Effects of Low and Moderate Antecedent Exercise on Counterregulatory Responses to Subsequent Hypoglycemia in Type 1 Diabetes

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Antecedent moderate-intensity exercise has been shown to blunt autonomic, neuroendocrine, and metabolic counterregulatory responses to subsequent hypoglycemia in nondiabetic individuals. The aims of the current study were to determine *1*) whether this occurs in type 1 diabetic patients and *2*) whether the degree of blunting is dependent on exercise intensity. Twenty-seven type 1 diabetic patients (13 women and 14 men) were studied during a single-step, 2-h hyperinsulinemic (9 pmol · kg^{-1} · min^{-1})-hypoglycemic (~2.8 mmol/l) clamp 1 day after two 90-min exercise bouts at 30% (n = 11) or at 50% (n = 11) $V\text{O}_{2\text{max}}$ or after no prior stress (control subjects, n = 25). After prior exercise at both 30 and 50% $V\text{O}_{2\text{max}}$, epinephrine (1,959 ± 553 and 1,528 ± 424 vs. 3,420 ± 424 pmol/l, respectively; P < 0.05) and pancreatic polypeptide (97 ± 32 and 98 ± 8 vs. 223 ± 32 pmol/l, respectively; P < 0.05) responses to subsequent hypoglycemia were significantly lower compared with those of control subjects. Endogenous glucose production was significantly lower, while glucose utilization and, consequently, the exogenous glucose infusion rate needed to maintain hypoglycemia were significantly greater after both exercise intensities compared with that of control subjects. Muscle sympathetic nerve activity was significantly reduced by prior exercise of both intensities at baseline (16 ± 4 and 22 ± 4 vs. 31 ± 3 bursts/min) and during hypoglycemia (22 ± 4 and 27 ± 5 vs. 41 ± 3 bursts/min) compared with that of control subjects (P < 0.05). Total hypoglycemic symptoms were also significantly lower (P < 0.05) in both exercise groups compared with the control group. In summary, repeated episodes of prolonged exercise of both low and moderate intensities blunted key autonomic (epinephrine and pancreatic polypeptide) and metabolic (endogenous glucose production and peripheral glucose uptake) counterregulatory responses to next-day hypoglycemia in type 1 diabetes. *Diabetes* 53: 1798–1806, 2004

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EGP, endogenous glucose production; MSNA, muscle sympathetic nerve activity.

Intensive maintenance of normal glucose levels delays or prevents the development of microvascular complications associated with diabetes (1,2). Unfortunately, an approximate threefold increase in severe hypoglycemia in these intensively treated patients (1) limits the widespread implementation of this treatment paradigm. Although excess insulin is an important contributing factor to increased hypoglycemia, with increased duration of the disease, glucagon responses to hypoglycemia in type 1 diabetes are absent (3). This leaves type 1 diabetic patients dependent on epinephrine to counter falling glucose levels. However, recent antecedent episodes of hypoglycemia have significantly reduced autonomic counterregulatory responses to subsequent hypoglycemia in nondiabetic and type 1 diabetic patients (4–7). This has been termed hypoglycemia-associated autonomic failure (8,9) and is proposed to create a vicious cycle of hypoglycemia for the type 1 diabetic patient.

Exercise has numerous therapeutic benefits. Despite this, exercise often results in hypoglycemia in type 1 diabetic patients. Again, a relative or absolute excess of insulin during exercise is an important mechanism for causing hypoglycemia. However, we have also found that neuroendocrine and metabolic responses during prolonged exercise (90 min at 50% $V\text{O}_{2\text{max}}$) are reduced by ~50% after prior hyperinsulinemic hypoglycemia in nondiabetic (10) and type 1 diabetic (11) individuals. Conversely, two bouts of prior exercise for 90 min at 50% $V\text{O}_{2\text{max}}$ (12) and for 60 min at 70% $V\text{O}_{2\text{max}}$ (13) have been shown to blunt counterregulatory responses to subsequent next-day hypoglycemia occurring in nondiabetic subjects. Thus, blunted counterregulation by prior moderate-intensity prolonged exercise could be hypothesized to be one mechanism for subsequent hypoglycemia in type 1 diabetic individuals. Only one study has directly investigated this topic in type 1 diabetic patients. Rattarasarn et al. (14) found no effect of a 60-min bout of exercise at 60% $V\text{O}_{2\text{max}}$ on responses to subsequent hypoglycemia in type 1 diabetic patients. However, experimental designs were vastly different in the above studies. These included differences in exercise duration, frequency, intensity, and time between exercise and hypoglycemia (2 h versus the next day), thereby confounding comparisons between the nondiabetic and type 1 diabetic subjects. Thus, the aims of this study were twofold: *1*) to determine whether repeated

episodes of antecedent moderate exercise blunts counterregulatory responses to subsequent hypoglycemia and *2*) to determine the impact of low-to-moderate exercise intensity on the responses to subsequent hypoglycemia in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

We studied 27 patients with type 1 diabetes (14 men and 13 women) matched for age (29 ± 1 years), BMI (23 ± 1 kg/m^2), HbA_{1c} level (7.8 ± 0.2%, normal range 4.0–6.5), and duration of diabetes (14 ± 1 years). None of the patients reported a history of hypoglycemic unawareness, and all received insulin as their only medication. No patient had any clinical evidence of autonomic neuropathy or any other tissue-specific complication of diabetes. All patients had normal blood count, plasma electrolytes, and liver and renal function. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board, and all subjects gave informed written and verbal consent.

Preliminary exercise testing. At least 2 weeks before the initial study, subjects performed an incremental exercise test on a stationary cycle ergometer to determine $V_{O_{2max}}$. Expired gases were collected and analyzed using computerized open-circuit, indirect calorimetry (CardiO$_2$; Medical Graphics, St. Paul, MN). $V_{O_{2max}}$ was determined when at least two of the following three criteria were met: *1*) the subject was too tired to continue, *2*) the respiratory exchange ratio was >1.0, or *3*) there was a plateau in oxygen consumption with increasing workloads. Subjects studied ranged from sedentary to recreationally active (average $V_{O_{2max}}$ 28 ± 2 ml · kg^{-1} · min^{-1}, range 17–45).

