

Effects of Antecedent Hypoglycemia on Subsequent Counterregulatory Responses to Exercise

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Antecedent hypoglycemia can blunt counterregulatory responses to subsequent hypoglycemia. It is uncertain, however, if prior hypoglycemia can blunt counterregulatory responses to other physiologic stresses. The aim of this study, therefore, was to determine whether antecedent hypoglycemia attenuates subsequent neuroendocrine and metabolic responses to exercise. Sixteen lean, healthy adults (eight men and eight women, ages 28 ± 2 years, BMI 22 ± 1 kg/m², $\dot{V}O_{2\max}$ 43 ± 3 ml · kg⁻¹ · min⁻¹) were studied during 2-day protocols on two randomized occasions separated by 2 months. On day 1, subjects underwent morning and afternoon 2-h hyperinsulinemic (528 ± 30 pmol/l) glucose clamp studies of 5.3 ± 0.1 mmol/l (euglycemic control) or 2.9 ± 0.1 mmol/l (hypoglycemic study). On day 2, subjects underwent 90 min of exercise on a static cycle ergometer at 80% of their anaerobic threshold ($\sim 50\% \dot{V}O_{2\max}$). Glycemia was equated during day 2 exercise studies via an exogenous glucose infusion. Day 1 hypoglycemia had significant effects on neuroendocrine and metabolic responses during day 2 exercise. The usual exercise-induced reduction in insulin, together with elevations of plasma epinephrine, norepinephrine, glucagon, growth hormone, pancreatic polypeptide, and cortisol levels, was significantly blunted after day 1 hypoglycemia ($P < 0.01$). Commensurate with reduced neuroendocrine responses, key metabolic counterregulatory mechanisms of endogenous glucose production (EGP), lipolytic responses, and ketogenesis were also significantly attenuated ($P < 0.01$) after day 1 hypoglycemia. Significantly greater rates of glucose infusion were required to maintain euglycemia during exercise after day 1 hypoglycemia compared with day 1 euglycemia (8.8 ± 2.2 vs. 0.6 ± 0.6 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.01$). During the first 30 min of exercise, day 1 hypoglycemia had little effect on EGP, but during the latter 60 min of exercise, day 1 hypoglycemia was associated with a progressively smaller increase in EGP compared with day 1 euglycemia. Thus, by 90 min, the entire exercise-induced increment in EGP (8.8 ± 1.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was abolished by day 1 hypoglycemia. We con-

clude that 1) antecedent hypoglycemia results in significant blunting of essential neuroendocrine (glucagon, insulin, catecholamines) and metabolic (endogenous glucose production, lipolysis, ketogenesis) responses to exercise; 2) antecedent hypoglycemia may play a role in the pathogenesis of exercise-related hypoglycemia in type 1 diabetic patients; and 3) antecedent hypoglycemia can blunt counterregulatory responses to other physiologic stresses in addition to hypoglycemia. *Diabetes* 49:73–81, 2000

During exercise, a complex interplay of neuroendocrine and autonomic nervous system (ANS) counterregulatory responses are activated so that plasma glucose concentration may be preserved (1). In normal individuals, these counterregulatory responses are effective in preventing hypoglycemia in all but the most extreme examples of prolonged exercise (2). The situation in patients with type 1 diabetes, however, is very different. Hypoglycemia can occur in relation to almost any form of exercise and is a major limitation to daily activities (3). The *in vivo* mechanisms responsible for the increased prevalence of exercise-related hypoglycemia in type 1 diabetes have not been fully elucidated. Sonnenberg et al. (4) have suggested that hypoglycemia occurs because of an absolute or relative excess of insulin, increased sensitivity to the hormone, or both. It is somewhat intriguing that the relatively modest hyperinsulinemia present in most type 1 diabetes could produce severe hypoglycemia in the presence of fully functioning counterregulatory responses. Accordingly, two recent studies have provided evidence that blunted counterregulatory responses are involved in the pathogenesis of exercise-related hypoglycemia in type 1 diabetes. Bottini et al. (5) demonstrated that patients with classical diabetic autonomic neuropathy have attenuated epinephrine responses during exercise. Furthermore, Schneider et al. (6) reported that catecholamine responses are blunted when tightly controlled type 1 diabetic patients exercise during hypoglycemic conditions. Implicit in the latter study was that counterregulatory failure was acquired due to intensive metabolic control (rather than classical diabetic autonomic neuropathy).

It is now appreciated that intensive therapy can produce syndromes of acquired hypoglycemia counterregulatory failure in type 1 diabetic patients (7). Recent work has identified the central importance of antecedent hypoglycemia in the pathogenesis of these syndromes of counterregulatory failure (8–11). We reasoned, therefore, that since antecedent hypoglycemia results in blunted counterregulatory responses to hypoglycemia, it may also cause attenuated

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ANOVA, analysis of variance; ANS, autonomic nervous system; AT, anaerobic threshold; CRC, Clinical Research Center; CV, coefficient of variation; HPLC, high-pressure liquid chromatography; NEFA, nonesterified fatty acid; R_a , glucose appearance; RIA, radioimmunoassay; SNS, sympathetic nervous system.

counterregulatory responses to other related physiologic stresses such as exercise. Thus a unifying hypothesis can be formulated stating that antecedent hypoglycemia blunts counterregulatory responses to subsequent exercise and is therefore implicated in the pathogenesis of exercise-related hypoglycemia in type 1 diabetic patients. To date, there are data both for and against this hypothesis. Rattarasarn et al. (12) reported that prior hypoglycemia in a group of type 1 diabetic patients only blunted counterregulatory responses to subsequent hypoglycemia but had no effect on ANS responses to other stresses, including exercise. Challenging this report are data indicating that ANS counterregulatory responses to a variety of stresses can be reduced by prior elevations of glucocorticoids (13–19). The latter studies provide a rationale for determining whether other stresses (in addition to hypoglycemia) that result in endogenous elevations of glucocorticoids may also blunt subsequent ANS counterregulatory responses.

