Metabolic Brain Disease
https://doi.org/10.1007/s11011-021-00727-2

ORIGINAL ARTICLE

Placebo controlled phase II clinical trial: Safety and efficacy of combining intranasal insulin & acute exercise

Kathryn L. Gwizdala[1] · David P. Ferguson[1] · Jeffery Kovan[2] · Vera Novak[3] · Matthew B. Pontifex[1]

Received: 11 November 2020 / Accepted: 31 March 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

A growing number of investigations are exploring the utility of intranasal insulin as a means of mitigating cognitive decline. However, as a basic tenant of dementia prevention programs is increasing physical activity, it is essential to obtain a preliminary assessment of the safety profile of combining intranasal insulin with physical activity; to ensure that undue risks are not incurred. Utilizing a randomized double-blind placebo-controlled design, a sample of 116 non-diabetic, fasted college-aged adults were randomly assigned to receive a dose of 0-to-120 IU of NovoLog (Insulin Aspart) before being randomized to 20 min of exercise or sitting control condition. The safety of intranasal insulin was assessed by examining the incidence of potential symptoms of hypoglycemia and changes in peripheral blood glucose. The efficacy of a combination therapeutic approach was assessed using behavioral measures of inhibition and sustained attention alongside neuroelectric indices of attentional engagement. The frequency of symptoms reported following administration of intranasal insulin were not observed to interact with exercise so as to make their occurrence any more or less prominent, nor was the frequency observed to relate to the dose of intranasal insulin. However, doses of intranasal insulin of 100 IU or more were observed to result in a 7-fold increase in the likelihood of a level 1 hypoglycemic event for those individuals in the exercise condition. This study provides preliminary evidence to suggest that exercise is not associated with an increase in risk when combined with lower doses of intranasal insulin.

Clinical trial registration The trial is registered at ClinicalTrials.gov, number NCT04292535.

Keywords Physical activity · Inhibition · Interference control · Sustained attention · ERP · P3

As many as 1 in 9 individuals in the United States aged 45 and older are experiencing subjective cognitive decline, with projections suggesting that as many as 14 million individuals could suffer from Alzheimer's Disease by 2060 (Matthews et al. 2018). With the increasing prevalence of Alzheimer's Disease and related dementias, it is essential to understand potential therapeutic routes that decrease the risk of developing these disorders or at the very least delay its progression (Baharfuchs et al. 2013; Farina et al. 2002; Grossberg et al. 2013; in 't Veld et al. 2001; Morris et al. 2017; Morris et al. 2015; Scarmeas et al. 2006). One such approach is the utilization of intranasal insulin to mitigate the dysregulation of brain metabolism (e.g. decreased cerebral glucose uptake, reduced insulin signaling, etc.) associated with Alzheimer's Disease (Claxton et al. 2015; Craft et al. 2012; Heni et al. 2015; Kullmann et al. 2016; Reger et al. 2006, 2008). However, given that a basic tenant of dementia prevention programs is increasing exercise related physical activity to stave off memory related declines (Baker et al. 2010; Intlekofer and Cotman 2013; Morris et al. 2017); it is essential to understand how intranasal insulin combines with exercise given the known risks of exercise and insulin administration (Berger et al. 1978; Tuominen et al. 1995). Accordingly, while intranasal insulin gains access to the brain with little peripheral effects (Born et al. 2002; Craft et al. 2012; Novak et al. 2014; Schmid et al. 2018), it is unknown to what extent negative outcomes might manifest when intranasal insulin therapies are combined with exercise. The aim of the present investigation was to provide preliminary insight into the dose-

The results of this manuscript have not been previously presented.

✉ Matthew B. Pontifex
pontifex@msu.edu

[1] Department of Kinesiology, Michigan State University, 126E IM Sports Circle, East Lansing, MI 48824-1049, USA

[2] Department of Intercollegiate Athletics, Michigan State University, East Lansing, MI, USA

[3] Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Published online: 15 April 2021

response safety profile of intranasal insulin in combination with an exercise protocol while also providing preliminary estimates of the efficacy of such combination therapeutic approaches for enhancing cognition.

The growing interest in intranasal insulin administration stems from its ability to manipulate cerebral glucose uptake, which offers considerable potential benefits for regulating altered metabolic profiles in the brain present within neurodegenerative diseases such as Alzheimer's disease (Craft et al. 2012, 2020; Heni et al. 2015; Kullmann et al. 2016). When administered intranasally, it has been proposed that insulin is able to directly affect the central nervous system by taking an extracellular route through the olfactory epithelium, bypassing the blood brain barrier and entering the subarachnoid space in the brain (Bakirtzi et al. 2009; Born et al. 2002). Given that insulin has been found to have specific receptors in areas of the frontal cortex and hippocampus (Hill et al. 1986; Unger et al. 1991); it is unsurprising that intranasal insulin administration in healthy, non-diabetic individuals has been observed to transiently enhance select aspects of cognition including cognitive control, visuospatial attention/memory, and long term memory (Benedict et al. 2004, 2006, 2006; Brünner et al. 2015; Novak et al. 2014). Given the use of healthy, cognitively-intact individuals, however; enhancements in cognition may not always be readily observable through behavioral metrics of performance alone.

Accordingly, event-related brain potentials (ERPs) provide a means of gaining insight into a subset of cognitive operations occurring between stimulus encoding and response production. In an initial investigation, Kern and colleagues (Kern et al. 1999) observed reductions in neuroelectric indices of attentional engagement following a 20 IU dose of intranasal insulin. Specifically, a reduction in the amplitude and an increase in the latency of the P3 ERP component assessed in response to a simple stimulus discrimination task was observed in a sample of 18 healthy college-aged adults; suggesting that intranasal insulin may negatively impact upon the allocation of attentional resources during stimulus engagement and stimulus classification and evaluation speed, respectively. Given the paucity of literature in this area, it is important to not over-interpret such findings; however, clearly they suggest that further research is necessary to better characterize the potential effects — both positive and negative — of intranasal insulin on cognitive processes.

A burgeoning body of research has demonstrated that intranasal insulin administration has little impact upon peripheral blood glucose — at least in resting individuals. In a recent review of the safety of intranasal insulin, Schmid and colleagues (Schmid et al. 2018) characterized a total of 38 studies encompassing 1,092 unique individuals; observing minimal impact of intranasal insulin on peripheral blood glucose levels and no adverse events reported. However, exercise has the potential to alter the safety profile of intranasal insulin. The feed forward mechanism to maintain blood glucose homeostasis during exercise only keeps glucose levels constant for so long and as the exercise duration increases, glucose levels will start to decrease (Brooks et al. 2004). Due to the body's increased demand for energy, the introduction of an additional amount of insulin might upset the homeostatic balance by shuttling glucose to inactive tissues during exercise thereby creating competition for fuel and amplify the rate at which glucose availability starts to decrease. It is also important to consider the potential risk that insulin administered intranasally has for being transported into the lungs as a result of the increased ventilation during exercise. Specifically, when the droplet size of intranasal insulin falls below 30 μm it may be lost to the lungs; thereby posing as a risk to impact peripheral blood glucose in the same manner as Afrezza (FDA approved rapid-acting inhaled insulin powder, into the lungs, for glycemic control in individuals diagnosed with diabetes) (Afrezza 2020; Giroux, 2005). During exercise, the increased airflow through the nose and mouth could potentially alter the droplet size or even cause appropriately sized droplets to move into the lungs (Hui et al. 2009). Thus, given that individuals with or at risk for Alzheimer's disease and related dementias are recommended to engage in exercise (Buchman et al. 2012; Yu et al. 2015); the use of intranasal insulin without an understanding of its potential interactive effects with exercise could place patients at elevated risk for potentially serious hypoglycemic events.

The purpose of the present investigation was to provide a preliminary assessment of the safety profile of combining intranasal insulin with exercise; to ensure that undue risks are not incurred through combination therapeutic approaches. To this end, a randomized double-blind placebo-controlled design was employed to examine the extent to which symptoms of hypoglycemia might manifest when intranasal insulin administration was followed by a bout of exercise in a sample of healthy, non-diabetic, fasted college-aged adults. This approach enabled the characterization of the dose-response safety profile of intranasal insulin administered in doses ranging from 0 IU to 120 IU. A second aim of the investigation was to provide a preliminary assessment of the efficacy of combining intranasal insulin with exercise for enhancing inhibitory aspects of cognitive control and sustained attention alongside neuroelectric indices of attentional engagement. An understanding of the safety and efficacy of combination therapeutic approaches is vital to support the continued use of intranasal insulin as a potential aid for neurodegenerative diseases such as Alzheimer's disease (Craft et al. 2012; Galindo-Mendez et al. 2020).

Method

Participants

A sample of 116 college aged young adults ($M = 20.7 \pm 2.5$ years, 72 females, 17.2 % nonwhite) participated in this

investigation at Michigan State University. See Fig. 1a for CONSORT flow diagram of enrollment.All participants provided written informed consent; reported being free of neurological disorders or physical disabilities; reported that they were not diabetic, pregnant, or were experiencing any sinus inflammation at the time of testing; and indicated having normal or corrected-to-normal vision. Demographic data for all participants are provided in Table 1. This investigation was approved by the Michigan State University Human Research Protection Program.

