Effect of differing antecedent hypoglycemia on counterregulatory responses to exercise in type 1 diabetes

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Galassetti, Pietro, Donna Tate, Ray A. Neill, Antoinette Richardson, Szu-Yun Leu, and Stephen N. Davis. Effect of differing antecedent hypoglycemia on counterregulatory responses to exercise in type 1 diabetes. Am J Physiol Endocrinol Metab 290: E1109–E1117, 2006. First published January 10, 2006; doi:10.1152/ajpendo.00244.2005.—Hypoglycemia frequently occurs during or after exercise in intensively treated patients with type 1 diabetes mellitus (T1DM), but the underlying mechanisms are not clear. In both diabetic and nondiabetic subjects, moderate hypoglycemia blunts counterregulatory responses to subsequent exercise, but it is unknown whether milder levels of hypoglycemia can exert similar effects in a dose-dependent fashion. This study was designed to test the hypothesis that prior hypoglycemia of differing depths induces acute counterregulatory failure of proportionally greater magnitude during subsequent exercise in T1DM. Twenty-two T1DM patients (11 males/11 females, HbA1c 8.0 ± 0.3%) were studied during 90 min of euglycemic cycling exercise after two 2-h periods of previous day euglycemia or hypoglycemia of 3.9, 3.3, or 2.8 mmol/l (HYPO-3.9, HYPO-3.3, HYPO-2.8, respectively). Patients’ counterregulatory responses (circulating levels of neuroendocrine hormones, intermediary metabolites, substrate flux, tracer-determined glucose kinetics, and cardiovascular measurements) were assessed during exercise. Identical euglycemia and basal insulin levels were successfully maintained during all exercise studies, regardless of blood glucose levels during the previous day. After day 1 euglycemia, patients displayed normal counterregulatory responses to exercise. Conversely, when identical exercise was performed after day 1 hypoglycemia of increasing depth, a progressively greater blunting of glucagon, catecholamines, cortisol, endogenous glucose production, and lipolytic responses to exercise was observed. This was paralleled by a graduated increase in the amount of exogenous glucose needed to maintain euglycemia during exercise. Our results demonstrate that acute counterregulatory failure during prolonged, moderate-intensity exercise may be induced in a dose-dependent fashion by differing depths of antecedent hypoglycemia starting at only 3.9 mmol/l in patients with T1DM.

IN PATIENTS WITH TYPE 1 DIABETES MELLITUS (T1DM), hypoglycemia often occurs in association with physical exercise (10, 19). This unfortunately limits the beneficial effects of exercise in type 1 diabetes, such as improving insulin sensitivity (4, 26) glycemic control (29, 42), blood pressure (35), and prevention of cardiovascular disease (2). Despite the many recent advances in diabetes management, the continued high prevalence of hypoglycemia, in general, and exercise-associated hypoglycemia, in particular, remain unresolved clinical challenges. Consequently, patients and physicians often choose suboptimal therapeutic regimens, including less effective insulin dosages and reduced physical activity to reduce the number of hypoglycemic episodes (43).