The question of whether antecedent hypoglycemia can diminish counterregulatory responses to exercise is, of course, clinically relevant. If it can, then the deleterious effects of antecedent hypoglycemia on counterregulatory responses would be even more extensive than currently thought. A vicious cycle could arise where iatrogenic antecedent hypoglycemia would diminish counterregulatory responses during subsequent exercise, thereby resulting in hypoglycemia that would in turn create an increased risk for future episodes of hypoglycemia. Therefore, the aim of this study was to determine if antecedent hypoglycemia could modify neuroendocrine, ANS, and metabolic responses during subsequent exercise in a group of healthy men and women. This study could serve as a first step in determining whether prior hypoglycemia is involved in the pathogenesis of exercise-related hypoglycemia in type 1 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. We studied 16 healthy volunteers (eight men and eight women), ages 28 ± 2 years, with a BMI of 22 ± 1 kg/m² and HbA_{1c} of $4.6 \pm 0.1\%$ (normal range 4–6.5). None were taking medication or had a family history of diabetes. Each subject had normal blood count, plasma electrolytes, and liver and renal function. All 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 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 P.M. on the evening before an experiment. All subjects were studied after an overnight 10-h fast.

Experimental design. At least 2 weeks before the initial study, subjects performed an incremental work test on a stationary cycle ergometer to determine $\dot{V}O_{2max}$ and anaerobic threshold (AT). Air flow and O₂ and CO₂ concentrations in inspired and expired air were measured by a computerized open-circuit indirect calorimetry (Cardio2 cycle; Medical Graphics, Yorba Linda, CA) with a mouth-piece and nose clip system. AT was determined by the V-slope method (20). The 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 (21). At workloads below the AT, exercise can be continued for a prolonged period, whereas above the AT fatigue will occur considerably faster (22). Experimental work rate was established by calculating 80% AT, which corresponded to $47 \pm 4\%$ of the subjects $\dot{V}O_{2max}$. 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 actively participating in competitive sports. Mean $\dot{V}O_{2max}$ for the group was 43 ± 3 ml · kg⁻¹ · min⁻¹ (range 21–54).

Day 1 glucose clamp studies. Each subject attended two separate, randomized, 2-day experiments separated by at least 2 months. On the morning of the 1st day of each study (after an overnight fast), 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 the hand. This hand was placed in a heated box

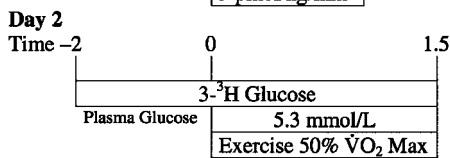
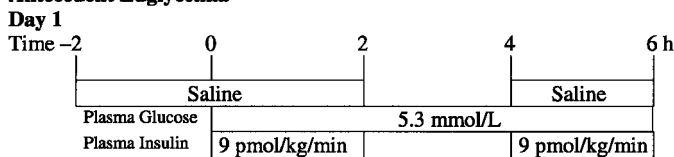
(55–60°C) so that arterialized blood could be obtained (23). The other cannula was placed in the contralateral arm so that 20% glucose could be infused via a variable-rate volumetric infusion pump (Imed, San Diego, CA).

Antecedent hypoglycemia experiments. On the morning of day 1, after insertion of venous cannulas, a period of 90 min elapsed followed by a 30-min basal control period and a 120-min hyperinsulinemic-hypoglycemic experimental period (Fig. 1). At time 0, a primed continuous infusion of insulin was administered at a rate of 9 pmol · kg⁻¹ · min⁻¹ for 120 min (24). Plasma glucose levels were measured every 5 min, and a variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant at 2.9 ± 0.1 mmol/l (25). Potassium chloride was infused at a rate of 5 mmol/h during each study. After completion of the initial 2-h test period, the insulin infusion was discontinued, and plasma glucose was rapidly restored to euglycemia with 20% dextrose. Plasma glucose was maintained at euglycemia for a further 2 h by adjusting an exogenous glucose infusion. At that point, insulin was restarted, and a 2nd hyperinsulinemic-hypoglycemic clamp, identical to the morning clamp, was performed. After completion of the 2nd glucose clamp, subjects consumed a large meal and bedtime snack and remained in the CRC. **Antecedent euglycemia experiments (control studies).** These experiments followed a format similar to the hypoglycemia experiments, with the exception that identical morning and afternoon hyperinsulinemic-euglycemic clamps (5.3 ± 0.1 mmol/l) were performed (26). At completion of the 2nd euglycemic clamp, subjects consumed an identical evening meal and snack as in the hypoglycemia experiments.

Day 2 exercise experiments. Day 2 experiments involved a standardized exercise protocol to assess the effects of day 1 glycemia on neuroendocrine, ANS, and metabolic counterregulatory responses. After a 10-h overnight fast, a 3rd glucose clamp study was performed. To measure glucose kinetics, a primed (18 μCi) constant infusion (0.18 μCi/min) of [³H]glucose (New England Nuclear, Boston, MA) was started at -120 min and continued for 120 min. At time 0, subjects commenced 90 min of continuous submaximal exercise (at 60 rpm) on an upright cycle ergometer at 80% of the individual's anaerobic threshold ($\sim 50\% \dot{V}O_{2max}$). Plasma glucose was maintained equivalent to baseline levels during all exercise studies by a glucose clamp technique. Infusion rates of [³H]glucose were doubled during the 1st 20 min of exercise to minimize changes in glucose specific activity and isotopic enrichment (27).

Rates of glucose appearance (R_a), EGP, and glucose utilization were calculated according to the methods of Wall et al. (28). EGP was calculated by determining the total rate of R_a (both endogenous glucose production and any exogenous glucose infused to maintain euglycemia) and subtracting the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, since underestimates of total R_a and glucose disposal (R_d) can be obtained (29).

