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Nutrition in acute and chronic diseases

Longer-term effects of the egg-protein hydrolysate NWT-03 on arterial stiffness and cardiometabolic risk markers in adults with metabolic syndrome: a randomized, double-blind, placebo-controlled, crossover trial

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BACKGROUND: Short-term intake of egg-derived protein hydrolysates, such as NWT-03, suggest improvements in arterial stiffness and metabolic profiles, but longer-term trials are lacking. This study therefore examined the longer-term effects of NWT-03 on arterial stiffness and cardiometabolic markers in men and women with metabolic syndrome.
METHODS: Seventy-six adults with metabolic syndrome (age 61 ± 10 years; BMI 31.7 ± 4.0 kg/m^2) participated in a randomized, controlled, double-blind, cross-over trial with 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 h following acute NWT-03 intake. Arterial stiffness was assessed by carotid-to-radial ($PWV_{c\text{-}r}$), carotid-to-femoral pulse wave velocity ($PWV_{c\text{-}f}$), and central augmentation index (CAIxHR75). Moreover, cardiometabolic markers were assessed.
RESULTS: Compared with control, longer-term NWT-03 supplementation did not affect fasting $PWV_{c\text{-}r}$ (0.1 m/s; −0.2 to 0.3; $P = 0.715$) or $PWV_{c\text{-}f}$ (−0.2 m/s; −0.5 to 0.1; $P = 0.216$). Fasting pulse pressure (PP) was however reduced by 2 mmHg (95% CI: −4 to 0; $P = 0.043$), but other fasting cardiometabolic 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 CAIxHR75 (−1.3%-point; −2.6 to −0.1; $P = 0.037$) and diastolic BP (−2 mmHg; −3 to 0; $P = 0.036$), but other cardiometabolic markers did not change.
CONCLUSION: Longer-term NWT-03 supplementation did not affect arterial stiffness, but modestly improved fasting PP in adults with metabolic syndrome. Acute intake of NWT-03 after the intervention also improved CAIxHR75 and diastolic BP.
TRIAL REGISTRATION: The study was registered at ClinicalTrials.gov as NCT02561663.

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INTRODUCTION

Metabolic syndrome is a clustering of (metabolic) risk markers that substantially promote arterial stiffening, and increases the risk to develop cardiovascular disease (CVD) and type 2 diabetes (T2D) [1]. Therefore, the need for effective nutritional approaches for reducing arterial stiffness and the improvement of metabolic profiles is key. Foods containing functional ingredients, such as protein hydrolysates, may be of potential interest in metabolic syndrome risk reduction [2, 3]. NWT-03 is a dietary egg-protein hydrolysate, derived from the digestion of lysozyme with alcalase. It has been identified as a potential inhibitor of angiotensin-converting enzyme (ACE) [4]. Recently, we have demonstrated a blood pressure (BP)-lowering effect in mild-hypertensive adults following the daily intake of 2 g NWT-03 for one week, but no effects were observed at lower (1 g) or higher doses (5 g) [5]. In adults with impaired glucose tolerance (IGT) or T2D, the administration of 5 g NWT-03 for two days did however not affect carotid-to-femoral pulse wave velocity ($PWV_{c\text{-}f}$), which is the gold standard to assess regional arterial stiffness [6, 7]. However, carotid-to-radial pulse wave velocity ($PWV_{c\text{-}r}$) was reduced, which suggests changes in arterial stiffness of peripheral muscular arteries that are more sensitive to vasoactive agents that central elastic arteries due to their differential composition [6, 8]. Interestingly, $PWV_{c\text{-}r}$ improvement was not accompanied by changed in BP, suggesting that the decrease in $PWV_{c\text{-}r}$ may be related to mechanisms other than ACE-inhibition [6]. In this context, in vitro studies have shown that NWT-03 also inhibits the enzyme dipeptidyl peptidase 4 (DPP-IV), which plays a major role in glucose and lipid metabolism [4, 9–11]. Accordingly, marked improvements in cardiometabolic risk markers (i.e., glucose, lipid

