Effects of Differing Antecedent Increases of Plasma Cortisol on Counterregulatory Responses During Subsequent Exercise in Type 1 Diabetes

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OBJECTIVE—Antecedent hypoglycemia can blunt neuroendocrine and autonomic nervous system responses to next-day exercise in type 1 diabetes. The aim of this study was to determine whether antecedent increase of plasma cortisol is a mechanism responsible for this finding.

RESEARCH DESIGN AND METHODS—For this study, 22 type 1 diabetic subjects (11 men and 11 women, age 27 ± 2 years, BMI 24 ± 1 kg/m², A1C 7.9 ± 0.2%) underwent four separate randomized 2-day protocols, with overnight normalization of blood glucose. Day 1 consisted of morning and afternoon 2-h hyperinsulinemic- (9 pmol·kg⁻¹·min⁻¹) euglycemic clamps (5.1 mmol/l), hypoglycemic clamps (2.9 mmol/l), or euglycemic clamps with a physiologic low-dose intravenous infusion of cortisol to reproduce levels found during hypoglycemia or a high-dose infusion, which resulted in further twofold greater elevations of plasma cortisol. Day 2 consisted of 90-min euglycemic cycling exercise at 50% VO₂max.

RESULTS—During exercise, glucose levels were equivalently clamped at 5.1 ± 0.1 mmol/l and insulin was allowed to fall to similar levels. Glucagon, growth hormone, epinephrine, norepinephrine, and pancreatic polypeptide responses during day 2 exercise were significantly blunted following antecedent hypoglycemia, low- and high-dose cortisol, compared with antecedent euglycemia. Endogenous glucose production and lipolysis were also significantly reduced following day 1 low- and high-dose cortisol.

CONCLUSIONS—Antecedent physiologic increases in cortisol (equivalent to levels occurring during hypoglycemia) resulted in blunted neuroendocrine, autonomic nervous system, and metabolic counterregulatory responses during subsequent exercise in subjects with type 1 diabetes. These data suggest that prior elevations of cortisol may play a role in the development of exercise-related counterregulatory failure in those with type 1 diabetes. Diabetes 58:2100–2108, 2009

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See accompanying commentary, p. 1951.
sponses to subsequent euglycemic exercise were studied during the following day.

RESEARCH DESIGN AND METHODS

We studied 22 patients with type 1 diabetes (11 men and 11 women), age 27 ± 2 years, BMI 24 ± 1 kg/m², and A1C 7.9 ± 0.2% (normal range 4–6.0%). Patients had been diagnosed with type 1 diabetes for 12 ± 2 years and had no clinical evidence of tissue complications of the disease such as retinopathy, renal impairment, or autonomic neuropathy. Patients were treated with either multiple daily injections of insulin or continuous subcutaneous insulin via a pump. Each patient had normal blood count, plasma electrolytes, and liver function. All gave written informed consent. Studies were approved by the Vanderbilt University human subjects institutional review board.

At least two weeks before the initial study, patients performed an incremental work test on a stationary cycle ergometer to determine VO₂max and anaerobic threshold (AT). Airflow, O₂, and CO₂ concentrations in inspired and expired air were measured by a computerized open-circuit indirect calorimetry cart (Parvo Medics, Kansas City, MO) with a mouthpiece and nose clip system. AT was determined by the V-slope method (25). AT determined by gas exchange corresponds to the onset of an increased lactate/pyruvate ratio in blood and indicates the level of exercise above which anaerobic mechanisms supplement aerobic energy production (25). At workloads below the AT, exercise can be continued for a prolonged period, whereas above the AT, fatigue will occur considerably faster (25). The experimental work rate was established by calculating 80% AT, which corresponded to 47 ± 2% of the subject’s VO₂max. 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 VO₂max for the group was 34 ± 2 ml · kg⁻¹ · min⁻¹ (range 24–54 ml · kg⁻¹ · min⁻¹).

Type 1 diabetic subjects were studied during four separate 2-day studies. Day 1 consisted of morning and afternoon 2-h hypoglycemic-euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with cortisol infusion at 1 μg · kg⁻¹ · min⁻¹ (anteCort) or 2 μg · kg⁻¹ · min⁻¹ (anteCort2). Day 2 was identical for all four protocols and consisted of 90 min euglycemic cycling exercise.

Patients were asked to avoid hypoglycemia during the 7 days preceding each visit. Patients checked blood glucose levels at least four times per day and reported values to the investigators before admission. Detection of any value <3.9 mmol/l resulted in rescheduling of the study. Patients were also asked to avoid exercise and consume a usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt Clinical Research Center on the afternoon before an experiment. Upon admission, patients were asked to discontinue usual insulin therapy, and two intravenous cannulas were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of one hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (26). The other cannula was placed in the contra lateral arm for infusion of dextrose, insulin, potassium chloride, hydrocortisone, and triligated glucose. Intravenous infusion of Humulin R (Eli Lilly, Indianapolis, IN) was started at a basal rate via a variable rate volumetric infusion pump (Imed, San Diego, CA). Patients then consumed a standardized dinner and 9:00 P.M. snack and were requested not to ingest any food after 10:00 P.M. The insulin infusion rate was increased during meal consumption. At 240 min, the insulin infusion was decreased to the basal rate; euglycemia was restored in anteHypo studies or maintained in anteEugly and anteCort studies. At 360 min, a second 2-h clamp identical to that in the morning was performed. At 480 min, the insulin infusion was decreased to the basal rate; euglycemia was again restored or maintained, and patients were allowed to consume a standardized meal. Evening and overnight procedures were then identical to those described for admission night.

