

# **A single bout of high-intensity interval training reduces awareness of subsequent hypoglycemia in patients with type 1 diabetes**

**Running title:** Effects of HIIT on awareness of hypoglycemia

**Authors:** Hanne M. Rooijackers<sup>1</sup>, Evita C. Wiegers<sup>2</sup>, Marinette van der Graaf<sup>2,3</sup>, Dick H Thijssen<sup>4,6</sup>, Roy P.C. Kessels<sup>5,7</sup>, Cees J. Tack<sup>1</sup>, Bastiaan E. de Galan<sup>1</sup>

**Affiliations:** Departments of <sup>1</sup>Internal Medicine, <sup>2</sup>Radiology and Nuclear Medicine, <sup>3</sup>Pediatrics, <sup>4</sup>Physiology, and <sup>5</sup>Medical Psychology, Radboud university medical center, Nijmegen, the Netherlands ; <sup>6</sup>Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom ; <sup>7</sup>Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands

**Corresponding author:**

Hanne M Rooijackers, MD  
Department of Internal Medicine 463  
Radboud university medical center  
PO Box 9101  
6500 HB  
Nijmegen  
the Netherlands  
Tel: +31 24 36 67203  
E-mail: Hanne.Rooijackers@radboudumc.nl

**Word count:** 3396

**Word count abstract:** 200

**Number of tables:** 2

**Number of figures:** 5

## Abstract

High-intensity interval training (HIIT) gains increasing popularity in patients with diabetes. HIIT acutely increases plasma lactate levels. This may be important, since administration of lactate during hypoglycemia suppresses symptoms and counterregulation, whilst preserving cognitive function. We tested the hypothesis that HIIT acutely reduces awareness of hypoglycemia and attenuates hypoglycemia-induced cognitive dysfunction. In a randomized crossover trial, patients with type 1 diabetes and normal awareness of hypoglycemia (NAH), patients with impaired awareness of hypoglycemia (IAH), and healthy participants (n=10 per group) underwent a hyperinsulinemic-hypoglycemic (2.6 mmol/L) clamp, either after a HIIT session or after seated rest. Compared to rest, HIIT reduced symptoms of hypoglycemia in patients with NAH, but not in healthy participants or patients with IAH. HIIT attenuated hypoglycemia-induced cognitive dysfunction, which was mainly driven by changes in the NAH subgroup. HIIT suppressed cortisol and growth hormone responses, but not catecholamine responses to hypoglycemia. The present findings demonstrate that a single HIIT session rapidly reduces awareness of subsequent hypoglycemia in patients with type 1 diabetes and NAH, but not in patients with IAH, and attenuates hypoglycemia-induced cognitive dysfunction. The role of exercise-induced lactate in mediating these effects, potentially serving as an alternative fuel for the brain, should be further explored.

Clinical trial registration number: NCT02308293, ClinicalTrials.gov

## Introduction

Regular exercise is recommended for patients with type 1 diabetes as it improves physical fitness, insulin sensitivity and general well-being, while reducing cardiovascular risk factors (1; 2). High-intensity interval training (HIIT) is a relatively new training modality that consists of repeated, brief bouts of exercise at high-intensity (i.e.  $\geq 85\%$  peak oxygen uptake), interspersed by periods of rest or low-intensity exercise (3; 4). HIIT is becoming increasingly popular, because it has similar or superior training effects compared to endurance training, despite lower time commitment (3). In patients with type 1 diabetes, high-intensity (interval) training has been reported to exert an acute glucose-stabilizing effect when compared to endurance exercise (5-7). Notwithstanding this short-term effect, the risk of late nocturnal hypoglycemia after HIIT may be increased (8). It is currently unknown whether HIIT affects awareness of subsequent hypoglycemic episodes.

Performance of HIIT markedly increases plasma lactate levels (9). This is highly relevant, since previous studies have demonstrated that the administration of lactate during hypoglycemia suppresses counterregulatory hormone responses and hypoglycemic symptoms (10-13). In addition, the infusion of lactate also attenuates the decline in cognitive function that normally occurs during hypoglycemia (10-13). These effects are presumed to result from increased utilization of lactate by the brain (12; 13), which can substitute glucose and act as an alternative energy source under hypoglycemic conditions. Elevated levels of endogenous lactate induced by HIIT may thus be expected to have similar effects as exogenous lactate, and suppress normal physiological responses to subsequent hypoglycemia (i.e. reduced counterregulatory hormones, reduced hypoglycemic symptoms and attenuated cognitive dysfunction).

Such a suppressive effect would be particularly worrisome for the ~25% of patients with type 1 diabetes and impaired awareness of hypoglycemia (IAH), who already have compromised

symptomatic and hormonal responses to hypoglycemia (14) and are at high risk of severe hypoglycemia (15). In these patients high levels of endogenous lactate after HIIT might even further impair counterregulation. Therefore, the aim of the present study was to investigate the effect of HIIT on hypoglycemic symptoms, counterregulatory hormone responses and cognitive function during subsequent hypoglycemia in patients with type 1 diabetes and normal awareness of hypoglycemia (NAH) and impaired awareness of hypoglycemia (IAH), but also in healthy participants.

## **Research design and methods**

### *Participants*

We recruited ten non-diabetic participants, ten type 1 diabetes patients with NAH, and ten type 1 diabetes patients with IAH. Patients with type 1 diabetes were eligible if they had an HbA<sub>1c</sub> below 9.0% (75 mmol/mol) and were free from macro- and microvascular complications, except for background retinopathy. Exclusion criteria included a history of cardiopulmonary disease, anxiety disorders, brain injury, age > 40 years, and the use of drugs other than insulin interfering with glucose metabolism. Awareness state was first assessed by a Dutch version of the Clarke questionnaire (16; 17), but final stratification was based on adrenaline and symptomatic responses to the hypoglycemic clamps. Eighteen out of twenty patients were rightly characterized as having IAH or NAH by the Clarke questionnaire. One patient initially classified as having intact awareness (NAH) had very low adrenaline and symptom responses to the hypoglycemic clamp, whereas another patient presumably with impaired awareness displayed normal responses to the clamp. The latter two patients switched groups. All participants were recreationally active. The institutional review board of the

Radboud university medical center approved the study and all study participants gave written informed consent before participation.

