# Oral Supplementation With Probiotic *L. reuteri* NCIMB 30242 Increases Mean Circulating 25-Hydroxyvitamin D: A Post Hoc Analysis of a Randomized Controlled Trial

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**Context:** Low serum 25-hydroxyvitamin D is a risk factor for osteoporosis, cardiovascular disease, diabetes, and cancer. Disruption of noncholesterol sterol absorption due to cholesterol-lowering therapies may result in reduced fat-soluble vitamin absorption.

**Objective:** We have previously reported on the cholesterol-lowering efficacy and reduced sterol absorption of probiotic bile salt hydrolase active *Lactobacillus reuteri* NCIMB 30242; however, the effects on fat-soluble vitamins was previously unknown and the objective of the present study.

**Design, Settings, Patients, and Intervention:** The study was double-blind, placebo-controlled, randomized, parallel-arm, multicenter lasting 13 weeks. A total of 127 otherwise healthy hypercholesterolemic adults with low-density lipoprotein-cholesterol >3.4 mmol/L, triglycerides <4.0 mmol/L, and body mass index of 22 to 32 kg/m$^2$ were included. Subjects were recruited from 6 private practices in Prague, Czech Republic, and randomized to consume *L. reuteri* NCIMB 30242 or placebo capsules over a 9-week intervention period.

**Outcome measures:** The primary outcome measure was the change in serum low-density lipoprotein-cholesterol over the 9-week intervention. Analysis of fat-soluble vitamins at weeks 0 and 9 were performed post hoc.

**Results:** There were no significant differences between *L. reuteri* NCIMB 30242 and placebo capsule groups in serum vitamin A, vitamin E, or $\beta$-carotene or dietary intake over the intervention period ($P > .05$). *L. reuteri* NCIMB 30242 increased serum 25-hydroxyvitamin D by 14.9 nmol/L, or 25.5%, over the intervention period, which was a significant mean change relative to placebo of 17.1 nmol/L, or 22.4%, respectively ($P = .003$).

**Conclusions:** To our knowledge, this is the first report of increased circulating 25-hydroxyvitamin D in response to oral probiotic supplementation. **(*J Clin Endocrinol Metab* 98: 2944–2951, 2013)**

Reduced serum levels of 25-hydroxyvitamin D [25(OH)D] is considered the best indicator of vitamin D status (1) and is highly prevalent in almost every region of the world, with more than 1 billion children and adults at risk (2). Low serum 25(OH)D is common in the general population and is a risk factor for osteoporosis (3), cardiovascular disease (CVD) (4), type 2 diabetes (5), and cancer (6). Poor vitamin D status is associated with decreased intestinal calcium resorption, low serum calcium and increased PTH secretion, which can result in the del-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received December 19, 2012. Accepted April 15, 2013.
First Published Online April 22, 2013

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Abbreviations: BMI, body mass index; BSH, bile salt hydrolase; CVD, cardiovascular disease; hs-CRP, high-sensitivity C-reactive protein; DHC, dehydrocholesterol; ITT, intention-to-treat; LDL, low-density lipoprotein; 25(OH)D, 25-hydroxyvitamin D; RAA, renin-angiotensin-aldosterone; VDR, vitamin D receptor.

eterious effects of secondary hyperparathyroidism. The Institute of Medicine has defined 4 categories of vitamin D status based on serum 25(OH)D: risk of deficiency (<30 nmol/L), risk of inadequacy (30–49 nmol/L), sufficiency (50–125 nmol/L), and above which there may be reason for concern (>125 nmol/L) (7). Furthermore, a classification system has been proposed based on physiologic concentrations of 25(OH)D required to maintain normal levels of PTH, including deficiency (<50 nmol/L), insufficiency (50–74 nmol/L), and sufficiency (75–250 nmol/L) (8). Additionally, an optimal target range of 75 to 100 nmol/L has been recommended for individuals at risk for musculoskeletal health problems, CVD, autoimmune diseases, and cancer (9). Vitamin D intoxication is rare and may be observed at 25(OH)D concentrations above 375 to 500 nmol/L (8).

