



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Effects of sleep deprivation on hypoglycemia-induced cognitive impairment and recovery in adults with type 1 diabetes

Citation for published version:

Inkster, BE, Zammitt, NN, Ritchie, SJ, Deary, IJ, Morrison, I & Frier, BM 2016, 'Effects of sleep deprivation on hypoglycemia-induced cognitive impairment and recovery in adults with type 1 diabetes', *Diabetes Care*, vol. 39, no. 5. <https://doi.org/10.2337/dc15-2335>

Digital Object Identifier (DOI):

[10.2337/dc15-2335](https://doi.org/10.2337/dc15-2335)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Diabetes Care

Publisher Rights Statement:

This is an author-created, uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Care Association (ADA), publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version is available in print and online at [10.2337/dc15-2335](https://doi.org/10.2337/dc15-2335).

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Effects of sleep deprivation on hypoglycemia-induced cognitive impairment and recovery in adults with type 1 diabetes

**Berit E. Inkster¹ MRCP(UK), Nicola N. Zammitt¹ MD, Stuart J. Ritchie² PhD,
Ian J. Deary² PhD, Ian Morrison³ PhD, Brian M. Frier¹ MD**

1. Department of Diabetes
Royal Infirmary of Edinburgh
51 Little France Crescent
Edinburgh
EH16 4SA,
Scotland, UK
2. Centre for Cognitive Ageing and Cognitive Epidemiology,
Department of Psychology,
University of Edinburgh
George Square
Edinburgh
EH8 9JZ,
Scotland, UK
3. Department of Neurology,
Ninewells Hospital,
Dundee
DD1 9SY
Scotland, UK

Corresponding Author:

Berit Inkster
Department of Diabetes
Royal Infirmary of Edinburgh
51 Little France Crescent
EH16 4SA,
Scotland, UK
Tel: +44 131 242 1467
Fax: +44 131 242 1367
Email: berit.inkster@luht.scot.nhs.uk

Word count: 4096
Number of tables: 2
Number of figures: 2

Abstract

Objectives: To ascertain whether hypoglycemia in association with sleep deprivation causes greater cognitive dysfunction than hypoglycemia alone, and protracts cognitive recovery after normoglycemia is restored.

Research design and methods: Fourteen adults with type 1 diabetes underwent a hyperinsulinemic, hypoglycemic clamp on two separate occasions. Before one glucose clamp the participants stayed awake overnight to induce sleep deprivation. Participants were randomized and counterbalanced to the experimental condition. Cognitive function tests were performed before and during hypoglycemia, and for 90 minutes after restoration of normoglycemia.

Results: Cognitive impairment during hypoglycemia did not differ significantly between the sleep-deprived and non-sleep-deprived conditions. However, in the sleep-deprived state, digit symbol substitution scores and choice reaction times were significantly poorer during recovery ($p < 0.001$) and hypoglycemia symptom scores were significantly higher ($p < 0.001$), even when symptoms that may have been caused by sleep deprivation, such as tiredness, were removed.

Conclusions: Hypoglycemia per se produced a significant decrement in cognitive function; co-existing sleep deprivation did not have an additive effect. However, after restoration of normoglycemia, preceding sleep deprivation was associated with persistence of hypoglycemic symptoms and greater and more prolonged cognitive dysfunction during the recovery period.

The neuroglycopenia resulting from acute hypoglycemia rapidly affects cognitive function; complex tasks such as working memory and choice reaction time are most impaired, and mood, motivation and psychomotor function are also affected (1). Recovery of several cognitive domains may be delayed for up to 70 minutes after normoglycemia has been restored (2).

Sleep deprivation, both total and partial, has detrimental effects on neurocognitive performance, but is characterized by wide inter- and intra-individual variability, so can be difficult to interpret (3,4). Sleep plays an important role in the encoding, consolidation and processing of memory (5,6). Sleep deprivation, both before and after learning tasks, results in deficits in performance (6). Attention, vigilance and alertness are all affected; individuals perform well initially but their performance deteriorates with duration of the task (4). Mood and emotion are also affected with an increase in negative mood states following sleep deprivation (3,7).