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and lipoprotein metabolism) were observed after intake of 5 g NWT-03 for two days in adults with IGT or T2D, which may contribute to the observed reduction in $PWV_{c\text{-}r}$ [6]. While our previous studies primarily focused on acute or short-term NWT-03 intake in overweight or obese adults with or without (pre)diabetes [5, 6], it is also relevant to examine the immediate physiological responses in adults with metabolic syndrome that exhibit a more heterogenous risk profile. Moreover, it is important to investigate also longer-term effects for a comprehensive understanding of the sustained impact on arterial stiffness and cardiometabolic markers to better evaluate the clinical relevance of NWT-03 supplementation in reducing CVD and T2D risk [12]. Therefore, the primary objective of this randomized, placebo-controlled, double-blind, cross-over trial was to evaluate the acute (2 h) and longer-term (27 days) effects of daily 5 g NWT-03 supplementation on arterial stiffness in adults with metabolic syndrome. Furthermore, effects on cardiometabolic risk markers were explored.

MATERIALS AND METHODS

Study population

Potential participants were recruited via posters in university and hospital buildings or advertisements in local newspapers. Additionally, participants of previous studies were approached if they had given written consent to contact them again. Potential volunteers were contacted by telephone and invited for a screening visit. Adults aged between 18 and 75 years were included when they met the International Diabetes Federation's (IDF) harmonized criteria for the presence of the metabolic syndrome [13], defined as having at least three of five risk components: abdominal obesity (waist circumference >94 cm for men or >80 cm for women) or a Body Mass Index (BMI) > 30 kg/m^2; raised fasting triacylglycerol (TAG) concentrations ≥1.7 mmol/L; reduced fasting HDL-cholesterol (HDL-C) concentrations (<1.03 mmol/L for men or <1.29 mmol/L for women); raised fasting plasma glucose concentrations (>5.6 mmol/L); raised BP (systolic BP [SBP] ≥130 mmHg or diastolic BP [DBP] ≥85 mmHg). The exclusion criteria were: hypersensitivity to study product; instable body weight (weight gain or loss ≤5% within 3 months); current smoker or smoking cessation <12 months; medical conditions that could interfere with the main study outcomes (e.g. chronic kidney disease, endocrinological or immunological disorders); use of medication or dietary supplements known to interfere with the main study outcomes; pregnancy or lactation; drug or alcohol abuse (men >21 units/week and women >14 units/week); blood donation within 8 weeks prior to screening or during the study; participation in another biomedical study within 60 days before or during the study. Written informed consent was obtained from all volunteers. The study was conducted according the guidelines of the Declaration of Helsinki and approved by the Medical Ethics Committee of Maastricht University Medical Center (METC153021). The study was registered on September 28, 2015 at ClinicalTrials.gov as NCT02561663.

Study design

The study had a randomized, placebo-controlled, double-blind, cross-over design with an intervention and control period of both 27 days, separated by a washout period of two to eight weeks (Fig. 1). Study participants were allocated by a research assistant to start either in the intervention or control period based on a computer-generated randomization scheme. During the intervention period, subjects consumed daily 5 g NWT-03, which was produced and provided by Newtricious R&D BV as described previously [5]. Both NWT-03 and the placebo (maltodextrin) were packaged in dry-powder sachets. Sachets had to be stored at room temperature (15–25 °C). Before consumption, the study product had to be dissolved in 200 mL water and was consumed 30 min before breakfast in the fasting state.

Both periods included visits at baseline and after 27 days. All measurements were performed in quiet and temperature-controlled (20 °C) rooms. Measurements were performed by several researchers, but measurements for a specific participant were always performed by the same observer. On the day preceding each visit, participants were provided a standardized meal (commercially available lasagna) to minimize the variation due to the influence of the last evening meal on metabolic and vascular outcomes. Participants were requested to fast overnight for at least 10 h and to abstain from alcohol before the study visits. Weight and height were measured at each visit using a wall-mounted stadiometer. Subsequently, participants had to rest in the supine position for at least 10 min before vascular measurements were performed. Moreover, a fasting venous blood sample was collected. Subjects were then requested to take their first daily dose of the investigational product under supervision of the study investigator. Acute effects were determined by repeating the same set of measurements two hours after intake. During the entire study period, all participants were requested to record daily in study diaries any signs or symptoms of illnesses, use of medication, alcohol consumption, any protocol deviations and any other complaints. Volunteers was asked to fill in a validated food-frequency questionnaire (FFQ) to assess energy and nutrient intakes over the past four weeks. Energy and nutrient intakes were calculated using the Dutch Food Composition Database (NEVO) [14].

