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Effects of Mild Hypoglycaemia on Cognitive Function in Type 2 Diabetes

Study protocol

Malin Nilsson, MD
Mette Zander, MD, PhD
Michael Gejl, MD, PhD
Kamilla Woznica Miskowiak MSc, PhD
Heidi Storgaard, MD, PhD
Jørgen Rungby, MD, PhD

Center for Diabetes Research
Department of Medicine
Gentofte Hospital, University of Copenhagen
Hellerup, Denmark

and

Department of Endocrinology
Bispebjerg University Hospital
Copenhagen, Denmark

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Study Protocol

Introduction and background

Hypoglycaemia in subjects suffering from type 2 diabetes may have substantial consequences including a significant negative impact on quality of life [1], [2]. Further, repeated minor hypoglycaemias may result in significant productivity losses. In healthy subjects a number of studies show that during a hypoglycaemic episode with plasma levels of 2.2 – 2.5 mmol/L (40-45 mg/dl) brain areas responsible for cognition have an altered neuronal function when measuring cerebral blood flow [3], [4]. This is accompanied by severely impaired cognitive function with a reduced ability to solve simple cognitive tasks. At higher levels of glucose (above 3 mmol/L (54 mg/dl)), it remains to be settled whether cognitive functions are also affected negatively and whether this may be accompanied by changes in brain metabolism. Apart from raising the blood glucose directly or indirectly via glucagon, no treatment for hypoglycaemia exists, but since GLP-1 based therapies used in type 2 diabetes may affect brain glucose consumption, therapeutic interventions to prevent negative results of hypoglycaemia may eventually become clinically possible [5].

Here, we propose to provide quantitative results on cognition during an acute mild hypoglycaemic episode (target plasma glucose 3 mmol/L). Data will be provided on executive function, attention and memory.

Objectives and endpoints

Objective

To investigate our hypothesis that acute mild hypoglycaemia in patients with diabetes type 2 volunteers is associated with cognitive impairment of executive function, attention and memory.

Primary endpoint

- Performance on the Symbol Digit Modalities Test (SDMT)

Secondary endpoints

- Additional measures of executive function, attention and memory (see section on neurocognitive tests).

Hypothesis

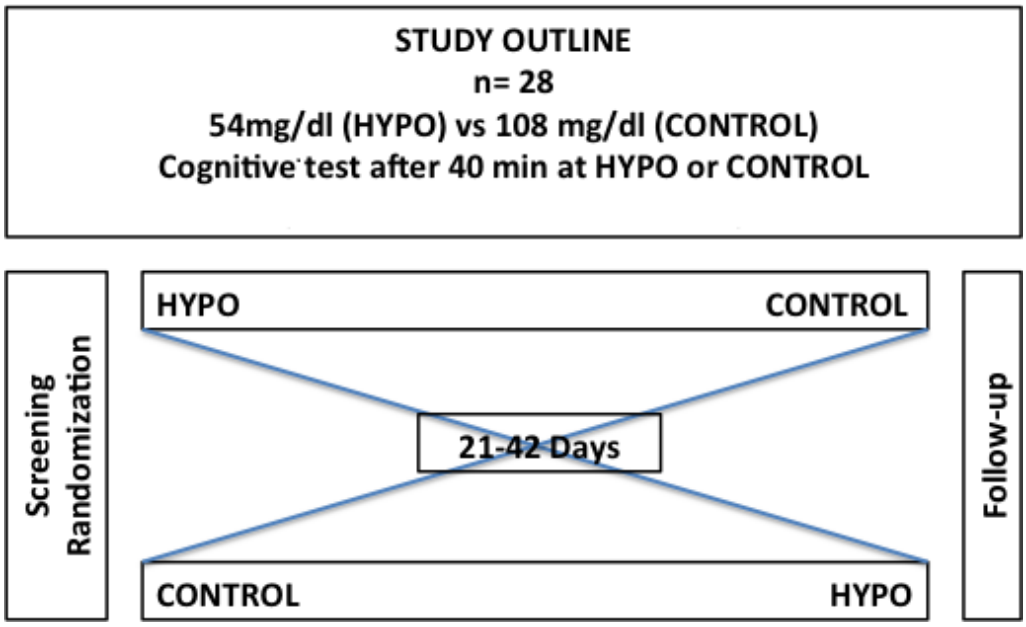
- Mild hypoglycaemia attenuates cognitive performance in type 2 diabetes.

Study design

Our study is a randomised, single-centre, single-blinded, two-period cross-over trial in subjects with type 2 diabetes examining the effect of an acute hypoglycaemic episode on cognitive function.

