Human longevity : crosstalk between the brain and periphery

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CHAPTER 5

EFFECT OF INTRANASALLY ADMINISTERED INSULIN ON CEREBRAL BLOOD FLOW AND PERFUSION; A RANDOMIZED EXPERIMENT IN YOUNG AND OLDER ADULTS

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ABSTRACT

Cerebral insulin acts as a vasoactive modulator regulating peripheral and cerebral blood flow. Insulin has been consistently linked to aging, lifespan and longevity. In this study, we assessed the effects of intranasally administered insulin on cerebral blood flow and perfusion in older and younger adults.

Eight healthy young men aged 20-26 years and eleven older men aged 60-69 years were intranasally administered insulin (40IU) or placebo after an overnight fast using a randomized, double-blinded, placebo-controlled crossover design. Changes in cerebral blood flow through three major cerebropetal arteries (basilar, left and right carotid arteries) were assessed by phase contrast MR angiography. Regional cortical tissue perfusion was assessed through pseudo continuous arterial spin labelling.

After intranasal administration of insulin as compared to placebo, total flow through the cerebropetal arteries was not changed, neither in young nor in older subjects. In young adults, intranasal insulin did not significantly change perfusion in any of the cortical regions tested. In older subjects, intranasal insulin compared to placebo increased perfusion through the occipital gray matter (65.2 ± 11.0 mL/100g/min vs 61.2 ± 10.1 mL/100g/min, P=0.001), the parietal gray matter (69.2 ± 11.5 mL/100g/min vs. 66.4 ± 10.9 mL/100g/min, P=0.034) and the thalamus (68.28 ± 6.75 versus 63.31 ± 6.84, P=0.003). Perfusion in the frontal and temporal gray matter was not changed.

Intranasal administration of insulin improved tissue perfusion of the parietal and occipital cortical brain regions and the thalamus in older adults. In younger adults, intranasal insulin had minimal effects, possibly because brain perfusion is not yet compromised.

INTRODUCTION

Cerebral blood flow is pivotal for providing oxygen and nutrients to the brain to maintain brain function. The brain is a highly metabolically active organ but has only limited possibilities for energy storage. Thus, cerebral blood flow is important because the brain depends on constant and regulated blood supply to function properly. With aging, pathophysiological changes occur in large arteries causing decreased ability of large arteries to absorb and dampen pulsatile blood flow, eventually leading to pulsatile stress and arterial stiffness [1]. Transmission of increased pulsatile stress to the brain microvasculature is thought to lead to increased microvascular brain damage via altered dampening of pulsatile blood flow in cerebral arteries, and is considered a contributing factor for some cerebrovascular diseases [2].

Insulin influences all aspects of human physiology, including central regulation of energy homeostasis, cognitive functions and neuronal activity [3, 4]. The central regulation of energy homeostasis involves a complex neuronal network, which comprises the hypothalamus, the thalamus as well as cortical brain structures. Insulin and its signaling have been consistently linked to aging, lifespan and longevity [5]. Aging is associated with reduced insulin action in peripheral tissues and possibly in the brain. Reduced insulin action in the aging brain may relate to age- associated cognitive defects and anomalies of metabolic homeostasis [5-9]. Recent data suggest that insulin is also a vasoactive modulator that regulates peripheral and cerebral blood flow, possibly via a direct vasodilatory effect [10]. Reduced insulin action has been associated with vascular impairment possibly via impaired insulin- mediated augmentation of endothelium- dependent vasodilation, enhanced generation of reactive oxygen species and/ or excessive free fatty acids release from adipose tissue [11-15].

When administered in humans via the nasal route, insulin has been shown to distinctly alter brain functions, without being absorbed into the blood stream or having direct systemic effects on peripheral blood glucose or insulin levels [16]. Thus, the nasal route provides a safe and effective way of conveying insulin to the brain to specifically modulate brain insulin action and exert beneficial effects.

In this randomized, placebo-controlled trial we investigated the effects of intranasal insulin administration on cerebral blood flow and perfusion in healthy young and elderly adults.

METHODS

Ethics statement

This study titled "Maintaining health in old age through homeostasis: Switchbox Phase II" was approved by the Medical Ethical Committee of Leiden University Medical Center under protocol P13.164. and the Dutch competent authority (Centrale Commissie Mensengebonden Onderzoek (CCMO)) with protocol code number NL45043.058.13. The study is registered in the European Clinical Trials Database under number 2012-005650-29. All investigations have been conducted according to the principles expressed in the Declaration of Helsinki. All participants provided written informed consent after written and verbal description of the study was given.

