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Draft guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health

(Revision 1)

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Abstract

EFSA asked the Panel on Dietetic Products, Nutrition and Allergies (NDA) to update the guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health published in 2011. The update takes into accounts experiences gained with evaluation of additional health claim applications related to antioxidants, oxidative damage and cardiovascular health, and the information collected from a Grant launched in 2014. This guidance is intended to assist applicants in preparing applications for the authorisation of health claims related to the antioxidants, oxidative damage and cardiovascular health. This draft guidance was discussed and endorsed by the NDA Panel on 28 June 2017 for release for public consultation before finalisation.

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Summary

The European Food Safety Authority (EFSA) has asked the Panel on Dietetic Products, Nutrition and Allergies (NDA) to revise the guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health published in 2011.

Since then, the NDA Panel has completed the evaluation of Article 13.1 claims (except for claims put on hold by the European Commission) and has evaluated additional health claim applications submitted pursuant to Articles 13(5), 14 and 19 which are in the area covered by this guidance. In addition, the NDA Panel has developed the general scientific guidance for stakeholders for health claims applications which addresses general issues that are common to all health claims. To further assist applicants, EFSA launched in 2014 a grant which aimed at gathering information in relation to claimed effects, outcome variables and methods of measurement in the context of the scientific substantiation of health claims, and the information collected helped to inform the NDA Panel in updating the present guidance.

This guidance is intended to assist applicants in preparing applications for the authorisation of health claims related to the antioxidants, oxidative damage and cardiovascular health. It focuses on key issues, particularly:

- claimed effects which are considered to be beneficial physiological effects, and
- characteristics of the human intervention studies which can provide evidence for the scientific substantiation of specific claims addressed in this guidance (e.g. appropriate outcome variables and methods of measurement, suitable study group(s), appropriate duration of the study, suitable controls).

The guidance does not intend to provide an exhaustive list of beneficial physiological effects and studies/outcome variables which could be acceptable, or address potential health relationships and related outcome measures which have not yet been considered by the Panel in the context of a particular application.

This draft guidance was discussed and endorsed by the NDA Panel on 28 June 2017 for release for public consultation before finalisation.

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Background and Terms of Reference as provided by EFSA

Background

Regulation (EC) No 1924/2006[1] harmonises the provisions related to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. According to the Regulation, health claims should only be authorised for use in the Community after a scientific assessment of the highest possible standard to be carried out by EFSA.

Owing to the scientific and technical complexity of health claims, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) has placed considerable effort into developing scientific criteria for the substantiation of health claims, and has published guidance on the scientific substantiation of health claims since 2007[2].

In the last years, the NDA Panel has gained considerable experience in the evaluation of health claim applications. To further assist applicants seeking approval of health claims, EFSA launched in 2014 a grant (GP/EFSA/NUTRI/2014/01) which aims at gathering information in relation to claimed effects, outcome variables and methods of measurement in the context of the scientific substantiation of health claims. The information collected will be published in a scientific report, which will help to inform the NDA Panel and serve as a basis for further guidance to applicants. The format(s) under which such guidance will be provided to applicants (e.g. guidance documents, and/or searchable, interactive databases) will be carefully considered by EFSA.

In this context, note is taken of the need to adapt the existing guidance on the scientific requirements for health claims to the new scientific and technical developments in specific areas taking into account lessons learned from the evaluation of health claim applications and the information collected from the grant.

To this end, the NDA Panel is asked to update the existing guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health published in 2011[3].

Terms of reference

The NDA Panel is requested by EFSA to update the existing guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health.

The guidance document shall clarify and address the scientific and technical developments in this area, taking into account the experience gained by the NDA Panel with the evaluation of health claims and the information collected from the grant.

The draft guidance shall be released for public consultation prior to finalisation, and shall be revised taking into account the comments received during the public consultation before adoption by the NDA Panel. A technical report on the outcome of the public consultation shall be published.

1. Introduction

The Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health (EFSA NDA Panel, 2011a), published in April 2011, laid down recommendations on specific issues that need to be addressed in applications submitted for the substantiation of health claims in this area. Since then, the European Food Safety Authority (EFSA)'s Panel on Dietetic products, Nutrition and Allergies (NDA Panel) has evaluated additional health claim applications related to antioxidants, oxidative damage and cardiovascular health.

Among the claim applications submitted to EFSA as of 17/02/2017, 59 were relevant to this guidance (13 were withdrawn during the evaluation, 44 were evaluated/finalised by the NDA Panel and two were under evaluation). Among those finalised[4], 18 applications were evaluated by the Panel with a

[1] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.
[2] https://www.efsa.europa.eu/en/applications/nutrition/regulationsandguidance
[3] https://www.efsa.europa.eu/en/applications/nutrition/regulationsandguidance
[4] **Three** were Article 13(5) claims (which are based on newly developed scientific evidence and/or which include a request for the protection of proprietary data), **three** were Article 14 children claims, **nine** were Article 14 disease risk reduction claims, and **three** were related to Article 19 applications for the modification of existing authorisations of health claims.

favourable opinion: three related to claims on the protection of cells and molecules against oxidative damage based on the essentiality of nutrients (on vitamin C[5], vitamin E[6] and selenium[7]), 12 referred to claims on cardiovascular health (on plant sterols[8], plant stanol esters[9], Danacol® low fat dairy product[10], oat beta-glucans[11], barley beta-glucans[12], water-soluble tomato concentrate[13], trans-free spreadable fats[14], cocoa flavanols[15], red yeast rice[16], Limicol®[17]), and three were requests for the modification of existing authorisations of health claims (on plant sterols and plant stanol esters [18], water-soluble tomato concentrate[19], and cocoa flavanols[20]).