### Experimental design

In a random order, all enrolled participants underwent two hyperinsulinemic-hypoglycemic glucose clamp (nadir, 2.6 mmol/L) experiments, one that was preceded by a HIIT session and one preceded by seated rest (duration equivalent of HIIT session). The two experiments were scheduled at least two weeks apart, except in the women participating, in whom experiments were conducted during similar phases of the menstrual cycle.

Participants presented between 8.00 and 8.30 AM at the clinical research facility after an overnight fast, having abstained from caffeine, alcohol and smoking for 24h, and from strenuous exercise for two days. Participants with diabetes received specific instructions to avoid (nocturnal) hypoglycemia the day before the clamp. Experiments were rescheduled in cases of hypoglycemia in the 24 hours before the clamp. Upon arrival, intravenous cannulae were inserted into the antecubital veins of both forearms. One forearm was placed in a heated box (55°C), so that arterialized venous blood could be obtained for frequent blood sampling. The cannula in the contralateral arm was used for infusion of glucose 20% (Baxter B.V., Deerfield, IL) and insulin (insulin aspart; Novo Nordisk, Bagsvaerd, Denmark). Baseline plasma glucose and lactate levels were determined (Biosen C-Line, EKF Diagnostics, Cardiff, UK) and hyperglycemia in patients with diabetes was corrected as needed with a small bolus of insulin. Subsequently, a two-step hyperinsulinemic (60 mU/m<sup>2</sup>/min) euglycemic (5.0 mmol/L)-hypoglycemic (2.6mmol/L) glucose clamp was initiated. Plasma glucose and lactate values were determined every five minutes. Blood samples for measurement of catecholamines and cortisol were taken at euglycemia, 5 minutes after exercise, and at 20-

minute intervals during hypoglycemia. Insulin, glucagon, growth hormone (GH) and pH were measured at euglycemia, after exercise (only GH and pH) and at the end of hypoglycemia. Under clamped, euglycemic conditions, participants performed HIIT or rested for the same period of time. Subsequently, plasma glucose levels were allowed to fall to 2.6 mmol/l over ~35 minutes and were maintained there for another 60 minutes.

### Exercise protocol

The HIIT session was performed on a cycle ergometer (Lode Corival, Procare, Groningen, The Netherlands) and had a total duration of approximately 15 minutes. Prior to the start, a short 10-second test sprint was performed to determine optimal resistance of the ergometer (equaling ~0.1kg/kg body mass, depending on the participant's physical activity level and cycling experience). The exercise protocol consisted of a 4-minute warm-up at 50 Watt (W), followed by three 30-second all-out sprints during which participants had to cycle as fast as possible, interspersed with 4 minutes active recovery (50W). The resistance applied during the all-out sprints was adjusted depending on the perceived intensity of the previous sprint, measured with the Borg scale (18), aiming at a Borg score >15 (ranging from 6, indicating no exertion to 20, indicating maximal exertion). The scale was displayed in front of the participants while exercising and after each all-out sprint, participants selected the number that best described their perceived level of exertion.

### Symptom scores

Participants were asked to complete a semiquantitative symptom questionnaire at euglycemia, and at 20, 40 and 60 minutes of hypoglycemia. Symptoms were scored from 0 (none) to 6 (severe) and included six autonomic (trembling, palpitations, anxiety, sweating, hunger,

tingling), six neuroglycopenic (blurred vision, difficulty speaking, feeling faint, difficulty thinking, fatigue, confused), four general (dry mouth, weakness, nausea, headache) and two dummy symptoms (yellow vision, pain in the legs). Total symptom scores (i.e. scores including all symptom subcategories) and subscores were averaged over the three time points (after 20, 40 and 60 minutes of hypoglycemia). Peak symptom scores were defined as the highest total symptom score during hypoglycemia, irrespective of the time point.

### Cognitive function tests

The following cognitive function tests were applied during the screening visit and during hypoglycemia, starting 15 minutes after hypoglycemia was reached. In cases of hypoglycemia (n=2) or hyperglycemia (n=1) during the screening visit, the first battery of cognitive function tests was applied during the euglycemic phase of the first test day. Non-specific practice effects were controlled for by counterbalancing the order of the rest and HIIT intervention, and parallel forms were used to avoid material-specific practice effects if applicable.

**Digit span forward and backward.** Subtest of the Dutch version of the Wechsler Adult Intelligence Scale (WAIS)-III that measures attention and working memory (19). Sequences of digits of increasing lengths were presented verbally and participants were asked to recall the digits in the presented and in the reverse order.

**Verbal fluency test.** Dutch version of the Controlled Oral Word Association Test (COWAT) that serves as an index of executive function (20). Participants were given 1 minute to name as many words as possible starting with a given letter. Parallel sets of letter triads (D-A-T, K-O-M, and P-G-R) were used for baseline, HIIT and rest measurements.

**Paced Auditory Serial Addition Test (PASAT).** Evaluates speed of information processing, divided and sustained attention (21). Continuous sequences of 61 digits are auditorily

presented and participants are asked to add each new digit to the one immediately prior to it and say the answer out loud. Sequences were presented at three rates of presentation, i.e. every 2.4, 2.0 or 1.6 seconds.

In order to provide a composite score of cognitive function, raw scores of each test were transformed into Z-scores, based on the distribution of test scores at baseline (including all three study groups), and were averaged (22).

### Analytical methods

Plasma insulin was assessed by an in-house radioimmunoassay (RIA) (23). After extraction (24), plasma glucagon was measured by RIA with a commercially available kit (Eurodiagnostica, Malmö, Sweden). Plasma GH and cortisol were determined using a routine analysis method with an Electrochemiluminescent Immunoassay on a Modular Analytics E170 (Roche Diagnostics, GmbH, Mannheim, Germany). Plasma adrenaline and noradrenaline were analyzed by HPLC combined with fluorometric detection (25). pH was measured by routine venous blood gas analysis in lithium heparin-anticoagulated blood immediately after withdrawal, using CG4+ cartridges and an i-STAT blood gas analyzer (Abbott, Libertyville, IL, USA).

