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Impaired Overnight Counterregulatory Hormone Responses to Spontaneous Hypoglycemia in Children with Type 1 Diabetes

Diabetes Research in Children Network (DirecNet) Study Group*

Abstract

To assess the changes in counterregulatory hormones overnight after an afternoon of structured exercise or sedentary activity in children with Type 1 Diabetes Mellitus (T1DM), the Diabetes Research in Children Network (DirecNet) studied 50 children (10-<18y) with T1DM in 5 clinical research centers on two separate days (with and without an afternoon exercise session) using a crossover design. Glucose, epinephrine, norepinephrine, cortisol, growth hormone (GH) and glucagon concentrations were measured hourly overnight. Nocturnal hypoglycemia (plasma glucose concentrations ≤70 mg/dL [3.9 mmol/L]) occurred more frequently on the nights following exercise (56% vs. 36%; p=0.008). Mean hourly concentrations of most hormones did not differ between sedentary or exercise nights or between nights with or without hypoglycemia. Spontaneous nocturnal hypoglycemia only stimulated small increases in plasma epinephrine and growth hormone concentrations and failed to cause a rise in norepinephrine, cortisol or glucagon levels in comparison to values during the hour before or after hypoglycemia or other times during those same nights. Counterregulatory hormone responses to spontaneous nocturnal hypoglycemia were markedly decreased regardless of whether there was antecedent afternoon exercise in children with T1DM. Sleep-induced impairments in counterregulatory hormone responses likely contribute to the increased risk of hypoglycemia during the entire overnight period in youth with T1DM.

Keywords

Hypoglycemia; Epinephrine; Norepinephrine; Diabetes Mellitus; Type I and Growth Hormone

Introduction

In non-diabetic subjects, falling plasma glucose concentrations activate a cascade of pancreatic, neuroendocrine and autonomic nervous system responses aimed at preventing hypoglycemia. In patients with type 1 diabetes mellitus (T1DM), glucagon responses to hypoglycemia are permanently lost shortly after the onset of the disease (1). However, in the absence of autonomic neuropathy, sympathetic nervous system responses are usually preserved and are able to mount an adequate counterregulatory response to hypoglycemia. Nonetheless, after repeated episodes of mild to moderate hypoglycemia, sympathetic responses to subsequent hypoglycemia are often reduced, increasing the likelihood of more severe hypoglycemic events. This phenomenon, known as "*hypoglycemia-associated autonomic failure*" has been observed in healthy volunteers, as well as in diabetic subjects (2–7). Intense or prolonged exercise also stimulates counterregulatory hormone responses, which serve to increase hepatic glucose production to meet the increased metabolic demands of exercising muscle and to prevent hypoglycemia. However, evidence suggests that stimulation of counterregulatory hormone

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concentrations by exercise itself can blunt sympathetic nervous system responses to subsequent insulin-induced hypoglycemia.

The DirecNet Study Group recently conducted an inpatient study in 50 children and adolescents with T1DM that examined whether moderate, prolonged (i.e., 75 minutes), aerobic exercise in the late afternoon increases the risk of delayed hypoglycemia during the subsequent night versus a night after no exercise (sedentary night). Although exercise stimulated consistent and significant increases in counterregulatory hormone concentrations in all of the subjects (8), 30% of the youngsters required treatment for hypoglycemia when plasma glucose levels fell to ≤60 mg/dL (3.3 mmol/L). Of even greater concern, nearly twice as many of the subjects developed hypoglycemia (48% versus 28%) on the night following exercise than on the night following the sedentary day (9). In order to examine whether counterregulatory hormone responses to spontaneous hypoglycemia are impaired during nights with versus nights without antecedent exercise, plasma epinephrine, norepinephrine, cortisol, GH and glucagon concentrations were frequently sampled during both the exercise and sedentary nights in the same group of subjects reported previously (8,9).

