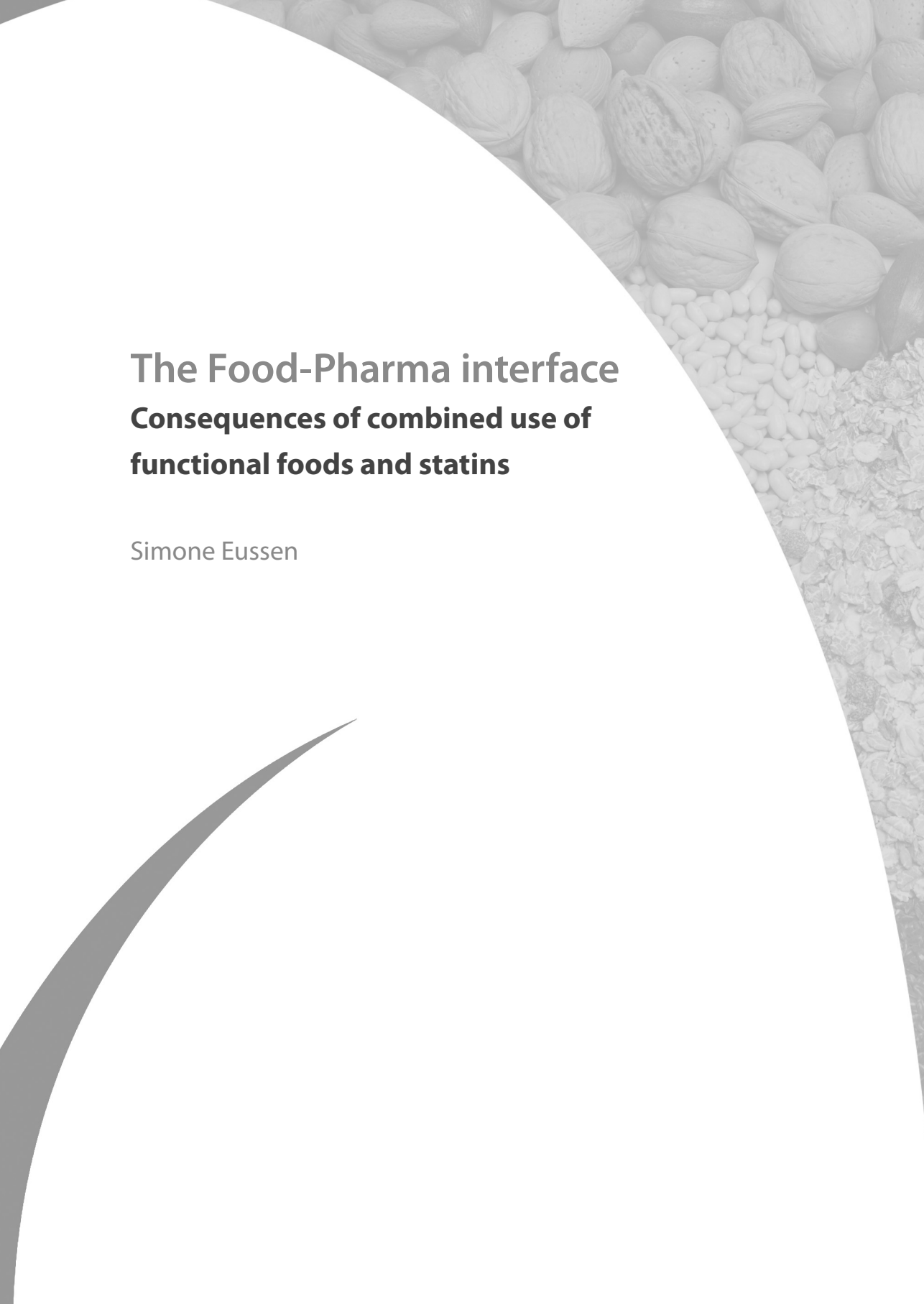

The Food-Pharma interface

Consequences of combined use of functional foods and statins

Simone Eussen

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The Food-Pharma interface

Consequences of combined use of functional foods and statins

De gevolgen van gecombineerd gebruik van functionele voedingsmiddelen en statines

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 12 december 2011 des middags te 2.30 uur

door

Simone Rosette Barbara Maria Eussen
geboren op 12 december 1982 te Maastricht

Promotoren: Prof. dr. J. Garssen
Prof. dr. H. van Loveren

Copromotoren: Dr. O.H. Klungel
Dr. C.J.M. Rompelberg

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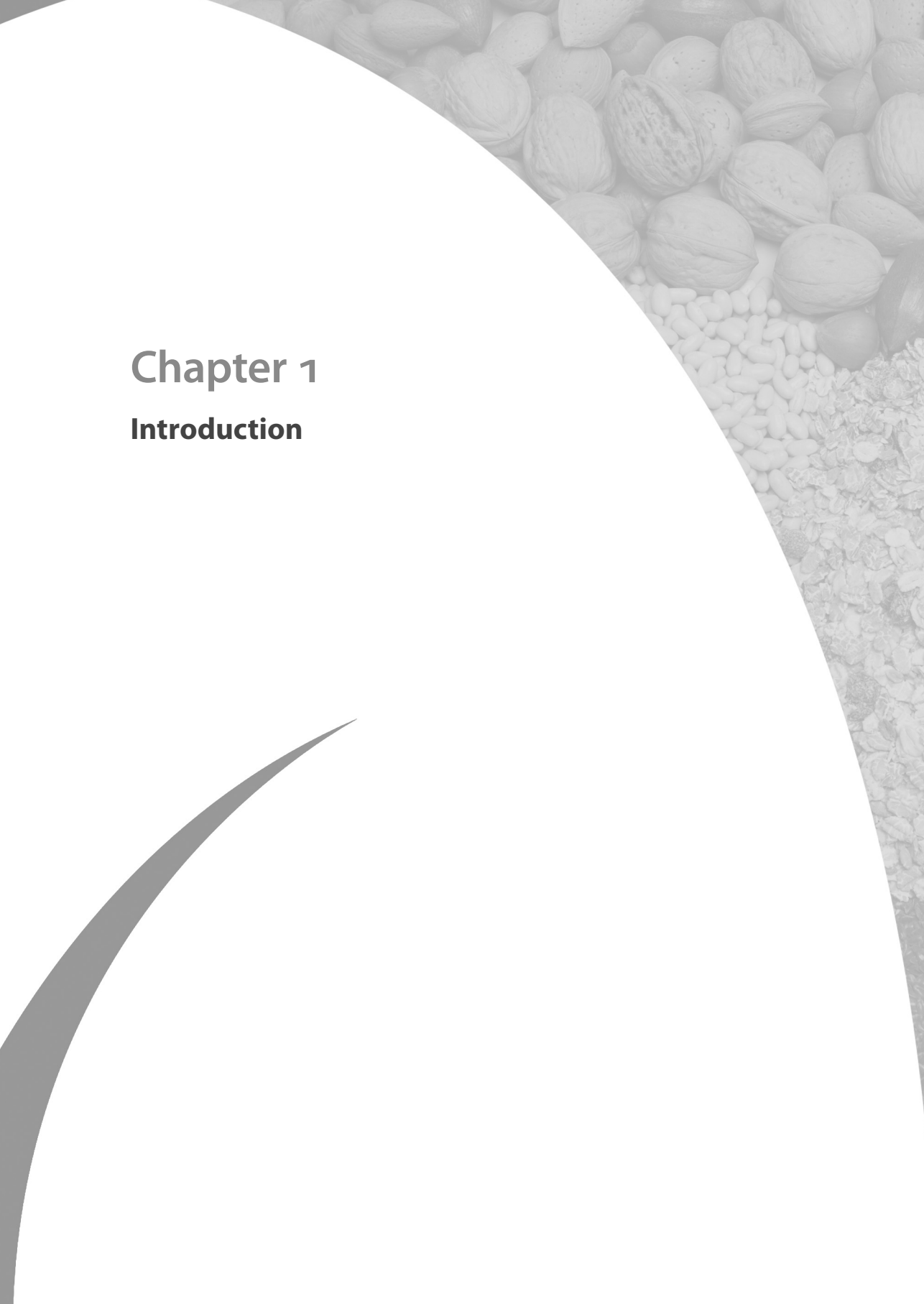
Contents



Chapter 1

Introduction

Chapter 1.1

General introduction

Part of this Chapter is based on:
Simone RBM Eussen, Hans Verhagen, Olaf H Klungel, Johan Garssen, Henk van Loveren, Henk J van Kranen, Cathy JM Rompelberg.

Functional foods and dietary supplements:
Products at the interface between pharma and nutrition.

Eur J Pharmacol 2011 [Epub ahead of print]

Introduction

Since ancient times, plants, herbs and other natural products have been used as healing agents. Advances in organic chemistry from the early 19th century onwards have enabled the preparation of numerous synthetic medicines. Yet, the majority of the medicinal substances available today have their origin in natural compounds. The best known example is aspirin (acetylsalicylic acid), originally derived from the bark of the white willow tree.[1,2] Also the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, have their roots in plant-based medicine.[3,4]

Traditionally, pharmaceuticals have been used to cure diseases or to alleviate the symptoms of disease. Nutrition, on the other hand, is primarily aimed to maintain health and to contribute to disease prevention by providing the body with the optimal balance of macro- and micronutrients needed for good health. Due to the emerging knowledge of disease, medicines are now increasingly being used to lower risk factors, and thereby to help prevent chronic diseases. Prime examples are lipid-lowering and blood pressure-lowering agents which reduce the risk of cardiovascular disease. The appearance of functional foods and dietary supplements on the market has further blurred the distinction between food and pharmaceuticals. These food items are considered to be positioned between traditional foods and medicines at the so-called 'Food-Pharma interface' (**Figure 1**).

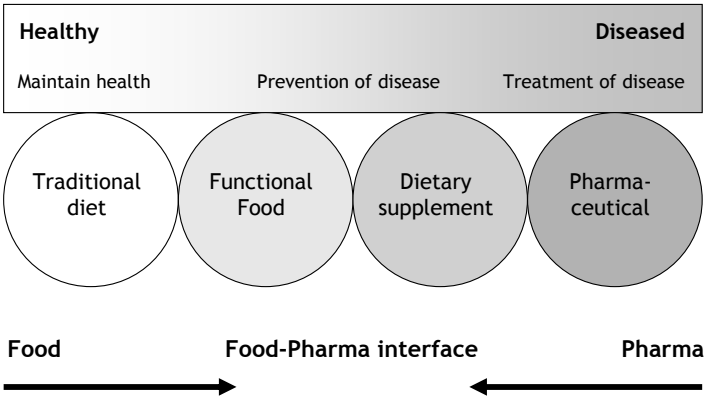


Figure 1. Schematic presentation of the 'Food-Pharma interface'

Functional foods are foods that are claimed to improve health, quality of life or well-being beyond basic nutritional functions.[5-7] Examples of functional foods are cereals fortified with soluble fibres, margarines enriched with cholesterol-lowering phytosterols and yoghurts with specific bacterial cultures added. Thus, functional foods resemble conventional food products in appearance and are consumed as part of the usual diet. In contrast, dietary supplements are typically marketed in the form of a capsule, pill, powder or gel and are not presented for use as a conventional food product.

Dietary supplements contain one or more dietary ingredients (e.g. vitamins, minerals, amino acids, herbs or other botanicals) and are intended to supplement the diet.[7,8]

Cardiovascular disease

In this thesis, we focus on pharmaceuticals, functional foods and dietary supplements used for reducing the risk of cardiovascular disease (CVD). CVD is the leading cause of death in the world. According to the World Health Organization, one-third of all deaths worldwide, i.e. about 17 million people per year, are attributed to CVD.[9] The most common form of CVD is coronary heart disease (CHD), resulting from the accumulation of atherosclerotic plaques in the walls of coronary arteries that supply blood to the myocardium. CHD is a multifactorial disease; numerous risk factors have been associated with a higher incidence of CHD. It has been estimated that at least one-third of all CHD is attributable to the following five risk factors:[10] tobacco use,[11] alcohol overconsumption,[12] obesity,[13] hypertension[14] and hyperlipidaemia.[15]

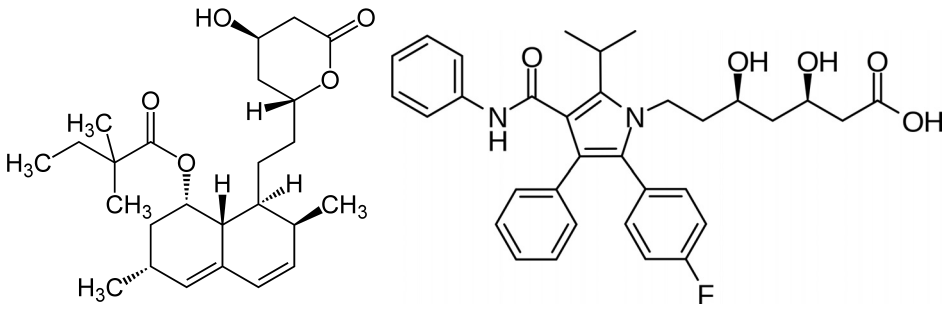
To control multifactorial diseases, such as CHD, a treatment approach where behavioural changes (e.g. stopping smoking and increasing physical activity), medicines and nutrition complement each other may prove to be the most successful. The role of medicines and (functional) foods in the management of hyperlipidaemia has been subject of increased interest for about 30 years, since in 1984 the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) provided strong evidence for a causal role of high lipid levels in the pathogenesis of CHD.[16,17] Hyperlipidaemia refers to a condition in which plasma levels of cholesterol and/or triglycerides are elevated. Subsequent clinical as well as epidemiological studies have consistently shown that high levels of total and low-density lipoprotein (LDL) cholesterol[18,19] and triglycerides,[20] and low levels of high-density lipoprotein (HDL) cholesterol[21] are strongly associated with an increased risk of CHD.[18]

Pharmacological management of hyperlipidaemia

Pharmacological treatment of hyperlipidaemia includes the use of statins, fibrates, nicotinic acid derivatives, bile acid binding resins and ezetimibe. In this thesis, we focus on statins because these are the drugs of first choice in hyperlipidaemic patients,[22] a large number of subjects are treated (suboptimally) with statins, and statins have a potential for interaction with functional foods or dietary supplements.

Statins

Statins are the most widely used medication in the treatment of hyperlipidaemia, both in primary and secondary prevention of CHD. In the Netherlands, there are currently five statins on the market: atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin, of which atorvastatin and simvastatin have the largest market share. **Figure 2** displays the chemical structure of these statin drugs. All statins act by a similar mechanism of action; they inhibit the enzyme HMG-CoA reductase, the



Simvastatin **Atorvastatin**

Figure 2. Chemical structure of the statin drugs, simvastatin and atorvastatin

rate-limiting enzyme in the mevalonate pathway of hepatic cholesterol synthesis. This results in the up-regulation of LDL receptors on the hepatocyte surface membranes, consequently leading to an increased removal of LDL cholesterol from the circulation.[23,24] It has also been shown that statins reduce circulating concentrations of apolipoprotein B-containing lipoproteins by decreasing the production of very low-density lipoprotein (VLDL) in the liver, and thereby the production of VLDL remnants and LDL.[25,26]

Statins are highly effective in lowering total and LDL cholesterol (by 18-55%) and to a lesser extent in increasing HDL cholesterol (by 5-15%) and reducing triglyceride levels (by 7-30%).[27-31] Over the years, also numerous (lipid-independent) pleiotropic effects of statins have been described.[36-38] For example, statins improve endothelial function and atherosclerotic plaque stability, decrease oxidative stress and inflammation, and inhibit the thrombogenic response.[39] The use of statins results in a reduction in CHD-related death of about 30% and reduces all-cause death by 20%.[32-35]

Nutritional management of hyperlipidaemia

In the last decade there has been more interest in the role of diet in influencing cholesterol and triglyceride levels. Apart from (disease-related) dietetic regimes, an increasing number of functional foods and dietary supplements has appeared on the market. Functional foods and dietary supplements that are currently marketed in the Netherlands for CHD risk reduction include cereals fortified with specific soluble fibres (e.g. β-glucans), food products or dietary supplements containing high levels of *n*-3 polyunsaturated fatty acids (PUFA) and dairy products enriched with phytosterols or phytostanols.

β-Glucans from soluble dietary fibre

β-Glucans are thought to reduce plasma cholesterol levels by interfering with cholesterol and/or bile acid (re)absorption, either by binding bile acids or by forming a thick unstirred water layer in the intestinal lumen.[40] This leads to an increased faecal output of bile acids, resulting in a reduction of bile

acids available for transport back to the liver. The compensatory up-regulation of the hepatic enzyme cholesterol 7-α-hydroxylase promotes the conversion of intracellular cholesterol to bile acids. This leads to an up-regulation of the LDL receptors and activation of the enzyme HMG-CoA reductase to re-establish hepatic cholesterol stores, ultimately resulting in an increased clearance of circulating LDL cholesterol.[40-42] Other proposed mechanisms by which β-glucans lower cholesterol levels are the inhibition of cholesterol synthesis by short-chain fatty acids (mainly propionate and butyrate) which are the major fermentation products of β-glucans, the increased intestinal viscosity causing reduced glucose absorption and thereby improved insulin sensitivity, and the increased satiety leading to a lower overall energy intake.[40,42] It has been estimated that the recommended intake of 3 g β-glucan-containing soluble fibre per day significantly lowers total and LDL cholesterol levels by 0.12 and 0.11 mmol/l (~2-3%), respectively. In the Netherlands, β-glucan is incorporated into bread and cookies.

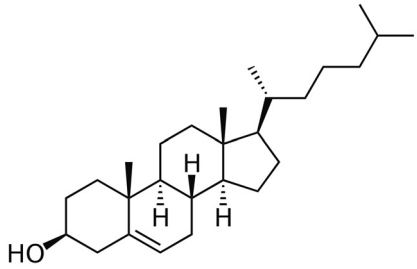
n-3 Polyunsaturated fatty acids

n-3 PUFA, especially the marine *n*-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the plant-derived α-linolenic acid (ALA), have been associated with a lower risk of CHD. The proposed mechanisms of action include improving heart rate variability, reducing serum triglycerides, and antithrombotic, anti-inflammatory and anti-atherogenic effects.[43-45] *n*-3 PUFA are sold on the Dutch market both as functional foods, e.g. margarines, eggs and bread, and as dietary supplements.

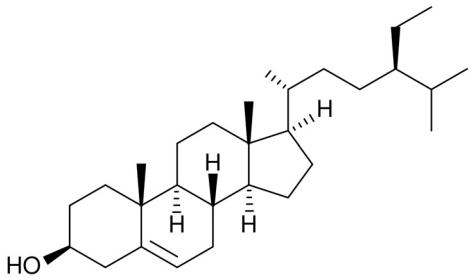
Phytosterols/-stanols

Phytosterols, also referred to as plant sterols, are chemically almost identical to cholesterol. Phytostanols or plant stanols are phytosterols without the double bond in the steroid skeleton. The most common phytosterols are β-sitosterol and campesterol and their stanol counterparts are sitostanol and campestanol (**Figure 3**). Phytosterols/-stanols reduce the intestinal absorption of cholesterol, presumably by competing with both dietary and biliary cholesterol for solubilisation into mixed micelles. Because phytosterols and -stanols are more hydrophobic than cholesterol, they have a higher affinity for the micelle.[46,47] Other mechanisms proposed are the interference with the cholesteryl ester-mediated hydrolysis process necessary for absorption and/or stimulation of the ATP-binding cassette (ABC) transporter expression by phytosterols/-stanols.[46,48-50] The ABCG5 and ABCG8 transporters actively transport dietary sterol out of the enterocytes back into the intestinal lumen, thereby limiting the amount of sterol absorbed. ABCA1 may also participate in this process.[51]

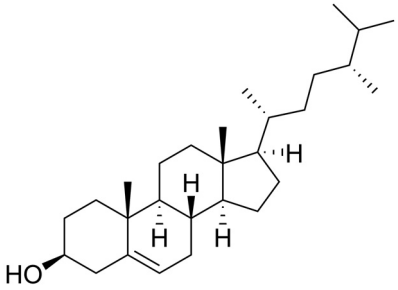
Margarines enriched with phytostanols have been launched on the Dutch market in 1999, shortly followed by margarines enriched with phytosterols in 2000. Over the years, also other food vehicles have been used, such as yogurt, yogurt drinks and milk. Functional foods enriched with phytosterols/-stanols are one of the most commonly used functional foods in the Netherlands. Dietary supplements with phytosterols/-stanols are also available on the Dutch market, but these are only marginally used.[52] In a meta-analysis, it was recently found that a daily dose of 2.15 g phytosterols/-stanols from functional foods reduces LDL cholesterol by 0.34 mmol/l or 8.8%.[53]



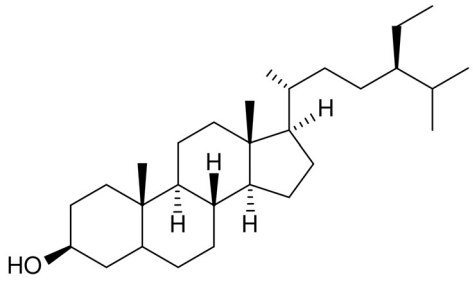
Cholesterol



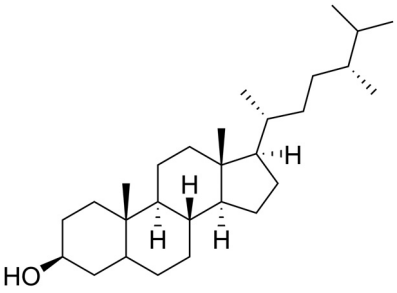
ß-sitosterol



Campesterol



Sitostanol



Campestanol

Figure 3. Chemical structure of cholesterol and the phytosterols, β-sitosterol and campesterol, and the phytostanols, sitostanol and campestanol

Regulatory framework

The European Commission has developed a regulatory framework that aims to ensure the safety and efficacy of pharmaceuticals and food products marketed to European consumers. The following section addresses the European regulation for pharmaceuticals and foods, and specifically focuses on the regulations that apply to the functional foods and dietary supplements described in this thesis, i.e. β-glucans, *n*-3 PUFA and phytosterols/-stanols.

Safety and efficacy of pharmaceuticals

An extensive legal regulatory system is in place for pharmaceutical products. Already shortly after the thalidomide affair in the 1960s, national and international regulatory authorities were established to monitor drug safety.[54] Since 1995, the European Medicines Agency (EMA) has been responsible for the scientific evaluation and monitoring of the safety and efficacy of pharmaceuticals in Europe. The agency was set up to reduce disparities in drug regulation across the different European Member States. Yet, the majority of the existing pharmaceuticals in the European Member States remain authorised nationally, whereas the majority of novel medicines are authorised through the European Medicines Agency.

Directive 2001/83/EC requires that all medicinal products are registered before they are placed on the European market.[55] Registration involves standard procedures to examine the efficacy, safety and quality of the product. An exception is made regarding the efficacy of traditional herbal medicinal products, for which a simplified registration procedure ('traditional-use registration') is in place. For traditional herbal medicinal products the provision of data from preclinical tests and clinical trials is not required, as long as their efficacy is plausible on the basis of longstanding use and experience.[55]

In exceptional circumstances, individual Member States may grant permission for the availability of pharmaceuticals without market authorisation under the compassionate use program.[56] This program makes promising therapies available to select patients with a seriously debilitating or life-threatening disease when no alternative authorised treatment exists.[56]

Safety of food

Food safety has long been a matter of national policy.[57] Following the bovine spongiform encephalopathy (BSE) crisis and other food scares in the 1990s, in January 2000 the European Commission published a White Paper on Food Safety.[58] The Paper outlines a comprehensive range of actions needed to complement and modernise existing European food legislation, and led to the introduction of the General Food Law (Regulation (EC) 178/2002).[59] This regulation formed the basis for the establishment of the independent European Food Safety Authority (EFSA) in 2002. This Authority is responsible for providing the European Commission with independent scientific advice on all matters with a direct or indirect impact on food safety.

Nowadays, the European Commission has established a legal framework regulating the dietary supplements market, the fortified food market and the market for so-called 'novel foods'.[60,61] The Food Supplements Directive 2002/46/EC specifies permitted vitamin and mineral substances, and provides maximum and minimum levels of vitamins and minerals in dietary supplements. Regulation (EC) 1925/2006 (Food Fortification Regulation) provides a positive list of vitamins, minerals and specific other substances (e.g. herbal extracts) that may be added to foods. When a functional food contains novel ingredients or is produced by a novel process it may fall under Regulation (EC) 258/97.[62] This regulation requires that all novel foods or novel food ingredients, i.e. food (ingredients) without a history of significant consumption in the European Union prior to 15 May 1997,[63] undergo a science-based safety assessment before being placed on the European market.

Efficacy of food: nutrition and health claims

Dietary supplements and functional foods are meant to benefit health. Consequently, such food products typically contain claims on their label stating their benefits. In order to harmonise those claims at the European level, in December 2006 the European Union published Regulation No 1924/2006 on nutrition and health claims made on foods.[64] This regulation distinguishes two categories of claims: nutrition claims and health claims. Nutrition claims are claims that state, suggest or imply that a food product has particular nutritional properties. Such claims may, e.g. state that a product contains calcium, or is low in salt or sugar. Health claims are statements that imply that a relationship exists between a food product and a health condition. Examples are general function claims (Article 13(1) and 13(5) claims), reduction of disease risk claims (Article 14(1)(a) claims) and claims referring to the growth and development of children (Article 14(1)(b) claims).[65] The EFSA evaluates the scientific data related to food and food ingredients that contain a nutrition or health claim into an opinion which is put forward to the European Commission for approval and authorisation. In the European Union medical claims, i.e. claims for the prevention, treatment or cure of human disease, are reserved for medical products.[66] Thus, the European Union differentiates between 'reduction of disease risk factor' and 'prevention' to acknowledge that diet and certain foods can make important contributions to maintain health and manage disease risk factors, but they may not bear a claim that they can prevent disease.[67]

Health claims on functional foods and dietary supplements described in this thesis

Various health claims in relation to cardiovascular disease risk of the functional foods or dietary supplements studied in this thesis have recently been evaluated by the EFSA.

β-Glucans from soluble dietary fibre

Based on all scientific data available, the EFSA concluded in 2009 that a cause and effect relationship has been established between the consumption of β-glucans and the reduction of blood cholesterol concentrations. The proposed health claim (Article 13(1)) states that 'Regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations'. The EFSA considers that, in order to bear the claim, foods should provide at least 3 g/d of β-glucans from oats or barley.[68] This advice has been forwarded to the European Commission for authorisation of this health claim.[65]

n-3 Polyunsaturated fatty acids

The EFSA has evaluated health claims (Article 13(1)) in relation to the *n*-3 PUFA, EPA and DHA, and the following cardiovascular effects: maintenance of normal HDL and LDL cholesterol concentrations, maintenance of normal blood concentrations of triglycerides and maintenance of normal blood pressure. The authority concluded that intakes of EPA and DHA of 2-4 g/d reduce blood triglycerides and intakes of 3 g EPA or DHA per day reduce blood pressure. According to the EFSA,

no cause and effect relationship has been established between the consumption of EPA or DHA and the maintenance of normal HDL or LDL cholesterol concentrations.[69]

Concerning ALA, the EFSA has provided positive advise to the European Commission for a health claim (Article 13(1)) stating 'ALA contributes to maintenance of normal blood LDL cholesterol concentrations', whereas evidence was considered insufficient for the relationship between dietary intake of ALA and the maintenance of normal blood pressure.[70] The EFSA has not yet evaluated health claims on ALA in relation to triglycerides.

Phytosterols/-stanols

In 2009 the European Commission authorised a health claim (Article 14(1)(a)) on phytosterols/-stanols and lowering/reducing blood LDL cholesterol based on a scientific opinion of the EFSA.[71,72] The health claim states that 'Phytostanol/-sterol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease'.[71-73] This does not explicitly mean that phytostanol and phytosterol esters prevent CHD. Nevertheless, this assumption may easily be made by most consumers.[74]

Objective of this thesis

The health claims for CHD risk reduction stated on the label of functional foods and dietary supplements resemble the documented efficacy of statin drugs. Therefore, it is not surprising that subjects may combine their statin therapy with the use of functional foods or dietary supplements. This combination may be beneficial, but may also increase the likelihood of the occurrence of food-drug interactions, either on a physiological level or a behavioural level. Physiological interactions are additive, synergistic or antagonistic effects when drugs are combined with functional foods or dietary supplements. Behavioural interactions arise when people who consume functional foods or dietary supplements alter the dosage of their prescribed drugs or stop the drug without consulting a general practitioner or pharmacist. The objective of this thesis is to gain further insight into both positive and negative aspects arising from the combined intake of statins and functional foods or dietary supplements.

Outline of this thesis

Chapter 1, the present chapter, gives an introduction to the topic of this thesis and includes a comprehensive review that elaborates on the beneficial effects of adding functional foods or dietary supplements to drug therapy (*Chapter 1.2*). In this review, we have focused on the addition of phytosterols/-stanols, soluble dietary fibres, *n*-3 PUFA and coenzyme Q_{10} to statin therapy.

Chapters 2 and **3** focus, respectively, on the physiological interactions and behavioural interactions that may arise after combined intake of functional foods/dietary supplements and statins. **Figure 4** summarises which type of interaction (physiological or behavioural) between statins and foods with either β-glucan dietary fibres, *n*-3 PUFA or phytosterols/-stanols, have been discussed in each subchapter. *Chapter 2.1* evaluates and presents the physiological interaction between β-glucans from oats and the statin drug, atorvastatin in a mouse model for atherosclerosis. Until now, the use of functional foods or dietary supplements with β-glucan dietary fibre is limited in the Netherlands and interactions between statins and β-glucans or other soluble fibres have rarely been examined. A first step towards improving our knowledge about this interaction is to conduct an animal study. *Chapter 2.2* presents the clinical efficacy of *n*-3 PUFA, either with or without statins, in the prevention of major cardiovascular events. In *Chapter 2.3*, we explore the cholesterol-lowering effectiveness of margarines enriched with phytosterols/-stanols in statin users and statin non-users under free-living conditions. Besides studying the effects of the combined use of statins and functional foods or dietary supplements using (pre)clinical and epidemiological data, modelling approaches are useful for a better interpretation of the experimental data. In *Chapter 2.4* we propose a mathematical model that simulates reductions in LDL cholesterol after separate and combined intake of phytosterols/-stanols and statins.

In **Chapter 3**, we first describe the results of a randomised controlled study aimed to improve patients' adherence to statins (*Chapter 3.1*). Next, *Chapters 3.2* and *3.3* assess the influence of the use of phytosterol/-stanol-enriched functional foods on adherence to statin therapy. Whereas in *Chapter 3.2* all persons using statins at the time of assessing functional food use are included, in the study described in *Chapter 3.3* only new statin users are enrolled.

In **Chapter 4** we determine the cost-effectiveness of the use of functional foods enriched with phytosterols/-stanols in addition to statins in the prevention of CVD. The aging of the population and the rising health care costs make it more and more important to consider the cost-effectiveness of different treatment strategies.

Finally, in **Chapter 5** the results presented in this thesis are discussed and placed in a broader perspective. Implications of this thesis for practice and further research are given.

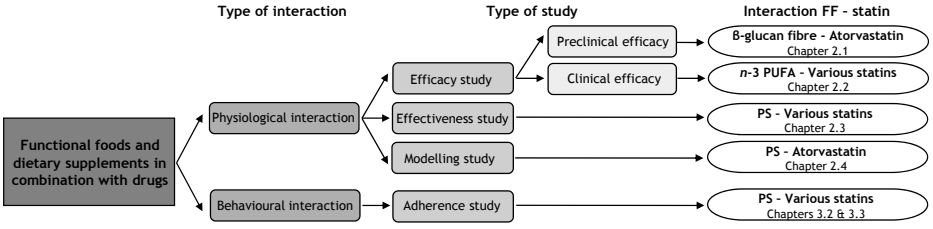


Figure 4. Overview of the different types of interactions between functional foods and statins that are studied in this thesis
FF, functional food; PS, Phystosterols/-stanols

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Chapter 1.2

Support of drug therapy using functional foods and dietary supplements: Focus on statin therapy

Simone RBM Eussen, Olaf H Klungel, Johan Garssen,
Hans Verhagen, Henk J van Kranen, Henk van Loveren and
Cathy JM Rompelberg

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Abstract

Functional foods and dietary supplements might have a role in supporting drug therapy. These products may 1) have an additive effect to the effect that a drug has in reducing risk factors associated with certain conditions, 2) contribute to improve risk factors associated with the condition, other than the risk factor that the drug is dealing with, or 3) reduce drug-associated side effects, for example, by restoring depleted compounds or by reducing the necessary dose of the drug. Possible advantages compared with a multidrug therapy are lower drug costs, fewer side effects and increased adherence. In the present review we have focused on the support of statin therapy using functional foods or dietary supplements containing phytosterols and/or phytostanols, soluble dietary fibre, *n*-3 polyunsaturated fatty acids (PUFA) or coenzyme Q_{10}.

We conclude that there is substantial evidence that adding phytosterols/-stanols to statin therapy further reduces total and low-density lipoprotein (LDL) cholesterol by roughly 6% and 10%, respectively. Adding *n*-3 PUFA to statin therapy leads to a significant reduction in plasma triglycerides of at least 15%. Data are insufficient and not conclusive to recommend the use of soluble fibre or coenzyme Q_{10} in patients on statin therapy and more randomised controlled trials towards these combinations are warranted.

Aside from the possible beneficial effects from functional foods or dietary supplements on drug therapy, it is important to examine possible (negative) effects from the combination in the long term, for example, in post-launch monitoring studies. Moreover, it is important to monitor whether the functional foods and dietary supplements are taken in the recommended amounts to induce significant effects.

Introduction

The world market for functional foods and dietary supplements is expanding rapidly. In 2010 functional foods are expected to represent 5% of the total global food market[1] and the market for dietary supplements is estimated at more than $60 billion worldwide.[2] In general, the target population of functional foods or dietary supplements is healthy individuals with slightly elevated risk factors or some physical discomfort. However, due to the fast growing market of functional foods and dietary supplements, and the accompanying strong advertising and marketing, also patients on medication may be stimulated to use functional foods or dietary supplements. This may have several consequences for the quality of drug treatment as stated by de Jong *et al.* with the example of the combined intake of phytosterols/-stanols and statins.[3] Whereas they addressed the additive effect of phytosterols/-stanols on reducing low-density lipoprotein (LDL) cholesterol values in patients on statin treatment, their main focus was the possible negative aspects of the combination, such as unfavourable effects on patient adherence with drug treatment and increasing the potential for food-drug interactions.

In the present review we will focus on the possible beneficial effects that functional foods or dietary supplements may have on drug therapy. Because of the large number of subjects treated suboptimally with statins (hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors)[4] and the availability of several functional foods and dietary supplements possibly contributing to the beneficial effects of statin treatment, we will put special emphasis on this group of drugs.

In theory, functional foods or dietary supplements may support drug therapy in three different ways. First, functional foods or dietary supplements may add to the effect that a drug has in reducing risk factors associated with certain conditions or diseases. For the example of statin therapy, statins reduce LDL cholesterol by 18-55% (mean absolute LDL cholesterol reduction: 1.8 mmol/l)[5-8] and phytosterols/-stanols and soluble dietary fibres are thought to reduce LDL cholesterol levels even further when added to the statin treatment.

Second, certain functional foods or dietary supplements may improve risk factors associated with the condition, other than the risk factor that the drug is dealing with. In our example, statins are highly effective in lowering total and LDL cholesterol, but statin monotherapy may not be sufficient to reach goals for triglyceride concentrations. Depending on the type of statin and its dose, triglycerides are lowered only by 7-30%.[5] Supplementing patients with *n*-3 polyunsaturated fatty acids (PUFA) will lower triglycerides and might improve statin therapy, since both cholesterol and triglyceride levels are lowered.

Third, functional foods or dietary supplements may be capable of reducing drug-associated side effects, for example, by restoring depleted compounds. With statin treatment, adverse events such as musculoskeletal complaints have been reported in 1-7% of statin users[9] and it has been hypothesised that statin-induced coenzyme Q_{10} deficiency is involved in this. Supplementing coenzyme Q_{10} might reduce musculoskeletal complaints. Besides, in patients who reach recommended goals for risk factors but experience side effects with drug use, combination therapy of the drug and a

functional food or dietary supplement might be an alternative with the potential of reducing the drug dose and as a result the side effects, while levels of risk factors remain constant. Subsequently, it is conceivable that patients experiencing fewer side effects will have a better adherence to drug treatment. Adherence might also be higher with the combination therapy of a functional food or dietary supplement and a statin compared with a multidrug therapy, as patients might be more willing to slightly modify their diet by replacing normal food items with comparable functional foods, compared with taking another drug; patients' perception of overmedication has been found to correlate with self-report of decreased adherence.[10] Other advantages of the combination therapy with functional foods or dietary supplements compared with multidrug therapy are the lower drug costs and the reduced risk for interactions and serious side effects.[11]

For the present study, we reviewed the data from clinical and observational studies that have investigated the effects of the use of functional foods or dietary supplements in patients on statin treatment. We selected four categories: functional foods or dietary supplements containing 1) phytosterols or phytostanols, 2) soluble dietary fibre, 3) *n*-3 PUFA, and 4) coenzyme Q_{10}. We investigated whether these functional foods or dietary supplements have been demonstrated to support statin therapy in one of the three ways described above.

This review should not be viewed as comprehensive in covering all possible beneficial combination therapies of functional foods or dietary supplements and statins. Rather, the authors' intent is to focus on different mechanisms of action by which functional foods or dietary supplements may support statin treatment and to provide a full coverage of the literature of the examples of combination therapies given.

Literature search

Computerised searches for relevant articles in the PubMed electronic database were performed between March and August 2008, using Medical Subject Heading (MeSH) terms or text words combi*, supple* or interact* with statin*, antilipemic agents, anticholesteremic agents or hydroxymethylglutaryl CoA reductase inhibitors, and combined to one of the search items for the specific functional foods or dietary supplements as noted in **Table 1**. The search was limited to articles written in English or Dutch and studies performed in human subjects. Studies conducted in patients with medical conditions other than hyperlipidaemia, for example, cancer or diabetics, were excluded.

Relevant articles were selected from the title and abstract. Moreover, additional articles were selected from citations in the publications found. Two authors of this report (S.E. and C.R.) independently reviewed the methodological quality of the included trials using the Jadad scoring system to evaluate the effect of study quality on the observed results. This validated scoring system assigns points for randomisation, double-blinding, and documentation of patient withdrawal, as well as additional points for the appropriateness of the randomisation and blinding methods.[12]

Table 1. Literature search

Functional food or dietary supplement	Literature search
Containing phytosterols/-stanols	phytosterols [MeSH], plant sterol*, plant stanol*, phytosterol*, phytostanol*, stanol ester* or sterol ester*
Containing soluble dietary fibre	dietary fiber [MeSH], dietary fiber, dietary fibre, soluble fiber, soluble fibre, beta-glucans [MeSH], psyllium [MeSH], oat*, yeast, barley or pectin
Containing *n*-3 PUFA	omega-3 fatty acids [MeSH], omega-3 fatty acid*, w-3 fatty acid*, n-3 fatty acid*, fish oil or marine oil
Containing coenzyme Q_{10}	ubiquinone [MeSH], ubiquinone, coenzyme Q_{10} or Q_{10}

Trials scoring 3 points or above, out of a maximum of 5, are generally considered to be of good methodological quality. Discrepancies between the two authors were settled through discussion.

In the following section we will first explain our current understanding of the mechanism of action by which the functional foods or dietary supplements may support statin treatment. Subsequently, the effects of the functional food or dietary supplement in the healthy population and approved health claims will be discussed and we will summarise the results of clinical and observational studies exploring the combination therapy. Finally, safety aspects of the combination are addressed.

Phytosterols and phytostanols

Mechanism of supporting statin therapy

Phytosterols and phytostanols lower serum levels of total cholesterol and LDL cholesterol through a different mechanism compared with statins. Whereas statins inhibit hepatic cholesterol synthesis, phytosterols/-stanols reduce the intestinal absorption of cholesterol. Therefore it is thought that both mechanisms work simultaneously when statins and phytosterols/-stanols are taken together. It is generally assumed that phytosterols/-stanols compete with both dietary and biliary cholesterol for solubilisation into mixed micelles. Because phytosterols/-stanols are more hydrophobic than cholesterol, they have a higher affinity for the micelle.[13,14] Other mechanisms proposed are the interference with the cholesteryl ester-mediated hydrolysis process necessary for absorption, and/or stimulation of the ATP-binding cassette (ABC) transporter expression by phytosterols/-stanols.[13,15-17] The ABCG5 and ABCG8 transporters actively transport dietary sterol out of the enterocytes back into the intestinal lumen, thereby limiting the amount of sterol absorbed. ABCA1 may also participate in this process.[18] However, studies in phytosterol- and phytostanol-treated ABCA1- and ABCG5/G8-deficient mice have not demonstrated the involvement of these ABC

transporters in the reduction of intestinal cholesterol absorption.[19] Differences in *ABCG5* and *ABCG8* genes between humans and murines might (partly) explain these results.[20]

Decreased cholesterol absorption is associated with a compensatory increase in cholesterol synthesis and an increase in LDL receptor expression. This elevated expression may not only lead to an increased clearance of LDL from the circulation, but also of intermediate-density lipoprotein (IDL). Because IDL is the precursor of LDL cholesterol, this may ultimately lead to a decreased LDL production. The net result of the lower cholesterol absorption, higher LDL expression and higher endogenous cholesterol synthesis is a reduction in serum total and LDL cholesterol concentration[13,17] (**Figure 1**).

Estimated effects of phytosterols/-stanols on lipid levels and health claims

Phytosterol/-stanol esters have been incorporated in dairy products such as low-fat margarine, milk and yoghurt. Also cereals, bread and orange juice containing esterified or non-esterified phytosterols/-stanols are available on the market. Since 2001, the Adult Treatment Panel of the US National Cholesterol Education Program has recommended the use of phytosterols or phytostanols (2 g/d) in conjunction with other lifestyle changes to enhance LDL cholesterol reduction. The panel states that daily intake of 2-3 g of phytosterol/-stanol esters will reduce LDL cholesterol by 6-15%.[5] Two recent meta-analyses evaluated the LDL cholesterol-lowering effects of phytosterols/-stanols. Both found that LDL cholesterol reduction was approximately 0.33 mmol/l for a mean daily intake of 2.1-2.5 g phytosterols/-stanols.[21,22] Phytosterols/-stanols do not have an effect on triglycerides or high-density lipoprotein (HDL) cholesterol levels.[23,24]

Phytosterols and phytostanols have approved health claims in the USA and in Europe. According to the United States Food and Drug Administration (FDA) there is significant scientific agreement for a consistent, clinically significant effect of phytosterols/-stanols on blood total and LDL cholesterol in both mildly and moderately hypercholesterolaemic (HC) populations. Therefore it has authorised the use of health claims on the association between phytosterol/-stanol esters and reduced risk of CHD on food labels. The claim states that 'Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 1.3 g of phytosterol esters or 3.4 g of phytostanol esters, may reduce the risk of heart disease'.[25,26] Based on the scientific evidence available at the time of evaluation, the FDA made a distinction between the amount of phytosterols and phytostanols necessary to lower total and LDL cholesterol. However, in a clinical trial comparing the cholesterol-lowering efficacy of phytosterols and phytostanols, published shortly after the claim authorisation, no significant difference between esterified phytosterols and phytostanols was found.[27]

Since January 2007, Regulation 1924/2006 applies to nutrition and health claims made in commercial communications in all European Union countries.[28] The European Food Safety Authority (EFSA) was requested to evaluate scientific data on phytosterols and phytostanols in accordance with the Regulation and approved in 2008 health claims stating: 'Phytosterols and phytostanol esters have been shown to lower/reduce blood cholesterol. Blood cholesterol-lowering may reduce

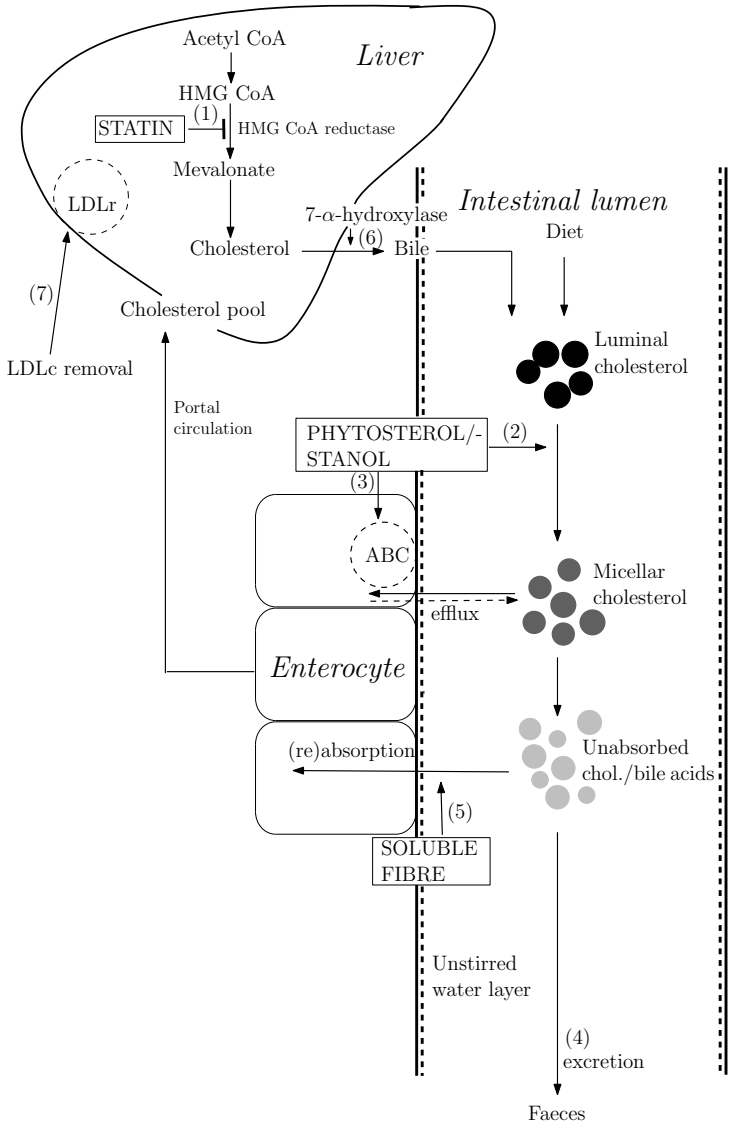

Figure 1. Postulated cholesterol-lowering mechanisms of statins, phytosterols/-stanols and soluble dietary fibre. Statins inhibit the enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase (1). Phytosterols and phytostanols compete with cholesterol for solubilisation into mixed micelles (2), leading to a reduced luminal absorption of cholesterol and/or they induce a higher expression of the ATP-binding cassette (ABC) transporter (3), resulting in an efflux of cholesterol back into the intestinal lumen. Both mechanisms lead to an increased faecal output (4). Soluble dietary fibre interrupts with cholesterol and/or bile acid (re)absorption (5), either by binding bile acids or by forming a thick unstirred water layer in the intestinal lumen, leading to an increased faecal output (4).[13,17] Compensatory up-regulation of the enzyme cholesterol 7-α-hydroxylase (6) increases the conversion of cholesterol into bile acids. All processes will result in a reduction in the cholesterol content of liver cells what will lead to an up-regulation of LDL receptors (LDLr) and ultimately in an increased clearance of circulating LDL cholesterol (LDLc) (7).[59,66,68]

the risk of coronary heart disease'.[29,30] This advice has been provided to the European Commission and member states who will adopt and authorise the health claims.[31] [a]

As concerns safety, the Scientific Committee on Food has assessed phytosterol-enriched foods under the novel foods procedure (European Union Regulation 258/97).[32] They concluded that a maximum level of 8% non-esterified phytosterols, consisting of 30-65% β-sitosterol, 10-40% campesterol, 6-30% stigmasterol and a total of 5% other phytosterols, is safe for human use, also stating that patients on cholesterol-lowering medication should only consume the enriched products under medical supervision.[33] Phytostanols were not assessed through the novel foods procedure as these products were consumed in Finland already before 1997.[34]

Effects of combination therapy with phytosterols/-stanols and statins

Vanhanen[35] was the first to conduct a clinical trial towards the effects of sitostanol esters on lipid levels in patients on pravastatin treatment. It was found that the daily addition of 1.5 g sitostanol ester did not lower serum total or LDL cholesterol after 6 weeks of supplementation. In contrast, subsequent studies, using higher doses, all reported that phytosterols/-stanols in combination with various statins have additive effects on total and LDL cholesterol reduction in patients with (familial) hypercholesterolaemia, as summarised in **Table 2**.

In **Table 2a**, results of clinical studies are presented that investigated the effects of adding phytosterols/-stanols, either in tablet form or incorporated into food products, on lipid levels in patients on (stable) statin treatment. In seven studies, using doses of phytosterols or phytostanols varying from 1.8 g/d to 6.0 g/d and with intervention periods between 4 and 16 weeks, effects were found ranging from a 6% to 10% decrease for total cholesterol and from a 6% to 15% decrease for LDL cholesterol. Absolute reductions in total and LDL cholesterol ranged from 0.31 to 0.62 mmol/l and from 0.30 to 0.67 mmol/l, respectively. The largest reductions were found in a cross-over trial conducted in patients with familial hypercholesterolaemia (FH),[36] although these reductions are probably partly caused by the low-fat spread as the results were not corrected for changes in a placebo-controlled group and no run-in period on placebo spread was used.

The results for total cholesterol were statistically significant for five out of seven studies,[36-40] and either borderline significant (P=0.052)[41] or non-significant[42] for the two remaining studies. Reductions in LDL cholesterol were not significantly different between the intervention and control group only in a single-blind study performed by Castro Cabezas *et al.*[42] This may have been due to the significant reduction in LDL cholesterol in both the intervention and the control group, caused by the nutritional guidelines and low-fat margarines given to both groups, which may have made it more difficult to find significant differences in reductions between the two groups. The methodological quality of this clinical trial was poor based on components assessed by the Jadad Scale.

a Since the paper was accepted, the European Commission and member states have adopted and authorised the health claims for phytosterols/-stanols

The majority of the studies did not find any significant effects of phytosterols/-stanols on HDL cholesterol or triglycerides, nor were the effects of phytosterols different compared with the effects of phytostanols. However, Ketomaki *et al.* found in a study consisting of two consecutive 4-week intervention periods with either a phytostanol ester or a phytosterol ester that only during the sterol ester period HDL cholesterol increased and triglyceride levels decreased significantly.[36] This study achieved a Jadad score of 3; no placebo-controlled group was included in this study, possibly leading to flawed results.

Table 2b shows the results of studies investigating the differences in effects that phytosterols/-stanols have on lipid levels in statin users and statin non-users. All studies have demonstrated that if phytosterols/-stanols are added to a statin, the effect on cholesterol reduction is similar[40,43] or even higher[44] compared with the effect observed with the use of the phytosterols/-stanols alone.

De Jong *et al.*[45] and Wolfs *et al.*[46] also investigated the cholesterol-lowering effects of phytosterol- and phytostanol-enriched margarine (no differentiation between phytosterols and phytostanols) between statin users and statin non-users in a post-launch monitoring setting over 5 years. These authors suggest that phytosterols/-stanols have an additive effect to the drug, although significance levels were not reached because of the small number of combination users in the studies. Moreover, Simons[40] performed a 2x2 factorial study with four parallel treatment arms, aiming to distinguish between an additive effect and an interactive effect between phytosterol ester margarine and cerivastatin. Statistical analysis showed no evidence of an interactive effect and therefore the authors concluded that, although a small interaction between the two compounds could not be excluded, it is unlikely that this interaction is of any clinical importance.