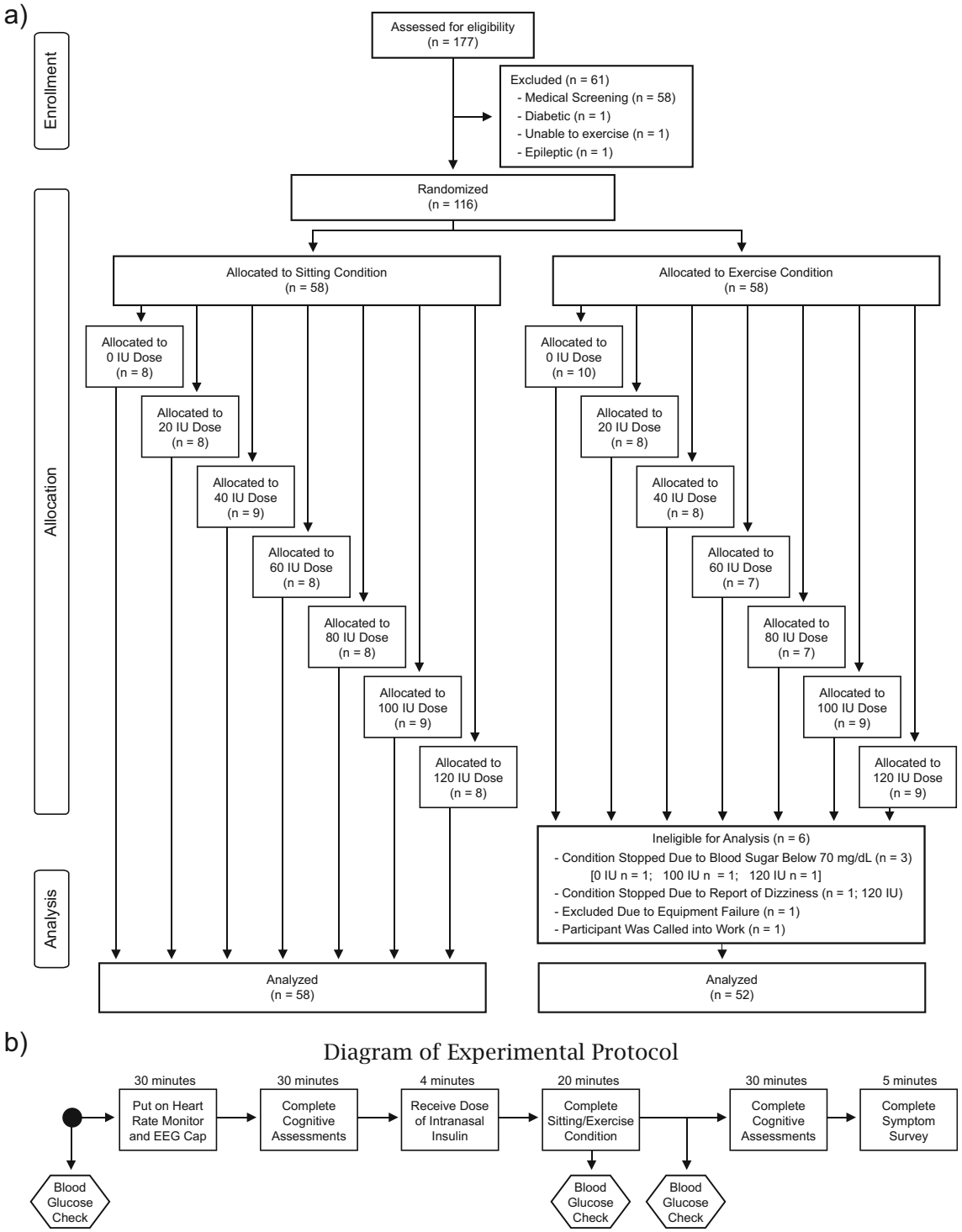

Fig. 1 **a** CONSORT flow chart of the experimental protocol. **b** Schematic diagram depicting the experimental protocol

Table 1 Demographics of all randomized participants (mean ± SD)

Measure	All Participants
N	116 (72 females)
Age (years)	20.7±2.5
Percent identifying as Nonwhite	17.2 %
Percent identifying as Hispanic or Latino	6.0 %
Education (years)	13.9±2.2
WASI-II (IQ)	107.5±9.9
Fasting blood glucose (mg/dL)	94.6±8.6
Aerobic Fitness Percentile	46.9±24.8

Note: WASI-II: Wechsler Abbreviated Scale of Intelligence 2nd Edition two-part subtest composite (Wechsler 2011). Aerobic Fitness Percentile was computed based upon maximal oxygen consumption (VO_2max) normative values (Shvartz and Reibold 1990)

Procedure

On the screening day of the testing protocol, following consenting to participate in this investigation; participants completed a brief health and history demographics questionnaire, a physical activity readiness questionnaire (Thomas et al. 1992), a medical screening questionnaire, the Wechsler Abbreviated Scale of Intelligence – 2nd Edition (WASI-II; (Wechsler 2011), and a test of maximal oxygen uptake (VO_2max; using the same protocols as reported in Chandler et al. 2019; Pontifex et al. 2016). The medical screening questionnaire ensured that all participants were free of medication for high blood pressure, cholesterol, asthma, heart issues, depression/anxiety; and were not currently on any beta-blockers, sulfonamide antibiotics, corticosteroids, protease inhibitors, or Clonidine. Participants who were eligible to continue in the study were then randomly assigned using a serial stratification approach accounting for biological sex to either an exercise [active experimental group] condition or sitting [control experimental group] condition. Within each condition; participants were subsequently randomly assigned using a serial stratification approach accounting for biological sex to receive a dose of either 0, 20, 40, 60, 80, 100, or 120 IU of fast acting insulin (Novolog Insulin Aspart 100 mg/mL) administered into the intranasal mucosa (see Fig. 1a for CONSORT flow diagram).

Participants were instructed to refrain from exercise or caffeine intake, and too fast for at least 3 h prior to participating in the second day of the testing protocol. Upon arriving to start the second day of testing (see Fig. 1b for diagram of experimental protocol), participants' blood glucose level was assessed via a glucometer (CVS Health Advanced Blood Glucose Meter, United States) to ensure that their blood glucose fell within the 70–115 mg/dL guideline to exercise safely. Following this, participants were outfitted with a polar heart rate monitor (Model H7, Polar Electro, Finland) to assess heart rate during the experimental condition and then were prepared for neuroelectric testing. Participants were subsequently seated in a sound-attenuated testing chamber approximately 1 m away from the computer monitor where they completed tests of inhibitory control and sustained attention while behavioral and neuroelectric measures were assessed. Once the pretest cognitive assessment was completed, participants were administered solution into their intranasal mucosa.

Using a double-blind, placebo-controlled approach; identical vials of saline solution and Novolog insulin aspart 100 mg/mL were obtained differentiated only by the color of the stopper top, and labeled as investigational drug A and investigational drug B. All participants were administered 6 doses of 0.2 mL of solution via the LMA MAD Nasal Intranasal Mucosal Atomization device (Teleflex 2013) into alternating nostrils with a total administration volume of 1.2 mL. The dose of intranasal insulin was controlled by varying the number of doses which contained saline relative to the doses which contained Novolog insulin aspart 100 mg/mL (see Fig. 2). The experimenters were aware of the number of doses of investigational drug A and investigational drug B administered; but were unaware which solution or stopper top was associated with insulin or saline. Following administration of the solution, participants completed the sitting/exercise experimental conditions.

