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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Effects of NWT-03, an egg-protein hydrolysate, on brain health and cognitive performance

Raquel Hermans

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :
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Effects of NWT-03, an egg-protein hydrolysate, on brain health and cognitive performance

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Association between cognition and brain health with NWT-03, an egg-protein hydrolysate.

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ABSTRACT

This study investigated the effects of NWT-03, an egg-protein hydrolysate, on cognitive performance and cerebral blood flow in older adults with subjective cognitive decline. A total of 40 participants were included in a 36-week randomised, placebo-controlled trial. Cognitive performance was assessed using the Cambridge Neuropsychological Test Automated Battery, cerebral blood flow was measured using arterial spin labelling magnetic resonance imaging and retinal microvasculature was measured using a retinal camera. No significant differences were found in cognitive performance, cerebral blood flow or retinal microvascular between NWT-03 and placebo. However, subgroup analyses based on sex revealed that NWT-03 supplementation improved executive function in women but not men. These findings suggest that NWT-03 does not improve cognitive performance overall but does improve executive function in women. Further research is needed to confirm these findings and investigate the underlying mechanisms.

INTRODUCTION

Age-related health conditions like cardiovascular disease, dementia and diabetes are among the most prevalent disorders worldwide (1). Cardiovascular disease is among the most common causes of death in older adults, although death rates due to Alzheimer's disease (AD) have also risen (1). Due to the improved life expectancies, the number of adults over age 65 has increased in the last ten years and will continue to rise for the next 20 years (1). Therefore, more and more people will suffer from these age-related health conditions like dementia. These age-related health conditions enormously impact the societal and economic levels. For instance, worldwide dementia prevalence may increase to 131 million in 2050 compared to 47 million in 2015 (1). Additionally, in 2015 the estimated cost of dementia was $818 billion and is expected to rise to 2 trillion dollars by 2030 (2).

Dementia is an umbrella term for cognitive impairment that has become severe enough to compromise social or occupational functioning (3). There is also mild cognitive impairment, a state between normal cognitive function and dementia. With AD being the first most prevalent degenerative dementia, vascular dementia (VD) is the second most common. Cognitive impairment in VD is usually due to cerebrovascular disease, which affects blood flow and vessels in the brain (4). When we talk about cognitive performance, we usually refer to the different areas or domains of functioning (5). Cognition is a broad field encompassing several domains, each responsible for specific cognitive functions (5). The domains of cognition include sensation, perception, motor skills, attention, concentration, memory, language/verbal skills, executive function and processing speed (5). Each domain comprises various subdomains corresponding to specific cognitive processes or abilities contributing to the larger domains (5). Neuropsychological test batteries are designed to measure a range of cognitive abilities in different domains, and each test is designed to measure one or more specific abilities within those subdomains (6).

Cerebral blood flow (CBF) is an important marker of brain vascular function related to

cognitive performance (7, 8). CBF can be defined as the amount of blood, measured in volume, that is delivered through the arteries to a specific unit mass of brain tissue per unit of time (9). A method to measure the delivery of arterial blood to different parts of the brain tissue is arterial spin labelling (ASL) MRI (9). This method relies on measuring the quantity of a tracer substance transported to the human brain tissue through blood flow (9).

Accordingly, a study of 275 patients with AD, mild cognitive impairment and subjective complaints showed that CBF was inversely associated with cognitive performance. Moreover, the more advanced stage of AD was associated with a lower CBF (10). Similarly, the Rotterdam Scan Study looked into impaired CBF and its association with specific domains of cognition. Eight hundred ninety-five older adults aged 60 to 91 participated in this study. Adults with lower CBF performed significantly worse on cognitive tasks (information-processing speed, executive function, and global cognitive function) than those with higher CBF (11). Thus, it can be hypothesised that greater CBF is essential for improved cognitive function.

The retinal vascular analysis is a valuable marker for assessing microvascular function (12). Retinal vessels are a promising biomarker since they share many similarities with the small blood vessels in other body parts, such as the brain (13). Not only do they share similar properties, but there are interrelationships between the retina and the brain (14). A systematic review showed that any retinopathy was significantly associated with dementia and cognitive decline, but more studies are needed (15). Further, there was an association between retinal perfusion and cognitive measures of new learning, information processing, and executive function (16).

