Vascular function and insulin sensitivity in the brain and periphery

Summary

A healthy diet is important in preventing the development of age-related disorders, such as cardiovascular disease (CVD), type 2 diabetes (T2D), and dementia, which are closely associated with cognitive decline. While previous dietary intervention trials have primarily focused on traditional CVD risk factors, underlying mechanisms involved in risk reduction of age-related disorders have not been fully explained. To gain a comprehensive understanding of the effects of diet, various markers of vascular function have emerged, including markers of endothelial function, arterial stiffness and retinal microvascular calibers. In addition, over the last decade the importance of evaluating brain health has been recognized in relation to dietary interventions. Arterial spin labeling magnetic resonance imaging (ASL-MRI) has emerged as a non-invasive tool to assess regional brain vascular function, which is closely related to cognitive performance. While many dietary interventions have focused on markers of peripheral insulin resistance using fasting values or an oral glucose tolerance test (OGTT), the effects of diet on brain insulin sensitivity remain an understudied area. Insulin signaling in the brain exerts region-specific effects on neural circuits involved in cognitive performance. Brain insulin responsiveness can be assessed with ASL-MRI combined with intranasal insulin application. The overall aim of this dissertation was to study the effects of dietary interventions on vascular function and insulin sensitivity in both the brain and periphery among adults.

Brain insulin resistance is an important hallmark of age-related conditions, including T2D and dementia. In **Chapter 2**, we therefore conducted a systematic review that summarized 58 randomized, placebo-controlled trials that investigated the acute effects of intranasal insulin on cerebral blood flow (CBF) using ASL-MRI in healthy and diseased populations to define brain insulin responsiveness. We also explored relationships between changes in brain insulin sensitivity and cognitive performance. Intranasal insulin did not affect whole-brain CBF in healthy adults, but increased regional CBF of the inferior frontal gyrus, dorsal striatum, and insular cortex, and reduced CBF around the middle frontal gyrus and hypothalamus. These regions have typically been related to cognitive functioning, and feeding and reward behaviors. Important determinants of the CBF response to the intranasal spray were obesity, T2D, and normal human aging, which indicates altered brain insulin sensitivity. Obese adults showed increased CBF following nasal insulin for the middle frontal gyrus but decreased CBF for hypothalamic and cortico-limbic regions. Furthermore, CBF responses were higher for the insular cortex in T2D patients and for occipital and thalamic regions in older adults. Intranasal insulin also improved memory and executive function, but a causal relation with regional CBF still needs to be established. Finally, nasal insulin at frequently used doses resulted in only a small amount of systemic spill-over, which is unlikely to have an impact on the observed findings. Future studies should investigate longer-term effects of nasal insulin and explore associations between effects on CBF and cognitive performance.

The next two chapters presented the findings of a randomized, single-blinded, controlled cross-over trial that investigated the effects of long-term mixed nut consumption. **Chapter 3** focused on the outcomes related to brain and peripheral insulin sensitivity, as well as cardiometabolic risk markers, while **Chapter 4** described the effects on brain and peripheral vascular function, and cognitive performance. The study involved twenty-eight older adults, aged 65 ± 3 years (mean ± SD), with overweight or obesity (BMI: 27.9 ± 2.3 kg/m^2). Participants were randomly assigned to either a sixteen-week mixed nut intervention (60 g/d mixed nuts: walnuts, pistachio, cashew, and hazelnuts) or a control period without nuts, separated by an 8-week washout. The main outcomes were measured at the end of both periods. Throughout the study, participants adhered to the Dutch food-based dietary guidelines. No serious adverse events or protocol deviations were reported in the diaries and mixed nut intake was well-tolerated. Compliance was excellent, with a median of 98% (IQR: 93-100%) of the sachets consumed. Body weight and composition did not change throughout the study. Food-frequency questionnaires revealed that total energy and protein intakes were not different between intervention periods. However, mixed nut consumption lowered carbohydrate (-4.3 En%; 95%CI: -5.5 to -3.1; P < 0.001) and cholesterol intake (-2.6 mg/MJ; 95%CI: -4.2 to -0.9; P = 0.004), and increased fiber intake (1.6 g; 95%CI: 0.3 to 3.0; P = 0.019) compared with the control. In contrast, total fat intake was 5.4 En% higher (95%CI: 4.1 to 6.8; P < 0.001), with lower intakes of saturated fatty acids, but higher intakes of *cis*-monounsaturated and *cis*-polyunsaturated fatty acids (all, P < 0.001). These dietary changes were further supported by the fatty-acid composition of plasma phospholipids.

