# Danacol® Monograph

2009

To help hypercholesterolemia management



DDB Danone Health - Danacol - December 2008.

# Table of contents

# Glossary

### Atherogenic disease
Cf. Atherosclerosis, Dyslipidemia, Triglycerides (Hypertriglyceridemia).

### Atherosclerosis
Disease caused by narrowed blood vessels due to fat deposits in arterial walls. Large deposits can block an artery thus inhibiting blood flow.

### Cardiovascular diseases
Pathologies associated with a dysfunction of the heart and the arteries supplying major organs (brain, heart, etc).

### Cardiovascular network
Key components are the pump, the heart and associated blood vessels: arteries, veins and capillaries.

### Carotenoids
Carotenoids are natural fat-soluble pigments found in certain plants. Carotenoids provide the bright red, orange, or yellow coloration of many vegetables, serve as antioxidants, and can be a source for vitamin A activity.

### Cholesterol
Cholesterol is a sterol and a lipid. It is a waxy substance carried in the blood stream that is also present in our cells. Cholesterol is crucial for cell function and hormone synthesis however, an excess of cholesterol is associated with an increased risk for cardiovascular disease.

### Dyslipidemia
Pathologies characterised by an excess of lipids, mainly cholesterol and triglycerides, in the blood stream. The main risk factor for cardiovascular disease.

### Lipoproteins
Spherical structures composed of fat and protein that transport fat in the blood stream. Lipoproteins resemble fat globules and act as carriers for different hydrophobic molecules like vitamins.

**HDL** - High Density Lipoproteins are responsible for the removal of excess cholesterol from the arteries and tissues. They are also referred to as the 'good' fraction of cholesterol.

**LDL** - Low Density Lipoproteins are responsible for the deposition of cholesterol on arterial walls and in tissues, and for the transport of cholesterol from the liver to the cells/tissues. They are also referred to as the 'bad' fraction of cholesterol.

### Risk for cardiovascular disease
Pathologies or life styles that could result in cardiovascular disease.

### Triglycerides
Triglycerides are molecules of glycerol with three fatty acids and are found in food and in the body, particularly in the plasma. Hypertriglyceridemia is an atherogenic disease.



# Introduction

Danacol® is a low-fat fermented milk product enriched with plant sterols, developed through research and expertise at Danone Research, Centre Daniel Carasso. The plant sterols used in Danacol® are naturally occurring substances found in low quantities in plants.

Enrichment with plant sterols of foods such as spreads, milk and in particular fermented milk like Danacol®, allows moderate consumption levels of plant sterols to be raised, enabling them to confer their clinically demonstrated hypocholesterolemic effects.

Hypercholesterolemia is a major public health issue in developed countries. As demonstrated in clinical trials, Danacol® can be safely and easily incorporated into dietary and lifestyle measures advised for hypercholesterolemic patients. Furthermore, the efficacy and health benefit of Danacol® have been officially recognised by the European Food Safety Authority (EFSA), who recently published a claim stating "phytosterols have been shown to lower/reduce blood cholesterol. High blood cholesterol is a risk factor in the development of coronary heart disease."

As Danacol® is a dairy-based food with health properties it can be called a functional food and cannot be compared to any drug. It is only for people who need to lower cholesterol levels in combination with a healthy diet and lifestyle. Functional foods like plant sterol-enriched foods are recommended for hypercholesterolemic patients by both European and US guidelines (e.g. European Society of Cardiology, Afssaps, NIH, International Atherosclerosis Society, American Heart Association).

It is advised that these foods be combined with low cholesterol and low fat dietary intakes as part of a balanced and varied diet rich in fruit and vegetables, together with moderate daily exercise. Consumers should always seek medical advice and dietary tips from their doctor.

Danacol® allows a reduction of 'bad' LDL-cholesterol without reducing the 'good' HDL-cholesterol. Medical advice should be given especially for individuals with cholesterol-lowering medication[78].

# 1. Cholesterol and hypercholesterolemia

# 1. Cholesterol and hypercholesterolemia

## 1.1 Cholesterol: sources and functions

Plasma cholesterol arises from two sources, endogenous cholesterol – synthesised in the liver – and exogenous cholesterol – from dietary intake. Typically, the liver accounts for approximately two-thirds of total cholesterol (~900 mg/day) and the diet accounts for the remaining one-third (~300 mg/day).

Cholesterol is transported through the body by lipoproteins. There are three main classes of lipoproteins, which differ according to their density and usually exist in the following proportions *(Figure 1)*:

**HDL** – High Density Lipoprotein (26%)

**LDL** – Low Density Lipoprotein (57%)

**VLDL** – Very Low Density Lipoprotein (17%)

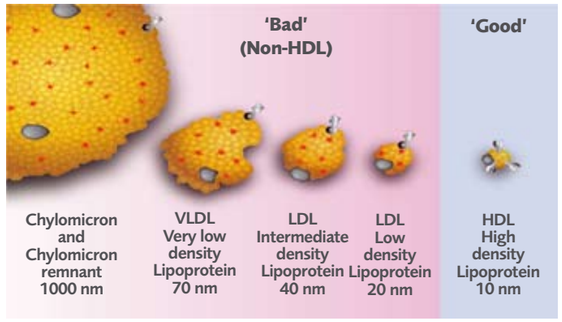



***Figure 1:** Classification of lipoproteins*

*A schematic of the main classes of lipoprotein*

In normal circumstances the total amount cholesterol remains constant because of feedback control: when dietary intake is high, synthesis of cholesterol by the liver is reduced and similarly when dietary intake is low, synthesis by the liver is increased.

Cholesterol is found in the tissues of all vertebrates and functions as a structural component of cell membranes. The main organs containing cholesterol are the brain, the reproductive organs and the liver. Cholesterol is necessary for the production of steroid hormones and forms the basis of corticoids such as cortisol and aldosterone in the adrenals.

Absorption of food, in particular fats, occurs primarily in the small intestine as this is the first site of contact with bile. Bile is produced in the liver, stored in the gall bladder and released into the small intestine *(Figure 2)*. It is composed of bile salts, bile pigments, cholesterol, phospholipids and electrolytes. Bile salts are cholesterol derivatives whose role is to facilitate fat and cholesterol absorption.

Monoglycerides and free fatty acids (FFA), that breakdown the products of fat digestion and cholesterol, are insoluble compounds. Therefore, they require bile salts to form lipid droplets called micelles that can be absorbed on the surface of the intestinal cells (enterocytes). Once in contact with the enterocytes, the FFA, monoglycerides and cholesterol diffuse from the micelles across the membrane to the enterocytes. A portion of cholesterol is immediately pumped back out of the enterocytes into the intestinal lumen.

Inside the enterocytes, the FFA and monoglycerides reform triglycerides and combine with cholesterol, phospholipids and FFA to form chylomicrons, the largest lipoproteins that primarily transport triglyceride fats and cholesterol from the intestinal mucosa.

After processing within the enterocyte, the chylomicrons enter the systemic circulation, where they are hydrolysed into FFA and glycerol, which are used by cells for energy or stored as fat. The residual chylomicrons return to the liver where they release triglycerides and a proportion of cholesterol that are first converted into Very Low Density Lipoproteins (VLDL) and then into Low Density Lipoproteins (LDL).

The LDLs carry triglycerides and cholesterol esters and deposit them in cells and different peripheral tissues. As LDL and VLDL have the ability to transport lipids to arteries, they represent the major contributors to the formation of atheromatous plaques. In contrast, the High Density Lipoproteins (HDL) transport cholesterol back to the liver for excretion, as it is the main organ able to eliminate the excess cholesterol within bile in its native form or as bile acids.

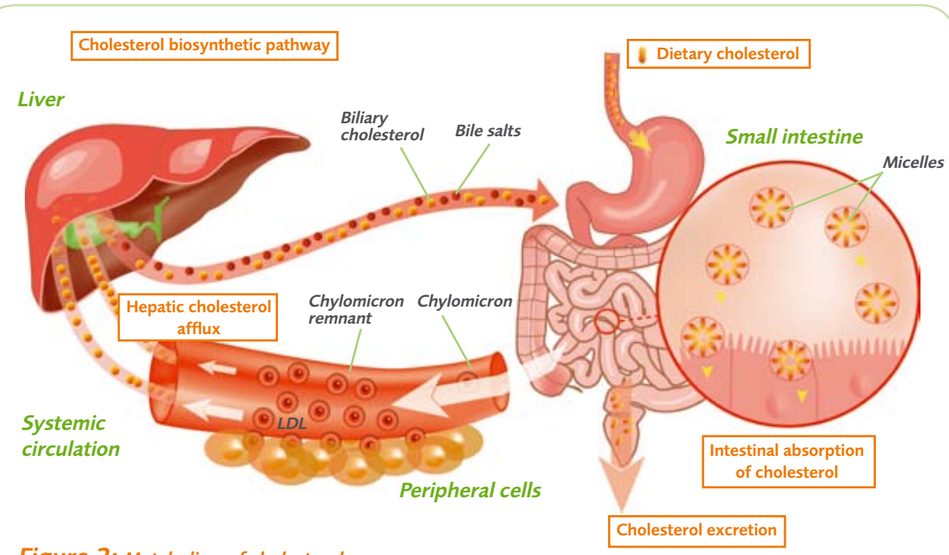



***Figure 2:** Metabolism of cholesterol*

*Endogenous cholesterol, synthesised in the liver, is released into the small intestine in the form of bile and assists with digestion. Exogenous or dietary cholesterol and emulsified fats along with the bile salts form micelles. Micelles interact with the enterocyte apical membrane, and their contents are transferred into the enterocyte. The cholesterol, fatty acids and monoglycerides are transferred to the enterocyte, esterified, and packaged into chylomicrons and transported via the systemic circulation. A portion of cholesterol and fats are released back into the intestinal lumen and eliminated with faeces. Cholesterol and fats that have been absorbed by the epithelial cells of the small intestine are further processed into chylomicrons before entering the systemic circulation and ultimately returning to the liver. In healthy individuals, absorption of cholesterol is limited by the membrane ABC (ATP-binding) transporters, which actively pump it from the enterocytes into the intestinal lumen. These same transporters also eliminate cholesterol in the liver by enhancing their excretion into bile. Unabsorbed cholesterol is eliminated with faecal waste.*

# 1. Cholesterol and hypercholesterolemia

## 1.2 Why do we care about hypercholesterolemia?

Hypercholesterolemia has been identified as a major cardiovascular risk factor on the basis of numerous epidemiological studies [5,24,110].

A relationship between diet and coronary heart disease was observed as early as the 1950's and 1960's, and in the 1970's and 1980's, hypercholesterolemia was identified as a major cardiovascular risk factor.

Cardiovascular disease (CVD) is a leading cause of mortality and is responsible for approximately one-third of deaths globally *(Table 1)*.

Today, as a result of these epidemiological studies, there is an increased awareness, both at national and individual levels, regarding the potentially harmful effects of hypercholesterolemia *(Figure 3)* and plasma lipid concentrations *(Figure 4)*.

**Cardiovascular mortality rates [101]:**

- **17 million deaths** per year (1/3 of all deaths)
- Cardiovascular mortality is mainly due to:
  - Ischaemic heart disease (**43%** of deaths)
  - Stroke (**33%** of deaths)

***Table 1:***
***Cardiovascular disease in the world***

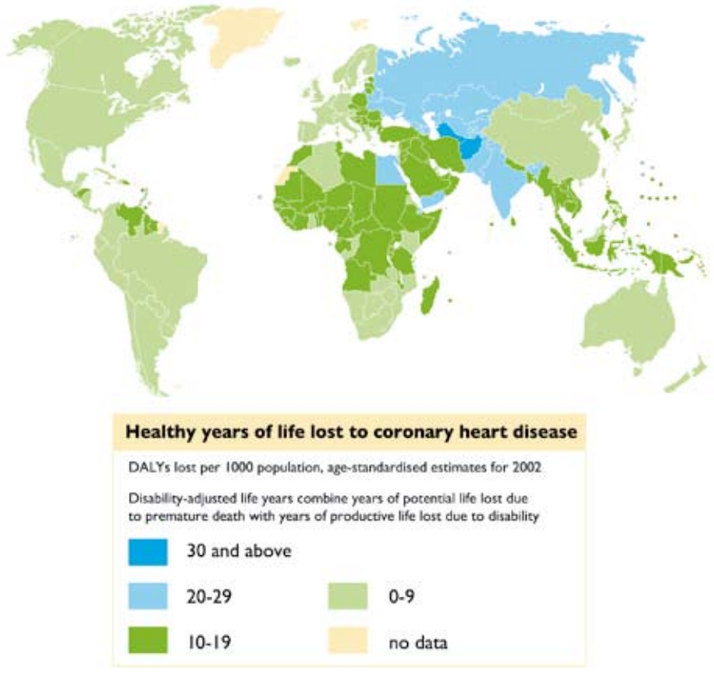



***Figure 3:** Global burden of coronary heart disease [67]*

*Disability-adjusted life years (DALYs) lost can be thought of as "healthy years of life lost." They indicate the total burden of a disease, as opposed to simply the resulting deaths. Cardiovascular disease is responsible for 10% of DALYs lost in low- and middle-income countries and 18% in high-income countries.*

*Coronary heart disease is decreasing in many developed countries, but is increasing in developing and transitional countries, partly as a result of increasing longevity, urbanisation, and lifestyle changes.*

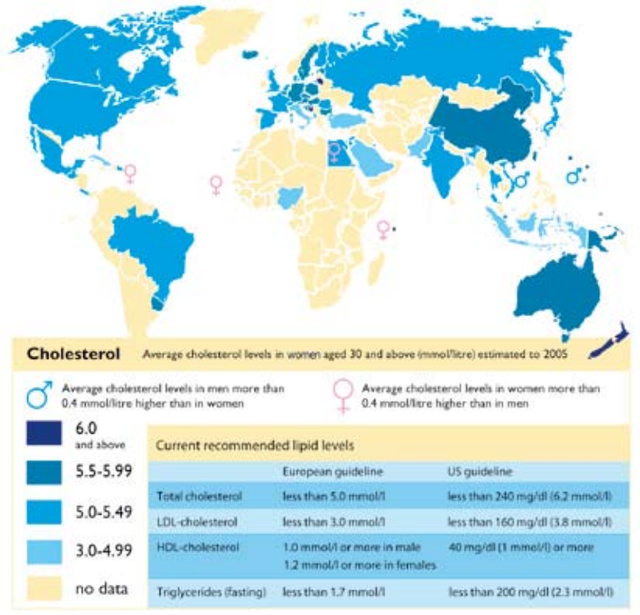



***Figure 4:** Geographic repartition of lipid plasma concentrations (at international level) [67]*

*High levels of LDL-cholesterol (LDL-C), and other abnormal lipids (fats), are risk factors for cardiovascular disease. Indeed, a high level of LDL-C can lead to clogging of the arteries, increasing the risk of heart attack and ischaemic stroke, while HDL-C reduces the risk of coronary heart disease and stroke. The female hormone oestrogen tends to raise HDL-C levels, which may explain why pre-menopausal women are relatively protected from developing coronary heart disease.*

### Dyslipidemia in Europe [3]

In Europe, most of the adult population have above-average total cholesterol (TC) levels. The world-renowned WHO MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) project provided estimates of mean TC for the European region for 2002. Among men aged 15 years or over, data suggest a mean TC between 4.5 mmol/l and 6.2 mmol/l (normal range being <5.0 mmol/l). Also, among women of the same age group, WHO data suggest the mean TC range to be between 4.6 mmol/l and 6.1 mmol/l.

