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/m^2, 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% $V_{O_{2max}}$.

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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Received 12 March 2009 and accepted 27 May 2009.

Published ahead of print at http://diabetes.diabetesjournals.org on 9 June 2009. DOI: 10.2337/db09-0382. Clinical trial reg. no. NCT00742521, clinicaltrials.gov.

See accompanying commentary, p. 1951.

The Diabetes Control and Complications Trial has definitively demonstrated that intensive glycemic control can reduce microvascular complications in those with type 1 diabetes (1). However, intensive therapy is associated with an increased incidence of hypoglycemia (2). Exercise is a cornerstone of diabetes management. It improves insulin sensitivity, helps in body weight maintenance, and lowers the risk of cardiovascular disease. Unfortunately, exercise is associated with an increased prevalence of hypoglycemia in patients with diabetes. Furthermore, fear of hypoglycemia results in a serious limitation to the widespread implementation of intensive glycemic control and exercise.

Previous studies have shown that antecedent hypoglycemia can blunt neuroendocrine, autonomic nervous system (ANS), and metabolic counterregulatory responses to subsequent exercise (3,4). Reciprocally, antecedent exercise can also blunt homeostatic counterregulatory responses to subsequent hypoglycemia (5,6). Therefore, vicious cycles can be created in type 1 diabetes where an episode of hypoglycemia or exercise can downregulate counterregulatory responses to a subsequent episode of either stress, thereby increasing the risk for further hypoglycemia. However, the mechanisms responsible for exercise-associated counterregulatory failure are not known. Multiple studies have demonstrated that antecedent increases of corticosteroids can blunt subsequent ANS and neuroendocrine responses to a wide spectrum of differing antecedent stress (7–21). Previous studies have also demonstrated that pharmacologic antecedent increases of cortisol can blunt counterregulatory responses to subsequent hypoglycemia in normal subjects (a stress that defends against a falling plasma glucose with similar counterregulatory responses compared with exercise) (7–8). Although, the question of whether physiologic levels of cortisol can blunt counterregulatory responses to subsequent hypoglycemia is still undecided (15,22–24). However, studies specifically investigating the effects of prior elevations of corticosteroids on counterregulatory mechanisms during exercise are lacking. Furthermore, no study has investigated the mechanisms responsible for exercise-related counterregulatory failure in the clinically relevant group of those with type 1 diabetes.

Therefore, the specific aim of this present study was to test the hypothesis that antecedent physiologic or pharmacologic elevations of cortisol could blunt counterregulatory responses during subsequent submaximal exercise in type 1 diabetic individuals. To test this hypothesis, hydrocortisone was administered intravenously on day 1 during hyperinsulinemic-euglycemic clamps and re-

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/m^2, and A1C 7.9 ± 0.2% (normal range 4–6.5%). 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 $V_{O_{2max}}$ and anaerobic threshold (AT). Airflow, O_2, and CO_2 concentrations in inspired and expired air were measured by a computerized open-circuit indirect colorimetric 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 responded to 47 ± 2% of the subject's $V_{O_{2max}}$. 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 $V_{O_{2max}}$ for the group was 34 ± 2 ml · kg^{-1} · min^{-1} (range 24–51 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 hyperinsulinemic-euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with cortisol infusion at 1 μg · kg^{-1} · min^{-1} (anteCort1) 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 tritiated glucose during the study. 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. Throughout the night, glucose was measured every 30 min and the insulin infusion rate was adjusted to maintain blood glucose between 4.4–6.7 mmol/l.

