

SCIENTIFIC OPINION

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MenaQ7[®] and maintenance of the elastic properties of the arteries: evaluation of a health claim pursuant to Article 13 (5) of Regulation (EC) No 1924/2006

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA)

Abstract

Following an application from NattoPharma ASA submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Ireland, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to MenaQ7[®] and maintenance of the elastic properties of the arteries. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food proposed by the applicant as the subject of the health claim is MenaQ7[®], which contains 97% menaquinone-7 by weight. The Panel considers that MenaQ7[®] is sufficiently characterised. The claimed effect proposed by the applicant is 'improves arterial stiffness'. Upon a request from EFSA, the applicant agreed that the claimed effect is maintenance of elastic properties of the arteries (by measuring the arterial stiffness). The Panel considers that maintenance of the elastic properties of the arteries is a beneficial physiological effect. No conclusions could be drawn from the three human intervention studies submitted for scientific substantiation of the claim. In the absence of evidence for an effect of MenaQ7[®] on maintenance of the elastic properties of the arteries *in vivo* in humans, the studies on the proposed mechanisms by which the food/constituent could exert the claimed effect cannot be used as a source of evidence for the scientific substantiation of the claim. The Panel concludes that a cause and effect relationship has not been established between the consumption of MenaQ7[®] and maintenance of the elastic properties of the arteries.

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Keywords: menaquinone-7, MenaQ7[®], arterial stiffness, arterial elasticity, health claim

Requestor: Competent Authority of Ireland following an application by NattoPharma ASA.

Question number: EFSA-Q-2019-00229

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Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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Competing interests: A waiver was granted to an expert of the working group, Jean-Louis Bresson. Pursuant to Article 21(6) of the afore-mentioned Decision, the concerned expert was allowed to take part in the discussion and in the drafting phase of the scientific output.

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Summary

Following a request from NattoPharma ASA submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Ireland, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to MenaQ7[®] and maintenance of elastic properties of the arteries.

The food proposed by the applicant as the subject of the health claim is 'MenaQ7[®], a vitamin K2 as menaquinone-7'. Upon a request from the European Food Safety Authority (EFSA), the applicant confirmed that the food/constituent being the subject of the claim is the food product MenaQ7[®] because MenaQ7[®] follows a specific manufacturing process and all the clinical studies submitted have been performed with MenaQ7[®]. MenaQ7[®] contains 97% menaquinone-7 by weight. The Panel considers that the food, MenaQ7[®] which is the subject of the health claim, is sufficiently characterized.

The claimed effect proposed by the applicant is 'improves arterial stiffness'. The proposed target population is 'healthy general population'. Upon a request from EFSA, the applicant agreed that the claimed effect is maintenance of elastic properties of the arteries (by measuring the arterial stiffness). The Panel considers that maintenance of the elastic properties of the arteries is a beneficial physiological effect.

Three human intervention studies were identified by the applicant as pertinent to the health claim. No conclusion can be drawn from one uncontrolled study for the scientific substantiation of the claim. Two other studies were carried out in the same centre and by the same research group. Both studies were randomised, two-arm, parallel, double-blind, placebo-controlled studies on MenaQ7[®] administration (during 1 and 3 years, respectively) and a number of vascular measures. The Panel considers that, owing to important methodological limitations, no conclusion can be drawn from these studies for the scientific substantiation of the claim. The Panel notes that no conclusions could be drawn from the three human intervention studies submitted for scientific substantiation of the claim.

In the absence of evidence for an effect of MenaQ7[®] on maintenance of elastic properties of the arteries *in vivo* in humans, the studies on the proposed mechanisms by which the food/constituent could exert the claimed effect cannot be used as a source of data for the scientific substantiation of the claim.

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of MenaQ7[®] and maintenance of the elastic properties of the arteries.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to MenaQ7[®] and maintenance of the elastic properties of the arteries.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of MenaQ7[®], a positive assessment of its safety, nor a decision on whether MenaQ7[®] is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'MenaQ7[®], vitamin K2 as menaquinone-7'.