The sitting experimental condition consisted of a 20 min duration where participants sat in a chair (HR = 73.2 bpm [95 % CI: 70.3 to 76.2]). The exercise experimental condition consisted of similar 20 min duration of exercise on a motor-driven treadmill at an intensity between 60 and 65 % of maximum heart rate achieved during the test of maximal oxygen uptake (HR = 118.1 bpm [95 % CI: 116.2 to 120.1]). During both the sitting and exercise experimental conditions, participants watched an emotionally neutral video (minutes 65–85 from Cooter and Holt (2011)). Midway through the sitting and exercise experimental conditions and again 5 min following the completion of the experimental conditions, peripheral blood glucose was assessed via a glucometer. If peripheral blood glucose fell below 70 mg/dL, testing was immediately discontinued (this occurred for 3 participants randomized to the exercise condition; 1 in the 0 IU dose, 1 in the 100 IU dose, and 1 in the 120 IU dose) for all measures. Following the peripheral blood glucose assessment, participants again completed the tests of inhibitory control and sustained attention while behavioral and neuroelectric measures were assessed. Finally, upon completion of the cognitive assessments, participants completed a symptom survey which assessed potential side effects of intranasal insulin that participants might

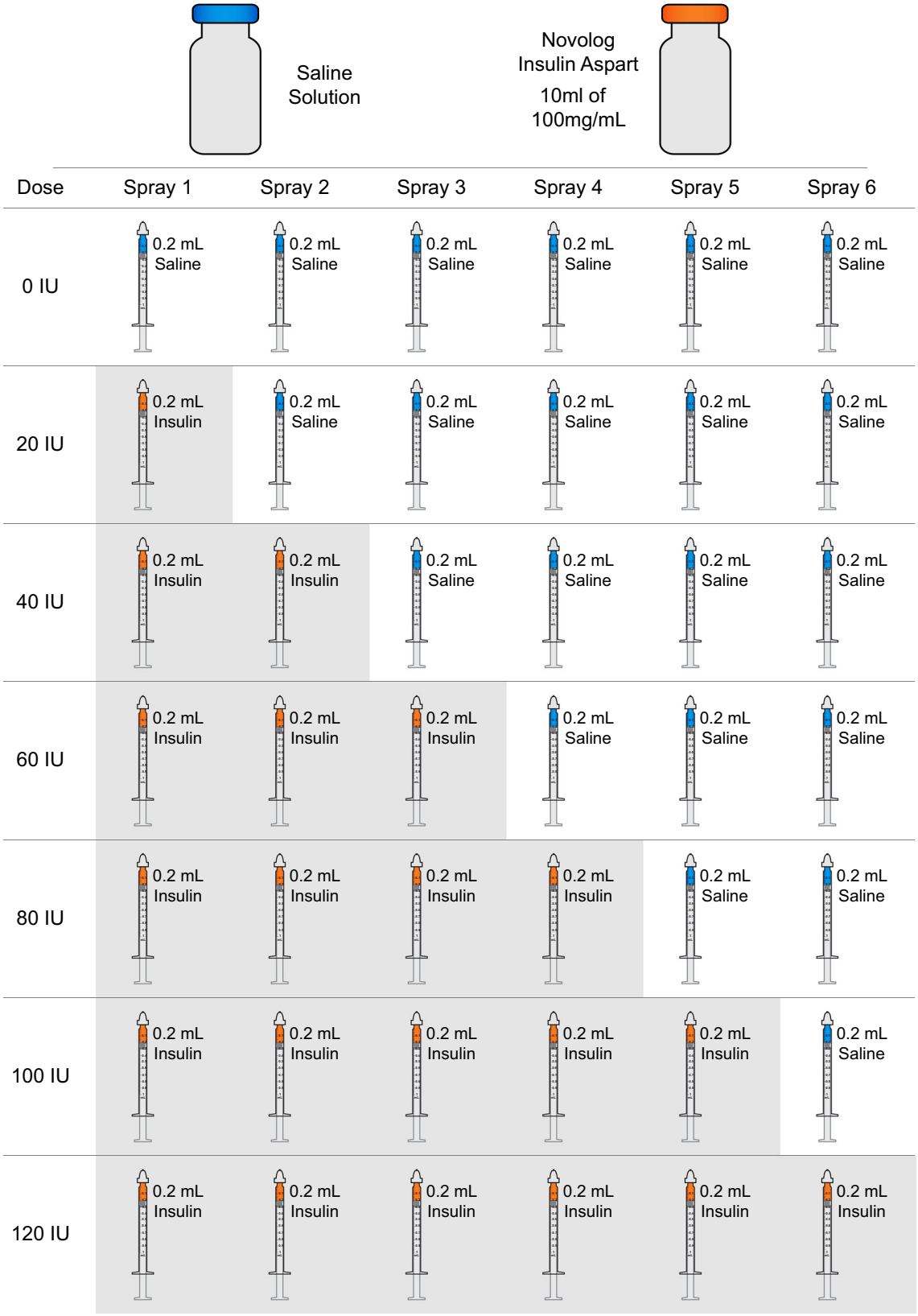

Fig. 2 Illustration of the intranasal insulin dosing protocol

have experienced at any time during the experimental protocol and if participants were still experiencing those symptoms. The protocol of this investigation is registered at ClinicalTrials.gov [number NCT04292535].

Inhibitory control task

Inhibitory control was assessed using a letter version of the Eriksen flanker task (Eriksen and Eriksen, 1974; McGowan et al. 2019), which is classified as a behavioral response selection construct of the inhibition/suppression focus and the performance monitoring focus of cognitive control according to the NIMH Research Domain Criteria (RDoC) classification system. Participants were instructed to attend to and to respond as accurately as possible to a centrally presented stimulus nested amid either congruous ('MMMMM') or incongruous ('NNMNN') flanking stimuli. Participants completed 80 practice trials at pretest followed by 160 trials grouped into two blocks of 80 trials, each consisting of equiprobable congruency and directionality; at each assessment period (160 trials pre-experimental condition and 160 trials post-experimental condition). For each block of trials, participants were presented with perceptually similar letter pairs (e.g., pretest block 1: M – N, pretest block 2: E – F, posttest block 1: I – T, posttest block 2: U – V) and were instructed to respond by pressing the button assigned to the letter presented in the middle of the flanking letters.

To ensure a high degree of task difficulty, at the midpoint of each block participants were given instructions to reverse the button-letter assignments (e.g., left button press for "M" through the first 40 trials and then right button press for "M" through the last 40 trials). Flanking letters were presented 300 ms prior to the onset of the target letter, and all five letters remained on the screen for a subsequent 100 ms (for a total stimulus duration of 400 ms) with a response window of 1000 ms and a variable inter-trial interval of 2300, 2400, 2500, 2600, or 2700 ms. All stimuli were 1.5 cm tall white block letters presented focally on a black background. Stimulus presentation and timing were controlled using PsychoPy 1.86 (Peirce 2009). Reaction time was quantified within each congruency as the mean speed of responding following the onset of the stimulus only for correct trials. Response accuracy was quantified within each congruency as the proportion of correct responses relative to the number of trials administered.

Sustained attention task

Sustained attention was assessed using the rapid visual information processing task (Chandler et al. 2020; Neale et al. 2015) which is classified as a behavioral construct of attention according to the NIMH Research Domain Criteria (RDoC) classification system. Participants were presented with a series of single digits (1–9) in a box in the center of the screen at a rate of 100 digits/min and were instructed to make a button response with their right thumb as soon as they detected any of the three target sequences: '2-4-6,' '3-5-7,' or '4-6-8.' To minimize working memory load, the three target sequences were presented on the bottom of the screen throughout the duration of the task. At pretest, participants completed a 1 min practice period prior to beginning the test trials which contained a series of 402 digits with 64 target sequences embedded. At posttest, participants completed another series of 402 digits with 64 target sequences embedded. Reaction time was quantified as the mean speed of responding following the presentation of the final digit of the target sequences only for correct sequence identifications. Response accuracy was quantified as the proportion of responses that correctly coincided with the 64 target sequences presented.

Neuroelectric indices of attentional engagement

During completion of the inhibitory control and sustained attention tasks, EEG activity was recorded from 32 electrode sites (Fpz, Fz, FCz, Cz, CPz, Pz, POz, Oz, F7/3/4/8, FT7/8, FC3/4, T7/8, C3/4, M1/2, CP3/4, TP7/8, P7/3/4/8, PO5/6) arranged in an extended montage based on the International 10–10 system (Chatrian et al. 1985) using a Neuroscan Quik-Cap (Compumedics, Inc., Charlotte, NC). Recordings were referenced to averaged mastoids (M1, M2), with AFz serving as the ground electrode. In addition, electrodes were placed above and below the left orbit and on the outer canthus of both eyes to monitor electrooculographic (EOG) activity with a bipolar recording. Continuous data was digitized at a sampling rate of 2048 Hz and amplified 500 times with a DC to 70 Hz filter using a Neuroscan Grael amplifier. The EEG data were then imported into EEGLAB (Delorme and Makeig 2004), downsampled to 1024 Hz and prepared for temporal ICA decomposition. Data more than 2 s prior to the first event marker and 2 s after the final event marker were removed to restrict computation of ICA components to task-related activity. The continuous data was filtered using a 0.05 Hz high-pass 2nd order Butterworth IIR filter to remove slow drifts (Pontifex et al. 2017), and the mastoids electrodes were removed prior to ICA decomposition. ICA decomposition was performed using the extended infomax algorithm to extract sub-Gaussian components using the default settings called in the MATLAB implementation of this function in EEGLAB with the block size heuristic (floor[sqrt(EEG.pnts/3)]) drawn from MNE-Python (Gramfort et al. 2013). Following the ICA decomposition, the eyeblink artifact components were identified using the icablinkmetrics function (Pontifex et al. 2017) and the EEG data was reconstructed without the eyeblink artifact.