Day 2 procedures. Day 2 procedures were identical for all four protocols. The experiments lasted 210 min and consisted of a tracer equilibration (0–90 min), basal (90–120 min), and exercise (120–210 min). A primed (18 μCi) continuous infusion (0.8 μCi/min) of high-performance liquid chromatography–purified [3-H] glucose (Perkin Elmer Life Sciences, Boston, MA; 11.5 mCi · μmol⁻¹ · 1) was started at 0 min and continued throughout the study to measure glucose kinetics. Exercise consisted of 90 min continuous cycling (at 60–70 rpm) on an upright cycle ergometer (Medical Graphics, Yorba Linda, LA) at 80% of the individual’s AT (60 ± 6 pmol/l) throughout the 90-min cycling exercise (3). Plasma glucose was measured every 5 min and maintained at basal levels (5 mmol/l) throughout the study via a variable rate infusion of 20% dextrose. After completion of the exercise protocol, patients consumed a meal and were discharged.

Tracer methodology. Rates of glucose appearance, endogenous glucose production (EGP), and glucose utilization were calculated according to the method of Wall et al. (28). EGP was calculated by determining the total rate of glucose appearance (which comprises both EGP and any exogenous glucose infused) and subtracting the rate of glucose disposal (which comprises exogenous glucose infused. It is now recognized that this approach is not fully quantitative because underestimates of total rate of glucose appearance and rates of glucose disposal can be obtained. This underestimate can be largely overcome by use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant glucose-specific activity). To minimize changes in specific activity, the tracer infusion rate was gradually reduced during the first 30 min of exercise. During the last 60 min of exercise, proportional additional increases of tracer delivery were made commensurate with changes of the exogenous glucose infusion rate.

Analytical methods. Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood for hormones and intermediary metabolites was drawn twice during the basal period and every 15–30 min during the clamp period. Glucagon was measured according to the method of Aguilar-Parada, Eisentraut, and Unger (29) with an interassay coefficient of variation (CV) of 15% free. Insulin was measured after polylethylene glycol extraction as previously described (30) with an interassay CV of 11%. Catecholamines were determined by high-pressure liquid chromatography (31) with an interassay CV of 12% for both epinephrine and norepinephrine. We made two modifications to the method of Eisentraut and Unger (29) with an interassay CV of 15% free. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (33) with an interassay CV of 9%. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured on deproteinized whole blood using the method of Lloyd et al. (34). Nonesterified fatty acids (NEFAs) were measured using the WAKO kit adopted for use on a centrifugal analyzer (35). Cardiovascular parameters (heart rate and systolic and diastolic blood pressure) were measured every 10 min during clamp studies. Hypoglycemic symptoms were quantified using a previously validated semiquantitative questionnaire (36). Each individual was asked to rate his/her experience of the symptoms twice during the control period and every 15 min during experimental periods. Symptoms measured included sweaty, tremor/shaky, hot, thirsty/dry mouth, agitation/irritability, palpitations, tired/fatigued, confusion/dizzy/difficulty thinking, blurriness of vision, and sleepiness. The ratings of the first six symptoms were summed to get the autonomic score, whereas the ratings from the last six symptoms provide a neuroglycopenic symptom score.

Statistical analysis. Data are expressed as means ± SE and were analyzed using parametric two-way analysis of variance with repeated measures. Tukey’s post hoc analysis or Student’s t tests were used to delineate statistical significance across time within each group and for anteHypo or anteCort group compared with the anteEugly control group. A P value of <0.05 was considered significant. The basal signals were all 90 min of exercise on day 2 were compared for most parameters. Baseline data represent an average of two time points (110 and 120 min), and the final 30-min data represent an average of three measures taken during this time (180, 195, and 210 min).

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EFFECTS OF CORTISOL ON EXERCISE

TABLE 1
Plasma glucose, insulin, and cortisol levels during two 2-h hyperinsulinemic-euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions of 1 μg · kg⁻¹ · min⁻¹ (anteCort1) or 2 μg · kg⁻¹ · min⁻¹ (anteCort2) on day 1