Physical exercise elicits a complex pattern of adaptive neuroendocrine and metabolic responses (referred to as counterregulatory responses) aimed at maintaining glucose homeostasis in the face of increasing energy substrate needs. If adequately activated, these responses, including increased secretion of glucagon, catecholamines, cortisol, growth hormone, and the consequent increase in endogenous glucose production (EGP), can effectively prevent the onset of hypoglycemia over a broad range of exercise intensities and durations. Conversely, inadequate counterregulatory responses during exercise increase the susceptibility to exercise-associated hypoglycemia. Some counterregulatory responses to stress may be permanently lost in diabetes, as occurs with the absence of the glucagon response to hypoglycemia relatively early in the disease (21), and with the reduction of the catecholamine responses to hypoglycemia after the onset of diabetic autonomic neuropathy (6). Additionally acquired blunting of counterregulatory responses to hypoglycemia and exercise is a temporary, potentially reversible phenomenon, induced by prior episodes of these stresses (11, 23, 41). Recent studies have demonstrated that antecedent hypoglycemia can blunt neuroendocrine and metabolic responses to subsequent exercise both in healthy subjects (13) and in patients with T1DM without evidence of autonomic neuropathy (20). In these studies only one, moderate level (2.8 mmol/l) of antecedent hypoglycemia was induced. Therefore, it could not be determined whether blunting of counterregulatory responses to subsequent exercise was because of a “threshold” value of prior hypoglycemia or whether the counterregulatory failure during exercise is dose dependent on the depth of prior hypoglycemia. During previous studies in which hypoglycemia was induced on two consecutive days, increasing depth but not duration of antecedent hypoglycemia resulted in progressively greater blunting of neuroendocrine and metabolic counterregulatory responses during next-day hypoglycemia in healthy subjects (14, 17). On the other hand, another recent study demonstrated that, in a group of patients with T1DM, antecedent exercise of varying intensities resulted in similar blunting of counterregulatory responses during next-day hypoglycemia (31). Thus it is un-
known whether differing levels of antecedent hypoglycemia will have differential effects on counterregulatory responses during subsequent exercise. Therefore, the present study was designed to test the hypothesis that antecedent hypoglycemia of increasing depth (3.9, 3.3, 2.8 mmol/l) would induce a greater magnitude of blunted neuroendocrine and metabolic counterregulatory responses during subsequent moderate, prolonged exercise in patients with T1DM.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Twenty-two patients with T1DM participated in the study. Gender distribution was 11 males/11 females, mean age 30 ± 3 yr, body mass index 22 ± 1 kg/m², and glycosylated hemoglobin (HbA₁c) 8.0 ± 0.4% (normal range 4.0–6.5%). Patients had been diagnosed with T1DM at least 5 yr before recruitment, with an average of 14 ± 3 yr. Patients were not affected by other chronic disease, as demonstrated by normal blood count, plasma electrolytes, and liver function tests; they also had no evidence of tissue complications of diabetes (retinopathy, renal impairment, hypertension) nor of diabetic autonomic neuropathy (as demonstrated by normal increase in R-R interval after Valsalva maneuver and no significant drop of systolic blood pressure 1 min after standing). All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board. Some of the patients’ data have been published in a previous report (20).

**General design.** After preliminary evaluation of physical fitness with an incremental exercise test, as described below, subjects participated in up to four 2-day studies during which resting hyperinsulinemic euglycemia (EUGLY) or hypoglycemia of 2.8, 3.3, or 3.9 mmol/l (HYPO-2.8, HYPO-3.3, and HYPO-3.9, respectively) was induced on day 1, and euglycemic cycling exercise was performed on day 2. Sixteen patients participated in the EUGLY visit, 16 in the HYPO-2.8 visit, 12 in the HYPO-3.3 visit, and 12 in the HYPO-3.9 visit. Participants in each group were carefully matched for age, glycemic control, level of fitness, and workload during testing (Fig. 1 and Table 1).

**Preliminary exercise testing.** At least 2 wk before the initial experimental visit, patients performed an incremental work test on a stationary cycle ergometer to determine VO₂ max and anaerobic threshold (AT). Air flow, O₂, and CO₂ concentrations in inspired and expired air were measured by a computerized open-circuit indirect calorimetry cart (Medical Graphics) with a mouthpiece and nose clip system. The AT was determined by the V-slope method (5, 5). The AT determined by gas exchange corresponds to the onset of an increased lactate-to-pyruvate ratio in blood and indicates the level of exercise above which anaerobic mechanisms supplement aerobic energy production (39). At work loads below the AT exercise can be continued for a prolonged period, whereas above the AT fatigue will occur considerably faster (38). The experimental work rate was established by calculating 80% AT. This work load 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 (Table 1).

**Experimental visits.** Each patient was further studied during two, three, or four separate visits, each lasting two consecutive days and

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**Table 1. Composition of experimental groups**

<table>
<thead>
<tr>
<th></th>
<th>EUGLY</th>
<th>HYPO-3.9</th>
<th>HYPO-3.3</th>
<th>HYPO-2.8</th>
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<tr>
<td>n</td>
<td>16</td>
<td>12</td>
<td>12</td>
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<td>31±2</td>
<td>32±2</td>
<td>30±3</td>
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<td>HbA₁c, %</td>
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<td>8.0±0.4</td>
<td>8.0±0.3</td>
<td>7.8±0.3</td>
</tr>
<tr>
<td>Watts during test</td>
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<td>2,212±250</td>
<td>2,286±238</td>
<td>2,232±217</td>
</tr>
<tr>
<td>VO₂ max, ml/min</td>
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</table>

Values are means ± SE; n, no. of subjects. EUGLY, euglycemia; HYPO-3.9, -3.3, and -2.8, hypoglycemia of 3.9, 3.3, and 2.8 mg/dl, respectively; HbA₁c, glycosylated hemoglobin.