The combined effect of sleep deprivation and hypoglycemia (blood glucose 2.5 mmol/l; 45 mg/dl) has been examined previously during normal sleep, partial sleep deprivation and total sleep deprivation in non-diabetic male adults (8). Reaction times and auditory evoked potentials were assessed at baseline and during hypoglycemia, but not after restoration of normoglycemia. Total sleep deprivation caused a significant deterioration in both measures at the normoglycemic baseline, but no significant deterioration was observed following partial sleep deprivation. Hypoglycemia caused significant deterioration from baseline following normal sleep, and after partial sleep deprivation, but no additional effect was observed following full sleep deprivation, suggesting that the detrimental neurocognitive impacts of sleep

deprivation and hypoglycemia were not additive. Alternatively, a ceiling effect may be present that limits the magnitude of cognitive deterioration that can be demonstrated with these neurocognitive tests, although the investigators asserted that the relatively short reaction times would prevail against this interpretation.

Sleep deprivation and hypoglycemia may share a final common pathway to influence the depletion of cerebral glucose (9). Adults with type 1 diabetes may experience these conditions concomitantly in everyday life, particularly if they are involved in shift work, and little is known about the effect of this dual insult on cognitive function. It is plausible that exposure to both conditions simultaneously could have an additive or even synergistic effect on cognitive impairment, and/or protract the delay in recovery after normoglycemia has been restored. In the present study, these hypotheses were tested in adults with type 1 diabetes, using a range of cognitive tests that are sensitive both to hypoglycemia and to sleep deprivation.

Research design and methods

Participants were recruited from hospital diabetes outpatient clinics in the Lothian region of Scotland. Written, informed consent was given by all participants, and ethical approval for the study was obtained from the local medical research and ethics committee. Those studied were adults aged between 18 and 40 years with type 1 diabetes for >1 year, normal awareness of hypoglycemia, BMI 20 to 30kg/m² and HbA1c of 6.5 to 10% (48 to 86 mmol/mol). Exclusion criteria included significant co-existent systemic disease or malignancy, a past history of a severe reaction to hypoglycemia (such as seizure or neurological deficit), cerebral injury, epilepsy,

chronic alcoholism or psychiatric disorder. People who were not fluent in written or spoken communication in English were excluded (as the cognitive tests employed are validated only in English), or if they were pregnant (pregnancy testing was performed on all potential female participants).

All patients in Lothian with type 1 diabetes were assessed (approximately 1500) and those meeting the age criteria were contacted by post and then by person when attending routine clinic appointments. Of a total of 129 patients who communicated an interest in participating (either verbally, by post or email), 24 did not meet the inclusion criteria and 90 decided not to participate. Of the remaining 15 subjects, all completed the study protocol apart from one subject who could not be made hypoglycemic using the standard glucose clamp technique.

Experimental procedure

Participants underwent hypoglycemic clamps on two separate occasions, performed at least two weeks apart. Two experimental conditions were randomized and counterbalanced: one following induction of short term total sleep deprivation by staying awake all night and one after a full night of sleep. Some participants were at work during the night preceding the glucose clamp, while those at home were asked to send a short text message to the investigator every 30 minutes to demonstrate that they were awake. The study was deferred if more than one text message was omitted during the night, or if they experienced symptomatic or biochemical hypoglycemia during the 24 hours preceding the study.

Participants attended at 08.00h and a modified hyperinsulinemic, clamp (12) was used to maintain blood glucose at pre-determined levels of 5.0mmol/l (90mg/dl) during euglycemia (run in and recovery) and 2.5mmol/l (45mg/dl) during hypoglycemia. Blood samples were taken every 5 minutes and analyzed at the bedside using a glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, OH, USA). During the run-in period the participants were asked to practise the cognitive test battery (digit symbol substitution test, choice reaction time and hypoglycemia symptom scale) to familiarize themselves with the tests and to eliminate a potential learning effect. The cognitive battery took approximately 5 minutes to complete and was repeated multiple times during the study (see Figure 1). After completion of baseline cognitive tests, blood glucose was lowered gradually to 2.5mmol/l (45mg/dl) over approximately 20 minutes, and maintained at this level for 1 hour, during which further cognitive tests were performed (see Figure 1). Once normoglycemia had been restored, the battery of cognitive tests was repeated at intervals for 90 minutes. Testing in the recovery period was carried out at 10, 20, 30, 40, 55, 70 and 85 minutes after blood glucose had risen to > 4.0mmol/l (72mg/dl), using the same time schedule as in a previous study by our group in which recovery of cognitive function following hypoglycemia was examined (2). Intravenous infusion was then discontinued and the participants received a meal.

