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Clinical Research Article

Relationship Between Hypoglycemia Awareness Status on Clarke/Gold Methods and Counterregulatory Response to Hypoglycemia

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Abstract

Context: Impaired awareness of hypoglycemia (IAH) is characterized by the diminished ability to perceive symptoms of hypoglycemia. Gold and Clark questionnaires are commonly used to identify patients with IAH. The relationship between IAH status on questionnaires and a person's symptom and epinephrine responses to hypoglycemia are not well understood.

Objective: We aimed to examine the relationship between hypoglycemia awareness status on Clarke and Gold questionnaires with both hormonal and symptomatic responses to experimental hypoglycemia.

Methods: In this university medical center study, we examined data from 78 subjects with type 1 diabetes (T1D) who completed both questionnaires and underwent a hyperinsulinemic hypoglycemic clamp (target glucose 50 mg/dL).

Results: Clarke and Gold scores were highly correlated with one another (r = 0.82) and each had a moderate negative relationship with epinephrine (Clarke: r = -0.51, Gold: r = -0.50) and total symptom response (Clarke: r = −0.59, Gold: r = −0.57). However, 32% of the subjects were classified inconsistently by Clark vs Gold. A clustering analysis was done to examine how disagreement between the 2 questionnaires on IAH classification relates to epinephrine and symptoms responses during hypoglycemia. Subjects who had partial loss of symptoms or of epinephrine response were more likely to be classified inconsistently.

Conclusion: Our results show that IAH classification may be discordant between Clark and Gold questionnaires and that hypoglycemia awareness status on Clarke and Gold questionnaires poorly predicts hormonal and symptomatic responses to hypoglycemia in subjects with T1D and moderate blunting of symptoms or epinephrine.

Key Words: Gold questionnaires, Clarke questionnaires, hypoglycemia, impaired awareness of hypoglycemia, type 1 diabetes, hypoglycemia unawareness

Abbreviations: HbA1c, glycated hemoglobin; IAH, impaired awareness of hypoglycemia; T1D, type 1 diabetes.

Iatrogenic hypoglycemia is a common and feared complication of treatment with insulin in patients with type 1 diabetes (T1D) [1, 2]. Such patients have beta cell failure and cannot attenuate serum insulin levels in the face of falling glucose, because of their reliance on exogenous insulin. In addition, they generally cannot mount a glucagon response in the setting of hypoglycemia and depend on the activation of the sympathetic nervous system and release of epinephrine from the adrenal medulla to develop the warning symptoms of hypoglycemia. The typical symptoms of hypoglycemia include sweating, hunger, shakiness/tremulousness, heart pounding, and nervousness/anxiety [3]. Subjective recognition of these warning symptoms is key for recognition of hypoglycemia so that the person can take corrective actions to prevent severe hypoglycemia [4]. Recognition of the onset of these symptoms constitutes awareness of hypoglycemia [4, 5].

Patients with diabetes who experience repeated episodes of iatrogenic hypoglycemia can develop impaired awareness of hypoglycemia (IAH) [6, 7]. IAH is an acquired complication of diabetes treatment in which patients can experience a partial or complete loss of ability to perceive typical symptoms associated with low blood glucose [7, 8]. IAH is associated with a reduction in hypoglycemia-induced epinephrine response and a reduction of the glucose threshold that is required to generate this response [9]. The prevalence of IAH has been reported to be around 25% in patients with T1D and 10% in people with advanced type 2 diabetes [10] and is associated with a six-fold greater frequency of severe hypoglycemia [11].

Accurate identification of presence of IAH in people with diabetes is critical in both the clinical and research settings. In the clinical setting, it identifies people at increased risk of severe hypoglycemia, in whom modification of glycemic

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targets and therapy may be needed to minimize risk of future hypoglycemia. Accurate classification of hypoglycemia awareness status in patients with diabetes is also critical in the research setting, particularly in the studies examining the pathophysiology or treatment of IAH. Standard practice has relied on questionnaires to determine the hypoglycemia awareness status of a patient with diabetes. The 2 most commonly used instruments are the Clarke and Gold questionnaires. The Clarke questionnaire is composed of 8 questions that examine the glycemic threshold at which subjects develop symptoms of hypoglycemia and also characterizes the subject's exposure to episodes of moderate and severe hypoglycemia [8]. The Gold scoring method is based on the response to a single question, "Do you know when your hypos are commencing?" with responses expressed in a Likert scale [11]. Despite differences between the structure and scoring of these 2 questionnaires, strong association has been previously shown between these 2 measures [4]. A previous study, which included 19 subjects with T1D, found that classification of IAH based on the Clarke method agreed reasonably well with classification of IAH based on the absence of adrenergic symptoms at a blood glucose level of 54 mg/dL during experimental hypoglycemia [12]. However, none of the currently available methods is considered to be fully reliable to identify IAH [4]. Previous studies that have assessed these questionnaires for identifying IAH did not include examination of counterregulatory hormone responses to hypoglycemia.