Each subject undergoes two experimental days in randomized order with a 21-42 day interval, Day A and Day B. At each visit they will undergo a clamp procedure where their plasma glucose levels will be clamped at either a hypoglycaemic (PG < 3.1 mmol/L, goal during clamp 2,8-3,2) or at an euglycaemic level (6 mmol/L, goal during clamp 5,8- 6,2). During the two conditions (hypoglycaemia or euglycaemia) cognitive perfomance will be assessed by validated cognitive tests.

The trial consists of a screening visit (Day 0) 2-35 days prior to two following experimental visits (Day A and B), that will be separated by a period of 21-42 days during which the participants will resume their normal medical and lifestyle treatments.
An information visit prior the screening visit and a phone call follow-up after completion of the second experimental day are included in the trial as well.



Participants

Participant Selection

Number of participants: Based on previous studies and the following power calculation, we estimate that a study will be informative with 28 participants.

Inclusion criteria:

- Informed and written consent
- Clinically diagnosed type 2 diabetes mellitus for at least 3 months (diagnosed according to the criteria of the World Health Organization (WHO)).
- Normal haemoglobin ≥ 8.0 mmol/L (male) or ≥ 6.4 mmol/L (female)
- Male or female participants aged 35-70 years, both inclusive.
- Treated with diet or any antidiabetic medication except sulfonylureas, meglitinides or insulin.
- HbA1c ≤ 9.0 % by local laboratory analysis.
- BMI >23 kg/m^2 and <35 kg/m^2

Exclusion criteria:

- Receipt of any investigational medicinal product within 3 months before screening in this trial.
- Liver disease (alanine aminotransferase (ALAT) and/or serum aspartate aminotransferase (ASAT) >2 times normal values) or history of hepatobiliary disorder.
- Nephropathy (serum creatinine levels ≥ 126 μmol/L (male) or ≥ 111 μmol/L (female)).
- Cardiac problems defined as decompensated heart failure (New York Heart Association (NYHA) class III and IV) at any time and/or angina pectoris within the last 12 months and/or acute myocardial infarction at any time.
- Active or recent malignant disease.
- Treatment with drugs that cannot be paused for 12 hours.
- Repeted resting blood pressure at screening outside the range 90−140 mmHg for systolic or 50−90 mmHg for diastolic. This exclusion criterion also pertains to subjects taking antihypertensives.
- Visual impairment or auditory impairment.
- Known abnormalities of the central nervous system or any endocrinological (with the exception of diabetes mellitus and euthyroid goiter), haematological, neurological, psychiatric diseases or other major disorders that in the opinion of the investigator precludes compliance with the protocol, evaluation of the results or represent an unacceptable risk for the participant's safety.
- Proliferative retinopathy (funducsopy performed within 3 months before the screening is acceptable) and/or severe neuropathy.
- Current treatment with systemic drugs, which may interfere with glucose metabolism.
- Significant history of alcoholism or drug/chemical abuse as per investigator's judgement.
- Current tobacco user (smoking or nicotinic product use 3 months prior to screening).
- Severe hypoglycaemic event during the past 6 months.
- Known hypoglycaemia unawareness.
- Participants with mental incapacity or language barriers precluding adequate understanding or co-operation or who, in the opinion of the investigator or their general practitioner, should not participate in the trial.
- For females only: Pregnancy, breast-feeding status or intention of becoming pregnant during the trial.
- Any chronic disorder or severe disease that in the opinion of the investigator might endanger participant's safety or compliance with the protocol.

Methods

This trial consists of a screening visit (Day 0) followed by two experimental days (Day A and B) with procedures as explained below.

Information visit

Prior to any protocol-related procedures, an information visit is arranged where potential participants will be provided with oral and written information about the trial and all the procedures involved. At this visit the medical doctor responsible for the project explains about the project in undisturbed and confidential surroundings. A suitable room for this is available at the Department of Endocrinology at Bispebjerg Hospital. The potential participant will prior to the information visit be informed about the possibility of bringing an assessor. Subjects will be offered a period of one week for consideration before deciding on

participation. If the person immediately decides to participate, written consent is obtained and screening subsequently performed.

Screening visit (Day 0)

The screening visit will take place 2-35 days prior to the first experimental day (Day A). The signed and dated informed consent is assessed and inclusion and exclusion criteria are checked. Demography (date of birth, ethnicity, sex) is assessed along with current medication and medical history are recorded. Height, weight, body mass index (BMI) and blood samples (creatinine, electrolytes (Na^+, K^+, Ca), ALAT/ASAT, alkaline phosphatase, albumin, bilirubin, haemoglobin, plasma glucose, C-reactive protein (CRP), HbA_1c, coagulation tests (INR and coagulation factor II, VII and X). Date of the diabetes diagnosis and current diabetes treatment is obtained, and finally a physical examination is performed and vital signs and an ECG will be assessed.
On the basis of the screening results, eligible participants are included in the study, and dates for the two experimental days are planned.