Cognitive assessments and symptom evaluation

National adult reading test (NART)

This test contains 50 words which, if unfamiliar to the reader, would be mispronounced when read aloud. The NART is used to provide an estimate of premorbid intelligence (10).

Willpower questionnaire

This six item, scaled questionnaire gauges an individual's expectations about how well they would engage with mental work, and was modified from the questionnaire of Job et al (13).

Modified hypoglycemia symptom scale

A modified version of the validated Edinburgh hypoglycemia score (14,15) was used to record symptoms on a 7 point Likert scale throughout both study sessions. In recognition that two of the hypoglycemia symptoms (sleepiness and drowsiness) might be influenced disproportionately by sleep deprivation, these were excluded from the symptom score analysis. The other 15 symptoms included autonomic symptoms (sweating, warmth, pounding heart, hunger, trembling), neuroglycopenic symptoms (confusion, difficulty speaking, inability to concentrate, blurred vision, anxiety, tingling of the lips) and non-specific symptoms (weakness, dizziness, nausea, and headache).

Cognitive tests

These tests were chosen as they are sensitive to the effects of hypoglycemia (2,16-18) and to sleep deprivation (19,20).

General cognitive test battery - performed at baseline, during hypoglycemia and at multiple time points during recovery

1. *Digit symbol substitution test.* This is a test of sustained attention, response speed and visuo-motor co-ordination, and is a subtest of the Wechsler Adult Intelligence Scale-revised (WAIS-R) (21). Rows of blank squares are displayed on a piece of paper. Each blank square is paired with a number from one to nine. A printed key pairs each number with a different symbol, and the participant fills the blank squares with the symbols that match the numbers. The score is the number completed within 2 minutes.
2. *Choice reaction time.* The subject presses one of four keys as quickly as possible in response to the appearance of a cross on the screen in a position corresponding to one of the keys (22). The subjects completed 40 trials with an inter stimulus interval of 1 to 3 seconds. The response was recorded if it occurred within 200 to 1500 ms. The mean response time for correct answers was used in the analysis.

Memory tests – performed during hypoglycemia only (logical memory repeated once during recovery)

1. *Logical memory test.* In this subtest from the Wechsler Memory Scales (23) a short story is read to the subject, who is then required to recount it. Points are obtained for recollection of specific details and story themes. Recollection was tested both immediately and at two further time points (see Figure 1).
2. *Working digit span test – backward* (23). In this test of working memory, a series of lists of numbers are presented verbally to the subject. Subjects are asked to recall the numbers in reverse numerical order. For example, for the

sequence 2-6-1-5-3 the correct response is 6-5-3-2-1. The test score is the number of lists that are remembered correctly.

3. *Letter/number sequencing test.* In this working memory test from the Wechsler Memory Scales (23), a series of sequences of numbers and letters that increase in length are presented verbally. The subject is asked to re-order and repeat, giving the numbers in ascending order, followed by the letters in alphabetical order. The score is the number of correctly re-ordered sequences.

Attention test – performed during hypoglycemia only

1. *Visual elevator.* This is an indirect test of cognitive flexibility assessing visual switching (24). Subjects are asked to imagine they are travelling up and down in an elevator, represented by a series of pictures of elevator doors. Arrows indicate the direction of counting between pictures and the subject is timed as they count up and down the floors.

Statistical Analysis

A repeated measures analysis of variance (ANOVA) was used to assess the effect of sleep deprivation on the cognitive tests performed *during* hypoglycemia. Experimental condition (sleep deprivation or normal sleep) was the within-subjects factor, and order of session (sleep-deprived or control study first) as a between-subjects factor. Cohen's *d* and partial eta squared were calculated to assess effect size (threshold for a large effect size is 0.8 and 0.5 respectively).

In order to address the issue of multiple comparisons resulting in spurious significant findings, the cognitive test battery was then analyzed by linear mixed models, estimated using the *lme4* package for R (25). The first set of models tested the effect of sleep deprivation on the two measurements of cognitive function during hypoglycemia, also testing whether sleep deprivation affected either the first or the second cognitive measurement more strongly (i.e. a test for a time \times condition interaction). The second set of models was similar, but tested the effect of sleep deprivation on cognitive function across the seven measurements during recovery. All models included a random (individual-specific) intercept for each participant. The time \times condition interaction in this situation tested whether the trajectory of cognitive ability differed according to the two sleep deprivation conditions. In both sets of models, the effect of sleep deprivation on the hypoglycemia symptom scale was also tested at each of the measurement points. All models adjusted for the baseline (pre-hypoglycemia) cognitive or symptom score (as applicable) in each condition by including this variable as a predictor.

