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(54) Title: THEOBROMINE FOR INCREASING HDL-CHOLESTEROL

(57) Abstract: Theobromine for use in the treatment of increasing HDL-cholesterol and/or increasing the ratio HDL-cholesterol : LDL-cholesterol in humans and the use of theobromine for increasing HDL-cholesterol in humans, and/or for increasing the ratio HDL-C / LDL-C, and compositions comprising theobromine.

## THEOBROMINE FOR INCREASING HDL-CHOLESTEROL

### Field of the invention

5 The present invention relates to a method for increasing HDL-cholesterol in humans, and/or for increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and to theobromine for use in the treatment of increasing HDL-cholesterol in humans, and/or to theobromine for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and to the use of theobromine for increasing HDL-cholesterol in humans, and/or to the use of  
10 theobromine for increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.

### Background of the invention

Since several decades it is widely known, both among physicians as well as a large part of the general (adult) public, especially in the Western world (Europe, North America) that high  
15 cholesterol (in blood) is a marker or indication for an increased risk on cardiovascular diseases. Hence, various medicaments have been developed to reduce the level of cholesterol (in blood). Since more recently, it is generally believed that not all cholesterol in this connection is "bad".

20 More specifically, it is the low density lipoprotein cholesterol (or even more specifically the non-high density lipoprotein cholesterol) that is preferably kept below a certain value. As it is believed that a relatively high level of high density lipoprotein cholesterol can help reducing the level and/or alleviate the negative effects of low density (or non-high density-) lipoprotein cholesterol, it is believed that it may be desired that the level of high density lipoprotein  
25 cholesterol is relatively high, or can be increased, and/or that the ratio high density lipoprotein cholesterol over low density lipoprotein cholesterol (or the ratio high density lipoprotein cholesterol over non-high density lipoprotein cholesterol) preferably kept above a certain ratio or can be increased in many humans.

30 High density lipoprotein cholesterol is often abbreviated to HDL-cholesterol (or herein: HDL-C) and low density lipoprotein cholesterol is often abbreviated to LDL-cholesterol (or herein: LDL-C), and non-high density lipoprotein cholesterol is often abbreviated as non-HDL-cholesterol (or herein: non-HDL-C). When referred to levels of the various forms of cholesterol, it is meant herein cholesterol in blood in humans. Such cholesterol levels are usually measured as serum  
35 (LDL-, HDL-, non-HDL-, or total-) cholesterol or plasma (LDL-, HDL-, non-HDL-, or total-) cholesterol. Non-HDL-C is herein total cholesterol (TC) minus HDL-C.

Hence, it is believed that health benefits can be achieved in humans by lowering low density lipoprotein cholesterol (or LDL-cholesterol, herein abbreviated to LDL-C) and/or by increasing the level of high density lipoprotein cholesterol (or HDL-cholesterol, herein HDL-C) and/or by increasing (molar or weight) ratio HDL-C over LDL-C. There are medicaments (prescription drugs) available that are aimed for one or more of these.

Apart from such medicaments, there are also food products that are enriched in edible actives, often actives that do exist in natural sources, that can achieve or are believed to achieve a lowering of LDL-C and/or an increase of HDL-C. In this connection, since the 1960's there exist food products (e.g. spreads and margarines) that contain polyunsaturated fatty acids that help reducing cholesterol in blood (of humans). More recently, products have appeared on the market in Europe and the USA that contain plant sterols or plant stanols (or esters of these sterols or stanols) which are also believed to be able to reduce total cholesterol or LDL-C.

Still, there is a desire for (further) actives that specifically increase HDL-C, and more preferably increase the ratio HDL-C / LDL-C, and/or increase HDL-C / non-HDL-C, e.g. as such can lead to improved blood lipids (including an improved blood lipids profile). Such active could be a medicament, but, in order to help people maintain a healthy lifestyle, such does not need to be a medicament. Such a component that increases HDL-C, preferably not at the expense of increasing also LDL-C or non-HDL-C, can be consumed on its own or conveniently be combined with one or more actives which does not have an effect on HDL-C, but which is already known to reduce LDL-C or non-HDL-C.

A large range of naturally occurring components have been investigated to achieve such benefit, such as (methoxy) flavonoids. The effect of cocoa, as a rich source of flavonoids, has been studied quite extensively in this respect.

Wan et al (Am J. Clin. Nutr. 74, pp 596-602, 2001) discloses a study in which humans were fed ("long term", without indicating how long) a diet containing 22 g cocoa powder + 16 g dark chocolate per daily dosage. Such diet contained 614 mg theobromine per day, which was however controlled for in the control diet (550 mg theobromine / day). They report a HDL-cholesterol increase of about 4%, and a LDL-cholesterol increase of 4.6%. Hence, the ratio HDL-C/LDL-C is not substantially changed.

De Graaf et al (British Journal of Nutrition, 88, pp 479-488, 2002) disclose chocolates enriched in plant sterols, and their study is on humans (for 4 weeks), with a dosing of 31.5 g chocolate and 1.8 g phytosterols per day. No effect is reported on HDL-C.

- 5 Mursu et al (Free Radical Biology and Medicine, 37(9), pp 1351-1359, 2004) shows that 75 g dark chocolate daily for 3 weeks can increase serum HDL-C (> 11%), when compared to a placebo of white chocolate not containing polyphenols.

10 Baba et al (Am. J. Clin. Nutr., 85 pp 709-717, 2007) show that a daily diet containing 26 g cocoa powder for 12 weeks showed an increase of plasma HDL-C (17%) compared to the control group. LDL-C was reduced by 8% compared to the control group. The same research group reported data from a dose-response study with low (13 g), moderate (19.5 g) and high levels of cocoa powder (26 g) (J. Nutr. 137 pp 1436-1441, 2007). HDL-C increased compared to placebo by 3, 5 and 7%, respectively. The placebo was adjusted to control for theobromine  
15 content in the cocoa powders.

Crews et al (Am. J. Clin. Nutr. 87, pp 872-880, 2008) disclose that the effect of 6 week daily consumption of 37 g dark chocolate (which they say contains 60% cacao, equaling 11 g natural cocoa) plus 237 ml of a cocoa beverage also containing about 11 g natural cocoa (i.e.  
20 daily 22 g cocoa) on HDL-C or LDL-C was not statistically significant (only an increase of heart rate was noted).

MU Eteng et al (Nutrition Research, Vol. 20 No 10, pp1513-1517, 2000), disclose that theobromine, when given to Wistar rats in an amount of 700 mg/kg body weight, decreased  
25 total cholesterol, LDL-cholesterol, and triglycerides, and elevated HDL-cholesterol in said rats.

MU Eteng et al (Discovery and Innovation, 18(3), pp 191-196, 2006) disclose that theobromine-rich cocoa powder, when given in the diet to Wistar rats in a dose of 3% to 15% cocoa powder (containing to 56-265 mg theobromine, respectively) daily in the diet for 4  
30 weeks, lead to body weight changes and changes in the lipid profile in said rats.

MU Eteng et al (Journal of Pure and Applied Sciences, 6(1) pp89-93, 2000) refers to trials with Wistar rats which were given 600 or 700 mg theobromine per kg body weight.

JP patent application 2006/151,878 refers to the combined use of curcuma or curcuma plant extract together with a xanthine derivative as hypoglycaemic agent for treating diabetes mellitus, and for the treatment of hyperlipedemia.

- 5 JP patent application 2001/169,753 discloses that soy protein and chitosan reduce blood cholesterol, but that one should take large dosages of such. This document aims to provide a composition that can:
- suppress a rise in total cholesterol, and
  - suppress a reduction in HDL-C,
- 10 wherein not so high dosages of soy protein or chitosan are needed. It refers that this can be achieved by a composition with 4 components together: a xanthine derivative (caffeine being preferred), a dietary fibre, an amino acid promoting glucagon secretion, and a vegetable protein.
- 15 H Yokogoshi et al, Nutritional Reports International, October 1983, volume 28, no. 4, pp 805-814, disclose that in Wistar rats, fed on a diet containing 19.4% cocoa for 2 weeks, the level of HDL-C increases when compared to a diet not containing cocoa. Serum total cholesterol of rats fed a diet supplemented with 0.3% of theobromine was significantly increased.
- 20 AM Jalil et al, J Sci Food Agric 89, pp 130-137 (2009) disclose that intake of a cocoa extract supplemented with polyphenols and methylxanthines in obese-diabetic rats, significantly reduced plasma total cholesterol, triglycerides, and LDL-C, but did not result in a significant difference in HDL-C.
- 25 RR Allen et al, J. Nutr. 137, pp725-731 (2008) disclose that consumption per day of 2 cocoa-flavonol containing dark chocolate bars, enriched in 1.1 g sterol esters per bar, in humans with elevated serum cholesterol, reduces blood cholesterol levels.
- 30 JA Polagruto et al, Journal of the American Dietetic Association, vol. 206, no. 11, pp 1804-1813 (2006) disclose that a consumption by humans of cocoa flavanol-enriched snack bars containing phytosterols effectively lowers total cholesterol and LDL-C, and that it does not affect HDL-C levels.