Materials and Methods

The study was approved by the DirecNet Data and Safety Monitoring Board and the Institutional Review Boards at each of the five DirecNet centers. A parent or guardian and each subject gave written consent and assent, respectively. Subject eligibility criteria included age from 10 to <18 years, type 1 diabetes for at least 18 months, and HbA1c ≤10.0%. The study protocol is reported in detail elsewhere (9). To briefly summarize, each subject was hospitalized in a clinical research center for approximately 24 hours on two occasions separated by one to four weeks. Subjects had a 75-minute exercise session on a treadmill in the late afternoon of one of the study days (exercise day), and no exercise on the other study day (sedentary day). Subjects were maintained on the same pre-meal bolus and basal insulin dose regimen as they normally received on a sedentary day without exercise. The order of the study days was chosen at random in a crossover design. Blood samples for central laboratory determination of overnight glucose concentrations and counterregulatory hormone levels were obtained from an intravenous catheter hourly from 2200 to 0600 h, plasma or serum was separated promptly and immediately frozen. Glucose in blood (with concentrations expressed as plasma equivalents) was also measured at the bedside using the One Touch® Ultra® meter (LifeScan, Inc., Milpitas, CA) every 30 min during the overnight period. If the meter glucose concentration was ≤60 mg/dL (3.3 mmol/L) between the pre-planned hourly sampling points, an extra sample for central laboratory glucose and counterregulatory hormone measurements was obtained and the subject was treated for hypoglycemia.

Overnight glucagon, cortisol and GH concentrations were measured for all 50 subjects for both visits. The glucagon concentration was unavailable for 9 (1%) of the expected 900 measurements, cortisol was missing for 12 (1%) and GH was missing for 12 (1%). Epinephrine and norepinephrine were measured for 34 (68%) subjects on both visits and was missing for 7 (1%) of the expected 612 measurements.

Glucagon, cortisol, GH and glucose concentrations were measured at the DirecNet Central Laboratory (University of Minnesota, Minneapolis, MN). Glucagon was measured by a radioimmunoassay (Linco Research, St. Charles, MO) with the primary antibody from guinea pig, and the secondary from goat. The lower limit of detection was 20 pg/mL (6 pmol/L). Coefficients of variation (CVs) were 6.5 – 8.8% on 3 controls. Cortisol was assayed with a competitive chemiluminescence assay (Bayer Advia Centaur, Bayer HealthCare, Diagnostics Division, Tarrytown, NY), using a polyclonal rabbit antibody and a mouse monoclonal antibody coupled with paramagnetic particles. Lower limit of detection was 0.5 μg/dL (14

nmol/L). CVs were 11 – 12% on 2 controls. GH was measured by a sandwich chemiluminescence assay (DPC Immulite, Diagnostic Products Corporation, Los Angeles, CA). Monoclonal mouse antibody was coated on the bead with a rabbit polyclonal antibody in the reagent. Lower limit of detection was 0.1 ng/mL (4 pmol/L). CVs were 5.9 – 9.1%. Glucose determinations were made using a hexokinase enzymatic method in the central laboratory (10,11) and measured by the OneTouch® Ultra at the bedside (12).

Epinephrine and norepinephrine concentrations were measured at the Mayo Clinic Laboratory (Rochester, MN) using a reverse phase (C18) HPLC column to separate norepinephrine and epinephrine, which were detected coulometrically, using an ESA Coulochem II instrument. The lower limit of detection in this assay is 10 pg/mL (0.06 nmol/L and 55 pmol/L, respectively). CVs were 7–11% and 6–7% on 3 controls. Samples for catecholamines were collected in EDTA tubes and immediately frozen. As part of the validation it was determined that there was no difference in the sample values when the samples collected in EDTA tubes were immediately frozen vs. when collected in EDTA-Na bisulfite tubes.

Statistical Analysis

A mean value was calculated for each hormone concentration for each night. The presented standard deviations denote the between night variation of these concentrations. Median values and quartiles of these nightly means were also calculated. A value of 10 pg/mL was imputed for the 12% of norepinephrine measurements below the detection limit and a value of zero was imputed for cortisol and growth hormone measurements below the detection limit (1% for both hormones). Because the large majority of epinephrine measurements were below the detection level, results are presented as the percentage detectable. A separate repeated measures regression model was fit for each hormone (one observation per night) to compare the mean level for nights following sedentary vs. exercise days and nights where the glucose did vs. did not drop ≤70 mg/dL (3.9 mmol/L). Each regression model controlled for any period effect in the crossover design. A square root transformation was used for growth hormone to reduce the skewness of the residual values. Permutation tests were used for epinephrine to account for a high percentage of measurements below the detection level.