Results

Participants

A total of 14 participants (five female) completed the study, with a median (range) age of 27.5 (20-38) years. The median (range) duration of diabetes was 10 (3-26) years, and mean HbA1c was $8.0 \pm 0.9\%$ ($64 \pm 9\text{mmol/mol}$). Seven had background retinopathy, two had microalbuminuria (raised albumin creatinine ratio of ≥ 3.5 mg/mmol (female) or ≥ 2.5 mg/mmol (male)); none had peripheral neuropathy. Mean

body mass index was $25.9 \pm 3.0 \text{ kg/m}^2$. Participants were of higher than average intelligence as measured by a mean NART (10) score of 31.5 ± 6.5 correct answers, equivalent to an IQ of 112.

Blood glucose

Blood glucose (mean \pm SD) during the baseline period was $5.4 \pm 0.6 \text{ mmol/l}$ ($97 \pm 11 \text{ mg/dl}$) in the control condition, and $5.2 \pm 0.5 \text{ mmol/l}$ ($94 \pm 9 \text{ mg/dl}$) in the sleep-deprived condition. During hypoglycemia mean blood glucose was $2.5 \pm 0.2 \text{ mmol/l}$ ($45 \pm 4 \text{ mg/dl}$) for both study conditions. Mean blood glucose level at recovery was $5.2 \pm 0.6 \text{ mmol/l}$ ($94 \pm 11 \text{ mg/dl}$) for both study conditions.

Tests performed during baseline and hypoglycemia

Although there was a trend towards poorer performance during the sleep-deprived condition on the baseline cognitive tests (i.e. before hypoglycemia), the difference between the control and sleep-deprived scores did not reach statistical significance for any test (Table 1). Scores on the willpower questionnaires were unaffected by the sleep condition. As expected, the general cognitive test battery scores deteriorated during hypoglycemia compared to baseline ($p < 0.01$).

During hypoglycemia, the performance on cognitive tests including memory, willpower and attention testing did not differ significantly between the two sleep conditions (Table 1). The linear mixed models showed that the results of the cognitive test battery did not differ significantly between the two conditions (Table 2). No significant interactions were observed between time and condition; the variables did

not change more rapidly between the two measurements taken during hypoglycemia in one condition more than the other (p -values for the time-condition interaction = 0.41, 0.53, and 0.22 for the hypoglycemia symptom scale, choice reaction time and digit symbol substitution test respectively).

The effect of the order in which studies were performed had an impact only on the modified hypoglycemia symptom scale. Symptom scores were higher at the start of the period of hypoglycemia during the first study visit, independent of sleep condition.

Recovery tests

The hypoglycemia symptom score results are shown in Figure 2. Even after controlling for baseline the modified symptom scores were significantly higher in the sleep deprived condition during recovery (Table 2). However, no interaction occurred between time and condition ($p = 0.22$), indicating that the trajectory of the symptom scores did not differ between the sleep-deprived and the control conditions. When the symptoms were subdivided into autonomic, neuroglycopenic, and non-specific groups, the results remained significantly different for both the autonomic and neuroglycopenic symptoms, but not for the non-specific symptoms.

Figure 2 shows that performance was consistently better on the choice reaction and digit symbol tests in the control condition during recovery: sleep deprivation slowed performance on both tasks, and this remained significant after adjustment for baseline score. Performance on the digit symbol but not the choice reaction decreased significantly across each of the tests, but no significant interactions between time and

condition were observed; the test scores in the sleep-deprived condition did not decline any more steeply than the control scores (p -values for the time-condition interaction: 0.38 and 0.90 for choice reaction and digit symbol respectively).

Conclusions

In the present study of young adults with type 1 diabetes, the impairment of cognitive function that was associated with hypoglycemia was not exacerbated by sleep deprivation. This is consistent with the report of a previous small study in 7 non-diabetic adults in which these forms of stress were examined in combination (8). One possible explanation is that hypoglycemia per se exerts a ceiling effect on the degree of cognitive dysfunction as is possible to demonstrate with conventional tests. It is also possible that the mechanism causing cognitive dysfunction during sleep deprivation differs from that during hypoglycemia, so that no additive effect is evident. Both this and the previous study were small, and had limited power to detect an effect, which may also have influenced these observations.

