

SUPPLEMENTAL MATERIAL

Supplemental Analysis

To control for the potential effects of seasonal variation and differences in the interval between testing, regression analyses were applied to the longitudinal study population. Seasonal adjustments to all four lipid measurements (total, LDL and HDL cholesterol and triglycerides) were made using Berliner Verfahren (BV) trend-cycle technique with age and gender as covariates.¹ The study population data spanned an 18 month period – which was insufficient for detecting seasonal patterns. Instead, we estimated the BV trend-cycle parameters using a random sample of 10 million lipid panel results from the larger Quest Diagnostics database measured over 36 months (January 2008 to December 2010). The BV trend-cycle decomposition regression is reported in supplemental Table S1. Seasonality was detected for all lipid panel components. Comparisons of actual and fitted mean values are illustrated in supplemental Figure S1.

The measured trend-cycle component was then subtracted from the actual lipid profile measurements for each visit of the study population. The resulting values are age-, gender-, trend-, and seasonally-adjusted lipid measurements (“adjusted lipid values”). Changes in the adjusted lipid values were regressed against dummy variables indicating repletion or control group status, as well as the number of weeks between the initial and final tests, which varied from 4 to 26 weeks. Dependencies in lipid measurements implied by the Friedewald equation were accommodated using a three-staged system fit regression model that explicitly accounted for covariance in the lipid measurements.² The statistical significance of differences in the parameter estimates measuring lipid changes for the repletion and control groups was assessed using two-sample, unpaired t-tests with

unequal variances (Table S2). The seasonally-adjusted inter-group differences were not statistically significant when accounting for the interval between testing ($P < 0.05$).

Therefore, the findings that vitamin D repletion has minimal clinical impact on lipid profile and implied cardiovascular risk hold even after correcting for seasonality and the interval between testing.

References for Supplemental Analysis:

1. The BV4.1 Procedure for Decomposing and Seasonally Adjusting Economic Time Series. Edited and distributed by Statistisches Bundesamt, Wiesbaden. Published in April 2006.

<http://www.destatis.de/jetspeed/portal/cms/Sites/destatis/Internet/EN/Content/Wissenschaftsforum/MethodsApproaches/Infos/BV41Procedure,property=file.pdf>
accessed November 28, 2011.

2. Henningsen, A, Hamann, J. Systemfit: A Package for Estimating Systems of Simultaneous Equations in R. *Journal of Statistical Software*, 2007;23(4):1-40.

Supplemental Table S1

Table S1. BV trend-cycle decomposition regression equation and parameter estimates

Lipid Parameter (mg/dl)	Intercept	Trend	Age	Gender	cos(2πt/12)	sin(2πt/12)	R ²
Total Cholesterol	222.86	-0.008	-0.479	-16.41	1.298	-0.023	0.056
LDL Cholesterol	140.073	-0.012	-0.535	-6.867	1.125	0.094	0.035
HDL Cholesterol	55.244	0.029	0.029	-11.448	0.140	0.159	0.124
Triglycerides	137.704	-0.121	-0.121	9.604	0.125	1.397	0.005

* All parameter estimates reported are significant at p < 0.05 level.

$$y_{l,t} = \alpha_l + \lambda_l t + \omega_l^1 \cos\left(\frac{2\pi t}{12}\right) + \omega_l^2 \sin\left(\frac{2\pi t}{12}\right) + \beta_l^1 AGE + \beta_l^2 GENDER + \varepsilon_{l,t}$$

where y = dependent variable

$l \in$ (Total cholesterol, LDL cholesterol, HDL cholesterol, Triglycerides)

$t \in$ (1, 2, ..., 36) = month of testing

α = intercept

$\lambda_l t$ = linear trend component

$\omega_l^1 \cos\left(\frac{2\pi t}{12}\right) + \omega_l^2 \sin\left(\frac{2\pi t}{12}\right)$ = monthly cycle (seasonal) component

$AGE \in$ (40 – 80 years)

$GENDER = \begin{cases} 0 & \text{if female} \\ 1 & \text{if male} \end{cases}$

ε = random error term

Supplemental Table S2

Table S2. Three Stage System Fit Regression Results Measuring Changes in Lipid Values (mg/dl) for Repletion and Control Groups

Lipid Parameter (mg/dl)	Group I (Repletion)	Group II (Control)	Inter-group Difference	95% CI
Total Cholesterol	-4.02	-4.91	0.90	(-0.43, 2.23)
LDL Cholesterol	-2.76	-3.26	0.50	(-0.28, 1.27)
HDL Cholesterol	0.13	-0.37	0.49	(-0.28, 1.26)
Triglycerides	-6.96	-6.44	-0.52	(-5.08, 4.43)

Supplemental Figure 1. Comparison of actual vs fitted mean lipid measurements

- To convert values of total cholesterol, LDL cholesterol, and HDL cholesterol to mmol/l multiply by 0.02586
- To convert values of triglycerides to mmol/l multiply by 0.1129