Additional repeated measures regressions were fit with one observation per hormone measurement with hypoglycemia and the hour before and hour after hypoglycemia as time-dependent indicators. An autoregressive covariance structure was used to model the correlations among the hourly measurements.

Results

Clinical characteristics of study subjects

Fifty subjects, 10 at each of the DirecNet centers, participated in the study. Their average age was 14.8 ± 1.7 years; 44% were female; 90% were Caucasian, 4% African-American, 2% Hispanic, and 4% Asian. The mean duration of diabetes was 7.0 ± 3.7 years. An insulin pump was used by 54% and injections consisting of glargine and short acting insulin by the other 46%. Mean HbA1c was 7.8 ± 0.8%. A severe episode of hypoglycemia (resulting in seizure or loss of consciousness) in the 6 months prior to the study was reported by 3 subjects (6%).

Glucose and counterregulatory hormone levels during the overnight periods

Mean hourly plasma glucose levels were lower during the exercise (131 ± 58 mg/dL) than the sedentary (154 ± 69, p=0.003) night and the number of nights with nadir glucose ≤70 mg/dL (3.9 mmol/L) was increased (28 nights after antecedent exercise versus 18 after sedentary days). The time of occurrence of these low glucose concentrations was distributed throughout the overnight period (Figure 1). Despite lower glucose levels and more frequent hypoglycemia

on the exercise than on the sedentary night, the overnight profiles of each of the counterregulatory hormones were very similar on both nights. Subjects with lower glucose concentrations during exercise were more likely to get low overnight (nadir Glucose over night vs. nadir Glucose during exercise on exercise visits (N=50): Pearson correlation is 0.72 ($p<0.001$)). In addition, patients whose 4PM (meter) glucose was low on the sedentary day were also more likely to get low that night (nadir Glucose over night vs. Glucose at 4PM on Sedentary Visits: Pearson correlation is 0.45 ($p=0.001$)).

There were no significant differences in the mean hormone concentrations between the exercise and sedentary nights for any of the hormones measured (epinephrine: 7% vs. 4% detectable, $p=0.30$, glucagon: 62 ± 17 vs. 63 ± 17 pg/mL [18 ± 5 vs. 18 ± 5 pmol/L], $p=0.24$; norepinephrine: 104 ± 43 vs. 102 ± 45 pg/mL [0.61 ± 0.25 vs. 0.60 ± 0.27 nmol/L], $p=0.91$; GH: 8.2 ± 4.3 vs. 8.6 ± 5.4 ng/mL [362 ± 190 vs 380 ± 238 pmol/L], $p=0.52$; cortisol: 5.0 ± 2.0 vs. 5.1 ± 2.0 μg/dL [138 ± 55 vs. 141 ± 55 nmol/L], $p=0.68$, exercise vs. sedentary respectively). When the nights were stratified depending on whether or not the nadir overnight glucose was ≤70 mg/dL (3.9 mmol/L), only epinephrine showed a significant difference 10% detectable on nights with hypoglycemia vs. 2% on nights without hypoglycemia; $p=0.002$). There were no significant differences in overall means for any of the other hormones measured.

Mean plasma glucose and counterregulatory hormone concentrations during the hour before, during and after the spontaneous nocturnal hypoglycemic events (plasma glucose <70 mg/dL), as well as other times during the same night, are shown in the Table. As expected, hypoglycemia failed to stimulate increases in circulating glucagon levels and plasma cortisol concentrations were also unchanged during hypoglycemia. Plasma epinephrine was detectable more frequently during hypoglycemia (21% vs. 6%; $p=0.002$) but norepinephrine concentrations did not differ meaningfully from values during the hour before and after hypoglycemia or at other times of the same night. In contrast to the catecholamine responses to nocturnal hypoglycemia, afternoon exercise stimulated a brisk rise in norepinephrine (from 221 to 572 pg/mL) and the percentage of epinephrine concentrations above detection rose from 12% to 82% during exercise in these same subjects. Nocturnal hypoglycemia was able to stimulate a significant increase in GH concentrations (Table). When the data were analyzed by gender the only conclusive difference is for norepinephrine where boys (N=14) had higher concentrations than girls (N=15) ($p=0.002$).