**Summary of the invention**

Hence, there is a desire for an active, preferably naturally occurring, which, when ingested regularly (preferably daily, or at least 5 times a week), and preferably at least for some time (preferably at least for 2 weeks, more preferably at least for 3 weeks, more preferably longer),  
5 can help in increasing HDL-C in humans. Preferably, such is achieved whilst the concentration of LDL-C and/or non-HDL-C is not increased, at least not by more than 5%, preferably not by more than 3%. The amount of HDL-C increase is preferably at least 5%, more preferably at least 8%, even more preferably at least 10%, when compared to a placebo (all increases and decreases herein in a statistically significant amount). There is also a desire for an active that,  
10 when ingested regularly (preferably daily, or at least 5 times a week), and preferably at least for some time (preferably at least for 2, more preferably at least for 3 weeks, more preferably longer), can help in increasing the ratio HDL-C / LDL-C in humans and/or that can help in increasing the ratio HDL-C / non-HDL-C in humans. Preferably such increase is at least 5%, more preferably at least 8%, even more preferably at least 10%, when compared to a placebo  
15 (preferably in a statistically significant amount). There is also a desire for a method or use of such active for increasing HDL-C and/or increasing the ratio HDL-C / LDL-C and/or increasing the ratio of HDL-C / non-HDL-C. Such raise of HDL-C in combination with lower increase of LDL-C and/or improved ratio of HDL-C / LDL-C is believed to improve the blood lipids (or in other words improve the blood lipids profile), which are both desirable objectives. Preferably,  
20 the effects on HDL-C, the ratio HDL-C/LDL-C, and/or the ratio HDL-C/non-HDL-C as set out above are achieved without significantly raising peripheral blood pressure. Preferably, the effects on HDL-C, the ratio HDL-C/LDL-C, and/or the ratio HDL-C/non-HDL-C as set out above are achieved without significantly raising the level of triglyceride in blood or serum, as such is considered undesired. There is also a desire for suitable edible compositions,  
25 preferably in the format of food products, that comprise such active. Preferred food formats in this context are emulsions such as e.g. spreads like butter alternative.

The active desired is preferably to be consumed regularly, and is preferably also not seen as a medicament. Hence, there is a desire for food products containing the desired active that fit in  
30 many diets, and preferably such food product should be such that consumers usually consume an amount which is fairly constant and predictable, so as to limit the chance of over- and under-dosing (over and below the daily amount for a good and safe effect). Also, such food products are preferably such that they are already seen as carriers for cardiovascular benefits, and preferably blood lipid-regulating food products. Typical examples of this in the  
35 diets in Europe and North America are spreads (margarine, for e.g. spreading on bread), and beverages, especially minidrinks (typically "shots" of a volume of e.g. between 50 and 150 ml

or even between 50 and 125 ml, which are frequently offered as carrier for ingredients with a real or perceived health benefit, such as to provide pro- or pre-biotics, for regulating LDL-cholesterol, for regulating blood pressure) and dairy-like products (i.e. fermented) as drinkable yoghurt-like products (the same or similar sort of uses).

5

It has now surprisingly been found that such objectives may be achieved, at least in part, by the ingestion of theobromine. It was found that by the ingestion of theobromine, HDL-C (as measured in serum or plasma) in humans can be increased (raised) by at least 5%. It was also found that such can be achieved in the absence of a rise in LDL-C or non-HDL-C (in serum or plasma), or at least by a rise in LDL-C or non-HDL-C (in serum or plasma, or blood) in humans by less than 5%. This is beneficial for the ratio HDL-C / LDL-C or HDL-C / non-HDL-C in humans (in serum or plasma, or in blood), or in other words for improving blood lipids, or the blood lipids profile.

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Hence, in a first aspect the present invention relates to theobromine for use in the treatment of improving blood lipids (including improving blood lipids profile).

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In a second aspect the present invention relates to theobromine for use in the treatment of increasing HDL-cholesterol in humans.

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In a third aspect, the present invention relates to theobromine for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.

25

In a fourth aspect the present invention relates to theobromine for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans.

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More specifically, in the above four aspects said treatment involves ingestion of theobromine by humans. Hence, the invention relates to theobromine for use in the treatment of improving blood lipids (including blood lipids profile) and/or increasing HDL-C in humans by ingestion of theobromine by humans, and/or to theobromine for use in the treatment of increasing the ratio HDL-C / LDL-C in humans by ingestion of theobromine by humans, and/or to theobromine for use in the treatment of increasing the ratio HDL-C / non-HDL-C in humans by ingestion of theobromine by humans.

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In a further aspect, the present invention relates to theobromine for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.

In a further aspect the present invention relates to theobromine for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans.

5 The invention further relates to a method for improving blood lipids and/or blood lipids profile by ingestion of theobromine. More specifically, the invention further relates to a method for improving blood lipids and/or blood lipids profile for a person in need of the treatment of improving blood lipids and/or blood lipids profile, by ingestion of theobromine. The invention further relates to a method for increasing HDL-cholesterol in a human, which method  
10 comprises administering to the human an HDL cholesterol raising effective amount of theobromine. The invention further relates to a method for increasing HDL-cholesterol in humans comprising ingestion of an HDL cholesterol raising effective amount of theobromine. More specifically, the invention further relates to a method for increasing HDL-cholesterol in humans, for a person in need of treatment of increasing HDL-cholesterol, by ingestion of an  
15 HDL-cholesterol raising effective amount of theobromine.

The invention also relates to a method for increasing the ratio HDL-cholesterol / LDL-cholesterol in humans by ingestion of theobromine. More specifically, the invention further relates to a method for increasing the ratio HDL-cholesterol / LDL-cholesterol in a human,  
20 which method comprises administering to the human an HDL-cholesterol / LDL-cholesterol ratio raising effective amount of theobromine. The invention also relates to a method for increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans by ingestion of theobromine. More specifically, the invention further relates to a method for increasing the ratio HDL-cholesterol / non-HDL-cholesterol in a human, which method comprises  
25 administering to the human an HDL-cholesterol / non-HDL-cholesterol ratio raising effective amount of theobromine.

In these methods, the effective amount is from 300 to 2000 mg theobromine per day, preferably from 400 to 1800 mg theobromine per day, more preferably from 600 to 1400 mg of  
30 theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day. These amounts per day in these methods are daily dosages. Preferably, in these methods this is achieved by administering to the human a foodstuff (including beverages) comprising at least 0.1% by weight of theobromine on the total composition, preferably at least 0.2%, more preferably at least 0.5%  
35 by weight of theobromine on the composition. Preferred embodiments of foodstuffs that can be used for such are set out in the detailed description of the invention, hereafter. The



theobromine in these methods (and the compositions in such methods) may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons, it is preferred that the levels of polyphenols (e.g. from cocoa) ingested in these methods and present in the compositions for use in these methods are not more than 5  
5 times the amount of theobromine ingested in these methods and present in such compositions, or in other words: the amount of polyphenols (e.g. from cocoa) ingested in the methods and present in the compositions for use in these methods referred to herein is preferably between 0% and 500% on the weight of theobromine ingested or present in the compositions, more preferably from 0% to 200% on the weight of theobromine ingested or  
10 present in the compositions, even more preferably from 0% to 50% on the weight of theobromine ingested or present in the compositions. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred if the amount of epicatechin ingested in these methods and present in the compositions for use in these methods is not more than the amount of theobromine ingested  
15 in these methods and present in the compositions for use in these methods, or in other words: the amount of epicatechin (e.g. from cocoa) ingested in the methods and present in the compositions for use in these methods referred to herein is preferably between 0% and 100% on the weight of theobromine ingested or present in the compositions, more preferably from 0% to 50% on the weight of theobromine ingested or present in the compositions, even more  
20 preferably from 0% to 20% on the weight of theobromine ingested or present in the compositions.

In the methods as set out herein, the increase in HDL-cholesterol in humans (in blood and/or serum) that can be achieved (when comparing humans not following the method of the  
25 present invention with humans following the present invention for at least 2 weeks) is preferably at least 5%, preferably at least 8%, more preferably at least 10%, e.g. depending on the daily dosing of theobromine. Likewise, the increase in the ratio HDL-cholesterol / LDL-cholesterol in humans (in blood and/or serum) that can be achieved is preferably at least 5%, preferably at least 8%, more preferably at least 10%, e.g. depending on the daily dosing of  
30 theobromine.

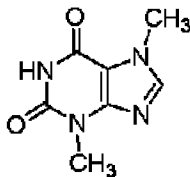
The invention also relates to edible compositions comprising theobromine and plant sterols, edible compositions comprising theobromine and PUFA's (polyunsaturated fatty acids), edible compositions comprising theobromine and statins.

35

**Detailed description of the invention**

Cocoa and chocolate have frequently been studied for its cardiovascular effects, e.g. on blood lipid composition. However, if effects were found on blood lipids, they were so far generally attributed to one or more of the polyphenols that are present in cocoa or chocolate. Hence, for that reason usually dark chocolate is taken for such studies, as such is richer in cocoa polyphenols than milk chocolate or white chocolate. The present inventor has found such is not the case, at least not to a large extent.

“Theobromine” herein relates to the molecular structure as set out below, and is chemically known as 2,6-dihydroxy-3,7-dimethylpurine or 3,7-dimethylxanthine (chemical formula:  $C_7H_8N_4O_2$ ,  $M_w = 180.16$ ), including the edible salts thereof.



In the present invention, when referring to cholesterol (HDL-C, LDL-C, non-HDL-C, or TC) in humans, such is herein to be understood as to encompass levels of cholesterol (HDL-C, LDL-C, non-HDL-C or TC) in blood in humans. Such is generally measured as HDL-C, LDL-C, non-HDL-C and TC levels in serum and/or plasma, but for ease of reference such are herein all encompassed when referring to (levels of) HDL-cholesterol, (levels of) LDL-cholesterol, level of non-HDL-cholesterol and level of total cholesterol.

Herein, “HDL-C” is to be understood to mean high density lipoprotein cholesterol.

Herein, “LDL-C” is to be understood to mean low density lipoprotein cholesterol.

Herein, “TC” is to be understood to mean total cholesterol.