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Fig. 1. Schematic diagram of experimental protocols. Hypo, hypoglycemia.
including two overnight stays on our Clinical Research Center. During day 1 (Fig. 1), patients underwent either morning and afternoon 2-h hyperinsulinemic-hypoglycemic clamps, with the level of hypoglycemia set at either 2.0, 3.3, or 3.9 mmol/l (HYPO-2.8, HYPO-3.3, HYPO-3.9), or morning and afternoon hyperinsulinemic-euglycemic control experiments (EUGLY). During day 2 of all visits, all patients performed an identical 90-min exercise protocol under euglycemic conditions. The sequence of visits was randomized, and at least 6 wk were allowed to elapse between any two visits.

Patients were asked to avoid hypoglycemia during the 14 days preceding each visit. Patients checked their blood glucose four times per day and two times weekly at night and reported the recorded values to the investigators before admission. Detection of any value <3.9 mM resulted in rescheduling of the study. Patients were also asked to avoid any exercise and consume their usual weight-maintaining diet for 3 days before each study. Intermediate or long-acting insulin was administered in the arms for 3 days before a study to eliminate exaggerated insulin absorption from a working muscle during cycle exercise. Each subject was admitted to the Vanderbilt Clinical Research Center at 4:00 PM on the afternoon before an experiment. Upon admission, patients were asked to discontinue their usual insulin therapy, and two intravenous cannulas were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion in a vein on the back of the left hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained. The other cannula was placed in the contralateral arm so that insulin and 20% glucose (when needed) could be infused via a variable-rate volumetric infusion pump (I-med, San Diego, CA). An insulin infusion was immediately started at a basal rate. Patients then consumed an evening meal and a 7:30 PM snack and were requested not to ingest any food after 10:00 PM. The insulin infusion rate was increased during meal consumption. Throughout the night, blood glucose was measured every 30 min, and the insulin infusion rate was adjusted constantly to maintain glycemic levels of 4.4–6.7 mM.

Day 1 procedures. Day 1 procedures started at 8:00 AM after a 10-h overnight fast and lasted 480 min divided into an equilibration period (0–120 min), a morning hyperinsulinemic clamp period (120–240 min), a rest period (240–360 min), and an afternoon hyperinsulinemic clamp period (360–480 min). At 120 min, all studies were divided into a primed continuous infusion of insulin (9 pmol·kg⁻¹·min⁻¹) started (33). In prior hypoglycemia studies, plasma glucose was allowed to fall over a 30-min period to the target hypoglycemic plateau. Plasma glucose was measured every 5 min and maintained at the desired level via a variable-rate infusion of 20% dextrose (3). In prior euglycemia studies, plasma glucose was held constant at basal levels by a similar technique (18). At 240 min, the insulin infusion was decreased to the morning basal rate and euglycemia was restored in prior hypoglycemia studies and maintained in the prior euglycemia studies. At 360 min (~2:00 PM), a second 2-h euglycemic or hypoglycemic clamp identical to that performed in the morning was carried out. At 480 min, the insulin infusion was decreased to the morning basal rate, euglycemia was restored in prior hypoglycemia studies, and all patients were allowed to consume a standardized meal. Evening and overnight procedures were then identical to those of the admission night.

Day 2 procedures. Day 2 procedures started at 8:00 AM after a 10-h overnight fast and lasted 210 min (time 0 min to 210 min), divided into an equilibration period (0–90 min), a basal period (90 to 120 min), and an exercise period (120–210 min). A primed (18 µCi) infusion (0.18 µCi/min) of [³H]glucose was started at 0 min and continued throughout the experiment. Exercise consisted of 90 min continuous pedaling (at 60–70 revolutions/min) on an upright cycle ergometer (Medical Graphics, Yorba Linda, CA) at 80% of the individual’s AT (~50% Vo₂ max). Plasma glucose was measured every 5 min and maintained equivalent to baseline levels throughout the study via variable-rate infusion of 20% dextrose. In an attempt to reproduce the drop in insulin levels that physiologically occurs with exercise of this intensity, the basal insulin infusion rate was decreased by 40% after the first 30 min of exercise, providing that the resulting reduced rate was at least 6 nmol/h (1 U/h). In cases in which a 40% reduction of the basal rate would have resulted in an insulin infusion rate of <6 nmol/h, a minimum rate of 6 nmol/h was maintained. Potassium chloride was also infused (5 mmol/h) during exercise. After completion of the exercise protocol, patients consumed a meal and were discharged.