### Statistical analysis

Data were tested for normality using the Shapiro-Wilk test and QQ-plots. Differences in means within groups were analyzed with paired Student's *t*-tests or Wilcoxon's signed rank test when data were not normally distributed. Differences in means between groups were analyzed by ANOVA followed by Bonferroni's post hoc tests to delineate statistical



significance, and for non-parametric data with the Kruskal-Wallis test and post hoc Mann-Whitney U tests. Serial data (hormone responses and glucose infusion rates) were analyzed by two-way repeated measures ANOVA, in which missing data were replaced with the last observed value. The cognitive tests were analyzed with a repeated measures general linear model ANOVA with study group (healthy controls, patients with NAH or patients with IAH) as a between-subject factor and intervention (HIIT or rest) as a within-subject factor, followed by post hoc paired Student's *t*-tests. All data are expressed as mean  $\pm$  standard error of the mean (SEM), unless otherwise specified. Alpha was set at 0.05 throughout. Statistical analyses were performed with IBM SPSS Statistics 20.

## Results

The three groups of participants were well matched for age, sex and BMI (Table 1). Diabetes duration did not differ significantly between the two diabetes groups, HbA<sub>1c</sub> was borderline significantly lower in patients with IAH compared to those with NAH ( $p=0.05$ ). Glucose levels during the clamps are shown in figure 1A and B.

### Physiological effects of exercise

Average workload during all-out sprints for the whole study population was  $437\pm 17$  W and mean perceived exertion was  $16\pm 0.4$  on the Borg scale, with no difference between groups. HIIT markedly increased plasma lactate levels from  $1.2\pm 0.1$  to  $13.1\pm 0.5$  mmol/L (Fig. 1D) and decreased pH from  $7.36\pm 0.00$  to  $7.19\pm 0.01$  to a similar extent in all groups. After reaching peak levels, plasma lactate levels fell gradually, but remained well above baseline levels during the subsequent hypoglycemic clamp in both patient groups and for 40 minutes of hypoglycemia in healthy participants. Immediately after HIIT, plasma glucose levels increased slightly in all three groups, but remained within the normoglycemic range (Fig. 1B).

HIIT increased plasma levels of adrenaline by  $0.11 \pm 0.04$  nmol/L, noradrenaline by  $4.1 \pm 0.4$  nmol/L and GH by  $14 \pm 4.7$  mU/L ( $p$  all  $< 0.05$ ), whereas plasma cortisol levels tended to increase (mean increase  $0.04 \pm 0.02$   $\mu$ mol/L,  $p = 0.06$ ). Hormonal responses to exercise were similar between all groups. All hormone levels returned to levels indistinguishable from those at rest days prior to the beginning of hypoglycemia (approximately 40 minutes after exercise), with the exception of plasma cortisol, which remained slightly elevated in diabetes patients with IAH.

#### Responses to hypoglycemia after HIIT or rest

**Hypoglycemic symptoms.** Compared to seated rest, HIIT reduced mean total hypoglycemic symptom scores ( $p = 0.01$ , Fig. 2) and peak symptom scores in patients with type 1 diabetes and NAH ( $p = 0.04$ , Table 2). These decreases were mainly due to reduced neurogenic and general symptom responses ( $p = 0.008$  and  $p = 0.04$ , Table 2). In healthy participants, HIIT numerically decreased all symptom categories, but this failed to reach statistical significance ( $p = 0.19$ , Fig. 2, Table 2). On both test days, diabetes patients with IAH had the lowest hypoglycemic symptom scores compared to the other two groups; their scores were not affected by prior HIIT ( $p = 0.80$ , Fig. 2, Table 2).

**Counterregulatory hormones and glucose infusion rate.** HIIT did not affect plasma adrenaline or noradrenaline responses to hypoglycemia in either subgroup (Fig. 3A-F). Plasma levels of glucagon increased to a similar extent in response to hypoglycemia on both test days in healthy participants, but – as expected – did not change on either day in the two patient groups (data not shown). HIIT suppressed the plasma cortisol response to hypoglycemia in healthy participants and patients with NAH (Fig. 3G-I) and suppressed the plasma GH responses in all groups (Fig. 4A-C). Glucose infusion rates during hypoglycemia

were not different between HIIT and rest days in any group ( $5.6 \pm 0.5$  versus  $4.8 \pm 1.0$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $4.4 \pm 0.6$  versus  $3.7 \pm 0.7$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and  $4.4 \pm 0.4$  versus  $4.6 \pm 0.4$   $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for healthy participants, patients with NAH, and patients with IAH, respectively). The plasma adrenaline response to hypoglycemia was significantly lower in patients with IAH compared to patients with NAH and healthy participants, on both study days ( $p < 0.005$ , Fig. 3A-C).

**Cognitive function.** All three subgroups performed worse on cognitive tests during hypoglycemia compared to the screening visit, with mean decreases in Z-scores of  $0.6 \pm 0.1$  and  $0.9 \pm 0.1$  on the HIIT and rest day, respectively (all  $p < 0.05$ , Fig. 5). Hypoglycemia-induced cognitive dysfunction was less pronounced after prior HIIT than after prior rest (main effect of intervention,  $p = 0.01$ ). There was no significant interaction effect (timepoint  $\times$  study group,  $p = 0.27$ ). Post hoc analysis revealed that the attenuating effect of HIIT on hypoglycemia-induced cognitive deterioration was only significant in patients with diabetes and NAH ( $p < 0.05$ ), but did not reach significance in the other two subgroups ( $p = 0.11$  for healthy participants and  $p = 0.46$  for patients with IAH).

## Discussion

The main finding of the present study is that a bout of high-intensity interval training suppresses symptoms of subsequent hypoglycemia in patients with type 1 diabetes and NAH. HIIT also causes less hypoglycemia-induced cognitive deterioration, an effect that was mainly driven by the NAH patient subgroup. HIIT did not affect hypoglycemic awareness in patients with IAH, likely because of a 'floor' effect in that symptom responses could not be further suppressed than they already were. In healthy participants, HIIT numerically decreased symptoms of hypoglycemia, but this failed to reach statistical significance. These data demonstrate that one short HIIT session is able to rapidly blunt hormonal and symptomatic

defenses against hypoglycemia in patients with NAH, which may increase the risk of post-exercise hypoglycemia.