Herein, “non-HDL-C” is to be understood to mean non-high density lipoprotein cholesterol, and that such is equal to TC minus HDL-C.

Herein, the ratio HDL-C / LDL-C is to be understood to mean ratio of HDL-C to LDL-C as set out by Mensink, R. P., Zock, P. L., Kester, A. D. M. & Katan, M. B. (2003) in: Effects of dietary

fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 77: 1146-1155, and likewise for the ratio HDL-C / non-HDL-C.

5 Herein, "plant sterols" (including its singular "plant sterol") mean components which can be classified in three groups: 4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols. In oils and more specifically vegetable oils they mainly exist as free sterols and sterol esters of fatty acids although sterol glucosides and acylated sterol glucosides are also present. Rice bran oil contains mainly 4,4'-dimethylsterols such as cycloartenol and 24-methylene  
10 cycloartenol, which commonly are in the form of ferulic acid esters. These 4,4'-dimethylsterol ferulic acid esters are also called oryzanol. Amongst the 4-desmethylsterols three are major plant sterols namely beta-sitosterol, stigmasterol and campesterol. There are three major plantsterols namely beta-sitosterol, stigmasterol and campesterol. Schematic drawings of the components meant are as given in "Influence of Processing on Sterols of Edible Vegetable  
15 Oils", S.P. Kochhar; Prog. Lipid Res. 22: pp. 161-188. The respective 5 alpha- saturated derivatives such as sitostanol, campestanol and ergostanol and their derivatives are also encompassed in the term plant sterol. Hence, "plant sterols" and "plant sterol" herein encompass plant sterols, esters of plant sterols, plant stanols, and esters of plant stanols and the specific compounds referred to herein. Unless specified otherwise, any specific amount of  
20 plant sterol, e.g. gram, weight ratio or weight %, is to be understood to be on the basis of free plant sterol equivalent.

Preferably the plant sterol herein is selected from the group comprising  $\beta$ -sitosterol,  $\beta$ -sitostanol, campesterol, campestanol, stigmasterol, brassicasterol, brassicastanol or a mixture  
25 thereof. Suitable sources of plant sterols are for example derived from soy bean oil, tall oil, rapeseed oil, rice bran oil or combinations of these oils.

Herein, "increase in HDL-C" is in a statistically significant amount. Herein, "increase in the ratio HDL-C / LDL-C" and "increase in the ratio HDL-C / non-HDL-C" is preferably in a  
30 statistically significant amount.

Herein, the term "polyphenols" means organic chemicals characterised by the presence of multiples of phenolic groups. Polyphenols can be found in certain plant material. It encompasses anthocyanins, flavonoids, flavanols, and flavonols. Specific examples of  
35 polyphenols herein are: catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, quercetin, rutin, hesperidin, naringin, naringenin, genistein,

and dimers, trimers, tetramers and oligomers thereof. Herein, the amount of polyphenols in a composition, when not specified otherwise e.g. by a supplier, existing analytical data or otherwise, is the amount as can be measured in such composition according to the Folin-Ciocalteu method and reported in epicatechin equivalents per gram of composition. An  
5 example of this for chocolate is set out by KA Cooper et al, J. Agric. Food Chem. 2008, 56, 260-265. The amount of epicatechin in a composition, when not specified otherwise e.g. by a supplier, existing analytical data or otherwise, can be quantified e.g. by a method as reported by KA Cooper et al, J. Agric. Food Chem. 2008, 56, 260-265.

10 Herein, "non-fat cocoa solids" (or NFCS) is to be understood as set out by KA Cooper et al, J. Agric. Food Chem. 2008, 56, 260-265.

Herein, "statin" (and "statins") is to be understood as a HMG-CoA reductase inhibitor, and as such encompasses the group of: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin,  
15 rosuvastatin, simvastatin, cerivastatin, mevastatin, and mixtures thereof. "Statin" and "statins" are herein both used interchangeably, as names, and refer to both the singular and plural, i.e. a single statin as well as mixtures (that is: a composition comprising "statins" also refers to compositions comprising only one specific statin as identified herein, next to other non-statin components).

20 Unless defined otherwise, any percentage or ratio of components is to be understood herein as percentage by weight, and weight ratio.

It is preferred, for reasons of e.g. efficacy and/or safety, that in the methods and for the  
25 theobromine for use in the treatment of increasing HDL-C in humans as set out herein and/or for the theobromine for use in the treatment of increasing the ratio HDL-C / LDL-C in humans as set out herein and/or for the theobromine for use in the treatment of increasing the ratio HDL-C / non-HDL-C in humans as set out herein, that such treatment or method comprises ingestion by a human of from 300 to 2000 mg theobromine per day. Such amount can be  
30 consumed all at once at a single point in the day, but also partial dosing can be done, e.g. 3 times 150 mg per day adds up to a daily dosing of 450 mg.

For reasons of e.g. balancing efficacy and safety, it may also be preferred that in the methods and for the theobromine for use in the treatments as set out herein, that such treatments or  
35 methods comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day,

even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day.

It is believed that the effects described herein are best achieved if theobromine is taken  
5 regularly and preferably also for some time. Hence, it is preferred for the theobromine for use in the treatments as set out herein that such theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks.

Theobromine, though naturally occurring in e.g. cocoa, is also available from synthetic origin.  
10 Both are believed to be active in this connection. Synthetic theobromine is a white crystalline powder. The taste of theobromine is very bitter. For this reason, it is preferred that in the methods and for the theobromine for use in the treatments as set out herein that such theobromine is ingested in the form of encapsulates (e.g. microcapsules which can be included in food products) or bound to components (e.g. tannins) so that the bitter taste of  
15 theobromine is suppressed.

Although the use as set out herein is not believed to be the treatment of a disease (but helps people to maintain a healthy life style without the need to take prescription drugs), the invention further relates to the use of theobromine for the manufacture of a medicament or  
20 foodstuff for increasing HDL-C in humans. Likewise, the invention also relates to the use of theobromine for the manufacture of a medicament or foodstuff for increasing the ratio HDL-C : LDL-C and/or the ratio HDL-C : non-HDL-C in humans. The preferred embodiments as set out above (e.g. on amounts and dosing) are also applicable to these uses for the manufacture of a medicament or foodstuff as specified in this paragraph.

25 The invention further relates to the use of theobromine for increasing HDL-C in humans and/or to the use of theobromine for increasing the ratio HDL-C / LDL-C in humans and/or to the use of theobromine for increasing the ratio HDL-C / non-HDL-C in humans.

30 The invention further relates to compositions containing theobromine. As theobromine can be used to increase HDL-C in humans selectively (i.e. without the effect of increasing also the LDL-C or non-HDL-C, which would be undesired), it is believed that theobromine can be easily combined with known agents that (selectively or not) lower LDL-C or non-HDL-C in humans, be it prescription drugs (such as statins) or actives that are not prescription drugs but are  
35 suitable (within limits) to be included in foodstuffs (e.g. plant sterols or plant stanols or esters of such sterols or stanols). Regarding the latter, the daily dosing of such sterols or stanols is

usually (for the benefit of cholesterol lowering) between 1 and 3 grams (e.g. on free plant sterol equivalent dosing). Hence, the invention further relates to an edible composition comprising theobromine and plant sterols or plant stanols or esters of plant sterols or plant stanols, wherein the amount of theobromine is between 300 and 2000, preferably between 500 and 2000 mg per daily dosing (preferably the amount of theobromine is between 400 and 1800 mg per daily dosing, more preferably from 500 to 1500 theobromine mg per daily dosing, even more preferably from 600 to 1400 mg theobromine per daily dosing, even more preferably from 700 to 1300 mg theobromine per daily dosing, most preferably from 750 to 1250 mg theobromine per daily dosing) and the amount of plant sterols or plant stanols or esters of plant sterols or plant stanols is 1 to 3 g per daily dosing (dosing preferably on free plant sterol equivalent). Other combinations of amounts of theobromine and plant sterols or plant stanols or esters of plant sterols or plant stanols may be used for some purposes, e.g. depending on price and desired effects. For this reason, it may be preferred that such edible composition comprises theobromine and plant sterols or plant stanols or esters of plant sterols or plant stanols, wherein the amount of theobromine is between 300 and 2000 mg per daily dosing (preferably wherein the amount of theobromine is between 400 and 1800 mg per daily dosing, more preferably from 500 to 1500 theobromine mg per daily dosing, even more preferably from 600 to 1400 mg theobromine per daily dosing, even more preferably from 700 to 1300 mg theobromine per daily dosing, most preferably from 750 to 1250 mg theobromine per daily dosing) and the amount of plant sterols or plant stanols or esters of plant sterols or plant stanols is more than 1.8 g per daily dosing, preferably from 2 to 3 g per daily dosing (dosing preferably on free plant sterol equivalent). It may also be preferred for e.g. reasons of efficacy, safety and or cost for an edible composition comprising theobromine and one or more from the group of plant sterols or plant stanols or esters of plant sterols or plant stanols, wherein the weight ratio theobromine : the group of plant sterols, plant sterols, esters of plant sterols or plant stanols taken together is from 0.3 : 1 to 1 : 1 (dosing plant sterols preferably on free plant sterol equivalent). For e.g. achieving the desired effect by ingestion by a human of a moderate amount of such composition, it is preferred that in such compositions, the amount of theobromine is at least 0.1% by weight of theobromine on the total composition, preferably at least 0.2% by weight of theobromine on the total composition, more preferably at least 0.5% by weight of theobromine on the total composition. The theobromine in these compositions may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these compositions is not more than 5 times the amount of theobromine in these compositions, or in other words: the amount of polyphenols (e.g. from cocoa) in the compositions referred to herein are preferably between 0% and 500% on the weight of

theobromine in the composition, more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these compositions is not more than the amount of theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the compositions referred to herein is preferably between 0% and 100% on the weight of theobromine in such compositions, more preferably from 0% to 50% on the weight of theobromine in such compositions, even more preferably from 0% to 20% on the weight of theobromine in such compositions.

