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

Verhulst, Clementine E. M.; Fabricius, Therese W.; Nefs, Giesje; Kessels, Roy P. C.; Pouwer, Frans; Teerenstra, Steven

Published in:
Diabetes Care

DOI:
10.2337/dc21-2502

Publication date:
2022

Document Version
Peer reviewed version

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

Clementine E.M. Verhulst, MD[1, *], Therese W. Fabricius, MD[2, *], Giesje Nefs, PhD[3, 4, 5], Roy P.C. Kessels, PhD[6,7,8], Frans Pouwer, PhD[9,10,11], Steven Teerenstra, PhD[12], Cees J. Tack, MD, PhD[1], Melanie M. Broadley, PhD[9], Peter L. Kristensen, PhD[2, 13], Rory J. McCrimmon, MD[14], Simon Heller, MD, PhD[15], Mark L. Evans, MD[16], Ulrik Pedersen-Bjergaard, DMSc[2, 13, ‡], Bastiaan E. de Galan, MD, PhD[1, 17, 18, ‡], on behalf of the Hypo-RESOLVE consortium

Institution of origin:

[1]Department of Internal Medicine, Radboud university medical center, Nijmegen, The Netherlands. [2]Department of Endocrinology and Nephrology, Nordsjællands Hospital, Hillerød, Denmark. [3]Department of Medical Psychology, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands. [4]Department of Medical and Clinical Psychology, Center of Research on Psychological disorders and Somatic diseases (C*o*RPS), Tilburg University, Tilburg, The Netherlands. [5]Diabeter; Rotterdam, The Netherlands. [6]Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands. [7]Department of Medical Psychology and Radboudumc Alzheimer Center, Radboud university medical center, Nijmegen, The Netherlands. [8]Vincent van Gogh Institute for Psychiatry, Venray, The Netherlands.[9]Department of Psychology, University of Southern Denmark, Odense, Denmark. [10]School of Psychology, Deakin University, Geelong, VIC, Australia. [11]Steno Diabetes Center Odense, Odense, Denmark. [12]Section Biostatistics, Department for Health Evidence, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, the Netherlands. [13]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. [14]School of Medicine, University of Dundee, Dundee, Scotland. [15]Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom. [16]Wellcome Trust/ MRC Institute of Metabolic Science, University of Cambridge, United Kingdom. [17]Department of internal medicine, Maastricht University Medical Centre, MUMC+ Maastricht, The

Netherlands. [18]CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, the Netherlands.

*Shared first authorship. ‡Shared last authorship.

Running title: Hypoglycemia and cognitive function

Corresponding author:

Clementine Verhulst

Department of internal medicine (389)

Radboud university medical centre

P.O. box 9101

6500 HB, Nijmegen, The Netherlands

Tel: +31 – 24 361 90 86

Email: clementine.verhulst@radboudumc.nl

Word count article: 3865

Word count abstract: 245

Number of tables: 2

Number of figures: 2

Abbreviations

CV	Coefficient of Variation
ESM	Electronic Supplementary Material
EQF	European Qualifications Framework
HbA_{1c}	Glycated hemoglobin
IAH	Impaired Awareness of Hypoglycemia
IHSG	International Hypoglycemia Study Group
ISI	Inter Stimulus Intervals
NAH	Normal Awareness of Hypoglycemia
PASAT	Paced Auditory Aerial Addition Test
PSQI	Pittsburgh Sleep Quality Index
TAP	Tests of Attentional Performance

Key words

Hyperinsulinemic-hypoglycemic clamp, cognitive function, PASAT, TAP, diabetes, hypoglycemia, Type 1 diabetes, Type 2 diabetes.

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”. The present study 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 controls 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 proportion of correct answers on the PASAT by 8.4 ± 12.8%, increased the mean 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 cut-off value of hypoglycemia <3.0 mmol/L (<54 mg/dL) as being clinically relevant for most people with diabetes.

INTRODUCTION

People with type 1 diabetes or with type 2 diabetes treated with insulin are at risk of hypoglycemia, with a reported average of 2-3 episodes per week and two events per month, respectively (1-3). Glucose is the principal fuel for the brain and since the brain is neither capable of 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. 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. Using 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 being 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). It remains unknown 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.