Diet is considered an essential modifiable lifestyle factor that can help reduce or prevent the risks of age-related diseases like age-related cognitive impairment (17-19). For instance, proteins and their amino acids are essential in the human diet. The consumption of high-protein diets is becoming more popular due to their beneficial effects on body composition and muscle protein synthesis (20). Some studies suggest that high-protein diets might improve cognition in participants. For instance, a 3-week randomised controlled dietary intervention trial in healthy men showed that the high-protein group (3.0 g protein/kg body weight) improved their reaction time. It was assessed using three tests with varying degrees of complexity from the Test battery for Attentional Performance (21). A systematic review found no significant links between and global cognitive performance. The mean protein consumption was higher than the Recommended Dietary Allowance, ranging from 0.90 g/kg of body weight to 1.1 g/kg. However, significant associations were found between dietary protein intake and memory, visuospatial memory, verbal fluency, processing speed, and sustained attention (22).

Nevertheless, the exact mechanisms of dietary proteins on cognitive performance are still unclear. So far, limited studies have explicitly looked into the association between proteins and amino acids and cognitive impairment. Therefore, more research is needed to understand the relationship between high-protein diets and cognitive function.

Foods containing functional ingredients, including egg-protein hydrolysates, may be potentially interested in reducing the risk of age-related cognitive decline (23). Egg-protein hydrolysates, such as NWT-03, are a type of protein supplement that is derived from eggs. Hydrolysis breaks down proteins into smaller peptides, making them more accessible for the body to absorb and utilise. This process is typically achieved through enzymes, which cleave the protein molecules into smaller units. It has been shown that egg protein hydrolysate could improve cardiovascular health. A possible mechanism can be the inhibition of DPP4, which is involved in glucose metabolism (24-26). The brain's sensitivity to insulin may serve as a potential connection between cognitive performance and NWT-03. In a randomised, placebo-controlled cross-over design, evidence illustrated that consuming 2g of NWT-03 per day for seven days lowered the blood pressure in subjects with mild hypertension (27). Another study showed that intake of 5 g/d NWT-03 improved peripheral vascular function in two days (28). Moreover, egg-derived hydrolysates contain bioactive peptides, which can have anti-obesity and anti-diabetic effects. However, despite the existing research, there remains a lack of studies specifically investigating the mechanisms of action and absorption of the peptides. (29). However, evidence

for the effects of egg-derived proteins on cognitive performance is lacking. A previous study using the hen egg-white hydrolysate, LumiVida, showed improvements in executive function in middle-aged women (30). Gravesteijn *et al.* suggest that 5 g/day NWT-03 for four weeks could improve cognitive function within the executive function domain (31). To our knowledge, no current studies investigate the association between egg-protein hydrolysate and CBF and its association with cognitive performance. This will be the first study to investigate the effects of NWT-03 cognition for a more extended time (36 weeks) in overweight or obese adults with subjective cognitive decline, providing an opportunity for improvement through interventions.

The primary aim of this study was to investigate the effects of long-term NWT-03 on cognitive performance. As a secondary outcome, we also observed the effects of CBF and the microvasculature. We hypothesise that 36-week NWT-03 consumption improves cognitive function in older adults with subjective cognitive decline and that these improvements in cognitive performance are related to changes in CBF and retinal microvascular function.

EXPERIMENTAL PROCEDURES

Study participants – Adults were recruited through advertisements in local newspapers, flyers in the university, the hospital, and public buildings in Maastricht, and among people who had participated in earlier studies. Adults were invited for a screening visit when they were between 60 and 75 years old, and had a BMI between 25 and 35 kg/m^2. The anthropometrics and blood pressure were measured during the screening visit, a fasting blood sample was drawn, and the subjective cognitive decline was assessed with a cognitive failure questionnaire (32). Other inclusion criteria included: fasting plasma glucose below 7.0 mmol/L, fasting serum total cholesterol below 8.0 mmol/L, systolic blood pressure below 160 mmHg, diastolic blood pressure below 100 mmHg, having a stable body weight (no weight or loss of more than 3 kg in the past three months), willing to give up being a blood donor from 8 weeks before the start of the study, during the study and for four weeks after completion of the study and have no difficult venipuncture. The exclusion criteria were: left-handedness, current smoker or smoking cessation of less than 12 months, diabetic patients, familial hypercholesterolemia, abuse of drugs, having more than three alcoholic consumptions per day, use of products or dietary supplements known to interfere with the primary outcomes as judged by the principal investigators, use medication to treat blood pressure, lipid, glucose metabolism, use of an investigational product within another biomedical intervention trial within the previous 1-month, having severe medical conditions that might