Following removal of the eye blink components, stimulus-locked epochs were created for correct trials from − 500 to 1,500 ms around the stimulus, baseline corrected using the − 100 to 0 ms pre-stimulus period, and filtered using a zero phase shift low-pass filter at 30 Hz. Trials with artifact exceeding ± 100 μV were rejected. To ensure the integrity of the signal, stimulus-locked epochs were visually inspected blind to the experimental condition and dose prior to computing

mean waveforms. Following visual inspection, the mean number of trials included in the waveforms was 112.0 [95 % CI: 107.2 to 116.7] trials stimulus-locked to the congruent and incongruent trials (separately) of the Inhibitory Control task and 41.6 [95 % CI: 40.1 to 43.2] trials stimulus-locked to the final stimulus of the target sequences of the Sustained Attention task. Attentional engagement (as indexed by the P3 ERP component) was evaluated as the mean amplitude within a 50 ms interval surrounding the largest positive going peak within a 275 to 700 ms latency window following stimulus onset for the flanker task, and a 275 to 600 ms window for the RVIP task (Chandler et al. 2020; McGowan et al. 2019; Pontifex et al. 2015). ERP latency was quantified as the time at which maximum peak amplitude occurred. Given the well-established nature of the P3 ERP component, analyses were conducted using a nine-channel region-of-interest approach centering around the topographic maxima of the P3 (i.e., the CP3/Z/4, P3/Z/4, PO5/Z/6 electrodes).

Results

Safety of intranasal insulin

The safety of intranasal insulin in combination with exercise was assessed by examining the incidence of participants reporting potential side effects of intranasal insulin (Schmid et al. 2018) at any time during the experimental protocol and the persistence of those symptoms. Additionally, the extent to which peripheral blood glucose was altered was also assessed.

Symptom manifestation at any point during the protocol Symptoms reported at any point during the session are represented in Fig. 3, part a. Between the exercise and control condition, burning/tingling of the nose and watering/tearing of the eyes during the nasal spray were the most cited symptoms across conditions. Only one person in the nosebleed/runny nose category had a minor nosebleed after spray administration. Additionally, one participant who received the 120 IU dose exhibited symptoms of dizziness that were sufficiently severe as to necessitate cessation of the exercise condition (blood glucose level = 97 mg/dL).

Symptom manifestation following the protocol Symptoms reported at the end of the session are represented in Fig. 3, part b. There were minimal differences across conditions for the frequency of reported symptoms. The most cited symptom between conditions was having a runny nose.

Alterations in peripheral blood glucose Blood glucose was assessed via glucometer at the beginning of the session, during the middle of the exercise/sitting condition, and following the exercise/sitting condition (see Fig. 1b). The change in peripheral blood glucose during the course of the experiment was computed relative to the 0 IU placebo groups within each experimental condition and is represented in Fig. 4. All dose groups stayed within the euglycemic range, regardless of condition, during the course of the session. However, for the doses past 80 IU, there was an overall downward trend for the exercise condition with no increase once exercise stopped. Additionally, of particular note is that the only individuals whose participation in this investigation was stopped due to peripheral blood sugar falling below 70 mg/dL (a level 1 hypogycemic event) were individuals randomly assigned to the exercise condition (the 70 mg/dL criteria was applied uniformly throughout the study regardless of condition). Specifically, one participant who received the 100 IU dose and another who received the 120 IU dose had the exercise condition stopped due to this safety protocol. Neither participant reported any other symptoms of hypoglycemia.

Efficacy of intranasal insulin

As the present investigation was a Phase II Clinical Trial, the research design was not sufficiently powered for hypothesis testing. Rather, analyses were conducted on measures of inhibitory control, sustained attention, and attentional engagement for the purpose of extracting standardized measures of effect size to understand the extent to which exercise engagement might alter the dose-response profile of intranasal insulin. Effect sizes were computed for each participant as the standardized change relative to the pretest assessment using the within-subject (d_{rm}) variance correction for Cohen's d (Lakens 2013). To ensure the integrity of the effect size estimates, within-subject effect sizes exceeding 3 times the interquartile range were identified as outliers and removed from analysis. Within each experimental condition, the effect of intranasal insulin was corrected by subtracting the effect size observed for the 0 IU placebo group to isolate the effects of intranasal insulin.

Change in behavioral indices of inhibitory control Effect size estimates with 95 % confidence intervals for both control and exercise conditions collapsed across congruences of the Flanker task are reported in Table 2 and see Fig. 5. For reaction time, the exercise condition appears to have blunted the facilitative effects of intranasal insulin relative to those effects observed in response to the control condition where the largest effects were observed for the 40 IU dose. The effect size for the interaction between condition and dose was Cohen's f^2 = 0.04 [95 % CI: 0.0 to 0.14] which is equivalent to Cohen's d = 0.2 [95 % CI: 0.0 to 0.75]. For response accuracy, the effects of intranasal insulin were more variable largely centering around no effect regardless of dose. The effect size for the interaction between condition and dose was Cohen's f^2 <

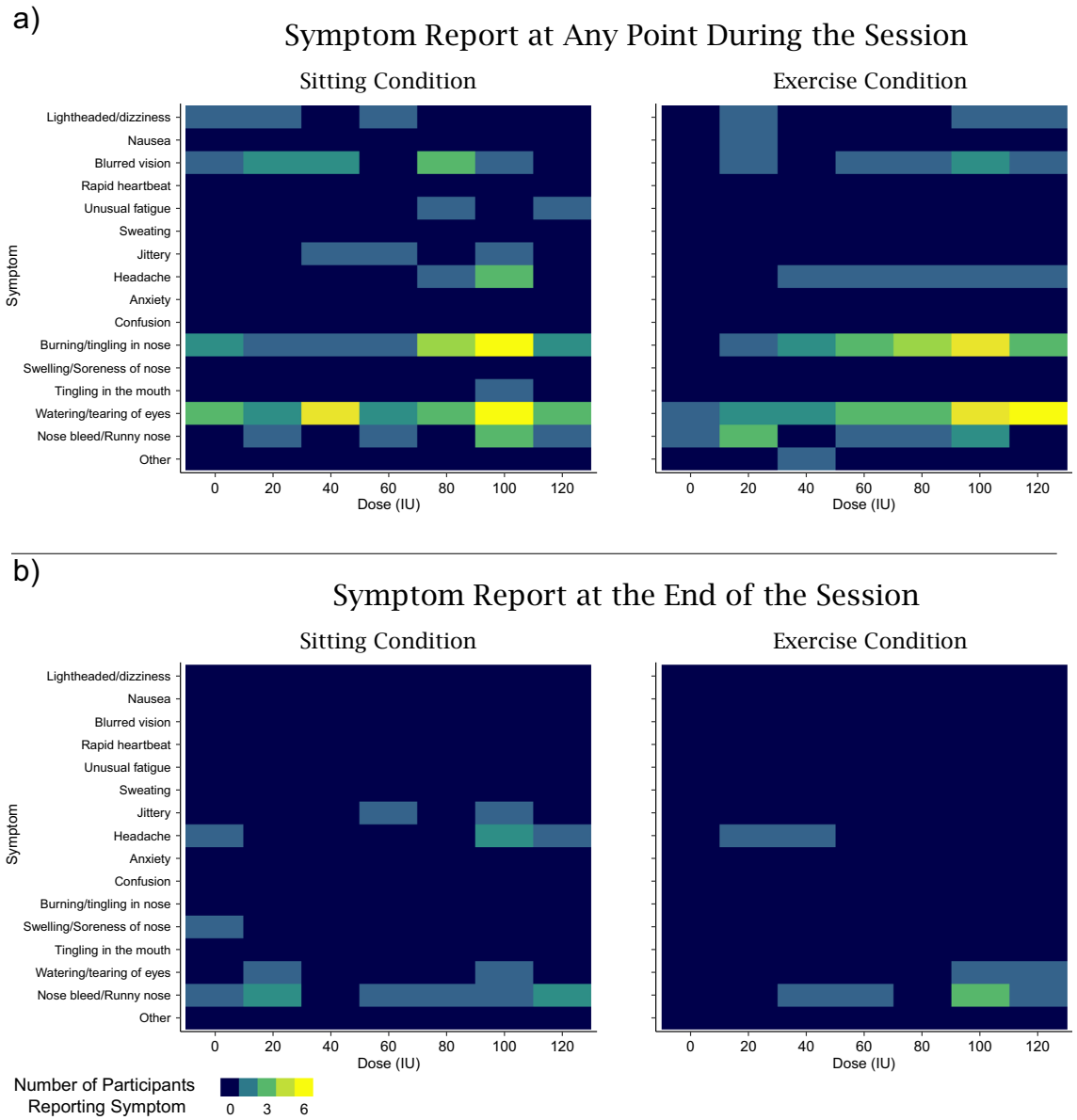

Fig. 3 Heatmap illustrating the frequency of symptoms reported as a function of condition (Left: sitting; Right: exercise) and dose for symptoms experienced at any point during the experimental session (**a**) and symptoms persisting at the completion of the protocol (**b**)

0.01 [95 % CI: 0.0 to 0.02] which is equivalent to Cohen's d < 0.2 [95 % CI: 0.0 to 0.3].