Experimental design. Eleven (7 men and 4 women) subjects underwent randomized single-blind studies consisting of one episode of hyperinsulinemic hypoglycemia (2.8 ± 0.1 mmol/l) following two 90-min exercise bouts at either 30% $V_{O_{2max}}$ (ANTE EX30 group) or 50% $V_{O_{2max}}$ (ANTE EX50 group). These workloads were chosen because these intensities are at the low and high end, respectively, of what subjects in this fitness range are capable of for prolonged exercise. Nine (7 men and 2 women) of the total 11 subjects in both exercise groups also underwent a 2-day control study that consisted of day 1 of rest followed by hyperinsulinemic hypoglycemia on day 2. These results were added to previously published historical control data from 16 (7 men and 9 women) subjects (15).

Day 1 studies. All study patients were asked to avoid any exercise and to consume their usual weight-maintaining diet for 3 days before each study. All patients performed intensive home blood glucose monitoring (before each meal, at bedtime, and on two occasions at 3:00 A.M.) for 2 weeks before a study. An experiment was not conducted if blood glucose readings fell to <3.9 mmol/l. On the day preceding an experiment, intermediate or long-acting insulin was discontinued and replaced by injections of regular insulin before breakfast and lunch. Each subject was admitted to the Vanderbilt General Clinical Research Center (CRC) at 5:00 P.M. on the evening before an experiment. At this time, two intravenous cannulae were inserted under 1% lidocaine as a 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 (16). The other cannula was placed in the contralateral arm for infusions. Patients then received an evening meal, and a continuous low-dose infusion of insulin was started to normalize plasma glucose. The insulin infusion was adjusted overnight to maintain blood glucose between 4.4 and 7.2 mmol/l.

After an overnight 10-h fast, at ~10:00 A.M. and after a 30-min basal period, subjects were randomized to perform 90 min of exercise at 30 or 50% $V_{O_{2max}}$ or to sit in a chair (control subjects). Exercise protocols were performed in a single-blind fashion. This was followed by a 180-min resting period and a second 90-min exercise period at the same exercise intensity as performed in the morning. Exercise was performed at 60–70 rpm on an upright cycle ergometer (Medical Graphics, Yorba Linda, CA). During exercise and while resting in the chair (control subjects), insulin was infused at 1 unit/h. Potassium chloride was infused at a rate of 5 mmol/h. Plasma glucose was measured every 5 min during both exercise and control periods and every 20 min during the rest period between exercise and control periods and was maintained at ~5 mmol/l with a 20% dextrose infusion. During the first 30 min of the break period between morning and afternoon exercise or control (resting in a chair) periods, a drink containing 0.6 (ANTE EX30 or control subjects) or 1.5 (ANTE EX50) g carbohydrate/kg body wt was administered orally to replenish glycogen stores depleted during morning exercise and as a control for this oral carbohydrate consumption in the control subjects. Insulin infusion was increased to cover the carbohydrate load in all subjects. After completion of the afternoon exercise or control period, subjects consumed a standard meal and bedtime snack and remained for another night in the general CRC. Blood glucose levels were monitored every 30 min overnight, and the intravenous insulin was adjusted to maintain levels between 4.4 and 7.2 mmol/l.

Day 2 hyperinsulinemic-hypoglycemic clamp experiments. Day 2 experiments involved a standardized hyperinsulinemic-hypoglycemic glucose clamp. Each study consisted of a tracer equilibration period (0–90 min), a basal period (90–120 min), and an experimental period (120–240 min). A primed (18 μCi) infusion (0.18 μCi/min) of high-performance liquid chromatography–purified [3-^{3}H]glucose (11.5 mCi · mmol^{-1}· l^{-1}; Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) starting at 0 min. Also at this time, isolation of the peroneal nerve for microneurography (technique described below) was started. An insulin infusion solution was prepared with normal saline containing 3% (vol/vol) of the subject's own plasma. At time 120 min, a primed constant (9.0 pmol · kg^{-1} · min^{-1}) infusion of insulin (Eli Lilly, Indianapolis, IN) was started via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) and continued until 240 min. The rate of fall of glucose was controlled (0.06 mmol/min), and the hypoglycemic nadir (2.9 mmol/l) was achieved using a modification of the glucose clamp technique (17). During the clamp periods, plasma glucose was measured every 5 min, and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant (2.8 ± 0.1 mmol/l). Potassium chloride (20 mmol/l) was infused during the clamp to reduce insulin-induced hypokalemia.

Tracer calculations. Rates of glucose appearance (R_a), endogenous glucose production (EGP), and glucose utilization were calculated according to the methods of Wall et al. (18). EGP was calculated by determining the total R_a (this comprises both EGP and any exogenous glucose infused to maintain the desired hypoglycemia) and subtracting it from the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, since underestimates of total R_a and rate of glucose disposal (R_d) can be obtained. The use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminates most, if not all, of the problems. In addition, to maintain a constant specific activity, isotope delivery was increased commensurate with increases in exogenous glucose infusion. During this study, only glucose flux results from the basal and the final 30-min periods of the hypoglycemic clamps are reported.

Direct measurement of muscle sympathetic nerve activity. Muscle sympathetic nerve activity (MSNA) was recorded in the present study, as this has been demonstrated to reflect increased sympathetic activity during insulin-induced hypoglycemia (7,12,19–21). Muscle sympathetic nerve activity was measured from the peroneal nerve at the level of the fibular head or popliteal fossa, and the data were processed as described previously (22).

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 were drawn twice during the control period and every 15 min during the experimental period. Catecholamines were determined by high-pressure liquid chromatography (23) with an interassay coefficient of variation (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 a one-point standard calibration curve; and *2*) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Insulin (24) (CV 11%), cortisol (Clinical Assays Gamma Coat Radioimmunoassay Kit; interassay CV 6%), growth hormone (25) (interassay CV 8%), pancreatic polypeptide (26) (interassay CV 8%), and glucagon (Linco Research) (interassay CV 15%) were all measured using radioimmunoassay techniques. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured on deproteinized whole blood using the method of Lloyd et al. (27). Nonesterified fatty acids were measured using the WAKO kit adopted for use on a centrifugal analyzer (28).

