Impaired Awareness of Hypoglycemia in Adults With Type 1 Diabetes Is Not Associated With Autonomic Dysfunction or Peripheral Neuropathy

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OBJECTIVE

Impaired awareness of hypoglycemia (IAH) is a risk factor for severe hypoglycemia in people with insulin-treated diabetes; autonomic neuropathy has been suggested to underlie its development. The aim was to evaluate a putative association between IAH and autonomic dysfunction using novel and sensitive measures of autonomic neural function.

RESEARCH DESIGN AND METHODS

Sixty-six adults with type 1 diabetes were studied, 33 with IAH and 33 with normal awareness of hypoglycemia (NAH), confirmed by formal testing. Participants were matched for age, sex, and diabetes duration. Clinical and laboratory evaluations included extensive autonomic function testing, peripheral nerve conduction studies, and quantitative sensory testing. Composite abnormality Z scores were used for group comparisons.

RESULTS

The IAH and NAH group had similar median (interquartile range) age of 48 (14.5) vs. 47 (14.5) years, diabetes duration of 30 (13.5) vs. 31 (13.5) years, and mean ± SD HbA_{1c} 7.8 ± 2.2% vs. 8.1 ± 1.9%, respectively. The autonomic composite Z score did not differ between the two groups (mean difference −0.15, 95% CI −0.46, 0.16; *P* = 0.33), nor did the thermal detection (mean difference 0.15, 95% CI −0.31, 0.61; *P* = 0.51) or nerve conduction scores (mean difference 0.03, 95% CI −0.43, 0.49; *P* = 0.89).

CONCLUSIONS

In adults with type 1 diabetes, IAH was not associated with autonomic dysfunction or peripheral neuropathy.

Impaired awareness of hypoglycemia (IAH), defined as a diminished ability to perceive the onset of hypoglycemia, is associated with an increased risk of severe hypoglycemia in people with insulin-treated diabetes (1–3). Elucidation of the pathogenesis of IAH may help to minimize the risk of severe hypoglycemia.

The glycemic thresholds for counterregulatory responses, generation of symptoms, and cognitive impairment are reset at lower levels of blood glucose in people who have developed IAH (4). This cerebral adaptation appears to be induced by

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A slide set summarizing this article is available online.

recurrent exposure to hypoglycemia, and failure of cerebral autonomic mechanisms may be implicated in the pathogenesis (4). Awareness may be improved by avoidance of hypoglycemia (5–7), but this is very difficult to achieve and does not restore normal awareness of hypoglycemia (NAH) in all people with IAH. Because the prevalence of IAH in adults with type 1 diabetes increases with progressive disease duration (2,8,9), mechanisms that involve diabetic complications have been suggested to underlie the development of IAH.

Because activation of the autonomic nervous system is a fundamental physiological response to hypoglycemia and provokes many of the symptoms of hypoglycemia, autonomic neuropathy was considered to be a cause of IAH for many years (10). Deficient autonomic responses were also observed to be associated with an increased risk of severe hypoglycemia in a large European study (11). Although most people with type 1 diabetes and autonomic neuropathy experience autonomic symptoms during hypoglycemia (12,13), some studies have suggested that autonomic neuropathy may be associated with attenuated autonomic symptomatic responses (14–16) and accompanied by delayed or diminished catecholamine secretion to hypoglycemia (14,17). However, catecholamine secretion is not essential for symptom generation (18), and hypoglycemia awareness depends primarily on the generation of symptoms and their interpretation rather than symptom intensity (10,19). Studies of people with type 1 diabetes that have examined the glycemic thresholds for symptom generation in those with and without autonomic neuropathy (13,14,16) have found no differences, and autonomic symptom generation was not delayed.

Awareness of hypoglycemia is difficult to define, but its identification partly depends on the generation of physiological and neuroglycopenic responses and their appropriate interpretation to warn individuals of a falling blood glucose. Self-reporting of hypoglycemia awareness accommodates both of these aspects and is used by scoring systems such as the questionnaires by Gold et al. (20) and Clarke et al. (21). Earlier studies examined the association between self-reported awareness of hypoglycemia and autonomic neuropathy (22,23), but no convincing association was found. Subsequently, clinical methods to assess hypoglycemia awareness have been developed and validated (24), and methods of evaluating neuropathy have been refined (25) and supplemented with tests that have greater sensitivity to detect autonomic dysfunction (26). We therefore considered it appropriate and novel to undertake a detailed reexamination of the relationship between IAH and autonomic dysfunction by using a larger battery of autonomic tests and tests that have greater sensitivity than those applied in previous studies. If autonomic neuropathy is shown to contribute to the development of IAH, then measures to preserve autonomic function may help to prevent IAH.