Antecedent Euglycemia



Antecedent Hypoglycemia

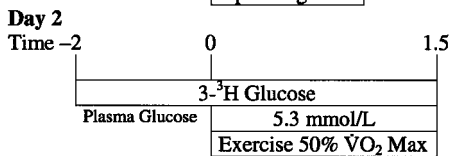
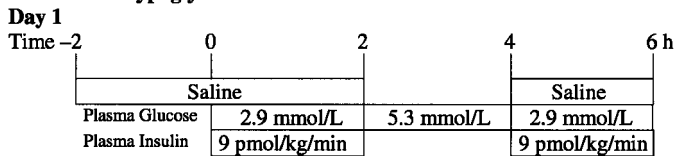


FIG. 1. Schematic diagram of experimental protocol for overnight-fasted male and female normal individuals (n = 16). Each 2-day study was separated by 2 months and performed in a randomized fashion.

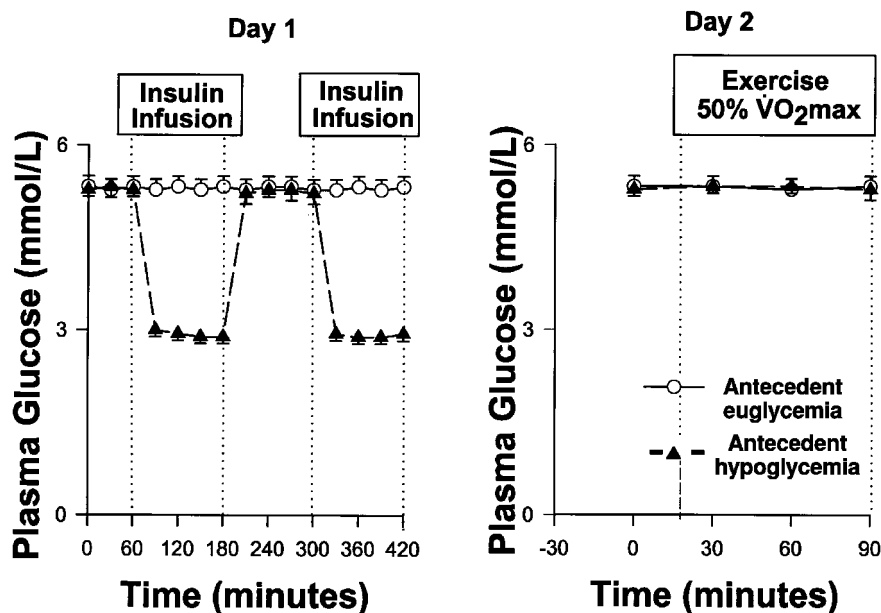


FIG. 2. A: Glucose levels during day 1 hyperinsulinemic-euglycemic and hypoglycemic clamps. B: Glucose levels during day 2 exercise at 50% $\dot{V}O_{2max}$.

This underestimate can be largely overcome by using a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity), as was done in the present experiments.

Analytical methods. The collection and processing of blood samples have been described (30). Plasma glucose concentrations were measured in triplicate using 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. (31), with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (32), with an interassay CV of 9%. Catecholamines were determined by high-pressure liquid chromatography (HPLC) (33), 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 5-point rather than a 1-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 could be made of the relevant respective catecholamine peaks. Cortisol was assayed using the Clinical Assays Gamma Coat radioimmunoassay (RIA) kit (Diagnostic Products, Los Angeles, CA), with an interassay CV of 6%. Growth hormone was determined by RIA (34), with an interassay CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (35), with an interassay CV of 8%. Lactate, glycerol, alanine, and β -hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (36). Nonesterified fatty acids (NEFAs) were measured using the Wako kit (Wako, Richmond, VA) adopted for use on a centrifugal analyzer (37).

Blood samples for glucose flux were taken every 10 min throughout the control period and every 15 min during the experimental period. Blood to measure hormones and intermediary metabolites was drawn twice during the control period and every 15 min during the experimental period. Cardiovascular parameters (pulse, systolic and diastolic blood pressure, and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each 210-min study. Gas exchange measurements were performed during the control period and during the final 30 min of exercise.

Materials. HPLC-purified [^3H]glucose was used as the glucose tracer ($11.5\text{mCi} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$). Human regular 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 mean \pm SE, unless otherwise stated, and analyzed using standard, parametric, two-way analysis of variance (ANOVA) with repeated-measures design. This analysis was coupled with Duncan's post hoc test to delineate at what time statistical significance was reached. A P value of <0.05 indicated significant difference.

RESULTS

Plasma glucose and insulin levels from day 1 studies. Steady-state insulin levels were similar during morning and afternoon euglycemic and hypoglycemic studies (528 ± 40 pmol/l). Plasma glucose levels reached steady state by

30 min (Fig. 2) and were maintained at 5.3 ± 0.1 and 2.9 ± 0.1 mmol/l during the remainder of the 120-min euglycemic and hypoglycemic studies, respectively.

Insulin, glucose, and counterregulatory hormone levels from day 2 exercise studies. Plasma glucose levels were equivalent at the start of exercise after day 1 euglycemia (5.3 ± 0.1 mmol/l) and hypoglycemia (5.3 ± 0.2 mmol/l). Glycemia was maintained at baseline during exercise and was equivalent (5.3 ± 0.2 mmol/l) after day 1 euglycemia or hypoglycemia (Fig. 2). Insulin levels were identical at the start of exercise (48 ± 6 pmol/l), but by the final 30 min, the usual fall in insulinemia was significantly blunted by prior hypoglycemia compared with euglycemia (decrease of 25 ± 3 vs. $50 \pm 4\%$; $P < 0.01$).