Cardiovascular parameters (heart rate, systolic and diastolic blood pressure, and mean arterial pressure) were measured manually during day 1 exercise and noninvasively by a Dinamap (Critikon, Tampa, FL) on day 2 every 10 min throughout the study. Symptoms of hypoglycemia were assessed every 15 min during the hypoglycemic clamps using a previously validated semiquantitative questionnaire (29). Each subject was asked to rate symptoms of tiredness, confusion, hunger, dizziness, difficulty thinking, blurred vision, sweaty, tremor, agitation, hot/thirsty, and palpitations. The score for the first six symptoms were summed for neuroglycopenic and for the last five symptoms for autonomic symptom scores.

Expired gases were collected and analyzed during the basal period and at the end of the exercise and hypoglycemic clamp periods using computerized open-circuit indirect calorimetry (CardiO$_2$, Medical Graphics). Whole body fat and carbohydrate oxidation were calculated using the equations of Frayn (30) after correction for protein oxidation.

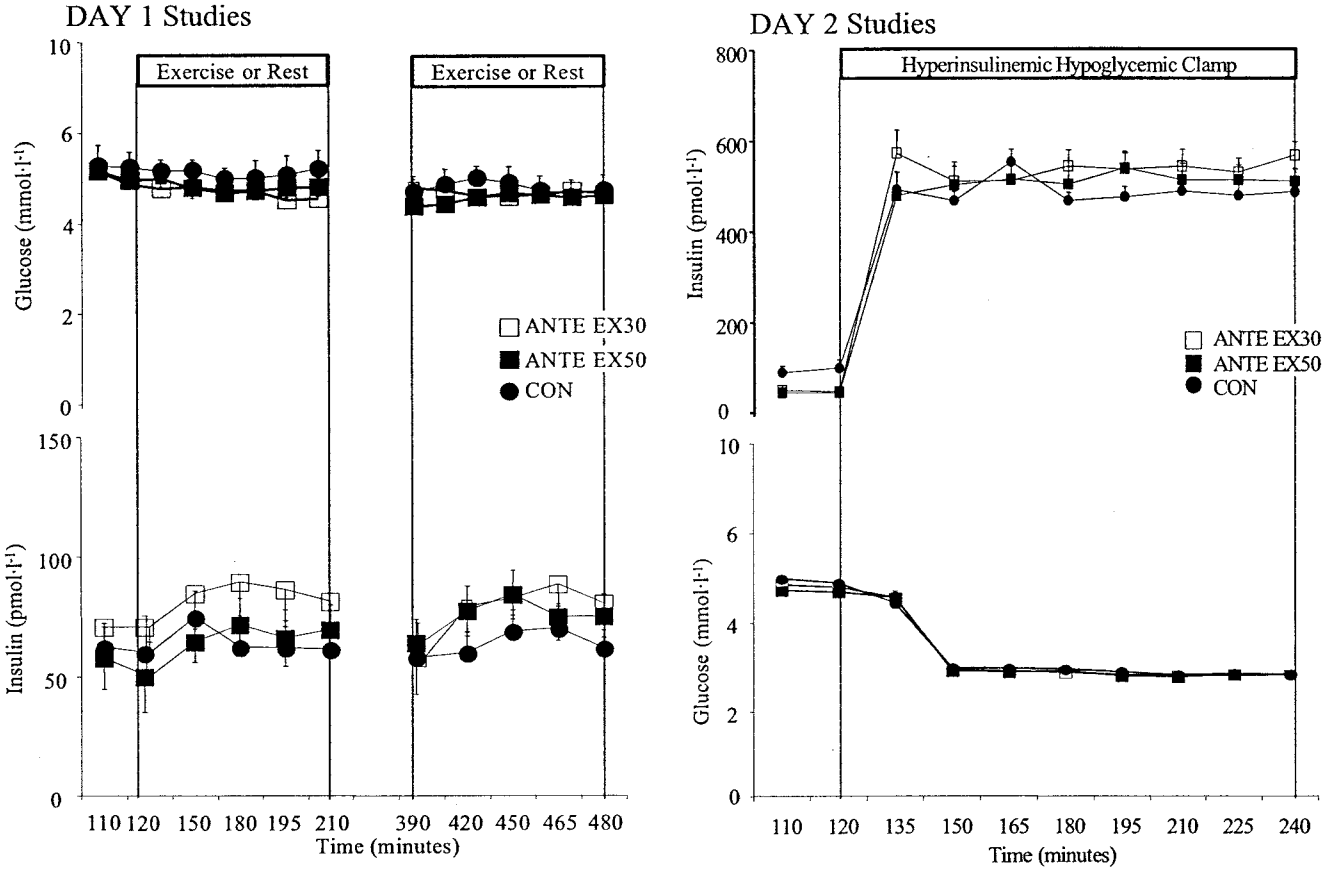

FIG. 1. Glucose and insulin levels during day 1 and day 2 studies in overnight fasted, euglycemic, type 1 diabetic subjects who performed no prior exercise (CON) or who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) $V\text{O}_{2\text{max}}$ on day 1. Levels were similar during day 1 and day 2 studies, respectively, between all three groups. Values are means ± SE.

Statistical analysis. Data are expressed as means ± SE and were analyzed using standard, parametric, two-way ANOVA with repeated measures where appropriate. A Tukey's post hoc analysis was used to delineate statistical significance. A $P \leq 0.05$ was accepted as statistical significance.

RESULTS

Day 1. Subjects performed relative exercise intensities of 33 ± 1 and 54 ± 1% $V\text{O}_{2\text{max}}$ for the ANTE EX30 and EX50 groups, respectively. Exercise caused heart rate to increase from 72 ± 1 to 104 ± 1 bpm in the ANTE EX30 group and from 76 ± 1 to 136 ± 1 bpm in ANTE EX50 group. Systolic blood pressure significantly increased from 116 ± 1 to 123 ± 1 mmHg in the ANTE EX30 group and from 117 ± 1 to 145 ± 2 mmHg in the ANTE EX50 group during exercise. Diastolic blood pressure did not significantly change during exercise of 30% $V\text{O}_{2\text{max}}$ but did significantly decrease during exercise of 50% $V\text{O}_{2\text{max}}$ (from 76 ± 1 to 68 ± 1 mmHg).