In **Table 2c**, the pooled results are given of studies not differentiating between statin users and statin non-users. Moreover, statin use was not quantified and cholesterol-lowering effects of phytosterols/-stanols in normal, HC or FH patients were put together. All studies described significant reductions in total and LDL cholesterol levels and suggested that the phytosterols/-stanols were effective in both statin users as well as statin non-users.[47-49]

In summary it can be concluded that phytosterol and phytostanol esters are an effective approach to lower cholesterol levels in addition to statin treatment in both HC and FH patients on statin treatment. Cholesterol-lowering is at least equally effective in statin users compared with non-users. The addition of 2-5 g phytosterol/-stanol esters per day to statins will result in an additive LDL and total cholesterol reduction of roughly 10% (or 0.40 mmol/l) and 6% (or 0.35 mmol/l) respectively, without significant changes in HDL cholesterol or triglyceride levels. Effects in FH patients might even be slightly greater, although well-designed randomised double-blind trials are needed to confirm this hypothesis. Differences in cholesterol-lowering effects of phytosterols/-stanols between the different studies might be explained by baseline cholesterol levels, because it has been hypothesised that patients with high baseline cholesterol levels experience a larger reduction in cholesterol levels after sterol or stanol ester consumption.[36] Moreover, it is suggested that patients with high ratios of serum cholestanol and phytosterols to cholesterol (markers for cholesterol absorption) may benefit the most from phytosterol/-stanol intake.[50] A synergistic effect between statins and phytosterols/-stanols should not be expected.[51]

Table 2. Clinical studies towards the effects on lipid levels of the combination therapy with statins and phytosterols or –stanols

Table 2a. Effects of phytosterols/-stanols in statin users

Author	Type of study	Jadad score	Subjects	Phytosterol/-stanol / Control intervention
Vanhanen (1994)[35]	DB, PC, R	3	HC on pravastatin ≥ 1 yr (*n*=14)	1.5 g/d sitostanol ester mayonnaise (*n*=7) / P: rapeseed oil-based mayonnaise (*n*=7)
Richter (1996)[39]	R, OL	1	HC on lovastatin for 16 weeks (*n*=30)	6.0 g/d beta-sitosterol tablets (*n*=15) /- (*n*=15)
Blair *et al.* (2000)[37]	DB, PC, R	4	HC on stable statin therapy ≥ 3 mo (*n*=167)	5.1 g/d phytostanol ester spread (*n*=83) / P: canola oil-based spread (*n*=84)
Simons (2002)[40]	DB, PC, R	4	HC (*n*=75) §	Cerivastatin + 2 g/d phytosterol ester spread (*n*=37) / Cerivastatin + P: regular spread (*n*=38)
Ketomaki *et al.* (2005)[36]	DB, R, AC, CO	3	FH on stable statin therapy ≥ 2 mo (*n*=18)	2 g/d phytostanol and 2 g/d phytosterol ester spread, CO (*n*=18)
Castro Cabezas *et al.* (2006)[42]	SB, PC, R	1	HC on stable statin therapy ≥ 6 mo (*n*=20)	3 g/d phytostanol ester spread (*n*=11) / P: regular spread (*n*=9)
Goldberg *et al.* (2006)[38]	DB, PC, R	4	HC on stable statin therapy ≥ 3 mo (*n*=26)	1.8 g/d soy stanol tablets (*n*=13) / P: starch containing tablets (*n*=13)
de Jong *et al.* (2007)[41]	DB, PC, R	4	HC on statins (*n*=41)	2.5 g/d phytostanol (*n*=15) or sterol ester spread (*n*=15) / P: 'light' spread (*n*=11)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; SB, single blind; OL, open-label; PC, placebo controlled; R, randomised; AC, active controlled; CO, cross-over; FH, familial hypercholesterolaemic; HC, hypercholesterolaemic; P, placebo; sta, phytostanol; ste, phytosterol

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns, not significant

† The net change in lipid levels was calculated by subtracting the mean change from baseline after control intervention from the mean change from baseline after phytosterol/-stanol intervention, except for the study of Ketomaki *et al.*[36] where the net change is the mean change from baseline after phytosterol and -stanol intervention

‡ Borderline significant

§ The study of Simons[40] is a 2x2 factorial design study with 4 parallel arms. In Table 2a the net change is calculated by subtracting the mean change from baseline after statin intervention from the mean change from baseline after combined intervention of phytosterols and statins

|| No significant difference between sterol and stanol ester

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
6 wk		-0.17		-0.07		ns		ns
12 wk	-7.4	-0.54*	-10.3	-0.55*		ns		ns
8 wk	-6.9	-0.41***	-10.0	-0.36***		ns		ns
4 wk	-5.7*		-6.1*			ns		ns
4 wk, 4 wk (CO)	-9.8	-0.62*‖	-14.8	-0.67*‖	ste: 8.7	sta: ns ste: 0.11**	ste: -11.8	sta: ns ste: -0.14**
6 wk	-6.6	-0.40	-7.9	-0.30		ns		ns
6 wk	-5.7	-0.31*	-9.1	-0.32**		ns		ns
16 wk	-6.9	-0.39‡‖	-10.3	-0.34*‖		ns		ns

Safety aspects of combination therapy with phytosterols/-stanols and statins

In none of the studies were adverse effects found related to the use of phytosterol/-stanol-enriched products in combination with statin therapy. However, in studies towards the effects of phytosterols alone, it has been found that serum phytosterol concentration is elevated after consumption of phytosterols (unlike phytostanols) with potential atherogenic effects.[52] Normally, only 5-15% of the phytosterols are absorbed in the intestinal tract.[51,53] Patients with the rare autosomal recessive disease phytosterolaemia, however, are hyperabsorbers of phytosterols and should therefore not consume products containing high amount of phytosterols, whether added to statin therapy or not. In healthy subjects, it is assumed that the beneficial effects on cholesterol levels of phytosterols outweigh any potential atherosclerotic risk, although additional research on this topic is urgently warranted.[54,55]

Table 2. Clinical studies towards the effects on lipid levels of the combination therapy with statins and phytosterols or –stanols

Table 2b. Difference in effects of phytosterols/-stanols between statin users and statin non-users

Author	Type of study	Jadad score	Subjects		Phytosterol/-stanol / Control intervention
			Statin therapy	No statin therapy	
Gylling *et al.* (1997)[43]	DB, R	2	CHD ♀ on simvastatin ≥ 1 yr (*n*=10)	CHD ♀ (*n*=11)	3 g/d sitostanol ester rapeseed oil-based spread (*n*=21)
Vuorio *et al.* (2000)[44]	OL	1	FH on simvastatin ≥ 90 d (*n*=12)	FH (*n*=4)	2.2 g/d stanol ester rapeseed oil-based spread (*n*=16)
Simons (2002)[40]	DB, PC, R	4	HC (*n*=76) ‡		Cerivastatin + 2 g/d phytosterol ester spread (*n*=37) / 2 g/d phytosterol ester spread + P: placebo drug (*n*=39)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; PC, placebo controlled; OL, open-label; R, randomised; FH, familial hypercholesterolaemic; HC, hypercholesterolaemic; CHD, patients with coronary artery disease; P, placebo
**P*<0.05; ***P*<0.01; ****P*<0.001; ns, not significant
† The net change in lipid levels was calculated by subtracting the mean change from baseline after phytosterol/-stanol intervention in statin non-users from the mean change from baseline after phytosterol/-stanol intervention in statin users.
‡ The study of Simons[40] is a 2x2 factorial design study with 4 parallel arms. In Table 2b the net change is calculated by subtracting the mean change from baseline after phytosterol intervention in patients on placebo drug from the mean change from baseline after phytosterol intervention in patients on cerivastatin
§ In both groups significant reduction
|| Simvastatin-treated patients 7 weeks, not treated patients 12 weeks
¶ Simvastatin-treated patients 6 weeks, not treated patients 12 weeks

Furthermore, both phytosterols and phytostanols are associated with reductions in plasma concentrations of α-carotene, β-carotene, lycopene and α-tocopherol. Reductions in all vitamins, except for β-carotene, can be explained by reductions in LDL cholesterol, the main lipoprotein carrier. Negative health effects related to these reductions are not expected,[51] although it might be a concern for groups with high nutritional needs such as elderly and pregnant women. These groups can be advised to add an extra amount of fruits and vegetables to the diet.

Post-launch monitoring of phytosterols/-stanols upon request of the European Commission did not indicate adverse effects,[56] thereby supporting the safety of these products.[33]

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
7 wk, 12 wk‖	1.7	0.24§	2.4	0.23§		ns		ns
6 wk, 12 wk¶	-3.1	0.18**§	-8.5	0.08***§		ns		ns
4 wk	1.8		4.1			ns		ns

Soluble dietary fibre

Mechanism of supporting statin therapy

Dietary fibres are associated with a reduced risk of CHD. Soluble fibre appears to be primarily responsible for the cholesterol-lowering effect of dietary fibre intake.[57] Studies in HC patients without treatment with cardiovascular drugs showed that the addition of soluble fibres (psyllium[58,59], β-glucan[60-63], guar gum[64,65], pectin[66]) to a low-fat, low-cholesterol diet was an effective approach to reduce total and LDL cholesterol. The mechanisms involved are not completely understood, but it is suggested that soluble fibres reduce plasma cholesterol by interrupting with cholesterol and/or bile acid (re)absorption.[67] Some authors suggest that soluble fibres bind bile acids; others assume that water-soluble fibres form a thick unstirred water layer in the intestinal lumen. Both proposed mechanisms will lead to an increased faecal output of bile acids, resulting in a reduction in bile acids available for transport back to the liver. Compensatory up-regulation of hepatic enzymes such as cholesterol 7-α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, results in a reduction in the cholesterol content of liver cells. This leads to an up-regulation of the LDL receptors and the enzyme HMG-CoA reductase to re-establish hepatic cholesterol stores, ultimately resulting in an increased clearance of circulating LDL cholesterol[59,66,68] (**Figure 1**). Other suggested mechanisms include the inhibition of cholesterol synthesis by short-chain fatty acids (mainly propionate), which are the major fermentation products of soluble fibre, the increased intestinal viscosity causing lowered glucose absorption and thereby improved insulin sensitivity, and the increased satiety leading to lower overall energy intake.[66,68] These postulated mechanisms differ from the cholesterol-lowering mechanism of statins, and therefore both compounds may decrease cholesterol levels simultaneously.

Table 2. Clinical studies towards the effects on lipid levels of the combination therapy with statins and phytosterols or –stanols

Table 2c. Effects of phytosterols/-stanols in combined group of statin users and statin non-users

Author	Type of study	Jadad score	Subjects		Phytosterol/-stanol / Control intervention
			Statin therapy	No statin therapy	
Neil *et al.* (2001)[48]	DB, PC, R	5	FH on statins (*n*=30)	HC (*n*=32)	2.5 g/d phytosterol spread (*n*=31) / P: mixed oil-based spread (*n*=31)
Amundsen *et al.* (2004)[47]	OL	1	FH on statins (*n*=19)	FH (*n*=1)	1.5 g/d phytosterol ester spread (*n*=20)
O'Neill *et al.* 2004)[49]	DB, PC, R	4	FH on statins (*n*=69)	Unaffected (*n*=65)	2.6 g/d phytostanol ester spread and bar (*n*=46) / 1.6 g/d phytosterol ester spread + P: regular bar (*n*=46) / 1.6 g/d phytostanol ester spread + P: regular bar (*n*=42)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; PC, placebo controlled; OL, open-label; R, randomised; FH, familial hypercholesterolaemic; HC, hypercholesterolaemic; P, placebo

*P<0.05; **P<0.01; ***P<0.001; ns, not significant

† The net change in lipid levels was calculated by subtracting the mean change from baseline after control intervention from the mean change from baseline after phytosterol/-stanol intervention in a combined group of statin users and statin non-users, except for the studies of Amundsen *et al.*[47] and O'Neill *et al.*[49] where the net change is the mean change from baseline after phytosterol/-stanol intervention

‡ No significant difference between sterol or stanol ester or between high and low dose stanol

Estimated effects of soluble dietary fibre on lipid levels and health claims

In a meta-analysis, it was estimated that 2-10 g soluble fibre per day significantly lowers total and LDL cholesterol concentrations by 0.045 mmol/l and 0.057 mmol/l, respectively.[66] Various soluble fibres, including oat products, psyllium, pectin and guar gum, reduce total and LDL cholesterol by similar amounts; the effects depend on the food matrix used, the method of food processing and the concentration, water-solubility and molecular weight of the fibres.

In 1997 the FDA adopted health claims on the labels of foods containing β-glucan soluble dietary fibre from whole oats noting that these foods, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease by reducing total and LDL cholesterol. Since then, this claim has been extended by adding psyllium seed husk, whole-grain barley products and barley β-fibre as additional eligible sources of soluble fibre. The FDA states that the food products must provide at least 0.75 g of β-glucan soluble fibre or 1.7 g of psyllium soluble fibre per serving.[69,70]

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
8 wk	-7.8	-0.57**	-10.0	-0.51***		ns		ns
26 wk	-9.1	-0.53**	-11.0	-0.45*	-10.6	-0.13***		ns
8 wk	-8.5	-0.5**‡	-8.1	-0.31*‡		ns		ns

In Europe, member states like Sweden, the Netherlands and UK, have also approved claims linking oat soluble fibre consumption and reduced total and LDL cholesterol.[71-73] This has led to the introduction of several food products enriched with soluble fibre, including bread, cereals and cookies. However, in the context of Regulation 1924/2006, the claims currently used in the different member states need to be reviewed by the EFSA.[28 a]

Effects of combination therapy with soluble dietary fibre and statins

Results of studies exploring the combination therapy with soluble dietary fibre and statins are shown in **Table 3**. All studies scored less than 4 points on the Jadad scale.

Table 3a shows the effects on lipid levels of soluble fibre in statin users. One of the first studies performed towards this combination found that in three female HC patients, the addition of pectin to treatment with lovastatin resulted in an average rise in LDL cholesterol of 42%. After the intake of pectin was stopped, levels returned to normal. Also after the addition of oat bran to lovastatin, LDL cholesterol levels rose strikingly in two patients in the same study. The authors concluded that both fibres might reduce the bioavailability of the statin.[74] Further studies towards this specific combination have not been performed, but Uusitupa *et al.* studied the effects of guar gum in a population of both FH and non-FH patients on lovastatin treatment and reported that adding guar

a Since the paper was accepted, the European Food Safety Authority (EFSA) has evaluated and approved health claims for oat and barley β-glucans

Table 3. Clinical studies towards the effects on lipid levels of the combination therapy with statins and soluble dietary fibre

Table 3a. Effects of soluble dietary fibre in statin users

Author	Type of study	Jadad score	Subjects	Soluble dietary fibre
Richter *et al.* (1991)[74]	OL	- ‡	HC ♀ (*n*=3) on lovastatin	Pectin 15 g/d (*n*=3)
Uusitupa *et al.* (1991)[75]	OL	1	HC (*n*=31) on lovastatin 80 mg/d for 18 wk	Guar gum tablets 5-20 g/d (*n*=31)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; OL, open-label; HC, hypercholesterolaemic
*P<0.05; **P<0.01; ***P<0.001; nm, not measured/calculated; ns, not significant
† The net change in lipid levels is the mean change from baseline after soluble dietary fibre intervention
‡ Jadad Score was not estimated because description of the study design has not been published (study was interrupted after 3 patients)

Table 3b. Difference in effects of a statin plus soluble dietary fibre versus a statin alone

Author	Type of study	Jadad score	Subjects	Soluble dietary fibre / Control intervention
Moreyra *et al.* (2005)[77]	DB, PC, R	3	HC (*n*=46)	Simvastatin 10 mg/d + psyllium-powder drink 15 g/d (*n*=23) / simvastatin 10 mg/d + P (*n*=23)
Jayaram *et al.* (2007)[78]	OL, R	2	HC (*n*=97)	Atorvastatin 10 mg/d + psyllium-powder drink 11.2 g/d (*n*=49) / atorvastatin 10 mg/d (*n*=48)
Agrawal *et al.* (2007)[79]	OL, R	3	Unaffected ♂ (*n*=24)	Lovastatin 20 mg/d + psyllium-powder drink 10 g/d (*n*=12) / lovastatin 20 mg/d (*n*=12)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; PC, placebo controlled; OL, open-label; R, randomised; HC, hypercholesterolaemic; P, placebo
*P<0.05; **P<0.01; ***P<0.001
† The net change in lipid levels was calculated by subtracting the mean change from baseline after control intervention from the mean change from baseline after soluble dietary fibre intervention

gum resulted in significant reductions in serum total and LDL cholesterol. However, the results might be flawed because no placebo-group was included and no correction for food intake was made.[75,76]

Table 3b presents the results of three studies comparing the effects on lipid values of the combination therapy of a statin and soluble dietary fibre *vs.* statin treatment alone. Whereas total and

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
4 wk	nm		42		nm		nm	
18 wk	-14	-1.0***	-18	-0.9***	ns		-7.7	-0.1

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
8 wk	-3.9	-0.24*	-5.1	-0.21*	-9.1	-0.13**	8.7	0.07
12 wk	-4.4	-0.28	-8.6	-0.35*	-6.4	-0.06	0.99	0.06
4 wk	-6.7	-0.3	-8.6	-0.2	-7.1	-0.07	6.7	0.08

LDL cholesterol-lowering effects of the soluble fibres reached statistical significance in the studies performed by Moreya *et al.*[77] and Jayaram *et al.*,[78] only trends towards an additive effect were observed by Agrawal *et al.*[79] It should be noted that this last trial was conducted in healthy adult men, and therefore results may differ from effects observed in the other studies performed in HC patients. Of note is that in two out of the three studies a blunting of the statin-associated increase in HDL cholesterol was observed after addition of the soluble dietary fibre.[77,79]

In conclusion we can say that studies towards the possible beneficial effects of soluble dietary fibre on statin therapy are scarce. Most clinical studies have reported negative associations between

the use of soluble fibre supplements in combination with statins and LDL or total cholesterol concentrations. However, also unfavourable reductions in statin bioavailability and reductions in HDL cholesterol have been described after high intakes of soluble fibre. At this moment, there is not sufficient evidence to recommend the use of functional foods or dietary supplements enriched with soluble fibres to patients using statins. Clinical studies are warranted to further elucidate the potentials of the combination therapy with soluble dietary fibre and statins. Research should focus on the effects of different sources of soluble fibre in combination with various statins on lipoprotein subclasses and drug bioavailability. Caution should be taken to interpret the direct effects of fibre supplements instead of possible accompanying effects of reduced dietary fat and cholesterol intake. Also studies investigating the mechanisms of combined action and a possible dose-response relationship between the combination therapy and cholesterol levels are needed.

Safety aspects of combination therapy with soluble dietary fibre and statins

Soluble fibre supplementation is generally considered as well tolerated. Side effects observed are mostly related to the gastrointestinal tract, such as abdominal distention, flatulence and diarrhoea. Also some negative nutritional impacts of high soluble fibre intake have been reported, as soluble fibres may interact with vitamins and minerals, resulting in a lower bioavailability of these compounds. However, there are insufficient data to firmly draw conclusions about this matter. Most likely, the effect of the fibre depends on the type of mineral or vitamin, the intestinal transit time and the degree of bacterial fibre degradation in the gut.[64,80,81]

The combination therapy with soluble fibre and statins may also have some safety limits, while unfavourable reductions in HDL cholesterol have been described and, in one study, reduced statin absorption from the gut was suggested after a high intake of soluble fibre.[74] Studies towards the effects of soluble fibres on the bioavailability of statins and other drugs are scarce and results depend greatly upon the type of drug and fibre. Also the time of drug administration in relation to food intake may influence the bioavailability of the drug. Soluble fibres may influence the bioavailability of statins and other drugs by direct binding or by altering luminal pH, gastric emptying, intestinal transit, mucosal absorption and metabolism of the drug.[58,82]

n-3 PUFA

Mechanism of supporting statin therapy

In recent years a lot of research has been performed towards the association between intake of *n*-3 PUFA and reduction in CHD. *n*-3 PUFA operate via several mechanisms. One of the most important is the favourable effect of *n*-3 PUFA on very low-density lipoprotein (VLDL) cholesterol and triglyceride levels. In a meta-analysis of seventeen population-based prospective studies it was estimated that after adjustment for other risk factors, a 1 mmol/l increase in serum triglycerides is associated with a 14% increase in CVD risk in men and 37% in women.[83] Statins efficiently reduce

total and LDL cholesterol, but have only limited triglyceride-lowering effects. Thus, a combined intake of *n*-3 PUFA and a statin might be beneficial in improving the lipid profile in patients with high triglyceride levels. The favourable decrease in triglyceride levels caused by *n*-3 PUFA is probably due to reduced hepatic VLDL and triglyceride synthesis and secretion, and enhanced triglyceride clearance from chylomicrons and VLDL particles. Reduced synthesis might be due to increased rates of mitochondrial and/or peroxisomal β-oxidation or a decreased expression of sterol regulatory element-binding protein-1c, a transcription factor involved in the regulation of fatty acid-synthesising enzymes. Both mechanisms will result in a reduction in the availability of the substrate, i.e. fatty acids. Increased clearance is possibly caused by increased lipoprotein lipase activity due to increased peroxisome proliferator-activated receptor (PPAR)-γ and/or PPAR-α gene expression. Activation of PPAR leads to increased fatty acid β-oxidation in the liver and skeletal muscle.[84-86]

Other mechanisms by which *n*-3 PUFA may lower the risk of CHD include reductions in platelet aggregation, blood viscosity and ischemia and their anti-thrombotic, fibrinolytic and anti-inflammatory activities. Moreover, *n*-3 PUFA appear to play an important role in the prevention of arrhythmias.[87,88]

Estimated effects of n-3 PUFA on lipid levels and health claims

In a recent meta-analysis of twenty-one randomised controlled trials it was estimated that *n*-3 PUFA consumption resulted in significant changes in triglycerides of -0.31 mmol/l, in HDL cholesterol of +0.04 mmol/l and in LDL cholesterol of +0.16 mmol/l. There was no effect on total cholesterol.[89] It has been suggested that the unfavourable increase in LDL cholesterol is attributable to the increased conversion of VLDL to IDL and LDL, and the conversion of IDL to LDL after *n*-3 PUFA supplementation.[90,91]

In September 2004 the FDA announced a qualified health claim for food products containing the *n*-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[92] According to the FDA there is supportive, but not conclusive, scientific evidence that suggests a reduction in CHD as a result of eating food or supplements rich in *n*-3 PUFA. The FDA judged that *n*-3 PUFA generally reduce triglycerides and VLDL cholesterol, and have no effect on total or HDL cholesterol in both general and diseased populations. The EFSA has not yet evaluated health claims on *n*-3 PUFA and cardiovascular function.[a]

Effects of combination therapy with n-3 PUFA and statins

Results of clinical studies that have investigated the combination therapy with *n*-3 PUFA and statins are summarised in **Table 4**. Contacos *et al.* were the first to demonstrate a beneficial effect of the combination of *n*-3 PUFA and statin therapy in HC patients.[93] They found that in patients

a Since the paper was accepted, the European Food Safety Authority (EFSA) has evaluated health claims for *n*-3 PUFA

Table 4. Clinical studies towards the effects on lipid levels of the combination therapy with statins and *n*-3 polyunsaturated fatty acids (PUFA)

Table 4a. Effects of *n*-3 PUFA in statin users

Author	Type of study	Jadad score	Subjects	*n*-3 PUFA / Control intervention
Contacos *et al.* (1993)[93]	OL	1	HC on pravastatin for 6 wk (*n*=9)	3 g/d PUFA oil§ (*n*=9)
Nordoy *et al.* (1998)[101]	DB, PC, R	4	HC on simvastatin for 5 or 10 wk (*n*=42)	4 g/d PUFA capsules‖ (*n*=22) / P: corn oil capsules (*n*=20)
Nakamura *et al.* (1999)[104]	OL	0	HC on various statins for 30 ± 6 mo (*n*=14)	0.9 – 1.8 g/d EPA capsules (*n*=14)
Durrington *et al.* (2001)[97]	DB, PC, R	4	CHD on stable statin therapy ≥ 3 mo (*n*=59)	4 g/d PUFA capsules‖ (*n*=30) / P: corn oil capsules (*n*=29)
Nordoy *et al.* (2001)[105]	DB, PC, R	4	HC on atorvastatin for ≥ 10 wk (*n*=42)	2 g/d PUFA capsules‖ (*n*=22) / P: corn oil capsules (*n*=20)
Hong *et al.* (2004)[99]	DB, PC, R	4	HC on simvastatin for 6-12 wk (*n*=40)	3 g/d PUFA capsules (*n*=20) / P: rapeseed oil capsules (*n*=20)
Meyer *et al.* (2007)[100]	DB, PC, R	2	HC on stable statin therapy ≥ 3 mo (*n*=27)	2.16 g/d DHA oil (*n*=13) / P: olive oil (*n*=14)
Davidson *et al.* (2007)[103]	DB, PC, R	5	HC on stable statin therapy ≥ 2 mo (*n*=254)	Simvastatin + 4 g/d PUFA capsules ‖ (*n*=122) / simvastatin 40 mg/d + P: vegetable oil capsules (*n*=132)¶

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; OL, open-label; PC, placebo controlled; R, randomised; HC, hypercholesterolaemic; CHD, patients with coronary artery disease; P, placebo; nm, not measured or calculated

*P<0.05; **P<0.01; ***P<0.001; ns, not significant

† The net change in lipid levels was calculated by subtracting the mean change from baseline after control intervention from the mean change from baseline after *n*-3 PUFA intervention, except for the studies of Contacos *et al.*[93] and Nakarmura *et al.*[104] where the net change is the mean change from baseline after *n*-3 PUFA intervention

‡ Borderline significant

§ EPA 67%, DHA 33%

‖ EPA 45-48%, DHA 36-39%

¶ At inclusion simvastatin replaced any previous statin

randomised to either pravastatin, *n*-3 PUFA or placebo for 6 weeks, an additional 12 weeks of combination therapy with *n*-3 PUFA and pravastatin further decreased plasma triglycerides and LDL cholesterol by 33% (P<0.05) and 26% (P<0.01), respectively, in patients in the placebo-group, whereas in patients already on pravastatin only triglyceride levels were non-significantly reduced

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
12 wk	-5.0	-0.3	9.7	0.3	6.9	0.07	-33.0	-1.6
5 wk	-9.8	-0.55‡		nm	11.3	0.13	-43.3	-1.2**
3 mo	-11	-0.61*		nm	8.9	0.11*	-48	-0.99**
24 wk	-13.9	-0.8	-10.5	-0.4	-27.3	-0.3	-26.5	-1.2**
5 wk	4.7	0.3	4.4	0.15	5.7	0.06*	6.0	0.22
8 wk	-3.7	-0.19	-5.0	-0.12	5.0	0.05	-16	-0.59**
3 wk	-8.3	-0.38	-10.0	-0.25	-9.9	-0.10	-17.2	-0.44*
8 wk	-3.2	-0.17**	5.3	0.09‡	5.2	0.06***	-24.7	-0.78***

by 33% and in patients in the *n*-3 PUFA group only LDL cholesterol levels were reduced by 24% ($P<0.05$). Total cholesterol levels showed similar changes to LDL cholesterol after combination therapy. This study indeed showed that statins particularly lowered total and LDL cholesterol, whereas *n*-3 PUFA lowered triglycerides and not cholesterol levels. Combination therapy reduced both cholesterol and triglyceride concentrations. These beneficial effects of *n*-3 PUFA on triglyceride levels have been confirmed in later studies.[94-104]

Table 4a shows the results of studies examining the effects of supplementing patients on statin therapy with *n*-3 PUFA.[93,97,99-101,103-105] All studies used EPA and/or DHA, in doses varying from 0.9 to 1.8 g/d and 0.78 to 2.16 g/d for EPA and DHA, respectively. All studies found significant reductions in triglycerides, ranging from 16% (or 0.44 mmol/l) to 48% (or 1.2 mmol/l), after supplementing *n*-3 PUFA, except one study performed by Nordøy *et al.* in which no triglyceride-lowering effect was attributable to the *n*-3 PUFA.[105] In this study relatively low doses of *n*-3 PUFA (0.9 g/d EPA, 0.78 g/d DHA) were used, which could explain these results. However, one small,

Table 4. Clinical studies towards the effects on lipid levels of the combination therapy with statins and *n*-3 polyunsaturated fatty acids (PUFA)

Table 4b. Difference in effects of a statin plus *n*-3 PUFA versus a statin alone

Author	Type of study	Jadad score	Subjects	*n*-3 PUFA / Control intervention
Davidson *et al.* (1997)[96]	DB, PC, R	2	HC (*n*=19)	Simvastatin 10 mg/d + 5 g/d PUFA capsules§ (*n*=9) / simvastatin + P (*n*=10)
Grekas *et al.* (2001)[98]	OL	1	Renal trans-plant HC (*n*=24)	Pravastatin 20 mg/d (*n*=24) and pravastatin 20 mg/d + 1 g/d PUFA oil (*n*=24)Σ
Chan *et al.* (2002)[95]	DB, PC, R	2	IR obese ♂ (*n*=24)	Atorvastatin + 4 g/d PUFA capsules‖ (*n*=11) / atorvastatin + P: corn oil capsules (*n*=13)
Yokoyama *et al.* (2007)[102]	OL, R	3	HC (*n*=18,645)	Prava- or simvastatin + 1.8 g/d EPA capsules (*n*=9326) / prava- or simvastatin (*n*=9319)

TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglycerides; DB, double blind; OL, open-label; PC, placebo controlled; R, randomised; HC, hypercholesterolaemic; CHD, patients with coronary artery disease; IR, insulin-resistant; P, placebo; nm, not measured or calculated
**P*<0.05; ***P*<0.01; ****P*<0.001; ns, not significant
† The net change in lipid levels was calculated by subtracting the mean change from baseline after control intervention from the mean change from baseline after *n*-3 PUFA intervention
‡ Borderline significant
§ EPA 60%, DHA 40%
‖ EPA 45-48%, DHA 36-39%
¶ Values are non-HDL cholesterol
Σ All patients received the same therapeutic protocol consisting of 4 weeks diet, 8 weeks diet+statin, 4 weeks diet, 8 weeks diet+statin+PUFA

uncontrolled study (Jadad score: 0), in which twelve patients were supplemented with 0.9 g EPA per day and two patients with 1.8 g EPA per day, showed highly significant reductions in triglycerides. In addition, in this study it was found that total cholesterol levels were significantly reduced and HDL cholesterol was significantly increased after EPA supplementation.[104] Most studies performed in patients on statin therapy did not find any significant changes in total, LDL or HDL cholesterol, although in some studies VLDL cholesterol was decreased.[93,97,100] In the COMBOS (**COMB**ination of prescription **O**mega-3 with **S**imvastatin) study,[103] administration of *n*-3-acid ethyl esters plus simvastatin improved, besides triglyceride levels, also total, HDL and VLDL cholesterol to a greater extent than simvastatin alone. On the unfavourable side, a trend was observed towards a greater reduction in LDL cholesterol in the simvastatin-only group (0.7% *vs.* -2.8%, *P*=0.052).

Table 4b shows the results of studies comparing the effects on lipid values of a combination therapy of a statin and *n*-3 PUFA *vs.* statin treatment alone. Davidson *et al.*[96] found that after treating HC patients with *n*-3 PUFA and/or simvastatin for 12 weeks, the triglyceride responses

Study duration	Net change in lipid levels†							
	TC		LDL		HDL		TG	
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l
12 wk	-0.36	-0.11	1.0	-0.10¶	3.2	0.05	-10.3	-0.14‡
8 wk	8.3	0.67	-0.09	0.05	4.3	0.05	-14.6	-0.26**
6 wk	-0.2	-0.2	4.9	0.08	9.6	0.11*	-13.7	-0.3***
5 yr	ns		ns		ns		-5.0***	

were similar in the EPA/DHA-group (-25.3%) and the combined group (-28.8%), and borderline significantly lower in the simvastatin group (-18.5%), whereas decreases in non-HDL cholesterol and increases in HDL cholesterol were statistically significant only for the combined (non-HDL: -24.8%, HDL: +10.4%) and simvastatin group (non-HDL: -25.8%, HDL: +7.2%). All other studies found significant improvements of triglycerides with a combination therapy compared with the statin therapy alone.[94,95,98,102,103] Study populations included, besides HC patients, renal transplant patients with persistent hypercholesterolaemia[98] and insulin-resistant obese men with dyslipidaemia.[94] In this last study, also atorvastatin alone significantly decreased triglyceride levels. The authors suggest that the two compounds reduce triglyceride levels through different mechanisms. Whereas *n*-3 PUFA reduced the hepatic secretion of VLDL-apoB, atorvastatin enhanced the clearance of all apo B-containing lipoproteins, resulting in an additive effect.[94,95]

Aligeti *et al.* performed a retrospective cohort study in which they compared the change in plasma triglyceride levels between patients taking fish oil as monotherapy and patients who added fish oil to their usual lipid-lowering drugs, including statins.[106] They found that adding fish oil to a statin alone or to multiple lipid-lowering drugs (combination of niacin, statin and/or fibrates) did not alter the triglyceride-lowering effects of fish oil and effects are therefore additive.

One study compared the effects of the combination therapy and statins as monotherapy on clinical endpoints and found in the combined group a statistically significant 19% relative reduction in major coronary events, particularly unstable angina and non-fatal coronary events. This applied in both patients taking statins for primary prevention as for secondary prevention.[102]

Few studies have examined the effects of combined treatment of *n*-3 PUFA and statins in patients with FH. Sandset *et al.* found no additional effects of adding *n*-3 PUFA (4 g/d for 6 weeks) to simvastatin (40 mg/d) in a small uncontrolled study (*n*=13); triglycerides even tended to increase on additional *n*-3 PUFA.[107] Also in another small study (*n*=14) in FH patients on chronic simvastatin treatment, triglycerides were not significantly decreased after *n*-3 PUFA supplementation (5.1 g/d).[108] Small sample sizes or the population under study may explain these results.

In conclusion we can say that all clinical studies conducted in HC patients suggest that after combined intake of *n*-3 PUFA and statins no diminution of the separate effects of the compounds is expected, but that they improve lipid levels simultaneously through different mechanisms. Whereas statins alone have little effect on triglyceride levels, adding *n*-3 PUFA to the statin regimen lowered triglycerides significantly in most of the studies. Higher doses of *n*-3 PUFA and higher baseline triglyceride levels appear to be associated with greater reductions. In some, but not all studies, HDL cholesterol was significantly increased and VLDL cholesterol was significantly decreased after supplementation with *n*-3 PUFA. None of the studies found a significant favourable effect of *n*-3 PUFA on LDL cholesterol and in some studies LDL cholesterol even tended to increase after *n*-3 PUFA supplementation, contributing to the hypothesis that *n*-3 PUFA increase the conversion of VLDL to LDL. Effects of combined treatment with *n*-3 PUFA and statins in FH patients are less clear and studies examining these effects in larger populations are warranted.

Safety aspects of combination therapy with n-3 PUFA and statins

In none of the studies were adverse effects seen after combination therapy of *n*-3 PUFA and statins other than the adverse effects caused by monotherapy of the compounds. *n*-3 PUFA were usually well tolerated and serious events have not been observed. Potential adverse effects related to *n*-3 PUFA include an increased bleeding time because of interference with platelet function, gastrointestinal disturbances and increases in LDL cholesterol.[103,109-111]

COENZYME Q_{10}

Mechanism of supporting statin therapy

Statins act by inhibiting the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis that catalyses the conversion of HMG-CoA to mevalonate. Besides being an intermediate in cholesterol synthesis, mevalonate is also a precursor of coenzyme Q_{10}; statins thus lower coenzyme Q_{10} levels[112,113] (**Figure 2**). Coenzyme Q_{10} is known for its enzymatic role in the production of energy within human cells, so coenzyme Q_{10} deficiency may impair muscle energy metabolism and contribute to the development of myalgia, a frequently reported adverse effect of statin treatment.[114] Supplementation with coenzyme Q_{10} can raise the circulating levels of coenzyme Q_{10} and might therefore be efficient in alleviating myopathic symptoms.

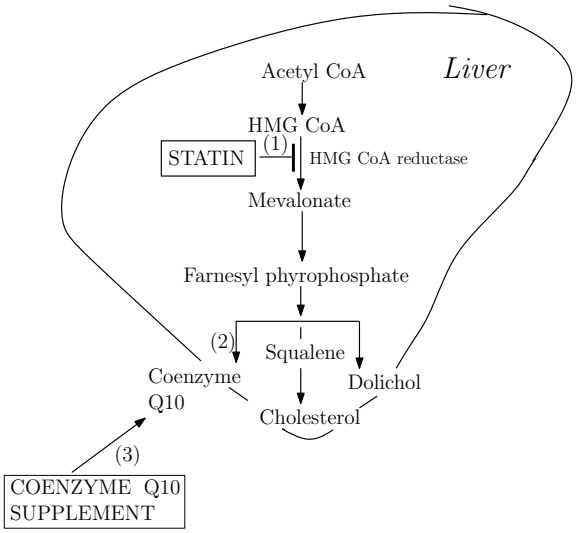

Figure 2. Proposed mechanism by which coenzyme Q_{10} supplements may support statin therapy. Statins inhibit the enzyme hydroxymethylglutaryl CoA (HMG-CoA) reductase (1) in the mevalonate pathway. The same pathway is shared by coenzyme Q_{10} and as a consequence coenzyme Q_{10} synthesis is inhibited (2).[112,113] Coenzyme Q_{10} supplements may raise the levels of coenzyme Q_{10} (3) in plasma and platelets.

Coenzyme Q_{10} and health claims

Dietary supplements containing coenzyme Q_{10} have not been evaluated for safety and effectiveness and there are no approved health claims for the use of coenzyme Q_{10}, neither in the USA nor in Europe.

Effects of combination therapy with coenzyme Q_{10} and statins

Although studies have repeatedly demonstrated reduced levels of plasma coenzyme Q_{10} with statin therapy[113] and restored levels after oral coenzyme Q_{10} supplementation,[113,115-118] large randomised controlled trials towards the impact of coenzyme Q_{10} supplementation on statin-induced myalgia in patients with hyperlipidaemia are lacking. Only two double-blind controlled clinical trials investigating this area have been performed. The first study, assigned a Jadad score of 2, was a pilot study in forty-four patients with self-reported myalgia. Patients were randomised to supplementation with 200 mg coenzyme Q_{10} per day or placebo in combination with an upward dose of simvastatin (starting dose of 10 or 20 mg/d up to 40 mg/d) for 12 weeks. Results showed no difference between the groups in severity of myalgia, in the number of patients tolerating the highest dose of simvastatin, or in the number of patients remaining on therapy.[119] The second study, assigned a Jadad score of 4, was performed in thirty-two patients with myopathic symptoms taking varying doses of statins, and supplemented with coenzyme Q_{10} (100 mg/d) or vitamin E (400 IU/d) for 30 days.[114] This study showed a significant 40% and 38% reduction in pain severity and pain interference with daily activities, respectively, in the group treated with coenzyme Q_{10}. Vitamin E did not affect

pain severity or pain interference. In this study, the benefit of coenzyme Q_{10} supplementation on improving pain was not stratified by statin type or dose.

In a third trial towards the effects of coenzyme Q_{10} supplementation on statin-induced myopathic symptoms, statin therapy was discontinued upon initial visit in all patients and no control group was included, so it is not clear what role coenzyme Q_{10} had in decreasing the incidence of myalgia.[120]

In summary it can be concluded that although some trial evidence exists about the effectiveness of coenzyme Q_{10} supplementation on myopathic symptoms, it is too early to recommend its routine use in clinical practice. The only randomised controlled clinical trials investigating this area showed contrasting results and further well-performed clinical trials are needed to investigate whether coenzyme Q_{10} can be used to support statin therapy. Although several studies have shown that plasma coenzyme Q_{10} levels are decreased after statin therapy, existing evidence also suggests that skeletal muscle coenzyme Q_{10} levels are not affected or even increased after statin use.[113,115,116]

Alternative explanations for the myotoxic adverse effects of statins include instability of skeletal muscle cells due to reduction in the cholesterol content of the membranes and inhibited production of GTP-binding proteins involved in cell growth and apoptosis. Apoptosis is a critical mechanism in the remodelling and maintenance of tissue structure and inappropriate apoptosis can produce pathological conditions.[121,122] Some of the decrease in coenzyme Q_{10} can probably be explained by the reduction in LDL cholesterol levels after statin therapy, since coenzyme Q_{10} is transported in the LDL particle.[121]

Safety aspects of combination therapy with coenzyme Q_{10} and statins

Coenzyme Q_{10} is widely recognised as safe with no reported toxicity.[123] It has been shown that coenzyme Q_{10} supplementation (100 mg/d) in HC patients treated with atorvastatin (10 mg/d) did not have an effect on statin-induced reductions in total or LDL cholesterol, or triglyceride levels.[124]

Discussion

The main objective of this review was to present options for the support of drug therapy with functional foods or dietary supplements. We focused on the support of statin therapy with phytosterols/-stanols, soluble fibre, *n*-3 PUFA or coenzyme Q_{10}, because many subjects are treated suboptimally with statins and there are indications supporting combined use with one of these functional foods or dietary supplements.

There is substantial evidence that adding phytosterols/-stanols to statin therapy reduces total and LDL cholesterol, and that adding *n*-3 PUFA to statins reduces plasma triglycerides. Both combination treatments are without any changes in HDL cholesterol. Neither supplementation with phytosterols/-stanols nor supplementation with *n*-3 PUFA had any known clinical significant side effects, although *n*-3 PUFA supplementation tended to increase LDL cholesterol and

phytosterol/-stanol supplementation is associated with a reduction of β-carotene. Also the potential atherogenicity of elevated serum phytosterol concentrations needs to be further investigated.

Information about the combination therapy with either soluble dietary fibres or coenzyme Q_{10} and statins is less clear. Soluble dietary fibre and statins may have additive effects on reducing total and LDL cholesterol levels. However, also an antagonistic effect of soluble fibre supplementation on statin therapy might be expected due to a reduced drug bioavailability. Furthermore, soluble fibre supplementation has been associated with a blunting of the HDL cholesterol increasing effect of statins. Coenzyme Q_{10} may counteract the adverse myalgic effect produced by statins, but further studies are needed to confirm this hypothesis. Despite the safety and low costs of coenzyme Q_{10}, thus far it should not be recommended as a routine supplement with statin therapy in clinical practice. In the present review we discussed the (limited) available literature on the effectiveness of coenzyme Q_{10} supplementation in reducing myopathic symptoms. Also other functional foods or dietary supplements might be helpful in reducing statin-induced side effects. Selenium supplementation has been suggested to reduce both statin-induced liver injury[125,126] and myotoxicity,[126] and L-carnitine might improve statin-associated myotoxicity.[127] However, current research is limited to cell culture and animal experiments, and human studies should be performed to assess the potential protective effects of these compounds in man. In the present review we have limited our literature search to human studies.

In conclusion it can be stated that using functional foods or dietary supplements might be an effective and safe approach to support drug therapy, especially when drugs alone are insufficient to achieve desirable effects on risk factors or when drug use is associated with side effects. In our example, functional foods or dietary supplements fortified with phytosterols/-stanols or *n*-3 PUFA are a good option for supporting statin therapy. However, every combination of a drug and a functional food or dietary supplement has to be investigated separately to draw conclusions about the type of effect: additive, synergistic, antagonistic or no effect. In our example of statin therapy, it is possible that various statins have different effects when combined to functional foods or dietary supplements, as statins vary in intestinal absorption and bioavailability. Also studies towards the effects of genetic polymorphisms are warranted as indicated by, for example, the association between variants in *SLCO1B1 (solute carrier organic anion transporter family, member 1B1)* and increased risk of statin-induced myopathy,[128] the association between *ABCA1* expression and cholesterol absorption after intake of phytostanols,[16] and the association between polymorphisms in the fatty acid desaturase *(FADS)* genes and fatty acid concentrations in plasma and erythrocyte membranes.[129-132]

More research is needed towards the effect that a functional food or dietary supplement has on side effects caused by drugs, and whether side effects can be reduced by replacing some dose of the drugs with functional foods or dietary supplements, without altering the effects on risk factors. Post-launch monitoring studies are required to assess the long term safety of the combination therapies and the safety in specific risk groups; clinical trials do often not attain adequate power for evaluating rare events and interactions. Moreover, the effectiveness of the combination

therapies under customary conditions should be addressed as compliance to drugs is known to be suboptimal[133,134] and recommended doses of functional foods and dietary supplements might not be consumed.[45,46,135]

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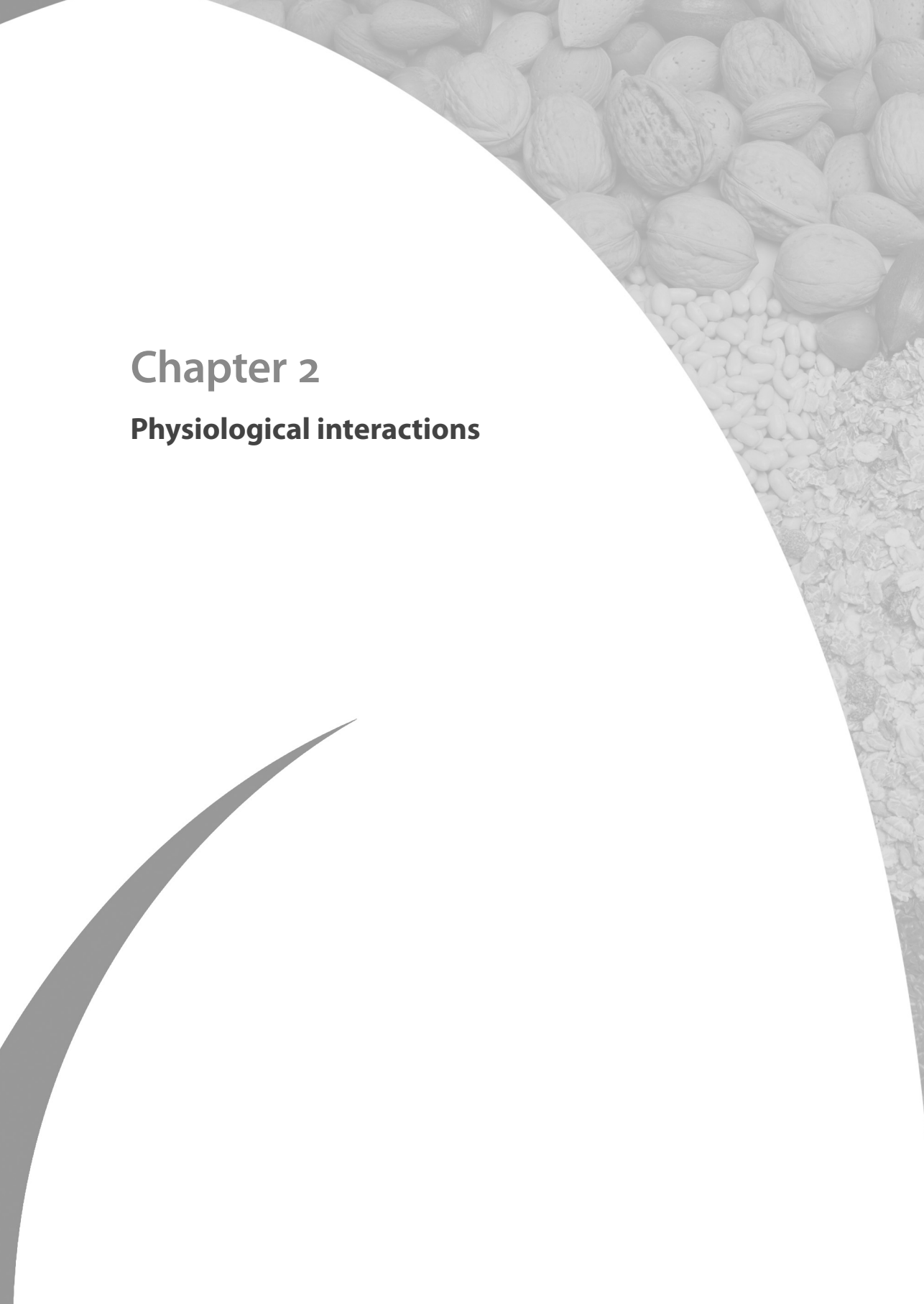

Chapter 2

Physiological interactions

Chapter 2.1

Simultaneous intake of oat bran and atorvastatin reduces their efficacy to lower lipid levels and atherosclerosis in LDLr-deficient mice

Simone RBM Eussen, Cathy JM Rompelberg, Kristina E Andersson, Olaf H Klungel, Per Hellstrand, Rickard Öste, Henk J van Kranen and Johan Garssen

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Abstract

Background Both oat bran, rich in the water-soluble fibre β-glucan, and statins lower serum total cholesterol. When used simultaneously both compounds may lower cholesterol levels additively. However, there might be a risk for a lower statin bioavailability when oat bran is added to the diet.

Objective To investigate the effects of separate and simultaneous dietary intake of atorvastatin (ATO) and oat bran on serum and hepatic lipid levels and the degree of atherosclerosis in mice.

Methods Ninety female LDL-receptor-deficient mice were fed a Western-type diet containing either a low dose (0.0025%), a high dose (0.01%) or no ATO, with or without oat bran (27%) (n=15 per group) for 16 weeks.

Results Both ATO and oat bran were effective in reducing serum total cholesterol levels (low ATO: -5.48 mmol/l, high ATO: -9.12 mmol/l, oat bran: -3.82 mmol/l, compared with control (no ATO/ no oat bran), all P<0.0001). When oat bran was added to a low dose ATO, the cholesterol-lowering effect of this combination was 50% smaller compared with the effect of the low dose ATO diet alone (between-group difference: 2.77 mmol/l, P=0.002), whereas total cholesterol decreased to a similar extent in the groups fed a high dose ATO, with or without oat bran (between-group difference: 1.10 mmol/l, P=0.21). Serum LDL and HDL cholesterol, triglycerides, hepatic lipid levels and atherosclerotic lesion development showed a similar pattern.

Conclusions The efficacy of oat bran and atorvastatin to lower lipid levels and atherosclerosis is reduced after simultaneous intake. We hypothesise that oat bran inhibits the intestinal absorption of atorvastatin and consequently its cholesterol-lowering effects. The effects are likely dependent on the type of statin and dietary fibre, and on the relative timing of intake of the statin and the dietary fibre. Future studies should focus on these aspects to provide further insight into the exact mechanism of this food-drug interaction.

Introduction

Increased total and low-density lipoprotein (LDL) cholesterol levels are one of the main risk factors for developing coronary heart disease. By interfering with de novo cholesterol synthesis, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) can effectively lower both total and LDL cholesterol levels. Recently, also several functional foods that carry a cholesterol-lowering health claim, have been launched on the EU and US market. These functional foods include cereals, bread and beverages containing oat β-glucans.[1,2] Oat β-glucans are dietary soluble fibres and are thought to reduce plasma cholesterol levels by interference with cholesterol and/or bile acid (re)absorption, either by binding bile acids or by forming a thick unstirred water layer in the intestinal lumen.[3] This leads to an up-regulation of cholesterol 7-α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol into bile acids, thereby reducing cholesterol levels. Other proposed mechanisms by which β-glucans lower cholesterol levels are the inhibition of cholesterol synthesis by short-chain fatty acids (SCFA, mainly propionate and butyrate) which are the major fermentation products of β-glucans, the increased intestinal viscosity causing lowered glucose absorption and thereby improved insulin sensitivity, and the increased satiety leading to lower overall energy intake.[3,4]

The market for functional foods is expanding rapidly worldwide[5] and therefore it is conceivable that an increasing number of patients suffering from hypercholesterolaemia will start to combine their prescribed statin treatment with the use of functional foods enriched with oat β-glucans. Since the postulated mechanisms by which β-glucans lower cholesterol levels differ from the cholesterol-lowering mechanism of statins, the two compounds may work additively to reduce total and LDL cholesterol levels.[6,7] However, there might be a risk for a reduced drug bioavailability when statins and oat β-glucans are used simultaneously. In a study with a limited number of hypercholesterolaemic patients, Richter *et al.* found that LDL cholesterol levels rose strikingly after oat bran was added to lovastatin treatment. Levels returned back to normal when intake of oat bran was stopped.[8]

The present study was designed to investigate the effects of separate and simultaneous intake of oat bran and atorvastatin on serum and hepatic cholesterol and triglyceride levels and the degree of atherosclerotic plaque formation in female LDL-receptor-deficient (LDLr-/-) mice.[9]

Materials and methods

Animals and diets

The study was performed in accordance with the approval of the Animal Experimentation Committee of the Faculty of Sciences of Utrecht University and in line with EC directive 86/609/EEC. Upon arrival, 8-week-old female LDLr-/- mice (The Jackson Laboratory, ME, USA) were randomly divided into six groups (15 mice per group, housed in cages of 7 or 8 mice). After a two-week run-in period on normal chow, mice were ad libitum-fed one of six Western-type diets (Research

Diets Inc., NJ, USA) providing 16% protein, 43% carbohydrates and 41% fat (energy percent) for 16 weeks. The diets contained either low dose (0.0025%), high dose (0.01%) or no atorvastatin (ATO). Either 27% oat bran (*Avena sativa* cv. Sang, Lantmännen AB, Sweden) or microcrystalline cellulose (control fibre) was added as dietary fibre source. This resulted in six different diets: (1) no ATO cellulose diet, (2) no ATO oat diet, (3) low ATO cellulose diet, (4) low ATO oat diet, (5) high ATO cellulose diet and (6) high ATO oat diet (**Supplementary Table 1**). The doses of ATO and oat bran were based on findings from a dose-escalation experiment which was performed prior to the start of this study. Diets were matched with respect to dietary fibre, energy, macronutrient and fatty acid contents (**Supplementary Table 2**). In the cellulose diets some of the fat from the lipid content of the oat bran was compensated for by adding a mixture of 75% soy oil, 15% Trisun oil and 10% palm oil to ensure that the fatty acid distribution was equal for all diets. Diets were refreshed twice a week.

Homogeneity and stability of β-glucan in diets

At baseline, halfway through and at the end of the study, three randomly chosen (500 mg) samples of each diet were removed. One sample of each diet was left in the animal housing facilities and the remaining two samples of each diet were kept in the fridge (4-7 °C). After 4 days (corresponding to the maximal number of days between refreshment of the diets) the amount of β-glucan in each sample was quantified according to McCleary and Codd.[10] All samples were analysed in duplicate.

Body weight, body fat percentage and food intake

Body weight of all animals was recorded weekly. Body fat percentage was measured with Dual Energy X-ray Absorptiometry scanning in anaesthetised mice at the end of the study. Food consumption per cage was determined twice a week.

Blood and tissue collection

Submandibular blood samples were collected after a 4-5 h fast at baseline and at 2, 4, 8, and 12 weeks of age. At 16 weeks of age all mice were sacrificed under urethane anaesthesia and blood samples were immediately obtained by heart puncture. Sera were extracted and stored at -70 °C until analysed. The livers were dissected and liver lobes were snap-frozen in liquid nitrogen and stored at -70 °C for the analysis of total and free cholesterol and triglycerides. The aortas were perfused with PBS followed by Histochoice Tissue Fixative (Amresco, Solon, USA) by left ventricular injection. The thoracic and abdominal aortas were dissected free from adventitial fat under the microscope, were cut longitudinally and mounted en face on ovalbumin-coated slides. Slides were stored in Histochoice at 4 °C until stained.[11]

Cholesterol and triglyceride levels in serum

Total and high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured routinely in serum samples on a clinical autoanalyser (LX20-Pro, Beckman Coulter, The Netherlands) using standard kits. LDL cholesterol was estimated using the Friedewald equation.[12]

Cholesterol and triglycerides in the liver

Lipids were extracted from liver samples according to the method described by Folch *et al.*[13] In short, approximately 200 mg of liver tissue (wet weight) was homogenised with 4 ml chloroform-methanol (2:1) in a Potter-Elvehjem homogeniser. The extract was mixed thoroughly with 0.8 ml 0.05% H_2SO_4 and centrifuged at 3,000 rpm for 10 min to separate the phases. One ml of the lower phase containing the lipids was added to an equivolume Triton X-100 (1% in chloroform) and evaporated under nitrogen. After resuspending the dried extract in 0.5 ml deionised H_2O, lipids were analysed using free cholesterol (Wako Diagnostics, VA, USA), total cholesterol and triglyceride (Human GmbH, Germany) kits.[14] The cholesterol ester amount was calculated by subtracting the free cholesterol amount from total cholesterol.

Assessment of atherosclerotic lesion formation

En face preparations of the aortas were stained with Oil Red O using an approach described by Branen *et al.*[11] The extent of atherosclerosis was determined blindly using Image-ProPlus Software 6.3. The lesion area was calculated as the ratio of the lesion area to the total aortic area.

Statistical analysis

Data are means ± standard error of the mean (SEM), unless indicated otherwise. The homogeneity and stability of β-glucan in diets was analysed with a three-way analysis of variance (ANOVA). Paired *t*-tests were used to test for changes in body weight and lipid levels over time within groups. A two-way mixed ANOVA was used to examine the effect of oat bran and different doses of atorvastatin on body weight, food consumption, serum and hepatic cholesterol and triglyceride concentrations, and atherosclerotic lesion area. Some variables were ln-transformed before analysis and back-transformed for presentation to fulfil ANOVA requirements for normally distributed residuals. Differences between means or medians were considered statistically significant when $P<0.05$. All statistical analyses were performed in Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA)

Results

Homogeneity and stability of β-glucan in diets

β-Glucan analysis of the diets revealed that the cellulose diet contained 0.09% β-glucans, whereas the 27% oat bran diet contained 2.0% β-glucans. No differences were detected in the β-glucan

content under different conditions of storage (P=0.19) or during the time of the study (P=0.36) (**Supplementary Table 3**).