Basal levels of counterregulatory hormones were equivalent at the start of exercise. During exercise, neuroendocrine responses were significantly blunted by day 1 hypoglycemia (Figs. 3–5). By the final 30 min of exercise, plasma epinephrine levels were 437 ± 93 and 753 ± 153 pmol/l ($P < 0.01$) after day 1 hypoglycemia and euglycemia, respectively. Norepinephrine levels were also significantly blunted after day 1 hypoglycemia compared with euglycemia (4.7 ± 0.8 vs. 7.5 ± 1.2 nmol/l, $P < 0.01$). Glucagon levels did not increase during exercise after day 1 hypoglycemia (43 ± 4 and 46 ± 4 ng/l) but increased significantly (from 42 ± 3 to 52 ± 3 ng/l; $P < 0.01$) after day 1 euglycemia. Plasma cortisol levels (Fig. 5) also remained similar to baseline after day 1 hypoglycemia (276 ± 55 and 345 ± 69 nmol/l) but increased significantly during exercise after day 1 euglycemia (from 276 ± 28 to 469 ± 69 nmol/l; $P < 0.01$). Pancreatic polypeptide (Fig. 5) increased twofold (from 20 ± 4 to 38 ± 6 nmol/l) during exercise after day 1 hypoglycemia, a blunted response ($P < 0.05$) compared with the threefold increase (from 15 ± 2 to 45 ± 7 nmol/l) after day 1 euglycemia. Growth hormone levels (Fig. 5) increased from 2.5 ± 1.0 to 6.3 ± 1 $\mu\text{g/l}$ after day 1 hypoglycemia, a reduced response ($P < 0.01$) compared with the increase after day 1 euglycemia (from 2.5 ± 1.0 to 12.0 ± 3 $\mu\text{g/l}$).

Glucose kinetics. Glucose specific activity (disintegrations per minute per millimole) was in a steady state during the control period and final 30 min of each exercise protocol

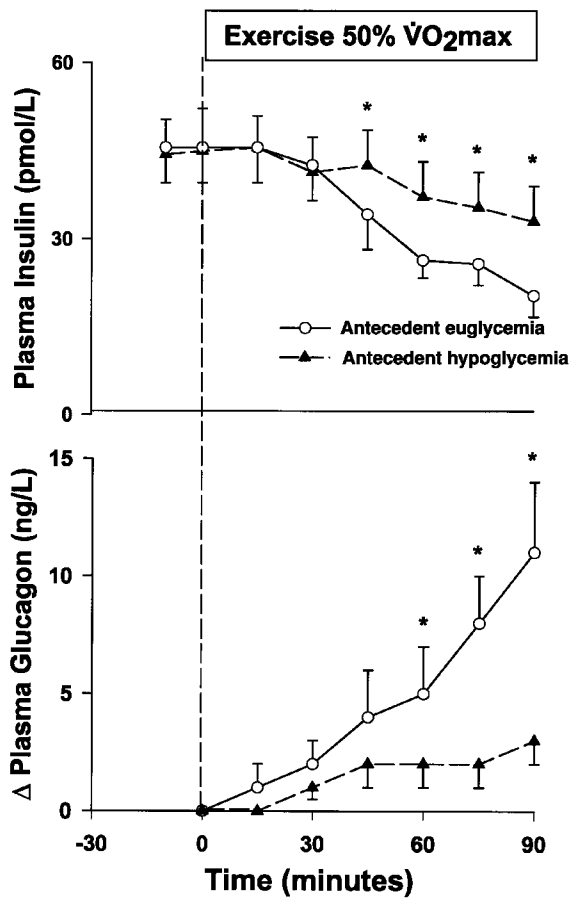


FIG. 3. Effects of 90 min of exercise at 50% $V_{O_{2max}}$ on plasma insulin levels and incremental glucagon responses. The fall in plasma insulin was significantly attenuated by day 1 hypoglycemia ($P < 0.01$, ANOVA). Glucagon responses were blunted by day 1 hypoglycemia compared with day 1 euglycemia ($P < 0.02$, ANOVA). *Significant differences ($P < 0.05$) in individual time points after day 1 hypoglycemia or euglycemia.

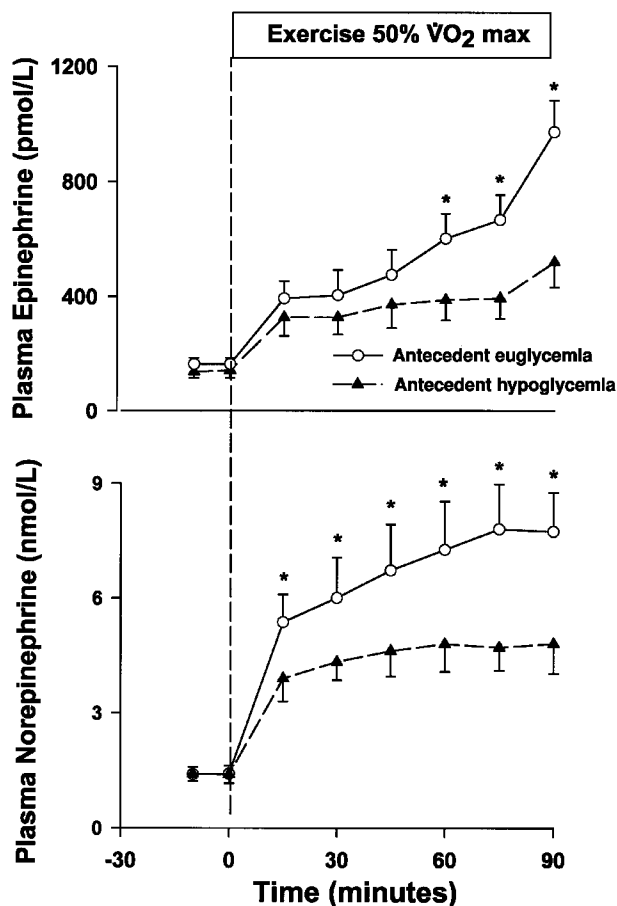


FIG. 4. Effects of 90 min of exercise at 50% $V_{O_{2max}}$ on plasma epinephrine and norepinephrine levels. Epinephrine and norepinephrine levels were significantly blunted by day 1 hypoglycemia compared with day 1 euglycemia ($P < 0.01$, ANOVA). *Significant differences ($P < 0.05$) in individual time points after day 1 hypoglycemia or euglycemia.