In this study, we sought to determine the concordance between the Gold and Clarke methods of categorizing hypoglycemia awareness status in subjects with T1D who completed the questionnaires before participating in a hypoglycemic clamp study where symptoms and epinephrine responses to hypoglycemia were measured. We also aimed to assess the magnitude of symptom and epinephrine response to hypoglycemia in these subjects with the goal of identifying which of the survey instruments performed best in identifying those subjects with blunted symptom and epinephrine responses. This is the first study to our knowledge to correlate Gold and Clarke scores with both hormonal and symptomatic responses to experimental hypoglycemia.

Methods

Participants

Subjects were drawn from 2 separate studies conducted between 2015 and 2020 to examine brain responses to experimental hypoglycemia in subjects with T1D. Inclusion criteria for these studies were the presence of T1D (defined on clinical grounds). Exclusion criteria were presence of proliferative retinopathy, comorbid neurological or severe psychiatric diseases, magnetic resonance imaging (MRI) contraindications, and significant vascular or space-occupying lesions on MRI scans.

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. The study protocol designated that subjects should complete both the Clarke and Gold questionnaires on the study day to ascertain their awareness status. The Clarke questionnaire is comprised of 8 questions [8] where each question's response corresponds to "aware" (value 0) or "unaware" (value 1); scores are then summed. Scores of 0-2 are categorized as "aware", 4-7 as "unaware", and 3 as "indeterminant". The Gold questionnaire has only 1 question: "Do you know when your hypoglycemic episodes are commencing?" [11] and is scored on a 7-point Likert scale. Scores of 1-2 are categorized as "Aware", 4-7 as "unaware", and 3 as "indeterminant". Starting from a cohort of 89 subjects enrolled during 2015 to 2020, we identified 78 participants who completed both Clarke and Gold questionnaires and had data for epinephrine or symptom response to hypoglycemia. Eleven participants participated in both studies; their study dates were separated on average by 7 months (range, 1-11 months) and both of their data sets were included in the analysis.

Hormonal and Symptomatic Responses During Hypoglycemia Clamp

On the morning of the study, subjects arrived at the research center after an overnight fast. After completing the Clarke and Gold questionnaires, they underwent a hyperinsulinemic (2 mU/kg/min) hypoglycemic clamp where glucose was allowed to drop and maintained at the hypoglycemic target of 50 mg/dL for approximately 30 minutes. Blood samples for epinephrine were collected at baseline and every 10 minutes during hypoglycemia. Samples for epinephrine were sent to the Vanderbilt Diabetes Research and Training Center core laboratory for analysis. Plasma epinephrine was measured by high-performance liquid chromatography (Dionex, Sunnyvale, CA, USA; formerly ESA). The epinephrine response during hypoglycemia was calculated by taking the difference between the average of the 3 values during hypoglycemia and the average of the 2 baseline values.

Symptoms of hypoglycemia were quantified by using a previously validated questionnaire [13] at baseline and during hypoglycemia at the end of the insulin clamp. Subjects were asked to score from 0 (none) to 6 (severe) for each of 12 symptoms (total symptoms), including 6 adrenergic (autonomic) symptoms (heart pounding, shaky/tremulous, nervous/anxious, sweaty, hungry, tingling) and 6 neuroglycopenic symptoms (difficulty thinking, tired/drowsy, weak, warm, faint, dizzy). The symptomatic response was calculated as the difference between the responses during hypoglycemia and baseline. Seventy-six out of 78 participants had epinephrine response data; 77 out of 78 participants had symptom response data.

Statistical Methods

Agreement analysis

Demographic and clinical factors were summarized descriptively for all participants. Using the data from the 78 participants who completed both questionnaires, agreement between the Clarke and Gold awareness statuses was calculated using linear weighted Cohen's Kappa statistic [14] and 95% CI to measure the inter-questionnaire agreement. Scatterplots were used to illustrate the relationship between the Clarke and Gold scores, as well as the relationship between the epinephrine and total symptom responses and the Clarke and Gold scores. Spearman correlation coefficients were calculated to measure the strength of these relationships.

Clustering analysis

To determine if the relationship between the epinephrine and adrenergic or total symptom responses cluster in certain types

of patterns, a clustering analysis was performed in participants who had complete epinephrine and symptom responses using the "mclust" package (version 5.4.3) in R [15]. The analysis is based on finite normal mixture modeling and clusters were selected according to the Bayesian Information Criteria. The clusters are displayed in a scatterplot, along with each person's questionnaire classifications to explore how awareness status relates to the joint epinephrine and adrenergic or total symptom responses.

