mouthpiece and nose clip system. The AT was determined by the V-slope method (5). The AT determined by gas exchange corresponds to the onset of an increased lactate-to-pyruvate ratio in blood and indicates the level of exercise above which anaerobic mechanisms supplement aerobic energy production (44). At workloads below the AT, exercise can be continued for a prolonged period, whereas above the AT, fatigue will occur considerably faster (43). The experimental work rate was established by calculating 80% AT. The AT was detected at 59 ± 3% of VO₂ max, and 80% AT corresponded to 47 ± 2% of the subject’s VO₂ max. This workload was chosen because it is close enough to the AT to produce a physically challenging stress (i.e., large experimental signal) but is sustainable for a prolonged period of time. Subjects studied ranged from sedentary to regularly exercising, although not actively participating in competitive sports. Mean VO₂ max for the group was 31 ± 2 ml·kg⁻¹·min⁻¹ (range 21–43 ml·kg⁻¹·min⁻¹).

Day 1 Studies

On the morning of day 1, after an overnight fast, two intravenous cannulas were inserted in subjects under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (23). The other cannula was placed in the contralateral arm so that 20% glucose could be infused via a variable-rate volumetric infusion pump (Imed, Gemini model, San Diego, CA).

priorEXE experiments. After insertion of the venous can- nulas, a period of 90 min was allowed to elapse, followed by a 30-min basal period and a 90-min morning exercise period (EXE 1) (Fig. 1). Morning exercise was followed by a 180-min resting period and a second 90-min exercise period (EXE 2) (Fig. 1). EXE 1 and 2 each consisted of 90 min of continuous submaximal exercise (at 60–70 rpm) on an upright cycle ergometer (Medical Graphics, Yorba Linda, CA) at 80% of the individual’s AT (~50% VO₂ max). Plasma glucose was maintained equivalent to baseline levels throughout the study by a glucose clamp technique, according to which glucose levels

![Fig. 1. Schematic diagram of experimental protocols.](http://ajpendo.physiology.org/ by 10.220.33.1 on November 7, 2017)
were measured every 5 min during both exercise periods and every 20 min during the rest period between exercise periods. A variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant at the desired level (3). Potassium chloride was infused at a rate of 5 mmol/h during EXE 1 and EXE 2. During the first 45 min of the rest period between exercise bouts, a drink containing 1.5 g carbohydrate/kg of the subject's body weight was administered orally to replenish glycogen stores depleted during EXE 1. At completion of EXE 2, subjects consumed a large meal and (later) a bedtime snack and remained in the CRC.

Prior REST experiments (control studies). These experiments followed a similar format to the previously described exercise experiments, with the exception that identical morning and afternoon hyperinsulinemic-euglycemic clamps were performed in lieu of the exercise bouts. At completion of the second euglycemic clamp, subjects consumed an evening meal and snack identical to those of the prior EXE experiments.

Day 2 Hypoglycemia Experiments

Day 2 experiments involved a standardized hyperinsulinemic-hypoglycemic glucose clamp to assess the effects of day 1 physical activity on neuroendocrine, ANS, and metabolic counterregulatory responses to subsequent hypoglycemia. Day 2 procedures started at 8 AM after a 10-h overnight fast and lasted 240 min (120 min to 120 min), divided into an equilibration period (120 to –30 min), a basal period (–30 to 0 min), and an experimental period (0–120 min). A primed (18 μCi) constant infusion of [1-13C]glucose was started at t = –120 min and continued throughout the experimental period. At t = 0 min, a primed continuous infusion of insulin (1.5 mU·kg⁻¹·min⁻¹) was started (38), and the rate of fall of plasma glucose was controlled so that the target hypoglycemic plateau (~54 mg/dl) was reached by t = 30 min and was maintained until the end of the experimental period.

Rates of glucose appearance (Ra), endogenous glucose production (EGP), and glucose utilization were calculated according to the methods of Wall et al. (41). EGP was calculated by determining the total rate of Ra, (this comprises both EGP and any exogenous glucose infused to maintain euglycemia) and subtracting from it the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, because underestimates of total Ra and glucose disposal (Kg) can be obtained. This underestimate can be largely overcome by use of a HPLC-purified tracer and measurements taken under steady-state conditions (i.e., constant specific activity). In this study, only data recorded at baseline and during the last 30 min of the hypoglycemic clamps, when a steady state existed, were used in calculating glucose turnover.