Table 1 – Anthropometrics during the NWT-03 and control intervention throughout the intervention trial

	Control period (n = 20)		Intervention period (n = 19)		P-value[1]
	Baseline	Follow-up	Baseline	Follow-up	
Weight (kg)	84.9 ± 9.3	85.2 ± 9.5	82.9 ± 10.3	82.9 ± 10.4	0.17
BMI (kg/m^2)	28.2 ± 2.8	28.3 ± 2.9	27.9 ± 2.8	28 ± 2.7	0.40
W-H ratio	0.92 ± 0.07	0.92 ± 0.07	0.93 ± 0.07	0.94 ± 0.07	0.35
Systolic Blood Pressure (mmHg)	126 ± 12	126 ± 14	124 ± 10	125 ± 7	0.83
Diastolic Blood Pressure (mmHg)	78 ± 7	76 ± 7	74 ± 6	74 ± 6	0.53
Heart rate (bpm)	61 ± 8	65 ± 9	59 ± 6	61 ± 6	0.13

The values are given as mean ± SD.
BMI = Body Mass Index, W-H ratio = Waist-Hip ratio
[1]One-way ANCOVA was performed for anthropometrics to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) anthropometrics as the dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) anthropometrics as the covariate.

Table 2 – Anthropometrics during the NWT-03 and control intervention throughout the intervention trial in men and women

	Men (n =23)		Women (n = 16)		P-value[1]
	Baseline	Follow-up	Baseline	Follow-up	
Weight (kg)	87.3 ± 8.3	87.2 ± 8.8	79.8 ± 9.7	80.4 ± 10.3	0.16
BMI (kg/m^2)	27.6 ± 1.9	27.5 ± 2.2	29.2 ± 3.5	29.4 ± 3.7	0.47
W-H ratio	0.97 ± 0.04	0.97 ± 0.04	0.88 ± 0.06	0.88 ± 0.06	0.47
Systolic Blood Pressure (mmHg)	125 ± 11	127 ± 11	125 ± 13	120 ± 12	0.06
Diastolic Blood Pressure (mmHg)	77 ± 8	76 ± 8	75 ± 6	73 ± 6	0.61
Heart rate (bpm)	60 ± 8	63 ± 8	60 ± 7	62 ± 8	0.57

The values are given as mean ± SD.
BMI = Body Mass Index, W-H ratio = Waist-Hip ratio
[1]One-way ANCOVA was performed for anthropometrics to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) anthropometrics as the dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) anthropometrics as the covariate.

interfere with the study, such as epilepsy, asthma, kidney failure or renal insufficiency, chronic obstructive pulmonary disease, inflammatory bowel diseases, autoinflammatory diseases and rheumatoid arthritis, have an active cardiovascular disease such as congestive heart failure or cardiovascular events, such as an acute myocardial infarction or cerebrovascular accident, and if they have contra-indications for MRI imaging.

Study design – A randomised, placebo-controlled trial with a parallel design was performed. In this study, the participants received NWT-03 (Newtricious R&D), which provided 5.0 g of NWT-03 for 36 weeks. In the control intervention, they received a placebo similar in taste and colour. The empty and full bags needed to be returned each visit to assess compliance. Participants were also asked to maintain their habitual diet and physical exercise. Each visit anthropometric measurements such as weight, height, BMI, waist-to-hip ratio, and blood pressure were performed, and a fasting blood sample was drawn at each visit. At baseline and after 36 weeks, participants had to attend the research facilities to perform the follow-up measurements. Participants were asked not to exercise heavily or drink alcoholic beverages on the days before blood sampling. On the morning of the blood sampling, the participants had to have fasted for 12 hours (from 20:00) and were only allowed to drink water.

