

# Heterogeneity in Epinephrine Response to Experimental Hypoglycemia in Type 1 Diabetes and Controls

Yuan Zhang,<sup>1</sup> Gülin Öz,<sup>2</sup> Amir Moheet,<sup>3</sup> Anjali Kumar,<sup>3</sup> Alison Alvear,<sup>3</sup> Silvia Mangia,<sup>2</sup> Lynn Eberly,<sup>1</sup> and Elizabeth R. Seaquist<sup>3</sup>

<sup>1</sup>Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN 55455, USA;

<sup>2</sup>Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, MN 55455, USA; and

<sup>3</sup>Division of Endocrinology and Diabetes, Department of Medicine, University of Minnesota, Minneapolis, MN 55455, USA

**Correspondence:** Elizabeth Seaquist, MD, Division of Diabetes, Endocrinology and Metabolism, MMC 101, 420 Delaware St SE, Minneapolis, MN 55455, USA.  
Email: [Seaqu001@umn.edu](mailto:Seaqu001@umn.edu).

## Abstract

**Context:** The epinephrine response (Epi) to a first episode of hypoglycemia (HG) has been proposed to be predictive of Epi in subsequent HG and to provide insight into the risk for developing HG-associated autonomic failure (HAAF) in healthy controls (HCs).

**Objective:** To determine if Epi and symptom response (SR) to the first episode of HG predicts who will develop HAAF after exposure to recurrent HG in volunteers with type 1 diabetes (T1D) and in HCs.

**Design:** Review of data collected between 2013 and 2019.

**Setting:** Academic clinical research unit.

**Patients or Participants:** Volunteers with T1D and HCs.

**Interventions:** Subjects participated in a preinduction protocol where they were exposed to three 2-hour episodes of clamped HG over 2 days. Data collected during clamp 1 were compared with data collected during clamp 3.

**Main outcome measure:** Difference in Epi and SR.

**Results:** Using the standard definition of HAAF in which HG-induced Epi during clamp 3 is at least 20% lower than during clamp 1, 21/28 HCs and 13/19 volunteers with T1D developed HAAF. Epi during clamp 1 was significantly higher in those subjects who developed HAAF than in those who did not in both groups ( $P = 0.02$ ). If HAAF is defined as achieving a 20% reduction in HG-induced SR measured during clamp 3 compared with clamp 1, 10/27 HCs and 10/19 volunteers with T1D developed SR-based HAAF.

**Conclusion:** There was heterogeneity in the response to the preinduction protocol. Epi during clamp 1 was higher than in clamp 3 in HCs and in those with T1D who developed HAAF.

**Key Words:** hypoglycemia, impaired awareness of hypoglycemia, epinephrine, type 1 diabetes

**Abbreviation:** HAAF, hypoglycemia-associated autonomic failure.

Hypoglycemia is common in the lives of people with insulin-treated diabetes. It can be disruptive to activities of daily living and can be associated with unconsciousness, seizures, and even death. Recurrent episodes of hypoglycemia in a short period leads to impaired awareness of hypoglycemia or hypoglycemia-associated autonomic failure (HAAF) in which people may not detect hypoglycemia until neuroglycopenic symptoms develop that render them unable to help themselves overcome the episode. This occurs because the glucose level at which the counterregulatory hormone and symptom response is elicited is dropped to a lower and lower level by recurrent hypoglycemia, whereas the glucose level responsible for neuroglycopenia is unchanged [1].

The mechanisms responsible for developing impaired awareness of hypoglycemia remain elusive despite years of investigation. One widely used experimental approach is the preinduction protocol in which an individual is exposed to repeated episodes of prolonged hypoglycemia maintained using

the clamp method over 2 days. The response to the hypoglycemia during the first exposure is then compared with the response during the final exposure. Perturbations during the periods of hypoglycemia provide opportunities to understand what component of the counterregulatory response to hypoglycemia is responsible for blunting the response to subsequent hypoglycemia. Hormones and metabolites such as cortisol [2], lactate [3], and beta endorphins [4] have been proposed, but many investigators have focused on the role of epinephrine, which is secreted from the adrenal medulla in response to hypoglycemia. Years ago, Ramanathan and Cryer [5] demonstrated that adrenergic blockade with nonselective  $\alpha$ - and  $\beta$ -adrenergic antagonists during the first episode of hypoglycemia prevented the expected blunting of the epinephrine response that occurred during a second episode of hypoglycemia, suggesting that the epinephrine released during hypoglycemia may itself be causing impaired awareness of hypoglycemia. More recently, Lontchi-Yimagou and