|                      | Morning clamp |                      | Afternoon clamp |                      |
|----------------------|---------------|----------------------|-----------------|----------------------|
|                      | Basal         | Final 30 min of exercise | Basal          | Final 30 min of exercise |
| **Plasma glucose (mmol/l)** |               |                       |                 |                       |
| anteEugly            | 5.4 ± 0.1     | 5.0 ± 0.1             | 5.3 ± 0.1       | 5.2 ± 0.1             |
| anteHypo             | 5.3 ± 0.1     | 2.9 ± 0.1*            | 5.2 ± 0.1       | 2.9 ± 0.1*            |
| anteCort1            | 5.4 ± 0.1     | 5.0 ± 0.1             | 5.5 ± 0.1       | 5.1 ± 0.1             |
| anteCort2            | 5.4 ± 0.1     | 5.1 ± 0.1             | 5.5 ± 0.1       | 5.2 ± 0.1             |
| **Plasma insulin (pmol/l)** |               |                       |                 |                       |
| anteEugly            | 94 ± 15       | 588 ± 33              | 60 ± 12         | 588 ± 42              |
| anteHypo             | 90 ± 15       | 576 ± 36              | 58 ± 9          | 606 ± 39              |
| anteCort1            | 108 ± 17      | 606 ± 77              | 93 ± 19         | 588 ± 72              |
| anteCort2            | 93 ± 15       | 582 ± 41              | 66 ± 15         | 602 ± 47              |
| **Plasma Cortisol (nmol/l)** |             |                       |                 |                       |
| anteEugly            | 419 ± 69      | 328 ± 48              | 290 ± 46        | 301 ± 35              |
| anteHypo             | 374 ± 47      | 686 ± 96*             | 351 ± 46        | 702 ± 98*             |
| anteCort1            | 341 ± 39      | 720 ± 39*             | 349 ± 39        | 703 ± 41*             |
| anteCort2            | 469 ± 55      | 1,302 ± 70†           | 579 ± 60†| 1,216 ± 70†          |

Data are means ± SEM. n = 22 patients (11 men/11 women) with type 1 diabetes. *Significant difference versus anteEugly. †Significant difference versus anteHypo and anteCort1 (P < 0.05).

RESULTS

Day 1

**Plasma glucose, insulin, cortisol levels, epinephrine, and symptom responses.** Basal plasma glucose levels were similar among the four experimental groups (Table 1). During the final 30 min of clamp studies, plasma glucose levels were similar between anteEugly, anteCort1, and anteCort2. Plasma insulin levels were also similar among the four experimental groups during basal periods and during the final 30 min of clamp studies (Table 1). Morning basal cortisol levels were similar among the four experimental groups (Table 1). Antecedent hypoglycemia and low-dose cortisol infusion (anteCort1) resulted in approximately twofold greater levels of the hormone during the final 30 min of morning and afternoon clamps compared with anteHypo and anteCort1. During anteHypo, plasma epinephrine increased from basal levels of 148 ± 37 to 2,718 ± 427 pmol/l and symptoms increased from 17 ± 3 to 54 ± 18.

Day 2

**Glucose, insulin, and counterregulatory hormone levels.** Basal plasma glucose levels were similar among the four experimental groups (anteEugly [5.2 ± 0.1 mmol/l], anteHypo [5.2 ± 0.1 mmol/l], anteCort1 [5.5 ± 0.1 mmol/l], and anteCort2 [5.4 ± 0.1 mmol/l]) and were maintained equivalently throughout exercise (Fig. 1). Basal plasma insulin levels were similar among the four experimental groups (anteEugly [98 ± 16 pmol/l], anteHypo [102 ± 16 pmol/l], anteCort1 [108 ± 22 pmol/l], and anteCort2 [107 ± 14 pmol/l]). During exercise, plasma insulin levels were allowed to fall similarly in a physiologic manner among the four groups (anteEugly [82 ± 11 pmol/l], anteHypo [84 ± 11 pmol/l], anteCort1 [90 ± 23 pmol/l], and anteCort2 [89 ± 10 pmol/l]; Fig. 1).

Basal values of glucagon, growth hormone, and cortisol were similar at the start of each protocol (Table 2). Incremental increases of glucagon were reduced during the final 30 min of exercise in anteHypo (4 ± 2 ng/l), anteCort1 (1 ± 3 ng/l), and anteCort2 (2 ± 1 ng/l) compared with anteEugly (9 ± 2 ng/l; P < 0.05; Fig. 2). Incremental increases of growth hormone were also reduced during the final 30 min of exercise in anteHypo (3 ± 1 μg/l), anteCort1 (4 ± 1 μg/l), and anteCort2 (4 ± 2 μg/l) compared with anteEugly (9 ± 2 μg/l; P < 0.05; Fig. 3). Exercise-induced increments in cortisol were less (P < 0.05) in anteHypo (74 ± 44 nmol/l), anteCort1 (35 ± 20 nmol/l), and anteCort2 (73 ± 52 nmol/l) compared with anteEugly (204 ± 63 nmol/l) (Fig. 2).

Basal values of epinephrine, norepinephrine, and pancreatic polypeptide were similar at the start of each protocol (Table 2). ANS responses during exercise were also reduced by day 1 antecedent hypoglycemia and cortisol infusions. Incremental increases of epinephrine were reduced during the final 30 min of exercise in anteHypo (316 ± 60 pmol/l), anteCort1 (212 ± 31 pmol/l), and anteCort2 (354 ± 66 pmol/l) compared with anteEugly (582 ± 101 pmol/l; P < 0.05; Fig. 3). Exercise-induced increments of norepinephrine were also less in anteHypo (2.2 ± 0.4 nmol/l), anteCort1 (2.0 ± 0.2 nmol/l), and anteCort2 (2.0 ± 0.3 nmol/l) compared with anteEugly (4.0 ± 0.5 nmol/l; P < 0.05; Fig. 3). Incremental increases of pancreatic polypeptide from baseline were also blunted during the final 30 min of exercise in anteHypo (7.4 ± 2 pg/l), anteCort1 (4.0 ± 1.4 pg/l), and anteCort2 (7.5 ± 2 pg/l) compared with anteEugly (17 ± 5 pg/l; P < 0.05; Fig. 2).