### Dyslipidemia in the United States [13]

Elevated serum TC is a major and modifiable risk factor for heart disease, the leading cause of death in the United States.

Results from the the National Health and Nutrition Examination Survey (NHANES) have shown that between 1999 and 2002, the age-adjusted mean serum cholesterol level among adults aged 20-74 years was 203 mg/dl.

This survey also showed that the percentage of US adults over the recommended TC level of <240 mg/dl* was 17%. Reducing mean serum TC levels among adults to <200 mg/dl and reducing the proportion who have levels ≥ 240 mg/dl to less than 17% are part of the US Healthy People 2010 objectives.

** Recommended levels may vary between countries. Check your local guidelines.*

## 1.3 LDL-cholesterol: the primary target of therapy

Several studies including the Framingham study, Multiple Risk Factor Intervention Trial (MRFIT) and the Parisian Prospective study have helped to demonstrate the role of LDL-C in the pathogenesis of atherosclerosis. LDL-C has come to be considered as the most important parameter in testing for hypercholesterolemia, however HDL-C also provides additional predictive information [5,24,78,110]. The risk of CVD for each individual is determined by the degree and extent of the various risk factors of this multifactor disease, as shown by the INTERHEART case-study, the largest cardiovascular epidemiological study in the world. The study demonstrated that 90% of first heart attacks can be attributed to nine specific risk factors, both modifiable and non-modifiable. In addition to this, INTERHEART also identified that the impact these risk factors have is consistent among both men and women across different regions, and by ethnic group. Studies such as this show a clear need for a uniform preventative strategy against CVD across the world [123]. These risk factors include non-modifiable criteria such as age, gender (males are more prone to CVD), family history, dyslipidemia, diabetes and hypertension, as well as modifiable criteria such as smoking, obesity and sedentary lifestyle *(Tables 2 and 3)*.

***Table 2:***

**Prevalence of cardiovascular risk factors in developed countries**

- Smoking: 12.2% of population
- Hypercholesterolemia: 7.6%
- Hypertension: 10.9%
- Obesity: 7.4%
- Alcohol consumption: 9.2%
- Physical inactivity: 3.3%

*The Atlas of Heart Disease and Stroke,WHO and CDC, Mackay, J and Mensah, G.A. [67]*

***Table 3:***

**Prevalence of cardiovascular risk factors in France**

- Smoking: 34% of the population
- Hypercholesterolemia: from 20 to 30% of the population*,**
- Hypertension: 16.5% of the population aged over 20 years
- Obesity: 10%
- Diabetes: 3.1%

**Programme National de Réduction des Risques Cardiovasculaires 2002-2005*
*** [101]*

The risk of coronary artery disease is even higher when several of these risk factors co-exist *(Tables 4)* [8]. Indeed, this table shows that several risk factors increase coronary heart disease (CHD) risk in a multiplicative manner. There are several ways for people to assess their CHD risk taking these numerous risk factors into account. The American Heart Association (AHA), for example, provides a risk calculator which can be completed online [2].

| Hypertension* | Diabetes Mellitus† | Hyperlipidemia# | Incidence of CHD (per 1000 subjects in 4 years) |
|---|---|---|---|
| | | | 6 |
| ✔ | | | 14 |
| | ✔ | | 15 |
| ✔ | ✔ | | 48 |
| | | ✔ | 96 |
| ✔ | | ✔ | 114 |
| | ✔ | ✔ | 114 |
| ✔ | ✔ | ✔ | 114 |

***Table 4:***

***Probability of a CHD event occurring within 4 years according to the number of risk factors present [8]***

** defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥ 95 mmHg.*

*† defined as fasting blood glucose >130 mg/dl or known diabetes mellitus.*

*# defined as (1) total cholesterol ≥ 300 mg/dl or (2) HDL-C <35 mg/dl plus total cholesterol between 200 and 300 mg/dl and/or triglycerides ≥ 200 mg/dl.*

## Hypercholesterolemia and other risk factors

It is therefore noteworthy that elevated cholesterol concentrations are more prevalent amongst adults with **type 2 diabetes** than in subjects with normal glucose tolerance. For example the mean LDL-cholesterol level is 151 mg/dl among American adults aged 20-74 years with type 2 diabetes and 135 mg/dl among those without [19].

The prevalence of abnormal (≥160 mg/dl) LDL-cholesterol levels is 30.9% among diabetic men and 43.8 % among diabetic women *(Figure 5)*. Similar percentages are observed for total cholesterol ≥240 mg/dl [19].

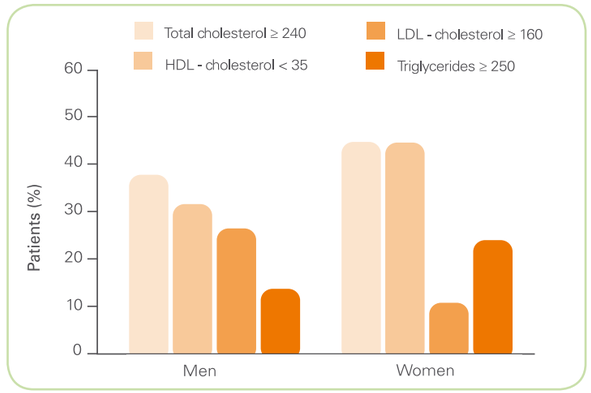



***Figure 5:*** *Percentage of diabetic persons aged 20 to 74 years with abnormal lipid concentrations (mg/dl)*
*US 1976-1980 [19]*

Similarly, there is a notable correlation between the prevalence of hyperlipidemia and **hypertension**. In the United States, an estimated 30 million Americans are diagnosed with both risk factors, equating to 15% of the US population. Moreover, 62% of patients suffering from hypertension are known to have dyslipidemia, and 44% of patients suffering from dyslipidemia suffer from hypertension *(Figure 6)* [6].

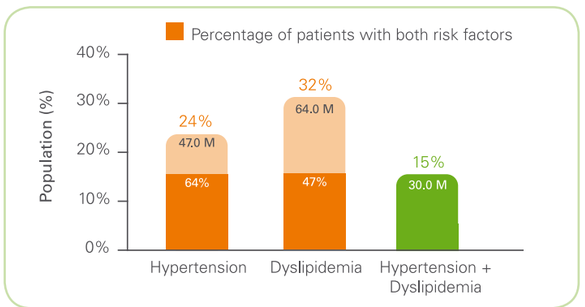



***Figure 6:*** *Concurrent hypertension and dyslipidemia in the US [6]*

Lipoprotein levels also vary significantly with **age and gender**. In the US more than 25% of women aged 20-74 have approximately 10 mg/dl higher values of total cholesterol than those considered normal for men. Furthermore, studies have shown that LDL-C serum levels increase progressively as women age and go through the menopause, so that by the time they reach their 50s, their average LDL-C concentrations are higher than those of men the same age. The National Cholesterol Education Program (NCEP) guidelines was the first instance of serum lipid recommendations with respect to gender. The report noted that being a women over 55 years of age was a risk factor in itself for coronary artery disease [64].

# 1. Cholesterol and hypercholesterolemia

Various nutritional factors are involved in protection against atherosclerosis, namely lipids, carbohydrates, fibre, proteins, minerals and vitamins. Current recommendations are based upon a reduction in cholesterol, saturated fatty acids (SFA) and total lipids in the diet and an increase in consumption of complex carbohydrates and fibre. LDL-C excess is a modifiable cardiovascular risk factor for which proof of the efficacy of therapeutic management has been clearly documented. Evidence has been provided by many morbidity and mortality studies concerning both dietary and drug management in primary prevention, as well as secondary prevention of hypercholesterolemia [5,78,109,110] and essential hypertension [27,50].

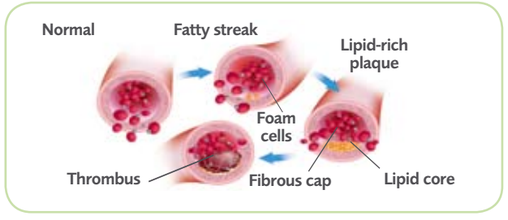
## Genesis and evolution of the atherosclerotic plaque

Atherosclerosis is the underlying cause of most CVD. It is developed from low-density lipoprotein molecules becoming oxidised (LDL-ox) by free radicals [42]. **An atherosclerotic plaque takes years to form before manifesting as acute myocardial infarction (MI), stroke or angina pectoris.** The fatty streak is the earliest identifiable morphological change during plaque formation. As progression continues, pools of extracellular lipids disrupt the intimal lining of the vessel eventually converging to form a clearly defined lipid core. Simultaneously, a secretory phenotype develops in the surrounding smooth muscle cells, resulting in stimulated synthesis and decreased reabsorption of collagen, the product of which is a collagen-rich fibrous cap. Lesions with a fibrous cap are characteristic of advanced atherosclerosis. These lesions may expose the underlying thrombotic surface to flowing blood, leading to the formation of thrombus which may result in the clinically recognised acute coronary syndromes *(Table 5 and Figure 7)* [92,111].

***Table 5:*** *Projected prevalence of atherosclerotic cardiovascular disease*

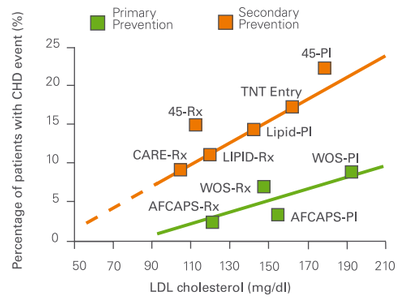
| Atherosclerotic morbidity [93]: |
|---|
| According to the Framingham Heart Study cohort, 67% of men and 59% of women, aged 40 years of over, will develop atherosclerotic cardiovascular disease. |

***Figure 7****: Stages of atherosclerotic plaque development [103]*




## Overview of primary and secondary prevention trials

The fact that lowering LDL-C is of major importance in lowering the risk of developing coronary artery disease (CAD) is now an accepted principle in medicine. Indeed, numerous primary and secondary preventative trials have shown that cholesterol-lowering therapies lead to a significant reduction in the morbidity and mortality associated with CAD. *Figure 8* demonstrates this linear relationship [54] showing: **the lower the better.** These findings, amongst others, have led to the revision of certain guidelines that now suggest LDL-C should be reduced to below 100 mg/dl, or even as low as 70 mg/dl in patients at particularly high risk for coronary heart disease (CHD) [12].




## "The lower the better"

***Figure 8:*** *Primary and secondary prevention trials [54]*

- *CARE: Cholesterol And Recurrent Events*
- *4S: Scandinavian Simvastatin Survival Study*
- *AFCAPS/TexCAPS: AirForceTEXasCoronary-Atherosclerosis Prevention Study*
- *WOS: West Of Scotland Coronary Prevention Study*
- *Rx: Statin treated*
- *PI: Placebo treated*

## 1.4 Hypercholesterolemia and cardiovascular disease: a major public health issue

Risk for CVD remains high despite increased knowledge concerning contributing factors and the existence of clear therapeutic guidelines for the prevention of atherogenesis.

Prevalence of risk factors is already high and constantly rising. Notable increases in infantile obesity [25], trends towards more sedentary lifestyles, bad eating habits and ageing of the population have been observed as well as potential and probable increases in the number of sufferers of cardiovascular disease. This has already been observed in relation to type 2 diabetes [57].

Public authorities have responded to these concerns by implementing national initiatives such as anti-smoking campaigns, national nutrition and health programmes and recommendations for the reduction of risk of CVD [50,122].

These programmes have been introduced in response to the need for widespread information for the population. They aim to instill the responsibility with the individual, and to encourage people to actively take care of their health.

France, for example, has established the French National Nutrition and Health Programme (PNNS) which was initially planned for 2001-2005 and has now been extended to 2010. The aim of this programme is to focus on nine main objectives related to major determinants of health status: diet, physical activity and nutrition.

National initiatives, such as the PNNS, require the involvement of multiple disciplines to work effectively, namely, stakeholders from ministries, research and educational institutions, the food industry, healthcare, and consumers. The introduction of the PNNS has already led to the accomplishment of a large percentage of the public health objectives, which specifically for cholesterol is a 5% reduction in cholesterolemia in the general adult population [97].

# 2. Hypercholesterolemia and dietary measures

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## 2.1 The role of dietary management

Current national and international recommendations for the management of hypercholesterolemia, such as those of the National Institute of Health and Afssaps (*Agence française de sécurité sanitaire des produits de santé* or French agency for the sanitary safety of health products), emphasise the importance of dietary management *(Table 6)*[1,77].

The French guidelines state that if closely respected, these dietary measures can circumvent the need to initiate drug treatment in many cases[1,77]. They also stress the need for both the prescriber and the patient to be sufficiently motivated.