Trial design and participants

The design is a double-blinded, randomised, cross-over, placebo controlled trial in volunteers from the general population. This study was aimed at examining the effects of intranasal insulin on circulatory metabolic and endocrine parameters, as well as on resting and functional brain activities using MRI. Twenty-three (fourteen elderly and nine young) relatively healthy men aged 18-85 years (age criteria 18-35 years for young adults and 60-85 years for older adults), with a body mass index (BMI) between 20 kg/m2 and 27 kg/m2 were recruited from the general population. Exclusion criteria included fasting plasma glucose above 7 mmol/l, anemia (haemoglobin < 7.1 mmol/l), presence of anatomic deviations of the nose, any significant endocrine, neurological and cardiovascular diseases, or use of medication known to influence lipolysis, thyroid function, glucose metabolism, GH/IGF-1 secretion or any other hormonal axis. Furthermore, persons with a current smoking or alcohol addiction or history of substance abuse were excluded.

Of the 23 participants that were contacted via telephone, three (older) participants were excluded after screening. The reasons for exclusion were uncontrolled high blood pressure (n=1) and renal insufficiency (n=2). Of these, all participants except for one (young), successfully completed the study. The reason for drop-out was phobia for cannulas/ blood withdrawal.

Randomisation, masking and intervention

Randomisation was carried out by the pharmaceutical *t*rial coordinator, while the researchers were blinded to what the participants received. Concealment of treatment allocation was ensured by delivery of trial medication in a standard plain vial (same for all treatment groups) that had been prepared and re-labelled by the hospital pharmacy. Deblinding was done at the end of the study.

The study interventions consisted of intranasal application of 40 IU insulin (Actrapid; Novo Nordisk, Mainz, Germany) or placebo (Sterile saline), delivered using the ViaNase Electronic Atomizer (Kurve Technology inc.). The ViaNase device had been specifically designed to deliver drugs to the olfactory region to maximize drug transport to the central nervous system. The device released a metered insulin (40 IU) or saline dose directly into the subject's nostrils. A total volume of 0.4 mL (0.2 ml per nostril) was administered, which was inhaled by breathing evenly over a 2-minute period. In both right and left nostril, two doses each were delivered resulting in a total insulin dose of 40 IU. This method allowed administration of smaller particle sizes to increase drug deposition in the upper nasal cavity without transporting the drug to the lungs [17].

For the current study, results from two study visits, spaced apart by a week, on one of which they received insulin and on the other placebo, in a randomised manner were compared. All participants underwent exactly the same protocol during both study visits.

Experimental protocol

In the morning after a 10-hour overnight fast, participants arrived at the MRI facilities at 08.00 in the morning. A catheter was placed in a vein of the forearm of the non-dominant hand. First, a baseline venous blood sample was collected in a serum-separator (SST)-tube thereafter, every 10 minutes, 4 ml of blood was collected into a SST-tube and 2 ml into K3-EDTA tube.

Ten minutes after the first blood withdrawal, the trial medication (placebo or 40 IU of insulin) was administered intranasally, under the supervision of the experimenter, with a ViaNase Electronic Atomizer. About 20 minutes after the intranasal application, participants were transferred into the MRI for the scanning procedure (figure 1). About 30 min after in the intranasal application, the CBF measurement was performed in the MRI scanner. This time interval was chosen to allow insulin to reach maximal concentrations in the CSF [18], since previous studies have shown that it takes insulin 30 minutes to reach maximal

concentrations/effects in the CSF, and this approximate time window has been used in other studies where brain – related effects of intranasal insulin were studied [19-21].