**Glucose kinetics.** During the final 30 min of exercise, the exogenous glucose infusion rates used to maintain euglycemia were significantly higher in anteHypo (19 ± 2 μmol · kg⁻¹ · min⁻¹), anteCort1 (20 ± 4 μmol · kg⁻¹ · min⁻¹), and anteCort2 (19 ± 3 μmol · kg⁻¹ · min⁻¹) compared with anteEugly (11 ± 2 μmol · kg⁻¹ · min⁻¹; P < 0.05; Fig. 4). EGP was significantly lower in anteCort1 (11 ± 2 μmol · kg⁻¹ · min⁻¹) and anteCort2 (14 ± 2 μmol · kg⁻¹ · min⁻¹) compared with anteEugly (22 ± 3 μmol · kg⁻¹ · min⁻¹; P < 0.05; Fig. 4).

**Intermediary metabolism.** Basal levels of lactate, alanine, β-hydroxybutyrate, and NEFA levels were...
Plasma lactate responses were also reduced during exercise following day 1 anteHypo and anteCort1 ($P < 0.05$). β-Hydroxybutyrate levels did not significantly increase during exercise following anteHypo and anteCort1 and fell following anteCort2 (Table 3).

**Cardiovascular parameters.** Heart rate and systolic and diastolic blood pressure were similar at baseline and changed similarly during exercise in all four groups (Table 4).

**DISCUSSION**

This study has investigated the mechanism(s) responsible for exercise-associated counterregulatory failure in those with type 1 diabetes. Our results demonstrate that antecedent physiologic and pharmacologic levels of plasma cortisol similar to prior hypoglycemia result in widespread blunting of neuroendocrine, ANS, and metabolic responses during next-day moderate-intensity exercise in subjects with type 1 diabetes.

Exercise-associated hypoglycemia is a significant clinical problem in patients with type 1 diabetes. In fact, recent work has reported that patients will overcompensate following episodes of hypoglycemia by excessively decreasing insulin and subsequently compromising glycemic control with increases in A1C. These actions then lead to paradoxical deterioration of glycemic control rather than the expected improvements in A1C following exercise (37). We and others have demonstrated that antecedent hypoglycemia and exercise can establish reciprocal feedforward vicious cycles that cause downregulation of ANS and neuroendocrine counterregulatory response during each subsequent stress (4,6,38,39). However, the specific mechanisms responsible for the failure of these physiologic counterregulatory mechanisms are not known. Thus, this study was conducted with the aim of providing knowledge regarding the mechanisms responsible for the inability of type 1 diabetic individuals to defend plasma glucose during exercise following antecedent hypoglycemia.

Glucose levels were carefully controlled at all times during the 2-day studies. Hypoglycemia was avoided during the overnight stays in our clinical research center so that the effects of antecedent cortisol on subsequent exercise could be clearly determined. Additionally, plasma glucose levels were clamped equivalently during all four protocols. This is important because during exercise hyperglycemia reduces, whereas even minimal reductions in plasma glucose can amplify, some neuroendocrine responses (40). Insulin levels were also carefully controlled during the clamp studies. During exercise the insulin levels were equated and were reduced to simulate the expected improvements in A1C following exercise. These actions then lead to paradoxical deterioration of glycemic control rather than the expected improvements in A1C following exercise (37).

**FIG. 1.** Plasma glucose (A) and insulin (B) concentrations (means ± SE) during day 2 euglycemic exercise studies in 22 patients (11 men/11 women) with type 1 diabetes. On the previous day, patients had undergone two 120-min euglycemic clamps (•, anteEugly), hypoglycemic clamps (■, anteHypo), or euglycemic clamps with intravenous cortisol infusions at 1 μg·kg$^{-1}$·min$^{-1}$ (▲, anteCort1) or 2 μg·kg$^{-1}$·min$^{-1}$ (△, anteCort2) on day 1. Plasma glucose and insulin levels were comparable among the four experimental groups during basal and exercise periods.

**TABLE 2**

Basal value for counterregulatory hormones at the start of day 2 exercise after two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions of 1 μg·kg$^{-1}$·min$^{-1}$ (anteCort1) or 2 μg·kg$^{-1}$·min$^{-1}$ (anteCort2) on day 1

|        | anteEugly | anteHypo | anteCort1 | anteCort2 |
|--------|-----------|----------|-----------|-----------|
| Epinephrine (pmol/l) | 279 ± 55  | 225 ± 38 | 290 ± 55  | 230 ± 33  |
| Norepinephrine (nmol/l) | 1.6 ± 0.2 | 1.4 ± 0.2 | 1.3 ± 0.2 | 1.2 ± 0.1 |
| Pancreatic polypeptide (pmol/l) | 16 ± 3 | 19 ± 6 | 21 ± 6 | 20 ± 4 |
| Glucagon (ng/l) | 44 ± 3 | 45 ± 4 | 54 ± 10 | 43 ± 3 |
| Growth hormone (μg/l) | 4 ± 3 | 4 ± 2 | 2 ± 1 | 2 ± 1 |
| Cortisol (nmol/l) | 359 ± 55 | 367 ± 47 | 286 ± 62 | 442 ± 59 |

Data are means ± SEM. $n = 22$ type 1 diabetic subjects (11 men/11 women).
The tight regulation of insulin is an important element of our experimental design because it allows determination of the effects of the antecedent corticosteroid on subsequent exercise in the presence of low (and reducing) levels of insulin. Secondly, the inability to suppress insulin levels during exercise in those with type 1 diabetes is recognized as an important causative factor for exercise-induced hypoglycemia (41).