In **Chapter 3**, we reported that mixed nut consumption improved regional brain insulin action in six brain clusters, as assessed by quantifying acute effects of nasal insulin on regional CBF, a marker for brain insulin sensitivity, using ASL-MRI. Five clusters were located in the left (-4.5 ± 4.7 mL/100g/min; P < 0.001; -4.6 ± 4.8 mL/100g/min; P < 0.001; and -4.3 ± 3.6 mL/100g/min; P = 0.007) and right occipital lobe (-4.3 ± 5.6 mL/100g/min; P = 0.028). Another cluster was part of the left frontal lobe (-4.9 ± 4.6 mL/100g/min; P < 0.001). Markers of peripheral insulin sensitivity during the oral glucose tolerance test were not affected. Intrahepatic lipid content (-0.7 %-point; (-1.3 to -0.1; P = 0.027), serum low-density lipoprotein cholesterol concentrations (-0.24 mmol/L; 95%CI: -0.44 to -0.04; P = 0.019), and systolic blood pressure (-5 mmHg; 95%CI: -8 to -1; P = 0.006) were reduced after the intervention as compared to the control period. In **Chapter 4**, we observed that mixed nut consumption resulted in a higher resting CBF in the right frontal and parietal lobes (5.0±6.5 mL/100g/min; P < 0.001), left frontal lobe (5.4 ± 7.1 mL/100g/min; P < 0.001), and bilateral prefrontal cortex (5.6 ± 6.6 mL/100g/min; P < 0.001). Effects on endothelial function, arterial stiffness, and the retinal microvasculature were also assessed. Carotid artery reactivity (0.7 %-point; 95%CI: 0.2 to 1.2; P = 0.007), brachial flow-mediated vasodilation (1.6 %-point; 95%CI: 1.0 to 2.2; P < 0.001) and retinal arteriolar calibers

were higher (2 μm; 95%CI: 0 to 3; P = 0.037), and carotid-to-femoral pulse wave velocity lower (-0.6 m/s; 95%CI: -1.1 to -0.1; P = 0.032). Finally, cognitive performance was measured using the Cambridge Neuropsychological Test Automated Battery, for which visuospatial memory (-4 errors [16%]; 95%CI: -8 to 0; P = 0.045) and verbal memory (+1 correct [16%]; 0 to 2; P = 0.035) improved, but executive function and psychomotor speed did not change. Based on these two chapters, we concluded that longer-term mixed nuts consumption as part of a healthy diet improved insulin sensitivity in specific brain regions involved in metabolic and cognitive processes in older adults with overweight and obesity. Regional brain vascular function also improved, which may relate to the observed beneficial effects on memory performance. Furthermore, different vascular function markers along the peripheral arterial tree also improved, and beneficial effects on intrahepatic lipid content, cholesterol concentrations, and blood pressure were observed.

In **Chapter 5**, the results from another randomized, double-blinded, placebo-controlled, cross-over trial investigating the longer-term effects of NWT-03 supplementation, an egg-protein hydrolysate, on arterial stiffness and cardiometabolic markers were reported. The study involved seventy-six adults with metabolic syndrome, aged 61 ± 10 years and a mean BMI of 31.7 ± 4.0 kg/m^2. Participants were randomly assigned to either a 27-day intervention (5 g/day NWT-03) or placebo period, separated by two-to-eight weeks of washout. At the start and end of both periods, measurements were performed in the fasting state and 2-hours following acute NWT-03 intake. Compared with the placebo, longer-term NWT-03 intake did not affect pulse-wave velocity, a marker of arterial stiffness. Fasting pulse pressure was however reduced by 2 mmHg (95%CI: -4 to 0; P = 0.043), but other fasting cardiometabolic risk markers were not affected. No effects were observed following acute NWT-03 intake at baseline. However, acute intake of NWT-03 after the intervention significantly lowered the central augmentation index (-1.3 %-point; -2.6 to -0.1; P = 0.037), suggesting a decreased pressure wave reflection, and diastolic blood pressure (-2 mmHg; -3 to 0; P = 0.036), but other cardiometabolic markers did not change. Longer-term NWT-03 intake did not affect arterial stiffness, but modestly improved fasting pulse pressure in adults with metabolic syndrome. Acute intake of NWT-03 after the intervention also improved CAIxHR75 and diastolic BP.

In conclusion, this dissertation provides further evidence that dietary intervention strategies can reduce the risk of age-related metabolic disorders by effects on vascular function and insulin sensitivity in both the brain and periphery. These observed findings may contribute to beneficial effects on cognitive functioning. Additionally, mixed nut consumption also improved traditional risk factors such as blood pressure and cholesterol levels, as well as various markers of vascular function in periphery, thereby reducing the risk of CVD.