Epidemiologic studies have reported reduced 25(OH)D concentrations in patients with CVD and cerebrovascular disease and that low levels are associated with an increased risk of CVD-related events and mortality (11). A prospective study including >40 000 individuals showed that low serum 25(OH)D significantly increased future CVD risk (12). Vitamin D deficiency may increase the risk of CVD by altering the balance of anti- and proinflammatory cytokines (13), promoting cell proliferation through reduced vitamin D receptor (VDR) activation (14) and increasing blood pressure (BP) and inflammation mediated by the renin-angiotensin-aldosterone (RAA) system (15). At present, large intervention studies evaluating the effects of vitamin D on CVD have been initiated.

The microbiome represents the totality of the intestinal microflora, its genetic composition, and its environmental interactions (16). It has been shown that the intestinal microflora serves important functions in immunity, inflammatory signaling, metabolism, and maintenance of normal epithelial cell function (17, 18). A dysregulated or dysfunctional microbiome is a condition termed dysbiosis and has been implicated in obesity, diabetes, inflammatory bowel disease, irritable bowel syndrome, and colorectal cancer (19, 20). It has recently been proposed that decreased vitamin D intake may be associated with changes to the microbiome (16, 21). VDR in the gut is critical in regulating intestinal homeostasis by preventing pathogenic bacterial invasion, inhibiting inflammation, and maintaining cell integrity (22). Attempts have been made to alter the gut microbiome by delivering probiotic bacteria, defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (23).

It has been reported that 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, including atorvastatin, simvastatin, and rosuvastatin may increase vitamin D in a cholesterol-independent or pleiotropic manner (24), although several studies have presented contradictory results (25). Previously, we have reported on the cholesterol-lowering efficacy of a *Lactobacillus reuteri* NCIMB 30242 yogurt formulation (26) as well as the cholesterol-lowering efficacy and reduced sterol absorption of an *L. reuteri* NCIMB 30242 capsule (27). However, the effects of oral supplementation with bile salt hydrolase (BSH)-active *L. reuteri* NCIMB 30242 on absorption of fat-soluble vitamins was previously unknown. To our knowledge, this is the first report of increased levels of circulating 25(OH)D in response to oral supplementation with a probiotic.

## Subjects and Methods

### Subjects

The present study was conducted according to the principles of the Declaration of Helsinki. Otherwise healthy hypercholesterolemic adult men and women were recruited from 6 private practices in Prague, Czech Republic. The protocol was approved by the Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol, Czech Republic (US Food and Drug Administration Office for Human Research Protections Institution or Organization Registration IORG0000612). The study protocol was carefully explained to all subjects before obtaining informed consent. The trial was registered in a public registry.

### Subject inclusion

Otherwise healthy hypercholesterolemic adult men and women between the ages of 20 and 75 years old (inclusive) with low-density lipoprotein (LDL)-cholesterol >3.4 mmol/L, triglycerides <4.0 mmol/L, and body mass index (BMI) of 22 to 32 kg/m$^2$ were included. Persons with diabetes or subjects receiving cholesterol-lowering prescription drugs within the last 6 months (with the exception of stable statin monotherapy for >3 months) or cholesterol-lowering nonprescription supplements within the last 3 months or having experienced any cardiovascular event in the last 6 months were excluded. Other exclusion criteria included history of chronic use of alcohol; use of systemic antibodies, corticosteroids, androgens, or phenytoin; current involvement in a clinical trial; history of angina, congestive heart failure, inflammatory bowel disease, pancreatitis, gastrointestinal, renal, pulmonary, hepatic, or biliary disease, or cancer; chronic use of probiotics, fiber laxative, or stimulant laxatives; and history of eating disorders. Furthermore, subjects exercising greater than 15 miles/wk or 4000 kcal/wk or who were pregnant, breastfeeding, or intended to get pregnant were excluded.

### Preparation of *L. reuteri* NCIMB 30242 and placebo capsules

*Lactobacillus reuteri* NCIMB 30242 (Cardioviva) was propagated in a 5000-L fermenter, concentrated, and lyophilized in compliance with standard operating procedures and quality control procedures at Probiotical S.p.A (Novara, Italy). Placebo and *L. reuteri* capsules (opaque white size 0 DRcaps [Capsugel, Colmar, France]) were produced by Probiotical. Microbiological analyses and bacterial culture purity were confirmed after pro-

duction. Placebo capsules contained 300 mg maltodextrin/silicon dioxide excipient, and *L. reuteri* capsules contained 130 mg lyophilized bacteria and 170 mg maltodextrin/silicon dioxide excipient. *L. reuteri* capsules contained $2.9 \times 10^9$ colony-forming units per capsule *L. reuteri* NCIMB 30242 at the study baseline and $2.0 \times 10^9$ colony-forming units per capsule *L. reuteri* NCIMB 30242 at the study endpoint as measured by Exova (Portland, Oregon). Placebo and *L. reuteri* capsules were identical in appearance and taste and were bottled in identical high-density polyethylene bottles with desiccant and sealed.