To further assist applicants, EFSA launched in 2014 a grant (GP/EFSA/NUTRI/2014/01) which aimed at gathering information in relation to claimed effects, outcome variables and methods of measurement in the context of the scientific substantiation of health claims. The information collected helped to inform the NDA Panel and served as a basis for updating this guidance to applicants.

2. Objectives and scope

This guidance is intended to assist applicants in preparing applications for the scientific substantiation of health claims related to antioxidants, oxidative damage and cardiovascular health.

The document focuses on key issues, particularly:

- claimed effects which are considered to be beneficial physiological effects, and
- characteristics of human intervention studies which can provide evidence for the scientific substantiation of specific claims addressed in this guidance (e.g. appropriate outcome variables and methods of measurement, suitable study group(s), appropriate duration of the study, suitable controls).

Issues related to scientific substantiation that are common to all health claims (e.g. characterisation of the food/constituent, characterisation of the claimed effect) are addressed in the general scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a) and will not be reiterated in this document.

Examples of claims evaluated by the Panel with a favourable opinion will be used to provide guidance to applicants on the scientific requirements for the substantiation of health claims in specific areas, whereas examples of claims evaluated by the Panel with an unfavourable opinion will be used to illustrate the shortcomings that prevented the substantiation of these claims.

The Panel, however, cannot provide guidance to applicants on the scientific requirements for the substantiation of health claims (e.g. type of human intervention studies needed for substantiation) in specific areas where no examples of favourable evaluations are available. This guidance does not intend to provide an exhaustive list of beneficial physiological effects, or of studies/outcome variables which could be acceptable for claim substantiation, or address potential health relationships and related outcome measures which have not yet been considered by the Panel in the context of a particular application. The guidance will be kept under review and will be amended and updated in the light of experiences gained from the evaluation of additional health claim applications in this area.

This guidance should be read in conjunction with the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a), the Scientific and technical guidance for the

[5] Vitamin C (EFSA-Q-2008-175, Art 14(b))
[6] Vitamin E (EFSA-Q-2008-179, Art 14(b)
[7] Selenium (EFSA-Q-2008-159, Art 14(b))
[8] Plant sterols (EFSA-Q-2008-085, Art 14(a))
[9] Plant stanol esters (EFSA-Q-2008-118, Art 14(a); EFSA-Q-2011-00851, Art 14(a))
[10] Danacol (EFSA-Q-2008-779, Art 14(a))
[11] Oat beta-glucan (EFSA-Q-2008-681, Art 14(a))
[12] Barley beta-glucan (EFSA-Q-2011-00798, Art 14(a); EFSA-Q-2011-00799, Art 14(a))
[13] Water-soluble tomato concentrate (EFSA-Q-2009-00229, Art 13(5))
[14] Trans free spreadable fats (EFSA-Q-2009-00458, Art 14(a))
[15] Cocoa flavanols (EFSA-Q-2012-00002, Art 13(5))
[16] Red yeast rice (EFSA-Q-2012-00736, Art 13(5))
[17] Limicol® (EFSA-Q-2012-00968, Art 14(a))
[18] Plant sterols and plant stanol esters (EFSA-Q-2011-01241, Art 19)
[19] Water soluble tomato concentrate (EFSA-Q-2010-00809, Art 19)
[20] Cocoa flavanols (EFSA-Q-2013-00832, Art 19)

preparation and presentation of a health claim application (EFSA NDA Panel, 2017a), Regulation (EC) No 1924/2006 on Nutrition and Health Claims made on foods[21], the Guidance on the implementation of Regulation (EC) No 1924/2006 (Standing Committee on the Food Chain and Animal Health, 2007), Commission Regulation (EC) No 353/2008[22], the Commission Implementing Decision of 24 January 2013[23], and future guidelines and regulations, as applicable.

3. Assessment

3.1. Function claims related to antioxidants and the protection of body cells and molecules (i.e. proteins, lipids, DNA) from oxidative damage, including photo-oxidative (UV-induced) damage

The protection of body cells and molecules such as proteins, lipids, and DNA from oxidative damage, including photo-oxidative (UV-induced) damage, is generally considered a beneficial physiological effect, assuming that any significant oxidative modification of the target molecule is potentially harmful.

Claims made on the antioxidant content or properties of foods/constituents based on their capability of scavenging free radicals *in vitro* refer to a property of the foods/constituents measured in model systems, and it is not established that this capability exerts a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

Claims referring to antioxidant status and antioxidant defence have been proposed. The references provided for the scientific substantiation of these claims included *in vivo* human studies which assessed changes in the overall antioxidant capacity of plasma using methods such as the total reactive antioxidant potential (TRAP), the trolox-equivalent antioxidant capacity (TEAC), the ferric reducing antioxidant potential (FRAP), the oxygen radical absorbance capacity (ORAC) or ferrous oxidation-xylenol orange (FOX) assays. It is not established that changes in the overall antioxidant capacity of plasma exert a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

Claims referring to the "protection of cells from premature ageing" or to "healthy aging" in relation to the antioxidant properties of food/constituents are not sufficiently defined for a scientific evaluation, are considered to be general and non-specific, and thus do not comply with the criteria laid down in Regulation (EC) No 1924/2006.

