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Full article

Plant sterol ester diet supplementation increases serum plant sterols and markers of cholesterol synthesis, but has no effect on total cholesterol levels

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ABSTRACT

This double-blind, randomized, placebo-controlled, cross-over intervention-study was conducted in healthy volunteers to evaluate the effects of plant sterol ester supplemented margarine on cholesterol, non-cholesterol sterols and oxidative stress in serum and monocytes. Sixteen volunteers, average age 34 years, with no or mild hypercholesterolemia were subjected to a 4 week period of daily intake of 3 g plant sterols per day supplied via a supplemented margarine on top of regular eating habits. After a wash-out period of one week, volunteers switched groups. Compared to placebo, a diet supplementation with plant sterols increased serum levels of plant sterols such as campesterol (+0.16 ± 0.19 mg/dL, $p = 0.005$) and sitosterol (+0.27 ± 0.18 mg/dL, $p < 0.001$) and increased markers of cholesterol synthesis such as desmosterol (+0.05 ± 0.07 mg/dL, $p = 0.006$) as well as lathosterol (+0.11 ± 0.16 mg/dL, $p = 0.012$). Cholesterol serum levels, however, were not changed significantly (+18.68 ± 32.6 mg/dL, $p = 0.052$). These findings could not be verified in isolated circulating monocytes. Moreover, there was no effect on monocyte activation and no differences with regard to redox state after plant sterol supplemented diet. Therefore, in a population of healthy volunteers with no or mild hypercholesterolemia, consumption of plant sterol ester supplemented margarine results in increased concentrations of plant sterols and cholesterol synthesis markers without affecting total cholesterol in the serum, activation of circulating monocytes or redox state.

1. Introduction

Hypercholesterolemia is a risk factor for cardiovascular diseases and therefore a major target for primary and secondary prevention [1,2]. Plant sterols have been added in different food matrixes to serve as cholesterol lowering agents for many years. Clinical trials have demonstrated that a diet supplementation with 2% of plant sterols or plant stanols confers a plasma LDL-cholesterol (LDL-C) lowering of about 10% [3,4]. Cater and colleagues showed that a daily intake of 2, 3, and 4 g of plant stanols as their esters reduce LDL cholesterol by 12, 13, and 14% [5]. However, clinical interventions show a significant inter-individual variability in the extent of cholesterol reductions and some studies show that not all individuals respond to an equal degree to standard doses of plant sterols [6,7]. This heterogeneity of responsiveness appears to be patient-specific, with individuals showing consistency of lipid level response to a diet supplementation with plant sterols across repeated challenges.

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Table 1
Study design. Sixteen volunteers were randomized either to a 4 week period to a pre-packed margarine supplemented with 3 g of plant sterols (red lable) a day or alternatively to a placebo-margarine (green lable). After a wash-out period of 1 week volunteers switshed groups.

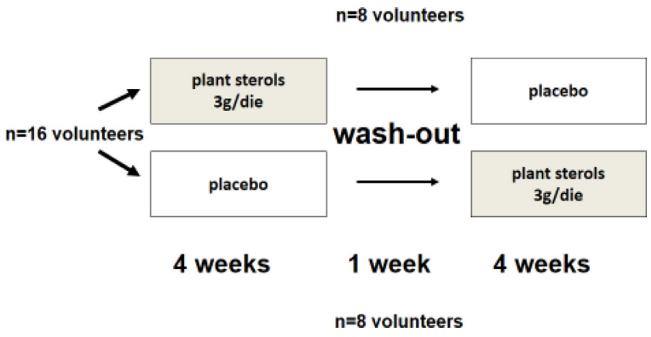

Table 2
Volunteers characteristics.

