Impact of Polymorphism in the β_2-Receptor Gene on the Metabolic Response to Epinephrine After Repeated Hypoglycemia

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The β_2-receptor mediates the metabolic response to epinephrine. This study investigates the impact of the β_2-receptor gene (*ADRB2*) polymorphism Gly16Arg on the metabolic response to epinephrine before and after repetitive hypoglycemia. Twenty-five healthy men selected according to *ADRB2* genotype being homozygous for either Gly16 (GG) (n = 12) or Arg16 (AA) (n = 13) participated in 4 trial days (D1–4): $D1_{pre}$ and $D4_{post}$ with epinephrine 0.06 μg $kg^{-1} \cdot min^{-1}$ infusion and $D2_{hypo1–2}$ and $D3_{hypo3}$ with three periods of hypoglycemia by an insulin-glucose clamp. At $D1_{pre}$, the insulin (mean ± SEM of area under the curve 44 ± 8 vs. 93 ± 13 pmol · L^{-1} h; P = 0.0051), glycerol (79 ± 12 vs. 115 ± 14 μmol · L^{-1} h; P = 0.041), and free fatty acid (724 ± 96 vs. 1,113 ± 140 μmol · L^{-1} h; P = 0.033) responses to epinephrine were decreased in AA participants compared with GG participants but without a difference in glucose response. There were no differences in response to epinephrine between genotype groups after repetitive hypoglycemia at $D4_{post}$. The metabolic substrate response to epinephrine was decreased in AA participants compared with GG participants but without a difference between genotype groups after repetitive hypoglycemia.

ARTICLE HIGHLIGHTS

- This study investigates the impact of the β_2-receptor gene (*ADRB2*) polymorphism Gly16Arg on the metabolic response to epinephrine before and after repetitive hypoglycemia.
- Healthy men homozygous for either Gly16 (n = 12) or Arg16 (n = 13) participated in the study.
- Healthy people with the Gly16 genotype have increased metabolic response to epinephrine compared with the Arg16 genotype but without a difference between genotypes after repetitive hypoglycemia.

Hypoglycemia is the main adverse effect of insulin treatment of people with type 1 diabetes, which is the limiting factor in reaching glycemic control (1). Early after the diagnosis of type 1 diabetes, the glucagon response is grossly reduced (2), rendering patients dependent on epinephrine as their primary counterregulatory response (3). However, the epinephrine response to hypoglycemia also becomes blunted in many patients, at least partly because of exposure to recurrent hypoglycemia, resulting in counterregulatory failure (4).

The metabolic response to epinephrine during hypoglycemia counterregulation involves both α-, and β-receptors but is reported to be mediated mainly through the β_2-receptor (5–7), resulting in increased glycogenolysis and gluconeogenesis supported by increased lipolysis, ketogenesis, and turnover of glucogenic amino acids. A potential modulating role of the β_2-receptor on the metabolic result of the adrenaline

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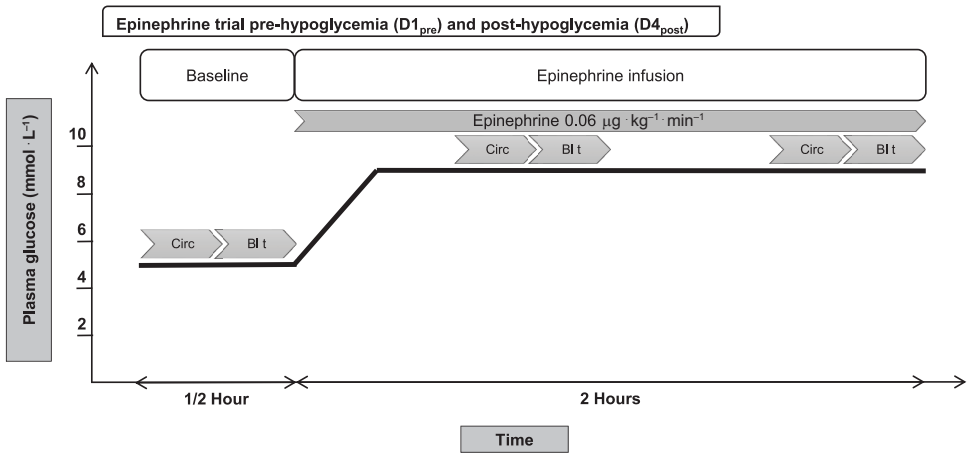

