Low-Carbohydrate Diet Impairs the Effect of Glucagon in the Treatment of Insulin-Induced Mild Hypoglycemia: A Randomized Crossover Study

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Diabetes Care 2017;40:132–135 | DOI: 10.2337/dc16-1472

OBJECTIVE

This study compared the ability of glucagon to restore plasma glucose (PG) after mild hypoglycemia in patients with type 1 diabetes on an isocaloric high-carbohydrate diet (HCD) versus a low-carbohydrate diet (LCD).

RESEARCH DESIGN AND METHODS

Ten patients with insulin pump–treated type 1 diabetes randomly completed 1 week of the HCD (≥250 g/day) and 1 week of the LCD (≤50 g/day). After each week, mild hypoglycemia was induced by a subcutaneous insulin bolus in the fasting state. When PG reached 3.9 mmol/L, 100 μg glucagon was given subcutaneously, followed by 500 μg glucagon 2 h later.

RESULTS

Compared with the HCD, the LCD resulted in lower incremental rises in PG after the first (mean ± SEM: 1.3 ± 0.3 vs. 2.7 ± 0.4 mmol/L, *P* = 0.002) and second glucagon bolus (4.1 ± 0.2 vs. 5.6 ± 0.5 mmol/L, *P* = 0.002). No differences were observed between the diets regarding concentrations of insulin, glucagon, and triglycerides.

CONCLUSIONS

The LCD reduces the treatment effect of glucagon on mild hypoglycemia. Carbohydrate intake should be considered when low-dose glucagon is used to correct hypoglycemia.

In individuals with type 1 diabetes, hypoglycemia is a barrier for achieving optimal glycemic control (1). Low doses of glucagon can effectively treat hypoglycemia (2) and even reduce the risk of hypoglycemia by automated insulin-glucagon delivery systems (3). However, glucagon treatment cannot eliminate all hypoglycemic events (4). Thus, identifying potential factors affecting glucagon efficacy is important (5). No human studies have investigated the glycemic response to glucagon during diets with different carbohydrate content.

We compared the ability of glucagon to increase plasma glucose (PG) in individuals with type 1 diabetes after 1 week of a high- (HCD) versus low-carbohydrate diet (LCD). We hypothesized that the hyperglycemic response to glucagon would be similar on both diets.

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Received 7 July 2016 and accepted 1 October 2016.

Clinical trial reg. no. NCT02578498, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc16-1472/-/DC1.

RESEARCH DESIGN AND METHODS

This was a randomized (1:1) open-label crossover study. From our outpatient clinic, we recruited patients with type 1 diabetes >3 years, insulin pump treatment >1 year, age 18–70 years, glycated hemoglobin (HbA_{1c}) <69 mmol/mol (<8.5%), BMI 20–27 kg/m^2, hypoglycemia awareness (self-reported), and practicing carbohydrate counting. Key exclusion criteria were impaired renal or liver function and use of drugs other than insulin affecting glucose metabolism. All patients completed two randomly ordered 1-week dietary periods ending with a study visit. The study was approved by the Regional Committee on Health Research Ethics (H-1509662) and the Danish Data Protection Agency, and was conducted in accordance with the Helsinki Declaration.

Before diet interventions, patients' insulin pump settings were optimized over a 2- to 3-week period (6). The dietitian used 3-day diet recordings to estimate each patient's daily calorie intake and designed individual 7-day isocaloric diets with high carbohydrate (HCD ≥250 g/day) or low carbohydrate (LCD ≤50 g/day) content. Patients emailed photographs of all meals and snacks to the dietitian, who assessed compliance with carbohydrate restrictions. One patient did not take photographs. A daily deviation of a maximum of 25 g carbohydrates was allowed. Greater deviations should be compensated for the next day.

Each diet week ended with a study visit. Hypoglycemia (continuous glucose monitor <3.5 mmol/L or capillary meter glucose ≤3.9 mmol/L), exercise, and alcohol consumption were avoided for 24 h before visits. Otherwise, the visit was postponed by ≥2 days. After a fast of 10 to 12 h, patients arrived in the morning, aiming for a PG level of 7.0 mmol/L. First, we gave an insulin aspart bolus (NovoRapid; Novo Nordisk, Bagsværd, Denmark) through the insulin pump. Bolus size was calculated to lower PG to 3.0 mmol/L and was based on the current PG value and the individual's insulin-correction factor (6). Once PG reached 3.9 mmol/L, a 100-μg glucagon bolus (GlucaGen, Novo Nordisk) was administered subcutaneously, followed by a 500-μg glucagon bolus 2 h later. The glucagon doses were selected because of their known efficacy in treating mild (6) and severe hypoglycemia (7), respectively.