Day 1 glucose levels at baseline (5.5 ± 0.1 and 5.3 ± 0.1 mmol/l) and during the final 30 min of exercise (5.4 ± 0.2 and 5.2 ± 0.1 mmol/l) were similar between morning and afternoon studies for all groups (Fig. 1). Similarly, day 1 insulin levels at baseline (50 ± 6 and 55 ± 9 pmol/l) and during the final 30 min of exercise with an insulin infusion rate of 1 unit/h (70 ± 9 and 67 ± 7 pmol/l) were similar between morning and afternoon studies for all groups (Fig. 1).

Plasma cortisol increased with exercise in the morning (from 362 ± 35 to 409 ± 36 and 560 ± 65 nmol/l in the ANTE EX30 and EX50 groups, respectively; $P < 0.05$) and in the afternoon (from 292 ± 27 to 357 ± 43 and 384 ± 47 nmol/l in the ANTE EX30 and EX50% groups, respectively; $P < 0.05$). This increase was greater in the ANTE EX50 vs. EX30 groups in the morning only. Lactate increased with morning and afternoon exercise in an intensity-dependent manner (from 0.7 ± 0.1 to 0.82 ± 0.1 vs. 1.41 ± 0.15 mmol/l during morning and to 0.8 ± 0.1 vs. 1.3 ± 0.2 mmol/l during afternoon exercise in the ANTE EX30 vs. ANTE EX50 groups, respectively; $P < 0.05$). β-Hydroxybutyrate did not significantly change with exercise and was similar between exercise and control groups. Besides the diurnal fall in cortisol, none of the above variables changed significantly over time in the control group that rested instead of exercising on day 1.

Day 2

Glucose and insulin levels. Day 2 steady-state plasma glucose (2.8 ± 0.1, 2.8 ± 0.1, and 2.9 ± 0.1 mmol/l for the ANTE EX30 and EX50 and control groups, respectively) and insulin levels (547 ± 29, 512 ± 30, and 485 ± 20 pmol/l for the ANTE EX30 and EX50 and control groups, respectively) were similar among the three groups (Fig. 1).

Counterregulatory hormone levels. In both the ANTE EX30 and EX50 groups, epinephrine (1,959 ± 553 and 1,528 ± 424 vs. 3,420 ± 424 pmol/l; $P < 0.05$) and pancreatic polypeptide (97 ± 32 and 98 ± 8 vs. 223 ± 32 pmol/l; $P < 0.05$) responses to subsequent hypoglycemia were significantly lower during the final 45 and 60 min of

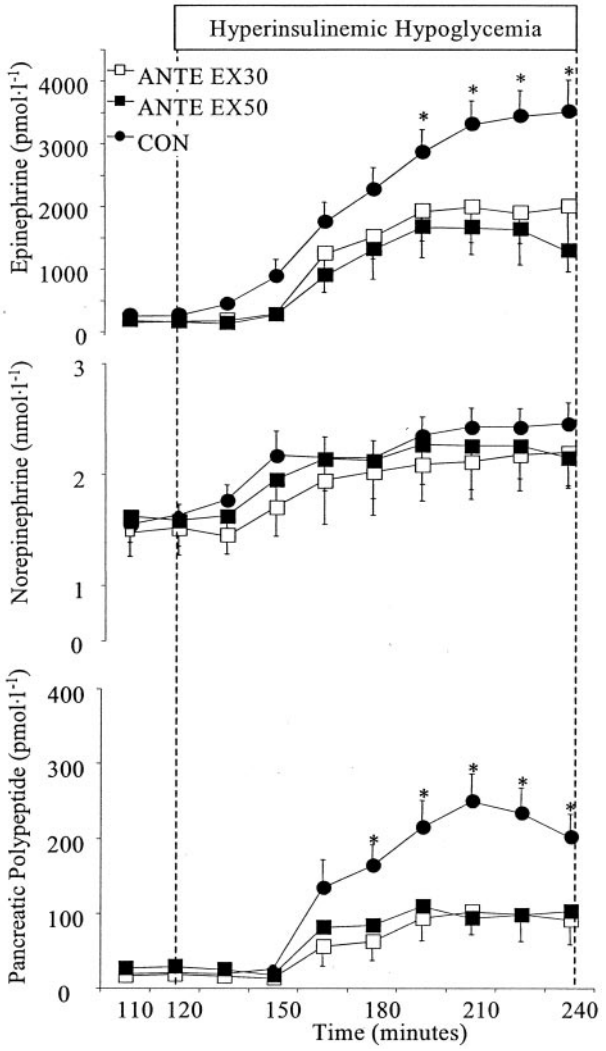

FIG. 2. Epinephrine, norepinephrine, and pancreatic polypeptide responses to day 2 hyperinsulinemic (9 pmol · kg^{-1} · min^{-1}) hypoglycemia in overnight-fasted, euglycemic, type 1 diabetic subjects who performed no prior exercise (CON) or who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) $V\text{O}_{2max}$ on day 1. *Epinephrine and pancreatic polypeptide levels were significantly greater in CON vs. both ANTE EX30 and ANTE EX50 ($P < 0.05$). Values are means ± SE.

hypoglycemia, respectively, compared with the control group (Fig. 2). Norepinephrine (Fig. 2), growth hormone, cortisol, and glucagon (Table 1) responses to hypoglycemia were similar among the three groups.

Glucose kinetics. Glucose-specific activity (disintegrations per min/mmol) did not significantly change during both the control period and the final 30 min of the hypoglycemic clamp (Table 2; CV 4 and 5% for basal and final 30 min of hypoglycemia, respectively). Endogenous glucose production was significantly lower during the final 30 min of hyperinsulinemic hypoglycemia in the ANTE EX30 and EX50 groups compared with the control group, respectively (3 ± 1 and 3 ± 2 vs. 7 ± 2 μmol · kg^{-1} · min^{-1}; $P < 0.05$) (Fig. 3). In contrast, glucose R_d was significantly greater in the ANTE EX30 and EX50 groups compared with the control group (17 ± 2 and 18 ± 2 vs. 12 ± 2 μmol · kg^{-1} · min^{-1}, respectively; $P < 0.05$) (Fig. 3). As a consequence, the exogenous glucose infusion rate necessary to maintain the hypoglycemic level of 2.8 mmol/l was significantly greater in both the ANTE EX30 and EX50 groups compared with the control group (14 ± 2 and 15 ± 2 vs. 8 ± 1 μmol · kg^{-1} · min^{-1}; $P < 0.05$) (Fig. 3). Indirect calorimetry data indicated that fat oxidation was significantly lower and glucose oxidation was significantly greater in the ANTE EX30 and EX50 groups compared with the control group (0.4 ± 0.2 and 0.4 ± 0.1 vs. 0.8 ± 0.1 mg · kg^{-1} · min^{-1} and 1.9 ± 0.4 and 1.6 ± 0.4 vs. 1.1 ± 0.2 mg · kg^{-1} · min^{-1} for the ANTE EX30 and EX50 groups versus the control group, respectively; $P < 0.05$) (Table 3). The increase in nonoxidative glucose metabolism during hyperinsulinemic hypoglycemia was similar among the three groups (Table 3).