A detailed evaluation of autonomic function produces many variables and increases the risk of chance findings. By combining normal deviates from several parameters into one average Z score, the number of tested variables is substantially reduced, random error is lessened, and test sensitivity may be increased. Previously, averaged Z scores have been used in nerve conduction studies (27) but not in autonomic function testing.

The aim of the current study was therefore to evaluate a putative association between IAH and the presence of autonomic neuropathy using composite Z (cZ) scores based on a battery of contemporary methods, including heart rate variability during paced breathing, the cardiovascular response to tilting and the Valsalva maneuver, and quantitative light reflex measurements by pupillometry. Validated methods to classify hypoglycemia awareness have been applied, with a matched design to control for potential confounding variables. In addition, somatic small- and large-fiber function was assessed using measures for thermal detection and nerve conduction. Three main variables that measure overall autonomic function, cardiovascular autonomic (CAN) function, and pupillary autonomic function were compared between participants with IAH and NAH. Three secondary composite variables that reflected thermal detection thresholds, pain thresholds, and nerve conduction measures were also analyzed.

RESEARCH DESIGN AND METHODS

Participants

Adults with type 1 diabetes and IAH (Gold score ≥4) (20) or NAH (Gold score 1–2) were identified in a cross-sectional survey of the outpatient adult population with type 1 diabetes at St. Olavs Hospital, Trondheim, Norway (9). Participants (aged 19–65 years) were recruited from this population. Exclusion criteria were pregnancy; breast-feeding; addiction to alcohol or other substances; mental, neurological, or systemic illness; reduced vision or hearing; or routine use of medication that could influence the test results (adrenoceptor β- and α-blockers, tricyclic antidepressants, anticonvulsants, antihistamines, and analgesics). Of the 56 people with IAH who met the inclusion criteria, 33 (59%) agreed to participate. For each person with IAH, one NAH participant was selected at random from eligible subjects of the same sex, similar age, and diabetes duration (±5 years) and requested to participate. To corroborate the IAH or NAH classification, participants completed the Gold score (20) again and the Clarke score (21) on the day of testing.

To supplement the reference ranges for the autonomic tests, 35 participants (21 female; mean [SD] age 46.4 ± 10.3 years) without diabetes were recruited by advertisement (via the intranet of our hospital and university). The study was approved by the regional medical ethics committee (2012/439). All participants gave informed consent.

Preparations and Precautions

Because antecedent hypoglycemia may attenuate cardiovascular reflexes (28), participants were recommended to set targets for blood glucose that were slightly higher than usual for 24 h before autonomic testing to avoid hypoglycemia, and tests were postponed if an episode of severe hypoglycemia (requiring external assistance) had occurred within the 24 h preceding the study. The participants were requested to avoid exercise for 24 h before testing; to avoid nicotine, caffeine, and analgesics from midnight; and not to eat or drink for 2 h before the CAN reflex tests, unless their blood glucose was low. Testing was not performed if blood glucose was <4.5 mmol/L (81 mg/dL). Room

temperature was maintained between 22° and 24°C, and participants were instructed to dress appropriately to stay comfortably warm with a stable body temperature during tests.

Clinical Assessment

To evaluate symptoms and signs of peripheral neuropathy and autonomic dysfunction, the neurological symptom score (NSS), the Survey of Autonomic Symptoms (SAS) (29), and the neuropathy impairment score (NIS) were used. Participants were also classified using the staged approach for estimating neuropathy severity as suggested by the Toronto Consensus Panel on Diabetic Neuropathy (30).

CAN Reflex Tests

Participants were supine on a tilt table during tests and underwent standard electrocardiography, respiratory monitoring, and continuous blood pressure monitoring using Finapres Pro (Finapres Medical System, Amsterdam, the Netherlands). Respiration was monitored with a thermistor attached under the nose (Embla S-AF-010; Flaga) and controlled with a metronome with visual feedback during paced breathing. A PowerLab data acquisition device with LabChart 8 software (both from ADInstruments, Dunedin, New Zealand) was used for data acquisition and analysis.