3.1.1. Claims based on the essentiality of nutrients

Some vitamins and essential minerals have a role in the function of enzymes which belong to the human antioxidant network that protects cells and molecules from oxidative damage. Claims on the protection of DNA, proteins and lipids from oxidative damage related to vitamin C (EFSA NDA Panel, 2017b), vitamin E (EFSA NDA Panel, 2016b), and selenium (EFSA NDA Panel, 2014a) were evaluated by the Panel with a favourable opinion. The scientific substantiation of these claims was based on the essentiality of these nutrients[24], i.e. on the well-established biochemical role of such nutrients, and/or on deficiency symptoms involving in the human antioxidant network and/or the antioxidant defence system. Their role in the human antioxidant network/the antioxidant defence system has been established based on a large body of scientific evidence. For these claims, the NDA Panel did not review the primary scientific studies submitted and it did not weigh the evidence.

[21] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1924:20100302:en:PDF

[22] Commission Regulation (EC) No 353/2008 of 18 April 2008 establishing implementing rules for applications for authorisation of health claims as provided for in Article 15 of Regulation (EC) No 1924/2006 of the European Parliament and of the Council (Text with EEA relevance) (OJ L 109, 19.4.2008, p. 11): http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2008R0353:20091221:EN:PDF

[23] Commission Implementing Decision of 24 January 2013 adopting guidelines for the implementation of specific conditions for health claims laid down in Article 10 of Regulation (EC) No 1924/2006 of the European Parliament and of the Council. OJ L 22, 25.1.2013, p. 25–28. Available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32013D0063

[24] See General scientific guidance for stakeholders on health claim applications, section 6.1

3.1.2. Claims other than those based on the essentiality of nutrients

In the context of an adequate supply of vitamins and essential minerals with a role in the function of enzymes which belong to the human antioxidant network, induction of antioxidant enzymes cannot be used alone as evidence for claims on the protection of cells and molecules from oxidative damage for non-essential food constituents. The same principle applies to non-specific changes in the overall antioxidant capacity of plasma.

A specific induction of antioxidant enzymes (e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), haeme oxygenase (HO)), or limiting the decrease in glutathione and glutathione/glutathione disulphide (GSH/GSSG) ratio, are considered to be a beneficial physiological effect only if such changes provide (additional) protection of cells and molecules from oxidative damage. Such protection from oxidative damage should be demonstrated *in vivo* in humans. The same principle applies to non-specific changes in the overall antioxidant capacity of plasma assessed *in vivo* in humans using methods such as TRAP, FRAP, TEAC, ORAC or FOX assays.

The scientific substantiation of health claims on the protection of body cells and molecules from oxidative damage, including photo-oxidative (UV-induced) damage, requires at least one appropriate marker of oxidative modification of the target molecule assessed *in vivo* in human studies (as defined in sections 3.1.2.1 to 3.1.2.3), preferably in combination with other marker(s). These other markers of oxidative damage to molecules cannot be used alone for substantiation, either because they represent a result of two processes (oxidative damage and repair), because they suffer from technical limitations (interferences from other unrelated processes or substances), or both. However, they can provide supportive evidence for the scientific substantiation of these claims. Different markers of oxidative damage to molecules should preferably be determined in the same study, but their determination in similar studies could be acceptable on a case-by-case basis.

3.1.2.1. Protection of proteins from oxidative damage

Direct measurements of oxidative damage to proteins *in vivo* (e.g. measurement of oxidative changes of amino acids in proteins) can be obtained by means of LC-MS and other methods, as long as identification and separation of such molecules in plasma from other substances is successfully achieved (e.g. from protein tyrosine nitration products).

Measures of protein oxidation products (e.g. protein carbonyls) using ELISA methods (with an anti-dinitrophenyl (DNP) antibody after dinitrophenylhydradzine (DNPH) derivatisation) can only be used in combination with at least one direct marker of oxidative damage to proteins *in vivo* if assessed directly in blood or target tissue (e.g. skin). Conventional assays (e.g. colorimetric assay involving DNPH derivatisation of carbonyl groups) can be used for plasma samples in combination with at least one direct marker of oxidative damage to proteins *in vivo*.

3.1.2.2. Protection of lipids from oxidative damage

Direct measurements of oxidative damage to lipids (i.e. lipid peroxidation) can be obtained *in vivo* by measuring changes in F2-isoprostanes in 24-hour urine samples, which is a better matrix than plasma, using appropriate chromatographic techniques coupled with mass spectrometry (e.g. GC-MS or LC-MS). Immunological techniques, owing to their lack of specificity due to possible cross reactions with other prostanoids, are not appropriate for measuring F2-isoprostanes.