Tracer methodology. Rates of glucose appearance (Ra), EGP, and glucose utilization were calculated according to the methods of Wall et al. (37). EGP was calculated by determining the total rate of Ra (this comprises both EGP and any exogenous glucose infused to maintain euglycemia) and subtracting from it the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, since underestimates of total Ra and glucose disposal can be obtained. This underestimate can be largely overcome by use of HPLC-purified tracer and taking measurements under steady-state conditions (i.e., constant 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 the tracer infusion rate were made commensurate with the changes of the exogenous glucose infusion rate (GIR). In this study, only data recorded at baseline and during the last 30 min of exercise (when a metabolic steady state existed) were used in calculating glucose turnover.

Analytical methods. The collection and processing of blood samples have been described elsewhere (9). 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. (1) with an interassay coefficient of variation (CV) of 12%. Free insulin was measured as previously described (40) with an interassay CV of 9%. Catecholamines were determined by HPLC (8) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. We made the following 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 accurate identification of the relevant respective 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 determined by RIA (25) with a CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (22) with an interassay CV of 8%. Lactate, glyceral, alanine, and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (27). NEFA were measured using the WAKO kit adapted for use on a centrifugal analyzer (24).

On day 2, blood samples for glucose flux were taken every 10 min throughout the basal period and every 15 min during exercise. Blood for hormones and intermediary metabolites was drawn two times during the basal period and every 15 min during exercise period. Cardiovascular parameters (pulse, systolic and diastolic arterial pressure) were measured every 10 min from 30 min to 90 min. Respiratory quotients and carbohydrate and lipid oxidation were measured by gas exchange during the basal period and the final 10 min of exercise.

Materials. HPLC-purified [³H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi/mM). 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 subject’s own plasma.

Statistical analysis. Data are expressed as means ± SE unless otherwise stated. A standard, parametric, two-way ANOVA test with repeated-measures design was performed between day 2 EUGLY data and data from the group exposed to the deepest level of antecedent hypoglycemia (HYPO-2.8). For variables in which statistical signifi-
cance was detected between these two groups, the hypothesis was then tested that the change in that variable over exercise time was dependent on antecedent glucose levels. To evaluate this hypothesis, data were analyzed with a mixed-model approach, which allows accommodation of the correlations between longitudinal measurements over exercise time and repeated measurements at different antecedent glucose levels for each subject. Applying this model, the change over exercise appeared to be linear in six of the eight variables [glucagon, cortisol, epinephrine, norepinephrine, free fatty acid (FFA), lactate, endogenous glucose production (EGP), and glucose infusion rate (GIR)], whereas it was best described as a curve, with the addition of a quadratic form of time, in the remaining two (norepinephrine and lactate). With this analysis for each variable of interest, an equation could be derived that allowed calculation of the change over exercise time of that variable at any antecedent glucose levels (between 2.8 and 3.9 mmol/l). Calculated changes over exercise time for key variables are shown in Fig. 2. Further analysis of this model allows detection of whether a statistically significant interaction between the antecedent glucose level and the change of that variable during exercise was present. A positive estimate for the interaction implied that the change over time of the variable of interest increased significantly over time proportionally to the level of antecedent glucose, and a negative estimate implied that the change of the variable decreased with increasing levels of antecedent glucose; a $P$ value of $<0.05$ in this analysis indicated that the interaction between antecedent glucose levels and changes in a given variable during exercise was statistically significant.

