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ORIGINAL ARTICLE

High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile

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Background/Objectives: Low serum 25-hydroxyvitamin D (25(OH)D) concentrations are related to increased mortality. One possible explanation could be an association between serum 25(OH)D and serum lipids.
Subjects/Methods: The study was performed at the University of Tromsø, Northern Norway. In total, 8018 nonsmoking and 2087 smoking subjects were included in a cross-sectional study performed in 2008, and 1762 nonsmoking and 397 smoking subjects in a longitudinal study from 1994/1995 to 2008. Nonfasting serum 25(OH)D, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), LDL-C/HDL-C ratio and triacylglycerol (TAG) were measured.
Results: After adjustment for gender, age, sample month and body mass index in the cross-sectional study, there was a significant increase in serum TC, HDL-C and LDL-C, and a significant decrease in serum LDL-C/HDL-C ratio and TAG across increasing serum 25(OH)D quartiles. For serum HDL-C and TAG in nonsmokers the differences between the means for the highest and lowest serum 25(OH)D quartiles were 6.0 and 18.5%, respectively. In the longitudinal study, an increase in serum 25(OH)D was associated with a significant decrease in serum TAG.
Conclusions: There is a cross-sectional association between serum 25(OH)D and serum lipids, and a longitudinal association over 14 years between serum 25(OH)D and TAG, which may contribute to explain the relation between low serum 25(OH)D concentrations and mortality.
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Introduction

The serum concentration of 25-hydroxyvitamin D (25(OH)D) is generally accepted for evaluating vitamin D status and has been shown to relate inversely to the risks of all-cause and cardiovascular mortality (Dobnig *et al*., 2008; Melamed *et al*., 2008; Pilz *et al*., 2009). Whether this reflects a causal relationship or just that subjects with good health spend more time outdoors in the sun and therefore produce more vitamin D in the skin, and/or eat more fatty fish and use more vitamin D supplements (DeLuca, 2004), is not known. However, the relationship was recently substantiated in a meta-analysis of 18 randomized controlled trials including more than 57 000 subjects, which strongly suggests that these associations are causal (Autier and Gandini, 2007). In that meta-analysis the relative risk for mortality was 0.93 for those supplemented with vitamin D as compared to the controls, but the difference was of borderline statistical significance with a confidence interval of 0.87–0.99.

It appears that the increased mortality in subjects with low serum 25(OH)D concentrations is particularly related to cardiovascular diseases (Dobnig *et al*., 2008; Pilz *et al*., 2009), which could be explained by the well-documented associations between low serum 25(OH)D concentrations and increased blood pressure (Scragg *et al*., 2007; Witham *et al*., 2009), blood glucose (Need *et al*., 2005) and body mass index (BMI) (Snijder *et al*., 2005). However, the relation between serum 25(OH)D and serum lipids, which are among the

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major risk factors for cardiovascular disease (Kannel *et al*., 1971), is more uncertain (Ford *et al*., 2005; John *et al*., 2005; Martins *et al*., 2007; Forouhi *et al*., 2008; Hyppönen *et al*., 2008; Gannagé-Yared *et al*., 2009; Lee *et al*., 2009). We have therefore examined the cross-sectional relation between serum 25(OH)D and serum lipids in 12 000 subjects from the Tromsø study, and also examined the longitudinal relation between change in serum 25(OH)D concentrations and change in serum lipids over a 14 years period.

Participants and methods

Participants

The Tromsø study, conducted by the University of Tromsø in cooperation with the National Health Screening Service, is a longitudinal population-based multipurpose study focusing on lifestyle-related diseases (Thelle *et al*., 1976). The fourth survey in 1994–1995 (1994 in the following for simplicity) was performed in two phases, and all residents in the Tromsø municipality aged 25 years or older were invited to participate in the first phase of the study. A total of 27 158 persons participated, providing an attendance rate of 77% among eligible inhabitants. All men aged 55–74 years, all women aged 50–74 years, and a 5–10% sample of the remaining age groups between 25 and 84 years were preselected to a second phase of the survey, and 7965 persons, or 78% of those invited, attended (Jorde and Bonaa, 2000). The sixth survey was performed in 2008 and the following groups were invited: those who participated in the second phase of the fourth survey, a random 10% sample of subjects 30–39 years old, all subjects 40–42 years old, a 40% random sample of subjects 43–59 years old and all subjects 60–87 years old. In total, 19 762 subjects were invited and 12 984 subjects (65.7%) attended. Subjects with diabetes and subjects using lipid-lowering drugs were analyzed separately.