Figure 1—Epinephrine trial overview. For epinephrine trial days $D1_{pre}$ and $D4_{post}$, the participant initially rested in the supine position for 30 min, and baseline measures were obtained. Blood samples from the arterial catheter (Bl t) (plasma glucose, lactate, FFAs, β-hydroxybutyrate, glycerol, alanine, insulin, C-peptide) and circulatory variables (Circ) (heart rate, stroke volume, cardiac output, SVR, systolic pressure, diastolic pressure, MAP) were assessed using Modelflow methodology. Epinephrine was infused at 0.06 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 2 h while the participant rested in the supine position, and blood samples and circulatory variables were obtained every hour.

response and the potential effect of recurrent hypoglycemia hereon have not been studied previously.

Several functional polymorphisms have been described in the β_2-receptor gene (*ADRB2*) of which one is associated with receptor function and desensitization (8). Thus, people homozygous for the Arg16 allele (AA) of the nonsynonymous 46 G > A (G16R) single nucleotide polymorphism with subsequent amino acid substitution Gly16Arg show decreased adrenergic responsiveness (9) and increased receptor desensitization after adrenergic stimulation compared with those homozygous for the Gly16 allele (GG) (10). In addition, AA study participants with type 1 diabetes demonstrated an increased risk of severe hypoglycemia (11). We hypothesized that the substrate mobilization in response to epinephrine is increased in people carrying the GG genotype of the *ADRB2* Gly16Arg polymorphism compared with carriers of the AA genotype and that exposure to repeated hypoglycemia decreases the metabolic substrate response to epinephrine in those with the AA genotype.

RESEARCH DESIGN AND METHODS

This study is an additional analysis of a previously published study (12). A total of 184 healthy Caucasian male participants (age 18–30 years, BMI <25 $kg \cdot m^{-2}$) were genotyped for the *ADRB2* Gly16Arg (rs1042713) polymorphism (11). Exclusion criteria were the use of medication, tobacco, illegal drugs, or alcohol (>21 units a week); endurance training more than three times a week; allergy to iodine; or claustrophobia. All trial participants gave written informed consent, and the study was approved by the Capital Region's Committee on Health Research Ethics, (J.nr. H-17017942) and the Danish Data Protection Agency (J.nr. 2012-58-0004/NOH-2017-020/05936).

Trial Overview

On day 1 of the trial ($D1_{pre}$), an epinephrine infusion 0.06 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was performed to obtain preconditional metabolic and circulatory response values (Fig. 1). After 1 week, trial days 2–4 were conducted over 3 consecutive days with a 2-day hypoglycemia protocol including three periods of 120 min of hypoglycemia (two periods at day 2 [$D2_{hypo1-2}$] and one period at day 3 [$D3_{hypo3}$]) to secure significant hypoglycemic conditioning as previously described (Supplementary Fig. 1) (12). Finally, on day 4 ($D4_{post}$), an epinephrine infusion was performed to evaluate postconditional responses (Fig. 1).

Preparation and Conditions for the Trial Days

Trial participants were asked to refrain from strenuous exercise 1 week before the first trial day and during the trial period and to refrain from alcohol intake for 24 h before the test day. Trial participants met in the morning after an overnight fast (>6 h) on all trial days.

Day 1_{pre}: Response to Epinephrine Infusion Before Hypoglycemia

For drug administration, a peripheral catheter was placed in a cubital vein. An arterial catheter (1.1 mm, 20 gauge) was inserted in the brachial artery for the determination of arterial pressure and heart rate. For assessment of cardiac stroke volume, cardiac output, and systemic vascular resistance (SVR), the arterial pulse wave was analyzed by Modelflow methodology (Finometer; FMS, Amsterdam, the Netherlands) after adjusting for weight, height, and age. Data were converted from analog to digital and sampled at 100 Hz (PowerLab; ADInstruments, Colorado Springs, CO), and the mean values from the antecedent 10-min period were registered at baseline and every hour during epinephrine infusion.