Cognitive performance – Our primary outcome, cognitive performance was tested in three cognitive domains: attention, memory, and executive function. The cognitive performance was tested using the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Cambridge Cognition). The Motor Screening Task (MOT) evaluates attention and psychomotor speed. The Reaction Time Task (RTI), where the motor and mental response speeds are measured. The outcome measures of the RTI are reaction time (RT) and movement time (MT). The Multitasking Test (MTT) and Spatial Working Memory (SWM) assessed executive function. Memory is evaluated with Spatial Span (SSP), Delayed Matching to Sample (DMS) and Paired Associated Learning (PAL).

MRI acquisition and processing – CBF measurements were performed at the Scannexus research facilities in Maastricht on a 3T MAGNETOM Prisma Fit MRI system using a 64-channel head/neck coil (Siemens Healthcare). Participants were placed in the scanner with their head-first in the supine position. One high-resolution anatomical (Magnetization-Prepared Rapid Acquisition Gradient Echo) MPRAGE scan was performed (repetition time 2400 ms, echo time 2.18 ms, inversion time 1040 ms, 1.0mm isotropic resolution, 8 degrees flip angle and 160 sagittal slices, duration 6min). Perfusion-weighted images were acquired using pseudo-continuous arterial spin labelling (PCASL) with a background-

suppressed segmented 3-dimensional gradient and spin echo readouts (repetition time 4000 ms, echo time 13.6 ms, Generalised Autocalibrating Partially Parallel Acquisitions 2, labelling duration 1750 ms, post-labelling delay 2000 ms and ten label-control repetitions, duration: 9 min). Nineteen slices with a voxel resolution of 3.0 mm isotropic were obtained. Pairwise subtraction of label and control images was performed on the PCASL data to generate perfusion-weighted images. Preparatory to CBF quantification, images were voxel-wise calibrated using the M0 image without magnetisation preparation and with a repetition time of 20 s. The software FSL version 6.0 (Analysis Group, FMRIB, Oxford, UK) was used to estimate the quantitative CBF maps from the ASL data. Quantifying the perfusion-weighted images was obtained by the FSL BASIL toolbox (version 4.0.15) following the recommendations of the ASL White Paper (33). Volbrain, an online web interface, was used to perform brain extraction and tissue segmentation for the anatomical MPRAGE image (34). The calibrated ASL images with the CBF images were co-registered using Boundary-Based Registration to the brain-extracted MPRAGE image. After that, mean CBF values were calculated for the global brain, grey matter, (sub)cortical and both hemispheres based on the high-resolution anatomical scan.

Retina – Measuring the microvasculature of the retina, participants were seated with the head resting on a chinrest and images were taken with a retinal camera (Topcon TRC-NW-300; Topcon Co.). Afterwards, images were analysed using the software IVAN. This software allowed measuring the diameter of three retinal arterioles and venules. Next, the arteriolar-to-venular ratio (AVR) was calculated using the Parr-Hubbard formula (35).

Statistical analyses – The software SPSS (IBM SPSS Statistics version 28.0) was used for the statistical analyses. Descriptive statistics summarised the baseline characteristics and are presented as means ± SDs for normally distributed parameters or medians (ranges) for non-normal distributed parameters. We compared the intervention group with the control group by performing One-way ANCOVA with post-intervention (follow-up) anthropometrics as the

Table 3 – Outcomes of cognitive tests between baseline and follow-up

Outcome	Control period (n = 20)	Intervention period (n = 19)	P-value[1]
Attention & Psychomotor Speed			
RTI Median Five-Choice Movement Time	310 ± 10	292 ± 10	0.25
RTI Median Five-Choice Reaction Time	391 ± 41	395 ± 40	0.84
Executive Function			
MTT Incongruency cost (median)	118 ± 68	123 ± 61	0.66
MTT Reaction latency (median)	760 ± 109	749 ± 110	0.21
MTT Multitasking cost (median)	205 ± 148	241 ± 107	0.79
MTT Total incorrect	14 ± 15	5 ± 8	0.14
SWM Strategy (6-8 boxes)	8 ± 0.4	9 ± 0.4	0.27
SWM Between Errors	16 ± 9	14 ± 10	0.20
Memory			
PAL First Attempt Memory Score	11 ± 5	10 ± 3	0.85
PAL Total Errors (Adjusted)	21 ± 15	20 ± 12	0.91