Discussion

We have performed a careful assessment of several counterregulatory hormones in children with T1DM sampled overnight after a sedentary afternoon and after exercise in the afternoon in order to examine whether antecedent afternoon exercise blunts counterregulatory hormone responses to spontaneous nocturnal hypoglycemia during the following night. Despite lower mean glucose levels and an increased incidence of hypoglycemia overnight after exercise, circulating concentrations of glucagon, GH, cortisol, and norepinephrine did not differ in these young subjects between the two nights. Most important, with the exception of epinephrine and GH, there was very little difference in counterregulatory hormone concentrations during hypoglycemia in comparison to values during the hour before and after hypoglycemia and to values at other times during the same night. Even though epinephrine concentrations rose above detectable levels significantly more frequently during nocturnal hypoglycemia in our cohort (21 vs 6%), epinephrine levels remained below the detection limits of the assay during hypoglycemia in the large majority of patients (i.e., 79%). These findings are remarkable and suggest that whether or not there was antecedent exercise, diabetic children have overall diminished counterregulatory hormone responses to spontaneous overnight hypoglycemia.

Detailed physiological studies have shown that hypoglycemia and exercise may reciprocally impair each other's hormonal counter-regulatory responses. Antecedent hypoglycemia blunts the counter-regulatory responses to exercise, and antecedent exercise blunts the counter-regulatory responses to subsequent hypoglycemia in both healthy volunteers and subjects with T1DM (4,5,13–16). Our findings of decreased hormone counterregulation during spontaneous hypoglycemia overnight are nearly identical to those previously reported by Jones and colleagues (17) who used the hypoglycemic clamp technique to demonstrate that in children with T1DM deep sleep markedly impairs catecholamine and cortisol responses to hypoglycemia in comparison to the responses to the same hypoglycemic stimulus when subjects are awake during the day or during the night. In our study, the decreased catecholamine responses to nocturnal hypoglycemia stand in sharp contrast to the vigorous plasma norepinephrine and more consistent epinephrine responses to moderate afternoon exercise in the same subjects. In the Jones study, the impairment in counterregulatory responses during deep sleep was nearly identical in the non-diabetic and diabetic children, providing further evidence that the defects in counterregulatory hormone responsiveness were due to the sleep state itself. Their studies were performed in a sleep laboratory and hypoglycemia was induced relatively early in the night as soon as the subjects achieved stage 3–4 sleep. Our findings extend those of Jones, et al. by demonstrating that sleep-induced impairments of catecholamine and cortisol responses occurred throughout the overnight period at presumably different stages of sleep. In addition, we observed diminished catecholamine and cortisol responses to nocturnal hypoglycemia under basal rather than hyperinsulinemic conditions and in response to spontaneous rather than acute, induced reductions in plasma glucose.

Pathophysiologic mechanisms leading to the development of hypoglycemia-associated autonomic failure have not been established and are controversial. Davis and colleagues have suggested that repeated activation of the hypothalamic-pituitary-adrenal axis leading to increases in plasma cortisol plays an important role in the development of hypoglycemia-associated autonomic failure in diabetic patients (18,19). In these studies, daytime hypoglycemic clamps were used to stimulate brisk cortisol responses. On the other hand, recent data showed a comparable blunting of epinephrine responses to hypoglycemia in patients with T1DM whether they had hydrocortisone treatment or metyrapone-induced cortisol blockade before hand (20). In our recent work, our subjects showed small, clinically and statistically insignificant increases in cortisol concentrations during episodes of spontaneous nocturnal hypoglycemia. As previously reported, there was no significant increase in cortisol concentrations during antecedent exercise, whether or not hypoglycemia occurred during exercise (8). Our data imply that episodes of hypoglycemia during the overnight period may be less likely to contribute to hypoglycemia-associated autonomic failure than daytime hypoglycemic events that stimulate a substantial rise in cortisol concentrations.