Experimental days (Day A and B)

If a participant meets one or more of the following exclusion criteria on any of the experimental days (Day A and B), he or she will be excluded from the visit or withdrawn from the trial:

Exclusion criteria:

- Consumption of alcohol within 24 hours prior to an experimental day.
- Extensive physical exercise within 24 hours prior to an experimental visit that will interfere with trial results, as judged by the investigator.
- Any use of systemic drugs that may interfere with glucose metabolism.
- Hypoglycaemia (detected as a plasma glucose (PG) ≤ 3.9 mmol/L) within 48 hours prior to an experimental day.

Participants will be instructed to maintain a regular diet and avoid alcohol and excessive eating for 3 days before each experimental day.

Day A

Subjects arrive at the laboratory after an overnight (10 hours) fast (water, coffee, tobacco, medicine) having avoided strenuous physical activity from the day before. They are placed in a recumbent position and will have a cannula inserted into a cubital vein. Another cannula is inserted into a contralateral cubital vein for insulin and glucose infusions.
On the first experimental day a hyperinsulinaemic hypoglycemic clamp is performed. At time 0 minutes, insulin infusion is initiated (Actrapid, Novo Nordisk). Plasma glucose is measured bedside every 5 minutes, allowing the plasma glucose level to be clamped on 3 mmol/L using an adjustable continuous infusion of 20 % glucose. Neurocognitive testing is initiated when glucose levels have been stabilized for 40 minutes and lasts for approximately 45 minutes.

Day B

Hyperinsulinaemic hyperglycemic clamp. Equal to Day A except that the plasma glucose level is clamped on 6 mmol/L.

Blood specimens:
Blood will be drawn at 30 after goal PG is reached, into chilled tubes containing heparin for analyses of insulin, C-peptide, epinephrine, glucagon, cortisol and growth hormone. Plasma samples are stored at −20°C until analysis. For bedside measurements of plasma glucose, blood is added to fluoride tubes and centrifuged at room temperature immediately for 30 seconds at 7400*g*.

Analytical procedures:
Plasma glucose concentrations are measured by the glucose oxidase method. Plasma insulin and C-peptide concentrations are measured using a two-sided electrochemiluminescence immunoassay (Roche/Hitachi Modular Analytics; Roche Diagnostic GmbH).

Performance of Cognitive Tests:

Neurocognitive test battery:
The test battery includes the following neurocognitive tests: Rey Auditory Verbal Learning Test (RAVLT) [6] (verbal memory), Trail Making Test (TMT) [7] part A and B (psychomotor speed and executive function), Symbol Digit Modalities Test (SDMT) [8] (psychomotor speed and executive function), WAIS-III Letter-Number Sequencing test [9] (executive function), verbal fluency test (letters S and D) (executive function) and Rapid Visual Processing (RVP) test from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (sustained attention) [10], [11]. To avoid learning effects from baseline to follow-up testing, we will use alternate versions of the tests that are associated with greatest learning effects (incl. the RAVLT). Verbal IQ is estimated with the Danish Adult Reading Test (DART) (equivalent to the National Adult Reading Test; NART). The total duration of neuropsychological testing on each occasion is approximately 45 min.

All experiments are carried out at the Department of Endocrinology, Bispebjerg University Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark, where the necessary equipment is available.

Randomisation procedure
The participants of this randomised single-blinded study will be treated in a randomized sequence; either hypoglycaemia followed by euglycaemia or euglycaemia followed by hypoglycaemia. The investigator will be blinded to the treatment sequence until randomisation of the participant.

Calculations and statistics

Data is processed and presented with the use of standard descriptive statistics. AUCs are calculated by use of the trapezoid rule. Repeated measurement analysis of variance is used for statistical analysis of repeated measurements in the same subject. Data that is not normally distributed is compared using the Mann-Whitney U-test.

A recent trial applying the cognitive DSST score (part of the above neurocognitive test battery) in type 1 diabetes revealed a standard deviation (*SD*) between eu- and hypoglycaemia of 9 [12]. We suggest a similar level of variance in this study and from previous trial opt to detect differences of 6 or more. *P* values ≤ 0.05 will be considered as statistically significant, i.e. significance level of 5%, which will allow a sample size of 28 to yield a more than 90% high chance of detecting a true difference in cognition.

Only data from trial participants who have completed the entire study will be included in the statistical analysis.

Side effects, risks and disadvantages for participants

The 10 hour fast prior to each research day is a part of the natural nocturnal fast (10 PM - 8 AM), and is neither associated with discomfort or danger.