However, throughout the recovery period in the present study a significant deterioration in cognitive function was evident during the sleep-deprived state, even after adjustment for baseline values, and using a mixed model which was not susceptible to error through multiple comparisons. This contrasts with the results of a previous study by our group, in which choice reaction time in adults with type 1 diabetes remained prolonged for 70 minutes after restoration of normoglycaemia, but had recovered fully by 85 minutes (2). It is possible that this could have resulted from exacerbation of the sleep deprivation towards the end of the study, by which time the

sleep deficit had been protracted by approximately a further six hours. The absence of a significant time interaction between the two conditions would argue against such an explanation; if greater sleep deprivation was the main causative factor promoting the poor scores during recovery from hypoglycemia, it would be expected that these scores would have worsened over time in the sleep-deprived study to a greater degree than in the control study, which was not observed. The recovery period also differed during the sleep-deprived study in that the hypoglycemia symptom scores were higher. This difference was not observed at baseline, so cannot be attributed to the symptom questionnaire being sensitive to sleep deprivation per se. As the autonomic symptom scores remained elevated following restoration of normoglycemia, this suggests that the autonomic response to hypoglycemia was enhanced and/or prolonged when the participants were in a sleep deprived state. This is in contrast to the attenuated epinephrine secretion that occurred in response to hypoglycemia during sleep in a study of 8 adults with type 1 diabetes (26). Interestingly, epinephrine concentrations were higher when hypoglycemia occurred overnight when these subjects were awake (1299 ± 213 pmol per liter vs 1616 ± 327 pmol per liter) than if hypoglycemia was induced during the daytime. In that study (26), no statistical analysis comparing these results was reported, but suggests that the epinephrine response to hypoglycemia may be amplified during sleep deprivation. Plasma catecholamine concentrations were not estimated in the present study as a measure of the intensity of the sympatho-adrenal response. While catecholamines do not generate the autonomic symptoms per se, they can heighten the magnitude of the symptom response (27,28).

The higher neuroglycopenic symptom scores during the recovery period were unexpected. The physiological cause is unclear, but the brain does store a small but significant quantity of glycogen (29), which presumably is utilized in response to acute neuroglycopenia. Sleep deprivation leads to depletion of cerebral glycogen (30,31), and an important restorative function of sleep may be to replenish stores of this substrate (9,31,32). This effect on neuroglycopenic symptoms may have been exacerbated by depletion of neuronal glycogen reserves associated with sleep deprivation. This would be consistent with prolonged impairment of cognitive function.

No significant difference in performance at baseline was observed between the sleep-deprived and the non-sleep-deprived conditions, suggesting that the battery of cognitive tests was either not sensitive to the sleep-deprived condition, or that the degree of sleep deprivation was insufficient. However, the tests were selected because they are known to assess domains of cognitive function that are affected by sleep deprivation. Cognitive impairment in sleep-deprived people is characterized by high intra-individual variation (4), which is problematic when using a repeated measures design that assumes that intra-individual variation is minimal, although the multi-level model should have accounted for this. In addition, there is wide inter-individual variation in response to sleep deprivation (33), which may have reduced the power of the study to identify significant outcomes, particularly as the number of participants was small. This low level of power is also a concern for the between-condition differences shown in Table 1: only large effect sizes could have produced significant

differences in these values. However, the repeated testing increases our power to detect effects in the longitudinal analysis.

The study design can be criticized for the simple method used to induce sleep deprivation resulting in two distinct populations (night shift workers, and those who stayed awake at home) and for the lack of characterization of participants before, and during, the study. No assessment was made at recruitment for sleep disorder, sleepiness, or chronotype. It is possible that some of the participants may have switched to a nocturnal chronotype, which could have affected their ability to cope with the tests. The degree of sleep deprivation induced was not assessed by polysomnography nor with a simple scoring system such as the Epworth score (34) or the Karolinska sleepiness scale (35). The degree of sleep deprivation may therefore have differed between individuals depending on the number of preceding night shifts worked, their usual sleep patterns, and any degree of sleep deprivation before participation in the study. However, this flexibility in approach was necessary to encourage recruitment for what was a demanding research design and protocol; the relative simplicity of the method that was applied was considered to be representative of real life conditions. By completion of the present study, the participants had been deprived of sleep for around 24 hours, which is of similar duration to that employed in most studies of sleep deprivation (20).