Insulin-induced hypoglycemia is a powerful secretagogue of GH that has long been used as a stimulation test of pituitary function in children with short stature. As in the Jones study (17), nocturnal hypoglycemia was able to stimulate GH responses in our subjects. Nevertheless, mean GH concentrations were not different on the nights following exercise or sedentary days or whether hypoglycemia occurred or not. Whether or not some of the nocturnal spikes in GH in our subjects were stimulated by hypoglycemia, the net effect was that there was no discernable difference in GH concentrations on nights with versus nights without hypoglycemia. Since GH plays only a minor role in acute glucose counterregulation (21), it is not surprising that the nocturnal increases in circulating GH concentrations that were observed in our subjects did not provide an effective defense against hypoglycemia. The failure to see a rise in glucagon concentrations during nocturnal hypoglycemia was also expected (1). It is possible that higher hormonal responses could have been observed with more frequent sampling after the hypoglycemic episode, however since we designed the studies to treat the

patients after any reported low glucose, the potential yield of such sampling was likely quite low.

The counterregulatory hormone responses to hypoglycemia may be influenced by gender and a greater blunting of hormonal counterregulation to hypoglycemia after antecedent exercise in males than females with T1DM has been reported (22). Out of 29 subjects that developed overnight hypoglycemia we had 14 boys and 15 girls. We observed no significant gender differences in the responses except for norepinephrine. The clinical significance of this is unknown.

Numerous studies have shown that peripheral utilization of glucose is increased by exercising versus resting muscle in subjects with diabetes, even in the face of similar circulating concentrations of insulin. A recent study in adolescents with type 1 diabetes indicates that such increases in peripheral glucose utilization extend well into the night following exercise in the late afternoon (23). Thus, children with type 1 diabetes on fixed basal insulin replacement regimens are at triple jeopardy for hypoglycemia on nights following increased physical activity than on sedentary days: peripheral glucose utilization is increased, counterregulatory hormone responses are impaired and insulin concentrations are unchanged. These data support the use of lower basal insulin doses on nights following exercise, which in patients receiving continuous subcutaneous insulin infusion therapy can be pre-programmed as an alternate basal rate into the pump. These observations also underscore the urgent need for real-time continuous glucose sensing systems that can accurately predict and alert the patient or the parent of impending hypoglycemia, especially during the overnight period.

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Appendix

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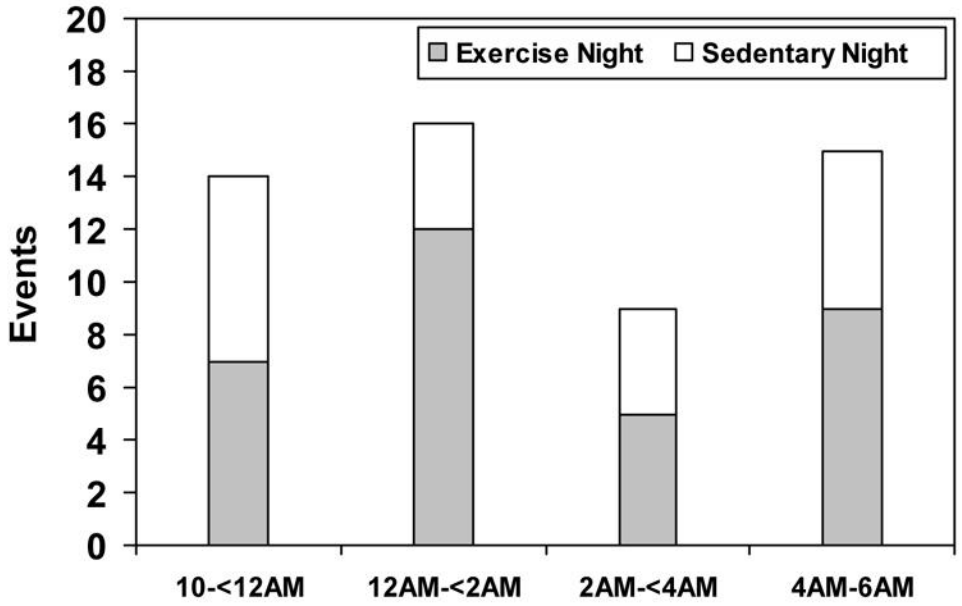

Figure 1.
Distribution of Overnight Hypoglycemic Events

Table 1

Mean ± SD hormone concentrations* on 42† nights (in 29 patients) where the glucose concentration dropped ≤70 mg/dL (3.9 mmol/L).