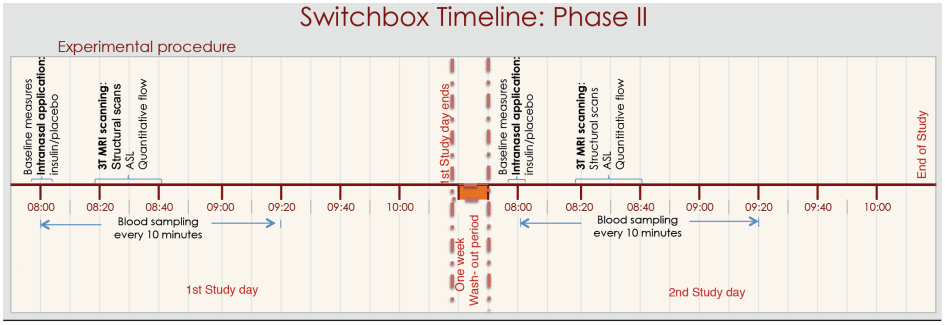

FIGURE 5.1 | Flowchart of experimental procedures related to cerebral blood flow and perfusion measurements.

After an overninght fast and baseline measures, the study day started at 08.00 with blood sample withdrawal for baseline measures, after which subjects received either the intranasal insulin or intranasal placebo treatment. MRI scanning began 20 mins later, including survey/structural MRI scans, flow and perfusion scans.

Anthropometrics.

During the first study day, height, weight, percentage of body fat, and waist and hip circumference of participants were measured. Weight (in kilograms) was divided by the squared height (in meters) to calculate the BMI.

Blood sampling and Chemical analysis of blood samples

After each withdrawal, the K3-EDTA tubes were immediately placed on ice before centrifugation, whereas the serum tubes were kept at room temperature and centrifuged when the samples were clotted, usually between 30–60 minutes. Samples were centrifuged at 3520 RPM at 4 °C for 10 minutes. The EDTA plasma and serum samples were stored in two aliquots of 500 µl during the rest of the sampling at – 20 °C. After the sampling they were transferred to a – 80 °C freezer and stored until analysis.

All laboratory measurements were performed with fully automated equipment and diagnostics from Roche Diagnostics (Almere, The Netherlands). Glucose levels were measured using Hitachi Modular P800 from Roche (Almere, the Netherlands), with coefficient of variation (CV) for measurement less than 1%. Insulin levels were measured using the Immulite 2500 from DPC (Los Angeles, CA). CV was less than 6%.

Brain MRI protocol

The perfusion measurement reported in the current study was part of a longer scanning protocol (48 minutes in young participants and 24 minutes in older participants) in which we aimed to successively assess several MRI endpoints. Scans were performed on a whole body magnetic resonance system with a 3 Tesla field strength (Philips Medical Systems, Best, the Netherlands). 3D T1-weighted images were acquired with the following imaging parameters: echo time (TE) 4.6ms, repetition time (TR) 9ms, Flip 8°, field of view (FOV) = 224 × 177 × 168mm, scan duration ~5minutes. The pseudo Continuous Arterial Spin Labeling (pCASL) with TE/TR/Flip: 14ms/4.0s/90°, FOV 240 x 133 x 240mm, matrix 80 x 80mm, slices 19, scan duration 6 minutes and the M0-scan with TE/TR/flip: 14ms/6.0s/90, FOV 240x133x240, Matrix 80 80, slices 19, scan duration 30sec. The phase-contrast quantitative flow (QF) scan was planned using 2 localizer angiograms in the sagittal and coronal planes and acquired with the following parameters: TR=11ms; TE 7.5ms; flip angle=10°; slice thickness=5mm; field of view 150x103mm; voxel size 1.17x1.17mm; velocity sensitivity=200cm/s, 20 signal averages. Time allocated to measure perfusion was 6 minutes.

Phase contrast MR Angiography

Quantitative flow measurements were performed using ungated phase-contrast MRA, based on 2 localizer MR angiograms in the sagittal and coronal orientation as shown in figure 2. A region of interest (ROI) was drawn around the vessel lumen on the magnitude images by an experienced rater using Philips Software on a PACS (Philips Medical Systems, Best, The Netherlands) workstation with ROI measurement tools. Flux was measured for basilar artery and both internal carotid arteries separately. Total flux was calculated for all three vessel together.

