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Consistent Effects of Hypoglycemia on Cognitive Function in People With or Without Diabetes

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PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

Hypoglycemia poses an immediate threat for cognitive function. Due to its association with acute cognitive impairment, the International Hypoglycemia Study Group (IHSG) defines a blood glucose level <3.0 mmol/L as "level 2 hypoglycemia." In the current study we investigated whether having diabetes, type of diabetes, or hypoglycemia awareness moderates this association.

RESEARCH DESIGN AND METHODS

Adults with type 1 diabetes with normal ($n = 26$) or impaired ($n = 21$) hypoglycemic awareness or with insulin-treated type 2 diabetes ($n = 15$) and age-matched control subjects without diabetes ($n = 32$) underwent a hyperinsulinemic-euglycemic-hypoglycemic glucose clamp (2.80 ± 0.13 mmol/L [50.2 ± 2.3 mg/dL]). At baseline and during hypoglycemia, calculation ability, attention, working memory and cognitive flexibility were measured with the Paced Auditory Serial Addition Test (PASAT) and the Test of Attentional Performance (TAP).

RESULTS

For the whole group, hypoglycemia decreased the mean ± SD proportion of correct answers on the PASAT by 8.4 ± 12.8%, increased reaction time on the TAP Alertness task by 32.1 ± 66.6 ms, and increased the sum of errors and omissions on the TAP Working Memory task by 2.0 ± 5.5 (all $P < 0.001$). Hypoglycemia-induced cognitive declines were largely irrespective of the presence or type of diabetes, level of symptomatic awareness, diabetes duration, or $HbA_{1c.}$

CONCLUSIONS

IHSG level 2 hypoglycemia impairs cognitive function in people with and without diabetes, irrespective of type of diabetes or hypoglycemia awareness status. These findings support the cutoff value of hypoglycemia <3.0 mmol/L (<54 mg/dL) as being clinically relevant for most people with diabetes.

People with type 1 diabetes or with type 2 diabetes treated with insulin are at risk for hypoglycemia, with a reported average of two to three episodes per week and two events per month, respectively (1–3). Glucose is the principal fuel for the brain, and since the brain is capable of neither producing nor storing glucose in sufficient amounts, a constant supply of glucose is needed to maintain its function. Hypoglycemia is an immediate threat for brain function, with symptomatology ranging from mild cognitive manifestations sufficient to affect daily activities (e.g.,

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driving) to seizures, coma, or even (brain) death depending on the duration and depth of the event (4).

What defines a glucose level sufficiently low to cause cognitive decline and whether this applies across clinical forms of diabetes are matters of debate. With use of the hyperinsulinemic glucose clamp technique, several, but not all (5,6), studies have shown deterioration of cognitive function in response to glucose levels between 3.0 and 2.0 mmol/L, with complex higher-order cognitive processes affected at higher glucose and to a greater extent than lower-level cognitive functions (7,8). The International Hypoglycemia Study Group (IHSG) reviewed the literature in 2017 and defined "level 2 hypoglycemia" at <3.0 mmol/L (<54 mg/dL) as clinically important, based in part on the evidence that glucose below this level impairs cognitive function (9). Whether vulnerability to the effects of hypoglycemia on cognitive function differs according to diabetes presence, diabetes type, diabetes duration, baseline glucose levels, hypoglycemia awareness status, and HbA_{1c} level remains unknown.

This leaves the universality of the 3.0 mmol/L glucose cutoff inconclusive. Therefore, we investigated the impact of level 2 hypoglycemia on cognitive function in individuals with type 1 diabetes with normal and impaired awareness of hypoglycemia, in individuals with type 2 diabetes treated with insulin, and in age-matched individuals without diabetes.

RESEARCH DESIGN AND METHODS

Study Design

This was a multicenter intervention study performed at the Internal Medicine outpatient clinics of Radboud University Medical Center in Nijmegen, the Netherlands, and Nordsjællands Hospital. The study was approved by both local institutional review boards and performed according to the principles of the Declaration of Helsinki. All participants gave written informed consent before participation.