Day 1 procedures. The protocol consisted of an initial period (0–120 min to simulate the isotope equilibration period of day 2), a morning hyperinsulinemic glucose clamp (120–240 min), a rest period (240–360 min), and an afternoon hyperinsulinemic glucose clamp (360–480 min). At 120 min in all studies, a primed continuous infusion of insulin (9 pmol · kg^{-1} · min^{-1}) was started via a Medfusion 3010 pump (Medex-A Furon Healthcare Company, Deluth, GA). Potassium chloride (5 mmol/h) was also infused during each clamp period to reduce insulin-induced hypokalemia. Plasma glucose was measured every 5 min and maintained at the desired level via a variable rate infusion of 20% dextrose via an Imed pump (27). In anteEugly studies, plasma glucose was held constant at basal levels (~5 mmol/l). In anteHypo studies, plasma glucose was allowed to fall over a 30-min period to a target hypoglycemic plateau of ~2.9 mmol/l. In anteCort studies, plasma glucose was held at euglycemia together with a constant 2-h intravenous physiologic infusion of cortisol of 1 μg · kg^{-1} · min^{-1} (anteCort1) or pharmacologic infusion at 2 μg · kg^{-1} · min^{-1} (anteCort2). Thus, in the two latter protocols, the effects of an increase of plasma cortisol, independent of hypoglycemia, on subsequent counterregulatory responses during exercise could be determinined. 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 of 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-^{3}H] glucose (Perkin Elmer Life Sciences, Boston, MA; 11.5 mCi · mmol^{-1} · l^{-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 (~50% $V_{O_{2max}}$). During exercise, the basal insulin infusion rate was adjusted down or maintained at 1 unit/h. This rate of insulin infusion has been shown to reproduce basal levels of insulin (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 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 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 doubled 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 polyethelene 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 procedure for catecholamine determination: *1*) we used a five-point rather than one-point standard calibration curve; *2*) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Cortisol was assayed using the Clinical Assays Gamma Coat Radioimmunoassay (RIA) kit with an interassay CV of 6%. Growth hormone was determinined by RIA (32) with an interassay CV of 8%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (33) with an interassay CV of 8%. 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 sleepy. 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 accepted as statistically significant. The baseline and final 30 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).

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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (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	696 ± 96*	351 ± 46	702 ± 98*
anteCort1	341 ± 39	720 ± 39*	343 ± 39	703 ± 41*
anteCort2	469 ± 55	1,202 ± 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). During antecedent hypoglycemia and low-dose cortisol infusion (anteCort1), there were equivalent increases in day 1 morning and afternoon plasma cortisol levels. High-dose cortisol (anteCort2) 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 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), anteCort1 (20 ± 4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and anteCort2 (19 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) compared with anteEugly (11 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P < 0.05$; Fig. 4). EGP was significantly lower in anteCort1 (11 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and anteCort2 (14 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) compared with anteEugly (22 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P < 0.05$; Fig. 4).

Intermediary metabolism. Basal levels of lactate, alanine, β-hydroxybutyrate glycerol, and NEFA levels were