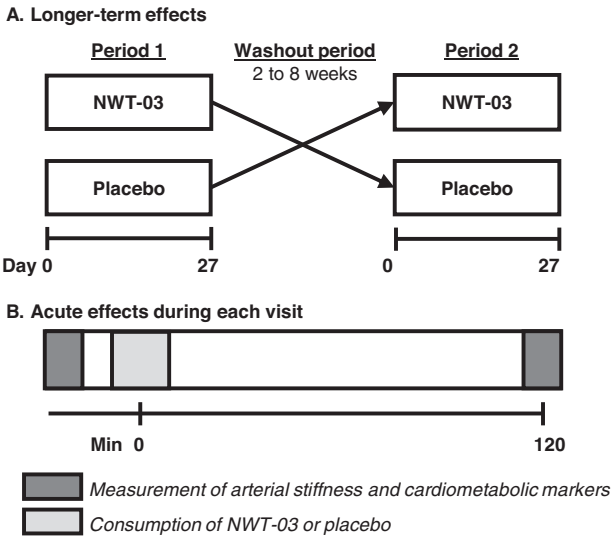

Fig. 1 Overview of the study design. A Longer-term effects were determined by comparing the difference in fasting values after the 27-day NWT-03 of placebo intervention. **B** During each visit, acute effects were determined 2 h after the intake of NWT-03 or placebo.

Arterial stiffness

Regional arterial stiffness was assessed in triplicate using a tonometer (SphygmoCor v9, AtCor Medical, West Ryde, Australia) by detecting the delayed pulse wave arrival compared to the R-wave of the electrocardiogram at the carotid and radial artery for $PWV_{c\text{-}r}$, and at the carotid and femoral artery for $PWV_{c\text{-}f}$. These parameters were calculated automatically by the program provided by the manufacturer by dividing the timeframe of delay by the direct carotid-to-radial and carotid-to-femoral distance [15, 16]. Furthermore, radial artery pulse wave analysis was performed in triplicate using the same tonometer applied to the radial artery near the wrist of the arm. The central arterial waveform was derived from the peripheral waveform with a validated transfer function. The central augmentation index adjusted for heart rate (CAIxHR75) was defined as the difference between the first and second peak of the waveform, expressed as a percentage of the pulse pressure (PP) and further corrected for heart rate [16].

Cardiometabolic markers

Using a validated semi-automatic device (Omron M7 IntelliSenseTM, Omron, Hoofddorp, The Netherlands) office brachial SBP and DBP were measured in a supine position four times, separated by at least one minute between measurements. The first measurement was discarded and the last three measurements were averaged. PP was calculated by subtracting SBP from DBP, and mean arterial pressure (MAP) was calculated by the following formula: MAP = 1/3 * SBP + 2/3 * DBP.

Venous blood samples were drawn into serum separator tubes and sodium fluoride (NaF)-containing tubes (Becton, Dickinson and Company, Franklin Lanes, New York, USA). Serum tubes were allowed to clot at room temperature for 30–60 min after withdrawal and centrifuged (15 min at 1300 g at 21 °C). Plasma tubes were directly placed on ice after withdrawal and immediately centrifuged (15 min at 1300 g at 4 °C). After centrifugation, plasma and serum samples were distributed in aliquots, snap frozen

in liquid nitrogen, and stored at −80 °C until further analysis. Serum total cholesterol (TC) concentrations (CHOD-PAP method; Roche Diagnostics System, Mannheim, Germany), HDL-C concentrations (precipitation method, Roche Diagnostics System, Mannheim, Germany), TAG concentrations corrected for free glycerol (GPO-Trinder, Sigma Diagnostics, St Louis, USA), and high-sensitivity C-reactivity protein (hsCRP; immunoturbidimetric assay, Horiba ABX, Montpeillier) were measured in all samples. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald formula [17]. Serum insulin concentrations (Millipore Corporation, Billerica, USA) and plasma glucose concentrations (Horiba, ABX, Montpellier, France) were measured in all samples. $HOMA_{IR}$, a marker for peripheral insulin sensitivity was calculated [18]. All technicians were blinded to the treatments of the subjects.