Study Population

We recruited the following groups of participants: *1*) individuals with type 1 diabetes and normal awareness of hypoglycemia (NAH); *2*) individuals with type 1 diabetes and impaired awareness of hypoglycemia (IAH); and *3*) people with type 2 diabetes treated with insulin for at least 1 year. Using advertisements in local newspapers and social media, we also recruited two control groups without diabetes, who were age, sex, and BMI matched to either the participants with type 1 diabetes (type 1 control subjects) or to those with type 2 diabetes (type 2 control subjects). Key exclusion criteria were age >80 years, use of antidepressive drugs, pregnancy, breastfeeding, and taking no birth control measures for women of child-bearing age. Individuals with diabetes with HbA_{1c} >11.3% (100 mmol/mol) were also excluded, as were individuals with any medical condition considerably interfering with perception of hypoglycemia, defined from medical record review and/or as judged by the treating physician. A complete overview of inclusion and exclusion criteria can be found in Supplementary Material.

Study Procedure

A total of 471 individuals were approached: 130 were invited for screening (Supplementary Fig. 1), of whom 94 participants were eligible and agreed to participate. Participants with diabetes completed Clarke, Gold and Pedersen-Bjergaard questionnaires for the assessment of awareness of hypoglycemia (10–12). With use of published cutoffs (Supplementary Material), a participant was classified as having IAH when results of at least two of these questionnaires fit that classification. Participants were asked about highest completed educational level and current job (if applicable). Answers were transformed to the European Qualifications Framework for Lifelong Learning (EQF) number from low (level 1) to high (level 8) (13). Blood was sampled for HbA_{1c} and kidney function if these data were not available in clinical records over the previous 3 months.

Hyperinsulinemic Glucose Clamp

On the experimental day, all participants underwent a hyperinsulinemic-euglycemic-hypoglycemic glucose clamp. Participants attended the research facility in fasting condition at 0700–0800 h, having abstained from alcohol and caffeine for at least 24 h and from strenuous exercise for 48 h. In addition, the six participants who were smokers were asked to abstain from smoking for at least 24 h. Participants with diabetes received an intermittently scanned continuous glucose monitoring device (FreeStyle Libre 1) for 2 weeks starting 7 days prior to the experimental day. Participants with diabetes were instructed to reduce their basal insulin replacement to avoid nocturnal hypoglycemia the night before the clamp and to omit their usual morning insulin dose. Experiments were rescheduled in case of glucose <3.0 mmol/L in the 24 h before the clamp, measured with CGM. Participants were asked about their sleep quality the night before with the following question of the Pittsburgh Sleep Quality Index (PSQI): "During the past month, how would you rate your sleep quality?" (14). The result was then categorized as "good," "fairly good," "fairly bad," or "bad," in line with the user instructions for the PSQI. Subsequently, an intravenous catheter was inserted into an antecubital vein of the dominant arm for continuous administration of insulin (Novo Nordisk, Bagsværd, Denmark) and variable infusion of glucose 20% (Baxter B.V., Deerfield, IL). The insulin infusion was set at a rate of 1.5 $mU \cdot kg^{-1} \cdot min^{-1}$

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for participants with type 1 diabetes and type 1 control subjects. For participants with type 2 diabetes and the type 2 control subjects, an infusion rate of 3.0 $mU \cdot kg^{-1} \cdot min^{-1}$ was used to overcome potential insulin resistance in these individuals (15). In six of the type 2 control subjects, the study was repeated with the lower insulin infusion rate to exclude an effect of insulin per se (data not shown). In the dorsal vein of the nondominant hand, a second catheter was inserted in retrograde fashion for blood sampling, with the hand placed in a heated box (temperature $\sim 55^{\circ}C$) to arterialize venous blood. Baseline plasma glucose level was determined (Biosen C-Line; EKF Diagnostics, Cardiff, U.K.). In case of hyperglycemia (glucose $\geq$10 mmol/L, 180 mg/dL) on arrival, an optional small bolus of insulin of maximal 2 units was administered before the continuous infusion was started. This had no effect on the time between baseline and start of euglycemic phase or on the achieved insulin levels during the clamp. Plasma glucose levels were subsequently determined at 5- to 10-min intervals and allowed to fall to 5.0 mmol/L (90 mg/dL), with glucose 20% infused to maintain plasma glucose at this level for 30 min. Thereafter, plasma glucose levels were allowed to drop gradually to 2.8 mmol/L (50 mg/dL) and maintained at this level for another 60 min. Then, the insulin infusion was stopped, participants received a meal, and glucose infusion was increased and then tapered until stable euglycemic levels were reached. Participants were allowed to leave the facility when they were judged fit enough to do so.