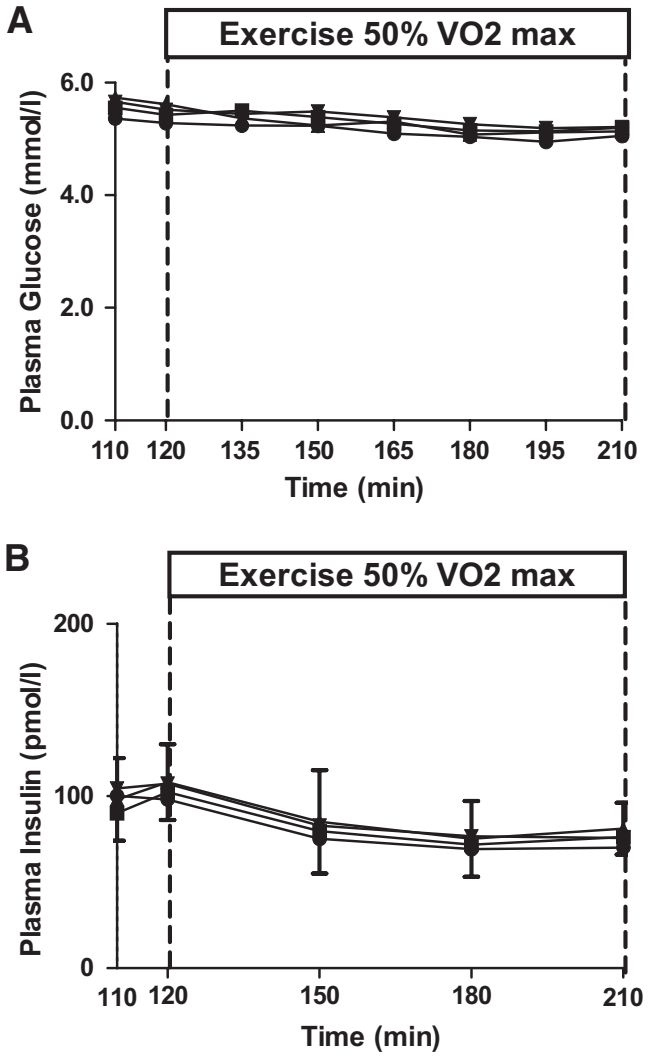

FIG. 1. Plasma glucose (*A*) and insulin (*B*) concentrations (means ± SE) during day 2 euglycemic exercise studies in 22 patients (11 men/11 women) with type 1 diabetes. On the previous day, patients had undergone two 120-min euglycemic clamps (●, anteEugly), hypoglycemic clamps (■, anteHypo), or euglycemic 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.

similar and are shown in Table 3. There were significantly different responses of intermediary metabolites during day 2 exercise. Following day 1 hypoglycemia, anteCort1, and anteCort2, glyerol and NEFA responses during exercise were reduced compared with anteEugly ($P < 0.05$). 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 usual physiologic fall that occurs in nondiabetic individu-

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} (anteCort) 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).

