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Effects of Glutamine on Glycemic Control During and After Exercise in Adolescents with Type 1 Diabetes Mellitus (T1DM): a Pilot Study

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Running Title: Glutamine and Exercise in T1DM Adolescents

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Objective: To investigate if oral glutamine ameliorates exercise- and post-exercise nighttime hypoglycemia in T1DM.

Research Design & Methods: 10 adolescents (15.2 ± 1.4 yrs (SD)), HbA1C 6.9 ± 0.9%, on insulin pumps were studied. Subjects were randomized to receive a glutamine or placebo drink pre-exercise and bedtime (0.25 g/kg/dose). A 3PM exercise session consisted of 4, 15-min treadmill/5-min rests cycles. Pre-exercise BG was 140-150mg/dl and monitored throughout the night. Studies were randomized crossover over 3 weeks.

Results: BGs dropped comparably (52%) during exercise both days. However, the overnight number of hypoglycemic events was higher on glutamine than placebo (≤70 mg/dl, p=0.03 and ≤60, p=0.05). The cumulative probability of nighttime hypoglycemia was increased on glutamine (80%) vs. placebo days (50%) (p=0.02).

Conclusions: Glutamine increased the cumulative probability of post-exercise overnight hypoglycemia compared to placebo in adolescents with T1DM. Whether glutamine may enhance insulin sensitivity post exercise requires further study in T1DM.

We observed a higher incidence of hypoglycemia during - and the night following exercise *vs*. after a sedentary day in adolescents with T1DM (1, 2). Although hypoglycemia decreased by discontinuing the insulin pump during exercise, significant rebound hyperglycemia was observed (3).

Glutamine, the body's most abundant free amino acid is thought to regulate intestinal protein synthesis and be a major source of carbon for gluconeogenesis (4). However, conflicting data reported suggests it can impair or accelerate recovery from hypoglycemia (5-7). In this pilot study we investigated if oral glutamine could ameliorate hypoglycemia during -and the nighttime after exercise in children with T1DM.

METHODS

Subjects. Written consent was obtained after approval by the Wolfson Children's Hospital Institutional Review Committee. Ten adolescents with T1DM on insulin pump therapy, mean (SD) age 15.2 ± 1.4 years (5 boys/5 girls) were recruited. Mean diabetes duration was: 6.2 ± 3.3 years, HbA1C: 6.9 ± 0.9%, with normal BMI: 76.9 ± 12.2 percentile. Subjects were on no other medications or dietary supplements.

Design. Regular dietary intake was maintained for at least 3d prior to each admission to the clinical research center (CRC) and diabetes managed as routine. Subjects were admitted mid-day, an iv placed and blood glucose (BG) titrated (mid 100mg/dl) prior to 3PM exercise. Children were randomized to receive a drink containing glutamine or placebo (PL, calorie and nitrogen-free) before exercise, and at bedtime (0.25g/kg/dose). Patients, CRC staff were blinded to type of drinks consumed. Sessions consisted of 4, 15-min treadmill cycles (heart rate ~140 bpm) with 5-min rest breaks when BG concentrations were checked for a total of 75 min. If BG <60mg/dl subjects consumed 15-30gm of carbohydrate and were not allowed back on the treadmill until BG >70mg/dl. Insulin basal rates were continued during exercise. Afterwards, subjects ate a snack and then dinner. BGs were monitored

hourly overnight from 8PM to 8AM. BG ≤60mg/dl prompted treatment with oral carbohydrate; treatment repeated as needed until >80mg/dl. Fasting glutamine and ammonia concentrations were measured 16hr post exercise the following morning. Subjects were discharged after breakfast and returned within 3w for an identical study with either placebo or glutamine. The caloric/protein intake of the 2 CRC days was identical**;** visits were randomized crossover.

Assays. BG concentrations were measured by Freestyle® meter (Abbott Diabetes Care, Alameda, CA) and by glucose oxidase methods with a Beckman glucose analyzer (Beckman, Brea, CA). Plasma glutamine concentrations were measured by GCMS and ammonia using an automated chemistry analyzer.

Statistics. During exercise hypoglycemia was considered if BG ≤70 mg/dl**;** if treated, the most recent previous BG was carried forward 1 hour in the calculations. Repeated measures regression models were performed to compare glutamine concentrations, glucose values prior to - and percentage drop during exercise, the overnight mean glucose and the percentage hypoglycemic values each visit. Rank scores (van der Waerden) were used to normalize skewed distributions. The proportions of subjects developing hypoglycemia during exercise on both visits were compared using a permutation test as was the time from 10PM until the first laboratory glucose value ≤70mg/dl on the 2 days**;** all analyses controlled for a visit effect. SAS version 9.1 (SAS Institute, Cary, NC) was used; 2-sided t-tests used.

RESULTS

Plasma glutamine concentrations were >50% higher the morning after glutamine administration (316μmol/L) as compared to placebo (200μmol/L, p<0.001).

Exercise. Mean BG concentrations prior to exercise were comparable on the glutamine and placebo day (143 ± 31 mg/dl vs. 162 ± 54 respectively, p=0.44) with a similar % drop from baseline during exercise of 52 ± 15% glutamine vs. 52% ± 9% placebo (p=0.84). There was a comparable number of subjects that developed hypoglycemia during exercise on the glutamine (N=6) or placebo day (N=7).