Change in behavioral indices of sustained attention Effect size estimates with 95 % confidence intervals for both control and exercise conditions in response to the target trial of the Rapid Visual Information Processing task are reported in Table 3 and see Fig. 5. Across the control and exercise conditions there was a high degree of similarity in the observed effects with a generally small positive effect for reaction time. The effect size for the interaction between condition and dose was Cohen's f^2 = 0.01 [95 % CI: 0.0 to 0.05] which is equivalent to Cohen's d = 0.2 [95 % CI: 0.0 to 0.5]. The effects for response accuracy were more variable and largely centered around a null effect; impairments in response accuracy were observed for the 40 IU dose regardless of condition. The effect size for the interaction between condition and dose was Cohen's f^2 = 0.01 [95 % CI: 0.0 to 0.05] which is equivalent to Cohen's d = 0.2 [95 % CI: 0.0 to 0.5].

Change in attentional engagement Effect size estimates with 95 % confidence intervals for both control and exercise conditions for P3 amplitude are reported in Table 4 and see Fig. 5. In response to the inhibitory control task,

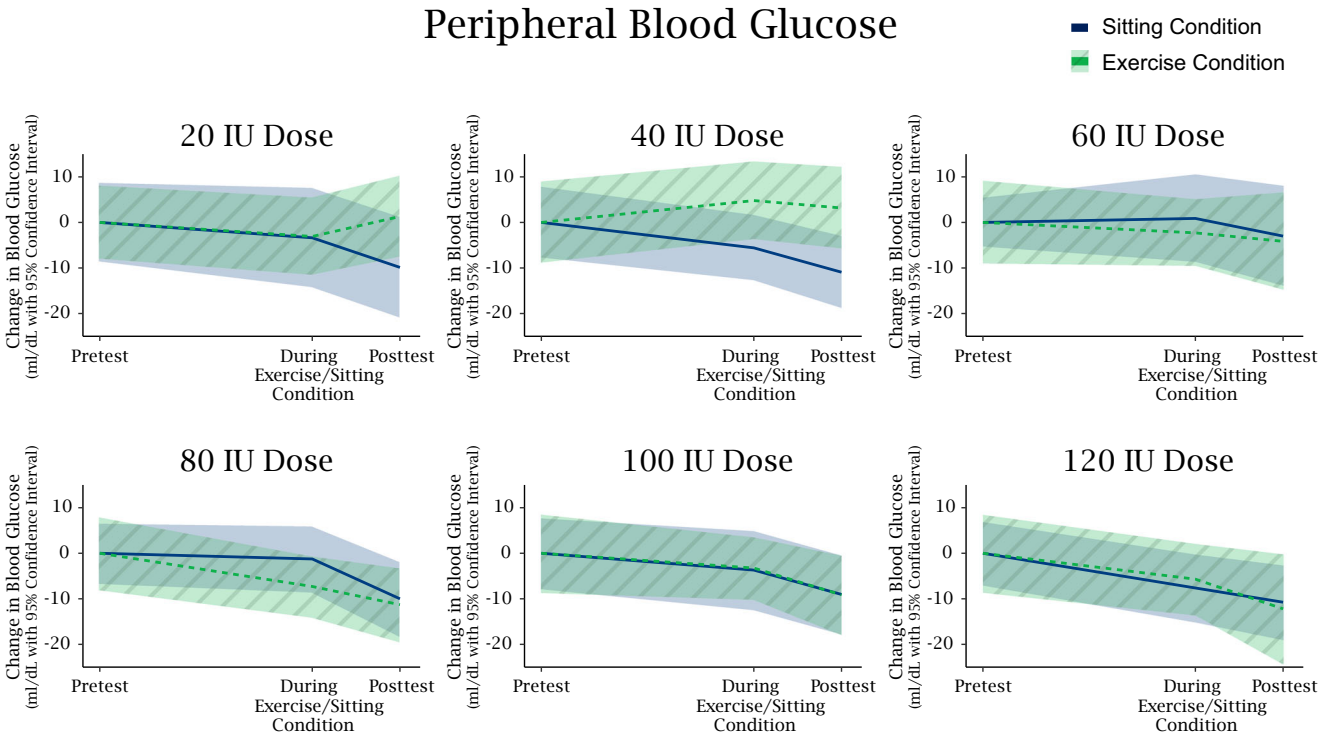

Fig. 4 Illustration of the change in peripheral blood glucose levels during the experimental session — over and above that of the effect induced by the 0 IU placebo dose — as a function of experimental condition (sitting vs. exercise) and dose

there was a general trend regardless of dose for reductions in attentional engagement as indexed by P3 amplitude for the control condition, whereas attentional engagement was sustained to a greater degree in the exercise condition. The effect size for the interaction between condition and dose was Cohen's f^2 = 0.01 [95 % CI: 0.0 to 0.03] which is equivalent to Cohen's d = 0.2 [95 % CI: 0.0 to 0.35]. In contrast, for the sustained attention task, enhancements in attentional engagement were observed for the 60 IU dose regardless of the experimental condition. The effect size for the interaction between condition and dose was Cohen's f^2 = 0.02 [95 % CI: 0.0 to 0.07] which is equivalent to Cohen's d = 0.3 [95 % CI: 0.0 to 0.53].

Change in attentional processing speed Effect size estimates with 95 % confidence intervals for both control and exercise conditions for P3 latency are reported in Table 5 and see Fig. 5. There was a general trend across experimental conditions for delays in attentional processing speed in response to the inhibitory control task, with the greatest delays occurring at 80 IU and above in response to the exercise condition. The effect size for the interaction between condition and dose was

Table 2 Effect size estimates with 95 % confidence intervals for the change in behavioral indices of inhibitory control

Condition	20 IU	40 IU	60 IU	80 IU	100 IU	120 IU
Reaction Time						
Control	-0.24 [-0.46 to -0.03]	-0.49 [-0.78 to -0.21]	-0.06 [-0.34 to 0.23]	-0.37 [-0.59 to -0.15]	-0.21 [-0.50 to 0.07]	-0.06 [-0.28 to 0.17]
Exercise	-0.12 [-0.37 to 0.13]	-0.17 [-0.40 to 0.06]	-0.25 [-0.55 to 0.04]	-0.11 [-0.37 to 0.15]	-0.19 [-0.50 to 0.13]	-0.28 [-0.49 to -0.07]
Response Accuracy						
Control	0.19 [-0.24 to 0.62]	-0.01 [-0.38 to 0.37]	-0.16 [-0.51 to 0.19]	-0.03 [-0.43 to 0.37]	-0.10 [-0.64 to 0.44]	0.14 [-0.46 to 0.74]
Exercise	-0.31 [-0.81 to 0.19]	-0.14 [-0.56 to 0.29]	0.01 [-0.35 to 0.36]	-0.06 [-0.54 to 0.42]	0.04 [-0.37 to 0.45]	-0.22 [-0.60 to 0.15]

