

# EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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## SCIENTIFIC OPINION

# Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

**EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

Following an application from Barry Callebaut Belgium nv, submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation. Cocoa flavanols are sufficiently characterised. The claimed effect is "help maintain endothelium-dependent vasodilation which contributes to healthy blood flow". The target population proposed by the applicant is the general healthy adult population. The Panel considers that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect. In weighing the evidence, the Panel took into account that cocoa flavanols consumed for 12 weeks have been shown to increase fasting ED-FMD significantly in the target population in one human intervention study, that in another study the effect was dose-dependent and occurred after one week of consumption, that the effect was supported by two additional studies, and that it was also observed in two out of three studies in patients under pharmacological treatment for coronary artery disease, although the mechanisms by which regular consumption of cocoa flavanols may induce a sustained effect on fasting ED-FMD are unknown. The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation. The following wording reflects the scientific evidence: "Cocoa flavanols help maintain endothelium-dependent vasodilation, which contributes to normal blood flow". In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate, both of which can be consumed in the context of a balanced diet. The target population is the general population.


## KEY WORDS

Cocoa flavanols, endothelium-dependent vasodilation, health claims

[1] On request from the Competent Authority of Belgium following an application by Barry Callebaut Belgium nv, Question No EFSA-Q-2012-00002, adopted on 27 June 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

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## SUMMARY

Following an application from Barry Callebaut Belgium nv, submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.

The food constituent that is the subject of the health claim is cocoa flavanols. Flavanols are flavonoids and belong to a larger group of polyphenols. The flavanols in cocoa (*Theobroma cacao* L.) consist of monomeric catechins (mainly epicatechin) and oligomeric flavanols (procyanidins) ranging from dimers to decamers. The Panel considers that cocoa flavanols are sufficiently characterised.

The claimed effect is "help maintain endothelium-dependent vasodilation which contributes to healthy blood flow". The target population proposed by the applicant is the general healthy adult population. The capacity of blood vessels to respond to an increase in blood flow by dilating is designated as flow-mediated dilation (FMD). Endothelium-dependent vasodilation contributes to the maintenance of an adequate blood flow to body cells and tissues. The Panel considers that a sustained increase of endothelium-dependent vasodilation in fasting conditions in response to an intervention (e.g. regular consumption of a food/constituent) is a beneficial physiological effect. The Panel considers that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect.

The human intervention studies identified by the applicant as being strictly pertinent to the claim (five published and one unpublished randomised controlled trials) were aimed at assessing the effects of cocoa flavanols on ED-FMD in fasting conditions during regular consumption of the food constituent (1-12 months) in non-diseased populations. One of the studies also assessed the acute effects of cocoa flavanol consumption on ED-FMD. The Panel considered that no conclusions could be drawn from two of these studies for the scientific substantiation of the claim due to important methodological limitations. One intervention study showed an effect of cocoa flavanols consumed for 12 weeks on fasting ED-FMD, another intervention study showed a dose-dependent effect of cocoa flavanols on fasting ED-FMD after one week of consumption, and the effect was observed under the conditions of use proposed by the applicant, and two additional intervention studies which may not have been adequately controlled for other food constituents in cocoa also supported an effect of cocoa flavanols on ED-FMD.

In addition, the applicant provided three human intervention studies as supportive evidence for the scientific substantiation of the claim which investigated the effects of cocoa flavanols on ED-FMD in fasting conditions during repeated consumption of the food constituent (for 30 days to 6 weeks) in subjects with coronary artery disease (CAD) under pharmacological treatment for this condition. Two out of the three studies showed an effect of cocoa flavanols consumed for 30 days on fasting ED-FMD in subjects with CAD who were under pharmacological treatment, and the effect occurred after seven days of consumption of cocoa flavanols.

A number of human intervention studies conducted in different population subgroups (e.g. smokers, older adults, obese subjects, subjects with type 2 diabetes and CAD) and which addressed the acute effects of cocoa flavanols when consumed on a single occasion on ED-FMD were also provided by the applicant as supportive evidence for the scientific substantiation of the claim. One of the studies also assessed the acute effects of cocoa flavanols on ED-FMD during repeated consumption (for seven days) of the food/constituent. Consumption of cocoa flavanols on a single occasion induced an acute, dose-dependent increase in ED-FMD which was maximal two hours after consumption of the

food constituent, parallels blood concentrations of the flavanol metabolites, returns to baseline six hours after ingestion, and is sustained with repeated consumption of cocoa flavanols.

The evidence provided in support of mechanisms by which repeated consumption of cocoa flavanols may induce longer-term effects on fasting ED-FMD was weak. Cocoa flavanols (mostly epicatechins) may exert an acute effect on ED-FMD by enhancing nitric oxide (NO) production in the endothelium each time they are consumed.

In weighing the evidence, the Panel took into account that cocoa flavanols consumed for 12 weeks have been shown to increase fasting ED-FMD significantly in the target population in one human intervention study, that in another human intervention study the effect was dose-dependent and occurred after one week of consumption under the conditions of use proposed by the applicant, that the effect was supported by two additional intervention studies, and that it was also observed in two out of three studies in patients under pharmacological treatment for CAD, whereas the mechanisms by which regular consumption of cocoa flavanols may induce a sustained effect on fasting ED-FMD are unknown. The Panel also took into account that consumption of cocoa flavanols on a single occasion induces an acute and dose-dependent increase in ED-FMD which is sustained with regular consumption of the food/constituent, and that this acute effect may be mediated by the enhancement of NO production in the endothelium each time cocoa flavanols are consumed.

The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

The Panel considers that the following wording reflects the scientific evidence: "Cocoa flavanols help maintain endothelium-dependent vasodilation, which contributes to normal blood flow".

In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate. These amounts of cocoa powder or dark chocolate can be consumed in the context of a balanced diet. The target population is the general population.

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## BACKGROUND

Regulation (EC) No 1924/2006[4] 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).

## STEPS TAKEN BY EFSA

- The application was received on 14/12/2011.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.
- The scientific evaluation procedure started on 20/01/2012.
- On 28/03/2012, 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, and the clock was stopped on 29/03/2012, in compliance with Art. 18(3) of Regulation (EC) No 1924/2006.
- On 13/04/2012, EFSA received the requested information as submitted by the applicant and the clock was restarted.
- On 27/04/2012, the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application, and the clock was stopped on 02/05/2012, in compliance with Art. 18(3) of Regulation (EC) No 1924/2006.
- On 17/05/2012, EFSA received the requested information as submitted by the applicant and the clock was restarted
- During its meeting on 27/06/2012, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

## 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: cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

[4] 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.

## EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of cocoa flavanols, a positive assessment of its safety, nor a decision on whether cocoa flavanols 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.

## INFORMATION PROVIDED BY THE APPLICANT

**Applicant's name and address**: Barry Callebaut Belgium nv., Aalstersestraat 122, B-9280 Lebbeke-Wieze, Belgium.

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006 for one unpublished study report (Grassi et al., 2011).

### Food/constituent as stated by the applicant

According to the applicant, the food constituent for which the claim is made is cocoa flavanols.

### Health relationship as claimed by the applicant

According to the applicant, regular consumption of at least 200 mg of cocoa flavanols leads to a sustained and physiologically relevant increase in endothelium-dependent vasodilation.

### Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "cocoa flavanols help maintain endothelium-dependent vasodilation which contributes to healthy blood flow".

### Specific conditions of use as proposed by the applicant

According to the applicant, the effect of cocoa flavanols on endothelium-dependent vasodilation is obtained by daily consumption of at least 200 mg cocoa flavanols, either in the form of 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate. The target population as proposed by the applicant is the general healthy adult population.

## ASSESSMENT

### 1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is cocoa flavanols.

Flavanols are flavonoids and belong to a larger group of polyphenols. The flavanols in cocoa (*Theobroma cacao* L.) consist of monomeric catechins (mainly epicatechin) and oligomeric flavanols (procyanidins) ranging from dimers to decamers. The variety and country of origin, as well as the fermentation and roasting processes applied to cocoa beans, affect the flavanol content in cocoa. Flavanols are measurable in foods by established methods. In cocoa beans fermented for several days, the amount of monomeric flavanols account for 34-37 % of total flavanol content (Wollgast and Anklam, 2000).

The Panel noted that, as stated by the applicant, only monomers and rarely dimers (of epicatechin subunits) are absorbed after oral consumption, whereas procyanidins are unchanged in the stomach and unabsorbed in the small intestine. The Panel also noted that in some of the studies provided for the scientific substantiation of the claim the total dose of cocoa flavanols used was not reported, but rather the amount of monomeric catechins. Upon EFSA's request, the applicant confirmed that the food constituent which is the subject of the health claim is cocoa flavanols with a degree of polymerisation (DP) of 1-10.

The Panel considers that the food constituent, cocoa flavanols, which is the subject of the health claim, is sufficiently characterised.

## 2. Relevance of the claimed effect to human health

The claimed effect is "help maintain endothelium-dependent vasodilation which contributes to healthy blood flow". The target population proposed by the applicant is the general healthy adult population.

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 (Corretti et al., 2002). 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.

Endothelium-dependent vasodilation can be assessed *in vivo* at different points of the arterial tree using well established methods (e.g. the FMD technique). 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. The Panel considers that a sustained increase in endothelium-dependent vasodilation in fasting conditions in response to an intervention (e.g. regular consumption of a food/constituent) is a beneficial physiological effect.

The Panel considers that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect.

## 3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed 08/03/2011 to identify randomised controlled trials, systematic reviews, meta-analyses and observational studies published in English on the effects of cocoa flavanols on endothelium-dependent vasodilation assessed as changes in flow-mediated dilation using various combinations of the search terms "cocoa", "chocolate", "intake", "flavanol", "endothelium", "vascular", "vasodilation", "flow-mediated dilation", "nitric-oxide", and "FMD". Extensive hand searching was also applied. A total of 25 potentially pertinent publications were identified. After applying the exclusion criteria defined by the applicant (e.g. acute interventions lasting less than one day, studies performed on diseased populations under medical supervision and pharmacological treatment for various conditions, endothelial function assessed using other techniques than FMD in locations of the vascular tree other than the brachial artery), 18 publications were excluded. Two additional studies were excluded by the applicant due to the lack of a control group (Heiss et al., 2007), or to the high exclusion rates for endothelial function analysis (seven subjects (44 %) in the high flavanol group and eight subjects (50 %) in the low flavanol groups), or when power calculations were not performed (Wang-Polagruto et al., 2006). The applicant identified five published (Davison et al., 2008; Engler et al., 2004; Grassi et al., 2005, 2008; Njike et al., 2011) and one unpublished (claimed as proprietary by the applicant, Grassi et al., 2011) randomised controlled trials (RCT) in healthy subjects as being strictly pertinent to the claim.

**Randomised controlled trials in healthy subjects**

The human intervention studies identified by the applicant as being strictly pertinent to the claim were aimed at assessing the effects of cocoa flavanols on ED-FMD in fasting conditions during regular consumption of the food constituent (1-12 months) in non-diseased populations. One of the studies also assessed the acute effects of cocoa flavanol consumption on ED-FMD (Davison et al., 2008).

In a randomised, controlled, single-blind, cross-over study (Njike et al., 2011), 44 overweight men and women (age ~52 years) consumed either a sugar-free cocoa beverage (22 g/day cocoa, 805 mg procyanidins including 48 mg epicatechin and 21 mg catechin), a sugar-sweetened cocoa beverage (22 g/day cocoa, 91 g/day sugar) or a sugar-sweetened cocoa-free (placebo) beverage (91 g/day sugar) for six weeks each with four-week wash-out periods in between. Subjects were asked to refrain from consuming flavonoid-rich foods 24 h prior to each test day. The ED-FMD was apparently assessed before and after each treatment after an eight-hours overnight fast and then 2 h following each treatment. However, results were only provided as percentage changes overtime for fasting ED-FMD. Upon EFSA's request for clarification, the applicant stated that this study was not standalone, but rather the continuation of a previous study on the acute effects of dark chocolate on ED-FMD (Faridi et al., 2008), that the authors could not clarify whether baseline FMD values were obtained before and after each intervention phase, and that it was unclear how acute (changes in FMD 2 h after cocoa consumption) and longer-term (changes in fasting FMD after consumption of cocoa for six weeks) effects could have been calculated from the raw data collected. The applicant subsequently requested that this particular study should not be considered as pertinent to the claim. The Panel agrees that no conclusions can be drawn from this study for the scientific substantiation of the claim.