As an alternative to or in addition to sterols or stanols, theobromine may be combined with another agent known to have beneficial effects on blood cholesterol levels such as polyunsaturated fatty acids (including esters thereof) like EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). In order to contribute to cardiovascular health, the combined EPA and DHA intakes of adolescents (aged 10-18) and adults (aged 19>) should be 500 mg/day. When balancing cost, safety and efficacy, compositions that comprise theobromine and EPA and/or DHA comprise such components in a weight ratio of from 1 : 4 to 1 : 0.3, more preferably from 1 : 3 to 1 : 0.5, most preferably from 1 : 2 to 1 : 0.5 (for theobromine : EPA + DHA combined). For e.g. achieving the desired effect by ingestion by a human of a moderate amount of such composition, it is preferred that in such compositions, the amount of theobromine is at least 0.1% by weight of theobromine on the total composition, preferably at least 0.2% by weight of theobromine on the total composition, more preferably at least 0.5% by weight of theobromine on the total composition. The theobromine in these compositions may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these compositions is not more than 5 times the amount of theobromine in these compositions, or in other words: the amount of polyphenols (e.g. from cocoa) in the compositions referred to herein are preferably between 0% and 500% on the weight of theobromine in the composition, more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these compositions is not more than the amount of theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the compositions referred to herein is preferably between 0% and 100% on the

weight of theobromine in such compositions, more preferably from 0% to 50% on the weight of theobromine in such compositions, even more preferably from 0% to 20% on the weight of theobromine in such compositions.

5 As mentioned, theobromine may also be combined with prescription drugs for influencing blood cholesterol levels. For this reason, it may be preferred to combine theobromine with widely used actives like statins, e.g. to boost overall health effect and/or to be able to use lower dosings of actives like statins. For this reason, the invention further relates to an edible composition comprising theobromine (preferably in an amount of 300-2000 mg per day) and a  
10 statin (preferably 10-80 mg per day). When balancing safety and efficacy, compositions that comprise theobromine and a statin comprise such components in a weight ratio of from 200 : 1 to 5 : 1, more preferably from 100 : 1 to 10 : 1, most preferably from 50 : 1 to 20 : 1 (for theobromine : statin). The statin herein can be one or more of: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, cerivastatin, mevastatin, and  
15 mixtures thereof. For e.g. achieving the desired effect by ingestion by a human of a moderate amount of such composition, it is preferred that in such compositions, the amount of theobromine is at least 0.1% by weight of theobromine on the total composition, preferably at least 0.2% by weight of theobromine on the total composition, more preferably at least 0.5% by weight of theobromine on the total composition. The theobromine in these compositions  
20 may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these compositions is not more than 5 times the amount of theobromine in these compositions, or in other words: the amount of polyphenols (e.g. from cocoa) in the compositions referred to herein are preferably between 0% and 500% on the weight of  
25 theobromine in the composition, more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these compositions is not more than the amount of  
30 theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the compositions referred to herein is preferably between 0% and 100% on the weight of theobromine in such compositions, more preferably from 0% to 50% on the weight of theobromine in such compositions, even more preferably from 0% to 20% on the weight of theobromine in such compositions.



The theobromine in the present case may be included in known food formats, such as e.g. spreads (butter alternatives aimed for e.g. spreading on bread). "Spread" is herein to be understood as an oil and water containing emulsion (W/O, O/W, or W/O/W), comprising 20-85% by weight of oil (oil is herein defined as including fat) and 15 to 80% water. Preferably, the spread herein has a pH of 4.8-6 (as measured by melting the spread, separating the molten fat phase and water phase, and measuring the pH of the water phase). Preferably (for good product properties), the spread herein is a W/O type emulsion. Preferably (for good product properties, e.g. spreadability), the spread herein has a Stevens value (when measured as set out below) of between 100 and 500 gram at 15 degrees Celsius. Stevens values give an indication about the hardness (also called firmness) of a product. The Stevens value herein is determined as follows: the product is stored for 24h at 15 degrees Celsius before measurements are done. The hardness of the product is measured with a Stevens penetrometer (Brookfield LFRA Texture Analyser (LFRA 1500), ex Brookfield EnLabs, UK) equipped with a stainless steel probe with a diameter of 6.35 mm and operated in "normal" mode. The probe is pushed into the product at a speed of 2 mm/s, a trigger force of 5 gram from a distance of 10 mm. The force required is read from the digital display and is expressed in grams.

Hence, the invention further relates to an edible emulsion (typically the spread as set out above) comprising (by weight) 20 to 85% of oil (preferably from 35% to 50%), water (preferably 15 to 80%, more preferably 50% to 65%), 0.5 to 10% theobromine (preferably from 0.8 to 5%). Preferably, such emulsion has a pH (the water phase) of 4.8-6. Preferably such emulsion is a W/O emulsion. Preferably the emulsion has a Stevens value as set out above. Preferably, said emulsion further comprises (by weight on the emulsion) 2-20% (preferably 3-15%) of plant sterols (dosing preferably on free plant sterol equivalent). The theobromine in these spreads may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these spreads is not more than 5 times the amount of theobromine in these spreads, or in other words: the amount of polyphenols (e.g. from cocoa) in the spreads referred to herein are preferably between 0% and 500% on the weight of theobromine in such spreads, more preferably from 0% to 200% on the weight of theobromine in such spreads, even more preferably from 0% to 50% on the weight of theobromine in the such spreads. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these spreads is not more than the amount of theobromine present in these spreads, or in other words: the amount of epicatechin (e.g. from cocoa) in the spreads referred to herein is preferably between 0% and 100% on the weight of theobromine

in such spreads, more preferably from 0% to 50% on the weight of theobromine in such spreads, even more preferably from 0% to 20% on the weight of theobromine in such spreads.

Spreads herein may comprise other ingredients commonly used for spreads, such as  
5 flavouring ingredients, thickeners, gelation agents, colouring agents, vitamins, emulsifiers, pH regulators, stabilizers etc. Common amounts of such ingredients as well as suitable ways to prepare margarines or spreads are well-known to the skilled person.

The invention further relates to an edible emulsion (including spreads) as set out above, for  
10 use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and/or for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans. For these emulsions in the use specified, it is preferred that the treatment comprises ingestion by a human of from 300 to 2000 mg theobromine per day. Likewise, for these emulsions in the use  
15 specified, it is preferred that the treatment comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day. Also, these emulsions in the use specified, it is preferred that in the treatment mentioned herein, e.g. as  
20 mentioned above, theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks. The theobromine in these emulsions (including spreads) may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) is not more than 5 times the amount of theobromine in these  
25 emulsions (including spreads), or in other words: the amount of polyphenols (e.g. from cocoa) in such emulsions (including spreads) referred to herein are preferably between 0% and 500% on the weight of theobromine present in the emulsions (including spreads), more preferably from 0% to 200% on the weight of theobromine present in the emulsions (including spreads, even more preferably from 0% to 50% on the weight of theobromine present in the emulsions  
30 (including spreads). Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these emulsions (including spreads) is not more than the amount of theobromine present in these emulsions (including spreads), or in other words: the amount of epicatechin (e.g. from cocoa) in the emulsions (including spreads) referred to  
35 herein is preferably between 0% and 100% on the weight of theobromine in such emulsions (including spreads), more preferably from 0% to 50% on the weight of theobromine, even

more preferably from 0% to 20% on the weight of theobromine in such emulsions (including spreads).

As an alternative to e.g. spreads, theobromine may also be included in other food products, such as e.g. beverages, including mini-drinks ("shots", of e.g. 50-150 ml, preferably 50-125 ml, more preferably 60-120 ml packaged unit dose volume). Preferably, in such beverages, the theobromine, which raises HDL-C, is combined with an active that can lower LDL-C. Hence, the present invention further relates to a liquid composition comprising water in an amount of more than 70% by weight on the total composition (preferably more than 80% by weight based on the total composition) and less than 99.8% by weight on the total composition, theobromine in an amount of from 0.3 % by weight on the total composition to 2% by weight on the total composition, plant sterols in an amount such that theobromine and plant sterols are present in a weight ratio theobromine : plant sterols of from 1 : 1 to 1 : 10 (preferably from 1 : 2 to 1 : 5) (dosing preferably on free plant sterol equivalent). The theobromine in these liquid compositions may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these liquid compositions is not more than 5 times the amount of theobromine in these liquid compositions, or in other words: the amount of polyphenols (e.g. from cocoa) in the liquid compositions referred to herein are preferably between 0% and 500% on the weight of theobromine in the liquid composition, more preferably from 0% to 200% on the weight of theobromine in the liquid composition, even more preferably from 0% to 50% on the weight of theobromine in the liquid composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these liquid compositions is not more than the amount of theobromine present in these liquid compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the liquid compositions referred to herein is preferably between 0% and 100% on the weight of theobromine in such liquid compositions, more preferably from 0% to 50% on the weight of theobromine in such liquid compositions, even more preferably from 0% to 20% on the weight of theobromine in such liquid compositions.

In the liquid composition as set out above, it is preferred that the composition comprises from 85 to 99% by weight on the total composition of water. In the liquid composition as set out above, for reasons of efficacy, safety, and product formulation, it is preferred that such composition comprises theobromine in an amount of 0.4 to 1.5 % by weight on the total composition.