***Table 6:*** *Recommended therapeutic lifestyle changes for hypercholesterolemic subjects*

| National Institute of Health (NIH)[77] | Afssaps[1] |
|---|---|
| "ATP II recommends a multifaceted lifestyle approach to reduce risk for CHD. This approach is designated as therapeutic lifestyle changes (TLC). Its essential features are:<br>• Reduced intakes of saturated fats (<7% of total calories) and cholesterol (<200 mg per day)<br>• Therapeutic options for enhancing LDL lowering such as plant stanols/sterols (2 g/day) and increased viscous (soluble) fiber (10-25 g/day)<br>• Weight reduction<br>• Increased physical activity" | "Dietary measures for the nutritional management of dyslipidemic patients can be divided into 4 categories:<br>1. Limited intake of saturated fatty acids (fats of animal origin), in favour of mono- or polyunsaturated fatty acids<br>2. Increased consumption of omega-3 polyunsaturated fatty acids (fish)<br>3. Increased consumption of fibre and natural micronutrients (fruits, vegetables and cereal products)<br>4. Limited dietary cholesterol, or use of products enriched with plant sterols" |

Reduction of the intake of SFA and cholesterol should be encouraged when LDL-C levels exceed 1.6 g/l (in general population) or 1.3 g/l (for individuals with more than 2 other risk factors or as secondary prevention). After 3 months of dietary management, if high LDL-C levels persist then hypocholesterolemic medication is required.

There are two different types of hypocholesterolemic medication with different modes of action: statins, which inhibit endogenous synthesis of cholesterol and Ezetimibe, which inhibits cholesterol absorption in the intestine. According to hypercholesterolemia management guidelines, dietary advice should always be dispensed together with drug treatment, and efficacy of treatment should be reinforced by lifestyle changes. It is also important that therapeutic lifestyle changes, such as eating a low cholesterol diet and doing regular exercise, are continued in the long-term in order to maintain cholesterol levels.

Current nutritional recommendations for hypercholesterolemia are supported by extensive proof of the effect of diet on the risk of CVD *(Table 7)*. In particular, the combination of several dietary modifications can reduce LDL-C levels to an extent equivalent to the use of standard doses of statins[78]. A sizable number of clinical trials have been carried out to test whether lowering serum cholesterol levels with dietary modification reduces risk for CVD. A meta-analysis of dietary trials revealed that the lowering of serum cholesterol via dietary measures resulted in a reduction in the risk of CVD commensurate with the degree of cholesterol lowering[77].

***Table 7:*** *Principle of hypocholesterolemic diets recommended by the World Health Organisation†*

| Dietary factors | Goal *(% of total energy, unless otherwise stated)* |
|---|---|
| 1. Total Fat | 15-30% |
| - Saturated fatty acids (SFA) | <10% |
| - Polyunsaturated fatty acids (PUFAs) | 6-10% |
| - n-6 Polyunsaturated fatty acids (PUFAs) | 5-8% |
| - n-3 Polyunsaturated fatty acids (PUFAs) | 1-2% |
| - Trans fatty acids | <1% |
| - Monounsaturated fatty acids (MUFAs) | By difference* |
| 2. Cholesterol | <300 mg per day |
| 3. Total carbohydrate | 55-75%** |
| Non-starch polysaccharides (NSP) | >25 g per day of total dietary fibre |

* This is calculated as: total fat – (saturated fatty acids > polyunsaturated fatty acids > trans fatty acids)

** The percentage of total energy available after taking into account that consumed as protein and fat, hence the wide range

† *The World Health Report 2002. Reducing Risks, Promoting Healthy Life, WHO (2002) - Geneva*

# 2. Hypercholesterolemia and dietary measures

## 2.2 Basis for the recommendations

The relationship between the quantity of dietary cholesterol and fatty acids absorbed and the risk of CVD has been demonstrated by several epidemiological studies.

The Keys study [56], involving 7 countries over a period of 15 years, demonstrated a positive correlation between coronary mortality, intake of saturated fats and blood cholesterol levels. The highest mortality rates were seen in Finland, where saturated fatty acid (SFA) intake accounts for at least 25% of the total calorie consumption. The lowest mortality rates were seen in Crete, where SFA intake was of the order of 8%, thus favouring unsaturated fatty acids. This study led to an increased interest in the Cretan diet and consequently in the Mediterranean diet.

Other studies illustrating the negative effects of SFA include the Framingham study [5] and other population-specific studies, all of which demonstrated that for a comparable carbohydrate intake, a 5% increase in SFA consumption resulted in a 17% increase in the risk of CVD. However, an inverse correlation was also observed concerning consumption of monounsaturated fatty acids and polyunsaturated fatty acids and cardiovascular mortality.

The effects of foods rich in cholesterol on LDL-C are variable. Under normal circumstances in individuals with normal cholesterol absorption, blood cholesterol levels vary linearly for a cholesterol intake between 100-300 mg/day with only slight variations for consumption of higher levels [18].

Protective effects against cardiovascular diseases and diabetes [46,66,100] have been exhibited by whole grain cereals (unrefined therefore containing all fibre, minerals and vitamins). In addition, data exist showing the benefits of a diet enriched in fruit and vegetables [10,80], which provide vitamin C, carotenoids, polyphenols, vitamin B9, potassium, plant sterols and dietary fibres and oleaginous fruits rich in plant sterols, $\alpha$-linolenic acid, vitamin E and minerals [102].

As oxidation of LDL is important in the physiopathology of atherosclerosis, interest has increased in diets rich in antioxidants such as vitamin E (e.g. cereals and nuts), vitamin C (e.g.oranges), ß-carotene and carotenoids (e.g.carrots), polyphenols (e.g.blueberries), zinc (e.g. meat) and selenium (e.g. fish). More evidence is required, as no studies demonstrating the involvement of antioxidants in the reduction of atherosclerosis exist to date.

In a single phase I study, 66 hyperlipidemic subjects were prescribed diets high in plant sterols (1.0 g/1000 kcal), soy protein (22.5 g/1000 kcal), viscous fibres (10 g/1000 kcal), and almonds (23 g/1000 kcal) for one year. These data were compared with published results on 29 of the participants who had also undergone separate one-month metabolic trials of a diet and a statin. Participants who ate a dietary portfolio of cholesterol-lowering foods under real-world conditions were able to lower LDL-C concentrations by >20%, which was not significantly different from their response to a first-generation statin taken under metabolically-controlled conditions [49].



# 3. Plant sterols

# 3. Plant sterols

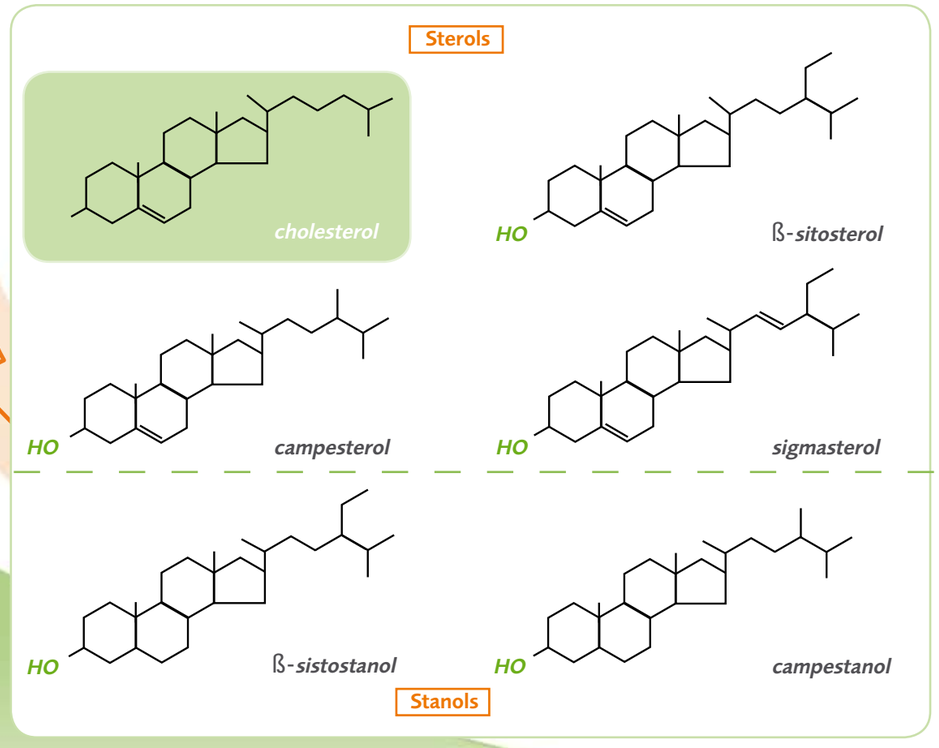
## 3.1 What are they?

Plant sterols/stanols (or phytosterols) are natural components of plant cells. They perform biological functions in plant cells similar to those of cholesterol in mammalian cells: maintaining the structure and function of cells' membrane. Plant sterols and stanols exhibit chemical structural similarities to cholesterol (tetracyclic ring common to sterols). However they also possess substitutions in their side chain consisting of an additional methyl or ethyl group or an additional double bond, which results in their poor intestinal absorption compared to cholesterol. *Figure 9* shows the most well-known plant sterols: sitosterol, campesterol and stigmasterol and stanols.

The structure of the nucleus may also be saturated, as in the stanol subgroup, whose main members are sitostanol and campestanol. Plant stanols occur in smaller quantities than plant sterols and are found in only trace amounts in most plants, with the exception of certain cereals such as rye, wheat, barley and oats [75]. However, plant sterols form essential components of plant cell membranes and are structurally related to cholesterol.

***Figure 9:*** *Comparison of the structures of cholesterol and main plant sterols and stanols*

*Structural similarities exist between cholesterol and both plant sterols and stanols. All sterols possess a tetracyclic nucleus however differences exist in the side chains. In the case of the stanol family, the nucleus is saturated, i.e. they have no double bond in the sterol ring*




## 3.2 Where are they found?

Various plant species have been shown to contain sterols. At least 44 different plant sterols from 7 different classes of plants have been described.

Foods richest in plant sterols are maize and soya oil and certain seeds and nuts such as sesame and almonds *(Table 8)*. Fruit and vegetables contain only very small quantities of sterols (approximately 5-30 mg/100 g) [91,119]. Plant stanols (saturated sterols) also occur naturally in grains and fruit, however at much lower concentrations than plant sterols. Plant stanols are much less abundant than sterols and are found in only trace amounts in most plant systems, with the exception of certain cereals [75]. They are naturally present in the free form, esterified to fatty acid or as steryl glycosides. After years of consuming a diet naturally rich in plant sterols (400-500 mg/d), no adverse events have been reported.

In standard diets, plant sterol intake levels vary between different populations and are dependent upon levels of plant product intake. Western diets contain relatively few plant sterols compared with other diets [89].

The EPIC study showed that in Britain, the mean (SD) intake of total plant sterols is 300 (108) mg/d for men and 293 (100) mg/d for women. Bread and other cereals, vegetables and added fats are the three major food sources representing 18.6 (8.9), 18.4 (8.5) and 17.3 (10.4)% of the total plant sterol intake respectively [59].

No data concerning the sterol content of the standard French diet are available. The Finnish diet contains a mean 300 mg/d of plant sterols, with the main sources being vegetable oils, margarines and cereal products, particularly those containing rye [99]. In Japan, the dietary content of plant sterols is approximately 373 mg/d, comprising 54% ß-sitosterol, 14% campesterol, 10% brassicasterol and 7.5% stigmasterol [40]. The Mexican Indian diet (Tarahumara), which is particularly rich in beans and maize, provides around 400 mg/d of sterols [14]. In the different diets studied, plant stanols consistently account for around 10% of total plant sterol intake.

***Table 8:***
***Plant sterol contents of certain foods***

| | Sterols (mg/100g) |
|---|---|
| **Oils*** | |
| Maize oil | 830 - 2530 |
| Soya oil | 250 - 418 |
| Sunflower oil | 325 - 515 |
| Rapeseed oil | 540 - 880 |
| **Oleaginous seeds and nuts**** | |
| Sesame seeds | 714 |
| Groundnuts | 141 |
| Cashew nuts | 158 |
| Almonds | 143 |
| **Cereals **** | |
| Rice bran | 1325 |
| Maize | 178 |
| Sorghum | 178 |
| Durum wheat | 154 |
| Soft wheat | 89 |
| **Fruit and vegetables**** | |
| Beetroot | 25 |
| Asparagus | 24 |
| Cauliflower | 18 - 24 |
| Onion | 15 |
| Fig | 31 |
| Orange | 24 |
| Apricot | 18 |
| Grapefruit | 17 |

* *From Manuel des corps gras, 1992*
** *From Oka et al. (1973-1974)*

# 3. Plant sterols

*Table 9* shows the quantity of various foods that would need to be consumed on a daily basis to obtain 1.6 g of plant sterols, which corresponds to the amount contained in just one bottle of Danacol®.

| Food | Quantity required to provide 1.6 g of plant sterols |
|---|---|
| Beetroot, | 6.4 kg |
| Asparagus | 6.7 kg |
| Cauliflower | 6.7 - 8.9 kg |
| Onion | 10.7 kg |
| Fig | 5.2 kg |
| Orange | 6.7 kg |
| Apricot | 8.9 kg |
| Grapefruit | 9.4 kg |
| Sesame seeds | 220 g |
| Almonds | 1.1 kg |
| Cashews | 1 kg |
| Wholemeal bread* | 1.9 kg |
| White bread* | 5.5kg |
| Cornflakes* | 6.2 kg |
| Rice (cooked)* | 5.2 kg |
| Chocolate (milk)** | 1.7 kg |

***Table 9:*** *Amounts of various foods required to provide an equivalent daily plant sterol intake to 1 serving of Danacol®*

** (86)*
*** (87)*

## 3.3 What is the role of plant sterols in the potential treatment of hypercholesterolemia?

The first paper reporting the cholesterol reducing effects of plant sterols was published in 1953 [96]. Since then, more than 200 articles including around 100 clinical studies and reviews have been published [55,95]. Clinical data consistently show that daily consumption of 1-3 g of plant sterols/stanols in free form reduces blood concentrations of LDL-C by 5-15% without affecting HDL-C or triglycerides to a significant extent [4,16,34,81]. These results have been further supported by the European Food Safety Authority (EFSA) whose scientific opinion panel recommends and confirms the cause-effect relationship between the consumption of plant sterols and the lowering of LDL-C [106].