Body weight, body fat percentage and food intake

During the time of the study all mice increased in body weight. However, mice fed the oat diet containing no atorvastatin or a low dose atorvastatin gained significantly more weight (7.26 grams, 95% confidence interval (CI): 3.56 to 11.0, or 39%, P<0.001) during the study compared with mice fed the cellulose diets and mice fed the oat diet combined with a high dose atorvastatin (**Figure 1**,

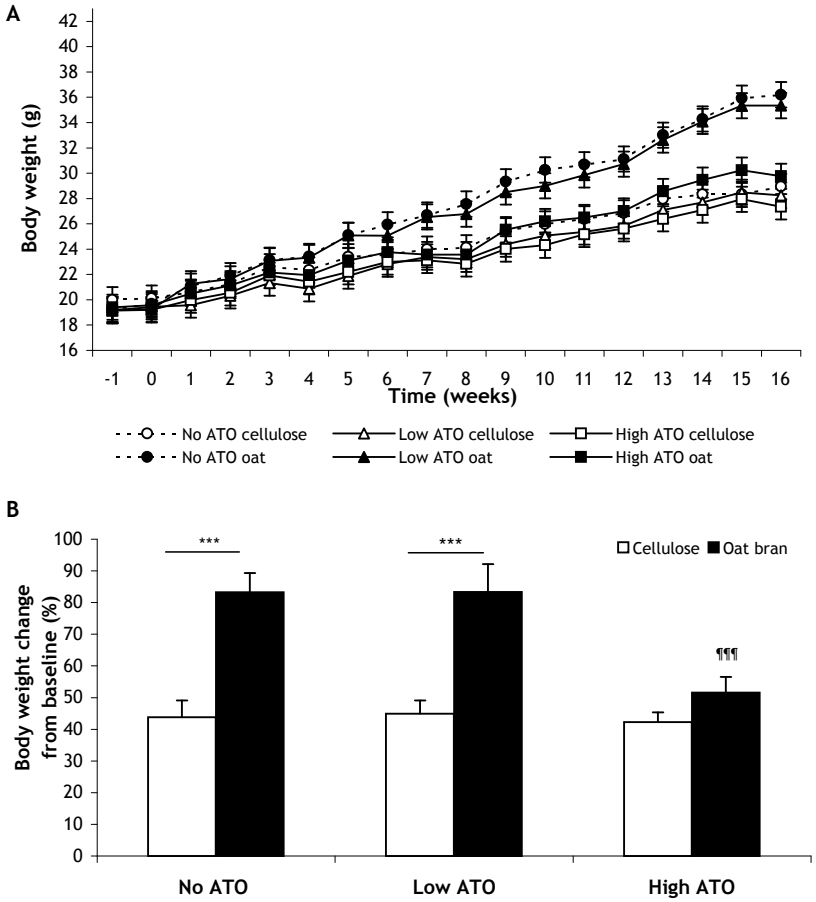

Figure 1. Mean body weight (A) and body weight change from baseline to 16 weeks (B) in LDLr-/- mice fed a diet with cellulose (open symbols) or oat bran (full symbols) dietary fibres and containing no atorvastatin (no ATO, circles), low dose atorvastatin (low ATO, triangles) or high dose atorvastatin (high ATO, squares) for 16 weeks

Values are means ± SEM of 15 mice

***P<0.001 for oat bran *vs.* cellulose in no ATO, low ATO and high ATO group; ¶¶¶P<0.001 for high ATO *vs.* no ATO in oat bran group

panel A). ATO alone did not affect body weight (low ATO, *P*=0.71; high ATO, *P*=0.39), but the addition of a high dose ATO to the oat diet led to 32% lower body weights, resulting in similar body weights in mice fed the high ATO oat diet and mice fed the diets without oat bran (**Figure 1**, **panel B**). Percentage body fat showed similar patterns (data not shown). All groups had similar food intake during the study (data not shown).

Cholesterol and triglyceride levels in serum

Inclusion of atorvastatin in a Western-type diet without oat bran lowered levels of total, HDL and LDL cholesterol, and triglycerides in a dose-dependent manner. At the end of the study, total cholesterol levels were 18.0 mmol/l in the no ATO cellulose group, compared with 12.5 mmol/l in the low ATO cellulose group (low ATO effect: -5.48 mmol/l, 95% CI: -7.21 to -3.75 or -30%, *P*<0.0001) and 8.88 mmol/l in the high ATO cellulose group (high ATO effect: -9.12 mmol/l, 95% CI: -10.8 to -7.39 or -51%, *P*<0.0001). Also the inclusion of oat bran in a diet without ATO resulted in significantly lower levels of total, HDL and LDL cholesterol, and triglycerides after 16 weeks. Total cholesterol levels were -3.82 mmol/l (95% CI: -5.55 to -2.10) or -21% (*P*<0.0001) lower in the group supplemented with oat bran (**Table 1** and **Figure 2**). Effects of atorvastatin and oat bran were significant from week 2 onwards, with *P*-trend<0.0001 for both. A low dose ATO in combination with oat bran resulted in total cholesterol levels that were -2.71 mmol/l (95% CI: -4.44 to -0.98) or -15% (*P*=0.003) lower compared with control (no ATO/no oat bran). Thus, when oat bran was incorporated into the diet with a low dose ATO, the cholesterol-lowering effect was approximately 50% smaller compared with the low ATO cellulose diet. This resulted in significantly higher levels of total cholesterol at the end of the study in the group given the low ATO diet with oat bran, compared with the group given the low ATO diet without oat bran (low ATO oat diet: 15.3 mmol/l, 95% CI: 14.1 to 16.5; low ATO cellulose diet: 12.5 mmol/l, 95% CI: 11.3 to 13.7; between-group difference: 2.77 mmol/l, 95% CI: 1.04 to 4.50, *P*=0.002). In contrast, total cholesterol levels were similar in the groups supplemented with a high dose of ATO whether or not oat bran was included in the diet (high ATO oat diet: 9.98 mmol/l, 95% CI: 8.76 to 11.2; high ATO cellulose diet: 8.88 mmol/l, 95% CI: 7.66 to 10.1; between-group difference: 1.10 mmol/l, 95% CI: -0.62 to 2.83, *P*=0.21).

Cholesterol and triglycerides in the liver

Total and free cholesterol levels in the liver revealed a similar tendency as was observed in serum, showing the efficacy of both oat bran and atorvastatin in improving lipid profile in the liver (**Figure 3**). Although statistical significance was reached for the reduction in free cholesterol both by oat bran and atorvastatin, total cholesterol was only significantly reduced by a low and high dose of atorvastatin. Also in the liver, atorvastatin was more effective in reducing cholesterol levels in the cellulose groups, compared with the oat groups. In contrast to serum cholesterol levels, liver total and free cholesterol levels were similar in the groups given a low ATO cellulose diet and a low ATO oat diet. For free cholesterol, the interaction terms between oat bran and low or high dose ATO were found to be similar (2.62 and 2.31 mg/g wet liver weight, respectively) and significant, meaning

Table 1. Serum total, HDL and LDL cholesterol and triglycerides at baseline and after 16 weeks in LDLr-/- mice fed diets varying in atorvastatin (ATO) content and with and without oat bran

	No ATO cellulose (*n*=15)	**No ATO oat** (*n*=15)	**Low ATO cellulose** (*n*=15)	**Low ATO oat** (*n*=15)	**High ATO cellulose** (*n*=15)	**High ATO oat** (*n*=15)
Total cholesterol, mmol/l						
Baseline	6.11 ± 0.12	6.70 ± 0.18	6.56 ± 0.19	6.35 ± 0.15	6.19 ± 0.17	6.70 ± 0.30
Week 16[a]	18.00 ± 0.95	14.18 ± 0.63***	12.52 ± 0.42###	15.29 ± 0.69**	8.88 ± 0.38###	9.98 ± 0.41¶¶¶
HDL cholesterol, mmol/l						
Baseline	2.45 ± 0.05	2.53 ± 0.05	2.58 ± 0.05	2.43 ± 0.04	2.39 ± 0.06	2.55 ± 0.07
Week 16[b]	5.13 ± 0.26	3.97 ± 0.14***	3.51 ± 0.17###	4.55 ± 0.19*** ¶	2.93 ± 0.11###	3.00 ± 0.12¶¶¶
LDL cholesterol, mmol/l						
Baseline	3.47 ± 0.08	3.96 ± 0.14	3.77 ± 0.15	3.73 ± 0.13	3.59 ± 0.12	3.94 ± 0.23
Week 16[a]	12.26 ± 0.71	9.93 ± 0.52***	8.70 ± 0.33###	10.17 ± 0.52*	5.70 ± 0.32###	6.64 ± 0.39¶¶¶
Triglycerides, mmol/l						
Baseline	0.98 ± 0.04	1.02 ± 0.06	1.09 ± 0.06	0.95 ± 0.04	1.04 ± 0.04	1.08 ± 0.07
Week 16[c]	3.05 ± 0.27	1.38 ± 0.12***	1.56 ± 0.11###	2.87 ± 0.25*** ¶¶¶	1.27 ± 0.09###	1.74 ± 0.16

Plus-minus values are means ± SEM of 15 mice

To convert mmol/l cholesterol to mg/dl, multiply by 38.7

[a b c] Values for all dietary groups were significantly different from baseline; [a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$,

*$P<0.05$, **$P<0.01$, ***$P<0.001$ for oat bran vs. cellulose in no ATO, low ATO and high ATO group

###$P<0.001$ for low ATO and high ATO vs. no ATO in cellulose group

¶$P<0.05$, ¶¶¶$P<0.001$ for low ATO and high ATO vs. no ATO in oat bran group

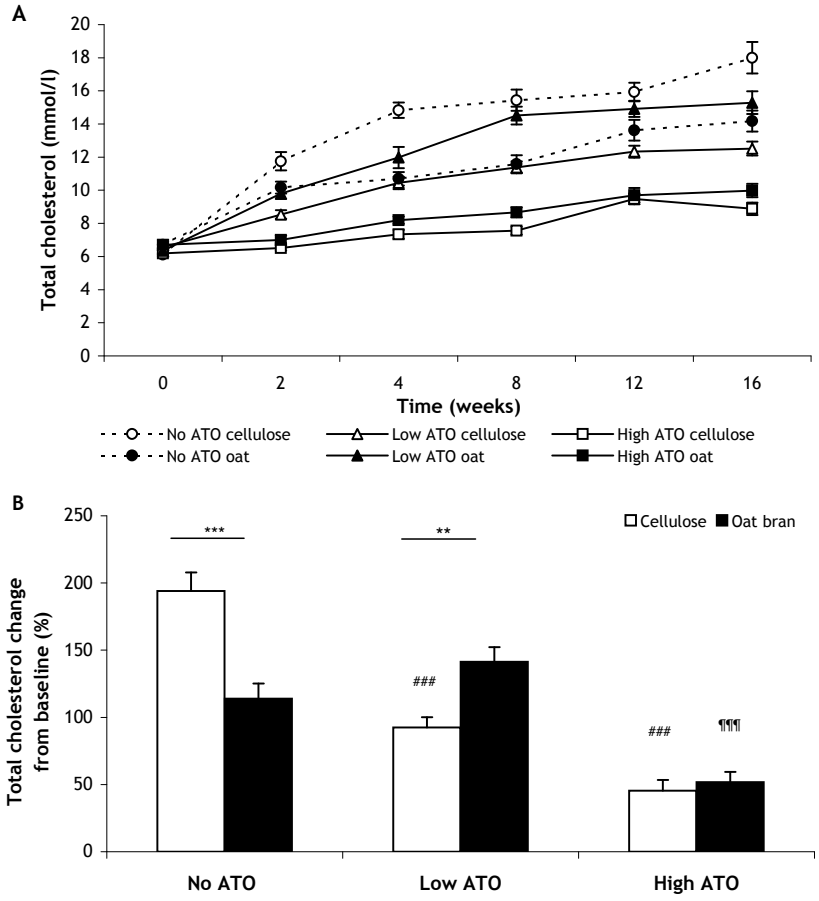

Figure 2. Mean total cholesterol level (A) and total cholesterol change from baseline to 16 weeks (B) in LDLr-/- mice fed a diet with cellulose (open symbols) or oat bran (full symbols) and containing no atorvastatin (no ATO, circles), low dose atorvastatin (low ATO, triangles) or high dose atorvastatin (high ATO, squares) for 16 weeks

Values are means ± standard error of the mean of 15 mice

***P*<0.01, ****P*<0.001 for oat bran *vs.* cellulose in no ATO, low ATO and high ATO group; ###*P*<0.001 for low ATO and high ATO *vs.* no ATO in cellulose group; ¶¶¶*P*<0.001 for low ATO and high ATO *vs.* no ATO in oat bran group

that the reductions in free cholesterol levels produced by combined intake were approximately 2.5 mg/g wet liver weight lower than what would have been expected if effects of oat bran and atorvastatin were additive. The interaction terms were not significant for hepatic total cholesterol levels. Triglyceride levels in the liver were not affected by either atorvastatin or oat bran.

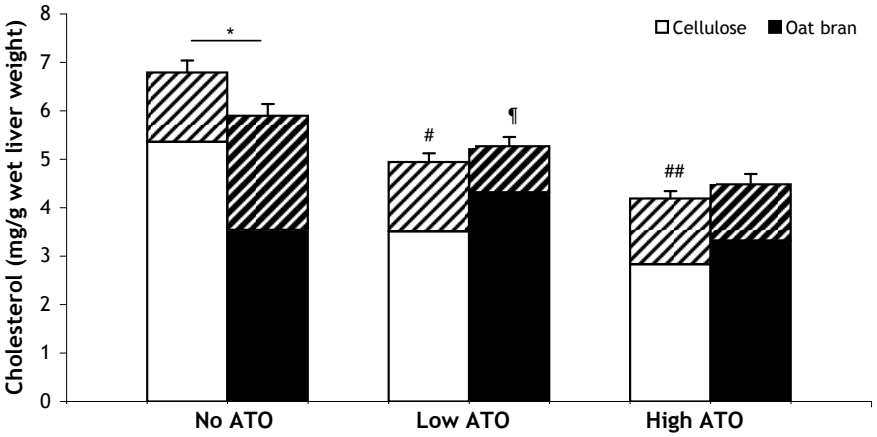

Figure 3. Mean total liver cholesterol, divided into free cholesterol (solid bar) and cholesterol esters (striped bar), of LDLr-/- mice fed a diet with cellulose (□) or oat bran (■) and containing no atorvastatin (no ATO), low dose atorvastatin (low ATO) or high dose atorvastatin (high ATO) for 16 weeks
Values are means ± SEM of 15 mice
*P<0.05 oat bran effect for free cholesterol in no ATO, low ATO and high ATO group; #P<0.05, ##P<0.01, low ATO and high ATO *vs.* no ATO effect for free and total cholesterol in cellulose group; ¶P<0.05, low ATO *vs.* no ATO effect for cholesterol esters in oat bran group

Atherosclerotic lesions

Median atherosclerotic lesion area as percentage of the total aortic area was significantly lower in the cellulose groups with a low and high dose ATO compared with the cellulose groups without ATO (**Figure 4**). A low and high dose of ATO lowered the atherosclerotic lesion area by -1.62% (95% CI: -2.00 to -1.24, P<0.0001) and -2.41% (95% CI: -2.80 to -2.03, P<0.0001), respectively. In the oat groups, a high dose of ATO still decreased atherosclerotic lesions significantly by -1.52% (95% CI: -1.92 to -1.13, P<0.0001), but a low dose of ATO had no effect on lesion area (-0.22%, 95% CI: -0.60 to 0.17, P=0.26). Oat bran tended to lower lesion area in the group without ATO (-0.31%, 95% CI: -0.69 to 0.069, P=0.10), but increased lesion area both in the low ATO (1.09%, 95% CI: 0.71 to 1.47, P<0.0001) and high ATO (0.58%, 95% CI: 0.18 to 0.97, P=0.005) group.

Discussion

The present study demonstrates that in female LDLr-/- mice both atorvastatin and oat bran are effective in reducing serum total and LDL cholesterol and triglyceride levels, but the efficacy of the compounds is reduced when given together as part of a diet. Similar effects as were observed for serum lipid levels, were seen for free and total cholesterol in the liver, and atherosclerotic lesion area. Unlike the results from Andersson *et al.*,[15] effects of oat bran on atherosclerotic lesion area did not reach statistical significance. This discrepancy is most likely explained by differences between

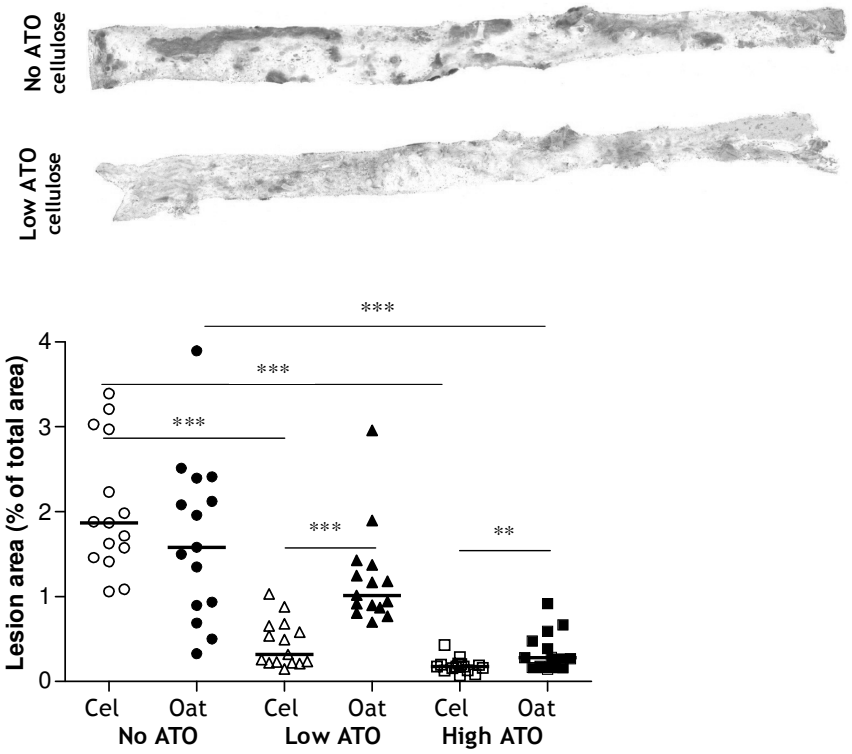

Figure 4. Atherosclerosis in LDLr-/- mice. (A) Representative examples of en face preparations of the descending aorta of LDLr-/- mice fed a diet with cellulose and containing no atorvastatin (no ATO) or low dose atorvastatin (low ATO). (B) Median atherosclerotic lesion area of the aorta artery (%) in LDLr-/- mice fed a diet with cellulose (Cel, open symbols) or oat bran (Oat, full symbols) and containing no atorvastatin (no ATO, circles), low dose atorvastatin (low ATO, triangles) or high dose atorvastatin (high ATO, squares) for 16 weeks

The lines represent the medians of 15 mice; $^{**}P<0.01$, $^{***}P<0.001$ between groups

the studies in the amount of oat bran added to the diets (40% used by Andersson *et al.*, compared with 27% in the current study) or the number of mice used per group (n=20 used by Andersson *et al.*, compared with n=15 in the present study).

As hypothesised before,[8] it is most likely that the intestinal absorption of atorvastatin is reduced when oat bran is included in the diet. The mechanisms by which oat β-glucans are thought to reduce serum cholesterol levels, i.e. by forming an unstirred water layer or by binding bile acids/cholesterol and thereby decreasing the (re)absorption of cholesterol and bile acids,[3,15,16] might also decrease the intestinal absorption of atorvastatin and consequently decrease its cholesterol-lowering efficacy. An alternative explanation for the reduced atorvastatin absorption may be the influence of oat bran on the gut microbiota composition, thereby constituting the gut barrier function that regulates the passage of exogenous substances, i.e. statins.[17] In order to test our hypothesis

that the intestinal absorption of atorvastatin is reduced in the presence of oat bran, a (preferably human) pharmacokinetic study should be performed in which time course data of atorvastatin concentrations are collected.[18] In addition, excretion studies are needed to provide quantitative information regarding atorvastatin and cholesterol in faeces.

In the present study, mice fed the diet containing only a low or high dose atorvastatin for 16 weeks had post-treatment serum total cholesterol levels that were respectively 30% and 51% lower compared with mice fed the diet without either atorvastatin or oat bran. When oat bran was added to the diet with a low dose atorvastatin, the cholesterol-lowering effect was approximately 50% smaller compared with the diet with a low dose atorvastatin alone. In contrast, a high dose atorvastatin lowered serum total cholesterol levels to the same extent, whether or not oat bran was included in the diet. Thus, when the amount of atorvastatin provided in the diet is high enough, i.e. in this study 0.01%, enough atorvastatin is still absorbed to significantly reduce cholesterol levels despite the presence of oat bran. We observed that post-treatment serum total cholesterol levels were somewhat higher after concomitant administration of a low dose of atorvastatin and oat bran compared with oat bran alone, although not statistically significantly. This suggests that also the effect of oat bran is diminished when atorvastatin is present. This might be explained by the fact that, when atorvastatin is present in the gut, the cholesterol-/bile-binding effects of oat β-glucans are reduced since they bind to atorvastatin, and consequently the excretion of cholesterol and/or bile acids is decreased.

In addition, it was noticed that the increases in body weight and body fat produced by oat bran were counterbalanced or outweighed by a high dose of atorvastatin. Body weight gain after oat bran supplementation has been reported previously in both mice[15,19] and rats.[20,21] Energy and macronutrients were well balanced between the different diets, and it seems unlikely that the small differences in the type of fatty acids explain the differences in weight gain observed during this study. We hypothesise that the uptake and metabolism of SCFA in the gastrointestinal tract, derived from the fermentation of oat, are a significant energy source. Augenlicht *et al.*[19] showed that mice that were wild-type for SCFA-metabolism gained significantly more weight when fed a wheat bran-supplemented diet compared with control diet, whereas this weight gain was absent in mice deficient in the gene encoding short-chain-acyl-dehydrogenase-enzyme, which catalyses the first step in SCFA β–oxidation. The binding or complex-forming between atorvastatin and oat bran might affect the effect oat bran has on SCFA-metabolism. Or, alternatively, a high dose atorvastatin affects the intestinal microbiota[22] and thereby SCFA concentrations. The difference in cholesterol-lowering effects observed in the high dose ATO with oat bran group, compared with the low dose ATO with oat bran group, is probably the result of the two mechanisms mentioned above. First, using a high dose of atorvastatin will result in an absorbed fraction of atorvastatin that is high enough to produce significant cholesterol-lowering effects. Second, the effect that oat bran has on body weight gain is limited in the presence of a high dose of ATO and thereby the increase in cholesterol levels produced by the weight gain is restricted.[23]

LDLr-/- mice have been used extensively in atherosclerosis studies.[24] It is generally accepted that statins lower LDL cholesterol by inhibition of hepatic cholesterol synthesis and up-regulation of LDL receptors on liver cell membranes. Therefore, cholesterol-lowering effects of statins would not be anticipated in LDLr-/- mice. However, besides the up-regulation of LDL receptors, statins also reduce circulating concentrations of apolipoprotein B-containing lipoproteins by decreasing the production of very low-density lipoprotein (VLDL) in the liver, and consequently the production of VLDL remnants and LDL.[25,26] Additionally, there have been suggestions for a physiologically important role of scavenger receptors (SR-BI) in LDL metabolism, with a selective uptake of LDL cholesteryl esters responsible for hepatic cholesterol uptake in the absence of LDL receptors.[27] Several studies have demonstrated the direct effect of statins on lowering LDL cholesterol in mice devoid of LDL receptors.[28-30] Wang *et al*. showed that the addition of simvastatin (0.15% wt/wt) to a high cholesterol diet reduced total cholesterol by 57% in male LDLr-/- mice.[29] This is consistent with the 50% reduction as found in the present study in which a lower dose of a more potent statin was used.[31-33] Also Guo *et al*. reported cholesterol-lowering effects around 50% in male LDLr-/- mice fed a high cholesterol diet containing 0.005% rosuvastatin (~0.01% atorvastatin).[30] Bisgaier *et al*. showed that atorvastatin was effective in reducing total cholesterol levels in female LDLr-/- mice.[28] Their observed effects were lower compared with those in the present study, explained by the fact that mice were fed chow instead of a Western-type diet. Also humans with both heterozygous and homozygous familial hypercholesterolaemia respond with reductions in LDL cholesterol levels after statin treatment (for review see Hemphill *et al.*),[34] which further shows that statins can reduce LDL cholesterol by a mechanism independent of the up-regulation of the LDL receptors.

Experimental animal studies have the advantage of allowing to study the effects on plaque formation in the aorta and on lipid levels in the liver. Moreover, long-term dietary intervention trials in humans are not easy because of practical and ethical reasons. Additionally, these trials might be biased by non-adherence to the dietary regimen or confounding factors. However, obviously there are uncertainties related to the extrapolation of the results from experimental animal species to the human situation. Studies towards the combined effects of oat bran and statins in humans are scarce. As mentioned before, Richter *et al.* observed markedly increased LDL cholesterol levels after oat bran was added to lovastatin treatment. However, for safety reasons, this study was interrupted after only two patients had finished the protocol. Three other lovastatin-treated patients were supplemented with the soluble fibre pectin, and also in these patients cholesterol levels rose strikingly.[8] Other human trials, using simvastatin, atorvastatin or lovastatin combined with either psyllium, guar gum or hydroxypropylmethylcellulose as soluble fibre, found either significant reductions in LDL cholesterol levels after soluble fibre supplementation,[6,7,35,36] or no effect.[16] Besides the differences in the physical-chemical attributes between the various fibres and statins used, the moment of ingestion of the statin and fibre might even be more important to explain the discrepancies between the studies. In the present study, atorvastatin and oat bran were added simultaneously to the same diet, thereby increasing the likelihood that both are present in the gut at the same time. In most human trials, participants were instructed to consume the dietary fibres at least 2

hours before or after taking the statin,[7,35,37] whereas others were not specific about administration time.[6,8,36] Atorvastatin can be taken with or without food and, due to the long elimination half-life of atorvastatin, it can be administered at any time of the day,[18] making the study of the combined effects of atorvastatin and oat bran relevant to clinical practice. Future studies, in animals as well as humans, should focus on comprehending the influence of intake timing. Also in vitro studies should be performed in order to better understand the mechanism behind the observed results, to reveal the influence of type of statin drug and dietary fibre and to identify the components of fibre responsible for the effect.

For this study, 27% oat bran (2% β-glucans) was used. This amount will probably not be reached in an average human diet, nor in a diet that includes functional foods enriched with oat β-glucans. The amounts of atorvastatin added were in the normal human range. Since the present study shows that the ratio between oat bran and atorvastatin amount might be decisive in deriving the effect of combined intake, various relevant dose-combinations should be explored in human trials.

In conclusion, the present study shows that in female LDLr-/- mice both atorvastatin and oat bran are effective in reducing serum and hepatic lipid levels and atherosclerosis, but the efficacy of the compounds is reduced when given together as part of a diet. Though our findings suggest that oat bran limits the absorption of atorvastatin in mice, confirmatory studies in humans are warranted and the mechanisms underlying this food-drug interaction should be explored. Future studies should focus on the influence of the relative timing of intake of the statin and the dietary fibre, and the type of statin and dietary fibre as these factors are likely to contribute to the observed effects.

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Supplementary Table 1. Formulation of the six experimental diets containing either low dose (0.0025%), high dose (0.01%) or no atorvastatin (ATO) and either oat bran (oat) or cellulose (control) dietary fibres

	No ATO cellulose diet g/kg diet	**No ATO oat diet** g/kg diet	**Low ATO cellulose diet** g/kg diet	**Low ATO oat diet** g/kg diet	**High ATO cellulose diet** g/kg diet	**High ATO oat diet** g/kg diet
Protein						
Casein, 80 Mesh†	200	140	200	140	200	140
DL-Methionine‡	3.00	4.50	3.00	4.50	3.00	4.50
Carbohydrates and atorvastatin						
Corn Starch	286	147	286	147	286	147
Maltodextrin 0% ATO	100	100				
Maltodextrin 0.0025% ATO			100	100		
Maltodextrin 0.01% ATO					100	100
Sucrose	100	96.7	100	96.7	100	96.7
Cellulose	44.2	0	44.2	0	44.2	0
Oil						
Anhydrous butter oil	174	174	174	174	174	174
Corn oil	10.0	10.0	10.0	10.0	10.0	10.0
Soy oil	19.2	0	19.2	0	19.2	0
Trisun oil	3.84	0	3.84	0	3.84	0
Palm oil	2.56	0	2.56	0	2.56	0
Other						
Mineral mix 1	10.0	10.0	10.0	10.0	10.0	10.0
DiCalcium Phosphate	13.0	13.0	13.0	13.0	13.0	13.0
Calcium Carbonate	5.50	5.50	5.50	5.50	5.50	5.50
Potassium Citrate, 1 H20	16.5	16.5	16.5	16.5	16.5	16.5
Vitamin mix 1	10.0	10.0	10.0	10.0	10.0	10.0
Choline Bitartrate	2.00	2.00	2.00	2.00	2.00	2.00

Supplementary Table 1. continued

	No ATO cellulose diet g/kg diet	**No ATO oat diet** g/kg diet	**Low ATO cellulose diet** g/kg diet	**Low ATO oat diet** g/kg diet	**High ATO cellulose diet** g/kg diet	**High ATO oat diet** g/kg diet
Oat bran	0	270	0	270	0	270
Nutrient contents (g/270 g oat bran)§						
Protein		53.2		53.2		53.2
Sucrose		3.30		3.30		3.30
Starch		136		136		136
Fat		25.6		25.6		25.6
Dietary fibres (whereof β-glucans)‖		44.2 (19.3)		44.2 (19.3)		44.2 (19.3)
Ash		7.81		7.81		7.81

† Casein is 88% protein

‡ DL-Methionine was added to provide sufficient supply of amino acids and to compensate for differences in methionine contents between oat bran protein and casein[a,b]

§ Nutrient contents of oat bran were analysed by Eurofins Food Lidköping, Sweden

‖ β-glucan content was analysed using a Megazyme kit (K-BGLU-kit, Megazyme International, Wicklow, Ireland), see **Supplementary Table 3**

a American Association of Cereal Chemists. 1986. Morphological and chemical characterization of the oat kernel. *In* Oats: Chemistry and Technology. F. Webster, editor. St Paul. 47

b National Research Council. 1995. Nutrient Requirements of the Mouse. *In* Nutrient Requirements of Laboratory Animals. National Academy Press, Washington. 80.

Supplementary Table 2. Energy and macronutrient content of the experimental oat diets (no ATO oat diet, low ATO oat diet, high ATO oat diet) and cellulose diets (no ATO cellulose diet, low ATO cellulose diet, high ATO cellulose diet)

	Oat diets†	**Cellulose diets†**
Energy (kJ/g diet)	19	19
Protein (en%)	16	16
Carbohydrates (en%)	43	43
Fat (en%) ‡	41	41
Saturated fatty acids	26	25
Monounsaturated fatty acids	11	11
Polyunsaturated fatty acids	4	5

† Atorvastatin (ATO) has no nutritional value and is therefore not accounted for
‡ Fatty acid profile of oat diet was analysed by Danone Research (Wageningen, The Netherlands)

Supplementary Table 3. Content, homogeneity and stability of β-glucan in mouse feed

	% β-glucan		
0% oat diets†	Day 0	Day 56	Day 112
Fridge, sample 1	0.123 ± 0.0481	0.123 ± 0.112	0.158 ± 0.0186
Fridge, sample 2	0.0983 ± 0.0421	0.0162 ± 0.0853	0.0873 ± 0.0605
Stables	0.125 ± 0.0415	0.0646 ± 0.149	0.0464 ± 0.0619
27% oat diets‡			
Fridge, sample 1	2.040 ± 0.171	2.048 ± 0.163	1.815 ± 0.376
Fridge, sample 2	2.083 ± 0.299	2.107 ± 0.438	1.900 ± 0.411
Stables	1.857 ± 0.215	2.020 ± 0.127	2.125 ± 0.243

Plus-minus values are means ± standard deviation of two duplicate determinations
† There was a negligible amount of β-glucan present in the cellulose diets.
‡ Target concentrations were 2% β-glucan

Chapter 2.2

Statins modify the effects of *n*-3 fatty acids on major cardiovascular events in patients after myocardial infarction

Simone RBM Eussen, Johanna M Geleijnse, Erik J Giltay,
Cathy JM Rompelberg, Olaf H Klungel and Daan Kromhout

Submitted

Abstract

Background Recent secondary prevention trials have failed to demonstrate a beneficial effect of *n*-3 polyunsaturated fatty acids (PUFA) on cardiovascular outcomes, which may be due to the growing use of statins since the mid 1990s.

Objective The aim of the present study was to assess whether statins modify the effects of *n*-3 PUFA on major adverse cardiovascular events in patients after myocardial infarction.

Methods Patients who participated in the Alpha Omega Trial were divided into consistent statin users (*n*=3740) and consistent statin non-users (*n*=413). In these two groups of patients the effects of an additional daily amount of 400 mg eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), 2 g α-linolenic acid (ALA), or both on major cardiovascular events were evaluated. Multivariate Cox proportional hazard models were used to calculate adjusted hazard rate ratios (HR_{adj}).

Results A total of 495 statin users (13%) and 62 statin non-users (15%) developed a major cardiovascular event. In statin users an additional amount of *n*-3 PUFA did not reduce cardiovascular events (HR_{adj} 1.02, 95% CI: 0.80 to 1.31, *P*=0.88). In statin non-users, however, only 9% of those who received EPA-DHA plus ALA experienced an event compared with 18% in the placebo group (HR_{adj} 0.46, 95% CI: 0.21 to 1.01, *P*=0.051). The effect was most pronounced in statin non-users with a high (≥4) baseline total to HDL cholesterol ratio (HR_{adj} 0.40, 95% CI: 0.18 to 0.89, *P*=0.025).

Conclusions In patients after myocardial infarction who were not treated with statins, supplementation with *n*-3 PUFA reduced major cardiovascular events, especially in those with dyslipidaemia.

Introduction

The landmark Scandinavian Simvastatin Survival Study (4S)[1] and subsequent randomised controlled trials[2] showed beneficial effects of statins on mortality and morbidity in patients with and without previous myocardial infarction (MI) or other coronary heart disease (CHD). Ever since, statins have been the first choice of drug treatment for preventing and treating cardiovascular disease (CVD). The benefits of statins were first attributed solely to their ability to inhibit hepatic cholesterol synthesis, thereby improving serum lipid levels. Depending on type, dose and baseline levels, statins reduce low-density lipoprotein (LDL) cholesterol by 18-55% and triglycerides by 7-30%, and increase high-density lipoprotein (HDL) cholesterol up to 15%.[3] Yet, over the years multiple lipid-independent pleiotropic effects of statins have been described. Statins have, for example, beneficial effects on endothelial function, inflammation and coagulation, independent of lipid-lowering.[4]

A healthy lifestyle is promoted for CVD prevention. Lifestyle changes include smoking cessation, increased physical activity level and adopting a healthier diet. Dietary guidelines emphasise the importance of *n*-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[5] A meta-analysis of both prospective cohort studies and trials showed that 250 mg per day of EPA-DHA reduced fatal CHD by 36% compared with no EPA-DHA.[6] Fish consumption, the major source of EPA-DHA in the diet, was inversely related to incident stroke in a meta-analysis of cohort studies.[7] Less evidence exists for a protective effect of α-linolenic acid (ALA), the plant-derived *n*-3 PUFA, against CVD.[8,9]

Although adding *n*-3 PUFA to statin therapy leads to significant reductions in triglyceride levels,[10] it has also been suggested that the use of guideline-concordant statin therapy dilutes the effects of *n*-3 PUFA such that no additional protective effect is observed.[11] This hypothesis is supported by the reduction in cardiovascular events through either fatty fish or EPA-DHA in trials in which less than one third of participants was on statin therapy (DART[12] and GISSI-Prevenzione[13]). *n*-3 PUFA did not reduce major cardiovascular events in three recently conducted trials with a large number of statin users. The OMEGA trial showed that guideline-adjusted drug treatment – including statin use in >90% of the post-MI patients – resulted in a low risk of cardiovascular events which could not be further reduced by 840 mg EPA-DHA daily.[14] Also in the SU.FOL.OM3 trial, no significant difference was detected in major vascular events between coronary artery disease patients allocated to 600 mg EPA-DHA daily and those allocated to placebo.[15] Finally, we showed in the Alpha Omega Trial no reduction in cardiovascular events in 4837 post-MI patients who were randomised to an additional amount of EPA-DHA (400 mg/d) and/or ALA (2 g per day), compared with placebo.[16]

The aim of the present study was to assess whether the effects of EPA-DHA and/or ALA on major cardiovascular events in the Alpha Omega Trial differed between statin users and statin non-users.

Methods

Study population

The Alpha Omega Trial is a multi-centre, double-blind, placebo-controlled trial of low doses of *n*-3 PUFA (400 mg/d EPA-DHA and/or 2 g/d ALA) on risk of fatal and non-fatal major cardiovascular events. Details of the study have been described elsewhere.[16,17] Briefly, 4837 free-living Dutch post-MI patients aged 60-80 years were randomised to receive one of four margarines: an EPA-DHA-enriched, an ALA-enriched, an EPA-DHA plus ALA-enriched margarine or a placebo margarine. Patients were enrolled from April 2002 through December 2006 and were followed for an average of 41 months.

At baseline, anthropometric measures were obtained and blood pressure, heart rate, lipid and glucose levels were determined. Information on demographic variables, lifestyle habits, current health status and medical history were collected by self-administered questionnaires. Baseline measurements were repeated after 20 months of the intervention in a random sample of 810 participants, and after 41 months in the 2531 participants who completed the trial before 1 January 2009. For the remaining participants, due to budgetary constraints, physical examination and blood sampling were not repeated and data was collected by questionnaires at the end of follow-up.[17]

Assessment of statin use

Questionnaires on medication use were filled out by all participants at baseline and after 41 months. Subjects were asked to record changes in medication use in a structured diary, and medication was checked during structured telephone interviews after 12 and 24 months. All drugs were coded according to the Anatomical Therapeutic Chemical (ATC) classification[18] by two of the authors (S.E. and O.K.). Subjects who reported use of statins (ATC codes C10AA and C10B) at all measurements (at baseline and at 12-, 24- and after on average 41-month follow-up) were classified as statin users. Those who were not using statins at any time point were classified as non-users. Subjects who initiated or stopped statin therapy during the trial and inconsistent statin users who used statins at some, but not all, time points were excluded.

Endpoint

The vital status of the participants was monitored via a computerised link with municipal registries. For patients who experienced a fatal event during follow-up, general practitioners, hospitals and family members were approached to ascertain the primary and contributing causes of death. The occurrence of non-fatal major cardiovascular events (MI, cardiac arrest and stroke) and cardiac interventions (percutaneous coronary intervention and coronary-artery bypass grafting) was monitored by annual telephone interviews conducted by research staff and verified against hospital records. The primary endpoint of this study was the rate of major cardiovascular events, which comprised fatal cardiovascular diseases, non-fatal MI, non-fatal cardiac arrest, non-fatal stroke and cardiac interventions (percutaneous coronary intervention and coronary-artery bypass grafting).[16]

Statistical analysis

Demographic and health characteristics of the participants who received the different margarines, stratified for statin users and non-users, were compared by using Student's *t*-test or the Mann–Whitney *U* test for continuous variables and the χ^2 test for nominal variables. Uni- and multivariate Cox proportional hazard models were used to estimate hazard rate ratios (HR) for major cardiovascular events with the placebo group as reference. Fixed effects in the models were the *n*-3 PUFA and the use of statins. To test whether the effect of EPA-DHA and/or ALA differed between patients with and without statins, the product term of *n*-3 PUFA and statins was added to the model. A similar statistical approach was used to examine differential effects of *n*-3 PUFA in statin (non-) users with a high (≥4) or low (<4) baseline ratio of total cholesterol to HDL cholesterol (total/HDL cholesterol ratio).[19]

In the multivariate model, we adjusted for age, gender and diabetes mellitus.[16] In addition, we checked whether inclusion one by one of other potential confounding variables altered the relationship of EPA-DHA and/or ALA with major cardiovascular events by ≥10%. We selected the following potential confounders: baseline levels of body mass index, current smoking (yes/no), physical activity level (≥ or <5 d/wk engaged in physical activity with a metabolic equivalent score>3), dietary EPA-DHA intake, alcohol consumption (≥ or <1 glass/wk), total/HDL cholesterol ratio, serum triglyceride levels, systolic blood pressure, current use of blood pressure-lowering medication (ATC codes C02, C03, C07, C08 and C09), antithrombotic agents (B01) and hormone replacement therapy (G03).

Results

Demographic and health characteristics of the patients

Of the 4837 patients who were enrolled in the Alpha Omega Trial, 3740 (77%) patients were consistent statin users and 413 (9%) patients were consistent statin non-users. Baseline serum lipid levels were measured in 4046 (97%) patients, of whom 3645 were statin users and 401 were statin non-users. For statin users as well as for statin non-users, the four study groups receiving placebo, EPA-DHA only, ALA only or EPA-DHA plus ALA were similar for most characteristics (**Table 1**). Among statin users, significant differences between study groups were observed for the use of blood pressure-lowering drugs, triglyceride levels and consumption of fish. Among statin non-users, significant differences between study groups were observed for the percentage of patients with diabetes mellitus, self-reported stroke, the use of antithrombotic drugs, physical activity, and plasma glucose and serum triglyceride levels.

Effect of n-3 PUFA in statin users and statin non-users

No patient was lost to follow-up. During 12,048 persons-years of follow-up, 495 (13%) statin users had a major cardiovascular event. For statin non-users, 1234 persons-years of follow-up were

Table 1. Baseline characteristics of users and non-users of statins randomised to placebo or *n*-3 polyunsaturated fatty acid supplementation in the Alpha Omega Trial

	Statin users (*n*=3740)				**Statin non-users** (*n*=413)			
	Placebo (*n*=943)	**EPA-DHA** (*n*=920)	**ALA** (*n*=930)	**EPA-DHA + ALA** (*n*=947)	**Placebo** (*n*=113)	**EPA-DHA** (*n*=102)	**ALA** (*n*=102)	**EPA-DHA + ALA** (*n*=96)
Age, yrs	68.7 ±5.6	68.7 ±5.5	68.5 ±5.3	68.8 ±5.5	70.4 ±5.8	71.5 ±5.2	71.8 ±6.3	71.2 ±5.9
Male gender, *n* (%)	745 (79)	718 (78)	734 (79)	751 (79)	85 (75)	72 (71)	78 (76)	66 (69)
Median time since MI, yrs	3.4 (1.5-6.2)	3.6 (1.7-6.3)	3.6 (1.7-6.3)	3.6 (1.6-6.2)	4.8 (2.8-6.3)	4.7 (2.8-7.3)	5.1 (1.8-7.2)	4.6 (2.2-7.2)
Self-reported history of stroke, *n* (%)	58 (6)	61 (7)	67 (7)	60 (6)	11 (10)[ab]	10 (10)[ab]	6 (6)[b]	14 (15)[a]
Use of cardiovascular medication, *n* (%)								
Antithrombotic agents	930 (99)	909 (99)	919 (99)	925 (98)	105 (93)[ab]	92 (90)[ab]	98 (96)[b]	82 (85)[a]
Antihypertensive agents	856 (90)[ab]	851 (90)[ab]	835 (90)[b]	856 (90)[ab]	97 (86)	84 (88)	97 (86)	85 (83)
Blood pressure, mmHg								
Systolic	141.6 ±20.9	142.3 ±21.3	141.2 ±20.9	140.8 ±22.2	141.5 ±23.6	141.7 ±23.7	141.7 ±23.7	143.5 ±26.6
Diastolic	80.0 ±10.5	80.5 ±11.3	80.3 ±11.0	80.2 ±11.1	80.2 ±13.2	77.4 ±12.2	77.4 ±12.2	80.2 ±11.8
Plasma glucose, mmol/l	6.25 ±2.10	6.21 ±2.10	6.17 ±1.95	6.18 ±2.27	6.07 ±1.61[a]	5.64 ±1.42[b]	6.09 ±1.73[a]	6.46 ±2.25[a]
Serum lipids, mmol/l								
Total cholesterol	4.59 ±0.88	4.61 ±0.86	4.56 ±0.84	4.55 ±0.84	5.61 ±1.15	5.58 ±1.14	5.51 ±1.03	5.50 ±1.00
LDL cholesterol	2.44 ±0.73	2.48 ±0.73	2.43 ±0.71	2.44 ±0.70	3.49 ±1.03	3.42 ±0.84	3.34 ±0.94	3.28 ±0.82
HDL cholesterol	1.29 ±0.34	1.29 ±0.35	1.29 ±0.34	1.29 ±0.32	1.25 ±0.41	1.30 ±0.37	1.26 ±0.34	1.33 ±0.41
TC/HDL ratio†	3.76 ±1.05	3.76 ±1.03	3.73 ±1.03	3.68 ±0.95	4.75 ±1.23	4.55 ±1.25	4.58 ±1.15	4.47 ±1.40
TC/HDL ratio ≥ 4, *n* (%)†	329 (36)	317 (36)	317 (35)	296 (32)	78 (72)	69 (68)	67 (68)	55 (59)

Median triglycerides, mmol/l (range)	1.68 (1.22-2.38)[b]	1.62 (1.24-2.29)[ab]	1.61 (1.19-2.28)[ab]	1.59 (1.18-2.20)[a]	1.75 (1.37-2.42)[b]	1.59 (1.17-2.18)[a]	1.79 (1.31-2.46)[ab]	1.52 (1.22-2.25)[ab]
BMI, kg/m^2	27.9 ±3.8	27.7 ±3.7	27.8 ±3.7	27.8 ±3.9	27.2 ±4.7	27.3 ±3.8	27.9 ±4.5	27.6 ±4.8
Diabetes mellitus, *n* (%)	190 (20)	204 (22)	201 (22)	180 (19)	15 (13)[b]	18 (18)[ab]	20 (20)[ab]	24 (25)[a]
Physical activity‡<5 d/w, *n* (%)	197 (21)	188 (20)	194 (21)	217 (23)	22 (19)[ab]	23 (23)[a]	11 (11)[b]	25 (26)[a]
Current smoker, *n* (%)	169 (18)	153 (17)	165 (18)	142 (15)	24 (21)	21 (21)	19 (19)	13 (14)
Alcohol use ≥1 glass/w, *n* (%)	673 (71)	646 (70)	661 (71)	691 (73)	74 (65)	56 (55)	68 (67)	60 (63)
Median fish consumption, g/d (range)	14.3 (5.9-18.4)[b]	15.0 (7.5-19.8)[ab]	15.3 (7.8-22.4)[a]	15.1 (6.4-19.4)[ab]	15.0 (5.3-19.1)	11.7 (5.2-18.3)	13.5 (6.0-17.1)	13.9 (5.9-18.3)
Intake of fish ≥20 g/d, *n* (%)	288 (31)	286 (31)	296 (32)	292 (31)	39 (35)	41 (40)	28 (27)	33 (34)
Median intake of EPA-DHA§, mg/d (range)	120 (50-210)[b]	130 (60-205)[ab]	130 (60-220)[a]	130 (50-210)[ab]	120 (60-200)	90 (40-190)	105 (45-180)	115 (40-220)

Plus-minus values are means ± SD; Range is the interquartile range

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; BMI, body mass index; MI, myocardial infarction; TC/HDL ratio, total to HDL cholesterol ratio

† Numbers vary because of missing values. Percentages are calculated without missing values

‡ Physical activity with a metabolic equivalent score >3

[a b] Values within a row with unlike superscripts were significantly different ($P < 0.05$)

§ Intake of EPA-DHA outside the study treatment

accumulated and 62 (15%) major cardiovascular events occurred. Among statin users, there was no significant difference in the rate of major cardiovascular events between the four groups (**Table 2**). Supplementation with EPA-DHA only or with ALA only did not reduce major cardiovascular events in statin non-users. However, 9% of the statin non-users who received EPA-DHA plus ALA had a major cardiovascular event during the 41-months follow-up period compared with 18% of the patients in the placebo group. Statin non-users receiving EPA-DHA plus ALA had a 54% lower incidence of major cardiovascular events compared with the placebo group, which was borderline significant (HR_{adj} 0.46, 95% CI: 0.21 to 1.01, *P*=0.051).

In statin non-users, 67% had a total/HDL cholesterol ratio ≥4 (**Table 3**). In this high-risk group, EPA-DHA reduced the number of major cardiovascular events by 29% (HR_{adj} 0.71, 95% CI: 0.36 to 1.38, *P*=0.31) and ALA by 26% (HR_{adj} 0.74, 95% CI: 0.38 to 1.45, *P*=0.38), compared with placebo. For EPA-DHA plus ALA, the risk was reduced by 60% (HR_{adj} 0.40, 95% CI: 0.18 to 0.89, *P*=0.025). The additive effect of EPA-DHA and ALA alone (HR: 0.71*0.74=0.53) was within the confidence limits for EPA-DHA plus ALA.

Discussion

The present study suggests that statin treatment modifies the effects of *n*-3 PUFA on the incidence of major cardiovascular events. In statin users additional *n*-3 PUFA had no effect. Statin non-users randomised to EPA-DHA plus ALA had 54% fewer major cardiovascular events than those on placebo. This effect amounted to 60% in statin non-users with a high total to HDL cholesterol ratio. These results suggest that the effect of *n*-3 PUFA is modified by statin treatment and the total to HDL cholesterol ratio.

The Alpha Omega Trial is the first double-blind placebo-controlled trial in which the additional effect of adding 2 g ALA per day to EPA-DHA on major cardiovascular events was investigated. The reduction in major cardiovascular events due to ALA increased from 6% in the total patient population to 26% in statin non-users with dyslipidaemia, but these results were not statistically significant. However, the combination of EPA-DHA plus ALA reduced major cardiovascular events by 60%. This is consistent with the hypothesis that the effects of EPA-DHA and ALA alone are additive and independent, although this has been disputed in a recent review.[20]

Other large randomised controlled trials have concentrated on the effect of consuming EPA-DHA alone.[21] Apart from the Alpha Omega Trial also the recently published OMEGA trial[14] and the SU.FOL.OM3 trial[15], both carried out in cardiac patients, failed to show a reduction in major cardiovascular events after a moderate additional intake of respectively 840 and 600 mg EPA-DHA per day. In all three trials at least 85% of the patients were treated with statins.[21] However, in the 11 years earlier published GISSI-Prevenzione (GISSI-P) trial,[13] treatment with 850-882 mg daily of EPA-DHA decreased major cardiovascular events defined as fatal cardiovascular disease plus non-fatal MI and non-fatal stroke by 20% in patients after a recent MI. In this trial, the percentage of

Table 2. Unadjusted and adjusted hazard rate ratios (HR) for major cardiovascular events among statin users and statin non-users randomised to *n*-3 polyunsaturated fatty acid supplementation in the Alpha Omega Trial with the placebo group as reference

	Statin users (*n*=3740)					**Statin non-users** (*n*=413)				
	no./total (%)	HR (95% CI)	*P*-value	HR_{adj}† (95% CI)	Adj *P*-value	no./total (%)	HR (95% CI)	*P*-value	HR_{adj}† (95% CI)	Adj *P*-value
Placebo (reference)	123/943 (13)	1.00		1.00		20/113 (18)	1.00		1.00	
EPA-DHA	127/920 (14)	1.06 (0.83; 1.36)	0.65	1.05 (0.82; 1.34)	0.72	16/102 (16)	0.84 (0.44; 1.62)	0.60	0.82 (0.42; 1.58)	0.55
ALA	119/930 (13)	0.98 (0.76; 1.27)	0.89	0.98 (0.76; 1.26)	0.87	17/102 (17)	0.94 (0.49; 1.80)	0.85	0.90 (0.47; 1.72)	0.75
EPA-DHA + ALA	126/947 (13)	1.02 (0.79; 1.31)	0.89	1.02 (0.80; 1.31)	0.88	9/96 (9)	0.48 (0.22; 1.06)	0.070	0.46 (0.21; 1.01)	0.051

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

† HR_{adj}, Hazard rate ratio adjusted for age, gender and type 1 and 2 diabetes mellitus

Table 3. Unadjusted and adjusted hazard rate ratios (HR) for major cardiovascular events in 401 statin non-users with a low or high ratio of total to HDL cholesterol (TC/HDL ratio) randomised to *n*-3 polyunsaturated fatty acid supplementation in the Alpha Omega Trial with the placebo group as reference

	Statin non-users (*n*=401)									
	Subjects with low TC/HDL ratio† (*n*=132)					**Subjects with high TC/HDL ratio** (*n*=269)				
	no./total (%)	HR (95% CI)	*P*-value	HR_{adj}‡ [95% CI)	Adj *P*-value	no./total (%)	HR [95% CI)	*P*-value	HR_{adj}‡ [95% CI)	Adj *P*-value
Placebo (reference)	3/30 (10)	1.00		1.00		17/78 (22)	1.00		1.00	
EPA-DHA	5/33 (15)	1.12 (0.53; 2.39)	077	1.07 (0.50; 2.29)	0.85	11/69 (16)	0.73 (0.37; 1.42)	0.35	0.71 (0.36; 1.38)	0.31
ALA	5/31 (16)	1.35 (0.64; 2.87)	0.44	1.26 (0.59; 2.69)	0.55	11/67 (16)	0.77 (0.39; 1.51)	0.44	0.74 (0.38; 1.45)	0.38
EPA-DHA + ALA	3/38 (8)	0.73 (0.31; 1.73)	0.47	0.68 (0.29; 1.61)	0.38	6/55 (11)	0.42 (0.19; 0.94)	0.035	0.40 (0.18; 0.89)	0.025

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

† Subjects were categorised to have a low TC/HDL ratio when an individual's baseline ratio of total to HDL cholesterol was lower than 4.[19]

‡ HR_{adj}, Hazard rate ratio adjusted for age, gender and type 1 and 2 diabetes mellitus

statin users increased from 5% at baseline to 29% after 6 months and to 46% at the end of the trial. Baseline total/HDL cholesterol ratio was 5.1 and clinically important changes in total and HDL cholesterol were not observed during the course of the trial. In the Alpha Omega Trial, supplementation with 400 mg daily of EPA-DHA did not reduce major cardiovascular events. Eighty-five percent of the participants in this trial were on statin treatment and baseline ratio of total to HDL cholesterol ratio was 3.9, i.e. 1.2 unit lower than in GISSI-P (**Table 4**). Yet, statin non-users with dyslipidaemia who had an average total/HDL cholesterol ratio of 5.2 experienced 29% fewer major cardiovascular events, a finding which was not statistically significant but in the same order of magnitude as in the GISSI-P trial.