Statistical analyses

Data were presented as means ± standard deviations (SDs) unless otherwise indicated. Based on previous research, it was determined that 72 participants were needed to detect a true difference of at least 0.84 m/s (expected SD = 1.76 m/s) in $PWV_{c\text{-}r}$, which was the primary study outcome, with 80% power and a two-sided alpha of 0.05 [6]. Linear mixed models were used to examine differences between the intervention and control periods over time. Treatment, period, and gender were included as fixed factors and a subject-specific random intercept was included. Long-term effects were determined as the difference between fasting values at the end of the treatments using day 0 values as covariate. Acute effects were evaluated at baseline and after the intervention by comparing differences between treatments before and two hours after intake of the study product using the corresponding fasting values as covariate. Carry-over effects were examined by including the order of the treatment as fixed factor in all models, but no significant effects were found and treatment order was therefore omitted from all models. SPSS was used to perform all statistical analyses (IBM Corp., IBM SPSS Statistics, V26, Armonk, NY, USA). A P-value < 0.05 was considered to be statistically significant.

RESULTS

A Consolidated Standards of Reporting Trials flow (CONSORT) diagram of the study is shown in Fig. 2. After screening, a total of 79 subjects fulfilling the criteria of the metabolic syndrome were eligible for participation in this trial. Two participants dropped out during the first period due to personal reasons or digestive problems, and one subject was excluded from analyses due to unjustified inclusion not meeting the IDF harmonized criteria for the metabolic syndrome. Therefore, 76 participants (46 men and 30 women) completed the study. Baseline subject characteristics are presented in Table 1. The mean age of participants was 61 ± 10 years and their BMI was 31.7 ± 4.0 kg/m^2. NWT-03 supplementation did not significantly change weight (P = 0.806) or BMI (P = 0.862) compared to placebo. No (serious) adverse events were reported regarding the intake of NWT-03 or placebo. Total energy and nutrient intakes were comparable during the experimental and placebo periods (Supplementary Table 1).

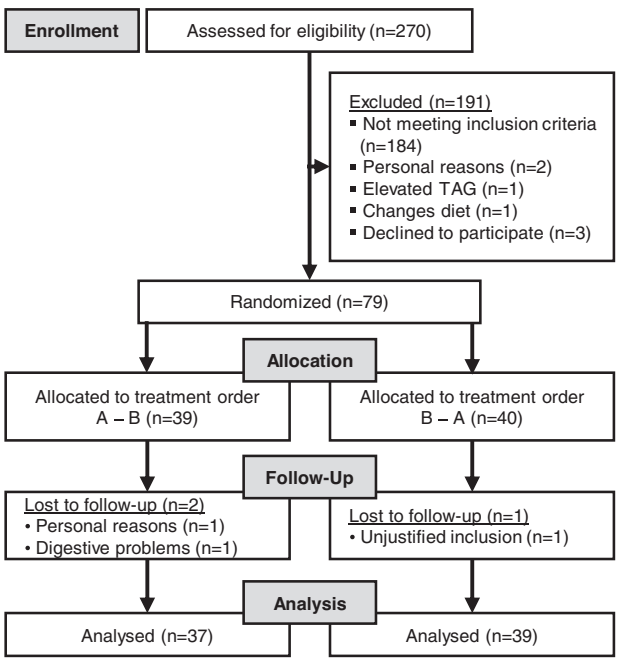

Fig. 2 CONSORT flow diagram. In total, 79 subjects were eligible to participate who were randomized for treatment order (A = Placebo, B = NWT-03). During the intervention three participants dropped out, resulting in a total of 76 subjects for the analysis.

Arterial stiffness

No significant differences were observed in fasting $PWV_{c\text{-}r}$, $PWV_{c\text{-}f}$ or CAIxHR75 after longer-term supplementation with NWT-03 (Table 2). However, CAIxHR75 was 1.3%-point lower (95% CI: −2.6 to −0.1; P = 0.037) after acute intake of NWT-03 compared to placebo at day 27, whereas no acute effects were observed for $PWV_{c\text{-}r}$ or $PWV_{c\text{-}f}$ (Table 3). There were no acute effects of NWT03 intake on markers of arterial stiffness at baseline (Supplemental Table 2).

Cardiometabolic markers

The longer-term NWT-03 intervention significantly lowered fasting PP by 2 mmHg (95% CI: −4 to 0; P = 0.043), but other fasting cardiometabolic markers did not change (Table 2). After 27 days, DBP was 2 mmHg (95% CI: −3 to 0; P = 0.036) lower following acute intake of NWT-03 compared to placebo (Table 3). Furthermore, the acute intake of NWT-03 after the 27-day intervention tended to lower MAP (−1 mmHg; −3 to 0; P = 0.095), TC (−0.07 mmol/L; −0.15 to 0.01; P = 0.097), and LDL-C (−0.06 mmol/L; 95% CI: −0.14 to 0.01; P = 0.089). No acute effects were observed for glucose, insulin, $HOMA_{IR}$, HDL-C, and TAG. Also, no acute effects on cardiometabolic markers were observed at baseline (Supplementary Table 2).

