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The "Mevalonate hypothesis": a cholesterol-independent alternative for the etiology of atherosclerosis

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Abstract

The "cholesterol hypothesis" is the leading theory to explain the cause of atherosclerosis. The "cholesterol hypothesis" assumes that plasma (LDL) cholesterol is an important causal factor for atherosclerosis.

However, data of at least seven placebo controlled randomized prospective trials with various cholesterol lowering drugs show that plasma cholesterol lowering does not necessarily lead to protection against cardiovascular disease. Therefore an alternative hypothesis for the etiology of cardiovascular disease is formulated. This alternative hypothesis, the "mevalonate hypothesis", assumes that after stimulation of the mevalonate pathway in endothelial cells by inflammatory factors, these cells start producing cholesterol and free radicals. In this hypothesis, only the latter play a role in the etiology of atherosclerosis by contributing to the formation of oxidized cholesterol which is a widely accepted causal factor for atherosclerosis.

Regardless of how the mevalonate pathway is activated (by withdrawal of statin drugs, by inflammatory factors or indirectly by reduced intracellular cholesterol levels) in all these cases free radical production is observed as well as cardiovascular disease. Since in the "mevalonate hypothesis" cholesterol is produced at the same time as the free radicals causing atherosclerosis, this hypothesis provides an explanation for the correlation which exists between cardiovascular disease and plasma cholesterol levels. From an evolutionary perspective, concomitant cholesterol production and free radical production in response to inflammatory factors makes sense if one realizes that both activities potentially protect cells and organisms from infection by gram-negative bacteria.

In conclusion, data have been collected which suggest that activation of the mevalonate pathway in endothelial cells is likely to be a causal factor for atherosclerosis. This "mevalonate hypothesis" provides a better explanation for results obtained from recent clinical studies with cholesterol lowering drugs than the "cholesterol hypothesis". Furthermore, this hypothesis explains how cholesterol can be correlated with cardiovascular disease without being a causal factor for it. Finally it provides a logical explanation for the etiology of this disease.

Introduction

In a series of review articles [1-5], Daniel Steinberg explains how the world got convinced of the "cholesterol hypothesis". This hypothesis states that plasma cholesterol is a causal factor for atherosclerosis. Authorities, including the European Food and Safety Authority (EFSA), appear to accept the "cholesterol hypothesis" as they approved health claims for certain ingredients in the area of cardiovascular health based on the sole fact that those ingredients lower plasma cholesterol values. Also, well known scientists in the field of nutrition reason that functional foods which increase plasma cholesterol levels can be dangerous for the cardiovascular system [6].

However, there have been contradictory observations in the recent years regarding the role of plasma cholesterol in cardiovascular disease, and this was the reason to further look into the actual evidence that plasma cholesterol would be the major causal factor for cardiovascular disease. In order to do this, evidence concerning the cholesterol dependent mechanisms for atherosclerosis were closely evaluated as well as pharmacological evidence

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obtained from trials on the efficacy of cholesterol lowering drugs. Also, epidemiological evidence for the relationship between plasma cholesterol and cardiovascular disease was taken into account. Ultimately, an alternative hypothesis was presented which explains why cholesterol is related to cardiovascular disease without being the cause of it.

The "cholesterol hypothesis"

To be able to understand the "cholesterol hypothesis" it is useful to go back to the evidence behind this hypothesis. Already in 1901, Windaus discovered that aortas of patients with atherosclerosis contain more cholesterol than aortas of healthy people [7]. Not long thereafter in 1913, Anitschkow showed that feeding cholesterol to rabbits increased their plasma cholesterol and caused atherosclerosis [8]. In the years thereafter, he established that the process of atherosclerosis starts with the formation of fatty streaks. Fatty streaks consist of white blood cells which have infiltrated the arterial wall. Most lipids in the cells of the fatty streaks are contained in foam cells. Later work of Anitschkow showed that these fatty streaks further develop into advanced lesions containing connective tissue. These lesions show high similarity to early atherosclerotic lesions in humans. In 1939, Muller discovered that familial cases of hypercholesterolemia (FH) exist and that these people suffer much more from cardiovascular disease than people without increased plasma cholesterol levels [9], suggesting that plasma cholesterol could be a causal factor in atherosclerosis.