The JELIS trial[22] was carried out in patients with a serum total cholesterol level of 6.5 mmol/l or more and contained a primary and secondary prevention group. The latter one consisted of 3664 cardiac patients who were followed for an average of 55 months. Mean serum total cholesterol at baseline was 6.97 mmol/l and mean HDL cholesterol level was 1.43 mmol/l. During follow-up total cholesterol decreased by 19% and HDL increased by 3%. The average total/HDL cholesterol ratio decreased from 4.9 to 3.8. At this low ratio an additional amount of 1.8 g EPA per day reduced the number of major cardiovascular events by 19% ($P<0.05$). The statin users in the Alpha Omega Trial had a very similar average total/HDL cholesterol ratio of 3.7, but in these patients an additional amount of 400 mg EPA-DHA, 2 g ALA or both did not reduce the number of major cardiovascular events (**Table 2**). These results suggest that in cardiac patients with hypercholesterolaemia who are effectively treated with statins and obtain a low total/HDL cholesterol ratio, an additional high dose of 1.8 g EPA is needed to significantly reduce the number of major cardiovascular events.

Statins and *n*-3 PUFA have different effects on blood lipids. Statins reduce cardiovascular disease risk through improving the total/HDL cholesterol ratio.[2,3,23] *n*-3 PUFA in large doses (>1 g/day) lower effectively serum triglycerides but their effect on cardiovascular disease risk is less convincing than that of statins.[24] Besides these effects on lipids, both statins and *n*-3 PUFA have anti-inflammatory effects, improve endothelial function and inhibit platelet aggregation.[4,20] Statins and *n*-3 PUFA share mechanisms such as plaque stabilisation that influence atherosclerosis and its complications positively.[25] The results of the present analysis suggest that in patients who do not use statins, the *n*-3 PUFA EPA-DHA and ALA in amounts comparable to the Recommended Dietary Allowances[26] effectively reduce major cardiovascular events.

Current guidelines recommend statin treatment to all subjects with established CVD unless their LDL cholesterol level is below 2.5 mmol/l.[27,28] In the present study, 86% of the statin non-users had an LDL cholesterol level exceeding 2.5 mmol/l, indicating a considerable level of undertreatment. One could argue that, when guidelines are followed more closely, additional use of *n*-3 PUFA is redundant. However, for the subset of patients in our trial who do not tolerate statins, an additional amount of 400 mg EPA-DHA plus 2 g ALA daily could be an attractive alternative to reduce their risk of future cardiovascular events.

Some limitations of our study should be acknowledged. First, the use of statins was assessed by questionnaires and telephone interviews. These methods of medication information collection

Table 4. Effect of EPA-DHA and/or ALA on major cardiovascular events in post-myocardial patients in the GISSI-Prevenzione Trial[13] and the Alpha Omega Trial[16]

	GISSI-Prevenzione	All patients in Alpha Omega	Statin non-users in Alpha Omega	Statin non-users with high TC/HDL ratio in Alpha Omega
Patients	11,324	4837	401	269
Intake of fish ≥1 serving/w or ≥20 g/d† (%)	86	31	34	32
Dose EPA (mg)	289	226	218	223
Dose DHA (mg)	577	150	145	149
Dose ALA (mg)	0	1882	1815	1857
Medication use (%)‡				
Anti-platelet drug	88	84	74	74
ACE inhibitors	41	42	36	35
β-blockers	41	69	58	61
Statins	29	85	0	0
Serum lipids, mmol/l				
Total cholesterol	5.45 ±1.10	4.73 ±0.97	5.55 ±1.08	5.69 ±1.12
HDL cholesterol	1.07 ±0.29	1.29 ±0.34	1.29 ±0.38	1.11 ±0.23
TC/HDL ratio§	5.08	3.87 ±1.13	4.59 ±1.26	5.24 ±0.98
Triglycerides, mmol/l	1.83 ±0.97	1.92 ±1.04	1.96 ±1.03	2.28 ±1.08
RR reduction in MCE‖				
EPA-DHA	0.80 (0.68; 0.95)	1.05 (0.85; 1.29)	0.82 (0.42; 1.58)	0.71 (0.36; 1.38)
ALA		0.94 (0.76; 1.17)	0.90 (0.47; 1.72)	0.74 (0.38; 1.45)
EPA-DHA plus ALA		0.91 (0.74; 1.13)	0.46 (0.21; 1.01)	0.40 (0.18; 0.89)

Plus-minus values are means ±SD

ACE, angiotensin-converting-enzyme inhibitors; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MCE, major cardiovascular event, RR, relative risk; TC/HDL ratio, total to HDL cholesterol ratio

† Fish intake was categorised into ≥ or <1 serving/week in GISSI-Prevenzione and as ≥ or <20 g/d in the Alpha Omega Trial

‡ Medication use in GISSI-Prevenzione at 6 months

§ Total to HDL cholesterol ratio was not presented in GISSI-Prevenzione but was derived by dividing total cholesterol level by HDL cholesterol level

‖ MCE comprised fatal cardiovascular disease, non-fatal myocardial infarction and non-fatal stroke in GISSI-Prevenzione. In the Alpha Omega Trial, MCE comprised fatal cardiovascular disease, non-fatal myocardial infarction, non-fatal stroke, non-fatal cardiac arrest and cardiac interventions (percutaneous coronary intervention and coronary-artery bypass grafting)

have the disadvantage that they are sensitive to recall bias. Nonetheless, previous validation studies indicated that for drugs used chronically such as statins, the specificity and sensitivity of questionnaires compared with pharmacy records is high.[29] Second, due to the high level of statin use in our cohort, the number of statin users to non-users was unbalanced. Nevertheless, despite the small number of statin non-users (*n*= 413) and of non-users with a high total/HDL cholesterol ratio (*n*=269) the effects of *n*-3 PUFA on major cardiovascular events reached statistical significance. However, these results need to be confirmed in larger high-risk patient populations before definitive conclusions can be drawn.

In conclusion, the present study indicates that supplementation with *n*-3 PUFA reduces the risk of major cardiovascular events in statin non-users with a history of MI, especially in those with dyslipidaemia. These results contribute to the explanation of the inconsistent results of *n*-3 PUFA in secondary prevention trials.

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Chapter 2.3

Dose-dependent cholesterol-lowering effects of phytosterol/-stanol-enriched margarine in statin users and statin non-users under free-living conditions

Simone RBM Eussen, Nynke de Jong, Cathy JM Rompelberg, Johan Garssen, WM Monique Verschuren and Olaf H Klungel

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Abstract

Background The efficacy of the use of phytosterol/-stanol-enriched margarine has been demonstrated in statin users as well as in statin non-users. However, inadequate intake levels of the margarines might influence the effectiveness of phytosterol/-stanol consumption.

Objective To assess the effectiveness of the use of phytosterol/-stanol-enriched margarines to lower total and non-HDL cholesterol levels in users and non-users of statins.

Methods Data for this retrospective cohort study were obtained from questionnaires on health and food intake from a population-based longitudinal cohort linked to pharmacy-dispensing records. The analysis included 3829 men and women (aged 31-71 years) who were examined during 1998-2002 and re-examined at 5-year follow-up during 2003-2007. Multivariate analysis of variance was performed to estimate the effectiveness of enriched margarines and/or statins to lower total and non-HDL cholesterol levels.

Results Recommended doses of margarines were consumed by only 9% of the subjects. Serum total cholesterol decreased by respectively -0.16 mmol/l (95% CI: -0.26 to -0.05), -1.40 mmol/l (95% CI: -1.51 to -1.30) and -1.64 mmol/l (95% CI: -1.91 to -1.37) in subjects who started to use phytosterols or phytostanols only, statins only or a combination of both compounds at some point in time between examination and re-examination, compared with subjects who did not start using phytosterols/-stanols or statins. Cholesterol-lowering effects of the phytosterols/-stanols were similar in statin users and statin non-users and increased with increasing intakes of enriched margarine (no intake, 0; low intake, -0.017 mmol/l, 95% CI: -0.16 to 0.13; medium intake, -0.089 mmol/l, 95% CI: -0.22 to 0.038; high intake, -0.32 mmol/l, 95% CI: -0.50 to -0.14).

Conclusions Although recommended intake levels of the enriched margarines were not reached by all persons, these data show that under customary conditions of use phytosterols/-stanols are effective in lowering cholesterol levels in both statin users and statin non-users.

Introduction

Patients with elevated total cholesterol and low-density lipoprotein (LDL) cholesterol levels are at high risk of developing atherosclerosis and coronary heart disease (CHD).[1] First-line treatment normally focuses on lowering LDL cholesterol, often accomplished by the use of statins. However, growing evidence suggests that non-high-density lipoprotein (non-HDL) cholesterol is a stronger predictor of CHD death than LDL cholesterol.[2-7] Apart from statins, changes in lifestyle factors, such as quitting smoking,[8] becoming more physically active and eating a healthy diet, can also influence LDL cholesterol and non-HDL cholesterol. In the last decade there has been more interest in changing dietary habits, with the appearance of functional foods. Since 1999, margarines containing phytosterols or phytostanols have become available on the US and EU market.[9] Phytosterols/-stanols, which are structurally related to cholesterol, are thought to compete with cholesterol for solubilisation into mixed micelles. This leads to a reduced absorption of cholesterol and/or to an enhanced efflux of cholesterol back into the intestinal lumen due to a higher expression of the ABC transporter. Both mechanisms ultimately result in an increased faecal output of cholesterol.[10-14]

Randomised controlled trials (RCT) have shown the efficacy (extent to which an intervention produces a beneficial effect under ideal conditions) of phytosterols/-stanols in lowering serum cholesterol levels: it is estimated that phytosterols/-stanols reduce total and LDL cholesterol by roughly 6% and 10%, respectively.[15-17] It has been shown that phytosterols/-stanols are equally effective when used alone as part of the diet or when used as an adjuvant to ongoing statin therapy. Adding phytosterols/-stanols to statin therapy appears to be more effective than doubling the statin dose[17,18] and, therefore, these products might especially be beneficial for persons who do not reach LDL cholesterol goals with statin monotherapy and for those who experience side-effects from high doses of statins.

Although RCT are widely accepted as the gold standard of medical intervention research, their design may include short-term interventions, frequent follow-up visits, extensive monitoring and the use of restricted patient populations with high adherence to therapy. These factors limit extrapolation to daily practice populations.[19,20] Because of the high adherence to therapy in RCT and the fact that poor adherence is thought to contribute to the failure of patients to achieve therapy targets,[21,22] the reductions of 6% in total cholesterol as found in RCT may not be accomplished in persons who use the enriched margarines and statins under customary conditions.

The aim of the present study was to assess the effectiveness (extent to which an intervention works in daily medical practice) of the use of phytosterol/-stanol-enriched margarine in subjects using or not using statins in a real-world setting. As there are currently no standard databases available that integrate food intake and drug monitoring, data from an ongoing free-living cohort study containing information on functional food use was linked to a pharmacy-dispensing database for the purpose of the present study.

Subjects and Methods

Study setting

Subjects from the Dutch Doetinchem Cohort Study and the Pharmacomorbidity-Record Linkage System (PHARMO-RLS) were linked using information on gender, date of birth and postcode in order to obtain information on the use of phytosterol/-stanol-enriched margarines and statins of the same subjects.

The Doetinchem Cohort Study was approved according to the guidelines of the Helsinki Declaration by the external Medical Ethics Committee of the Dutch TNO Research Institute. Linkage has been performed only for those participants who have agreed to it in their informed consent. The main objective of this ongoing cohort study is to investigate changes in lifestyle and risk factors for chronic diseases within patients over time in consecutive 5-year intervals. Details of the overall cohort study have been described elsewhere.[23] Participants who were examined between 1998 and 2002 and were re-examined at 5-year follow-up between 2003 and 2007 were included in the present analysis. On both examination days, respondents completed a general questionnaire and a validated food frequency questionnaire.[24,25] The general questionnaire contained questions on demographic and lifestyle factors. The 178-item semi-quantitative food frequency questionnaire assessed habitual dietary intake. Daily energy and nutrient intake were computed using an adapted version of the 1996 computerised Dutch food composition table.[26] In addition, non-fasting blood samples were obtained on each examination day.

PHARMO-RLS comprises a database in which pharmacy-dispensing data are collected of a representative sample of more than 200 community pharmacies in fifty geographically defined areas in the Netherlands.[27-29] Data used for the present study were the person's age and gender, the prescribed drug, the anatomical therapeutic chemical (ATC) classification, the defined daily dose (DDD),[30] the dispensing date and the amount dispensed.

Exposure definition

The food frequency questionnaire of the Doetinchem Cohort Study contained an open question on the brand name of the spread used on bread. The amount of margarine used was calculated by multiplying the number of bread slices consumed daily by the amount of margarine per slice, estimated from photographs of four differently sized portions. On each examination day, users of phytosterols/-stanols were defined as those with an intake of phytosterol/-stanol-enriched margarine greater than zero. To evaluate the effects of different levels of margarine use, the average margarine intake was categorised into no, low (>0 to <10 g/d), medium (≥10 to <20 g/d) or high (≥20 g/d). This represents no, low (>0 to <0.75 g/d), medium (≥0.75 to <1.5 g/d) or high (≥1.5 g/d) intake of phytosterols/-stanols. From the pharmacy-dispensing records, all prescriptions for statins (ATC classification C10AA and C10B) dispensed between 1 January 1998 and 1 October 2008 were selected. The type and dose of statin used were converted into a single equipotency score according to Penning-van Beest *et al.*[31] Subjects were considered to be users of statins at the examination

day, re-examination day or both if they were, according to the PHARMO-RLS, exposed to the drug on that specific day. Linking the Doetinchem Cohort data to PHARMO-RLS resulted in four categories of users on each examination day: (1) non-users, (2) subjects using phytosterols/-stanols without statins, (3) subjects using statins without phytosterols/-stanols and (4) subjects who combined phytosterols/-stanols and statins (combination users).

Outcome definition

Total and HDL cholesterol were determined from non-fasting blood samples using standardised enzymatic methods.[32] Non-HDL cholesterol was calculated as the difference between total and HDL cholesterol. The effectiveness of the phytosterol/-stanol-enriched margarine and/or statins was assessed by the change in total and non-HDL cholesterol, and the ratio of total cholesterol to HDL cholesterol (total/HDL cholesterol ratio) between the examination and the re-examination day.

Potential confounding variables

The following variables were considered as possible confounders: age, gender, body mass index (BMI), waist/hip circumference ratio (WHR), energy intake, (un)saturated and total fat intake, dietary cholesterol intake, alcohol intake, smoking behaviour, physical activity level, systolic and diastolic blood pressure, type 2 diabetes mellitus and educational level. Variables that altered the regression coefficient of the usage indicator variable by ≥10% were entered in the model as confounding factors.[33]

Statistical analyses

General characteristics

Demographic and health characteristics of the four groups of users were compared using ANOVA or the Kruskal-Wallis test for continuous variables and the χ^2 test for nominal variables. Analyses were based on the re-examination data of the Doetinchem study (2003-2007), as the phytosterol/-stanol-enriched margarines were available on the Dutch market only from 1999 onwards.

Effectiveness of phytosterols/-stanols and statins

A general linear regression model was used to assess differences in total cholesterol change over time between subjects not using cholesterol-lowering products at any moment and subjects who started to use phytosterols/-stanols without statins, statins without phytosterols/-stanols or both compounds at some point in time between the two examination days (analysis I, see **Figure 1**). Multivariate ANOVA was carried out to adjust for confounders at examination (1998-2002). All models were adjusted for cholesterol levels at examination as it has been shown that patients with high baseline cholesterol levels experience larger reductions in cholesterol levels after phytosterol/-stanol or statin intake.[34]

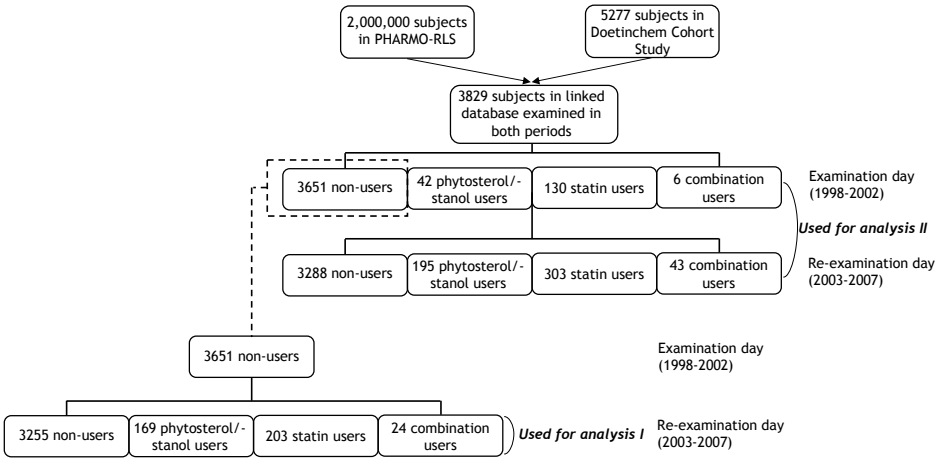

Figure 1. Flowsheet of subject numbers in the linked database used for analysis I and II

In order to describe the cholesterol-lowering effects of the use of phytosterols/-stanols more thoroughly, to include persons already using phytosterol/-stanol-enriched margarines in the years 1999-2002 and to be able to adjust for time-varying confounders, repeated-measures analysis of covariance (ANCOVA) was used (analysis II, see **Figure 1**). The following fixed effects were included in the model: use of phytosterol/-stanol-enriched margarine, use of statin and time. Furthermore, an interaction term for enriched margarine and statins was entered in the model to test whether there was a difference between the effect of the enriched margarine given with statins and the effect of the enriched margarine given without statins. Use of enriched margarine was entered in the model as a dichotomous variable (yes/no), as a continuous variable (enriched margarine use in g/d) and as a categorical variable (no/low/medium/high intake). Models were checked for collinearity and residuals were checked for homoscedasticity, outliers and normal distribution. Non-HDL cholesterol and total/HDL cholesterol ratio were analysed in the same way.

P-values were considered statistically significant at the 0.05 level. The Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

General characteristics

From the linked database, complete records were available for 3829 subjects (**Figure 1**). These subjects were examined in the Doetinchem Cohort Study during the years 1998-2002 and re-examined during the years 2003-2007. At re-examination, 195 (5.1%) of these subjects used phytosterol/-stanol-enriched margarine only, whereas 43 subjects (1.1%) combined the use of enriched margarine with statins. A total of 303 subjects (7.9%) used statins only.

Phytosterol/-stanol-enriched margarine, with or without statins, was more frequently used among the higher educated and phytosterol/-stanol users consumed more alcohol daily (**Table 1**). The vast majority of subjects who used enriched margarine used phytosterol-enriched margarine (98%). Median intake of margarine was 13 g/d, ranging from 0.12 g/d to 60 g/d (0.01 to 4.5 g phytosterols/-stanols per day). There was no significant difference in intake amount between subjects who did or did not combine their phytosterol/-stanol intake with statins. Only 9% of the subjects used the recommended margarine intake of 27 g/d (2 g phytosterols/-stanols per day).

Statin users, whether or not in combination with phytosterols/-stanols, were more likely to be male, had a higher WHR and perceived their health more often as moderate or poor compared with statin non-users. Users of cholesterol-lowering products, either phytosterol/-stanol-enriched margarine and/or statins, were older and consumed less dietary (saturated) fat compared with non-users.

Table 1. Demographic and health characteristics of non-users, phytosterol/-stanol users, statin users and combination users in the linked database (*n*=3829)

	Non-users† (*n*=3288)	**Phytosterol/-stanol users** (*n*=195)	**Statin users**† (*n*=303)	**Combination users** (*n*=43)
Age, yrs	55.0 ± 9.7[a]	58.2 ± 7.7[b]	62.7 ± 7.5[c]	60.9 ± 8.5[c]
Male gender, %	48[a b]	44[a]	56[c]	63[b c]
Low education level, %	48[a]	39[b]	55[c]	44[a b c]
History of CVD, %	3[a]	3[a]	24[b]	19[b]
Family history of CVD, %	33[a]	47[b]	43[b]	42[a b]
Comorbidities				
Hypertension, %	30[a]	43[b]	59[c]	63[c]
Diabetes mellitus, %	3[a]	4[a b]	23[c]	9[b]
Asthma, %	4	3	5	0
Ever diagnosed with HC, %	16[a]	48[b]	90[c]	98[c]
Median BMI (range), kg/m^2	25.9 (23.7-28.5)[a]	26.0 (23.7-28.3)[a]	27.4 (25.2-29.8)[b]	27.7 (24.8-29.4)[a b]
WHR	0.91 ± 0.08[a]	0.91 ± 0.08[a]	0.95 ± 0.08[b]	0.93 ± 0.07[b]
Mean blood pressure				
Systolic, mmHg	134.6 ± 18.6[a]	139.6 ± 18.6[b]	142.0 ± 20.8[b]	140.7 ± 13.6[b]
Diastolic, mmHg	84.9 ± 10.2[a]	87.6 ± 10.6[b]	85.4 ± 9.9[a]	87.4 ± 9.1[a b]
Currently smoking, %	21[a]	18[a b]	17[a b]	7[b]

Table 1. Continued

	Non-users† (*n*=3288)	Phytosterol/-stanol users (*n*=195)	Statin users† (*n*=303)	Combination users (*n*=43)
Median dietary intake (range)				
Energy, MJ/d	8.56 (7.28-10.1)[a]	8.22 (6.79-9.59)[b]	8.00 (6.67-9.19)[b]	8.25 (6.37-9.84)[a b]
Total fat, g/d	80.1 (65.2-98.3)[a]	71.0 (54.8-83.4)[b]	73.0 (61.0-88.0)[b]	73.3 (58.8-89.9)[b]
Monounsaturated fat, g/d	31.1 (24.9-38.0)[a]	27.2 (21.3-32.7)[b]	27.5 (22.5-34.1)[b]	26.7 (22.3-34.0)[b]
Polyunsaturated fat, g/d	15.9 (12.5-20.3)[a]	14.1 (10.7-17.0)[b]	15.4 (12.1-20.2)[a]	15.7 (12.3-18.8)[a b]
Saturated fat, g/d	32.1 (25.7-39.7)[a]	28.1 (22.7-33.7)[b]	28.8 (23.4-33.9)[b]	28.8 (21.4-33.5)[b]
Cholesterol, mg/d	212 (172-263)[a]	195 (156-232)[b]	206 (163-249)[b]	211 (179-271)[a b]
Alcohol, g/d	7.4 (1.4-18.7)[a]	11.4 (2.9-24.1)[b]	7.1 (1.0-20.0)[a]	11.1 (3.7-24.2)[b]
Dietary fat intake				
Total fat, % of energy	35.7 ± 5.0[a]	32.7 ± 5.0[b]	34.9 ± 4.7[c]	33.7 ± 4.7[b c]
Saturated fat, % of energy	14.3 ± 2.5[a]	13.0 ± 2.2[b]	13.7 ± 2.3[c]	13.0 ± 2.0[b c]
Low self-perceived health, %	13[a]	9[a]	24[b]	37[b]
Low physical activity level, %	18	17	18	14
Median phytosterol/-stanol margarine intake (range), g/d	na	13.2 (7.77-18.5)	na	13.1 (8.41-17.7)
Statin				
Simvastatin, %	na	na	46	46
Pravastatin, %	na	na	14	18
Atorvastatin, %	na	na	31	25
Fluva-/Rosuvastatin, %	na	na	9	11

Plus-minus values are means ± SD; Range is the interquartile range
CVD, cardiovascular disease; BMI, body mass index; WHR, waist/hip circumference ratio; HC, hypercholesterolaemia; na, not applicable
[a b c] Values within a row with unlike superscripts were significantly different ($P<0.05$)
† Numbers vary due to missing values

Effectiveness of phytosterols/-stanols in statin users and statin non-users

Table 2 presents the results of the univariate and multivariate linear regression analysis (analysis I). Calculations are based on a total of 169 phytosterol/-stanol only users, 203 statin only users, 24 combination users and 3255 non-users. These persons did not use phytosterol/-stanol-enriched margarine or statins at examination (1998-2002) and started to use one or both of these products at some point in time during the 5-year interval until re-examination. From **Table 2a** it appears that at examination, thus before the start of phytosterols/-stanols and/or statins, mean serum total cholesterol levels of future phytosterol/-stanol-enriched margarine only users were significantly higher than those of non-users (6.15 mmol/l *vs.* 5.62 mmol/l, $P<0.0001$), but significantly lower than those of future statin only users (6.15 mmol/l *vs.* 6.66 mmol/l, $P<0.0001$) and combination users (6.15 mmol/l *vs.* 6.69 mmol/l, $P=0.015$). Total and non-HDL cholesterol, and total/HDL cholesterol ratio decreased significantly during the 5-year follow-up period in all users, compared with the reference group (non-users; **Table 2b**). The largest difference in total cholesterol change compared with the non-users was found in combination users (-1.64 mmol/l, 95% CI: -1.91 to -1.37), followed by statin only users (-1.40 mmol/l, 95% CI: -1.51 to -1.30). Statistical significance was not reached for change in total cholesterol between these groups ($P=0.11$), but there was a significant difference in change in non-HDL cholesterol and total/HDL cholesterol ratio between combination users and statin only users.

Table 2. Serum cholesterol levels at examination (1998-2002) (Table 2a) and change in cholesterol levels between examination (1998-2002) and re-examination (2003-2007) day (Table 2b) in subjects who started to use phytosterols/-stanols without statins ($n=169$), statins without phytosterols/-stanols ($n=203$) or a combination of both compounds ($n=24$) between examination and re-examination, as compared with subjects who neither started using phytosterols/-stanols nor statins (non-users, $n=3255$) (*analysis I*). Data from the linked database

Table 2a. Serum cholesterol levels at examination (1998-2002)

	Non-users (reference) ($n=3255$)	**Phytosterol/-stanol users** ($n=169$)	**Statin users** ($n=203$)	**Combination users** ($n=24$)
Total cholesterol, mmol/l				
At examination	5.62 ± 0.98[a]	6.15 ± 1.00[b]	6.66 ± 1.23[c]	6.69 ± 1.05[c]
Non-HDL cholesterol, mmol/l				
At examination	4.24 ± 1.04[a]	4.75 ± 1.07[b]	5.44 ± 1.23[c]	5.47 ± 0.98[c]
Total/HDL cholesterol ratio				
At examination	4.39 ± 1.51[a]	4.74 ± 1.54[b]	5.80 ± 1.78[c]	5.97 ± 1.97[c]

Values are means ± SD

[a b c d] Values within a row with unlike superscripts were significantly different ($P<0.05$)

Table 2b. Change in cholesterol levels between examination (1998-2002) and re-examination (2003-2007) day

	Non-users (reference) (n=3255)		**Phytosterol/-stanol users** (*n*=169)		**Statin users** (*n*=203)		**Combination users** (*n*=24)	
	Crude†	Adjusted‡	Crude†	Adjusted‡	Crude†	Adjusted‡	Crude†	Adjusted‡
Total cholesterol, mmol/l								
5 yrs difference	0[a]	0[a]	-0.13	-0.16	-1.43	-1.40	-1.63	-1.64
(95% CI)			(-0.24, -0.024)[b]	(-0.26, -0.050)[b]	(-1.53, -1.33)[c]	(-1.51, -1.30)[c]	(-1.91, -1.36)[c]	(-1.91, -1.37)[c]
Non-HDL cholesterol, mmol/l								
5 yrs difference	0[a]	0[a]	-0.17	-0.18	-1.47	-1.45	-1.72	-1.74
(95% CI)			(-0.28, -0.067)[b]	(-0.29, -0.077)[b]	(-1.57, -1.37)[c]	(-1.55, -1.35)[c]	(-2.00, -1.45)[d]	(-2.01, -1.46)[d]
Total/HDL cholesterol ratio								
5 yrs difference	0[a]	0[a]	-0.21	-0.19	-1.33	-1.29	-1.72	-1.69
(95% CI)			(-0.34, -0.074)[b]	(-0.32, -0.051)[b]	(-1.46, -1.21)[c]	(-1.42, -1.16)[c]	(-2.07, -1.37)[d]	(-2.04, -1.34)[d]

[a b c d] Values within a row with unlike superscripts were significantly different ($P<0.05$)

† Adjusted for cholesterol level at examination

‡ Adjusted for age, body mass index, waist/hip circumference ratio, saturated fat intake, alcohol intake, diastolic blood pressure, type 2 diabetes mellitus and cholesterol level at examination

Table 3. Effectiveness of phytosterols/-stanols on change in total cholesterol, non-HDL cholesterol, and total/HDL cholesterol ratio between examination (1998-2002) and re-examination (2003-2007), according to repeated-measures analysis of covariance (*analysis II*). Data from the linked database

Phytosterols/-stanols	Total cholesterol, mmol/l		Non-HDL cholesterol, mmol/l		Total/HDL cholesterol ratio	
	β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value
With use as a yes/no variable						
Crude†	-0.077 (-0.17, 0.014)	0.098	-0.13 (-0.22, -0.040)	0.0047	-0.22 (-0.34, -0.10)	<0.001
Adjusted‡	-0.11 (-0.20, -0.025)	0.020	-0.16 (-0.25, -0.072)	<0.001	-0.22 (-0.34, -0.11)	<0.001
With use as a continuous variable, g/d						
Crude†	-0.0075 (-0.013, -0.0022)	<0.001	-0.0089 (-0.014, -0.0036)	0.0010	-0.011 (-0.018, -0.0041)	0.0013
Adjusted‡	-0.0094 (-0.014, -0.0043)	<0.001	-0.011 (-0.016, -0.0058)	<0.0001	-0.013 (-0.019, -0.0061)	<0.001
With use as a categorical variable						
Crude†						
No intake (0 g/d)	0		0		0	
Low intake (>0 to <10 g/d)	0.014 (-0.13, 0.16)	0.85	-0.061 (-0.21, 0.086)	0.42	-0.18 (-0.37, -0.0095)	0.063
Medium intake (≥10 to <20 g/d)	-0.058 (-0.19, 0.074)	0.39	-0.13 (-0.27, -0.0023)	0.046	-0.26 (-0.44, -0.090)	0.0028
High intake (≥20 g/d)	-0.27 (-0.46, -0.082)	0.0050	-0.25 (-0.44, -0.057)	0.011	-0.20 (-0.45, 0.044)	0.11
Adjusted‡						
No intake (0 g/d)	0		0		0	
Low intake (>0 to <10 g/d)	-0.017 (-0.16, 0.13)	0.82	-0.068 (-0.21, 0.073)	0.34	-0.14 (-0.32, 0.047)	0.15
Medium intake (≥10 to <20 g/d)	-0.089 (-0.22, 0.038)	0.17	-0.016 (-0.28, -0.032)	0.014	-0.26 (-0.42, -0.097)	0.0018
High intake (≥20 g/d)	-0.32 (-0.50, -0.14)	<0.001	-0.31 (-0.49, -0.13)	<0.001	-0.29 (-0.52, -0.051)	0.017

† Adjusted for equipotency score of statin

‡ Adjusted for age, body mass index, waist/hip circumference ratio, saturated fat intake, alcohol intake, diastolic blood pressure, type 2 diabetes mellitus and equipotency score of statin

Results of the repeated-measures ANCOVA are shown in **Table 3** (analysis II). After adjustment for age, BMI, WHR, saturated fat intake, alcohol intake, diastolic blood pressure, type 2 diabetes mellitus and statin use, the intake of phytosterols/-stanols was significantly associated with a decrease in total cholesterol of -0.11 mmol/l (95% CI: -0.20 to -0.025). Similarly, non-HDL cholesterol and total/HDL cholesterol ratio decreased significantly over time when phytosterols/-stanols were used. There was no evidence of an interactive effect between enriched margarine use and statin use, as the interaction term was not significant.

Each gram increase in enriched margarine use resulted in a decrease in total cholesterol of -0.0094 mmol/l (95% CI: -0.014 to -0.0043). Also non-HDL cholesterol and total/HDL cholesterol ratio were significantly reduced by phytosterols/-stanols. The effectiveness of phytosterols/-stanols to lower total cholesterol increased progressively across the four categories of intake amounts (0; -0.017 mmol/l, 95% CI: -0.16 to 0.13; -0.089 mmol/l, 95% CI: -0.22 to 0.038; -0.32 mmol/l, 95% CI: -0.50 to -0.14). Similar patterns were found for non-HDL cholesterol and total/HDL cholesterol ratio, although these outcome measures were significantly reduced following an intake of ≥10 g enriched margarine per day, whereas total cholesterol was significantly reduced only after high intake (≥20 g/d).

Discussion

Our results indicate that the use of margarine enriched with phytosterols/-stanols is effective in lowering total and non-HDL cholesterol, and total/HDL cholesterol ratio in both users and non-users of statins under free-living conditions. In the present study, serum total cholesterol decreased by respectively 0.16 mmol/l, 1.40 mmol/l and 1.64 mmol/l in subjects who started to use phytosterols/-stanols only, statins only or a combination of both compounds at some point in time between the examination and re-examination day, compared with subjects who did not start using phytosterols/-stanols or statins. Statistical significance was not reached for change in total cholesterol between combination users and statin only users (P=0.11), but there was a significant difference in change in non-HDL cholesterol and total/HDL cholesterol ratio between these groups. Repeated-measures ANCOVA showed slightly lower levels of effectiveness, most likely explained by the fact that the greatest reductions in cholesterol levels are achieved in subjects who started the use of the enriched margarine. The cholesterol-lowering effect of the margarine when added to statin therapy was similar to the effect observed when the margarine was used alone. This additive effect of the enriched margarine to statin therapy has also been found in prior studies.[35,36] Intake amounts above 20 g margarine per day (1.5 g phytosterols/-stanols per day) were necessary to reduce total cholesterol level significantly. In our study, only 20% of the subjects used this intake level.

In the model with continuous variables, each gram intake of margarine was associated with reductions in total cholesterol of 0.0094 mmol/l. People who consume the recommended intake of

enriched margarine of 27 g/d (2 g phytosterols/-stanols per day) may reduce their total cholesterol level by 0.25 mmol/l (0.0094 mmol/l x 27 g), which is about 4%. Although no trials have investigated the direct relationship between the intake of phytosterols/-stanols and CHD risk reduction, data from RCT and prospective studies indicate that a 4% decrease in serum total cholesterol levels would reduce the incidence of CHD by approximately 10 to 15%.[37,38] A recently conducted meta-analysis of RCT on the LDL cholesterol-lowering effects of phytosterols/-stanols found that LDL cholesterol was reduced by 0.34 mmol/l (or 8.8%) for a daily intake of 2.15 g phytosterols/-stanols.[16] In the present study, a daily intake of 2 g phytosterols/-stanols reduced LDL cholesterol levels by approximately 0.25 mmol/l or 5%, given that the cholesterol-lowering effect of phytosterols/-stanols affects only LDL cholesterol and about 80% of the circulating cholesterol in the human body is carried bound to LDL.[39] This level of effect is considerably lower than the effects expected from RCT. As could be expected, effects from statins were substantially larger compared with the effects achieved by the use of enriched margarines.

In this Dutch cohort, 98% of the enriched margarine users consumed phytosterol-enriched margarine. Yet, it is reasonable to assume that our results are also applicable to countries or situations where phytostanols are more commonly used. Phytosterols and phytostanols have been found to reduce cholesterol levels equally in both short[40-43] and longer[44] term RCT,[16] albeit it has been suggested that the cholesterol-lowering effect of phytosterols attenuates over time due to down-regulation of bile acid synthesis.[45]

Two other related studies explored the effectiveness of phytosterols/-stanols and statins in a real-life setting.[35,46] The first study did not find any significant differences in effects between cholesterol-lowering drugs only and combined intake.[46] On the other hand, phytosterols/-stanols appeared to reduce cholesterol levels additively to cholesterol-lowering drugs in the second study.[35] A major limitation of these studies was the small number of combination users; only twelve and fifteen subjects combined enriched margarine and cholesterol-lowering drugs in the first and second study, respectively. Moreover, those studies did not distinguish between statins and other cholesterol-lowering drugs and questionnaires were used for the determination of the drug usage. Administrative databases, such as PHARMO-RLS, have the advantage that patient-related recall bias and non-response bias are reduced, precise information about prescribed drugs can be obtained and the drug history is available over a long period. Pharmacy data have the advantage over medical records of being able to obtain information regarding what medication were acquired instead of what medication was prescribed. However, uncertainty still exists whether or not the drug is actually taken. Another limitation of the present study is that no information was available about the use of other phytosterol/-stanol-containing products. This might have led to an overestimation of the effect of phytosterol/-stanol-enriched margarines, as phytosterol/-stanol-enriched margarine users might be inclined to use other phytosterol/-stanol-enriched products as well. In addition, no information on food intake was gathered in the 5-year interval between examination and re-examination, and it should be acknowledged that this is an observational study which might be subject to residual confounding due to potential unmeasured differences in cardiovascular risk

profile and patient characteristics between users and non-users of phytosterol/-stanol-enriched margarine and/or statins. The restriction of the present study to a particular area of the Netherlands might constrain the generalisability of the results. Doetinchem is a rural area in the eastern part of the Netherlands and smokers and the lower educated appear to be under-represented in the cohort. However, although it is conceivable that this affects the number of subjects using enriched margarine or the baseline lipid values, it is unlikely that it has an influence on the estimated associations.

For the purpose of this study, a database was used which included pharmacy-dispensing data and questionnaire data on health and food intake. There are no standard databases available that integrate food intake and drug monitoring data, and thus methods that link large health survey data and pharmacy data are necessary to investigate effects of a combination of (functional) foods and drugs. By using such databases items like type of consumers, overall effectiveness of therapies, adherence to food and drug therapies, potential interactions on a behavioural or physiological level and long-term safety can be studied. In the near future this will become more and more important as the market for functional foods and dietary supplements with a health claim is expanding rapidly worldwide and consequently an increasing number of persons will use these products and combine them with their prescribed drugs.

Conclusions

In the present study, we found that phytosterol/-stanol-enriched margarine is effective in lowering total and non-HDL cholesterol, and total/HDL cholesterol ratio under customary conditions in both statin users and statin non- users. Recommended intake levels were achieved by only 9% of the subjects and resulted in a 4% decline in total cholesterol levels. Phytosterol/-stanol-enriched margarine can be recommended to statin non-users with normal to moderately increased serum total and non-HDL cholesterol concentrations who wish to maintain their cholesterol levels at, or reduce their cholesterol levels to, healthy levels. Statin users who wish to reduce their total and non-HDL cholesterol levels through diet can use the phytosterol/-stanol-enriched margarines as an adjunct to their ongoing statin therapy. This might be especially beneficial for those subjects who do not achieve recommended total and non-HDL cholesterol target levels with statin monotherapy. Dietetics professionals should advise consumers on the appropriate intake level of the enriched margarines and should teach consumers how to use phytosterol/-stanol-enriched margarine as part of a balanced diet.

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Chapter 2.4

Modelling approach to simulate reductions in LDL cholesterol levels after combined intake of statins and phytosterols/-stanols in humans

Simone RBM Eussen, Cathy JM Rompelberg,
Olaf H Klungel and Jan CH van Eijkeren

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Abstract

Background To examine the effects on LDL cholesterol of the combined use of statins and phytosterols/-stanols, in vivo studies and clinical trials are necessary. However, for a better interpretation of the experimental data as well as to possibly predict cholesterol levels given a certain dosing regimen of statins and phytosterols/- stanols a more theoretically based approach is helpful. This study aims to construct a mathematical model to simulate reductions in low-density lipoprotein (LDL) cholesterol in persons who combine the use of statins with a high intake of phytosterols/-stanols, e.g. by the use of functional foods.

Methods and Results The proposed model includes the cholesterol pool size in the liver and serum levels of very low-density lipoprotein (VLDL) cholesterol. Both an additional and a multiplicative effect of phytosterol/-stanol intake on LDL cholesterol reduction were predicted from the model. The additional effect relates to the decrease of dietary cholesterol uptake reduction, the multiplicative effect relates to the decrease in enterohepatic recycling efficiency, causing increased cholesterol elimination through bile. From the model, it was demonstrated that a daily intake of 2 g phytosterols/-stanols reduces LDL cholesterol level by about 8% to 9% on top of the reduction resulting from statin use. The additional decrease in LDL cholesterol caused by phytosterol/-stanol use at the recommended level of 2 g/d appeared to be similar or even greater than the decrease achieved by doubling the statin dose.

Conclusion We proposed a simplified mathematical model to simulate the reduction in LDL cholesterol after separate and combined intake of statins and functional foods acting on intestinal (re)absorption of cholesterol or bile acids in humans. In future work, this model can be extended to include more complex (regulatory) mechanisms.

Introduction

Increased total cholesterol and low-density lipoprotein (LDL) cholesterol levels represent a major risk for atherosclerosis and coronary heart disease (CHD). Lipid-lowering drugs, of which the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have shown to be the most effective, reduce morbidity and mortality in patients with CHD.[1-3] Since the last decade of the 20th century, more interest has been given to changing dietary habits, for example with the appearance of the so-called functional foods. Dairy products enriched with phytosterols/-stanols are one of the best known and most used functional foods to lower elevated total and LDL cholesterol levels. Phytosterols/-stanols are thought to compete with cholesterol for solubilisation into mixed micelles, the transport vehicles for cholesterol across the intestinal wall. Consequently, the intestinal (re)absorption of cholesterol is reduced, faecal output is increased and total and LDL cholesterol levels are lowered by 6% and 10%, respectively.[4,5] Due to the rising public awareness of health and nutritional improvement, and the mounting evidence of the effectiveness of phytosterols/-stanols, it is conceivable that in the near future an increasing number of people will combine their statin therapy with these functional foods.

To examine the effects on total and LDL cholesterol levels of the combined intake of statins and phytosterols/-stanols, in vivo studies and clinical trials are necessary. However, for a better interpretation of the experimental data as well as to possibly predict cholesterol levels given a certain dosing regimen of statins and phytosterols/-stanols a more theoretically based approach is helpful.

The present study focuses on the combined effect of atorvastatin and phytosterols/-stanols. However, our model can easily be applied to other statins and similar acting functional foods (e.g. soluble dietary fibres) as well. Moreover, based on certain genetic variants associated with cholesterol absorption and production an individual's specific reduction in total and LDL cholesterol can be predicted.

Methods

We propose a simplified mathematical model to estimate reductions in LDL cholesterol after separate and combined intake of statins and phytosterols/-stanols (**Figure 1**). A list of model variables and abbreviations is presented in the **Supplementary Table**. Since LDL is the product of very low-density lipoprotein (VLDL) delipidation and VLDL also transports, although to a lesser extent than endogenous triglycerides,[6] cholesterol from the liver to the blood circulation, our model includes the modelling of the metabolism of VLDL cholesterol as well. Also a hepatic cholesterol pool is accounted for in the model, because VLDL cholesterol secretion depends on cholesterol pool size. In the next section, we first describe a basic model which includes the modelling of the cholesterol pool, VLDL cholesterol and LDL cholesterol. Subsequently, this basic model is reformulated to express reductions in LDL cholesterol level dependent of statin and/or phytosterol/-stanol intake.

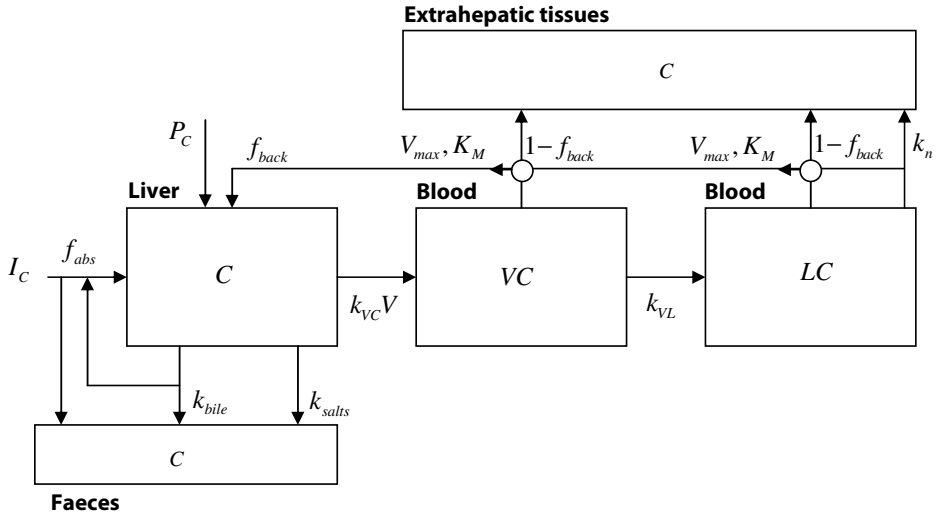

Figure 1. Simplified scheme of LDL cholesterol metabolism in humans. For detailed description of the model see text. The definition of the model variables are summarised in the Supplementary Table

Published scientific literature was used to estimate specific model parameters. In the second part of the present study (Results section), we tested the appropriateness of our model using available published experimental data.

Basic cholesterol model

Modelling of the cholesterol pool

A mass balance is considered with cholesterol input from endogenously produced cholesterol P_C and from cholesterol taken up from the diet, I_C. Only a fraction f_{abs} of dietary cholesterol is assumed to be taken up across the gut wall, and consequently the uptake of dietary cholesterol is $U_C = f_{abs} \cdot I_C$. The mass balance output consists of produced VLDL cholesterol, cholesterol cleared by elimination of excess cholesterol through bile and cholesterol needed to produce bile salts. For simplicity, we neglect the reverse cholesterol transport mediated by high-density lipoprotein (HDL) and the existence of a hepatic cholesteryl ester pool that might be involved in cholesterol homeostasis. Moreover, up- and down-regulation of LDL receptors is not considered.

The model considers only steady state levels of cholesterol, VLDL cholesterol and LDL cholesterol. For a steady cholesterol level, the input of cholesterol should be balanced by its output. Clearance of cholesterol by VLDL cholesterol formation, bile excretion and bile salts formation is assumed to be non-saturated and described by the product of clearance rates and steady cholesterol level.

Of the daily amount of VLDL cholesterol formation, $k_{VC}\ V \cdot C$, the product of steady cholesterol level C with VLDL particles V and association rate k_{VC}, a fraction f_{back} is reabsorbed into the liver. It consists of VLDL cholesterol that is not used for LDL cholesterol production and of LDL cholesterol. The other fraction $1 - f_{back}$ is taken up by the extrahepatic tissues, of which part is excreted through HDL cholesterol, which will not be considered in this modelling approach. As a consequence of this recycling, the effective clearance rate of cholesterol to VLDL cholesterol is $(1\text{-}f_{back}) \cdot k_{VC}V$. The amount of cholesterol eliminated through bile salts formation is $k_{salts} \cdot C$.

Likewise, because it is assumed that not only dietary cholesterol but also cholesterol cleared by bile with a daily amount of $k_{bile} \cdot C$ is reabsorbed through enterohepatic recycling, the effective clearance rate of cholesterol through bile is $(1\text{-}f_{abs}) \cdot k_{bile}$.

As we consider the effect of statins and phytosterols/-stanols on LDL cholesterol levels, the model becomes slightly more complicated. First, it is assumed that reduced cholesterol production P_C is related to the external daily dose S of statins, $P_C = P_C\ (S)$. Second, it is assumed that the reduced cholesterol fraction absorbed from dietary cholesterol intake relates to the amount of intake of phytosterols/-stanols (PS), $f_{abs} = f_{abs}\ (PS)$.

In the steady state the input cholesterol $P_C\ (S) + f_{abs}\ (PS) \cdot I_C$ is balanced by cleared cholesterol, which is the product of the effective clearance rates and the steady cholesterol level $((1\text{-}f_{back}) \cdot k_{VC}V + (1\text{-}f_{abs}\ (\text{PS})) \cdot k_{bile} + k_{salts}) \cdot C(S, PS)$. Thus, the steady cholesterol level is:

$$C\ (S, PS) = \frac{P_C\ (S) + f_{abs}\ (PS) \cdot I_C}{(1\text{-}f_{back}) \cdot k_{VC}V + (1\text{-}f_{abs}\ (PS)) \cdot k_{bile} + k_{salts}} \quad (1)$$

It should be noted that it is implicitly assumed that there is no interaction between statins and phytosterols/-stanols consumed, i.e. both compounds work simultaneously, independent of each other.

Modelling of VLDL cholesterol level

In the modelling of the cholesterol pool (equation (1)) it is assumed that the production of VLDL cholesterol P_{VC} is proportional to both the concentration of VLDL particles and the free cholesterol level: $P_{VC} = k_{VC}V \cdot C(S, PS)$. Like for cholesterol, a steady state level $VC(S,PS)$ of VLDL cholesterol follows from the balance between its production and its clearance. VLDL cholesterol is assumed to be cleared due to the production of LDL cholesterol with daily clearance of $k_{VL} \cdot VC(S,PS)$ and due to saturated receptor-mediated uptake from blood into the liver and extrahepatic tissues.[6] Receptor-mediated uptake is assumed to follow Michaelis-Menten kinetics with a maximum clearance rate V_{max} and a saturation constant K_M .

Therefore, a steady state VLDL cholesterol level leads to the following mass balance for LDL cholesterol:

$$P_{VC} = k_{VC}V \cdot C(S, PS) = k_{VL} \cdot VC(S, PS) + \frac{V_{max} \cdot VC(S, PS)}{K_M + VC(S, PS)} \quad (2)$$

The steady state VLDL cholesterol level can be obtained by solving the implicit equation for V*C(S, PS)*. The explicit expression for *VC* is deduced in **Supplementary Appendix 1**. Note that of the Michaelis-Menten saturated clearance of VLDL cholesterol from blood a fraction f_{back} goes into the liver. The complementary fraction 1 – f_{back} goes into extrahepatic tissues (**Figure 1**).

Modelling of LDL cholesterol level

In the modelling of the VLDL cholesterol level (equation (2)), we assumed that the production of LDL cholesterol P_{LC} is proportional to the steady VLDL cholesterol level: $P_{LC} = k_{VL} \cdot VC(S, PS)$. LDL cholesterol is assumed to be cleared with rate k_n through a non-saturated process and by saturated uptake from blood into the liver and extrahepatic tissues by the same receptors as for the saturated uptake of VLDL cholesterol.[6] Hence, the mass balance for steady state LDL cholesterol is:

$$P_{LC} = k_{VL}VC(S, PS) = k_n \cdot LC(S, PS) + \frac{V_{max} \cdot LC(S, PS)}{K_M + LC(S, PS)} \quad (3)$$

from which the LDL cholesterol level can be obtained by solving the implicit equation (3) for *LC(S, PS)*. The explicit expression for *LC* can be found in **Supplementary Appendix 1**. Similar as for VLDL cholesterol, a fraction f_{back} of the Michaelis-Menten saturated clearance of LDL cholesterol from blood goes into the liver. The complementary fraction 1 – f_{back} goes into extrahepatic tissues (**Figure 1**). V_{max}, K_M are the same maximum elimination rate and saturation constant of the Michaelis-Menten saturated uptake of VLDL cholesterol from blood into the liver. These constants are assumed to be the same, but this assumption is not essential.

Cholesterol reduction model

Modelling cholesterol reduction by statins and phytosterols/-stanols

When it is assumed that statins and phytosterols/-stanols work independently of each other, the reduction in the cholesterol pool size can be expressed in terms of a reduction: $R_P(S) = P_C(S) / P_{C,0}$ in cholesterol production caused by statin use and a reduction: $R_U(PS) = f_{abs}(PS) / f_{abs,0}$ in dietary cholesterol absorption across the gut wall caused by phytosterol/-stanol use. $P_{C,0}$ and $f_{abs,0}$ denote the cholesterol production rate and absorption fraction in absence of statins or phytosterols/-stanols. From equation (1) it is derived in **Supplementary Appendix 2** that the corresponding reduction $R_C(S, PS)$ in the steady state cholesterol level is the product of the reduction due to a decrease in enterohepatic efficiency (first factor at the right side of equation (4)) and a weighted mean of the reduction due to a decrease in cholesterol production and uptake (second factor at the right side of equation (4)):

$$R_C(S, PS) = \frac{C(S, PS)}{C_0} = \underbrace{\frac{\rho_k + 1 - f_{abs,0}}{\rho_k + 1 - f_{abs}(PS)}}_{\text{first factor}} \times \underbrace{\left\{ \frac{\overbrace{R_p(S)}^{\text{first term}}}{1 + U_{C,0} / P_{C,0}} + \frac{\overbrace{R_U(PS)}^{\text{second term}}}{1 + P_{C,0} / U_{C,0}} \right\}}_{\text{second factor}} \quad (4)$$

Here, like for cholesterol production and absorption, C_0 and $U_{C,0}$ denote, respectively, the cholesterol pool concentration and dietary uptake in absence of statins and phytosterols/-stanols. In the first factor at the right side, the ratio ρ_k denotes the proportion of cholesterol elimination through VLDL cholesterol production and bile salts production to cholesterol elimination through bile excretion, as introduced in **Supplementary Appendix 2**.

The following remarks should be made regarding this model. First, the effectiveness of statins or phytosterols/-stanols to lower cholesterol production is determined by the ratio of the contribution of endogenous produced cholesterol and the contribution of dietary cholesterol uptake to the cholesterol pool. Thus, when dietary cholesterol uptake is increased, the effectiveness of statins (first term in the second factor) is reduced with respect to the effectiveness of phytosterols/-stanols. Obviously, the opposite holds true for the effectiveness of phytosterols/-stanols.

Second, the reduction in the absorbed fraction of cholesterol has an additional effect in total cholesterol pool reduction (the second term in the second factor at the right side of equation (4)) and a multiplicative one (first factor at the right side of equation (4)). The additional effect relates to the decrease of dietary cholesterol uptake reduction, whereas the multiplicative effect relates to the decrease in enterohepatic recycling efficiency, causing increased cholesterol elimination through bile.

Third, the additional reduction caused by statin and phystosterol/-stanol use is a weighted sum of the reduction in cholesterol production and cholesterol absorption because $1/(1 + P_{C,0} / U_{C,0}) + 1/(1 + U_{C,0} / P_{C,0}) = 1$.

Formulating reduction as a Michaelis-Menten process

In the model described above, we aim to associate reductions in the cholesterol pool size to reductions in LDL cholesterol level. In order to estimate the reduction in the cholesterol pool size following statin intake, $R_P(S)$, a reduction model has to be assumed. From experimental *in vitro* data from Shum *et al.*[7] a reasonable model assumption is obtained as follows. Shum *et al.* related the concentration of atorvastatin in plasma to the inhibition of the enzyme HMG-CoA reductase *in vitro*. We assumed a Michaelis-Menten saturated inhibition process. This model was fitted to their experimental data and provided a nearly perfect fit (**Figure 2**).

From this experimental *in vitro* result, it is proposed that the reduction in endogenously produced cholesterol is most likely Michaelis-Menten saturated with administered statin dose as well:

$$R_P(S) = 1 - \frac{R_{P,max} \cdot S}{S_{P,1/2} + S} \quad (5)$$

where $R_{P,max} \leq 1$ determines the maximum achievable reduction and $S_{P,1/2}$ is the half maximum reduction statin dose.