colleagues [6] demonstrated that healthy volunteers who met the standard criteria for HAAF during the third exposure to hypoglycemia demonstrated higher levels of hypoglycemia-induced epinephrine response during the first exposure to hypoglycemia in our preinduction protocol, although they did find marked interindividual variability in the responses to hypoglycemia. They hypothesized that this variability may explain an individual's susceptibility to developing impaired awareness.

We recently examined the concordance between hypoglycemia-induced epinephrine secretion and symptoms scores in adults with type 1 diabetes and found a wide variability in responses [7]. We also noted a loose correlation between epinephrine levels and symptoms, even among individuals who were categorized as having impaired awareness of hypoglycemia by the Cox or Gold questionnaires [7]. This observation, coupled with the hypothesis proposed by Lontchi-Yimagou and colleagues [6], prompted us to reexamine the data collected during a series of experiments in which healthy volunteers and volunteers with type 1 diabetes were submitted to a preinduction protocol of recurrent hypoglycemia to induce HAAF. We sought to determine if the epinephrine response to the first episode of hypoglycemia was predictive of who would show the greatest blunting of the hormonal response during episode 3, as was demonstrated by Lontchi-Yimagou and colleagues in healthy volunteers [6]. To verify that levels of hypoglycemia-induced epinephrine secretion were physiologically relevant in blunting the counterregulatory response in clamp 3, we examined the relationships between the glucose infusion rates required to maintain target glycemia in the 2 hypoglycemic episodes. We also aimed to determine if the magnitude of symptom responses during the first exposure to hypoglycemia would be predictive of who would show the greatest blunting at the end of the third bout of hypoglycemia. Finally, we examined the impact of epinephrine response during the first bout of hypoglycemia on the symptoms experienced during the third episode of hypoglycemia.

## Methods

### Research Subjects

Adults were recruited for participation in a series of experiments designed to define the mechanisms contributing to the loss of the hormonal and symptomatic response to hypoglycemia in persons exposed to recurrent hypoglycemia. These studies were done between 2013 and 2019 and the results of the healthy volunteers were previously reported [8-10]. All of the subjects with type 1 diabetes were classified as being hypoglycemia aware on a Clarke questionnaire before the study [11]. Subjects who provided complete sets of glucose and epinephrine data during the preinduction were included in this report. The investigation was approved by University of Minnesota Institutional Review Board: Human Subjects Committee. All procedures performed were in accordance with the 1975 Declaration of Helsinki and its later amendments. Exclusion criteria included history of ischemic heart disease, arrhythmia, seizure disorder, or being on medications known to alter blood flow or carbohydrate metabolism, in addition to magnetic resonance safety criteria (claustrophobia, weight >300 pounds, and presence of paramagnetic metal in the body).