In 1950, Gofman showed that most of the cholesterol in FH patients resided in LDL and IDL fractions. He also showed that there was a correlation between the levels of these lipoproteins in the blood and cardiovascular disease [10].This was the starting point of numerous successful studies done later to confirm the positive correlation between LDL cholesterol and cardiovascular disease.

Not much later in 1951, Barr et al discovered that another cholesterol containing lipoprotein, HDL, was negatively related to cardiovascular disease [11]. This is relevant in view of later discoveries which clarified that cellular uptake of cholesterol in tissues is mediated by receptors which recognize LDL-cholesterol [12], whereas reverse transport of cholesterol to the liver is mediated by HDL cholesterol [13]. Since in patients with cardiovascular disease, plasma LDL-cholesterol levels were increased and plasma HDL-cholesterol levels were decreased, there appeared to be an increased transport of cholesterol into the cardiovascular lesions and a reduced transport of cholesterol from the lesions back to the liver in patients with this disease.

In 1961, the first data of the Framingham study were presented. This study showed for the first time a clear relationship between moderately increased levels of plasma cholesterol and the occurrence of cardiovascular disease [14]. In order to prove that plasma cholesterol was causally involved in cardiovascular disease, it was necessary to show that a decrease in plasma cholesterol would result in a decrease in cardiovascular disease. In 1952, Kinsell had shown that saturated fats increase plasma cholesterol and that polyunsaturated fats decrease plasma cholesterol [15]. One of the first long term studies which indicated that such a cholesterol lowering diet would reduce cardiovascular disease was performed by Leren in 1966 [16]. This positive trial added to the hypothesis that plasma cholesterol can cause cardiovascular disease.

The 7 countries study, published in 1980, showed that the lower the mean plasma cholesterol levels of the population, the lower the cardiovascular death incidence in countries [17]. This further added to that view, although the author had only included countries in this evaluation which supported this hypothesis. In 1984, the now famous LRC CPPT trial was published [18]. This trial showed that cholestyramine, a drug which lowered plasma cholesterol by about 10% by binding cholesterol inside the intestinal tract, lowered the relative risk of coronary heart disease by almost 20%. Although the statistical evaluation of this study was subject to discussion, this result supported the hypothesis that plasma cholesterol can cause cardiovascular disease. Ten years later the well-known 4S study was published [19]. In this study simvastatin was used to reduce plasma cholesterol levels. Simvastatin is an inhibitor of HMGCoA reductase, which is the rate limiting enzyme of the mevalonate pathway which is responsible for the formation of cholesterol. In this study plasma cholesterol was decreased by about 25% and reduced the relative risk of death due to coronary heart disease by 42%.

Together these data made Steinberg to conclude that "the Cholesterol controversy" had ended [5] as in his view plasma cholesterol had been proven to play an important causal role in cardiovascular disease. Many pharmaceutical companies, food companies, university scientists and regulatory authorities followed that view. However, while these arguments seem convincing, several issues remain unresolved. These will be discussed below.

Doubts on the "cholesterol hypothesis"

In familial hypercholesterolemia (FH) patients have high plasma cholesterol levels and suffer frequently from cardiovascular disease. This may suggests that high plasma cholesterol levels are directly involved in formation of cholesterol loaded foam cells. However, cells of FH patients are defective in cholesterol receptors which in controls are involved in cellular uptake of cholesterol, so

increased uptake of native cholesterol due to higher cholesterol concentrations plays no role in the formation of foam cells. Alternatively, it is now widely accepted that macrophages, as predecessors of foam cells in fatty streaks, do not take up native cholesterol but mainly oxidatively modified LDL cholesterol (ox-LDL) using scavenger receptors [3]. For FH this suggests that not the uptake of native cholesterol in macrophages is increased but the uptake of oxidatively modified cholesterol. Apparently oxidatively modified cholesterol, and not native cholesterol, plays an important role in the induction of atherosclerosis.

Steinberg agrees that ox-LDL plays an important role in the etiology of atherosclerosis. However, in the "cholesterol-hypothesis" the relationship between increased plasma cholesterol levels and increased levels of ox-LDL is not entirely clear. It has been argued that in the reaction of LDL-cholesterol with oxygen radicals, plasma LDL cholesterol would be the driving force for the production of oxidized cholesterol. However, this is not very likely: In the reaction between LDL cholesterol and oxygen radicals, oxygen radical concentrations are very low due to their high reactivity, whereas LDL-cholesterol levels in plasma are very high. Under those conditions, pseudo first order kinetics occur. This means that the rate of formation of ox-LDL is determined by the rate of formation of oxygen radicals and not by the concentration of LDL cholesterol since this concentration hardly changes during this reaction. Therefore free radicals are more likely to be a causal factor for atherosclerosis than increased plasma levels of native LDL cholesterol.