Based on the fact that cholesterol uptake is receptor-mediated,[8] we assumed that the reduction in the fraction absorbed cholesterol is like:

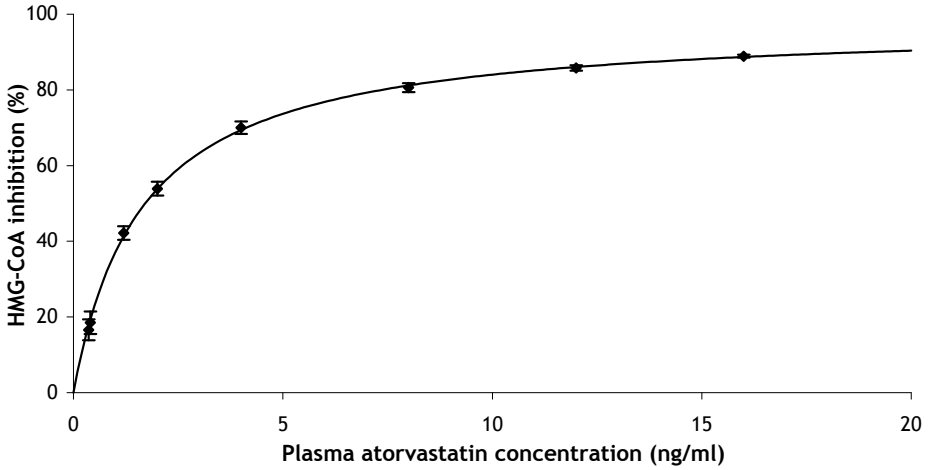

Figure 2. Simulated dose-response relation between *in vitro* plasma atorvastatin concentration (ng/ml) and hydroxymethylglutaryl-coenzyme A (HMG-CoA) inhibition (%) in humans. Maximum inhibition is 97.8% and the concentration at half maximum inhibition is 1.64 (ng/ml). Solid symbols present data by Shum *et al.*[7]

$$R_U(PS) = 1 - \frac{R_{U,max} \cdot PS}{PS_{U,1/2} + PS} \quad (6)$$

where $R_{U,max} \leq 1$ determines the maximum achievable reduction and $PS_{U,1/2}$ is the half maximum reduction phytosterol/-stanol dose.

Thus, the total reduction in cholesterol pool size after combined intake of statins and phytosterols/-stanols is obtained by substituting equations (5) and (6) in equation (4):

$$R_C(S, PS) = \frac{\rho_k + 1 - f_{abs,0}}{\rho_k + 1 - (1 - R_{U,max} \cdot PS / (PS_{U,1,2} + PS)) \cdot f_{abs,0}} \times \left\{1 - \frac{P_{C,0}}{P_{C,0} + U_{C,0}} \cdot \frac{R_{P,max} \cdot S}{S_{P,1/2} + S} - \frac{U_{C,0}}{P_{C,0} + U_{C,0}} \cdot \frac{R_{U,max} \cdot PS}{PS_{U,1/2} + PS}\right\} \quad (7)$$

In this derivation we used $1/(1+U_{C,0}/P_{C,0}) + 1/(1+P_{C,0}/U_{C,0}) = 1$

Parameter value estimation

The reduced steady state cholesterol concentration can be obtained from a given daily dose of statins or phytosterols/-stanols by applying the reduction model proposed in equation (7). From that reduced concentration, the reduced steady VLDL cholesterol level can be derived by solving equation (2), and subsequently the reduced LDL cholesterol level can be derived by solving equation (3). However, to be applicable in practice, model parameters should be known. In this section all parameters of our model are quantified based on data in the literature.

Basic cholesterol model parameters

It is assumed that the liver produces $P_{C,0}$ = 1000 mg cholesterol per day.[9-12] Furthermore, we assumed a dietary cholesterol intake of I_C = 400 mg/d, of which a fraction of 50% ($f_{abs,0}$ = 0.5)[9,13] is taken up in the liver ($U_{C,0}$ = 200 mg/d). The same fraction is supposed to be recycled through enterohepatic recycling of cholesterol excreted with bile. The final contribution to liver cholesterol input is assumed to be 70% of produced VLDL cholesterol (f_{back} = 0.7).[14]

It is assumed that the amount of cholesterol excreted with bile is 1000 mg/d[9,13,15] and consequently, 500 mg/d re-enters the liver. The rate of excretion through the formation of bile salts is 400 mg/d.[16] Concerning the local liver balance, the input is 1000 (produced cholesterol, $P_{C,0}$) plus 200 (uptake, $U_{C,0}$) plus 500 (recycled, $(1\text{-}f_{abs,0}) \cdot k_{bile} \cdot C_0$) plus 700 (back transport, $f_{back} \cdot k_{VC}V \cdot C_0$) making a total of 2400 mg/d. The output is 1000 (bile, $k_{bile} \cdot C_0$) plus 400 (bile salts, $k_{salts} \cdot C_0$), and making a total of 2400 mg/d, plus the production of 1000 mg VLDL cholesterol per day ($k_{VC}V \cdot C_0$). Moreover, as the elimination from the liver is proportional to the production rates of bile salts, cholesterol in bile and VLDL cholesterol, the ratio of these production rates is $k_{salts} : k_{bile} : k_{VC}V$ = 0.4 : 1 : 1.

From Sahlin *et al.*[17,18] we estimated the free cholesterol content in the liver to be 55 nmol/mg microsomal protein. Together with a microsomal protein content of 45 mg/g liver[19] this amounts to 2500 µmol/kg liver which equals 960 mg free cholesterol/kg liver. From this free cholesterol concentration and the daily bile excretion, one can derive k_{bile} =1000/960 = 1.04, $k_{VC}V$ = 1.04 and k_{salts} = 0.416.

Dietschy *et al.*[6] report LDL cholesterol model parameter values in humans. When assuming a subject of 70 kg, these values are V_{max} = 1340 mg/d, K_M = 90 mg/dl, k_n = 5 dl/d and P_{LC} = 910 mg/d. Based on these values, a steady cholesterol level LC = 67 mg/dl results from equation (3).

The VLDL:LDL:HDL cholesterol ratio was estimated to be 1:8:3.[20] Thus the corresponding VLDL level is 8.4 mg/dl. As the LDL cholesterol production rate is equal to $k_{VL} \cdot VC$ (equation (3)), k_{VL} = 108 (dl/d). From equation (2) a VLDL cholesterol production of 1020 mg/d can be calculated. Above it is assumed to be 1000 mg/d which shows a consistency error of 2% only.

Cholesterol reduction model parameters

From the ratio between the effective clearances, introduced in the basic cholesterol model parameters section, one can derive that the ratio ρ_k in equation (4) is 0.7. The four remaining parameters $R_{P,max}$, $S_{P,1/2}$, $R_{U,max}$, $PS_{U,1/2}$ are unknown and were fitted to optimise their likelihood in comparing modelled LDL cholesterol reduction induced by cholesterol reduction to LDL cholesterol reduction data. Thus, given an estimation of the four cholesterol reduction model parameters, the reduction in steady state cholesterol is calculated, the resulting reduction in VLDL cholesterol is determined from equation (2) and the resulting reduction in LDL cholesterol is determined from equation (3).

To simulate the appropriateness of our model, reduced levels are compared with the experimental data for separate intakes of atorvastatin[21] and phytosterols/-stanols.[22] For this procedure we use for $R_{P,max}$, $S_{P,1/2}$ data from a recent meta-analysis of Berry *et al.*[21] that shows experimentally

determined *in vivo* LDL cholesterol reduction due to atorvastatin dose. For $R_{U,max}$, $PS_{U,1/2}$ we use data presented in Demonty *et al.*[22] showing experimentally determined *in vivo* LDL cholesterol reduction due to intake of free phytosterols/-stanols, i.e. phytosterols/-stanols not in esterified form. Finally, we simulate reductions after combined intake of atorvastatin and phytosterols/-stanols.

Results

Separate intake

LDL cholesterol reduction by atorvastatin

We applied equation (7) together with the corresponding VLDL and LDL cholesterol levels equations (2) and (3) to data in Berry *et al.*[21] in an LDL cholesterol reduction model using the model parameters given above. The unknown parameter values in equation (7) were estimated through fitting the maximum reduction $R_{P,max}$ and the statin dose $S_{P,1/2}$ when half maximum reduction is reached.

Figure 3 shows the comparison of the resulting LDL cholesterol reduction model to the data in Berry *et al.*[21] Parameter values are $R_{P,max}$ = 0.544 (standard error (SE) = 0.033) and half reduction dose $S_{P,1/2}$ = 6.7 (SE = 1.4) mg/d. Given the parsimony of dose levels and scattering of data, a good comparison between the model and the experimental results is obtained (R^2 = 0.70).

LDL cholesterol reduction by phytosterols/-stanols

We applied equation (7) together with the corresponding VLDL and LDL cholesterol levels equations (2) and (3) to data in Demonty *et al.*[22] in an LDL cholesterol reduction model. The unknown parameter values in equation (7) were estimated through fitting the maximum reduction $R_{U,max}$ and the free phytosterol/-stanol dose $PS_{U,1/2}$ when half maximum reduction is reached.

Figure 4 shows the comparison of the resulting LDL cholesterol reduction model to the data of Demonty *et al.*[22] Parameter values are $R_{U,max}$ = 0.221 (SE = 0.039) and half reduction dose $PS_{U,1/2}$ = 1.78 (SE = 0.69) mg/d. The model shows reasonable agreement with the published experimental data (R^2 = 0.17).

Combined intake

LDL cholesterol reduction by combined use of atorvastatin and phytosterols/-stanols

The model is applied to LDL cholesterol reduction due to the combined intake of statins and phytosterols/-stanols. For subjects with a daily intake of 0, 20, 40 and 80 mg atorvastatin, respectively, we show in **Figure 5** the total reduction in LDL cholesterol as a function of daily phytosterol/-stanol intake. For the daily recommended intake level of 2 g free phytosterols/-stanols (equivalent to 3.3 g/d phytosterol/-stanol esters), the additional reduction is 4.2% for a daily statin dose of

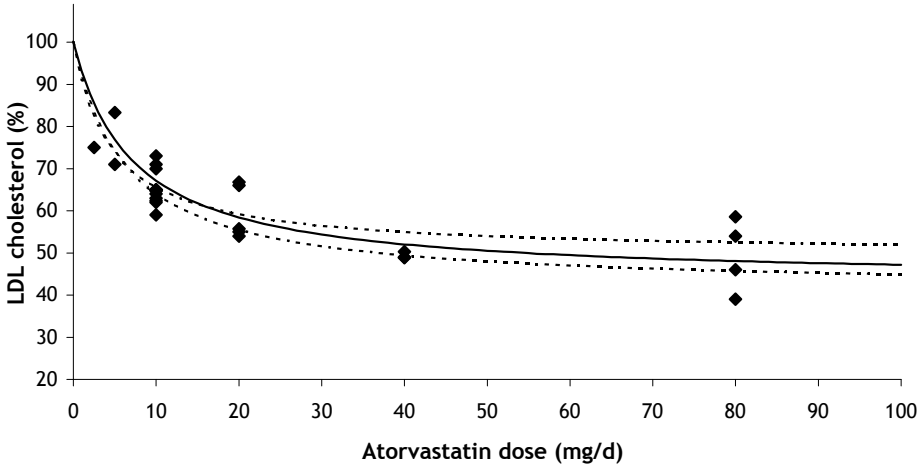

Figure 3. Simulated reduction (%) in LDL cholesterol after treatment with different doses of atorvastatin. The solid line shows the fit to the model and symbols represent experimental data from Berry *et al.*[21] Values for the Michaelis-Menten parameters are: effective maximum LDL cholesterol reduction ($R_{P,\,max}$) = 0.544 and half maximum reduction statin dose ($S_{P,\,1/2}$) = 6.7 mg/d. The dashed lines show the 5% and 95% uncertainty range in reduction obtained by correlated sampling (correlation coefficient ρ = 0.88) of $R_{P,\,max}$ and $S_{P,\,1/2}$ from their covariance matrix

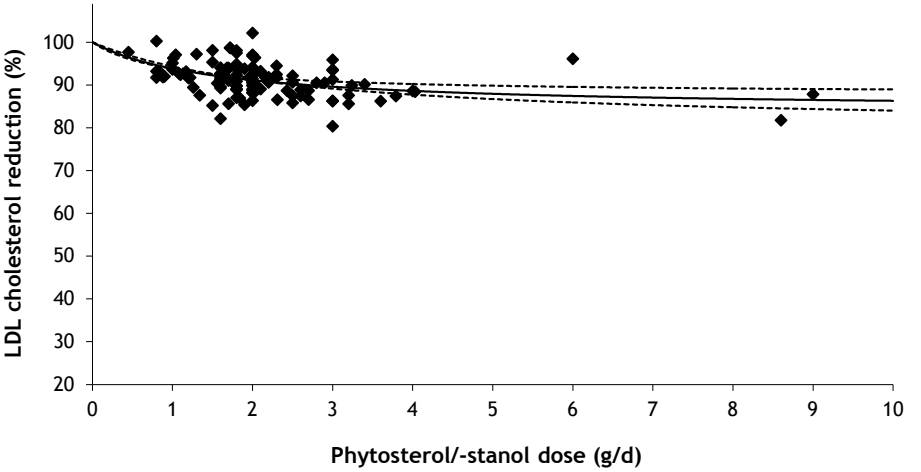

Figure 4. Simulated reduction (%) in LDL cholesterol after treatment with different doses of free phytosterols/-stanols. The solid line shows the fit to the model and symbols represent experimental data from Demonty *et al.*[22] Values for the Michaelis-Menten parameters are: effective maximum LDL cholesterol reduction ($R_{U,max}$) = 0.221 and half maximum reduction phytosterol/-stanol dose ($PS_{U,1/2}$) = 1.78 mg/d. The dashed lines show the 5% and 95% uncertainty range in reduction obtained by correlated sampling (correlation coefficient ρ = 0.98) of $R_{U,\,max}$ and $PS_{U,1/2}$ from their covariance matrix.

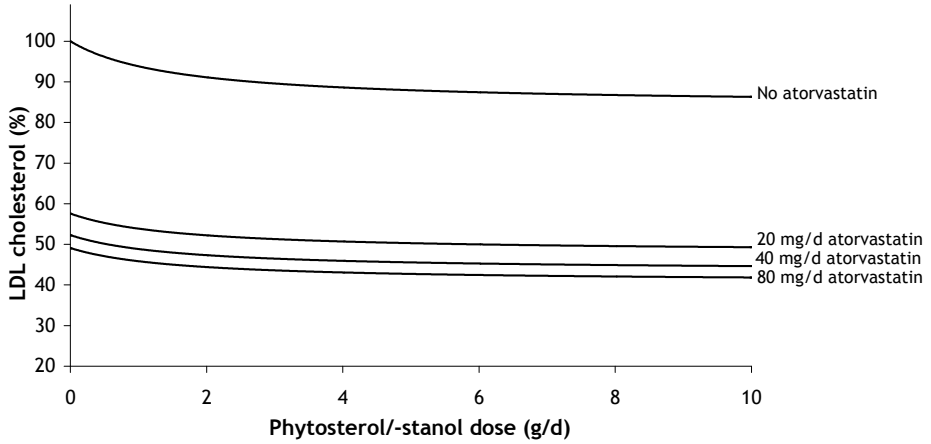

Figure 5. Simulated reduction (%) in LDL cholesterol after combined treatment with different doses of free phytosterols/-stanols and atorvastatin in humans. The lines from upper to lower show LDL cholesterol reduction for subjects that are exposed to no atorvastatin, or daily doses of 20, 40 or 80 mg atorvastatin, respectively

80 mg, 4.5% for a daily statin dose of 40 mg, 4.8% for a daily statin dose of 20 mg and 7.8% with no statin intake. Thus, the reduction in LDL cholesterol caused by additional phytosterol/-stanol intake decreases with increasing daily atorvastatin dose.

However, when considering the additional decrease as a percentage of the LDL cholesterol level already reduced due to statin intake, the additional decrease ranges from 7.8% (no statin intake) to 8.6% (80 mg daily statin dose). Randomised controlled trials in which patients on statin therapy were treated daily with 1.8 to 6 g phytosterol or –stanol esters have shown reductions in the same order of magnitude, i.e. between 6.1% and 10.3%.[23-28]

The reduction in enterohepatic recycling contributes for 68%, 58%, 56% and 55% of the total decrease in LDL cholesterol levels at daily statin doses of 0, 20, 40 and 80 mg, respectively. At the same recommended phytosterol/-stanol intake level of 2 g/d, the additional decrease in LDL cholesterol by physterols/-stanols for a daily statin dose of 20 mg (4.8%) is equal to the additional decrease by doubling daily statin dose to 40 mg (5.3%). For a daily statin dose of 40 mg the additional decrease in LDL cholesterol by physterols/-stanols (4.5%) is 30% larger than the additional decrease by doubling daily statin dose to 80 mg (3.2%).

Discussion

In this paper, a mathematical model is presented that simulates the reductions in LDL cholesterol after separate and combined intake of atorvastatin and phytosterols/-stanols in humans. We demonstrated that a daily intake of 2 g phytosterols/-stanols reduces LDL cholesterol level by about 8% to 9% on

top of the reduction resulting from statin use. This level of reduction is consistent with the findings of randomised controlled trials.[23-28] The additional decrease in LDL cholesterol caused by phytosterol/-stanol use at the recommended level of 2 g/d appeared to be similar or even greater than the decrease achieved by doubling the statin dose, a finding that has been observed previously in human trials.[23,29] The reduction in LDL cholesterol level due to phytosterol/-stanol use results from a decrease in the intestinal uptake of dietary cholesterol (additional effect) and a reduction in enterohepatic recycling (multiplicative effect). For daily statin doses of 20 mg or more, the contribution of the enterohepatic recycling reduction is 55% or more. When no statin is used, this contribution is 68%.

Mathematical models provide a valuable means of interpreting experimental data and improving the ability to predict the response to a given treatment. Other modelling studies have focused on cholesterol metabolism, but are merely aimed at answering questions on the cellular level or tend to focus on specific areas of cholesterol metabolism, such as the fluid dynamics of lipid accumulation on the arterial wall or the chemical kinetics of LDL oxidation.[30-32]

In the present study, the separate and combined effects of the cholesterol-lowering drug atorvastatin and functional foods with phytosterols/-stanols in humans were modelled. Yet, this model can easily be applied to other statins and similar acting (functional) foods as well. Products with soluble dietary fibres, for example, are also known to lower total and LDL cholesterol by reducing the intestinal (re)absorption of cholesterol and bile acids, although they work by a different mechanism as phytosterols/-stanols.[4,33,34] Moreover, individuals' specific reductions in total and LDL cholesterol can be predicted, based on certain genetic variants.[35] For example, the ratio of cholesterol synthesis to cholesterol absorption varies between individuals and is an important determinant for the cholesterol pool size.[36] Also mutations in the LDL receptor gene causing familial hypercholesterolaemia can be modelled by varying the parameter V_{max} .

There are a few possible directions for improving our model. First, the model could be extended by including the up- and down regulatory mechanisms involving the LDL receptors. Nonetheless, since we assumed that the clearing of (V)LDL cholesterol from the blood follows Michaelis-Menten kinetics, we implicitly included receptor-mediated uptake in the model. Also other regulatory control pathways were disregarded, such as the existence of a hepatic cholesteryl ester pool that might be involved in cholesterol homeostasis and the regulatory loop in the synthesis of LDL receptors.[30] Another extension would include reverse cholesterol transport mediated by HDL.[20] Moreover, the proposed model assumes that the reducing effects of statins and functional foods are independent of each other. Although this is likely the case for the combination of phytosterols/-stanols and statins,[22,24,37,38] it is uncertain whether this applies for other food-drug combinations. It has, for example, been proposed that soluble dietary fibres reduce the intestinal uptake of statins.[39,40] Our model should be extended to include such an interaction.

In conclusion, we proposed a simplified mathematical model to simulate the reduction in LDL cholesterol after separate and combined intake of statins and functional foods acting on intestinal (re)absorption of cholesterol or bile acids in humans. In future work, this model can be extended to include more complex (regulatory) mechanisms.

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Supplementary Appendix 1

Steady VLDL cholesterol concentration

The mass balance equation (2) can be rewritten as a quadratic equation in *VC*:

$$k_{VL} \cdot VC^2 + (V_{max} + k_{VL} \cdot K_M - k_{VC}V \cdot C) \cdot VC - K_M \cdot k_{VC}V \cdot C = 0$$

Here, we suppress the dependencies on statin administration *S* and dietary phytosterol/-stanol intake *PS*.

A quadratic equation has two solutions, but the only physicochemical relevant solution for which the VLDL cholesterol concentration is non-negative is:

$$VC = \frac{1}{2k_{VL}} \begin{pmatrix} k_{VC}V \cdot C - k_{VL} \cdot K_M - V_{max} \\ + \sqrt{(k_{VC}V \cdot C - k_{VL} \cdot K_M - V_{max})^2 + 4k_{VL} \cdot K_M \cdot k_{VC}V \cdot C} \end{pmatrix}$$

Steady LDL cholesterol concentration

Like for VLDL cholesterol, the mass balance equation (3) can be rewritten as:

$$k_n \cdot LC^2 + (V_{max} + k_n \cdot K_M - k_{VL} \cdot VC) \cdot LC - K_M \cdot k_{VL} \cdot VC = 0$$

with as solution:

$$LC = \frac{1}{2k_n} \begin{pmatrix} k_{VL} \cdot VC - k_n \cdot K_M - V_{max} \\ + \sqrt{(k_{VL} \cdot VC - k_n \cdot K_M - V_{max})^2 + 4k_n \cdot K_M \cdot k_{VL} \cdot VC} \end{pmatrix}$$

Supplementary Appendix 2

Reduction in steady state cholesterol

The reduction in steady state cholesterol level is:

$$
\begin{aligned}
R_C(S, PS) &= \frac{C(S, PS)}{C_0} \\
&= \frac{(P_C(S) + f_{abs}(PS) \cdot I_C) / ((1\text{-}f_{back}) \cdot k_{VC}V + (1 - f_{abs}(PS)) \cdot k_{bile} + k_{salts})}{(P_{C,0} + f_{abs,0} \cdot I_C) / ((1 - f_{back}) \cdot k_{VC}V + (1 - f_{abs,0}) \cdot k_{bile} + k_{salts})} \\
&= \frac{(1 - f_{back}) \cdot k_{VC}V + (1 - f_{abs,0}) \cdot k_{bile} + k_{salts}}{(1 - f_{back}) \cdot k_{VC}V + (1 - f_{abs}(PS)) \cdot k_{bile} + k_{salts}} \cdot \frac{P_C(S) + f_{abs}(PS) \cdot I_C}{P_{C,0} + f_{abs,0} \cdot I_C} \\
&= \frac{\rho_k + 1 - f_{abs,0}}{\rho_k + 1 - f_{abs}(PS)} \cdot \left(\frac{P_C(S)}{P_{C,0} + U_{C,0}} + \frac{f_{abs}(PS) \cdot I_C}{P_{C,0} + f_{abs,0} \cdot I_C} \right) \\
&= \frac{\rho_k + 1 - f_{abs,0}}{\rho_k + 1 - f_{abs}(PS)} \cdot \left(\frac{R_P(S)}{1 + U_{C,0} / P_{C,0}} + \frac{R_U(PS)}{1 + P_{C,0} / U_{C,0}} \right)
\end{aligned}
$$

In the third line, the ratio of clearance rates $\rho_k = ((1\text{-}f_{back}) \cdot k_{VC}V + k_{salts})/k_{bile}$ is introduced. In this line also one instance of $f_{abs,0} \cdot I_C$ is substituted by $U_{C,0}$. In the fourth line the definition of production reduction and, after dividing out intake I_C, of uptake reduction is substituted.

Supplementary Table. Model variables and abbreviations used in the study

Model variable	Abbreviation
Endogenously produced cholesterol	P_C
Dietary cholesterol intake	I_C
External daily statin dose	S
External daily free phytosterol/-stanol dose	PS
(Steady state) concentration of free cholesterol in the liver	C
VLDL particles	V
Absorbed cholesterol fraction	f_{abs}
Fraction of produced VLDL cholesterol that re-enters the liver	f_{back}
Association rate of VLDL particles and free cholesterol to VLDL cholesterol	k_{VC}
Excretion of cholesterol from the cholesterol pool by bile	k_{bile}
Excretion of cholesterol through the formation of bile salts	k_{salts}
Reduction in cholesterol pool size	R_C
Cholesterol pool concentration in absence of statins and phytosterols/-stanols	C_0
Endogenous produced cholesterol in absence of statins and phytosterols/-stanols	$P_{C,0}$
Uptake of dietary cholesterol in absence of statins and phytosterols/-stanols	$U_{C,0}$
Absorbed cholesterol fraction in absence of statins and phytosterols/-stanols	$f_{abs,0}$
Reduction in endogenous cholesterol production	R_P
Reduction in fraction of cholesterol uptake from the diet	R_U
Ratio of exponential rates of different cholesterol elimination routes	ρ_k
VLDL cholesterol production rate	P_{VC}
Transformation rate of VLDL cholesterol to LDL cholesterol	k_{VL}
(Steady state) VLDL cholesterol concentration	VC
Maximum rate of change in (V)LDL cholesterol due to saturated uptake process	V_{max}
Michaelis-Menten constant in (V)LDL cholesterol model	K_M
(Steady state) LDL cholesterol concentration	LC
LDL cholesterol production from VLDL cholesterol	P_{LC}
Clearance rate of LDL cholesterol through non-saturated process	k_n
Maximal achievable reduction in endogenous cholesterol production	$R_{P,max}$
Half maximum reduction statin dose	$S_{P,1/2}$
Maximal achievable reduction in fraction of cholesterol uptake from the diet	$R_{U,max}$
Half maximum reduction phytosterol/-stanol dose	$PS_{U,1/2}$

Chapter 3

Behavioural interactions

Chapter 3.1

A pharmaceutical care program to improve adherence to statin therapy: A randomised controlled trial

Simone RBM Eussen, Menno E van der Elst, Olaf H Klungel,
Cathy JM Rompelberg, Johan Garssen, Marco H Oosterveld,
Anthonius de Boer, Johan J de Gier and Marcel L Bouvy

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Abstract

Background Despite the well-known beneficial effects of statins, many patients do not adhere to chronic medication regimens.

Objective To implement and assess the effectiveness of a community pharmacy-based pharmaceutical care program developed to improve patients' adherence to statin therapy.

Methods An open-label, prospective, randomised controlled trial was conducted at 26 community pharmacies in the Netherlands. New users of statins who were aged 18 years or older were randomly assigned to receive either usual care or a pharmacist intervention. The intervention consisted of 5 individual counselling sessions by a pharmacist during a 1-year period. During these sessions, patients received structured education about the importance of medication adherence, lipid levels were measured and the association between adherence and lipid levels was discussed. Adherence to statin therapy was assessed as discontinuation rates 6 and 12 months after statin initiation and as the medication possession ratio (MPR), and was compared between the pharmaceutical care and usual care group.

Results A total of 899 subjects (439 in the pharmaceutical care group and 460 in the usual care group) were evaluable for effectiveness analysis. The pharmaceutical care program resulted in a significantly lower rate of discontinuation within 6 months after initiating therapy *vs.* usual care (Hazard rate ratio (HR) 0.66, 95% CI: 0.46 to 0.96). No significant difference between groups was found in discontinuation at 12 months (HR 0.84, 95% CI: 0.65 to 1.10). Median MPR was very high (>99%) in both groups and did not differ between groups.

Conclusions These results demonstrate the feasibility and effectiveness of a community pharmacy-based pharmaceutical care program to improve medication adherence in new users of statins. Frequent counselling sessions (every 3 months) are necessary to maintain the positive effects on discontinuation. Although improvements are modest, the program can be applied easily to a larger population and have a large impact, as the interventions are relatively inexpensive and easy to implement in clinical practice.

Introduction

Despite the well-known beneficial effects of statins, adherence to statin treatment is poor in daily medical practice. One-year persistence with statins has been estimated to be about 60% in patients with previous cardiovascular events.[1-3] In primary prevention, discontinuation rates are likely to be even higher.[3,4] Poor adherence is a major barrier to successful treatment. Therefore, potential benefits of statins as established in randomised controlled trials (RCT) may not be accomplished in clinical practice. Both the World Health Organization and the European Council have advocated for a multidisciplinary approach in addressing non-adherence. In this approach, the community pharmacist has an important role to play in ensuring that drug therapy is appropriate and the patient has an optimal chance of success with therapy.[5,6] Community-based pharmacists are the most easily accessible health care providers, have extensive knowledge about drug therapy and disease management, and can provide information and education to the patient and monitor adherence.

Several RCT have been conducted in which pharmaceutical interventions to enhance medication adherence have been implemented.[7-17] Evaluated interventions range from giving patients more information and education on the goals and benefits of drug therapy to the simplification of the drug regimen and intensification of patient care by telephone reminders, home visits, and follow-up interviews. Most RCT showed beneficial effects on adherence,[10,15,17] lipid levels,[7,8,13,14,16] or both.[9] Moreover, overall health care expenditures in the intervention and control group seem to be similar, despite increased visits to the pharmacist and laboratory costs.[8]

It has been shown that the most critical need for adherence interventions is during the first few months of therapy, as adherence levels drop shortly after initiation of statin treatment.[18] Hence, persons who have been newly prescribed medications comprise an interesting subgroup when pharmaceutical care programs are implemented. Most studies aimed at improving adherence among users of statins were hospital pharmacy-based,[7-10,14,16] sometimes with complex interventions[10,14,17] and mostly not focusing solely on patients initiating statin treatment.[7,8,10-14,16] We therefore developed a large multicenter community pharmacy-based pharmaceutical care program for new statin users. This program was aimed at improving adherence to statin therapy by giving patients education and feedback on achieved lipid levels. These interventions are easy to implement in the community pharmacies, are relatively inexpensive, and have been shown to be effective in clinical trials with various patient populations.[13,14] The purpose of the present study was to examine the feasibility and effectiveness of this program.

Methods

Study population

This study, the **ST**atin **I**ntervention research **P**roject**T** (STIPT) was a community pharmacy-based, multicenter, open-label, randomised controlled trial to improve medication adherence in new users of statins. Patients were recruited from 26 community pharmacies (both independent and chain stores) in the Netherlands and were eligible for inclusion if they were new users of statins, were aged 18 years or older, and were capable of visiting the pharmacy. New users were defined as those who had not filled a prescription for statins in the preceding 6 months, verified by the pharmacist through a patient record check. Virtually all Dutch inhabitants are registered with a single community pharmacy, independent of prescriber. Consequently, pharmacy records are nearly complete with regard to prescription drugs.[19] The study was approved by the Medical Committee of Ethics of the University Medical Centre Utrecht and all patients signed written informed consent prior to the study. Study enrolment started in September 2004 and was completed in March 2006.

Study design

Once the informed consent form was received, each participant was randomly assigned to either the intervention or control group by a procedure that was built into the computer system and used a set of random numbers in a 1:1 ratio. Patients in the intervention (pharmaceutical care) group were invited to visit the pharmacy for 5 individual counselling visits, each lasting 10-15 minutes. Counselling visits were scheduled at first prescription, at second prescription (after 15 days), and at subsequent refill dates at 3, 6 and 12 months after the start of statin therapy. In the Netherlands, the first prescription for statins is limited to 15 days[20] and subsequent prescriptions are generally dispensed in 3-month supplies. Because it has been shown that patients are most likely to discontinue statins in the first months after therapy initiation,[18] counselling sessions were scheduled more frequently during the first months of treatment. Counselling at time of first prescription comprised structured education on indication, effects and adverse effects of statin therapy, dosage, importance of medication adherence, and intended duration of treatment. Additionally, a drug information letter that summarised the verbal information was given to each patient. At the time of the second prescription, patients were asked about their experience with statin therapy, potential drug-related problems and difficulties in adhering to the dosing regimen. At 3, 6 and 12 months, total and high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured from fasting fingerstick whole blood samples using Cholestech LDX Analysers (Cholestech Corp., Hayward, CA, USA) and low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald formula.[21] Measured lipid levels and treatment goals were recorded on a wallet card that was kept by all patients to monitor their progress in lowering lipid levels. In addition, medication adherence was assessed via unused pill counts and the association between adherence and lipid levels was discussed to encourage patients to adhere to the prescribed dosing regimen.

Patients in the control group were provided usual care, consisting of verbal and written drug information according to the standard protocol in the pharmacies. Patients in the usual care group did not receive lipid measurements or counselling sessions.

In both the pharmaceutical care and usual care groups, patients were asked to fill out a questionnaire at baseline and after 6 and 12 months. The baseline questionnaire included items on sociodemographics, (family) history of cardiovascular disease (CVD), comorbidities, self-perceived health, lifestyle factors (smoking habits, alcohol consumption, dietary habits), and the application of other lipid-lowering strategies (e.g. eating healthier or becoming more physically active). Questionnaires at 6 and 12 months contained questions about changes in self-perceived health and lifestyle modifications to lower lipid levels.

All questions about the study or treatment from patients in both treatment arms were answered as forthrightly as possible. Participants and those administering the interventions were not blinded to the treatment assignment. Conversely, those assessing differences in outcomes between the pharmaceutical care and usual care groups remained blinded throughout the study.

Outcome definition

Electronic pharmacy-dispensing records of all patients were collected after the end of follow-up. Adherence to statins was evaluated in terms of discontinuation of treatment and the medication possession ratio (MPR).[22] The primary endpoint of this study was discontinuation of treatment assessed 1 year after the start of statin therapy. Secondary endpoints were discontinuation rates 6 months after statin initiation, the MPR and the relation between MPR and total and LDL cholesterol levels. Patients were considered to have discontinued therapy if they failed to refill their statin agents within 90 days or 1 time the theoretical duration of the prescription, whichever was the lowest number of days.[23] Time to discontinuation was defined as the number of days between the start of statin therapy and the discontinuation day. When a patient refilled a prescription for the same type of statin before the theoretical end date of the previous prescription, we assumed that the new prescription began after the end date of the previous one.[24] Patients who switched from one type of statin to another were considered to be continuous users. Patients were censored at the end of the study period or when they changed to a pharmacy not participating in the study or died before the end of follow-up. The patient's MPR was assessed from the pharmacy-dispensing records at the end of study or, for patients who stopped statin therapy earlier, at the time of discontinuation. The MPR was calculated as the ratio of the sum of the days' supply of all statin medication dispensed divided by the length of therapy. A patient with an MPR of 0.9 or more was defined as being adherent to the prescribed dosing regimen. Medication adherence assessed by pill counts during the counselling sessions was not regarded as an outcome of this study but was used solely to instantly address an individual's adherence at the counselling session.

Statistical analysis

Necessary sample size was estimated with the assumption of a 1-year discontinuation rate of 33% in the control group, as suggested by a previous study in a comparable patient population,[25] and of 24% in the pharmaceutical care group. The pharmaceutical care group discontinuation rate was chosen conservatively based on previous effects of community pharmacy-based programs.[15,17] With an 80% power of detecting a significant difference (P=0.05, 2-sided) between the two groups and an expected loss to follow-up of 20%, a sample size of 493 patients in each group (986 total) was required.

Patient characteristics were compared between the pharmaceutical care and usual care groups using an independent sample Student's t-test or χ^2 test as appropriate. Discontinuation was estimated by using Kaplan-Meier analysis and was compared between the groups with a log-rank test. Univariate Cox proportional hazard models were used to compare further the probability of discontinuation between the groups. In addition, Cox proportional hazard models were used to estimate the probability of discontinuation at 12 months in various exploratory subgroups that were defined by factors potentially associated with discontinuation. Those factors were age, gender, level of education, comorbidities (hypertension, diabetes mellitus, history of CVD), familial hypercholesterolaemia, the application of other lipid-lowering strategies, and the number of medications used (at Anatomical Therapeutic Chemical (ATC) classification level 3).[26] A treatment-by-subgroup interaction term was added to the model to test whether different subgroups had different risks. The MPR between the two study groups was analysed using the non-parametric Mann-Whitney U test and the percentage of subjects having a high (≥90%) or low (<90%) MPR was compared using the χ^2 test. The number of subjects switching to a statin with a different equipotency score (measure for the potency of a statin to lower total cholesterol according to type and dose)[27] was computed and compared between the pharmaceutical care and usual care groups with the Mann-Whitney U test.

Lipid levels were measured only in the intervention group as part of the pharmaceutical care program. Therefore, the effect of differences in MPR on lipid levels could be estimated only in these subjects. Patients were considered to have met lipid treatment goals if they achieved fasting total cholesterol levels of <5 mmol/l and LDL cholesterol levels of <3 mmol/l.[28,29] The percentage of subjects reaching lipid goals among patients with high (≥90%) or low (<90%) MPR was compared using the χ^2 test. Spearman correlation was used to determine the relationship between the MPR and lipid levels.

The results were considered statistically significant at a 2-sided probability level of P<0.05. All statistical analyses were performed according to the intention-to-treat principle using the Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Patient enrolment and baseline characteristics

A total of 1016 subjects were enrolled in the trial, 513 (50%) of whom were randomised to the pharmaceutical care group and 503 (50%) to the usual care group (**Figure 1**). A total of 117 patients were excluded because no pharmacy-dispensing data were available for these subjects, due to mismatch between data from the electronic records and the handwritten study entry forms. Thus, 899 patients (439 in the pharmaceutical care group and 460 in the usual care group) were eligible for analysis. Of the patients in the pharmaceutical care group, 62 (14%) did not attend any follow-up counselling session, whereas 29 (7%), 43 (10%) and 305 (69%) patients attended 3, 4, and all 5 counselling sessions, respectively.

Baseline characteristics of the patients are shown in **Table 1**. Mean age of all participants was 60.1 ± 11.1 years and 49% were male. Most patient characteristics were similar between the groups. However, significantly more patients in the usual care group had a history of CVD, and those in the usual are group classified their health status more often as moderate/poor. Significantly more patients in the pharmaceutical care group were prescribed atorvastatin, whereas fewer pharmaceutical care patients were prescribed rosuvastatin. Most patients (52%) started statin therapy at a medium equipotency score, equivalent to a simvastatin dose of 20 mg/d or an atorvastatin dose of 10 mg/d.

Discontinuation of statin treatment

Figure 2 presents the Kaplan-Meier curve, comparing discontinuation of statin therapy over time between patients in the pharmaceutical care and usual care groups. Of the 899 patients, 58 were censored (20 in the pharmaceutical care group and 38 in the usual care group) because they died or left the study pharmacy before the end of follow-up. A total of 47 (11%) patients in the pharmaceutical care group and 72 (16%) patients in the usual care group discontinued statins within 6 months after the initiation of treatment (*P*-value for log-rank test=0.026). The corresponding percentages at 1 year after the start of therapy were 23% and 26%, respectively, in the pharmaceutical care and usual care groups (*P*-value for log-rank test=0.21). The hazard rate ratio of discontinuing statin therapy, as determined by the Cox proportional hazard analysis, showed that patients in the pharmaceutical care group had a statistically significantly lower rate of discontinuation within 6 months

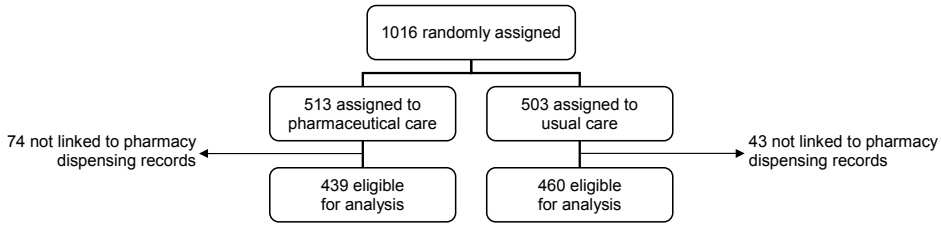


Figure 1. Patients enrolment in the **ST**atin **I**ntervention research **P**roje**T** (*n*=899)

Table 1. Baseline patient characteristics in the pharmaceutical care (*n*=439) and usual care (*n*=460) group

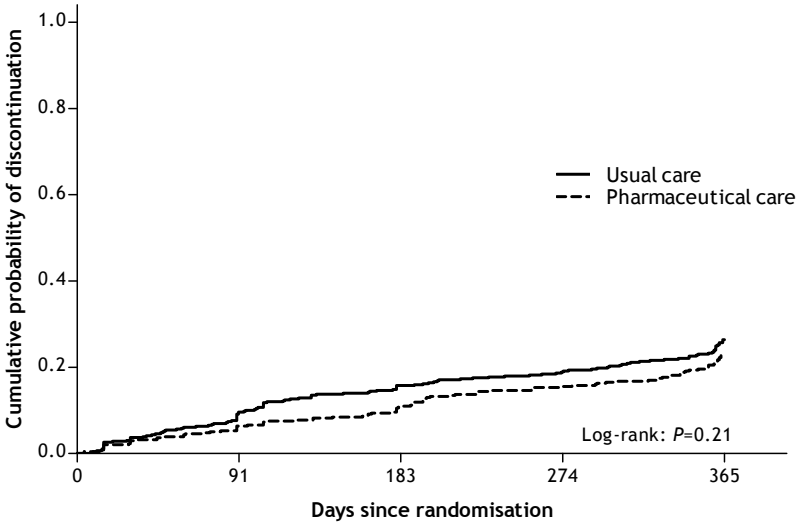
	Pharmaceutical care (*n*=439)	Usual care (*n*=460)
Age, yrs	60.2 ± 10.9	60.1 ± 11.3
Male gender, *n* (%)	207 (47)	230 (50)
Dutch origin, *n* (%)†	359 (91)	380 (93)
Marital status, *n* (%)†		
Married/living together	297 (80)	325 (83)
Unmarried/widowed/divorced	74 (20)	67 (17)
Level of education, *n* (%)† ‡		
Low	156 (42)	162 (42)
Intermediate	160 (43)	158 (40)
High	53 (14)	70 (18)
Comorbidities, *n* (%)†		
Hypertension	169 (43)	200 (49)
Diabetes Mellitus	115 (29)	111 (27)
Respiratory disease	30 (8)	35 (9)
History of CVD, *n* (%)† §	117 (30)	146 (37)
Family history of hypercholesterolaemia, *n* (%)†	96 (24)	112 (27)
Lifestyle factors, *n* (%)†		
Current smoker	93 (24)	88 (22)
Alcohol use ≥ 1 times p/w	73 (20)	68 (17)
Following a specific diet	155 (40)	160 (40)
Other lipid-lowering strategies, *n* (%)†		
Smoking cessation or reduction	53 (14)	42 (10)
Reducing alcohol consumption	52 (13)	50 (12)
Eating healthier	184 (47)	199 (49)
Becoming more physically active	148 (38)	169 (42)
Using plant sterol/stanols	154 (39)	166 (41)
Self-perceived health, *n* (%)† §		
(Very)good	276 (74)	273 (69)
Moderate/poor	96 (26)	125 (31)
Statin, *n* (%)‡		
Simvastatin	157 (36)	153 (33)
Pravastatin	40 (9)	59 (13)
Atorvastatin§	169 (39)	139 (30)
Rosuvastatin§	68 (15)	98 (21)
Fluvastatin	4 (1)	11 (2)

Plus-minus values are means ± SD; CVD, cardiovascular disease

† Numbers vary due to missing responses in the questionnaire. Percentages are calculated without missing values.

‡ Due to rounding, percentages may not total 100%

§ Statistically significant difference, $P<0.05$, X^2 test



Number of subjects at risk					
Usual care	460	415	384	360	304
Pharm care	439	415	391	366	319

Figure 2. Kaplan-Meier curve for discontinuation of statin agents in patients in the pharmaceutical care group and in the usual care group (*n*=899)

after initiating therapy than did patients in the usual care group (HR 0.66, 95% CI: 0.46 to 0.96). Thus, patients in the pharmaceutical care group were 34% less likely to discontinue treatment, or 1.52 (95% CI: 1.04 to 2.17) times more likely to persist with treatment compared with patients in the usual care group. Twelve months after therapy was initiated, this difference in discontinuation rate was not statistically significant (HR 0.84, 95% CI: 0.65 to 1.10).

Analyses of discontinuation rates by subgroups are shown in **Figure 3**. We noted a significant treatment-by-subgroup interaction between patients using ≤5 or >5 medications at the ATC3-level (treatment-by-subgroup interaction, *P*=0.028), which indicated that patients using more medications were less likely to benefit from the pharmaceutical care program. Although patients aged 50 years or younger, females, the higher educated, and patients who did not implement other lipid-lowering strategies seemed to gain more benefit from receiving pharmaceutical care, the differences in effect of the pharmaceutical care program between the subgroups were not statistically significant.

Medication possession ratio and statin adjustments

The median MPR (25^{th}–75^{th} percentile) was 99.5% (96.9-100%) in the pharmaceutical care group and 99.2% (95.6-100%) in the usual care group (*P*=0.14). Only 37 patients (8%) in the pharmaceutical care group and 54 patients (12%) in the usual care group had an MPR <90% (χ^2: *P*=0.10). There was no significant difference between the groups in the percentage of patients switching to a statin with a different equipotency score.

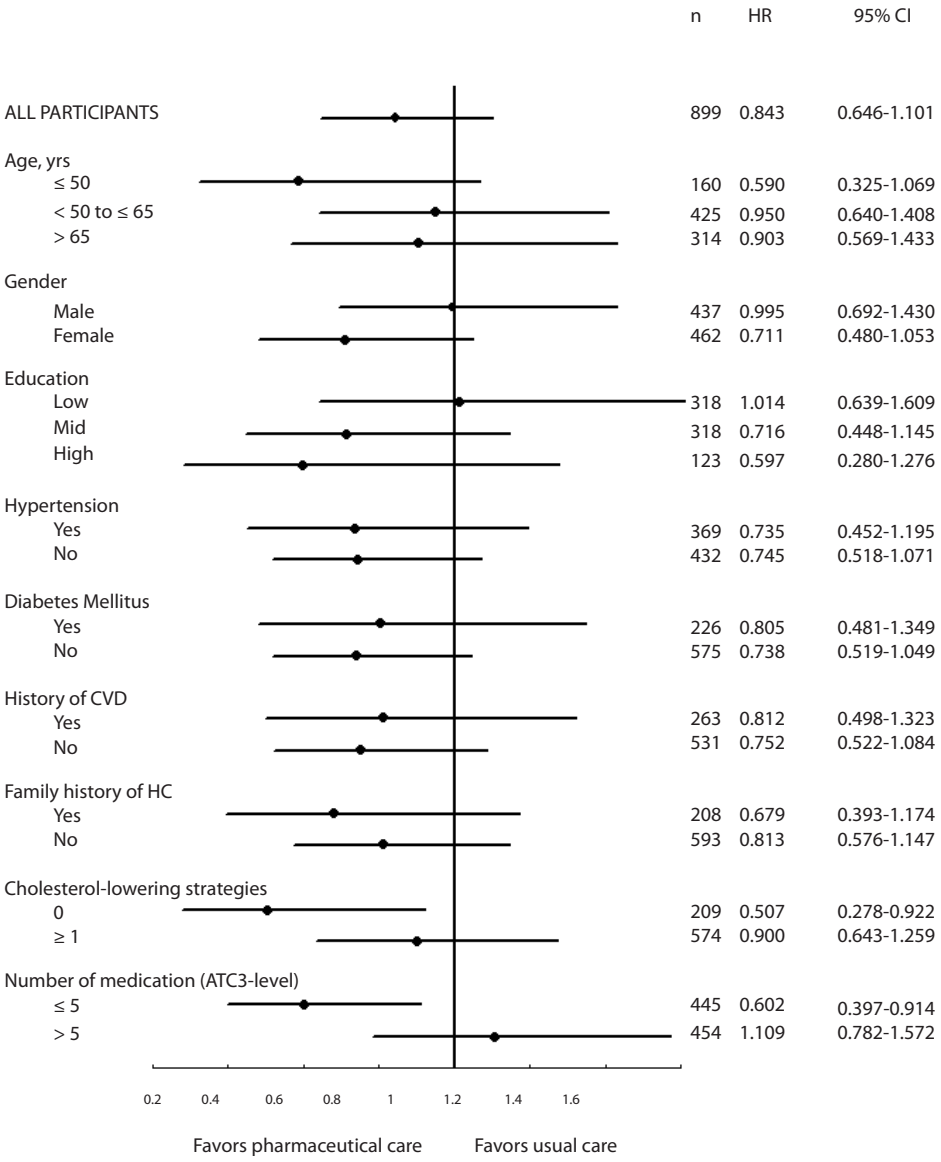


Figure 3. Incidence of discontinuation of statin agents in subgroups of the **ST**atin **I**ntervention research **P**roject**T** (*n*=899) according to Cox proportional hazard analyses
CVD, cardiovascular disease; HC, hypercholesterolaemia; ATC, Anatomical Therapeutic Chemical

Lipid levels

In patients receiving pharmaceutical care, both mean total and LDL cholesterol levels declined significantly during the study. The average reduction in total cholesterol and LDL cholesterol was 0.44 mmol/l (95% CI: 0.32 to 0.57) and 0.24 mmol/l (95% CI: 0.13 to 0.36), respectively. Three

months after initiating statin therapy, 65% of the subjects reached the target LDL cholesterol level below 3 mmol/l. At 6 and 12 months after treatment, these percentages were 72% and 77%, respectively. A higher percentage of adherent patients (MPR ≥90%) than non-adherent patients reached target LDL cholesterol levels after 3 months (67% *vs.* 45%, respectively, *P*=0.01) and 6 months (74% *vs.* 50%, respectively, *P*=0.01). Spearman's correlation showed a significant negative association between the MPR and total cholesterol (r= -0.16, *P*=0.002) and a trend toward a negative association between the MPR and LDL cholesterol level (r= -0.10, *P*=0.08).

Discussion

Patients who understand the benefits of treatment and are satisfied with health care provider communication, and those with frequent follow-up lipid tests, have been shown to be more adherent to statin therapy.[30] In the present study, a community pharmacy-based pharmaceutical care program composed of patient counselling and feedback on achieved lipid levels was associated with modestly lower discontinuation rates of statin therapy. Compared with patients in the usual care group, those in the pharmaceutical care group were 34% less likely to discontinue treatment within 6 months (*P*=0.03) and 16% less likely to discontinue treatment within 1 year after initiating statin therapy (*P*=ns). This difference in effect on discontinuation rates between 6 and 12 months might imply that frequent counselling sessions (every 3 months) are necessary to maintain the positive effects. However, the fact that the difference between groups in discontinuation rates at 12 months did not reach statistical significance could also be explained by other factors. Most importantly, discontinuation rates in the usual care group were lower than anticipated. The margin for improvement was therefore less than hypothesised in the power calculation. This might be due to the fact that adherent patients and pharmacies that had already been involved in advanced provision of pharmaceutical care were more willing to participate in the program. Moreover, adherence to therapy in the usual care group might have been enhanced because the subjects were aware that their behaviour was being monitored. Several studies aimed at improving adherence have shown unexpected high adherence in usual care groups.[31,32] The fact that patients included in the study reported a relatively high proportion of health-promoting behaviour modifications suggests that study patients were more aware of their lipid levels and cardiovascular risk. Therefore, the effect of this pharmaceutical care program on adherence might be higher in routine medical practice.

Another reason for the lack of effect of the pharmaceutical care program on 1-year discontinuation rates is that 19% and 31% of the patients randomised to the pharmaceutical care group did not attend the follow-up counselling session at 6 and 12 months, respectively. Patients not adhering to the study protocol cannot benefit optimally from the program, leading to a diluted treatment effect. When this program is being implemented in daily medical practice, an effort should be made (e.g. by sending reminders and contacting patients who did not show up for their scheduled counselling session) to ensure that patients adhere to the counselling sessions. In the present study,

we present only results obtained from an intention-to-treat analysis. Analysing results according to the per-protocol principle of including only patients who had at least 1 follow-up counselling visit could introduce selection bias,[33] due to associations between discontinuation of statin therapy and study drop-out.

Finally, significantly more patients in the usual care group reported a history of CVD. This might have affected our results, as it is known that persistence with statin therapy is better among patients with pre-existing CVD.[34] However, including CVD status as a confounder in Cox proportional hazard analysis did not change our results.

Although not statistically significant, the pharmaceutical care program seemed to be more effective in younger patients, females, the higher educated, and patients not taking many other medications, i.e. patients generally classified as having a lower cardiovascular risk profile. Several observational studies have shown lower statin adherence among these patients.[35-38] Therefore, the margin of improvement might be greater in these subgroups.

Despite the high MPR, we found significant associations between differences in the MPR and total and LDL cholesterol-lowering effects. Because lipid levels were measured only in the pharmaceutical care group, we were not able to study the effects of the pharmaceutical care intervention on lipid levels. Measuring lipid values in the usual care group probably would have influenced patients' behaviour and thereby would have increased adherence in the usual care group. As a result, the effect of the intervention would have been diluted. However, as discussed earlier, is it still conceivable that adherence to therapy is higher in the usual care group compared with daily medical practice.

We did not observe more patients switching to another type or dose of statin in the pharmaceutical care group. Apparently, measuring lipid levels can be seen primarily as a method to give feedback to patients on the effect of statin treatment and does not result in adjustments of drug therapy. However, a lack of feedback from the pharmacist to the physician might also be a reason for the absence of dosage or drug adjustments.

In the present study we used pharmacy-dispensing data to calculate patient adherence to statin medication. These data present many advantages over both self-reported adherence and medical records. Dispensing data are not suspect to patient-related recall bias and reduce non-response bias. However, uncertainty still exists as to whether dispensed drugs are actually being taken according to the prescribed regime. In a study monitoring patient adherence to lipid-lowering therapy in clinical practice, it was found that, during the monitoring period of 6 months, approximately 60% of patients erroneously took multiple doses of statins per day.[39] In addition, we did not have information for many patients about the reason for discontinuation, and therefore we were unable to assess whether statin therapy was discontinued for clinical reasons. However, this would seem uncommon, as statin therapy is mostly indicated over a patient's lifetime and statins have a relatively mild adverse event profile.[40] Another limitation of this study is that we could not perform a double-blind study because of the nature of the intervention studied in this trial.

The authors recognise that randomisation at the patient level, rather than at the pharmacy level, may have contaminated the care received by the patients in the usual care group by pharmacists' knowledge of the pharmaceutical care program. This would have increased the risk of a type II error, i.e. incorrectly accepting the null hypothesis, and therefore could have diluted the effect size. In this study, however, extra time was scheduled for patients in the pharmaceutical care group for measuring the lipid levels and for counselling. Patients randomised to the usual care group visited the pharmacy only to refill their statin prescription. The alternative of a cluster-randomised trial would have given rise to other problems, such as recruitment bias, since participants are recruited after the clusters have been randomised.[41-43]

In conclusion, we demonstrated the feasibility and effectiveness of a community pharmacy-based pharmaceutical care program to improve medication adherence in new users of statins. Although improvements in adherence were modest, the program is convenient for the patients because counselling sessions are linked to the prescription refill dates. Moreover, the interventions are relatively inexpensive and easy to implement; the lipid tests cost about €55,- per patient and counselling sessions take an additional hour per patient. Therefore, the program can be applied easily to a larger population and have a large impact on population level. Health economic studies should be performed to fully assess the cost-effectiveness of this pharmaceutical care program.