This leaves the universality of the 3.0 mmol/L glucose cut-off inconclusive. Therefore, we investigated the impact of level 2 hypoglycemia on cognitive function in people with type 1 diabetes with normal and impaired awareness of hypoglycemia, in people

with type 2 diabetes treated with insulin, and in age-matched people without diabetes.

RESEARCH DESIGN AND METHODS

Study Design

This was a multi-center intervention study performed at the Internal Medicine outpatient clinics of Radboud University Medical Center in Nijmegen, the Netherlands and Nordsjællands Hospital in Hillerød, Denmark. 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) people with type 1 diabetes and normal awareness of hypoglycemia (NAH); 2) people with type 1 diabetes and impaired awareness of hypoglycemia (IAH); and 3) people with type 2 diabetes treated with insulin for at least one year. Using advertisements in local newspapers and social media, we also recruited two control groups without diabetes who were age-, sex- and body mass index (BMI)-matched to either the participants with type 1 diabetes (type 1 controls) or to those with type 2 diabetes (type 2 controls). Key exclusion criteria were age over 80 years, use of anti-depressive drugs, pregnancy, breastfeeding and taking no birth control measures for women of child-bearing age. People with diabetes with HbA_{1c} above 11.3% (100 mmol/mol) were also excluded, as were people with any medical condition considerably interfering with the 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 the electronic supplementary material (ESM) (ESM Methods).

Study Procedure

A total of 471 people were approached: 130 were invited for screening (ESM Figure 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). Using published cut-offs (ESM Methods), a participant was classified as having IAH when results of at least two of these questionnaires fitted that classification. Participants were asked about their highest completed educational level and current job (if applicable). Answers were transformed to the European Qualifications Framework (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 three 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 0700-0800h, having abstained from alcohol and caffeine for at least 24 hours, and from strenuous exercise for 48 hours. In addition, the six participants who were smokers were asked to abstain from smoking for at least 24 hours. Participants with diabetes received an Intermittently Scanned Continuous Glucose Monitoring (isCGM) device (Freestyle Libre 1®) for two 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 below 3.0 mmol/L in the 24 hours before the clamp, measured with CGM. Participants were asked about their sleep quality the night before, using 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 $mU \cdot kg^{-1} \cdot min^{-1}$ in participants with type 1 diabetes and type 1 controls. In participants with type 2 diabetes and the type 2 controls, an infusion rate of 3.0 $mU \cdot kg^{-1} \cdot min^{-1}$ was used to overcome potential insulin resistance in these people (15). In six of the type 2 control participants, 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 non-dominant hand, a second catheter was inserted in retrograde fashion for blood sampling, with the hand placed in a heated box (temperature ~55° C) to arterialize venous blood. Baseline plasma glucose level was determined (Biosen C-Line; EKF Diagnostics, Cardiff, UK). In case of hyperglycemia (glucose ≥10 mmol/L, 180 mg/dL) upon arrival, an optional small bolus of insulin of maximal 2 units was administered, before starting the continuous infusion. 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 5-10 min intervals and allowed to fall to 5.0 mmol/L (90 mg/dL), with glucose 20% being infused to maintain plasma glucose at this level for 30 minutes. Thereafter, plasma glucose levels were allowed to drop gradually to 2.8 mmol/L (50 mg/dL) and were maintained at this level for another 60 minutes. 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 of 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 using an audio clip on a laptop with interstimulus intervals (ISI) of either 2.8 or 2.0 seconds. 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, the test duration was 4.5 minutes and 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 seconds during a period of five minutes. 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 pre-test at baseline to ensure they understood the tests correctly and to minimize non-specific practice effects. The total duration of the test battery was on average 20 minutes. 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 c702 (Roche Diagnostics) or a Vista1500 (Siemens). HbA_{1c} was assessed by the TOSOH

G8 and G11 HPLC-analyzer (Sysmex). Plasma adrenaline and noradrenaline were measured by HPLC in combination with fluorometric detection. Plasma glucagon was measured by RIA-analysis (Euro-Diagnostica). Plasma insulin was analyzed with an in-house radioimmunoassay. Plasma cortisol and growth hormone were measured by 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. Non-normally distributed data are shown as median (IQR) and log transformed for analyses. One-way ANOVA with Bonferroni post-hoc test was used to compare continuous data and the chi-square test to compare dichotomous baseline characteristics. Symptom scores at baseline, euglycemia and hypoglycemia were analyzed using paired t-test and the difference among subgroups between euglycemia and hypoglycemia with one-way ANOVA. Scores on the four cognitive function tests at baseline and hypoglycemia were compared using paired samples t-tests and Cohen's 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. IBM SPSS statistics, version 25.0 (IBM Corp., Armonk, NY) was used for analysis. Alpha was set at 0.05 throughout.