Hypoglycemia Symptom Score

The validated Edinburgh Hypoglycemia Score (16) was modified and administered at baseline (i.e., before the onset of insulin infusion), during euglycemia, and twice during hypoglycemia to assess the nature and intensity of hypoglycemic symptoms. Symptoms included autonomic symptoms (sweating, anxious, tingling of hands and feet, palpitations, hunger, trembling and shivers), neuroglycopenic symptoms (feeling warm, confused, inability to concentrate, blurry vision, tiredness, difficulty speaking, weakness, double vision, dizziness, drowsiness) and general symptoms (headache and nausea). Symptoms were ranked from 1 (none) to 7 (severe).

Cognitive Function Tests

Four widely used validated cognitive function tests, selected because they are well validated, contain sufficient complexity to detect the effect of hypoglycemia, and have minimal learning effects, were applied at baseline (started before the onset of insulin infusion) and during hypoglycemia. We thus administered the Paced Auditory Serial Addition Test (PASAT) (17), which measures auditory information processing speed and working memory, as well as calculation ability. A series of 60 single digits was presented via an audio clip on a laptop with interstimulus intervals of either 2.8 or 2.0 s. Participants were requested to continuously add each new digit to the prior one and provide the answer verbally, with the outcome parameter being the percentage of correct answers. We also administered three subtasks of the Test of Attentional Performance (TAP) (version 2.3.1), i.e., Alertness, Working Memory, and Verbal Flexibility, to measure aspects of attention and executive function (18). In the Alertness task, processing speed is examined with or without an auditory warning signal. Participants were asked to press a button as quickly as possible when an "X" was presented on the screen of a laptop. In total, the X was presented on the screen 80 times and the test duration was 4.5 min; the outcome was the mean reaction time. During the Working Memory 2-Back task, a total of 100 single digits were presented on a screen with an interval of 3 s during a period of 5 min. When a digit was identical to the one before the previous digit (two digits back), participants needed to press a button, the outcome parameter being the sum of omissions and errors. In the Flexibility task, a letter and a number were presented to the right and left of the center of the screen, respectively. Participants needed to press the left or the right button according to whether the number or letter was presented on the screen. For the simple task, participants pressed the button only on the side of the number (first block) or the letter (second block). In the last block, the complex task, participants switched between letter and number and pressed the button alternatively corresponding to the position of either the number or the letter. The outcome parameter was the ratio of the mean reaction time of the two simple tasks and the reaction time of the complex task.

All cognitive tests were performed with participants in sitting position, the order of which was randomized at baseline and during hypoglycemia. The cognitive function tests were explained to the participants, and all were asked to perform a short pretest at baseline to ensure they understood the tests correctly and to minimize nonspecific practice effects. The total duration of the test battery was on average 20 min. Due to a logistic error, the TAP Flexibility task was not performed by 18 participants.

Laboratory Measurements

Serum creatinine was determined with an enzymatic assay on a cobas 8000 c 702 (Roche Diagnostics) or a Vista 1500 (Siemens Healthineers). HbA_{1c} was assessed with the TOSOH G8 and G11 HPLC Analyzer (Sysmex). Plasma adrenaline and noradrenaline were measured with high-performance liquid chromatography in combination with fluorometric detection. Plasma glucagon was measured with radioimmunoassay analysis (Euro Diagnostica). Plasma insulin was analyzed with an in-house radioimmunoassay. Plasma cortisol and growth hormone were measured via a routine analysis method with an electrochemiluminescent immunoassay on a MODULAR ANALYTICS E170 (Roche Diagnostics, GmbH, Mannheim, Germany).