Direct Measurement of Muscle Sympathetic Nerve Activity

During day 2 hypoglycemic clamps, muscle sympathetic nerve activity (MSNA) was assessed directly via microneurography. Microneurographic activity was recorded from the peroneal nerve at the level of the fibular head (42). The approximate location of this nerve was determined by transdermal electrical stimulation (10–60 V, 0.01-ms duration). The stimulation produced painless muscle contraction of the foot. After this, a reference tungsten electrode with a shaft diameter of 200 μm was placed subcutaneously. A similar electrode, with an uninsulated tip (1–5 μm), was inserted into the nerve and used for recording MSNA. A recording of MSNA was considered adequate when 1) electrical stimulation produced muscle twitches but not paresthesia, 2) stretching of the tendons in the foot evoked proprioreceptive afferent signals, whereas cutaneous stimulation by slight stroking of the skin did not, 3) nerve activity increased during phase II of the Valsalva maneuver (hypotensive phase) and was suppressed during phase IV (blood pressure overshoot), and 4) nerve activity increased in response to held expiration.

Two types of sympathetic fibers (skin and muscle) can be identified from recordings of peripheral nerves. MSNA was recorded in the present study, as this has been demonstrated to reflect increased sympathetic activity during insulin-induced hypoglycemia (22), 2-deoxyglucose-induced neuroglycopenia (21), and hyperinsulinemic euglycemia in normal humans (4).

Sympathetic nerve activity is expressed as bursts per minute. Measurements of MSNA were made from the original tracings with a digitizer tablet (HIPAD, Houston Instruments, Austin, TX) coupled to Sigma Scan Software (Jandel Scientific, Coite Modena, CA) in a microcomputer. MSNA bursts were analyzed by an observer blinded to the specific experimental protocol.

Analytical Methods

The collection and processing of blood samples have been described elsewhere (11). Plasma glucose concentrations were measured in triplicate by use of the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured according to a modification of the method of Aguilar-Parada et al. (1) with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (45), with an interassay CV of 9%. Catecholamines were determined by HPLC (9) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. We made two modifications to the procedure for catecholamine determination: 1) we used a five-point rather than a one-point standard calibration curve; and 2) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant respective catecholamine peaks could be made. Cortisol was assayed using the Clinical Assays Gamma Coat RIA kit with an interassay CV of 6%. Growth hormone was determined by RIA (26) with a CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (24) with an interassay CV of 8%. Lactate, glycerol, alanine, and β-hydroxybutyrate (β-OHB) were measured in deproteinized whole blood by use of the method of Lloyd et al. (30). Nonesterified fatty acids were measured using the WAKO kit adapted for use on a centrifugal analyzer (25).

Blood samples for glucose flux were taken every 10 min throughout the basal period and every 15 min during the experimental period. Blood for hormones and intermediary metabolites was drawn twice during the basal period and every 15 min during the experimental period. Cardiovascular parameters (pulse and systolic, diastolic, and mean arterial pressures) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min from t = –120 min to t = 120 min. Gas exchange measurements were performed during the basal period and during the final 30 min of exercise.

Hypoglycemic symptoms were quantified using a previously validated semiquantitative questionnaire (12). Each individual was asked to rate his/her experience of the symptoms twice during the basal period and every 15 min during the hypoglycemic clamp. Symptoms measured included tiredness, confusion, hunger, dizziness, difficulty thinking, blurry vision, sweating, tremor, agitation, sensation of heat/thirst,
and pounding heart. The ratings of the first six symptoms were summed to get a neuroglycopenic score, and the ratings from the last five symptoms provided an autonomic symptom score.

Materials

HPLC-purified [3-3H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi/mM). Regular human insulin was purchased from Eli Lilly (Indianapolis, IN). The insulin infusion solution was prepared with normal saline and contained 3% (vol/vol) of the subjects’ own plasma.

Statistical Analysis

Data are expressed as means ± SE unless otherwise stated and are analyzed using standard parametric two-way ANOVA with repeated-measures design. This was coupled with the Duncan post hoc test to delineate at which time points statistical significance was reached. A value of $P < 0.05$ indicated significant difference.