Following catheterization, the participant rested in the supine position for a minimum of 25 min to secure steady state; thereafter, baseline circulatory measures and blood samples were obtained. Epinephrine was infused at 0.06 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 120 min (Fig. 1).

Biochemical Analyses

The substrate and hormonal response of glucose, lactate, glycerol, free fatty acids (FFAs), β-hydroxybutyrate, alanine, insulin, and C-peptide were measured in arterial samples at baseline and every hour during epinephrine infusion. The analysis of blood samples was as previously described (12).

$D4_{post}$: Response to Epinephrine Infusion After Repeated Hypoglycemia

The peripheral venous and arterial catheters inserted on the first day of the first hypoglycemia trial day were still in situ and used for the trial procedure that was conducted according to the same protocol as described for $D1_{pre}$.

Statistical Analysis

Variables are presented as mean ± SEM. Responses were analyzed as the area under the curve using the trapezoidal rule and calculated as the change in values from the preceding baseline to values during a 2-h period with epinephrine infusion. Data distribution was assessed by Shapiro-Wilks test. Genotype groups and epinephrine responses before and after repetitive hypoglycemia were compared by unpaired, paired Student *t* tests for normally distributed data, by Mann-Whitney *U* tests for unpaired comparisons, or Wilcoxon signed rank test for paired comparisons for non-normally distributed data. Comparison of change in epinephrine response after recurrent hypoglycemia between genotype groups were by linear mixed models. Data were analyzed using Python and the packages pingouin (version 0.3.8) and numpy (version 1.18.1). $P < 0.05$ (two-sided) was considered statistically significant.

Data and Resource Availability

Data and resources are available upon request.

RESULTS

Participant Characteristics

Twenty-five men matched for height and weight participated in the study after being selected according to *ADRB2* genotype from a previous cohort of genotyped study participants as being homozygous for either GG (n = 12) or AA (n = 13). There were no differences in baseline characteristics between the genotype groups (Supplementary Table 1) (12).

Baseline Measures $D1_{pre}$

At $D1_{pre}$, no baseline measures differed, except mean arterial pressure (MAP) was slightly increased in AA participants (Supplementary Table 2).

Glucose, Insulin, Substrate, and Circulatory Responses to Epinephrine Infusion $D1_{pre}$

There was no difference between genotype groups in the glucose response to epinephrine infusion at $D1_{pre}$, but the insulin response in AA participants was less than half that of GG participants (44 ± 8 vs. 93 ± 13 pmol · L^{-1} h; P = 0.0051) (Fig. 2 and Supplementary Table 3). The FFA response to epinephrine at $D1_{pre}$ was ∼35% lower in AA participants than in GG participants (724 ± 96 vs. 1,113 ± 140 μmol · L^{-1} h; P = 0.033), with the same reduction in the glycerol response (79 ± 12 vs. 115 ± 14 μmol · L^{-1} h; P = 0.041)