RESULTS

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

Hypoglycemic Glucose Clamp

The mean glucose levels during the clamps are shown in Figure 1. Baseline glucose levels were higher in the participants with either type 1 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] compared to 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, the mean glucose level in the euglycemic phase was 5.20 ± 0.40 mmol/L (93.7 ± 7.3 mg/dL) with mean CVs 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 CVs 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 when compared to 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 controls than in people 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 group performed significantly better at baseline than participants with type 2 diabetes (p = 0.042) and participants with type 1 diabetes and NAH (p = 0.007, ESM Figure 2).

The mean 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 's 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 (Figure 2A).

In the TAP Alertness task, the mean 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 's d_z 0.48). The increase in reaction times during hypoglycemia was consistent across subgroups with no significant differences in performance between the subgroups (Figure 2B).

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

On the TAP Flexibility task, the ratio between reaction times on the simple and the complex tasks for the entire group averaged 0.68 ± 0.17 at baseline and 0.71 ± 0.22 during hypoglycemia (p = 0.053, Cohen's d_z 0.20), with consistent effects across subgroups (Figure 2D).

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 (-11.1 ± 14.4 versus -5.6 ± 10.2, $p = 0.035$, ESM Table 1), whereas higher age and 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 with the effect of hypoglycemia on cognitive function (ESM 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 ($p = 0.001$).

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 (ESM Figure 3).

CONCLUSIONS

The present study describes the acute effects of IHSG-defined level 2 hypoglycemia on cognitive function in a large series 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 supports the glucose cut-off (<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, using 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, makes it difficult to compare results across studies (21). This study enrolled clinically distinct subgroups of people with diabetes and, controls, using the same methodology allowing for direct comparison between different diabetic phenotypes and non-diabetic people. Also, we deliberately chose a glucose target level just below 3.0 mmol/L to test the validity of IHSG level 2 hypoglycemia on 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 that investigated 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 their 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 the 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. One previous study applying the same test, reported a similar increase of reaction times by hypoglycemia (2.5 mmol/L), but did not report the ratio (23). Another study 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-Word Test, while 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 one night or the use of cannabis (25-27). Consuming two units of alcohol, a level that exceeds the recommended driving limits in many countries, results even in 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 cut-off 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 using 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’. Because of ethical 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, this single-step hypoglycemic clamp could not investigate whether participants developed cognitive impairment above a glucose level of 3.0 mmol/L, yet it underscores this glucose cut-off to be generalizable as a criterium for hypoglycemia causing cognitive decline. Third, we cannot exclude that the order of the intervention (hypoglycemia following baseline measurements) played a role. However, a previous study applying cognitive tests with a euglycemic time-control design 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 persons over the age of 75 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.

Acknowledgements. The authors thank Stephanie A. Amiel for discussing the data and providing feedback on the manuscript and, Namam Ali, Josephin Zielmann and Pieter Drijver for testing the cognitive function, and Evertine Abbink, Linda Drenthen, Karin Saini, Marjolein Eybergen, Emma Lenssen and Esther Eggenhuizen for assistance during the clamps in the Netherlands, and Stine Tving Kjøller, Charlotte Hansen, Pernille Banck-Petersen, Rikke Carstensen, for assisting as research nurses and Charlotte Pietraszek and Susanne Månsson for preparation of blood and other practicalities during the clamp in Denmark. Clementine E. M. Verhulst is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial support. This study has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 777460. The JU receives support from the European Union's Horizon 2020 research and innovation program and EFPIA and T1D Exchange, JDRF, International Diabetes Federation (IDF), The Leona M. and Harry B. Helmsley Charitable Trust.

Clinical Trial information. Clinicaltrials.gov no. NCT03976271 (registered 5 June 2019).