Data obtained in a meta-analysis comprising almost 300,000 people showed that HDL cholesterol does not protect against cardiovascular disease [20], and also people with a genetically lowered HDL cholesterol level did not suffer from increased incidences of myocardial infarction [21]. These data undermine the arguments for a protective role of HDL cholesterol in atherosclerosis. Of course it is useless to deny the existence of an inverse correlation between the incidence of cardiovascular disease and plasma HDL cholesterol levels. The data presented in references 20 and 21 do not deny this inverse correlation but just indicate that this inverse correlation does not involve any causality in relation to cardiovascular disease.

The arguments above seriously question a causal role of native LDL cholesterol and HDL cholesterol in atherosclerosis. Further evidence that neither LDL nor HDL cholesterol plays an important role in the development of atherosclerosis comes from several large, randomized, placebo controlled trials which show that various drugs which considerably reduce plasma LDL cholesterol and/or increase plasma HDL cholesterol do not protect against atherosclerosis or cardiovascular disease:

In a trial testing the effect of hormone replacement therapy (HRT) on cardiovascular disease, 2763 women with coronary disease were included [22]. Patients were followed for more than 4 years after having started HRT or placebo treatment. HRT treatment resulted in a decrease of LDL cholesterol levels by 11% and an increase of HDL cholesterol levels by 10%. Despite these "favorable" changes in plasma cholesterol, the overall rate of cardiovascular events did not change.

In a trial testing the effect of torcetrapib, a cholesterol ester transfer protein inhibitor, 850 patients with familial hypercholesterolemia were included [23]. Patients were treated with statins or with statins plus torcetrapib for 2 years. Thereafter carotid intima-media thickness for the common carotic artery was measured as a surrogate marker for atherosclerosis. Treatment with torcetrapib decreased LDL cholesterol levels by about 25% and increased HDL cholesterol by about 35% compared to treatment by statins alone. Despite these "favorable" changes in plasma cholesterol, treatment with torcetrapib/statin resulted in an annual increase of the carotid intima-media thickness, whereas for the statin-only group a small decrease was reported. Torcetrapib therefore appeared to worsen atherosclerosis in this study.

Another example concerns a study with ezetimibe. Ezetimibe inhibits intestinal cholesterol uptake. In this study, 720 patients with familial hypercholesterolemia were included [24]. Patients were treated with statins or with statins plus ezetimibe for 2 years. Thereafter carotid intima-media thickness for the common carotic artery was measured as a surrogate marker for atherosclerosis. Treatment with ezetimibe decreased LDL cholesterol levels by 16.5%. HDL cholesterol did not change. Again these changes in plasma cholesterol after treatment with ezetimibe/statin did not result in positive effects on the intima thickness compared to the statin-only group.

In conclusion, it was shown that unbalanced native cholesterol transport to atherosclerotic lesions is unlikely to play a role in atherosclerosis. This view was confirmed by the results of clinical studies on atherosclerosis using various cholesterol modifying drugs [22-24]. Together these data represent serious doubts that plasma cholesterol has a causal role in atherosclerosis.

The effect of statins on cardiovascular disease
The protective effects of statins against cardiovascular disease is seen by Steinberg as final proof for the "cholesterol hypothesis" [5]. Statins inhibit HMGCoA reductase activity and thereby reduce plasma cholesterol levels. However, not all trials with statins show protection against cardiovascular disease even though in all these trials clear reductions in plasma LDL cholesterol were achieved [25-28]. These data confirm data of studies with other cholesterol lowering drugs [22-24] that

LDL cholesterol is unlikely to be an important causal factor for cardiovascular disease.

At least two different reasons can be considered as explanation for the failure of statins in recent trials: 1) Statins may also have pharmacological effects which may reduce its protective effect and 2) Possibly not all subpopulations are responders to statin treatment.