The inspiration-to-expiration heart rate difference was ascertained using the mean of the heart rate difference from five consecutive cycles during paced breathing (6 breaths/min).

The Valsalva maneuver was performed by blowing into a mouthpiece with a small air leak, holding a pressure of ~40 mmHg for 15 s. The maneuver was repeated three times or up to five times if maneuvers were suboptimal. Mean values from three maneuvers were used unless participants were unable to perform three successful maneuvers, or flat top responses (31) occurred. Because of the potential risk of intraocular hemorrhage (32), the Valsalva maneuver was not performed if untreated proliferative retinopathy was present or if an ophthalmological assessment had not been performed during the year preceding the study.

Brachial blood pressure was recorded with 1-min intervals for 5 min with the subject supine and for 10 min after the subject was tilted to 60°.

Pupillometry

The participants sat in a dark room for 15 min before pupillary light reflex tests were performed. A light-emitting diode was used with stimulus intensity of 50 lux to the right eye and two different stimulus durations (0.2 and 1.0 s) with a 5-min interval between tests. Direct and indirect response curves lasting 15 s were recorded by infrared cameras using a frame rate of 30 Hz. Sympathetic (basal diameter, late redilatation time) and parasympathetic parameters (latency to onset and peak, early redilatation, response amplitude) were calculated.

Thermal Quantitative Sensory Thresholds

Tests were performed using Somedic SENSELab MSA II equipment with a handheld rectangular 25- × 50-mm Peltier element thermode (Somedic Sales AB, Hörby, Sweden). Warmth detection threshold (WDT) and cold detection threshold (CDT) were established as a mean of five repetitions separated by 4–6 s on the left thenar and distal to the left and right medial malleolus. The baseline skin temperature was kept stable by the metallic thermode at 32°C, the change rate was 1°C/s, upper limit was 50°C, and the lower limit was 10°C. Participants reported a perceptible change of temperature by pressing a button.

Nerve Conduction Studies

Standard nerve conduction studies (NCS) were performed with Keypoint G4 EMG apparatus with Keypoint Classic 5.13 software (Medtronic, Copenhagen, Denmark) using pregelled adhesive surface electrodes with recording area of 9 mm × 6 mm (Alpine Biomed ApS, Skovlunde, Denmark) after all other investigations. Skin temperature was maintained ≥33°C. Motor amplitude, distal latency, conduction velocity, and F responses of the median, ulnar, peroneal, and posterior tibial nerves were measured, as well as sensory amplitude and conduction velocity of the median, ulnar, sural, superficial peroneal, and medial plantar nerves in the left arm and leg. Most participants also had recordings from the right leg, but the Z-score analysis was based solely on left-sided recordings. If a traumatic neuropathy was suspected, the contralateral extremity was analyzed. The NCS were performed by experienced technicians and later evaluated by a senior consultant neurophysiologist (T.S.). The individual clinical neurophysiological interpretation was based on all measured variables, and abnormality was defined as one or more abnormal Z scores (defined as $Z > 2.0$) in two or more nerves. In the composite scores used for group comparisons, a subset of eight of these variables was used: ulnar and tibial mean F-M wave latency, peroneal motor conduction velocity, tibial distal motor amplitude over abductor halluces brevis, peroneal and medial plantar conduction velocity, and ulnar and sural sensory amplitude (Supplementary Table 1). NCS variables generally unaffected by common entrapments and known to be sensitive markers for distal symmetric polyneuropathy were selected.

Blinding

During tests and analysis of test results, all investigators were blinded with respect to the hypoglycemia awareness status of the participants with diabetes. The investigators were also blinded during autonomic, clinical, and thermal tests with respect to diabetes status and hypoglycemia awareness status.

Statistical Analyses

The database of normal values from our laboratory, supplemented with current data from healthy participants without diabetes, was used to calculate age- and height-adjusted reference ranges. Available subjects in the control database varied between tests; 378 for large-fiber NCS assessment and 192 for small-fiber assessment ($n = 118$ for thermal thresholds, $n = 28$ for pupillometry, $n = 37$ for tilt and deep breathing, $n = 33$ for Valsalva).