RESULTS

Day 1: Plasma glucose levels. Basal plasma glucose levels were comparable in all experimental groups during both morning and afternoon clamps (Fig. 3). During the last 30 min of the clamp periods, plasma glucose was $5.1 \pm 0.1$ mM in the morning and $5.2 \pm 0.1$ mM in the afternoon in EUGLY, $3.8 \pm 0.1$ mM in the morning and $3.9 \pm 0.1$ mM in the afternoon in HYPO-3.9, $3.3 \pm 0.1$ mM in the morning and $3.3 \pm 0.1$ mM in the afternoon in HYPO-3.3, and $2.8 \pm 0.1$ mM in the morning and $2.8 \pm 0.1$ mM in the afternoon in HYPO-2.8.

Plasma insulin concentrations were similar in the four groups at all times during day 1 procedures.

Day 2: Insulin, glucose, and counterregulatory hormone levels. Preexercise plasma glucose levels were similar in the four experimental groups both at baseline and during the last 30 min of exercise ($5.1 \pm 0.1$ to $5.2 \pm 0.1$ mM). Similarly, plasma insulin was comparable in the four groups at baseline (EUGLY 78 $\pm$ 6 pmol/l, HYPO-2.8 72 $\pm$ 12 pmol/l, HYPO-3.3 78 $\pm$ 18 pmol/l, HYPO-3.9 84 $\pm$ 12 pmol/l) and during
exercise (last 30 min: EUGLY 60 ± 6 pmol/l, HYPO-2.8 60 ± 6 pmol/l, HYPO-3.3 66 ± 6 pmol/l, HYPO-3.9 72 ± 12 pmol/l).

In the EUGLY group, plasma glucagon increased by 10 ± 2 ng/l during exercise (Table 2 and Fig. 4). In the other three groups, there was a reduction in the glucagon response that was significantly (P = 0.023) dependent on the depth of antecedent hypoglycemia (HYPO-3.9 6 ± 3 ng/l, HYPO-3.3 4 ± 3 ng/l, HYPO-2.8 0.5 ± 1 ng/l). A similar pattern was also observed in the cortisol response to exercise, which was 11 ± 2 μg/dl in EUGLY, and was reduced in the prior hypoglycemia groups (HYPO-3.9 8 ± 3 mg/l, HYPO-3.3 4 ± 3 mg/l, HYPO-2.8 2 ± 2 mg/l) in a manner significantly dependent (P = 0.0015) on the depth of antecedent hypoglycemia.

The exercise-induced increment in plasma epinephrine during EUGLY was 110 ± 19 pg/ml but was progressively reduced after antecedent hypoglycemia (HYPO-3.9 72 ± 21 pg/ml, HYPO-3.3 69 ± 22 pg/ml, HYPO-2.8 48 ± 7 pg/ml). The exercise-induced increase in plasma norepinephrine was 560 ± 110 pg/ml in EUGLY, 443 ± 112 pg/ml in HYPO-3.9, 514 ± 172 pg/ml in HYPO-3.3, and 363 ± 98 pg/ml in HYPO-2.8. This progressive reduction in catecholamine responses to exercise was significantly dependent on the depth of antecedent hypoglycemia for epinephrine (P = 0.002), although there was a trend to reach statistical significance for norepinephrine (P = 0.087).

The exercise-induced increase in plasma growth hormone and pancreatic polypeptide was not significantly blunted by any levels of antecedent hypoglycemia.

Day 2: Glucose kinetics. By the last 30 min of exercise, EGP reached 16 ± 3 μmol·kg⁻¹·min⁻¹ in the EUGLY group, a value not dissimilar from that previously observed in healthy subjects during comparable exercise (Fig. 5). However, EGP was reduced in each of the three antecedent hypoglycemic groups during exercise (HYPO-3.9 12 ± 6 μmol·kg⁻¹·min⁻¹, HYPO-3.3 6 ± 2 μmol·kg⁻¹·min⁻¹, HYPO-2.8 6 ± 3 μmol·kg⁻¹·min⁻¹). Conversely, the infusion rate of exogenous glucose required to maintain euglycemia at the end of exercise was lowest in EUGLY (9 ± 2 μmol·kg⁻¹·min⁻¹) and progressively greater in the groups exposed to prior hypoglycemia (HYPO-3.9 15 ± 3 μmol·kg⁻¹·min⁻¹, HYPO-3.3 20 ± 4 μmol·kg⁻¹·min⁻¹, HYPO-2.8 24 ± 5 μmol·kg⁻¹·min⁻¹). For both variables, the change in the exercise response was significantly dependent on the depth of antecedent hypoglycemia (EGP P = 0.0024, GIR P < 0.0001).