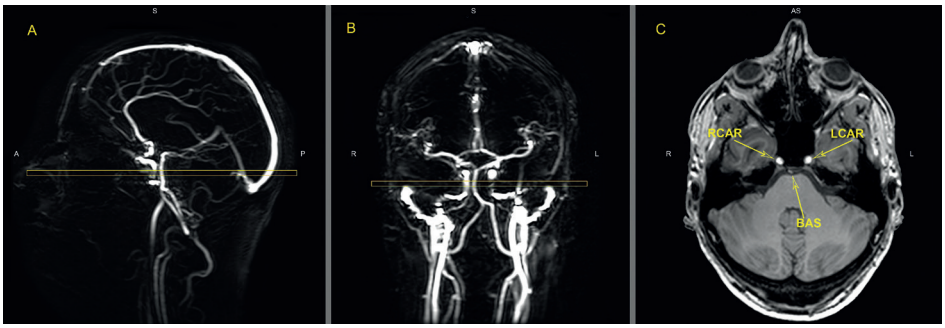

FIGURE 5.2 | MR images of the vasculature measured with phase contrast MR angiography imaging for Quantitive Flow (QF).

Typical placement of the QF phase-contrast slice (C) through the right internal carotid artery (RCAR), left internal carotid artery (LCAR) and the basilar artery (BAS) using the sagittal (A) and coronal (B) localizer angiograms. ROI's were drawn around the three arteries in slice C to measure flow.

pCASL quantitative analysis

MR images were analyzed using FMRIB Software Library (FSL) (Analysis Group, FMRIB, Oxford, UK). [22] pCASL cans were performed in a total time of 6 minutes. pCASL images were first corrected for slice timing, gradient nonlinearities and subject motion using in-house software and different tools of the FMRIB Software Library (FSL) version 5.0.6. Files were smoothed and after subtraction a mean CBF over the entire 6 minute pCASL scan was calculated, yielding one average perfusion map for the 6- minute scan. This map was then translated to MNI152 space using FLIRT and FNIRT with the conversion matrix based on the 3DT1 scans. The perfusion was measured in the corrected gray matter volume. This corrected gray matter was segmented in four cortical areas based on the Harvard Oxford probabilistic cortical atlas threshold 25 percent [23]. These ROI templates were subsequently used with each subject's gray matter mask to calculate the mean gray matter CBF in units of mL/100g/min for each anatomical region. Perfusion in the hypothalamus and thalamus was calculated in a similar way by measuring perfusion on the CBF maps in thalamic/hypothalamic ROI using Harvard oxford atlas ROI templates for these regions.

One participant was noticed to have extremely tortious blood vessels. For this participant, it was difficult to draw a consistent region of interest for phase contrast MR Angiography. For same reason, he also had strongly decreased ASL labelling efficiency. He was excluded from subsequent analyses.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of both study groups. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by the product of the fasting insulin level (mU/L) and the fasting glucose level (mmol/L) divided by 22.5.

The relation between intranasal insulin and cerebral blood flow was determined using paired t-tests, and presented as means with standard deviation with corresponding p-values. Homogeneity of variance assumption was tested using Levene's test. Correction for inter- individual and intra- individual differences in perfusion was done by calculating normalized gray matter volumes according to MNI standard space registration. For statistical analyses, Statistical Package for Social Sciences (SPSS) software for windows (version 20.0) was used. Statistical significance was set as $P<0.05$.

RESULTS

Characteristics of the study subjects are shown in table 1. The mean age of the younger adults was 22.3 years (range 20-26 years) while the mean age of the older adults was 65.2 (range 60-69) years. The average BMI was similar in both groups of adults. For the younger adults, mean systolic blood pressure (SBP) was 125 mmHg (SD 9.3) and diastolic blood pressure (DBP) 74.5 mmHg (SD 7.2). For the older adults, mean SBP was 156 mmHg (SD 22.7) and mean DBP was 90.4 mmHg (SD 7.9). Fasted glucose, fasted insulin and HOMA index of insulin resistance were all within normal reference ranges for both groups of adults.

Plasma glucose and insulin trajectories

We measured the peripheral (venous) glucose and insulin trajectories throughout an experimental period of 90 minutes, which comprised measurements before, during and after MRI scanning. As shown in figure 3, the trajectories of glucose and insulin values were similar and stable in insulin compared to placebo conditions throughout the experimental period, both in young and older adults. In the young adults, the mean (SD) glucose and insulin levels over the 90- minute period were 4.57 (0.3) mmol/L and 5.25 (2.7) mU/L respectively under placebo condition, while the mean (SD) glucose and insulin levels were

4.61 (0.5) mmol/L and 5.71 (2.6) mU/L respectively under insulin condition. Likewise, in the older adults, the mean (SD) glucose and insulin levels over the 90- minute period were 4.87 (0.5) mmol/L and 5.75 (3.4) mU/L respectively under placebo condition, while the mean (SD) glucose and insulin levels were 4.87 (0.5) mmol/L and 5.30 (3.6) mU/L respectively under insulin condition.