The values are given as mean ± SD (After adjustment for pre-intervention cognitive test outcome). MTT = Multitasking Test; PAL = Paired Associates Learning; RTI = Reaction Time; SWM = Spatial Working Memory.

[1]One-way ANCOVA was performed for cognitive performance to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) cognitive test outcome as the dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) cognitive test outcome as the covariate.

Table 4 – Outcomes of cognitive test differences between baseline and follow-up in men.

Outcome	Control period (n = 12)	Intervention period (n = 11)	P-value[1]
Attention & Psychomotor Speed			
RTI Median Five-Choice Movement Time	303 ± 80	301 ± 85	0.40
RTI Median Five-Choice Reaction Time	391 ± 45	407 ± 37	0.54
Executive Function			
MTT Incongruency cost (median)	133 ± 55	129 ± 68	0.78
MTT Reaction latency (median)	734 ± 102	747 ± 110	0.77
MTT Multitasking cost (median)	187 ± 107	215 ± 80	0.73
MTT Total incorrect	13 ± 13	4 ± 6	0.14
SWM Strategy (6-8 boxes)	7 ± 3	9 ± 1	0.54
SWM Between Errors	18 ± 9	10 ± 9	0.69
Memory			
PAL First Attempt Memory Score	11 ± 5	9 ± 2	0.56
PAL Total Errors (Adjusted)	22 ± 18	24 ± 12	0.96

The values are given as mean ± SD (After adjustment for pre-intervention cognitive test outcome). MTT = Multitasking Test; PAL = Paired Associates Learning; RTI = Reaction Time; SWM = Spatial Working Memory.

[1]One-way ANCOVA was performed for cognitive performance to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) cognitive test outcome as the dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) cognitive test outcome as the covariate.

dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) anthropometrics as the covariate.

RESULTS

Participant characteristics – Sixty-one older men and women were assessed for eligibility. Seventeen persons were excluded due to not meeting the inclusion criteria (n = 15) or due to other reasons (n = 2). Forty-four older men and women with subjective cognitive decline started the study. Four persons dropped out during the study. One dropped out before the first test day; one dropped out due to the product taste, one due to sudden death and one due to taking high blood pressure medication. A total of 40 participants (23 men and 17 women) completed the study and were included in the statistical analyses. Due to missing measurements of CBF, 1 participant could not be analysed. Participants had a mean age of 68 ± 4 years (men: 68.4 ± 4.3 years and women: 67.7 ± 3.7 years), and the mean BMI was 28.1 ± 2.9 kg/m^2 (men: 27.6 ± 1.9 kg/m^2 and women: 28.9 ± 3.5 kg/m^2). No serious adverse events were reported, and NWT-03 intake was well tolerated. Body weight, BMI, W-H ratio, systolic and diastolic

blood pressure, and heart rate did not significantly differ between interventions (See Table 1) as well between gender (See Table 2)

Cognitive performance – No significant intervention effects were found for cognitive parameters between NWT-03 or placebo supplementation (See Table 3). After performing subgroup analyses, differences were found in the responses based on sex. There is a statistically significant difference in post-intervention latency response between the intervention when adjusted for pre-intervention latency response. The median latency response in women was statistically significantly lower in the NWT-03 intervention group (751 ± 118 ms) compared to the control group (799 ± 114 ms), with a mean difference of 78 ms (95% CI: 15.92 to 141.67 ms; p = 0.018) (See Table 4). There is also a statistically significant difference in post-intervention errors between the interventions when adjusted for pre-intervention latency response. The between error in women was statistically significantly lower in the NWT-03 intervention group (20 ± 9) compared to the control

Table 5 – Outcomes of cognitive test differences between baseline and follow-up in women .