Measurements of oxidative damage to lipids (i.e. lipid peroxidation) can also be obtained *in vivo* by measuring oxidised LDL particles (Ox-LDL) in blood using immunological methods (i.e. specific monoclonal antibodies).

Lipid hydroperoxides (e.g. phosphatidylcholine hydroperoxides (PCOOH)) measured in blood or tissue by chemiluminescence-based liquid chromatography (CL-LC) is also an acceptable marker of lipid peroxidation *in vivo*. However, the combined measurement of PCOOH and F2-isoprostanes is preferable.

Other outcome variables proposed are not reliable *in vivo* markers of lipid peroxidation (e.g. thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), HDL-associated paraoxonases, conjugated dienes, breath hydrocarbons, auto-antibodies against LDL particles, and *ex*

vivo LDL resistance to oxidation). However, concentrations of MDA in blood or tissue can be used as supportive evidence (i.e. in addition to measurements of F2-isoprostanes and/or *in vivo* LDL oxidation) if appropriate techniques are used for MDA analysis (e.g. LC).

3.1.2.3. Protection of DNA from oxidative damage

Direct measurements of oxidative damage to DNA (i.e. oxidised DNA bases) can be obtained *in vivo* by using modifications of the comet assay (e.g. performed with endonuclease III to detect oxidised pyrimidines and with formamidopyrimidine DNA glycosilase (FPG) to remove oxidatively damaged purines). Although the assay provides no absolute values, it allows quantitative comparison with an appropriate control. This assay directly reflects DNA oxidative damage within cells when assessed, for example, in circulating lymphocytes.

Measures of DNA damage using the traditional comet assay (single-cell microgel electrophoresis, SCGE), which detect DNA strand breaks by the appearance of tailing, are not specific for oxidative damage. Other variants of the comet assay determine resistance against oxidative modification using *ex vivo* pro-oxidant challenges. Neither of these measurements is appropriate for assessing *in vivo* oxidative damage to DNA.

Analyses of 8-hydroxy-2-deoxy-guanosin (8-OHdG) in blood (e.g. lymphocytes), tissue (e.g. skin) and urine have been used to assess oxidative damage to DNA. Free 8-OHdG results from oxidative damage and excision-repair; it may also result from oxidation of free bases or nucleotides, from oxidation of other nucleic acids, and from artefacts during sample work up. Urinary 8-OHdG does not directly reflect DNA oxidation within cells, but can be used in combination with direct measurements of oxidative damage to DNA if appropriate techniques are used for analysis (e.g. LC).

3.2. Function claims related to the protection of DNA from strand breaks

DNA strand breaks occur spontaneously during the DNA repair process but can also be induced by e.g. environmental factors (such as mutagenic or pro-oxidant chemicals, radiation). Such DNA strand breaks alter DNA properties, may induce anomalies during DNA replication and translation, and require repair for maintenance of cell functioning and survival. Protection of DNA from strand breaks is a beneficial physiological effect.

Direct measurements of DNA strand breaks by the appearance of tailing can be obtained *in vivo* by using the traditional comet assay (SCGE).

A health claim on the reduction of spontaneous DNA strand breaks has been evaluated by the Panel with an unfavourable opinion (EFSA NDA Panel, 2011b).

3.3. Function claims related to cardiovascular health

Claims referring to cardiovascular health in general are not sufficiently defined for a scientific evaluation, as they are considered to be general and non-specific, and thus do not comply with the criteria laid down in Regulation (EC) No 1924/2006 unless they are accompanied by a specific claim. Examples of specific claims are addressed in sections 3.3.1 to 3.3.8 of this guidance.

A number of outcome variables have been proposed for the scientific substantiation of general claims on cardiovascular health, including beneficial changes in the blood lipid profile, arterial blood pressure, endothelial function, plasma homocysteine concentrations, platelet aggregation and venous blood flow. Such outcome variables can be assessed *in vivo* in humans by well-established methods, and allow the scientific evaluation of specific function claims in the area of cardiovascular health.

Evidence for a beneficial change in two of these outcome variables (i.e. for a reduction in blood LDL-cholesterol (LDL-c) concentrations; for a reduction in arterial (systolic) blood pressure (SBP)) is sufficient for the scientific substantiation of both function claims (i.e. on the maintenance of normal blood LDL-c concentrations or arterial (systolic) blood pressure) and reduction of disease risk claims related, for example, to the risk of coronary heart disease (CHD) (see section 3.4).

A reduction in the incidence of coronary events has also been proposed for the scientific substantiation of function claims referring to cardiovascular health in general. Evidence for a reduction

in the incidence of coronary events (e.g. myocardial infarction) can be used alone for the substantiation of function claims relating to the maintenance of normal cardiac function. However, such evidence is not sufficient for the scientific substantiation of reduction of disease risk claims in relation, for example, to the risk of CHD, because evidence for a beneficial alteration of one or more outcome variables (other than LDL-c and/or SBP) which can be considered as risk factors for the disease in the context of a particular application is also needed (see section 3.4).

3.3.1. Claims on maintenance of normal cardiac function

A function claim on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and maintenance of normal cardiac function has been evaluated by the Panel with a positive opinion (EFSA NDA Panel, 2010a).