Repeated measures analysis

To determine the intraindividual variability of the cluster analysis, using data of the 11 participants who were studied twice, we compared the assignments made at each visit for the 9 of them who provided full datasets. To determine the interindividual variability of the cluster analysis we randomly selected 9 sets of 2 non-repeat subjects to determine what proportion had the same cluster, and then repeated this process 1000 times to define the mean and 95% CI of these values. We also compared the variability (SD) of epinephrine level, total symptoms response, and Clarke and Gold questionnaires scores among the 9 subjects with repeat visits and complete datasets to the variability in 1000 replicated averages of 9 sets of 2 random non-repeat subjects.

P values less than 0.05 were considered statistically significant. All analyses were performed in R (R Core Team, Version 3.6).

Results

Demographic and clinical characteristics of the study participants are shown in Table 1. Fifty-six percent of the participants were female; the group had a long duration of diabetes (mean diabetes duration 20 years) and a mean glycated hemoglobin (HbA1c) of 7.4% (Table 1). There was a strong relationship between the Clarke and Gold scores, with a Spearman correlation coefficient of 0.82 [95% CI: (0.74, 0.88), $P \leq 0.001$] (Fig. 1). Among the participants who completed both questionnaires, 32% were classified inconsistently by the questionnaires. The agreement table of classification as determined by Clarke and Gold is shown in Table 2. Misclassification was defined as the percent of classifications that did not agree. Cohen's Kappa was calculated to be 0.63, which can be considered a moderate measure of inter-instrument reliability. The Clarke questionnaire was more likely to consider a patient aware and less likely to consider a patient unaware than the Gold questionnaire.

Both questionnaires had moderately strong negative relationships to the epinephrine response (Clarke: r = –0.51, Gold: r = –0.50, $P \leq 0.001$) and total symptom response to hypoglycemia (Clarke: r = –0.59, Gold: r = –0.57, $P \leq 0.001$; Fig. 2). A clustering analysis was done to examine patterns of epinephrine and adrenergic symptom responses, and how those responses relate to the questionnaire classifications. Participants who had both epinephrine and symptom responses were included in the cluster analysis (n = 75). According to the clustering algorithm, there were 3 general types of epinephrine and adrenergic symptomatic response patterns: 1) high epinephrine and high symptom responses; 2) low epinephrine and moderate-to-high symptom responses; and 3) low epinephrine and low symptom responses (Fig. 3). In general, those in cluster 1 were mostly aware on both questionnaires and those in cluster 3 were mostly unaware cluster 2 contained a mix of awareness statuses. In general, participants who had at least 1 aware status (Clarke or Gold or both) tended to have a larger epinephrine and/or adrenergic

Table 1. Demographic and clinical characteristics of the 78 participants present in the study sample

Characteristic	All participants N = 78	
	Mean (SD) or N (%)	min - max
Sex		
Female	44 (56.4%)	
Male	34 (43.6%)	
Age (years)	36.7 (12.7)	18 - 67
Years of diabetes	20.1 (11.3)	1.6 - 46.4
BMI (kg/m^2)	26.1 (4.6)	17.6 - 42.7
HbA1c	7.4 (1.2)	5.4 - 13.6
Clarke Awareness		
Aware	35 (44.9%)	
Indeterminant	17 (21.8%)	
Unaware	26 (33.3%)	
Gold Awareness		
Aware	25 (32.1%)	
Indeterminant	19 (24.4%)	
Unaware	34 (43.6%)	
Epinephrine response[a] (ng/dL) (N = 76)	243.1 (206.7)	23.5 - 911.2
Symptom response[b] (N = 77)	19.3 (13.5)	–3.0 - 46.0

[a]The epinephrine response during hypoglycemia was calculated by taking the difference between the average of the 3 values during hypoglycemia and the average of the 2 baseline values.
[b]The symptom response was calculated as the difference between the responses during hypoglycemia and baseline.

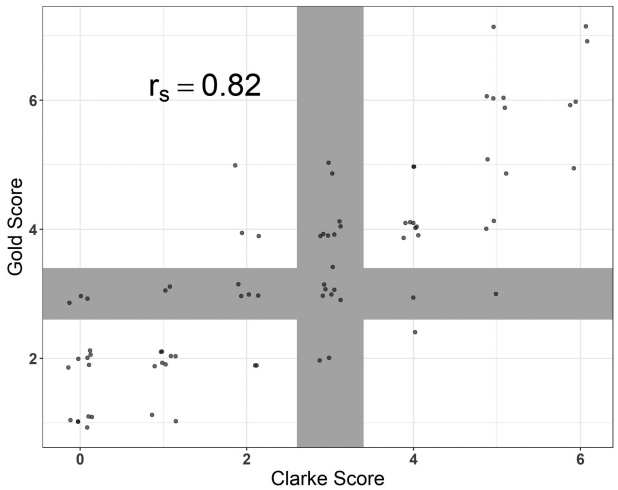

Figure 1. The relationship between the total Clarke and Gold questionnaire scores for each participant. Scores of 0-2 are categorized as "aware" and 4-7 as "unaware" on Clark and scores of 1-2 are categorized as "Aware" and 4-7 as "unaware" on the Gold questionnaire. Shaded area in the figure represents score of 3 which is classified as "indeterminate" by both the Clark and Gold questionnaires. Data points were jittered for visibility. The Spearman correlation coefficient was calculated.

