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Basic Research

Changes in intestinal and liver global gene expression in response to a phytosterol-enriched diet

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Abstract

Background: Dietary phytosterols are a recommended therapeutic option for decreasing plasma cholesterol. The increased activity of ATP-binding cassette (ABC) transporters ABCA1, ABCG5 and ABCG8, or, alternatively, a decrease in Niemann–Pick C1 Like 1 (NPC1L1) could mediate the reduction in intestinal cholesterol absorption caused by phytosterols. Other biological properties such as a direct immune modulatory activity have recently been ascribed to these plant compounds.
Methods: To gain insight into the molecular effects of phytosterols, global genome-wide gene profiling and real-time RT-PCR studies were conducted in small intestines and livers of phytosterol-treated apolipoprotein E-deficient (apoE$^{-/-}$) mice. Re-testing of the main results was performed in C57BL/6J and LDL receptor-deficient (LDLR$^{-/-}$) mice.
Results: Intestinal cholesterol absorption was decreased in all mouse models but plasma cholesterol was only decreased in apoE$^{-/-}$ and LDLR$^{-/-}$ mice. ABCA1, ABCG5, ABCG8 and NPC1L1 mRNA levels were slightly reduced in the intestine of phytosterol-treated apoE$^{-/-}$ and LDLR$^{-/-}$ mice, but increased in C57BL/6J-treated mice. Phytosterols changed genes involved in immune regulation in apoE$^{-/-}$ mice. However, these changes were less extensive in LDLR$^{-/-}$ mice and were not found in C57BL/6J mice.
Conclusions: Inhibition of intestinal cholesterol absorption by phytosterols is not mediated via transcriptional changes in ABCA1, ABCG5, ABCG8 or NPC1L1. Changes suggestive of immunomodulation are associated with the hypocholesterolemic effect of phytosterols and with apoE deficiency.

Keywords: Phytosterols; Cholesterol; ATP-binding cassette transporter; Atherosclerosis; Immune system; Cancer

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG5, ATP-binding cassette transporter G5; ABCG8, ATP-binding cassette transporter G8; ALT, alanine aminotransferase; apoE, apolipoprotein E; apoE$^{-/-}$, apoE-deficient mice; ETV6, ETS variant gene 6; FDPS, farnesyl diphosphate synthase; FoxQ1, forkhead box Q1; FPLC, fast protein liquid chromatography; GC–MS, gas–liquid chromatography–mass spectrometry; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; IgK-V, immunoglobulin kappa chain variable; LDL, low-density lipoprotein; LDLR$^{-/-}$, LDL receptor-deficient mice; LFC, limit fold change; LXR, liver X receptor; MARCO, macrophage receptor with collagenous structure; NCEP, National Cholesterol Education Program; NPC1L1, Niemann–Pick C1 Like 1; Reg, regenerating islet-derived; RT-PCR, reverse-transcriptase polymerase chain reaction; SREBP, sterol regulatory element binding protein; SAA3, serum amyloid A3; VLDL, very-low-density lipoprotein

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1. Introduction

Phytosterols, and their saturated forms known as stanols, are the most abundant plant sterols. The cholesterol-lowering effect of phytosterols has been demonstrated in both humans and animals [1,2] and the most recent guidelines for cholesterol management of the National Cholesterol Education Program (NCEP) encouraged plant sterol/stanol consumption as a therapeutic dietary option to decrease LDL cholesterol [3]. Further, phytosterols can now be acquired in many countries without medical prescription. Thus, full understanding of their effects is highly desirable.