In a double-blind, placebo-controlled, parallel intervention study (Engler et al., 2004), 21 normotensive men and women (age ~32 years) were randomised to consume after lunch dark chocolate bars (46 g/day) with either a high (213 mg/day procyanidins, including 46 mg/day epicatechins; n=11) or low (traces; n=10) flavanoid content for two weeks. The ED-FMD was assessed at the beginning (after a 12-h fast) and end (2 h after consumption of the chocolate bar) of the study and expressed as the peak (%) change in the brachial artery diameter from baseline within 2 minutes of hyperaemia. The Panel notes that the study design did not allow an assessment of the effects of cocoa flavanols on ED-FMD either 2 h after consumption of cocoa nor in fasting conditions after regular administration of cocoa for two weeks because the measurement conditions at baseline and at the end of the study were not comparable with respect to the time at which cocoa flavanols were administered. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

A total of 65 obese and overweight males and females (age 18-65 years) were randomly assigned in a double-blind, parallel, controlled trial (Davison et al., 2008) to consume either a high-flavanol (HF) cocoa drink (902 mg flavanols including epicatechin, catechin and procyanidins) or a low-flavanol (LF) cocoa drink (36 mg flavanols) daily, each with or without exercise (four intervention arms), for 12 weeks. The test drinks were comparable for energy, macronutrient composition and other cocoa constituents such as caffeine and theobromine. To monitor compliance with the intervention, subjects recorded their drink consumption in a diary each day and were asked to return empty sachets at weeks 6 and 12. The ED-FMD was assessed at baseline after a ≥12-h overnight fast and also at 2 h after consumption of one packet (half daily dose) of the assigned drink, and at weeks 6 and 12 after a ≥12-h overnight fast. Images of the artery were taken before cuff inflation, 10 s before cuff release, 10 s after cuff release and then every 30 s for an additional 3 min. The coefficient of variation (CV) of the technique was reported to be 5 %. The Panel notes that this procedure is not in agreement with international guidance for the assessment of ED-FMD, which recommends the continuous recording of the artery from 30 s before to 2 min after cuff deflation (Corretti et al., 2002). The Panel also notes that endothelium-independent vasodilation (EIVD) of the brachial artery after sublingual glyceryl trinitrate (GTN) administration as control was not measured in this study. Upon EFSA's request to clarify the reason for deviation from the standard protocol, the applicant argued that multiple studies

have shown the peak increase in diameter of the brachial artery to occur between 60 and 90 seconds post cuff occlusion, which is within the time recordings that were taken in the study (3 min), and that although discrete recording (as in this study) versus continuous recording (as recommended) may have increased the margin of error in the detection of peak change in diameter, this should not have compromised the validity of the FMD results obtained, particularly when the CV of the technique was reported in the study. The applicant also argued that the vasodilator response to nitroglycerin may only be expected to be impaired in subjects at high cardiovascular risk, but not in healthy subjects as in the present study, and that all previous studies which have assessed the effects of cocoa flavanols on EIVD using GTN showed no effect of the intervention (e.g. Balzer et al., 2008; Grassi et al., 2005, 2008), suggesting that this test may not be essential to validate ED-FMD measurements. The Panel shares the view of the applicant on these points. RM-ANOVA was used to determine the effect of the treatments, time of measurement and their interactions on the dependent variables. Where ANOVA showed no interactive effect between exercise and cocoa, factorial analysis was performed for each of the separate treatments to detect independent effects. Where ANOVA showed a statistically significant main effect, pair-wise comparisons were performed using Tukey's Honestly Significant Differences test to determine differences between means. A total of 49 subjects (LF+exercise=13; HF+exercise=13; LF=11; HF=12) completed the study and entered data analysis. The Panel notes a dropout rate of 25 %, and that statistical analyses were performed in the sample of completers only. FMD increased significantly ($p=0.02$) in the HF group compared to LF 2 h after consuming a single 450 mg dose of the cocoa drink. With respect to the ED-FMD measurement in fasting conditions, there were no significant three-way interactions (cocoa x exercise x time). Fasting ED-FMD significantly increased (by 1.6 %) at weeks 6 and 12 relative to baseline in the HF group compared to LF (cocoa x time interaction, $p=0.002$). The Panel considers that this study shows an effect of cocoa flavanols on fasting ED-FMD at doses of about 900 mg/day consumed for 12 weeks in overweight subjects. The Panel notes that this study also shows an acute effect (two hours after consumption) of cocoa flavanols on ED-FMD.

In an unpublished randomised, double-blind, cross-over study by the same authors (Grassi et al., 2011, unpublished, claimed as proprietary by the applicant), 20 male and female subjects (age ~54 years) received, in a random order, 0 mg, 80 mg, 200 mg, 500 mg or 800 mg cocoa flavanols (monomers and oligomers, DP 1-10) in a beverage containing 10 g of cocoa powder, for one week each and with a one-week wash-out in between. The test drinks were comparable for energy, macro- and micronutrient composition, as well as for other cocoa constituents such as caffeine and theobromine. Subjects were asked to avoid flavonoid-rich foods (tea, red wine and chocolate), dietary supplements or aspirin, and to maintain their usual daily intake of fruit and vegetables. ED-FMD was measured after each treatment and in fasting conditions as described in Grassi et al. (2005). EIVD was not assessed. Results were evaluated using a linear mixed model with Proc Mixed Procedure with subjects treated as a random factor and treatment and sequence as fixed factors. Estimates for differences between mean responses at each dose [0 (control), 80, 200, 500 and 800 mg cocoa flavanols (CF) per day] were computed using Dunnett-Hsu correction for multiple comparisons. Compared to the control beverage (6.2±1.05 %), FMD was significantly higher after the 80 mg/day dose (7.27±1.61 %; $p<0.0001$), the 200 mg/day dose (7.62±1.41 %; $p<0.0001$) the 500 mg/day dose 8.16±1.41 %; $p<0.0001$), and the 800 mg/day dose (8.21±1.29 %; $p<0.0001$) of cocoa flavanols, corresponding to absolute increases in FMD of 1.07 % (80 mg), 1.42 % (200 mg CF), 1.96% (500 mg CF) and 2.01% (800 mg), respectively. Significant differences were also observed between the FMD obtained after the 800 mg/day dose compared to the 80 mg/day ($p=0.0003$) and the 200 mg/day ($p=0.05$) doses, but not compared to the 500 mg/dose. Upon EFSA's request, the applicant provided a formal assessment of dose-response effects, which were statistically significant when using both linear and e-power regression analyses. The optimum curve fit was found by using a pseudo-Hill equation, which showed a plateau. It was calculated that the plateau was reached at a dose of approximately 500 mg/day cocoa flavanols, and that EC50 was reached at approximately 90 mg/day cocoa flavanols. The Panel notes that the curve starts to flatten off at approximately 200 mg cocoa flavanols per day. The Panel considers that this study shows a dose-dependent effect of cocoa flavanols on fasting ED-FMD after

one week of consumption in healthy subjects, and that the effect occurs under the conditions of use proposed by the applicant.