The scientific basis for the substantiation of the claim was a wealth of human observational studies showing a consistent association between the consumption of the food/constituent and a reduction in the risk of CHD outcomes in the target population, as well as human intervention studies showing an effect of the food/constituent in reducing the risk of CHD outcomes in patients under medication (i.e. secondary prevention).

3.3.2. Claims on a beneficial change in the blood lipid profile

A reduction in fasting blood LDL-c concentrations (for claims on maintenance of normal blood concentrations of LDL-c), and/or fasting triglycerides (TG), and/or an increase in fasting blood HDL-cholesterol (HDL-c) concentrations (as long as blood LDL-c concentrations are not increased) are all considered beneficial physiological effects. In this context, changes in the blood lipid profile within the normal range are also considered beneficial physiological effects.

In principle, if a claim is best described by a number of outcome variables which are interrelated (e.g. total cholesterol and LDL-c, LDL-c and HDL-c; TG and HDL-c), and which in combination could provide information about the claim and eventually about the underlying mechanism of action, the Panel will consider the information provided on all these variables to evaluate the claim. However, the selection of the outcome variable(s) to be tested in a study and the decision to treat such variable(s) as primary or as secondary outcomes would depend, among other considerations, on the study objectives (e.g. exploratory, confirmatory), the outcome variable(s) on which the power calculation was based, the study group, and the information which is already available (in the literature, or to the applicant) regarding the relationship between the consumption of the food/constituent and the claimed effect (e.g. whether a mechanism of action by which the food/constituent could exert the claimed effect is already known) (General scientific guidance, section 7.2.1).

The characterisation of the claimed effect for claims related to beneficial changes in the blood lipid profile requires identification of the particular marker(s) which should be considered for the scientific evaluation (e.g. LDL-c, HDL-c, fasting TG, or a combination thereof).

The scientific evidence for the substantiation of health claims related to a beneficial change in the blood lipid profile can be obtained from human intervention studies showing a reduction in LDL-c concentrations, and/or an increase in HDL-c concentrations with a reduction or no change in LDL-c concentrations, and/or a reduction in blood TG concentrations as compared to an appropriate food/constituent or exceptionally to no intervention (e.g. control group on usual diet). All measurements should be performed in fasting conditions using well-accepted methods and following standardised conditions and protocols (e.g. Miller M et al., 2011[25]; Catapano AL et al., 2016[26]).

Even if a significant effect on one or more of these outcome variables is observed within short periods of time (e.g. three to four weeks), evidence on the sustainability of the effect with continuous consumption of the food/constituent over longer periods of time (i.e. at least eight weeks) should be provided.

Claims for a beneficial effect of the absence (or reduced content) of a food constituent in a food or category of food on LDL-c concentration have been proposed. Substantiation may be based on evidence for an independent role of the food constituent in increasing LDL-c concentration. For

[25] Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association.
[26] 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias.

example, for claims on a reduced content of saturated fatty acids (SFAs) in relation to blood LDL-c concentration, SFAs in mixed diets have been shown to increase blood LDL-c concentration when compared to carbohydrates which have a neutral effect on LDL-c concentration, and therefore SFAs in mixed diets have an independent role in increasing LDL-c concentration (EFSA NDA panel, 2011c).

Claims for a beneficial effect of a food constituent when used in replacement of a food constituent with an independent role in increasing LDL-c concentration have also been proposed. Substantiation may be based on evidence for an independent role of the replaced food constituent in increasing LDL-c concentration, together with evidence for the lack of an effect or a reduced effect of the food constituent which is used for replacement (e.g. claims for unsaturated fats and reduced LDL-c concentration when replacing saturated fats) (EFSA NDA Panel, 2011d).

With respect to the study population, results from studies conducted in hypercholesterolaemic and /or hypertriglyceridaemic subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in hypercholesterolaemic subjects under pharmacological treatment with cholesterol-lowering medications (e.g. statins), and/or in hypertriglyceridaemic subjects under treatment with "triglyceride-lowering" medications (e.g. fibrates), to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

3.3.3. Claims on the reduction of post-prandial blood concentration of triglycerides

An increase in blood concentrations of TG after consumption of a fat-containing meal and/or food is a normal physiological response that varies in magnitude and duration, and which may be influenced by the chemical and physical nature of the food or meal consumed, as well as by individual factors (Jackson et al., 2012). Claims on the reduction of post-prandial blood concentrations of TG refer to the ability of a food/constituent to reduce the blood TG rise after consumption of a food or meal rich in fat (i.e. in comparison to a reference food or meal). A reduction of post-prandial blood concentrations of TG is, in general, a beneficial physiological effect for the adult population.

In principle, the scientific evidence for the substantiation of these claims can be obtained from human intervention studies showing a reduction of post-prandial blood concentrations of TG at different time points during an appropriate period of time after consumption of the test food in comparison to the reference food. Post-prandial blood concentrations of TG should be measured under well-defined conditions (Jagla A and Schrezenmeir J, 2001; Mihas C et al., 2011; Nordestgaard BG and Freiberg JJ, 2011). The Panel notes, however, that there is no consensus on cut-off values to define normal post-prandial blood concentrations of TG and that no standard protocols for the assessment are available yet (Kolovou et al., 2011; Mihas et al., 2011).