RESULTS

Day 1: Plasma Glucose, Insulin, and Cortisol Levels

Plasma glucose was similarly maintained at basal levels during day 1 exercise and day 1 rest (Fig. 2). Basal plasma insulin levels were similar in priorEXE and priorREST both in the morning (6 ± 1 vs. 7 ± 1 μU/ml) and in the afternoon (8 ± 1 vs. 10 ± 2 μU/ml). Steady-state insulin levels decreased physiologically during exercise (4 ± 1 μU/ml in the morning and 5 ± 1 μU/ml in the afternoon), whereas they increased, according to our experimental design, in priorREST (82 ± 10 μU/ml in the morning and 86 ± 6 μU/ml in the afternoon). Plasma cortisol increased significantly over basal levels during each exercise bout (from 15 ± 2 to 21 ± 2 μg/dl in the morning and from 9 ± 1 to 16 ± 2 μg/dl in the afternoon) in the priorEXE group, whereas in the priorREST group, cortisol levels remained close to basal levels throughout day 1 (morning baseline, 11 ± 2 μg/dl; morning steady state, 11 ± 2 μg/dl; afternoon baseline, 10 ± 3 μg/dl; afternoon steady state, 11 ± 2 μg/dl).

Day 2: Insulin, Glucose, and Counterregulatory Hormone Levels

Insulin infusion resulted in equivalent steady-state levels by 30 min in both groups (87 ± 5 μU/ml). Plasma glucose fell at an equivalent rate (1.4 mg·dl$^{-1}$·min$^{-1}$) and reached steady-state hypoglycemic plateaus of 53 ± 1 mg/dl in priorEXE and 54 ± 2 mg/dl in priorREST (Fig. 2).

Basal levels of counterregulatory hormones were equivalent in priorEXE and priorREST groups at the start of day 2 hypoglycemic clamps. During hypoglycemia, neuroendocrine responses were significantly blunted by day 1 exercise (Figs. 3 and 4). By the final 30 min of hypoglycemia, plasma epinephrine levels were 478 ± 52 and 749 ± 79 pg/ml ($P < 0.005$) in priorEXE and in priorREST, respectively (Fig. 3). Norepinephrine levels were also significantly blunted in priorEXE compared with priorREST (263 ± 17 vs. 329 ± 25 pg/ml, $P = 0.02$; Fig. 3). Glucagon levels increased from 48 ± 6 to 91 ± 13 pg/ml during hypoglycemia in priorEXE; these were significantly blunted ($P < 0.01$) compared with the increase after priorREST (from 50 ± 5 to 158 ± 22 pg/ml; Fig. 3). Pancreatic polypeptide (Fig. 4) increased from 94 ± 18 to 629 ± 67 pg/ml during hypoglycemia after day 1 exercise, which was also significantly blunted ($P < 0.001$) compared to day 1 exercise.
E912 PRIOR EXERCISE AND SUBSEQUENT HYPOGLYCEMIA

Figure 3. Plasma epinephrine, norepinephrine, and glucagon levels from arterialized venous blood during 120-min hypoglycemia (64 ± 1 mg/dl) in 30 healthy human subjects. The increase measured after day 1 rest (from 145 ± 33 to 1,154 ± 127 pg/ml). Day 2 growth hormone levels were also significantly blunted (P < 0.0001, Fig. 4) after day 1 exercise compared with day 1 rest (15 ± 2 vs. 37 ± 4 ng/ml). The hypoglycemia-induced increase in ACTH on day 2 was significantly smaller after day 1 exercise (28 ± 5 pg/ml) compared with day 1 rest (81 ± 24, P < 0.001, Fig. 4). Plasma cortisol (Fig. 4), unlike other counterregulatory hormones, displayed similar increases during day 2 hypoglycemia in both groups (8 ± 1 to 25 ± 1 μg/dl in priorEXE, and 9 ± 1 to 24 ± 1 μg/dl in priorREST).

Day 2: MSNA

Basal MSNA was similar in the two groups of subjects (32 ± 1 bursts/min in priorEXE and 34 ± 4 bursts/min in priorREST). During the last 30 min of hypoglycemia, MSNA remained unchanged in priorEXE (33 ± 1 bursts/min), which was significantly blunted (P < 0.02) compared with the increase observed after priorREST (44 ± 3 bursts/min).

Day 2: Glucose Kinetics

Glucose specific activity (disintegrations per min per mg) was in a steady state during the basal control period and final 30 min of day 2 hypoglycemic clamps (Table 1). EGP was significantly greater after priorREST compared with priorEXE (1.7 ± 0.2 vs. 0.7 ± 0.3 mg·kg⁻¹·min⁻¹, P < 0.002) during the last 30 min of day 2 hypoglycemia (Table 1). Minimal exogenous glucose was required (0.2 ± 0.1 mg·kg⁻¹·min⁻¹) to maintain glucose at the target hypoglycemic level in priorREST. The amount of glucose required to maintain the hypoglycemic plateau in priorEXE was significantly greater (1.7 ± 0.5 mg·kg⁻¹·min⁻¹, P < 0.01) despite similar tracer-determined glucose R₃ values.