## 3.4 How do they work?

Plant sterols/stanols are metabolised differently to cholesterol. When consumed in similar quantities to mean dietary cholesterol levels, the rate of absorption of plant sterols is 0.02-3.5% compared to 35-70% for cholesterol [90]. This difference is due to several factors including the side chain substitution in the plant sterols and the lower affinity of plant sterols, within the enterocyte, for the enzyme system required to transport molecules into the cells [28]. **The hypocholesterolemic effect of plant sterols is based on the reduction of intestinal absorption of exogenous cholesterol from the diet as well as endogenous cholesterol originating in the bile and undergoing enterohepatic recycling [7,117]** *(Figure 10)*.

In the intestine, plant sterols are hydrolysed into free plant sterols. One portion associates with cholesterol in soluble particles and is eliminated in faeces while another portion displaces cholesterol from micelles. The similarity of structure and the higher affinity of plant sterols for micelles compared to cholesterol results in a competition for the incorporation into the micelles. Since the micelle capacity is limited, less cholesterol is taken up [7,43,83].

In addition to this mechanism of action, a number of studies suggest that plant sterols increase the activity of proteins responsible for trans-membrane excretion of cholesterol and plant sterols in the intestine and liver. In this way, release of both cholesterol and plant sterols, into the intestinal lumen by enterocytes and into the bile ducts by hepatocytes, is enhanced [55]. In addition, the half-life of plant sterols reaching the blood circulation is relatively short since they are rapidly excreted into bile after their first passage through the liver. **Reduction of intestinal absorption of cholesterol by plant sterols stimulates expression in the liver of LDL receptors [95], thereby enhancing hepatic uptake of circulating LDL and helping to reduce plasma concentrations of LDL-C.**

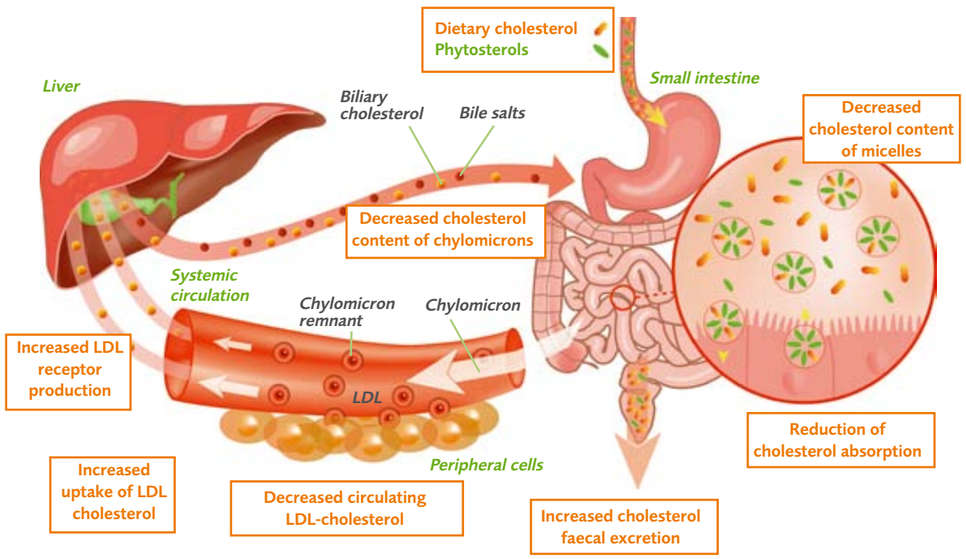



***Figure 10:*** *Action of plant sterols*

*Cholesterol from both endogenous and exogenous sources is absorbed in the small intestine. Plant sterols are preferentially incorporated into micelles, due to their hydrophobic nature, which is essential for absorption by the epithelial cells of the small intestine. Micelles interact with enterocyte apical membranes, and their contents are transferred into the enterocyte. Cholesterol, fatty acids and monoglycerides are transferred to the enterocyte, esterified, and packaged into chylomicrons to be secreted into the lymphatic channels. A portion of cholesterol and fats are released back into the intestinal lumen that are ultimately excreted in the faeces after passage through the gastrointestinal tract. Once inside the epithelial cell, plant sterols are packaged into chylomicrons and enter the systemic circulation and ultimately the liver. In response to reduction of intestinal absorption of cholesterol or depletion of the hepatic cholesterol pool by plant sterols, expression of LDL receptors in the liver is stimulated thereby enhancing hepatic uptake of circulating LDL and helping to reduce plasma concentrations of LDL-C.*

# 4. Clinical studies using phytosterols

# 4. Clinical studies using phytosterols

## 4.1 Overview of clinical experience

The hypocholesterolemic effects of plant sterols have been studied under a wide variety of conditions, including using normolipidemic and hypercholesterolemic adults, with and without cardiovascular disease, hypocholesterolemic medication, type-2 diabetes and in children with familial hypercholesterolemia.

Several different food vectors have been used to deliver plant sterols. Initially, margarine-type products were chosen, as, for technical reasons, they were the only plant sterol-enriched products available. However, oils, salad dressings, milk, yoghurts, meat-based products and cereal-based products were subsequently explored. Clinical trials performed with different products including bread, yoghurt, chocolate and meat-based products showed reductions in serum concentrations of LDL-C and in total cholesterol [22,45,53,72,98,115,109].

Studies performed with non-fatty drinks such as orange juice or drinks enriched with plant sterols demonstrated equivalent reductions in LDL-C levels. Clinical studies performed with low-fat yoghurt-type dairy products enriched with plant sterols showed that the hypocholesterolemic effects were equivalent to the margarine-type vectors e.g. significant reduction in plasma concentrations of total cholesterol and LDL-C with no change in levels of HDL-C or triglycerides [22,41,48,53,84].

Three recent studies, performed on moderately hypercholesterolemic adults, using the low-fat fermented milk product Danacol®, in both spoonable and drinkable forms, demonstrated equivalent reductions of total and LDL-C plasma levels *(see section 5 for details)*.

## 4.2 Plant sterols in hypercholesterolemic adults

Clinical data in both hypercholesterolemic and normolipidemic subjects agree that plasma LDL-C levels can be reduced by 5-15% with daily consumption of 1-3 g of plant sterols/stanols. However, within the dose range of 1.5 to 2 g no rigorous dose-response relationship can be established *(see section 4.4)*. The majority of these data concern European and US populations however similar findings have been revealed in Asian populations [44,69,70].

Most of the studies have been for short periods of time with subjects consuming products for between 6 and 8 weeks. Under these conditions, plasma concentrations of total cholesterol were also significantly reduced while no significant effects on plasma concentrations of HDL-C or triglycerides were observed. The hypocholesterolemic effects of plant sterol-enriched products appear to be independent of the sterol or stanol family [33] *(Table 10)*.

Studies using various vectors to deliver plant sterols such as yoghurt drinks or frying oil containing different amounts of fat demonstrated that irrespective of the fat content of the vector, total plasma and LDL-C were reduced [23,36]. Since small LDL particles are associated with a higher risk of cardiovascular disease, several authors have investigated the impact of plant sterols on their number and size.

They showed that the fat content of the vector did not alter the size of the LDL particles in the blood [15]. In a study [61] combining a low saturated fat diet with plant sterol-enriched products, a reduction of the number of small LDL particles and hence a reduction in risk of cardiovascular disease were observed. Based on literature, the Scientific Committee on Food (SCF) concluded that plant sterol/stanol-enriched products induce the same range of efficacy (5 to 15% of LDL-C reduction) depending on population and on interindividual variability. The variability of the response is independent of the active ingredient [105].

***Table 10:*** *Overview of some recent studies in hypercholesterolemic adults*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Devaraj 2004 [22] | • **Study product: orange juice enriched with sterols**<br>• Dose of phytosterol studied: 2 g/d<br>• Study duration: 8 weeks<br>• Randomised, placebo-controlled study<br>**N=72 mildly hypercholesterolemic healthy subjects** | Reduction in relation to control:<br>TC=-7.2%<br>**LDL-C=-12.4%**<br>Significant differences ($p$<0.01) |
| Hayes 2004 [36] | • **Study product: tortilla chips fried in oil with or without phytosterols (2 x 28 g servings)**<br>• Dose of phytosterol studied: 0 or 1.5 g/d<br>• Study period: 4 weeks<br>• Crossover design<br>**N=7 subjects** | During consumption of the PS-enriched chips, **significant reductions in TC (10%) and LDL-C (15%)** were achieved without affecting HDL-C. |
| Lamarche 2004 [61] | Treatment as part of a diet very low in saturated fat:<br>• **Concurrent incorporation of plant sterols (1 g/4.2 MJ), soyabean protein (23 g/4.2 MJ), viscous fibre (9 g/4.2 MJ) and almonds (15 g/4.2 MJ)**<br>• Study period: 4 weeks<br>**N=12 mildly hypercholesterolemic subjects** | **Reduction in plasma LDL-C of 30%** ($p$<0.0001) attributed to concurrent reductions in the serum TC concentrations of large (>26.0 nm-30, $p$<0.001), medium (25.5-26.0 nm-29, $p$<0.001) and small (<25.5 nm-21, $p$<0.01) LDL particles, with near maximal reductions seen by week 2. |
| Hyun 2005 [41] | Treatment as part of a regular diet:<br>• **Low-fat yoghurt enriched with plant stanol *vs.* control without plant stanols**<br>• Dose of phytosterol studied: 2 g/d<br>• Study period: 4 weeks<br>• Double blind, randomised, placebo-controlled study<br>**N=51 young adults with normo-cholesterolemia and mild hypercholesterolemia** | **Reduction in plasma LDL-C of 30%** ($p$<0.0001) attributed to concurrent reductions in the serum TC concentrations of large (>26.0 nm-30, $p$<0.001), medium (25.5-26.0 nm-29, $p$<0.001) and small (<25.5 nm-21, $p$<0.01) LDL particles, with near maximal reductions seen by week 2. |

# 4. Clinical studies using phytosterols

*Table 10: (cont.)*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Noakes 2005 [84] | Treatment:<br>Two studies:<br>**1) N=39 moderately hypercholesterolemic subjects.** Single blind crossover, placebo-controlled design with 4 phases of 3 weeks treatment: 300ml/d of placebo or PSteE–milk (2.0 g plant sterols/d) alone or combined with 25 g/d of placebo or PsteE–spread.<br>**2) N=40 moderately hypercholesterolemic subjects.** Randomised, double blind crossover, placebo-controlled design with 3 phases of 3 weeks:<br>• 2 portions (150 g tubs each) of placebo, PSteE–yoghurt (1.8 g plant sterols/d) or PStaE–yoghurt (1.7 g plant stanols/d). | Result study 1:<br>**PSteE–milk and PSteE–spread were equally efficacious in lowering total and LDL-C by 6–8% and 8–10% respectively,** *vs.* placebo.<br>No significant additional cholesterol–lowering was observed with the combination of PSteE–milk and PSteE–spread (4 g plant sterols/d).<br><br>Result study 2:<br>PSteE– and PStaE–enriched yoghurts reduced **LDL–cholesterol significantly by 0.27 0.05 mmol/l (6%) and 0.23 0.05 mmol/l (5%), respectively** compared to placebo.<br>No effect on HDL-C triacylglycerol. |
| Charest 2005 [15] | • 3 experimental diets:<br>**- nonfat placebo**<br>**- nonfat with phytosterols (NFPS)**<br>**- low-fat with phytosterols (LFPS)**<br>• Participants consumed 3 beverages daily at meal time<br>• Dose of phytosterol studied 1.8 g/d<br>• Double-blind, randomised, crossover, placebo-controlled study<br>**N=15 moderately hypercholesterolemic subjects** | NFPS and LFPS beverage induced **no significant changes in LDL size phenotype** compared to the control diet. |
| Jauhiainen 2006 [48] | Treatment as part of a regular diet:<br>• **Low fat hard cheese enriched with plant stanols 2 g/d *vs.* control without plant stanols**<br>• Study period: 5 weeks<br>• Double-blind, randomised, placebo-controlled, parallel study<br>**N=67 mildly hypercholesterolemic subjects** | **TC and LDL-C both decreased by 5.8% ($p<0.001$)** and 10.3% ($p<0.001$) respectively in the stanol ester group *vs.* the control group.<br>**No significant changes were observed for HDL-C,** triglycerides or apolipoprotein B concentrations between the groups. |
| Korpela 2006 [60] | Treatment as part of a regular diet:<br>• **Dairy products (yoghurt, low-fat hard and fresh cheese) enriched with plant sterol 2 g/d *vs.* control without plant sterols**<br>• Study period: 3 weeks run-in > 6 weeks<br>• Double-blind, randomised, placebo-controlled, parallel study<br>**N=164 mildly or moderately hypercholesterolemic subjects – Finland** | Serum total cholesterol decreased by 6.5% in the sterol group while no change was observed in the control group ($p<0.0005$).<br>**LDL-C was reduced by 10.4%** in sterol group and by 0.6% in control group ($p<0.00005$).<br>No change observed for HDL-C or triacylglycerol concentrations. |
| Doornbos 2006 [23] | • **100-g Single-dose yoghurt drink enriched with plant sterol *vs.* control**<br>• 3 g of phytosterols/d<br>Two studies:<br>1) (i) drink A (0.1% dairy fat, 2.2% total fat) with a meal, (ii) drink A without a meal<br>2) (iii) drink B (1.5% dairy fat, 3.3% total fat) with a meal, (iv) drink B without a mealand (v) control drink with a meal<br>• Study period: 4 weeks run-in and 4 weeks intervention period<br>• Double-blind, randomised, placebo-controlled, parallel study<br>**N=184 moderate hypercholesterolemic subjects (>57 y.old)** | When yoghurt-drink taken with a meal, **LDL-C was significantly reduced by:**<br>9.5%, $p<0.001$ (drink A i)<br>9.3%, $p<0.001$ (drink B iii)<br><br>When yoghurt-drink taken without a meal, **smaller effect but LDL-C was still reduced by:**<br>5.1%, $p<0.05$ (drink A ii)<br>6.9%, $p<0.01$ (drink B iv)<br>All *vs.* control (v) |

## 4.3 Studies performed in combination with hypocholesterolemic medication

Clinical trials with hypercholesterolemic patients being treated with hypocholesterolemic drugs of the statin or fibrate families have demonstrated that drug treatment combined with consumption of foods enriched with phytosterols results in a valuable additional reduction in plasma concentrations of total cholesterol and LDL-C *(Table 11)* [9,30,32,47,73,76,79,82,108,118].