(Table 1). EGP increased from 11 ± 0.6 to $20.0 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during exercise after day 1 euglycemia but, after an initial increase, declined to baseline ($9.9 \pm 2.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) after day 1 hypoglycemia (Fig. 6). Minimal exogenous glucose was required ($0.6 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) to maintain glucose at baseline during exercise after day 1 euglycemia. The amount of glucose required to maintain euglycemia during exercise after day 1 hypoglycemia was significantly greater ($P < 0.01$) and increased to $9.4 \pm 2.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by the final 15 min of exercise (Table 1). Tracer-determined glucose disappearance rates were similar during all day 2 exercise studies.

Intermediary metabolism. The usual exercise-induced metabolic responses were significantly blunted by day 1 hypoglycemia (Table 2). Blood lactate levels remained similar to baseline (980 ± 140 to $1,163 \pm 150 \mu\text{mol/l}$) after day 1 hypoglycemia, a reduced response ($P < 0.01$) compared with increases (990 ± 120 to $1,825 \pm 320 \mu\text{mol/l}$) after day 1 euglycemia. Increases in blood glycerol levels after day 1 hypoglycemia (50 ± 5 to $150 \pm 10 \mu\text{mol/l}$) were also attenuated ($P < 0.01$) compared with elevations after day 1 euglycemia (50 ± 7 to $180 \pm 15 \mu\text{mol/l}$). The ketone body β -hydroxybutyrate remained at basal levels during exercise after day 1 hypo-

glycemia (24 ± 4 to $24 \pm 5 \mu\text{mol/l}$) but increased after antecedent euglycemia (25 ± 3 to $38 \pm 7 \mu\text{mol/l}$; $P < 0.01$). Basal levels of NEFAs were different at the start of exercise; therefore, plasma NEFA responses were analyzed as proportional changes relative to baseline. Consistent with other intermediary metabolic results, there was a greater incremental increase in NEFA levels during exercise after day 1 euglycemia compared with day 1 hypoglycemia (64 ± 5 vs. $35 \pm 6\%$, $P < 0.02$). Blood alanine levels were similar during exercise after day 1 euglycemia or hypoglycemia.

Gas exchange measurements. Basal gas exchange values were similar at the start of day 2 exercise studies: \dot{V}_{O_2} , 215 ± 15 and $215 \pm 12 \text{ ml/min}$; \dot{V}_{CO_2} , 188 ± 12 and $185 \pm 11 \text{ ml/min}$; and respiratory quotient, 0.86 ± 0.01 and 0.84 ± 0.01 after day 1 euglycemia and hypoglycemia, respectively. Work rate ($90 \pm 10 \text{ W}$) and gas exchange measurements were equivalent and in steady state during the final 30 min of exercise: \dot{V}_{O_2} , $1,422 \pm 115$ vs. $1,385 \pm 118 \text{ ml/min}$; \dot{V}_{CO_2} , $1,308 \pm 110$ vs. $1,274 \pm 115 \text{ ml/min}$; and respiratory quotient, 0.92 ± 0.1 vs. 0.92 ± 0.01 after day 1 euglycemia and hypoglycemia, respectively. Cardiovascular parameters. Heart rate, systolic and diastolic blood pressure, and mean arterial pressure increased equivalently during exercise after day 1 hypoglycemia or euglycemia (Table 3).

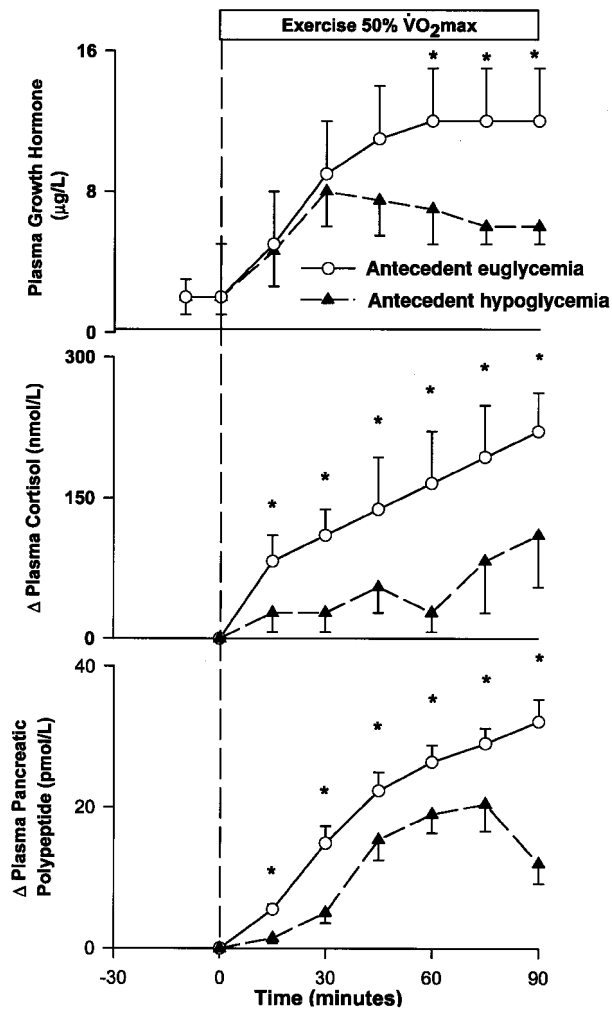


FIG. 5. Effects of 90 min of exercise at 50% $\dot{V}O_{2max}$ on plasma growth hormone, incremental cortisol, and pancreatic polypeptide responses. Growth hormone, cortisol, and pancreatic polypeptide responses were significantly blunted by day 1 hypoglycemia compared with day 1 euglycemia ($P < 0.01$, ANOVA). *Significant difference ($P < 0.05$) in individual time points after day 1 hypoglycemia or euglycemia.

DISCUSSION

The aim of this study was to determine the effects of antecedent hypoglycemia on neuroendocrine and metabolic responses during subsequent exercise in healthy humans. Our results demonstrate that two episodes of prior moderate

hypoglycemia (2.9 ± 0.1 mmol/l) can substantially reduce (by ~50%) exercise-induced counterregulatory hormone and metabolic responses the next day.