Intermediary metabolism. The fall from baseline in the nonesterified fatty acid levels (−220 ± 34 and −224 ± 46 vs. −62 ± 41 μmol/l, respectively) was significantly greater during hyperinsulinemic hypoglycemia in the ANTE EX30 and EX50 groups compared with the control group ($P < 0.05$) (Fig. 4). Glycerol levels decreased from baseline in both exercise groups but increased in the control group (−16 ± 7 and −3 ± 6 vs. 14 ± 7 μmol/l, respectively; $P < 0.05$) (Fig. 4). Lactate levels significantly increased with hyperinsulinemic hypoglycemia similarly among the three groups (Table 3). β-Hydroxybutyrate and alanine responses to hyperinsulinemic hypoglycemia were similar during the final 30 min of the hyperinsulinemic-hypoglycemic clamp (Table 3).

Cardiovascular responses. Heart rate, systolic blood pressure, and mean arterial pressure significantly increased with hyperinsulinemic hypoglycemia in all groups (Table 4). Diastolic blood pressure decreased with hyperinsulinemic hypoglycemia in all groups (Table 4).

Symptom responses. Total hypoglycemic symptom scores were lower in both exercise groups compared with the control group (39 ± 4 vs. 26 ± 5 and 25 ± 4, respectively; $P < 0.05$) (Fig. 5). Most of the increase in symptoms in the control group was due to elevated autonomic symptoms ($P < 0.05$) (Fig. 5), whereas the neuroglycopenic symptom scores were similar among the groups (Fig. 5).

MSNA. MSNA significantly increased with hypoglycemia

TABLE 1
Neuroendocrine responses to day 2 hyperinsulinemic hypoglycemia after no prior stress (CON) or exercise of 30 (ANTE EX30) or 50% (ANTE EX50) $V\text{O}_{2max}$

	CON		ANTE EX30		ANTE EX50	
	Basal	Final 30 min	Basal	Final 30 min	Basal	Final 30 min
Glucagon (ng/l)	44 ± 4	54 ± 7	43 ± 3	46 ± 5	46 ± 5	45 ± 7
Growth hormone (μg/l)	4 ± 1	24 ± 4*	3 ± 1	24 ± 6*	5 ± 1	25 ± 5*
Cortisol (nmol/l)	333 ± 36	607 ± 45*	349 ± 39	605 ± 66*	347 ± 50	594 ± 76*

Data are means ± SD. *$P < 0.05$ vs. basal period.

TABLE 2
Glucose-specific activity for basal and final 30-min periods of the day 2 hyperinsulinemic-hypoglycemic clamp after no prior stress (CON) or exercise of 30 (ANTE EX30) or 50% (ANTE EX50) Vo_{2max}

	−20 min	−10 min	0 min	90 min	105 min	120 min
CON	324 ± 5	316 ± 5	311 ± 5	317 ± 5	312 ± 5	312 ± 4
ANTE EX30	396 ± 7	402 ± 7	401 ± 6	314 ± 5	303 ± 5	303 ± 5
ANTE EX50	394 ± 4	395 ± 4	386 ± 4	298 ± 3	297 ± 4	296 ± 4

Data are means ± SD.

but was significantly lower at baseline and during hypoglycemia in both exercise groups compared with the control group ($P < 0.05$) (Table 4).

DISCUSSION

This study has examined the counterregulatory responses to hyperinsulinemic hypoglycemia after both low and moderate exercise intensity in type 1 diabetic patients. We found that both exercise intensities blunted autonomic, neuroendocrine (epinephrine and pancreatic polypeptide), metabolic (EGP and fat oxidation), and symptomatic responses to subsequent hypoglycemia compared with control studies. These data demonstrate that when prolonged, even low-intensity exercise can blunt subsequent counterregulatory responses to hypoglycemia in type 1 diabetes.