In particular, two recent studies were performed with a diet providing a daily dose of phytosterols of between 1 and 3 g, as recommended by the SCF.

- In the first study, 11 hypercholesterolemic coronary patients on a low-fat, low-cholesterol baseline diet added simvastatin for 3 months, and then dietary plant stanol ester-containing margarine (2.25 g stanols/d) for 8 weeks, followed by cholestyramine 8 g/d for a further 8 weeks [30]. The hypocholesterolemic drug was found to reduce LDL-C by 39% ($p<0.001$) and the stanol ester-containing margarine by an additional 13% ($p<0.05$), whereas treatment with all three hypocholesterolemic treatments reduced LDL-C by 67% compared to baseline and increased HDL-cholesterol by 15% ($p<0.01$). Serum lathosterol/cholesterol ratio was increased indicating an increase in cholesterol synthesis. The combination of stanol esters with moderate doses of statin and resin appears to effectively control LDL-C levels in hypercholesterolemic subjects.
- The second study was an interventional study with plant sterols and was performed in more than 150 patients of whom 75 were undergoing treatment with cerivastatin [108]. When the drug treatment was combined with 'control' margarine, a significant reduction (32%) in LDL-C was observed ($p<0.0001$) and consumption of margarine enriched with esterified plant sterol alone resulted in an 8% decrease in LDL-C ($p<0.0001$). In subjects receiving both the statin treatment and the margarine enriched with sterols, a mean reduction of 39% in LDL-C was observed, thus demonstrating a cumulative effect of the two active products.

In conclusion, it has been demonstrated that plant sterols have a complementary effect when combined with statins (which use a different mechanism of action to lower excess cholesterol). However, medical advice should always be sought whether using plant sterols or drugs to lower cholesterol.

# 4. Clinical studies using phytosterols

***Table 11:** Overview of recent studies with subjects undergoing treatment with hypercholesterolemic agents*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Gylling 1997 [32] | • **Study product: margarine enriched with esterified plant stanols**<br>• Dose of plant stanol studied: 3 g/d<br>• Study duration: 7 weeks (group I) and 12 weeks (group II)<br>**N=32 menopausal women with a history of MI**<br>22 women on a hypocholesterolemic diet alone (group I) and 10 women on simvastatin (group II) | Group I: non-treated women<br>Reduction in relation to diet alone:<br>TC=-13%<br>**LDL-C=-20%**<br>Group II: women treated with simvastatin<br>Reduction in relation to medication alone:<br>TC=-11%<br>**LDL-C=-16%** |
| Vurio 2000 [118] | • **Study product: margarine enriched with esterified plant stanols**<br>• Dose of plant stanol studied: 2.24 g/d<br>• Study duration: 6 weeks<br>**N=12 subjects treated with a statin** | Reduction in LDL-C:<br>**LDL-C=-20%** |
| Blair 2000 [9] | • **Study product: margarine enriched with esterified plant stanols**<br>• Dose of plant stanol studied: 5.1 g/d<br>• Study duration: 8 weeks<br>• Randomised, double-blind, placebo-controlled study<br>**N=167 subjects treated with a statin** | Enriched margarine group:<br>TC=-12%<br>**LDL-C=-17%**<br>Significant differences *vs.* placebo ($p$<0.0001) |
| Miettinen 2000 [73] | • **Study product: margarine**<br>• Dose of plant stanol studied: 2 g/d<br>• Study duration: 7–18 days<br>**N=11 colectomised patients** | TC and the ratio of plant sterol to cholesterol decreased significantly after only 1 day of consumption of stanol esters and the **LDL-C reduction after 7 days was 16% ($p$<0.01).**<br>Plant stanols found in serum and bile but effectively eliminated. |
| Nigon 2001 [82] | • **Study product: margarine enriched with esterified plant sterol**<br>• Dose of phytosterol studied: 1.6 g/d<br>• Study duration: 8 weeks<br>• Randomised, double-blind, placebo-controlled, crossover study<br>**N=53 subjects treated with fibrates or untreated** | Overall results:<br>TC=-6.4%<br>**LDL-C=-8.8%**<br>Significant differences vs. placebo ($p$<0.05)<br>Subgroup not on fibrates:<br>TC=-5.5%<br>**LDL-C=-7.7%**<br>Subgroup on fibrates:<br>TC=-8.5%<br>**LDL-C=-11.1%** |
| Neil 2001 [79] | • **Study product: margarine enriched with esterified plant sterols**<br>• Dose of phytosterol studied: 2.5 g/d<br>• Study duration: 8 weeks<br>• Randomised, double-blind, controlled study<br>**N=62 subjects including 30 treated with statins** | Reduction in LDL-C:<br>**LDL-C=-10%**<br>No significant difference between treated and untreated groups |

***Table 11:** (cont.)*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Simons 2002 [108] | • Randomised, double-blind study in 4 parallel groups:<br>**- Control margarine + placebo (N=38)**<br>**- Margarine enriched with esterified plant sterol + placebo (N=39)**<br>**- Control margarine + statin (N=38)**<br>**- Margarine enriched with esterified plant sterols + statin (N=37)**<br>• Dose of plant sterol studied: 2 g/d<br>• Study duration: 4 weeks<br>**N=152 subjects** | Effect of statin *vs.* control margarine:<br>**LDL-C=-32%**<br>Effect of enriched margarine *vs.* control:<br>**LDL-C=-8%**<br>Significant differences ($p$<0.0001)<br>Effects of statin + enriched margarine group:<br>**LDL-C=-39%** |
| Gylling 2002 [30] | Treatment (2 phases)<br>• **Low-fat, low-cholesterol diet with simvastatin (20 mg/d) - 3 months**<br>• **Followed by dietary plant stanol ester margarine for 8 weeks**<br>• Cholestyramine 8 g/d for another 8 weeks<br>• Dose of plant sterol studied: 2.25 g/d<br>**N=11 hypercholesterolemic coronary patients** | Reduction in LDL-C:<br>With Simvastatin: LDL-C=-39% ($p$<0.001),<br>**With stanol ester margarine by a further 13% ($p$<0.05).**<br>**Triple treatment led to 67% reduction from baseline ($p$<0.001), with all LDL-C values being < 2.6 mmol/l,** and increased HDL-C by 15% ($p$<0.01). Increase in lathosterol/ cholesterol ratio ($p$<0.01), and sitosterol. |
| Jakulj 2005 [47] | • Double blind, placebo-controlled, crossover study for plant sterol component with open-label ezetimibe treatment.<br>Randomised to 4 groups:<br>**- Ezetimibe (10 mg/d) + control spread (25 g/d)**<br>**- Ezetimibe (10 mg/d) + PS-enriched spread (25 g/d)**<br>**- PS-enriched spread (25 g/d)**<br>**- Control spread (25 g/d)**<br>• Dose of plant sterol studied: 2 g/d<br>• Study duration: 4 x 4 weeks<br>**N=40 mildly hypercholesterolemic subjects** | Reduction in LDL-C:<br>**Ezetimibe + control spread: -22.2%**<br>**Ezetimibe + PS-enriched spread: -25.2%**<br>**PS-enriched spread: -4.7%**<br>Increase in latherosterol/cholesterol ratio in all groups |

# 4. Clinical studies using phytosterols

## 4.4 Relationship between plant sterol daily intake and cholesterol-lowering effect

**Consumption of between 1-3 g/d of plant sterols results in a reduction of plasma LDL-C, from 5 - 15%**. The upper limit of 3 g/d of plant sterols is stated by the SCF[105]. A dose higher than 3 g corresponds to a plateau of efficacy, above which any increase in the quantity of plant sterols consumed results in no additional reduction in LDL-C levels.

Although several clinical studies have investigated the relationship between daily dose of plant sterol/stanol and the extent of LDL-C reduction *(Table 12),* [16,34,39,65,81] no strict dose-response relationship has been observed within the narrow effective dose range (1 to 3 g/d). The impossibility to establish a rigorous dose-response relationship, is due to the fact that the study designs simultaneously differed in several respects (e.g. age of the population, baseline cholesterol, trial duration, phytosterol dose, background diet, food matrices).

To illustrate this difficulty, the calculated correlation coefficient between the amount of phytosterols in dairy matrices and the corresponding reduction in LDL-cholesterol appears as low as 0.11. This is probably linked to the small dose range (1.6 to 2.3 g/d), which increases the effect of confounding factors (duration, population, food matrices) and can easily overshadow the effect of phytosterol doses.

In other words, whilst data suggest some relationship between the dose of phytosterols and the magnitude of the LDL-cholesterol decrease, the calculation of a true dose-response is not possible with the available data and has so far not been attempted. Whilst a daily dose of 2 to 2.4 g of phytosterols has been evaluated as able to trigger a decrease of approximately 9% in LDL-cholesterol, a specifically developed food product with a lower phytosterol content, such as Danacol®, can lead to similar or more significant decreases in LDL-cholesterol. Indeed, the recommended daily serving of Danacol® provides 1.6 g of plant sterols and a normal balanced diet typically provides an additional 0.2 to 0.4 g.

***Table 12:*** *Overview of studies on the dose-efficacy relationship of plant sterols*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Hendriks 1999 [39] | • **Study product: Plant sterol enriched spread**<br>• Doses of plant sterols studied: 0, 0.83, 1.61, 3.24 g/d<br>• Study duration: 4 x 3.5 weeks<br>• Randomised, double-blind, placebo-controlled, balanced incomplete, Latin square design trial<br>**N=100 healthy normocholesterolemic and mildly hypercholesterolemic subjects** | Reductions in levels of LDL-C<br>• **0.83 g/d=-0.20 mmol/l**<br>• **1.61 g/d=-0.26 mmol/l**<br>• **3.24 g/d=-0.30 mmol/l** |
| Nguyen 1999 [81] | • Study: 3 groups:<br>- **Placebo**<br>- **Margarine enriched with esterified plant stanols (2 g/d)**<br>- **Margarine enriched with esterified plant stanols (3 g/d)**<br>• Study duration: 8 weeks<br>**N=318 mildly hypercholesterolemic patients** | Dose-dependent response in the group receiving 3 g/d<br>TC=-6.4%<br>**LDL-C=-10.1%** |
| Hallikainen 2000 [34] | Placebo-controlled study with **4 different doses of esterified plant stanols in association with a margarine:**<br>- 0.8 g/d<br>- 1.6 g/d<br>- 2.4 g/d<br>- 3.2 g/d<br>• Study duration: 4 weeks<br>**N=22 hypercholesterolemic subjects** | Corresponding mean reductions in LDL-C:<br>• **1.7%**<br>• **5.6%**<br>• **9.7%**<br>• **10.4%** |
| Christiansen 2001 [16] | • **Study product: margarine enriched with sterol**<br>• Doses of plant sterols studied: 1.5 g/d and 3 g/d.<br>• Study duration: 24 weeks<br>• Randomised, double-blind, placebo-controlled study<br>**N=155 subjects** | Reductions in levels of LDL-C<br>**between -7.5% and -11.6%**<br><br>No significant difference between the 2 doses. |
| Li 2007 [65] | • **Study product: milk tea powder containing plant sterol esters**<br>• Doses of plant sterols studied: 0, 1.5 g/d or 2.3 g/d<br>• Study duration: 5 weeks<br>• Randomised, double-blind, placebo-controlled trial<br>**N=309 Chinese subjects**<br>(62% with hypercholesterolemia) | Reductions in LDL-C<br>• **2.3 g/d=-0.17 mmol/l**<br>• **1.5 g/d=-0.15 mmol/l ($p$>0.4)** |

## 4.5 Efficacy of long term use of plant sterol-enriched products

Several studies of more than 12 weeks' duration have demonstrated that the LDL-cholesterol lowering action of phytosterols is sustainable over time [17,20,37,72,116] and of a similar magnitude to that observed in shorter trials (i.e. around 10%).

Hendriks *et al.*, 2003 monitored the decrease in LDL-cholesterol relative to control values and showed unambiguously that it was maintained after 3, 6, 9 and 12 months of regular intake of 1.6 g phytosterols [37]. This is consistent with the findings of Christiansen *et al.*, 2001 [17], who established that the effect of 1.5 g/d phytosterols was sustained during a 6-month long study, and of Miettinen *et al.*, 1995 [72] who observed that the effect was maintained after 6 and 12 months of daily intake of 1.8 g and 2.6 g phytosterols *(Table 13)*.

***Table 13:*** *Overview of long-term studies demonstrating the efficacy of plant sterols*

| 1st author, year of publication | Elements of methodology | Main results |
|---|---|---|
| Christiansen 2001 [17] | • **Study product: Plant sterol ester-enriched spread *vs.* control spread**<br>• **Doses of plant sterol studied: 1.5 or 3 g/d**<br>• Randomised, double-blind, placebo-controlled trial<br>• Duration: 6-month experimental period<br>**N=155 hypercholesterolemic subjects** | Reductions in LDL-C:<br>**LDL-C=-7.5 to -11.6% in PS group *vs.* control** |
| De Jong 2008 [20] | • **Study product: Plant sterol/stanol ester-enriched margarine *vs.* control margarine**<br>• Dose of plant sterol/stanol studied: 2.5 g/d<br>• Study duration: 85-week experimental period<br>• Randomised, double-blind study<br>**N=54 hypercholesterolemic subjects on statins** | Reductions in LDL-C:<br>**Stanol group: -8.7% ($p$=0.08)**<br>**Sterol group: -13.1% ($p$=0.006)** |
| Hendriks 2003 [37] | • **Study product: Plant sterol ester-enriched spread *vs.* control spread**<br>• Dose of plant sterols studied: 1.60 g/d<br>• Study duration: 1 year<br>• Randomised, double-blind, placebo-controlled parallel trial<br>**N=185 healthy volunteers** | Reductions in TC and LDL-C:<br>TC=-4%<br>**LDL-C=-6%** |
| Miettinen 1995 [72] | • **Study product: Sitostanol ester-enriched spread *vs.* control spread**<br>• Dose of plant stanol studied: 1.8 or 2.6 g/d<br>• Study duration: 1 year<br>• Randomised, double-blind trial<br>**N=153 mildly hypercholesterolemic subjects** | Reductions in TC and LDL-C:<br>TC=-10.3%<br>**LDL-C=-13.0%** |
| Vanhanen 1994 [116] | • **Study product: Rapeseed oil fat mayonnaise with and without sitostanol ester**<br>• Dose of plant stanol studied: 0.8 g/d for 9 weeks then 2.0 g/d for 6 weeks<br>• Study duration: 15 weeks<br>• Randomised, double-blind trial study<br>**N=15 mildly hypercholesterolemic subjects** | Reductions in LDL-C:<br>**0.8 g/d=-7.4% ($p$=NS)**<br>**2.0 g/d=-15.7%** |

The sustainability of the LDL-cholesterol-lowering effect of phytosterols is consistent with their mechanism of action. By reducing the absorption of endogenous and exogenous intestinal cholesterol, phytosterols lead to enhanced endogenous cholesterol synthesis, but also an enhanced hepatic uptake of LDL. Following modification of external inputs (medication, dietary changes) cholesterol metabolism is known to reach a new steady-state within a few days and to be maintained if these inputs do not change [11]. Thus, although very long term trials have not yet been published, we can hypothesise that the LDL-cholesterol-lowering effect of phytosterols in general, and Danacol® in particular, is sustainable for years, provided that phytosterols are consumed daily.