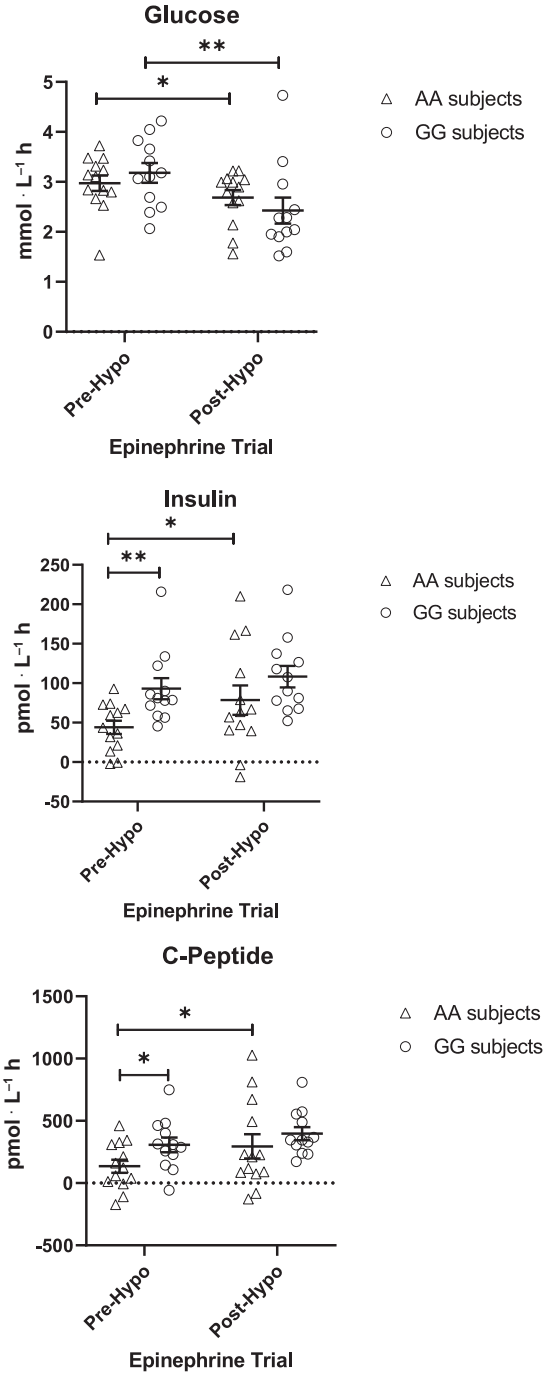

Figure 2—The glucose, insulin, and C-peptide response to epinephrine before (pre-hypo) and after (post-hypo) repeated hypoglycemia in AA and GG participants. Interleaved scatter plot of the area under the curve values of the plasma glucose, insulin, and C-peptide response to epinephrine during a 2-h period before and after repeated hypoglycemia, with lines and error bars representing mean and SEM, respectively, in AA and GG participants. No values were missing (N = 25). *$P < 0.05$, **$P < 0.01$.