Day 2: Intermediary Metabolism

Blood lactate levels increased by a significantly greater amount during hypoglycemia after priorEXE (0.8 ± 0.1 vs. 0.2 ± 0.1 mM) compared with priorREST (1.0 ± 0.1 to 1.5 ± 0.1 mM, P < 0.01) (Table 2). Free fatty acid (FFA) basal levels were similar in the two groups (425 ± 38 μM in priorEXE and 470 ± 62 μM in priorREST). FFA levels decreased in both groups during hypoglycemia, but steady-state levels were significantly lower after priorEXE (129 ± 17 μM) than after priorREST (193 ± 26, P < 0.02). The basal levels of the ketone body β-OHB were significantly higher (P = 0.01) after priorEXE (70 ± 13 μM) than after priorREST (33 ± 7 μM). β-OHB levels subsequently decreased to similar steady-state levels during hypoglycemia in both groups (14 ± 1 μM in priorEXE and 10 ± 2 μM in priorREST). Blood glycerol and alanine levels were similar during day 2 hypoglycemia in the two groups.

Day 2: Hypoglycemic Symptoms

Baseline total hypoglycemic symptom scores were 16 ± 2 in priorEXE (7 ± 1 autonomic, 9 ± 1 neuroglycopenic) and 15 ± 1 in priorREST (8 ± 1 autonomic, 7 ± 1, neuroglycopenic). By the final 30 min of hypoglycemia, total symptom scores increased to 35 ± 4 (19 ± 2 autonomic, 16 ± 4 neuroglycopenic) in priorEXE and to 38 ± 10 (20 ± 3 autonomic, 18 ± 4, neuroglycopenic) in priorREST.

Day 2: Cardiovascular Parameters

No significant difference was measured between the groups in heart rate (priorEXE: baseline 61 ± 2, steady-
state 68 ± 3; priorREST: baseline 66 ± 4, steady state 74 ± 5), systolic blood pressure (priorEXE: baseline 110 ± 2, steady state 118 ± 3; priorREST: baseline 110 ± 3, steady state 119 ± 5), diastolic blood pressure (priorEXE: baseline 62 ± 1, steady state 57 ± 1; priorREST: baseline 66 ± 2, steady state 59 ± 4), and mean arterial pressure (priorEXE: baseline 78 ± 1, steady state 77 ± 2; priorREST: baseline 78 ± 2, steady state 80 ± 4) responses to day 2 hypoglycemia.

**DISCUSSION**

The aim of this study was to ascertain whether antecedent exercise could reduce neuroendocrine and metabolic responses to subsequent hypoglycemia in healthy humans. Our results demonstrate that two episodes of prior moderate and prolonged exercise (90 min at ~50% VO2 max), separated by 3 h of rest, can markedly blunt counterregulatory hormone and metabolic responses to hypoglycemia on the following day.

During hypoglycemia, the release of neuroendocrine factors enhances EGP and restricts glucose uptake. In the present study, after previous-day exercise, glucagon, catecholamines, pancreatic polypeptide, ACTH, and growth hormone increased to only about one-half the expected levels during hypoglycemia, thus indicating a widespread blunting effect on multiple neuroendocrine counterregulatory responses.

The present data also highlight the marked blunting effects of antecedent exercise on subsequent ANS responses to hypoglycemia. Not only were epinephrine and norepinephrine responses attenuated after exercise, but pancreatic polypeptide (an indicator of para-

**Table 1. Day 2 whole body glucose kinetics during 120 min of hyperinsulinemic hypoglycemia in healthy humans after day 1 antecedent exercise or rest**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60</th>
<th>90</th>
<th>105</th>
<th>120</th>
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<tbody>
<tr>
<td>Glucose SA, dpm/mg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>priorEXE</td>
<td>97 ± 8</td>
<td>90 ± 4</td>
<td>90 ± 4</td>
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<td>86 ± 3</td>
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<td>priorREST</td>
<td>83 ± 5</td>
<td>88 ± 8</td>
<td>87 ± 6</td>
<td>89 ± 7</td>
<td>87 ± 7</td>
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<tr>
<td>Glucose Rₖ, mg·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>priorEXE</td>
<td>1.8 ± 0.1</td>
<td>2.4 ± 0.5</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.6</td>
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<tr>
<td>priorREST</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Glucose inf rate, mg·kg⁻¹·min⁻¹</td>
<td>No Infusion</td>
<td>2.0 ± 0.6</td>
<td>1.4 ± 0.5</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>priorEXE</td>
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<td>0.2 ± 0.1*</td>
<td>0.2 ± 0.1*</td>
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<tr>
<td>priorREST</td>
<td>1.7 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.3</td>
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</tr>
<tr>
<td>EGP, mg·kg⁻¹·min⁻¹</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.2*</td>
<td>1.7 ± 0.2*</td>
<td>1.7 ± 0.2*</td>
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</table>