DISCUSSION

The intake of egg-protein-derived hydrolysates, such as NWT-03, has already been shown to exert several cardiometabolic benefits in animal studies [3, 4]. However, the number of well-designed human intervention trials investigating these functional ingredients is limited and mostly restricted to short-term interventions. We evaluated here for first time the longer-term effects of NWT-03 on regional arterial stiffness and cardiometabolic markers in adults

Table 1. Baseline subject characteristics[a].

	Subjects (n = 76)
Men/women, n (%)	46 (61)/30 (39)
Age (years)	60.5 ± 10.1
BMI (kg/m^2)	31.8 ± 4.0
Waist circumference (cm)	109.3 ± 11.4
SBP (mmHg)	133 ± 12
DBP (mmHg)	88 ± 8
Glucose (mmol/L)	5.7 ± 0.7
HDL-C (mmol/L)	1.24 ± 0.27
TAG (mmol/L)	1.99 ± 0.76

BMI Body Mass Index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TAG* triacylglycerol.
[a]Values are means ± SDs.

Table 2. Longer-term effects of NWT-03 or placebo intake on fasting arterial stiffness and cardiometabolic markers in adults with metabolic syndrome[a].

	NWT-03		Placebo		Treatment effect [95% CI][b]	*P*-value
	Day 0	Day 27	Day 0	Day 27		
Arterial stiffness						
$PWV_{c\text{-}r}$ (m/s)	7.5 ± 1.3	7.5 ± 1.1	7.5 ± 1.2	7.5 ± 1.1	0.1 [−0.2, 0.3]	$P = 0.715$
$PWV_{c\text{-}f}$ (m/s)	8.7 ± 1.7	8.6 ± 1.6	8.6 ± 1.5	8.7 ± 1.6	−0.2 [−0.5, 0.1]	$P = 0.216$
CAIxHR75 (%)	21.7 ± 8.4	21.7 ± 8.1	22.7 ± 8.3	21.5 ± 8.1	0.8 [−0.5, 2.1]	$P = 0.186$
Cardiometabolic markers						
SBP (mmHg)	131 ± 13	129 ± 13	130 ± 13	130 ± 14	−2 [−4, 1]	$P = 0.180$
DBP (mmHg)	86 ± 6	85 ± 8	85 ± 8	85 ± 9	0 [−1, 2]	$P = 0.778$
PP (mmHg)	45 ± 10	44 ± 10	45 ± 10	45 ± 10	−2 [−4, 0]	**$P = 0.043$**
MAP (mmHg)	101 ± 9	100 ± 9	100 ± 9	100 ± 9	0 [−2, 1]	$P = 0.616$
Glucose (mmol/L)	6.0 ± 0.7	6.0 ± 0.7	6.0 ± 0.7	6.0 ± 0.7	−0.1 [−0.1, 0.0]	$P = 0.203$
Insulin (mIU/L)	15.7 ± 6.3	16.4 ± 8.8	15.0 ± 6.2	16.2 ± 8.3	−0.4 [−2.1, 1.3]	$P = 0.636$
$HOMA_{IR}$	4.3 ± 2.0	4.5 ± 2.7	4.0 ± 1.9	4.4 ± 2.7	−0.2 [−0.7, 0.4]	$P = 0.519$
TC (mmol/L)	5.59 ± 0.98	5.55 ± 1.03	5.60 ± 1.00	5.59 ± 1.01	−0.03 [−0.18, 0.12]	$P = 0.682$
HDL-C (mmol/L)	1.10 ± 0.24	1.11 ± 0.27	1.08 ± 0.31	1.11 ± 0.25	−0.01 [−0.04, 0.03]	$P = 0.607$
LDL-C (mmol/L)	4.09 ± 0.91	4.07 ± 0.94	4.12 ± 0.95	4.11 ± 0.91	−0.01 [−0.14, 0.12]	$P = 0.887$
TAG (mmol/L)	2.03 ± 1.01	1.84 ± 0.82	1.94 ± 0.92	1.82 ± 0.80	−0.02 [−0.04, 0.11]	$P = 0.817$
hsCRP (mg/L)	2.2 [1.2, 4.0]	2.2 [1.0, 4.1]	2.0 [1.0, 3.8]	2.4 [1.2, 4.1]	−0.5 [−1.1, 0.1]	$P = 0.103$