The invention further relates to a liquid composition as set out above, for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and/or for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans. For these liquids in the use specified, it is preferred that the treatment comprises ingestion by a human of from 300 to 2000 mg theobromine per day. Likewise, for these liquids in the use specified, it is preferred that the treatment comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day. Also, for these liquids in the use specified, it is preferred that in the treatment, theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks. The theobromine in this may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these liquid composition for the use as specified herein are not more than 5 times the amount of theobromine in said liquid compositions, or in other words: the amount of polyphenols (e.g. from cocoa) in said liquid compositions referred to herein are preferably between 0% and 500% on the weight of theobromine in said composition, more preferably from 0% to 200% on the weight of theobromine in said composition, even more preferably from 0% to 50% on the weight of theobromine in said composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these liquid compositions in the use as specified is not more than the amount of theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in said compositions referred to herein is preferably between 0% and 100% on the weight of theobromine in said compositions, more preferably from 0% to 50% on the weight of theobromine in said compositions, even more preferably from 0% to 20% on the weight of theobromine in said compositions.

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Another preferred (e.g. for taste reasons) food composition for offering theobromine to consumers are fermented, dairy-like, products. Hence, the invention further relates to a fermented food composition comprising from 70 to 99 wt% water (preferably 80-98%), from 0.1 to 10 wt% protein (preferably dairy protein), at least 0.3 wt% lactic acid, and 0.2 to 2 wt% theobromine (herein: % is by weight on the total composition). A fermented food composition herein is a product in which microorganisms are present, either alive or dead, depending on

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subsequent processing. The fermentation will result in the formation of lactic acid, hence its presence in the composition. For example, "yoghurt" contains in most countries live microorganisms of specific species, but milk, when fermented, and subsequently subject to e.g. an UHT preservation treatment contains mostly dead microorganisms: both are

5 comprised herein by the term fermented (i.e. comprising such microorganisms dead or alive, preferably alive). Preferably, the fermented food compositions as set out herein comprise at least 10 million bacteria (dead or alive, preferably alive) per gram composition, from the group of: *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus* species, *Lactococcus* species, *Bifidobacterium* species, and mixtures thereof. More preferably, such fermented food

10 compositions comprise at least 10 million live bacteria per gram composition, from the group of: *Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* subsp. *bulgaricus*. To achieve both an increase in HDL-C and a decrease in LDL-C, it is preferred that the fermented compositions herein further comprise plant sterols, and preferably in an amount such that theobromine and plant sterols are present in a weight ratio theobromine : plant sterols of from

15 1 : 1 to 1 : 10 (dosing preferably on free plant sterol equivalent). Preferably, the fermented food compositions as set out herein has a pH of between 2 and 7, more preferably between 3 and 5. The dairy protein present preferably comprises dairy protein. A preferred fermented food composition in this context is yoghurt containing the specified amounts of theobromine, and optionally plant sterols. The theobromine in these fermented food compositions may be

20 from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the level of polyphenols (e.g. from cocoa) in the fermented food compositions as set out herein are not more than 5 times the amount of theobromine in the composition, or in other words: the amount of polyphenols (e.g. from cocoa) in the fermented food compositions referred to herein are preferably between 0%

25 and 500% on the weight of theobromine in the fermented food composition, more preferably from 0% to 200% on the weight of theobromine in the fermented food composition, even more preferably from 0% to 50% on the weight of theobromine in the fermented food composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa)

30 present in the fermented food compositions as set out herein is not more than the amount of theobromine present in these fermented food compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the fermented food compositions referred to herein is preferably between 0% and 100% on the weight of theobromine in such fermented food compositions, more preferably from 0% to 50% on the weight of theobromine in such

35 fermented food compositions, even more preferably from 0% to 20% on the weight of theobromine in such fermented food compositions.

The invention further relates to a fermented food composition as set out above, for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and/or for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans. For these fermented food compositions in the use specified, it is preferred that the treatment comprises ingestion by a human of from 300 to 2000 mg theobromine per day. Likewise, for these fermented food products in the use specified, it is preferred that the treatment comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day. Also, for these fermented food products in the use specified, it is preferred that in the treatment, theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks. The theobromine in this may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the level of polyphenols (e.g. from cocoa) in the fermented food compositions in the use specified is not more than 5 times the amount of theobromine in the fermented food composition in the use specified, or in other words: the amount of polyphenols (e.g. from cocoa) in the fermented food composition in the use as specified herein are preferably between 0% and 500% on the weight of theobromine in the composition, more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these fermented food compositions in the use as specified herein is not more than the amount of theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the fermented food compositions in the use as referred to herein is preferably between 0% and 100% on the weight of theobromine in such compositions, more preferably from 0% to 50% on the weight of theobromine in such compositions, even more preferably from 0% to 20% on the weight of theobromine in such compositions.

The invention further relates to a liquid composition comprising water in an amount of more than 70% by weight on the total composition, preferably more than 80% by weight on the total composition and less than 99.8% by weight on the total composition, theobromine in an amount of from 0.3 % by weight on the total composition to 2% by weight on the total

composition, plant sterols in an amount such that theobromine and plant sterols are present in a weight ratio theobromine : plant sterols of from 1 : 1 to 1 : 10 (preferably from 1 : 2 to 1 : 5) (dosing preferably on free plant sterol equivalent), for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans, and/or for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans. For these liquid compositions in the use specified, it is preferred that the treatment comprises ingestion by a human of from 300 to 2000 mg theobromine per day. Likewise, for these liquid compositions in the use specified, it is preferred that the treatment comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day. Also, for these liquid compositions in the use specified, it is preferred that in the treatment, theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks. The theobromine in this may be from cocoa, but does not need to be. Cocoa contains next to e.g. theobromine also polyphenols. E.g. for colour reasons it is preferred that the levels of polyphenols (e.g. from cocoa) in these liquid compositions (including for the use thereof as specified) are not more than 5 times the amount of theobromine in the composition, or in other words: the amount of polyphenols (e.g. from cocoa) in these liquid compositions (including for the use thereof as specified) referred to herein are preferably between 0% and 500% on the weight of theobromine in the liquid composition (including for the use thereof as specified), more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Epicatechin is a polyphenol present in substantial amounts in cocoa, and e.g. for reasons of taste (e.g. undesired bitterness) it is preferred that the amount of epicatechin (e.g. from cocoa) present in these liquid compositions (including for the use thereof as specified) is not more than the amount of theobromine present in these compositions, or in other words: the amount of epicatechin (e.g. from cocoa) in the compositions (including for the use thereof as specified) referred to herein is preferably between 0% and 100% on the weight of theobromine in such compositions, more preferably from 0% to 50% on the weight of theobromine in such compositions, even more preferably from 0% to 20% on the weight of theobromine in such compositions.

Although the compositions as referred to herein, can be made by using high amounts of cocoa, as cocoa contains a small percentage of theobromine, this is not preferred, e.g. due to its strong colour and taste. Hence, it is preferred that the compositions (and their use)

according to this invention comprise no or only a low amount of non-fat cocoa solids. More specifically, it is preferred that the compositions (and their use) according to this invention comprise less non-fat cocoa solids than 500% by weight on theobromine (i.e. at maximum 5 times the amount of theobromine in the composition). In other words: the amount of non-fat cocoa solids in the compositions referred to herein are preferably between 0% and 500% on the weight of theobromine in the composition, more preferably from 0% to 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition. Likewise, the amount of cocoa in the compositions (including their uses) and methods referred to herein is preferably between 0% and 500% on the weight of theobromine in said composition or method, more preferably from 0% to 200% on the weight of theobromine in said composition or method, even more preferably from 0% to 50% on the weight of theobromine in said composition or method. For reasons of e.g. colour and taste, the amount of cocoa-polyphenols in the compositions as set out herein, in the uses, treatments, methods and processes as set out herein is preferably less than 500% (i.e. at maximum 5 times the amount of theobromine in the composition, uses, treatments, methods and processes), more preferably less than 200% by weight on theobromine (in other words: the amount of cocoa polyphenols in the compositions, uses, treatments, methods and processes referred to herein is preferably between 0% and 500%, more preferably between 0% and 200% on the weight of theobromine in the composition, even more preferably from 0% to 50% on the weight of theobromine in the composition, e.g. for ease of product formulation.

In the compositions disclosed herein, their uses, treatments with such compositions, as well as in the methods and processes that use compositions disclosed herein, it is preferred that such compositions comprise less than 0.001% (by weight, based on the total composition) curcumin (and extracts of a plant belonging to the *Curcuma* genus of the *Zingiberaceae* family).

Preferably, in the compositions disclosed herein, their uses, treatments with such compositions, as well as in the methods and processes that use compositions disclosed herein, it is preferred that such compositions comprise no other ingredient or active that has proven and/or suggested to be able to provide an increase in HDL-C in blood or serum in humans (e.g. in an amount of at least 5%), other than theobromine.

Regardless of the composition to be ingested (be it food, be it a medicament, and regardless of a possible combination with other actives known to or believed to influence cholesterol levels in blood), the theobromine in it may preferably be present in an encapsulated form, e.g. microcapsules, optionally included in a food product.