[[|]] [[Plasma Concentration|]]	[[Hour Before|]]	[[Nocturnal Hypoglycemic Events|]] [[During Hypoglycemia|]]	[[Hour After|]]	[[|]] [[Other Times|]]	[[|]] [[P-value |]] ‡
Glucose (mg/dL)	[[83 ± 14|]]	[[59 ± 7|]]	[[95 ± 21|]]	[[115 ± 27|]]	[[N/A|]]
[[Exercise|]]	[[85 ± 15|]]	[[58 ± 6|]]	[[96 ± 24|]]	[[118 ± 28|]]	[[|]]
[[Sedentary|]]	[[81 ± 11|]]	[[61 ± 7|]]	[[93 ± 17|]]	[[110 ± 24|]]	[[|]]
Epinephrine (%detectable)	[[6%|]]	[[21%|]]	[[5%|]]	[[6%|]]	[[0.002|]]
[[Exercise|]]	[[6%|]]	[[21%|]]	[[8%|]]	[[7%|]]	[[|]]
[[Sedentary|]]	[[6%|]]	[[23%|]]	[[0%|]]	[[4%|]]	[[|]]
Norepinephrine (pg/mL)	[[129 ± 54|]]	[[119 ± 63|]]	[[115 ± 44|]]	[[115 ± 41|]]	[[0.56|]]
[[Exercise|]]	[[136 ± 54|]]	[[119 ± 52|]]	[[128 ± 42|]]	[[119 ± 42|]]	[[|]]
[[Sedentary|]]	[[114 ± 54|]]	[[120 ± 83|]]	[[93 ± 41|]]	[[106 ± 39|]]	[[|]]
Cortisol (ug/dL)	[[3.5 ± 3.2|]]	[[5.8 ± 5.1|]]	[[5.9 ± 4.3|]]	[[5.6 ± 3.6|]]	[[0.11|]]
[[Exercise|]]	[[3.5 ± 3.4|]]	[[6.1 ± 5.2|]]	[[5.7 ± 4.5|]]	[[5.9 ± 3.5|]]	[[|]]
[[Sedentary|]]	[[3.5 ± 3.1|]]	[[5.2 ± 5.0|]]	[[6.2 ± 4.1|]]	[[5.0 ± 3.8|]]	[[|]]
Glucagon (pg/mL)	[[64 ± 20|]]	[[59 ± 19|]]	[[61 ± 25|]]	[[58 ± 18|]]	[[0.002|]]
[[Exercise|]]	[[62 ± 19|]]	[[57 ± 15|]]	[[60 ± 25|]]	[[54 ± 14|]]	[[|]]
[[Sedentary|]]	[[69 ± 23|]]	[[62 ± 24|]]	[[65 ± 26|]]	[[65 ± 21|]]	[[|]]
GH (ng/mL)	[[9 ± 10|]]	[[13 ± 10|]]	[[9 ± 8|]]	[[8 ± 8|]]	[[0.006|]]
[[Exercise|]]	[[9 ± 9|]]	[[13 ± 10|]]	[[8 ± 8|]]	[[7 ± 7|]]	[[|]]
[[Sedentary|]]	[[9 ± 13|]]	[[12 ± 12|]]	[[9 ± 7|]]	[[10 ± 8|]]	[[|]]

*To convert cortisol to nmol/L, multiply by 27.59; growth hormone to pmol/L, multiply by 44.15; glucagon to pmol/L, multiply by 0.2848; norepinephrine to nmol/L, multiply by 0.005911; epinephrine to pmol/L, multiply by 5.458.

†Excludes 4 nights where subject was treated based on a meter value ≤70 mg/dL (3.9 mmol/L), but the central laboratory measurement was >70 mg/dL.

‡P-value for 3 degree of freedom comparison of hour before vs. during vs. hour after hypoglycemia vs. other times.