These reference data were used to calculate Z scores for the isolated parameters from CAN tests, pupillometry, quantitative sensory thresholds, and for the NCS. Data were assessed for normality and transformed with power or logarithmic functions when necessary to fit a normal distribution before Z scores were calculated. If reference data correlated significantly with age and/or height, Z scores were adjusted accordingly by linear regression. The Z score sign was adjusted to ensure that abnormality (i.e., hypofunction in autonomic,

NCS, and thermal detection parameters, and hyperfunction in thermal pain thresholds) always produced high positive values.

Z scores from isolated parameters were combined to form cZ scores for overall autonomic function, CAN tests, pupillometry, and somatic small- and large-fiber functions, respectively (Table 1), as an average of Z scores of the included variables. If more than one variable could be given a similar physiological interpretation (i.e., blood pressure response to tilt after 1 and 3 min), the variable's standard weight = 1 was replaced with reduced weight = 0.5 before the cZ was calculated by weighted averaging. Weights were also adjusted to equalize the contributions from sympathetic and parasympathetic variables. Missing variables resulting from technical errors or unmeasurable nerve conduction velocities were imputed with Z = 0, and unrecordable sensory and motor amplitudes in the NCS were scored as 0 μV (giving a high positive Z score). The variables included in the different composite scores and their weighting are listed in Supplementary Table 1. For the composite scores, variables that best distinguished between control subjects without diabetes and participants with diabetes were selected (Table 1). Abnormality rates based on cZ >2 SD for control subjects without diabetes and *P* values for a two-group comparison between all participants with diabetes and all control subjects were also calculated as sensitivity indicators (Table 1).

Paired Student *t* tests were used to compare IAH and matched NAH participants for the different composite scores and also for post hoc analyses of parameters that constitute the Z scores (Supplementary Table 1). Paired comparisons ($n = 33$) have 80% power to detect a medium-sized group mean differences equal to 66% of the population SD. For comparison of categorical data, the Fisher exact test was used.

Nine participants in the current study (performed in 2012 and 2013) reported different awareness status than in the 2011 survey (9). In subgroup analyses, we repeated all analyses after excluding participants with altered awareness status and matched participants with diabetes. However, participants were not excluded if the Clarke score indicated the same awareness status as in 2011.

RESULTS

The participants with IAH and NAH were of similar age, had similar diabetes duration and mean HbA_{1c}, and had similar insulin regimens and frequency of self-monitoring of blood glucose (Table 2).

During the year preceding the study, 13 IAH participants (39.4%) and 6 NAH participants (18.2%) had experienced one or more episodes of severe hypoglycemia. During the preceding month, 17 IAH participants (51.5%) and only 1 NAH participant (3.0%) had experienced more than one episode of asymptomatic hypoglycemia (blood glucose <3.9 mmol/L [54 mg/dL]) per week, and 9 IAH (27.3%) vs. 24 NAH participants (72.7%) had experienced no such episodes (Table 2). On the test day, 33.3% of IAH participants and 21.2% of NAH participants were taking an ACE inhibitor or an angiotensin II receptor antagonist. Ophthalmological assessment, within 12 months before to 11 months after participation in the study, was performed in 32 IAH and 33 NAH subjects. No diabetic retinopathy was present in 55% and 49% of the IAH and NAH participants, respectively, mild nonproliferative retinopathy was observed in 30% vs. 31%, and previously treated but quiescent proliferative retinopathy was present in 15% vs.20%.

Clinical Assessment

Eight participants with IAH and four with NAH reported neuropathic pain ($P = 0.20$). The NSS, the NIS, total score of the SAS, and clinical grading of neuropathy were similar between IAH and NAH participants (Table 3).

Autonomic Neuropathy

No differences were observed between the participants with IAH and NAH in the autonomic composite score or in the composite scores for the CAN and pupillometric tests (Table 4). The post hoc analyses of the isolated parameters that constitute these composite scores did not reveal any differences between IAH and NAH participants for CAN tests. However, small but significant differences for latency until maximal pupillary contraction for the pupillary light reflex emerged when performing separate *t* tests for each of the 32 pupillometric subparameters, although these differences were not in the hypothesized direction (Supplementary Table 1).