Our findings of the suppressive effect of antecedent HIIT on defenses against hypoglycemia extend those of other exercise studies (26-29). However, there is substantial variation in the specific counterregulatory responses that are affected by antecedent exercise. Antecedent low- to moderate- intensity exercise (at 30 and 50% peak oxygen uptake) has been shown to cause a universal suppression of hormonal and symptomatic responses to next-day hypoglycemia (28). Remarkably, two bouts of more vigorous exercise (~70% peak oxygen uptake) had limited effects, in that only adrenaline responses to subsequent hypoglycemia were attenuated (27). We now demonstrate that an even more intensive exercise test causes blunting of symptomatic, cortisol and GH responses to subsequent hypoglycemia, but not of catecholamine responses. Similar defects in counterregulatory hormones were observed in antecedent hypoglycemia studies in which the interval between the stimulus and subsequent hypoglycemia was relatively short (30; 31). Taken together, it appears that antecedent exercise, similar to antecedent hypoglycemia, is able to induce a range of counterregulatory defects, the magnitude and components of which seem to depend on the particular exercise protocol used (e.g. exercise intensity, duration) and the timeframe between exercise and subsequent hypoglycemia.

The mechanisms underlying exercise-induced attenuation of counterregulatory hormone and symptom responses are currently not known. We hypothesize that elevated lactate levels in response to HIIT mediate a suppressive effect. High plasma lactate levels lead to an increase in brain lactate utilization, both after intravenous administration (32) and after vigorous exercise (33; 34). An increase in brain lactate oxidation during hypoglycemia has been

suggested to preserve brain metabolism (10; 12). This may prevent hypoglycemia-induced cognitive dysfunction, since the brain is no longer deprived of fuel. Maintenance of brain metabolism may simultaneously impede hypoglycemia sensing by the brain and thus suppress the consequent initiation of protective counterregulatory and symptom responses (35). Previous work found that administration of exogenous lactate during hypoglycemia suppresses symptoms and hormone responses, while preserving cognitive function, thus mimicking the situation seen in patients with IAH (10-13). In these patients, several cerebral adaptations have been observed during hypoglycemia, including increased capacity to use lactate (35-38). Alternatively, lactate may act as a metabolic regulator in the brain rather than as a fuel per se (39; 40).

Several arguments support a role for elevated lactate levels in the suppression of physiological responses to hypoglycemia after HIIT. First, we showed that the deterioration of cognitive function during hypoglycemia was less after HIIT than rest, which is in accordance with previous studies that infused lactate during hypoglycemia (10; 12). In addition, a recent exercise study found that higher lactate levels after HIIT were associated with better executive function during post-exercise recovery, under euglycemic conditions (41). We also observed fast suppression of hypoglycemic symptoms, cortisol and growth hormone responses after HIIT (i.e. less than 1 h after the initial stimulus). Whereas central nervous system adaptations to hypoglycemia are thought to take hours to days to become manifest (42), the suppressive effects of lactate are known to occur rapidly and probably do not involve an adaptation process (13). Interestingly, blunting of symptoms was most pronounced in participants with the highest symptom scores. Changes in symptoms were not correlated to changes in adrenaline responses. Increased capacity to transport lactate over the blood-brain barrier in patients with type 1 diabetes (as a result of prior exposure to hypoglycemia) may in part

explain differential effects of HIIT in patients with NAH and healthy controls (43). However, quantitative comparisons with studies using exogenous lactate during hypoglycemia need to acknowledge the influence of exercise itself, pH differences and the duration and stability of plasma lactate elevations.

Habituation of the brain in response to recurrent hypoglycemia is thought to underlie the development of IAH (44) and restoration of IAH can be achieved by scrupulous avoidance of hypoglycemia (45; 46). It has recently been speculated that ‘re-sensitizing’ the brain to hypoglycemia might also be achieved by exposition to high-intensity exercise as a novel stimulus in an animal model of IAH (47). In this model, antecedent hypoglycemia-induced defects in glucose counterregulation were restored 24 hours after a bout of high-intensity exercise. This differs from our findings, in that we observed no improvements in counterregulatory responses to hypoglycemia after HIIT in patients with type 1 diabetes and IAH. However, differences in species and study design, particularly the timeframe between exercise and hypoglycemia, should be acknowledged when explaining these seemingly contradictory results.

Our study design differed from most studies that addressed the impact of antecedent exercise on defenses against hypoglycemia, since the timeframe between exercise and hypoglycemia was short. This approach was chosen because we wanted to assess the effect of high endogenous plasma lactate levels during hypoglycemia, in view of the known effects of exogenous lactate during hypoglycemia. While we provide arguments for increased lactate levels as an explanation for the suppression of awareness of hypoglycemia and attenuation of cognitive dysfunction, our studies can not prove a cause and effect relationship. Although less likely, other mechanisms than lactate may explain the acute beneficial effects of HIIT on cognition, including increased arousal (48; 49). Participants could not be blinded for the

intervention (HIIT vs. rest): while unlikely, some influence of participant expectations on the results cannot be excluded. Strengths of our study include the randomized, cross-over design that allowed us to compare responses to HIIT and rest in the same participants, under similar conditions.

In conclusion, a short bout of high-intensity interval exercise suppresses symptoms of subsequent hypoglycemia in patients with type 1 diabetes, but does not affect awareness in patients with IAH. The role of exercise-induced lactate in mediating the suppressive effects of HIIT on hypoglycemic awareness should be further explored. Reduced symptomatic responses may increase the risk of hypoglycemia after intensive exercise.

## **Acknowledgements**

We are indebted to Iris Knoester and Lisa Simons (medical students, Radboud university medical center) for assistance during the glucose clamps. We thank Bregina Kersten for assistance with the exercise protocol (Department of Physiology, Radboud university medical center), Sandra Vos and Ilja Klabbers-Helsper (Department of Medical Psychology, Radboud university medical center) for their advice on cognitive testing, and lastly, all the volunteers for their participation and enthusiasm.

### Funding

Research support from the Dutch Diabetes Research Foundation (DFN 2012.00.1542) and the European Foundation for the Study of Diabetes is gratefully acknowledged.

### Contributors

HR, EW and BdG designed the study with input from MvdG, DT, RK and CT. HR performed the experiments and collected all the data. EW assisted with the experiments and data collection. HR analyzed the data and wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages. BdG 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.

### Duality of interest

The authors declare no potential conflicts of interests relevant for this study.