1. *Statins may have pharmacological effects which reduce its protective effect.* Due to HMGCoA reductase inhibition, which is a rate limiting enzyme of the of the mevalonate pathway, statins have many downstream biological effects. One of those is the inhibition of the formation of Coenzyme Q10 [29]. This is highly relevant as coenzyme Q10 is a major anti-oxidant for circulating LDL cholesterol [30], and many cardiac patients are known to have low circulating Coenzyme Q10 levels [31]. A further reduction of Coenzyme Q10 in such patients may have a more negative impact on atherosclerosis than in patients with a proficient antioxidant system. Also, kidney patients and diabetics suffer from oxidative stress. It is therefore quite possible that plasma Coenzyme Q10 lowering due to statin treatment contributed to the failure of several recent statin trials in such patient groups [25-28].

2. *Not all subpopulations of patients are responders to statin treatment.* Statins are probably most efficacious in patients with an overactive HMGCoA reductase system. An elegant study of Miettienen et al [32,33] actually confirmed that the efficacy of statin treatment is indeed linked to reduction of an overactive HMGCoA reductase system. He divided a group of sufferers from cardiovascular disease who had been treated with statins in four quartiles with increasing cholesterol production. After 5 years of treatment, the effect of statins on protection against cardiovascular disease was compared in the two most extreme different quartiles: Only the group with increased cholesterol synthesis showed reduced frequency of cardiovascular disease on statins.

Together these data would suggest that not plasma cholesterol, but an increased activity of the mevalonate pathway, is an important causal factor for atherosclerosis.

Steinberg concluded that the clinical data obtained with statins had ended "the cholesterol controversy" and that these data prove that plasma cholesterol is a causal factor for atherosclerosis [5]. However, the recent failed studies with statins [25-28] appear to prove the opposite: Plasma cholesterol is unlikely to play a causal role in atherosclerosis. The increased activity of HMGCoa reductase enzyme activity appears to be a more likely causal factor for atherosclerosis.
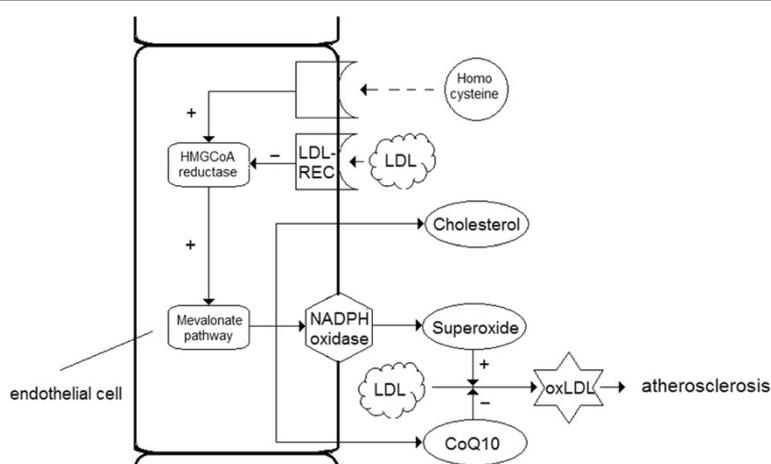
The "mevalonate hypothesis"

As discussed above, there are several serious doubts on the validity of the "cholesterol hypothesis" for the etiology of atherosclerosis. Therefore an attempt was made to define a better hypothesis. Based on the evidence discussed above it was considered that an alternative hypothesis had to fulfill the following requirements: 1) HMGCoA-reductase should play a crucial role in the induction or progression of cardiovascular disease; 2) The alternative hypothesis should explain the correlation which exists between plasma cholesterol and the incidence of cardiovascular disease and 3) Plasma-cholesterol should be no causal factor for atherosclerosis.

The simplest hypothesis which would fulfill these criteria is the "mevalonate hypothesis" (shown in Figure 1). This hypothesis states that an overactive mevalonate pathway in endothelial cells is probably an important causal factor in atherosclerosis since this would result in the activation of NADPH-oxidase. NADPH oxidase produces superoxide free radicals which transform native LDL cholesterol into ox-LDL, a widely accepted causal factor for atherosclerosis [34].

The "mevalonate hypothesis" fulfills the requirements as listed above: In this hypothesis, activation of HMGCoA reductase results in the formation of free radicals by NADPH-oxidase which transform native LDL into ox-LDL. Since after activation of HMGCoA reductase cholesterol is produced, a correlation between plasma cholesterol and atherosclerosis is ensured. Furthermore in this hypothesis cholesterol-production is not related to causing atherosclerosis.