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Chapter 3.2

Effects of the use of phytosterol/ -stanol-enriched margarines on adherence to statin therapy

Simone RBM Eussen, Nynke de Jong, Cathy JM Rompelberg, Johan Garssen, WM Monique Verschuren and Olaf H Klungel

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Abstract

Background The use of margarines enriched with phytosterols or phytostanols is recommended as an appropriate adjunctive therapy for patients with certain lipid profiles, but may result in a behavioural modification leading to a change in person's adherence to lipid-lowering drug treatment.

Objective This study aimed to examine the influence of the use of margarines enriched with phytosterols/-stanols on adherence to statin therapy.

Methods Retrospective data from food frequency questionnaires were used to assess phytosterol/-stanol-enriched margarine intake from a population based, longitudinal cohort between 1998 and 2007. Intake data were linked to pharmacy-dispensing records. Multivariate Cox proportional hazards models were used to calculate adjusted hazard rate ratios (HR_{adj}) for discontinuation of statin therapy. The medication possession ratio was compared between users and non-users of enriched margarine using the Mann-Whitney *U* test. Predefined subgroup analyses were performed to evaluate differences in adherence between prevalent statin users and starters of statins.

Results Among 4848 subjects, 522 used statins only and 60 combined these drugs with phytosterol/-stanol-enriched margarine. Overall statin discontinuation rates were not significantly different between the users and non-users of enriched margarine (HR_{adj} 1.37, 95% CI: 0.82 to 2.31), but more combination users discontinued statin therapy within 12 months in the subgroup of starters (HR_{adj} 2.52, 95% CI: 1.06 to 6.00). The medication possession ratio was high in both users and non-users of enriched margarine and was slightly lower in combination users ($P<0.10$).

Conclusions These results imply that persons who combine enriched margarines with statins may neglect taking their drug according to the prescription. Further investigations in larger populations are important, especially among patients susceptible to a low adherence to drug therapy.

Introduction

The popularity of functional foods with nutritional or health claims is growing and consequently an increasing number of persons will use these products in the near future. As certain functional foods have the same health claim as pharmaceutical products, it is conceivable that more and more persons will combine these foods with their prescribed drugs. Combined intake of functional foods and drugs might result in unexpected effects due to physiological interactions between the functional ingredients and active drug constituents, or behavioural modifications, potentially leading to changes in adherence to drug therapy. On the one hand it is conceivable that persons lower the dose of their drugs, or that they take their drug less consistently, as they have implemented an additional therapy with potentially less side effects.[1] On the other hand one can speculate that combined use of functional foods and drugs may have a stimulating impact on drug taking behaviour as subjects who are highly motivated to lower their cholesterol levels will be more adherent to their drug therapy and these subjects are also prone to buy the expensive functional foods. Insight into factors affecting statin adherence is highly relevant, since poor adherence to statin therapy is common in daily medical practice[2] and is associated with significant health risks.[3]

A key-example for food-drug interaction is simultaneous use of phytosterol/-stanol-enriched margarines and statins. Both randomised controlled trials[4-8] and post-launch monitoring studies[9,10] have found that the simultaneous intake of statins and phytosterols/-stanols produces a purely additive effect (i.e. no interactive effect) on cholesterol reduction: it is estimated that adding phytosterols/-stanols to statin therapy further reduces total and LDL cholesterol by roughly 6% and 10%, respectively.[11] To our knowledge, no studies have been performed examining patients' behaviour toward statin adherence when phytosterol/-stanol-enriched margarines are added to the diet. There are currently no standard databases available that integrate food intake and drug monitoring, and therefore we linked data from an ongoing free-living cohort containing information on functional food use to a pharmacy-dispensing database. The aim of the present study was to examine the influence of the use of margarines enriched with phytosterols/-stanols on persons' adherence to statin therapy.

Methods

Study setting

Patients' data from the Dutch Doetinchem Cohort Study[12] and the Pharmacomorbidity-Record Linkage System (PHARMO-RLS)[13-15] were linked using information on gender, date of birth and postcode in order to obtain information on the use of margarines enriched with phytosterols/-stanols and statins of the same subjects.

The Doetinchem Cohort Study was approved according to the guidelines of the Helsinki Declaration by the external Medical Ethics Committee of the Dutch TNO Research Institute. Linkage has

been performed only for those participants who have agreed on that in their informed consent.[12] In a validation sample of subjects who consented to use their complete information on name and address, it was assessed that about 95% of the subjects were linked correctly. The main objective of the Doetinchem Cohort Study is to investigate changes in lifestyle and risk factors for chronic diseases within patients over time in consecutive 5-year intervals.[12] From the Doetinchem Cohort Study, detailed nutrition and health related data were available from 5277 subjects who were examined in the years 1998-2002 and/or (re-)examined at 5-year follow-up during 2003-2007. On the examination days, demographic and health characteristics were collected using a standardised questionnaire, including items regarding smoking habits, educational level and physical activity. A validated 178-item semi-quantitative food frequency questionnaire assessed habitual dietary intake.[16,17]

The PHARMO-RLS includes pharmacy-dispensing records from a representative sample of more than 200 community pharmacies in 50 geographic defined areas in the Netherlands. The database comprises records of about 2,000,000 people. The computerised records include information regarding the patient (gender and date of birth), the prescribed drug, the anatomical therapeutic chemical (ATC) classification, the defined daily dose (DDD),[18] the dispensing date, and the amount dispensed. Since virtually all Dutch inhabitants are registered with a single community pharmacy, independent of prescriber, pharmacy records are nearly complete with regard to prescription drugs.[19]

Exposure definition

The food frequency questionnaire contained one open question on the brand name of bread spread used. On each examination day, users of phytosterols or phytostanols were defined as those with an intake of phytosterol/-stanol-enriched margarine (e.g. Becel pro.activ or Benecol) greater than zero. From the pharmacy-dispensing records, all prescriptions for statins (ATC classification C10AA and C10B) dispensed between 1 January 1998 and 1 October 2008 were selected. Subjects were considered to be users of statins if they were exposed to the drug at the (re-)examination day in the Doetinchem study, or at some moment in time in the year after the (re-)examination day. These subjects were followed in the PHARMO-RLS for a period of maximally 365 days, starting either on the day of (re-)examination (prevalent users) or, when statins were not used on that moment, on the day of first statin prescription in the year after the day of (re-)examination (starters of statins). Starters of statins had to receive their first prescription within a year after they had filled out the food frequency questionnaire to reduce the probability of misclassifying non-users of phytosterol/-stanol-enriched margarines as users and *visa versa*. In subjects who used statins on both the examination and the re-examination day, adherence was only assessed based on the 365-day interval from the re-examination day (**Figure 1**). Subjects were divided into statin only users and users that combined statins and phytosterol/-stanol-enriched margarine.

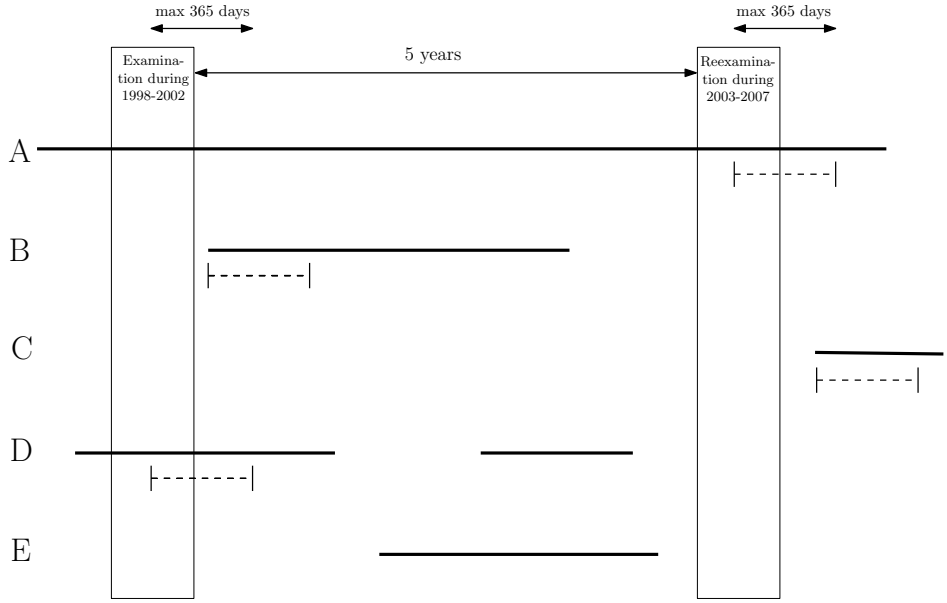


Figure 1. Examples for definitions of exposure to statin therapy
Episode of statin use is represented by the heavy solid line. Pharmacy-dispensing data were gathered in a 365-day period (├-------┤) that started either on the day of (re-)examination (example A and D) or, when statins were not used on that moment, on the day of first prescription in the year after the day of (re-) examination (example B and C). If statins were used on both examination days, the 365-day period started on the day of re-examination (example A). The subject in example E is classified as a statin non-user

Outcome definition

Adherence to statin therapy was assessed in terms of discontinuation of treatment and the medication possession ratio (MPR), i.e. whether patients execute the dosing regimen or not. Patients discontinued therapy if they failed to refill their statin agents within 90 days or one time the duration of the dispensation after the expected end date of the previous prescription, whichever was the lowest number of days.[20] When a patient refilled a prescription for the same type of statin before the theoretical end date of the previous prescription, it was assumed that the new prescription started after the end date of the previous one. Time to discontinuation was defined as the period from the day of (re-)examination or, when statins were not used on that moment, from the first statin prescription after the (re-)examination day, to the day of discontinuation of statin treatment with a maximum of 365 days. Subjects switching from one type of statin to another were considered as continuous users. The MPR was calculated from the PHARMO-RLS as the percentage of pills dispensed, relative to the number of pills that should have been dispensed in the 365 days after the day of (re-)examination or first statin prescription. If a subject discontinued therapy prior to the end date of the 365-day interval, the MPR was assessed during the period of treatment.

Potential confounding variables

Factors that could potentially influence the relationship between the use of phytosterol/-stanol-enriched margarines and adherence to statin therapy were taken into account. These factors included age, gender, comorbidities (cardiovascular disease, type 1 and 2 diabetes mellitus, hypertension and asthma), educational level (low, primary school or lower vocational education; medium, high school or intermediate vocational education; high, higher vocational education or university degree), current smoking status, physical activity level, self-perceived health status, equipotency score of the statin according to Penning-van Beest *et al.*,[21] the number of daily doses of medication and total cholesterol level. Variables that altered the regression coefficient of the usage indicator variables by ≥ 10% were entered in the model as confounding factors.[22]

Statistical analysis

Demographic and health characteristics of the statin only users and combination users were compared using Student's *t*-test or the Mann-Whitney *U* test for continuous variables and the χ^2 test for nominal variables. The number of subjects switching to another equipotency score of statin in the 365-day period after the day of (re-)examination in the Doetinchem study was computed and compared between the statin only users and combination users with the non-parametric Mann-Whitney *U* test. The time course of discontinuation of statin use is illustrated by Kaplan-Meier survival curves and the equality of curves between the statin only users and the combination users was tested with the log-rank test. Observations were censored if they exceeded the end of the study period or if the subject moved out of the cohort or died in the 365-day period after the day of (re-)examination in the Doetinchem study. Multivariate Cox proportional hazards models were used to calculate hazard rate ratios (HR) and 95% confidence intervals (CI) for the comparison of the probability of discontinuation between the statin only and combination users while adjusting for potential confounders. The MPR is expressed as median (25%-75% quartile) and was compared between statin only and combination users with the Mann-Whitney *U* test. Predefined subgroup analyses were performed in order to examine whether there was a difference in adherence between subjects that initiated statin therapy during the 365-day interval after the Doetinchem (re-)examination day (starters of statins) and subjects already on statin therapy at the (re-)examination day (prevalent users).

P-values were considered statistically significant at the 0.05 level. The Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

General characteristics

From a total of 4848 subjects, 582 subjects used statins. Of these subjects, 522 used statins only, whereas 60 subjects combined their statins with phytosterol/-stanol-enriched margarine.

Approximately 75% of both statin only users and combination users was using statins on the Doetinchem (re-)examination day, whereas 25% initiated statin therapy in the year after the (re-) examination day (**Figure 2**).

From **Table 1** it appears that combination users had higher high-density lipoprotein (HDL) cholesterol levels, used more alcohol per day, consumed less dietary (saturated) fat and were more physically active than statin only users. Overall, combination users tended to have a healthier risk profile. Moreover, phytosterol/-stanol-enriched margarine was more frequently used among subjects diagnosed with hypercholesterolaemia and among the higher educated.

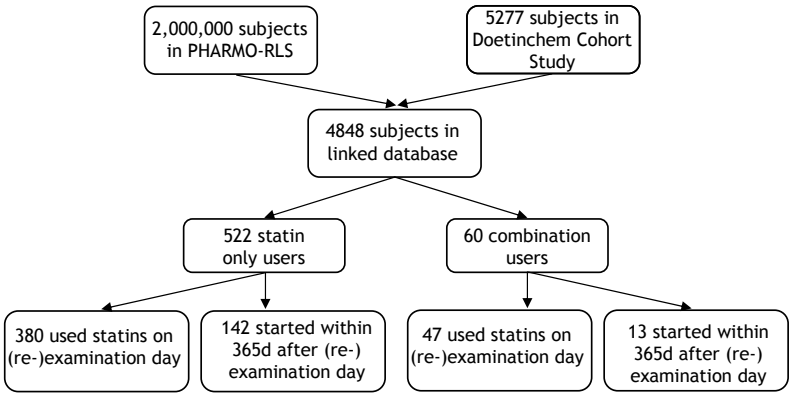


Figure 2. Flowsheet of subject numbers in the Doetinchem Cohort Study, the Pharmacomorbidity-Record Linkage System (PHARMO-RLS) and, consequently, in the linked database

Dosing and switching

The majority of subjects in both the statin only group and the combination group were prescribed simvastatin (respectively 48% and 47%, *P*=0.88), followed by atorvastatin (30% and 25%, *P*=0.47). A trend towards a higher number of combination users prescribed rosuvastatin was found (5% and 10%, *P*=0.07). Both statin only users and combination users were most often prescribed a medium equipotent dose of statins (56% and 62%, *P*=0.88), equivalent to a simvastatin dose of 20 mg/d or an atorvastatin dose of 10 mg/d. The rate of switching to a new agent or a different dose was 10%. This resulted in a higher equipotency in 28 (48%) subjects, a lower equipotency in 19 (33%) subjects and an unchanged equipotency in 11 (19%) subjects. No differences between groups were found in these switching rates.

Adherence to statin therapy

During the 365-day interval after the Doetinchem (re-)examination day, 23% of the statin users and 28% of the combination users discontinued treatment (log-rank: *P*=0.37) (**Figure 3**, **panel A**). In **Figure 3**, **panel B** and **C**, predefined subgroup analyses are shown. Panel B shows that no

Table 1. Demographic and health characteristics of statin only users (*n*=522) and combination users (*n*=60) in the linked database

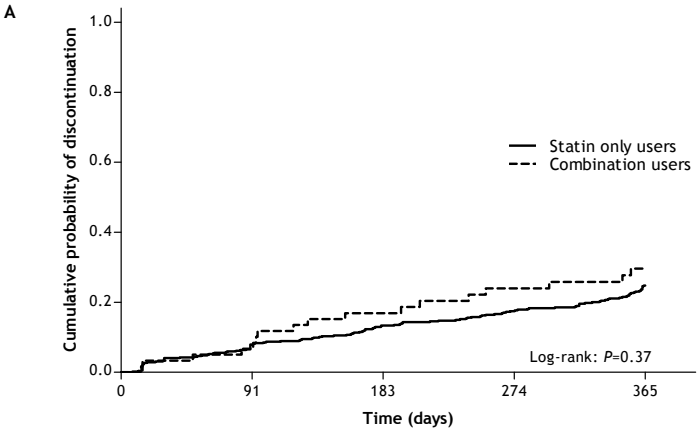
	Statin users† (*n*=522)	**Combination users** (*n*=60)	*P*-value‡
Mean age ± SD, yrs	61.4 ± 8.2	60.0 ± 8.4	ns
Male gender, %	58	60	ns
Low education level, %	61	42	0.005
History of CVD, %	23	17	ns
Family history of CVD, %	42	50	ns
Comorbidities			
Hypertension, %	54	57	ns
Diabetes Mellitus, %	20	8	0.02
Asthma, %	4	0	ns
Ever diagnosed with HC, %	80	90	0.05
Mean total cholesterol ± SD, mmol/l	5.39 ± 1.21	5.41 ± 1.28	ns
Mean HDL cholesterol ± SD, mmol/l	1.27 ± 0.37	1.38 ± 0.39	0.03
Median BMI (range), kg/m^2	27.6 (25.4-30.3)	27.6 (24.6-29.3)	ns
Mean WHR ± SD	0.95 ± 0.08	0.93 ± 0.07	0.07
Mean blood pressure ± SD, mmHg			
Systolic	141.2 ± 20.1	139.6 ± 16.4	ns
Diastolic	85.5 ± 10.6	87.6 ± 9.1	ns
Current smoker, %	21	12	0.10
Median dietary intake (range)			
Energy (MJ/d)	8.05 (6.67-9.41)	8.63 (6.40-9.84)	ns
Total fat (g/d)	74.4 (59.6-88.1)	73.6 (58.1-90.5)	ns
Monounsaturated fat (g/d)	28.1 (22.3-34.4)	27.4 (21.7-34.0)	ns
Polyunsaturated fat (g/d)	15.4 (11.7-19.7)	15.8 (11.8-18.7)	ns
Saturated fat (g/d)	29.5 (23.4-35.0)	28.6 (21.6-33.9)	ns
Cholesterol (mg/d)	209 (167-257)	211 (175-245)	ns
Alcohol (g/d)	6.99 (0.97-20.0)	11.2 (3.47-25.4)	0.01
Mean dietary fat intake ± SD			
Total fat (en%)	35.0 ± 5.1	33.1 ± 5.0	0.006
Saturated fat (en%)	13.9 ± 2.5	12.9 ± 2.2	0.002
Moderate/poor self-perceived health, %	27	27	ns
Low physical activity pattern, %	22	12	0.05
Median phytosterol/-stanol-enriched margarine intake (range), g/d	na	13.4 (7.88-18.0)	

Plus-minus values are means ± SD; CVD, cardiovascular disease; HC, hypercholesterolaemia; BMI, body mass index; WHR, waist-hip circumference ratio; ns, not significant

† Numbers vary due to missing values

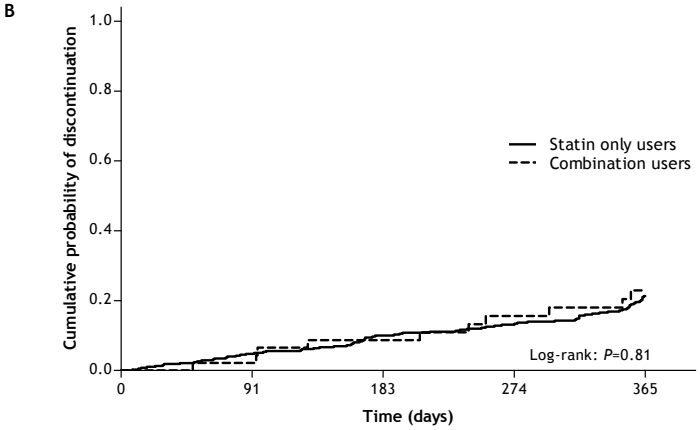
‡ Mann-Whitney *U*, Student's t-test or chi-square test

significant difference in adherence was found between combination users and statin only users in the subgroup of patients already using statins at the (re-)examination day (log-rank: *P*=0.81). However, among subjects who initiated statin therapy after the (re-)examination day (starters), 54% of the combination discontinued statin therapy compared with 32% of the statin only users (log-rank: *P*=0.08) (**Figure 3, panel C**).



Number (%) of subjects at risk

Statin only	522 (100)	477 (91)	430 (82)	392 (75)	346 (66)
Combination	60 (100)	56 (93)	50 (83)	43 (72)	37 (62)



Number (%) of subjects at risk

Statin only	380 (100)	358 (94)	325 (86)	303 (80)	265 (70)
Combination	47 (100)	46 (98)	43 (91)	37 (79)	31 (66)

Figure 3. Time to discontinuation of statin therapy for statin only users and users combining statins with phytosterol/-stanol-enriched margarine in all subjects (A), in subjects already using statins at the (re-) examination day, i.e. prevalent users (B), and in subjects initiating statin therapy after the (re-)examination day, i.e. starters (C). Data from the linked database

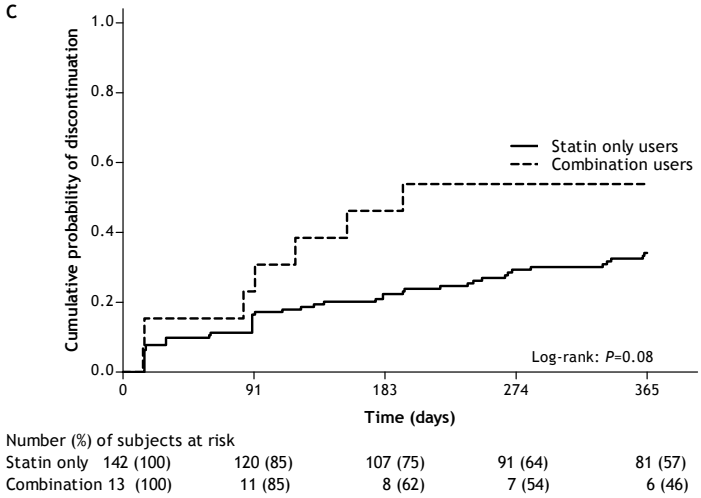


Figure 3. Continued

Cox proportional hazard analysis revealed that the HR_{adj} for discontinuation was 1.37 (95% CI: 0.82 to 2.31, *P*=0.23) for combination users compared with statin only users, after adjustment for age, level of education, total cholesterol level, and the number of daily doses of medication. Subgroup analysis showed that for subjects who initiated statin therapy after the (re-)examination day significantly more combination users discontinued statin therapy compared with statin only users (HR_{adj} 2.52, 95% CI: 1.06 to 6.00, *P*<0.05), whereas the HR_{adj} was 1.04 (95% CI: 0.53 to 2.03, *P*=0.92) for prevalent users.

There was a slight trend towards a lower MPR in combination users; median MPR was 98.1% (93.9-100%) in combination users compared with 100% (94.1-100%) in statin only users (*P*=0.09). In persons already using statins at the (re-)examination day this was borderline significant. In this subgroup combination users had a median MPR of 97.5% (93.8-100%) and the statin only users had a median MPR of 100% (93.8-100%) (*P*=0.06).

Discussion

Adherence to statin therapy was slightly lower in patients who combined their statin therapy with the use of phytosterol/-stanol-enriched margarines, compared with statin only users. This difference was most pronounced among persons who initiated statin therapy after the (re-)examination day. In this subgroup of starters, combination users were 2.5-fold more likely to discontinue statin therapy compared with patients who only used statins (and no phytosterol/-stanol-enriched margarines). Starters have been shown to have lower adherence to drug therapy compared with

prevalent users, since prevalent users are 'survivors' of the early period of pharmacotherapy.[23-25] The MPR was not relevantly lower among combination users compared with statin only users.

Our results imply that persons who combine different cholesterol-lowering therapies might be more negligent in taking the drug according to the prescription. Nevertheless, it is not conceivable that an increase of 5% in discontinuation rates, as observed in our overall study population, results in reductions in the cholesterol-lowering effects of statins. Although cholesterol concentrations were measured in the Doetinchem Cohort Study, it was not feasible in this setting to link the data on adherence to effectiveness. However, two studies using related databases showed indeed beneficial cholesterol-lowering effects of phytosterol/-stanol-enriched margarine when used in combination with cholesterol-lowering drugs.[9,10] Nonetheless, apart from starters, impact on cholesterol values might also be higher in other patient populations with lower adherence to therapy, like younger persons and males.[26]

One of the strengths of this study is the use of an administrative database for person's adherence assessment. Such databases have the advantage that patient-related recall bias and non-response bias are reduced, precise information about prescribed drugs can be obtained and the drug history is available over a long period of time. Pharmacy data have the advantage over medical records of being able to obtain information regarding what medication were acquired instead of what medication was prescribed. However, uncertainty still exists whether or not the drug is actually taken. Moreover, in the present study no information about the reason for discontinuation was available and therefore we could not control for the fact that statin therapy may be discontinued by the prescriber for clinical reasons. This seems uncommon, however, since statin therapy is mostly indicated lifelong and statins have a relatively mild adverse event profile.[27]

A limitation of this study is that the linkage of data from the Doetinchem Cohort Study and the PHARMO database yielded only 60 users who combined statin therapy and the use of enriched margarine. This number is limited what might have influenced the significance of the results due to a lack of power. One might argue that any potential behavioural interaction between statins and phytosterol/-stanol-enriched margarine is of less priority as the number of combination users is low. However, it takes time for a new product to make a way into a consumer's habitual dietary pattern and it is expected that usage rates will increase in the near future. Another limitation of this study is that we did not know whether the persons were using other phytosterol/-stanol-containing products, besides the enriched margarines. Moreover, we could not ascertain that all phytosterol/-stanol-enriched margarine users continued the enriched margarine use in the year after the (re-) examination day in the Doetinchem study.

In conclusion, the effects of phytosterol/-stanol-enriched margarine on adherence to statin therapy found in this study are indicative, but should be verified in other studies. Larger populations should be explored before firm conclusions can be drawn about the importance of this behavioural interaction and the potential influence on the effectiveness of the combined therapy to lower cholesterol values. In addition, further combinations of functional foods with a health claim and prescribed drugs should be explored to investigate other potential food-drug behavioural interactions.

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Chapter 3.3

Influence of the use of functional foods enriched with phytosterols/-stanols on adherence to statin therapy

Simone RBM Eussen, Marcel L Bouvy, Cathy JM Rompelberg, Menno E van der Elst, Johan Garssen, Marco H Oosterveld, Anthonius de Boer, Johan J de Gier, Henk J van Kranen and Olaf H Klungel

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Abstract

Background Subjects using functional foods with approved health claims may be more likely to be non-adherent with prescribed drug therapy.

Objective This study aimed to assess the influence of the use of phytosterol/-stanol-enriched functional foods on adherence to statin therapy among patients initiating treatment.

Methods We used data from the **ST**atin **I**ntervention research **P**roje**T** (STIPT), a randomised controlled trial aimed at improving adherence to statins. In the trial, new statin users were randomised to receive either usual care or extensive pharmaceutical care consisting of five individual counselling sessions. Customary use of phytosterol/-stanol-enriched products was identified by questionnaires filled out by all participants. Automated pharmacy-dispensing records were used to assess adherence in terms of discontinuation of therapy and the medication possession ratio. Analyses were performed for the overall population, as well as stratified for receiving pharmaceutical or usual care.

Results The use of functional foods enriched with phytosterols/-stanols was not related to discontinuation of statin therapy, neither in the overall population (overall population adjusted hazard rate ratio (HR_{adj}): 0.80, 95% CI: 0.59 to 1.08), nor when stratified by randomisation arm (pharmaceutical care HR_{adj} 0.77, 95% CI: 0.49 to 1.23); usual care HR_{adj} 0.81, 95% CI: 0.54 to 1.21). The median medication possession ratio was significantly lower in users of phytosterols/-stanols in the usual care group, but the difference was not clinically relevant.

Conclusions Customary use of phytosterol/-stanol-enriched functional foods did not affect adherence to statins in new users that are well informed on the beneficial effects of statin therapy. In daily medical practice, general practitioners and pharmacists should urge subjects not to take phytosterol/-stanol-enriched functional foods as replacement for their prescribed medication.

Introduction

Coronary heart disease (CHD) is among the leading causes of death worldwide. Abnormal blood lipid levels are one of the main risk factors for CHD.[1] The hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) lower total and low-density lipoprotein (LDL) cholesterol by inhibiting cholesterol synthesis[2] and have been shown to reduce the 5-year incidence of major coronary events by about one third.[3] In recent years, functional foods enriched with phytosterols or phytostanols, claimed to lower cholesterol levels, have gained huge popularity.[4] It is therefore conceivable that an increasing number of people will combine their statin therapy with phytosterol/-stanol-enriched products. In several randomised controlled trials (RCT) positive additive effects of phytosterols/-stanols on the cholesterol-lowering effect of statins have been demonstrated (for review, see Scholle *et al*).[5] Phytosterols or phytostanols are thought to further reduce LDL cholesterol by approximately 10% when added to ongoing statin therapy.[6] RCT typically follow strict protocols to maximise patients' adherence to statins and phytosterols/-stanols. In daily medical practice, however, the use of functional foods which claim to lower cholesterol levels might reduce adherence to statin therapy. Patients may assume that statins are no longer necessary when they are using functional foods or they may regard the functional foods as an alternative with the potential of reducing statin dose and possibly the side effects. Consequently, the benefits shown in RCT may not be replicated in daily medical practice because poor adherence contributes to the failure of patients to achieve therapy targets.[7,8] Alevizos *et al*.[9] interviewed over 400 patients on statin treatment and found that more than 90% of the patients thought phytosterols were at least equally effective as statins. Moreover, patients were convinced that, in contrast to statins, phytosterols had no adverse effects. We showed in a retrospective study towards this potential behavioural interaction between statins and phytosterols/-stanols that adherence to statin therapy was slightly lower in persons who combined their statins with the use of phytosterol/-stanol-enriched margarines. The effect was most pronounced in the subgroup of new users of statins. In this subgroup, combination users were 2.5-fold more likely to discontinue statin therapy compared with patients who only used statins. Limitations of this study were the limited number of new statin users (155 new statin users of which 13 combined statins and phytosterols/-stanols) and the fact that persons were inquired about the use of phytosterols/-stanols incorporated in *margarine* only.[10] We have recently conducted an RCT towards the effects of a pharmaceutical care program on improving adherence in a large population of new users of statins, in which customary use of phytosterol/-stanol-enriched products was also monitored.

In the present study, we aimed to determine the influence of the use of functional foods enriched with phytosterols or phytostanols on adherence to statin therapy in the setting of this large RCT among new statin users. Additionally, changes in total and LDL cholesterol over the time of the trial were compared between users and non-users of these functional foods.

Methods

Data for this study were obtained from the **ST**atin **I**ntervention research **P**rojec**T** (STIPT), a pharmacy-based, multi-centre and open-label RCT enrolling new users of statins. The main focus of STIPT was to improve patients' adherence to statin therapy through education and feedback on achieved cholesterol levels.

Study population

As described in detail elsewhere,[11] new users of statins aged 18 years and above were included in the trial and randomised to either the pharmaceutical care or the usual care program. New users were defined as those who had not filled a prescription for statins in the preceding 6 months, verified through a patient record check. The pharmaceutical care program consisted of 5 individual counselling sessions with their pharmacist, scheduled at first prescription, second prescription (after 15 days) and 3, 6 and 12 months after the start of statin therapy. During these sessions, patients received structured education on indication, effects and side effects of statin therapy, dosage and the importance of adherence, and they were asked about their experience with treatment. Total and high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured and the association between adherence and lipid levels was discussed to encourage patients to adhere to the prescribed dosing regimen. Patients in the usual care group received standard care from their pharmacist, consisting of verbal and written drug information. These patients did not receive counselling sessions or lipid measurements.

Exposure definition

At baseline, all patients were asked to fill out a questionnaire about socio-demographic characteristics, (family)history of CHD, comorbidities, smoking habits, alcohol consumption, self-perceived health status, dietary habits and the use of phytosterol/-stanol-enriched products. Users and non-users of phytosterol/-stanol-enriched products were identified by the following questions: *'What kind of bread spread do you usually use'?* and *'Are you currently using other methods to lower your cholesterol level besides medication'?* Patients who ticked the answer *'Cholesterol-lowering margarine enriched with phytosterols or phytostanols, for example, Becel pro.activ or Benecol'* or *'Specific cholesterol-lowering products containing phytosterols or phytostanols, for example, Becel pro.activ, Benecol or Vifit Choless Control'* were identified as users. Becel pro.activ (Unilever N.V., Vlaardingen, The Netherlands), Benecol (Raisio Group, Raisio, Finland) and Vifit Choless Control (FrieslandCampina N.V., Amersfoort, The Netherlands) are Dutch brand names for phytosterol/-stanol-enriched margarines and yoghurt (drinks) marketed in the Netherlands at the time of the study. Intake of phytosterols/-stanols from dietary supplements was also considered, but supplements with phytosterols/-stanols were only marginally used in the Netherlands at the time of the study.[12] After 6 and 12 months of inclusion, all patients completed another questionnaire in

which they were asked to indicate whether their health status and/or dietary habits (e.g. the use of phytosterol/-stanol-enriched products) had changed since the last questionnaire.

Outcome definition

In the pharmaceutical care group, total and HDL cholesterol, and triglyceride levels were measured at 3, 6 and 12 months on a Cholestech LDX analyser (Cholestech Corp., Hayward, CA, USA) in a standardised way. LDL cholesterol was estimated by using the Friedewald formula.[13] Information from the questionnaires and lipid measurements of each individual patient was linked to automated pharmacy-dispensing records. Retrievable information from the records included the name of the prescribed drug, the defined daily dose, the dispensing date and the amount dispensed. Adherence to statins was evaluated in terms of time to discontinuation and the medication possession ratio (MPR),[14] and was assessed in the year after the start of statin therapy. Discontinuation was defined as a continuous gap between an expected refill and actual refill of 90 days, or one time the duration of the previous dispensation, whichever was the lowest number of days.[15] Time to discontinuation was defined as the number of days between the start of statin therapy and the discontinuation date. When a patient filled a prescription for the same type of statin before the theoretical end date of the previous prescription, we assumed that the new prescription began after the end date of the previous one.[16] Patients who switched from one type of statin to another were considered to be continuous users. The MPR was calculated from the records as the ratio of the sum of the days' supply of all statin medication dispensed divided by the length of therapy.

Potential confounding variables

Variables that could potentially confound the association between the use of phytosterols/-stanols and adherence to statin therapy were age, gender, level of education, comorbidities (type 1 and 2 diabetes mellitus, hypertension, respiratory disease and history of CHD), familial hypercholesterolaemia, lifestyle factors, application of other cholesterol-lowering strategies, self-perceived health status, equipotency score of statins (the potency of a statin to lower total cholesterol according to type and dose),[17] and the number of medications (at Anatomical Therapeutic Chemical (ATC) Classification level 3)[18] used. For the analysis of the effects of the use of phytosterol/-stanol-enriched functional foods on total and LDL cholesterol levels the following covariates were regarded as potential confounders: age, gender, comorbidities (type 1 and 2 diabetes mellitus, hypertension, respiratory disease, history of CHD), lifestyle factors, dietary habits (e.g. the use of low-fat bread spread), application of other cholesterol-lowering strategies and the equipotency score of statins.

Statistical analysis

Subjects were divided into users and non-users of phytosterol/-stanol-enriched products based on the baseline questionnaire. Time to discontinuation was assessed by Kaplan-Meier curves, and univariate and multivariate Cox proportional hazard models were used to estimate hazard rate ratios (HR) for discontinuation between users and non-users of phytosterols/-stanols. Patients were

censored at the end of the study period or when they changed to a pharmacy not participating in the trial or died before the end of follow-up. Patients dropping out of the study were not excluded from this primary analysis, as pharmacy-dispensing records of these patients remained available. As a sensitivity analysis, Cox proportional hazard models with time-varying use of phytosterols/-stanols and covariate variables were run. This last analysis was only carried out in the subset of patients that completed the study, as it appeared that there was a high correlation between study drop out (and consequently missing questionnaires at 6 and 12 months) and discontinuation of statin therapy. Results are presented for the overall population and, in order to check whether results were similar in the different study arms, also separately for the pharmaceutical care and usual care group.

The Mann-Whitney *U* test was used to compare differences in median MPR and in total and LDL cholesterol level at 3, 6 and 12 months between users and non-users of phytosterol/-stanol-enriched products. Changes in total and LDL cholesterol over the time of the study were compared between users and non-users of enriched products using repeated-measures analysis of covariance (ANCOVA), including all potential confounders that altered the regression coefficient for phytosterol/-stanol use by at least 10%.[19] The number of patients switching to another type or dose of statin during the study was computed and compared between users and non-users with the χ^2 test. All data were analysed with the Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA). Two-sided *P*-values below 0.05 were considered as statistically significant.

Results

Patient enrolment and baseline characteristics

Pharmacy-dispensing records were available from 899 (88%) out of the 1016 patients who signed informed consent.[11] Valid baseline questionnaires, used for the identification of users and non-users of phytosterol/-stanol-enriched products were returned to the pharmacy by 794 (88%) patients of which 390 (49%) were randomised to the pharmaceutical care group and 404 (51%) to the usual care group (**Figure 1**).

General and health characteristics as reported by the patients in the baseline questionnaire are presented in **Table 1**. Phytosterol/-stanol-enriched products were used by approximately 40% of the subjects. Users of enriched products were similar to non-users for most characteristics, although, as could be expected, more users were following a low-fat/low-cholesterol diet.

Users were also more likely to apply strategies other than medication and functional foods to reduce cholesterol, such as losing weight and becoming more physically active. The majority of patients, users as well as non-users of enriched products, were using simvastatin (36%) or atorvastatin (35%). Most patients (53%) initiated statin therapy at an equipotency of 4, equivalent to a simvastatin dose of 20 mg/d or an atorvastatin dose of 10 mg/d.

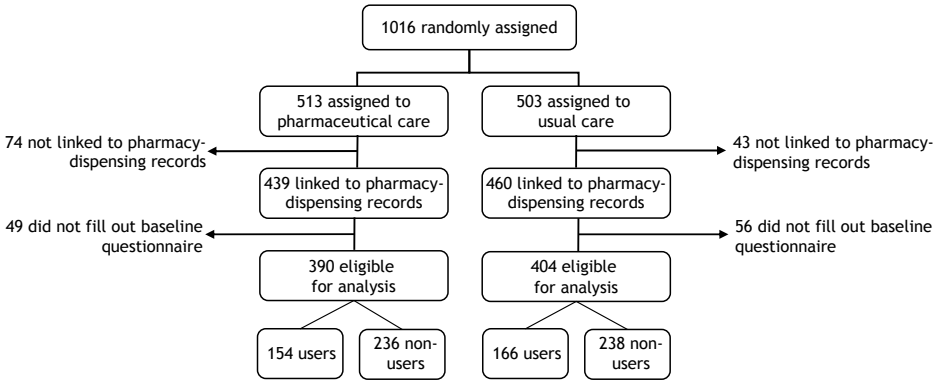

Figure 1. Flow chart of subjects enrolled in the **ST**atin **I**ntervention research **P**roject**T** (STIPT)

Table 1. Baseline general and health characteristics of users and non-users of phytosterol/-stanol-enriched products in the **ST**atin **I**ntervention research **P**roject**T** (STIPT). All participants were statin users

	Phytosterol/ -stanol users† (*n*=320)	**Phytosterol/ -stanol non-users**† (*n*=474)	*P*- value‡
Randomisation arm STIPT			
Pharmaceutical care, *n* (%)	154 (48)	236 (50)	ns
Usual care, *n* (%)	166 (52)	238 (50)	ns
Age, yrs	60.8 ± 10.6	60.0 ± 11.5	ns
Male gender, *n* (%)	145 (45)	236 (50)	ns
Dutch origin, *n* (%)	298 (93)	432 (91)	ns
Marital status, *n* (%)			
Married/living together	240 (79)	380 (83)	ns
Unmarried/widowed/divorced	64 (21)	76 (17)	
Level of education, *n* (%)			
Low	130 (43)	188 (42)	ns
Intermediate	122 (40)	194 (43)	
High	53 (17)	70 (15)	
Comorbidities, *n* (%)			
Hypertension	146 (46)	219 (47)	ns
Diabetes mellitus	82 (26)	140 (30)	ns
Respiratory disease	19 (6)	45 (10)	0.07

Table 1. Continued

	Phytosterol/ -stanol users† (*n*=320)	Phytosterol/ -stanol non-users† (*n*=474)	*P*- value‡
History of CVD, *n* (%)	101 (32)	156 (34)	ns
Family history of HC, *n* (%)	82 (26)	120 (25)	ns
Lifestyle factors, *n* (%)			
Current smoker	73 (23)	103 (22)	ns
Alcohol use ≥ 1 times p/w	56 (18)	83 (18)	ns
Following a specific diet			
Salt-restrictive	52 (16)	53 (11)	0.04
Sugar-restrictive	60 (19)	80 (17)	ns
Low-fat/low-cholesterol	152 (48)	144 (30)	<0.0001
Weight reducing	40 (13)	49 (10)	ns
Other cholesterol-lowering strategies, *n* (%)			
Smoking cessation or reduction	47 (15)	44 (9)	0.017
Reducing alcohol consumption	41 (13)	56 (12)	ns
Eating healthier	195 (62)	177 (38)	<0.0001
Becoming more physically active	146 (47)	164 (35)	0.001
Losing weight	99 (32)	110 (23)	0.012
Self-perceived health, *n* (%)			
(Very) good	223 (73)	324 (71)	ns
Moderate/poor	83 (27)	133 (29)	
Statin, *n* (%)			
Simvastatin	119 (37)	163 (34)	ns
Pravastatin	27 (8)	58 (12)	0.09
Atorvastatin	114 (36)	158 (33)	ns
Rosuvastatin	55 (17)	86 (18)	ns
Fluvastatin	5 (2)	9 (2)	ns

Plus-minus value is mean ± SD; CVD, cardiovascular disease; HC, hypercholesterolaemia; ns, not significant
† Numbers vary because of missing values. Percentages are calculated without missing values
‡ Mann-Whitney *U*, Student's *t*-test or chi-square test

Valid follow-up questionnaires were returned by 528 (66%) and 209 (26%) patients at 6 and 12 months, respectively. Of the 390 patients that were randomised to the pharmaceutical care group, a total of 338 (87%), 324 (83%) and 280 (72%) patients received counselling and had their lipid levels assessed at respectively 3, 6 and 12 months after the start of statin therapy.

Adherence to statin therapy and switching of statins

Within the first year of statin therapy, 67 (21%) users and 117 (25%) non-users of phytosterols/-stanols discontinued statin therapy. Kaplan-Meier curves show no significant difference in the likelihood of discontinuation between users and non-users of phytosterol/-stanol-enriched products (log-rank: P=0.19) (**Figure 2**). The adjusted HR (HR_{adj}) was 0.80 (95% CI: 0.59 to 1.08) for users as compared with non-users and did not differ between the pharmaceutical or usual care group (**Table 2**). Sensitivity analysis, using time-varying exposure in the subgroup of patients that completed the study, led to the same results.

Median MPR was high (>98%) in both users and non-users of phytosterol/-stanol-enriched products. A slight tendency was observed towards a lower MPR in the users of enriched products (P=0.09), which was statistically significant in the usual care group (P=0.045). The rate of switching to another statin was 10%, whereas in 4% of the patients the dose of the statin was altered, without changing the type of statin. The switching rates did not differ significantly between users and non-users of phytosterol/-stanol-enriched products.

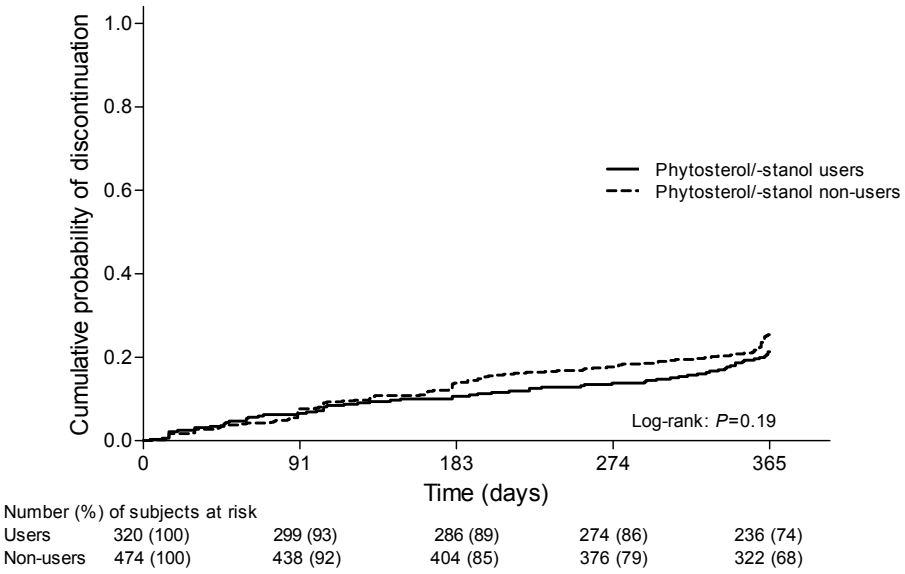

Figure 2. Kaplan-Meier curve for discontinuation of statin agents in users and non-users of phytosterol/-stanol-enriched products in the **ST**atin **I**ntervention research **P**roje**T** (STIPT)

Table 2. Unadjusted and adjusted hazard rate ratios (HR) among users and non-users of phytosterol/-stanol-enriched products in the overall population, and in the pharmaceutical care and usual care groups of the **ST**atin **I**ntervention research **P**roject**T**

	Cox proportional hazard models					
	Overall		Pharmaceutical care group		Usual care group	
	HR (95% CI)	HR_{adj} † (95% CI)	HR (95% CI)	HR_{adj} † (95% CI)	HR (95% CI)	HR_{adj} † (95% CI)
Phytosterol/-stanol users	0.82 (0.61, 1.11)	0.80 (0.59, 1.08)	0.82 (0.52, 1.29)	0.77 (0.49, 1.23)	0.81 (0.54, 1.21)	0.81 (0.54, 1.21)
Phytosterol/-stanol non-users (reference)	1.00	1.00	1.00	1.00	1.00	1.00

† HR_{adj}: Hazard rate ratio adjusted for age, gender, hypertension and current smoking status

Lipid levels

Adjusted levels for total and LDL cholesterol decreased significantly during the study period in both users (total cholesterol: -0.22 mmol/l per 3 months (95% CI: -0.30 to -0.15); LDL cholesterol: -0.11 mmol/l per 3 months (95% CI: -0.18 to -0.046)) and non-users (total cholesterol: -0.21 mmol/l per 3 months (95% CI: -0.28 to -0.14); LDL cholesterol: -0.19 mmol/l per 3 months (95% CI: -0.25 to -0.12)) of phytosterol/-stanol-enriched products ($P<0.001$). There was no significant difference between the users and non-users in cholesterol changes over time, nor in median total or LDL cholesterol levels at 3, 6 and 12 months.

Discussion

The aim of the present study was to examine the influence of the use of functional foods enriched with phytosterols/-stanols on adherence to statin therapy in new statin users. We hypothesised that persons using enriched products are more negligent in taking the drug according to the prescription as they have implemented an additional cholesterol-lowering therapy.

In contrast to our earlier findings,[10] however, users of phytosterol/-stanol-enriched products did not have higher discontinuation rates. In a real life setting, patients might assume that they may well stop their statin therapy as they have implemented another lipid-lowering strategy. In the present study, half of the subjects were educated about the importance of adhering to statin therapy. Although no special attention has been paid towards increasing adherence among phytosterol/-stanol users, these patients might be the ones that benefit the most from the pharmaceutical care program as subjects that use the (expensive) phytosterol/-stanol-enriched products are likely persons who are more conscious about their health. The reason why phytosterol/-stanol users in

the usual care group did not have lower adherence rates might relate to the fact that these patients also knew they were enrolled in a study aimed to improve medication adherence. Several studies have shown unexpectedly high adherence in usual care groups.[20-22] Moreover, consenting patients appear to be different from non-consenting patients.[20-22]

Users of phytosterols/-stanols tended to have a slightly lower MPR, but this was not clinically relevant.

Total and LDL cholesterol levels were not significantly lower in users of phytosterols/-stanols compared with non-users. The most likely explanation for this is that in our study, users of phytosterol/-stanol-enriched products did not consume sufficient intake levels. We have previously shown that recommended intake amounts of phytosterols/-stanols, i.e. 2 g/d, were reached by only 9% of all users.[23] A daily intake of 2 g phytosterols/-stanols is expected to result in 9% lower LDL cholesterol levels.[24] Lower intake levels will result in smaller cholesterol-lowering effects and these effects are likely to be overwhelmed by those produced after the initiation of statin therapy. In the present study, it was found that approximately 40% of the enrolled subjects used phytosterol/-stanol-enriched products. Previous studies reported usage rates of 5% and 10% in the general population[25] and among statin users,[10] respectively. Higher usage rates in the current study are understandable, given that the earlier studies assessed the intake of phytosterol/-stanol-enriched *margarines* only, and both earlier studies started just after the introduction of the phytosterol/-stanol-enriched products on the market. Nevertheless, it is conceivable that not all users in the present study will consume the phytosterols/-stanols on a daily basis and in sufficient amounts. Moreover, it is likely that physicians will partly adjust dosage of statins according to cholesterol levels, thereby diluting the already small effects of phytosterols/-stanols.

A major strength of this study is the large representative sample of new statin users from 26 community pharmacies in the Netherlands. Based on our sample size of nearly 800 patients, this study achieved a 98% power to detect a true difference of 22%[10] in discontinuation rates. Limitations of this study include uncertainty about the exact intake amounts of phytosterol/-stanol-enriched products and the mismatch between the time of administering the first questionnaire and the time of first lipid measurement. As a result, misclassification cannot be ruled out: some subjects who were regarded as users at 3 months might actually not have used phytosterols/-stanols at that time, and *vice versa*. Moreover, in spite of careful attempts to control confounding, the existence of residual confounding cannot be fully excluded. Finally, our results may not be generalisable to daily medical practice because all subjects participated in an RCT. However, the results of the present study add to the findings from our previous study in an unselected general population. Patients that are well informed on the beneficial effects of statins do not seem to have reduced adherence to statins when using phytosterols/-stanols.

Ideally, the influence of the use of phytosterols/-stanols on adherence to statin therapy should be examined in a dataset that links pharmacy-dispensing records to individuals' purchase behaviour of phytosterol/-stanol-enriched functional foods. In this way, besides statin use, functional food consumption can also be assessed without recall bias or non-response bias, and more detailed

information can be gathered on the functional food type, amount of use and time of acquiring the product. Moreover, interesting subgroups can be easily differentiated, for example, starters of phytosterols/-stanols who might take the functional foods as a replacement for their statins.

In conclusion, customary use of phytosterol/-stanol-enriched products did not affect adherence rates in new statin users who were educated about the importance of adhering to therapy. In daily medical practice, general practitioners and pharmacists should ask patients about any functional foods they may be using. Patients using these foods should be urged not to take them as a replacement for their prescribed medication, without consulting a general practitioner or pharmacist.

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Chapter 4

Cost-effectiveness of functional foods

Chapter 4.1

Costs and health effects of adding functional foods containing phytosterols/-stanols to statin therapy in the prevention of cardiovascular disease

Simone RBM Eussen, Talitha L Feenstra, Ido B Toxopeus,
Jeljer Hoekstra, Olaf H Klungel, Hans Verhagen,
Henk J van Kranen and Cathy JM Rompelberg

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Abstract

Objective The present modelling study aimed to evaluate if and by how much functional foods containing phytosterols/-stanols add to the benefits of statins in the prevention of cardiovascular disease (CVD) in terms of cost-effectiveness.

Methods Long-term health effects, measured as quality-adjusted life-years (QALYs) gained, and costs for scenarios with additional phytosterol/-stanol use were compared with scenarios without extra use. Phytosterols/-stanols were given only to persons who were eligible for use according to their 10-year absolute risk of fatal CVD (SCORE-risk). Intake levels and discontinuation rates as observed in daily practice were included in the model. Two situations were compared: 1) A real-life situation in which persons at high SCORE-risk were identified through clinical case-finding and, 2) A theoretical maximum situation where universal screening was implemented resulting in known SCORE-risks for the whole Dutch population aged 35-75 years (8.4 million people). Sensitivity analyses were performed for variations in the cholesterol-lowering effect and intake level of phytosterols/-stanols, indirect health care costs, time horizon and discount rates.

Results At the model's start year, a total of 1.0 (real-life situation) to 3.3 (maximum situation) million persons qualified for phytosterol/-stanol use based on their SCORE-risk (both statin users and statin non-users). Over the model's time horizon, this resulted in a gain of 2700 to 16,300 QALYs, and yielded cost-effectiveness ratios that ranged between €92,000 and €203,000 per QALY.

Conclusions This simulation study showed that the cost-effectiveness of phytosterols/-stanols as monotherapy and as add-on to statins is above thresholds for cost-effectiveness, generally ranging between €20,000 and €50,000, and is thus a non-cost-effective strategy to reduce CVD.

Introduction

Despite the steady decline in death rates from cardiovascular disease (CVD) during the last decades, CVD continues to be one of the biggest health care problems in terms of burden of disease and health care costs. The beneficial effects of statins in the primary and secondary prevention of CVD are well established.[1,2] These benefits are primarily attributed to the lipid-lowering properties of statins: it has been estimated that statins reduce low-density lipoprotein (LDL) cholesterol levels by 18-55%.[3-5] In addition to this cholesterol-lowering activity, statins possess multiple pleiotropic effects.[6,7] In Europe, current recommendations for cardiovascular risk management are based on the **S**ystematic **CO**ronary **R**isk **E**valuation (SCORE)-risk charts.[8,9] In 2006, for the Netherlands an adapted SCORE-risk chart has been developed using national data.[10] From the charts, the 10-year absolute risk of fatal CVD can be derived, taking into account several risk factors (gender, age, smoking, systolic blood pressure, and serum total cholesterol or total/HDL cholesterol ratio). According to the Dutch guidelines, treatment with a statin in the primary prevention of CVD is recommended for all persons with a 10-year SCORE-risk of fatal CVD ≥10%, unless LDL cholesterol is less than 2.5 mmol/l. For subjects with type 2 diabetes mellitus or established CVD, treatment is recommended for all persons with LDL cholesterol ≥ 2.5 mmol/l.