Measurements

In both surveys the participants filled in questionnaires covering medical history, lipid-lowering medication and lifestyle factors including smoking. Blood samples were drawn in a nonfasting state. Serum lipids were analyzed consecutively both in the fourth survey in 1994 and in the sixth survey in 2008. In the fourth survey serum total cholesterol (TC) and triacylglycerol (TAG) levels were analyzed by enzymatic colorimetric methods with commercial kits (CHOD-PAP for TC and GPO-PAP for TAG; Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein cholesterol (HDL-C) was measured after the precipitation of lower-density lipoproteins with heparin and manganese chloride. The serum low-density lipoprotein cholesterol (LDL-C) was calculated using the formula LDL-C = TC−HDL-C−(TAG × 0.46), provided the serum TAG value was <4.0 mmol/l. In the sixth survey serum TC, TAG and HDL-C were analyzed with enzymatic colorimetric assays (CHOD-PAP, GPO-PAP, HDL-C plus 3rd generation, LDL-C plus 2nd generation, respectively, from Roche Diagnostics, Mannheim, Germany) using an automated clinical chemistry analyzer (Modular P; Roche Diagnostics). The current reference intervals were those established in collaboration with the Nordic Reference Interval Project, and were as follows for TC: 18–29 years, 2.9–6.1 mmol/l; 30–49 years, 3.3–6.9 mmol/l and $\geqslant 50$ years, 3.9–7.8 mmol/l; for TAG: 0.5–2.6 mmol/l; for HDL-cholesterol: women, 1.0–2.7 mmol/l and men, 0.8–2.1 mmol/l; and for LDL-cholesterol: 18–29 years, 1.2–4.3 mmol/l; 30–49 years, 1.4–4.7 mmol/l and $\geqslant 50$ years, 2.0–5.3 mmol/l.

Sera from the second phase of the fourth Tromsø study were stored at $-70\,^{\circ}C$, and after a median storage time of 13 years, thawed in March 2008 and analyzed for 25(OH)D whereas sera from the sixth Tromsø study were analyzed for 25(OH)D consecutively by immunometry (electrochemiluminescence immunoassay), using an automated clinical chemistry analyzer (Modular E170; Roche Diagnostics; Leino *et al*., 2008; Roth *et al*., 2008; Wagner *et al*., 2009). According to the manufacturers, the assay has, for total analytical precision, a coefficient of variation $\leqslant 7.8\%$ as judged in any of three concentrations (48.6–73.8–177.0 nmol/l). The cross-reactivity with 25(OH)D_2 was $<10\%$ and the analytical sensitivity was 10 nmol/l. At present, the laboratory has no reference values for 25(OH)D, but the manufacturer provides a population-based reference range of 27.7–107.0 nmol/l for adults as a guideline. This analysis has been approved by the Norwegian Accreditation Authority. With this method we have found smokers to have 15–20% higher serum 25(OH)D concentrations than nonsmokers, which we have not found when measuring serum 25(OH)D with other immunological, high pressure liquid chromatography or liquid chromatography-tandem mass spectrometry methods (Grimnes *et al*., 2010). For this discrepancy we have at present no explanation, and have therefore decided to present nonsmokers and smokers separately.

Statistics

Normal distribution was evaluated with visual inspection of histograms and determination of skewness and kurtosis, and except for serum TAG all variables used as dependent variables were considered normally distributed. After log transformation serum TAG attained normal distribution and was used as such in the statistical analyses. Linear trends across serum 25(OH)D quartiles were tested with linear regression with use of covariates as described in the tables. In the cross-sectional analyses month of blood sampling was adjusted for using dummy variables. Analysis of covariance was used to calculate adjusted means of serum lipids. A linear regression model with gender, age, BMI and month of blood sampling (with the use of dummy variables) as covariates was used for testing 25(OH)D as a continuous variable for prediction of serum lipids. Comparisons between summer and winter months were performed with a general linear

model with covariates as described in the tables. In the longitudinal study the changes (Δ values) were calculated as the value in 2008 minus the value in 1994. For change in serum 25(OH)D, we performed the analyses using both unadjusted serum 25(OH)D values as well as *z*-scores for serum 25(OH)D values calculated within each month in 1994 and 2008 to eliminate effects of season and storage. Unless otherwise stated, all data are expressed as mean ± s.d. All tests were two sided, and $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics, North Norway. Each participant gave written informed consent before the examinations.