$PWV_{c\text{-}r}$ carotid-to-radial pulse wave velocity, *$PWV_{c\text{-}f}$* carotid-to-femoral pulse wave velocity, *cAIxHR75* central augmentation index corrected for heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MAP* mean arterial pressure, *$HOMA_{IR}$* homeostatic model assessment of insulin resistance, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerol, *hsCRP* high-sensitive C-reactive protein.

[a]Values are means ± SDs or median [IQR]; $n = 76$.

[b]Linear mixed model analysis with treatment, period and gender as fixed factors, participant as random factor and day 0 values as covariate. *P*-values for the effect of treatment (mean difference [95% CI] between the NWT-03 and placebo intervention) were reported. $P < 0.05$ was considered statistically significant (in bold).

with unfavorable metabolic profiles. Our findings indicate that supplementation of 5 g NWT-03 for 27 days did not affect fasting regional arterial stiffness. However, longer-term beneficial effects on fasting PP were observed, whereas the acute intake of NWT-03 after the 27 days intervention significantly improved CAIxHR75 and DBP.

The current study showed that the 27-day consumption of NWT-03 did not affect markers of arterial stiffness in this population consisting of adults with metabolic syndrome. In line with the current findings, no effects were observed on $PWV_{c\text{-}f}$ in our previous study following two days of 5 g NWT-03 intake in subjects with IGT or T2D [6]. Lucey et al. also failed to show any effects on $PWV_{c\text{-}f}$ in adults with mild hypertension after the 6-week supplementation of an egg ovalbumin-derived protein hydrolysate [19]. Previous studies using milk-derived protein hydrolysates showed improvements in $PWV_{c\text{-}f}$ with supplementation periods longer than 6 weeks, suggesting that longer trials may be necessary to observe beneficial effects [20]. In contrast, in a previous trial we reported an improved $PWV_{c\text{-}r}$ after two days of 5 g NWT-03 intake in adults with IGT or T2D [6], but no effects were observed on $PWV_{c\text{-}r}$ in the current study. Effects on $PWV_{c\text{-}r}$ suggest changes in stiffness of peripheral muscular arteries that are more sensitive to vasoactive agents (i.e., nitric oxide (NO)) than central elastic arteries due to their differential composition [8]. Thus, increased NO-availability leads to a greater vasodilation, thereby improving $PWV_{c\text{-}r}$ [21]. Unfortunately, no human trials to date have focused on NO-dependent vasodilation as a potential explanation for the observed discrepancies between the outcomes of the different studies evaluating effects of NWT03. Animal models have already reported beneficial effects of NWT-03 on endothelium-dependent vasodilation and vascular resistance [4, 22]. Moreover, trials using protein hydrolysates from other food sources already have demonstrated beneficial effects on the NO-dependent endothelial function after 2 weeks of supplementation [20]. Altogether, based on these findings, effects of egg-derived protein hydrolysates on arterial stiffness are not convincing and further longer-term trials are warranted to study whether NWT-03 intake affects markers of endothelial function in humans.

In our current study, we observed a reduction in fasting brachial PP following NWT-03 supplementation. Although the magnitude of the PP reduction may appear to be small, it is important to recognize that lower PP has been independently associated with a reduced risk of CVD and all-cause mortality [23]. In the context of our findings, it can however be questioned whether the observed decrease in brachial PP translates to a clinically relevant CVD risk reduction. The underlying physiological explanation driving the PP change remains to be elucidated. With advancing age, PP amplification occurs as a result of arterial stiffening, which was not affected by NWT-03 [21, 23]. An alternative explanation for the observed changes in PP could however be potential effects on other markers of the vasculature, such as endothelial function, which was not assessed in the current study. Regarding the potential link between the PP reduction and cardiovascular health, it is important to note that the effects of egg-derived protein hydrolysates on BP regulation have yielded inconsistent findings. While animal studies have shown that egg-derived protein hydrolysates exert modest ACE-inhibitory effects thereby reducing BP [24], these effects are more controversial in humans. In two studies, no effects were observed [6, 19], while in one trial a daily supplement of 2 g NWT-03 for 7 days decreased 36-h ambulatory

Table 3. Acute effects of NWT-03 or placebo intake on arterial stiffness and cardiometabolic markers following the longer-term intervention in adults with metabolic syndrome[a].