## Study design

The study design was double-blind, placebo-controlled, randomized, parallel-arm, multicenter lasting 13 weeks. This included a 2-week washout period, a 2-week run-in period in which subjects consumed placebo capsules twice daily at breakfast or dinner, and a 9-week intervention period in which subjects consumed either placebo or *L. reuteri* capsules twice daily at breakfast and dinner. Dietary recommendations were advised, via the National Cholesterol Education Profile Adult Treatment Panel III guidelines (28) for the entire 13-week study period. Subjects met with the investigational team at 7 different time points: visit 0 (week −4), 1 (week −2), 2-1 (week 0 −1 d), 2-2 (week 0, randomization and intervention baseline), 3 (week 3), 4 (week 6), and 5 (week 9, intervention endpoint).

## Dietary intake

Dietary intake was measured at weeks 0 and 9 of the intervention period by 2-day subject-reported diet journal and was evaluated by the NutriDan software program (Danone Institute, Prague, Czech Republic) for total energy, total lipids, total proteins, and total carbohydrates as well as micronutrients including vitamin A, retinol, carotenoids, vitamin E, and vitamin D.

## Sample analyses

Twelve-hour fasting blood samples were obtained by venipuncture at weeks 0 and 9. Serum and plasma were transported immediately from each center to a central laboratory (Prevedig s.r.o., Prague, Czech Republic) for analysis. Serum LDL-cholesterol, calcium, and phosphate were assessed on a Dimension RxL biochemistry analyzer using appropriate reagent kits (Siemens Dade Behring, Munich, Germany). Serum high-sensitivity C-reactive protein (hs-CRP) was assessed by highly sensitive immunoassay. Serum vitamin A, vitamin E, and $\beta$-carotene were assessed at weeks 0 and 9 by HPLC at the University Hospital Regensburg (Regensberg, Germany). Serum 25(OH)D was assessed at weeks 0 and 9 by RIA at the University Hospital Regensburg.

## Statistical analysis

The number of subjects was calculated based on LDL-cholesterol as the primary endpoint by taking into account a difference in LDL-cholesterol of 0.34 (0.64) mmol/L between the placebo and *L. reuteri* groups with $\alpha = 5\%$ and a power of 80%. Given these constraints, 57 evaluable subjects per group, or 114 in total, were required. To take into account possible premature withdrawal, 131 subjects were included for random assignment. A blinded statistician prepared 16 unique randomization lists using the completely randomized design generated by SAS software package version 9 procedure PLAN (SAS Institute, Cary, North Carolina). The capsule producer chose 1 list and prepared the capsule bottles accordingly. Records of randomization number, corresponding to placebo or *L. reuteri*, were accessible only to the capsule producer until database lock.

The primary null hypothesis was that an *L. reuteri* capsule is not more effective than a placebo capsule in reducing serum LDL-cholesterol after 9 weeks, and analysis of fat-soluble vita-