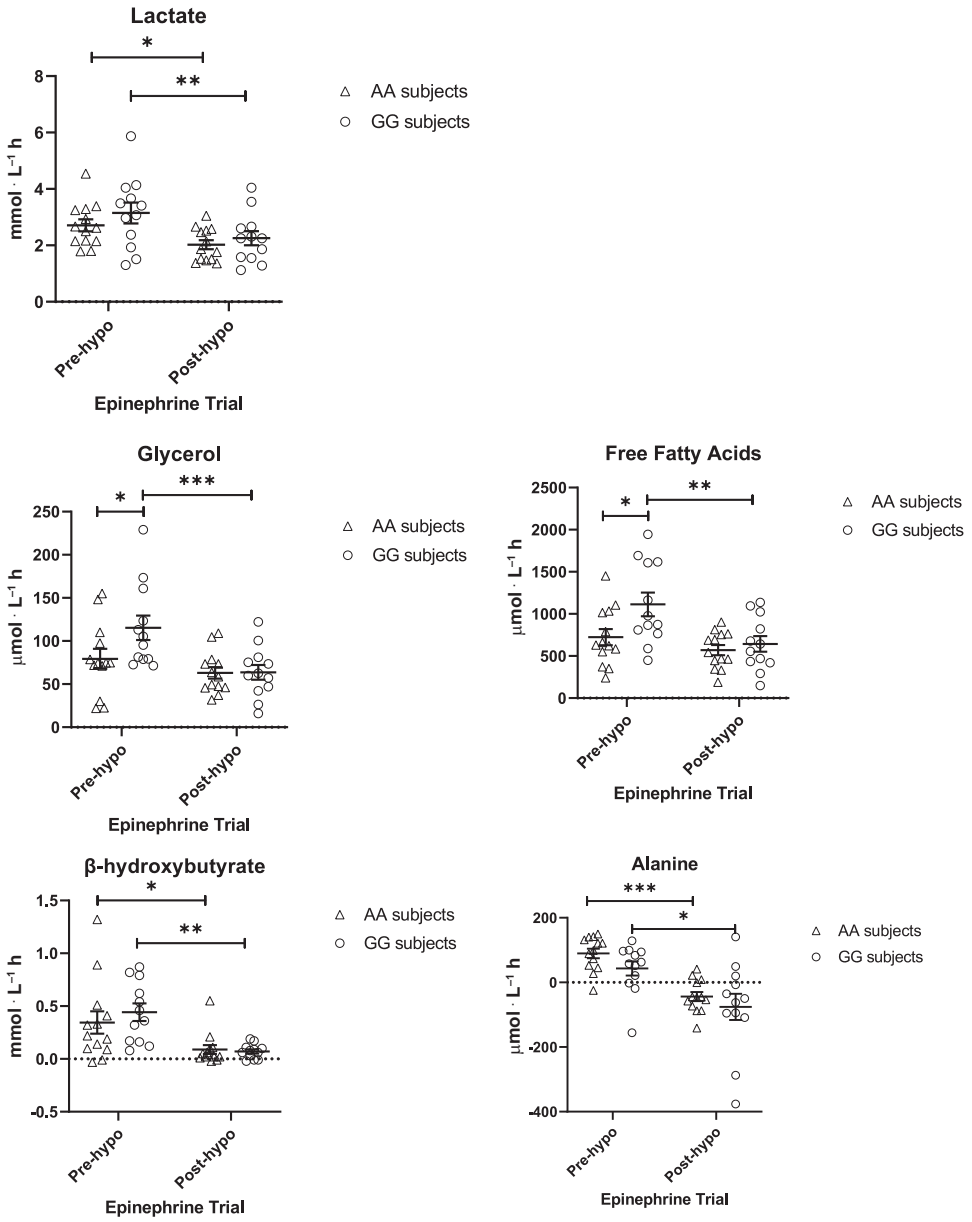

Figure 3—The substrate response to epinephrine before (pre-hypo) and after (post-hypo) repeated hypoglycemia in AA and GG participants. Interleaved scatter plot of the area under the curve values of the substrate response to epinephrine during a 2-h period before and after repeated hypoglycemia, with lines and error bars representing mean and SEM, respectively, in AA and GG participants. No values were missing ($N = 25$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

but no differences between genotype groups in the lactate, β-hydroxybutyrate, or alanine responses were observed (Fig. 3 and Supplementary Table 3). There were no differences between genotype groups in circulatory (heart rate, stroke volume, cardiac output, SVR, systolic pressure, diastolic pressure, MAP) responses to epinephrine at $D1_{pre}$ (Fig. 4 and Supplementary Table 3).

Baseline Measures $D4_{post}$

At $D4_{post}$, no baseline measures differed between genotype groups except for glycerol (Supplementary Table 2). Significant and comparable changes in baseline metabolic and circulatory measures were observed in both genotype groups at $D4_{post}$ compared with $D1_{pre}$ except for diastolic pressure and MAP, which were slightly reduced in AA participants (Supplementary Table 4).

Glucose, Insulin, Substrate, and Circulatory Responses to Epinephrine Infusion $D4_{post}$

At $D4_{post}$, there was no difference between genotype groups in responses to epinephrine, glucose, insulin, or substrates (lactate, glycerol, FFAs, β-hydroxybutyrate, alanine), or circulatory measures (heart rate, stroke volume, cardiac output, SVR, systolic pressure, diastolic pressure, MAP) (Figs. 2–4 and