With respect to the study population, results from studies conducted in hypercholesterolaemic and /or hypertriglyceridaemic subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in hypercholesterolaemic subjects under pharmacological treatment with cholesterol-lowering medications (e.g. statins), and/or hypertriglyceridaemic subjects under treatment with "triglyceride-lowering" medications (e.g. fibrates) to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

A health claim on the reduction of post-prandial lipaemic response has been evaluated by the Panel with an unfavourable opinion (EFSA NDA Panel, 2013a).

3.3.4. Claims on the maintenance of normal (arterial) blood pressure

Maintenance of normal arterial blood pressure (BP) is a beneficial physiological effect. The scientific evidence for the substantiation of health claims on the maintenance of normal BP can be obtained from human intervention studies showing a reduction in SBP (e.g. point SBP, 24-h SBP), or a reduction in diastolic blood pressure (DBP) (e.g. point DBP, 24-h DBP) if accompanied by a reduction in SBP, as compared to an appropriate food/constituent or exceptionally to no intervention (e.g.

control group on usual diet). In this context, also reductions in BP within the normal range are considered beneficial physiological effects.

Even if a significant effect on BP is observed within short periods of time (e.g. three to four weeks), evidence on the sustainability of the effect with continuous consumption of the food/constituent over longer periods of time (i.e. at least eight weeks) should be provided.

Studies should be designed to account for intra-individual variability, and BP should be measured using well-accepted methods according to standardised conditions and protocols (Mancia G et al., 2013[27]).

Owing to the lack of standardisation, self (home) measurement of BP (e.g. using an electronic device) is not an appropriate method for measuring point SBP and point DBP in research settings; validation of the device and protocols used are required. Measurement with a calibrated sphygmomanometer (Mancia G et al., 2013; Tolonen H et al., 2015)[28] is the standard method for the assessment of office BP.

Ambulatory blood pressure monitoring (ABPM) allows measuring BP over a 24-hour period and provides an insight to BP changes during everyday activities not covered by single measurements. The ABPM is an appropriate method for measuring mean 24-h SBP and 24-h DBP.

With respect to the study population, results from studies conducted in hypertensive subjects treated with lifestyle measures only (e.g. diet) could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in hypertensive subjects under treatment with blood pressure-lowering medications (e.g. ACE-inhibitors, blockers of beta adrenergic receptors, calcium channel blockers and diuretics) to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

3.3.5. Claims on the improvement of endothelial functions

Endothelial function *per se* is not sufficiently defined for a scientific evaluation, because endothelium-derived active factors play a role in the maintenance of several functions of the vascular system. These include vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis and oxidation, which can be assessed by indirect methods. The characterisation of the claimed effect requires identification of the specific function which should be considered for the scientific evaluation (e.g. endothelium-dependent vasodilation).

Some claims referred to the improvement of endothelium-dependent vasodilation, which is a specific endothelial function that can be measured *in vivo* in humans using well-accepted methods. The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow, or more precisely shear stress, by dilating. This phenomenon is designated as flow-mediated dilation (FMD). A principal mediator of FMD is endothelium-derived nitric oxide (NO). Endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels. Endothelium-derived prostanoids and the putative endothelium-derived hyperpolarizing factor have also been implicated as back-up mechanisms mediating changes in arterial diameter in response to shear stress, so that there may be some redundancy in the system in order to ensure an appropriate response of blood vessels to shear stress. Endothelium-dependent vasodilation contributes to the maintenance of an adequate blood flow to body cells and tissues (EFSA NDA Panel, 2012a).

Endothelium-dependent vasodilation can be assessed *in vivo* at different points of the arterial tree using well-established methods (e.g. the FMD technique[29]). Endothelium-independent vasodilation (EIVD) of the brachial artery induced by exogenous NO donors (e.g. after the sublingual administration of nitroglycerin) is also measured as control (Corretti et al., 2002; Thijssen et al., 2011).

[27] 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).
[28] The sale of mercury sphygmomanometers has been banned since April 2014 (EU regulation 847/2012).
[29] Corretti et al., 2002; Thijssen et al., 2011.

The effect of a food/constituent on endothelium-dependent vasodilation can be expressed as changes in endothelium-dependent FMD (ED-FMD) either in fasting conditions after regular consumption of the food/constituent, or as acute changes in ED-FMD occurring shortly after consumption of the food/constituent. A sustained increase in endothelium-dependent vasodilation in fasting conditions in response to an intervention (regular consumption of a food/constituent for at least four weeks) is a beneficial physiological effect.

Markers of plasma nitric oxide status (e.g. nitrite/nitrosyl species (RXNO) measured by reductive gas-phase chemiluminescence) can provide evidence on the mechanisms by which the food/constituent could exert the claimed effect, but cannot be used alone for the substantiation of the claim.

Claims on maintenance of normal endothelium-dependent vasodilation response have been evaluated by the Panel with a favourable opinion (e.g. cocoa flavanols (EFSA NDA Panel, 2012a)).