The use of functional foods enriched with phytosterols and phytostanols is an alternative strategy to lower elevated total and LDL cholesterol levels. Phytostanol- and phytosterol-enriched margarines were launched on the Dutch market in 1999 and 2000, respectively and its use has increased in the past years in both users and non-users of statins (Eussen *et al.*, unpublished data). In a recent meta-analysis, Demonty *et al.*[11] found that a daily dose of 2.15 g phytosterols/-stanols reduces LDL cholesterol by 8.8%. Furthermore, phytosterols and -stanols seem to be equally effective in both statin users and statin non-users.[12] It is generally assumed that phytosterols/-stanols will decrease coronary heart disease by lowering cholesterol levels, although there are no studies yet to confirm this.[13] The guidelines for cardiovascular risk management recommend that all persons with a 10-year SCORE-risk ≥5% should be given lifestyle recommendations, including the encouragement of the use of phytosterols/-stanols as part of a healthy diet.[10,14]

There is currently no universal screening for risk factors of CVD in the Netherlands, nor in any other EU country. Consequently, the detection of high cholesterol values and other CVD risk factors occurs primarily through clinical case-finding. As a result many people are unaware that they are at high risk for CVD and could benefit from statin and/or phytosterol/-stanol use.[15]

The aging of the population together with the rising health care costs requires considering the cost-effectiveness and budgetary impact of different intervention strategies. In cost-effectiveness analyses the costs and health effects of an intervention are compared to determine whether the intervention provides value-for-money.[16] Statins have been assessed for cost-effectiveness in a range of publications,[17,18] and were found to be cost-effective for high risk patients.[17,19] In contrast, to date only two studies evaluated the cost-effectiveness of phytosterols or -stanols.[20,21] In both studies it was concluded that phytosterols and -stanols are (potentially) cost-effective under optimal

conditions of use, i.e. taking the daily recommended amount of 2 g phytosterols/-stanols (without discontinuation). However, neither study included an economic evaluation in which real-life consumption patterns of phytosterols/-stanols were taken into account, nor were all health benefits and costs considered. Moreover, the incremental costs and health effects of phytosterols/-stanols in addition to statins have not been evaluated.

Therefore, the present study aimed to evaluate the health benefits, i.e. the prevention of CVD, and health care costs of functional foods enriched with phytosterols/-stanols in addition to statin therapy, taking into account the intake levels and discontinuation rates as observed in daily practice.

Methods

The cost-effectiveness of the use of functional foods with phytosterols/-stanols as monotherapy and as add-on to statin therapy was estimated both in a real-life situation and in a theoretical maximum situation. The real-life situation assumed passive clinical case-finding to identify subjects eligible for treatment with statins. The theoretical maximum situation assumed that free population-based screening was implemented resulting in known 10-year SCORE-risks for the whole Dutch population between 35 and 75 years of age and all subjects with a SCORE-risk ≥10% were treated with statins. This theoretical situation gives information about the maximum health benefits that can be achieved with phytosterols/-stanols in addition to optimal statin therapy.

In both the real-life and theoretical maximum situation, long-term disease prevalence and mortality rates, as well as health care resource use, were simulated and compared for two scenarios using the RIVM Chronic Disease Model described below. The first scenario is the current situation in which functional foods enriched with phytosterols/-stanols are used as customary in the Dutch population. A large part of the population does not use phytosterols/-stanols, whereas others use them on their own initiative or on general practitioner's (GP's) advice. In the second scenario an increase in phytosterol/-stanol use is considered, both as a monotherapy for subjects with a modestly elevated risk (SCORE-risk ≥5%, <10%), and as add-on to statin therapy for subjects with a highly elevated risk (SCORE-risk ≥10%) (**Table 1**).

Scenarios

Real-life (RL) situation

In a clinical case-finding or real-life situation the SCORE-risk is only known for subjects who have their cholesterol level and blood pressure assessed, presumably the ones that are susceptible to a high risk of CVD events and/or health-conscious people.

Table 1. Overview of scenarios in the real-life and theoretical maximum situation

Situation	Scenario		Phytosterol/-stanol use	Statin use
Real-life (RL)				
	RL reference	Real-life	(no change in phytosterol/-stanol use)	(no change in statin use)
	RL plus PS (min)	Minimum real-life plus PS	By all current real-life statin users	(no change in statin use)
	RL plus PS (max)	Maximum real-life plus PS	By all current real-life statin users and all subjects with a 10-year SCORE-risk ≥5%, <10%	(no change in statin use)
Theoretical maximum (TM)				
	TM reference	Maximum statin use	(no change in phytosterol/-stanol use)	By all subjects with a 10-year SCORE-risk ≥10%
	TM plus PS	Maximum statin and PS use	By all current real-life statin users and all subjects with a 10-year SCORE-risk ≥5%	By all subjects with a 10-year SCORE-risk ≥10%

PS, Phytosterols/-stanols

RL reference: Real-life situation with customary phytosterol/-stanol use

The *RL reference* scenario assumed no additional phytosterol/-stanol use in a real-life situation. It reflects the real-life consumption patterns of phytosterols/-stanols, including actual daily intake levels and discontinuation rates. In this scenario population numbers, morbidity rates and health care costs of the Dutch population that was between 35 and 75 years of age in 2007 were simulated over a time horizon of 50 years. Data from the population-based Doetinchem Cohort Study were used to estimate subjects' 10-year SCORE-risk and current phytosterol/-stanol use in the Dutch population.[22] In this ongoing cohort study, participants are examined in consecutive 5-year intervals. The most recent data were used for the current study, collected during the years 2003-2007, which included about 4500 persons. Current statin and combined users of both statins and phytosterols/-stanols were identified by linking the data of each participant of the Doetinchem Cohort Study to their pharmacy-dispensing records using the Pharmacomorbidity-Record Linkage System.[23]

RL plus PS: Real-life situation with additional phytosterol/-stanol use

In the *RL plus PS* scenario, subjects who have a known SCORE-risk ≥5% were assumed to start phytosterol/-stanol use. We assumed that in the Dutch population all current statin users had their SCORE-risk assessed at the beginning of their therapy and their SCORE-risk was ≥10%, conforming to the guidelines. These subjects start using phytosterols/-stanols. In addition, we assumed that subjects with a known modestly elevated risk (SCORE-risk ≥5%, <10%) start using phytosterols/-stanols. However, in a real-life setting it is difficult to identify which fraction of the

Dutch population has their SCORE-risks assessed and no GP data were available on which to make a reliable estimate. Therefore, we defined a minimum and maximum scenario for phytosterol/-stanol use. In the *minimum* real-life plus phytosterols/-stanols scenario *(RL plus PS* (*min))*, only current statin users start using phytosterols/-stanols, which results in a minimum number of additional phytosterol/-stanol users. In the *maximum* real-life plus phytosterols/-stanols scenario (*RL plus PS (max))*, both current statin users and all subjects with a SCORE-risk ≥5%, <10% start using phytosterols/-stanols, resulting in a maximum number of additional phytosterol/-stanol users (**Table 1**). The true number of additional phytosterol/-stanol users in the general population lies somewhere between these two extremes.

The cost-effectiveness of additional phytosterol/-stanol use in a real-life situation was obtained by subtracting the results of the real-life scenario (*RL reference*) from the scenarios with added phytosterols/-stanols *(RL plus PS (min)* and *RL plus PS (max))*.

Theoretical maximum (TM) situation

In the theoretical maximum situation it is assumed that the SCORE-risk for the whole Dutch population aged between 35 and 75 years is known. In this situation, all subjects with a 10-year SCORE-risk ≥10% start using statins in both scenarios. Subjects already using statins before the start of the scenario were assumed to continue taking their current medication.

TM reference: Maximum situation with customary phytosterol/-stanol use

The *TM reference* scenario assumed customary use of phytosterols/-stanols in a situation with maximum statin use. It reflects the real-life consumption patterns of phytosterols/-stanols, including actual daily intake levels and discontinuation rates.

TM plus PS: Maximum situation with additional phytosterol/-stanol use

In this scenario, we assumed phytosterol/-stanol use in all subjects with a 10-year SCORE-risk ≥5% and combined use of phytosterols/-stanols and statins in all subjects with a 10-year SCORE-risk ≥10%. Because all current statin users supposedly have or had a SCORE-risk ≥10%, they were also assumed to start using phytosterols/-stanols (**Table 1**).

The cost-effectiveness of additional phytosterol/-stanol use in the maximum situation was obtained by subtracting the results of the scenario without added phytosterols/-stanols *(TM reference)* from the scenario with added phytosterols/-stanols *(TM plus PS)*.

The Chronic Disease Model

The RIVM Chronic Disease Model is a Markov-type, dynamic population-based model developed at the National Institute for Public Health and the Environment (RIVM) with the purpose to evaluate effects of public health policy on the incidence and prevalence of chronic diseases in the Dutch population.[24-26] The model links lifestyle and lifestyle related risk factors to morbidity and mortality using relative risks for disease incidence. It contains data on smoking, alcohol, cholesterol levels,

blood pressure and food intake, as well as data on 13 chronic diseases.[26] For the current application, the modelling of cholesterol in relation to CVD is especially important. The Chronic Disease Model includes different relative risk estimates for acute myocardial infarction, stroke, and chronic heart failure, and accounts for interactions between these diseases, with for instance myocardial infarction increasing the risk of chronic heart failure (**Figure 1**). For an example of a recent application of the model in the evaluation of nutritional effects on the risk of CVD see Engelfriet *et al.*[27]

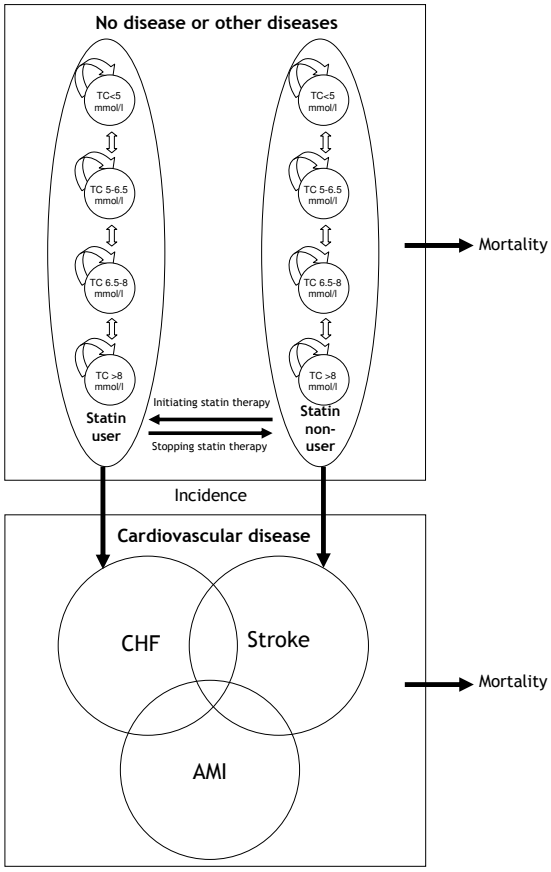

Figure 1. The modelling of cholesterol in relation to cardiovascular disease in the Chronic Disease Model**.** In the Chronic Disease Model the model population is stratified into eight classes of cardiovascular risk, based on total cholesterol levels (TC) and the use of statins (upper part of figure). After a change in cholesterol level, subjects may either transit to another cholesterol class (⇳) or remain in the same cholesterol class ( ). After initiating statin therapy, subjects transit from one of the right four classes (Statin non-user) to one of the left four classes (Statin user). Subjects in all classes are at risk of cardiovascular disease, with different classes having different risks of developing cardiovascular disease (lower part of figure). The model includes different relative risk estimates for chronic heart failure (CHF), stroke and acute myocardial infarction (AMI), and accounts for interactions between these diseases. Subjects are always of risk of death from cardiovascular disease-related and other-cause mortality. This risk depends on the class the subject belongs to.

The Chronic Disease Model simulates effects on health and costs over the model's time horizon for a model population, accounting for a background rate of new phytosterol/-stanol and statin users and background changes in cholesterol level (e.g. due to aging) over time. The model population was stratified into four classes based on total cholesterol level, with cut-off values of 5.0, 6.5 and 8.0 mmol/l. Each class was further subdivided into two groups based on the use of statins (yes/no), resulting in eight different classes of cardiovascular risk. The incidence of CVD is increased for higher classes of total cholesterol and absence of statin use (due to the pleiotropic effects of statins), and also, for example with higher age and male gender. Transitions between the classes are possible, reflecting starting or stopping the use of statins, and changes in total cholesterol level, e.g. due to increased phytosterol/-stanol use in our scenarios (**Figure 1**).

Model input data

General demographic data and data on risk factors and diseases

General demographic data on total mortality, birth rates and population size were obtained from Statistics Netherlands (*http://statline.cbs.nl/statweb/*). Age- and sex-specific initial prevalences of risk factors, including cholesterol levels, and transitions between risk factor classes were obtained from large representative Dutch Health monitoring studies.[22,28-30] Finally, data on disease specific prevalence, incidence, remission and mortality were obtained from four GP registrations.[30-34]

Intake and effect of phytosterols/-stanols and statins

The average per person daily intake of phytosterols/-stanols in the scenarios with additional phytosterol/-stanol use was derived from the averages assessed in the Doetinchem Cohort Study by a food frequency questionnaire. The questionnaire contained an open question on the brand name of bread spread used (e.g. phytosterol/-stanol-enriched bread spreads) and photographs of 4 differently sized portions. The average intake level was 1.05 g phytosterols/-stanols per day. This intake level would cause a reduction in total cholesterol of 4.7% (95% CI: -7.2 to -3.2) based on the dose-response relation in the meta-analysis by Demonty *et al.* (**Supplementary Appendix 1**).[11]

The use of different types and dosages of statins in the Netherlands in 2009 was derived from the GIP-databank, a drug information system of the Dutch Health Care Insurance Board (*http://www.gipdatabank.nl/*), containing reimbursement data on almost the whole Dutch population. The average hypocholesterolaemic effect of these different statins was estimated to be 24.6% (**Supplementary Appendix 1**).[35-37]

It was assumed that statins and phytosterols/-stanols had additive cholesterol-lowering effects, i.e. 29.3%, when used in combination.[12,38,39]

Discontinuation of phytosterols/stanols and statins

In daily life, many subjects discontinue the use of phytosterols/-stanols and/or statins.[40,41] Suffering from side effects such as myalgia, for example, is considered a reason for stopping statin

therapy.[42] To adapt our scenarios to this daily life experience, we have included discontinuation rates for new users of phytosterol/-stanol and new statin users. For phytosterols/-stanols these were estimated from the percentage of subjects who stopped the use of phytosterols/-stanols between subsequent rounds in the Doetinchem Cohort Study. We assumed that subjects who discontinued phytosterols/-stanols, stopped in the first and second year with discontinuation rates of 33% after one year and 44% after two years. Subjects who adhere to the use of phytosterols/-stanols for at least two years, were assumed to continue use during the rest of their lives. In subjects who discontinued the use of phytosterols/-stanols, the total cholesterol level was assumed to return to the same level as before the start of the scenario.

Discontinuation rates for statins were 38.5% after one year and 53.5% after two years.[40] As for phytosterols/-stanols, we assumed total cholesterol levels to increase to the same level as before the start of the scenario in subjects who discontinued statin therapy. New combined users of statins and phytosterols/-stanols were assumed to stop both with a probability as if they were statin only users.

Health effects

Health effects were computed in terms of quality-adjusted life-years (QALYs), a measure of the life expectancy of a person (in years) adjusted for the quality of life,[43] by using data from the Global and Dutch Burden of Disease studies.[26,44-47] Total QALYs lived by the model population in each year of the simulation were found by tracking population sizes and disease prevalence. Net present values of QALYs were calculated by adding annual QALYs over the model's time horizon of 50 years, discounting future QALYs at 1.5% according to Dutch guidelines for pharmacoeconomic research.[48] Similarly, net present values of life-years saved were obtained.

Intervention costs and health care costs

We have calculated all intervention costs as well as both directly and indirectly related health care costs. Costs are expressed in Euros and are based on Dutch unit prices of 2010. Future costs were discounted at 4% annually according to the Dutch guidelines.[48]

Intervention costs included all costs related to the intervention, i.e. costs related to phytosterols/-stanols and, additionally for the theoretical maximum situation, all costs related to statin use. With respect to intervention costs for phytosterols/-stanols, we assumed that phytosterols/-stanols were incorporated into a bread spread. The additional costs of using the phytosterol/-stanol-enriched margarine instead of regular bread spread without phytosterols/-stanols was estimated at €6.20/kg (€9.68/kg for enriched margarine minus €3.48/kg for regular margarine) which amounts to €31.68/yr for current phytosterol/-stanol intake levels (1.05 g phytosterols/-stanols per day equals 5.1 kg margarine per year). In addition, we assumed that all phytosterol/-stanol users had one doctor visit (€24,80)[49] and one lipid test (€24,98)[50] every 5 years costing in total €10,-/yr. Annual statin drug costs were estimated at €150/yr, based on the distribution of the different types and dosages of statin use in the Netherlands and the corresponding costs. Statin users were assumed to have one

doctor visit, one lipid test[10] and three repeat prescriptions every year (€12.40 each),[49] summing up to a total of €237,-/yr.

Health care costs included future savings related to diseases averted by using phytosterols/-stanols and/or statins and those resulting from surviving longer (indirect health care costs).[51] Lifetime health care costs were calculated in the Chronic Disease Model based on disease prevalence combined with age and gender specific data from the Dutch Cost of Illness Study.[26,52,53]

Calculation of cost-effectiveness

Cost-effectiveness ratios were calculated by dividing incremental costs (Euros) by health benefits (QALYs) gained due to the additional use of phytosterols/-stanols. First, intervention costs per QALY gained were computed and second, total costs per QALY gained, i.e. intervention costs plus all differences in health care costs. These cost-effectiveness ratios represent the value-for-money provided by adding treatment with phytosterols/-stanols to current statin use (real-life situation) as well as to maximal statin use resulting from screening (maximum situation).

Uncertainty and sensitivity analysis

Probabilistic uncertainty analysis was used to evaluate the combined effect of uncertainty regarding the effectiveness of phytosterols/-stanols and the use of the Doetinchem Cohort data to estimate the cholesterol levels in the Dutch population. For this uncertainty analysis, Monte Carlo simulation was used, with 100 independent simulations drawing for each simulation new parameter-values for the dose-response curve from their 95% confidence interval (CI). Each simulation used a new distribution over the eight cholesterol classes, assuming Dirichlet distributions for the conversion of the cholesterol distribution of the Doetichem Cohort to the Dutch population.

A series of univariate sensitivity analyses were performed to evaluate the impact of other important model assumptions and parameters on the results. The daily phytosterol/-stanol intake amount was set at the recommended level of 2 g/d. We assumed this was obtained by an increased concentration in bread spread at equal costs. Discontinuation rates for phytosterols/-stanols and statins were set to zero and indirectly related health care costs were disregarded. Furthermore, discount rates on costs and effects of 0%, 3% and 5% were used, and a discount rate of 4% for costs combined with 0% for effects. Finally, time horizons of 10, 20 and 30 years were evaluated.

Results

Number of phytosterol/-stanol and statin users

At the start of the simulation (year 2007), about 615,000 members (7%) of the Dutch population aged between 35 and 75 years used functional foods enriched with phytosterols/-stanols in the reference scenarios (*RL reference* and *TM reference*) (**Table 2**). Statins were used by approximately 1.2 million (14%) and 1.5 million (18%) persons in the real-life and theoretical maximum situation, respectively.

Due to the implementation of the scenarios the number of phytosterol/-stanol users increased. The number of extra phytosterol/-stanol users in the real-life situation ranged between 1.0 million *(RL plus PS (min))* and 2.6 million *(RL plus PS (max))*. In the theoretical maximum situation, a total of 3.3 million subjects started phytosterol/-stanol use *(TM plus PS)*.

Table 2. The number of phytosterol/-stanol (PS) and statin users and the effect of additional phytosterol/-stanol use on health effects, costs, and the cost-effectiveness ratios in the real-life (RL) and theoretical maximum (TM) situation, cumulative over the 50-year period of the simulation (as compared with the reference scenario for each situation, *RL reference* and *TM reference*). Costs were discounted at 4%, and life-years and QALYs at 1.5%. Data for the Dutch population aged 35-75 years (8.4 million people).

	Real-life (RL)			**Theoretical maximum (TM)**	
		Effect of additional PS use			Effect of additional PS use
	RL reference	*RL plus PS (min)*	*RL plus PS (max)*	*TM reference*	*TM plus PS*
No. of subjects† (x 1000)	8407	+0	+0	8407	+0
No. of PS users† (x 1000)	615	+1048	+2571	615	+3333
No. of statin users† (x 1000)	1193	+0	+0	1514	+0
Health effects (x 1000)					
Life-years	199,100	+3.6	+15.5	199,100	+19.5
QALYs	132,400	+2.7	+12.4	132,400	+16.3
Costs (mln €)					
Intervention	0	+473	+993	0	+1255
Health care (direct and indirect)‡	895,800	+29	+118	896,500	+133
Total	895,800	+502	+1111	896,500	+1388
Cost-effectiveness (€ per QALY gained)					
Intervention costs		**192,200**	**86,900**		**83,900**
Total costs		**203,000**	**96,400**		**92,200**

PS, Phytosterols/-stanols; QALY, quality-adjusted life-year

† At onset of scenario

‡ Direct costs include all future savings related to diseases averted by using PS and/or statins; indirect costs include all costs resulting from surviving longer

Health effects

For both the real-life and theoretical maximum situation, the discounted total health effects at the end of the simulation (after 50 years), expressed as life-years and QALYs gained, in the scenarios with additional phytosterol/-stanol use as compared with the reference scenarios (*RL reference* and *TM reference*) are shown in **Table 2**. In the real-life situation, a total of 3600 life-years or 2700 QALYs (on average 0.0034 life-year or 0.0026 QALY per extra phytosterol/-stanol user) were gained if all current statin users would start the use of phytosterols/-stanols *(RL plus PS (min))*. A total of 15,500 life-years or 12,400 QALYs (on average 0.0060 life-year or 0.0048 QALY per extra phytosterol/-stanol user) were gained when additionally also all subjects with a modestly elevated risk (≥5%, <10%) would start using phytosterols/-stanols *(RL plus PS (max))*. Additional phytosterol/-stanol use in the theoretical maximum situation resulted in a total of 16,300 QALYs (19,500 life-years) gained, or 0.0049 QALY (0.0059 life-year) per extra phytosterol/-stanol user *(TM plus PS)*. **Figure 2** shows the discounted extra QALYs gained per year by additional phytosterol/-stanol use compared with the reference scenario for each situation (*RL reference* or *TM reference*). In both situations, the QALYs gained by extra phytosterol/-stanol use reached a maximum after some 20 years when most people in the cohort are old but still alive, and some CVD events can be delayed or prevented by the use of phytosterols/-stanols. Further in time, more and more people die and fewer events can be prevented.

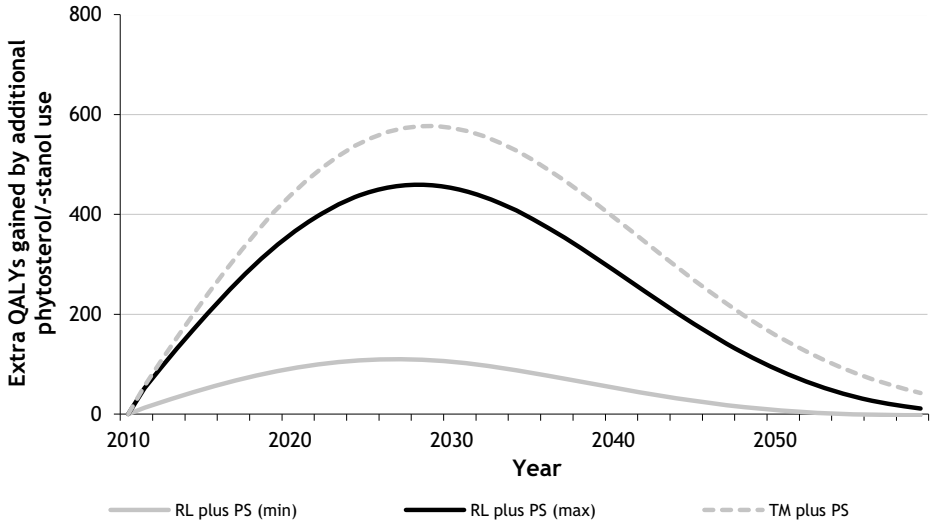

Figure 2. Effect of additional phytosterol/-stanol (PS) use on discounted (1.5%) quality-adjusted life-years (QALYs) gained per year in the real-life (RL) and theoretical maximum (TM) situation. Data are expressed as extra QALYs gained compared with the reference scenario for each situation (*RL reference* and *TM reference*).

Intervention costs and health care costs

The effect of additional phytosterol/-stanol use on cumulative discounted intervention and health care costs over the 50-year period in both situations are presented in **Table 2**. Discounted intervention costs were about a factor 10 higher than health care costs, and ranged between €0.47 billion for added phytosterols/-stanols in the *minimum* real-life situation *(RL plus PS (min))* to €1.26 billion for added phytosterols/-stanols in the theoretical maximum situation (*TM plus PS*). Intervention costs were the highest at the beginning of the simulation, declined steadily during the first two years due to discontinuation of phytosterol/-stanol and statin use, and gradually reached zero near the end of the simulation when most of the cohort has died (**Figure 3, Panel A**). **Figure 3, Panel**

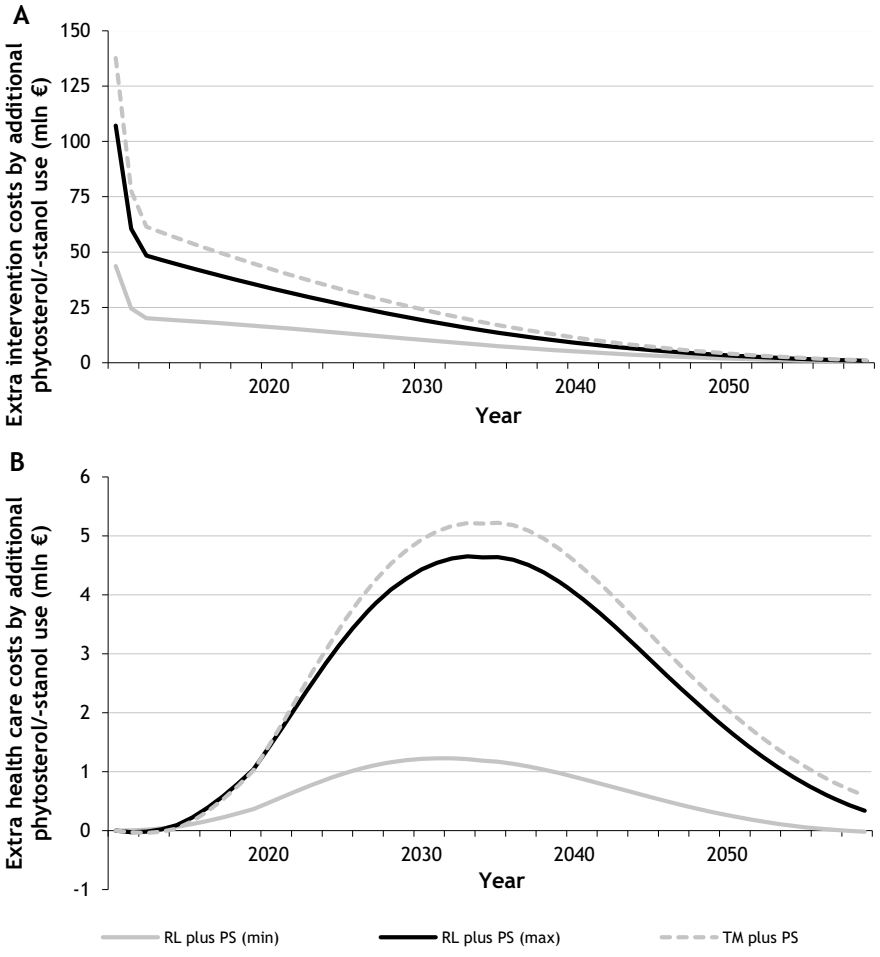

Figure 3. Effect of additional phytosterol/-stanol (PS) use on discounted (4%) annual intervention costs (A) and health care costs (B) in the real-life (RL) and theoretical maximum (TM) situation. Data are expressed as extra costs compared with the reference scenario for each situation (*RL reference* and *TM reference*).

B shows the difference in discounted health care costs per year in the scenarios with additional phytosterol/-stanol use compared with the reference scenarios (*RL reference* or *TM reference*). Apart from minor savings in health care costs during the first three years, health care costs were higher with additional phytosterol/-stanol use than without the additional use. This can be explained by the fact that subjects with a healthier cholesterol level live longer. During their longer lifetime they develop more diseases, with associated costs.[51] The costs of these indirectly related health effects turn out to be higher than the prevented costs of CVD events. Consequently, the more people that start using phytosterols/-stanols, the higher the health care costs.

Cost-effectiveness

Mean incremental total costs per QALY for additional phytosterol/-stanol use varied between €96,000 and €203,000 in the real-life situation, and were about €92,000 in the theoretical maximum situation (**Table 2**). When only the costs of the intervention itself were considered, mean costs were approximately €10,000 lower per QALY, resulting in mean costs per QALY between €84,000 and €192,000. **Figure 4** shows the cost-effectiveness of additional phytosterol/-stanol use for the different scenarios. The cost-effectiveness ratios were compared to threshold values of €20,000, €50,000 and €80,000 per additional QALY.[54,55] In the *maximum* real-life situation *(RL plus PS (max))* and the maximum situation *(TM plus PS)*, the addition of phytosterols/-stanols had a probability

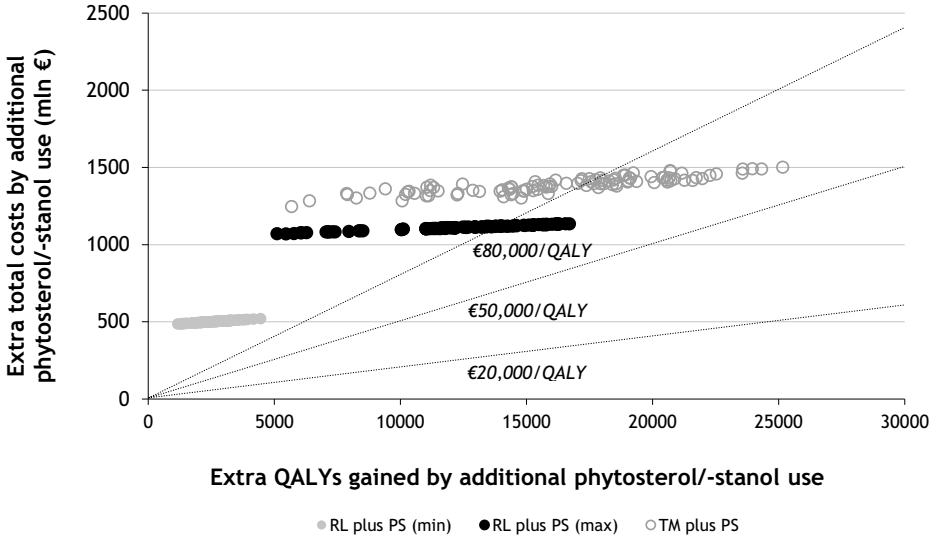

Figure 4. Cost-effectiveness of additional phytosterol/-stanol (PS) use in the real-life (RL) and theoretical maximum (TM) situation. Costs and quality-adjusted life-years (QALYs) are expressed as extra total costs and extra QALYs gained, cumulative for the years 2007-2057 (as compared with the reference scenario for each situation, *RL reference* and *TM reference*). The symbols are the cost-effectiveness ratio of each model run in the uncertainty analysis (100 runs in total). The lines represent cost-effectiveness ratios of €20,000, €50,000 and €80,000.

between 30% and 44% of being cost-effective at a threshold value of €80,000. When a threshold for cost-effectiveness of €20,000 or €50,000 was considered, the addition of phytosterols/-stanols was not cost-effective in any of the 100 uncertainty simulation runs, neither in the real-life situation, nor in the theoretical maximum situation.

Table 3. Results for cost-effectiveness ratio of the uncertainty and sensitivity analyses. The influence of changes in effectiveness and intake level of phytosterols/-stanols, discontinuation, indirect health care costs, discount rates and time horizon on the cost-effectiveness ratio (total extra costs per QALY gained) of additional phytosterol/-stanol use in the real-life (RL) and theoretical maximum (TM) situation (as compared with the reference scenarios). Data are cumulative over the 50-year period of the simulation.

		Real-life (RL)		**Theoretical maximum (TM)**
		RL plus PS (min)	*RL plus PS (max)*	*TM plus PS*
Variable	**Values**	*Cost-effectiveness ratio (€ per QALY gained)*†		
	Reference	203,000	96,400	92,200
Effectiveness of PS on reducing TC‡	Lower bound of 95% CI (-3.2%)	349,300	171,700	151,800
	Upper bound of 95% CI (-7.2%)	134,400	69,700	63,200
PS intake§ (g/d)	2	168,600	78,800	86,400
Discontinuation‖ (%)	0	193,600	101,100	121,500
Indirect health care costs	0	210,500	96,700	88,100
Discount rates¶ (%)	0, 0	334,000	155,300	122,300
	3, 3	348,300	174,000	145,500
	5, 5	368,300	191,400	165,300
	4, 0	177,100	83,400	65,600
Time horizon (years)	10	589,900	330,500	374,000
	20	335,200	155,500	167,100
	30	238,200	123,000	123,400

PS, Phytosterols/-stanols; QALY, quality-adjusted life-year. TC, total cholesterol

† Cost-effectiveness ratio is expressed as extra costs per extra QALY gained compared to costs and QALYs of the reference scenario for each situation (*RL reference* and *TM reference*)

‡ Combined effect of uncertainty regarding the effectiveness of PS (**Supplementary Appendix 1**) and the use of the Doetinchem Cohort data to estimate the cholesterol levels in the Dutch population

§ Intake of PS was increased without additional costs

‖ Discontinuation rates for both PS and statins

¶ Discount rates for costs and effects, respectively

Uncertainty and sensitivity analyses

Results of the uncertainty and sensitivity analyses are shown in **Table 3**. As expected, an increase in effectiveness of phytosterols/-stanols (total cholesterol reduction increased from 4.7% to 7.2%, the upper bound of the 95% CI) and an increased intake of phytosterols/-stanols (from 1.05 g/d to the recommended levels of 2 g/d) resulted in more favourable cost-effectiveness ratios. Assuming no discontinuation of phytosterols/-stanols or statins did only marginally affect the cost-effectiveness ratio. Apparently, lifetime health benefits of the phytosterols/-stanols seem to be counterbalanced by the lifetime payment for phytosterols/-stanols. Disregarding indirect health care costs did not change the results, due to the fact that health care costs (both direct and indirect) were only 10% of the total costs. Considering a greater difference in discount rates for costs and effects, i.e. a higher discount rate for costs and a lower rate for effects, resulted in a more favourable cost-effectiveness ratio, explained by the fact that the costs of the intervention are largely made in the first years, whereas a longer time-span is required to achieve effects of the intervention. Shorter time horizons led to a less cost-effective intervention, because much intervention costs are made at the start, whereas most of the health gains appear later.

Discussion

The present study suggests that the use of functional foods enriched with phytosterols/-stanols as monotherapy and as add-on to statin therapy is a non-cost-effective strategy to reduce CVD. In a situation in which persons eligible for use were identified through passive clinical case-finding, the cost-effectiveness of phytosterols/-stanols ranged from about €96,000 to €203,000 per QALY. A slightly lower (more favourable) cost-effectiveness ratio of €92,000 was obtained when subjects qualifying for phytosterols/-stanols were found through a (hypothetical) universal screening program for CVD (costs of the universal screening program were not included in the analyses). In both situations, cost-effectiveness ratios are well above established threshold values for cost-per-QALY, which generally range between €20,000 and €50,000.[54,55] These threshold values for cost-effectiveness ratios were also not reached in sensitivity analyses in which treatment effect or intake level of phytosterols/-stanols was increased, or future health care costs were not taken into account.

This is the first study evaluating whether functional foods enriched with phytosterols/-stanols are a cost-effective strategy in addition to the beneficial effects of statins in the prevention of CVD. Two studies have been performed to assess the cost-effectiveness of phytosterols/-stanols alone.[20,21] Gerber *et al.*[20] found that €52 per person could be saved when phytosterol/-stanol-enriched margarine was consumed by the entire German population between 30 and 79 years of age. In contrast to our study Gerber *et al.* disregarded intervention costs, i.e. costs of the functional foods enriched with phytosterols/-stanols and costs for doctor visits and lipid tests. Martikainen *et al.*[21] found cost-effectiveness ratios between €7436 and €112,151, conditional on age and gender, and

concluded that phytosterol/-stanol-enriched functional foods were a cost-effective option for high-risk persons (adult men and women aged 60 years or older).

One of the reasons for the difference between the results of these two studies and the present one is that, although phytosterols/-stanols are recommended for subjects with elevated cholesterol levels[3,56] or elevated SCORE-risks,[10,14] all persons in a certain age group were treated with phytosterols/-stanols in the previous studies, regardless of a person's cholesterol level or SCORE-risk.[20,21] Moreover, both previous studies assumed perfect adherence to phytosterols/-stanols, i.e. the continuous use of the recommended daily amount of 2 g phytosterols or –stanols. Actual adherence is, however, known to be less than optimal.[41,57] People do stop the use of phytosterols/-stanols and consume less than the recommended intake amount. Finally, neither previous study considered costs caused by diseases other than cardiovascular disease, acquired later in the life-years saved. Yet, it is more and more recommended that these indirectly related health care costs should be included in economic evaluations.[51,58-60] Nevertheless, with respect to the latter two aspects, sensitivity analyses which disregarded discontinuation of phytosterols/-stanols or indirect health care costs did not substantially alter the results.

We assumed that phytosterols/-stanols were incorporated into a bread spread. Although enriched bread spreads are the most commonly used source for phytosterols/-stanols today, the market is expanding to include other dairy products, like yoghurt (drinks) and milk. Nevertheless, costs (in Euros 2010) for recommended daily intake levels of enriched yoghurt drinks and milk are €0.60 and €1.05, respectively, which is notably higher than costs for recommended intake levels of the bread spread (€0.25). Consequently, this would result in even more unfavourable cost-effectiveness ratios.

In the present study, the cost-effectiveness of phytosterols/-stanols was evaluated both in a real-life situation and in a theoretical maximum situation. In the maximum situation, persons eligible for statin treatment or lifestyle modifications (phytosterols/-stanols) were selected following the Dutch guidelines for cardiovascular risk management. However, it is known that not all GPs follow these guidelines and use the SCORE risk calculation charts that accompany the guidelines.[61,62] Besides GP-related factors, also patient-related factors may have contributed to the fact that in the present study, one fifth (n=321,000) of the subjects eligible for statin use were not using them. Patients may refrain from starting statin therapy, or may discontinue the medication because of side effects or lack of effect.[63] In addition, a few deviations between the guidelines and the implementation of the guidelines in our scenarios should be mentioned. First, the guidelines offer separate recommendations for subjects with and subjects without type 2 diabetes mellitus or established CVD. In the current analysis, all patients suffering from type 2 diabetes or CVD were considered to have the same probability of receiving phytosterols/-stanols and statins as the general population. Thus, we underestimated the chance of being treated for these patients. Furthermore, the guidelines consider subjects with a 10-year SCORE-risk of fatal CVD ≥10% eligible for statin treatment, unless their LDL cholesterol level is < 2.5 mmol/l. We were not able to include this limitation as in the Doetinchem Cohort Study, which was assumed to represent the Dutch population,

only subjects' total and HDL cholesterol level was assessed. However, under the assumption that 80% of the circulating cholesterol in the human body is bound to LDL,[64] less than 1% of the Dutch population with a SCORE-risk ≥10% has an LDL cholesterol level below 2.5 mmol/l. This would not have affected the estimated cost-effectiveness of phytosterols/-stanols.

We have used the Chronic Disease Model to project future effects on health and health care costs. Some limitations of the use of this model need to be addressed. Most importantly, continuous risk factors, such as total cholesterol level, in the Chronic Disease Model are categorised into four classes (**Figure 1**). As a consequence, subjects already in the lowest cholesterol risk factor class before the start of the simulation cannot gain benefits from the phytosterols/-stanols. This may result in an underestimation of the effects of phytosterols/-stanols. Nonetheless, there is currently no evidence that lowering total cholesterol levels below the established target values of 5 mmol/l is associated with lower mortality.[65,66] Moreover, estimates of relative risks of CVD in the Chronic Disease Model are based on studies from different countries. Although this results in the best approximation of the available data, it is unknown whether this approach gives the best values for the Dutch relative risk estimate. Finally, in using the Chronic Disease Model, some assumptions had to be made. First, it was assumed that the association between cholesterol-lowering effects of phytosterols/-stanols and reduction in CVD was similar to the associations seen for other cholesterol-lowering strategies and CVD risk reduction. Second, we assumed that subjects entering the model were similar to those enrolled in the Doetinchem Cohort Study with respect to SCORE-risk and phytosterol/-stanol and statin use. However, the Doetinchem Cohort is not entirely representative for the Dutch population. Smokers and the lower educated appear to be underrepresented in the cohort.[22] Since smoking is associated with increased total cholesterol levels[67] and phytosterol/-stanol-enriched margarines are less often used by the lower educated,[68] SCORE-risks and the percentage of phytosterol/-stanol and statin users in the Dutch population are likely to be slightly different than those estimated from the Doetinchem Cohort Study.

In conclusion, this simulation study shows that the intake of functional foods enriched with phytosterols/-stanols for those with elevated CVD-risk, as encouraged in the guidelines for cardiovascular risk management, is above Dutch and international thresholds for cost-effectiveness, and is thus a non-cost-effective strategy to reduce CVD. This study demonstrates the importance of incorporating cost-effectiveness assessments in health care resource allocation decision-making. Comparing the cost-effectiveness of phytosterol/-stanol-enriched functional foods to other (functional) foods and drugs is suggested to be a critical step in assessing their broader applicability.

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Supplementary Appendix 1

Calculation of total cholesterol-lowering effect of phytosterols/-stanols

Predicted LDL cholesterol change (%) = $-a \cdot (1-\exp(-\frac{\text{dose}}{b/1n(2)})$,[a] where a is -12.68% (95% CI: -15.38 to -9.99) and b is 1.12 g/d (95% CI: 0.62 to 1.63).

The average daily intake level of phytosterols/-stanols, estimated from the food frequency questionnaire used in the Doetinchem Cohort Study, was 1.05 g phytosterols/-stanols per user.

From the distributions in a and b 10,000 random drawings were taken, resulting in a predicted LDL cholesterol change of -5.85% (95% CI: -8.94 to -4.03). Under the assumptions that the cholesterol-lowering effect of phytosterols/-stanols only affects LDL cholesterol and that 80% of the circulating cholesterol in bound to LDL,[b] this results in a predicted total cholesterol reduction of 4.7% (95% CI: -7.2 to -3.2).

Calculation of total cholesterol-lowering effect of statins

Estimated reductions in total cholesterol resulting from the defined daily dose (DDD), i.e. the average maintenance dose per day for a drug in adults,[69] were taken from Penning-van Beest *et al.*[c] (**Supplementary Table 1**). Information about the number of users of various types of statins and the DDD consumed in the Netherlands was taken from the GIP databank. Subsequently, the reduction in total cholesterol resulting from the average consumed dose was calculated per type of statin (**Supplementary Table 2**).

The average reduction in total cholesterol of all different types and doses of statins that were consumed was calculated by multiplying the percentages of the various statins used by the reduction in total cholesterol at the consumed dose, and was found to be 24.6%.

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a Demonty I, Ras RT, van der Knaap HC, Duchateau GS, Meijer L, Zock PL, *et al.* Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. *J Nutr* 2009; 139: 271-84

b Crowley LV, An Introduction to Human Disease. Pathology and Pathophysiology Correlations, Sudbury (2009)

c Penning-van Beest FJ, Termorshuizen F, Goettsch WG, Klungel OH, Kastelein JJ, Herings RM. Adherence to evidence-based statin guidelines reduces the risk of hospitalizations for acute myocardial infarction by 40%: a cohort study. *Eur Heart J* 2007; 28: 154-9

Supplementary Table 1. Average reduction in total cholesterol (TC) per type of statin resulting from the defined daily dose (DDD) of the statin

	DDD (mg)†	Reduction in TC (%) at the defined DDD‡
Simvastatine	30	29.5
Pravastatine	30	24.5
Fluvastatine	60	24.5
Atorvastatine	20	32.0
Rosuvastatine	10	32.0

DDD, defined daily dose; TC, total cholesterol
† Information taken from *http://www.whocc.no/atc_ddd_index/*
‡ Adapted with permission from Penning-van Beest *et al.*

Supplementary Table 2. Number of statin users per type of statin, the average consumed daily dose of statins, and the reduction in total cholesterol (TC) resulting from the consumed dose in the Netherlands in 2009

	Number (%) of users† ‡	Average consumed dose (mg) per user†	Reduction in TC (%) at the average consumed dose
Simvastatine	864,970 (51.8)	21.5	21.1
Pravastatine	175,200 (10.5)	24.8	20.3
Fluvastatine	24,654 (1.5)	42.0	17.3
Atorvastatine	417,750 (25.0)	19.9	31.9
Rosuvastatine	188,210 (11.3)	9.3	29.8

TC, total cholesterol
† Information taken from *http://www.gipdatabank.nl/*
‡ Due to rounding, percentages may not total 100%

Chapter 5

General discussion

Introduction

It is increasingly being recognised that most chronic diseases are multifactorial in origin. In this thesis, we have focused on cardiovascular disease (CVD), a multifactorial disease in which a combination of genetic and environmental factors contributes to the aetiology and progression of the disease. To control such diseases and adverse health conditions, a treatment approach in which medicines and nutrition complement each other may prove to be the most successful. In the domain of nutrition, apart from (disease-related) dietetic regimes, an increasing number of functional foods and dietary supplements, each with their own health claim, are marketed. These food items are considered to be positioned between traditional foods and medicines at the so-called 'Food-Pharma interface'.

The attention of the European Union regarding functional foods and dietary supplements has been principally directed to food safety and (claims of) efficacy (*Chapter 1.1*), and most of the research focuses on these two areas. Currently little is known about physiological or behavioural interactions between functional foods or dietary supplements and pharmaceuticals. In addition, the cost-effectiveness of functional foods and dietary supplements is largely unexamined. This thesis aims to start filling the gaps in knowledge in this field and adds to our understanding of the beneficial and harmful effects of the combined use of functional foods/dietary supplements and medicines.

In this general discussion, the main findings of this thesis are discussed and put into a broader perspective, several methodological issues are considered and implications for clinical practice as well as for future research are given.

Main findings

Physiological interactions

Due to the elevated amounts of specific bioactive ingredients in functional foods and dietary supplements, there is an increased risk for physiological food-drug interactions. Physiological interactions are additive, synergistic or antagonistic effects when drugs are combined with functional foods or dietary supplements.[1] In this thesis, physiological interactions between statins and β-glucans from oats (*Chapter 2.1*), statins and *n*-3 PUFA (*Chapter 2.2*) and statins and phytosterols/-stanols (*Chapters 2.3* and *2.4*) were examined.

Oat β-glucans and statins

Based on results from a study with a limited number of hypercholesterolaemic patients, it has been proposed that oat β-glucans might decrease the intestinal absorption, and thereby the cholesterol-lowering effects of statins.[2] Yet, other human trials, using simvastatin, atorvastatin or lovastatin combined with either psyllium, guar gum or hydroxypropylmethylcellulose as soluble fibre, found

either significant reductions in LDL cholesterol levels after soluble fibre supplementation,[3-6] or no effect.[7]

We performed an in vivo study in animals to investigate the physiological interaction between oat β-glucans and atorvastatin (*Chapter 2.1*). In this study, LDL-receptor-deficient mice were fed a diet containing either a low dose, a high dose or no atorvastatin with or without oat bran (*n*=15 per group) for 16 weeks. We found that both atorvastatin and oat bran were effective in reducing serum total cholesterol levels (*P*<0.0001). When oat bran was added to a low dose atorvastatin, the cholesterol-lowering effect of this combination was 50% smaller compared with the effect of the diet with a low dose atorvastatin only. In contrast, total cholesterol decreased to a similar extent in the groups fed a high dose atorvastatin, with or without oat bran. Thus, when the amount of atorvastatin provided in the diet was high enough, a sufficient amount of atorvastatin was still absorbed to significantly reduce cholesterol levels despite the presence of oat bran. Similar effects were seen for other lipid fractions, free and total cholesterol in the liver and atherosclerotic lesion area. The observed effects are likely dependent on the type and dose of statin and oat β-glucans, and on the relative timing of intake of the statin and the dietary fibre.

As with statins, the absorption and bioavailability of other drugs may also be reduced by dietary soluble fibres. Canga *et al.*[8] and Schmidt *et al.*[9] reviewed the literature for interactions between different types of fibres and several drugs. Dietary fibre has been found to interact with several drugs (e.g. with lithium, tricyclic antidepressants, hypoglycaemic drugs such as glibenclamide and metformin, and digoxin), whereas other drugs (e.g. bile acid sequestrants and valproic acid) do not interact with fibre. The mouse model we used seems to be an appropriate model for rapid screening of potential interactions between dietary fibres and cholesterol-lowering drugs.

n-3 Polyunsaturated fatty acids and statins

There is evidence that the addition of *n*-3 polyunsaturated fatty acids (PUFA) to statins improves statin therapy, since both cholesterol and triglyceride levels are lowered (*Chapter 1.2*). Nevertheless, it has also been proposed that the use of concomitant statin therapy may dilute the effects of *n*-3 PUFA because subjects receiving guideline-concordant statin therapy are at relatively low risk of future cardiovascular events, such that extra protection of *n*-3 PUFA is difficult to prove.[10,11]

Using data from the randomised controlled Alpha Omega Trial, it was found that statin use and (residual) cardiovascular risk indeed modified the effects of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid (ALA) (*Chapter 2.2*). Fewer major cardiovascular events were observed among statin non-users who received the combination of EPA-DHA and ALA than among statin non-users who received placebo (adjusted hazard rate ratio (HR_{adj}) 0.46, 95% CI: 0.21 to 1.01, *P*=0.051). In contrast, the number of events did not differ significantly between the *n*-3 PUFA and placebo groups among statin users (HR_{adj} 1.02, 95% CI: 0.80 to 1.31, *P*=0.88; between-group difference: *P*=0.057).

These results support the hypothesis that concomitant statin therapy lowers the risk of future cardiovascular events, which cannot be further reduced by *n*-3 PUFA. This has been suggested

previously by Rauch *et al.*[10] who conducted a randomised controlled trial (RCT) in which they showed that *n*-3 PUFA, if given in addition to guideline-adjusted treatment, did not reduce the rate of major adverse cardiovascular and cerebrovascular events. To our knowledge, apart from our study, no other studies have directly compared the cardiovascular effects of *n*-3 PUFA between users and non-users of concomitant statin therapy, nor between patients at high and low cardiovascular risk.

Phytosterols/-stanols and statins

Several RCT have suggested that the combination of statins with phytosterols/-stanols gives an additive reduction in total and LDL cholesterol of 6% and 10%, respectively (*Chapter 1.2*). However, RCT may have low external validity, which limits the extrapolation to daily practice populations.[12] In *Chapter 2.3*, we assessed the effectiveness of the use of phytosterol/-stanol-enriched margarine in subjects using or not using statins in a real-life setting.

Phytosterol/-stanol-enriched margarine appeared to be effective in lowering total and non-HDL cholesterol levels under customary conditions of use in both statin users and statin non-users. The cholesterol-lowering effect of the margarine when added to statin therapy was similar to the effect observed when the margarine was used alone and increased with increasing intake levels of the enriched margarine. The recommended daily intake level of 27 g margarine per day (2 g phytosterols/-stanols per day) was consumed by only 9% of the subjects and resulted in a 4% decline in total cholesterol levels.

Two other studies have explored the effectiveness of combined customary use of phytosterols/-stanols and cholesterol-lowering drugs to lower total and LDL cholesterol levels.[13,14] In accordance with our results, de Jong *et al.*[14] showed that phytosterols/-stanols reduced cholesterol levels additively to cholesterol-lowering drugs. On the other hand, in the study performed by Wolfs *et al.*[13] no significant difference in change in cholesterol levels was found between cholesterol-lowering drug only users and combination users. The reason why Wolfs *et al.* did not find any significant differences between the groups may be due to the fact that the authors did not adjust for baseline cholesterol levels, which were (non-significantly) lower in the combination users compared with the cholesterol-lowering drug only users. It has been shown that patients with higher baseline levels experience larger reductions in cholesterol levels after intake of phytosterols/-stanols or statins.[15] Moreover, Wolfs *et al.* did not distinguish between statins and other cholesterol-lowering drugs and the study had only a limited number of combination users (n=12), which may have resulted in a lack of power to detect a significant effect. Yet, the latter two aspects also apply to the study by de Jong *et al.*[14]

We proposed a simplified, mathematical model to describe the reductions in LDL cholesterol after separate and combined intake of phytosterols/-stanols and statins (*Chapter 2.4*). It was demonstrated that the additional decrease in LDL cholesterol caused by daily consumption of the recommended dose of phytosterols/-stanols (i.e. 2 g/d) is similar or even greater than the decrease achieved by doubling the statin dose. This finding has been observed previously in human clinical

trials.[16,17] The model can easily be applied to other similar acting (functional) foods, such as products with soluble dietary fibres. Moreover, individuals' specific reductions in total and LDL cholesterol can be predicted, based on certain genetic variants in, for example, the ratio of cholesterol synthesis to cholesterol absorption and the number of LDL receptors.[18]

Behavioural interactions

Phytosterols/-stanols and statins

Behavioural interactions arise when people consuming functional foods or dietary supplements alter the dosage of their prescribed drugs or stop the drug without consulting a general practitioner or pharmacist. As reported in **Chapter 3**, we performed two studies to determine whether the use of functional foods enriched with phytosterols/-stanols influenced adherence to statin therapy. In the first study, phytosterol/-stanol intake data was derived from the food frequency questionnaire of the Doetinchem Cohort Study and was linked to pharmacy-dispensing records (*Chapter 3.2*). It was found that among starters of statins, combination users were 2.5-fold more likely to discontinue statin therapy compared with patients who only used statins (HR_{adj} 2.52, 95% CI: 1.06 to 6.00, *P*=0.036). In the overall population, statin discontinuation rates were not significantly different between users and non-users of phytosterol/-stanol-enriched margarine (HR_{adj} 1.37, 95% CI: 0.82 to 2.31, *P*=0.23). We attempted to confirm this finding in a large population of new users of statins using data from the randomised controlled **ST**atin **I**ntervention research **P**rojec**T** (STIPT). STIPT was aimed to improve patients' adherence to statin therapy through education and feedback on achieved cholesterol levels (*Chapter 3.1*). In contrast to our earlier findings, the use of functional foods enriched with phytosterols/-stanols was not related to discontinuation of statin therapy (HR_{adj} 0.80, 95% CI: 0.59 to 1.08, *P*=0.15) (*Chapter 3.3*). Apparently, subjects that are well informed on the beneficial effects of statins do not have reduced statin adherence rates when using functional foods enriched with phytosterols/-stanols.