**EXAMPLE**

## Example 1

- 5 In a randomised controlled cross-over study with 42 untreated prehypertensive males and females the effect of cocoa-containing drinks either high in polyphenols or high in polyphenols and theobromine was assessed on blood pressure (BP). As a secondary objective the effect of the drinks was assessed on plasma lipid profile.
- 10 The subjects consumed for 3 weeks daily (in the morning before breakfast) 200 ml acidified dairy drink as in table 1, with added to that 1) none (control drink), 2) 3.65 g cocoa powder (Acticoa, Barry-Callebaut) containing 500 mg polyphenols and 79 mg theobromine (amount in product specification Acticoa) and 3) 3.65 g cocoa powder containing 500 mg polyphenols and 79 mg theobromine plus added thereto 918 mg pure theobromine (Fagron, Ph.Eur. 5.7) (in
- 15 total about 1 g theobromine, when the naturally present 79 mg theobromine is added up to the additional 918 mg theobromine). The synthetic theobromine used was: theobromine complying with European Pharmacopeia 5.7, as available from Fagron, Waregem, Belgium.

Table 1: composition of the dairy drink (wt% of major components)<sup>1</sup>

	placebo	cocoa	cocoa+theobromine
skimmed milk	30.5	30.5	30.5
cream	0.97	0.97	0.97
Demin. water	62.9	61.5	58.7
cocoa powder (Acticoa)	0	1.82	1.82
theobromine in NaOH <sup>2</sup>	0	0	2.7
sucrose	3	3	3
lactic acid	0.4	0.4	0.4
flavours	0.55	0	0
colourant	0.09	0.08	0.07
Total	98.38	98.27	98.16

20 <sup>1</sup> 200g dairy product was consumed daily.

<sup>2</sup> Pure theobromine powder was dissolved in 1 M NaOH to a stock concentration of approximately 170 mg theobromine per ml. This solution was diluted 38.5 times with the dairy drink (1 part stock solution + 37.5 parts drink).

Inclusion criteria: prehypertensive or grade I hypertensive subjects, age 40-70, with  $\leq 2$  additional cardiovascular risk factors, not on active anti-hypertensive treatment. Details are set out in table 2.

5 Table 2

Parameters		Mean	SD	95% CI
n		42		
Age	yr	62	4.5	60.7-63.5
Male	no (%)	32 (76%)		
Office SBP	mmHg	142	14.0	137-146
Office DBP	mmHg	84	7.9	82-87
Length	cm	177	8.1	175-180
Weight	kg	82	9.0	79-85
BMI	kg/m <sup>2</sup>	25.9	2.4	25.2-26.7
Fasting glucose	mmol/L	4.9	0.6	4.7-5.1
TC	mmol/L	5.77	0.77	5.52-6.01
LDL-C	mmol/L	3.72	0.66	3.52-3.93
HDL-C	mmol/L	1.55	0.42	1.42-1.68
Triglycerides	mmol/L	1.06	0.41	0.93-1.19
Smoking	no (%)	1 (2%)		

### Methodology

10 Venous blood samples were drawn from the antecubital vein in EDTA containing tubes in fasting condition (morning, before breakfast) for lipid profile (HDL-C, LDL-C, total cholesterol, triglycerides, HDL-C being herein high density lipoprotein cholesterol, and LDL-C herein being low density lipoprotein cholesterol). The blood samples were taken at baseline and were repeated after the end of each treatment period. All measurements were done with standard laboratory techniques.

### 15 Descriptive statistics

Plasma lipid outcomes were done in the Per Protocol population. Linear mixed models were performed using compound symmetry repeated covariance type with treatment as a fixed factor and with baseline parameters as a covariate. We performed pairwise comparisons with

no adjustment for multiple testing to assess differences between placebo and the two treatment arms. A 2 sided p-value < 0.05 was considered significant.

Results

5 85 subjects were screened and 42 of them were found eligible to enter the study. The population characteristics at baseline were as follows (average values with between brackets standard deviation): 32 males (76%), age 62 (4.5), office SBP 142 (14) and DBP 84 (8), BMI 25.9 (2.4), total cholesterol 5.77 (0.77), 2% smokers. Data from four subjects were discarded for various reasons (e.g. arrhythmia, holiday, BP measurement failure). The remaining  
 10 population was defined as the Per Protocol population.

Compliance was >99% as estimated from counting returned empty bottles.

The results are set out in table 3 and 4.

15 In table 3: tests of fixed effects and mean estimates, standard error (SE), 95% confidence interval (95% CI), and significant difference between interventions of plasma total cholesterol, HDL-C and LDL-C. Baseline values were included as covariable.

Table 3

Parameter	Covariable	Treatment	Mean	SE	95% CI	<i>p value</i>
Total cholesterol (mM)	Baseline	Placebo	5.61	0.10	5.40 - 5.81	0.46
	Total cholesterol	Cocoa	5.68	0.10	5.48 - 5.88	
		Theobromine*	5.72	0.10	5.52 - 5.92	
HDL (mM)	Baseline HDL	Placebo	1.55	0.03	1.48 - 1.62	>0.001
		Cocoa	1.60	0.03	1.54 - 1.67	
		Theobromine*	1.74	0.03	1.67 - 1.81	
LDL (mM)	Baseline LDL	Placebo	3.55	0.08	3.39 - 3.71	0.35
		Cocoa	3.58	0.08	3.42 - 3.74	
		Theobromine*	3.47	0.08	3.31 - 3.64	

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\*Cocoa with pure theobromine added.

In table 4: pairwise comparisons between placebo and cocoa and cocoa plus theobromine interventions of total cholesterol, HDL-C and LDL-C plasma values.

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Table 4.

Parameter	treatment A	treatment B	Change (A-B)	SE	95%CI	<i>p value</i>
Total cholesterol (mM)	Placebo	Cocoa	0.072	0.09	-0.11 - 0.25	0.43
		Theobromine*	0.113	0.09	-0.07 - 0.29	0.22
HDL-C (mM)	Placebo	Cocoa	0.050	0.03	-0.01 - 0.11	0.12
		Theobromine*	0.183	0.03	0.12 - 0.25	0.000
LDL -C (mM)	Placebo	Cocoa	0.033	0.08	-0.12 - 0.18	0.66
		Theobromine*	-0.073	0.08	-0.22 - 0.08	0.33

\*Cocoa with pure theobromine added.

- 5 Thus, plasma HDL-C increased significantly in the cocoa-theobromine intervention from 1.55 (placebo) and 1.60 (cocoa) to 1.74 mM (cocoa+theobromine). Compared to placebo this is a 12% increase and compared to cocoa a 9% increase. Plasma LDL-C decreased slightly, which had an additional positive effect on the HDL-C : LDL-C ratio, increasing from 0.44 (placebo) and 0.45 (cocoa) to 0.50 (cocoa + theobromine).

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#### Conclusion

Consumption of theobromine increases plasma HDL-C and HDLC : LDL-C ratio in humans.

#### 15 Example 2

In a randomized controlled intervention study with 153 healthy males and females the effect of drinks containing cocoa, added theobromine or a combination of cocoa and added theobromine was assessed on HDL cholesterol (HDL-C). As a secondary objective the effect of the various drinks on total cholesterol (TC), LDL cholesterol (LDL-C), Triglycerides (TG) and blood pressure (BP) was assessed.

20

The study had a bi-centric, double-blind, randomized, placebo-controlled, 2 by 2 full factorial parallel design. After a run-in period of 2 weeks the subjects consumed for 4 weeks daily (in the morning one hour before breakfast) 200 ml acidified dairy drink as in table 5, with added to that 1) none (control drink), 2) 5.9 g cocoa powder (Acticoa, Barry-Callebaut) containing about 500 mg polyphenols and 150 mg theobromine 3) about 850 mg synthetic theobromine (Fagron) and 4) 5.9 g cocoa powder containing 500 mg polyphenols and 150 mg theobromine plus added thereto about 850 mg synthetic theobromine (Fagron) (in total about 1 g

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theobromine). The synthetic theobromine used was: theobromine complying with European Pharmacopeia 5.7, as available from Fagron, Waregem, Belgium.

For preparing the test products containing theobromine, theobromine was dissolved in a 1 M NaOH solution to create a theobromine stock solution, containing approximately 170 mg / ml of theobromine. The various drinks used in the trial have been made using the following processing:

- mixing of the pectin with all of the sugar
- dispersing the pectin/sugar mix with part of the demi water at 80°C to prepare a pectin slurry
- dissolving smp in the remainder of the demi water at a temperature of 40°C
- adding the cream
- adding the xanthan gum under vigorous mixing for 15 minutes
- adding the pectin slurry
- adding sucralose
- (depending on the drink: adding the cacao slowly under vigorous mixing)
- (depending on the drink: adding the theobromine solution)
- adding the sunflower oil
- adding the flavours and colorants
- acidifying the premix with lactic acid until pH 4.2

The process to prepare bottles containing the drink so prepared in a pasteurised form further contained the step of homogenisation after pasteurisation.

Table 5: Composition of the dairy drink (wt% of major components)<sup>1</sup>

	placebo	cocoa	theobromine	cocoa+theobromine
skimmed milk powder	3.06	3.06	3.06	3.06
cream	0.97	0.97	0.97	0.97
Demineralised water	91.85	88.10	88.18	84.8
cocoa powder (Acticoa)	0	2.9	0	2.9
theobromine in NaOH <sup>2</sup> (solution)	0	0	3.06	3.06
sucrose	2.0	3.0	2.0	3.0
lactic acid	0.4	0.4	0.65	0.65
flavours	0.5	0	0.5	0
colorants	0.08	0.06	0.08	0.06
HM pectin	0.4	0.32	0.40	0.4
xanthan gum	0.1	0.08	0.10	0.1
sunflower oil	1.0	1.0	1.0	1.0
sucralose	0.01	0.01	0.01	0.01
Total	100.37	99.9	100.01	100.01

<sup>1</sup> 200ml dairy product was consumed daily.