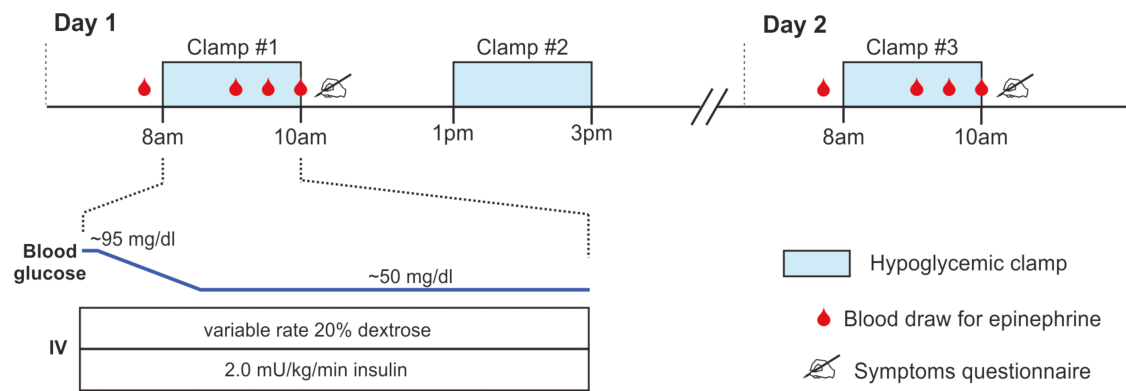
### Preinduction Protocol

To induce impaired awareness of hypoglycemia, subjects underwent hypoglycemic preconditioning that consisted of two 2-hour hypoglycemic clamps, in the morning (8:00-10:00 am) and afternoon (12:00-2:00 pm) on day 1, and a third 2-hour hypoglycemic clamp in the morning (8:00-10:00 am) on day 2 as previously described [12] (Figure 1). The insulin regimen used by the subjects with type 1 diabetes was adjusted so that their basal insulin was not present during the clamp studies. Subjects arrived at the Clinical and Translational Science Institute in the morning after an overnight fast. Insulin was administered IV at a rate of 2.0 mU/kg/min and potassium phosphate administered at a rate of 4 mEq/h. Arterialized blood samples [13] were collected every 5 minutes for the measurement of plasma glucose. Plasma glucose was allowed to fall to 50 mg/dL and then maintained at this level by a variable infusion of 20% dextrose. Samples for epinephrine were drawn at baseline (30 minutes after the placement of the last IV catheter) and at 60, 90, and 120 minutes after starting insulin during the clamps 1 and 3. Volunteers with type 1 diabetes were maintained at euglycemia on an insulin infusion of 0.5 mu/kg/min between clamps 1 and 2; controls were taken off insulin and returned to euglycemia after clamp 1. After clamp 2, subjects were fed a meal and sent home. Volunteers with diabetes were instructed how to administer insulin to maintain normoglycemia between clamps 2 and 3. Hypoglycemia-induced epinephrine secretion was defined as the average of epinephrine measures collected at 90 and 120 minutes in each clamp study. Each clamp's basal epinephrine level was not subtracted from that clamp's average. Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography (Dionex Corp, Sunnyvale, CA) in the Vanderbilt Diabetes Research Center Core laboratory. Glucose infusion rates were calculated as the average amount infused during the last 30 minutes of the clamp study.

After clamps 1 and 3, hypoglycemia symptoms were quantified by using a previously validated questionnaire [14]. Subjects were asked to score from 0 (none) to 6 (severe) each of 12 symptoms: 6 autonomic symptoms (heart pounding, shaky/tremulous, nervous/anxious, sweaty, hungry, and tingling) and 6 neuroglycopenic symptoms (difficulty thinking, tired/drowsy, weak, warm, faint, and dizzy).

### Statistical Analysis

The average responses (epinephrine, symptom scores, and glucose infusion rate) during the last 30 minutes in hypoglycemic clamps 1 and 3 were recorded and used for analysis. HAAF is defined in 2 ways: volunteers were classified as epinephrine-based HAAF if there was at least a 20% drop in the epinephrine response in clamp 3 compared with clamp 1; and volunteers were classified as symptom-based HAAF if there was at least a 20% drop in the total symptom scores in the in clamp 3 compared with clamp 1. For each definition of HAAF, and separately for volunteers with and without type 1 diabetes, within-group comparison of responses between clamps 1 and 3 were carried out separately for subjects who developed HAAF and those who did not using paired-sample *t* tests. Comparison of responses between those who developed HAAF and those who did not were carried out using 2-sample *t* tests.



**Figure 1.** Preinduction protocol. The preinduction protocol consisted of 3 hypoglycemia clamps done over 2 days. For each, subjects were infused with insulin 2.0 mU/kg/min and blood glucose was targeted at 50 mg/dL. Samples for epinephrine were drawn at baseline (30 minutes after placement of the last IV catheter) and at 60, 90, and 120 minutes after starting insulin during the first and third clamps.