## 4.6 Effects of long term use of plant sterol-enriched products

The LDL cholesterol lowering effect of plant sterol-enriched foods can be sustained in the long-term by continued daily consumption. Indeed, this effect has been shown up to 85 weeks*.

Plant sterols/stanols are recognised by the SCF as safe ingredients. To date, no serious adverse effects have been observed except a slight decrease of certain carotenoids carried by LDL particles. Concern over the long term effects of the use of plant sterols exists due to their mechanism of action (inhibition of intestinal absorption of cholesterol) and to their potential persistence in the body leading to potential pathogenic effects. Some carotenoids (precursors of vitamin A) are transported by lipoproteins, in particular LDL. The concentrations of these substances might be affected as a result of reduced plasma levels of LDL after several weeks of consumption of plant sterol-enriched products. A normal balanced diet containing carotenoid-rich fruit and vegetables is able to compensate for their modest decrease in the plasma [40].

Several clinical studies examining the effects of medium and long-term continuous consumption of plant sterol-enriched products [31,38,39,71,72,85] have shown reductions in plasma concentrations of ß-carotene (from -10 to -20% but still within the normal range) and to a lesser extent lycopene (-7% ) and tocopherol (only minor change in a few cases). However, these studies confirm the absence of other nutritional effects and support the safety of phytosterols. **In a setting of consumption of plant sterol-enriched foods, a fruit and vegetable-rich diet could help compensate for a decrease in plasma levels of carotenoids.**

It has been hypothesised that the decrease in vitamins and anti-oxidants, such as ß-carotene, associated with plant sterol consumption may be a possible determinant of increased oxidative burden [68]. However, a recent study [68] demonstrated that consuming plant sterols for 6 weeks significantly decreases levels of 8-isoprostane *(see section 4.4 for details)*. This suggests that phytosterols have anti-oxidative rather than pro-oxidative properties [68]. Isoprostanes are prostaglandin-like compounds formed in vivo via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid. Hence, they are a very reliable indicator of oxidative damage *in vivo* [68].

Cholesterol plays an important role in the rigidity of membranes. In red blood cells, membrane rigidity is an important parameter affecting the passage of erythrocytes through narrow capillaries. Several studies have shown that while plant sterols and stanols have an effect of lowering plasma LDL-C levels, examination of erythrocyte fragility showed no changes. **The use of plant sterols and stanols did not alter the stability of the membrane of the red blood cells [21,26,52].**

* The EFSA Journal. 2009. 1175: 1-9

# 4. Clinical studies using phytosterols

The C-reactive protein (CRP) is a biomarker for inflammation and elevated levels are considered to be one of the risk factors for CVD [121]. One study involving 34 hypercholesterolemic subjects looked at the effects of three different treatments: Low-saturated fat diet, low-saturated fat diet with statin treatment or plant sterol-enriched diet. The statin treatment and plant sterol treatment reduced the CRP to a similar extent [51]. Studies originally conducted in mice [74] and rabbits [88] and later extended to human subjects have demonstrated that elevated plasma levels of plant sterols are not correlated with atherosclerosis [120]. In a particular set of patients with inherited phytosterolemia, elevated serum plant sterols may be a risk factor for premature atherosclerosis, however whether this is also a risk for CVD has not been established [113]. One study addressing the issue of atherosclerosis caused by elevated plasma levels of plant sterols states that there are insufficient data to date to support a correlation [114]. Similarly, insufficient data exist to support claims that plant sterols and stanols prevent or promote colon cancer [55].

## 4.7 Plant sterol-enriched foods and dietary recommendations

Plant sterols are endorsed by numerous national and international guidelines and recommendations *(Table 14)*.

***Table 14:*** *Guidelines recommending plant sterols as means of lowering blood cholesterol levels*

| Name of recommending body | Website | Statement |
|---|---|---|
| European Society of Cardiology (ESC) | http://www.guideline.gov/summary/summary.aspx?doc-id=12541&nbr=6457&ss=6&xl=999#s23 | "…phytosterols may help to reduce plasma concentrations of LDL-cholesterol" |
| National Institutes of Health (NIH) | http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3full.pdf | Essential features of therapeutic lifestyle changes are…"Therapeutic options for enhancing LDL lowering such as plant stanols/sterols (2 g/d)" |
| International Atherosclerosis Society (IAS) | http://www.lassa.org.za/guidelines/IASHarmonGuidelines.pdf | Consideration must "be given to adding other non-drug options for enhanced lowering of LDL-cholesterol levels e.g.…Plant stanols/sterols (2 g/d)" |
| American Heart Association and American College of Cardiology | http://circ.ahajournals.org/cgi/reprint/113/19/2363 | "Adding plant stanols/sterols (2 g/d)… will further lower LDL-C" |
| Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps) | http://afssaps.sante.fr/pdf/5/rbp/dysarg.pdf | Treatment of dyslipidemic patients necessitates "Limitation of dietary cholesterol or use of foods enriched with plant sterols" |



# 5. Hypocholesterolemic effects of low-fat, plant sterol-enriched yoghurt in hypercholesterolemic adults: Danacol®

# 5. Hypocholesterolemic effects of Danacol®

## 5.1 Key clinical findings

**Danacol® resulted in an overall LDL-cholesterol reduction of 9.5% compared to the control group at 3 weeks of daily consumption ($p$<0.001).**
**This decrease was maintained at 6 weeks (-8.8% *vs.* control; $p$<0.001).**

## 5.2 Introducing the Danacol® clinical trials

Most of the earlier studies on plant sterol effects were performed using spread/margarine and until recently, only a few studies had been performed with low-fat food matrices.

Danone recently undertook three studies in moderately hypercholesterolemic subjects using Danacol® in both the spoonable and drinkable forms [35,68,94]. These three studies, performed in Italy, France and Spain, were all multicentre, randomised, placebo-controlled, double-blind studies. All three studies started with a run-in period to allow standardisation of subjects' diet. Subsequently, hypercholesterolemic adults were randomly assigned to either Danacol® (1.6 g/d plant sterol in the form of 1 bottle or 2 pots) or a control fermented milk product for 6 weeks after *(Table 15)* in combination with therapeutic lifestyle changes (TLC) [35,68,94].

***Table 15:** Methodology of Danacol®'s key clinical trials*

| | Plana *et al.*, 2008 [94] | Mannarino *et al.*, 2008 [68] | Hansel *et al.*, 2008 [35] |
|---|---|---|---|
| **Population** | • 82 adults (18 - 75 years)<br>• LDL-C >3.3 mmol/l if 10 year risk ≤20% and no ischaemic heart disease or >2.6 mmol/l if 10 year risk >20% or ischaemic heart disease<br>• BMI >30 kg/m² | • 116 adults (20 - 75 years)<br>• Stabilised hypercholesterolemia: LDL-C = 130 - 190 mg/dl for > 3 months<br>• BMI = 19-30 kg/m² | • 194 adults (18 - 75 years)<br>• LDL-C = 130 - 190 mg/dl<br>• BMI = 19-30 kg/m² |
| **Country** | **Spain** | **Italy** | **France** |
| **Intervention** | • Mediterranean diet during 4-week run-in and 6-week experimental phases<br>• Danacol® drinkable (1.6 g/d free sterol) (N=43) or control fermented milk product (N=40) for 6 weeks<br>• No lipid-lowering treatments except statins (N=9 in each group) | • NCEP-ATP III dietary recommendations for 4 weeks<br>• One low-fat dairy product with main meal for 2-week run-in period<br>• Danacol® drinkable (1.6 g/d free sterol) (N=60) or control fermented milk product (N=56) for 6 weeks<br>• No lipid-lowering treatments except stable statins (>3 months N=8 in Danacol® group and N=7 in control group) | • General dietary recommendations, no PS-enriched foods + 2 low-fat yoghurts/d during 4-week run-in<br>• 2 x Danacol® spoonable (2 x 0.8 g/d free sterol) (N=95) or 2 x control fermented milk product (N=96) for 6 weeks<br>• No lipid-lowering treatments except statins |

## 5.3 LDL-lowering effect of Danacol®

LDL-cholesterol was measured at baseline and after 3 and 6 weeks. In the Spanish study for example, a decrease of 10.2% (-0.41 mmol/l) in the plasma level of LDL-C was observed in the group consuming Danacol®, whereas an increase of 2.0% (+0.05 mmol/l) was observed in the control group after 3 weeks *(Figures 11 & 12)* [94]. Hence, **LDL-C decreased by 12.2%** in the Danacol® group compared to the control group.

After 6 weeks, a 10.5% (-0.43 mmol/l) reduction in LDL-C was observed in the Danacol® group and a 0.1% (-0.07 mmol/l) increase in the placebo group, giving an overall **decrease of 10.6%** in LDL-C in the Danacol® group compared to the control group. *(Figures 11 & 12)* [94].

***Figure 11:** Relative change in LDL-cholesterol concentrations after 3 and 6 weeks of Danacol® consumption*

***A randomised, double-blind, placebo-controlled, parallel-group clinical study on the effects of a fermented dairy product enriched with phytosterols on blood cholesterol levels in hypercholesterolemic adult subjects. After 3 weeks of daily consumption of drinkable Danacol® (1.6 g/d) a 12.2% reduction in LDL-cholesterol levels compared to the control population can be seen. This reduction is maintained even at 6 weeks (10.6%)[94].***

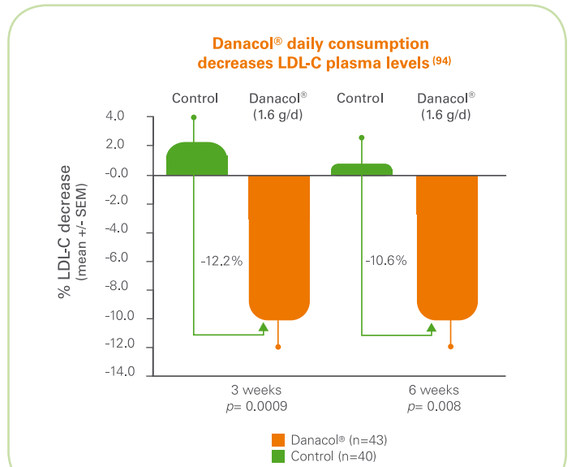
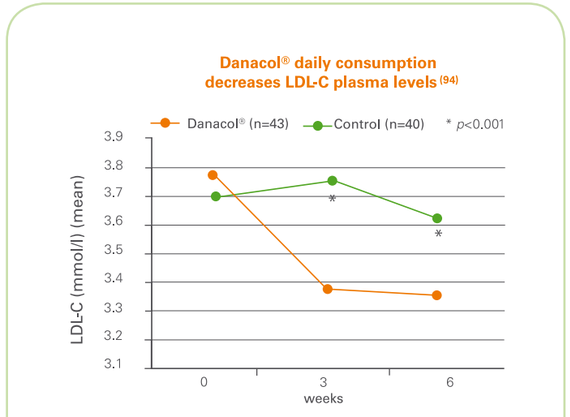



***Figure 12:** Absolute change in LDL-cholesterol concentrations after 3 and 6 weeks of Danacol® consumption*

***A significant reduction in LDL-C was observed from 3 weeks (-0.41 mmol/l) and maintained throughout the 6-week study period (-0.43 mmol/l) in the Danacol® group[94].***



# 5. Hypocholesterolemic effects of Danacol®

Similar results were obtained with both spoonable and drinkable Danacol® [35,67,91]. Pooled analysis demonstrated an overall LDL-cholesterol reduction of 9.5% compared to the control group at 3 weeks of daily consumption *(Table 16)*; this reduction was maintained at 6 weeks (-8.8% *vs.* control).

***Table 16:*** *Pooled analysis of the LDL-cholesterol-lowering effect of Danacol® after 3 and 6 weeks of consumption*

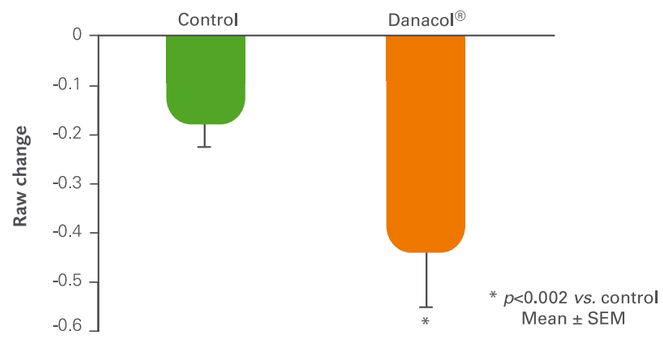
| | 3 WEEKS | | 6 WEEKS | |
|---|---|---|---|---|
| Study Name | % Difference in means | *p*-value | % Difference in means | *p*-value |
| Hansel *et al.*, 2007 [35] | -9.5 | 0.001 | -7.8 | 0.001 |
| Plana *et al.*, 2008 [94] | -12.2 | <0.001 | -10.6 | <0.001 |
| Mannarino *et al.*, 2008 [68] | -8.4 | ≤0.001 | -9.5 | ≤0.001 |
| **Pooled results** | **-9.5** | **0.0000** | **-8.8** | **<0.001** |

None of the studies revealed any changes in the levels of HDL-C [35,67,91]. Accordingly, the Italian study revealed a two-fold greater reduction in LDL-C/HDL-C ratio among subjects who had consumed Danacol® for 6 weeks *(Figure 13)* [68]. This is notable because the LDL-C/HDL-C ratio is a good predictor of cardiovascular risk in hypercholesterolemic patients.