**Table 1.** Demographic and Clinical Characteristics at Week 0

| | Mean (SD) | | |
|---|---|---|---|
| | **Placebo (n = 61)** | ***L. reuteri* (n = 66)** | ***P*** |
| Caucasian, % | 100% | 100% | 1.00[b] |
| Male, % | 44% | 42% | .859[b] |
| Age, y | 47.59 (12.88) | 50.48 (14.03) | .230[c] |
| Body weight, kg | 81.66 (12.41) | 78.55 (11.38) | .145[c] |
| BMI, kg/m$^2$ | 27.62 (2.81) | 26.83 (3.05) | .133[d] |
| Systolic BP, mm Hg | 131.56 (11.58) | 130.12 (11.22) | .359[d] |
| Diastolic BP, mm Hg | 77.61 (6.85) | 78.48 (5.35) | .296[d] |
| Pulse, beats/min | 72.59 (6.06) | 73.30 (7.12) | .614[d] |
| hs-CRP, mg/L[a] | 1.63 (0.90–3.25) | 2.00 (0.80–4.43) | .161[d] |
| Vitamin A, $\mu$mol/L | 1.96 (0.53) | 1.90 (0.47) | .561[d] |
| 25(OH)D, nmol/L | 75.12 (27.68) | 67.91 (22.14) | .277[d] |
| Vitamin E, $\mu$mol/L | 32.24 (4.90) | 33.15 (2.89) | .546[d] |
| $\beta$-Carotene, $\mu$mol/L | 0.55 (0.40) | 0.60 (0.49) | .545[d] |
| Calcium, mmol/L | 2.35 (0.09) | 2.34 (0.07) | .674[d] |
| Phosphate, mmol/L | 1.18 (0.22) | 1.19 (0.18) | .314[d] |

[a] Geometric mean (interquartile range).

[b] Fisher's exact test.

[c] One-way ANOVA.

[d] Mann-Whitney Wilcoxon test.

mins was performed post hoc. All analysis was performed according to the intention-to-treat (ITT) principle. Descriptive statistics are presented as mean (SD) or as geometric mean or median and interquartile ranges for continuous variables or as a percentage for categorical variables. The Shapiro-Wilk test was used to determine whether variables were parametrically distributed. Differences between groups for baseline characteristics were analyzed using a 1-way ANOVA or a nonparametric Mann-Whitney Wilcoxon test for continuous variables or Fisher's exact test for categorical variables. Differences between groups in dietary intake of macronutrients and micronutrients as well as in serum fat-soluble vitamins were assessed by using 2-factor repeated-measures ANOVA with time and intervention as the 2 factors. Variables not parametrically distributed were logarithmically transformed before statistical analysis and were determined to be sufficiently log-normal to permit the respective logarithm to be used in a parametric test. A Spearman rank correlation was used to assess the association between individual changes in serum 25(OH)D and changes in serum vitamin A, vitamin E, β-carotene, calcium, phosphate, systolic BP, diastolic BP, LDL-cholesterol, and hs-CRP. Differences between groups in study entry by calendar week were assessed by Pearson's $\chi^2$ goodness-of-fit test. All analyses were performed using SPSS software package version 17.0 (SPSS Inc, Chicago, Illinois).

## Results

### Study population

The baseline characteristics for the 127 subjects in the ITT population are presented in Table 1. The 2 groups produced by randomization presented largely homogeneous demographic and clinical characteristics. Male and female study subjects were equally distributed with 44% males and 56% females in the placebo group and 42% males and 58% females in the *L. reuteri* group. There were no significant differences between groups at week 0 in age, body weight, BMI, systolic BP, diastolic BP, and pulse. Also, there were no significant differences between groups at baseline in serum vitamin A, 25(OH)D, vitamin E, β-carotene, calcium, phosphate, or hs-CRP. There were no significant differences in the seasonality of the intervention period (study entry by calendar week) in subjects consuming placebo as compared with *L. reuteri* ($P > .05$).

### Dietary assessment

A dietary assessment of total energy, total lipids, total proteins, total carbohydrates, vitamin A, retinol, carote-