Large- and Small-Fiber Neuropathy

No differences were observed between IAH and NAH participants with respect to the nerve conduction composite score or thermal threshold tests (Table 4). The post hoc analyses of isolated parameters that constitute the composite score showed no significant differences (Supplementary Table 1).

Subgroup Analyses

No differences were demonstrated in neurophysiological test results between the IAH and NAH participants when matched IAH and NAH pairs were excluded in which one subject had an

Table 1—Discriminating ability (all subjects with diabetes vs. control subjects) of cZ scores

	Number of variables in cZ score*	Subjects with diabetes ($n = 66$) Mean ± SD	Control subjects ($n = 35$) Mean ± SD	*P* value	Abnormal† number of subjects with diabetes, *n (%)*
Autonomic score‡	40	0.49 ± 0.56	0.05 ± 0.37	0.000005	16 (24)
CAN score	12	0.46 ± 0.71	0.06 ± 0.40	0.0005	20 (30)
Pupillometry score	28	0.50 ± 0.64	0.04 ± 0.43	0.00004	11 (17)
Thermal detection score	6	0.76 ± 0.96	0.13 ± 0.84	0.001	15 (23)
NCS	8	1.70 ± 1.14	0.44 ± 0.86	3.7E-07	48 (73)

P values from two-group Student *t* test. †Based on normal cZ mean + 2 SD. ‡Combining CAN and pupillometry scores.

Table 2—Clinical and biochemical characteristics of participants with type 1 diabetes

	All with diabetes	Impaired awareness	Normal awareness
Sex, *n*			
Men	28	14	14
Women	38	19	19
Age, median (IQR), years	47 (15.0)	48 (14.5)	47 (14.5)
Diabetes duration, median (IQR), years	31 (13.3)	30 (13.5)	31 (13.5)
Current HbA_{1c}, median (IQR), %	8.0 (1.8)	7.8 (2.2)	8.1 (1.9)
Current HbA_{1c}, median (IQR), mmol/mol	64.0 (19.7)	62.0 (24.0)	65.0 (20.8)
Insulin regimen, *n* (%)			
Long + rapid-acting analogs	34 (51.5)	18 (54.5)	16 (48.5)
NPH insulin + rapid-acting analog	12 (18.2)	7 (21.2)	5 (15.2)
Insulin pump with rapid-acting analog	19 (28.8)	8 (24.2)	11 (33.3)
Other	1 (1.5)	0 (0)	1 (3)
ACE inhibitor use, *n* (%)			
Yes	7 (10.6)	4 (12.1)	3 (9.1)
No	59 (89.4)	29 (87.9)	30 (90.9)
Angiotensin II receptor antagonist use, *n* (%)			
Yes	11 (16.7)	7 (21.2)	4 (12.1)
No	55 (83.3)	26 (78.8)	29 (87.9)
Frequency of blood glucose measurement, *n* (%)			
>4 times/day	34 (51.5)	17 (51.5)	17 (51.5)
1–4 times/day	20 (30.3)	10 (30.3)	10 (30.3)
1–6 times/week	12 (18.2)	6 (18.2)	6 (18.2)
<1 time/week	—	—	—
Number of severe hypoglycemia episodes in the preceding year, *n* (%)			
None	47 (71.2)	20 (60.6)	27 (81.8)
1–2	15 (22.7)	9 (27.3)	6 (18.2)
≥3	4 (6.1)	4 (12.1)	0 (0)
During last month, number of blood glucose measurements <3.9 mmol/L (<54 mg/dL) without symptoms, *n* (%)			
Never	33 (50.0)	9 (27.3)	24 (72.7)
1–3 times/month	12 (18.2)	5 (15.2)	7 (21.2)
1 time/week	3 (4.5)	2 (6.1)	1 (3.0)
>1 time/week	18 (27.2)	17 (51.5)	1 (3.0)
Laboratory values			
Plasma thyroid-stimulating hormone, median (IQR), mU/L	1.4 (0.8)	1.4 (1.0)	1.4 (0.8)
Plasma creatinine, median (IQR), μmol/L	61.0 (22.0)	60.5 (19.8)	63.0 (25.5)
Plasma cholesterol, median (IQR), mmol/L	4.9 (0.6)	4.9 (1.2)	4.8 (0.6)
Plasma HDL cholesterol, median (IQR), mmol/L	1.6 (0.8)	1.6 (0.7)	1.6 (0.9)
Plasma LDL cholesterol, median (IQR), mmol/L	2.5 (1.0)	2.5 (1.0)	2.5 (1.0)
Plasma triglycerides, median (IQR), mmol/L	0.9 (0.6)	0.9 (0.6)	0.9 (0.7)
Urinary albumin-to-creatinine ratio, median (IQR), mg/mmol	0.8 (1.5)	0.9 (1.5)	0.8 (1.4)
<3 mg/mmol, *n* (%)	51 (77.3)	25 (75.8)	26 (78.8)
≥3 mg/mmol, *n* (%)	15 (22.8)	8 (24.2)	7 (21.2)
Myocardial infarction/angina, *n* (%)			
Yes	1 (1.5)	0	1 (3.0)
No	66 (98.5)	33 (100)	32 (97.0)