## Results

Twenty-nine healthy adults without diabetes (10 women, 19 men; mean age  $36 \pm 3$  years; body mass index,  $25.6 \pm 0.6$  kg/m<sup>2</sup>) and 20 volunteers with type 1 diabetes (10 women, 8 men; mean age  $32 \pm 3$  years; mean glycated hemoglobin,  $6.9 \pm 0.2\%$ ; mean duration of diabetes,  $15 \pm 2$  years; body mass index,  $26.3 \pm 1.2$  kg/m<sup>2</sup>) were included in this study. They represent all the volunteers who successfully completed a preinduction protocol between 2013 and 2019 in our laboratory and had at least 1 complete set of counterregulatory responses (symptom scores, epinephrine response, glucose infusion rate) for clamp 1 or clamp 3. One person from each group was missing 1 clamp's epinephrine data, whereas 2 volunteers with diabetes and 1 volunteer without diabetes were missing 1 clamp's symptom data; they were excluded from the corresponding statistical comparisons.

Using the standard definition of HAAF induction in which the hypoglycemia-induced epinephrine secretion during clamp 3 is at least 20% lower than during clamp 1 [15, 16], 21 of 29 (72%) healthy volunteers and 13 of 20 (65%) volunteers with type 1 diabetes developed HAAF as a result of the preinduction protocol. Seven healthy volunteers and 6 volunteers with type 1 diabetes did not (Figure 2). As expected, the epinephrine response during clamp 1 was significantly higher than the response during clamp 3 in both subject groups who developed HAAF (healthy volunteers:  $P < 0.0001$ ; volunteers with type 1 diabetes:  $P < 0.0001$ ). The response during clamp 3 was significantly higher than the response in clamp 1 for healthy controls who did not develop HAAF ( $P = 0.016$ ) but not for volunteers with type 1 diabetes who did not develop HAAF. Epinephrine responses during clamp 1 were significantly higher in those subjects who developed HAAF than they were in those who did not in both groups ( $P = 0.02$ ). If epinephrine response was reported as peak level obtained during the final 30 minutes of the clamp, the results were unchanged (data not shown).

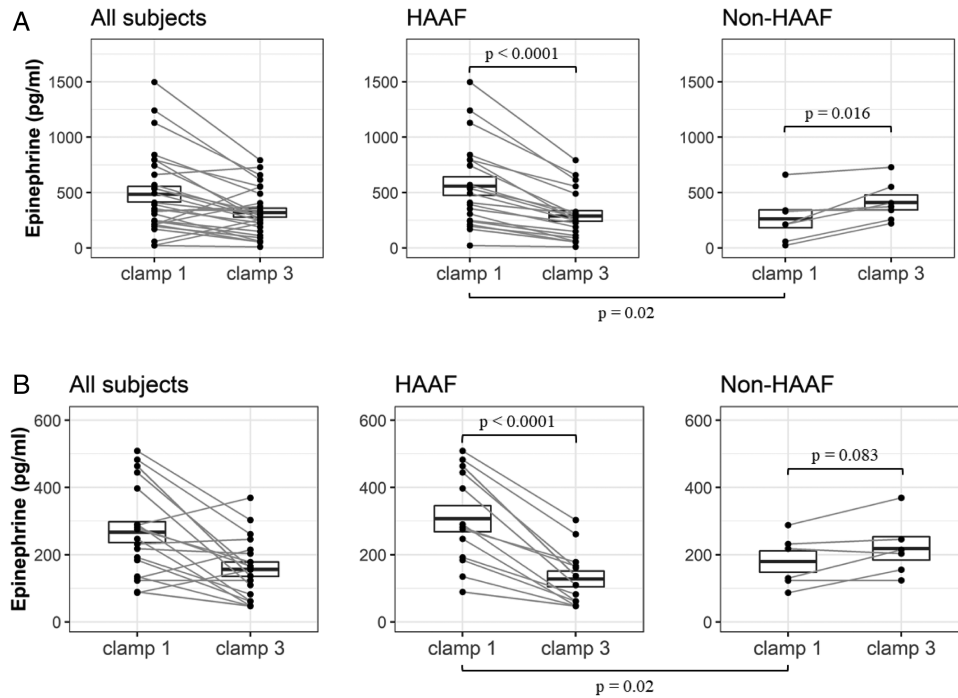
If HAAF is defined as achieving a 20% reduction in hypoglycemia-induced symptoms measured during clamp 3 compared with clamp 1, 10 healthy volunteers and 10 volunteers with type 1 diabetes developed symptom-based HAAF as a result of the preinduction protocol. Seventeen healthy volunteers and 9 volunteers with type 1 diabetes did not (Figure 3). However, there was no difference noted in the mean symptom scores measured during clamp 1 between subjects

