Fortification of vitamin A in a phytosterol enriched milk maintains plasma beta-carotene levels

Maria Petrogianni · Spyridon Kanellakis ·
George Moschonis · Yannis Manios

Revised: 6 April 2013 / Accepted: 8 July 2013 / Published online: 4 August 2013
© Association of Food Scientists & Technologists (India) 2013

Abstract The aim of the current study was to investigate if the supplementation of vitamin A via a phytosterol enriched low-fat milk could prevent the reduction of plasma beta-carotene concentrations than often occurs after plant sterols' or stanols' intake. A sample of 108 hypercholesterolaemic adults (40–60 years old) was randomized to an enriched milk group that contained among other nutrients phytosterols (0.5 g/100 ml) and vitamin A (111 µg/100 ml) (EMG: $n=40$), a placebo plain milk group (PMG: $n=37$), and a control group (CG: $n=31$) following their usual diet; the EMG and the PMG consumed 500 ml milk per day and in order to ensure compliance with the intervention scheme, attended health and nutrition counselling sessions biweekly over a 3-month period. Dietary intake of vitamin A significantly increased in the EMG compared to the PMG and the CG ($P<0.001$) and all groups significantly increased their vegetable consumption. However, no significant differences were found among groups regarding changes in dietary intake of beta-carotene and consumption of fruits. Regarding biochemical indices, total cholesterol, LDL-cholesterol and apolipoprotein-B decreased significantly within all study groups, with the decreases being significantly higher in the EMG compared to the CG (−25.4 vs. −9.9; −21.7 vs. −8.2 and −13.2 vs. −3.4 mg/dl, respectively; $P<0.05$). Plasma concentrations of beta-carotene did not change in any of the three groups. Extra fortification of a phytosterol enriched milk with vitamin A seems to be useful in maintaining plasma beta-carotene levels of hypercholesterolemic adults after consumption of 2.5 g/d of phytosterols over a 3-month intervention period.

Keywords Beta-carotene · Enriched milk · Phytosterols · Vitamin A

Introduction

Although intervention schemes providing food-products enriched with plant sterols have shown an important hypocholesterolemic effect, they have also been found to reduce plasma levels of carotenoids (Tikkanen 2005). This is an undesirable effect since carotenoids have a significant antioxidant effect and they have been associated with a decreased risk of cancer and cardiovascular disease (Voutilainen et al. 2006), while they are also involved in the pathway of vitamin A synthesis (Colgan et al. 2004). On the other hand in other intervention studies, motivation of study participants that consumed fortified food products to also increase their fruits and vegetable consumption, has been proven effective in preserving plasma carotenoids levels (Ntanos and Duchateau 2002) at a desirable normal range. Although food legislation and practical issues do not allow enrichment of milk with carotenoids, fortification of milk with vitamin A might be a practical alternative to overcome this side-effect of plant sterols.

A secondary aim of the current intervention study, initially intended to examine the changes on CVD risk factors (Petrogianni et al. 2012), was to explore whether the supplementation of vitamin A via a phytosterol enriched low-fat milk could prevent the reduction of plasma beta-carotene concentrations that often occurs after plant sterols' or stanols' intake.

Materials and methods

Two screenings phases identified 115 volunteers that were considered eligible to participate in the study. Inclusion

M. Petrogianni · S. Kanellakis · G. Moschonis · Y. Manios (✉)
Department of Nutrition and Dietetics, Harokopio University,
Athens, Greece
e-mail: manios@hua.gr

criteria were age 40–60 years, BMI <35 kg/m², TC >200 but <310 mg/dl, and no use of lipid-lowering medications as well as absence of metabolic disorders other than hypercholesterolemia (i.e., diabetes, thyroid, renal, and hepatic disease). The experimental protocol was approved by the Ethics Committee of Harokopio University and all subjects signed informed consents. Subjects were randomized in one of three intervention arms, with the intervention period lasting for 3 months: i) a group consuming low fat milk also enriched with phytosterols (2.5 g per day) and vit-A (555 µg per day) (EMG, *n*=41); ii) a group consuming the same labelled product that was a plain regular low-fat milk (PMG, *n*=38); iii) a control group (CG, *n*=36). Subjects in the EMG and PMG were instructed to consume two portions (250 ml each) of milk daily. To ensure compliance with the intervention scheme, health and nutrition counselling sessions were also held biweekly. The CG received no milk and

attended no sessions. Subjects in the EMG and PMG attended the sessions together in small groups of five to seven persons. Product bottles were encoded with white and red lids blinded for both researcher and subjects. The study products were all packaged in 1000 ml bottles and produced by FrieslandCampina, Netherlands. Compliance to the milk consumption was examined via food consumption diaries filled in and returned by study participants to the research group and was found to be about 80 %.