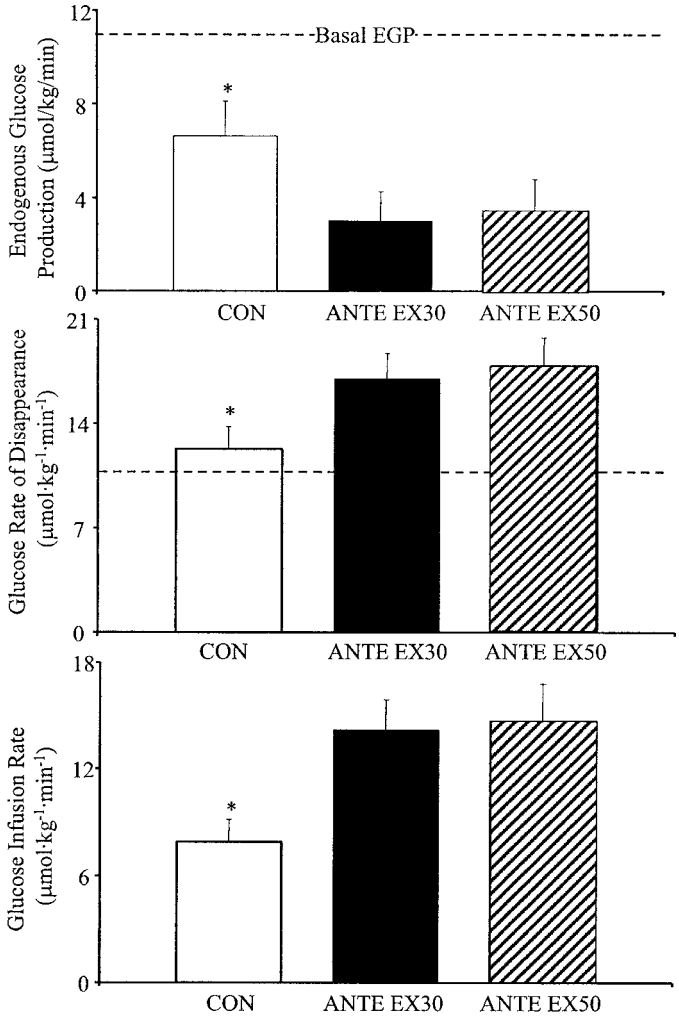

FIG. 3. EGP, glucose utilization, and glucose infusion rate on day 2 during the 2-h hyperinsulinemic (9 pmol · kg^{-1} · min^{-1})-hypoglycemic clamp in overnight-fasted, euglycemic, type 1 diabetic subjects who performed no prior exercise (CON) or who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) Vo_{2max} on day 1. *EGP was significantly lower and glucose R_d and glucose infusion rate were significantly higher, respectively, in subjects who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) Vo_{2max} on day 1 vs. control subjects (CON) ($P < 0.01$). Values are means ± SE.

The loss of glucagon responses to hypoglycemia with increasing duration of type 1 diabetes (3) leaves these individuals dependent on the autonomic nervous system for effective counterregulation. However, in these studies, prior exercise blunted both limbs of the autonomic nervous system. Epinephrine (sympathoadrenal branch of the sympathetic nervous system) as well as pancreatic polypeptide (index of parasympathetic nervous system) during hypoglycemia were reduced by prior exercise. Although plasma norepinephrine responses were similar between groups, changes in circulating norepinephrine levels imprecisely reflect sympathetic nerve activity due to increased reuptake at the synaptic cleft and clearance by various tissues. Interestingly, MSNA (a more direct measurement of sympathetic nervous system activity) was significantly reduced after antecedent exercise at baseline and during hypoglycemia. The change from baseline in MSNA, although lower after antecedent exercise (7 ± 2 and 8 ± 3 vs. 10 ± 2 bursts/min), did not reach statistical significance. The physiological significance of blunted baseline levels of sympathetic nerve activity is unknown. Similarly, it is unknown whether it is the absolute nerve activity or the change in activity from baseline that determines effects of the sympathetic nervous system on target tissues. Consistent with the above, autonomic symptom responses were also reduced by prior exercise. Thus, the combination of the reduced epinephrine, autonomic symptom, and pancreatic polypeptide responses together with the reduced MSNA during hypoglycemia suggest that a spectrum of autonomic nervous system action was blunted by antecedent exercise in type 1 diabetes.

The blunted epinephrine responses during day 2 hypoglycemia had widespread metabolic effects, as both glucose and fat metabolism were altered by prior exercise. During hypoglycemia, increases in epinephrine and/or glucagon (in normal subjects) upregulate EGP. Epinephrine also limits peripheral glucose uptake by inhibition of hexokinase activity and glucose phosphorylation (31,32) and stimulates lipolysis. Thus, following prior exercise and with reduced epinephrine responses, EGP was blunted and glucose rate of disappearance and subsequently glucose oxidation (as indicated by indirect calorimetry) were enhanced. Lipolysis (as indicated by free fatty acids and glycerol levels) and fat oxidation (indicated by the indirect calorimetry data) were also decreased during hypoglyce-

TABLE 3
Indirect calorimetry and metabolite responses to day 2 hyperinsulinemic hypoglycemia after no prior stress (CON) or exercise of 30 (ANTE EX30) or 50% (ANTE EX50) Vo_{2max}

	CON		ANTE EX30		ANTE EX50	
	Basal	Final 30 min	Basal	Final 30 min	Basal	Final 30 min
Fat disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	0.7 ± 0.1	0.8 ± 0.1*	0.7 ± 0.1	0.4 ± 0.2	0.7 ± 0.2	0.4 ± 0.1
Oxidative glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.4 ± 0.2	1.1 ± 0.2*	0.8 ± 0.3	1.9 ± 0.4	0.9 ± 0.3	2.0 ± 0.3
Nonoxidative glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	0.5 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.5	1.0 ± 0.3	1.6 ± 0.4
Lactate (mmol/l)	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	1.1 ± 0.2
β-Hydroxybutyrate (mmol/l)	0.08 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	0.04 ± 0.02	0.13 ± 0.03	0.05 ± 0.02
Alanine (mmol/l)	0.27 ± 0.02	0.22 ± 0.01	0.28 ± 0.03	0.23 ± 0.01	0.31 ± 0.06	0.28 ± 0.03

Data are means ± SD. *$P < 0.05$ vs. final 30-min periods in both ANTE EX30 and EX50.

mia after prior exercise. Interestingly, free fatty acid elevations have also been shown to independently increase EGP and to reduce glucose utilization during hypoglycemia in nondiabetic volunteers (33). Consequently, a fall in free fatty acids, in addition to the blunted epinephrine, could have contributed to a blunted EGP and increased glucose utilization during subsequent hypoglycemia in the exercise groups. All of the above metabolic mechanisms would serve to reduce the ability of the type 1 diabetic individual to defend against hypoglycemia.

In addition to blunting counterregulatory responses, prior exercise also enhances insulin sensitivity. A single bout of exercise in rats (34) and humans (35) enhanced insulin sensitivity for 6 and 12 h, respectively. Thus, it is likely that insulin sensitivity was enhanced in the exercise groups who were exposed to hypoglycemia ~15 h after the