Table 2. The agreement table of the Clarke and Gold questionnaire awareness status (N = 78)

		Clarke questionnaire			
		Aware	**Indeterminant**	**Unaware**	**Total**
Gold questionnaire	**Aware**	**23 (92.0%/ 65.7%)**	2 (8.0%/11.8%)	0 (0%/ 0%)	25
	Indeterminant	9 (47.4%/ 25.7%)	**7 (36.8%/ 41.2%)**	3 (15.8%/ 11.5%)	19
	Unaware	3 (8.8%/ 8.6%)	8 (23.5 %/ 47.1%)	**23 (67.6%/ 88.5%)**	34
	Total	35	17	26	78

The first % represents the row percentage, i.e., the percent of each Clarke status among that Gold status. The second % represents the column percentage, i.e., the percent of each Gold status among that Clarke awareness status. These 2 measures have a linear weighted Kappa statistic of 0.63 (0.31, 0.95).

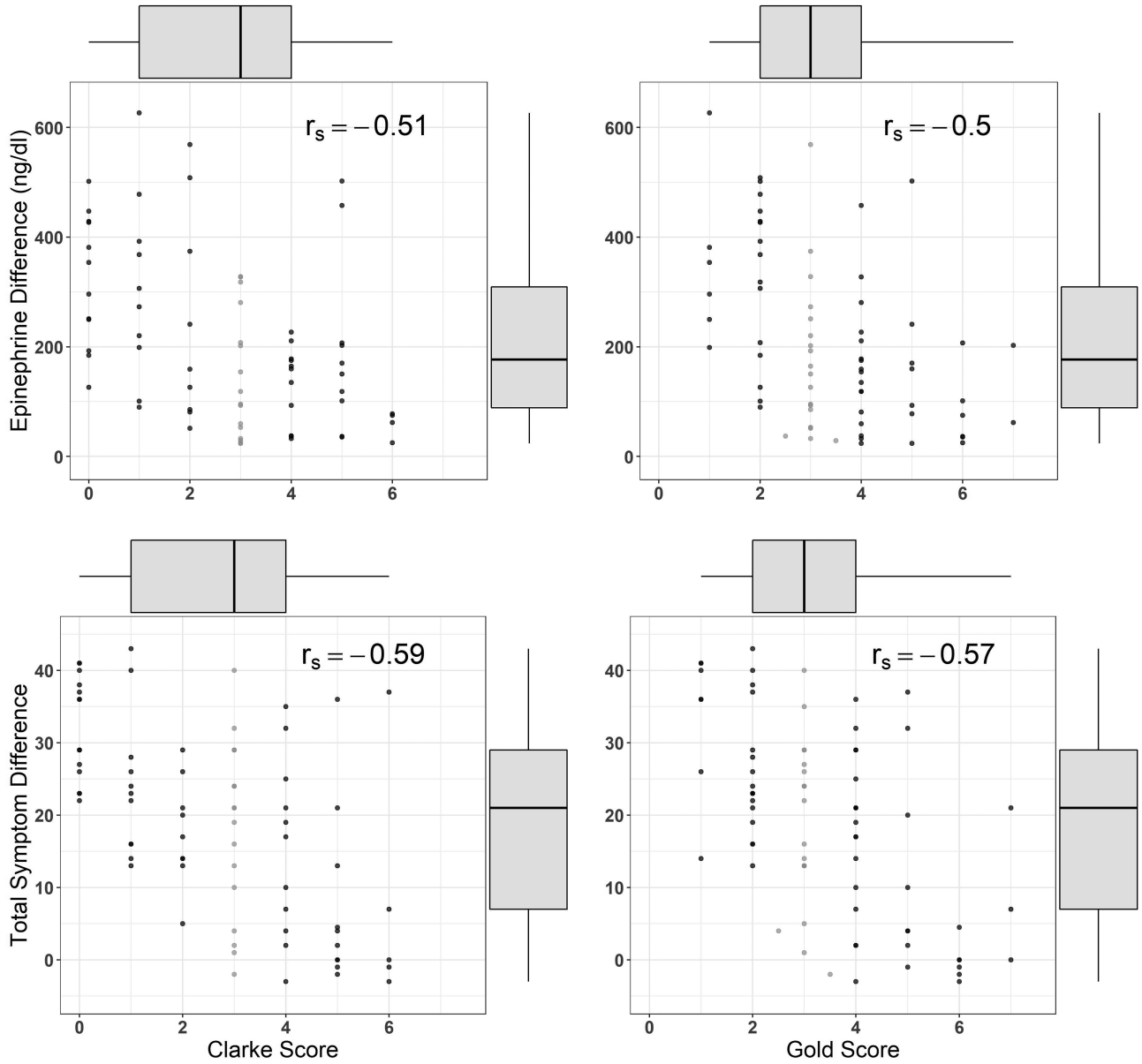

Figure 2. The epinephrine (top row) and the total symptom score (bottom row) response to hypoglycemia were compared to both the Clarke (left column) and the Gold (right column) scores. Boxplots to display the distribution of each measure are located at the margins of each graph. The light gray points represent the "indeterminant" scores, whereas the black points represent either aware or unaware values. Spearman correlations were calculated for each of the 4 scatterplots.

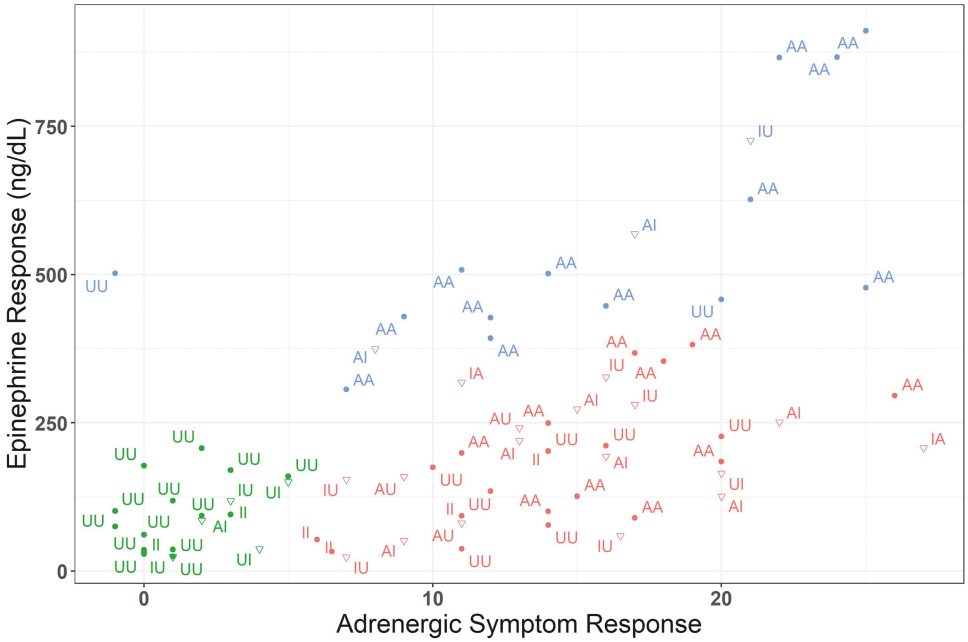

Figure 3. The relationship between the epinephrine and adrenergic (autonomic) symptom response to hypoglycemia is graphed (N = 75), along with the 3 response type clusters by color (cluster 1, Blue; cluster 2, Red; cluster 3, Green). The letters corresponding to each data point show the awareness status (A = aware, I = indeterminant, U = unaware) for both the Clarke (first letter) and Gold (second letter) scores. Solid points represent concordant classification between Clarke and Gold, whereas the transparent triangles represent discordant classifications.