Statistical Analysis

All normally distributed data are shown as mean ± SD. Nonnormally distributed data are shown as median (interquartile range) and log transformed for analyses. One-way ANOVA with Bonferroni post hoc test was used to compare continuous data and the χ^2 test to compare dichotomous baseline characteristics. Symptom scores at baseline and during euglycemia and hypoglycemia were analyzed with paired *t* test, and the difference among subgroups between euglycemia and hypoglycemia was analyzed with one-way ANOVA. Scores on the four cognitive function tests at baseline and during hypoglycemia were compared with paired samples *t* tests and Cohen d_z (19), to determine the size of the

effect (small 0.2–0.4, medium 0.5–0.7, large ≥0.8). We used univariate and multivariate linear regression analyses to assess the associations between clinical characteristics and the effect of hypoglycemia on cognitive function. This analysis was performed for the whole group and separately for participants with type 1 or 2 diabetes. In this linear regression model, the dependent variable was the difference in score between baseline and end of hypoglycemia for each cognitive function task separately. The independent factors for the whole group were age, sex, EQF, sleep, adrenaline response, and baseline glucose levels, and for the participants with diabetes these were age, sex, diabetes duration, HbA_{1c} level, and increase of total symptomatic response by hypoglycemia. Independent *t* tests were used in the sensitivity analyses to test the impact of hypoglycemia awareness status and symptom responses during the clamp on the outcome of the cognitive function tests. Symptom response was present during the clamp when the hypoglycemic level exceeded the 95% CI from the mean of baseline and euglycemia values. IBM SPSS Statistics, version 25.0 (IBM, Armonk, NY), was used for analysis. α was set at 0.05 throughout.

RESULTS

A total of 94 participants were included in this study (Supplementary Fig. 1). Except for somewhat lower BMI of control subjects, participants with type 1 diabetes and the type 1 diabetes control subjects were well matched for age and sex (Table 1). Participants with type 2 diabetes were older compared with participants with type 1 diabetes, but the type 2 diabetes control subjects were well matched to the type 2 subgroup.

Hypoglycemic Glucose Clamp

The mean glucose levels during the clamps are shown in Fig. 1. Mean ± SD baseline glucose levels were higher in the participants with either type 1 diabetes, 11.7 ± 3.6 mmol/L (211.5 ± 65.3 mg/dL), or type 2 diabetes, 9.6 ± 4.7 mmol/L (173.4 ± 84.8 mg/dL), in comparison with those without diabetes, 5.7 ± 0.6 mmol/L (102.5 ± 10.2 mg/dL) (both $P < 0.001$), with no significant differences between participants with type 1 or type 2 diabetes ($P = 0.083$). During the clamp, glucose level in the euglycemic phase was 5.20 ± 0.40 mmol/L (93.7 ± 7.3 mg/dL) with mean coefficients of variation ranging from 4.7 to 6.5% and no significant between-group differences (all $P > 0.90$). Mean glucose level in the hypoglycemic phase was 2.79 mmol/L (50.2 mg/dL) with mean coefficients of variation ranging from 6.2 to 6.8% and no significant differences between groups (all $P > 0.70$).

All subgroups had significant symptomatic responses to hypoglycemia (Table 2). Participants with type 1 diabetes and IAH had a lower symptomatic response to hypoglycemia compared with participants with type 2 diabetes ($P < 0.05$). There were no other differences between the subgroups, although symptom responses were numerically, but not significantly, lower in type 1 control subjects than in participants with type 1 diabetes and NAH.

Cognitive Function

At baseline, no differences were present between the subgroups with respect to performance on the three TAP subtasks. On the PASAT, the type 1 control subjects performed significantly better at baseline than participants with type 2 diabetes ($P = 0.042$) and participants with

Table 1—Participant characteristics

	Type 1 diabetes + NAH	Type 1 diabetes + IAH	Type 2 diabetes	Type 2 control subjects	Type 1 control subjects
Participants, *n*	26	21	15	16	16
Male	13 (50.0)	10 (47.6)	9 (60.0)	9 (56.3)	7 (43.8)
Age, years	35.0 [22.3–63.3]	59.0 [48.5–63.0]*	62.0 [55.0–68.0]*	57.0 [52.3–61.8]*	47.5 [24.5–64.5]**
EQF	4.6 ± 1.6	4.6 ± 1.7	4.6 ± 1.4	4.6 ± 1.4	5.7 ± 1.2
Diabetes duration, years	19.8 ± 15.2	25.4 ± 11.3	15 ± 7.7‡	—	—
HbA_{1c}, mmol/mol	60.6 ± 9.9†	62.8 ± 10.1†	63.5 ± 11.2†	35.6 ± 2.2	33.6 ± 3.5
HbA_{1c}, %	7.7 ± 0.9†	7.9 ± 0.9†	8.0 ± 1.0†	5.4 ± 0.2	5.2 ± 0.3
BMI, kg/m^2	26.7 ± 3.6¶	26.2 ± 3.8	29.0 ± 4.3¶	28.0 ± 4.4¶	22.6 ± 2.8
Diabetes complications	5 (19.2)	9 (42.9)	3 (20.0)		
Retinopathy, *n*	5	7	2		
Neuropathy, *n*	3	6	2		
Nephropathy, *n*	0	1	1		
Glucose-lowering medication					
Oral	0 (0.0)	0 (0.0)	11 (73.3)		
CSII	11 (42.3)	10 (47.6)	1 (6.7)		
MDI	15 (57.7)	11 (52.4)	14 (93.3)		
Insulin dose, IU/day	53.6 ± 23.0	45.7 ± 23.8	71.3 ± 54.6		