Dietary intake data were collected at baseline and follow-up with the use of three 24-h recalls respectively as described elsewhere (Manios et al. 2006). Fasting TC, high-density lipoprotein cholesterol (HDL-C) and triglycerides were determined by photometric method. Apolipoprotein-B (apo-B) concentrations were determined by an immunoturbidimetric method. All analyses were carried out by an automated centrifugal analyzer with commercially available assays (Roche/Hitachi

Table 1 Comparisons of changes in beta-carotene serum levels and lipid profile between Enriched Milk Group (*n*=40), Placebo Milk Group (*n*=37) and Control Group (*n*=31) subjects following the 3-month intervention programme

	Baseline Mean ± SD	3 months Mean ± SD	3-month change Mean change (95 %C.I)	<i>P</i> ^a
Total cholesterol (mg/dl)				0.030
CG	255.7±31.1	245.8±32.7	−9.9(−19.2 to −0.7)* [†]	
PMG	251.5±30.8	238.2±36.5	−13.3(−21.8 to −4.8)*	
EMG	250.4±32.0	225.0±28.5	−25.4(−33.6 to −17.3)** [‡]	
P (treatment effect) ^b	0.763	0.027		
LDL cholesterol (mg/dl)				0.018
CG	174.0±31.2	165.8±31.0	−8.2(−16.2 to −0.1)* [†]	
PMG	172.7±29.8	163.6±34.2	−8.1(−16.5 to −1.8)*	
EMG	171.0±27.3	149.3±25.9	−21.7(−28.8 to −14.6)** [‡]	
P (treatment effect)	0.910	0.044		
HDL cholesterol (mg/dl)				0.079
CG	55.3±17.1	54.9±16.9	−0.4(−2.5 to 1.8)	
PMG	53.2±13.7	49.8±12.2	−3.4(−5.4 to −1.4)*	
EMG	56.5±16.8	53.3±13.2	−3.2(−5.1 to −1.3)*	
P (treatment effect)	0.649	0.298		
Triglycerides (mg/dl)				0.925
CG	130.6±56.0	124.7±61.1	−5.9(−19.5 to 7.8)	
PMG	128.1±41.4	123.4±49.3	−4.6(−17.1 to 7.8)	
EMG	113.9±45.0	111.5±48.5	−2.4(−14.4 to 9.7)	
P (treatment effect)	0.264	0.493		
Apolipoprotein B (mg/dl)				0.022
CG	119.1±24.4	115.6±24.0	−3.4(−9.0 to 2.1) [†]	
PMG	119.9±20.7	114.3±24.8	−5.6(−10.7 to −0.5)* [†]	
EMG	115.3±18.3	102.1±17.4	−13.2(−18.1 to −8.3)** [‡]	
P (treatment effect)	0.594	0.017		
beta-carotene (µg/dl) ^c				0.842
CG	37.15±18.17	39.71±22.29	2.55(−3.21 to 8.32)	
PMG	40.74±21.34	43.53±26.32	2.79(−2.48 to 8.07)	
EMG	33.22±17.94	34.02±15.12	0.8(−4.27 to 5.87)	
P (treatment effect)	0.234	0.154		

CG Control Group; PMG Placebo Milk Group; EMG Enriched Milk Group; LDL-cholesterol Low Density Lipoprotein-cholesterol; HDL-cholesterol High Density Lipoprotein-cholesterol

*Significantly different from baseline, *p*<0.05(paired-sample *t* test)

^a *P* values were derived from one-way ANOVA. Means in a column with different symbols ([†], [‡]) are significantly different, *P*<0.05

^b Treatment x time interaction effect

^c Differences remained insignificant also after correction with fruit and vegetable intake

modular system P.800 module, Japan). LDL-C was calculated with the Friedewald equation (Friedewald et al. 1972). Beta-carotene was measured in plasma by HPLC with UV detection method (HPLC System-Chromsystems, Munich Germany) (Cavina et al. 1988).

Seven subjects dropped out for personal reasons. At the end of the study 108 subjects were re-examined i.e. 40 in the EMG, 37 in the PMG and 31 in the CG. No statistically significant differences were observed at baseline values. Data were analysed by using analysis of variance (ANOVA) for repeated measurements with time as within-subject factor (baseline vs. follow-up) and groups as between-subject factor (CG vs. PMG vs. EMG). Statistical analysis was carried out with SPSS 13 statistical software. Statistical significance was set to P -value < 0.05.