3.3.6. Claims on the reduction of platelet aggregation

Platelet hyperactivity and hypercoagulability states are more commonly observed in subjects with cardiovascular (CV) risk factors. Healthy subjects at very low risk of CV disease normally have non-activated circulating platelets. A reduction in platelet aggregation (i.e. the percentage of inhibition in platelet aggregation using light transmission aggregometry (LTA) according to well-accepted and standardised protocols[30]) in subjects with platelet activation during sustained exposure to the food/constituent (at least four weeks) is a beneficial physiological effect.

Other outcome variables, such as thromboxane A2 (TXA2), or plasma soluble P-selectin (P-sel), are not well-established markers of platelet aggregation, but can be used as supportive evidence for the scientific substantiation of these claims.

A claim on maintenance of normal platelet aggregation has been evaluated by the Panel with a favourable opinion (Water-soluble tomato concentrate (EFSA NDA Panel, 2009a)).

3.3.7. Claims on the maintenance of normal blood homocysteine concentrations by contributing to normal homocysteine metabolism.

Maintenance of normal homocysteine metabolism is a beneficial physiological effect. It is well established that homocysteine metabolism is closely linked with both folate metabolism and one-carbon metabolism.

Evidence for the scientific substantiation of such a claim may come from the well-established role of a food/constituent in contributing to the remethylation or degradation of homocysteine in the liver (e.g. some vitamins), or from human intervention studies showing a reduction of homocysteine concentrations (measured, for example, by liquid chromatography tandem mass spectrometry (LC-MS/MS)) as compared to an appropriate food/constituent or exceptionally to no intervention (e.g. control group on usual diet), or both.

Even if a significant effect on blood homocysteine concentrations is observed within short periods of time (e.g. three to four weeks), evidence on the sustainability of the effect with continuous consumption of the food/constituent over longer periods of time (i.e. at least eight weeks) should be provided.

Several claims on the maintenance of normal homocysteine metabolism, which have been evaluated by the Panel with a favourable opinion, were based on the essentiality of nutrients (e.g. Folate (EFSA NDA Panel, 2009b), Vitamin B12, (EFSA NDA Panel, 2010b), Vitamin B6 (EFSA NDA Panel, 2010c)). Claims on betaine (and choline, which can be a precursor of betaine) were substantiated taking into account that betaine can act as a methyl donor in the remethylation of homocysteine in the liver by the enzyme betaine-homocysteine methyltransferase, and that human intervention studies consistently showed a significant decrease in plasma homocysteine concentrations following betaine (or choline) administration (EFSA NDA Panel, 2011e and 2011f).

[30] Cattaneo M, et al, 2013

3.3.8. Venous blood flow

Healthy veins contain bicuspid valves assisting unidirectional flow from the lower limbs towards the heart. The valves of the venous system may become incompetent and blood can flow backwards (venous reflux). Venous reflux is a pathological condition characterised by impaired return of blood and increased venous pressure that may lead to venous stasis and eventually microangiopathy.

Maintenance of normal venous blood flow is a beneficial physiological effect. Blood flow (e.g. venous reflux) in blood vessels, including the veins, can be assessed *in vivo* by standard dynamic ultrasound techniques (e.g. Duplex Doppler). The great saphenous diameter and the popliteal vein diameter are *per se* not appropriate outcome variables for the substantiation of the claim.

Results from studies conducted in non-diseased population subgroups could be used for the scientific substantiation of health claims on the maintenance of normal venous blood flow. Information on the selection criteria applied and on the characteristics of the study group in relation to the claimed effect, as well as a rationale for extrapolation of the results (e.g. subjects with chronic venous insufficiency (CVI)) to the target population (healthy subjects without CVI) for which the claim is made should be provided, and will be considered on a case-by-case basis.

Studies in patients with varicose veins and associated chronic venous diseases, and which relate to the treatment of symptoms of the disease cannot be considered for the scientific substantiation of claims on venous function in the general population.

A health claim on the maintenance of normal venous blood flow has been evaluated by the Panel with an unfavourable opinion (e.g. EFSA NDA panel, 2012b).

Also health claims on the maintenance of normal physiological venous tone and the maintenance of normal venous-capillary permeability have been proposed, and were evaluated by the Panel with an unfavourable opinion (e.g. EFSA NDA Panel, 2014b and 2014c). The studies provided for the substantiation of these claims included measurement of a reduction in the feeling of heaviness, burning, cramps and formication in the lower limbs, which is not a direct measure of "venous tone", or measurement of the changes of the volume of foot, ankle and leg, which is not a direct measure of "venous-capillary permeability".

3.4. Reduction of disease risk claims related to cardiovascular diseases

Regulation (EC) No 1924/2006 defines a 'reduction of disease risk claim' as any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease. This is because health claims referring to the reduction of the risk of a disease directly (i.e. to the prevention of a disease) cannot be made on food. From a scientific perspective, the reduction in the risk of a disease (i.e. an effect of the food/constituent on disease outcomes) provides stronger evidence for the substantiation of reduction of disease risk claims than the reduction of a risk factor for the disease. However, evidence on the reduction of the risk of a disease (e.g. coronary events) is not sufficient for the substantiation of these claims. Evidence that the consumption of the food/constituent also reduces (or beneficially affects) one or more risk factors for the disease should be provided to fulfil the requirements for the wording of the claim laid down in Regulation (EC) No 1924/2006.