Competition between plant sterols and intestinal cholesterol for incorporation into mixed micelles has been proposed as the mechanism underlying the hypocholesterolemic effect of plant sterols [4]. However, one of the potential explanations for this competition, the cocrystallization of cholesterol and phytosterols or phystostanols in the intestinal lumen has been recently shown to be unlikely [5]. Further, recent studies have suggested that plant sterols may exert an unknown molecular action inside enterocytes and hepatocytes, especially considering that plant sterols/stanols do not need to be present in the intestinal lumen simultaneously with cholesterol to inhibit its absorption [6]. Recently, several adenosine triphosphate binding cassette (ABC) transporters have been proposed as carriers exchanging cholesterol and phytosterols in intestine and liver and sitostanol treatment of Caco-2 cells has been shown to increase ABCA1 expression [7]. On the other hand, mutations in ABCG5 and ABCG8 genes cause sitosterolemia, a rare autosomal recessive disorder characterized by elevated plasma levels and tissue accumulation of both plant and animal sterols [8]. As ABCA1, ABCG5 and ABCG8 genes are targets of liver X receptor (LXR) [9,10] and the overexpression of ABCG5 and ABCG8 in mice promotes biliary cholesterol secretion and reduces dietary cholesterol absorption [11], phytosterols or their derivatives could act as LXR ligands [12]. Also recently, Niemann–Pick C1 Like 1 (NPC1L1) protein has been shown to play a critical role in the absorption of intestinal cholesterol [13,14]. Ezetimibe, a drug that inhibits cholesterol absorption acting through the NPC1L1 pathway decreases the levels of plant sterols in patients with sitosterolemia [15]. Thus, dietary phytosterols could act also reducing the intestinal expression of NPC1L1.

Although most studies have focused on the cholesterol-lowering activity of phytosterols, other biological properties such as immunomodulation have been ascribed to these compounds [16,17]. β-Sitosterol and its glucoside act as potentially positive immune modulators in humans by increasing cytokines derived from T_{H1} helper cells [16–18]. Interestingly, LXR-dependent gene expression has been shown to play a role in the innate immune response [19]. Moreover, experimental studies suggest that phytosterols could protect against common cancers [20–23]. Possible mechanisms of protection include effects on membrane structure/function, signal transduction and immune function [21,24].

Apolipoprotein E-deficient (apoE$^{-/-}$) mice have been used as an animal model to show the antihypercholesterolemic and antiatherogenic effects of phytosterols [25,26]. In the present study, microarray-based technology and quantitative real-time RT-PCR were used to identify phytosterol-regulated genes in intestine and liver of apoE$^{-/-}$ mice and the main findings were re-tested in C57BL/6J and in LDL receptor-deficient (LDLR$^{-/-}$) mice.

2. Materials and methods

2.1. Mice and diets

All animal procedures were in accordance with published recommendations for the use of laboratory animals [27]. Use of apoE$^{-/-}$ mice with a C57BL/6J background has been described previously [28]. Mice were maintained in a temperature-controlled (20 °C) room with a 12-h light:12-h dark cycle and food and water were provided ad libitum. Eight to ten-week-old female mice were randomized in four groups and fed either a control Western-type diet (200 g/kg fat, polyunsaturated/saturated = 0.07, 0.8 g/kg cholesterol, 170 g/kg protein, 105 g/kg fiber; Mucedola srl, Settimo Milanese, Italy) or a 0.5, 1 or 2% phytosterol-enriched Western-type diet (w/w) for 4 weeks. Homozygous LDLR$^{-/-}$ in C57BL/6J background and wild-type C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbour, ME) and were fed either a control Western-type diet or a 2% phytosterol-enriched Western-type diet (w/w) for 4 weeks. The phytosterol product was composed of 20% campesterol, 22% stigmasterol and 41% β-sitosterol (Lipofoods, S.L., Barcelona, Spain).

2.2. Lipid analyses of plasma, liver, small intestine, bile and stools

The methods used for plasma and liver lipid analyses have been described in detail elsewhere [28,29]. α-Tocopherol, α-carotene, β-carotene and retinol plasma content were quantified by reverse-phase HPLC [30]. Plasma and tissue phytosterols were analyzed by gas–liquid chromatography–mass spectrometry (GC–MS) [31]. Liver and intestine mixtures were extracted with isopropyl alcohol–hexane (2:3, v/v) and quantitative results were obtained by GC–MS and single ion monitoring; the m/z^+ values used were those described elsewhere [32].

Bile was removed from the gallbladders of anesthetized mice using a 30.5-gauge needle. Concentrations of cholesterol and phospholipids were determined enzymatically using commercial kits adapted to a BM/HITACHI 911 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Bile acids were measured by the 3α-hydroxysteroid dehydrogenase method (Sigma Diagnostics, St. Louis, MO, USA). Gallbladder, liver and small intestine bile acids were measured and the bile acid pool size was calculated as the sum of all bile acids.