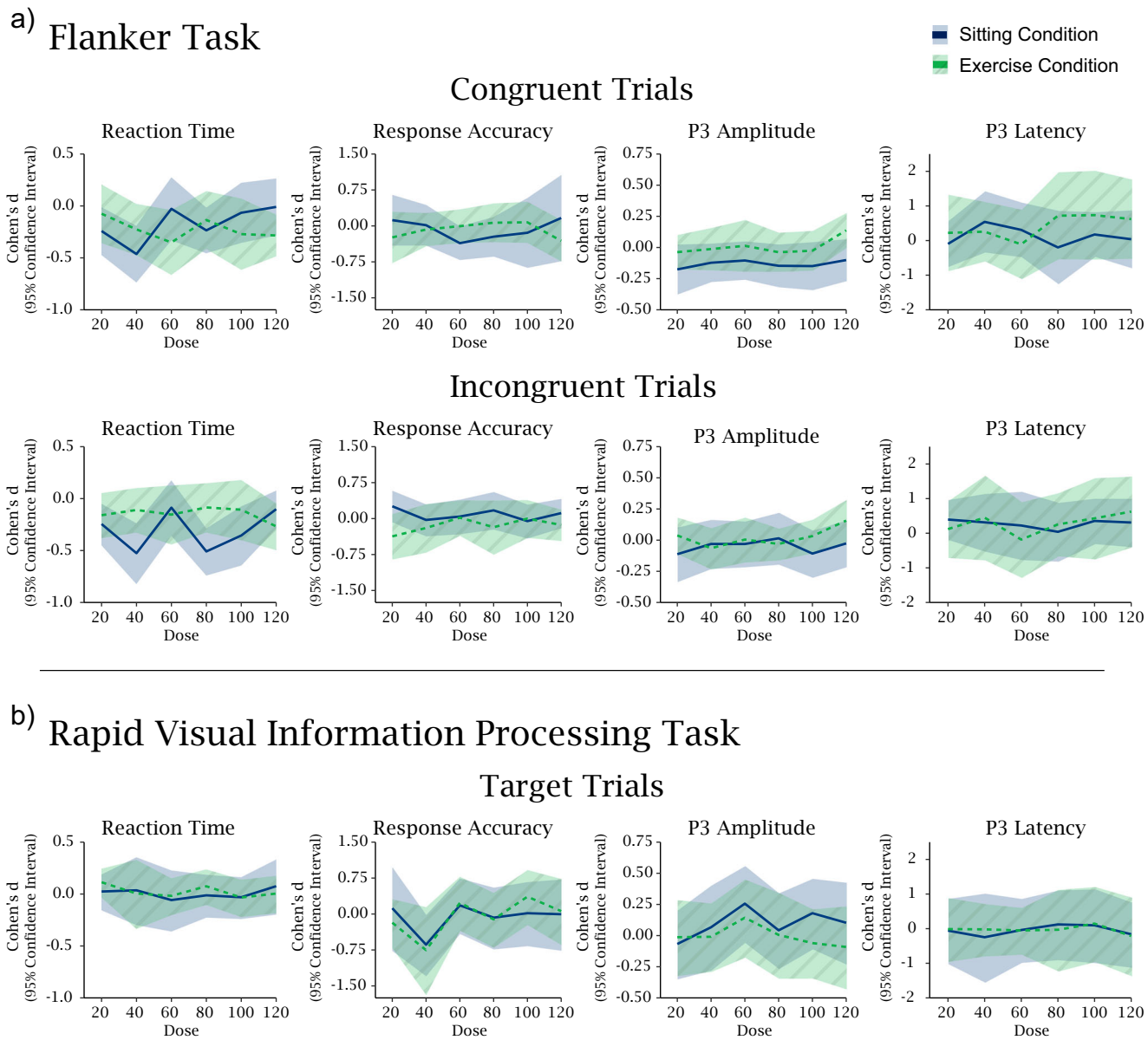

Fig. 5 Illustration of the effect (cohen's d_{rm} with 95 % confidence intervals) of dose of intranasal insulin administration — over and above that of the effect induced by the 0 IU placebo dose — as a function of condition for inducing changes in inhibitory control (**a**), and sustained attention (**b**)

Cohen's $f^2 = 0.02$ [95 % CI: 0.0 to 0.07] which is equivalent to Cohen's d = 0.3 [95 % CI: 0.0 to 0.53]. For the sustained attention task, there was a high degree of variability in the effects of intranasal insulin that largely centered around a null effect. The effect size for the interaction between condition and dose was Cohen's $f^2 < 0.01$ [95 % CI: 0.0 to 0.03] which is equivalent to Cohen's d < 0.2 [95 % CI: 0.0 to 0.35].

Discussion

The present investigation was a Phase II Clinical Trial designed to provide a preliminary assessment of the safety and efficacy of combining intranasal insulin with an exercise protocol. Although exercise did not appear to alter the frequency of symptoms reported; at doses of 100 IU and higher, there does appear to be a potential risk of intranasal insulin impacting upon peripheral blood glucose when immediately followed by exercise. Specifically, of the 58 individuals randomized into the exercise condition, 4 individuals presented with issues related to hypoglycemia (i.e., peripheral blood sugar falling below 70 mg/dL [mean = 61.2 ± 5.4 mg/dL] or severe dizziness) with 3 of those individuals receiving doses of intranasal insulin of 100 IU or greater. Accordingly, doses of intranasal insulin of 100 IU or more were observed to increase the likelihood of a hypoglycemic event for those

Table 3 Effect size estimates with 95 % confidence intervals for the change in behavioral indices of sustained attention

Condition	20 IU	40 IU	60 IU	80 IU	100 IU	120 IU
Reaction Time						
Control	0.02 [-0.15 to 0.20]	0.04 [-0.29 to 0.36]	-0.06 [-0.35 to 0.24]	-0.01 [-0.22 to 0.19]	-0.03 [-0.23 to 0.17]	0.08 [-0.19 to 0.34]
Exercise	0.11 [-0.02 to 0.25]	0.01 [-0.33 to 0.34]	-0.02 [-0.19 to 0.16]	0.07 [-0.10 to 0.25]	-0.03 [-0.21 to 0.15]	0.0 [-0.17 to 0.18]
Response Accuracy						
Control	0.12 [-0.75 to 0.99]	-0.64 [-1.28 to 0.0]	0.18 [-0.40 to 0.76]	-0.08 [-0.72 to 0.57]	0.02 [-0.65 to 0.68]	0.0 [-0.75 to 0.74]
Exercise	-0.18 [-0.69 to 0.32]	-0.75 [-1.67 to 0.16]	0.23 [-0.33 to 0.79]	-0.11 [-0.68 to 0.46]	0.36 [-0.21 to 0.93]	0.06 [-0.63 to 0.75]

individuals in the exercise condition, Odds Ratio = 7.49 [95 % CI: 0.55 to 418.52]; whereas no severe issues manifested in the sitting condition regardless of the dose. Consistent with prior findings (Schmid et al. 2018), the most cited symptoms associated with intranasal insulin were non-severe burning/tingling of the nose and watering/tearing of the eyes which largely resolved by the end of the testing session. The relative frequency of these events appears unrelated to the dose of intranasal insulin and were not observed to interact with exercise so as to make their occurrence any more or less prominent.

Accordingly, while the nature of a Phase II Clinical Trial is such that the present investigation was not sufficiently powered for hypothesis testing; the findings reported herein suggest that caution is warranted when combining high-dose intranasal insulin therapies with exercise. Patients, investigators, and clinicians should be aware that there appears to be an increased potential for hypoglycemic events to occur during aerobic exercise with intranasal insulin doses of 100 IU or more. Speculatively, this may relate to the atomization of the insulin into the nasal passageways. While the MAD Nasal Atomizer is a simple, easy-to-use and low cost device, a recent investigation utilizing adult and pediatric nasal airway replicas observed that as much as 30 % of the dose of insulin could be lost to the throat when using such a device (Hosseini et al. 2019). At rest, such misplacement of the insulin is likely to have little impact as the droplets descend into the gastrointestinal tract (Carino and Mathiowitz 1999). However, speculatively, the increased inspiratory flow during exercise may cause any residual droplets of insulin in the intranasal passageways that have not yet been absorbed to be transported into the lung where the insulin would be likely to impact upon the periphery, particularly given the increased cardiac output and lung perfusion associated with exercise.

Compounding this, exercise is also associated with decreases in mucosal thickness and availability (Baraniuk and Merck 2008), which may enable a greater portion of the dose of insulin to bypass the nasal passageway. Under low doses of intranasal insulin; this misplacement of insulin may not be of sufficient concentration to meaningfully impact upon peripheral blood glucose; whereas the relatively greater distribution of insulin at doses of 100 IU

Table 4 Effect size estimates with 95 % confidence intervals for the change in attentional engagement

Condition	20 IU	40 IU	60 IU	80 IU	100 IU	120 IU
Inhibitory Control Task						
Control	-0.14 [-0.35 to 0.06]	-0.08 [-0.25 to 0.10]	-0.07 [-0.24 to 0.10]	-0.07 [-0.25 to 0.12]	-0.13 [-0.32 to 0.06]	-0.06 [-0.24 to 0.11]
Exercise	0.0 [-0.14 to 0.14]	-0.04 [-0.21 to 0.13]	0.01 [-0.18 to 0.20]	-0.03 [-0.18 to 0.11]	0.0 [-0.14 to 0.15]	0.15 [-0.01 to 0.30]
Sustained Attention Task						
Control	-0.07 [-0.34 to 0.21]	0.07 [-0.27 to 0.41]	0.26 [-0.05 to 0.56]	0.04 [-0.26 to 0.34]	0.18 [-0.10 to 0.46]	0.10 [-0.22 to 0.43]
Exercise	-0.01 [-0.32 to 0.29]	-0.01 [-0.28 to 0.26]	0.14 [-0.17 to 0.46]	0.01 [-0.34 to 0.35]	-0.06 [-0.34 to 0.22]	-0.09 [-0.42 to 0.24]

Table 5 Effect size estimates with 95 % confidence intervals for the change in attentional processing speed

Condition	20 IU	40 IU	60 IU	80 IU	100 IU	120 IU
Inhibitory Control Task						
Control	0.15 [-0.47 to 0.76]	0.43 [-0.43 to 1.28]	0.27 [-0.62 to 1.15]	-0.08 [-1.03 to 0.87]	0.26 [-0.38 to 0.91]	0.17 [-0.59 to 0.94]
Exercise	0.17 [-0.79 to 1.13]	0.35 [-0.68 to 1.39]	-0.15 [-1.19 to 0.90]	0.49 [-0.59 to 1.57]	0.58 [-0.64 to 1.80]	0.62 [-0.46 to 1.70]
Sustained Attention Task						
Control	-0.06 [-1.00 to 0.88]	-0.25 [-1.53 to 1.03]	-0.04 [-0.96 to 0.88]	0.12 [-0.88 to 1.13]	0.10 [-0.96 to 1.16]	-0.16 [-1.10 to 0.78]
Exercise	-0.01 [-0.93 to 0.91]	-0.02 [-0.77 to 0.73]	-0.05 [-0.72 to 0.62]	-0.03 [-1.21 to 1.14]	0.15 [-0.93 to 1.23]	-0.21 [-1.35 to 0.92]

or more may enable the manifestation of changes in peripheral blood glucose. Clearly, further research is necessary to better understand the mechanisms by which high doses of intranasal insulin impact the likelihood of hypoglycemia when combined with aerobic exercise. Additional investigations should determine the likelihood of hypoglycemia when non-aerobic exercise (e.g. strength training) is combined with intranasal insulin. However, recent evidence in animal models suggests that skeletal muscle contraction (regardless of exercise type) stimulates glucose uptake through the AMPK and TBC1D1 mechanisms, and this occurs primarily in the period following exercise (Kjøbsted et al. 2019). Thus, while it is advised to evaluate these relationships during different types of exercise (strength and aerobic), it is anticipated that hypoglycemic incidents are more likely to occur after exercise regardless of exercise type.