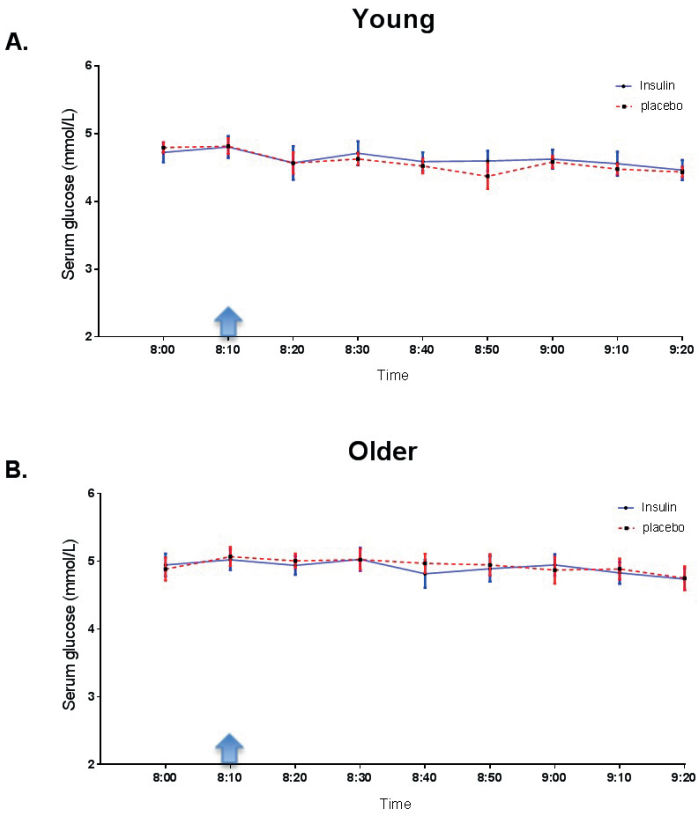

FIGURE 5.3 | Glucose trajectory during the experimental period.

Concentrations (every 10 minutes) of glucose in blood serum over a 90- minute period, comprising measurements before and after intranasal application of insulin (40IU in sulin Actrapid, blue line) or placebo (saline, red dotted line) using ViaNase nasal atomizer. Blue arrow indicate timing of intranasal insulin administration. Data is presented as mean with standard error in A.) younger and B.) older adults.

TABLE 5.1 | Characteristics of study subjects

	Young	Older
Demographics	N = 8	N = 11
Age in years (SD)	22.3 (1.8)	65.2 (3.3)
BMI in kg/m^2	23.6 (2.2)	24.1 (2.2)
Alcohol intake (units/week)	14.4 (2.2)	9.77 (1.7)
Systolic BP (mmHg)	125 (9.3)	156 (22.7)
Diastolic BP (mmHg)	74.5 (7.2)	90.4 (7.9)
Hypertension, n (%)	0 (0)	2 (18.2)
Metabolic		
Fasted glucose in mmol/L	4.83 (0.32)	5.31 (0.51)
Fasted Insulin in pmol/L, median (IQR)	5.3 (4.83, 6.58)	4.63 (2.9, 8.23)
HOMA-IR index, median (IQR)	1.21 (1.0, 1.49)	1.05 (0.65, 2.03)

Unless otherwise stated, values are means (standard deviation). BMI: body mass index.

Quantitative flow

The mean flow through the main cerebropetal arteries supplying the brain after intranasal application of insulin compared to placebo in both the young and older adults is presented in Table 2. Intranasal application of insulin did not significantly change mean blood flow through the cebebropetal arteries in either young nor in older adults. The mean difference in total quantitative flow under insulin minus placebo conditions in the younger adults was 0.35 ml/sec (SD 2.59), while that of the older adults was -0.24 ml/sec (SD 1.66).

The variance (standard deviations) around the mean flow were consistently lower after insulin administration. A Levene's test verified the inequality of the variances in total quantitative flow after intranasal application of insulin compared to placebo when data from young and old adults were combined (P=0.047). Similar trends towards lower variances in cerebral blood flow after intranasal insulin application were observed in young and older adults.