Results-discussion

Consumption of products enriched with plant sterols/stanols, in the context of a healthy lifestyle, can reduce LDL-C levels by 10–14 % (Plat and Mensink 2005). Similar to these findings, the current intervention was effective in significantly reducing total and LDL-cholesterol as well as apo-B by 10.1, 12.7 and 11.4 % respectively in the EMG (Table 1). Apo-B concentrations were reduced significantly ($P < 0.05$) in EMG and PMG. However, the relative decreases in the EMG were significantly greater than those in the PMG and CG. There were no significant differences among and within groups with regards to triglycerides concentrations (Table 1).

Although the changes in fruits and vegetables consumption were not found to be significantly different among groups, still vegetables consumption within all groups

Table 2 Comparisons of changes in 24-h recalls vitamin A and beta-carotene intake, fruits vegetables and vegetables rich in beta-carotene intake between Enriched Milk Group ($n=40$), Placebo Milk Group ($n=37$) and Control Group ($n=31$) subjects following the 3-month intervention programme

	Baseline Mean $\pm$ SD	3 months Mean $\pm$ SD	3-month change Mean change (95 %C.I.)	P^a
VitA intake (retinol equivalent/day) ^c				<0.001
CG	769 $\pm$ 608	586 $\pm$ 395	−204(−473 to 65) [†]	
PMG	859 $\pm$ 848	647 $\pm$ 408	−193(−463 to 78) [†]	
EMG	817 $\pm$ 646	1316 $\pm$ 598	501(243 to 759) ^{*‡}	
P (treatment effect) ^b	0.999	<0.001		
beta-carotene intake (μ g/day) ^c				0.389
CG	482 $\pm$ 610	409 $\pm$ 469	−73(−279 to 134)	
PMG	480 $\pm$ 695	406 $\pm$ 695	−74(−264 to 115)	
EMG	484 $\pm$ 526	430 $\pm$ 283	−54(−236 to 127)	
P (treatment effect)	0.922	0.242		
Fruits consumption (portion/day) ^c				0.096
CG	1.58 $\pm$ 2.12	1.25 $\pm$ 1.23	−0.33(−1.15 to 0.48)	
PMG	2.22 $\pm$ 2.21	1.91 $\pm$ 2.11	−0.31(−1.05 to 0.43)	
EMG	1.68 $\pm$ 2.10	2.25 $\pm$ 2.52	0.57(−0.15 to 1.29)	
P (treatment effect)	0.154	0.263		
Vegetables consumption (portion/day)				0.787
CG	1.82 $\pm$ 1.55	2.66 $\pm$ 1.74	0.84(0.05 to 1.62) [*]	
PMG	2.01 $\pm$ 1.68	2.86 $\pm$ 2.11	0.84(0.12 to 1.57) [*]	
EMG	2.08 $\pm$ 1.26	3.23 $\pm$ 2.15	1.15(0.44 to 1.85) [*]	
P (treatment effect)	0.764	0.480		
Green & orange vegetables (portion/day) ^c				0.346
CG	0.68 $\pm$ 0.78	0.30 $\pm$ 0.65	−0.37(−0.94 to −0.01) [*]	
PMG	0.72 $\pm$ 0.99	0.46 $\pm$ 0.65	−0.26(−0.60 to 0.08)	
EMG	1.27 $\pm$ 1.19	0.81 $\pm$ 0.91	−0.46(−0.79 to −0.13) [*]	
P (treatment effect)	0.020	0.012		
Carrot consumption (portion/day) ^c				0.637
CG	0.13 $\pm$ 0.24	0.04 $\pm$ 0.13	−0.09(−0.21 to 0.03)	
PMG	0.07 $\pm$ 0.20	0.01 $\pm$ 0.04	−0.06(−0.18 to 0.05)	
EMG	0.19 $\pm$ 0.46	0.02 $\pm$ 0.08	−0.17(−0.28 to −0.06) [*]	
P (treatment effect)	0.343	0.750		

CG Control Group; PMG Placebo Milk Group; EMG Enriched Milk Group

*Significantly different from baseline, $p < 0.05$ (paired-sample t test)

^a P values were derived from one-way ANOVA. Means in a column with different symbols ([†], [‡]) are significantly different, $P < 0.05$

^b Treatment $\times$ time interaction effect

^c Value was rank transformed

increased significantly (Table 2). This within-group increase could be possibly explained by seasonal differences in the increased availability and the frequency consumption of vegetables, from winter (baseline) to spring (follow-up measurements). However, beta-carotene intake did not change significantly, despite the increase in vegetable consumption (Table 2). A possible reason for that could be again the effect of seasonality on the availability and consumption of vegetables rich in beta-carotene (i.e. green and orange vegetables) that seems to decrease from winter to spring months and replaced by other vegetables not equally rich in beta-carotene (i.e. tomatoes, cucumbers). Concerning vitamin A intake, there was a significant increase only in the EMG compared to the other two groups and this was obviously due to the consumption of the fortified milk (Table 2).