symptomatic response within the cluster. Similarly, those with at least 1 unaware status tended to be located on the lower end of the epinephrine and/or symptomatic response scale. However, given the range of the responses, and the discordant awareness classifications, a distinct pattern is difficult to discern for cluster 2. Subjects in cluster 3 were older and had longer duration of diabetes, whereas there was no significant difference in gender, body mass index, or glycemic control among the 3 clusters (Table 3). A clustering analysis was also done with using total symptom score, which showed a similar pattern as seen with adrenergic symptoms (supplemental Figure 1 and supplemental Table 1) [16].

Eleven participants in this cohort participated in 2 study visits separated on average by 7 months (range, 1-11 months). Nine participants in this group provided cluster information for both visits and 7 (77.8%) remained in the same cluster for both visits. There was no significant change in HbA1c in these subjects between the 2 visits [mean difference (SD) –0.0% (0.4)]. Among the non-repeat participants, there was, on average, a 59% (95% CI: 49%-70%) chance that any 2 random participants would have the same cluster. Analysis of the differences in the variability (SD) (in the 11 with repeat sample vs the 11 sets of non-repeats) of epinephrine level (89.4 vs 283.6), symptoms response (9.2 vs 19.2), Clarke (1.5 vs 2.7) and Gold questionnaires scores (0.8 vs 2.3) again demonstrated lower intra- vs interindividual variability.