TABLE 5.2 | Quantitative flow through cerebropetal arteries

	Young			Older		
	Placebo Mean ± SD	**Insulin Mean ± SD**	***p*-value**	**Placebo Mean ± SD**	**Insulin Mean ± SD**	***p*-value**
Arterial Flow						
Basilar artery	3.36 ± 0.74	3.15 ± 0.42	0.376	2.91 ± 0.91	2.78 ± 0.63	0.313
Left carotid artery	4.44 ± 1.12	4.60 ± 0.78	0.745	4.48 ± 0.86	4.63 ± 0.69	0.537
Right carotid artery	4.24 ± 1.13	4.66 ± 0.81	0.263	4.64 ± 1.26	4.39 ± 0.75	0.532
Total flow	12.05 ± 2.73	12.40 ± 1.68	0.713	12.04 ± 2.26	11.8 ± 1.10	0.667

Cerebral blood flow (in ml/sec) ± Standard deviation (SD) through cerebropetal arteries in insulin conditions compared to placebo in young and older adults.

ASL perfusion

Perfusion through specific cortical regions of the gray matter is shown in Table 3. In addition to cortical regions, we also assessed perfusion in the thalamus and the hypothalamus. In younger adults, perfusion through the whole brain gray matter, frontal, parietal, temporal and occipital gray matter regions seemed slightly higher after intranasal administration of insulin, but were not significantly different when compared to placebo. In younger adults, intranasal administration of insulin did not change perfusion in the thalamus or in the hypothalamus.

In older adults, intranasal administration of insulin significantly increased perfusion through the occipital gray matter with 6.5% when compared to the administration of a placebo (Table 3, P=0.001). Perfusion through the parietal gray matter was 4.3% increased after intranasal administration of insulin (P=0.034). Perfusion in the frontal and the temporal gray matter regions was not significantly changed after intranasal administration of insulin (Table 3). In older adults, intranasal administration of insulin significantly increased perfusion in the thalamus (P=0.003), but no significant changes were found for perfusion in the hypothalamus. The change in perfusion after intranasal application of insulin for one representative participant is visualized in the gray matter perfusion map shown in figure 4.

TABLE 5.3 | ASL Perfusion through specific brain regions

	Young			Older		
	Placebo Mean ± SD	**Insulin Mean ± SD**	***p*-value**	**Placebo Mean ± SD**	**Insulin Mean ± SD**	***p*-value**
Brain regions						
Whole brain	65.79 ± 11.69	67.53 ± 5.58	0.488	57.53 ± 8.0	59.0 ± 9.77	0.292
Frontal GM	74.93 ± 13.67	76.64 ± 5.52	0.603	63.11 ± 8.19	63.90 ± 10.72	0.653
Temporal GM	62.48 ± 10.82	64.39 ± 7.47	0.405	55.83 ± 7.22	55.74 ± 9.22	0.959
Parietal GM	77.2 ± 14.87	78.66 ± 7.04	0.653	66.35 ± 10.95	69.22 ± 11.51	0.034
Occipital GM	67.67 ± 13.21	68.27 ± 8.97	0.837	61.20 ± 10.11	65.15 ± 11.0	0.001
Thalamus	65.43 ±10.27	65.84 ± 9.89	0.831	63.31 ± 6.84	68.28 ± 6.75	0.003
Hypothalamus	43.97 ± 8.64	42.31 ± 6.43	0.603	38.31 ± 5.25	42.51 ± 6.43	0.156

Perfusion through the four major cortical gray matter regions (ml/100g/min) ± Standard deviation (SD) in insulin conditions compared to placebo in young and older adults as measured by continuous arterial spin labelling. GM: gray matter.

In addition to the whole gray matter regions, we also looked at the perfusion through the right and left hemisphere of each ROI separately. The directions of observed effects in the left/right ROIs were comparable to those observed for the whole lobe ROIs. For example, in older participants perfusion in the right parietal region under placebo compared to insulin condition was 65.58 ± 11.70 compared to 68.31 ± 11.17 (p=0.061); and perfusion in the left parietal region under placebo compared to insulin condition was 67.12 ± 10.41compared to 70.12 ± 12.71 (p=0.221). Similarly, in older participants perfusion in the right occipital region under placebo compared to insulin condition was 60.92 ± 11.07 compared to 65.15 ± 10.97 (p=0.006); and perfusion in the left occipital region under placebo compared to insulin condition was 61.49 ± 9.35 compared to 65.13 ± 11.61 (p=0.005). Likewise, in older participants perfusion in the right thalamus under placebo compared to insulin condition was 63.79 ± 6.72 compared to 68.52 ± 6.14 (p=0.008); and perfusion in the left thalamus under placebo compared to insulin condition was 62.84 ± 7.11 compared to 68.03 ± 7.63 (p=0.005).