The "mevalonate hypothesis" followed from the knowledge that this pathway plays a central role in the activation of endothelial NADPH oxidase. This enzyme is activated by the small GTPase Rac1, which for its activity is dependent on isoprenylation, regulated by the mevalonate pathway [35]. NADPH oxidase is the strongest contributor to the formation of free radicals by endothelial cells. Inflammatory factors which activate endothelial NADPH oxidase probably play an important role in atherosclerosis [36]. These endothelial NADPH-oxidase activating and pro-atherogenic factors include at least TNF alpha, angiotensin, homocysteine, interferon gamma, interleukin1, interleukin 6 and interleukin 8. Finally, endothelial activation of NADPH-oxidase is associated with arterial stiffness [37], suggesting a role for this enzyme activity in atherosclerosis.

There is ample experimental evidence to show that activation of HMGCoA reductase results in activation of NADPH-oxidase: Chronic exposure of cells to statins (pharmacological inhibitors of HMGCoA reductase) leads to a major upregulation of HMGCoA reductase activity [38]. After sudden statin withdrawal, a strong increase in the NADPH oxidase dependent production of

Figure 1 The mevalonate –hypothesis. Inflammatory factors like homocysteine can activate the mevalonate pathway. Also reduced uptake of LDL cholesterol (LDL) by LDL receptors (LDL-REC) can activate the mevalonate pathway. The mevalonate pathway regulates numerous biological activities including the formation of coenzyme Q10, the formation of cholesterol and the activation of NADPH oxidase. Activation of NADPH oxidase can contribute to the formation of ox-LDL cholesterol which is probably an important trigger for the induction of atherosclerosis. Coenzyme Q10 plays an important role as an anti-oxidant in the protection of LDL cholesterol from oxidation.

free radicals was observed in the aorta of mice [39]. A similar effect was seen after statin withdrawal from human endothelial cells in culture [39]. These data confirm that stimulation of the mevalonate pathway can result in the formation of NADPH-oxidase. Chronic treatment with statins also potently up-regulate the HMGCoA reductase activity in humans [40,41]. The clinical consequence of suddenly stopping statin treatment after such up-regulation of HMGCoA reductase is an increase in ischemic stroke [42] and an increased event rate in patients with acute coronary syndromes [43]. These adverse cardiac effects were independent of changes in plasma cholesterol levels. Interestingly, statin withdrawal in humans also resulted in a rebound inflammatory response during the acute phase of myocardial infarction [44] and in endothelial dysfunction [45]. In combination, these data confirm that activation of HMGCoA reductase can be responsible for activation of endothelial NADPH-oxidase and that this can contribute to cardiovascular disease.

There are additional human models in which increased activity of endothelial HMGCoA reductase is observed: In familial hypercholesterolemia, due to a genetic defect, all cells lack functional LDL-receptors which are necessary for cholesterol uptake. Due to a lack of intracellular cholesterol, cells of people with familial hypercholesterolemia have increased plasma cholesterol levels and increased levels of HMGCoA reductase. For fibroblasts this latter effect was quantified [46], but effects on HMGCoA reductase in the same direction are expected in endothelial cells. Cells of people with FH not only have increased levels of HMGCoA reductase, cells in the arterial wall of these patients also show at least a

doubling of NADPH-oxidase activity [47], suggesting that an increased mevalonate pathway is responsible for that. As mentioned previously, in these people the incidence of atherosclerosis is clearly increased. These clinical data in patients with FH confirm that an increased activity of the mevalonate pathway can lead to cardiovascular disease.

Further evidence for the theory that increased HMGCoA reductase leads to increased NADPH oxidase activity and cardiovascular disease comes from studies on people with mutant forms of ApoE. Cells of patients with these mutations cannot take up cholesterol since ApoE is required for that. As a consequence of this and as explained above, these cells will have increased HMGCoA reductase activities, and people with this mutation have increased plasma cholesterol values. A direct effect of this mutation of endothelial NADPH oxidase activity was not measured yet in humans, but in animals this mutation indeed leads to increased HMGCoA reductase activity [47]. It is therefore likely that endothelial cells of people with mutant ApoE phenotypes also have an increased activity of the mevalonate pathway. This may explain the increased incidence of cardiovascular disease in patients with these mutant ApoE phenotypes [48].