Day 2: Intermediary metabolism. Blood lactate (Table 3 and Fig. 6) increased by a greater extent during exercise in EUGLY (1.34 ± 0.14 mM) than in the three hypoglycemic groups (HYPO-3.9 0.81 ± 0.20 mM, HYPO-3.3 0.66 ± 0.13 mM, HYPO-2.8 0.77 ± 0.20 mM); the magnitude of the blunted response was significantly dependent on the depth of prior hypoglycemia (P < 0.003).

Values were calculated via mixed-model analysis integrating longitudinal measurements over exercise time and repeated measurements at different prior glucose levels. GIR, glucose infusion rate; EGP, endogenous glucose production; FFA, free fatty acid.

Table 2. Estimated slopes of glucagon, epinephrine, cortisol, GIR, EGP, and FFA responses to exercise derived from data measured in 22 type 1 diabetic patients who exercised after previous-day euglycemia or hypoglycemia of 2.8, 3.3, or 3.9 mM

<table>
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<th>P Value, Interaction Between Time and Level</th>
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<td>Glucagon, ng·l⁻¹·min⁻¹</td>
<td>HYPO-2.8 -0.0058</td>
<td>HYPO-3.3 0.017</td>
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<tr>
<td></td>
<td>HYPO-3.9 0.041</td>
<td>EUGLY 0.087</td>
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<td></td>
<td>E1113PRIOR HYPOGLYCEMIA AND EXERCISE IN DIABETES</td>
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<tr>
<td>Epinephrine, pM/min</td>
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<tr>
<td>Cortisol, nM/min</td>
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<td>GIR, μmol·kg⁻¹·min⁻¹·min⁻¹</td>
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<td>0.041</td>
</tr>
<tr>
<td>EGP, μmol·kg⁻¹·min⁻¹·min⁻¹</td>
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<tr>
<td>FFA, μM/min</td>
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Fig. 4. Plasma incremental (baseline to final 30 min of exercise) catecholamine, glucagon, and cortisol levels during day 2 exercise in (90 min cycling at ~50% VO₂max) 22 patients (11 males/11 females) with T1DM. Data are group means ± SE. On the previous day, patients had undergone two 120-min hyperinsulinemic clamps at either euglycemia or hypoglycemia of 3.9, 3.3, or 2.8 mmol/l. *Epinephrine, glucagon, and cortisol responses during exercise are significantly reduced (P < 0.05) by increasing day 1 antecedent hypoglycemia.
indexes of lipolysis during exercise also displayed dose-dependent blunting after prior hypoglycemia. FFA increased during exercise by 224 ± 68 μM in EUGLY but only by 152 ± 39 μM in HYPO-3.9, 105 ± 51 μM in HYPO-3.3, and 80 ± 62 μM in HYPO-2.8. Blood glycerol increased during exercise by 103 ± 14 μM in EUGLY and by 51 ± 9 μM in HYPO-3.9, 49 ± 15 μM in HYPO-3.3, and 66 ± 12 μM in HYPO-2.8. Blunting of the FFA response to exercise was significantly dependent on the depth of antecedent hypoglycemia (P = 0.002); exercise-induced increases in glycerol levels were also significantly lower (P < 0.02–0.001) than in EUGLY for each of the three hypoglycemic groups.

No difference in the circulating levels of alanine or of the ketone body β-hydroxybutyrate was measured among the four groups either at baseline or during exercise. Resting energy rate, respiratory quotient, and fat and carbohydrate oxidation were also similar during exercise in all groups.

Day 2: Cardiovascular parameters. No significant difference occurred in heart rate or systolic, diastolic, and mean arterial pressure among any of the four experimental groups at baseline or during exercise.

**DISCUSSION**

This study has determined that 1) counterregulatory failure during exercise can be caused by even mild antecedent hypoglycemia (3.9 mmol/l) and 2) the magnitude of neuroendocrine and metabolic counterregulatory failure during exercise increases commensurate with the depth (2.8–3.9 mmol/l) of antecedent hypoglycemia.