<sup>2</sup> Pure theobromine powder was dissolved in 1 M NaOH to a stock concentration of approximately 170 mg theobromine per ml. This solution was then diluted with the dairy drink.

5

Inclusion criteria: generally healthy men and pre-menopausal women, age 40-70, with a 10-year risk of developing CHD <10%, not on active cholesterol-lowering or anti-hypertensive treatment and non-smoking. Details of the subject characteristics are set out in table 6.

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Table 6: Subject characteristics

Parameters		Mean	SD	range
n		153		
Age	yr	54.9	8.5	40-70
Male	no (%)	77 (50.3%)		
Office SBP	mmHg	124.0	13.3	94.0-166.0
Office DBP	mmHg	80.9	9.0	59.0-112.0
Length	cm	168.8	9.0	149-191
Weight	kg	70.0	12.1	46.0-100.7
BMI	kg/m <sup>2</sup>	24.4	2.8	18.8-30.8
Fasting blood glucose	mmol/L	5.08	0.50	3.70-6.33
TC	mmol/L	5.98	1.05	3.1-8.26
LDL-C	mmol/L	3.76	0.95	1.29-6.24
HDL-C	mmol/L	1.72	0.50	0.93-3.1
Triglycerides	mmol/L	1.12	0.65	0.32-4.97

### Methodology

5 Venous blood samples were drawn from the antecubital vein in SST II advance BD tubes in fasting condition (morning, before breakfast) for blood lipid profile (HDL-C, LDL-C, total cholesterol (TC), triglycerides, HDL-C being herein high density lipoprotein cholesterol, and LDL-C herein being low density lipoprotein cholesterol). The blood samples were taken twice at baseline and were repeated twice after the end of the intervention period. All measurements were done with standard laboratory techniques.

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### Descriptive statistics

Serum lipid outcomes were done in the Intention to treat (ITT) and Per Protocol (PP) population.

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The statistical analysis was done according to a full 2x2 factorial design including interaction of theobromine and cocoa. Gender within treatment and baseline blood lipids were included in the model as covariates. A 2 sided p-value < 0.05 was considered significant.

## Results

203 subjects were screened and 153 of them were found eligible to enter the study. The population characteristics at baseline were as follows (average values with between brackets standard deviation): 77 males (50%), age 54.9 yrs (8.5), weight 70.0 kg (12.1), height 168.8  
5 cm (9.0), BMI 24.4 (2.8), total cholesterol 5.98 mmol/L (1.05), SBP 124 mmHg (13.3) and DBP 80.9 mmHg (9.0). Ten people withdrew from the study prematurely; data from nine more subjects were discarded from the PP analysis as they lost or gained more than 2 kg body weight during the study, which could have had an impact on the blood lipid profile. Reported are the results of the ITT population (n= 143) as it showed similar results as the PP analysis.

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Compliance was >99.7% as estimated from counting returned empty bottles and consumption diaries. The results are set out in table 7 and 8.

Table 7: Tests of fixed effects and mean estimates, standard error (SE), 95% confidence  
15 interval (95% CI), and significant difference between interventions of plasma total cholesterol, HDL-C and LDL-C, HDL/non-HDL-cholesterol ratio, HDL/LDL-cholesterol ratio, triglycerides, diastolic and systolic blood pressure. Baseline values and gender were included as co-variables.

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Table 7

Parameter	Covariable	Treatment	Mean	SE	95% CI	<i>p</i> value
Total cholesterol (mmol/L)	Baseline	Placebo	5.86	0.07	5.73 - 6.00	0.81
	Total cholesterol	Cocoa	5.89	0.07	5.75 - 6.02	
		Theobromine	5.82	0.07	5.69 - 5.96	
	Gender	TB+C	5.82	0.07	5.67 - 5.96	
HDL-C (mmol/L)	Baseline	Placebo	1.60	0.03	1.54 - 1.65	<0.001
	HDL	Cocoa	1.66	0.03	1.60 - 1.71	
		Theobromine	1.75	0.03	1.70 - 1.81	
	Gender	TB+C	1.87	0.03	1.81 - 1.93	
LDL-C (mmol/L)	Baseline	Placebo	3.48	0.05	3.38 - 3.58	<0.05
	LDL	Cocoa	3.46	0.05	3.35 - 3.56	
		Theobromine	3.30	0.05	3.19 - 3.40	
	Gender	TB+C	3.27	0.06	3.16 - 3.39	
HDL-C/non-HDL-C ratio	Baseline	Placebo	0.38	0.02*	0.36 - 0.39	<0.0001
	ratio	Cocoa	0.39	0.02*	0.38 - 0.41	
		Theobromine	0.43	0.02*	0.41 - 0.44	
	Gender	TB+C	0.46	0.02*	0.44 - 0.48	
HDL-C/LDL-C ratio	Baseline	Placebo	0.47	0.02*	0.45 - 0.48	<0.0001
	ratio	Cocoa	0.48	0.02*	0.46 - 0.50	
		Theobromine	0.52	0.02*	0.50 - 0.54	
	Gender	TB+C	0.55	0.02*	0.53 - 0.57	
Triglyceride (mmol/L)	Baseline	Placebo	1.03	0.04*	0.96 - 1.10	0.48
	triglyceride	Cocoa	1.01	0.04*	0.95 - 1.09	
		Theobromine	1.05	0.03*	0.98 - 1.12	
	Gender	TB+C	0.94	0.04*	0.87 - 1.01	
DBP (mm Hg)	Baseline	Placebo	75.3	0.95	73.3 - 77.2	0.99
	DBP	Cocoa	74.4	0.97	72.4 - 76.4	
		Theobromine	75.0	0.95	73.1 - 77.0	
	Gender	TB+C	76.3	1.03	74.2 - 78.5	
SBP (mm Hg)	Baseline	Placebo	118.7	1.81	115.0 - 122.4	0.47
	SBP	Cocoa	117.7	1.88	113.9 - 121.6	
		Theobromine	121.2	1.79	117.6 - 124.9	
	Gender	TB+C	121.8	1.96	117.8 - 125.8	

\* SE are on log-transformed data.

Table 8: Pair-wise comparisons between placebo and cocoa and cocoa plus theobromine interventions of total cholesterol, HDL-C and LDL-C, HDL/non-HDL-cholesterol ratio,

5 HDL/LDL-cholesterol ratio, tryglycerides, diastolic and systolic blood pressure.

Parameter	treatment A	treatment B	Change (A-B)	SE	95%CI	<i>p</i> value
Total cholesterol (mmol/L)	Placebo	Cocoa	0.027	0.10	-0.16 - 0.22	0.77
		Theobromine	-0.038	0.10	-0.23 - 0.15	0.70
		TB+C	-0.046	0.10	-0.25 - 0.15	0.65
HDL-C (mmol/L)	Placebo	Cocoa	0.061	0.04	-0.02 - 0.14	0.13
		Theobromine	0.159	0.04	0.08 - 0.24	<0.0001
		TB+C	0.272	0.04	0.19 - 0.35	<0.0001
LDL -C (mmol/L)	Placebo	Cocoa	-0.022	0.07	-0.17 - 0.12	0.76
		Theobromine	-0.184	0.08	-0.33 - -0.04	0.02
		TB+C	-0.207	0.08	-0.36 - -0.05	<0.01
HDL-C/non-HDL-C ratio	Placebo	Cocoa	0.040	0.03*	-0.02 - 0.10	0.17
		Theobromine	0.126	0.03*	0.07 - 0.18	<0.0001
		TB+C	0.198	0.03*	0.14 - 0.26	<0.0001
HDL-C/LDL_c ratio	Placebo	Cocoa	0.033	0.03*	-0.02 - 0.08	0.20
		Theobromine	0.117	0.03*	0.07 - 0.17	<0.0001
		TB+C	0.165	0.03*	0.11 - 0.22	<0.0001
Triglycerides (mmol/L)	Placebo	Cocoa	-0.013	0.05	-0.11 - 0.08	0.79
		Theobromine	0.018	0.05	-0.08 - 0.12	0.72
		TB+C	-0.092	0.05	-0.20 - 0.01	0.08
DBP (mm Hg)	Placebo	Cocoa	-0.84	1.39	-3.69 - 2.01	0.55
		Theobromine	-0.20	1.35	-2.97 - 2.57	0.88
		TB+C	1.10	1.39	-1.77 - 3.96	0.44
SBP (mm Hg)	Placebo	Cocoa	-0.95	2.71	-6.52 - 4.63	0.73
		Theobromine	2.55	2.55	-2.69 - 7.80	0.33
		TB+C	3.12	2.64	-2.31 - 8.55	0.25

\* SE are on logtransformed data.

The analysis showed that theobromine (TB) alone had a significant effect on HDL-C levels resulting in an increase of 0.16mmol/L which is about a 10% increase compared to placebo ( $p < 0.0001$ ). The combination of theobromine and cocoa (TB +C) significantly increased the HDL-C levels by 0.27 mmol/L, i.e. about a 17% increase compared to placebo ( $p < 0.0001$ ).

5 This increase is more than the separate effects of theobromine and cocoa combined, although no significant interaction was found (+0.05mmol/L,  $p = 0.3735$ ). Cocoa alone did not significantly increase HDL-C levels (+0.06mmol/L,  $p = 0.1288$ ).

A significant increase in ApoA1 concentrations, the main apoprotein found in HDL particles, was seen with theobromine alone (+0.11,  $p < 0.0001$ ) and the combination of theobromine and  
10 cocoa. No significant interaction effect was found.