**Table 2.** Dietary Total Energy and Nutrient Intake

| | Mean (SD) | | |
|---|---|---|---|
| | **Placebo (n = 61)** | ***L. reuteri* (n = 66)** | ***P*** |
| Energy, kcal/d | | | |
| wk 0 | 2033.1 (642.5) | 2074.8 (690.0) | .85[b] |
| wk 9 | 1999.7 (621.0) | 2024.6 (586.5) | |
| Lipids, kcal/d | | | |
| wk 0 | 710.0 (258.5) | 770.5 (333.5) | .22[b] |
| wk 9 | 744.8 (301.7) | 741.0 (276.5) | |
| Proteins, kcal/d | | | |
| wk 0 | 345.6 (115.5) | 351.3 (116.4) | .87[b] |
| wk 9 | 339.7 (104.3) | 345.8 (100.1) | |
| Carbohydrates, kcal/d | | | |
| wk 0 | 977.5 (343.6) | 953.0 (361.2) | .44[b] |
| wk 9 | 915.2 (321.8) | 937.8 (295.7) | |
| Vitamin A, μg/d[a] | | | |
| wk 0 | 554.5 (401.7–843.4) | 575.1 (450.9–834.3) | .70[c] |
| wk 9 | 540.8 (394.7–832.0) | 542.9 (413.4–772.4) | |
| Retinol, μg/d[a] | | | |
| wk 0 | 308.5 (206.6–595.3) | 345.2 (243.6–484.0) | .68[c] |
| wk 9 | 348.8 (224.2–522.4) | 339.4 (241.9–477.7) | |
| Carotenoids, mg/d[a] | | | |
| wk 0 | 2.51 (1.05–3.81) | 1.86 (0.96–4.27) | .90[c] |
| wk 9 | 2.20 (0.96–3.21) | 2.13 (0.83–3.61) | |
| Vitamin D, μg/d[a] | | | |
| wk 0 | 1.26 (0.52–2.25) | 1.31 (0.72–2.29) | .26[c] |
| wk 9 | 1.10 (0.57–2.07) | 1.09 (0.69–1.70) | |
| Vitamin E, mg/d[a] | | | |
| wk 0 | 9.96 (7.17–11.72) | 10.12 (7.62–13.27) | .51[c] |
| wk 9 | 9.59 (6.71–12.66) | 9.90 (7.53–12.78) | |

[a] Median (interquartile range).

[b] Two-factor repeated-measures ANOVA.

[c] Two-factor repeated-measures ANOVA on log-transformed values.

noids, vitamin E, and vitamin D was performed at weeks 0 and 9 of the intervention period. There were no significant differences between placebo and *L. reuteri* groups over the intervention period in dietary intake ($P > .05$) (Table 2).

### Effect of *L. reuteri* NCIMB 30242 on fat-soluble vitamins

Serum vitamin A, 25(OH)D, vitamin E, and β-carotene were assessed at weeks 0 and 9 and are presented in Figure 1. There were no significant differences between placebo and *L. reuteri* groups over the intervention period in serum vitamin A, vitamin E, β-carotene, calcium, or phosphate over the intervention period. Subjects consuming *L. reuteri* NCIMB 30242 capsules increased serum 25(OH)D over the intervention period ($P = .003$), which was statistically significant after Bonferroni adjustment for multiple comparisons. The significance of increased serum 25(OH)D in subjects consuming *L. reuteri* NCIMB 30242 capsules was also observed after adjustment for seasonality of the intervention period as a confounder ($P = .001$) As shown in Figures 1 and 2, the *L. reuteri* NCIMB 30242 group increased 25(OH)D by 14.9 nmol/L, or 25.5%, over the intervention period, which was a significant mean change relative to placebo of 17.1 nmol/L, or 22.4%, respectively. Figure 2 shows a side-by-side comparison of individual 25(OH)D changes from week 0 to week 9, indicating a treatment effect across the spectrum of responses. A significant negative correlation was seen between individual changes in serum 25(OH)D and hs-CRP of $r = -0.208$ ($P = .023$). No significant correlations ($P > .05$) were observed between individual changes in serum 25(OH)D and vitamin A ($r = -0.048$), vitamin E ($r = 0.024$), β-carotene ($r = 0.116$), calcium ($r = 0.071$), phosphate ($r = -0.028$), systolic BP ($r = -0.081$), diastolic BP ($r = -0.033$), or LDL-cholesterol ($r = -0.017$).

## Discussion

Although we have previously shown that BSH-active *L. reuteri* NCIMB 30242 reduces cholesterol and the absorption of noncholesterol sterols in hypercholesterolemic adults (27), its effect on the absorption of fat-soluble vitamins was previously unknown. Thus, the present study was undertaken to assess the effect of *L. reuteri* NCIMB 30242 capsules on vitamin A, vitamin E, 25(OH)D, and β-carotene in hypercholesterolemic adults over 9 weeks. Results show that subjects consuming *L. reuteri* NCIMB 30242 capsules had similar levels of vitamin A, vitamin E, and β-carotene and significantly increased levels of serum 25(OH)D, despite no differences in dietary intake or seasonality of the intervention period at the 9-week study endpoint as compared with placebo. To our knowledge, this is the first report of fat-soluble vitamin status in response to a BSH-active probiotic and the first report of increased levels of circulating 25(OH)D in response to oral supplementation with any probiotic.