Results

Cross-sectional study 2008

A total of 12 984 subjects attended the study and 12 817 had valid serum 25(OH)D measurements. Among these, 622 subjects had diabetes, and a further 2090 subjects were using lipid-lowering drugs. Among the remaining 10 105 subjects, 8018 were nonsmokers and 2087 were current smokers. The baseline characteristics of these subjects are shown in Table 1.

After adjusting for confounders, there was (in the 8018 nonsmokers) a significant increase in age, a decrease in BMI, an increase in TC, HDL-C and LDL-C, and a decrease in LDL-C/HDL-C ratio and TAG across increasing serum 25(OH)D quartiles (Table 2) as well as when the cohort was stratified according to serum 25(OH)D concentrations of 25, 50 and 75 nmol/l (Table 3). These significant trends were found in both men and women (data not shown). After adjustment for confounders the difference between the lowest and the highest serum 25(OH)D quartiles was most pronounced for HDL-C and TAG, being for nonsmokers 6.0 and 18.5%, respectively (Table 2).

Except for serum HDL-C and LDL-C, similar results were observed for the 2087 smokers (Table 4). In nonsmoking subjects with diabetes but not using lipid-lowering drugs ($n=239$) and in nonsmoking subjects without diabetes but using lipid-lowering drugs ($n=1243$), there was a significant decrease in serum TAG across the serum 25(OH)D quartiles whereas no significant trend was observed for serum TC (Table 4).

When serum 25(OH)D was used as a continuous variable in a multiple regression model with gender, age, BMI and month of blood sampling, the associations with HDL-C and TAG were found in both sexes and in all age and BMI groups tested in nondiabetic nonsmokers not taking lipid-lowering medication (Table 5).

For serum 25(OH)D, the expected seasonal variation was observed with 26.3% higher concentrations during the summer as compared with the winter. The serum HDL-C values were higher and the serum TAG concentrations were lower during the summer than during the winter, but the differences did not reach statistical significance (Table 6).

Longitudinal study

Among the 8018 nonsmoking subjects in the cross-sectional study in 2008, 1762 attended the second phase of the fourth survey in 1994 and had valid serum 25(OH)D measurements, and among the 2087 smokers included in the cross-sectional study in 2008, 397 were smokers in 1994 and had valid serum 25(OH)D measurements. Their characteristics are shown in Table 1.

When this cohort was divided in quartiles of change (Δ) in serum 25(OH)D from 1994 to 2008 (value in 2008 minus value in 1994), there was, in both the nonsmokers and the smokers, a significant decrease in Δ serum TAG across increasing Δ serum 25(OH)D quartile after adjustment for gender, age, serum TAG concentrations in 1994 and Δ BMI.

Table 1 Baseline characteristics of the subjects without diabetes and not using lipid-lowering drugs in the longitudinal study that lasted from 1994 to 2008, and characteristics of the subjects without diabetes and not using lipid-lowering drugs in the cross-sectional study in 2008

	Subjects in the longitudinal study (characteristics in 1994)		*Subjects in the cross-sectional study (characteristics in 2008)*	
	Nonsmokers	*Smokers*	*Nonsmokers*	*Smokers*
Males/females	554/1208	112/285	3685/4333	916/1171
Age (years)	55.5 ± 9.8	52.1 ± 10.3	55.9 ± 12.6	53.6 ± 11.4
BMI (kg/m^2)	25.6 ± 3.5	24.1 ± 3.1	26.7 ± 4.1	25.9 ± 4.2
Serum TC (mmol/l)	6.30 ± 1.16	6.38 ± 1.17	5.69 ± 1.04	5.81 ± 1.04
Serum HDL-C (mmol/l)	1.67 ± 0.43	1.56 ± 0.42	1.55 ± 0.44	1.46 ± 0.42
Serum LDL-C (mmol/l)	4.02 ± 1.05	4.14 ± 1.09	3.62 ± 0.91	3.73 ± 0.93
Serum TAG (mmol/l)	1.35 ± 0.77	1.48 ± 0.99	1.45 ± 0.86	1.58 ± 0.95
Serum 25(OH)D (nmol/l)	54.1 ± 16.2	75.4 ± 20.9	55.0 ± 17.7	68.7 ± 20.8

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TC, total cholesterol.