Age	34.20		
BMI	25. 10		
	mean (n = 16)	female (n = 8)	male (n = 8)
GC–MS			
cholesterol	202 ± 41	194 ± 33	210 ± 49
sitosterol	0.23 ± 0.13	0.27 ± 0.17	0.20 ± 0.08
campesterol	0.31 ± 0.15	0.35 ± 0.20	0.27 ± 0.09
cholestanol	0.29 ± 0.06	0.30 ± 0.07	0.29 ± 0.05
lathosterol	0.39 ± 0.20	0.34 ± 0.25	0.44 ± 0.13
desmosterol	0.19 ± 0.07	0.16 ± 0.08	0.21 ± 0.05
Enzymatic			
Cholesterol	205 ± 42	194 ± 45	216 ± 38
LDL-C	121 ± 40	102 ± 38	141 ± 34
HDL	66 ± 18	77 ± 18[a]	55 ± 11
Triglycerides	118 ± 51	100 ± 39	136 ± 58

Volunteer characteristics are displayed in Table 2.
[a] Difference $p < 0.05$.

In this prospective study we enrolled healthy volunteers with no or only mild hypercholesterolemia who were not on any lipid-lowering medications. We analysed concentrations of sterols in serum and in circulating monocytes before and after a diet supplementation with 3 g of plant sterol esters and assessed the effects on monocyte activation, subpopulations and redox state.

2. Materials and methods

2.1. Study population and study design

The protocol was registered at Clinical-Trials.gov (Identifier NCT00928616) and approved by the ethics committee of the Saarland, Germany (number 159/07). Sixteen subjects (8 males, 8 females) with normal or only mildly elevated serum cholesterol levels were included in the study. *See also* Table 1 *"Study design"*. The presence of angina pectoris, inflammatory gastrointestinal disease, diabetes mellitus, lipid-lowering medication, or consumption of functional foods were exclusion criteria. The volunteers' characteristics and their baseline laboratory values are outlined in Table 2. The study was a prospective, randomized, double-blind, placebo-controlled, crossover study. The volunteers were on their usual diet throughout the entire study period. Volunteers were randomized to margarine with plant sterol esters or placebo for a time period of 4 weeks. A detailed lipid composition of the margarines is provided in Table 3. After a wash out period of one week, volunteers switched groups. The margarines were provided by RAISIO (Turku, Finland). Routine laboratory measurements were taken to ensure normal health before entry. Both at baseline and after 4 weeks, routine laboratory measurements, total cholesterol, LDL-C, HDL-C, TGs, non-cholesterol sterols and monocyte subpopulations in serum were determined. Moreover, total cholesterol, non-cholesterol sterols and oxidative stress were assessed in circulating monocytes.

2.2. Sterol and oxysterol extraction from monocytes

The cells were diluted in 200 μl phosphate-buffered saline. Fifteen microliters were taken for protein analysis. Ten μl epicoprostanol (stock solution: 100 μg/mL in ethanol) and 25 μl 5α-cholestane (stock solution: 1 mg/mL in ethanol) were added to the remaining cell solution prior to alkaline hydrolysis. Free sterols were extracted by cyclohexane and derivatized to trimethylsilyl ethers as described previously [8,9].

2.3. Sterol and oxysterol analysis in serum and in monocytes

Serum sterols were extracted and derivatized to trimethylsilyl ethers as described previously [8,9]. Serum cholesterol was quantified using gas chromatography-flame ionization detection (GC-FID) and non-cholesterol sterols such as plant sterols and cholesterol precursors as well as cholesterol in monocytes by GC-mass spectrometry-selected ion monitoring [8,9]. Moreover, total cholesterol, LDL-C, HDL-C and triglycerides were determined enzymatically.

Table 3
Margarine composition.

	[μg/mg] cholestanol	[μg/mg] campesterol	[μg/mg] campestanol	[μg/mg] stigmasterol	[μg/mg] sitosterol	[μg/mg] sitostanol
green-labeld margarine	0,017	0,944	0,011	0,020	1,482	0,027
red-labeled margarine	0,020	24,162	3,752	0,472	102,602	30,463

Detailed lipid composition of placebo and plant sterol margarine.