Blood samples, blood pressure, heart rate, hypoglycemic symptoms (Edinburgh Hypoglycemia Scale [8]), and visual analog scales for adverse effects to glucagon were measured throughout the visit. Blood samples were analyzed for PG, plasma glucagon, serum insulin aspart, serum free fatty acids, plasma ketones (Wako Pure Chemical), and serum triglycerides by assays previously described (6).

Primary outcome was peak change in PG from 0 to 120 min after the first glucagon administration. Secondary outcomes were peak change in PG caused by the second glucagon bolus, time-to-peak, and the positive incremental area under the curve (AUC) from 0 to 120 min ($AUC_{0–120}$) after both glucagon boluses.

Ten patients were needed as a result of the following assumptions: no difference in peak PG between visits (paired), standard deviation of 0.8 mmol/L, noninferiority margin of 1.0 mmol/L, two-sided α = 0.05, and 90% power.

Paired *t* tests, linear mixed effect models, and logistic regressions with patients as random effects were used to compare data after HCD and LCD. Outcomes not prespecified were Bonferroni adjusted. SAS 9.4 (SAS Institute, Inc., Cary, NC) and GraphPad Prism 6.01 (GraphPad Software, La Jolla, CA) software were used. We considered $P < 0.05$ as statistically significant. Data are presented as mean ± SEM unless otherwise stated.

RESULTS

Ten patients (4 women) completed 2 diet weeks with an interval of 7 (1–18) days. Patients were a median (range) age of 48 (32–60) years, HbA_{1c} was 7.0% (6.0–8.1; 53 [42–65] mmol/mol), diabetes duration was 23 (10–30) years, and BMI was 24.5 (21.9–27.9) kg/m^2. The emailed photographs and the carbohydrate intake registered by insulin pumps showed that patients adhered to the isocaloric carbohydrate diets (mean ± SD: 225 ± 30 vs. 47 ± 10 g carbohydrates daily) (Supplementary Table 1). Patients' weight reduction from screening to both visits did not differ (Fig. 1).

PG levels were similar on both visits at the time of administration of insulin and 100 μg glucagon. Changes in PG over time after the first glucagon administration were significantly different between visits (Fig. 1). Thus, after the HCD, 100 μg glucagon elicited a greater increase than after the LCD (mean ± SEM: 2.7 ± 0.4 vs. 1.3 ± 0.3 mmol/L, P = 0.002). Further, the HCD resulted in a significantly higher peak PG concentration and $AUC_{0–120}$ after 100 μg and 500 μg glucagon administrations (Supplementary Table 2).

The mean insulin aspart doses required to achieve hypoglycemia were similar between the HCD and LCD (2.7 ± 1.5 vs. 2.4 ± 1.0 IU, $P > 0.05$). Insulin profiles and total AUC were similar on both visits (Fig. 1*C*).

Fasting levels of plasma glucagon were significantly lower after the HCD compared with LCD (5.0 ± 0.8 vs. 7.0 ± 1.3 pmol/L, P = 0.01). Time course and pharmacokinetic parameters of glucagon were similar on both visits (Fig. 1 and Supplementary Table 2).

Fasting levels and $AUC_{0–120}$ of free fatty acids and ketones were significantly higher after the LCD than after the HCD (Fig. 1). No differences were observed between the HCD and LCD regarding the concentration of triglycerides, intensity of hypoglycemia symptoms, nausea, and occurrence of vomiting (Supplementary Table 3). No subjects required rescue carbohydrate during visits.

CONCLUSIONS

In individuals with insulin pump–treated type 1 diabetes, the glycemic responses to subcutaneous glucagon boluses of 100 μg and 500 μg were smaller after 1 week of the LCD compared with 1 week of the HCD. To our knowledge, this is the first study demonstrating that an LCD attenuates the glycemic response to a subcutaneous glucagon bolus in individuals with type 1 diabetes.

Pharmacokinetic parameters of plasma glucagon and insulin were similar on both visits and, therefore, cannot explain the altered response to glucagon (9). Thus, the reduced glycemic response to glucagon may, in fact, be mainly explained by the diet interventions. The LCD has been shown to reduce hepatic glycogen stores in healthy individuals (10) and may be similar in individuals with type 1 diabetes (11). Differential storage of hepatic glycogen is, therefore, a likely explanation for the difference in glycemic response to glucagon after the two diets (12,13).