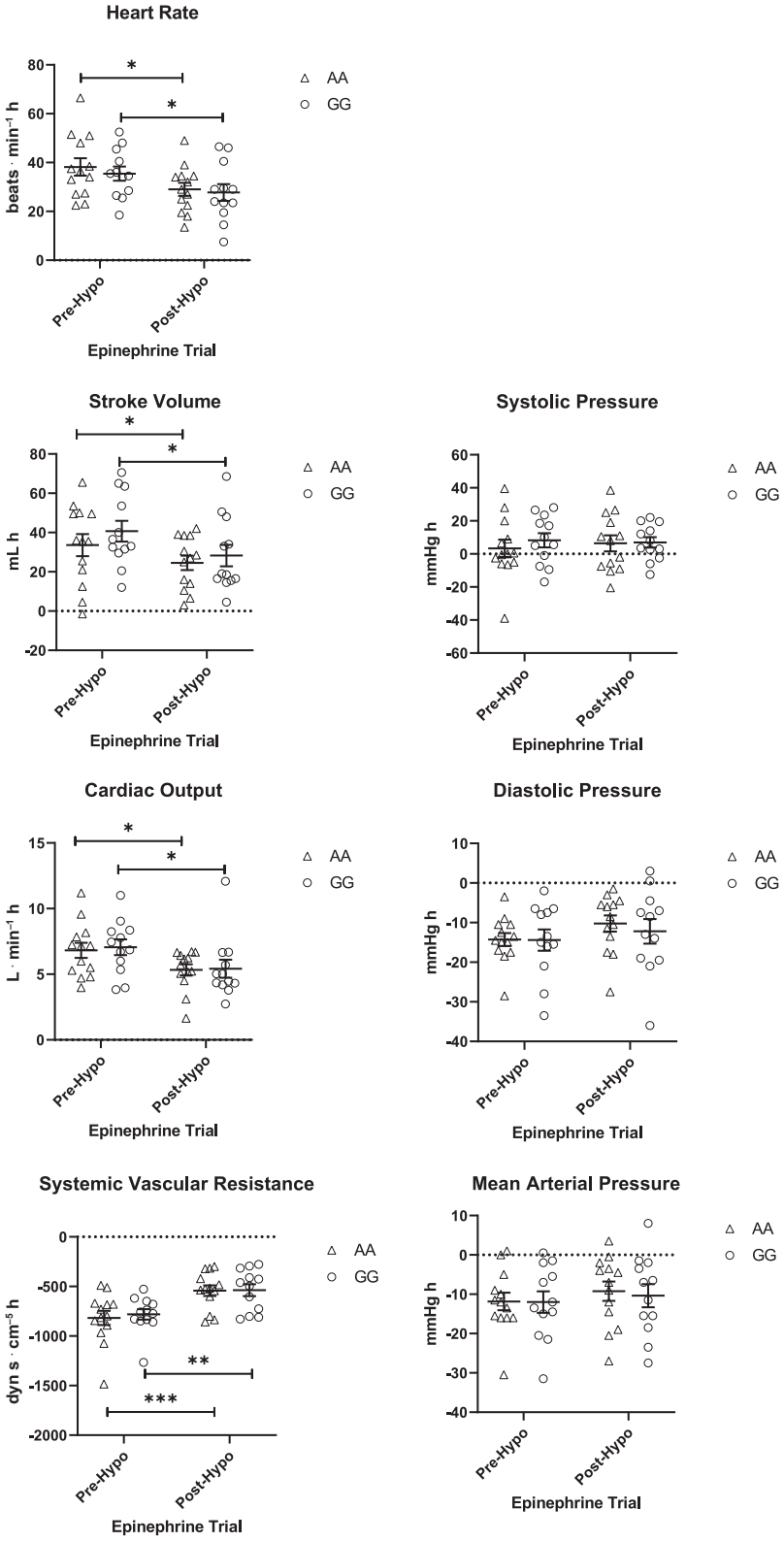

Figure 4—The circulatory response to epinephrine before (pre-hypo) and after (post-hypo) repeated hypoglycemia in AA and GG participants. Interleaved scatter plot of the area under the curve values of the circulatory response to epinephrine during a 2-h period before and after repeated hypoglycemia, with lines and error bars representing mean and SEM, respectively, in AA and GG participants. No values were missing ($N = 25$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Supplementary Table 5). The change in glucose, insulin, and C-peptide responses to epinephrine infusion from $D1_{pre}$ to $D4_{post}$ followed the same pattern in both genotype groups (Fig. 2 and Supplementary Table 5). In both groups, the lactate, β-hydroxybutyrate, and alanine responses to epinephrine infusion were equally decreased at $D4_{post}$, but in GG participants, the glycerol response was significantly reduced by almost 50% compared with $D1_{pre}$ (Fig. 3 and Supplementary Table 5). In both genotype groups, heart rate, stroke volume, and cardiac output responses were decreased at $D4_{post}$ compared with $D1_{pre}$, with an increase of the SVR response in the same period (Fig. 4 and Supplementary Table 5).