Serum LDL-C decreased slightly by 0.18mmol/L upon treatment with theobromine alone, i.e. a decrease of about 5% compared to placebo. This consequently had an additional positive effect on the HDL-C : LDL-C and HDL-C: non-HDL-C ratios. Theobromine alone significantly increased the HDL-C: LDL-C ratio by 0.12 ( $p < 0.0001$ ) and the HDL-C: non-HDL-C ratio by  
15 0.13 ( $p < 0.0001$ ).

No effect on total cholesterol ( $p = 0.81$ ) and triglycerides ( $p = 0.48$ ) was found.

Theobromine alone showed no significant effect on blood pressure, neither on systolic BP (SBP +2.55 mmHg vs control;  $p = 0.33$ ) nor on diastolic BP (DBP -0.20 mmHg vs. control;  $p = 0.88$ ). Cocoa alone and the combination of theobromine and cocoa did not have an effect  
20 on BP, either.

#### Conclusion

Daily intake of 850 mg theobromine (TB) for 4 weeks significantly increases HDL-C compared  
25 to placebo. The effect is attributable to theobromine; there is no significant interaction effect with cocoa.

#### Example 3a

30 To a commercial 35% low fat spread (product as available in the Netherlands under the trademark Becel Light) theobromine (not the salt form, contrary to examples 1 and 2) was added (1.65% and 3.30%), mixed with a spoon and judged on appearance. White theobromine particles were visible in both spreads.

Example 3b

Two plant-sterol ester containing spreads were prepared on lab scale with a microvotator, one reference and one with theobromine in a concentration of 1.65 wt% (500 mg per serving of 30 gram). The composition is in table 9 below.

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Table 9: composition

Ingredient	Amount (wt%)
Refined sunflower oil	24.1%
Plant sterol esters	12.5%
Palm oil fraction and palm kernel oil (chemically interesterified)	5.4%
Emulsifier (DIMODAN HP)	0.3%
Sunflower lecithin (SUNLEC M)	0.1%
Demineralised water (Millipore)	52.9%
Tapioca starch	2.5%
Theobromine	1.7%
Butter milk powder	0.3%
Potassium sorbate	0.1%

Processing was done in a conventional way for making a spread using a microvotator, with the exception that the theobromine (not the salt form, unlike examples 1 and 2) was added to the water phase and mixed well for 3 minutes with an ultra turrax mixer at 6000 rpm.

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In the resulting product there were no particles visible in this spread with theobromine and there was no effect on the colour of the spread when compared to the one without.

15 Example 3c

A potassium-enriched low fat spread was produced using a standard formulation (as in table 10), but without colour and flavour. To this, per 20 g spread was added 0.5 g theobromine.

20

Table 10: composition

Ingredient	Amount (wt%)
Refined sunflower oil	31.6%
Palm oil fraction and palm kernel oil (chemically interesterified)	5.5%
Emulsifier (DIMODAN HP)	0.2%
Sunflower lecithin (SUNLEC M)	0.1%
Demineralised water (Millipore)	45.9%
Theobromine	2.5%
Polyglycerol polyricinoleate	0.4%
Potassium sorbate	0.1%
Potassium gluconate	13.6%

**CLAIMS**

1. Theobromine for use in the treatment of improving blood lipids.
- 5 2. Theobromine for use in the treatment of increasing HDL-cholesterol in humans.
3. Theobromine for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.
- 10 4. Theobromine for use in the treatment of increasing the ratio HDL-cholesterol / non-HDL-cholesterol in humans.
- 15 5. Theobromine for use in the treatment according to any of claims 1 to 3, wherein the treatment comprises ingestion by a human of from 300 to 2000 mg theobromine per day.
- 20 6. Theobromine for use in the treatment according to claim 4, wherein the treatment comprises ingestion from 400 to 1800 mg theobromine per day, preferably from 500 to 1500 theobromine mg per day, more preferably from 600 to 1400 mg theobromine per day, even more preferably from 700 to 1300 mg theobromine per day, most preferably from 750 to 1250 mg theobromine per day.
- 25 7. Theobromine for use in the treatment according to any of claims 1 to 5, wherein the theobromine is ingested for at least 5 days per week for at least 3 weeks, preferably for at least 5 days per week for at least 4 weeks.
- 30 8. Theobromine for use in the treatment according to any of claims 1 to 6, wherein theobromine is ingested in the form of encapsulates.
- 35 9. Edible composition comprising theobromine and plant sterols, wherein the amount of theobromine is between 500 and 2000 mg per daily dosing and the amount of plant sterols is 1 to 3 g per daily dosing.
10. Edible composition comprising theobromine and plant sterols, wherein the amount of theobromine is between 300 and 2000 mg per daily dosing and the amount of plant sterols more than 1.8 g per daily dosing, preferably from 2 to 3 g per daily dosing.

- 5 11. Edible composition comprising theobromine and plant sterols, wherein the weight ratio theobromine : plant sterols is from 0.3 : 1 to 1 : 1, and wherein the amount of polyphenols in said composition is between 0% and 500% on the weight of theobromine in the composition.
12. Edible composition comprising theobromine and EPA and/or DHA, wherein the weight ratio of theobromine : EPA and DHA combined is from 1 : 4 to 1 : 0.3.
- 10 13. Edible composition comprising theobromine and a statin, in a weight ratio of from 200 : 1 to 5 : 1, more preferably from 100 : 1 to 10 : 1 (for theobromine : statin).
14. Edible emulsion comprising (by weight) 20 to 85% of oil, 15 to 80% water and 0.5 to 10% theobromine.
- 15 15. Emulsion according to claim 14, wherein said emulsion further comprises (by weight) 2-20% of plant sterols.
16. Emulsion according to claim 14 or 15, wherein the amount of polyphenols in said emulsion is between 0% and 500% on the weight of theobromine in the emulsion.
- 20 17. Edible emulsion according to any of claims 14 to 16, for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.
- 25 18. Liquid composition comprising water in an amount of more than 70% by weight on the total composition, preferably more than 80% by weight on the total composition, and less than 99.8% by weight on the total composition, theobromine in an amount of from 0.3 % by weight on the total composition to 2% by weight on the total composition,
- 30 plant sterols in an amount such that theobromine and plant sterols are present in a weight ratio theobromine : plant sterols of from 1 : 1 to 1 : 10.
19. Edible composition according to claim 18, wherein the composition comprises from 85 to 99% by weight on the total composition of water.
- 35

20. Edible composition according to any of claims 18 or 19, wherein the composition comprises from 0.4 to 1.5 % by weight on the total composition of theobromine.
21. Edible composition according to claim 18 to 20, for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.
22. Fermented food composition comprising from 70 to 99 wt% water, from 0.1 to 10 wt% protein, at least 0.3 wt% lactic acid, and 0.2 to 2 wt% theobromine.
23. Fermented composition according to claim 22, which comprises at least 10 million bacteria per gram composition, from the group of: *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus* species, *Lactococcus* species, *Bifidobacterium* species, and mixtures thereof.
24. Fermented composition according to claim 22 or 23, which composition further comprises plant sterols in an amount such that theobromine and plant sterols are present in a weight ratio theobromine : plant sterols of from 1 : 1 to 1 : 10.
25. Fermented composition according to claim 22 to 24, for use in the treatment of increasing HDL-cholesterol in humans and/or for use in the treatment of increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.
26. Use of theobromine for increasing HDL-cholesterol in humans.
27. Use of theobromine for increasing the ratio HDL-cholesterol / LDL-cholesterol in humans.
28. A method for increasing HDL-cholesterol in a human, which method comprises administering to the human an HDL cholesterol raising effective amount of theobromine.
29. A method for increasing the ratio HDL-cholesterol / LDL-cholesterol in a human, which method comprises administering to the human an HDL-cholesterol / LDL-cholesterol ratio raising effective amount of theobromine.



30. A method for increasing the ratio HDL-cholesterol / non-HDL-cholesterol in a human, which method comprises administering to the human an HDL-cholesterol / non-HDL-cholesterol ratio raising effective amount of theobromine.
- 5 31. The method according to any of claims 28 to 30, wherein the effective amount is from 300 to 2000 mg theobromine per day.
32. The method according to any of claims 28 to 31, comprising ingestion of an amount of polyphenols of between 0% and 500% on the weight of theobromine ingested.
- 10 33. The method according to claim 28, 30, 31 or 32, wherein the increase in HDL-cholesterol in humans (in blood and/or serum) is at least 5%.
- 15 34. The method according to claim 29, 30, 31 or 32, wherein the increase in the ratio HDL-cholesterol / LDL-cholesterol in humans (in blood and/or serum) is at least 5%.

**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/EP2011/057816

**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. A61K31/522 A61K45/06 A61P9/10  
 ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
 EPO-Internal, WPI Data, PAJ, BIOSIS, FSTA, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ETENG M U ET AL: "Comparative effects of theobromine and cocoa extract on lipid profile in rats", NUTRITION RESEARCH, vol. 20, no. 10, October 2000 (2000-10), pages 1513-1517, XP002598824, ISSN: 0271-5317	1-7, 26-34
Y	page 1516; table 1 -----	8-25
X	ETENG M U ET AL: "Theobromine rich cocoa powder induces weight loss and changes in lipid profile of obese Wistar rats", DISCOVERY AND INNOVATION,, vol. 18, no. 3, 1 September 2006 (2006-09-01), pages 191-196, XP009102531, ISSN: 1015-079X	1-7, 26-34
Y	the whole document ----- -/--	8-25

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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- "&" document member of the same patent family

Date of the actual completion of the international search  17 June 2011	Date of mailing of the international search report  11/07/2011
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2011/057816

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	abstract	9-13, 15, 18-25
X	----- JP 2006 151878 A (NAT INST OF ADV IND & TECHNOL) 15 June 2006 (2006-06-15) abstract	1
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/057816

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