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Dietary plant sterols accumulate in the brain

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Abstract

Dietary plant sterols and cholesterol have a comparable chemical structure. It is generally assumed that cholesterol and plant sterols do not cross the blood–brain barrier, but quantitative data are lacking. Here, we report that mice deficient for ATP-binding cassette transporter G5 (Abcg5) or Abcg8, with strongly elevated serum plant sterol levels, display dramatically increased (7- to 16-fold) plant sterol levels in the brain. Apolipoprotein E (ApoE)-deficient mice also displayed elevated serum plant sterol levels, which was however not associated with significant changes in brain plant sterol levels. Abcg5- and Abcg8-deficient mice were found to carry circulating plant sterols predominantly in high-density lipoprotein (HDL)-particles, whereas ApoE-deficient mice accommodated most of their serum plant sterols in very low-density lipoprotein (VLDL)-particles. This suggests an important role for HDL and/or ApoE in the transfer of plant sterols into the brain. Moreover, sitosterol upregulated apoE mRNA and protein levels in astrocytoma, but not in neuroblastoma cells, to a higher extend than cholesterol. In conclusion, dietary plant sterols pass the blood–brain barrier and accumulate in the brain, where they may exert brain cell type-specific effects.

Keywords: Cholesterol; Plant sterol; Campesterol; Sitosterol; Apolipoprotein E; ABCG5; ABCG8

1. Introduction

Plant sterols comprise a group of sterols that enter the mammalian body only via the diet. Relatively large amounts of plant sterols are present in plant oils, nuts, and avocados. The structure of plant sterols is very similar to that of cholesterol. They differ from cholesterol only by an additional methyl-group (campesterol) or ethyl-group (sitosterol) at the C24-position, or by an additional double bond at the C22-position (brassicasterol, stigmasterol, respectively). Yet, this small structural difference leads to very divergent metabolic fates of plant sterols and cholesterol in mammals [1]. Both cholesterol and plant sterols are internalized by intestinal mucosa cells via the Niemann–Pick C1-Like1 (NPC1L1)-transporter [2]. Cholesterol is transported to the endoplasmic reticulum, where it is esterified by the action of acyl-CoA:cholesterol O-acyltransferase 2 (Acat2) for incorporation into chylomicrons [3]. However, plant sterols are poor Acat2 substrates and hence are transported back to the luminal membrane to be re-secreted into the lumen of the intestine by the heterodimeric ATP-binding cassette (Abc) half transporters g5 and g8 [4]. Abcg5 and Abcg8 are present at the apical membrane of enterocytes and are also expressed in liver [5]. In the liver, the Abcg5/Abcg8 transporter mediates efflux of plant sterols and cholesterol into bile [6,7]. The formation of this heterodimer becomes impossible in the absence of either Abcg5 or Abcg8 and thus leads to a dysfunction of the transporter system. Mutations in the genes encoding either of these half transporters lead to sitosterolemia, an inborn error of metabolism characterized

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by high levels of sitosterol, campesterol and stigmasterol [5,7,8]. It has been reported previously that Abcg5-deficient mice display massively elevated serum levels of campesterol and sitosterol and ~50% lower serum cholesterol levels compared to wild-type mice [7]. Mice deficient for both Abcg5 and Abcg8 also display dramatically elevated circulating levels of campesterol and sitosterol as well as reduced serum cholesterol levels as compared to wild-type mice [8].

It is widely assumed that, in contrast with other tissues, the brain does not retrieve cholesterol from the circulation, because this is normally prevented by the blood–brain barrier (BBB), and instead synthesizes all its cholesterol in situ [9]. Yet, indications of close communications between extracerebral and brain cholesterol have been reported previously [10]. In line with the fact that the structure of plant sterols is very similar to that of cholesterol, and the subsequent assumption that they do not seem to enter the brain, only very low levels of plant sterols in brain have been reported [8,11]. However, consequences of high dietary plant sterol intake on brain plant sterol levels have not been reported so far.

In the present study, we have determined levels of cholesterol and plant sterols (campesterol and sitosterol) in serum and brain of wild-type mice that were fed either a normal chow diet or a diet rich in plant sterols for 4 weeks. Interestingly, we observed a small but significant increase in brain campesterol levels in mice fed the plant sterol-enriched diet. Subsequently, we examined three mouse models with strongly elevated serum plant sterol levels, i.e., Abcg5-deficient mice, Abcg8-deficient mice and apolipoprotein E (ApoE)-deficient mice [4,5,7]. Surprisingly, we found that high plant sterol levels in serum were associated with significantly elevated plant sterol levels in brain of Abcg5- and Abcg8-deficient mice, but not in brain of ApoE-deficient mice. Plant sterols have been reported to affect HMG-CoA reductase activity, although the results are inconsistent [12,13]. Therefore, we investigated the effect of plant sterols on total brain cholesterol metabolism in vivo and their effects on ApoE protein and mRNA levels in vitro, using astrocytoma and neuroblastoma cells. Our results suggest that an accumulation of plant sterols in the brain may exert brain cell type-specific effects and as a consequence may affect brain functioning.