Data are presented as *n* (%), mean ± SD, or median [interquartile range] unless otherwise indicated. CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections. *$P < 0.05$ vs. type 1 diabetes + NAH. **$P < 0.05$ vs. type 2 diabetes. †$P < 0.05$ vs. both control groups. §$P < 0.05$ vs. type 1 diabetes + NAH. ‡$P < 0.05$ vs. type 1 diabetes + IAH. ¶$P < 0.05$ vs. type 1 control subjects.

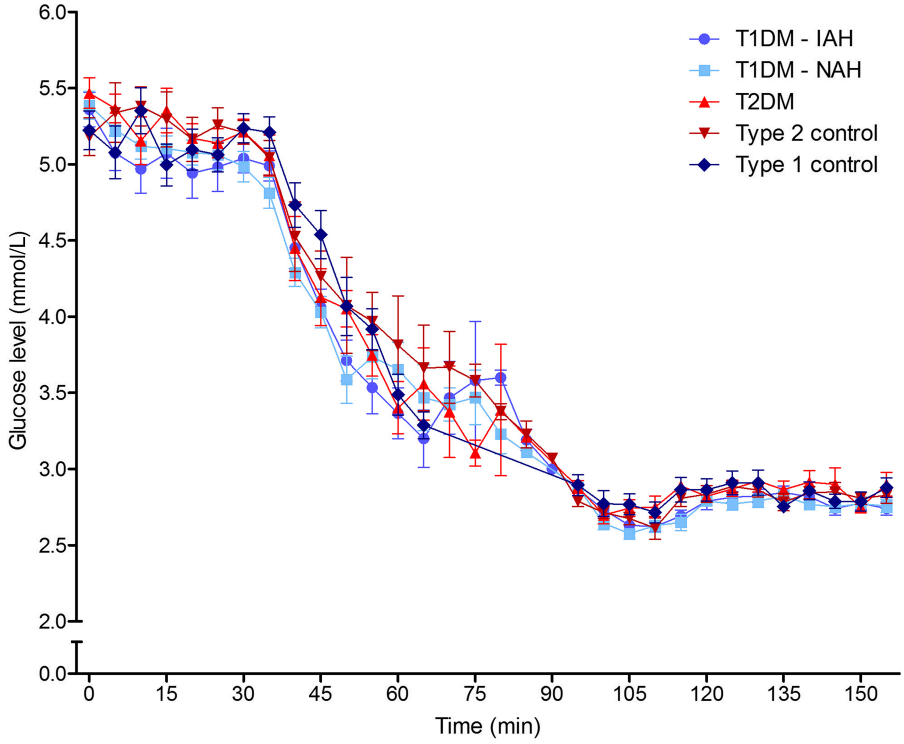

Figure 1—Glucose levels during the glucose clamp in the five subgroups. T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

type 1 diabetes and NAH ($P = 0.007$) (Supplementary Fig. 2).

Mean ± SD percentage of correct answers on the PASAT for all participants declined from 67.1 ± 17.9% at baseline to 58.7 ± 19.6% during hypoglycemia ($P < 0.001$, Cohen $d_z = 0.66$). The size of this decline was consistent across the different subgroups, ranging from −6.7 to −10.6% with no significant differences in performance between the subgroups (Fig. 2*A*).

In the TAP Alertness task, mean ± SD reaction time for the entire group increased from 285.4 ± 69.7 ms at baseline to 317.5 ± 85.0 ms during hypoglycemia ($P < 0.001$, Cohen $d_z = 0.48$). The increase in reaction times during hypoglycemia was consistent across subgroups with no significant differences in performance between the subgroups (Fig. 2*B*).