Discussion

There are 2 different ways of measuring the association between the responses on the Gold questionnaire and the responses on the Clarke questionnaire: 1) correlation between the total numeric versions of the questionnaire responses; and 2) concordance between the scores when classified into 1 of 3 awareness statuses. While correlation between the total numeric scores of the responses to Gold and to Clarke were strong (Fig. 1), as has been shown previously by others [4, 17], the concordance between the scores when classified into the 3 awareness statuses was not as good (Table 2). We interpret this to mean that the use of Clarke and Gold questionnaires, which rely on a person's previous experience with hypoglycemia, must be done with caution because they may poorly predict how the person will respond to an episode of hypoglycemia in the future.

Despite the strong correlation found between the raw numeric Clarke and Gold survey responses, we did find discrepancies between the status assigned by each questionnaire, particularly in those participants assigned to the indeterminate status. Forty-one percent of the individuals categorized as indeterminant by the Clarke questionnaire were identified as being unaware on the Gold survey, and less than 37% found to be indeterminant on the Gold were classified as such on the Clarke. Among the participants who completed both questionnaires, 32% were classified differently by the 2 instruments (Table 2). Participants were more likely to be classified as "unaware" on the Gold compared with the Clarke questionnaire. In a previous study of children older than 9 years of age, prevalence of IAH was higher when classified using Gold as compared to Clarke questionnaire, but the Clarke was noted to be superior in predicting risk of clinically significant hypoglycemia [18]. In other studies, in adults with T1D, the prevalence of IAH was comparable when classified by Gold or Clarke methods [4, 19]. This discrepancy in the strength of the association between the numeric versions of the 2 questionnaires and the association when classified into awareness status shows that the type of response data (numeric vs. categorical), as well as the thresholds used to classify awareness status both matter. Perhaps this also means that if classification of awareness status is to be done using a survey instrument, it would be better to use both the Clarke

Table 3. Demographic and clinical characteristics of participants with complete data by cluster assigned by the clustering algorithm

	Cluster 1 (N = 17)	Cluster 2 (N = 38)	Cluster 3 (N = 20)	Total (N = 75)	*P* value
Sex					0.375
Female	7 (41.2%)	23 (60.5%)	12 (60%)	42 (56%)	
Male	10 (58.8%)	15 (39.5%)	8 (40%)	33 (44%)	
Age (years)					**<0.001**
Mean (SD)	35.4 (13.5)	31.7 (10.2)	46.6 (11.6)	36.5 (12.9)	
Range	18- 64	19 - 60	25 - 67	18.0 - 67.0	
Years of diabetes					**<0.001**
Mean (SD)	15.5 (11.1)	17.8 (9.5)	28.8 (10.6)	20.3 (11.4)	
Range	3.5 - 42.7	4.2 - 41.8	13.6 - 46.4	3.5 - 46.4	
BMI (kg/m^2)					0.837
Mean (SD)	25.9 (4.9)	25.8 (4.2)	26.6 (5.3)	26.0 (4.7)	
Range	20.3 - 36.1	17.6 - 38.8	19.2 - 42.7	17.6 - 42.7	
HbA1c					0.774
Mean (SD)	7.3 (1.3)	7.5 (1.4)	7.3 (1.0)	7.4 (1.2)	
Range	5.5 - 11.4	6.0 -13.6	5.4 - 9.5	5.4 - 13.6	

and Gold instruments and assign a category only when both surveys are in agreement.

We observed moderate, negative relationships (Fig. 2) between scores on both questionnaires and the epinephrine response measured during hypoglycemia. Among the patients classified as unaware (Clarke/Gold score > 4), most show a reduced epinephrine response. However, among the patients classified as aware (Clarke/Gold < 2) there is a wide range of epinephrine responses, especially for aware classification based on Clarke questionnaire. Some participants found to be aware on the Clarke instrument were found to have hypoglycemia-induced epinephrine responses that are as low as those seen in participants categorized as unaware. We found a similar relationship between scores on the questionnaires and total symptom responses during hypoglycemia (Fig. 2). As would be expected, patients classified as unaware had lower hypoglycemia symptoms score responses than the aware patients (Fig. 2) but there were unaware participants who had symptoms scores equal to participants who had been categorized as aware by the survey instruments. These data show that hypoglycemia awareness status on Clarke and Gold questionnaires may poorly predict epinephrine and symptomatic responses to hypoglycemia in T1D.