Classically, exercise-induced severe hypoglycemia has been attributed to either an absolute or relative excess of insulin (6). Although this mechanism must play an important role, it appears unlikely that the relatively modest hyperinsulinemia present in most type 1 diabetic patients could cause severe hypoglycemia in the presence of fully functioning counterregulatory responses. Further emphasizing this point, neuroendocrine and ANS counterregulatory responses in nondiabetic individuals are actually significantly amplified when exercise occurs during mild hypoglycemic conditions (38). The increased counterregulatory responses would thus serve as a barrier against further severe hypoglycemia developing during exercise. Therefore, a hypothesis that blunted counterregulatory responses are involved in the pathogenesis of exercise-related severe hypoglycemia appears plausible and is supported by the experimental data of Schneider et al. (6).

Several studies have identified the central importance of antecedent hypoglycemia in the pathogenesis of blunted counterregulatory responses to subsequent hypoglycemia (7–13). Therefore, since many components of ANS and neuroendocrine counterregulatory responses to exercise and hypoglycemia are conceptually similar, we reasoned in this study that prior hypoglycemia may also blunt subsequent counterregulatory responses to exercise.

Neuroendocrine and ANS responses during exercise are significantly modulated by prevailing glycemia (38,39). On the one hand, mild hypoglycemia can amplify glucagon and catecholamine responses (38), whereas even mild hyperglycemia can attenuate these critical responses (39). In the present study, therefore, glycemia was carefully equated so that plasma glucose levels were identical during all day 2 exercise studies. To ensure that neuroendocrine responses were not attenuated by overreplacing with exogenous glucose, however, glycemic targets during our clamp experiments were set at 0.2 mmol/l below baseline. By setting glycemic targets below baseline in the posthypoglycemia experiments, we avoided the possibility of creating an increased signal for insulin secretion while conservatively allowing for a possible increased drive for neuroendocrine responses.

During exercise, glycemia is preserved within narrow limits because increased glucose production matches the demand created by elevated skeletal muscle glucose utilization. Increased glucose production results primarily from

TABLE 1

Effects of day 1 hyperinsulinemic ($9.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) euglycemia (5.3 ± 0.1 mmol/l) and hypoglycemia (2.9 ± 0.1 mmol/l) on glucose specific activity and glucose infusion rates during day 2 50% $\dot{V}O_{2max}$ exercise

	Control period	Duration of exercise (min)			
		30	60	75	90
Glucose specific activity (dpm/mmol)					
Antecedent euglycemia	428 ± 20	406 ± 23	397 ± 19	402 ± 19	398 ± 18
Antecedent hypoglycemia	459 ± 22	439 ± 18	441 ± 21	443 ± 22	434 ± 21
Glucose infusion rates ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)					
Antecedent euglycemia	0	0	0	0.6 ± 0.6	1.1 ± 1.1
Antecedent hypoglycemia	0	$1.8 \pm 0.6^*$	$5.5 \pm 1.8^*$	$8.8 \pm 2.2^*$	$10.0 \pm 2.2^*$

Data are means \pm SE. *Significantly increased ($P < 0.01$) compared with antecedent euglycemia. dpm, disintegrations per minute.

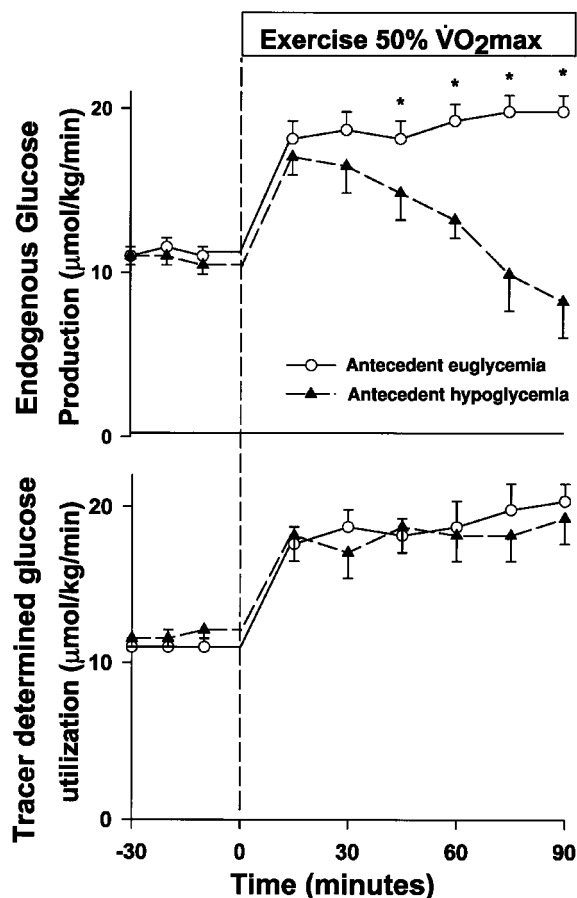


FIG. 6. Effects of 90 min of exercise at 50% $\dot{V}O_{2max}$ on tracer-determined glucose kinetics. EGP was significantly reduced after day 1 hypoglycemia compared with day 1 euglycemia ($P < 0.01$). *Significant differences ($P < 0.05$) in individual time points after day 1 hypoglycemia or euglycemia.

increased glucagon release, decreased insulin secretion, and a smaller contribution from elevated adrenergic drive (1). After day 1 hypoglycemia, each of these three key responses was blunted during day 2 exercise. Glucagon, a primary stimulus for increased hepatic glucose production, remained at basal levels during exercise after day 1 hypoglycemia but increased significantly after antecedent euglycemia. It should be stressed, however, that quantitatively peripheral levels of glucagon during exercise are a very conservative indicator of the physiologically relevant prehepatic (i.e., portal vein) values (40). During exercise, because of a combination of hepatic extraction and reduced hepatic blood flow, glucagon levels are ~2.5-fold greater in the portal vein relative to the peripheral circulation (40). That being the case, it can be inferred that portal vein levels of glucagon during exercise were dramatically reduced two- to threefold after day 1 hypoglycemia compared with antecedent euglycemia. In other words, the physiologic effects of day 1 hypoglycemia on blunting glucagon responses during exercise were likely to have been far greater than apparent from just the differences in peripheral levels of the hormone.