Outcome	Control period (n = 8)	Intervention period (n = 8)	P-value[1]
Attention & Psychomotor Speed			
RTI Median Five-Choice Movement Time	295 ± 69	304 ± 81	0.50
RTI Median Five-Choice Reaction Time	390 ± 38	380 ± 41	0.99
Executive Function			
MTT Incongruency cost (median)	97 ± 83	116 ± 53	0.60
MTT Reaction latency (median)	799 ± 114	751 ± 118	0.01
MTT Multitasking cost (median)	231 ± 200	276 ± 133	0.92
MTT Total incorrect	16 ± 18	7 ± 10	0.53
SWM Strategy (6-8 boxes)	9 ± 2	9 ± 1	0.80
SWM Between Errors	14 ± 8	20 ± 9	0.005
Memory			
PAL First Attempt Memory Score	11 ± 3	13 ± 3	0.45
PAL Total Errors (Adjusted)	19 ± 12	14 ± 9	0.89

The values are given as mean ± SD (After adjustment for pre-intervention cognitive test outcome). MTT = Multitasking Test; PAL = Paired Associates Learning; RTI = Reaction Time; SWM = Spatial Working Memory.

[1]One-way ANCOVA was performed for cognitive performance to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) cognitive test outcome as the dependent variable, the control and intervention groups as levels of the independent variable, and the pre-intervention (baseline) cognitive test outcome as the covariate.

group (14 ± 8), with a mean difference of 8 (95% CI: 3.01 to 14.41; p = 0.005) (See Table 4). In contrast, no significant differences were observed in men (See Table 5). No significant changes were observed for the attention & psychomotor speed test RTI, the memory tests PAL and the executive function test SWM.

CBF – No intervention effects were found in global CBF, grey matter CBF, cortical CBF, subcortical CBF, and the CBF in the hemisphere (See Table 6)

Retinal analyses – After excluding retinal images from four participants due to poor image quality, we performed the analysis. No intervention effects were found in central retinal venular equivalent (CRVE), central retinal arteriolar equivalent (CRAE) and Arteriolar-to-Venular diameter Ratio (See Table 7).

DISCUSSION

Our findings – This study aimed to examine the effects of longer-term NWT-03 intake on cognitive function, focusing on executive function, attention and psychomotor speed, memory, and its association with CBF. In this randomised, placebo-controlled trial with a parallel design, NWT-03 did not significantly improve cognitive function overall. After performing subgroup analyses, differences were found in the responses based on gender. NWT-03 significantly improved executive function in women but not men. The finding that NWT-03 improved executive function in women is significant and suggests that this supplement could be beneficial for people looking to enhance their cognitive abilities. Further, cognitive performance within the domain of attention and psychomotor speed, and memory was not changed.

Finally, the study found no significant changes in CBF and retinal microvasculature after the 36-week egg-protein hydrolysates intervention.

Table 6 – Outcomes of CBF between interventions.

Outcome	Control period (n = 8)	Intervention period (n = 8)	P-value[1]
Global CBF	37.6 ± 6.2	30.8 ± 8.7	0.97
Grey Matter CBF	43.8 ± 7.44	40.8 ± 9.9	0.82
Cortical CBF	47.9 ± 7.9	44.9 ± 11.3	0.99
Subcortical CBF	28.7 ± 8.5	26.3 ± 8.0	0.93
Hemi CBF	33.3 ± 5.2	31.4 ± 8.2	0.84

The values are given as mean ± SD. CBF: cerebral blood flow; hemi: hemisphere.
[11]One-way ANCOVA was performed for cognitive performance to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) cognitive test outcome as the dependent variable. the control and intervention groups as levels of the independent variable. and the pre-intervention (baseline) cognitive test outcome as the covariate.