***Figure 13:*** *Reduction of LDL-C/HDL-C ratio after 6 weeks of Danacol®*

*After 6 weeks, the LDL-C/HDL-C ratio had decreased by twice as much among patients consuming Danacol® vs. patients in the control group [68].*

**Danacol® daily consumption lowers the LDL-C/HDL-C ratio [68]**

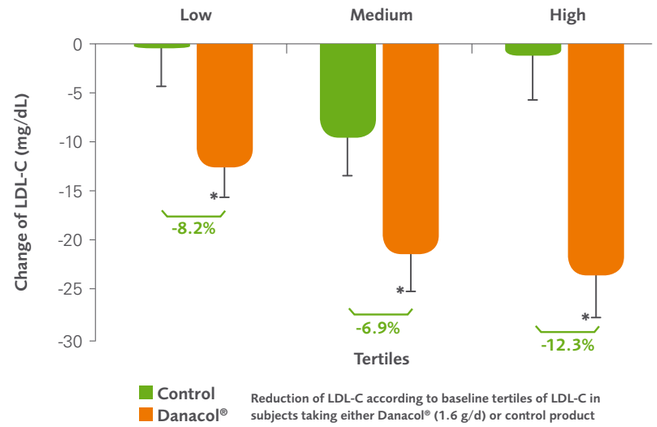



Furthermore, when patients were divided into tertiles according to their initial cholesterol levels, there appeared to be a trend ($p$<0.08) towards a greater decrease in LDL-C among those Danacol® patients with the highest baseline levels *(Figure 14)*. No such tendency was seen among control patients [68]. This may suggest a more important reduction in cholesterol absorption in patients with higher cholesterol levels.

***Figure 14:*** *Decrease in LDL-cholesterol in function of baseline LDL-C tertile after 6 weeks of Danacol® consumption*

*After 6 weeks of consumption, the LDL-C levels had decreased by 8.4%, 12.6% and 12.8% among Danacol® patients in the first, second and third baseline tertiles respectively (p=0.08) [68].*

**Danacol® daily consumption tends to have a greater LDL-C-lowering effect in subjects with higher baseline LDL-C levels [68]**




* $p$<0.05 *vs.* control

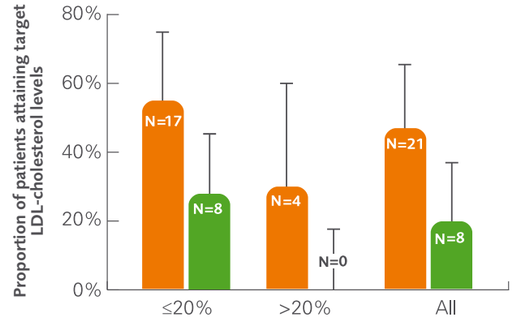
Reduction of LDL-C according to baseline tertiles of LDL-C in subjects taking either Danacol® (1.6 g/d) or control product

The clinical trials also showed that Danacol® helps hypercholesterolemic patients achieve their predefined cholesterol targets. In the Spanish study about 50% of Danacol® subjects attained their recommended LDL-C level compared to just 20% in the control group *(Figure 15)*. This percentage reached 70% among Danacol® subjects at high cardiovascular risk [94].

***Figure 15:*** *Proportion of subjects attaining their LDL-C goals after 6 weeks of Danacol® stratified according to cardiovascular risk*

*After 6 weeks of consumption, over twice as many subjects receiving Danacol® achieved pre-defined LDL-C targets compared to control subjects [94].*

**Daily consumption of Danacol® helps hypercholesterolemic subjects reach target LDL-C levels [94]**

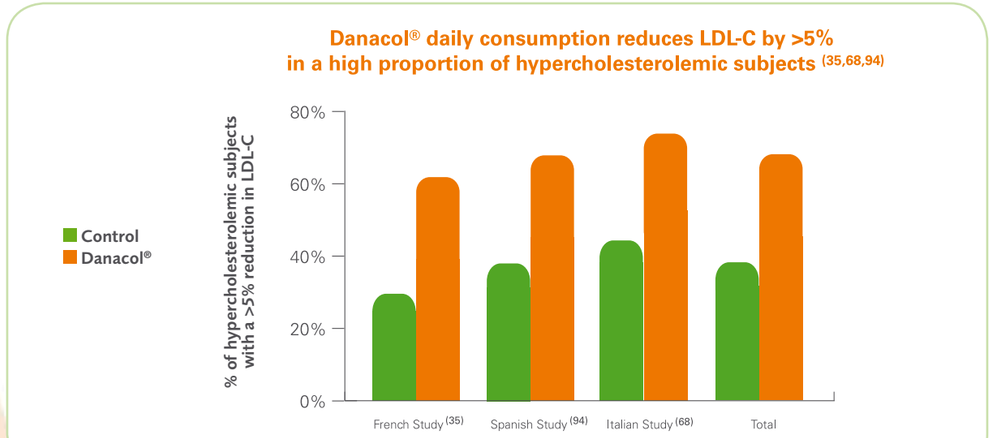


# 5. Hypocholesterolemic effects of Danacol®

In the Italian study, it was demonstrated that over 1 in 2 patients successfully lowered their LDL-C by 10% or more and over three quarters by 5% or more [67]. This suggests that most patients exhibit a clinically relevant LDL-cholesterol reduction derived from daily consumption of Danacol® [35].

The pooled analysis found that the probability of lowering LDL-C by over 5% was 1.84 times greater in the Danacol® group than in the control group *(Figure 16)* [35,68,94]. These findings suggest that daily consumption of Danacol®, in addition to traditional dietary recommendations, results in a clinically relevant LDL-cholesterol reduction.

***Figure 16:*** *Proportion of subjects lowering their LDL-C by >5% after 6 weeks of Danacol®*
*After 6 weeks, nearly 70% of subjects had lowered their LDL-C by over 5% in the Danacol® and approximately 35% in the control group [35,68,94].*

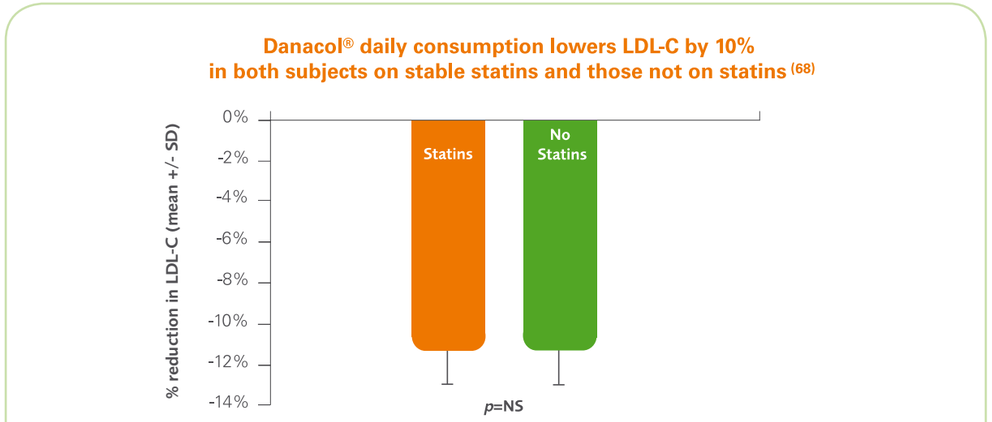



In summary, the three clinical trials show that daily consumption of 1.6 g of plant sterols in the form of drinkable or spoonable Danacol® is a simple and effective way of lowering LDL-cholesterol in hypercholesterolemic adults, enhancing the attainment of LDL-cholesterol goals [35,68,94].

## 5.4 The use of Danacol® with statins

Each of the three clinical trials included a small number of patients who had been receiving statins for at least three months before starting to consume Danacol® [35,68,94]. Subgroup analysis revealed that Danacol® exerted similar benefits on LDL-cholesterol regardless of whether patients were receiving statins or not at baseline *(Figure 17)*. Indeed, Danacol® led to a ~10% decrease in LDL-cholesterol even in patients on statins [35,68,94]. This confirms previous findings showing an additive effect rather than a synergistic effect of plant sterols in patients on statins. For comparative purposes, doubling the statin dose induces just a 6 to 8% extra LDL-C reduction.

***Figure 17:*** *LDL-cholesterol-lowering effect of Danacol® in patients on stable statin therapy at enrolment*
*LDL-cholesterol was decreased by -10.9 ± 2.0% in patients on statins and by -11.5 ± 1.6% by patients not on statin (p=NS) [68].*




Larger trials are needed to confirm and to quantify the additive effect. However, it may be of special interest to combine statin-mediated inhibition of cholesterol synthesis with plant sterol-mediated inhibition of cholesterol absorption in the same hypercholesterolemic subjects.

# 5. Hypocholesterolemic effects of Danacol®

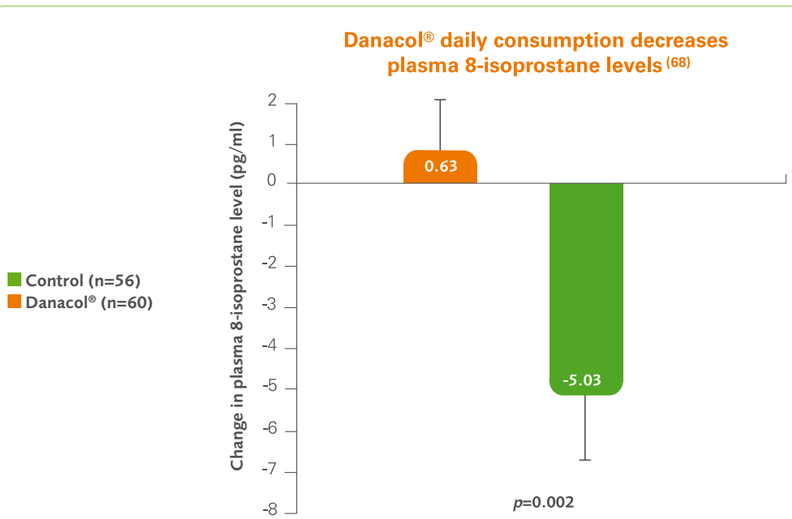
## 5.5 Danacol® and oxidative stress

It has been suggested that by lowering cholesterol absorption, plant sterols may also affect the absorption of liposoluble vitamins such as ß-carotene, and that this in turn may disrupt the oxidative balance with pro-atherogenic consequences. However, in the three clinical trials on Danacol®, although ß-carotene levels did indeed decrease slightly, this decrease was not significant when normalised to the LDL-cholesterol level [35,68,94].

ß-carotene levels are just one component of oxidative stress. For example, plasma oxidated LDL (oxLDL) is known to be an integrative marker of systemic oxidative stress and a strong predictor of acute coronary heart disease events. In our studies, plasma concentrations of oxLDL decreased significantly following consumption of Danacol® as opposed to consumption of the control yoghurt ($p$<0.05) [35]. This suggests that plant sterols do not affect LDL oxidation.

Likewise, plasma levels of **8-isoprostane**, a reliable measure of oxidative damage with a documented role in atherogenesis, decreased significantly in the Danacol® group but not in the control group *(Figure 18)* [68]. This is suggestive of possible anti-oxidative rather than pro-oxidative effects of Danacol®.

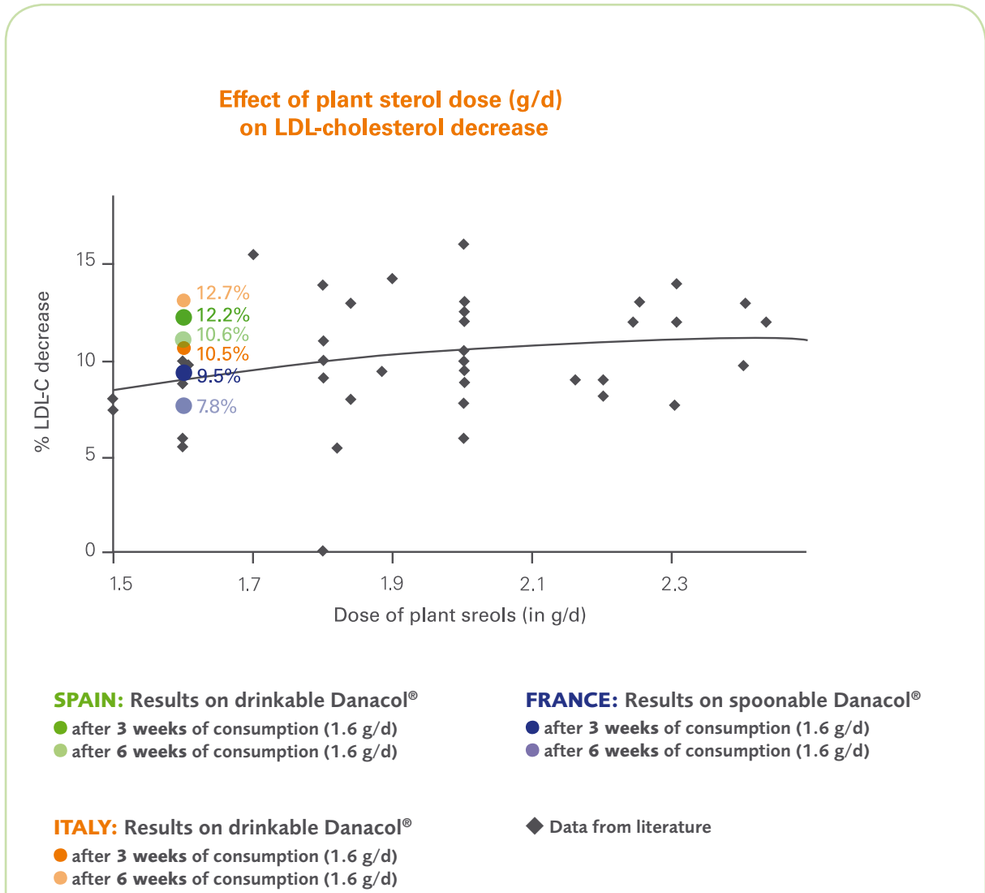
***Figure 18:*** *Change of plasma 8-isoprostane concentrations after 6 weeks of Danacol® consumption*
*After 6 weeks of daily consumption of drinkable Danacol® (1.6 g/d), plasma 8-isoprostane levels dropped from 43.07 ± 1.78 to 38.04 ± 1.14 pg/ml (-5.03 ± 1.64 pg/ml; p=0.018) whereas they did not change in the control group: 42.56 ± 2.12 to 43.19 ± 2.0 pg/ml (+0.63 ± 1.71 pg/ml; p=NS)[68].*




## 5.6 The efficacy of Danacol® in comparison with plant sterol studies

The results of the most important studies documented in the literature in addition to the recent studies using spoonable and drinkable Danacol®, can be used to determine a dose-response curve *(Figure 19)* showing that the hypocholesterolemic effect is clinically significant from a dose of sterols of 1.6 g/d, equating to a reduction of 9.5% in plasma LDL-C levels after 3 weeks consumption.