who did and did not develop symptom-based HAAF in both groups. There also was no difference in clamp 1 levels of epinephrine released in response to hypoglycemia between those who did and did not develop symptom-based HAAF, although in the group that developed HAAF the epinephrine levels during clamp 1 were significantly higher than during clamp 3 ( $P = 0.001$ ) (Figure 4). If adrenergic or neuroglycopenic symptoms were used for the analysis, the results were similar. If epinephrine response was reported as peak level obtained during the final 30 minutes of the clamp, the results were unchanged (data not shown).

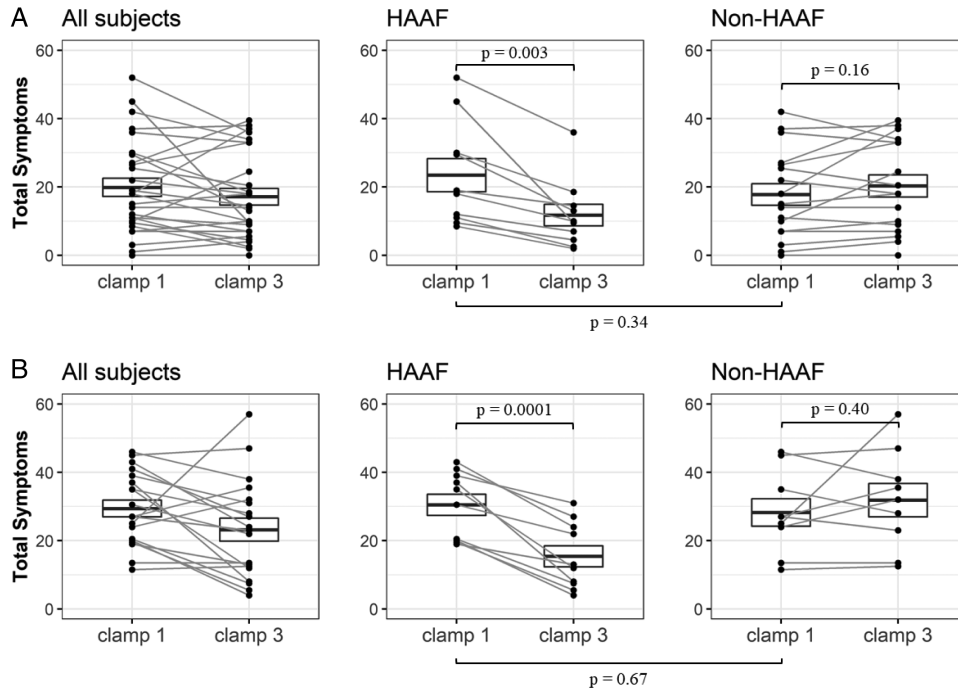
The glucose infusion rates required to maintain target glycemia during clamps 1 and 3 were not different in healthy volunteers regardless of whether they developed HAAF as defined by epinephrine or symptom reduction criteria (Table 1). The glucose infusion rates required to maintain target glycemia in volunteers with type 1 diabetes were marginally lower in clamp 3 than in clamp 1 in those who did not develop HAAF by either criterion ( $P = 0.04$  using epinephrine criteria and  $P = 0.05$  using symptom criteria) but were essentially the same in those who did develop HAAF using either criteria.