Day 27	NWT-03		Placebo		Treatment effect [95% CI][b]	*P*-value
	0 h	2 h	0 h	2 h		
Arterial stiffness						
$PWV_{c\text{-}r}$ (m/s)	7.5 ± 1.1	7.4 ± 1.1	7.5 ± 1.1	7.5 ± 1.1	0.0 [−0.3, 0.2]	$P = 0.778$
$PWV_{c\text{-}f}$ (m/s)	8.6 ± 1.6	8.8 ± 1.6	8.7 ± 1.6	8.9 ± 1.7	−0.2 [−0.3, 0.3]	$P = 0.785$
CAIxHR75 (%)	21.7 ± 8.1	21.9 ± 8.1	21.5 ± 8.1	23.0 ± 8.5	−1.3 [−2.6, −0.1]	**$P = 0.037$**
Cardiometabolic markers						
SBP (mmHg)	129 ± 13	133 ± 13	130 ± 14	135 ± 14	−1 [−3, 2]	$P = 0.532$
DBP (mmHg)	85 ± 8	86 ± 9	85 ± 9	87 ± 8	−2 [−3, 0]	**$P = 0.036$**
PP (mmHg)	44 ± 10	47 ± 11	45 ± 10	48 ± 10	1 [−1, 3]	$P = 0.507$
MAP (mmHg)	100 ± 9	102 ± 9	100 ± 9	103 ± 9	−1 [−3, 0]	$P = 0.095$
Glucose (mmol/L)	6.0 ± 0.7	5.7 ± 0.5	6.0 ± 0.7	5.6 ± 0.6	0.0 [0.0, 0.1]	$P = 0.268$
Insulin (mmol/L)	16.4 ± 8.8	12.9 ± 6.0	16.2 ± 8.3	13.1 ± 6.5	−0.3 [−1.4, 0.7]	$P = 0.507$
$HOMA_{IR}$	4.5 ± 2.7	3.3 ± 1.7	4.4 ± 2.7	3.3 ± 1.9	−0.1 [−0.4, 0.3]	$P = 0.765$
TC (mmol/L)	5.55 ± 1.03	5.46 ± 1.01	5.59 ± 1.01	5.57 ± 1.01	−0.07 [−0.15, 0.01]	$P = 0.097$
HDL-C (mmol/L)	1.11 ± 0.27	1.09 ± 0.24	1.11 ± 0.25	1.10 ± 0.26	−0.01 [−0.03, 0.02]	$P = 0.530$
LDL-C (mmol/L)	4.07 ± 0.94	3.99 ± 0.93	4.11 ± 0.91	4.10 ± 0.93	−0.06 [−0.14, 0.01]	$P = 0.089$
TAG (mmol/L)	1.84 ± 0.82	1.86 ± 0.83	1.82 ± 0.80	1.82 ± 0.83	0.02 [−0.06, 0.09]	$P = 0.685$
hsCRP (mg/L)	2.2 [1.0, 4.1]	2.1 [1.0, 4.0]	2.4 [1.2, 4.1]	2.2 [1.1, 3.8]	0.1 [−0.13, 0.27]	$P = 0.517$

$PWV_{c\text{-}r}$ carotid-to-radial pulse wave velocity, *$PWV_{c\text{-}f}$* carotid-to-femoral pulse wave velocity, *cAIxHR75* central augmentation index corrected for heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MAP* mean arterial pressure, *$HOMA_{IR}$* homeostatic model assessment of insulin resistance, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerol, *hsCRP* high-sensitive C-reactive protein.

[a]Values are means ± SDs or median [IQR]; $n = 76$.

[b]Linear mixed model analysis with treatment, period and gender as fixed factors, participant as random factor and 0 h values as covariate. *P*-values for the effect of treatment (mean difference [95% CI] between the NWT-03 and placebo intervention) were reported. $P < 0.05$ was considered statistically significant (in bold).