Health relationship as claimed by the applicant

According to the applicant, 'MenaQ7[®], vitamin K2 as menaquinone-7 improves arterial stiffness. Arterial stiffness (progressive loss of elastic properties) and remodelling (lumen enlargement and wall thickening) characterize arteriosclerosis, a typical manifestation of vascular ageing. Arterial stiffness has emerged as independent predictor of cardiovascular risk'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'improvement of arterial stiffness is achieved by reducing dp-ucMGP (circulating desphospho-uncarboxylated matrix Gla-protein)'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wordings for the health claim: 'MenaQ7[®], vitamin K2 as menaquinone-7, improves arterial stiffness'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is the 'healthy general population'. The daily consumption of 180 µg MenaQ7 is recommended.

Data provided by the applicant

Health claim application on MenaQ7[®] and maintenance of the elastic properties of the arteries pursuant to Article 13.5 of Regulation 1924/2006 is presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of a health claim application.

As outlined in the General scientific guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA General guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health are outlined in a specific EFSA guidance (EFSA NDA Panel, 2018).

Data claimed as confidential: variability from batch-to-batch analyses, manufacturing process, stability information, and Knapen et al. (2017) unpublished study. EFSA has issued its Decision on Confidentiality on 19/11/2019.

Data claimed as proprietary: Knapen et al. (2015, 2017) studies, batch-to-batch variability, manufacturing process, stability information.

3. Assessment

The approach used by the NDA Panel for the evaluation of health claims is explained in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

In assessing each specific food/health relationship which forms the basis of a health claim, the NDA Panel considers the following key questions:

- i) the food/constituent is defined and characterised;
- ii) the claimed effect is based on the essentiality of a nutrient; OR
the claimed effect is defined and is a beneficial physiological effect for the target population, and can be measured *in vivo* in humans;
- iii) a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three questions needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of questions (i) and/or (ii) precludes the scientific assessment of question (iii).

3.1. Characterisation of the food/constituent

The food/constituent proposed by the applicant as the subject of the health claim is 'MenaQ7[®], a vitamin K2 as menaquinone-7'.

Menaquinone-7 (MK-7) is a chemical compound with a molecular formula $C_{46}H_{64}O_2$, molecular weight 649.016 g/mol and chemical formula: 2-methyl-3-all-*trans*-polyprenyl-1,4-naphthoquinones. MK-7 belongs to the group of chemical compounds called menaquinones. Menaquinones (MK-n) have different numbers of isoprene units attached to the naphthoquinone ring structure (up to 13). Together with other menaquinones, MK-7 forms vitamin K2.

MK-7 is either manufactured by fermentation of a broth consisting of chickpea components and *Bacillus licheniformis* or obtained from chemical synthesis. Synthetic or natural MK-7 is then purified to obtain MenaQ7[®] formulation.

An overview of the manufacturing process (claimed as confidential) of MenaQ7[®] was provided. Information related to stability, batch to batch variability and bioavailability of MenaQ7[®] was also provided. The applicant claims that MenaQ7[®] contains < 3% impurities and that at least 97% by weight is MK-7.

Upon a request from EFSA, the applicant confirmed that the food/constituent being the subject of the claim is MenaQ7[®], and not vitamin K2 in general or menaquinone-7 from any source, because MenaQ7[®] follows a specific manufacturing process and all the clinical studies submitted have been performed with MenaQ7[®].

The Panel considers that the food/constituent MenaQ7[®], which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'improves arterial stiffness'. The proposed target population is the 'healthy general population'.

Upon a request from EFSA, the applicant agreed that the claimed effect is maintenance of the elastic properties of the arteries, and that the claimed effect can be assessed *in vivo* in humans by measuring indices of arterial stiffness.

The scientific evidence for the substantiation of health claims on the maintenance of the elastic properties of the arteries can be obtained from human intervention studies showing a reduction in indices of arterial stiffness. Evidence on the sustainability of the effect with continuous consumption of the food/constituent over extended periods of time (e.g. 4–8 weeks) should be provided.

Carotid-femoral pulse wave velocity (cfPWV) is the gold standard measurement of arterial stiffness. Non-invasive determinations of wave reflections can also be obtained by central pulse wave analysis through three major parameters: central pulse pressure, central systolic pressure and the augmentation index (AIx). Central pressure, the AIx and PWV cannot be used interchangeably as indices of arterial stiffness because, in contrast to PWV, which is a direct measure of arterial stiffness, central pressure and the AIx are only indirect, surrogate measures of arterial stiffness. There is consensus on the methodological aspects to be considered when measuring arterial stiffness *in vivo* in humans (EFSA NDA Panel, 2018).