Hyperinsulinemic hypoglycemia, glucose clamp of 54 ± 1 mg/dl. priorEXE and priorREST, day 1 antecedent exercise (n = 16) and rest (n = 14); SA, specific activity; Rₖ, glucose disposal rate; Inf, infusion; EGP, endogenous glucose production. *P < 0.01 vs. priorEXE.
Hyperinsulinemic hypoglycemia, glucose clamp of 54 ± 1 mg/dl. FFA, free fatty acids. priorEXE and priorREST experiments involved 16 and 14 subjects, respectively. *P < 0.05 vs. priorEXE.

Despite the diffuse blunting of neuroendocrine responses after antecedent exercise, hypoglycemic symp- tom scores were not different during hypoglycemia after rest or exercise. Although explanations for this observation remain speculative, it appears that blunting effects on counterregulatory responses to hypoglycemia induced by various antecedent stresses may be organized in hierarchical fashion. Support for this hypothesis comes from previous observations that cardiovascular responses to subsequent hypoglycemia were unaffected by prior hypoglycemia, unlike neuroendo- crine responses (19). Furthermore, after very short (5-min) antecedent hypoglycemia (15), metabolic, neu- roendocrine, and MSNA responses to subsequent hypoglycemia were blunted, but symptom scores, similar to the present study, were unaffected. Finally, Par- amore et al. (33) recently observed that, after anteced- ent hypoglycemia, the forearm sympathetic neural re- response to subsequent hypoglycemia was not blunted, whereas most other neuroendocrine and metabolic re- sponses were attenuated.

Higher circulating lactate levels were present during day 2 hypoglycemia in priorEXE compared with prior- REST, in apparent contrast with a reduced epineph- rine response. Increased muscle insulin sensitivity fav- ors muscle glycogen storage during postexercise feeding (31). Although epinephrine provides the principal stimulus for muscle glycogenolysis during hypo- glycemia, the dose–response relationship for epineph- rine action on peripheral muscle is unknown. Thus we speculate that the blunted epinephrine responses to hypoglycemia may still be above a threshold level that elicits a substantial glycogenolytic response, especially when we consider that it may have acted on muscle tissue particularly rich in glycogen. In addition, re- duced liver lactate uptake and changes in renal glu- cocorticogenic precursor balance may also have played a role in increasing circulating lactate levels (10).

Circulating levels of FFAs were moderately but con- sistently lower during hypoglycemia after antecedent exercise compared with day 1 rest. The lipolytic re- sponse to hypoglycemia, on the other hand, was similar in the two groups, as suggested by similar circulating glycerol. Hepatic FFA oxidation, as reflected by similar steady-state circulating levels of β-OHB, was similar in priorEXE and in priorREST. Due to significantly greater basal β-OHB levels in the priorEXE group, however, it appears that the suppression of β-OHB concentration, and therefore of hepatic FFA oxidation, was greater in priorEXE compared with priorREST. Possible explanations for the reduced FFA levels after exercise include increased FFA utilization by skeletal muscle and/or increased FFA reesterification.

Only two previous studies, to our knowledge, have investigated the effects of antecedent exercise on sub- sequent hypoglycemic responses (28, 35). Kozlowski et al. (28) exercised dogs on a treadmill to exhaustion (avg 3 h 20 min) and observed blunting of epinephrine responses to subsequent administration of 2-deoxy-D- glucose, a substance known to induce hormonal and metabolic neuroglycopenic responses. In a later study, Rattrasarn et al. (35) induced hypoglycemia in seven healthy men after either 60-min cycle exercise or rest, and these investigators detected no differences in counterregulatory responses to subsequent hypoglycemia.