For the whole group, mean ± SD sum of errors and omissions increased from 5.4 ± 6.2 at baseline to 7.5 ± 7.6 during hypoglycemia ($P < 0.001$, Cohen $d_z = 0.38$). This effect was also consistent across subgroups with no significant differences in performance between the subgroups (Fig. 2*C*).

On the TAP Flexibility task, mean ± SD ratio between reaction times on the simple and the complex tasks for the entire group was 0.68 ± 0.17 at baseline and 0.71 ± 0.22 during hypoglycemia ($P = 0.053$, Cohen $d_z = 0.20$), with consistent effects across subgroups (Fig. 2*D*).

Table 2—Symptom responses

	Baseline	Euglycemia	Hypoglycemia
Type 1 diabetes + NAH	26.7 ± 11.2	28.3 ± 10.8	50.6 ± 19.0†#
Type 1 diabetes + IAH	24.8 ± 7.1	26.9 ± 7.4	38.8 ± 15.6†#*
Type 2 diabetes	23.3 ± 4.8	28.3 ± 10.4†	58.7 ± 24.8†#
Type 1 control subjects	20.4 ± 2.1	21.9 ± 3.7	38.6 ± 7.7†#
Type 2 control subjects	23.1 ± 3.3	24.6 ± 5.4	50.2 ± 21.5†#

Data are presented as mean ± SD. Symptoms responses were measured during baseline, euglycemia, and hypoglycemia. †$P < 0.001$ for difference vs. baseline; #$P < 0.001$ for difference vs. euglycemia; *$P < 0.05$ for difference vs. type 2 diabetes.

Univariate and Multivariate Analysis

In univariate linear regression analysis, the effect of hypoglycemia on the performance of the PASAT for the whole group was greater in men than in women (mean ± SD score −11.1 ± 14.4 vs. −5.6 ± 10.2, $P = 0.035$) (Supplementary Table 1), whereas older age and higher adrenaline response were associated with longer reaction times during hypoglycemia on the TAP Alertness task ($P = 0.020$ and $P = 0.002$). Age was no longer statistically significant in the multivariate analyses and did not influence the effect of hypoglycemia on any of the other cognitive function tests. None of the tests showed an interaction between sleep quality, baseline glucose levels, or EQF and the effect of hypoglycemia in linear regression models.

In univariate and multivariate linear regression analyses restricted to people with diabetes, sex, age, duration of diabetes, and HbA_{1c} were unrelated to effect of hypoglycemia on cognitive function (Supplementary Table 1). In both analyses, the hypoglycemia-induced increase in reaction time on the TAP Alertness task was positively associated with hypoglycemic symptom scores during the clamp ($P = 0.001$).