Table 2 Gender, age, BMI and serum lipids in relation to serum 25(OH)D quartiles in nonsmokers without diabetes and not using lipid-lowering drugs in the cross-sectional study 2008

	Serum 25(OH)D quartiles				P *for trend*[a]
	Quartile 1 (<43 nmol/l)	*Quartile 2 (43–53 nmol/l)*	*Quartile 3 (54–65 nmol/l)*	*Quartile 4 (>65 nmol/l)*	
Males/females	907/1090	920/1088	910/1093	948/1062	
Age (years)	54.4 ± 13.6	55.5 ± 12.5	56.6 ± 12.3	57.1 ± 11.7	<0.01
BMI (kg/m^2)	27.4 ± 4.7	27.0 ± 4.0	26.7 ± 4.0	26.0 ± 3.7	<0.01
Unadjusted values					
Serum TC (mmol/l)	5.62 ± 1.09	5.69 ± 1.03	5.72 ± 1.01	5.74 ± 1.04	<0.01
Serum HDL-C (mmol/l)	1.48 ± 0.43	1.52 ± 0.41	1.57 ± 0.45	1.63 ± 0.45	<0.001
Serum LDL-C (mmol/l)	3.55 ± 0.93	3.63 ± 0.91	3.64 ± 0.88	3.67 ± 0.91	<0.01
Ratio LDL-C/HDL-C	2.62 ± 1.06	2.58 ± 0.98	2.52 ± 0.96	2.44 ± 0.93	<0.001
Serum TAG (mmol/l)	1.64 ± 1.05	1.48 ± 0.87	1.43 ± 0.77	1.27 ± 0.68	<0.001
Adjusted values[b]					
Serum TC (mmol/l)	5.64 ± 0.98	5.69 ± 0.99	5.70 ± 0.98	5.73 ± 0.99	<0.01
Serum HDL-C (mmol/l)	1.50 ± 0.40	1.52 ± 0.36	1.55 ± 0.36	1.59 ± 0.36	<0.001
Serum LDL-C (mmol/l)	3.56 ± 0.89	3.64 ± 0.85	3.64 ± 0.85	3.67 ± 0.85	<0.01
Ratio LDL-C/HDL-C	2.60 ± 0.89	2.59 ± 0.90	2.55 ± 0.89	2.50 ± 0.90	<0.001
Serum TAG (mmol/l)	1.62 ± 0.80	1.48 ± 0.81	1.45 ± 0.80	1.32 ± 0.81	<0.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TAG, triacylglycerol; TC, total cholesterol.
[a]Linear regression with gender, age, BMI and month of blood sampling as covariates.
[b]Adjusted for gender, age and BMI.

Table 3 Gender, age, BMI and serum lipids in relation to serum 25(OH)D <25 nmol/l, 25–49 nmol/l, 50–74 nmol/l and >74 nmol/l in nonsmokers without diabetes and not using lipid-lowering drugs in the cross-sectional study 2008