The Panel considers that maintenance of the elastic properties of the arteries is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in MEDLINE, Science Direct and Google Scholar using the following key words: "MenaQ7[®]", "vitamin K2", "menaquinone-7", "cardiovascular", "vascular". No restrictions were applied with respect to publication date and language. A manual review of literature was also performed to identify any additional relevant studies.

Three human intervention studies were identified as pertinent to the health claim, of which two were carried out in the same centre and by the same research group (Knapen et al., 2015, 2017, unpublished). All the studies were performed with the food/constituents complying with the specifications given for MenaQ7[®] in Section 3.1.

Mansour et al. (2017) report on a one-arm, uncontrolled intervention study in which MenaQ7[®] was consumed daily for 8 weeks by renal transplant recipients (n = 60). The Panel considers that no conclusions can be drawn from this uncontrolled study for the scientific substantiation of the claim.

Knapen et al. (2015) report on a randomised, two-arm, parallel, double-blind, placebo-controlled study in which healthy postmenopausal women consumed either MenaQ7[®] or placebo for 3 years. The results for the primary outcome of the study, indices of 'bone strength', were published elsewhere (Knapen et al., 2013).

Healthy postmenopausal women between 55 and 65 years of age were recruited for the study. Women < 2 years postmenopausal, with BMI > 30 kg/m², osteoporosis at baseline (bone mineral density (BMD) T-score ≤ - 2.5 SD), coagulation disorders, chronic diseases, metabolic bone diseases, gastrointestinal diseases, taking medication that interferes with vitamin K metabolism/status and/or blood coagulation, using corticosteroids, bisphosphonates, or hormone replacement therapy, and/or taking vitamin K supplements, were excluded.

Sample size (n = 244) was calculated based on indices of 'bone strength' (specific variable not reported in the publication), the primary outcome of the study. A power of 65% was calculated post hoc for aortic PWV (cPWV). The Panel notes that the power of the study to detect between-group differences in cfPWV, which is considered the gold standard measurement of arterial stiffness, has not been specified.

Participants were randomised (1:1 allocation) to consume MenaQ7[®] (one capsule daily containing 180 µg MK-7; n = 120) or placebo (similar capsules without MK-7; n = 124) for 3 years and attended the clinical centre every year (three visits post-baseline).

The following vascular outcomes were measured at each visit using established methods:

- a) Carotid-femoral and carotid-radial pulse wave velocity (cfPWV and crPWV, respectively);
- b) the following characteristics of the common carotid artery: intima-media thickness (IMT), diameter at end-diastole (D), change in diameter in systole (Δd), change of area from diastole to systole (ΔA), distensibility coefficient (DC), compliance coefficient (CC), Young's elasticity modulus, stiffness index β , PWV (cPWV);
- c) mean arterial pressure (MAP) and pulse pressure (ΔA) in the brachial artery.

The Panel notes that 14 outcome variables were assessed in this study in relation to the properties of the arterial tree.

T-tests for independent samples (for normally distributed variables) and chi-square tests (for categorical variables) were used to compare groups at baseline. Multivariate linear regression analysis was performed to evaluate treatment effects on the variables of interest adjusting for age, BMI and log(TG) in the intention-to-treat (ITT) analyses.

Mean age of participants at baseline was 59.5 ± 3.3 years. No significant differences were observed at baseline between the two study groups. Out of the 244 women randomised, 223 (112 in the MK-7 group and 111 in the placebo group) completed the study. The reasons for discontinuation are given.

The Panel notes that the primary outcome of this study was 'bone strength', that all vascular measures were secondary outcomes, and that corrections for multiple testing were not applied. In addition, the Panel notes that the statistical analyses performed do not take into account the repeated-measures design of study, in which the outcomes were measured at 1, 2 and 3 years during the intervention period.

Upon a request from EFSA to clarify to which extent the data presented by Knapen et al. (2015) could still be considered as confirmatory in relation to the claimed effect, the applicant acknowledged that 'bone strength' and changes in 'bone strength' were the primary outcomes of the study, but did not provide clarifications about the appropriateness of the statistical analyses in relation to the secondary outcomes.

The Panel considers that, owing to the inappropriate statistical analysis, which does not account for the repeated measures design or the multiplicity of secondary outcomes, no conclusions can be drawn from this study for the scientific substantiation of the claim.