Figure 5 shows the paired gray matter perfusion in the parietal and occipital lobes and in the thalamus for every individual subject separately to illustrate individual differences between the placebo and insulin.

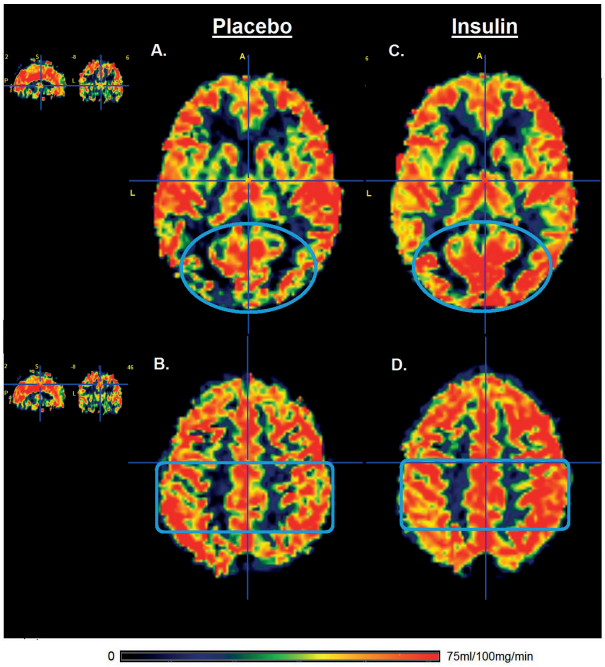

FIGURE 5.4 | Gray matter perfusion maps after intranasal administration of placebo and insulin.

Left panel (A. & B.) represents the gray matter perfusion map after intranasal placebo administration and the right panel (C. & D.) the perfusion map after intranasal insulin administration for one representative older participant. The top row shows an increase in the gray matter perfusion of occipital lobe (illustrated by blue oval) after intranasal administration of insulin compared to placebo. The bottom row shows an increase in gray matter perfusion in the parietal lobe (illustrated by blue rectangle) after intranasal administration of insulin compared to placebo. Only the gray matter was included for calculation of perfusion after intranasal administration of insulin compared to placebo.

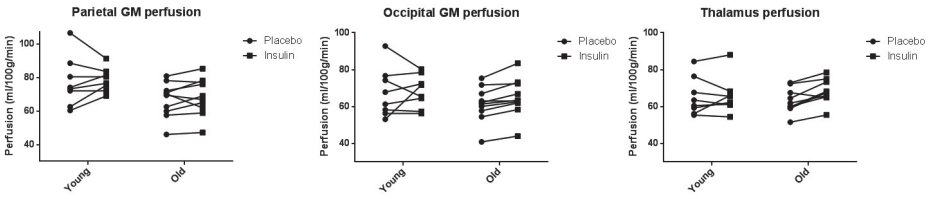

FIGURE 5.5 | Paired individual gray matter perfusion measurements for placebo and insulin conditions.

Left panel represents the gray matter perfusion in the parietal lobe as paired data for placebo and intranasal insulin per indivudual subject in the young and the older group. The right panel represents the gray matter perfusion in the occipital lobe.

DISCUSSION

This study was aimed at investigating the effects of intranasal application of insulin on cerebral blood flow and perfusion in healthy young and older adults. We found no effect of intranasal insulin application on total quantitative flow, or on flow in the basilar, left or right carotid arteries, in either young nor older adults. However, region specific analysis indicated that intranasal insulin application increased perfusion in parietal and occipital gray matter regions and in the thalamus in older but not in young adults.

Due to the presence of direct pathways from the nasal cavity to the CNS, insulin can be delivered non- invasively rapidly to the CNS through the intranasal route without spilling over to the periphery. The intranasal route of insulin administration thus bypasses uptake into the bloodstream and so circumvents the risk of systemic hypoglycemia that is associated with peripheral administration. We found that blood glucose and insulin levels did not change after intranasal administration. This is in line with previous studies that show that plasma glucose concentrations remain unchanged after intranasal application of 40 IU insulin [24, 25]. It further strengthens the fact that intranasal administration can safely be used for selectively increasing cerebral insulin levels.