In a randomised, single-blind, cross-over study (Grassi et al., 2005), 20 men and women (age ~44 years) with never-treated essential hypertension and impaired glucose tolerance, and 15 normotensive subjects consumed daily dark chocolate (100 g) with 500 mg polyphenols (including 66 mg epicatechin and 22 mg catechin) or flavanol-free white chocolate (90 g) for 15 days, each with a 7-day chocolate-free wash-out period in between and after a run-in period which excluded all cocoa foods. The authors state that the dark and white chocolate bars contained 480 kcal energy and similar amounts of cocoa butter, macronutrients, fibre, electrolytes and vitamins (values not reported). The Panel notes that the total amount of cocoa flavanols administered to the intervention group was not reported. However, the Panel also notes that considering the ratio of total cocoa polyphenols to total cocoa flavanols reported in the study by Grassi et al. (2008), and considering that the amount of monomeric flavanols account for 34-37 % of total flavanol content in cocoa beans, the amount of cocoa flavanols provided in this study may have been around 300 mg/day. It is unclear whether this study was controlled for other cocoa constituents, such as caffeine and theobromine, which have been shown to exert an effect on FMD in some intervention studies. Exclusion criteria were pregnancy, concomitant diseases, use of medications including dietary supplements, smoking, and alcohol consumption. Subjects were asked to refrain from flavanoid-rich foods and beverages (a detailed list was given to each participant), including wine as well as all other alcoholic beverages. FMD of the brachial artery was assessed before and after each intervention in fasting for ≥12 h. A cuff was placed around the forearm just below the elbow. After 1-minute acquisition to measure basal diameter, the cuff was inflated for 5 minutes at 250 mm Hg and then deflated to induce reactive hyperaemia. ED-FMD was defined as the maximal dilation of the brachial artery induced by increased flow expressed as the peak (%) change in the brachial artery diameter. The EIVD was obtained by administering 25 µg sublingual GTN. Differences between hypertensives and normotensives were analysed by paired Student's t-test. Within each treatment group, changes in ED-FMD and GTN-mediated vasodilation from baseline were analysed by 1-factor ANOVA. For multiple comparisons, data were analysed with a 2-factor RM-ANOVA with time and treatment as the two factors. Post-hoc comparisons were performed by Tukey's honestly significant difference test. Baseline ED-FMD of the brachial artery was significantly lower in hypertensive subjects compared with controls (7.4±1.4% *vs.* 9.9±0.9 %; $p<0.0001$) and significantly increased in hypertensive subjects after consumption of dark chocolate (8.9±1.4 %; $p<0.0001$) but not after consumption of white chocolate (7.5±1.3 %). ED-FMD also increased significantly in the control group after consumption of dark chocolate (11.8±1.3%; $p<0.0001$) but not after consumption of white chocolate (10.1±0.9 %). Upon EFSA's request, the applicant clarified that changes in ED-FMD during consumption of dark versus white chocolate were statistically significant ($p<0.0001$) in both groups of subjects. Baseline GTN-induced vasodilation remained unchanged after both the dark and white chocolate intake in both groups of subjects. It is unclear from the publication whether carry-over effects were tested. Upon EFSA's request for clarification, the applicant stated that no carry-over effects were detected between intervention phases in this study. The Panel considers that this study supports an effect of cocoa flavanols on fasting ED-FMD, and no effect on EIVD, at doses of about 300 mg/day when consumed for 15 days in healthy subjects and in subjects with untreated hypertension. However, the Panel notes that an effect of other food constituents in cocoa (e.g. caffeine, theobromine) on ED-FMD cannot be excluded.

In another randomised, controlled, cross-over study by the same research group with a similar design (Grassi et al., 2008), 19 men and women (age ~45 years) with hypertension and impaired glucose tolerance who were not on pharmacological treatment for hypertension consumed 100 g/day of dark (1008 mg total polyphenols, including 111 mg epicatechin and 36 mg catechin) versus white (flavanol-free) chocolate for 15 days each divided in two servings, with a one week wash-out period in between. The Panel notes that the total amount of cocoa flavanols administered to the intervention group was not reported. However, the Panel also notes that considering the ratio of total cocoa polyphenols to total cocoa flavanols reported in the study by Grassi et al. (2008), and considering that

the amount of monomeric flavanols account for 34-37 % of total flavanol content in cocoa beans, the amount of cocoa flavanols provided in this study may have been around 500 mg/day. This study was not controlled for the macronutrient composition of the intervention or for other cocoa constituents such as caffeine and theobromine. Subjects were asked to refrain from flavanoid-rich foods and beverages (a detailed list was given to each participant), including wine as well as all other alcoholic beverages. ED-FMD and EIVD were assessed as in Grassi et al. (2005) at the beginning and end of each intervention. Data were analysed using Proc Mixed Procedure with subject treated as a random factor and treatment, sequence, and baseline as fixed factors. Multiple comparisons were performed by Tukey's honestly significant difference test. Dark chocolate consumption significantly increased ED-FMD (from 5.0±1.2 to 6.3±1.3 %; $p<0.05$), whereas white chocolate did not affect ED-FMD (from 5.0±1.4 to 5.0±1.3 %). Changes in ED-FMD during consumption of dark versus white chocolate were statistically significant ($p<0.05$). Baseline GTN-induced vasodilation remained unchanged after both dark (from 8.40±0.95 to 8.20±1.34 %) and white (from 8.51±0.80 to 8.31±1.27 %) chocolate intake. Upon EFSA's request, the applicant stated that no carry-over effects were detected between intervention phases in this study. The Panel considers that this study supports an effect of cocoa flavanols on fasting ED-FMD, and no effect on EIVD, at doses of about 500 mg/day when consumed for 15 days in subjects with untreated hypertension and impaired glucose tolerance. However, the Panel notes that an effect of other food constituents in cocoa (e.g. caffeine, theobromine) on ED-FMD cannot be excluded.