There are numerous reports of prior increases of corticosteroids blunting subsequent physiologic responses to a wide variety of differing physiologic stress (7–21). It is unknown whether prior increases of corticosteroids can blunt subsequent homeostatic/counterregulatory responses to exercise. In this present study, we clearly demonstrate that antecedent increases of both physiologic (by matching the increase of cortisol occurring during prior hypoglycemia) and pharmacologic levels of cortisol can substantially reduce counterregulatory response during next-day submaximal exercise.

In subjects with type 1 diabetes, glucagon responses to hypoglycemia are gradually lost over the first few years following diagnosis. However, glucagon release during exercise is preserved, indicating that the pancreatic α-cell deficit is stimulus specific. After antecedent euglycemia, our patients were able to mount a glucagon response similar to that previously observed in nondiabetic subjects during exercise of similar duration and intensity (4). Day 1 hypoglycemia significantly reduced the glucagon response to subsequent exercise. Both prior physiologic (anteCort1) and pharmacologic (anteCort2) cortisol elevation without hypoglycemia also resulted in similar glucagon-blunting effects. The regulation of glucagon release during exercise is controversial. There are data both for and against ANS regulation of the hormone during exercise (42,43). However, of the studies reporting positive modulation of glucagon release by ANS during exercise, there is debate whether regulation occurs via sympathetic or parasympathetic nervous system mechanisms (42). In this present study, prior physiologic and pharmacologic increases of cortisol had similar effects to downregulate branches of ANS activity during exercise. Epinephrine (adrenomedullary), norepinephrine (sympathetic neural), and pancreatic polypeptide (also β-adrenoceptor sympathetic nervous system mediated during exercise [44]) were all blunted by antecedent low- and high-dose cortisol. Additionally, a direct effect of epinephrine on glucagon release has also been proposed. Thus, absent an effect of cortisol to directly inhibit glucagon release from pancreatic α-cells, it would appear that the blunted response of the hormone following low- and high-dose cortisol infusions was due to reduced direct ANS activation and/or via blunted epinephrine levels.
The current study also demonstrated that growth hormone responses to exercise were blunted after either prior hypoglycemia or both physiologic and pharmacologic antecedent cortisol elevations. The central pathways responsible for hypoglycemia and corticosteroid downregulation of neuroendocrine responses during subsequent exercise are not known. Much recent work has demonstrated the importance of AMP-activated protein kinase (AMPK) as a key regulator of carbohydrate and lipid metabolism during exercise (45). Of note are recent studies in rats demonstrating that hypothalamic AMPK can also regulate neuroendocrine responses during hypoglycemia (46,47). The study of Kola et al. (48) of humans with Cushing’s syndrome has determined that AMPK activity in visceral adipose tissue is reduced by 70%, thus providing an intriguing possibility that acute increases in corticosteroids may also have an effect to downregulate brain AMPK activity. Recent work has also demonstrated the action of central N-methyl-D-aspartate to stimulate secretion of epinephrine and norepinephrine (49). Liu et al. (50) have reported that corticosterone can rapidly inhibit N-methyl-D-aspartate receptor activity in cultured hippocampal neurons, thus providing another possible or complimentary central molecular target for corticosteroid downregulation of neuroendocrine and ANS activity during subsequent exercise.

The decreased ANS and neuroendocrine responses following day 1 physiologic and pharmacologic cortisol administration had profound effects on reducing metabolic responses during day 2 exercise. During exercise, plasma glucose levels are maintained when EGP matches the requirements of the working muscles. Following antecedent physiologic and pharmacologic elevations of cortisol,

**FIG. 3.** Plasma epinephrine (A) and norepinephrine (B) levels (means ± SE) during the final 30 min of day 2 exercise in 22 type 1 diabetic patients (11 men/11 women). On the previous day, patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions at 1 µg · kg\(^{-1}\) · min\(^{-1}\) (anteCort1) or 2 µg · kg\(^{-1}\) · min\(^{-1}\) (anteCort2) on day 1. There were significantly fewer increases of epinephrine and norepinephrine during the final 30 min of exercise from basal levels in anteHypo and anteCort compared with anteEugly. *P < 0.05.