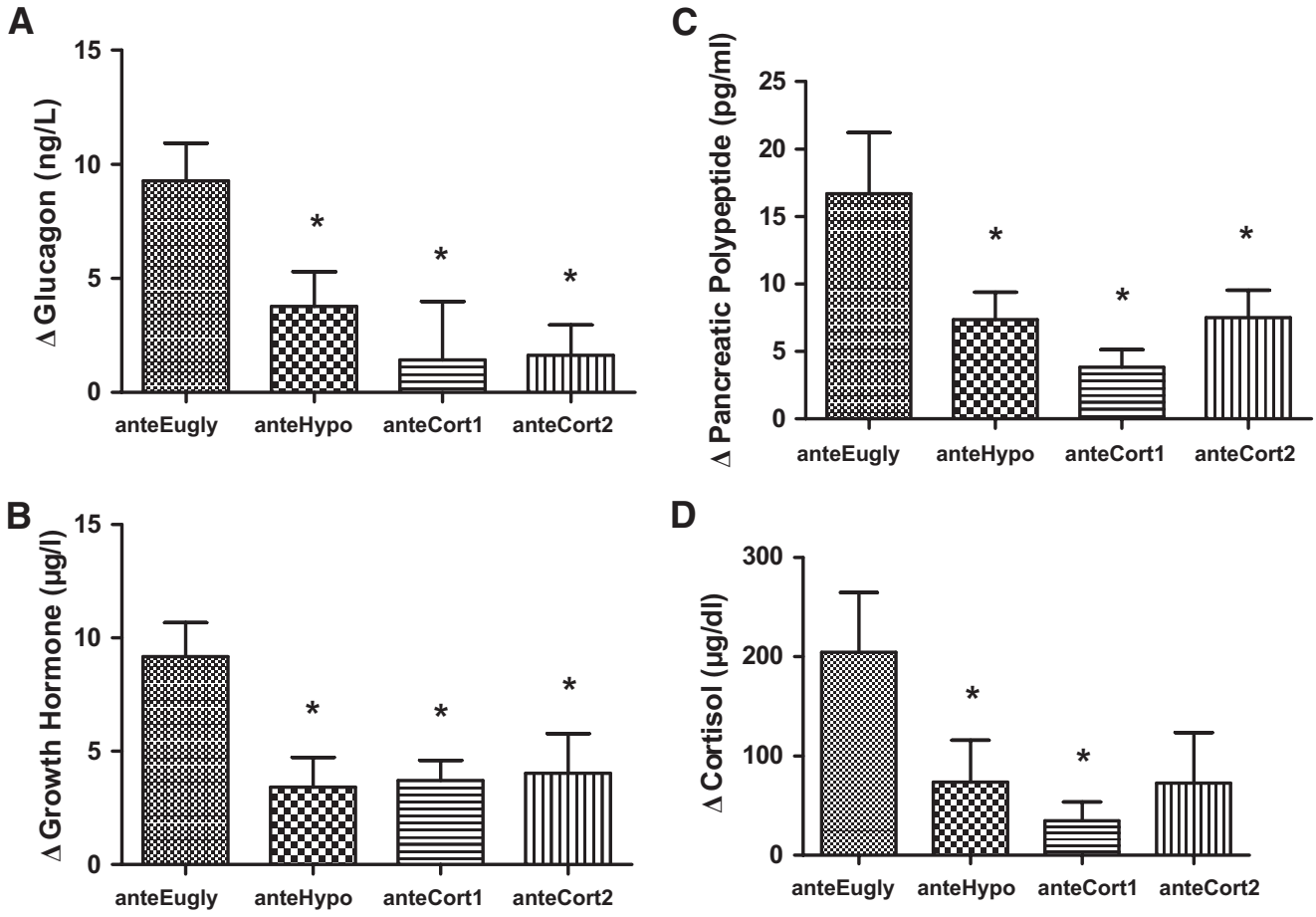

FIG. 2. Average plasma glucagon (*A*), growth hormone (*B*), pancreatic polypeptide (*C*), and cortisol (*D*) levels (means ± SE) during the final 30 min of day 2 exercise in 22 type 1 diabetic patients (11 men/11 women). On the previous day, patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort2) on day 1. There were significantly fewer increments of glucagon, growth hormone, and pancreatic polypeptide during the final 30 min of exercise from basal levels in anteHypo and anteCort compared with anteEugly. *$P < 0.05$.

als. 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-adrenoreceptor 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.

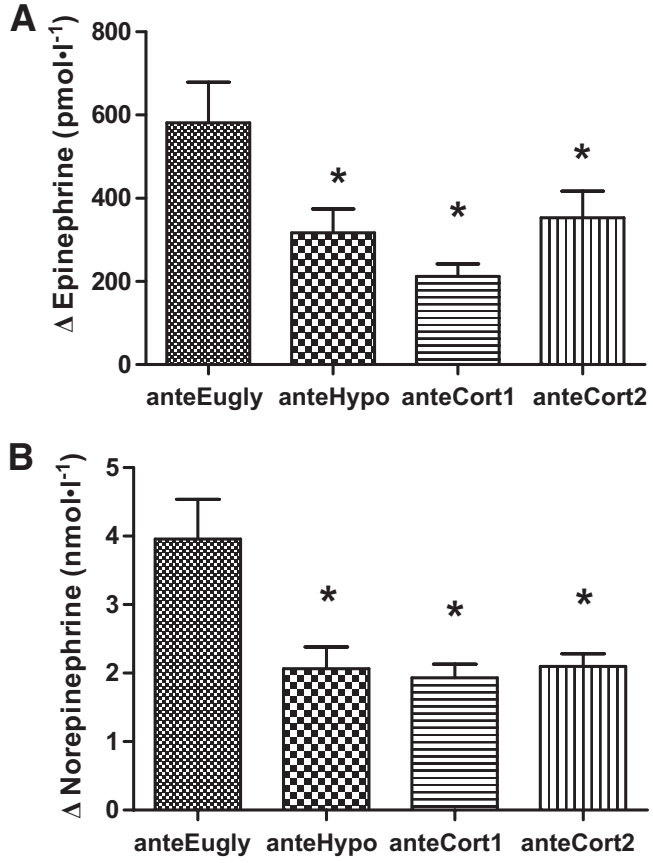

FIG. 3. Plasma epinephrine (*A*) and norepinephrine (*B*) levels (means ± SE) during the final 30 min of day 2 exercise in 22 type 1 diabetic patients (11 men/11 women). On the previous day, patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (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. *$P < 0.05$.