The Panel notes that, in healthy subjects, one intervention study showed an effect of cocoa flavanols consumed for 12 weeks on fasting ED-FMD (Davison et al., 2008), that one intervention study showed a dose-dependent effect of cocoa flavanols on fasting ED-FMD after one week of consumption, that the effect is observed under the conditions of use proposed by the applicant (Grassi et al., 2011, unpublished), and that two additional intervention studies which may have not been adequately controlled for other food constituents in cocoa also supported an effect of cocoa flavanols on ED-FMD (Grassi et al., 2005, 2008).

**Other human intervention studies**

***Human intervention studies in patients with coronary artery disease***

Three human intervention studies on the effects of cocoa flavanols on ED-FMD in fasting conditions during repeated consumption of the food constituent (for 30 days to 6 weeks) in subjects with coronary artery disease (CAD) (Balzer et al., 2008; Farouque et al., 2006; Heiss et al., 2010) under pharmacological treatment for this condition were provided by the applicant as supportive evidence for the scientific substantiation of the claim.

In an efficacy study performed after a dose-finding study described below under "acute human intervention studies" (Balzer et al., 2008), 41 diabetic subjects with established and stably-treated type 2 diabetes mellitus, previous history of CAD and under pharmacological treatment with blood-pressure lowering, cholesterol-lowering and antiplatelet medications were randomised to receive 75 mg or 966 mg of cocoa flavanols per day divided in three doses for 30 days. Subjects were asked to maintain their usual diet and physical activity patterns. No instructions were given to restrict flavanol intake from other sources in the diet. ED-FMD, which was the primary outcome of the study, was assessed at baseline and on days 8 and 30 of the study, both after an overnight fast and two hours after consumption of the test cocoa drinks assigned. Sample-size calculations, based on the feasibility study, indicated that 20 patients in each group were needed to detect an effect size of 0.9 % with a 5 % 2-sided significance and 80 % power. ED-FMD values at the start of the intervention (day 0) were not statistically different between the low and high flavanol groups (3.3±1.2% *vs.* 3.3±1.1%, respectively). In the high flavanol dose group, baseline ED-FMD values significantly increased from 3.3±1.1 % on day 0 to 4.1±1.1% ($p<0.001$) on day 8, and to 4.3±1.2% ($p<0.0001$) on day 30, whereas no significant changes were reported in the low flavanol group. Between-group differences were statistically significant. The acute effects on FMD (two hours after cocoa ingestion) in the treatment

group were of similar effect size at study entry (3.3±1.1% to 4.8±1.4%, p<0.0001) and after 8 (4.1±1.1% to 5.7±1.6%, p<0.0001) and 30 days (4.3±1.2 % to 5.8±1.6 %, p<0.0001) of cocoa intake, whereas no acute effects were observed in the low flavanol group. No effect of cocoa flavanols was observed on EIVD for any dose at any time point. Fasting plasma concentrations of flavanol metabolites increased from 1,473±671 nmol/l on day 0 before regular intake of flavanol-rich cocoa to values of 2,152±1,222 nmol/l on day 8 (p<0.01) and 2,178±995 nmol/l on day 30 (p<0.01). No significant changes in plasma flavanol metabolites were observed in the control group (day 0: 1,485±1,062 nmol/l; day 8: 1,287±846 nmol/l; day 30: 1,501±1,299 nmol/l). The Panel considers that this study shows an effect of cocoa flavanols on fasting ED-FMD, and no effect on EIVD, at doses of 966 mg/day consumed for 30 days in diabetic subjects with CAD, and that the effect is already observed after seven days.

In a randomised, controlled, double-blind, cross-over trial (Heiss et al., 2010), 20 patients on secondary prevention for CAD (64±3 years of age) received a dietary high-flavanol cocoa drink (HF, 375 mg) and a macronutrient- and micronutrient-matched low-flavanol cocoa drink (LF, 9 mg) twice daily (750mg/day and 18 mg/day, respectively) in random order over 30 days with one week of wash-out between interventions. The ED-FMD of the brachial artery and the number and function of circulating angiogenic cells (CACs) were the primary and secondary outcomes of the study, respectively. Measurements were taken at baseline and at the end of the intervention after overnight fasting. A total of 16 subjects completed the study and entered data analyses. The primary test for an effect was two-way RM-ANOVA (2 factors were drink [HF/LF] *vs.* time [pre/post]) and the Holm-Sidak test for post-hoc comparisons. The average baseline ED-FMD values were 4.4±0.5 % and 4.6±0.5 % (p=0.125) before starting the LF or HF cocoa drinks, respectively. By the end of the 30-day periods, FMD values significantly increased to 5.7±0.5 % (LF) and 8.4±0.8 % (HF, each p<0.001 *vs.* pre-intervention values), and the post-HF values were significantly greater than post-LF values (p<0.001 between groups). The baseline diameter of the brachial artery, the baseline and hyperaemic blood flow velocities, as well as corresponding blood flow at baseline and at reactive hyperaemia, were unaffected by treatments. After 30-day HF, $CD34^+/KDR^+$-CACs increased 2.2-fold and $CD133^+/KDR^+$-CACs increased 8.0-fold, relative to LF control (each p<0.001). The Panel considers that this study shows an effect of cocoa flavanols on fasting ED-FMD at doses of about 750 mg/day consumed for 30 days in subjects with CAD.

In a randomised, double-blind, placebo-controlled, parallel intervention (Farouque et al., 2006), 40 subjects (61±8 years; 30 male) with CAD (>50% stenosis in at least one epicardial coronary artery) were randomised to consume a flavanol-rich chocolate bar and cocoa beverage daily (total flavanols, 444 mg/day) or matching isocaloric placebos (total flavanols, 19.6 mg/day) for six weeks. Subjects were asked to consume their usual diet during the study. Brachial artery ED-FMD, EIVD (GNT) and systemic arterial compliance (SAC) were assessed after an overnight fast at baseline, and after three and six weeks of daily consumption of the test products, as well as at 90 min following consumption of the first beverage (day 0). Power calculations indicated that 16 and 20 subjects would be required in each treatment arm to detect a difference in FMD of 4–5 % between groups at a p value <0.01 with 90 % power. ED-FMD and SAC responses did not differ between groups at baseline. No acute or chronic changes in ED-FMD or SAC were observed in either group. The Panel notes that this study does not show an effect of cocoa flavanols on ED-FMD or EIVD in patients with CAD.