**FIG. 4.** Glucose kinetics (means ± SE) during the final 30 min of day 2 exercises in 22 type 1 diabetic patients (11 men/11 women). A: Glucose infusion rate. B: Glucose utilization. C: EGP. On the previous day, patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps, or euglycemic clamps with intravenous cortisol infusions at 1 µg · kg\(^{-1}\) · min\(^{-1}\) (anteCort1) or 2 µg · kg\(^{-1}\) · min\(^{-1}\) (anteCort2) on day 1. *P < 0.05 versus anteEugly.
TABLE 3
Blood lactate, alanine, β-hydroxybutyrate, NEFA, and glycerol levels during day 2 exercise euglycemic clamp studies after two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions of 1 µg · kg⁻¹ · min⁻¹ (anteCort1) or 2 µg · kg⁻¹ · min⁻¹ (anteCort2) on day 1

|                  | Basal period | Final 30 min of exercise |
|------------------|--------------|--------------------------|
| **Lactate (mmol/l)** |              |                          |
| anteEugly        | 0.8 ± 0.1    | 1.9 ± 0.2*               |
| anteHypo         | 0.8 ± 0.1    | 1.3 ± 0.1†               |
| anteCort1        | 0.7 ± 0.1    | 1.1 ± 0.2†               |
| anteCort2        | 0.9 ± 0.1    | 1.9 ± 0.1*               |
| **Alanine (µmol/l)** |          |                          |
| anteEugly        | 320 ± 34     | 418 ± 43*                |
| anteHypo         | 330 ± 32     | 367 ± 30*                |
| anteCort1        | 310 ± 60     | 360 ± 40*                |
| anteCort2        | 378 ± 32     | 419 ± 29*                |
| **β-hydroxybutyrate (µmol/l)** |        |                          |
| anteEugly        | 40 ± 10      | 61 ± 10*                 |
| anteHypo         | 48 ± 13      | 66 ± 18                  |
| anteCort1        | 37 ± 5       | 51 ± 10                  |
| anteCort2        | 50 ± 10      | 40 ± 10†                 |
| **NEFA (µmol/l)** |            |                          |
| anteEugly        | 143 ± 25     | 383 ± 72*                |
| anteHypo         | 227 ± 42‡    | 298 ± 48†                |
| anteCort1        | 148 ± 21     | 219 ± 61†                |
| anteCort2        | 149 ± 28     | 199 ± 39†                |
| **Glycerol (µmol/l)** |        |                          |
| anteEugly        | 44 ± 9       | 145 ± 18*                |
| anteHypo         | 48 ± 10      | 111 ± 16‡                |
| anteCort1        | 77 ± 20      | 111 ± 21‡                |
| anteCort2        | 50 ± 13      | 100 ± 14‡                |

Data are means ± SEM. n = 22 type 1 diabetic subjects (11 men/11 women). *Significant difference versus basal level (P < 0.05). †Significantly reduced versus anteEugly (P < 0.05). ‡Significantly increased compared with other groups (P < 0.05).

The key metabolic mechanism of EGP was reduced during day 2 exercise. Glucose infusion rates were also increased during exercise following both doses of day 1 cortisol and antecedent hypoglycemia. The increased glucose infusion rates represent an aggregate reduction in counterregulatory responses because more glucose was required to maintain euglycemia during exercise. Other metabolic counterregulatory responses were also similarly reduced following low- and high-dose cortisol and antecedent hypoglycemia. Plasma lactate, a marker of glycogenolysis and an important substrate for gluconeogenesis, was reduced following both antecedent infusions of cortisol, presumably due to the blunted sympathetic nervous system responses. Similarly, plasma glycerol and blood NEFA responses, indicators of lipolysis, were also blunted by the reduced sympathetic nervous system and growth hormone response. Lipolysis is a key metabolic counterregulatory mechanism during exercise. Glycerol is an important gluconeogenic substrate, and NEFA provides energy for the working muscle and hepatic gluconeogenesis and also inhibits insulin’s ability to suppress hepatic glycogenolysis (19).

Current clinical practice stresses the importance of physical activity in diabetes management. Exercise improves insulin sensitivity, helps in weight maintenance, and lowers the risk of cardiovascular disease. Consequently, growing numbers of those with type 1 diabetes participate in different forms of exercise such as soccer, tennis matches, outdoor hiking, or bike rides with intensity and duration similar to our exercise model. The present study, as well as our previous study (3), has shown that prior hypoglycemia in type 1 diabetes can blunt counterregulatory responses to subsequent exercise, thereby increasing the risk for future hypoglycemia. This present study provides evidence, for the first time, that antecedent physiologic and pharmacologic levels of corticosteroids on a background of hyperinsulinemic euglycemia can produce substantial reductions in counterregulatory responses (similar to prior hypoglycemia) during subsequent exercise in type 1 diabetes.

In summary, this study demonstrated, despite equivalent glucose, insulin, and relative workloads, that antecedent hypoglycemia, physiologic (anteCort1), and supraphysiologic (anteCort2) levels of cortisol blunted neuroendocrine (growth hormone and glucagon), ANS (epinephrine, norepinephrine, and pancreatic polypeptide), and metabolic (glucose kinetics, lipolysis, and glycogenolysis) counterregulatory responses during subsequent exercise in individuals with type 1 diabetes. We conclude that both prior physiologic and pharmacologic increases in cortisol can blunt a wide spectrum of homeostatic responses during subsequent exercise and may play a role in the development of exercise-related counterregulatory failure in those with type 1 diabetes.
ACKNOWLEDGMENTS
This work was supported by research grants from the National Institutes of Health (HL056693 and DK069803), Diabetes Research and Training Grant (DK20593), and General Clinical Research Center Grant (RR00095).

No potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the 69th Scientific Sessions of the American Diabetes Association, New Orleans, Louisiana, 5–9 June 2009.