	Serum 25(OH)D (nmol/l)				P *for trend*[a]
	<25	*25–49*	*50–74*	*>74*	
Males/females	75/123	1407/1689	1766/2004	437/517	
Age (years)	52.9 ± 14.9	54.7 ± 13.0	56.5 ± 12.1	57.7 ± 12.0	<0.001
BMI (kg/m^2)	27.4 ± 5.5	27.3 ± 4.4	26.5 ± 3.8	26.0 ± 3.7	<0.001
Unadjusted values					
Serum TC (mmol/l)	5.49 ± 1.19	5.65 ± 1.06	5.71 ± 1.03	5.77 ± 1.01	<0.01
Serum HDL-C (mmol/l)	1.47 ± 0.44	1.49 ± 0.42	1.57 ± 0.44	1.67 ± 0.47	<0.001
Serum LDL-C (mmol/l)	3.25 ± 1.02	3.43 ± 0.94	3.50 ± 0.92	3.53 ± 0.92	<0.001
Ratio LDL-C/HDL-C	2.55 ± 1.12	2.61 ± 1.04	2.52 ± 0.95	2.39 ± 0.93	<0.001
Serum TAG (mmol/l)	1.67 ± 1.05	1.58 ± 0.99	1.40 ± 0.77	1.23 ± 0.62	<0.001
Adjusted values[b]					
Serum TC (mmol/l)	5.55 ± 1.02	5.67 ± 0.99	5.70 ± 0.98	5.74 ± 0.99	<0.01
Serum HDL-C (mmol/l)	1.48 ± 0.39	1.51 ± 0.39	1.55 ± 0.36	1.62 ± 0.37	<0.001
Serum LDL-C (mmol/l)	3.47 ± 0.90	3.60 ± 0.88	3.65 ± 0.85	3.67 ± 0.87	<0.001
Ratio LDL-C/HDL-C	2.59 ± 0.91	2.60 ± 0.88	2.55 ± 0.85	2.46 ± 0.90	<0.001
Serum TAG (mmol/l)	1.67 ± 0.83	1.57 ± 0.83	1.42 ± 0.79	1.28 ± 0.81	<0.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TAG, triacylglycerol; TC, total cholesterol.
[a]Linear regression with gender, age, BMI and month of blood sampling as covariates.
[b]Adjusted for gender, age and BMI.

There was no significant relation between Δ serum 25(OH)D divided into quartiles and change in the other lipids measured (Table 7). Similarly, in a multiple linear regression model with Δ serum 25(OH)D as a continuous variable, Δ serum 25(OH)D was a significant and negative predictor for Δ serum TAG (data not shown). The use of Δ *z*-score for serum 25(OH)D (to eliminate effects of season and storage) did not change these results significantly (data not shown).

Table 4 Gender, age, BMI and unadjusted serum lipids in relation to serum 25(OH)D quartiles in smokers without diabetes and not using lipid-lowering drugs (1); nonsmoking subjects with diabetes but not using lipid-lowering drugs (2); and in nonsmoking subjects without diabetes but using lipid-lowering drugs in the cross-sectional study 2008 (3)