## References

1. Chimen M, Kennedy A, Nirantharakumar K, Pang TT, Andrews R, Narendran P: What are the health benefits of physical activity in type 1 diabetes mellitus? A literature review. *Diabetologia* 2012;55:542-551
2. Pivovarov JA, Taplin CE, Riddell MC: Current perspectives on physical activity and exercise for youth with diabetes. *Pediatr Diabetes* 2015;16:242-255
3. Gibala MJ, Little JP, Macdonald MJ, Hawley JA: Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol* 2012;590:1077-1084
4. Norton K, Norton L, Sadgrove D: Position statement on physical activity and exercise intensity terminology. *J Sci Med Sport* 2010;13:496-502
5. Marliss EB, Vranic M: Intense exercise has unique effects on both insulin release and its roles in glucoregulation: implications for diabetes. *Diabetes* 2002;51 Suppl 1:S271-283
6. Fahey AJ, Paramalingam N, Davey RJ, Davis EA, Jones TW, Fournier PA: The effect of a short sprint on postexercise whole-body glucose production and utilization rates in individuals with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2012;97:4193-4200
7. Guelfi KJ, Jones TW, Fournier PA: The decline in blood glucose levels is less with intermittent high-intensity compared with moderate exercise in individuals with type 1 diabetes. *Diabetes Care* 2005;28:1289-1294
8. Maran A, Pavan P, Bonsembiante B, Brugin E, Ermolao A, Avogaro A, Zaccaria M: Continuous glucose monitoring reveals delayed nocturnal hypoglycemia after intermittent high-intensity exercise in nontrained patients with type 1 diabetes. *Diabetes Technol Ther* 2010;12:763-768
9. Goodwin ML, Harris JE, Hernandez A, Gladden LB: Blood lactate measurements and analysis during exercise: a guide for clinicians. *J Diabetes Sci Technol* 2007;1:558-569
10. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans. *Diabetes* 1994;43:1311-1317
11. King P, Parkin H, Macdonald IA, Barber C, Tattersall RB: The effect of intravenous lactate on cerebral function during hypoglycaemia. *Diabet Med* 1997;14:19-28
12. Maran A, Cranston I, Lomas J, Macdonald I, Amiel SA: Protection by lactate of cerebral function during hypoglycaemia. *Lancet* 1994;343:16-20
13. Maran A, Crepaldi C, Trupiani S, Lucca T, Jori E, Macdonald IA, Tiengo A, Avogaro A, Del Prato S: Brain function rescue effect of lactate following hypoglycaemia is not an adaptation process in both normal and type I diabetic subjects. *Diabetologia* 2000;43:733-741
14. Cryer PE: Mechanisms of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med* 2013;369:362-372
15. Geddes J, Schopman JE, Zammitt NN, Frier BM: Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. *Diabet Med* 2008;25:501-504
16. Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W: Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. *Diabetes Care* 1995;18:517-522
17. Janssen MM, Snoek FJ, Heine RJ: Assessing impaired hypoglycemia awareness in type 1 diabetes: agreement of self-report but not of field study data with the autonomic symptom threshold during experimental hypoglycemia. *Diabetes Care* 2000;23:529-532
18. Borg GA: Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377-381
19. Uterwijk JMR: WAIS-III: Wechsler Adult Intelligence Scale III - Nederlandstalige bewerking. Lisse, Swets Test publishers, 2000

20. Schmand B, Groenink SC, van den Dungen M: [Letter fluency: psychometric properties and Dutch normative data]. *Tijdschr Gerontol Geriatr* 2008;39:64-76
21. Gronwall DM: Paced auditory serial-addition task: a measure of recovery from concussion. *Percept Mot Skills* 1977;44:367-373
22. Brands AM, Kessels RP, Hoogma RP, Henselmans JM, van der Beek Boter JW, Kappelle LJ, de Haan EH, Biessels GJ: Cognitive performance, psychological well-being, and brain magnetic resonance imaging in older patients with type 1 diabetes. *Diabetes* 2006;55:1800-1806
23. Abbink EJ, Walker AJ, van der Sluijs HA, Tack CJ, Smits P: No role of calcium- and ATP-dependent potassium channels in insulin-induced vasodilation in humans in vivo. *Diabetes Metab Res Rev* 2002;18:143-148
24. Hendriks T: Radioimmunoassay of clinically meaningful components of circulating glucagon: removal of big plasma glucagon prior to assay. *Clinica chimica acta; international journal of clinical chemistry* 1984;140:301-307
25. Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ: Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. *Clin Chem* 1995;41:1455-1460
26. Galassetti P, Mann S, Tate D, Neill RA, Costa F, Wasserman DH, Davis SN: Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol Endocrinol Metab* 2001;280:E908-917
27. McGregor VP, Greiwe JS, Banarer S, Cryer PE: Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure. *Diabetes* 2002;51:1485-1492
28. Sandoval DA, Guy DL, Richardson MA, Ertl AC, Davis SN: Effects of low and moderate antecedent exercise on counterregulatory responses to subsequent hypoglycemia in type 1 diabetes. *Diabetes* 2004;53:1798-1806
29. Sandoval DA, Guy DL, Richardson MA, Ertl AC, Davis SN: Acute, same-day effects of antecedent exercise on counterregulatory responses to subsequent hypoglycemia in type 1 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2006;290:E1331-1338
30. Davis MR, Shamon H: Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 1991;73:995-1001
31. Davis MR, Mellman M, Shamon H: Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 1992;41:1335-1340
32. Smith D, Pernet A, Hallett WA, Bingham E, Marsden PK, Amiel SA: Lactate: a preferred fuel for human brain metabolism in vivo. *J Cereb Blood Flow Metab* 2003;23:658-664
33. Dalsgaard MK, Quistorff B, Danielsen ER, Selmer C, Vogelsang T, Secher NH: A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J Physiol* 2004;554:571-578
34. van Hall G, Stromstad M, Rasmussen P, Jans O, Zaar M, Gam C, Quistorff B, Secher NH, Nielsen HB: Blood lactate is an important energy source for the human brain. *J Cereb Blood Flow Metab* 2009;29:1121-1129
35. Wiegers EC, Rooijackers HM, Tack CJ, Heerschap A, de Galan BE, van der Graaf M: Brain Lactate Concentration Falls in Response to Hypoglycemia in Patients With Type 1 Diabetes and Impaired Awareness of Hypoglycemia. *Diabetes* 2016;65:1601-1605
36. Bischof MG, Brehm A, Bernroider E, Krssak M, Mlynarik V, Krebs M, Roden M: Cerebral glutamate metabolism during hypoglycaemia in healthy and type 1 diabetic humans. *Eur J Clin Invest* 2006;36:164-169