Ideally the study design should also have included normoglycemia control arms, with and without sleep deprivation, but the existing protocol was demanding and time-consuming, and to require participants to attend for a further two glucose clamps would have made recruitment extremely difficult. Of the local clinic population of

adults with type 1 diabetes (approximately 1500) only 15 agreed to participate. The participants could not be blinded to the sleep condition, but a significant sleep order effect was observed only for the symptom score when hypoglycemia commenced. (Participants gave higher symptom scores when they first became hypoglycemic on their first study visit, regardless of sleep state). No significant order effect was seen at any other time, or for any of the cognitive tests.

The repetitive nature of the protocol meant that participants were asked to perform the same general cognitive tests ten times during each study session. This may have increased their skill at performing the task, and their performance would therefore be expected to improve during the study, and potentially from one study to the next. This effect was moderated by having participants practise the tests during the run in period to familiarize them with the tests. The counterbalanced design ensured that the learning effect would affect both arms of the study equally. The lack of improvement beyond baseline, and indeed a deterioration in choice reaction times, would suggest that a significant learning effect did not occur, or that the effect of fatigue, sleep deprivation or hypoglycemia was greater than any learning effect that had occurred.

Alternatively, fatigue and inattention due to the repetitive and lengthy nature of the protocol may in particular have had an impact on the sleep deprivation studies. Evidence for this can be seen in the results of the choice reaction time (see Figure 2), which suddenly improves at 55 minutes after a period of deterioration, similar to that observed in a previous study (2). In both of these studies, this recovery time point coincided with repetition of the logical memory test, so changing the routine of the cognitive test battery. This may have unmasked an element of underlying boredom in

the participants, whose interest and concentration were revived when a different test was introduced. The effect was more pronounced in the sleep-deprived condition. It is known that sleep deprivation leads to a classic 'fatigue effect' where initial performance is good but deteriorates with increasing duration of the task (4). This could explain why the baseline tests were not affected, but performance then deteriorated as the session progressed, particularly with the repetitive cognitive test battery.

The present study in adults with type 1 diabetes has shown that while the cognitive impairment induced by hypoglycemia is not exacerbated by sleep deprivation, the post-hypoglycemia recovery takes longer with persistence both of cognitive dysfunction and of hypoglycemia symptoms. As these combined forms of stress may be encountered at some time in everyday life by people with insulin-treated diabetes, the delayed post-hypoglycemia recovery could have important consequences in situations such as driving. People with diabetes should be advised that exposure to hypoglycemia while suffering from sleep deprivation could prolong the impairment of cognitive function considerably, despite prompt restoration of normoglycemia.

Acknowledgements

The authors would like to thank the staff of the Edinburgh Wellcome Trust Clinical Research Facility for their technical assistance and the participants who kindly gave their time and willingly endured a period of sleep deprivation for this study.

BMF, SJR and IJD are members of the University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and

Wellbeing Initiative (MR/K026992/1). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC) is gratefully acknowledged.

The authors have no conflicts of interest to declare. This research has not been published previously, but has been presented in poster for at EASD annual meeting, Stockholm, 2015. The guarantor for the work is BI.

BI recruited the study participants, performed data collection, and prepared the manuscript. BI and SJR performed the statistical analysis. NNZ, IJD, IM and BMF conceptualized the study and designed the protocol, and advised on data analysis. All authors helped to write the manuscript.

References

- (1) Inkster B, Frier BM. The effects of acute hypoglycaemia on cognitive function in type 1 diabetes. *Br J Diabetes Vasc Dis* 2012;12:218-223.
- (2) Zammit NN, Warren RE, Deary IJ, Frier BM. Delayed recovery of cognitive function following hypoglycemia in adults with type 1 diabetes: effect of impaired awareness of hypoglycemia. *Diabetes* 2008;57:732-736.
- (3) Walker MP. The role of sleep in cognition and emotion. *Ann N Y Acad Sci* 2009;1156:168-197.
- (4) Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. *Semin Neurol* 2005;25:117-129.
- (5) Walker MP. Sleep, memory and emotion. *Prog Brain Res* 2010;185:49-68.
- (6) Walker MP. The role of slow wave sleep in memory processing. *J Clin Sleep Med* 2009;5:S20-26.
- (7) Benedetti F, Colombo C. Sleep deprivation in mood disorders. *Neuropsychobiology* 2011;64:141-151.
- (8) Jauch-Chara K, Hallschmid M, Schmid SM, Bandorf N, Born J, Schultes B. Sleep loss does not aggravate the deteriorating effect of hypoglycaemia on neurocognitive function in healthy men. *Psychoneuroendocrinology* 2010;35:624-628.
- (9) Scharf MT, Naidoo N, Zimmerman JE, Pack AI. The energy hypothesis of sleep revisited. *Prog Neurobiol* 2008 11;86:264-280.
- (10) Nelson HE, Willison J. National adult reading test (NART) test manual. 2nd ed.: NFER-NELSON Publishing Company Ltd; 1991.
- (11) Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 1981;30:936-940.
- (12) DeFronzo R, Tobin J, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol Endocrinol Metab* 1979;237:E214-E223.
- (13) Job V, Dweck CS, Walton GM. Ego depletion-Is it all in your head? Implicit theories about willpower affect self regulation. *Psychol Sci* 2010;21:1686-1693.
- (14) Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. *Diabetologia* 1993;36:771-777.