	Serum 25(OH)D nmol/l				P *for trend*[a]
	<43	*43–53*	*54–65*	*>65*	
(1) Smokers					
Males/females	95/93	148/148	213/246	460/684	
Age (years)	53.0 ± 13.2	52.1 ± 11.5	53.6 ± 12.0	54.2 ± 10.9	<0.05
BMI (kg/m^2)	26.2 ± 5.4	26.6 ± 4.4	25.9 ± 4.3	25.7 ± 3.9	<0.001
Serum TC (mmol/l)	5.60 ± 0.99	5.74 ± 0.94	5.76 ± 1.05	5.87 ± 1.07	<0.001
Serum HDL-C (mmol/l)	1.46 ± 0.51	1.38 ± 0.42	1.43 ± 0.40	1.49 ± 0.41	NS
Serum LDL-C (mmol/l)	3.31 ± 0.94	3.52 ± 0.87	3.60 ± 0.97	3.70 ± 1.01	NS
Ratio LDL-C/HDL-C	2.65 ± 1.11	2.95 ± 1.22	2.83 ± 1.12	2.77 ± 1.10	<0.05
Serum TAG (mmol/l)	1.81 ± 1.16	1.83 ± 1.22	1.60 ± 0.88	1.47 ± 0.83	<0.001
(2) Subjects with diabetes					
Males/females	32/37	38/29	31/28	19/25	
Age (years)	61.5 ± 14.8	61.2 ± 11.1	61.5 ± 12.7	63.9 ± 10.4	NS
BMI (kg/m^2)	30.1 ± 5.0	30.1 ± 5.6	28.2 ± 4.2	27.9 ± 4.3	<0.001
Serum TC (mmol/l)	5.44 ± 1.01	5.42 ± 0.99	5.21 ± 0.86	5.63 ± 1.03	NS
Serum HDL-C (mmol/l)	1.34 ± 0.36	1.33 ± 0.37	1.31 ± 0.36	1.57 ± 0.43	NS
Serum LDL-C (mmol/l)	3.13 ± 0.89	3.25 ± 0.80	3.16 ± 0.84	3.36 ± 0.96	NS
Ratio LDL-C/HDL-C	2.67 ± 0.92	2.77 ± 0.94	2.74 ± 0.95	2.47 ± 1.04	NS
Serum TAG (mmol/l)	2.12 ± 1.38	1.82 ± 0.84	1.61 ± 0.82	1.53 ± 0.69	<0.001
(3) Using lipid-lowering drugs					
Males/females	129/136	163/133	203/145	187/147	
Age (years)	67.4 ± 10.5	66.6 ± 9.4	66.3 ± 8.4	66.9 ± 8.3	NS
BMI (kg/m^2)	28.4 ± 4.3	28.5 ± 4.3	27.8 ± 4.3	27.6 ± 4.1	<0.001
Serum TC (mmol/l)	4.92 ± 1.11	4.94 ± 1.00	5.01 ± 1.13	4.97 ± 0.96	NS
Serum HDL-C (mmol/l)	1.47 ± 0.45	1.44 ± 0.38	1.50 ± 0.45	1.51 ± 0.42	NS
Serum LDL-C (mmol/l)	2.64 ± 0.90	2.76 ± 0.90	2.81 ± 0.96	2.81 ± 0.82	<0.05
Ratio LDL-C/HDL-C	2.10 ± 0.81	2.21 ± 0.88	2.17 ± 0.95	2.13 ± 0.81	NS
Serum TAG (mmol/l)	1.76 ± 1.07	1.62 ± 0.79	1.52 ± 0.82	1.40 ± 0.70	<0.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, not significant; 25(OH)D, 25-hydroxyvitamin D; TAG, triacylglycerol; TC, total cholesterol.
[a]Linear regression with gender, age, BMI and month of blood sampling as covariates.

Table 5 Standardized β and *t*-values for serum 25(OH)D from multiple linear regression models with serum lipids as dependent variables and gender, age, BMI and month of blood sampling as covariates in relation gender, age and BMI groups, in nonsmokers without diabetes and not using lipid-lowering drugs in the cross-sectional study 2008

Subjects		*Dependent variables*							
		Serum TC		*Serum HDL-C*		*Serum LDL-C*		*Lg serum TAG*	
	N	β	t	β	t	β	t	β	t
All subjects	8018	0.04	3.31	0.08	7.95	0.06	4.91	−0.12	−10.9
Males	3685	0.06	3.29	0.13	7.81	0.07	3.95	−0.15	−9.37
Females	4333	0.04	2.76	0.06	4.00	0.04	2.90	−0.08	−5.51
Age (years)									
<50	2916	0.03	1.82	0.04	2.33	0.07	4.03	−0.08	−4.95
50–64	3024	0.01	0.59	0.08	4.84	0.03	1.60	−0.15	−8.26
>64	2078	0.04	1.87	0.10	4.82	0.04	1.79	−0.13	−5.91
BMI (kg/m^2)									
<25.0	2896	0.02	0.85	0.06	3.59	0.02	0.98	−0.10	−5.55
25.0–30.0	3639	0.03	1.99	0.12	7.21	0.04	2.36	−0.17	−9.83
>30.0	1483	0.08	2.93	0.09	3.48	0.10	3.47	−0.10	−3.67

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; TC, total cholesterol. *t*-values >1.96, >2.58, >3.29 correspond to $P<0.05$, <0.01 and <0.001, respectively.

Discussion

In this study there were highly significant positive associations between serum 25(OH)D and serum TC, HDL-C and LDL-C, and significant negative associations between serum 25(OH)D and both LDL-C/HDL-C ratio and TAG in the nonsmokers after adjustment for gender, age, BMI and month of blood sampling. In particular, the cross-sectional associations between serum 25(OH)D and serum HDL-C and TAG were strong and significant in both genders and in all age and BMI subgroups tested. In addition, an increase in serum 25(OH)D over time was associated with a decrease in serum TAG, which to the best of our knowledge has not been reported before.