Previously, we have reported that the LDL-cholesterol reduction observed in response to *L. reuteri* NCIMB 30242 supplementation was significantly correlated with increased plasma deconjugated bile acids resulting from intraluminal BSH activity (27). As well, absolute concentrations of plasma plant sterols were significantly reduced in sub-

**A**

| | Placebo (n 61) Mean (SD) | *L. reuteri* (n 66) Mean (SD) | *P* |
|---|---|---|---|
| Vitamin A (μmol/l) | | | |
| Week 0 | 1.96 (0.53) | 1.90 (0.47) | 0.360[a] |
| Week 9 | 2.01 (0.53) | 1.91 (0.39) | |
| 25(OH)D (nmol/l) | | | |
| Week 0 | 75.12 (27.68) | 67.91 (22.14) | 0.003[a] |
| Week 9 | 71.93 (22.05) | 82.64 (35.52) | |
| Vitamin E (μmol/l) | | | |
| Week 0 | 32.24 (4.90) | 33.15 (2.89) | 0.852[b] |
| Week 9 | 33.96 (5.61) | 34.63 (6.39) | |
| β-carotene (μmol/l) | | | |
| Week 0 | 0.55 (0.40) | 0.60 (0.49) | 0.790[a] |
| Week 9 | 0.55 (0.37) | 0.59 (0.46) | |
| Calcium (mmol/l) | | | |
| Week 0 | 2.35 (0.09) | 2.34 (0.07) | 0.100[a] |
| Week 9 | 2.36 (0.11) | 2.33 (0.07) | |
| Phosphate (mmol/l) | | | |
| Week 0 | 1.18 (0.22) | 1.19 (0.18) | 0.312[a] |
| Week 9 | 1.17 (0.18) | 1.16 (0.18) | |

[a] Two-factor repeated measures ANOVA on log-transformed values
[b] Two-factor repeated measures ANOVA
25(OH)D, 25-hydroxyvitamin D

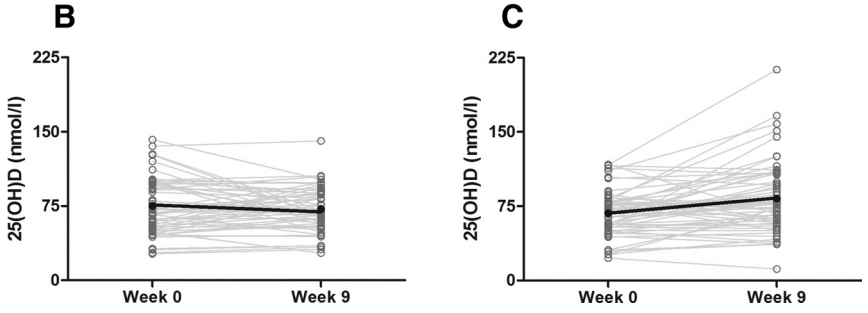


**Figure 1.** A, Serum fat-soluble vitamins, calcium, and phosphate at weeks 0 and 9. B and C, Distribution of serum 25(OH)D levels before and after treatment with placebo (B) or *L. reuteri* NCIMB 30242 capsules (C).

jects consuming *L. reuteri* NCIMB 30242 capsules, suggesting a reduction in the absorption of dietary and biliary cholesterol (27). It has previously been reported that disruption of noncholesterol sterol absorption, a surrogate marker for cholesterol absorption (29), may result in reduced absorption of fat-soluble vitamins, and the use of bile acid-binding resins have been shown to reduce absorption and circulating levels of fat-soluble vitamins (30). Here we provide evidence that oral supplementation with BSH-active *L. reuteri* NCIMB 30242 does not result in reduced absorption of fat-soluble vitamins and unexpectedly increases the circulating levels of 25(OH)D. Although the mechanism of action is not clear, several observations and statements can be made: 1) among fat-soluble vitamins assessed, the effect of BSH-active *L. reuteri* NCIMB 30242 was exclusive to 25(OH)D, and individual changes in 25(OH)D were not associated with changes in vitamins A, E, or $\beta$-carotene; 2) reports have shown that increased absorption of dietary vitamin D is associated with increased hydrogen ion concentration, or lower pH, in the intestine, but not with increased conjugated bile acid concentration (31); 3) increased hepatic 25-hydroxylase activity or 7-dehydrocholesterol (7-DHC) concentration may lead to increased 25(OH)D production (32, 33); and 4) increased hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity, often observed in response to reduced absorption of dietary and biliary cholesterol (34), may lead to increased synthesis of 7-DHC (33). Therefore, it is possible that oral supplementation of *L. reuteri* NCIMB 30242 may increase circulating 25(OH)D through increased intraluminal lactic acid production, increased synthesis of 7-DHC, or both. Alternatively, because individual changes in 25(OH)D were not associated with changes in LDL-cholesterol but rather with changes in hs-CRP, it is possible that the effect may be unrelated to cholesterol metabolism and pleiotropic.