Our findings are conceptually similar with previous data investigating the effects of increasing depth of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia. Davis et al. (17) investigated a group of healthy subjects during a 2-day protocol in which differing antecedent hypoglycemia of 3.9, 3.3, or 2.8 mmol/l was used to determine the magnitude of blunted counterregulatory responses to day 2 hypoglycemia. Similar to the present study, deeper day 1 hypoglycemia resulted in greater blunting of counterregulatory responses to next-day hypoglycemia. The same authors, in a separate study (14), also tested the effects of differing durations of antecedent hypoglycemia of ~2.8 mmol/l on counterregulatory responses to next-day hypoglycemia. Unlike the previous study increasing the duration of hypoglycemia (2 episodes of 5, 30, or 120 min) of 2.8 mmol/l, hypoglycemia caused very little difference in the magnitude of the blunting of neuroendocrine and metabolic counterregulatory responses to next-day hypoglycemia. Symptom responses were, however, reduced by the longer-duration but not by shorter-duration hypoglycemia. A more recent report (31) involved an experimental model that is almost the exact reciprocal of the present study, i.e., T1DM patients were challenged with day 1 exercise of different intensities (rest, 30 or 50% of maximal aerobic capacity), followed by day-night hypoglycemia of ~2.8 mmol/l. Interestingly, although both exercise levels resulted in a widespread significant blunting of counterregulatory responses during next-day hypoglycemia, the magnitude of the

**Table 3. Estimated peak- and end-exercise changes over baseline of plasma norepinephrine and lactate derived from data measured in 22 type 1 diabetic patients who exercised after previous-day euglycemia or hypoglycemia of 2.8, 3.3, or 3.9 mM**

<table>
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<tr>
<td></td>
<td>Time, min</td>
<td>HYPO-2.8 HYPO-3.3 HYPO-3.9 EUGLY</td>
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<tr>
<td>Norepinephrine, nM</td>
<td>70 (Peak)</td>
<td>319.9 353.2 386.6 453.3</td>
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<td></td>
<td>90</td>
<td>261.4 304.3 347.1 432.9</td>
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<tr>
<td>Lactate, mM</td>
<td>50 (Peak)</td>
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<td>90</td>
<td>−0.073 0.047 0.165 0.402</td>
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Values were calculated via mixed-model analysis integrating longitudinal measurements over exercise time and repeated measurements at different prior glucose levels.
blunting was similar for both exercise intensities. Unlike the present study, therefore, a dose-response relationship between intensity of the prior stress (exercise) and reduced response to the subsequent stress (hypoglycemia) was not observed. This difference may be because of the intrinsic characteristics of exercise as a form of stress by which a threshold-type activation of adaptive mechanisms may be in place independent of the intensity of exercise performed (at least within the range of nonexhaustive exercise intensities used in the above protocol).

A potentially confounding factor in the interpretation of our data would have been the presence of uncontrolled hypoglycemia at any time during the study or during the days preceding it. Hypoglycemia was prevented by intensive blood glucose testing by the patients and frequent phone calls by the study team during the 2 wk preceding the study (any reported hypoglycemic value during this period resulted in rescheduling of the study) and by careful control of glycemic levels at all times during our 2-day studies. During overnight stays at our clinical research center, hypoglycemia was avoided by constant adjustments of exogenous insulin and/or glucose. Additionally, euglycemia was also strictly maintained during exercise, when hyperglycemia could have inhibited counterregulatory responses while even moderate drops in blood glucose would have artificially enhanced the magnitude of the counterregulatory responses induced by exercise per se. Similarly, fluctuations in prevailing plasma insulin levels could have...
significantly impacted exercise responses. Typically, peripheral plasma insulin levels are reduced 40–50% during exercise, similar to those in our study. (12). In T1DM, however, a similar reduction in insulin levels during exercise may result in relative hypoinsulinemia, particularly in patients who need very low basal insulin infusion rates; as a consequence, it is not unusual to see glycemic levels increasing to 8–11 mmol/l. Furthermore, during our experimental model of exercise, a drop in insulin levels during exercise will not reflect the usual daily conditions of diabetic patients on multiple daily insulin basal/bolus injection regimens who are unable to rapidly reduce circulating insulin levels. Therefore, insulin concentrations during exercise were controlled at levels of 60–70 pmol/l that reflect typical postabsorptive insulinemia found in T1DM patients (30). Indeed, the inability of T1DM patients to suppress insulin levels during exercise may per se be a factor responsible for exercise-associated hypoglycemia.