The Panel notes that two out of three intervention studies showed an effect of cocoa flavanols consumed for 30 days on fasting ED-FMD in subjects with CAD who were under pharmacological treatment, and that the effect occurred after seven days of consumption of cocoa flavanols.

***Acute human intervention studies***

A number of human intervention studies conducted in different population subgroups (e.g. smokers, older adults, obese subjects, subjects with type 2 diabetes and CAD) and which addressed the acute

effects of cocoa flavanols when consumed on a single occasion on ED-FMD were also provided by the applicant as supportive evidence for the scientific substantiation of the claim. One of the studies also assessed the acute effects of cocoa flavanols on ED-FMD during repeated consumption (for seven days) of the food/constituent (Heiss et al., 2007).

In a feasibility study (Balzer et al., 2008), 10 patients (50-80 years) with established and stably-treated type 2 diabetes mellitus, previous history of CAD and under pharmacological treatment with blood-pressure lowering, cholesterol-lowering and antiplatelet medications were randomised in a double-masked, three-period cross-over design, and consumed a cocoa drink which provided 75 mg (control), 371 mg or 963 mg of flavanols on three different occasions with a wash-out period in between of at least three days. All cocoa drinks were closely matched for energy, macro- and micronutrients, theobromine and caffeine content. Subjects were asked to maintain their usual diet and physical activity patters. No instructions were given to restrict flavanol intake from other sources in the diet. ED-FMD was assessed at baseline (after an overnight fast) and at one, two, three, four and six hours after consumption of the test drinks according to the guidelines of the American College of Cardiology (Corretti et al., 2002). The EIVD was measured 4 min after sublingual application of 400 µg GTN. Based on previous reports showing that flavanol metabolites reach a maximum plasma concentration around two hours after ingestion, a blood sample was also taken at that time point for the measurement of plasma flavanol concentrations. Before the intervention, the mean ED-FMD for the study population was 3.8±0.3 %. Baseline values did not differ among the three interventions. Consumption of 75 mg of cocoa flavanols did not significantly affect ED-FMD values at any time point, whereas intake of 371 mg and 963 mg significantly increased ED-FMD values from baseline in a dose-dependent manner. ED-FMD returned almost to baseline values at six hours after cocoa ingestion. The ED-FMD values were significantly higher for the 963 mg dose compared to the 75 mg dose at two and three hours after cocoa consumption. The maximum biological effects as measured by ED-FMD were reached at around two hours, and their half-life at around four hours, after consumption of the cocoa drinks containing 371 or 963 mg of flavanols. No effect of cocoa flavanols was observed on EIVD for any dose. The sum of aglyconic flavanols and their respective conjugated flavanol metabolites in plasma increased dose-dependently within two hours from 509±69 nmol/l to 1,038±95 nmol/l when ingesting 371 mg of flavanols ($p<0.05$), and from 463±53 nmol/l to 1,427±80 nmol/l when a 963-mg flavanol dose was given ($p<0.01$). The consumption of the control cocoa drink (75 mg of flavanols) did not result in a statistically significant increase in flavanol plasma concentrations (472±54 nmol/l to 666±72 nmol/l). The Panel considers that this study shows an acute, dose-dependent increase in ED-FMD following consumption of cocoa flavanols which is maximal at two hours, that ED-FMD values almost return to baseline after six hours, and that changes in ED-FMD parallel blood concentrations of flavanol metabolites. The Panel notes that this acute effect was sustained overtime with repeated consumption of the food (at 8 and 30 days) in the efficacy study by the same authors described above (Balzer et al., 2008).

In an initial study involving six subjects with at least one risk factor for CAD, Heiss et al. (2003) assessed the time course of the effects of cocoa flavanols on ED-FMD, which was measured 0, 2, 4 and 6 hours after ingestion of 100 ml of cocoa drink containing 176 mg of flavan-3-ols (70 mg of epicatechin plus catechin, 106 mg of procyanidins, HF) (n=6) or a control drink (100 ml cocoa drink with <10 mg of flavan-3-ols or water, LF) (n=3). Consumption of the HF cocoa drink increased ED-FMD maximally at two hours, whereas the LF drinks did not affect ED-FMD. There was no significant difference in ED-FMD in the three individuals receiving either water or the LF cocoa drink. Then 20 subjects of similar characteristics received 100 ml of cocoa drinks with HF or LF on two consecutive days, in a random order. The ED-FMD of the brachial artery was measured each day at baseline and at 2 h after ingestion of the drinks. The EIVD of the brachial artery following sublingual application of 400 µg GNT was also assessed after the last FMD measurement. The sum of nitrosylated and nitrosated species (collectively referred to as RXNO) was measured two hours after ingestion on both days. In addition, a number of other vascular parameters such as blood pressure, heart rate and plasma concentrations of nitrite and nitrate, which would not be expected to change as a

result of acute flavanol intake, were also assessed. Ingestion of the HF cocoa drink significantly increased plasma levels of RXNO from 22 to 36 nmol/l and FMD from 3.4 % to 6.3 % ($p<0.001$ for both comparisons). Changes in plasma levels of RXNO and in FMD were correlated ($r=0.42$; $p=0.02$). No changes in FMD or RXNO were observed after the LF drink. No changes in any of the other parameters assessed were observed after consumption of any drink. The Panel considers that this study shows an acute effect of cocoa flavanols on ED-FMD which is maximal at about two hours after ingestion, and shows no effect on EIVD, in subjects at low risk of CAD.