Overnight. Mean nighttime post-exercise low glucose levels (≤70 mg/dl or ≤60 mg/dl) were more frequent after glutamine than after placebo (≤70 mg/dl, glutamine: 19%, placebo: 15%, P=0.03, and ≤60 mg/dl, glutamine: 7.7%, placebo: 3.6%, p=0.05). The cumulative probability of overnight hypoglycemia was increased on the glutamine day (80%) compared with placebo (50%, p=0.02, Fig 1).

Safety. Glutamine is tasteless and odorless and was well tolerated. Plasma ammonia concentrations the morning after the studies were similar (glutamine: 40.2 ± 15.3 μMol/L, placebo: 41.2 ± 16.1, p=NS).

DISCUSSION

Our pilot data suggest that in adolescents with T1DM performing heavy exercise, glutamine supplementation increases the likelihood of nighttime hypoglycemia after exercise.

Morning glutamine concentrations 16 hrs post exercise were >50% higher during the glutamine day vs. placebo, however these concentrations were still below normal as compared to healthy, age-matched controls or adolescents with T1DM measured at rest (p<0.001) (8), and similar to those of healthy adults after prolonged exercise (9). Possibly, glutamine maybe low due to increased use in gluconeogenesis, or as buffer; or increased cortisol secretion (which enhances splanchnic glutamine utilization (10). This relative glutamine depletion after exercise requires further study.

In our study, glutamine did not affect BGs during exercise but increased the cumulative probability of nighttime hypoglycemia post exercise. Indirect evidence suggests glutamine

may indeed enhance insulin sensitivity in other experimental and clinical situations associated with insulin resistance including in ICU and trauma patients (11, 12) and in children with cystic fibrosis on growth hormone (13). Glutamine increased insulin signaling/sensitivity in skeletal muscle in experimental animals (14) and in healthy exercising subjects oral glutamine increased non oxidative glucose disposal (15).

Our pilot study however, has several limitations, including its small sample size, the low concentrations of glutamine achieved, and no direct measurement of insulin sensitivity. These results are nonetheless intriguing as the investigation of nutritional, non pharmacological avenues to improve diabetes control is important. and deserve further study, particularly in teenagers in whom insulin resistance increases. In aggregate, these data are congruent with a positive effect of glutamine on insulin sensitivity.

In conclusion, oral glutamine administration was associated with a higher incidence of nighttime post exercise hypoglycemia as compared to placebo in a pilot group of adolescents with T1DM. Whether glutamine supplementation affects peripheral and/or hepatic insulin sensitivity requires further study in children with T1DM.

Author Contributions: Dr. Nelly Mauras is the principal investigator of the studies, she wrote the grant, recruited subjects, carried out the experiments, analyzed the data and wrote the paper. Dr. Xing is the principal biostatistician of the study and helped analyze data, create graphs and wrote the statistical section. Dr. Larry Fox is a co-investigator and helped recruit and treat patients in the CRC, and reviewed and critiqued the manuscript. Kim Englert, RN was the lead study coordinator in charge of all implementation aspects of the study. Dr. Dominique Darmaun is a co-investigator who assisted Dr. Mauras in grant writing, data analysis and interpretation and assisted in the writing of the paper.

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Figure 1 Cumulative probability of nighttime hypoglycemia (≤70mg/dl) if the same adolescents with T1DM took glutamine or placebo before afternoon exercise and at bedtime.

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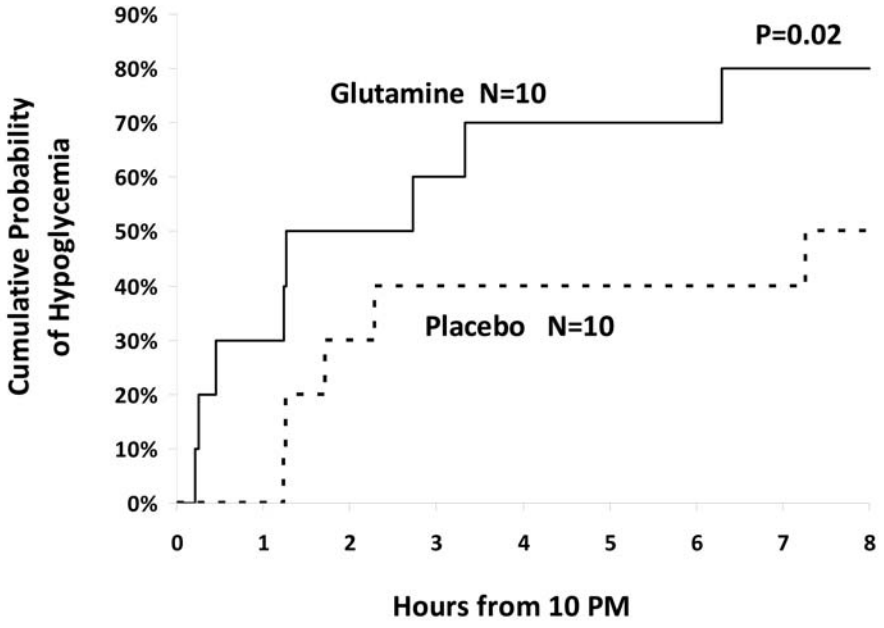
P=0.02
Glutamine N=10
Placebo N=10
Cumulative Probability of Hypoglycemia
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
Hours from 10 PM