A second aim of the investigation was to provide a preliminary assessment of the efficacy of combining intranasal insulin with exercise for enhancing inhibitory aspects of cognitive control and sustained attention alongside neuroelectric indices of attentional engagement. While the nature of a Phase II Clinical Trial is such that the present investigation was not sufficiently powered for hypothesis testing, inspection of effect size estimates suggests that relative to changes incurred by the placebo dose, there does not appear to be a clear dose-response relationship between intranasal insulin and changes in cognition, nor does exercise appear to modulate this relationship. Thus, while effect size estimates are provided, given the small sample size we remain cautious regarding over interpreting the findings given the absence of clear patterns. Nevertheless, the general pattern of reduced P3 amplitude and increased P3 latency observed within the present investigation is consistent with prior research by Kern and colleagues (Kern et al. 1999). Given the theoretical framework of the P3 ERP component as manifesting from the suppression of extraneous non-task related neural processes in the brain (Polich 2007), such changes in the P3 component suggest that intranasal insulin may be increasing the activity of other non-task related cognitive operations as insulin binds to receptors in neural regions not engaged with the task.

Despite the methodological strengths of the present investigation, it is important to acknowledge a few key limitations. In particular, given the potential risk of combining exercise with intranasal insulin the present investigation utilized a sample of healthy, non-diabetic, fasted college-aged adults to limit the potential for a serious adverse event. Future research is therefore necessary to ensure that the findings reported herein generalize to elderly populations and those at risk for dementia whom are likely to be targeted for the clinical use of intranasal insulin. Given the use of a non-continuous glucometer, it may also be that other more transient alterations in blood glucose occurred which were not captured within the assessment of blood glucose at only three time points.

The present investigation utilized a design centering around a single bout of aerobic exercise to specifically ensure that any effects observed were the result of the combination of exercise and intranasal insulin. However, further research is necessary to better determine how chronic administration of intranasal insulin and habitual physical activity engagement interact on the likelihood of hypoglycemic events occurring as this may differ from the acute effects given the impacts of hydration, circulating levels of blood glucose, and the relative timing/intensity/frequency of physical activity relative to the dose of intranasal insulin. While the efficacy of intranasal insulin was assessed during the initial peak onset of insulin in the cerebral spinal fluid; it may also be that the dose-response relationship between intranasal insulin and modulations in cognition follows a more protracted time course.

Ultimately, however, the present investigation provides a preliminary assessment of the safety and efficacy of combining intranasal insulin with aerobic exercise suggesting that additional attention to the risk of hypoglycemic events is particularly warranted for doses of 100 IU and greater. Under

doses less than 100 IU, exercise does not appear to be associated with an elevated risk of hypoglycemic events nor does it alter symptom manifestation of potential side effects of intranasal insulin. Although future research is necessary to ensure the long-term safety and efficacy of combination therapeutic interventions; findings from the present investigation suggest that exercise and intranasal insulin do not appear to interact to hinder nor enhance the immediate benefits of any particular therapeutic dose.

Acknowledgements Support for this investigation was provided by a Dissertation Completion Fellowship awarded to K. L. Gwizdala through the Department of Kinesiology at Michigan State University.

Author contributions **Kathryn L. Gwizdala**: Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Funding Acquisition. **David P. Ferguson**: Methodology, Writing – Review & Editing, Supervision, Project Administration. **Jeffery Kovan**: Methodology, Writing – Review & Editing, Supervision. **Vera Novak**: Methodology, Writing – Review & Editing. **Matthew B. Pontifex**: Methodology, Software, Validation, Formal Analysis, Data Curation, Writing – Original Draft, Visualization, Supervision, Project Administration, Funding Acquisition.

Data availability Data is available upon request.

Declarations

Conflict of interest No conflicting financial interests exist.

Ethical approval All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This investigation was approved by the Michigan State University Human Research Protection Program.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Afrezza (2020) https://afrezza.com/

Bahar-Fuchs A, Clare L, Woods B (2013) Cognitive training and cognitive rehabilitation for mild to moderate Alzheimer's disease and vascular dementia. Cochrane Database Syst Rev 2013(6). https://doi.org/10.1002/14651858.CD003260.pub2

Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, Plymate SR, Fishel MA, Watson GS, Cholerton BA, Duncan GE, Mehta PD, Craft S (2010) Effects of Aerobic Exercise on Mild Cognitive Impairment: A Controlled Trial. Arch Neurol, 67(1). https://doi.org/10.1001/archneurol.2009.307

Bakirtzi K, Belfort G, Lopez-Coviella I, Kuruppu D, Cao L, Abel ED, Brownell A-L, Kandror KV (2009) Cerebellar neurons possess a vesicular compartment structurally and functionally similar to Glut4-storage vesicles from peripheral insulin-sensitive tissues. J Neurosci 29(16):5193–5201. https://doi.org/10.1523/JNEUROSCI.0858-09.2009

Baraniuk JN, Merck SJ (2008) Nasal reflexes: Implications for exercise, breathing, and sex. Curr Allergy Asthma Rep 8(2):147–153

Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W (2004) Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29(10):1326–1334. https://doi.org/10.1016/j.psyneuen.2004.04.003

Benedict C, Hallschmid M, Schmitz K, Schultes B, Ratter F, Fehm HL, Born J, Kern W (2006) Intranasal insulin improves memory in humans: Superiority of insulin aspart. Neuropsychopharmacology 32(1):239–243. https://doi.org/10.1038/sj.npp.1301193

Berger M, Halban PA, Muller WA, Offord RE, Renold AE, Vranic M (1978) Mobilization of subcutaneously injected tritiated insulin in rats: Effects of muscular exercise. Diabetologia 15(2):133–140

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: A transnasal approach to the human brain. Nat Neurosci 5(6):514–516. https://doi.org/10.1038/nn0602-849

Brooks GA, Fahey TD, Baldwin KM (2004) Exercise physiology: Human bioenergetics and its applications (fourth). McGraw-Hill, New York

Brünner YF, Kofoet A, Benedict C, Freiherr J (2015) Central insulin administration improves odor-cued reactivation of spatial memory in young men. J Clin Endocrinol Metab 100(1):212–219

Buchman AS, Boyle PA, Yu L, Shah RC, Wilson RS, Bennett DA (2012) Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology 78(17):1323–1329

Carino GP, Mathiowitz E (1999) Oral insulin delivery. Adv Drug Deliv Rev 35(2–3):249–257

Chandler MC, McGowan AL, Payne BR, Hampton Wray A, Pontifex MB (2019) Aerobic fitness relates to differential attentional but not language-related cognitive processes. Brain Lang 198:104681. https://doi.org/10.1016/j.bandl.2019.104681

Chandler MC, McGowan AL, Ferguson DP, Pontifex MB (2020) Carbohydrate mouth rinse has no effects on behavioral or neuroelectric indices of cognition. Int J Psychophysiol. https://doi.org/10.1016/j.ijpsycho.2020.02.012

Chatrian GE, Lettich E, Nelson PL (1985) Ten percent electrode system for topographic studies of spontaneous and evoked EEG activity. American Journal of EEG Technology 25:83–92. https://doi.org/10.1080/00029238.1985.11080163

Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, Callaghan M, Arbuckle M, Behl C, Craft S (2015) Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. J Alzheimers Dis 44(3):897–906. https://doi.org/10.3233/JAD-141791

Cooter S, Holt C (2011) Wonders of the Universe [Film]. BBC America

Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B (2012) Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: A pilot clinical trial. Arch Neurol 69(1):29–38. https://doi.org/10.1001/archneurol.2011.233

Craft S, Raman R, Chow TW, Rafii MS, Sun C-K, Rissman RA, Donohue MC, Brewer JB, Jenkins C, Harless K, Gessert D, Aisen PS (2020) Safety, efficacy, and feasibility of intranasal insulin for the treatment of mild cognitive impairment and Alzheimer disease dementia: A randomized clinical trial. JAMA Neurol 77(9):1099–1109. https://doi.org/10.1001/jamaneurol.2020.1840

Delorme A, Makeig S (2004) EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics. J Neurosci Methods 134:9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009

Eriksen CW, Eriksen BA (1974) Effects of noise letters upon the identification of a target letter in a non-search task. Percept Psychophys 25:249–263. https://doi.org/10.3758/BF03203267

Farina E, Fioravanti R, Chiavari L, Imbornone E, Alberoni M, Pomati S, Pinardi G, Pignatti R, Mariani C (2002) Comparing two programs of

cognitive training in Alzheimer's disease: A pilot study. Acta Neurol Scand 105(5):365–371