To our knowledge, no other studies have compared adherence to drug treatment between users and non-users of functional foods or dietary supplements. However, Alevizos *et al.*[19] conducted a survey among 412 statin users regarding their attitude towards lipid-lowering treatment options. The survey found that almost 10% of the patients had discontinued statin therapy, because they considered the use of phytosterols/-stanols less detrimental to health and equally effective as drug therapy.

Cost-effectiveness of phytosterols/-stanols

Aging of the population, combined with the increasing health care costs, underlines the need to consider the cost-effectiveness of a therapy. It has been suggested that functional foods and dietary supplements, as part of healthy eating habits, can have a substantial effect on health care costs.[20] To help policy-makers in making reimbursement decisions, comparative cost-effectiveness analyses of pharmaceuticals *vs.* functional foods/dietary supplements in persons with a modestly elevated

risk profile are indicated. Comparing the cost-effectiveness of drugs plus functional foods/dietary supplements *vs.* drug therapy alone can be useful in assessing the additive value of a functional food or dietary supplement in patients with a high risk profile.

We evaluated the long-term health effects, measured as quality-adjusted life-years (QALYs) gained, and costs of functional foods enriched with phytosterols/-stanols as monotherapy and as add-on to statin therapy (*Chapter 4.1).* Phytosterols/-stanols were given only to persons who were eligible for use according to their 10-year absolute risk of fatal CVD. Intake levels and discontinuation rates as observed in daily practice were considered in the study. We showed that the cost-effectiveness of phytosterols/-stanols ranges between €92,000 and €203,000, which is above thresholds for cost-effectiveness (i.e. €20,000 to €50,000).[21,22] Thus, from this study it appears that functional foods enriched with phytosterols/-stanols are a non-cost-effective strategy to reduce CVD.

The cost-effectiveness of the use of functional foods or dietary supplements in the prevention of CVD is a largely unexplored area of research.[23] Two other studies have been published towards the cost-effectiveness of phytosterol/-stanol-enriched margarine,[24,25] one study examined the cost-effectiveness of grain fortification with folic acid[26], and another the cost-effectiveness of *n*-3 PUFA supplements.[27] Results ranged from cost-savings to €112,151 per QALY for phytosterols/-stanols, from cost-savings to €180,000 per QALY for folic acid and were about €10,000 per myocardial infarction avoided for *n*-3 PUFA supplements. Cost-effectiveness ratios between and within studies vary considerably due to different modelling approaches and assumptions relating to the population's risk profile (e.g. age, gender and history of CVD) and included costs (e.g. in- or excluding productivity costs and/or indirectly related health care costs).

Methodological issues

Type of study design

Different types of study have been used for the *Chapters 2.1, 2.2* and *2.3* in which we studied physiological interactions between statins and functional foods with either β-glucan soluble dietary fibre, *n*-3 PUFA or phytosterols/-stanols. In the next section, we will discuss the rationale of using these different study types and their strengths and limitations.

Functional foods with soluble dietary fibre are limitedly available in the Netherlands and fibre-rich dietary supplements are only used sparingly.[28] Moreover, studies towards the cardiovascular effects of soluble dietary fibres in patients on statin treatment are scarce and lack consistency in results (*Chapter 1.2*). The preclinical in vivo study described in *Chapter 2.1* was conducted as a first essential step in understanding the potential causes of these mixed results. Experimental animal studies have the advantage of allowing to study the effects of functional foods and statins on invasive or fatal measures. Moreover, long-term dietary intervention trials in humans are often not easy because of practical and ethical reasons and these trials might be biased by non-adherence

to the dietary regimen. Obviously, uncertainties related to the extrapolation of the results from experimental animal species to the human situation are the largest disadvantage of animal studies.

Functional foods or dietary supplements enriched with *n*-3 PUFA are used more in the Netherlands.[28] Several RCT have shown that the consumption of *n*-3 PUFA lowers triglyceride levels in statin users (*Chapter 1.2*) as well as in statin non-users.[29] Yet, it has also been hypothesised that statin users are at relatively low risk of future cardiovascular events, such that no additional protection of *n*-3 PUFA can be observed.[30] The aim of the study described in *Chapter 2.2* was to examine whether the use of statins modifies the effects of the *n*-3 PUFA as observed in the randomised, placebo-controlled Alpha Omega Trial. RCT are widely accepted as the gold standard of medical intervention research. The randomisation process reduces the risk of bias due to confounding by ensuring that all observed and unobserved characteristics of the participants are equally distributed between the intervention and control group. Nevertheless, their design may include short-term interventions, frequent follow-up visits, extensive monitoring and the use of restricted patient populations with high adherence to therapy; factors which limit extrapolation to daily practice populations.[19,20]

Phytosterols/-stanols have been on the market since 1999 and slowly found their way into the Dutch diet. Around 2005, user rates of phytosterol/-stanol-enriched margarines were about 6% for statin non-users and even twice as high for statin users. They are already examined extensively in both preclinical and clinical studies.[7,17,31] This makes the interaction between phytosterols/-stanols and statins especially suitable for study under customary conditions of use. Therefore, we performed an effectiveness study that used retrospective epidemiological data from the ongoing Dutch Doetinchem Cohort Study to study interactions between statins and phytosterols/-stanols (*Chapter 2.3*). Studies using retrospective epidemiological data have the advantage of being able to follow large numbers of subjects for a long period of time; the Doetinchem Cohort Study comprised functional food intake data of nearly 4000 subjects who were followed for two consecutive 5-year intervals. Moreover, effectiveness studies reflect the real-life situation more accurately than the RCT described in *Chapter 2.2*. On the disadvantage side, the observational Doetinchem Cohort Study might be subject to residual confounding due to potential unmeasured differences in cardiovascular risk profile and patient characteristics between users and non-users of functional foods and/or statins; factors that are accounted for in randomised trials.

Behavioural interactions should best be explored in a free-living situation, as persons who know their behaviour is being monitored are more likely to change this behaviour, especially if their existing behaviour is not the desired one.[32,33] For this reason, the behavioural interactions studied in this thesis focused on combined use of functional foods enriched with phytosterols/-stanols and statins (*Chapters 3.2* and *3.3*).

Assessment of functional food intake

In the observational Doetinchem Cohort Study (*Chapters 2.3* and *3.2*) as well as in the randomised controlled STIPT (*Chapter 3.3*), functional food intake was assessed by self-administered

questionnaires. The questionnaire used in the Doetinchem Cohort Study was a food-frequency questionnaire, and thus addressed the frequency of consumption and portion size of (functional) food intake. In contrast, intake levels were not considered in STIPT. Furthermore, persons were inquired about the use of phytosterols/-stanols incorporated in *margarine* only in the Doetinchem Cohort Study, whereas in STIPT the use of all products enriched with phytosterols/-stanols was addressed, including yoghurt and yoghurt drinks.

The use of questionnaires to assess food intake has a number of limitations. First, the questionnaires assessed habitual dietary intake over the previous 12 months. Intakes may therefore have been subjected to recall bias. Yet, studies generally report moderate to high correlations between food intake information derived from self-administrated (food-frequency) questionnaires and information estimated from 24h recalls or food diaries.[34-36] Second, the exact intake amount cannot be ascertained, even with the food-frequency questionnaire. And finally, the time of starting the use of functional food is not determined. This last point is of importance, because subjects who are on statin therapy and start the use of functional foods may be of special interest for research as they may take the functional foods as a replacement for their statin therapy.

In the Alpha Omega Trial, functional food intake was part of the study intervention and was therefore standardised for all subjects (*Chapter 2.2*).

Assessment of statin intake

For the purposes of the studies described in *Chapters 2.3* and *3.2* of this thesis, questionnaire data on health and (functional) food intake data of the Doetinchem Cohort Study was linked to pharmacy-dispensing records using the Pharmacomorbidity-Record Linkage System (PHARMO-RLS). Similar pharmacy-dispensing data were obtained from the pharmacies participating in STIPT (*Chapters 3.1* and *3.3*). In the Netherlands, virtually all inhabitants are registered with a single community pharmacy, independent of prescriber. Consequently, pharmacy records are nearly complete with regard to prescription drugs.[19] An advantage of the use of pharmacy-dispensing data over self-reported questionnaires as were used in the Alpha Omega Trial (*Chapter 2.2*) is that patient-related recall bias and non-response bias are reduced, precise information about prescribed drugs can be obtained and the drug history is available over a long period of time. Nonetheless, previous validation studies have indicated that for drugs used chronically such as statins, the specificity and sensitivity of questionnaires compared with pharmacy records is high.[37-39]

Pharmacy data have the advantage over medical records of being able to obtain information regarding what medication were acquired instead of what medication was prescribed. It has been found that pharmacy records are a reliable reflection of the drug exposure as estimated in a home inventory.[40] However, uncertainty still exists about whether or not the drug is actually being taken according to the prescribed regimen and no information is available about the reason for discontinuation.

Cost-effectiveness methodology

Variations in the cost-effectiveness methodology make it difficult to compare results from different studies. Studies differ with respect to assumptions about the effectiveness of a treatment, the association between risk factor reduction and risk of cardiovascular events, the cost of treatment and health care services, the duration of therapy and the discounting of effects and costs.[41] Dutch guidelines for pharmacoeconomic research recommend that future costs and health effects are discounted at 4% and 1.5% annually, respectively.[42] However, different discount rates are used in different countries and most guidelines prescribe discounting money and health against the same rate.[43] Moreover, whether and how indirect health care costs should be incorporated into cost-effectiveness analyses is still a point of debate.[44-46]

Other methodological considerations

Some additional methodological issues need to be considered when investigating the effects of functional foods and dietary supplements. Concerning epidemiological observational studies, beneficial effects of functional foods and/or dietary supplements may reflect a general healthier lifestyle and dietary intake by patients taking these food products. On the other hand, it is possible that persons who use functional foods may strive less to eat healthy. Adequate adjustment for confounding is thus essential to avoid over- or underestimation of the effects of functional foods and dietary supplements. This requires high quality data on potential confounding factors, such a collected in the Doetinchem Cohort Study. In RCT it is of great importance that a well-considered placebo group is included. This controls for the possible changes in nutritional intake, e.g. a reduced fat intake, associated with the nutritional supplementation.

Implications of this thesis

Implications for practice

Phytosterol/-stanol use as monotherapy and as add-on to statin treatment

We have shown that phytosterol/-stanol-enriched margarine is effective in lowering total and non-HDL cholesterol levels under customary conditions of use in both statin users and statin non-users. Phytosterol/-stanol-enriched functional foods can be recommended to statin non-users with normal to moderately increased serum total and LDL cholesterol concentrations who wish to maintain their cholesterol levels at, or reduce their cholesterol levels to, healthy levels. Statin users who wish to reduce their total and LDL cholesterol levels through diet can use the phytosterol/-stanol-enriched functional foods as an adjunct to their ongoing statin therapy. This might be especially beneficial for those subjects who do not achieve total and LDL cholesterol target levels with statin-monotherapy. Only 9% of the subjects in our study cohort used the phytosterols/-stanols at the recommended intake levels. Dietetics professionals should play a role in advising consumers on

the appropriate intake level of the phytosterol/-stanol-enriched functional foods and should teach consumers how to use these functional foods as part of a balanced diet.

Importance of adherence

In a real-life setting, behavioural factors may lead to a lower adherence to statin therapy among users of functional foods. When patients are well informed on the beneficial effects of statins, they do not seem to have reduced adherence to statins when using functional foods. This underlines the important role that general practitioners and pharmacists have in asking patients about any functional foods or dietary supplements they are using and urging them not to take the functional foods or dietary supplements as a replacement for their prescribed medication. Replacing medication by food products may have detrimental effects, as the use of functional foods or dietary supplements does not necessarily compensate for the lower dose of the drug.

Potential food-drug interactions

Besides the role of general practitioners and pharmacists in underlining the importance of adhering to drug treatment, they should also inform patients about possible negative food-drug interactions. Currently, only food-drug interactions that are mentioned on the label are covered in the pharmacy-surveillance system, such as the effect of grapefruit juice on statin therapy. In order to provide the general practitioners and pharmacists with more information about food-drug interactions, an easily accessible database should be set up which contains all potential relevant interactions between food and pharma. A post-launch monitoring system, as described below, may be a valuable tool in this context.[47]

Reimbursement decisions

Albeit margarines enriched with phytosterols/-stanols are effective in reducing total and non-HDL cholesterol levels in statin users as well as in statin non-users, the intake of these margarines does not seem to be a cost-effective strategy to reduce CVD. This finding may help policy makers in reimbursement decisions for phytosterol/-stanol-enriched functional foods.

Implications for further research

Hard endpoints

Statin users and statin non-users who consume the recommended daily intake of phytosterols/-stanols of 2 gram may reduce their total cholesterol level by about 4%. Extrapolating data on the association between LDL cholesterol-lowering and reduction in coronary heart disease (CHD) events obtained from drug trials suggests that a 4% decrease in serum total cholesterol levels reduces the incidence of CHD by approximately 10-15%.[48,49] However, no studies to date have directly tested the effect of phytosterol/-stanol intake on the incidence of CHD.[17] It is therefore not evident that phytosterols/-stanols reduce CHD rates in statin users. From our analysis towards the

effects of *n*-3 PUFA in the Alpha Omega Trial we found that the use of statin therapy dilutes the effects of the *n*-3 PUFA such that no additional protection can be observed. A similar situation may be observed with phytosterol/-stanol intake in statin users.

Participants in the Doetinchem Cohort Study were linked to the national population register, the mortality register of Statistics Netherlands and the national hospital discharge register.[50] This allows studying the effects of phytosterols/-stanols on cardiovascular events and mortality. Although statistical power is too low to address this topic at the moment, it is expected that usage rates of phytosterols/-stanols will increase in the near future, resulting in higher statistical power. Expanding the food frequency questionnaire to cover also other functional foods with phytosterols/-stanols (besides phytosterol/-stanol-enriched margarine) will also increase user rates and seems useful and warranted to make valid estimations about phytosterol/-stanol consumption.

Other areas of research

This thesis has focused on combinations of statins and functional foods with either β-glucan soluble dietary fibre, *n*-3 PUFA or phytosterols/-stanols. In addition to further exploring these combinations in larger (free-living) populations and with longer durations, other physiological or behavioural interactions between drugs and functional foods or dietary supplements should be explored, since results differ from one combination to another. Examples of interesting combinations to explore are food products with claimed pre- and probiotic activity and drugs affecting the gastrointestinal tract (e.g. prostaglandins, antidiabetics),[9] dietary supplements rich in vitamin K and anticoagulant or antiplatelet drugs,[51,52] and calcium-fortified foods and antibiotic drugs.[53,54]

(Pre)clinical trials are needed to determine the mechanism, efficacy and safety of the combined intake (**Figure 1**). Although certain food-drug combinations need to be studied in pre-launch

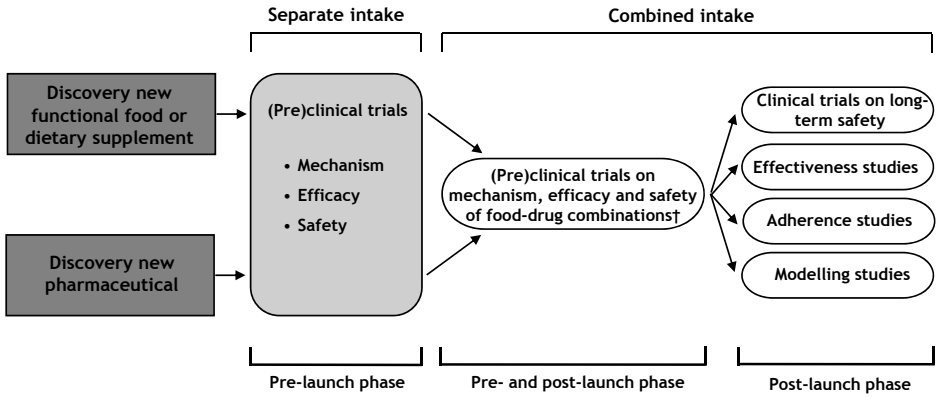

Figure 1. Overview of the study of combinations between functional foods or dietary supplements and drugs, divided into a pre-launch phase and a post-launch phase.
† Based on the possibility of interfering mechanisms (e.g. enzyme induction, drug transport) and concomitant intake clinically relevant food-drug interactions are addressed in the pre-launch phase. Other interesting food-drug interactions are addressed in the post-launch phase.

studies based on the possibility of interfering mechanisms and concomitant intake,[55] it is impossible to identify all relevant food-drug interactions in the pre-launch phase. Therefore, post-launch efficacy and safety data from (pre)clinical studies are needed to determine a product's full risk profile. Yet, (pre)clinical trials are expensive, time-consuming and difficult to run, they lack information about effectiveness, and effects on adherence are difficult to assess in the setting of a clinical trial. Therefore, studies exploring the effectiveness of the combination and the influence of functional foods or dietary supplements on drug adherence should complement the (pre)clinical trials. A post-launch monitoring system, as described below, can be a valuable tool to select interesting combinations between drugs and functional foods or dietary supplements for further exploration in (pre)clinical, effectiveness and adherence studies.

Post-launch monitoring system

The European Commission requests that all adverse drug reactions and drug-drug interactions are reported to the Union Pharmacovigilance database.[56] Moreover, post-authorisation safety studies on medicines are required to ensure adequate ongoing pharmacovigilance monitoring.[57] There is, however, no mandatory requirement for post-launch monitoring or post-launch safety studies of functional foods or dietary supplements in the European Union.[47,58] Such a monitoring system, that systematically monitors the safety and effectiveness of functional foods and dietary supplements after they have been placed on the market, may result in a (cost-)effective strategy to explore food-drug interactions in a free-living situation.[47] The post-launch monitoring system for functional foods, or even preferably a system that combines functional food and drug data, should include passive signalling of beneficial and harmful effects based on consumer feed-back, thereby providing the means to select relevant functional foods or dietary supplements for further research. Active research on food-drug interactions should focus on food-drug interactions that are considered clinically relevant based on the information obtained from the passive signalling process and from pre-launch studies. Active research should comprise the evaluation of the (long-term) exposure, effectiveness and safety under customary conditions of use, adverse effects in potential risk groups, unforeseen (long-term) health effects and interaction effects with nutrients and/or drugs. These aspects will become more and more important in the near future as the market for functional foods and dietary supplements with a health claim is expanding worldwide and consequently an increasing number of persons will use these products and combine them with their prescribed drugs.

Purchase behaviour data

Effectiveness and adherence studies during post-launch monitoring depend largely on the availability of food and drug intake data and outcome data on risk factors or disease. There are currently no standard databases available that integrate functional food intake and drug monitoring. Therefore, we linked food intake data from the Doetinchem Cohort Study to pharmacy-dispensing records. Other possibilities of linking data on drug monitoring and outcome with intake data on functional foods and dietary supplements should be explored, for example linking pharmacy-dispensing

records with other cohort studies (e.g. The Netherlands Cohort Study on Diet and Cancer)[59] or with data from National Food Consumption Surveys.[60] The limitation of most surveys and cohort studies is, however, that they contain only information about an individual's dietary intake at the time of filling out the questionnaire or food diary. Especially for adherence studies, it is of importance to monitor functional food and dietary supplement intake continuously as it is relevant to know whether persons start statin therapy while already using functional foods or *visa versa*. Continuous monitoring of consumer intakes of functional foods, dietary supplements and drugs, can be accomplished by linking pharmacy-dispensing records to individuals' purchase behaviour of functional foods and dietary supplements. In this way, interesting subgroups can be easily differentiated, i.e. new users of drugs who have been shown to have lower adherence to therapy[61-63] and new users of functional foods who might take the foods as a replacement for their drugs. The latter subgroup is also interesting for assessing whether functional food or dietary supplement users change their dietary pattern as they may strive less to eat healthy or, in contrast, may adopt a general healthier diet. Moreover, both functional food consumption and drug use are assessed in a longer time frame without recall bias or non-response bias, and more detailed information can be gathered on the functional food, dietary supplement and drug type, amount of use and time of acquiring the products. Nevertheless, purchase data is normally collected at the household level and should be accompanied by questionnaires or telephone interviews to allow for the extrapolation of household purchases to individual figures.[64,65]

Personalised therapy

A next step in the use of functional foods and dietary supplements as adjuvant to drug therapy is the concept of personalised therapy. Today's functional foods and dietary supplements are typically marketed to large (sub)groups of the total population. Functional foods enriched with phytosterols/-stanols, for example, are targeted to all adults with (moderately) elevated cholesterol levels. However, their use may not be beneficial to the entire (sub)group, as indicated by our results described in *Chapter 2.2*. Personalised therapy relies on targeted treatment based on a person's (genetic) risk profile and increases the probability that a person will benefit from this treatment.[66-68] Pharmaceuticals,[69,70] as well as several dietary components,[71] have been recognised to modulate gene and protein expression and thereby metabolic pathways, homeostatic regulation, and presumably health and disease. In addition, genes also contribute largely to different responses to diet or drug exposure, including interindividual variations in the occurrence of adverse drug reactions.[72,73]

Public understanding and perception of claims

The European Food Safety Authorisation (EFSA) is currently assessing the accuracy of health claims on (functional) foods to provide the European Commission with advice on the substantiation of the health claims (*Chapter 1.1*). The relevance of a health claim has been focused merely on the scientific substantiation of a health claim, whereas little is known about the relevance the health

claim may have on public health. The understanding and perception of a health claim by consumers is an important issue of concern as it appears that consumers cannot differentiate between unqualified and qualified health claims, or between a simple nutrition claim and a health claim.[74,75] Effort should be made to convey health claims that can be understood and are relevant to public health nutrition, without conflicting with official nutritional guidelines or being misleading.[76] Moreover, when available, information about positive and negative interactions between the food product and pharmaceuticals should be added to the label of functional foods and dietary supplements.

Health technology assessments

Economic evaluations can be a useful tool to support decisions at various levels (e.g. prescribing and reimbursement decisions) on therapies (food or pharmaceuticals), or combination of therapies (food and pharmaceuticals). To improve the comparability between studies that assess the cost-effectiveness of different therapies, a standardised methodology should be developed and adopted. This involves the use of agreed discount rates and consensus on which costs should be included in the analysis.

Besides considering the cost-effectiveness of a therapy, benefit-risk assessment is increasingly important in making decisions on medicines, and should also play a role in decisions on functional foods. The European Commission requires manufactures of pharmaceuticals to supply the European Medicines Agency with all information relevant for the evaluation of the benefits and risks related to a medical product. When therapeutic alternatives are available, one should perform a comparative benefit-risk assessment.[57,77,78] So far, no benefit-risk assessment is required for the approval and market introduction of (novel) functional foods or dietary supplements.[79] Nonetheless, potential health benefits of these products are currently being assessed under Regulation (EC) 1924/2006, and evaluating food safety is an established field of research.[80,81] The concept of integrated benefit-risk assessments of food (products) and nutrition is new.[82-84] The EFSA has recently developed guidance for performing benefit-risk assessment of foods.[85] This guidance is applicable to all foods, but might be especially appropriate for (novel) functional foods or dietary supplements that may be beneficial to health, but may also cause health risks due to the over- or underconsumption of specific (micro-)nutrients, unforeseen adverse health effects or long-term effects, and interactions. Due to the absence of a history of safe use, novel functional foods and dietary supplements may be more frequently related to adverse health effects. The changes in dietary intake level after the introduction of a (novel) functional food or dietary supplement allow for assessing a benefit-risk balance both before and after market introduction.

Conclusion

In conclusion, the present thesis shows that the use of functional foods and dietary supplements may offer opportunities to reduce health risk factors when combined with prescription drugs. We

have shown that functional foods enriched with phytosterols/-stanols lower total and LDL cholesterol by 4% and 5%, respectively, when used in combination with statins in a real-life setting. At the moment, however, it is not clear whether these reductions in cholesterol levels lead to a reduced CHD risk. For functional foods enriched with *n*-3 PUFA we found that patients on statin therapy were at a relatively low risk of future cardiovascular events, such that supplementation with *n*-3 PUFA did not provide additional protection against cardiovascular events.

There are also potential problems related to the use of functional foods and dietary supplements. First, their use may increase the risk for food-drug interactions due to the elevated amounts of specific functional ingredients in the diet, as we showed in the experimental animal study on the separate and combined effects of oat β-glucans and statins. More research is needed towards this and other relevant food-drug combinations. Linking data on functional food and dietary supplement intake (e.g. purchase behaviour data or data from questionnaires or food diaries) to pharmacy-dispensing records is helpful in this respect. Second, the use of functional foods or dietary supplements may lead people to indulge in self-medication, potentially resulting in lower adherence to drug therapy. Research towards this behavioural interaction is currently lacking and will become more important in the future as the world market for functional foods and dietary supplements is growing.[86]

General practitioners and pharmacists have an important role to play in providing information about possible food-drug interactions and in urging people not to take functional foods or dietary supplements as replacement for their prescribed medication. Tools such as a post-launch monitoring system based on both passive and active signalling of beneficial and harmful effects should be set up to provide easy accessible information about food-drug interactions. Also the development and use of modelling tools, such as the ones proposed in this thesis, will improve our knowledge about interactions between drugs and functional foods or dietary supplements.

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Chapter 6

Summary

Samenvatting

Summary

The popularity of functional foods and dietary supplements has increased significantly over the last decade. Functional foods are foods that are claimed to improve health, quality of life or well-being beyond basic nutritional functions. They resemble conventional food products in appearance and are consumed as part of the usual diet. In contrast, dietary supplements are typically marketed in the form of a capsule, pill, powder or gel and are not presented for use as a conventional food, meal or diet. Dietary supplements contain one or more dietary ingredients (e.g. vitamins, minerals, amino acids, herbs or other botanicals) and are intended to supplement the diet.

Functional foods and dietary supplements are meant to benefit health, and consequently such food products typically contain health claims on their label stating their beneficial effects. These claimed effects on disease risk reduction often resemble the effects of preventive medicines. It is therefore not surprising that numerous subjects combine their drug therapy with the use of functional foods or dietary supplements. This combination may be beneficial, as the food product and drug may additively reduce risk factors. However, combined intake also increases the likelihood of the occurrence of food-drug interactions, either on a physiological level or a behavioural level.

The studies described in this thesis aimed to gain further insight into both positive and negative aspects arising from the combined intake of drugs and functional foods or dietary supplements. Our research focused on functional foods, dietary supplements and drugs used to lower lipid levels, and thereby the risk of cardiovascular disease (CVD), the leading cause of death in the world. More specifically, we have focused on combinations of functional foods or dietary supplements enriched with oat β-glucans, *n*-3 polyunsaturated fatty acids (PUFA) or phytosterols/-stanols with statin drugs. Statins are the drugs of first choice to lower elevated lipid levels, an important risk factor for CVD, and are the most widely prescribed drugs in the world.

In *Chapter 1.2* we reviewed the literature with the aim of summarising the potential beneficial effects of adding functional foods or dietary supplements to statin therapy.

Functional foods and dietary supplements may have a role in supporting statin therapy in three different ways. First, functional foods or dietary supplements may add to the effect that a medicine has in reducing risk factors associated with CVD. For example, statins reduce low-density lipoprotein (LDL) cholesterol by 18-55% and phytosterols/-stanols reduce LDL cholesterol by 6-15%. Combination of statins with the consumption of phytosterols or –stanols results in additive LDL cholesterol-lowering effects. Thus, phytosterols and -stanols reduce LDL cholesterol levels even further when added to statin treatment.

Second, certain functional foods or dietary supplements may improve risk factors for CVD, which are different to the risk factor that the medicine is dealing with. Statins are highly effective in lowering total and LDL cholesterol, but only moderately effective in reducing triglyceride levels. Supplementing persons with *n*-3 PUFA will lower triglycerides and thereby improve statin therapy, since both cholesterol and triglyceride levels are lowered.

Third, functional foods and dietary supplements may be capable of reducing medicine-associated side effects. One to seven percent of the patients experience side effects with statin use. These are thought to be caused by a statin-related reduction in the amount of coenzyme Q_{10} in the body. The use of dietary supplements with coenzyme Q_{10} may resolve the side effects.

Chapter 2 describes four studies to investigate physiological interactions that may arise after combined intake of functional foods and statins. Physiological interactions are additive, synergistic or antagonistic effects when drugs are combined with functional foods.

In *Chapter 2.1* we investigated the separate and combined effects of the dietary fibre β-glucan from oat bran and different doses of atorvastatin in an animal experiment. It was found that both oat bran and atorvastatin were effective in reducing serum total cholesterol levels (low dose atorvastatin: −5.48 mmol/l, high dose atorvastatin: −9.12 mmol/l, oat bran: −3.82 mmol/l, compared with control (no atorvastatin/no oat bran), all $P<0.0001$). When oat bran was added to a low dose atorvastatin, the cholesterol-lowering effect of this combination was 50% smaller compared with the effect of the diet with a low dose atorvastatin only (between-group difference: 2.77 mmol/l, 95% confidence interval (CI): 1.04 to 4.50, $P=0.002$). In contrast, total cholesterol decreased to a similar extent in the groups fed a high dose atorvastatin, with or without oat bran (between-group difference: 1.10 mmol/l, 95% CI: -0.62 to 2.83, $P=0.21$).

Chapter 2.2 explored the effects of the *n*-3 PUFA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid (ALA), either with or without statins, on major cardiovascular events. Although there is substantial evidence that the addition of *n*-3 PUFA to statin therapy improves a patient's risk profile, as discussed in *Chapter 1.2*, it has also been proposed that patients who are using statins are at relatively low risk of future cardiovascular events, such that no additional protection of *n*-3 PUFA can be observed. Indeed, we observed no risk reduction in statin users who received additional *n*-3 PUFA (adjusted hazard rate ratio (HR_{adj}) 1.02; 95% CI: 0.80-1.31, $P=0.88$). However, 54% fewer major cardiovascular events occurred among statin non-users who received EPA-DHA plus ALA than among statin non-users who received placebo (HR_{adj} 0.46, 95% CI: 0.21 to 1.01, $P=0.051$). This effect was most pronounced in statin non-users with a high (≥4) baseline total to HDL cholesterol ratio (HR_{adj} 0.40, 95% CI: 0.18 to 0.89, $P=0.025$).

In *Chapter 2.3* we determined the cholesterol-lowering effects of margarines enriched with phytosterols/-stanols in statin users and statin non-users in a real-life setting. We found that phytosterol/-stanol-enriched margarine lowered total and non-high-density lipoprotein cholesterol under customary conditions of use in both statin users and statin non-users. The cholesterol-lowering effect of the margarine when added to statin therapy was similar to the effect observed when the margarine was used alone and increased with increasing intake levels of the enriched margarine (no intake, 0; low intake, -0.017 mmol/l (95% CI: -0.16 to 0.13); medium intake, -0.089 mmol/l (95% CI: -0.22 to 0.038); high intake, -0.32 mmol/l (95% CI: -0.50 to -0.14)). The recommended daily intake level of 2 g phytosterols/-stanols per day (27 g margarine per day) was consumed by only 9% of the subjects and resulted in a 4% decline in total cholesterol levels, i.e. a ~5% decline in

LDL cholesterol. This level of effect is considerably lower than the effects observed in randomised controlled trials, i.e. 6-15%.

In *Chapter 2.4* we proposed a simplified mathematical approach to model reductions in LDL cholesterol after separate and combined intake of statins and functional foods acting on the intestinal (re)absorption of cholesterol and bile acids, such as functional foods enriched with phytosterols/-stanols or β-glucan dietary fibres. For separate intakes, we demonstrated a moderate to high correlation between experimentally collected data derived from two recent meta-analyses and the simulated data. For combined intakes, we showed that a daily intake of 2 g phytosterols/-stanols reduces LDL cholesterol level by about 8% to 9% on top of the reduction resulting from statin use. A finding that is consistent with previously published data. In future work, this model can be extended to include more complex (regulatory) mechanisms and genetic factors, and may finally be used to identify potential food-drug-gene interactons.

Chapter 3 starts with a study that aimed to improve patients' adherence to statin therapy and subsequently describes two studies on the behavioural interaction between functional foods and statin drugs. Behavioural interactions arise when people consuming functional foods or dietary supplements change their adherence to drug treatment, e.g. they alter the dosage of the prescribed drugs or stop the drug when taking these food products.

Chapter 3.1 showed the feasibility and effectiveness of a community pharmacy-based pharmaceutical care program to improve medication adherence in new users of statins. The pharmaceutical care program consisted of five individual counselling sessions with a pharmacist during a 1-year period. During the sessions, patients received structured education about the importance of medication adherence, lipid levels were measured and the association between adherence and lipid levels was discussed. The results showed that patients in the pharmaceutical care group were 34% less likely to discontinue statin treatment within 6 months after the start of treatment compared with the usual care group (HR 0.66, 95% CI: 0.46 to 0.96, P=0.028). Twelve months after initiating therapy, the difference in discontinuation rate was not statistically significant (HR 0.84, 95% CI: 0.65 to 1.10, P=0.21).

In *Chapter 3.2* and *Chapter 3.3* we addressed the question whether the use of functional foods enriched with phytosterols/-stanols led to changes in adherence to statin therapy. On the one hand it is conceivable that persons lower the dose of their drugs, or that they take their drug less consistently as they have implemented an additional therapy with potentially less side effects. On the other hand one can speculate that the combined use of functional foods and drugs has a stimulating impact on drug taking behaviour as subjects who are highly motivated to lower their cholesterol levels will be more adherent to their drug therapy and these subjects are also prone to buy the relatively expensive functional foods. In *Chapter 3.2* we linked retrospective data on phytosterol/-stanol-enriched margarine intake from the population-based, longitudinal Doetinchem Cohort Study to pharmacy-dispensing records. Among 4848 persons, 522 (11%) used statins only and 60 (1.2%) combined these drugs with phytosterol/-stanol-enriched margarine. Overall statin

discontinuation rates were not significantly different between the users and non-users of enriched margarine (HR_{adj} 1.37, 95% CI: 0.82 to 2.31, *P*=0.23), but combination users were 2.5-fold more likely to discontinue statin therapy within 12 months in the subgroup of starters (HR_{adj} 2.52, 95% CI: 1.06 to 6.00, *P*=0.036). In *Chapter 3.3*, however, the use of functional foods enriched with phytosterols/-stanols was not related to discontinuation of statin therapy in a large population of new users of statins (HR_{adj} 0.80, 95% CI: 0.59 to 1.08, *P*=0.15). This discrepancy is likely due to differences in the population under study. Whereas in the study described in *Chapter 3.2* statin users from the general population were included, *Chapter 3.3* assessed adherence rates among new statin users, half of whom were following the pharmaceutical care program described in *Chapter 3.1*. Apparently, persons that are well informed on the beneficial effects of statins do not have reduced adherence to statins when using functional foods enriched with phytosterols/-stanols.

Chapter 4 focused on the cost-effectiveness of functional foods. The aging of the population together with the rising health care costs requires considering the cost-effectiveness and budgetary impact of different intervention strategies. In cost-effectiveness analyses the costs and health effects of an intervention are compared to determine whether the intervention provides value-for-money.

In *Chapter 4.1* we evaluated the cost-effectiveness of functional foods enriched with phytosterols/-stanols in persons who were eligible for use according to their 10-year absolute risk of fatal cardiovascular disease. The model estimated that the use of phytosterols/-stanols improved life expectancy and quality-adjusted life-years (QALYs) by 0.0034 to 0.0060 and 0.0026 to 0.0048 years per additional phytosterol/-stanol user, respectively, depending on model assumptions. Costs were increased by about €450 per additional user, yielding cost-effectiveness ratios that ranged between €92,000 and €203,000 per QALY. This level is well above Dutch and international thresholds for cost-effectiveness, which generally lie between €20,000 and €50,000. Consequently, it was concluded that the use of functional foods enriched with phytosterols/-stanols is a non-cost-effective strategy to reduce CVD.

In **Chapter 5,** the main findings of the studies described in this thesis are summarised and discussed. The chapter ends with implications for clinical practice and suggestions for areas of future research.

Samenvatting

Het aanbod en gebruik van functionele voedingsmiddelen en voedingssupplementen is de laatste jaren sterk toegenomen. Functionele voedingsmiddelen zijn eet- of drinkwaren waarin (bestanddelen van) ingrediënten in min of meerdere mate aanwezig zijn, op grond waarvan de fabrikant bepaalde gezondheidsbevorderende eigenschappen van het levensmiddel claimt. Deze producten zien er hetzelfde uit als andere voedingsproducten en worden geconsumeerd als onderdeel van de dagelijkse voeding. Daarentegen zijn voedingssupplementen veelal verkrijgbaar in de vorm van pillen, poeders of capsules en zijn deze bedoeld als aanvulling op de normale voeding. Ze bevatten één of meerdere ingrediënten met een mogelijke gezondheidsbevorderende werking, zoals vitamines, mineralen, vetzuren, aminozuren of kruiden.

Functionele voedingsmiddelen en voedingssupplementen zijn bedoeld om de gezondheid te bevorderen. Dit gezondheidsbevorderende effect staat dikwijls in de vorm van een voedings- of gezondheidsclaim vermeld op de verpakking van het product. De claim lijkt soms op de gedocumenteerde gezondheidseffecten van preventieve geneesmiddelen. Om deze reden is het niet verwonderlijk dat patiënten hun voorgeschreven geneesmiddelen steeds vaker combineren met het gebruik van functionele voeding of voedingssupplementen. Deze combinatie kan voordelen opleveren, omdat het geneesmiddel en voedingsproduct gezamenlijk de risicofactoren voor ziekte kunnen verlagen. Echter, gecombineerde inname verhoogt de kans op het ontstaan van een ongewenste wisselwerking (interactie) tussen het geneesmiddel en het voedingsproduct. Dit kunnen zowel fysiologische interacties zijn, als interacties op gedragsniveau. Bij een fysiologische interactie wordt bij gelijktijdig gebruik van een functioneel voedingsmiddel/voedingssupplement het effect van het geneesmiddel versterkt of verzwakt, of *visa versa*. Een interactie op gedragsniveau ontstaat wanneer personen, die een functioneel voedingsmiddel of voedingssupplement gebruiken, hun therapietrouw aan het geneesmiddel veranderen. Voorbeelden zijn het aanpassen van de dosering van een voorgeschreven geneesmiddel of helemaal stoppen met het gebruik van een geneesmiddel.

Het doel van het in dit proefschrift beschreven onderzoek was om meer inzicht te krijgen in zowel de positieve als negatieve interacties die optreden bij een gecombineerde inname van geneesmiddelen en functionele voedingsmiddelen of voedingssupplementen. Het onderzoek richtte zich op producten die helpen het cholesterolgehalte te verlagen. Een hoog cholesterolgehalte is één van de belangrijkste risicofactoren voor het ontstaan van hart- en vaatziekten. Meer specifiek is er gekeken naar combinaties van statines en functionele voedingsmiddelen of voedingssupplementen die zijn verrijkt met β-glucan, omega-3 vetzuren of plantensterolen/-stanolen. Statines zijn de eerste keuze bij de medicamenteuze behandeling van verhoogde cholesterolwaarden en zijn één van de meest voorgeschreven medicijnen ter wereld.

Na een algemene inleiding (**Hoofdstuk 1**) is in *Hoofdstuk 1.2* de literatuur samengevat over de mogelijke positieve effecten van de toevoeging van functionele voedingsmiddelen of voedingssupplementen aan statinetherapie.

Functionele voedingsmiddelen en voedingssupplementen kunnen op drie verschillende manieren een bijdrage leveren aan statinetherapie. Ten eerste kan het voedingsmiddel de risicofactor waar het geneesmiddel zijn werking op uitoefent extra verlagen. Functionele voedingsmiddelen met plantensterolen/-stanolen verlagen bijvoorbeeld het cholesterolgehalte in het bloed bovenop de cholesterolverlagende werking van statines. Ten tweede kan het functionele voedingsmiddel of het voedingssupplement een risicofactor voor een ziekte verlagen waarop het geneesmiddel weinig effect heeft. Zo zijn statines zeer effectief in het verlagen van het cholesterolgehalte, maar slechts matig effectief in het verlagen van triglyceriden, een andere belangrijke risicofactor voor hart- en vaatziekten. Het gebruik van functionele voedingsmiddelen of voedingssupplementen met omega-3 vetzuren door gebruikers van statines kan voor deze patiënten gunstig zijn, omdat naast het cholesterolgehalte dan ook het gehalte aan triglyceriden wordt verlaagd. Tenslotte kunnen functionele voedingsmiddelen of voedingssupplementen een rol spelen in het verminderen van bijwerkingen van statinetherapie. Eén tot 7% van de statinegebruikers ervaart bijwerkingen als gevolg van statinegebruik. Deze bijwerkingen zijn mogelijk een gevolg van een statine-gerelateerde verlaging in de hoeveelheid coenzym Q_{10} in het lichaam. Het gebruik van een voedingssupplement met coenzym Q_{10} zou daardoor mogelijk kunnen leiden tot minder bijwerkingen.

In **Hoofdstuk 2** worden fysiologische interacties onderzocht die kunnen optreden bij een gecombineerde inname van statines en functionele voedingsmiddelen verrijkt met β-glucan, omega-3 vetzuren of plantensterolen/-stanolen.

In *Hoofdstuk 2.1* zijn de fysiologische effecten onderzocht van afzonderlijke en gecombineerde inname van de voedingsvezel β-glucan uit haver en verschillende doseringen statines. Muizen kregen hiervoor gedurende 16 weken een voeding die verrijkt was met β-glucan en/of een lage of hoge dosering statine. De afzonderlijke inname van zowel β-glucan als van de statine was effectief in het verlagen van het cholesterolgehalte. Een hogere dosering statine leidde tot een groter cholesterolverlagend effect. Echter, bij de gecombineerde inname van β-glucan met een lage dosering statine werd een 50% kleiner cholesterolverlagend effect waargenomen in vergelijking met de inname van de lage dosering statine alleen. Gecombineerde inname van β-glucan met een hoge dosering statine resulteerde in een even groot effect als de hoge dosering statine alleen. Uit deze studie blijkt dat sommige functionele voedingsmiddelen de werking van geneesmiddelen kunnen tegenwerken.

In *Hoofdstuk 2.2* is onderzocht of het gebruik van functionele voedingsmiddelen met omega-3 vetzuren tot minder hart- en vaatziekten leidt bij gebruikers en niet-gebruikers van statines die een hartinfarct hebben doorgemaakt. Vette vis, walnoten en lijnzaad zijn belangrijke natuurlijke bronnen van omega-3 vetzuren. Hoewel er bewijs is dat door consumptie van omega-3 vetzuren het risicoprofiel van statinegebruikers verbetert (*Hoofdstuk 1.2)*, is het niet bewezen dat dit ook resulteert in minder hart- en vaatziekten. Het is mogelijk dat het gebruik van statines leidt tot een relatief laag

risico op hart- en vaatziekten, dat niet verder verlaagd kan worden door het gebruik van omega-3 vetzuren. Uit de studie beschreven in *Hoofdstuk 2.2* bleek inderdaad dat het aantal opgetreden hart- en vaataandoeningen onder statinegebruikers gelijk was, ongeacht de extra inname van margarine met toegevoegde omega-3 vetzuren. Echter, bij niet-statinegebruikers die extra omega-3 vetzuren kregen, kwamen 54% minder hart- en vaatziekten voor dan bij niet-statinegebruikers die geen extra omega-3 vetzuren kregen. Het gebruik van omega-3 vetzuren lijkt dus voornamelijk zinvol en effectief voor hartinfarctpatiënten die geen statines willen of kunnen gebruiken.

In *Hoofdstuk 2.3* zijn de cholesterolverlagende effecten van margarines met toegevoegde plantensterolen/-stanolen onderzocht bij gebruikers en niet-gebruikers van statines. Er werd geconcludeerd dat deze margarines effectief zijn in het verlagen van het cholesterolgehalte bij zowel statinegebruikers als niet-statinegebruikers (onder normale, ongecontroleerde gebruiksomstandigheden). Het cholesterolverlagende effect van de margarines was gelijk voor statinegebruikers en niet-statinegebruikers en nam toe bij een hogere inname van de verrijkte margarine. De door de fabrikant aanbevolen dagelijkse hoeveelheid van 2 gram plantensterolen/-stanolen (27 gram margarine per dag) werd slechts geconsumeerd door 9% van de gebruikers en resulteerde in een verlaging van het cholesterolgehalte van 5%. Dit effect is kleiner dan de geobserveerde effecten in gecontroleerde klinische studies (6-15%). Diëtisten en voedingskundigen hebben een rol bij de voorlichting over het juiste gebruik van functionele voedingsmiddelen met plantensterolen/-stanolen als onderdeel van een gezonde voeding.

In *Hoofdstuk 2.4* wordt een vereenvoudigd model beschreven dat dient om reducties in het cholesterolgehalte te schatten na inname van verschillende hoeveelheden statines en plantensterolen/-stanolen. Met het model wordt aangetoond dat de toevoeging van plantensterolen/-stanolen aan een statine resulteert in een extra verlaging van het cholesterolgehalte van 8% tot 9%, ongeacht de hoeveelheid ingenomen statine. In toekomstig werk kan het model uitgebreid worden met meer complexere (regel)mechanismen en genetische factoren.

Hoofdstuk 3 start met een studie gericht op het verbeteren van de therapietrouw van statinegebruikers en beschrijft vervolgens twee studies naar interacties tussen functionele voedingsmiddelen en statines op gedragsniveau.

Hoofdstuk 3.1 laat zien dat een farmaceutisch patiëntenzorgprogramma, uitgevoerd door openbare apothekers, effectief is in het verbeteren van de therapietrouw aan statines. Voor deze studie werden 899 nieuwe gebruikers van statines (patiënten die in het voorafgaande half jaar niet eerder statines voorgeschreven kregen) willekeurig verdeeld in een interventiegroep met uitgebreide patiëntenzorg en een controlegroep. Het patiëntenzorgprogramma bestond uit extra voorlichting en begeleiding door de apotheker bij de eerste en tweede uitgifte van het geneesmiddel. Na 3, 6 en 12 maanden kregen de patiënten een begeleidingssessie waarin naast voorlichting over het belang van een goede therapietrouw, ook cholesterolwaarden werden gemeten en de relatie tussen therapietrouw en gemeten cholesterolwaarden werd benadrukt. De patiënten in de controlegroep kregen de gebruikelijke zorg. De resultaten lieten zien dat het aantal patiënten dat binnen 6 maanden stopte

met de behandeling met statines, 34% lager was in de interventiegroep dan in de controlegroep. Na 12 maanden was dit verschil tussen de interventiegroep en controlegroep kleiner (16%).

In de *Hoofdstukken 3.2* en *3.3* is onderzocht of het gebruik van functionele voedingsmiddelen met toegevoegde plantensterolen/-stanolen leidt tot veranderingen in de therapietrouw aan statines. Enerzijds is het denkbaar dat personen hun dosering geneesmiddel verlagen of zelfs stoppen met het geneesmiddel, omdat zij een additioneel product met een soortgelijk effect denken te gebruiken. Anderzijds is het aannemelijk dat personen die functionele voedingsmiddelen gebruiken een hogere therapietrouw hebben, omdat dit wellicht personen zijn die bewuster met hun gezondheid bezig zijn en hierdoor ook het belang van een goede therapietrouw aan statines inzien. In *Hoofdstuk 3.2* zijn gegevens over de inname van margarines met toegevoegde plantensterolen/-stanolen, gekoppeld aan medicatie aflevergegevens van apotheken. Van de 4848 personen slikten 522 patiënten alleen statines en 60 patiënten combineerden het gebruik van statines met verrijkte margarines. In de totale populatie van statinegebruikers (582 patiënten) werd er geen significant verschil gevonden in het staken van het statinegebruik tussen beide groepen. In een subgroep van startende statinegebruikers stopten combinatiegebruikers echter 2,5 keer zo vaak met hun statine. In de studie beschreven in *Hoofdstuk 3.3,* die uitgevoerd werd in een grote populatie van startende statinegebruikers, werd daarentegen niet aangetoond dat het gebruik van functionele voedingsmiddelen met toegevoegde plantensterolen/-stanolen samenhing met het staken van de statinetherapie. Het verschil in uitkomst tussen beide studies is waarschijnlijk te verklaren door het verschil in studiepopulatie. Het onderzoek beschreven in *Hoofdstuk 3.2* vond plaats onder statinegebruikers in de algemene bevolking, terwijl in *Hoofdstuk 3.3* de therapietrouw werd bepaald van nieuwe statinegebruikers, waarvan de helft het farmaceutisch patiëntenzorgprogramma volgde. Op basis van deze studies werd geconcludeerd dat patiënten die functionele voedingsmiddelen gebruiken geen verlaagde therapietrouw aan statines hebben, mits zij goed geïnformeerd zijn over de voordelen van een goede therapietrouw.

Hoofdstuk 4 gaat in op de kosteneffectiviteit van functionele voedingsmiddelen. Een kosteneffectiviteitsanalyse is een methodiek waarin de effecten van een behandeling worden afgezet tegen de kosten. Een behandeling is kosteneffectief wanneer de verhouding tussen de kosten van de behandeling en de gezondheidswinst in gewonnen levensjaren lager (gunstiger) is dan een vooraf vastgestelde grenswaarde. Inzicht hebben in de kosteneffectiviteit van gezondheidsbevorderende maatregelen wordt steeds belangrijker als gevolg van de vergrijzing, de toegenomen levensverwachting en de sterk oplopende zorgkosten.

In *Hoofdstuk 4.1* is de kosteneffectiviteit van functionele voedingsmiddelen met toegevoegde plantensterolen/-stanolen bepaald. In de studie is met computermodellen gesimuleerd dat personen, aan wie het gebruik van voedingsmiddelen met toegevoegde plantensterolen/-stanolen wordt aangeraden, deze ook daadwerkelijk gaan gebruiken. Dit zijn personen met een 10-jaarsrisico op sterfte door hart- en vaatziekten van 5 procent of meer (zowel gebruikers als niet-gebruikers van

statines). Uit de studie blijkt dat functionele voedingsmiddelen met plantensterolen/-stanolen niet kosteneffectief zijn.

In **Hoofdstuk 5** zijn de belangrijkste bevindingen van dit proefschrift samengevat en in een bredere context geplaatst. Tot slot worden conclusies over de klinische relevantie en aanbevelingen voor toekomstig onderzoek geformuleerd.

Concluderend kan gesteld worden dat het gebruik van functionele voedingsmiddelen en voedingssupplementen als toevoeging aan statines, de therapie zowel op een positieve als negatieve wijze kan beïnvloeden. Statines werken mogelijk minder goed bij personen die functionele voedingsmiddelen of voedingssupplementen met de oplosbare voedingsvezel β-glucan gebruiken. Het onderzoek beschreven in dit proefschrift geeft aanleiding tot nader onderzoek, zodat er meer inzicht kan worden verkregen in de mechanismen en factoren (o.a. dosis, moment van inname) die bij deze fysiologische interactie een rol spelen. Functionele voedingsmiddelen of voedingssupplementen met toegevoegde omega-3 vetzuren of plantensterolen/-stanolen kunnen een aanvulling zijn op de statinetherapie, omdat het cholesterol- en triglycerideniveau extra verlaagd wordt door het gebruik van deze voedingsproducten. De waargenomen effecten hangen echter af van het uitgangsrisico en de therapietrouw van de gebruiker.

De huisarts en apotheker spelen een belangrijke rol bij het verlenen van informatie over mogelijke interacties tussen voeding en medicijnen en bij het benadrukken van het belang van een goede therapietrouw aan geneesmiddelen naast het gebruik van functionele voedingsmiddelen of voedingssupplementen. Om in de toekomst huisartsen en apothekers van informatie te voorzien over interacties tussen voeding en geneesmiddelen kan een zogenaamd 'Post-Launch Monitoring'-systeem waardevol zijn. Met een dergelijk systeem kunnen interacties tussen functionele voedingsmiddelen of voedingssupplementen en geneesmiddelen worden gevolgd nadat de voedingsmiddelen op de markt zijn gekomen. Hiervoor is onderzoek nodig naar nieuwe mogelijkheden voor het koppelen van inname-gegevens van functionele voedingsmiddelen en voedingssupplementen aan medicatie aflevergegevens.

Chapter 7

List of co-authors

List of publications

Dankwoord

About the author

List of co-authors presented in this thesis

Kristina E Andersson
Department of Experimental Medical Science, Unit of Vascular Physiology, Lund University, Lund, Sweden

Anthonius de Boer
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Marcel L Bouvy
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Jan CH van Eijkeren
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Menno E van der Elst
Medicines Evaluation Board, The Hague, The Netherlands

Talitha L Feenstra
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands;
Department of Epidemiology, University Medical Centre Groningen, Groningen, The Netherlands

Johan Garssen
Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands;
Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands

Johanna M Geleijnse
Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

Johan J de Gier
Department of Pharmacotherapy and Pharmaceutical Care, University of Groningen, Groningen, The Netherlands

Erik J Giltay
Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands

Per Hellstrand
Department of Experimental Medical Science, Unit of Vascular Physiology, Lund University, Lund, Sweden

Jeljer Hoekstra
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Nynke de Jong
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Olaf H Klungel
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Henk J van Kranen
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Daan Kromhout
Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

Henk van Loveren
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, The Netherlands

Aukje K Mantel-Teeuwisse
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Marco H Oosterveld
Waldeck Pharmacy, The Hague, The Netherlands

Rickard Öste
Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden; Aventure AB, Lund, Sweden

Cathy JM Rompelberg
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Ido B Toxopeus
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Hans Verhagen
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

WM Monique Verschuren
National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

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Vademecum Huisartsen 2010

Dankwoord

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About the author

Simone Eussen was born on 12 December 1982 in Maastricht, the Netherlands. She grew up in Mheer and completed secondary school (VWO) at the 'Scholengemeenschap Sophianum' in Gulpen. In September 2001, she started her studies in Biomedical Sciences at the Radboud University of Nijmegen. In 2007, she obtained her Master's degrees in Toxicology and Epidemiology with specialisations in Nutrition and Public Health. During her studies, she completed a research traineeship at the Department of Epidemiology, Maastricht University, the Netherlands as well as traineeships in Dar es Salaam, Tanzania and at TNO Quality of Life, Zeist, the Netherlands.

In October 2007, she started the PhD research described in this thesis at the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht Institute for Pharmaceutical Sciences, the Netherlands in collaboration with the Division of Pharmacology of Utrecht Institute for Pharmaceutical Sciences and the National Institute for Public Health and the Environment in Bilthoven, the Netherlands. During this work, she obtained a Postgraduate Degree in Epidemiology as well as in Toxicology and in Nutrition.

From August 2011, she holds a position as a Nutrition Scientist at Danone Research, Centre for Specialised Nutrition, Wageningen, the Netherlands.