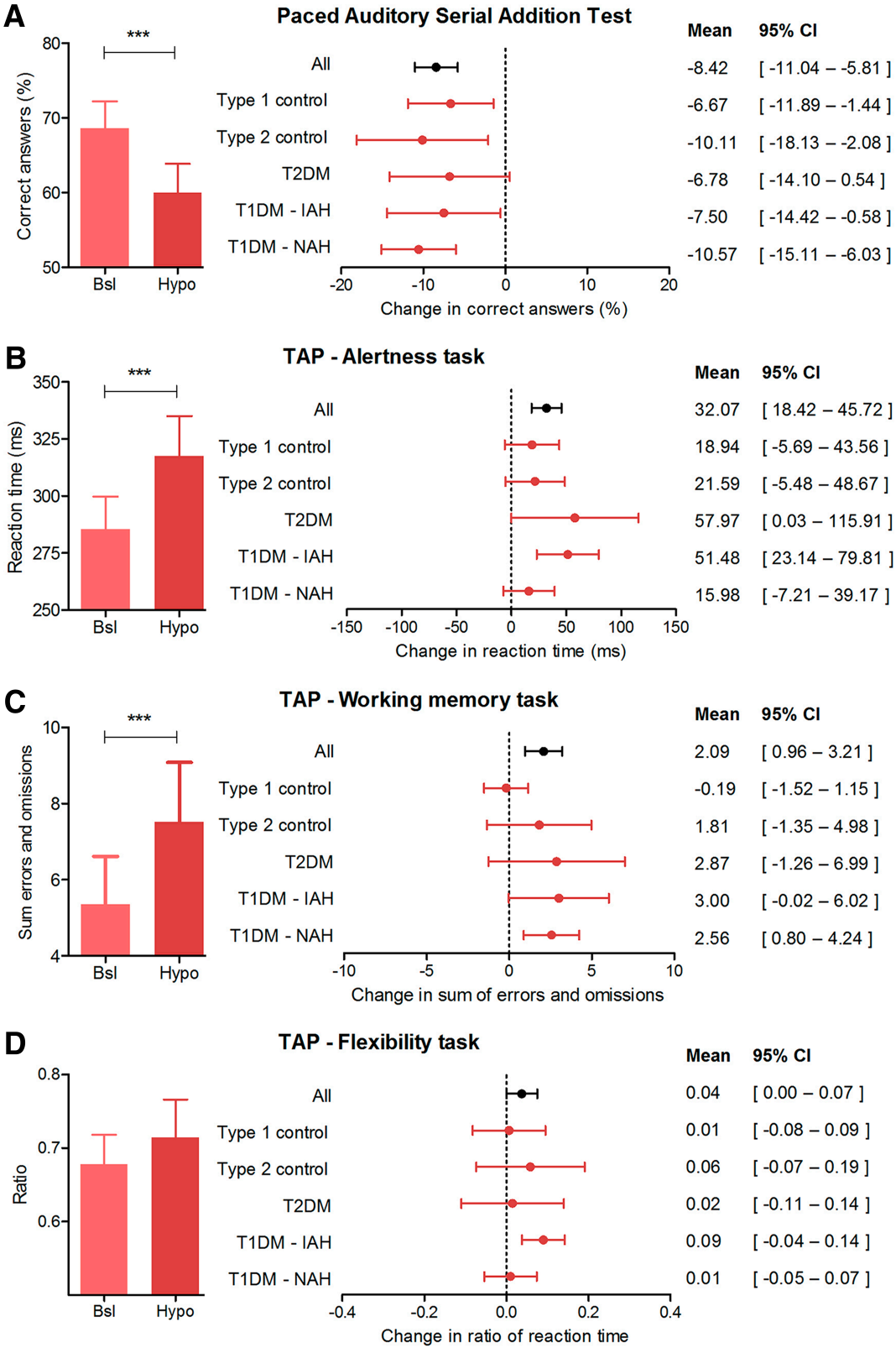

Figure 2—Effect of hypoglycemia on cognitive function in the whole group (left) and in subgroups (right). (*A*) PASAT. (*B*) TAP Alertness task. (*C*) TAP Working Memory task. (*D*) TAP Flexibility task. Values are presented as mean (95% CI). ***$P < 0.001$. Bsl, baseline; Hypo, Hypoglycemia; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

In a sensitivity analysis, we examined the impact of IAH in participants with type 1 diabetes based on the individual questionnaires and the total symptom response to hypoglycemia during the clamp. In neither analysis did awareness status impact on the effect of hypoglycemia on cognitive function (Supplementary Fig. 3).

CONCLUSIONS

In the current study we describe the acute effects of IHSG-defined level 2 hypoglycemia on cognitive function in large groups of people with and without diabetes. Hypoglycemia deteriorated cognitive performance in all groups of participants to a similar extent, with effect sizes ranging from small to medium. In general, this effect was irrespective of the presence of diabetes, diabetes type, awareness status, glycemic control (HbA_{1c}), or duration of diabetes. The consistency and size of this effect of hypoglycemia support the glucose cutoff (<3.0 mmol/L) for level 2 clinically important hypoglycemia as proposed by the IHSG for all individuals with diabetes.

The effect of hypoglycemia on cognitive performance has been examined since the 1980s, with use of hyperinsulinemic-hypoglycemic glucose clamps (20). Many studies have shown reduced cognitive performance in response to hypoglycemia in various subgroups. The range of hypoglycemia levels achieved and variety of tests used to assess cognitive function in these groups, however, make it difficult to compare results across studies (21). In this study we enrolled clinically distinct subgroups of people with diabetes, and control subjects, using the same methodology allowing for direct comparison between different diabetic phenotypes and subjects without diabetes. Also, we deliberately chose a glucose target level just below 3.0 mmol/L to test the validity of use of IHSG level 2 hypoglycemia as a threshold for cognitive function in people with diabetes (9).