Insulin when administered can lead to hypoglycaemic episodes. This will sometimes appear as certain symptoms like palpitations (increased heartbeat/pulse), hunger, tremor (slight shaking), pallor, nausea, headache, sudden perspiration, feelings of anxiety, visual and speech disorders, paraesthesia (tingling and numbness in the feet and hands), symptoms of paralysis, and unsteadiness. If the condition progresses to severe hypoglycaemia, it can lead to loss of self-control, unconsciousness and, in extreme cases, death.

The participants of this trial will be exposed to hypoglycaemia and they will be closely monitored during this procedure. During the development of hypoglycaemia a physician and at least one trained person should be monitoring the participant, while a second physician should be available in 1-2 minutes on-call. A glucose solution will be placed close to the participant as a safety precaution, and plasma glucose concentration must be monitored frequently during the hypoglycaemic episode. In general, mild to moderate hypoglycaemia (plasma glucose levels $\leq$ 3.9 mmol/L) can be treated by ingestion of carbohydrates, while severe hypoglycaemia should be treated by the investigator and with the best available medical practice (e.g. dextrose given intravenously, or glucagon given subcutaneously or intramuscularly).

As a theoretical complication associated with intravascular catheters (and any other penetration of the skin and blood vessels with sharp/pointed objects) superficial phlebitis should be mentioned. Superficial phlebitis is not dangerous and will be treated with antibiotics should any sign of infection be present. The risk of superficial phlebitis is small and minimized by compliance with clinical standards for the insertion of intravenous catheters including double cleansing of the skin with an ethanol swap and other sterile procedures.

Participants physical and mental integrity and privacy

Information regarding participants of the project will be protected in accordance with the applicable laws of Denmark. The protocol is reported to The Danish Data Protection Agency via the Capitol Region of Denmark.

Operating costs and economy

The responsible physician of the project is professor Jørgen Rungby from Department of Endocrinology, Bispebjerg University Hospital. The project is initiated by MD, PhD, Professor Jørgen Rungby and funded by the pharmaceutical company MSD with DKK 1.4 million. Neither Jørgen Rungby nor the rest of the research group have any financial interests in the conduct and outcome of the project or the company funding the project.
In case of additional costs we plan to apply for funding from public and private funds. The Research Ethics Committee will be informed if additional funding is achieved. Funds will be deposited in an account administered by Bispebjerg University Hospital.

Remuneration and reimbursement of costs for trial participants

Documented travel expenses related to the project will be reimbursed. For the inconvenience and the time spent (two study days of approximately 8 hours and one screening visit of

around 1 hour), each participant will be remunerated DKK 1200 (taxable income). If participants choose to withdraw prematurely from the study they will be reimbursed according to the time spent (DKK 600 per study day).

Availability of data for participants

All participants are guaranteed access to more information about the project.

Publication of study results

Data from the study will be processed and presented in one or more manuscripts for publication in international scientific journals. Inconclusive, negative and positive test results will be published, in accordance with the law concerning processing of personal data.

Adjournment of trial

The experiment is ended for each participant in case they wish to withdraw from the protocol, or in case exceptional circumstances make it impossible to complete the study. Likewise, any extraordinary event that makes it impossible to finish the project will result in termination of remaining study days. In any case of premature termination, the participants will be informed about the decision and the reason why.

Recruitment of participants

Patients with type 2 diabetes will be recruited among persons who have previously participated in trials and at that time accepted to be contacted again regarding other research projects, or among the patients attending the out-patient diabetes clinic in Department of Endocrinology at Bispebjerg Hospital. Alternatively, participants will be recruited through advertising in newspapers or on websites such as www.forsoegspersoner.dk. Potential participants will be contacted by telephone or email, except for patients attending the out-patient diabetec clinic, where the first contact will be in writing with an approved contact letter.

Exposition to the National Health Research Ethics Committee

All participants receive oral and written information, and oral and written consents are obtained prior to any protocol activity. A doctor who is not otherwise in charge of their treatment (if any) informs participants. The protocol is in compliance with the principles of Helsinki Declaration II.

All participants are assigned with a trial number and will on datasheets and tubes only appear with initials and trial number. The full name, social security number and trial number will be stored separately. If necessary, participants are covered by the patient insurance of Bispebjerg Hospital.

Participants are fully informed about possible adverse events and inconveniences related to trial procedures, and adequate precautions have been implemented in the study design in order to minimise the risks of participating in the trial.

Blood samples (100 ml per participant) will be stored at our department in a research bio-bank until analysis and completion of the study. Any excess plasma, blood and white blood cells will be stored at our department for up to 10 years after the completion of the study. In case of any re-analyses or need for further analyses a new approval by the National Health Research Ethics Committee is required.

The project will not benefit the individual participant (apart from the general medical examination, which is part of the screening visit), but it will add valuable information to our understanding of hypoglycaemia. Perhaps such knowledge can lead to new and improved strategies in the treatment of type 2 diabetes.

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