Author Contributions. C.E.M.V., T.W.-F., C.J.T., G.N., R.P.C.K., U.P.-B. and B.E.d.G. designed the study. C.E.M.V. and T.W.-F. performed the experiments and collected the data. C.E.M.V. analyzed the data and wrote the first version of the manuscript. All authors discussed the results and implications and provided feedback on the manuscript at all stages.

Disclosure Summary. C.E.M.V.: None. T.W.-F.: None. G.N.: None. R.P.C.K: None. F.P.: None. S.T.: None. C.J.T.: None. M.M.B.: None. P.L.K.: has received speakers

fee from Sanofi A/S, Novo Nordisk A/S and AstraZeneca A/S. R.J.M.: None. S.H.: None. M.L.E.: None. B.G. has received research support from Novo Nordisk. U.P.-B. has served on advisory boards for AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis, Novo Nordisk, and Zealand Pharma and has received lecture fees from AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis and Novo Nordisk.

References

1. Cryer PE. Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia. Diabetes 2014;63:2188-2195
2. Ostenson CG, Geelhoed-Duijvestijn P, Lahtela J, Weitgasser R, Markert Jensen M, Pedersen-Bjergaard U. Self-reported non-severe hypoglycaemic events in Europe. Diabetic medicine : a journal of the British Diabetic Association 2014;31:92-101
3. Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. Nature reviews Endocrinology 2014;10:711-722
4. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. The American journal of physiology 1991;260:E67-74
5. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E, et al. Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM. Diabetologia 1994;37:1265-1276
6. Heller SR, Macdonald IA. The measurement of cognitive function during acute hypoglycaemia: experimental limitations and their effect on the study of hypoglycaemia unawareness. Diabet Med 1996;13:607-615
7. Inkster B. The effects of acute hypoglycaemia on cognitive function in type 1 diabetes. The British Journal of Diabetes & Vascular Disease 2012;12:221-226
8. Deary IJ, Zammitt NN, Frier BM, Heller SR, McCrimmon RJ. Symptoms of Hypoglycaemia and Effects on Mental Performance and Emotions. Hypoglycaemia in clinical diabetes 2013;Chapter 2
9. Glucose Concentrations of Less Than 3.0 mmol/L (54 mg/dL) Should Be Reported in Clinical Trials: A Joint Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care 2017;40:155-157
10. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes care 1994;17:697-703
11. 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;30:1868-1870
12. Pedersen-Bjergaard U, Agerholm-Larsen B, Pramming S, Hougaard P, Thorsteinsson B. Activity of angiotensin-converting enzyme and risk of severe hypoglycaemia in type 1 diabetes mellitus. Lancet (London, England) 2001;357:1248-1253
13. Commission E. The European Qualifications Framework for Lifelong Learning. 2008;
14. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213
15. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 2004;88:787-835, ix
16. Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. Diabetologia 1993;36:771-777
17. Gronwall DM. Paced auditory serial-addition task: a measure of recovery from concussion. Percept Mot Skills 1977;44:367-373
18. Test for attentional performance (TAP) [article online], 1995. August 2012
19. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol 2013;4:863
20. Holmes CS, Hayford JT, Gonzalez JL, Weydert JA. A survey of cognitive functioning at difference glucose levels in diabetic persons. Diabetes care 1983;6:180-185
21. Fabricius TW, Verhulst CEM, Kristensen PL, Tack CJ, McCrimmon RJ, Heller S, Evans ML, Amiel SA, Pieber TR, de Galan BE, Pedersen-Bjergaard U. Hyperinsulinaemic-hypoglycaemic glucose clamps in human research: a systematic review of the literature. Diabetologia 2021;64:727-736
22. Warren RE, Frier BM. Hypoglycaemia and cognitive function. Diabetes, obesity & metabolism 2005;7:493-503