SBP and DBP, but only in mild-hypertensive adults [5]. The discrepancies in the effects of NWT-03 on BP may be attributed to several factors, including the dosage and characteristics of the study population. Indeed, positive effects of protein hydrolysates depend on the type of hydrolysate and characteristics of the study population, such as baseline BP [25]. Whereas trials with egg-derived protein hydrolysates show inconsistent effects on BP [6, 19], a recent meta-analysis has demonstrated beneficial effects of protein hydrolysates from other food sources, especially milk-derived lactotripeptides [26]. Interestingly, studies that observed improvements in $PWV_{c\text{-}f}$ following the longer-term intake of casein-derived lactotripeptides simultaneously observed changes in BP [27, 28]. In our study, we also showed that after the longer-term intervention but not at baseline, acute intake of NWT-03 reduced CAIxHR75 by 1.3%-point, which reflects a decreased wave reflection that may indicate a lower amplitude of pressure waves in peripheral arteries that is associated with lower CVD risk [29, 30]. However, the use of CAIxHR75 as a surrogate for arterial stiffness has been challenged, as the parameter is dependent on several confounding factors, including changes in reflection sites and DBP [29, 31]. Accordingly, we found that acute NWT-03 intake after the longer-term intervention also lowered DBP, which could to some extent explain the reduction in CAIxHR75 [31].

Our results did not show any effects on glucose homeostasis or insulin sensitivity. However, in a previous trial we found that 5 g NWT-03 for 2 days improved fasting glucose and insulin concentrations in adults with IGT or T2D [6]. In agreement with the present study, a 6-week supplementation of an egg-ovalbumin-derived protein hydrolysate did also not show any effects in a population with normal blood glucose profiles [19]. This may imply that NWT-03 exerts more pronounced glucoregulatory effects in those with IGT or T2D, in which the current study population may have been too heterogenous to detect changes. In vitro studies suggest that egg-derived proteins may beneficially affect glucose homeostasis by inhibition of α-glucosidase to reduce intestinal carbohydrate absorption, and by DPP-IV-inhibition, leading to increased incretin levels that inhibit glucagon synthesis and stimulate insulin production to lower plasma glucose [4, 9, 10]. However, animal studies show little to no change in blood glucose or insulin concentrations following intakes of egg-protein hydrolysates [32]. No longer-term effects of NWT-03 intake were observed on fasting lipid or lipoprotein concentrations in the current study, which is in line with Lucey et al. that did not show any effects [19]. At the end of the current intervention, acute NWT-03 intake did however tend to lower serum TC and LDL-C concentrations compared to placebo. Comparable results have already been reported in other studies with protein hydrolysates showing acute improvements in lipid and lipoprotein metabolism [6, 33]. Mechanisms of hypolipidemic activity of food protein hydrolysates have already been reviewed before [34], but it must be noted that evidence is mainly based on in vitro and animal studies. Protein hydrolysates are thought to alter the enterohepatic bile acid circulation and as such elevate cholesterol catabolism, thereby inducing a compensatory increase in hepatic cholesterol uptake and consequently lower serum TC and LDL-C concentrations. Other postulated mechanisms for the hypolipidemic activity include disruption of micellar solubility and the regulation of lipogenic proteins and genes [34].

A key strength of the current trial was the double-blinded, controlled design with low dropout rates, excellent compliance and no changes in the background diet of participants. In addition, the study used a comprehensive assessment of arterial

stiffness at both carotid-femoral and carotid-radial sites using the recommended guidelines. Moreover, the study focused on a well-defined population with metabolic syndrome who had not yet been diagnosed or treated for chronic or metabolic diseases. This also implies that it remains unknown whether our findings can be extrapolated to other population groups. Finally, the study intervention duration was relatively short. Nevertheless, a strength of the study was the inclusion of analyzing both acute and chronic effects of the intervention side by side.

CONCLUSION

In conclusion, the present findings indicate that NWT-03 supplementation for 27 days does not affect arterial stiffness in adults with metabolic syndrome. However, an improvement in fasting PP was observed, but it remains to be elucidated whether this moderate change is clinically relevant. Future well-designed trials involving diverse study populations are warranted to validate the potential longer-term cardiometabolic benefits of egg-protein hydrolysates.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

Conceptualization was performed by JP. All authors contributed to the data analysis, curation and visualization, and contributed to the original draft of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

The study was conducted according the guidelines of the Declaration of Helsinki and approved by the Medical Ethics Committee of Maastricht University Medical Center (METC153021).

ADDITIONAL INFORMATION

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