In a randomised, parallel, two-arm, double-blind, placebo-controlled study, Knapen et al. (2017, unpublished) investigated the effect of MenaQ7[®] on a number of vascular outcomes. Healthy subjects aged 40–70 years with serum dp-ucMGP (desphospho-uncarboxylated Matrix Gla-Protein, a marker of vitamin K2 status) concentrations > 400 pmol/L were included. Exclusion criteria were cardiovascular, metabolic or gastrointestinal diseases, and use of oral anticoagulants prior to the study.

Participants were randomised (1:1 ratio), stratified by sex, to consume either 180 µg MenaQ7[®] (one tablet daily) or placebo (similar tablet containing microcrystalline cellulose) for 1 year.

Power calculations were not performed. The vascular outcomes measured at the beginning and end of the study were IMT, D, Δd , ΔA , DC, CC, Young's elasticity modulus, stiffness index β and cPWV of the carotid artery, cfPWV and crPWV. Body composition was also measured. The applicant claims that DC, IMT and cfPWV were the primary outcomes of the study.

Between-group differences were tested by unpaired Student's t-test (comparison of absolute values at the end of the intervention and % changes from baseline). Pre-planned subgroup analyses for post-menopausal women and women with a high Stiffness Index β (>10.8) were also performed. The Panel notes that corrections for multiple testing were not made and that the analysis was not adjusted for baseline.

A total of 243 participants were randomised (166 women, mean age 61 ± 7.2 years, 121 in the MenaQ7[®] and 122 in the placebo group). Eight subjects dropped-out during the study (four in each group). The reasons were reported. The results were given for 235 completers.

Upon a request from EFSA to clarify to what extent the data presented by Knapen et al. (2017) could still be considered as confirmatory in relation to the claimed effect, the applicant replied that 'the primary outcome of the study is the measurements of changes between the groups (MenaQ7[®] group and the placebo-group) in the carotid-femoral pulse-wave velocity (cfPWV)'. The Panel notes, however, that several vascular variables were assessed in this study, that three were identified in the study

report as 'primary endpoints', and that it is unclear how, when and on which bases these were selected as primary outcomes, considering that no power calculations were performed.

The Panel considers that, owing to important methodological limitations (sample size calculation was not performed, unclear how primary outcome(s) were selected, no correction for testing of multiple primary outcomes was made, baseline was not included in the statistical analyses), no conclusions can be drawn from this study for the scientific substantiation of the claim.

The Panel notes that no conclusions could be drawn from the three human intervention studies submitted for substantiation of the claim.

In the absence of evidence for an effect of MenaQ7[®] on maintenance of elastic properties of the arteries *in vivo* in humans, the studies on the proposed mechanisms by which the food/constituent could exert the claimed effect cannot be used as a source of evidence for the scientific substantiation of the claim.

The Panel concludes that a cause and effect relationship has not been established between the consumption of MenaQ7[®] and maintenance of the elastic properties of the arteries.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent, MenaQ7[®], which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'improves arterial stiffness'. The target population proposed by the applicant is 'general healthy population'. Maintenance of the elastic properties of the arteries is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of MenaQ7[®] and maintenance of the elastic properties of the arteries.

Documentation as provided to EFSA

Health claim application on MenaQ7[®] and maintenance of the elastic properties of the arteries pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0483_IE). Submitted by NattoPharma ASA, Lilleakerveien 2b, 0283 Oslo NO-1326 Lysaker, Norway.

Steps taken by EFSA

- 1) This application was received by EFSA on 8/04/2019.
- 2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- 3) The scientific evaluation procedure started on 15/07/2019.
- 4) On 10/09/2019, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 26/09/2019 and was restarted on 11/10/2019, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- 5) During its meeting on 27/11/2019, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to MenaQ7[®] and maintenance of the elastic properties of the arteries.

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Abbreviations

AIx	augmentation index
BMI	body mass index
BMD	bone mineral density
cPWV	central pulse wave velocity
cfPWV	carotid–femoral pulse wave velocity
crPWV	carotid-radial pulse wave velocity
CC	compliance coefficient
D	diameter at-end diastole
DC	distensibility coefficient
dp-ucMGP	desphospho-uncarboxylated matrix Gla-protein
IMT	intima media thickness
ITT	intention-to-treat
MK-7	menaquinone-7
MPA	mean Arterial pressure
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
PWV	pulse wave velocity
TG	triglycerides