IQR, interquartile range.

altered awareness status from 2011 (9) to the current study, as explained above (Supplementary Table 1).

CONCLUSIONS

The current study has shown no difference in measures of autonomic function between adults with long-standing type 1 diabetes who had IAH, and carefully matched adults with type 1 diabetes with NAH. In addition, no differences between IAH and NAH participants were found with respect to the NCS, thermal thresholds, and clinical pain or neuropathy scores. Neither autonomic dysfunction nor somatic neuropathy was associated with IAH. We consider that this study provides considerable value and novelty in view of the rigorous methodology that has been used. Potential confounding variables have been controlled for by the use of well-matched groups of participants, validated methods for classification of awareness, a large battery of neurophysiological tests, and a novel statistical approach to provide very high sensitivity for the detection of between-group differences.

Studies of hypoglycemia awareness have been hampered by a lack of

Table 3—Clinical data

	All with diabetes	Impaired awareness	Normal awareness
Total NSS score, median (IQR)	1.0 (3.0)	1 (3.0)	1 (3.0)
Neuropathic pain, *n* (%)			
No	51 (77.3)	24 (72.2)	27 (81.8)
Possible	3 (4.5)	1 (3)	2 (6.1)
Yes	12 (18.2)	8 (24.2)	4 (12.1)
Total NIS sum, median (IQR)	10 (12.0)	10 (12.5)	10 (11.0)
Staged evaluation of DSPN,* *n* (%)			
Stage 0	14 (21.1)	6 (18.2)	8 (24.2)
Stage 1a	16 (24.2)	8 (24.2)	8 (24.2)
Stage 1b	17 (25.8)	9 (27.3)	8 (24.2)
Stage 2a	19 (28.8)	10 (30.3)	9 (27.3)
SAS			
Symptoms reported, *n*	1.0 (2.0)	2.0 (2.0)	1.0 (1.5)
Total impact score, median (IQR)	3.0 (5.0)	3.5 (4.0)	2.5 (5.0)

*Diabetic sensorimotor neuropathy graded according to the Toronto Consensus Panel on Diabetic Neuropathy. IQR, interquartile range.

consensus of how IAH should be defined. The Gold questionnaire (20) is based on having a diminished ability to perceive the onset of hypoglycemia, allowing for differing interpretations of what constitutes impaired awareness. In addition to the Gold questionnaire (20), other methods may be used to assess hypoglycemia awareness (21,33,34). The Gold and Clarke questionnaires have been validated and show good concordance in people with type 1 diabetes, and their use, separately or together, has been advocated for clinical and research application (24). To maximize detection of potential differences between the IAH and NAH groups, the current study did not include participants with a Gold score of 3 because their awareness status is uncertain (9).

A few studies have investigated the association between hypoglycemia awareness and autonomic neuropathy using experimentally induced hypoglycemia and have defined impaired awareness based on higher glycemic thresholds, defined as blood glucose at a lower level, before autonomic symptoms appear (14,16,17). Reasonable agreement has been shown between this definition and self-reported state of awareness (35). These studies did not demonstrate an association between autonomic neuropathy and an altered glycemic threshold for generation of autonomic symptoms (14,16,17), which mainly concurs with the conclusions of the current study. Although the magnitude of symptomatic responses may be lower in people with autonomic neuropathy (14–16), it is the initial symptoms that are important for hypoglycemia awareness (10), and we have demonstrated previously that impaired awareness is not associated with reduced intensity of autonomic symptoms (9).