In our study, hypoglycemia resulted in acute declines in cognitive functioning, which is in line with most other smaller studies with investigation of the effect of hypoglycemia on cognitive function (7,22), but not all (6,8). Although not studied to the same extent, most aspects of cognitive performance seem to become impaired when glucose levels fall below 3.0 mmol/L (8). However, at a hypoglycemic level of 2.0 mmol/L, simple motor tasks reportedly still remain almost intact (6). Thus, performance on a given cognitive task depends on the complexity of the task (and on underlying neurocognitive processes) as well as on the level of hypoglycemia.

The magnitude of the hypoglycemia-induced deterioration of cognitive function did not significantly differ between subgroups, as supported by the univariate and multivariate analyses, which showed that several clinical factors did not affect or only minimally modulated cognitive performance. Although symptom responses during hypoglycemia affected the performance of the TAP Alertness task, it is likely that the presence of symptoms interfered with accomplishing the task rather than contributing to "real" cognitive impairment. Overall, test results tended to be a little different in some subgroups, but no consistent direction was observed and differences may be partly explained by the limitations of the specific tests used.

Notably, performance on the TAP Flexibility task was not affected by hypoglycemia. The outcome of this task is calculated as the ratio between the reaction times of the simple and the complex task. Hypoglycemia increased both in all subgroups, to a similar extent, which explains why the ratio did not change. Investigators in a previous study with the same test reported a similar increase of reaction times by hypoglycemia (2.5 mmol/L) but did not report the ratio (23). In another study investigators found no effect of hypoglycemia (2.5 mmol/L) on flexibility, as reflected by the absence of additional time needed to switch between tasks on the Stroop Color and Word Test, while results of the Trail Making Test B, again, showed an increased reaction time in response to hypoglycemia (24). These data suggest that while hypoglycemia increases the reaction time of tasks of varied complexity, it does not affect flexibility per se. Whether cognitive flexibility is resistant to the effect of hypoglycemia or whether more profound hypoglycemia is needed to impair flexibility remains to be established.

The effect size of hypoglycemia-induced cognitive decline is about the same as that of sleep deprivation for 1 night or the use of cannabis (25–27). Consuming 2 units of alcohol, with a blood alcohol level that exceeds the recommended driving limits in many countries, results in even less cognitive decline (28). Given the well-known effects of alcohol on driving performance (29), the cognitive impairment caused by IHSG level 2 hypoglycemia may have implications that are relevant for both the individual and society. This supports the cutoff value of <3.0 mmol/L for level 2 hypoglycemia, as proposed by the IHSG (9).

A strength of our study is the standardized protocol for induction of hypoglycemia and examination of the effect of hypoglycemia with use of a broad array of cognitive measures and the involvement of subgroups of people with diabetes at higher risk of recurrent hypoglycemia. This allows generalizability to the larger population with diabetes. There are also limitations to consider. First, inducing a hypoglycemic event with high insulin levels through the clamp technique is highly controlled, which may differ from spontaneous hypoglycemia in real-life. For ethics reasons, all participants were aware of the fact that they would undergo a hypoglycemic event and that cognitive function would be tested, which may have introduced expectation bias. Second, with use of this single-step hypoglycemic clamp we could not investigate whether participants developed cognitive impairment above a glucose level of 3.0 mmol/L, yet this underscores this glucose cutoff to be generalizable as a criterion for hypoglycemia causing cognitive decline. Third, we cannot exclude that the order of the intervention (hypoglycemia following baseline measurements) played a role. However, in a previous study with application of cognitive tests with a euglycemic time-control design, investigators reported similar impairments of cognitive function during hypoglycemia in people with type 1 diabetes (30). Finally, our data cannot be extrapolated to the pediatric population and extrapolation to older adults should be done with great caution, due to the lack of participants over the age of 75 years in our study population.

In conclusion, clinically significant hypoglycemia (glucose <3.0 mmol/L) results in declines in important aspects of

cognitive function. The level of decline is rather consistent in adults with or without type 1 or type 2 diabetes and largely independent of clinical factors, including age, level of hypoglycemic awareness, and glycemic outcomes. Altogether, these findings underscore the clinical relevance of avoiding hypoglycemia of this magnitude for the broader population of people with diabetes and support the current classification proposed by the IHSG, in particular with respect to level 2 hypoglycemia.

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