Multiple key neuroendocrine, autonomic nervous system, and metabolic counterregulatory responses during exercise were blunted in a dose-response fashion by the differing day 1 hypoglycemia. We chose a prolonged model of exercise (90 min) that would be representative of many typical forms of exercise undertaken by T1DM (e.g., soccer, tennis, bike riding, running, yard work). The blunted glucagon responses appear particularly relevant for patients with T1DM. In these patients, although the ability to acutely increase circulating glucagon levels in response to hypoglycemia is permanently lost during the first 5 yr after diagnosis (21), secretion of the hormone during exercise is preserved (20). This suggests that the pancreatic α-cell deficit present in diabetes is stimulus specific, and preservation of this counterregulatory mechanism represents an effective defense against exercise-associated hypoglycemia. In our study, the EUGLY group was able to mount a glucagon response similar to that in age-matched non-diabetic subjects during comparable exercise (13). Prior hypoglycemia of increasing depth, however, progressively reduced this response until its complete ablation with the deepest hypoglycemic level (2.8 mmol/l), thereby proportionally increasing the risk of exercise-associated hypoglycemia. Similarly, catecholamines and FFAs were significantly and proportionally reduced during exercise by increasing depth of day 1 hypoglycemia (Fig. 2). The metabolic consequence of this progressively reduced response was the increasing amount of exogenous glucose needed to maintain euglycemia during exercise (Fig. 5, top). In fact, the key homeostatic metabolic counterregulatory mechanisms of increased EGP and limitation of glucose disposal were significantly blunted by increasing day 1 hypoglycemia. Analysis of the areas under each of the curves in Fig. 5 represents the average amounts of glucose that each group of patients, in the presence of basal arterial insulin levels, would have needed to ingest during exercise to prevent hypoglycemia from occurring.

The deficits in neuroendocrine and metabolic counterregulatory responses became progressively worse after 30 min of exercise. This demonstrates that antecedent hypoglycemia has a more pronounced effect on blunting counterregulatory mechanisms during prolonged exercise. The patients studied in the present protocols had a mean HbA1c of ≈7.9%. This value is similar to the average HbA1c values of T1DM in the United States. We therefore believe that the results of this study are translatable to many T1DM individuals. We do not know whether intensive glucose control with HbA1c values <7% would modify the results of this study.

The mechanisms responsible for exercise-associated hypoglycemia in T1DM remain unresolved. Although acute increases in insulin sensitivity caused by exercise, paralleled by the potential relative hyperinsulinemia resulting from the inability of diabetic patients to endogenously regulate insulin levels, have been hypothesized to play a role (34), this mechanism is likely to have only a modest impact (3). Additionally, both in well-controlled T1DM patients (i.e., exposed to frequent episodes of antecedent hypoglycemia) and in patients with classical diabetic autonomic neuropathy (32), reduced adrenergic adaptation to prolonged physical exercise has been reported (6). Furthermore, because of the many similarities existing between counterregulatory adaptation to hypoglycemia and exercise, it is possible that exercise also shares some of the mechanisms responsible for counterregulatory failure induced by repeated hypoglycemia. However, these mechanisms also remain partly speculative. These include alterations in cerebral glucose extraction (7), elevations of circulating levels of lactate (28, 36) and/or ketone bodies (36) during the subsequent stress, and prior hypercortisolemia (15, 15, 16). Therefore, elucidation of the mechanisms responsible for blunted counterregulatory responses during exercise after prior hypoglycemia requires additional investigation.

In summary, this study has demonstrated that two 2-h episodes of antecedent hypoglycemia of increasing depth (~3.9, ~3.3, and ~2.8 mmol/l) result in a widespread, progressive blunting of counterregulatory responses during next-day moderate, prolonged exercise in patients with T1DM. Among neuroendocrine counterregulatory responses, glucagon, epinephrine, and cortisol were all blunted in a clear dose-response pattern, and this reduction was paralleled by proportional dose-response blunting of EGP and lipolysis.

We conclude that 1) in patients with T1DM, even mild antecedent hypoglycemia of 3.9 mmol/l can produce counterregulatory failure during subsequent, prolonged exercise; and 2) there is a dose-response relationship between the depth of antecedent hypoglycemia and the magnitude of counterregulatory failure during subsequent exercise.

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