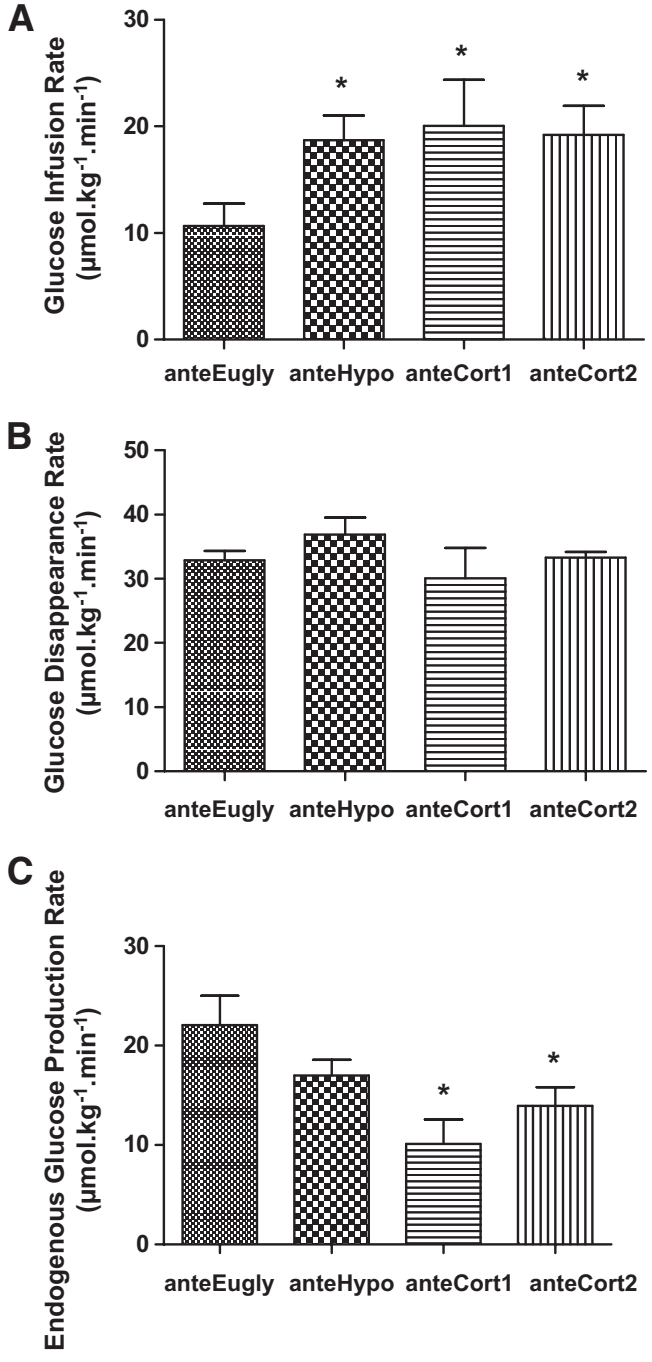

FIG. 4. Glucose kinetics (means ± SE) during the final 30 min of day 2 exercises in 22 type 1 diabetic patients (11 men/11 women). *A*: Glucose infusion rate. *B*: Glucose utilization. *C*: EGP. On the previous day, patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps, or euglycemic clamps with intravenous cortisol infusions at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort2) on day 1. *$P < 0.05$ versus anteEugly.

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,

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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (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	329 ± 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 ($P < 0.05$).

TABLE 4
Cardiovascular responses during day 2 exercise euglycemic clamp studies after patients had undergone two 120-min euglycemic clamps (anteEugly), hypoglycemic clamps (anteHypo), or euglycemic clamps with intravenous cortisol infusions of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort1) or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (anteCort2) on day 1

	Basal period	Final 30 min of exercise
Heart rate (bpm)		
anteEugly	70 ± 3	136 ± 4*
anteHypo	70 ± 3	132 ± 3*
anteCort1	71 ± 2	131 ± 4*
anteCort2	69 ± 3	135 ± 3*
Systolic blood pressure (mmHg)		
anteEugly	113 ± 3	134 ± 4*
anteHypo	115 ± 3	136 ± 5*
anteCort1	114 ± 3	141 ± 5*
anteCort2	111 ± 3	134 ± 4*
Diastolic blood pressure (mmHg)		
anteEugly	70 ± 3	69 ± 5
anteHypo	69 ± 2	68 ± 2
anteCort1	69 ± 3	75 ± 4
anteCort2	69 ± 3	65 ± 2
Mean blood pressure (mmHg)		
anteEugly	86 ± 3	89 ± 2
anteHypo	86 ± 2	91 ± 2
anteCort1	86 ± 7	85 ± 2
anteCort2	83 ± 2	87 ± 2

Data are means ± SEM. $n = 22$ type 1 diabetic subjects (11 men/11 women). *Significant difference versus basal level ($P < 0.05$).

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 Penaloza, 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.

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