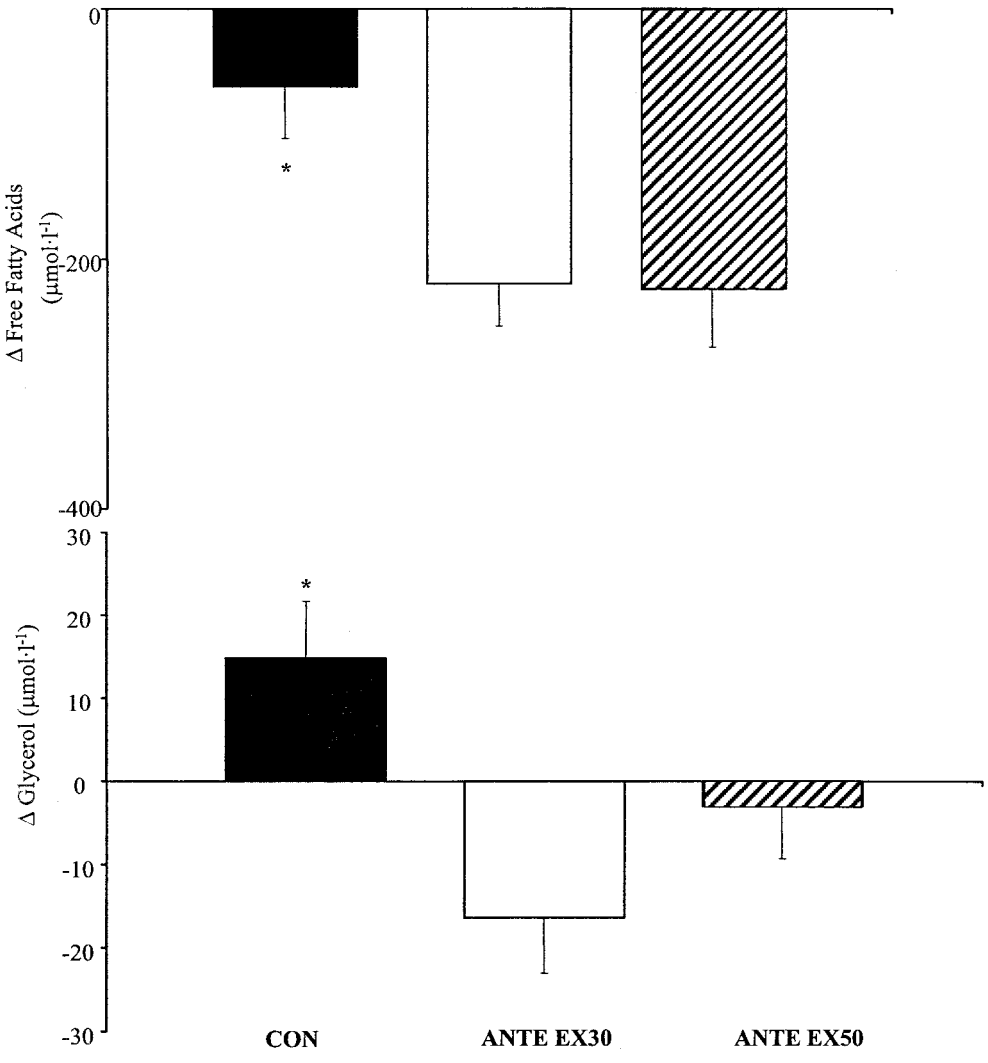

FIG. 4. The change in nonesterified fatty acids and glycerol levels during the day 2 hyperinsulinemic (9 pmol · kg^{-1} · min^{-1})-hypoglycemic clamp in overnight-fasted, euglycemic, type 1 diabetic subjects who performed no prior exercise (CON) or who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) Vo_{2max} on day 1. *Nonesterified fatty acids fell significantly less and glycerol levels significantly increased (compared with a decrease) during the hypoglycemic clamp in CON vs. ANTE EX30 and ANTE EX50 ($P < 0.05$). Values are means ± SE.

TABLE 4
Cardiovascular responses to day 2 hyperinsulinemic hypoglycemia after no prior stress (CON) or exercise of 30 (ANTE EX30) or 50% (ANTE EX50) $V\text{O}_{2max}$

	CON		ANTE EX30		ANTE EX50	
	Basal	Final 30 min	Basal	Final 30 min	Basal	Final 30 min
Heart rate (bpm)*	70 ± 2	75 ± 2	73 ± 4	81 ± 5	67 ± 4	73 ± 5
Systolic blood pressure (mmHg)*	115 ± 3	122 ± 3	121 ± 9	131 ± 16	117 ± 4	129 ± 9
Diastolic blood pressure (mmHg)*	68 ± 1	62 ± 2	71 ± 2	64 ± 3	68 ± 2	64 ± 28
Mean arterial blood pressure (mmHg)	85 ± 2	85 ± 2	87 ± 3	85 ± 3	84 ± 3	83 ± 2

Data are means ± SD. *$P < 0.05$ for final 30 min vs. basal in all groups.

prior afternoon bout of prolonged exercise. Enhanced insulin sensitivity in a type 1 diabetic individual would also contribute to a blunting of EGP (increased suppression of glucose production due to exogenous insulin delivery), an enhancement of glucose utilization, and a reduction of lipolysis during subsequent hypoglycemia.

Previous studies that have examined the impact of prior exercise on subsequent counterregulatory responses to hypoglycemia have shown mixed results. For example, in dogs, prolonged exercise reduced counterregulatory responses to immediate subsequent glucoprivation (36), while in another study counterregulatory responses to subsequent hyperinsulinemic hypoglycemia appeared unaffected (37). In humans, one bout of prolonged exercise (60 min at 60% $V\text{O}_{2max}$) in nondiabetic subjects had no effect on counterregulatory responses to subsequent (90 min postexercise) hypoglycemia (14). However, a later study found that two 60-min exercise bouts at 70% $V\text{O}_{2max}$ blunted epinephrine, glucagon, and growth hormone responses and increased the glucose infusion rate during next-day hypoglycemia in nondiabetic subjects (38). Lastly, our previous and current data in healthy and type 1 diabetic individuals showed that prolonged exercise at 50% $V\text{O}_{2max}$ did blunt counterregulatory responses to subsequent hypoglycemia. Taken together, it appears that differences in duration of exercise, number of prior bouts of exercise, and time between exercise and subsequent hypoglycemia directly impact comparison of these studies and most likey explain the discrepant results.

In comparison with our previous data in nondiabetic subjects (12), norepinephrine levels were not blunted by prior exercise and epinephrine levels were blunted to a greater extent in type 1 diabetes (~55 vs. 36%). The type 1 diabetic patients also had a greater increase in glucose R_d and lower lipolytic (free fatty acid and glycerol) responses after exercise compared with the nondiabetic subjects.