## Discussion

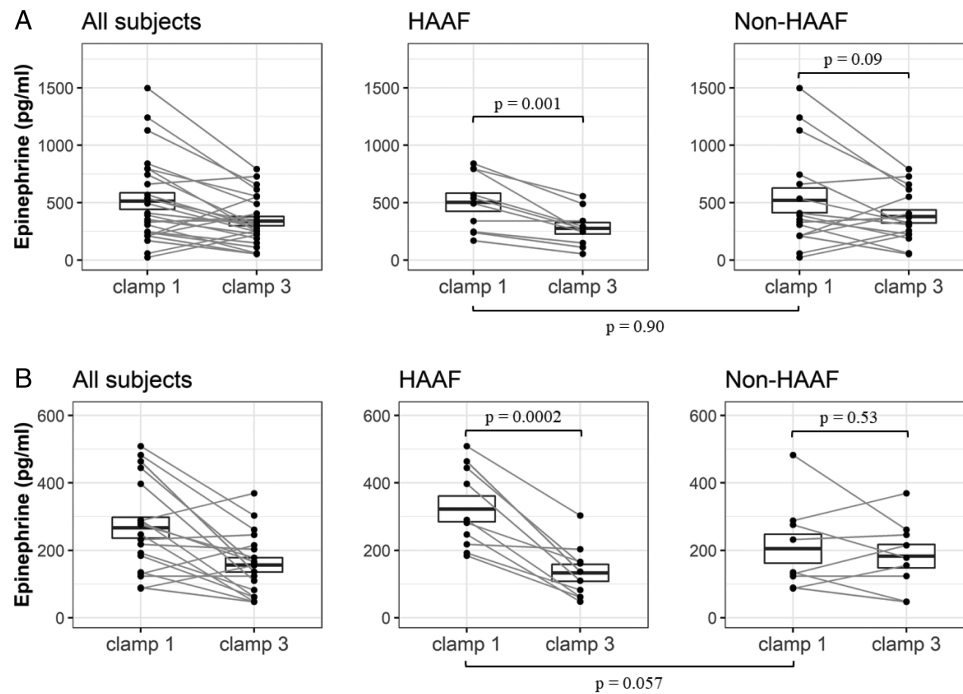
In this investigation, we confirmed the findings of Lontchi-Yimagou et al [6] that healthy adults who met the standard criteria for HAAF during the third exposure to hypoglycemia demonstrated higher levels of hypoglycemia-induced epinephrine response during the first exposure to hypoglycemia in our preinduction protocol. We also made the novel observation that subjects with type 1 diabetes who met the standard criteria for HAAF during the third exposure to hypoglycemia demonstrated higher levels of hypoglycemia-induced epinephrine response during the first exposure to hypoglycemia in our preinduction protocol than did similar subjects who did not develop HAAF. Additionally, we verified that the levels of hypoglycemia-induced epinephrine secretion during clamp 1 were physiologically relevant in blunting the counterregulatory response in clamp 3 because the glucose infusion rates during clamp 1 were lowest in those subjects with the highest epinephrine levels. These observations demonstrate that the ability to secrete epinephrine in response to the first episode of hypoglycemia is a major determinant of whether the response will be blunted during subsequent exposures.



**Figure 2.** Hypoglycemia-induced epinephrine responses during clamp 1 and clamp 3 of the preinduction protocol when epinephrine is used to define HAAF. (A) The response of the 28 healthy volunteers with complete epinephrine datasets is shown on the left. The middle graph shows the responses of the 21 who developed HAAF, as defined by a 20% reduction in epinephrine response during clamp 3 compared with clamp 1. The right graph shows the 7 who did not develop HAAF. (B) The response of the 19 volunteers with type 1 diabetes who had complete epinephrine datasets is shown on the left. The middle graph shows the responses of the 13 who developed HAAF, as defined by a 20% reduction in epinephrine response during clamp 3 compared with clamp 1. The right graph shows the 6 who did not develop HAAF. The boxes represent the average response  $\pm$  standard error of the mean. The table shows the epinephrine responses in pg/mL for each subject group.



**Figure 3.** Hypoglycemia-induced total symptom responses during clamp 1 and clamp 3 of the preinduction protocol when total symptoms are used to define HAAF. (A) The response of the 27 healthy volunteers with complete datasets for symptoms is shown on the left. The middle graph shows the responses of the 10 who developed HAAF, as defined by a 20% reduction in symptom response during clamp 3 compared with clamp 1. The right graph shows the 17 who did not develop HAAF. (B) The response of the 19 volunteers with type 1 diabetes who had complete datasets for symptoms is shown on the left. The middle graph shows the responses of the 10 who developed HAAF, as defined by a 20% reduction in symptom response during clamp 3 as compared to clamp 1. The right graph shows the 9 who did not develop HAAF. The boxes represent the average response  $\pm$  standard error of the mean. The table shows symptom responses by subject group.