We further did a cluster analysis to examine how disagreement between the Clarke and Gold methods on IAH classification relate to epinephrine and adrenergic symptoms responses during hypoglycemia. The participants were clustered according to their epinephrine and adrenergic symptom responses. The clustering analysis showed 3 general types of epinephrine and symptomatic response patterns: 1) high epinephrine and high symptom responses; 2) low epinephrine and moderate-to-high symptom responses; and 3) low epinephrine and low symptom responses (Fig. 3). One would expect "aware" participants to have both larger symptom and hormonal responses than the "unaware" participants. This is generally what we observe when we compare clusters 1 and 3. Participants in cluster 1 were mostly classified as aware on both the Clarke and Gold questionnaire. By contrast, cluster 3 includes those mostly categorized as unaware. Participants in cluster 3 were more likely to be older and have longer duration of diabetes (Table 3). This is not surprising, as both increasing age and longer duration of diabetes are associated with increased risk of IAH [10]. Cluster 2 includes participants with more mixed epinephrine and symptom responses. Participants in this cluster have partial loss of symptoms or epinephrine response and are more likely to be classified differently by the 2 questionnaires. This analysis highlights, not surprisingly, that these questionnaires are more accurate in classifying awareness status when participants are at the more extreme ends of the IAH spectrum with either intact or total loss of symptoms and epinephrine responses.

The observation that human subjects can have hypoglycemia-induced symptoms in the absence of a robust epinephrine response raises the question: what is causing the symptoms associated with hypoglycemia? This uncoupling of hypoglycemia-induced epinephrine and symptom responses has previously been reported to occur in patients with type 1 diabetes [20-22]; and in adrenalectomized subjects without diabetes [23]. A recent study by Nwokolo [24] suggests that different brain regions might be responsible for the regulation of different parts of the counterregulatory response. In their investigation, they used an educational intervention to restore awareness of hypoglycemia in patients with T1D and hypoglycemia unawareness and found that symptom restoration was not associated with restoration of hypoglycemia-induced epinephrine section. They also found that following the intervention, there was a greater increase in blood flow to the anterior cingulate cortex during hypoglycemia, without a blood flow change noted in other brain regions [24]. These investigators interpret their results to mean that the anterior cingulate may be particularly important in the regulation of hypoglycemia-associated symptoms but not in the regulation of the hormonal counterregulatory response; an interpretation that suggests that different brain regions are responsible for the regulation of different components of the counterregulatory response. In the present investigation, we did not consider regional blood flow so cannot comment on whether anterior cingulate cortex blood flow was greater during hypoglycemia in those participants who had high

symptoms but low epinephrine responses but plan to pursue this question in the future.

Despite these identified challenges with using the questionnaires to assign hypoglycemia awareness status to patients with T1D, we did find that this assignment tends to remain same over at least a 6-month period. We found that the intraindividual variability of the measures over time was less than the interindividual variability imposed by chance. Therefore, we can generally assume that patients with T1D who have IAH assigned by questionnaires administered twice over 6 months will have reduced epinephrine and symptoms responses during future episodes of hypoglycemia and that participants similarly assigned as aware will have robust responses during future hypoglycemia. Perhaps this means that participants should only be categorized as aware or unaware after they have shown a consistent response to both questionnaires over a 6-month period. This will put a burden on the research community but may provide confidence that the results from studies examining the pathogenesis and/or treatment of IAH using such a classification approach are relevant to the clinical situation.

Strengths of our study include the rigorous application of a protocol to assess the symptomatic and hormonal responses to hypoglycemia and a detailed statistical analysis to understand the relationship between the hypoglycemia awareness status identified by the Clarke and Gold instruments and the response of study participants to experimental hypoglycemia. Limitations include a relatively small sample size that could impact the reliability of our conclusions. Another limitation is the potential translatability of data obtained during experimental hypoglycemia clamps to patients' experiences of hypoglycemia in their daily lives.

In conclusion, our findings highlight that IAH can be difficult to classify. Despite the strong correlation between the scores of the Clarke and Gold instruments, we observe that there are considerable differences in how these questionnaires assign hypoglycemia awareness status. Our results also show that hypoglycemia awareness status on Clarke and Gold questionnaires may poorly predict hormonal and symptomatic responses to hypoglycemia in T1D. For clinical and research use, if classification of hypoglycemia awareness status is to be done using these survey instruments, using a combination of both questionnaires can provide more confidence in the awareness status classification.

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Author Contributions

N.R. designed the study, analyzed and interpreted the data, and wrote the initial draft. A.M. designed the study, participated in data collection, interpreted the data, and edited the manuscript. L.E. designed the study, interpreted the data, and reviewed the manuscript. A.K. recruited the study participants, participated in data collection, and reviewed the manuscript. S.M. secured funding, interpreted the data, and reviewed the manuscript. G.Ö. secured funding, interpreted the data, and reviewed the manuscript. E.R.S. designed the study, secured funding, and reviewed and wrote the manuscript. N.R., A.M., and E.R.S. 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

Disclosures

The authors have nothing to disclose.

Prior Presentation

Parts of this data were presented in abstract form at the American Diabetes Association's 78th Scientific Sessions, June 2018, Orlando, FL.

Clinical Trial Information

Clinical Trial Registration Numbers: NCT02747680, NCT03410277.