Galindo-Mendez B, Trevino JA, McGlinchey R, Fortier C, Lioutas V, Novak P, Mantzoros CS, Ngo L, Novak V (2020) Memory advancement by intranasal insulin in type 2 diabetes (MemAID) randomized controlled clinical trial: Design, methods and rationale. Contemp Clin Trials 89:105934. https://doi.org/10.1016/j.cct.2020.105934

Giroux M (2005) Controlled particle dispersion™: Effective nasal delivery from a versatile, flexible technology platform. ONdrugDelivery Ltd

Gramfort A, Luessi M, Larson E, Engemann DA, Strohmeier D, Brodbeck C, Goj R, Jas M, Brooks T, Parkkonen L, Hämäläinen M (2013) MEG and EEG data analysis with MNE-Python. Front Neurosci 7:1–13. https://doi.org/10.3389/fnins.2013.00267

Grossberg GT, Manes F, Allegri RF, Gutiérrez-Robledo LM, Gloger S, Xie L, Jia XD, Pejović V, Miller ML, Perhach JL, Graham SM (2013) The safety, tolerability, and efficacy of once-daily memantine (28 mg): A multinational, randomized, double-blind, placebo-controlled trial in patients with moderate-to-severe Alzheimer's disease taking cholinesterase inhibitors. CNS Drugs 27(6):469–478. https://doi.org/10.1007/s40263-013-0077-7

Heni M, Kullmann S, Preissl H, Fritsche A, Häring H-U (2015) Impaired insulin action in the human brain: Causes and metabolic consequences. Nat Rev Endocrinol 11(12):701

Hill JM, Lesniak MA, Pert CB, Roth J (1986) Autoradiographic localization of insulin receptors in rat brain: Prominence in olfactory and limbic areas. Neuroscience 17(4):1127–1138. https://doi.org/10.1016/0306-4522(86)90082-5

Hosseini S, Wei X, Wilkins JV Jr, Fergusson CP, Mohammadi R, Vorona G, Golshahi L (2019) In vitro measurement of regional nasal drug delivery with Flonase,® Flonase® Sensimist™ and MAD Nasal™ in anatomically correct nasal airway replicas of pediatric and adult human subjects. J Aerosol Med Pulm Drug Deliv 32(6):374–385

Hui DS, Chow BK, Chu LCY, Ng SS, Hall SD, Gin T, Chan MTV (2009) Exhaled air and aerosolized droplet dispersion during application of a jet nebulizer. Chest 135(3):648–654. https://doi.org/10.1378/chest.08-1998

In 't Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MMB, Stricker BHC (2001) Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 345(21), 1515–1521. https://doi.org/10.1056/NEJMoa010178

Intlekofer KA, Cotman CW (2013) Exercise counteracts declining hippocampal function in aging and Alzheimer's disease. Neurobiol Dis 57:47–55

Kern W, Born J, Schreiber H, Fehm HL (1999) Central nervous system effects of intranasally administered insulin during euglycemia in men. Diabetes 48(3):557–563

Kjøbsted R, Roll JLW, Jørgensen NO, Birk JB, Foretz M, Viollet B, Chadt A, Al-Hasani H, Wojtaszewski JF (2019) AMPK and TBC1D1 regulate muscle glucose uptake after, but not during, exercise and contraction. Diabetes 68(7):1427–1440. https://doi.org/10.2337/db19-0050

Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring H-U (2016) Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. Physiol Rev 96(4):1169–1209. https://doi.org/10.1152/physrev.00032.2015

Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. Front Psychol 4(863):1–12. https://doi.org/10.3389/fpsyg.2013.00863

Matthews KA, Xu W, Gaglioti AH, Holt JB, Croft JB, Mack D, McGuire LC (2018) Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015–2060) in adults aged ≥ 65 years. Alzheimers Dement 15(1):17–24. https://doi.org/10.1016/j.jalz.2018.06.3063

McGowan AL, Chandler MC, Brascamp JW, Pontifex MB (2019) Pupillometric indices of locus-coeruleus activation are not modulated following single bouts of exercise. Int J Psychophysiol 140:41–52. https://doi.org/10.1016/j.ijpsycho.2019.04.004

Morris MC, Tangney CC, Wang Y, Sacks FM, Bennett DA, Aggarwal NT (2015) MIND diet associated with reduced incidence of Alzheimer's disease. Alzheimers Dement 11(9):1007–1014. https://doi.org/10.1016/j.jalz.2014.11.009

Morris JK, Vidoni ED, Johnson DK, Van Sciver A, Mahnken JD, Honea RA, Wilkins HM, Brooks WM, Billinger SA, Swerdlow RH, Burns JM (2017) Aerobic exercise for Alzheimer's disease: A randomized controlled pilot trial. PLoS One 12(2). https://doi.org/10.1371/journal.pone.0170547

Neale C, Johnston P, Hughes M, Scholey A (2015) Functional activation during the rapid visual information processing task in a middle aged cohort: An fMRI study. PLoS One 10(10):e0138994. https://doi.org/10.1371/journal.pone.0138994

Novak V, Milberg W, Hao Y, Munshi M, Novak P, Galica A, Manor B, Roberson P, Craft S, Abduljalil A (2014) Enhancement of vasoreactivity and cognition by intranasal insulin in type 2 diabetes. Diabetes Care 37(3):751–759. https://doi.org/10.2337/dc13-1672

Peirce JW (2009) Generating stimuli for neuroscience using PsychoPy. Fron Neuroinformatics 2:10. https://doi.org/10.3389/neuro.11.010.2008

Polich J (2007) Updating P300: An integrative theory of P3a and P3b. Clin Neurophysiol 118:2128–2148. https://doi.org/10.1016/j.clinph.2007.04.019

Pontifex MB, Parks AC, Henning DA, Kamijo K (2015) Single bouts of exercise selectively sustain attentional processes. Psychophysiology 52(5):618–625. https://doi.org/10.1111/psyp.12395

Pontifex MB, Gwizdala KL, Parks AC, Pfeiffer KA, Fenn KM (2016) The association between physical activity during the day and long-term memory stability. Sci Rep 6:38148. https://doi.org/10.1038/srep38148

Pontifex MB, Gwizdala KL, Parks AC, Billinger M, Brunner C (2017) Variability of ICA decomposition may impact EEG signals when used to remove eyeblink artifacts. Psychophysiology 54(3):386–398. https://doi.org/10.1111/psyp.12804

Pontifex MB, Miskovic V, Laszlo S (2017) Evaluating the efficacy of fully automated approaches for the selection of eyeblink ICA components. Psychophysiology 54(5):780–791. https://doi.org/10.1111/psyp.12827

Reger MA, Watson GS, Frey WH, Baker LD, Cholerton B, Keeling ML, Belongia DA, Fishel MA, Plymate SR, Schellenberg GD, Cherrier MM, Craft S (2006) Effects of intranasal insulin on cognition in memory-impaired older adults: Modulation by APOE genotype. Neurobiol Aging 27(3):451–458. https://doi.org/10.1016/j.neurobiolaging.2005.03.016

Reger MA, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, Fishel MA, Plymate SR, Breitner JCS, DeGroodt W, Mehta P, Craft S (2008) Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 70(6):440–448. https://doi.org/10.1212/01.WNL.0000265401.62434.36

Scarmeas N, Stern Y, Tang M-X, Mayeux R, Luchsinger JA (2006) Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 59(6):912–921. https://doi.org/10.1002/ana.20854

Schmid V, Kullmann S, Gfrörer W, Hund V, Hallschmid M, Lipp H-P, Häring H-U, Preissl H, Fritsche A, Heni M (2018) Safety of intranasal human insulin: A review. Diabetes Obes Metab 20(7):1563–1577. https://doi.org/10.1111/dom.13279

Shvartz E, Reibold RC (1990) Aerobic fitness norms for males and females aged 6 to 75 years: A review. Aviat Space Environ Med 61:3–11

Teleflex (2013) LMA® MAD NASAL™ Intranasal mucosal atomization device

Thomas S, Reading J, Shephard RJ (1992) Revision of the physical activity readiness questionnaire (PAR-Q). Can J Sport Sci 17:338–345

Tuominen JA, Karonen S-L, Melamies L, Bolli G, Koivisto VA (1995) Exercise-induced hypoglycaemia in IDDM patients treated with a short-acting insulin analogue. Diabetologia 38(1):106–111

Unger JW, Livingston JN, Moss AM (1991) Insulin receptors in the central nervous system: Localization, signalling mechanisms and functional aspects. Prog Neurobiol 36(5):343–362. https://doi.org/10.1016/0301-0082(91)90015-S

Wechsler D (2011) Wechsler abbreviated scale of intelligence, 2nd ed. Pearson Assessments, Toronto

Yu F, Thomas W, Nelson NW, Bronas UG, Dysken M, Wyman JF (2015) Impact of 6-month aerobic exercise on Alzheimer's symptoms. J Appl Gerontol 34(4):484–500. https://doi.org/10.1177/0733464813512895

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.