37. van de Ven KC, Tack CJ, Heerschap A, van der Graaf M, de Galan BE: Patients with type 1 diabetes exhibit altered cerebral metabolism during hypoglycemia. *J Clin Invest* 2013;123:623-629
38. Terpstra M, Moheet A, Kumar A, Eberly LE, Seaquist E, Oz G: Changes in human brain glutamate concentration during hypoglycemia: insights into cerebral adaptations in hypoglycemia-associated autonomic failure in type 1 diabetes. *J Cereb Blood Flow Metab* 2014;34:876-882
39. Lauritzen KH, Morland C, Puchades M, Holm-Hansen S, Hagelin EM, Lauritzen F, Attramadal H, Storm-Mathisen J, Gjedde A, Bergersen LH: Lactate Receptor Sites Link Neurotransmission, Neurovascular Coupling, and Brain Energy Metabolism. *Cereb Cortex* 2013;
40. Herzog RI, Jiang L, Herman P, Zhao C, Sanganahalli BG, Mason GF, Hyder F, Rothman DL, Sherwin RS, Behar KL: Lactate preserves neuronal metabolism and function following antecedent recurrent hypoglycemia. *J Clin Invest* 2013;123:1988-1998
41. Tsukamoto H, Suga T, Takenaka S, Tanaka D, Takeuchi T, Hamaoka T, Isaka T, Ogoh S, Hashimoto T: Repeated high-intensity interval exercise shortens the positive effect on executive function during post-exercise recovery in healthy young males. *Physiol Behav* 2016;160:26-34
42. Frier BM: Impaired awareness of hypoglycaemia. In *Hypoglycaemia in clinical diabetes*, 3th ed. Frier BM, Heller SR, McCrimmon RJ, Eds. Chichester, John Wiley & Sons, 2014, p. 114-144
43. Mason GF, Petersen KF, Lebon V, Rothman DL, Shulman GI: Increased brain monocarboxylic acid transport and utilization in type 1 diabetes. *Diabetes* 2006;55:929-934
44. McCrimmon RJ: Update in the CNS response to hypoglycemia. *J Clin Endocrinol Metab* 2012;97:1-8
45. Dagogo-Jack S, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 1994;43:1426-1434
46. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, et al.: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 1993;42:1683-1689
47. McNeilly AD, Gallagher JR, Huang JT, Ashford ML, McCrimmon RJ: High Intensity Exercise as a Dishabituating Stimulus Restores Counterregulatory Responses in Recurrently Hypoglycemic Rodents. *Diabetes* 2017;
48. Lambourne K, Tomporowski P: The effect of exercise-induced arousal on cognitive task performance: a meta-regression analysis. *Brain Res* 2010;1341:12-24
49. Skriver K, Roig M, Lundbye-Jensen J, Pingel J, Helge JW, Kiens B, Nielsen JB: Acute exercise improves motor memory: exploring potential biomarkers. *Neurobiol Learn Mem* 2014;116:46-58

**Tables****Table 1: Participant characteristics**

	<b>Healthy controls</b>	<b>T1DM-NAH</b>	<b>T1DM-IAH</b>
Age, yrs	25.2±5.5 (19-37)	23.9±4.4 (19-34)	25.7±5.8 (19-35)
Sex, M:F	5:5	4:6	5:5
BMI, kg/m <sup>2</sup>	22.5±1.8	23.0±2.3	23.4±1.4
Exercise, hours/week	3.7±2.2	4.3±2.9	4.9±3.7
Score on modified Cox questionnaire (range)	-	0.8±1.3 (0-4)	3.7±1.3 (1-5)
HbA <sub>1C</sub> , % (mmol/mol)	-	7.5±0.6 (59.0±6.9)	6.9±0.7 (52.1±7.7)
Duration of diabetes, yrs	-	10.7±4.5	13.9±8.1
Insulin dose, Units/kg/day (range)	-	0.7±0.4 (0.4-0.9)	0.6±0.4 (0.4-0.8)
Insulin regimen, n	-		
Multi-injection therapy		5	2
Pump therapy		5	8

*Data are presented as number or means ± SD. Abbreviations: T1DM, type 1 diabetes; NAH, normal awareness of hypoglycemia; IAH, impaired awareness of hypoglycemia; BMI, body-mass index.*

**Table 2. Symptom scores during hypoglycemia**

	Healthy controls		T1DM-NAH		T1DM-IAH	
	Rest	HIIT	Rest	HIIT	Rest	HIIT
Peak	20.3 ± 3.5†	17.8 ± 2.7	31.1 ± 5.6†	21.9 ± 3.9*†	10.5 ± 1.6	10.9 ± 2.0
Neurogenic	7.7 ± 1.4†	6.6 ± 0.9†	10.9 ± 2.0†	7.0 ± 1.6*	3.3 ± 0.5	3.3 ± 0.6
Neuroglycopenic	5.6 ± 1.4†	4.3 ± 1.1	9.2 ± 1.8†	6.7 ± 1.3†	2.9 ± 0.5	3.0 ± 0.6
General	3.5 ± 0.9†	3.3 ± 0.7	5.2 ± 0.9†	3.8 ± 0.7*†	1.1 ± 0.3	1.6 ± 0.5

*Peak scores represent the highest total symptom score during hypoglycemia ( $\pm$ SEM), irrespective of the time point. All other symptom scores were averaged over the three time points (after 20, 40 and 60 minutes of hypoglycemia, mean  $\pm$  SEM), \* $p$ <0.05 for HIIT vs. Rest, † $p$ <0.05 versus T1DM-IAH.*

## Figure legends

**Figure 1:** Time courses of plasma glucose (A, B) and plasma lactate (C, D) during the rest day (left panel) or HIIT day (right panel). Dashed lines represent the beginning and end of the euglycemic phase, and the beginning of the hypoglycemic phase, respectively. During the euglycemic phase, participants performed HIIT or rested. Baseline values represent the first sample obtained upon arrival. Open circles, healthy controls; black squares, patients with type 1 diabetes (T1DM) and NAH (T1DM-NAH); black triangles, patients with IAH (T1DM-IAH).