In order to assess the potential of a dietary intervention using flavanol-rich foods to reverse endothelial dysfunction, and the dose needed to achieve the effect, plasma RXNO and ED-FMD were measured in a subgroup of 11 healthy volunteers, with smoking as the only major cardiovascular risk factor (Heiss et al., 2005). In a preliminary dose-founding study, RXNO and FMD were measured in four smokers before and two hours after ingestion of tap water (100 ml) or 50, 100 and 200 ml of high-flavanol cocoa drink (HFCD) on four separate days. The HFCD contained 176 to 185 mg of flavan-3-ols per 100 ml (40 % epicatechin/catechin, 60 % procyanidins). Dose-dependent increases in RXNO and FMD were observed two hours after consumption of HFCD. Plasma concentrations of free aglyconic flavanols, as well as those of conjugated flavanol metabolites, increased significantly only following the ingestion of >100 ml HFCD containing ≥176 mg of total flavanols. The increases in epicatechin, catechin, epicatechin-7-beta-D-glucuronide, 4'-O-methyl-epicatechin, and 4'-O-methyl-epicatechin-β-D-glucuronide correlated significantly with the increase in RXNO. The increases in FMD correlated with the increase in epicatechin ($r=0.66$, $p=0.020$) and catechin ($r=0.62$, $p=0.031$). A randomised double-blind cross-over study was conducted subsequently in 11 smokers. On two days, RXNO and FMD were measured before and two hours after ingestion of 100 ml HFCD or a control low-flavanol cocoa drink (LFCD, 100 ml cocoa drink with <11 mg of flavanols). Volunteers fasted overnight and were required to refrain from smoking 12 h before beginning and during the entire assessment period. RM-ANOVA with two within-subject factors (baseline *vs.* 2 h post-drink and high-flavanol drink *vs.* control drink) revealed a significant two-way interaction (FMD, $p=0.002$; RXNO, $p=0.03$). The pair wise comparison showed that RXNO and FMD significantly increased 2 h after ingestion of 100 ml HFCD (4.2±0.9 % to 6.9±0.9 %, $p<0.001$, and 20±3 nmol/l to 29±2 nmol/l; $p<0.001$) but was unaltered after the LFCD (4.5±0.9% to 3.6±0.9 %, $p=0.141$, and 23±4 nmol/l to 20±3 nmol/l, $p=0.452$), and baseline values were not significantly different on the two study days. In a subset of subjects (n=4), inhibition of endothelial nitric oxide synthase (eNOS) via intravenous infusion of L-NG-monomethyl-arginine (L-NMMA), mimicking acute endothelial dysfunction, abolished the cocoa-related improvement in endothelial dysfunction and the increase in the circulating NO pool. Intravenous infusion of ascorbic acid, which has been shown to increase endothelial function in smokers by increasing tetrahydrobiopterine, a cofactor of eNOS, did not improve further endothelial dysfunction when given in addition to the HFCD (administered at 0 h) at the 2-h time point. The Panel considers that this study shows an acute dose-dependent effect of cocoa flavanols on ED-FMD in smokers, and that the effect is observed at doses of about 176 mg of cocoa flavanols.

Another intervention study by the same authors (Heiss et al., 2007) was designed to investigate the time course of endothelial function during daily consumption of high-flavanol (HF) cocoa. The FMD was determined acutely (for up to six hours after single-dose ingestion), chronically (after 2, 4 and 7 days of cocoa consumption corresponding to days 3, 5 and 8 of the study), and after a 7-day wash-out (day 15), in 11 subjects with smoking-related endothelial dysfunction but normal GNT-mediated EIVD. Also plasma nitrite and nitrate were measured at baseline and days 8 and 15 of the study. The daily consumption of a flavanol-rich cocoa drink (3 x 306 mg flavanols/day) over seven days (n=6) resulted in continuous FMD increases at baseline (after overnight fast and before flavanol ingestion), and in sustained FMD increase at 2 h after ingestion. Fasted FMD responses increased from 3.7±0.4 % on day 1 to 5.2±0.6 %, 6.1±0.6 % and 6.6±0.5 % ($p<0.05$ for all) on days 3, 5 and 8, respectively. The FMD returned to 3.3±0.3 % after a wash-out week of cocoa-free diet (day 15). A significant increase of FMD of about 2.4±0.3 % 2 h after consumption of the HF drink was observed at all testing

days. Fasting plasma concentrations of nitrites (but not nitrates) significantly increased at day 8 and returned to baseline at day 15. Plasma concentrations of nitrites (but not nitrates) also increased significantly 2 h after cocoa administration at baseline and day 8. The Panel notes the absence of a control group but considers that this study supports an acute effect (two hours after consumption) of cocoa flavanols on ED-FMD which is sustained overtime with repeated consumption (for seven days) of the food.

In addition, Berry et al. (2010) showed that a single dose of high-flavanol (701 mg CF; 139 mg epicatechin) cocoa beverage significantly increased FMD compared to a single dose of a low-flavanol (22 mg CF; 0 mg epicatechin) cocoa beverage (from 3.4±0.5 % to 6.1±0.6 %) in 21 obese but otherwise healthy volunteers.

Another randomised, double-blind, placebo-controlled, cross-over study (Monahan et al., 2011) was designed to investigate whether the effect of acute cocoa ingestion on ED-FMD as measured by an increase in brachial artery FMD was dose-dependent in healthy older adults (n=23, mean age 63 years). Measurements were obtained on five different days, at least three days apart, before and 1 h and 2 h after consumption of 0 (placebo), 2, 5, 13 and 26 g of cocoa, providing about 0, 80, 200, 500 or 1000 mg of cocoa flavanols, respectively. The Panel notes that this study was not controlled for other components in cocoa (0, 0, 6, 12 and 27 mg of caffeine and 0, 27, 66, 138 and 279 mg of theobromine in increasing doses of cocoa, respectively) but considers that differences in the content of these constituents between the cocoa doses used may have been too low to account for a significant effect on FMD. Changes in brachial artery FMD 1-h and 2-h post-ingestion compared with baseline were used to determine the effects of cocoa. The FMD was unchanged 1 h and 2 h after placebo (0 g cocoa) but significantly increased 1 h and 2 h after consumption of 2, 5, 13 and 26 g of cocoa in a dose-dependent manner. The increase in FMD 1 h and 2 h after consumption of 5, 13 and 26 g of cocoa, but not after consumption of 2 g of cocoa, was statistically significant compared to placebo. The Panel considers that this study shows an acute dose-dependent effect of cocoa flavanols on ED-FMD in healthy older adults.

Other studies using white chocolate as control or with no control group have also reported an effect of cocoa flavanol-containing products on ED-FMD of the brachial artery at higher doses (>700 mg) (Faridi et al., 2008; Hermann et al., 2006; Schroeter et al., 2006; Vlachopoulos et al., 2005; Westphal and Luley, 2011), with or without sugar (Faridi et al., 2008), and that the effect correlated with plasma concentrations of RXNO species and could be inhibited by the administration of L-NMMA, an inhibitor of eNOS (Schroeter et al., 2006).

The Panel notes that consumption of cocoa flavanols on a single occasion induces an acute, dose-dependent increase in ED-FMD which is maximal 2 h after consumption of the food constituent, parallels blood concentrations of the flavanol metabolites, returns to baseline 6 h after ingestion, and is sustained with repeated consumption of cocoa flavanols.