Comparison with previous studies – Other studies have also demonstrated improvements in

cognitive function after supplementing egg-protein hydrolysates. A previous trial within our Nutrition and Movement Sciences department examined the effects of the egg protein hydrolysate NWT-03 on cognitive function in men and women with metabolic syndrome (31). This randomised, double-blind, placebo-controlled study looked at 76 men and women with metabolic syndrome (mean age of 60 ± 10 years) that received a placebo (5 g/day maltodextrin) or the supplement (5 g/day NWT-03) for four weeks separated by a wash-out period of 2-8 weeks. Only two cognitive tests were performed: an anti-cue reaction time test and a psychomotor vigilance test. Their results are consistent with ours as they found that NWT-03 improves cognitive function in the aspect of executive function. A possible explanation of why they did find an improvement in cognition in both men and women could be that, in this population, all the participants had metabolic syndrome, allowing for more improvement by the intervention. Another explanation could be that this study used other cognitive tests than ours. CANTAB's cognitive assessments target specific cognitive domains more effectively than traditional pen-and-paper assessments. By generating a more significant number of data points, these digital assessments minimise extraneous factors and increase sensitivity, resulting in highly accurate measurements.

Another study showed a significant improvement in executive function after the intervention of tryptophan-rich protein hydrolysate (30). In this randomised, placebo-controlled, parallel trial, 59 healthy women (45-65 years) received a placebo (n = 30; control drink: 0.5 g casein hydrolysate twice per day) or the supplement (n = 29; LumiVidae: 0.5 g twice per d, containing 70 mg Trp in total) for 19 days. Like our study, this study used CANTAB to assess the effects on cognitive performance but with other tests focused on Attention & Psychomotor speed and Memory. This study only found significant changes in the 'Simple reaction time task' and 'Match to Sample Visual Search task', more specifically in the aspects of executive function, which is consistent with our study. The study product differs from ours as it is a tryptophan-rich protein drink, while ours is derived from lysozyme protein. Both products were provided as a powder that needed to be dissolved in water as it makes it easier to consume for the elderly population. The dosage of NWT-03 (5.0 g) is impractically high for convenient consumption in capsule form.

Possible mechanisms – While there is limited research on the specific effects of egg-protein hydrolysates on cognition, there are several potential mechanisms through which they could impact brain function. This is the first study to observe the effects of NWT-03 on CBF and microvasculature and its association with cognitive performance. However, the study found no significant changes in CBF or in the retinal microvasculature. It is possible that the intervention did not directly impact CBF or retinal microvasculature. Different physiological processes may be involved, and the specific mechanisms of action may not have influenced these particular outcomes. A previous study found that systolic and diastolic blood pressure was reduced after NWT-03 but only in subjects with

Table 7 – Outcomes of retinal vascular between interventions.

Outcome	Control period (n = 8)	Intervention period (n = 8)	P-value[1]
CRAE	122.8 ± 17.3	124.6 ± 21.0	0.44
CVRE	219.1 ± 16.9	221.6 ± 27.6	0.38
AVR	0.6 ± 0.1	0.6 ± 0.07	0.77

The values are given as mean ± SD. AVR = Arteriolar-to-Venular diameter; CRAE = central retinal arteriolar equivalent; CVRE = central retinal venular equivalent.

[11]One-way ANCOVA was performed for cognitive performance to test for intervention differences over time. One-way ANCOVA with post-intervention (follow-up) cognitive test outcome as the dependent variable. the control and intervention groups as levels of the independent variable. and the pre-intervention (baseline) cognitive test outcome as the covariate.

elevated blood pressure at baseline (mild hypertension). Mild hypertension means a blood

pressure level of 140-159 mmHg systolic and/or 90-99 mmHg diastolic. Table 1 and 2 shows that the average systolic and diastolic blood pressure is way less than the standard values of people with mild hypertension. It is plausible that our study population included individuals who were less likely to show improvements in peripheral circulation compared to a population with hypertension. Further research should identify the mechanisms by which NWT-03 exerts its cognitive benefits, the optimal dosage and duration of intake, and whether it could have more significant effects in populations with dementia.