23. Hermanns N, Kubiak T, Kulzer B, Haak T. Emotional changes during experimentally induced hypoglycaemia in type 1 diabetes. Biol Psychol 2003;63:15-44
24. Graveling AJ, Deary IJ, Frier BM. Acute hypoglycemia impairs executive cognitive function in adults with and without type 1 diabetes. Diabetes care 2013;36:3240-3246
25. Bougard C, Moussay S, Espié S, Davenne D. The effects of sleep deprivation and time of day on cognitive performance. Biological Rhythm Research 2016;47:401-415
26. Fimm B, Blankenheim A. Effect of sleep deprivation and low arousal on eye movements and spatial attention. Neuropsychologia 2016;92:115-128
27. Arkell TR, Lintzeris N, Kevin RC, Ramaekers JG, Vandrey R, Irwin C, Haber PS, McGregor IS. Cannabidiol (CBD) content in vaporized cannabis does not prevent tetrahydrocannabinol (THC)-induced impairment of driving and cognition. Psychopharmacology (Berl) 2019;236:2713-2724
28. Scheel JF, Schielke K, Lautenbacher S, Aust S, Kremer S, Wolstein J. Low-Dose Alcohol Effects on Attention in Adolescents. Zeitschrift für Neuropsychologie 2013;24:103-111
29. Martin TL, Solbeck PA, Mayers DJ, Langille RM, Buczek Y, Pelletier MR. A review of alcohol-impaired driving: the role of blood alcohol concentration and complexity of the driving task. J Forensic Sci 2013;58:1238-1250
30. McAulay V, Deary IJ, Sommerfield AJ, Frier BM. Attentional functioning is impaired during acute hypoglycaemia in people with Type 1 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2006;23:26-31

Table 1 Participant characteristics

	Type 1 diabetes + NAH	Type 1 diabetes + IAH	Type 2 diabetes	Type 2 control	Type 1 control
Participants, *n*	26	21	15	16	16
Male, *n* (%)	13 (50.0)	10 (47.6)	9 (60.0)	9 (56.3)	7 (43.8)
Age, y	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, y	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
%	7.7 ± 0.9†	7.9 ± 0.9†	8.0 ± 1.0†	5.4 ± 0.2	5.2 ± 0.3
BMI, kg/m²	26.7 ± 3.6¶	26.2 ± 3.8	29.0 ±4.3¶	28.0 ± 4.4¶	22.6 ± 2.8
Diabetes complications, *n* (%)	5 (19.2)	9 (42.9)	3 (20.0)	-	-
Retinopathy	5	7	2	-	-
Neuropathy	3	6	2	-	-
Nephropathy	0	1	1	-	-
Glucose lowering medication					
Oral, *n* (%)	0 (0.0)	0 (0.0)	11 (73.3)	-	-
CSII, *n* (%)	11 (42.3)	10 (47.6)	1 (6.7)	-	-
MDI, *n* (%)	15 (57.7)	11 (52.4)	14 (93.3)	-	-
Insulin dose, IU/d	53.6 ± 23.0	45.7± 23.8	71.3 ± 54.6	-	-

*Data are presented as number (%), mean ± SD or median [IQR]. EQF, European quality framework, BMI, Body Mass Index, CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections.. *p < 0.05 versus type 1 diabetes + NAH.**p < 0.05 versus type 2 diabetes, †p < 0.05 versus both control groups. §p < 0.05 versus type 1 diabetes + NAH. ‡p < 0.05 versus type 1 diabetes and IAH. ¶p < 0.05 versus the type 1 controls.*

Table 2 Symptom responses

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

Symptoms responses were measured during baseline, euglycemia and hypoglycemia. Data are presented as mean ± SD. $^{\dagger}p < 0.001$ for difference versus baseline, $^{\#}p < 0.001$ for difference versus euglycemia.$p < 0.05$ for difference versus type 2 diabetes.*

LEGENDS OF FIGURES

Figure 1 –Glucose levels during the glucose clamp in the five subgroups. T1DM denotes type 1 diabetes, NAH normal awareness of hypoglycemia, IAH impaired awareness of hypoglycemia and T2DM type 2 diabetes.

Figure 2 –Effect of hypoglycemia on cognitive function in the whole group (left) and in subgroups (right). A. Paced auditory serial addition test (PASAT). B. TAP - Alertness task. C. TAP - Working memory task. D. TAP - Flexibility task. T1DM denotes type 1 diabetes, NAH normal awareness of hypoglycemia, IAH impaired awareness of hypoglycemia and T2DM type 2 diabetes. Values presented as mean (95%CI). ***$p < 0.001$.