**Summary of the evidence provided by human intervention studies**

The Panel notes that cocoa flavanols consumed for 12 weeks have been shown to increase fasting ED-FMD significantly in the target population (Davison et al., 2008), that this effect is dose-dependent and occurs after one week of consumption under the conditions of use proposed by the applicant (Grassi et al., 2011, unpublished), that the effect is supported by two additional intervention studies of shorter duration (Grassi et al., 2005, 2008), and that it is also observed in two out of three studies in patients under pharmacological treatment for CAD (Balzer et al., 2008; Heiss et al., 2010). The Panel also notes that consumption of cocoa flavanols on a single occasion induces an acute, dose-dependent increase in ED-FMD which is maximal 2 h after consumption of the food constituent, parallels blood concentrations of the flavanol metabolites, returns to baseline 6 h after ingestion, and is sustained with repeated consumption of cocoa flavanols (Balzer et al., 2008; Heiss et al., 2003, 2005, 2007; Monahan et al., 2011).

**Mechanisms of action**

With regard to the longer-term effects of cocoa flavanols on fasting ED-FMD, the applicant proposes "an increased expression/activity of eNOS, changes in NO bioavailability, changes in expression/activity of eNOS-related proteins that may influence cellular localization of eNOS, co-factor availability, or substrate accessibility, or eNOS-independent mechanisms as biologically plausible mechanisms". However, the applicant also acknowledges that the precise mechanisms involved in longer-term effects of cocoa flavanols on FMD, and their relative contribution to the effect, remain to be elucidated. The Panel notes that the evidence provided for any of these mechanisms is weak.

The applicant proposed an enhancement of NO production by eNOS as the most plausible and best documented mechanism by which cocoa flavanols could induce (at least in part) an acute effect on endothelium-dependent vasodilation. Some evidence was provided in support of this mechanism in humans. The FMD responses at two hours following consumption of cocoa flavanols appear to correlate with increased plasma concentrations of flavanol metabolites (mostly catechin and epicatechin) and RXNO in plasma (Balzer et al., 2008; Heiss et al., 2003, 2005, 2007; Schroeter et al., 2006), and the effect was inhibited, at least in part, by L-NMMA (Heiss et al., 2005; Schroeter et al., 2006), an inhibitor of eNOS. Intravenous infusion of ascorbic acid, which has been shown to increase endothelial function by increasing tetrahydrobiopterine, a cofactor of eNOS, did not appear to have an additional effect on ED-FMD in addition to cocoa flavanols (Heiss et al., 2005).

Upon EFSA's request for clarification about the food constituents which may be responsible for the claimed effect, the applicant clarified that only cocoa flavanol monomers and rarely dimers (mostly epicatechin) may be responsible for the acute effects of cocoa flavanols on FMD as they are absorbed in the small intestine and plasma concentrations of catechins, epicatechins and their metabolites correlate with the increase in FMD after a single dose of cocoa flavanols (Balzer et al., 2008; Heiss et al., 2003, 2005, 2007; Schroeter et al., 2006). The applicant also states that procyanidins and the fraction of epicatechin, which is not absorbed in the small intestine are extensively metabolised by gut microbiota into low molecular weight phenolics, which are then absorbed and could contribute to the effects of cocoa flavanols on fasting FMD, but not to the acute effect (Rios et al., 2003). Although no evidence has been provided that procyanidin microbial metabolites exert an effect on fasting FMD *in vivo* in humans, such an effect cannot be ruled out.

The Panel considers that the evidence provided in support of mechanisms by which repeated consumption of cocoa flavanols may induce longer-term effects on fasting ED-FMD is weak. The Panel also considers that cocoa flavanols (mostly epicatechins) may exert an acute effect on ED-FMD by enhancing NO production in the endothelium each time they are consumed.

**Weighing of the evidence**

In weighing the evidence, the Panel took into account that cocoa flavanols consumed for 12 weeks were shown to increase fasting ED-FMD significantly in the target population in one human intervention study, that in another human intervention study the effect was dose-dependent and occurred after one week of consumption under the conditions of use proposed by the applicant, that the effect is supported by two additional intervention studies, and that it is also observed in two out of three studies in patients under pharmacological treatment for CAD, whereas the mechanisms by which regular consumption of cocoa flavanols may induce a sustained effect on fasting ED-FMD are unknown. The Panel also took into account that consumption of cocoa flavanols on a single occasion induces an acute and dose-dependent increase in ED-FMD which is sustained with regular consumption of the food/constituent, and that this acute effect may be mediated by the enhancement of NO production in the endothelium each time cocoa flavanols are consumed.

The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

The Panel could not have reached its conclusions without the human intervention study claimed as proprietary by the applicant (Grassi et al., 2011, unpublished).

### 4. Panel's comments on the proposed wording

The Panel considers that the following wording reflects the scientific evidence: "Cocoa flavanols help maintain endothelium-dependent vasodilation, which contributes to normal blood flow".

### 5. Conditions and restrictions of use

In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate. These amounts of cocoa powder or dark chocolate can be consumed in the context of a balanced diet. The target population is the general population.

## CONCLUSIONS

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

- The food constituent, cocoa flavanols, which is the subject of the claim, is sufficiently characterised.
- Maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect.
- A cause and effect relationship has been established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.
- The following wording reflects the scientific evidence: "Cocoa flavanols help maintain endothelium-dependent vasodilation, which contributes to normal blood flow".
- In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate. These amounts of cocoa powder or dark chocolate can be consumed in the context of a balanced diet. The target population is the general population.

## DOCUMENTATION PROVIDED TO EFSA

Health claim application on cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0319_BE). December 2011. Submitted by Barry Callebaut Belgium nv.

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## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| CACs | Circulating angiogenic cells |
| CAD | Coronary artery disease |
| CF | Cocoa flavanols |
| CV | Coefficient of variation |
| DP | Degree of polymerisation |
| EC50 | Half maximal effective concentration |
| ED-FMD | Endothelium-dependent flow-mediated dilation |
| EIVD | Endothelium-independent vasodilation |
| eNOS | Endothelial nitric oxide synthase |
| FMD | Flow-mediated dilation |
| GTN | Glyceryl trinitrate |
| HF | High-flavanol |
| HFCD | High-flavanol cocoa drink |
| LF | Low-flavanol |
| L-NMMA | L-NG-monomethyl-arginine |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| RCT | Randomised controlled trials |
| RM-ANOVA | Repeated measures ANOVA |
| RXNO | Sum of nitrosylated and nitrosated species |
| SAC | Systemic arterial compliance |