Not only atherosclerosis as observed in FH or in patients with ApoE mutations is related to an increased activity of HMGCoA reductase. Industrial trans fatty acids, well known for their adverse cardiovascular effects [49], stimulate NADPH oxidase in endothelial cells [50] and can also stimulate endogenous cholesterol synthesis [51]. Therefore, atherosclerosis induced by industrial trans-fatty acids may also be dependent on activation of the mevalonate pathway. Bacterial lipopolysaccharide

(LPS) is produced by bacteria in the gut. Diets not only affect the amount and types of LPS produced but also the uptake of LPS from the gut. LPS is likely to contribute to atherosclerosis in humans [52] and circulates in the blood in concentration which are relevant with respect to its pro-inflammatory effects. LPS at relevant concentrations increased endothelial superoxide production in human saphenous veins, which led to superoxide- dependent binding of monocytes to the endothelia. This phenomenon which is seen as a model for early phase atherosclerosis, was inhibited by statins [52], suggesting the involvement of the mevalonate pathway. Together these data imply that diet-induced atherosclerosis may also follow a mechanism covered by the "mevalonate hypothesis".

The "mevalonate hypothesis" implies that high levels of inflammatory factors can lead to a concomitant increase in cholesterol production and NADPH-oxidase activity. This dual action may have a logical explanation. LPS, the archetype inflammatory factor, is detoxified in vivo by binding to LDL cholesterol [53]. Therefore evolutionary, the production of cholesterol by endothelial cells, the primary targets for LPS toxicity, in response to inflammatory factors appears to make sense. Concomitant activation of NADPH-oxidase is very useful since this has a direct toxic effect against invading bacteria [54].

Protection of plasma cholesterol against bacterial infections is not just theory. Large epidemiological studies show an inverse correlation between the occurrence of infectious disease and plasma cholesterol levels [55,56]. Furthermore, rats made hypolipidaemic by pharmacological tools had a marked increased endotoxin-induced mortality compared to control rats, whereas administration of exogenous lipoprotein reduced their mortality substantially [57].

The liver is known to be the primary organ for the regulation of plasma cholesterol. However since the endothelial cells represent a huge number of cells present throughout the entire body, probably representing the largest "organ" of the body, even a relatively low rate of cholesterol production by these cells can probably be sufficient to increase plasma cholesterol levels under pro-inflammatory conditions. Therefore, under chronic pro-atherogenic (pro-inflammatory) conditions it is quite possible that enhanced production of cholesterol by endothelial cells [58] will contribute to the increased plasma cholesterol levels.

Implications of the "mevalonate hypothesis"
For plasma cholesterol as a biomarker
If the "mevalonate hypothesis" would be correct, plasma cholesterol levels can still be used as a biomarker for cardiovascular disease in populations who are not exposed to cholesterol lowering drugs. However, plasma cholesterol levels of populations treated with plasma cholesterol-modifying drugs or functional foods are not necessarily useful as biomarkers for cardiovascular risk, since not all mechanisms which lead to decreased cholesterol plasma levels will inhibit the mevalonate pathway, and not all mechanisms which lead to increased cholesterol plasma levels will activate the mevalonate pathway.

For approval of functional foods by regulatory authorities
Health claims for several functional food ingredients have been approved by EFSA based on their effect on plasma cholesterol only. Most of these ingredients lower plasma cholesterol by lowering cholesterol uptake from the gut. However since reduced uptake of cholesterol (due to lower plasma cholesterol levels) is likely to stimulate HMGCoA reductase, these ingredients may actually worsen cardiovascular risk if the hypothesis of this paper would be correct. Therefore, these approvals may need reconsideration.

Abbreviations
FH: Familial hypercholesterolemia; LDL: Low density lipoprotein; IDL: Intermediate density lipoprotein; HDL: High density lipoprotein; EFSA: European Food and Safety Authority; LRC-CPPT: The Lipid Research Clinics Coronary Primary Prevention Trial; Ox-LDL: Oxidized LDL cholesterol; HMGCoA reductase: 3-hydroxy-3-methylglutaryl coenzyme A reductase; NADPH oxidase: Nicotinamide adenine dinucleotide phosphate oxidase.

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