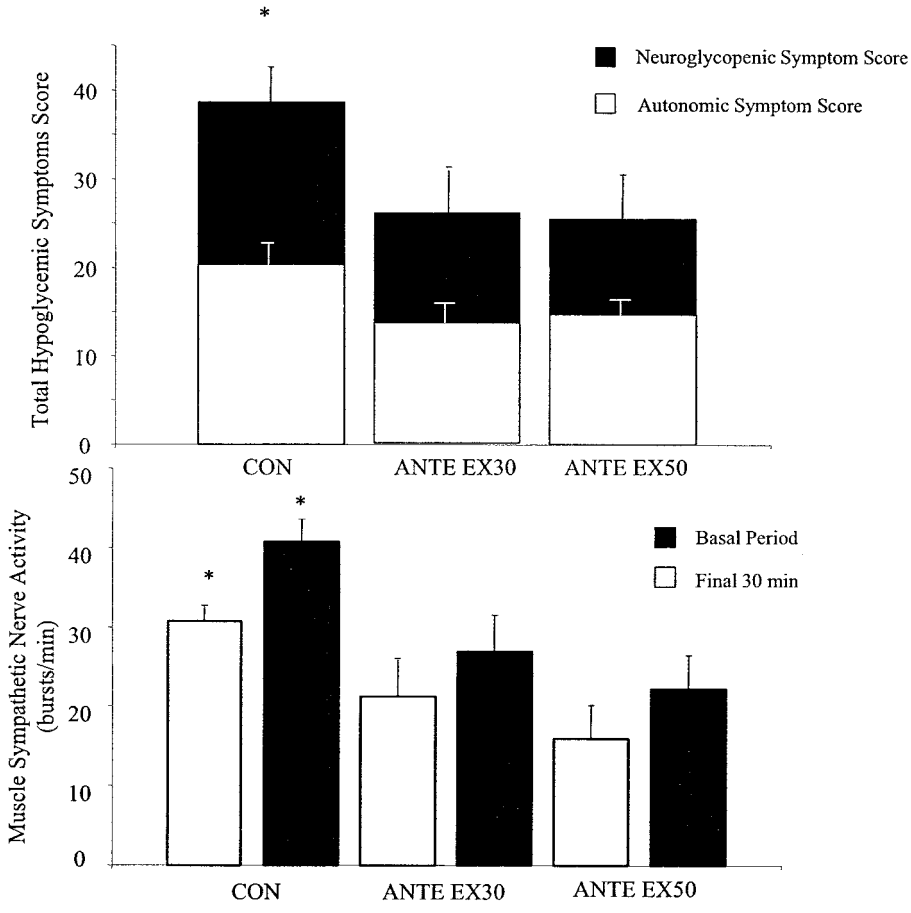

FIG. 5. Total, neuroglycopenic, and autonomic hypoglycemic symptom scores during the final 30 min and MSNA during the basal and final 30 min of the hyperinsulinemic (9 pmol · kg^{-1} · min^{-1})-hypoglycemic clamp in overnight-fasted, euglycemic, type 1 diabetic subjects who performed no prior exercise (CON) or who performed exercise at 30% (ANTE EX30) or 50% (ANTE EX50) $V\text{O}_{2max}$ on day 1. *Total and neurogenic symptom scores were significantly lower during the final 30 min and MSNA was significantly lower during the basal and final 30 min of hypoglycemia in the ANTE EX30 and ANTE EX50 groups on day 1 versus CON ($P < 0.05$). Values are means ± SE.

Growth hormone was not blunted by prior exercise in the type 1 diabetic patients but was blunted in the nondiabetic subjects. The absolute responses of growth hormone to day 2 hypoglycemia in the nonexercise groups were lower in the type 1 diabetic versus the nondiabetic subjects (~24 vs. ~37 μg/l), which may have truncated the experimental signal in the diabetic subjects. Neither the type 1 diabetic nor the nondiabetic subjects showed blunted cortisol levels in response to hypoglycemia after exercise. Thus, under similar experimental conditions, type 1 diabetic patients, as compared with nondiabetic subjects, may have slightly greater counterregulatory failure of their major defense mechanisms against hypoglycemia (i.e., epinephrine, glucose, and fat flux) induced by prior exercise.

These present results indicate that the autonomic nervous system in type 1 diabetic patients is exquisitely sensitive to the effects of prior exercise. This is illustrated by the fact that prolonged exercise of only 30% $V\text{O}_{2\text{max}}$, which is ~20% greater than resting $V\text{O}_2$, caused a similar degree of blunting compared with prolonged exercise of 50% $V\text{O}_{2\text{max}}$. The mechanism for this is unknown. It has been previously shown that prior peripheral infusion of lactate or β-hydroxybutyrate in humans (39) and intracerebroventricular infusion of lactate in rats (40) significantly reduced counterregulatory responses to hypoglycemia. While lactate responses to exercise on day 1 were significantly greater in the ANTE EX50 group, there were no changes in lactate levels in both the ANTE EX30 and control groups. In addition, β-hydroxybutyrate did not change with either intensity exercise on day 1. Thus, these data suggest that lactate and β-hydroxybutyrate, per se, may not be independent mechanisms for exercise-induced counterregulatory failure in this experimental model. Although there is controversy in the literature (41–43), cortisol infusion has also been shown to blunt counterregulatory responses to hypoglycemia in humans (13,44) and rats (45). In the current study, cortisol increased to a similar extent with prolonged exercise, regardless of intensity. Thus, the role of cortisol in exercise-induced hypoglycemia cannot be addressed by this study. Because of the important therapeutic benefits of exercise in diabetes, future studies are needed to delineate a mechanism for exercise-induced hypoglycemia.

In summary, two episodes of prolonged exercise of both low (30% $V\text{O}_{2\text{max}}$) and moderate (50% $V\text{O}_{2\text{max}}$) intensities caused significant blunting of epinephrine, pancreatic polypeptide, EGP, lipolysis, and increased glucose utilization during subsequent hypoglycemia in type 1 diabetes. This resulted in a significantly greater exogenous glucose infusion rate in order to maintain the glycemic level and to prevent more severe hypoglycemia. Thus, patients with type 1 diabetes may have to carefully monitor glucose levels and adjust insulin levels and glycemic targets both during and up to 24 h following prolonged exercise of both low and moderate intensities to prevent subsequent hypoglycemia.

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