During prolonged moderate-intensity exercise, insulin levels fall ~50% from baseline. Together with increased glucagon levels, decreased insulin secretion is an important contributory mechanism for elevated glucose production during exercise (1). In the present study, the normal exercise-induced fall in insulin levels was attenuated by prior hypoglycemia. Insulin levels fell by only $25 \pm 3\%$ after day 1 hypoglycemia compared with $50 \pm 4\%$ after antecedent euglycemia. Decreased insulin secretion during exercise is primarily mediated via increased α -adrenergic activity (1). We therefore speculate that prior hypoglycemia reduces sympathetic drive to the pancreas, thereby leading to increased insulin levels during day 2 exercise. Consistent with the premise of reduced sympathetic drive after day 1 hypoglycemia, plasma epinephrine and norepinephrine responses were also significantly blunted during day 2 exercise. Additionally, plasma pancreatic polypeptide responses (a marker

TABLE 2

Effects of day 1 hyperinsulinemic ($9.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) euglycemia ($5.3 \pm 0.1 \text{ mmol/l}$) and hypoglycemia ($2.9 \pm 0.1 \text{ mmol/l}$) on intermediary metabolite results during day 2 50% $\dot{V}O_{2max}$ exercise

	Control period	Duration of exercise (min)			
		30	60	75	90
Blood lactate ($\mu\text{mol/l}$)					
Antecedent euglycemia	990 ± 120	$2,760 \pm 600$	$1,998 \pm 310$	$1,880 \pm 305$	$1,785 \pm 290$
Antecedent hypoglycemia*	980 ± 140	$1,740 \pm 300^\dagger$	$1,250 \pm 180^\dagger$	$1,130 \pm 140^\dagger$	$1,100 \pm 140^\dagger$
Blood alanine ($\mu\text{mol/l}$)					
Antecedent euglycemia	400 ± 40	460 ± 45	470 ± 45	460 ± 40	450 ± 35
Antecedent hypoglycemia	405 ± 45	450 ± 40	430 ± 35	420 ± 30	405 ± 30
Blood glycerol ($\mu\text{mol/l}$)					
Antecedent euglycemia	50 ± 5	100 ± 10	155 ± 12	180 ± 14	205 ± 20
Antecedent hypoglycemia*	50 ± 7	105 ± 10	135 ± 15	$150 \pm 10^\dagger$	$165 \pm 11^\dagger$
β -Hydroxybutyrate ($\mu\text{mol/l}$)					
Antecedent euglycemia	25 ± 3	27 ± 3	29 ± 4	40 ± 8	48 ± 10
Antecedent hypoglycemia*	24 ± 4	21 ± 3	$22 \pm 3^\dagger$	$24 \pm 5^\dagger$	$27 \pm 6^\dagger$
Plasma NEFAs ($\mu\text{mol/l}$)					
Antecedent euglycemia	360 ± 40	363 ± 50	478 ± 55	590 ± 56	703 ± 80
Antecedent hypoglycemia ‡	422 ± 66	453 ± 63	496 ± 68	565 ± 69	654 ± 72

Data are means \pm SE. *Overall response blunted ($P < 0.01$, ANOVA) compared with antecedent euglycemia. † Time points significantly reduced ($P < 0.05$) compared with antecedent euglycemia. ‡ Incremental increase blunted ($P < 0.05$) compared with antecedent euglycemia.

TABLE 3

Effects of day 1 hyperinsulinemic ($9.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) euglycemia ($5.3 \pm 0.1 \text{ mmol/l}$) and hypoglycemia ($2.9 \pm 0.1 \text{ mmol/l}$) on cardiovascular responses during day 2 50% $\dot{V}O_{2\text{max}}$ exercise

	Control period	Duration of exercise (min)			
		30	60	75	90
Systolic blood pressure (mmHg)					
Antecedent euglycemia	107 ± 4	155 ± 6	159 ± 5	158 ± 3	158 ± 3
Antecedent hypoglycemia	109 ± 4	152 ± 5	151 ± 5	152 ± 8	153 ± 6
Diastolic blood pressure (mmHg)					
Antecedent euglycemia	65 ± 2	78 ± 3	74 ± 3	78 ± 4	75 ± 3
Antecedent hypoglycemia	64 ± 3	73 ± 3	73 ± 3	74 ± 4	72 ± 3
Mean arterial pressure (mmHg)					
Antecedent euglycemia	79 ± 3	104 ± 4	102 ± 3	105 ± 4	103 ± 4
Antecedent hypoglycemia	79 ± 3	99 ± 3	99 ± 4	100 ± 5	99 ± 5
Heart rate (beats/min)					
Antecedent euglycemia	68 ± 6	151 ± 8	153 ± 8	152 ± 6	154 ± 6
Antecedent hypoglycemia	69 ± 4	142 ± 7	145 ± 6	143 ± 8	145 ± 7

Data are means ± SE.

of parasympathetic input to the pancreas) were also reduced after day 1 hypoglycemia. Thus, it would appear that prior hypoglycemia produced a diffuse reduction in ANS drive (pancreas, adrenal gland, sympathetic nerve endings) during subsequent exercise.

The reduced neuroendocrine and sympathetic nervous system (SNS) responses after antecedent hypoglycemia combined to produce profound metabolic deficits during day 2 exercise. EGP increased similarly for the first 15 min during exercise after day 1 euglycemia or hypoglycemia. By 30 min, however, EGP responses after day 1 hypoglycemia began to decrease and fell continuously to basal levels during the remaining 60 min of exercise. Thus, by the end of exercise, EGP was nearly twofold greater after day 1 euglycemia compared with day 1 hypoglycemia.