2. Materials and methods

2.1. Animals

2.1.1. Experiment 1

Twelve male, three month old C57BL/6J mice were purchased from Harlan (Horst, The Netherlands). Animals were housed in temperature-controlled rooms (21 °C) with 12-h light cycles and received a semisynthetic diet and water ad libitum. All animals were fed the semisynthetic diet for 2 weeks (run-in period). Afterwards, mice were assigned to two treatment groups (6 animals per group) and were fed the specific diets (control or plant sterol-rich diet) for 4 weeks. Analysis of sterol composition revealed that the control diet contained 0.03% (w/w) sterols, mainly beta-sitosterol (53.8% w/w of all sterols). The plant sterol enriched diet contained 0.12% cholesterol (w/w) and 0.83% plant sterol esters (w/w); plant sterol esters were mainly beta-sitosterol, campesterol and stigmasterol (36.5%, 20.4% and 14.9%, respectively).

2.1.2. Experiment 2

Male Abcg5-deficient mice ($n=10$), Abcg8-deficient mice ($n=5$) and apoE-deficient mice ($n=7$) and their respective littermate controls were maintained on a standard laboratory chow diet and were allowed to eat and drink ad libitum. The Abcg5-deficient mice and littermate control mice were generated by Deltagen (Redwood City, CA, USA) and were fed a normal chow diet (Arie Blok, Woerden, The Netherlands) as described by Plösch et al. [7]. The Abcg8-deficient mice and littermate control mice were generated as described by Klett et al. [14]. The ApoE-deficient mice (Breslow mouse line) were obtained from Charles River (Someren, The Netherlands). The three different mouse models were from three different laboratories and sterol profiles were therefore examined in littermate controls from the respective institute. All animal procedures were performed with approval of the respective institutional ethical committees for the use of experimental animals of the Universities of Groningen, Amsterdam and Maastricht, according to governmental guidelines.

2.2. Tissue sample preparation

The mice were anaesthetised with Nembutal (CEVA, Maassluis, The Netherlands) in a dose of 180 μg/g body weight. In the Abcg5- and ApoE-deficient mice the whole body was perfused with phosphate buffered saline (137 mM NaCl; 2.7 mM KCl; 6.5 mM Na_2HPO_4; 1.5 mM KH_2PO_4; pH=7.3) (PBS) [15]. Blood was collected before perfusion and spun at 1400 rpm to obtain serum or plasma, which was snap frozen in liquid nitrogen. The Abcg8-deficient mice were not perfused. However, brains were carefully rinsed in PBS and peripheral blood was collected by puncturing of the heart. The brain hemispheres of all mice were immediately shock-frozen in liquid nitrogen, while submerged in isopentane, and stored at −80 °C. Prior to sterol analysis, brains were spun in a speed vacuum dryer (Savant AES 1000) at 12 mbar for 48 h to relate individual sterol concentrations to dry weight. The sterols were extracted from the dried tissues by placing them in a 5-ml mixture of chloroform–methanol (2:1) for 48 h.

2.3. Sterol profile determination

One ml of the brain sterol extracts was evaporated to dryness under a stream of nitrogen at 63 °C, and hydrolyzed for 1 h with 1 ml of 1 M NaOH in 90% ethanol at 50 °C. One ml of distilled water was added to the samples. To extract the neutral sterols 3 ml of cyclohexane was added twice. The combined cyclohexane phases were again evaporated to dryness under a stream of nitrogen at 63 °C, and the sterols were dissolved in 100 μl n-decane. After transfer to gas-chromatography (GC)-vials, the sterols were converted to trimethylsilylethers (TMSis) by adding 40 μl of TMSi reagent (pyridine–hexamethyldisilazan–trimethylchlorosilane, 9:3:1 v/v/v) and incubated at 60 °C for 1 h [11]. The sterols were extracted from 100 μl serum by the same protocol. Levels of cholesterol were determined in a gas-chromatograph-flame ionization detector (GC-FID) with fifty μl 5α-cholestane-solution (1 mg/ml 5α-cholestane in cyclohexane) as internal standard. Levels of plant sterols (campesterol, sitosterol), cholesterol precursors (lanosterol, lathosterol and desmosterol) and cholesterol metabolites (24S-OH-cholesterol and cholestanol) were determined using gas chromatography-mass spectrometry (GC-MS) as described previously [11] using epicoprostanol as internal standard.

2.4. FPLC

We determined the lipoprotein fractions in serum of ApoE-deficient mice ($n=4$), and plasma of Abcg5-deficient mice ($n=3$) and Abcg5-wild type controls ($n=5$) using fast protein liquid chromatography (FPLC). Equal volumes of serum or plasma (50 μl) were injected on a Superose 6 HR 10/30 column (Amersham Biosciences), which was connected to an AKTA Basic FPLC system (Amersham Biosciences). Lipoproteins were eluted at a constant flow rate of 0.05 ml/min with Dulbecco's phosphate buffered saline (Sigma-Aldrich, St. Louis, U.S.A.) containing 1 mM EDTA. Fractions 8–14 corresponded to VLDL-, 15–22 to LDL- and 23–31 to HDL-sized particles. These fractions were pooled for the different genotypes and analysed with GC-MS for cholesterol and plant sterol levels.

2.5. Cell culture

The human astrocytoma cell line CCF-STTG1 and human neuroblastoma cell line SH-SY-5Y were purchased from ECACC (Sallisbury, UK). Both cell