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Plasma cholesterol-raising potency of dietary free cholesterol versus cholesteryl ester and effect of β-sitosterol

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ABSTRACT

The present study (i) compared plasma cholesterol-raising activity of free cholesterol (FC) with that of cholesteryl palmitate (CP) and (ii) examined plasma cholesterol-reducing activity of β-sitosterol in FC-induced and CP-induced hypercholesterolemia. Male hamsters were divided into five groups and fed one of the five diets containing no cholesterol (NC), 2.6 mmol cholesterol (C), 2.6 mmol cholesterol plus 2.6 mmol β-sitosterol (C+S), 2.6 mmol cholesteryl palmitate (CP), and 2.6 mmol CP plus 2.6 mmol β-sitosterol (CP+S), respectively, for 8 weeks. Hamsters fed diet C had plasma TC of 317.5 mg/dl whereas hamsters fed diet CP has plasma TC of 281.3 mg/dl. β-Sitosterol reduced plasma TC by 17.4% and 11.6%, respectively, in FC-induced and CP-induced hypercholesterolemia (not significant). It was concluded that plasma cholesterol-raising activity of dietary cholesterol was a function of its chemical forms in diet, and β-sitosterol could similarly suppress the hypercholesterolemia induced by both dietary FC and CP.

1. Introduction

It is well documented that individuals with elevated concentrations of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) as well as reduced concentration of high-density lipoprotein cholesterol (HDL-C) have a higher risk of developing coronary heart disease (Daniels, Killinger, Michal, Wright, & Jiang, 2009; Sharrett et al., 2001). It is recommended that daily cholesterol consumption shall not be more than 300 mg per person. On an average, dietary surveys have demonstrated that humans consume cholesterol ranging 200–500 mg per day (Centers for Disease Control, 2000; Elmadfa & Weichselbaum, 2005; Food & Nutrition Department, 1994; Schmidhuber, 2007; Woo, Leung, Ho, Lam, & Janus, 1998; Zhao et al., 2009). Cholesterol in human diet exists in two forms, namely free cholesterol (FC) and cholesteryl esters (CE), with the latter accounting for approximately up to 30% total cholesterol (Awad, Bennink, & Smith, 1997; Bitman & Wood, 1980). Although adverse effect of dietary cholesterol on cardiovascular health has been extensively investigated, the effect of dietary CE versus dietary FC on plasma TC has not been fully explored.

Phytosterols have become popular as a health supplement in reducing plasma TC and LDL-C (Lingberg et al., 2008; Vanstone, Raeini-Sarjaz, Parsons, & Jones, 2002). In the intestinal lumen, they displace cholesterol from mixed micelles and inhibit cholesterol absorption due to their poor absorption and structural similarity with that of cholesterol (Smet, Mensink, & Plat, 2012). In humans, about 40–70% dietary cholesterol can be absorbed whereas only 1–2% dietary phytosterols is absorbed. Both cholesterol and phytosterols can enter enterocytes via transporter Niemann–Pick C1 like 1 (NPC1L1). However, phytosterols are prevented from being further absorbed because the ATP binding cassette transporters (ABCG5/8) return them to the lumen of the intestine (Ostlund & Lin, 2006). Intestinal acyl CoA: cholesterol acyltransferase (ACAT2) is responsible for conversion of FC to CE. Subsequently, CE is packed into chylomicrons for absorption by microsomal triglyceride protein (MTP). In this regard, ACAT2 prefers cholesterol to phytosterol for esterification, preventing phytosterols from the absorption (Temel, Gebre, Parks, & Rudel, 2003). Research has demonstrated plasma TC-lowering activity of phytosterol is mediated by not only its competition for incorporation into micelles but also its inhibition in gene expression of intestinal NPC1L1 (Jesch, Seo, Carr, & Lee, 2009). However, it remains unexplored how dietary phytosterols interact with dietary CE versus dietary FC in the intestinal absorption process.

β-Sitosterol is the major phytosterol while cholesteryl palmitate (CP) is the major CE in human diets in addition to free cholesterol. The present study was designed to (i) compare plasma TC-raising potency of dietary CP with that of dietary FC; and (ii) compare

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plasma TC-suppressing activity of β-sitosterol in dietary FC-induced hypercholesterolemia compared with that in CP-induced hypercholesterolemia.

2. Materials and methods

2.1. Diets

Diets were prepared by modifying the formulation as we had previously described (Jiao et al., 2013). The basal diet containing no cholesterol (NC) was prepared by mixing the following ingredients (per kilogram diet): cornstarch, 508 g; casein, 242 g; sucrose, 119 g; lard, 50 g; mineral mix 40 g; vitamin mix 20 g; gelatin, 20 g; DL-methionine, 1 g. The four experimental diets were prepared by adding 2.6 mmol of free cholesterol (C), 2.6 mmol free cholesterol plus 2.6 mmol of β-sitosterol (C+S), 2.6 mmol of cholesteryl palmitate (CP), and 2.6 mmol of cholesteryl plamitate plus 2.6 mmol of β-sitosterol (CP+S), into the basal diet, respectively (Table 1).