The biphasic response of EGP after day 1 hypoglycemia is intriguing. It would appear that an initial transient glycogenolytic burst of EGP was maintained after day 1 hypoglycemia, which may be related to the fact that insulin and glucagon responses were similar in the two groups for the first 30–45 min and only diverged during the latter 45 min of exercise. Thereafter, EGP could not be sustained and fell to basal levels. Interestingly, insulin, growth hormone, and blood lactate also exhibited a pattern of response similar to that of EGP. Rates of tracer-determined glucose disposal were equivalent during all day 2 exercise studies. Therefore, without an exogenous glucose infusion to support plasma glycemia during day 2 exercise, the reduced rates of EGP occurring after day 1 hypoglycemia would have resulted in significant hypoglycemia. This phenomenon was clearly evident, since during the final 15 min of posthypoglycemia studies, we had to match the entire usual exercise-induced increase in EGP by an external glucose infusion.

Consistent with reduced EGP, blood lactate, β -hydroxybutyrate, and glycerol responses were also blunted by day 1 hypoglycemia. Blood glycerol levels during exercise provide an accurate indication of lipolysis. Glycerol cannot reenter adipocytes, and splanchnic fractional extraction is not altered by exercise (41). Thus, peripheral levels of glycerol will reflect unidirectional transport of the metabolite from adipocytes essentially to the liver (although a small propor-

tion will also be taken up by the kidney). Therefore, the blunted glycerol responses observed after day 1 hypoglycemia can serve as an accurate marker of reduced lipolysis during day 2 exercise. Reductions in blood lactate and glycerol are most plausibly explained by blunted SNS activity. The decrease in β -hydroxybutyrate levels, however, is most likely to have been caused by the blunted glucagon responses resulting from day 1 hypoglycemia. Taken together, the above effects clearly underscore the wide-ranging deleterious consequences of prior hypoglycemia on subsequent metabolism during exercise.

Day 1 hypoglycemia also blunted growth hormone and cortisol responses during day 2 exercise. Thus, all major glucoregulatory and ANS hormones released during exercise were blunted by day 1 hypoglycemia. This response is conceptually similar to the physiologic effects of prior hypoglycemia on neuroendocrine responses during subsequent hypoglycemia (8–11). The present results strongly argue, therefore, that prior hypoglycemia can blunt counterregulatory responses to other stresses in addition to hypoglycemia. This is a novel finding and goes somewhat against conventional wisdom. Currently, the belief is that the blunting of subsequent counterregulatory responses is specific for hypoglycemia (12). However, there is accumulating evidence that prior elevations of glucocorticoids can reduce neuroendocrine and ANS counterregulatory responses to a variety of stresses (13–19). Implicit in the above studies is the belief that it is not a particular stress that specifically causes downregulation of subsequent counterregulatory responses but rather any intense activation of the hypothalamus-pituitary-adrenal axis. To our knowledge, only one previous study has investigated the effects of prior hypoglycemia on neuroendocrine responses during subsequent exercise (12). Interestingly, those researchers found no blunting of exercise-induced counterregulatory responses in a group of type 1 diabetic patients after afternoon hypoglycemia. It should be noted, however, that there are several differences between the experimental design used by Rattarasarn et al. (12) and the present study. First, Rattarasarn et al. induced afternoon hypoglycemia during two afternoons before morning exercise on the 3rd day. This schedule is different from the concentrated model of two episodes of hypoglycemia during day 1 in

this study. It is therefore possible that two episodes of hypoglycemia occurring during the previous 48 h may induce less blunting of subsequent counterregulatory responses than two episodes of hypoglycemia occurring during the previous 24 h. Second, Rattarasarn et al. studied patients with type 1 diabetes. The patients' counterregulatory responses to both hypoglycemia and exercise appeared to be attenuated even after the control experiment of prior euglycemia. To illustrate this point, pancreatic polypeptide levels did not increase in response to exercise (as opposed to the present study, in which pancreatic polypeptide values increased threefold during exercise). Additionally, during hypoglycemia, epinephrine increased only sixfold compared with 20-fold increases observed during comparable day 1 hypoglycemia in the present study. It is therefore possible that the patients of Rattarasarn et al. had a form of diabetic autonomic neuropathy (classical or acquired from previous hypoglycemia) not detectable by standard bedside testing but manifested by blunted ANS responses to stress. This phenomenon has been described by Bottini et al. (5), who demonstrated blunted responses to hypoglycemia and exercise in type 1 diabetic patients with both clinically detectable and undetectable autonomic neuropathy. Third, the duration of exercise was only 60 min during the study of Rattarasarn et al. compared with 90 min in the present experiments. This difference is pertinent because the magnitude of neuroendocrine responses increases with duration of exercise. In this present study, the biggest difference between groups occurred during the final 30 min of exercise. Furthermore, the patients in the study of Rattarasarn et al. exercised during hyperglycemic conditions (~150 mg/dl) that would have also tended to reduce epinephrine and glucagon responses. Therefore, the experimental design in the present study would have maximized any potential difference between groups. Conversely, because of a combination of several factors, the experimental design used by Rattarasarn et al. would have tended to limit the potential of observing blunting of neuroendocrine responses during exercise by prior hypoglycemia.

In summary, this study has demonstrated that two episodes of prior moderate hypoglycemia can blunt neuroendocrine and metabolic responses during exercise on the next day. All components of the neuroendocrine counterregulatory response were blunted: pituitary (growth hormone), pancreas (insulin, glucagon, pancreatic polypeptide), adrenal (epinephrine, cortisol), and sympathetic nerve terminals (norepinephrine). Commensurate with blunted neuroendocrine responses, key homeostatic metabolic mechanisms such as endogenous glucose production, ketogenesis, and lipolysis were also significantly reduced.

We conclude that in overnight-fasted healthy humans, prior hypoglycemia can blunt counterregulatory responses to subsequent exercise. Coupled with our previous results (13,42), we also conclude that prior hypoglycemia can attenuate counterregulatory responses to physiologic stresses other than hypoglycemia. These data may represent a first step in determining whether antecedent hypoglycemia is involved in the pathogenesis of exercise-related hypoglycemia in patients with type 1 diabetes.

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