It is well established that elevated blood **LDL-c** concentration is independently associated with an increased risk of CHD, and that reducing blood LDL-c concentration (by dietary modification and/or drugs) would generally reduce the risk of development of CHD. It is also well established that elevated arterial **SBP** is independently associated with an increased risk of CHD and stroke, and that reducing arterial SBP (by dietary modification and/or drugs) would generally reduce the risk of development of CHD and stroke. Therefore, the scientific substantiation of claims related to a decreased risk of CHD can be based on evidence for a reduction of either blood LDL-c concentrations or arterial SBP, and evidence for a reduction in the incidence of CHD is not required. Similarly, the scientific substantiation of claims related to a decreased risk of stroke can be based on evidence for a reduction of arterial SBP, and evidence for a reduction in the incidence of stroke is not required. In this context, blood LDL-c concentrations and arterial SBP are the risk factors for CHD and/or stroke, as required by Regulation (EC) No 1924/2006.

Several disease risk reduction claims related to CHD risk which have proposed elevated **LDL-c** concentrations as the risk factor for the disease have been evaluated by the Panel with a favourable opinion (e.g. Limicol (EFSA NDA Panel, 2013b); Plant sterols (EFSA NDA Panel, 2008a); Plant stanol esters (EFSA NDA Panel, 2008b); Danacol (EFSA NDA Panel, 2009c); Oat beta glucan (EFSA NDA Panel, 2010d); Barley beta-glucan (EFSA NDA Panel, 2011g); Trans free spreadable fats (EFSA NDA Panel, 2011h)). The scientific substantiation of all these claims has been based on evidence for a sustained reduction in LDL-c concentrations with continuous consumption of the food/constituent, whereas evidence for a reduction in the risk of the disease directly (i.e. on disease outcomes) has not been provided.

For proposed risk factors other than LDL-c and arterial SBP, the evidence on the relationship between the risk factor and the development of the disease may not be as strong. There is some evidence, for example, that low blood **HDL-c** concentration, elevated blood concentration of (fasting) **TG**, or elevated blood **homocysteine** concentration are associated with an increased risk of CHD. However, changes in any of these factors (by dietary modification and/or drugs) have not generally been shown to reduce the risk of CHD. Therefore, human studies on how the consumption of the food/constituent prospectively modifies the risk of CHD are required for the substantiation of these claims in order to validate the association between these variables and the risk of disease in the context of a particular nutritional intervention.

Evidence for a reduction in the incidence of the disease (e.g. CHD, stroke) is necessary, but not sufficient, for the scientific substantiation of reduction of disease risk claims. Evidence for a beneficial alteration of one or more risk factors (e.g. reduction in blood concentration of (fasting) TG, reduction in blood homocysteine concentration, or an increase in blood HDL-c concentration) with the consumption of the food/constituent is also required.

A disease risk reduction claim related to the reduction of arterial stiffness by reducing the risk of CV disease has been evaluated by the Panel with an unfavourable opinion (Evolus® (EFSA NDA Panel, 2008c)). There is insufficient evidence demonstrating that a reduction in arterial stiffness (evaluated through the calculation of augmentation index (Aix), measurement of the return time of reflected wave) univocally leads to a reduction in the risk of CV disease. In this context, a reduction in arterial stiffness can be considered as a risk factor in the development of the risk of CV disease, as long as evidence is provided that the consumption of the food that is the subject of the health claim reduces the proposed risk factor as well as the incidence of hypertension/CV disease.

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Glossary and Abbreviations

8-OHdG	8-hydroxy-2-deoxy-guanosin
ABPM	Ambulatory blood pressure monitoring
ACE	Angiotensin converting enzyme
CAT	Catalase
CHD	Coronary heart disease
CL-LC	Chemiluminescence-based liquid chromatography
CV	Cardiovascular
CVI	Chronic venous insufficiency
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DNP	Dinitrophenyl
DNPH	Dinitrophenylhydrazine
ED-FMD	Endothelium-dependent flow-mediated dilation
EIVD	Endothelium-independent vasodilation
ELISA	Enzyme-linked immunosorbent assay
FMD	Flow-mediated dilation
FOX	Ferrous oxidation-xylenol orange
FPG	Formamidopyrimidine DNA glycosilase
FRAP	Ferric reducing antioxidant potential
GC-MS	Gas chromatography-mass spectrometry
GSH/GSSG	Glutathione/glutathione disulphide
GSH-Px	Glutathione peroxidase
HO	Haeme oxygenase
HDL-c	High-density lipoprotein cholesterol
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDL-c	Low-density lipoprotein cholesterol
LTA	Light transmission aggregometry
MDA	Malondialdehyde
MS	Mass spectrometry
NO	Nitrite oxide
NOS	Nitric oxide synthase
ORAC	Oxygen radical absorbance capacity
Ox-LDL	Oxidised LDL
PCOOH	Phosphatidylcholine hydroperoxides

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P-sel	P-selectin
RXNO	Nitrite/nitrosyl species
SBP	Systolic blood pressure
SCGE	Single-cell microgel electrophoresis
SFA	Saturated fatty acid
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TEAC	Trolox-equivalent antioxidant capacity
TG	Triglyceride
TRAP	Total reactive antioxidant potential
TXA2	Thromboxane A2
UV	Ultraviolet

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