**Figure 4.** Hypoglycemia-induced epinephrine responses during clamp 1 and clamp 3 of the preinduction protocol when total symptoms are used to define HAAF. (A) The response of the 26 healthy volunteers with complete epinephrine and symptom datasets is shown on the left. The middle graph shows the epinephrine responses of the 10 who developed symptom-based HAAF, as defined by a 20% reduction in symptoms during clamp 3 compared with clamp 1. The right graph shows the 16 who did not develop symptom-based HAAF. (B) The response of the 19 volunteers with type 1 diabetes with complete epinephrine and symptom datasets is shown on the left. The middle graph shows the epinephrine responses of the 10 who developed symptom-based HAAF, as defined by a 20% reduction in symptoms during clamp 3 compared with clamp 1. The right graph shows the 9 who did not develop symptom-based HAAF. The boxes represent the average response  $\pm$  standard error of the mean. The table shows epinephrine responses in pg/mL by subject group.

**Table 1.** Glucose infusion rates required to maintain target glycemia

		Those who developed HAAF		Those who did not develop HAAF	
		Clamp 1	Clamp 3	Clamp 1	Clamp 3
HAAF defined by epinephrine criteria	Subjects with type 1 diabetes	5.3 $\pm$ 0.8	5.2 $\pm$ 0.7	3.0 $\pm$ 0.5	2.0 $\pm$ 0.4 <sup>a</sup>
	Volunteers without diabetes	2.1 $\pm$ 0.5	3.1 $\pm$ 0.6	3.0 $\pm$ 1.0	2.0 $\pm$ 0.6
HAAF defined by symptom criteria	Subjects with type 1 diabetes	5.6 $\pm$ 0.5	5.5 $\pm$ 0.8	3.5 $\pm$ 0.7	2.7 $\pm$ 0.7 <sup>b</sup>
	Volunteers without diabetes	1.5 $\pm$ 0.5	3.0 $\pm$ 0.8	2.9 $\pm$ 0.6	2.7 $\pm$ 0.6

Glucose infusion rates are in mg/kg/min and represent the average rate of glucose infused during the final 30 minutes of the clamp study.

HAAF, hypoglycemia-associated autonomic failure.

<sup>a</sup>P = 0.04 comparing rating in clamp 1 to clamp 3 in this group.

<sup>b</sup>P = 0.05 comparing rating in clamp 1 to clamp 3 in this group.

Hypoglycemia-induced epinephrine secretion has long been known to play an important role in the pathogenesis of impaired awareness of hypoglycemia. Ramanathan and Cryer [5] demonstrated that adrenergic blockade with nonselective  $\alpha$ - and  $\beta$ -adrenergic antagonists during the first episode of hypoglycemia prevented the expected blunting of the epinephrine response that occurred during a second episode of hypoglycemia. Some [6] but not all investigators [17] have demonstrated that infusion of physiological levels of epinephrine before exposure to hypoglycemia blunted the subsequent counterregulatory hormone response. Even though volunteers with the highest epinephrine secretory response to hypoglycemia during clamp 1 were significantly more likely to develop HAAF than were volunteers who had a lower response

during clamp 1, we are unable to identify the minimum level of response necessary for the subsequent development of HAAF. It is interesting that the volunteers with type 1 diabetes who developed HAAF as defined by a 20% reduction in epinephrine response had an epinephrine response during clamp 1 that is similar to the healthy volunteers who did not develop HAAF. Bolli et al [18] previously demonstrated that people with longstanding type 1 diabetes have a reduced epinephrine response relative to healthy controls, which some have suggested may reflect the frequency with which they experience hypoglycemia in the days or weeks before experimental investigation. This suggests there may be a range of responses that should be expected in the epinephrine response to experimental hypoglycemia based on prior experiences and

if the magnitude of that response is sufficient, one may experience a blunting of response during subsequent exposure. Understanding what experiences alter hypoglycemia-induced epinephrine secretion in real life away from the laboratory may be the key to understanding the pathogenesis of impaired awareness of hypoglycemia. It is possible that these experiences are merely hypoglycemia and patients truly are demonstrating a habituated response to this stress, as has been proposed by McNeilly et al [19]. However, as has been demonstrated with exercise [20, 21], it may be that all sorts of stresses play a role in determining this response. Future investigation should focus on understanding these stresses so we can use the information in the clinical management of patients with type 1 diabetes.