Fasting glucagon values were significantly higher after the LCD compared

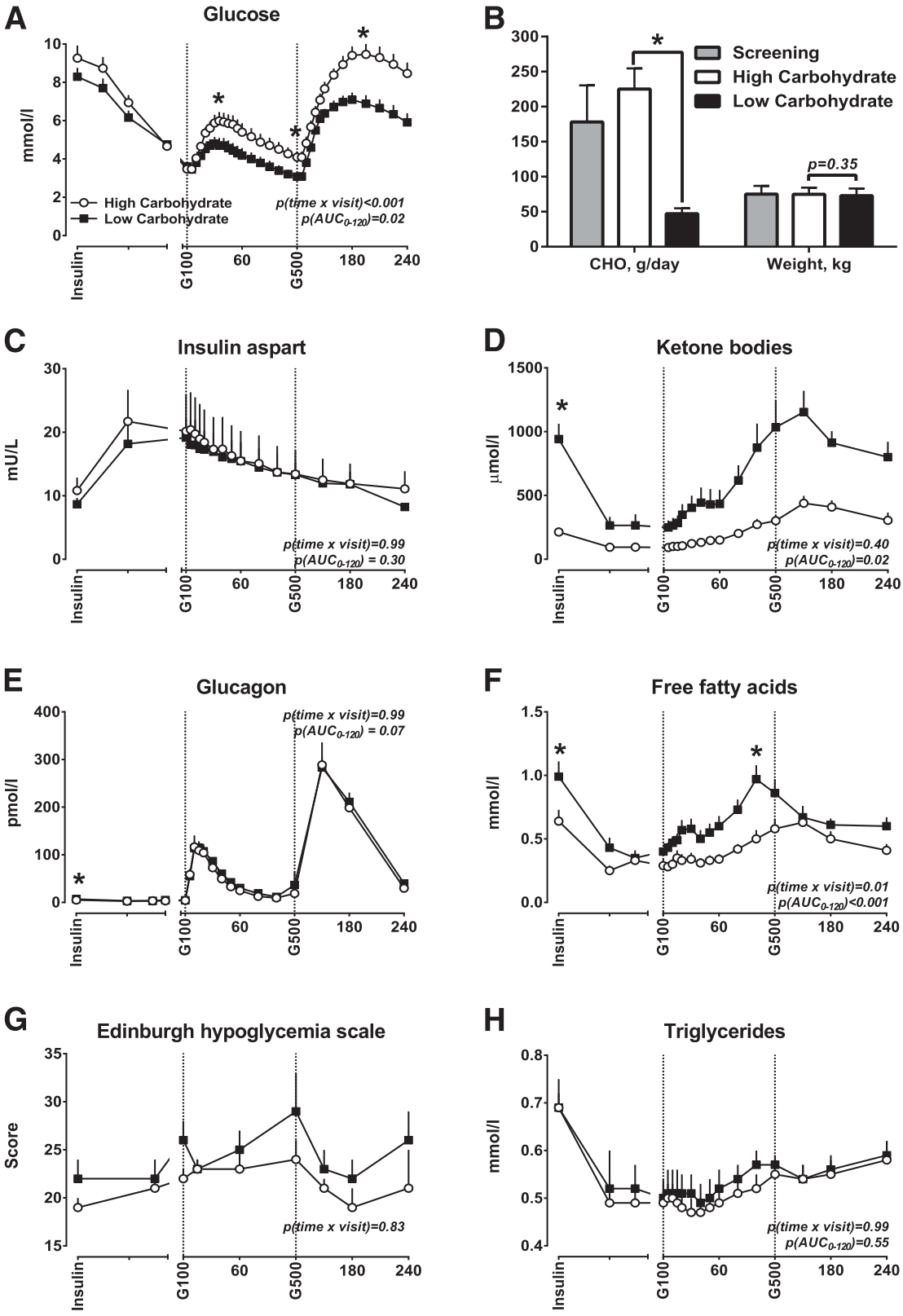

Figure 1—A subcutaneous insulin bolus was administered to induce hypoglycemia, which was treated with a subcutaneous injection of 100 µg glucagon (G100). A subcutaneous injection of 500 µg glucagon (G500) was administered 2 h later. Data on plasma glucose (*A*), serum insulin aspart (*C*), plasma ketones (*D*), plasma glucagon (*E*), serum free fatty acids (*F*), serum triglycerides (*H*), and intensity of hypoglycemia symptoms (*G*) are given as mean ± SEM for each study visit after 1 week of the HCD (≥250 g/day) and 1 week of the LCD (≤50 g/day). Panel *B* shows mean + SD carbohydrate consumption (CHO) per day registered by the insulin pump and body weight measured before screening and after each dietary week. Comparison over time between study visits (time × visit) was analyzed with a repeated-measurement ANOVA. A paired *t* test was used to compare the positive incremental (p)$AUC_{0–120}$ after the 100-µg glucagon injection. *$P < 0.05$.