DISCUSSION

This study found that healthy men with the GG genotype of the *ADRB2* Gly16Arg polymorphism had a greater metabolic response to epinephrine infusion compared with those with the AA genotype. This response included a more than twofold increased insulin response in GG participants, which considering an α-receptor mechanism for the epinephrine inhibition of insulin release (13) and the comparable glucose increment and baseline insulin sensitivity in the two genotype groups, could suggest an increased glucose response effectively counterregulated by an increased insulin response in GG participants. In contrast, there were no differences between the genotypes in circulatory responses. Following preconditioning with recurrent hypoglycemia, resulting in significant epinephrine responses (12), both metabolic and circulatory responses to epinephrine infusion were attenuated in both genotype groups. These results suggest that the GG participants had a greater metabolic β_2-receptor responsiveness prior to recurrent hypoglycemia, but change in the metabolic and circulatory response to epinephrine after recurrent hypoglycemia followed the same pattern in both genotype groups.

Findings from studies on the metabolic effect of β-blockade during epinephrine stimulation differ (14,15). The current study used an epinephrine dose comparable to the study of Rizza et al. (14) in which the applied nonselective β-blockade eliminated the insulin response. Also, the FFA response to epinephrine is diminished by nonselective β-blockade (15). Additionally, during glucose recovery following insulin-induced hypoglycemia in people with type 1 diabetes, nonselective β-blockade (propranolol) inhibited FFA levels to a greater extent than selective β_1-blockade (metoprolol), supporting a significant contribution from the β_2-receptor response (7). In the current study, insulin, glycerol, and FFA responses to epinephrine were attenuated in AA participants prior to hypoglycemia, in accordance with reduced β_2-receptor responsiveness.

There is an association between Arg16 homozygosity and increased risk of hypoglycemia in people with type 1 diabetes and hypoglycemia unawareness. In one study, the distribution of Gly16Arg genotype groups among patients with hypoglycemia unawareness did not differ from the distribution in the general population, but in patients with hypoglycemia unawareness, the prevalence of severe hypoglycemia was reported to be 55% in AA patients compared with 28% in GG patients (11). This genetic impact on risk of severe hypoglycemia in people with type 1 diabetes could potentially be explained by differences in metabolic responsiveness to epinephrine between Gly16Arg genotypes, leading to reduced lipolysis and endogenous glucose production in response to epinephrine during hypoglycemia. This is supported by studies that showed that in participants with type 1 diabetes, gluconeogenesis contributed more to glucose production than in healthy participants (16,17), substrate supply was an essential limiting factor (18), and β-blockade resulted in impaired glucose recovery after hypoglycemia, with normalization of glucose recovery during the substitution of lactate and glycerol (19).

Strengths and Limitations

This study was conducted under controlled conditions, with the exposure to preconditioning by repeated hypoglycemia being similar between the two genotype groups (12) and the all-male cohort (as adrenergic response differs between males and females [20]) reducing confounding. A potential limitation is the single-dose epinephrine protocol that precluded assessment of a dose-response relationship. Plasma epinephrine levels were not measured during epinephrine infusion, but plasma epinephrine of $\sim$0.7 ng · mL^{-1} has been reported during comparable conditions (21), equivalent to plasma epinephrine levels during the recurrent hypoglycemia periods (1.1 ± 0.07 and 0.7 ± 0.07 ng · mL^{-1}, respectively) (K.Z.R., J.J. Holst, N.V. Olsen, F.D., N.H.S., A. Juul, J. Faber, S. Wilberg, B.T., and U.P.-B., unpublished observations) (12). Preconditioning with a hyperinsulinemic euglycemic clamp could have supported differentiation between the hyperinsulinemic and hypoglycemic impact on the metabolic response to epinephrine.

Conclusions

Healthy people with the GG genotype have increased metabolic response to epinephrine compared with the AA genotype but without differences between the genotypes after repetitive hypoglycemia. This finding may contribute to the understanding of glucose counterregulation beyond the magnitude of the epinephrine response.

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Author Contributions. K.Z.R. contributed to the conceptual design of the study, recruited the participants, conducted the study, collected the data, performed the data analysis, and drafted the first version of the manuscript. F.D. and L.G. performed the data analysis. N.H.S., B.T., and U.P.-B. initiated

and designed the study and performed the data analysis. All authors contributed significantly to the data collection or analysis and reviewed and edited the manuscript. K.Z.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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