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.

References

1. Cryer PE. The barrier of hypoglycemia in diabetes. *Diabetes*. 2008;57(12):3169-3176. doi:10.2337/db08-1084
2. Khunti K, Alsifri S, Aronson R, *et al*; HAT Investigator Group. Rates and predictors of hypoglycaemia in 27 585 people from 24 countries with insulin-treated type 1 and type 2 diabetes: the global HAT study. *Diabetes Obes Metab*. 2016;18(9):907-915. doi:10.1111/dom.12689
3. Tesfaye N, Seaquist ER. Neuroendocrine responses to hypoglycemia. *Ann N Y Acad Sci*. 2010;1212:12-28. doi:10.1111/j.1749-6632.2010.05820.x
4. Geddes J, Wright RJ, Zammitt NN, Deary IJ, Frier BM. An evaluation of methods of assessing impaired awareness of hypoglycemia in type 1 diabetes. *Diabetes Care*. 2007;30(7):1868-1870. doi:10.2337/dc06-2556
5. McAulay V, Deary IJ, Frier BM. Symptoms of hypoglycaemia in people with diabetes. *Diabet Med*. 2001;18(9):690-705. doi:10.1046/j.1464-5491.2001.00620.x
6. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*. 2013;369(4):362-372. doi:10.1056/NEJMra1215228
7. Seaquist ER, Anderson J, Childs B, *et al*. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care*. 2013;36(5):1384-1395
8. Clarke WL, Cox DJ, Gonderfrederick LA, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM - a prospective-study of hypoglycemic frequency and associated symptoms. *Diabetes Care*. 1995;18(4):517-522.

9. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes*. 2005;54(12):3592-3601.
10. Graveling AJ, Frier BM. Impaired awareness of hypoglycaemia: a review. *Diabetes Metab*. 2010;36(Suppl 3):S64-S74.
11. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. *Diabetes Care*. 1994;17(7):697-703.
12. Janssen MM, Snoek FJ, Heine RJ. Assessing impaired hypoglycemia awareness in type 1 diabetes: agreement of self-report but not of field study data with the autonomic symptom threshold during experimental hypoglycemia. *Diabetes Care*. 2000;23(4):529-532.
13. Towler DA, Havlin CE, Craft S, Cryer P. Mechanism of awareness of hypoglycemia - perception of neurogenic (Predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes*. 1993;42(12):1791-1798.
14. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas*. 1960;20:37-46.
15. Scrucca L, Fop M, Murphy TB, Raftery AE. mclust 5: clustering, classification and density estimation using gaussian finite mixture models. *R J*. 2016;8(1):289-317.
16. Rubin *et al*. Supplement to: Relationship between hypoglycemia awareness status on Clarke/Gold methods and counterregulatory response to hypoglycemia. Dryad, Dataset. 2021. *Journal of the Endocrine Society*. 2022. Deposited July 24, 2022. Zenodo. https://doi.org/10.5281/zenodo.6897642
17. Sepúlveda E, Poínhos R, Nata G, *et al*. Differentiating hypoglycemia awareness status from hypoglycemia experience in tools for measuring impaired awareness of hypoglycemia. *Diabetes Technol Ther*. 2020;22(7):541-545.
18. Hatle H, Bjørgaas MR, Skrivarhaug T, *et al*. Assessing awareness of hypoglycemia in children and adolescents with type 1 diabetes: evaluation of established questionnaires. *Pediatr Diabetes*. 2020;21(2):300-309.
19. Ghandi K, Pieri B, Dornhorst A, Hussain S. A comparison of validated methods used to assess impaired awareness of hypoglycaemia in type 1 diabetes: an observational study. *Diabetes Ther*. 2021;12(1):441-451.
20. Dagogo-Jack S, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes*. 1994;43(4):1426-1434.
21. Nwokolo M, Amiel SA, O'Daly O, *et al*. Hypoglycemic thalamic activation in type 1 diabetes is associated with preserved symptoms despite reduced epinephrine. *J Cereb Blood Flow Metab*. 2020;40(7):787-798.
22. Kinsley BT, Weinger K, Bajaj M, *et al*. Blood glucose awareness training and epinephrine responses to hypoglycemia during intensive treatment in type 1 diabetes. *Diabetes Care*. 1999;22(12):1022-1028.
23. Altorfer RM, Ziegler WH, Froesch ER. Insulin hypoglycaemia in normal and adrenalectomized subjects: comparison of metabolic parameters and endocrine counter regulation. *Acta Endocrinol (Copenh)*. 1981;98(3):413-419.
24. Nwokolo M, Amiel SA, O'Daly O, Macdonald IA, Zelaya FO, Choudhary P. Restoration of hypoglycemia awareness alters brain activity in type 1 diabetes. *Diabetes Care*. 2021;44(2):533-540.