**Figure 2:** Average total hypoglycemic symptom scores. Average individual (individual dots) and average group total symptom scores (gray bars) during hypoglycemia after prior HIIT or seated rest in healthy controls (A), T1DM-NAH (B) and T1DM-IAH (C). Symptom scores after 20, 40 and 60 minutes of hypoglycemia were averaged to provide one composite score.

**Figure 3:** Adrenaline, noradrenaline and cortisol responses to hypoglycemia after prior rest (open circles) or prior HIIT (black squares) in healthy controls (left), T1DM-NAH (middle) and T1DM-IAH (right). Eu denotes euglycemia, Hypo denotes hypoglycemia.

**Figure 4:** Growth hormone responses to hypoglycemia after prior rest (open bars) or prior HIIT (black bars) in healthy controls (left), T1DM-NAH (middle) and T1DM-IAH (right). Eu denotes euglycemia, Hypo denotes hypoglycemia, \* $p < 0.05$ .

**Figure 5:** Hypoglycemia-induced change in cognitive function after seated rest (open bars) or HIIT (closed bars). Mean (with SEM) differences in Z-scores between the screening visit and hypoglycemia are depicted, \* $p < 0.05$ .

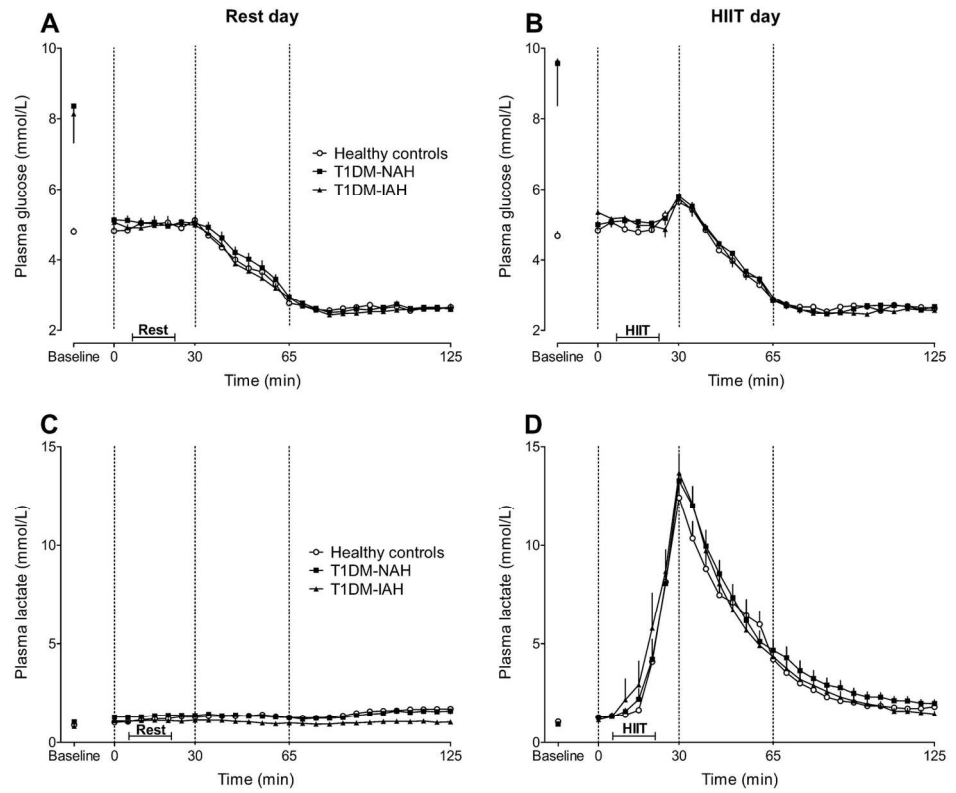


Figure 1: Time courses of plasma glucose (A, B) and plasma lactate (C, D) during the rest day (left panel) or HIIT day (right panel). Dashed lines represent the beginning and end of the euglycemic phase, and the beginning of the hypoglycemic phase, respectively. During the euglycemic phase, participants performed HIIT or rested. Baseline values represent the first sample obtained upon arrival. Open circles, healthy controls; black squares, patients with type 1 diabetes (T1DM) and NAH (T1DM-NAH); black triangles, patients with IAH (T1DM-IAH).

149x124mm (300 x 300 DPI)

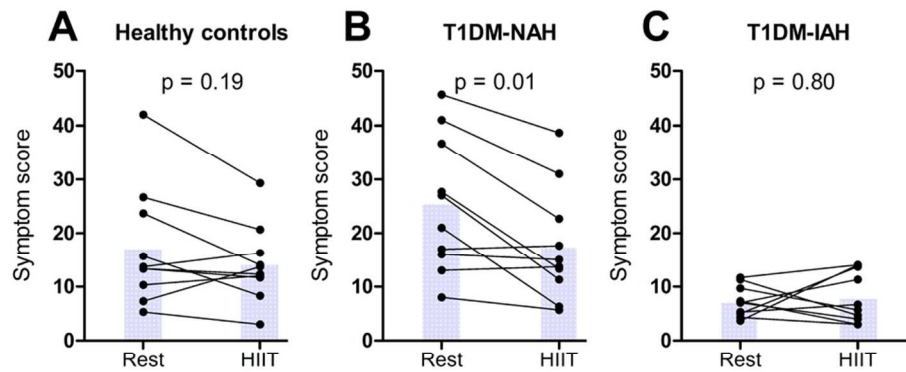


Figure 2: Average total hypoglycemic symptom scores. Average individual (individual dots) and average group total symptom scores (gray bars) during hypoglycemia after prior HIIT or seated rest in healthy controls (A), T1DM-NAH (B) and T1DM-IAH (C). Symptom scores after 20, 40 and 60 minutes of hypoglycemia were averaged to provide one composite score.