2.2. Hamsters

Male Golden Syrian hamsters ($n = 56$) were divided into five groups with NC having 8 hamsters and the other groups having 12 hamsters each. All hamsters were housed in an animal room at 23 °C with 12/12-h light–dark cycles. The fresh diets were given to the hamsters daily, and uneaten food was discarded. Food intake was measured daily and body weight was recorded twice a week. The hamsters were allowed free access food and water. Each hamster was bled from the retro-orbital sinus into a heparinized capillary tube under light anaesthesia using a mixture of ketamine, xylazine and saline (v/v/v; 4:1:5) after overnight fasting, respectively, at week 0 and 8. The blood was be centrifuged at 2300*g* for 10 min and the plasma was collected. At the end of week 8, all the hamsters were killed using carbon dioxide anaesthesia; the abdomen was cut open with blood being sampled from the aorta into syringe. The liver was then removed, washed with saline, weighed and frozen in liquid nitrogen. The first 5 cm of duodenum was discarded, and the next 30 cm of the small intestine was kept. All samples were stored at a −80 °C freezer prior to cholesterol analysis. All the faeces from each hamster were also collected and pooled at week 1 and 8 followed by being freeze-dried, ground and saved for neutral and acidic sterol analyses. Experiments were conducted following the approval and in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong.

2.3. Analysis of plasma lipids

Plasma TC and total triacylglycerols (TG) were measured using the commercial enzymatic kits from Infinity (Waltham, MA) and Stanbio Laboratories (Boerne, TX), respectively. For measurement of HDL-C, non-HDL cholesterol (non-HDL-C) was firstly precipitated with phosphotungstic acid and magnesium chloride using a commercial kit (Stanbio Laboratories) and HDL-C in the supernatant was determined similarly as for TC (Jiao et al., 2013).

Table 1
Composition of the five diets containing no cholesterol (NC), 2.6 mmol cholesterol (C), 2.6 mmol cholesterol plus 2.6 mmol β-sitosterol (C+S), 2.6 mmol cholesteryl palmitate (CP), and 2.6 mmol CP plus 2.6 mmol β-sitosterol (CP+S).

Ingredients (per kg diet)	NC	C	C+S	CP	CP+S
Corn starch (g)	508	508	508	508	508
Casein (g)	242	242	242	242	242
Sucrose (g)	119	119	119	119	119
Lard (g)	50	50	50	50	50
Mineral mixture AIN-76 (g)	40	40	40	40	40
Vitamin mixture AIN-76A (g)	20	20	20	20	20
Gelatin (g)	20	20	20	20	20
DL-Methionine (g)	1	1	1	1	1
Cholesterol (mmol)	0	2.6	2.6	0	0
Cholesteryl palmitate (mmol)	0	0	0	2.6	2.6
β-Sitosterol (mmol)	0	0	2.6	0	2.6

2.4. Determination of cholesterol in the liver

Hepatic cholesterol was determined according to the method as we previously described (Liang et al., 2011). Total lipids were extracted into chloroform:methanol (2:1, v/v) followed by saponification. Cholesterol in the fraction of non-saponifiable substances was converted to its TMS-ether derivative by a commercial TMS reagent (Sigma-Sil-A; Sigma). The analysis of cholesterol TMS-ether derivative was performed in a SAC^{TM}-5 column (Bellefonte, USA) using a Shimadzu GC-14 B GLC equipped with a flame ionisation detector. Hepatic cholesterol was quantified according to the amount of internal standard 5α-cholestane added during the extraction.

2.5. Determination of faecal neutral and acidic sterols

Neutral and acidic sterols in the faeces were quantified as we previously described (Chan et al., 1999). Total faecal sample from each hamster was freeze-dried, ground and well mixed. Faecal sample (300 mg) was weighed and then saponified. The total neutral sterols were extracted into cyclohexane and then were converted to their corresponding TMS-ether derivatives for GC analysis. The remaining aqueous layer was saponified using NaOH, then neutralise with HCl and extracted with diethyl ether twice. The acidic sterols were similarly converted to their TMS-ether derivatives for GC analysis. Total and individual neutral and acidic sterols were quantified according to internal standards 5α-cholestane and hyodeoxycholic acid added into the faecal sample during the extraction.

2.6. Measurement of atherosclerotic plaque

The atherosclerotic plaque in aorta was determined as previously described (Chan et al., 1999). In brief, aorta artery was cut opened vertically and then stained with 0.05 g oil red in 1 ml isopropanol for 30 min. The endothelial layer of aorta was washed with isopropanol and distiled water for 3 times and scanned with a table scanner. The area of atherosclerotic plaque was measured with the aid of computer images analysing program "Sigma Scan Pro 5.0" (SPSS, Inc., Chicago, USA).

2.7. Measurement of mRNA of intestinal NPC1L1, ABCG5, ABCG8, ACAT2 and MTP

Real-time PCR was employed to quantify mRNA of intestinal NPC1L1, ABCG5/8, ACAT2, and MTP (Ma et al., 2011). Total intestinal mRNA was extracted and converted to complementary DNA (cDNA) using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Reverse transcription was carried out in a thermocycler (Gene Amp® PCR system 9700, Applied Biosystems). Real-time PCR analysis was carried out on a Fast Real-time PCR System 7500 (Applied Biosystems). To measure intestinal NPC1L1, ABCG5, ABCG8, ACAT2, MTP and Cyclophilin, SYBR green was used as a fluorophore. Data were analysed using the Sequence Detection Software version 1.3.1.21 (Applied Biosystems).

2.8. Statistics

Data were expressed as mean ± standard deviation (SD). Following the analysis of variance (ANOVA), post hoc LSD test was