There are other possible underlying mechanisms. Firstly NWT-03 could influence cognitive function due to the inhibition of the Dipeptidyl peptidase-4 inhibitor (DPP-4). DPP-4 is an enzyme involved in glucose metabolism and has been linked to cognitive impairment. One proposed mechanism for the potential cognitive benefits of DPP-4 inhibitors involves GLP-1 (36). GLP-1 receptors are found in various regions of the brain (37). Activation of GLP-1 receptors has been associated with neuroprotective effects. By increasing GLP-1 levels, DPP-4 inhibitors may potentially exert these neuroprotective effects and improve cognitive function (36). Inhibition of DPP4 is associated with improvements in glucose control (reduction in glucose fluctuations), thereby protecting against worsening cognitive performance (24-26). DPP-4 inhibitors and GLP-1

receptor agonists primarily work by enhancing insulin secretion through the incretin effect. A previous study showed that there was a decreased plasma glucose and insulin concentrations after NWT-03 consumption (28), so this mechanism could explain this beneficial effect. Research suggests that the brain is sensitive to insulin (12), and it has been observed that insulin can cross the blood-brain barrier. One important function of insulin in the brain is its involvement in synaptic plasticity, which is essential for learning and memory (38). Insulin receptors are present in key brain regions involved in memory formation, such as the hippocampus. Insulin signaling helps facilitate the strengthening and formation of new synaptic connections, thereby promoting optimal cognitive function (38). This finding indicates that peripheral insulin levels could potentially play a role in cognitive performance, specifically in the area of memory (13). However, it should be noted that the relationship between NWT-03, DPP4 inhibition, and cognitive performance is still not well established. Further research is needed to determine the specific effects of DPP4 inhibition on cognitive function and to understand the potential role of NWT-03 in this process.

Secondly, egg-protein hydrolysates are a rich source of essential amino acids, including tryptophan, a precursor to serotonin. Serotonin regulates mood, appetite, and sleep and has been linked to cognitive processes such as memory and learning. Therefore, egg-protein hydrolysates may indirectly impact brain function by producing serotonin (39-41).

An alternative explanation for the cognitive benefits of NWT-03 could lie in the possibility that the small protein fragments present in it can cross the blood-brain barrier (42). The blood-brain barrier is a selective barrier composed of specialised cells lining the blood vessels in the brain (43). Its primary purpose is to protect the brain from potentially harmful substances while allowing the passage of essential nutrients and molecules (43). Once small protein fragments successfully cross the BBB, they can interact with various molecular

targets in the brain, including receptors and enzymes, and affect cognitive processes. This may also allow them to impact the brain processes involved in cognitive performance, as it can directly affect brain vasculature (42). NWT-03 could dissolve in small protein fragments and directly interact with receptors or signaling pathways in the brain, affecting neuronal function and synaptic transmission, thereby impacting cognitive processes such as learning, memory, attention, and executive function.

Strength and limitations – A strength of our study was that the study was double-blinded, reducing the risk of bias. Also, the study was more extended (36 weeks) compared to the two other studies examining cognitive function after supplementation of egg-protein hydrolysate (30, 31). Another strength of this study is the measurement of CBF, the MRI scan. MRI is commonly used to measure CBF due to its non-invasive nature and its ability to provide detailed anatomical and functional information about the brain (44). Additionally, measurements of CBF using arterial spin labelling have demonstrated the ability to differentiate between individuals with normal cognition, individuals at risk for Alzheimer's disease , and those diagnosed with AD (45).

One limitation was that it was a parallel study; a cross-over design would have been better for reducing participant variability. In a cross-over design, each participant serves as their own control, receiving all interventions or treatments. This allows for a direct within-subject comparison, reducing the impact of inter-individual variability on the study results. It also allows for the minimisation of confounding factors as each participant receives all interventions, any potential confounding factors that are constant within the participant are automatically balanced out.

CONCLUSION

In conclusion, the findings of this study indicate that NWT-03 did not significantly impact overall cognitive performance. However, a notable improvement in executive function was observed in women. This suggests that NWT-03 may have a differential effect on cognitive domains based on gender.

The observed improvement in executive function could not be explained by changes in CBF or microvascular factors, indicating that alternative mechanisms may be at play. The specific underlying mechanisms responsible for the observed gender-specific improvement in executive function remain unclear, highlighting the need for further investigation.

Additional studies are needed to elucidate the underlying mechanisms of NWT-03 and its effects on cognitive function. Future research should explore potential gender differences in response to NWT-03 and investigate alternative pathways that may be involved in the observed cognitive improvements.

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