90x45mm (300 x 300 DPI)



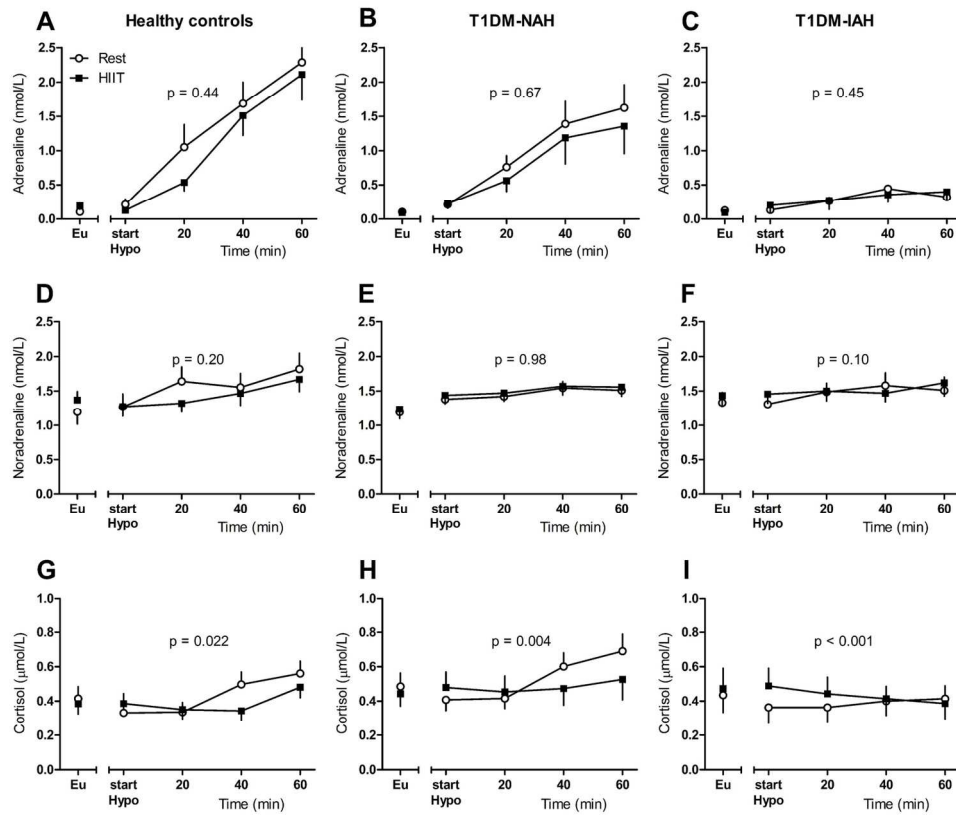


Figure 3: Adrenaline, noradrenaline and cortisol responses to hypoglycemia after prior rest (open circles) or prior HIIT (black squares) in healthy controls (left), T1DM-NAH (middle) and T1DM-IAH (right). Eu denotes euglycemia, Hypo denotes hypoglycemia.

149x124mm (300 x 300 DPI)

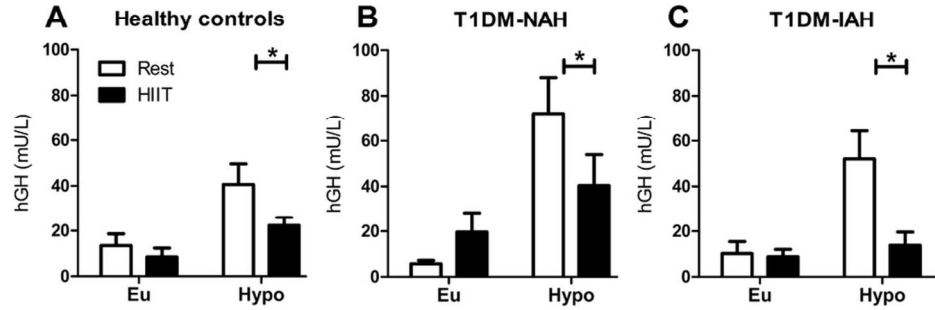


Figure 4: Growth hormone responses to hypoglycemia after prior rest (open bars) or prior HIIT (black bars) in healthy controls (left), T1DM-NAH (middle) and T1DM-IAH (right). Eu denotes euglycemia, Hypo denotes hypoglycemia, \* $p < 0.05$ .

88x44mm (300 x 300 DPI)

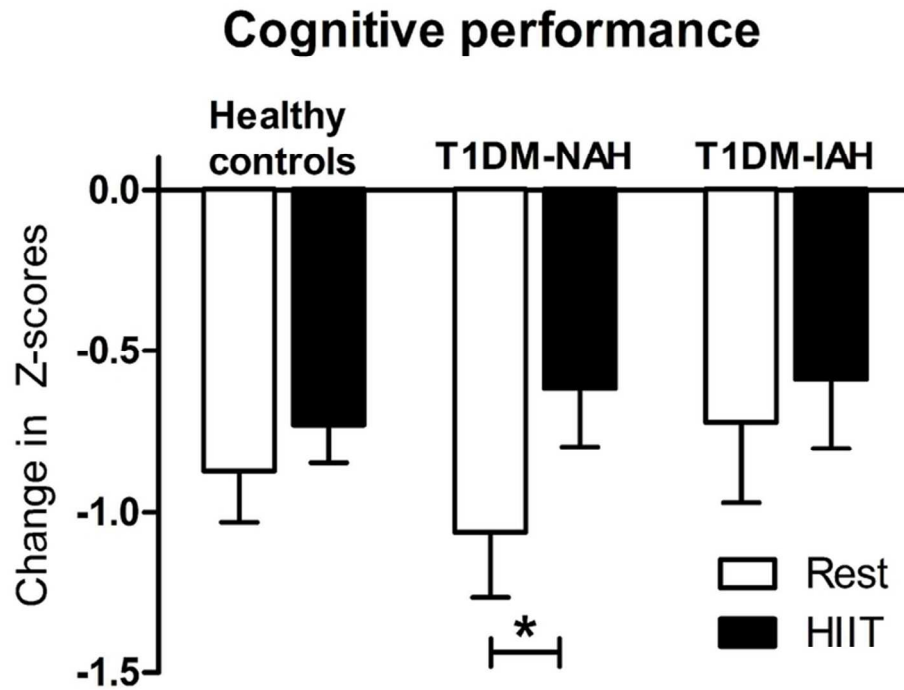


Figure 5: Hypoglycemia-induced change in cognitive function after seated rest (open bars) or HIIT (closed bars). Mean (with SEM) differences in Z-scores between the screening visit and hypoglycemia are depicted, \* $p < 0.05$ .

70x55mm (300 x 300 DPI)