- (15) Hepburn DA, Deary IJ, Frier BM, Patrick AW, Quinn JD, Fisher M. Symptoms of acute insulin-induced hypoglycemia in humans with and without IDDM factor-analysis approach. *Diabetes care* 1991;14:949-957.
- (16) Strachan MW, Deary IJ, Ewing FM, Frier BM. Recovery of cognitive function and mood after severe hypoglycemia in adults with insulin-treated diabetes. *Diabetes Care* 2000;23:305-312.
- (17) Warren RE, Allen KV, Sommerfield AJ, Deary IJ, Frier BM. Acute hypoglycemia impairs nonverbal intelligence. *Diabetes Care* 2004;27:1447-1448.
- (18) Geddes J, Deary IJ, Frier BM. Effect of acute insulin-induced hypoglycaemia on psychomotor function: people with type 1 diabetes are less affected than non-diabetic adults. *Diabetologia* 2008;51:1814-1821.
- (19) Jackson ML, Croft RJ, Kennedy GA, Owens K, Howard ME. Cognitive components of simulated driving performance: Sleep loss effects and predictors. *Accid Anal Prev* 2013;50:438-444.
- (20) Lim J, Dinges DF. A meta-analysis of the impact of short-term sleep deprivation on cognitive variables. *Psychol bull* 2010;136:375-389.
- (21) Wechsler D. WAIS-R : Wechsler adult intelligence scale--revised. New York, NY: Harcourt Brace Jovanovich [for] Psychological Corp.; 1981.
- (22) Deary IJ, Der G, Ford G. Reaction times and intelligence differences: A population-based cohort study. *Intelligence* 2001;29:389-399.
- (23) Wechsler D. WMS-R : Wechsler Memory Scale--Revised. San Antonio, Texas: Harcourt Brace Jovanovich for The Psychological Corporation; 1987.
- (24) Robertson IH, Ward T, Ridgeway V, Nimmo-Smith I. The structure of normal human attention: The Test of Everyday Attention. *J Int Neuropsychol Soc* 1996;2:525-534.
- (25) Bates D, Maechler M, Bolker B, Walker S. lme4: Linear mixed-effects models using Eigen and S4 R 2014;R package version 1.1-7.
- (26) Jones TW, Porter P, Herwin RSS, Davis EA, O'Leary P, Frazer F, et al. Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med* 1998;338:1657-1662.
- (27) DeRosa MA, Cryer PE. Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab* 2004;287:E32-E41.
- (28) McAulay V, Deary IJ, Frier BM. Symptoms of hypoglycaemia in people with diabetes. *Diabet Med* 2001;18:690-705.

- (29) Tesfaye N, Seaquist ER, Oz G. Noninvasive measurement of brain glycogen by nuclear magnetic resonance spectroscopy and its application to the study of brain metabolism. *J Neurosci Res* 2011;89:1905-1912.
- (30) Gruetter R. Glycogen: The forgotten cerebral energy store. *J Neurosci Res* 2003;74:179-183.
- (31) Brown AM. Brain glycogen re-awakened. *J Neurochem* 2004;89:537-552.
- (32) Benington JH, Heller HC. Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol* 1995;45:347-360.
- (33) Van Dongen HPA, Belenky G. Individual differences in vulnerability to sleep loss in the work environment. *Industrial health* 2009;47:518-526.
- (34) Johns MW. A new method for measuring daytime sleepiness the epworth sleepiness scale. *Sleep* 1991;14:540-545.
- (35) Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int J Neurosci* 1990;52:29-37.

Table 1. Results of tests performed at baseline, during hypoglycemia, and at the first recovery test.