with the HCD, which may be caused by an increased intake of protein combined with insulin-independent reduction of the glucose during the LCD (14,15). Elevated glucagon concentrations may downregulate glucagon receptors, thus leading to decreased glucagon sensitivity (16). Although this remains speculative, it could contribute to the attenuated glycemic response to glucagon observed in this study.

After the LCD, the first glucagon bolus led to a significantly higher increase in the concentrations of free fatty acids and ketone bodies compared with after the HCD. The LCD may have changed the metabolic flux toward more use of fat, resulting in increased fat oxidation and ketogenesis (17).

Glucagon may be used as an add-on to insulin in open-loop (18) and closed-loop settings (19). Our data indicate that diet carbohydrate content must be accounted for when treating hypoglycemia with low-dose glucagon. In closed-loop settings, the controller algorithms may adapt to the alterations caused by an LCD. Nevertheless, a higher dose of glucagon is required in both settings to restore hypoglycemia in patients on an LCD, increasing the risk of glucagon-related adverse events.

Study strengths include patients stringently following the diet plans. Consumptions were photographically documented, and carbohydrate intake was meticulously registered. Limitations of the study are the short duration of the diet interventions and the lack of estimating glycogen stores.

In conclusion, 1 week of an LCD reduces the glycemic responses to low-dose glucagon. Thus, a subject's carbohydrate intake should be considered when low-dose glucagon is used in open-loop and closed-loop pump settings.

Acknowledgments. The authors thank the study participants and acknowledge the laboratory assistance of Alis Sloth Andersen (Department of Endocrinology Research, Hvidovre University Hospital, Hvidovre, Denmark) and Gitte Kølander Hansen (Department of Obesity Biology, Novo Nordisk A/S, Måløv, Denmark), the glucagon analysis by Bolette Hartmann and Lene Brus Albæk (Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark), the insulin aspart analysis by Reingard Raml (Joanneum Research, Graz, Austria), and the analysis of triglycerides and free fatty acids by Jette Nymann (Department of Biochemistry, Hvidovre University Hospital). The manuscript was proofread by native English speaker Benjamin Leung.

Funding. This work was funded by a research grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation, the Danish Diabetes Association, and the Poul and Erna Sehested Hansen Foundation.

Duality of Interest. S.S. serves on the continuous glucose monitoring advisory board for Roche Diabetes Care and served as a consultant for Unomedical. C.D.-F. has received fees for speaking from Roche Diabetes Care. I.S. has received a research grant from Zealand Pharma and has received fees for speaking from Rubin Medical and Roche Diabetes Care. T.R.C. works for Novo Nordisk A/S and owns shares in Novo Nordisk A/S and Zealand Pharma A/S. J.J.H. has consulted for Merck Sharp & Dome, Novo Nordisk, and Roche. S.M. has served as a consultant or adviser to Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi, has received a research grant from Novo Nordisk, and has received fees for speaking from AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Merck Sharp & Dohme, Novo Nordisk, Novartis Pharma, and Sanofi. K.N. serves as adviser to Medtronic, Abbott, and Novo Nordisk, owns shares in Novo Nordisk, has received research grants from Novo Nordisk, Zealand Pharma, and Roche, and has received fees for speaking from Medtronic, Roche, Rubin Medical, Sanofi, Zealand Pharma, Novo Nordisk, and Bayer. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. A.R. performed the studies, analyzed the data, and wrote and edited the manuscript. S.S., C.D.-F., and I.S. performed some of the studies and reviewed the manuscript. T.R.C. provided data analysis and reviewed and approved the final manuscript. J.J.H. provided data analysis and reviewed, edited, and approved the final manuscript. S.M. and K.N. reviewed, edited, and approved the final manuscript. A.R and K.N. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of the study were presented at the 76th Scientific Sessions of the American Diabetes Association, New Orleans, LA, 10–14 June 2016. The abstract was printed in the American Diabetes Association Scientific Sessions Late Breaking Abstract book in June 2016. Parts of the study were presented as a poster at the 52nd European Association for the Study of Diabetes meeting, Munich, Germany, 12–16 September 2016.

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