***Figure 19:*** *Relationship between quantity of plant sterols ingested and concomitant reduction in LDL-cholesterol*
*Recent studies using Danacol® (spoonable and drinkable) agree with data from the literature that reductions in LDL-cholesterol are observed with a daily dose of plant sterols of 1.6 g/d after a period of 3 weeks.*



# 5. Hypocholesterolemic effects of Danacol®

## 5.7 Other parameters investigated in the Danacol® studies

- The daily consumption of Danacol® for 6 weeks resulted in no significant change in triglyceride levels [35,68,94].
- A slight but insignificant decrease in plasma CRP (C-Reactive Protein) levels, a powerful risk factor for cardiovascular diseases, was observed after 6 weeks of Danacol® [35,94].
- The ApoB/ApoA1 ratio, which is one of the main predictive factors for cardiovascular disease was significantly ($p$<0.001) reduced following consumption of Danacol®.
- The Italian study revealed no significant variations in plasma lathosterol, campesterol or ß-sitosterol concentrations after 6 weeks of treatment with Danacol®, allaying fears of a potentially atherogenic build up of sterols [68].

  The French and Spanish studies described modest increases in phytosterol concentrations. This effect was in accordance with the product composition and within the normal range reported in the literature [35,94].
- Last and by no means least, none of the studies using Danacol® resulted in any severe clinical adverse events [35,68,94].

## Summary of Danacol® Studies

**These studies show that the daily consumption of spoonable or drinkable Danacol® results in:**

- Significant reductions in total and LDL-C levels in the plasma after 3 weeks, maintained through 6 weeks.
- A trend towards a greater effect in patients with higher baseline levels of LDL-C.
- Preliminary evidence of an equal decrease in patients receiving stable statin treatment.
- No safety issues.
- No effect on HDL-C or triglyceride levels.
- No effect on plant sterols (ß-sterols or campesterols).
- No effect on ß-carotene/LDL-C ratio.
- An anti-oxidative effect through the reduction of plasma 8-isoprostane.
- No major adverse events.

# 6. Danacol® in practice

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## 6.1 A valuable food product for the management of hypercholesterolemic patients

Many patients do not fully realise the severity of hypercholesterolemia due to its asymptomatic nature and the difficulty in assessing the associated statistical risk. This means that the value of preventive treatment and the need to take such treatment for unlimited periods are often underestimated. Together with other reasons, these factors account for the poor compliance frequently seen [58,63]. The data in the literature on this subject are revealing. In three major primary prevention studies, between 25% and over 33% of subjects reduce their dose of lipid-lowering agents or discontinue their treatment within 1 to 5 years of beginning it [29,107,112]. These figures correspond to those of anti-hypertensive treatments, for which long-term compliance is poor in among 30-55% of patients [76].

It is also difficult to ensure compliance with dietary prescriptions in a large number of cases. Although doctors are able to 'encourage' compliance with dietary recommendations, patients frequently register only lists of 'prohibited' foods. When dietary habits are involved, as with anything associated with lifestyle, there is generally greater resistance to the type of lasting change required.

This is particularly apparent where patients are asked to make a number of simultaneous lifestyle changes such as increasing their physical activity and stopping smoking, which constitute necessary steps in the overall management of a group of concomitant cardiovascular risk factors [78,104].

**In this context, a food matrix with an adequate nutritional composition such as Danacol® from Danone represents a novel way of optimising dietary advice for hypercholesterolemic patients. Danacol® is a low-fat product containing probiotics and traditional yoghurt starters (Streptococcus thermophilus and Lactobacillus bulgaricus) enriched in plant sterols. Danacol® may be readily incorporated into a balanced, varied and pleasant diet, and it may be associated when necessary with hypocholesterolemic medication to optimise the cumulative lipid-lowering effects. It is important however to continue to regularly monitor cholesterol levels.**

## 6.2 Who should use Danacol®?

**Danacol® is recommended for any individuals who need to manage their blood cholesterol levels or who need to lower their excess blood cholesterol level. It is recommended for consumption in conjunction with a low cholesterol dietary intake, moderate daily exercise and, where advised by medical practitioners, the use of hypocholesterolemic medication.**

Cholesterol-reducing products are not recommended for pregnant or breast-feeding women, children or adolescents with specific nutritional requirements.

Danacol® may be suitable for use in diabetic subjects with hypercholesterolemia under careful medical supervision. Indeed, although Danacol® contains no added sugar, drinkable Danacol® still contains 4.4 g of carbohydrate per 100 g serving, of which 4.1 g sugars. This must be taken into account when calculating the daily sugar intake in diabetic subjects.

## 6.3 What dose of Danacol® should be recommended?

As indicated on the packaging the esterified plant sterol content of one bottle (drinkable)* of Danacol® is 1.6 g. Consumption of one bottle* of Danacol® daily thus provides a dose of plant sterols that produces effective hypocholesterolemic effects according to the SCF (1 to 3 g/d).

Furthermore, the quantity of plant sterols in Danacol® (1.6 g) is in line with the recent EFSA statement** which acknowledges that a daily intake of between 1.5 and 2.4 g plant sterols results in a 7 to 10.5% reduction in LDL cholesterol. This range reflects the fact that the product's effects can vary from one individual to another.

When consuming Danacol® it is important to take into account intake of any other foods enriched with plant sterols or stanols (e.g. margarines enriched with plant sterols). In effect, the maximum recommended total daily dose of plant sterols and/or stanols is 3 g, with higher quantities conferring no additional benefit.

It is recommended that Danacol® be consumed with one main meal in order to ensure optimal efficacy.

* or 2 pots (spoonable) of Danacol® in France.

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## 6.4 What additional dietary advice should be given?

Since the action of phytosterols can result in decreased absorption of certain liposoluble nutrients belonging to the carotenoid family (mainly ß-carotene), increased intake of fruit and vegetables rich in carotenoids is recommended for patients regularly consuming Danacol®. In practice, patients may be advised to consume adequate quantities of plant products naturally rich in ß-carotene in order to achieve the recommended daily intake level of 2100 µg/d *(Table 17)*. This advice clearly falls within the current dietary recommendations concerning increased intake of fruit and vegetables.

***Table 17:** Fruits and vegetables rich in ß-carotene*

| Fruit | ß-carotene/µ100 g |
|---|---|
| Dried apricot | 4700 |
| Apricot | 1500 |
| Mango | 3130 |
| Melon | 1750 |
| Kaki | 1420 |
| Papaya | 948 |
| Passion fruit | 500 |
| Peach | 500 |
| Prune | 450 |
| Mandarin | 334 |

| Vegetables | ß-carotene/µ100 g |
|---|---|
| Raw carrot | 11000 |
| Raw dandelion salad | 8400 |
| Fresh parsley | 6500 |
| Cooked spinach | 4460 |
| Pumpkin | 4240 |
| Red pepper | 3480 |
| Cress | 2900 |
| Lettuce | 360 |
| Raw tomato | 600 |
| Cooked broccoli | 430 |
| Cooked green beans | 336 |

## 6.5 Danacol® and diet/lifestyle recommendations for hypercholesterolemic subjects

A multifactorial lifestyle approach to reduce risk for CVD is recommended [13]. This approach is called Therapeutic Lifestyle Changes (TLC) and includes reducing intake of saturated fats and cholesterol, therapeutic dietary options for enhancing LDL lowering (plant stanols/sterols and increased viscous [soluble] fibre), weight reduction and increase in regular physical activity. At all stages of dietary therapy, physicians are encouraged to refer patients to registered dietitians or other qualified nutritionists for medical nutrition therapy, which is the term for the nutrition intervention and guidance provided by a nutrition professional.

In order to obtain maximum benefit from consumption of Danacol® *(Table 18)*, hypercholesterolemic patients should attempt to restrict their dietary intake of Saturated Fatty Acids in favour of foods that constitute sources of unsaturated fatty acids. In practice, you can provide personalised advice based on the information given in *Tables 19 to 21*. Finally, you should attempt to convince your patients of the value of regular, moderate physical activity, equivalent to walking for 30 minutes per day. Simply "moving around more on a daily basis" is already beneficial.

***Table 18:** Nutritional composition of Danacol®*

| | Drinkable Danacol® (plain*) per 100 g serving | Spoonable Danacol® (plain) per 125 g serving |
|---|---|---|
| Energy | 37 kcal/ 157 kJ | 60 kcal/ 255 kJ |
| Protein | 3.5 g | 6.1 g |
| Carbohydrates:<br>*of which sugars* | 4.4 g<br>4.1 g | 8.0 g<br>7.6 g |
| Fat (excluding sterols):<br>*of which saturates* | 0.6 g<br>0.6 g | 0.4 g<br>0.4 g |
| Plant sterol (equivalent as free sterols) | 1.6 g | 0.8 g |
| Fibre | 0.7 g | // |
| Calcium | 123 mg | 194 mg |
| Sodium | 40 mg | 90 mg |

*The only difference with fruit-containing varieties is the sugar content = 4.2 g/100 g

# 6. Danacol® in practice

*Table 19: Choosing fats for cooking, spreads and seasoning*

| Avoid | Prefer |
|---|---|
| **Animal fats:**<br>butter,<br>butter-based margarines,<br>lard,<br>full-fat fresh cream,<br>sources containing butter,<br>cream or eggs (e.g. mayonnaise) | **Vegetable fats:**<br>olive oil,<br>colza oil,<br>sunflower oil,<br>maize oil,<br>walnut oil,<br>hazelnut oil<br>(vary oils to ensure a balanced diet) |
| **Vegetable fats containing copra or palm oil**<br>**Hydrogenated fats** | **Margarines containing the above fats** |

*Table 20: Meat, poultry, eggs, fish and seafood*

| Avoid | Prefer |
|---|---|
| **Fatty cooked meats:**<br>pâtés, sausages, dried sausage, lard, etc. | Lean rindless ham, low fat poultry meats (fillets of turkey, chicken, etc, excluding goose and duck) |
| **Fatty meats:**<br>mutton, lamb, fatty cuts of beef at all cut of meat in general from which fat cannot be readily trimmed (chops, ribs, etc.) | Cuts of meat containing muscle and/or with clearly visible fat that can be easily trimmed (dishes without too much added fat: grilled meats, roast meats, etc.) |
| **Eggs** (yolk): not more than 3 per week | All fish, including fatty fish (salmon, herring, mackerel, sardines) except for eel (cooked in an oven, "en papillottes", in stock, microwaved, etc.) |
| **Offal (brain and liver)** | |
| **Pizzas, quiches, pies containing bacon pieces, eggs, sausage, etc.** | Shellfish and seafood |

*Table 21: Dairy products*

| Avoid | Prefer |
|---|---|
| **Whole-fat, non-skimmed** dairy products | **Low-fat dairy products:** milk, fromage blanc, yoghurt, milk-based desserts, milk-based products, etc. |
| **Fatty and cream cheeses, produced from whole-fat milk** | Low-fat cheeses (to be eaten with moderation) |

## 6.6 Are there any risks associated with phytosterol consumption?

Danone uses only safe ingredients that have been approved by the European Commission and the Novel Food Regulation. Phytosterols fall into this category. Despite having received authorisation on the basis of over 50 years of research, there is currently no medical consensus on the potential atherogenic side effects of plant sterols. However, it is noteworthy that phytosterols are naturally occurring substances, present in small quantities in foods such as nuts, seeds, fruits and oils. Indeed, the general population consumes an average 0.3 g of phytosterols per day in such foods.

Danacol® contains phytosterol, the health effect of which (reduction of hypercholesterolemia) has been proven in clinical studies and acknowledged by the EFSA. No side effects related to the product were observed in any of these studies. Indeed to date, no intestinal, digestive or metabolic side effects have been reported following the consumption of Danacol®.

Danone carefully analyses all scientific data published on this subject. Phytosterol consumption does not alter plasma HDL-C or triglyceride levels or modify the ß-carotene/ LDL-C ratio. Levels of oxidation biomarkers (LDL and isoprostanes) were unchanged, suggesting that phytosterols do not reduce antioxidant plasma capacity. Phytosterols may however lower blood concentration of beta carotene, however this is countered by regularly eating fruit and vegetables as stipulated in recommendations. This specific decrease in beta-carotene (approx 10 - 20%) does not affect other lipid-soluble vitamins such as vitamins A, D, E or K and remains within normal values.

Recently, European authorities (EFSA) reported that the general population typically consumes less than the recommended dose of phytosterols. This report stresses the importance of communicating on the fact that phytosterols are recommended only for people with hypercholesterolemia. These recommendations are fully in line with our packaging.

Danacol® is recommended for subjects with elevated cholesterol levels in combination with a balanced diet and physical activity. It is not recommended that pregnant and breast feeding women or children under the age of 5 years consume products enriched with plant sterols without specific medical advice given the particular needs of these populations. This product is also not recommended for people with sitosterolemia and careful medical follow up is necessary for high absorbers.

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