Condition	Test		<u>Control</u>		<u>Sleep deprived</u>		<u>Difference between conditions</u>		<u>Experimental Order Effect</u>	
			Mean	SD	Mean	SD	<i>p</i>	Cohen's <i>d</i>	<i>p</i>	η^2_p
baseline (normo-glycaemia)	Willpower		25.3	4.4	23.7	5.5	0.39	0.32	0.09	0.226
	Baseline battery	mod hypo symptom scale	24.8	8.4	28.8	11.3	0.31	-0.40	0.79	0.007
		Digit symbol substitution	87.7	18.3	81.8	17.4	0.16	0.33	0.14	0.186
		Choice reaction time	449.0	59.0	459.0	61.0	0.74	-0.17	0.07	0.286
Hypoglycaemia	Hypo 1 battery	mod hypo symptom score	36.6	14.0	38.3	16.0	0.98	-0.11	0.01	0.559
		Digit symbol substitution	68.7	14.2	67.5	13.4	0.80	0.09	0.80	0.006
		Choice reaction time	503.0	88.0	518.0	100.0	0.80	-0.16	0.11	0.232
	Logical memory (immediate)		14.0	4.7	11.6	4.6	0.18	0.52	0.09	0.226
	visual elevator (timing)		7.5	4.6	6.4	3.8	0.62	0.26	0.14	0.173
	Working digit span backwards		7.7	2.6	7.9	2.6	0.59	-0.08	0.13	0.185
	Letter number sequencing		9.4	2.9	9.6	2.7	0.81	-0.07	0.97	0.000
	Logical memory (delayed 1)		12.0	4.2	9.7	4.2	0.16	0.55	0.16	0.155
	Hypo 2 battery	mod hypo symptom scale	43.2	13.3	45.9	17.0	0.81	-0.18	0.09	0.242
		Digit symbol substitution	73.3	12.2	66.9	16.6	0.22	0.44	0.45	0.054
		Choice reaction time	515.0	109.0	513.0	100.0	0.59	0.02	0.65	0.022
	willpower		26.6	5.4	27.1	4.9	0.39	-0.10	0.06	0.262
	recovery (normo-glycaemia)	Rec 1 battery	mod hypo symptom scale	25.1	5.8	29.1	13.2	0.19	0.39	0.47
Digit symbol substitution			86.1	16.6	78.4	16.8	0.14	0.46	0.07	0.263
Choice reaction time			451.0	56.0	475.0	73.0	0.16	-0.37	0.86	0.003
Logical memory (delayed 2)		12.1	4.0	8.9	4.2	0.07	0.78	0.09	0.220	

Note: NART = National Adult Reading Test, Hypo battery = general cognitive tests performed at start (1) and end (2) of hypoglycemia, Hypo Rec 1 = First recovery time point (10 minutes), mod hypo symptom score = modified hypoglycemia symptom score (see text). Differences between conditions were calculated using a general linear model comparing sleep deprived with non sleep deprived, with experimental order as a between subjects factor.

Table 2. Results from linear mixed models of the cognitive test battery during hypoglycemia and during recovery.

Effect	Hypoglycemia Symptom Scale			Choice Reaction Time			Digit-Symbol Substitution		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>
Measurements made during hypoglycemia									
Condition	.04	.14	.77	-.03	.14	.83	-.05	.16	.72
Time	.19	.07	.01	.02	.07	.78	.07	.07	.34
Baseline	.46	.10	<.001	.62	.12	<.001	.63	.15	<.001
Measurements made during post hypoglycemia recovery									
Condition	.87	.07	<.001	.34	.08	<.001	-.23	.06	<.001
Time	-.22	.03	<.001	.02	.04	.61	.11	.03	<.001
Baseline	.22	.05	<.001	.09	.10	.34	.52	.08	<.001

Note: β = standardized beta value. Condition is a dummy variable, 0 = Control condition, 1 = Hypoglycemia condition.

Higher scores on the Choice reaction time measure indicate slower (poorer) performance.

All values come from models with no Condition*Time interaction. Condition*Time interactions were nonsignificant ($p>0.05$) for all three tests.

Figure legends

Figure 1. Outline of study design showing timing of cognitive tests in relation to blood glucose concentration.

Figure 2. Results from hypoglycemia scale (top), digit symbol score (middle) and choice reaction time (bottom). Error bars represent standard error. Overall effect of condition during recovery $p < 0.01$ for all tests.