It should be noted that Rattrasarn et al. induced hypoglycemia 1 h after a single 60-min exercise bout (as opposed to two 90-min bouts in our study), during which cortisol levels did not increase over baseline, suggesting that the degree of stress was mild. Also, in
the study of Rattarasarn et al., hypoglycemia (excluding the time needed to reach the hypoglycemic plateau) lasted only 30–40 min, too early for counterregulatory hormone steady state. In our study, the hypoglycemic plateau was reached at $t = 30$ min, and hypoglycemia was maintained for 90 min more, the last 30 min of which allowed the detection of steady-state differences in counterregulatory responses. We believe that the experimental protocols used in the study by Rattarasarn et al. may have attenuated the effects of prior exercise on counterregulatory responses to subsequent hypoglycemia.

Some aspects of our experimental design, on the other hand, may require some comments. Different subjects were used in the two experimental groups. Adequate sample size and careful matching of all subjects to avoid potentially confounding variables (such as age, weight, level of physical training) in the two groups prevented this from affecting our results. This study is one part of a long-term project, so that experimental and control studies only partly overlapped chronologically. We believe that the consistency and reproducibility of the experimental conditions and methodologies employed in our laboratory over the last several years (14, 17, 19) have minimized any effect of this variable. Finally, subjects from the priorREST group underwent on day 1 hyperinsulinemic euglycemia. Day 2 hypoglycemic counterregulatory responses in this group, however, were identical to those reported during hypoglycemia in subjects with no antecedent hyperinsulinemic euglycemia (14, 19). We therefore believe that the above factors exerted a negligible effect on the results of our study.

Increased cerebral glucose extraction (7) and elevation of lactate (40), ketone bodies (40), or cortisol (16, 18) have been hypothesized as mechanisms responsible for the blunting effects of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia. Lactate increased about threefold over basal levels during day 1 exercise. This increase is much greater than that observed during antecedent hypoglycemia (19), when lactate levels increase by only $\sim 50\%$ over basal values. It is therefore possible that, if lactate has a role in blunting subsequent hypoglycemic responses, this may be more marked when the antecedent stimulus is exercise rather than hypoglycemia. In the present study, peak cortisol levels during antecedent exercise were $21 \pm 2$ µg/dl, not dissimilar from the values of 25–26 µg/dl previously observed to cause blunting of counterregulatory responses to subsequent hypoglycemia (16, 18). Our data therefore support the possible role of elevated cortisol and/or lactate in blunting counterregulatory responses to subsequent stress, although other mechanisms cannot be excluded.

Cortisol was the only major counterregulatory hormone not blunted by antecedent exercise during day 2 hypoglycemia. The decreased growth hormone and ACTH responses during hypoglycemia after exercise indicate that the lack of effect on the cortisol responses was probably generated by a mechanism downstream of the anterior pituitary. Changes in both adrenal sensitivity to ACTH (13) and ACTH sensitivity to glucocorticoids (2) have been reported and may conceptually explain discordant cortisol and ACTH responses.

Our results support the concept that different forms of stress may similarly reduce counterregulatory responses to subsequent hypoglycemia. The blunting in counterregulatory responses induced by prior exercise (epinephrine $\downarrow 37\%$, norepinephrine $\downarrow 40\%$, glucagon $\downarrow 58\%$, growth hormone $\downarrow 62\%$, pancreatic polypeptide $\downarrow 44\%$, MSNA $\downarrow 90\%$, EGP $\downarrow 70\%$) was similar in magnitude to that previously reported after antecedent hypoglycemia (19) (Fig. 5). Similar magnitude in the blunting of counterregulatory responses is consistent with the hypothesis that common mechanisms are working to cause the observed blunting effects.
In summary, this study has demonstrated that two bouts of prior prolonged moderate cycling exercise can blunt neuroendocrine and metabolic responses during next-day hypoglycemia. The neuroendocrine counterregulatory response was diffusely blunted: growth hormone, ACTH (pituitary), glucagon, pancreatic polypeptide (pancreas), epinephrine, norepinephrine, MSNA (adrenal gland, sympathetic nerve terminals). Reduced neuroendocrine responses were paralleled by proportional blunting of EGP.

We conclude that, in overnight-fasted healthy humans, antecedent prolonged, moderate exercise can blunt counterregulatory responses to subsequent hypoglycemia. The blunting of hypoglycemic counterregulatory responses after antecedent exercise appears to be similar in magnitude to that observed after antecedent hypoglycemia.

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medullary activity, but, unlike the adrenomedullary response, the forearm sympathetic neural response is not reduced after recent hypoglycemia. *Diabetes* 48: 1429–1436, 1999.