One of the regions where insulin becomes readily available is the cerebral cortex, which is bathed by CSF, to which intranasally administered insulin has direct access [18]. There is evidence of bulk flow transport of peptides through extracellular pathways from the nasal cavity to the CNS, including cerebral cortex, gray matter and other parts of the CNS, via both olfactory and trigeminal pathways [26]. In the upper nasal cavity, olfactory neurons are exposed such that their axons project through the cribriform plate to the olfactory bulb. When inhaled, insulin accesses the CNS parenchyma either through the cribriform plate along the olfactory neurons through perivascular channels associated with the olfactory or trigeminal systems [27].

Very few studies have been performed to study the effect of intranasal application of insulin on regional blood flow in the brain. A recent study done in eight young healthy adults found no effect of intranasal insulin application on cerebral blood flow in the visual cortex, neither at baseline nor when cerebral blood flow was measured after specific tasks [19]. In line, we found no effect of intranasal insulin application on flux in the major vessels, neither in young healthy men, nor in older men.

While quantitative flow in the major arteries did not increase after intranasal application of insulin, we found that after intranasal insulin application tissue perfusion was increased by 6.5% in occipital cortex and 4.3% in parietal cortex in older but not in young adults. The occipital lobe is the visual processing center. It contains mainly the visual cortex and the ventral stream of vision that enables ability to focus on motor actions in response to outside stimuli. Next to the occipital lobe, the parietal lobe integrates sensory information among various modalities, including proprioception, mecano-reception, and visuo-spatial processing. The posterior parietal cortex, also referred to as the dorsal stream of vision, receives somatosensory and/or visual input that can be transmitted to motor signals. In line with the present data, another study found that perfusion was increased in the insular cortex, an area closely related to the parietal cortex, after intranasal insulin administration [21]. Somewhat similar to the parietal cortex, the insular cortex also integrates information from other sensory modalities and contains topographically organized visceral sensory representation. We also observed that intranasal insulin application increased perfusion of the thalamus. The thalamus receives information from almost all sensory systems and relays the information to associated cortical areas. From literature, increased cerebral blood flow has been linked to vasodilatation around the active area due to increased energy demand [28]. Also, insulin has been shown to be a vasoactive modulator that regulates peripheral and cerebral blood flow possibly via a direct vasodilatory effect [10]. Taken together, our finding of increased perfusion on the parietal and occipital cortex and in the thalamus in the older adults would support the hypothesis that intranasal insulin application might restore energy demand and neuronal activity in these regions in the older adults, possibly by overcoming age- induced deficits in local central insulin action [5, 29]. Similar finding was not demonstrated in the young men, probably because they already had optimal brain perfusion.

This is the first placebo-controlled, cross- over, study in young and older adults showing that intranasally administered insulin increased regional perfusion in the parietal and occipital brain regions and in the thalamus of older adults, as measured with a non-invasive ASL technique. A strength of our study is the cross-over design in which the adults were their own controls, thus minimizing the confounding effects of between- person differences. Moreover, both the young and older participant were included with strict in- and exclusion criteria. Thus, the characteristics of adults in both the young and older participant groups were homogenous, making them comparable within the group. On the

other hand, this study has some limitations. It is limited by its sample size, and the inclusion of males only. Thus, we cannot rule out that insulin has effect on many other brain areas, since several previous studies have demonstrated that insulin had beneficial effects on brain function including, memory, cognition [30-32], and brain mediated functions such as postprandial thermogenesis and other metabolic profiles [29, 33-35]. Thus, other brain regions could have gone undetected because of the small sample size. We also cannot exclude the possibility that effects of intranasal administration of insulin may be sex specific. Indeed, sex-specific effects of intranasal administration of insulin have been observed for other endpoints, including reduction of body weight [34]. It is also a limitation that the clinical significance of the observed increases in CBF remains to be determined. Thus, larger sufficiently powered studies are needed to delineate the exact effects of insulin on cerebral blood flow and perfusion in both the cortical brain regions and in subcortical structures in both males and females and in both young and older adults and to relate these to clinically significant outcomes.

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