Physiologic effects of 25(OH)D deficiency leading to serious cardiovascular consequences, believed to be at least partially due to suppression of the RAA system, have previously been reported at serum concentrations below 37.5 mmol/L (4). 25(OH)D concentrations required to maintain normal PTH levels are reportedly >50 mmol/L (8). Higher levels of PTH have been shown to activate the RAA system and are associated with higher systolic and diastolic BP and higher prevalence of hypertension (37). However, contradictory evidence exists showing that serum 25(OH)D and PTH levels are not independently associated with BP or risk of hypertension (10). In the current study, we show a significant increase in circulating 25(OH)D in response to *L. reuteri* NCIMB 30242 that is not attributable to an increase in dietary intake or differences in the seasonality of the intervention period and despite decreases in noncholesterol sterol absorption (27). Although there were no significant differences between groups in systolic or diastolic BP and individual changes were not significantly associated with changes in 25(OH)D, an investigation employing larger sample size or evaluation of at-risk subjects may be warranted. The

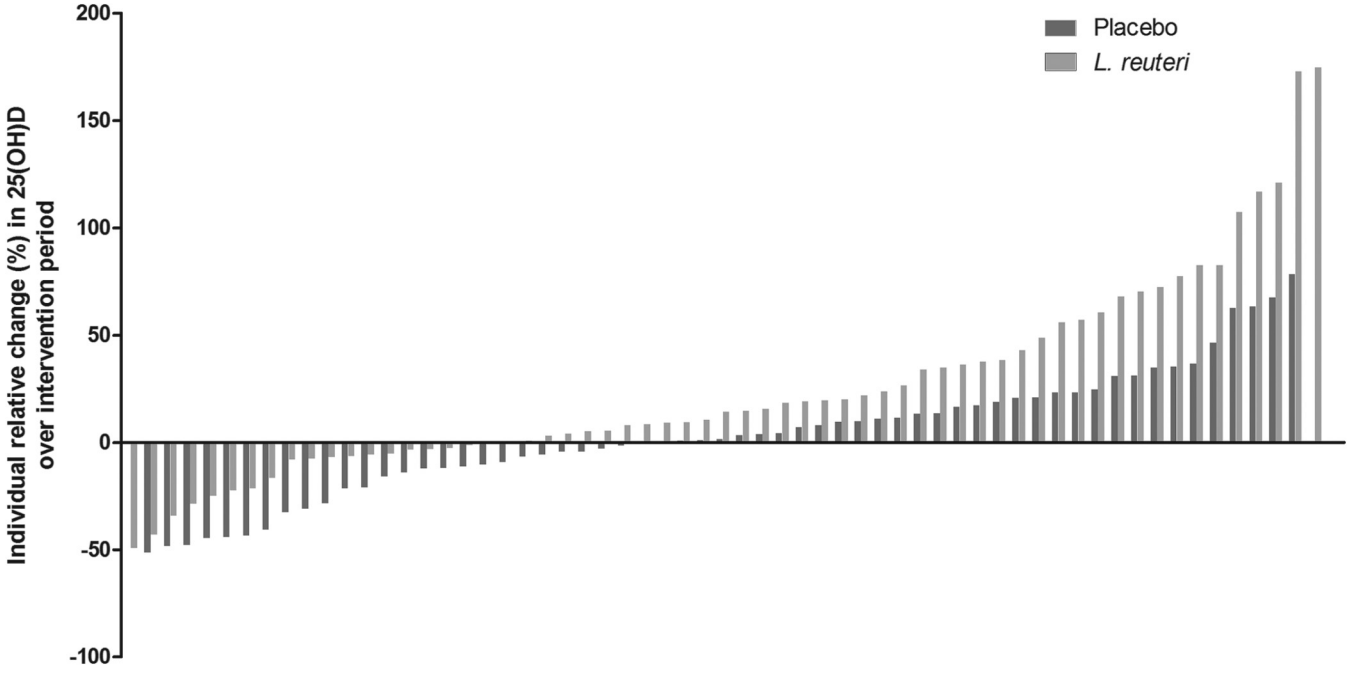


**Figure 2.** Spectrum of individual relative changes (percentage) in 25(OH)D from weeks 0 to 9 in subjects receiving placebo capsules or *L. reuteri* NCIMB 30242 capsules. Individual relative changes in 25(OH)D (percentage) = 100 × [25(OH)D (week 9) − 25(OH)D (week 0)/25(OH)D (week 0)].

current study included 10, 24, and 78 subjects with baseline serum 25(OH)D <37.5, <50, and <75 mmol/L, respectively. Future studies should focus on specific subsets of subjects with metabolic disease in combination with low concentrations of 25(OH)D, where study duration is determined by the likely effects of 25(OH)D on ongoing pathophysiology. It should be noted that despite a significant increase in mean 25(OH)D, approximately one-third of subjects receiving *L. reuteri* NCIMB 30242 did not show increased 25(OH)D over the intervention period. Such marked differences in 25(OH)D concentrations after dietary vitamin D supplementation have previously been ascribed to differences in calcium intake, race, body fat, age, and different genetic factors (35). Further work is required to understand the 25(OH)D responder rate to *L. reuteri* NCIMB 30242 supplementation, particularly in the context of varying basal 25(OH)D concentrations, daily sunlight exposures, and other potential confounders. Probiotics have also been shown to enhance VDR expression and activity (16), potentially affecting uptake by