We thank Eric Allen, Angelina Penalozza, Wanda Snead, Damon Maes, Antoinette Richardson, Anthony Neill, Ginger Barnett, Bin Gong, Nathan Jones, and Shampa Nasreen for expert technical assistance. We are grateful for the exceptional care and support provided by the nursing staff of the Vanderbilt General Clinical Research Center. We thank Jan Hicks for her expert assistance in preparation of this manuscript.

REFERENCES
1. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986
2. The Diabetes Control and Complications Trial Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes 1997;46:271–286
3. Galassetti P, Tate D, Neil RA, Morrey S, Wasserman DH, Davis SN. Effect of antecedent hypoglycemia on counterregulatory responses to subsequent euglycemic exercise in type 1 diabetes. Diabetes 2003;52:1761–1769
4. Davis SN, Galassetti P, Wasserman DH, Tate D. Effects of antecedent hypoglycemia on subsequent counterregulatory responses to exercise. Diabetes 2000;49:73–81
5. Sandoval DA, Guy DLA, Richardson MA, Ertl AC, Davis SN. Effects of low and moderate antecedent exercise on counterregulatory responses to subsequent hypoglycemia in type 1 diabetes. Diabetes 2004;53:1798–1806
6. Galassetti P, Mann S, Tate D, Neil RA, Costa F, Wasserman DH, Davis SN. Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. Am J Physiol Endocrinol Metab 2001;280:E908–E917
7. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R. Role of cortisol in the pathogenesis of deficient counter regulation after antecedent hypoglycemia in normal humans. J Clin Invest 1996;98:680–691
8. McGregor VP, Banarer S, Creyer PE. Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia. Am J Physiol 2002;282:E770–E777
9. Lamberts SW, de Quidiia M, Visscher TJ. Regulation of prolactin secretion in patients with Cushing’s disease. A comparative study on the effects of dexamethasone, lysine vasopressin and ACTH on prolactin secretion by the rat pituitary gland in vitro. Neuroendocrinology 1981;32:150–154
10. Dodb C, Keyser B, Moller M, Felm HL, Elam M. Acute suppression of muscle sympathetic nerve activity by hydrocortisone in humans. Hyper tension 2000;35:738–763
11. Sznajder K, Bagdy G, Stull R, Calogero AE, Kopin JJ, Goldstein D. Sympathoadrenomedullary inhibition by chronic glucocorticoid treatment in conscious rats. Endocrinology 1988;123:2358–2360
12. Golczynska A, Lenders JW, Goldstein DS. Glucocorticoid-induced sympathoinhibition in humans. Clin Pharmacol Ther 1995;58:90–98
13. Scheuer D, Milfin S. Glucocorticoids modulate baroreflex control of renal sympathetic nerve activity. Am J Physiol Regul Integr Comp Physiol 2001;280:E140–E149
14. Flugge G. Effects of cortisol on brain alpha2-adrenoceptors: potential role in stress. Neurosci Biobehav Rev 1999;23:949–956
15. Davis SN, Shavers C, Costa F. Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. J Clin Invest 1997;100:429–438
16. Mion D Jr, Rea RF, Anderson E, Kahn D, Sinkey C, Mark A. Effects of fluodrocortisone on sympathetic nerve activity in humans. Hypertension 1994;23:123–130
17. Komesaroff PA, Funder JW. Differential glucocorticoid effects on catecholamine responses to stress. Am J Physiol 1994;268:E118–E128
18. Sanders NM, Ritter S. Acute 2DG-induced glucoprivation or dexamethasone abolishes 2DG-induced glucoregulatory responses to subsequent glucoprivation. Diabetes 2001;50:2831–2836
19. Sandoval D, Ping L, Neil A, Morrey S, Davis SN. Cortisol acts through central mechanisms to blunt counterregulatory responses to hypoglycemia in conscious rats. Diabetes 2003;52:2198–2204
20. Brown MR, Fisher J. Glucocorticoid suppression of the sympathetic nervous system and adrenal medulla. Life Sci 1986;39:1003–1012
21. Tatarami PA, Larson DE, Sukieter S, Ravusin E. The effects of glucocorticoids in energy metabolism and food intake in humans. Am J Physiol 1996;271:E317–E325
22. Kale AY, Parajape S, Briski KP. L.c.v. administration of the nonsteroidal glucocorticoid receptor antagonist, Cs-47555, prevents exacerbated hypoglycemia during repeated insulin administration. Neuroscience 2006;155:555–565
23. Raju B, McGregor VP, Creyer PE. Cortisol elevations comparable to those that occur during hypoglycemia do not cause hypoglycemia-associated autonomic failure. Diabetes 2003;52:2083–2089
24. Goldberg P, Weiss R, McGrann K, Hintz D, Dzuraj J, Sherwin RS. Antecedent hypercortisolism is not primarily responsible for generating hypoglycemia-associated autonomic failure. Diabetes 2006;55:1121–1126
25. Wasserman K. The anaerobic threshold measurement to evaluate exercise performance. Am Rev Respir Dis 1984;129:535–546