Despite the important hypocholesterolaemic effect of plant sterols, still the use of sterols has been reported to be negatively associated with plasma concentrations of beta-carotene (Tikkanen 2005). In the current analysis, despite the favourable effects of the intervention on TC, LDL-C and apo-B, the concentrations of beta-carotene did not change significantly in any of the study groups (Table 1). In other similar intervention studies, the reduction of beta-carotene levels by plant sterols was insignificant only when the intervention subjects were encouraged to increase their consumption of fruits and vegetables (Plat et al. 2000; Ntanos and Duchateau 2002; Raeini-Sarjaz et al. 2002; Colgan et al. 2004). The fact that the current study did not show any decrease in beta-carotene levels in the EMG could be attributed to the fortification of milk with vitamin A. Increased vitamin A intake could probably have counterbalanced sterol-induced plasma beta-carotene reduction, probably via preservation of carotenoids' pool, which is used for provitamin A synthesis. However, as the analysis conducted in the present study examined the effectiveness of the intervention on beta-carotene blood levels as a secondary outcome (Petrogianni et al. 2012), future studies should be specifically designed and carried out to test this exact hypothesis (i.e. the effect of vitamin A fortification in a phytosterol product concerning plasma levels of beta-carotene).

In conclusion, the current study showed that extra fortification of phytosterol-enriched milk with vitamin A is effective enough to reduce serum TC, LDL-C and apo-B levels and at the same time to prevent plasma beta-carotene

level reduction that often occurs after plant sterols' or stanols' intake.

Acknowledgments The study was supported by a research grant from FrieslandCampina Hellas. The study sponsor had no role in the study design; the collection, analysis, or interpretation of the data; the writing of the manuscript; and the submission and revision of the paper. Y. M. works as a part-time scientific consultant for Friesland Campina Hellas. None of the other authors had any conflict of interest.

References

- Cavina G, Gallinella B, Porra R, Pecora P, Suraci C (1988) Carotenoids, retinoids and alpha-tocopherol in human serum: Identification and determination by reversed-phase HPLC. *J Pharm Biomed Anal* 6:259–269
- Colgan HA, Floyd S, Noone EJ, Gibney MJ, Roche HM (2004) Increased intake of fruit and vegetables and a low-fat diet, with and without low-fat plant sterol-enriched spread consumption: effects on plasma lipoprotein and carotenoid metabolism. *J Hum Nutr Diet* 17:561–569, quiz 571–564
- Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502
- Manios Y, Moschonis G, Grammatikaki E, Katsaroli I, Kanelou P, Tanagra S (2006) Nutrition education in postmenopausal women: changes in dietary and cardiovascular indices. *Maturitas* 55:338–347
- Ntanos FY, Duchateau GS (2002) A healthy diet rich in carotenoids is effective in maintaining normal blood carotenoid levels during the daily use of plant sterol-enriched spreads. *Int J Vitam Nutr Res* 72:32–39
- Petrogianni M, Grammatikaki E, Pitsavos C, Moschonis G, Alexopoulos EC, Manios Y (2012) Changes in CVD risk factors after combined dietary counselling and supplementation with lipid-lowering milk product: the effect of compliance. *e-SPEN J* 7:e205–e210
- Plat J, Mensink RP (2005) Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am J Cardiol* 96:15D–22D
- Plat J, van Onselen EN, van Heugten MM, Mensink RP (2000) Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. *Eur J Clin Nutr* 54:671–677
- Raeini-Sarjaz M, Ntanos FY, Vanstone CA, Jones PJ (2002) No changes in serum fat-soluble vitamin and carotenoid concentrations with the intake of plant sterol/stanol esters in the context of a controlled diet. *Metabolism* 51:652–656
- Tikkanen MJ (2005) Plant sterols and stanols. *Handb Exp Pharmacol*, 215–230
- Voutilainen S, Nurmi T, Mursu J, Rissanen TH (2006) Carotenoids and cardiovascular health. *Am J Clin Nutr* 83:1265–1271