enterocytes (35). Further understanding of VDR's contribution to the effect of *L. reuteri* NCIMB 30242 on increased circulating 25(OH)D and anti-inflammation is warranted. However, in the context of hypercholesterolemia and low-normal levels of 25(OH)D, the observed increases in mean circulating 25(OH)D may be physiologically relevant in this population, because there is good evidence that improved 25(OH)D status is associated with improved metabolic status and that low levels are an independent risk factor for cardiovascular events.

It has recently been proposed that decreased vitamin D intake may be associated with changes to the gut microbiome (16, 21) and that a dysbiosis may be responsible for reduced VDR-regulated maintenance of intestinal homeostasis (16, 19). We have previously reported improved inflammatory bowel disease symptomology and reduced inflammation in response to *L. reuteri* NCIMB 30242 (36) and have shown here increased circulating 25(OH)D as well as an association between individual changes in 25(OH)D and hs-CRP. Thus, we propose that dysbiosis of the gut microflora may be responsible for changes in vitamin D status in opposition to, or as well as, previous hypotheses that vitamin D status may be the cause of dysbiosis (21). This is particularly interesting when considering that microbiota-driven changes to vitamin D status may represent an important homeostatic signal to the host and indicate appropriate intestinal colonization, while acting to prevent pathogenic bacterial invasion, inhibit excess local inflammation, and maintain enterocyte cell integrity (21).

In conclusion, oral supplementation with *L. reuteri* NCIMB 30242 should be considered for improving vitamin D status, and this effect should be confirmed in future clinical studies examining at-risk subjects. Further research regarding the mechanism of action and whether dysbiosis may result in changes in vitamin D status are warranted.

## Acknowledgments

We thank J. Lahovský, G. Schmitz, S. Matysik, V. Voštiňáková, and M. Svobodová for their contribution to the study. We also thank all volunteers who participated in the study. G. Schmitz and S. Matysik offered advice and oversaw the analysis of fat-soluble vitamins. J. Lahovský, V. Voštiňáková, and M. Svobodová oversaw the clinical study.

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This work was supported by Micropharma Limited.

Disclosure Summary: M.L.J. and S.P. acknowledge a conflict of interest because they are cofounders and shareholders of Micropharma. C.J.M. is employed by and is a shareholder of Micropharma.

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