26. Abumrad NN, Rubin D, Diamond MC, Lacy WW. Use of a heated superficial hand vein as an alternative site for measurement of amino acid concentration and for the study of glucose and alanine kinetics in man. Metabolism 1981;30:936–940
27. DeFronzo RA, Tobin K, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E216–E223
28. Wall JS, Steele R, DeBodo RD, Altszuler N. Effect of insulin on utilization and production of circulating glucose. Am J Physiol 1957;189:43–50
29. Aguilar-Parada E, Eisentraut AM, Unger RH. Pancreatic glucagon secretion in normal and diabetic subjects. Am J Med Sci 1969;257:415–419
30. Hanning I, Home PD, Albert KG. Measurement of free insulin concentrations: the influence of the timing of extraction of insulin antibodies. Diabetologia 1985;28:831–835
31. Lawson R, Caruthers M, Rodnight R. Assay of plasma catecholamines by liquid chromatography with electrical detection. Anal Biochem 1981;116:223–226
32. Hunter W, Greenwood F. Preparation of [131I]-labeled human growth hormone of high specific activity. Nature 1962;194:495–496
33. Hagopian W, Lever E, Cen D, Emmounoud M, Polonsky K, Pugh W, Moosa A, Jaspans JB. Predominance of renal and absence of hepatic metabolism of pancreatic polypeptide in the dog. Am J Physiol 1982;243:171–177
34. Lloyd B, Bunnin J, Smythe P, Alberti KGMM. Enzymatic fluorometric continuous-flow assays for blood glucose lactate, pyruvate, alamine, glyc erol, 3-hydroxybutyrate. Clin Chem 1978;24:1724–1729
35. Ho RJ. Radiochemical assay of long chain fatty acid using 63Ni as tracer. Anal Biochem 1970;26:105–113
36. Deary I, Hieburn D, Macleod K, Frier BM. Partitioning the symptoms of hypoglycemia using multi-sample confirmatory factor analysis. Diabetologia 1990;33:771–777
37. Younk I, Tate D, Davis SN. Physical activity in adolescents with type 1 diabetes: is more better for glycemic control? Pediatric Diabetes 2009;10:231–233
38. McGregor VP, Greiwe JS, Banarer S, Creyer P. Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure (Abstract). Diabetes 2001;50(Suppl. 2):A138
39. Bisagno V, Cardoso C, Ortega K, Mion D, Forjac C. Previous exercise attenuates muscle sympathetic activity and increases blood flow during acute euglycemic hyperinsulinaemia. J Appl Physiol 2005;98:866–871
40. Wasserman DH. Control of glucose fluxes during exercise in the postabsorptive state. In Annual Review Physiology. Palo Alto, CA, Annual Reviews Inc., 1995, p. 191–218
41. Rahbaran Z, Shafai K, Alipour S, Mohammadi F, Khani S. Guidelines for premeal insulin dose reduction for postprandial exercise of different intensities and durations in type 1 diabetic subjects treated intensively with a basal-bolus insulin regimen (ultralente-lispro). Diabetes Care 2001;24:625–630
42. Lavoie J, Cardin S, Doiron B. Influence of hepatic vagus nerve on pancreatic hormone secretion during exercise. Am J Physiol 1989;257:E855–E859
43. Kjaer M, Engfred K, Fernandes A, Secher NH, Galbo H. Regulation of glucose production during exercise in the postabsorptive state. In Annual Review Physiology. Palo Alto, CA, Annual Reviews Inc., 1995, p. 191–218
44. Bergh D, Floy JC, Lampman RM, Fojans SS. The effect of adrenergic...
receptor blockade on the exercise-induced rise in pancreatic polypeptide in man. J Clin Endocrinol Metab 1980;50:33–39
45. Barnes B, Marklund S, Steiler TL, Walter M, Hjalm G, Amarger V, Mahlapuu M, Leng Y, Johansson C, Galuska D, Lindgren K, Abrink M, Stapleton D, Zierath JR, Andersson L. The 5′-AMP-activated protein kinase γδ isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. J Biol Chem 2004;279:38441–38447
46. McCrimmon RJ, Fan X, Cheng H, McNay E, Chan O, Shaw M, Ding Y, Zhu W, Sherwin RS. Activation of AMP-activated protein kinase within the ventromedial hypothalamus amplifies counterregulatory hormone responses in rats with defective counterregulation. Diabetes 2006;55:1755–1760
47. McCrimmon RJ, Fan X, Ding Y, Zhu W, Jacob RJ, Sherwin RS. Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. Diabetes 2004;53:1953–1958
48. Kola B, Christ-Crain M, Lolli F, Arnaldi G, Giacchetti G, Boscaro M, Grossman A, Korbonits M. Changes in adenosine 5′-monophosphate-activated protein kinase as a mechanism of visceral obesity in Cushing’s Syndrome. J Clin Endocrinol Metab 2008;93:4969–4973
49. Okada S, Yamaguchi-Shima N, Shimizu T, Arai J, Yorimitsu M, Yokotani K. Centrally administered N-methyl-D-aspartate evokes the adrenal secretion of noradrenaline and adrenaline by brain thromboxane A2-mediated mechanisms in rats. Eur J Pharmacol 2008;586:145–150
50. Liu L, Wang C, Ni X, Sun J. A rapid inhibition of NMDA receptor current by corticosterone in cultured hippocampal neurons. Neuro Sci Lett 2007;420:245–250