In this investigation, we also found that those subjects who had the greatest level of hypoglycemia-induced symptom scores during the first exposure to hypoglycemia in a preinduction protocol were most likely to demonstrate a more than 20% reduction in symptom scores during the third exposure. These observations demonstrate the correlation between epinephrine and symptoms responses and provide support for using the objective measure of epinephrine as a surrogate for the more subjective measures of symptoms in studying the pathogenesis of impaired awareness. This relationship is not a failsafe one, however, as demonstrated by Altorfer et al [22] in adrenalectomized subjects exposed to hypoglycemia and the studies in which hypoglycemia avoidance restored symptom but not epinephrine response in subjects with type 1 diabetes and hypoglycemia unawareness [23, 24].

We expected to find higher glucose infusion rates during clamp 3 than during clamp 1 in those participants who developed HAAF defined by a 20% or more reduction in epinephrine secretion in clamp 3 when compared with clamp 1. We saw a trend toward higher rates in healthy volunteers who developed HAAF but saw essentially no change in subjects with type 1 diabetes. Traditional thinking has been that hypoglycemia-induced epinephrine secretion contributes to restoration of euglycemia by reducing glucose disposal and enhancing endogenous glucose production. Perhaps the trend toward higher rates during clamp 3 in controls is due to glucagon secretion, which we did not measure in our studies, but we doubt that glucagon explains our infusion results in the subjects with diabetes. With a mean duration of 15 years, previous investigation has shown such patients have markedly reduced hypoglycemia-induced glucagon secretion [18]. Perhaps the counterregulatory response to hypoglycemia is dependent on more than epinephrine secretion in individuals with type 1 diabetes. Davis et al [25] have demonstrated that a preinduction protocol similar to our own suppresses sympathetic nervous activity as measured by microneurography. Assuming that occurred in our subjects, what is responsible for maintaining target glycemia during day 3 in these type 1 subjects with HAAF? Although our inability to find a significant difference in glucose infusion rates may merely be a function of our relatively small sample size, it is interesting to consider that as-yet undiscovered counterregulatory mechanisms may be responsible for our observations.

The preinduction protocol used in these experiments induced epinephrine-based HAAF in 75% of the healthy volunteers and 68% of the volunteers with type 1 diabetes. This level of success exceeds the 54% rate reported by Lontchi-Yimagou et al [6] but clearly demonstrates there is a great heterogeneity of response. Such heterogeneity must

be considered in the design of future experiments to evaluate impaired awareness of hypoglycemia.

In conclusion, we have confirmed that those subjects with the greatest level of hypoglycemia-induced epinephrine during the first exposure to hypoglycemia in a preinduction protocol were most likely to demonstrate sufficient blunting of epinephrine secretion to meet the standard criteria for HAAF during the third exposure to hypoglycemia. We have also extended these findings to volunteers with type 1 diabetes. Both groups showed a great heterogeneity of response to the preinduction protocol and there was overlap between the epinephrine responses seen in the volunteers with type 1 diabetes who develop HAAF and the healthy volunteers who did not. We propose that the responses individuals experience in real life before experimental hypoglycemia, including the response to recurrent hypoglycemia, may play a role in determining how great a response one may have to the experimental condition. If so, greater attention should be focused on understanding the recurrent stresses that occur in the real lives of patients at risk for impaired awareness to understand how to better prevent and treat this condition.

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## Clinical Trial Information

Cerebral Responses to Insulin Induced Hypoglycemia (1018) no. NCT02747680 (registered April 22, 2016; and no. Recurrent Hypoglycemia in Type 1 Diabetes (Aim 1) no. NCT03410277 (registered January 25, 2018).

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## Disclosures

The authors have no conflicts of interest to disclose.

## Data Availability

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References. Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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