Previous studies that have explored a possible association between self-reported reduced awareness to hypoglycemia and autonomic dysfunction also failed to support such an association (13,22,36). Furthermore, a recent study of patients with type 1 diabetes who received islet cell or whole-pancreas transplantation found that restoration of hypoglycemia awareness was not affected by the presence of autonomic neuropathy (37).

Strengths of the current study include the well-matched IAH and NAH groups, the use of validated methods to assess hypoglycemia awareness, blinding of the investigators, and the application of sensitive methodology to investigate autonomic function.

The definition of diabetic neuropathy for research purposes has been revised in recent years, and the use of a cZ score of normal deviates from several variables is strongly recommended (30). The traditional approach to test the autonomic nervous system function is to apply tests of cardiovascular reflexes as described by Ewing et al. (38). The sensitivity of the tests can be enhanced by adding quantitative assessment of the Valsalva maneuver and by the construction of age-adjusted reference values, as in the current study. Furthermore, use of cZ scores, based on a selection of variables that are prone to be affected by diabetes, will increase effect sizes, precision, and sensitivity of the tests. This also reduces the risk of a type I statistical error by reducing the total number of statistical tests. The post hoc finding of a small increase in latency of the pupillary light reflex was in the opposite direction of the hypothesis, was not supported by the results of the other autonomic or pupillary function tests, and is considered to be a chance finding.

Techniques to evaluate autonomic function are numerous, but CAN tests and sudomotor tests are most commonly used. A limitation of the current study is that quantitative sudomotor function testing was not performed. However, we included pupillary response tests,

Table 4—cZ scores for autonomic small-fiber function, thermal somatic small- and large-fiber function

	Impaired awareness, mean ± SD	Normal awareness, mean ± SD	Mean difference (95% CI)	*P* value
Autonomic score*	0.42 ± 0.52	0.58 ± 0.61	−0.15 (−0.46, 0.16)	0.33
CAN† score	0.40 ± 0.73	0.53 ± 0.69	−0.13 (−0.45, 0.19)	0.42
Pupillometry score	0.43 ± 0.53	0.59 ± 0.73	−0.16 (−0.51, 0.20)	0.39
Thermal detection score	0.91 ± 1.04	0.75 ± 0.94	0.15 (−0.31, 0.61)	0.51
Nerve conduction score	1.71 ± 1.19	1.68 ± 1.10	0.03 (−0.43, 0.49)	0.89

P values from paired Student *t* test. *Combining CAN and pupillometry scores. †Cardiovascular autonomic tests.

which traditionally have been incorporated into the evaluation of autonomic function in diabetes (39). Another limitation is that only 59% of the identified persons with IAH accepted the invitation to participate in the current study. However, it is unlikely that the hypothesized association between autonomic function and IAH would differ in those who participated in the study compared with those who declined to participate.

When hypoglycemia awareness status was assessed using the Gold method (20), a few of the participants reported a change in awareness status between 2011 and the present investigations, which were conducted in 2012 and 2013. However, we corroborated our classification with results from the Clarke questionnaire and could demonstrate that IAH participants had experienced more asymptomatic hypoglycemia episodes than NAH participants during the month preceding the study. In addition, more than twice as many IAH participants than NAH participants had experienced severe hypoglycemia during the preceding year, and IAH participants had more numerous episodes. Although these data support the original classification of awareness status, we acknowledge the possibility for misclassification in some participants. The results were therefore reanalyzed after exclusion of participants with an apparent change in awareness status, and the results were unchanged.

Another limitation of the current study is that the frequency of nonsevere hypoglycemia was not assessed on the day before testing. A decreased ability to report nonsevere hypoglycemic episodes is, however, a fundamental problem associated with the IAH syndrome, and postponing tests based on self-report of such episodes could have introduced a bias. Providing each of the participants with a continuous glucose monitoring device and the necessary training to use this effectively was not feasible. However, participants were asked to set glycemic targets slightly higher than usual for 24 h before the studies to limit the risk of hypoglycemia, and tests were postponed if severe hypoglycemia had occurred the preceding day.

In conclusion, by using detailed and sensitive measures of autonomic function and peripheral neuropathy, no differences were found between adults with type 1 diabetes who had IAH and matched individuals with type 1 diabetes and normal hypoglycemia awareness.

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