The positive association between serum 25(OH)D and HDL-C has previously been reported from our group in a study on 438 overweight and obese subjects (Jorde *et al*., 2009). The association between serum 25(H)D and lipids is also reported from other studies, but the results are not consistent. Thus, in a study on 6810 45-year-old subjects by Hyppönen *et al*. (2008), and in a study on 3069 older men by Lee *et al*. (2009), a negative association between serum 25(OH)D and TAG was found, but the association between serum 25(OH)D and HDL-C was not significant after adjustment for confounders. However, in both the studies subjects with high serum 25(OH)D concentrations tended to have higher HDL-C concentrations than those with lower serum 25(OH)D concentrations. Conversely, in a study on 201 men and 180 women, Gannagé-Yared *et al*. (2009) found no significant association between serum 25(OH)D and TAG, whereas in men there was a significant negative relation with serum LDL-C. A negative relation between serum 25(OH)D and both TC and LDL-C was also reported by Chiu *et al*. (2004) in a study on 126 subjects, whereas in that study no association between serum 25(OH)D and TAG was found. And finally, no significant association between fasting serum lipids and serum 25(OH)D was found in 524 subjects in the MRC-Ely study (Forouhi *et al*., 2008).

In the larger studies, however, that is, those by Hyppönen *et al*. (2008), Lee *et al*. (2009) and our own, it appears that the relation between serum 25(OH)D and TAG is consistent. However, our finding of a positive relation between serum 25(OH)D and serum TC, HDL-C and LDL-C needs further confirmation. Our observation supports the suggestion that a high serum 25(OH)D concentration is associated with a desirable lipid profile with low serum TAG concentration and a low LDL-C/HDL-C ratio (Miller *et al*., 1977; Bansal *et al*., 2007; Fernandez and Densie Webb, 2008). If confirmed this could contribute to an explanation of the association between low serum 25(OH)D concentrations and increased risk of mortality (Dobnig *et al*., 2008; Melamed *et al*., 2008; Pilz *et al*., 2009).

Table 6 Serum 25(OH)D and lipid concentrations during the winter (December, January, February) and summer (June, August, September) months in nonsmokers without diabetes and not using lipid-lowering drugs in the cross-sectional study 2008

	Winter (n = 2011)	*Summer (n = 1963)*
Serum 25(OH)D (nmol/l)	48.2 ± 17.0	61.5 ± 18.0[a]
Serum TC (mmol/l)	5.70 ± 1.08	5.72 ± 1.05
Serum HDL-C (mmol/l)	1.55 ± 0.44	1.59 ± 0.44
Serum LDL-C (mmol/l)	3.49 ± 0.95	3.48 ± 0.93
Serum TAG (mmol/l)	1.46 ± 0.84	1.42 ± 0.78

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; TAG, triacylglycerol; TC, total cholesterol.
[a]$P < 0.001$ versus winter, general linear model with adjustment for gender, age and BMI.

Table 7 Change in serum lipids from 1994 to 2008 in relation to quartiles of change in serum 25(OH)D from 1994 to 2008 in subjects without diabetes and not using lipid-lowering drugs stratified by smoking status

Δ serum 25(OH)D quartiles (nmol/l)	N	*Δ Serum TC (mmol/l)*	*Δ Serum HDL-C (mmol/l)*	*Δ Serum LDL-C (mmol/l)*	*Δ Ratio LDL-C/HDL-C*	*Δ Serum TAG (mmol/l)*
Nonsmokers						
Quartile 1 (< −10.3)	440	−0.31 ± 0.00	−0.01 ± 0.32	−0.24 ± 0.84	−0.10 ± 0.75	0.15 ± 0.77
Quartile 2 (−10.2–0.87)	441	−0.32 ± 0.91	−0.01 ± 0.32	−0.20 ± 0.81	−0.08 ± 0.77	0.06 ± 0.78
Quartile 3 (0.88–12.0)	440	−0.25 ± 0.92	−0.01 ± 0.31	−0.10 ± 0.82	−0.06 ± 0.68	0.00 ± 0.71
Quartile 4 (>12.0)	441	−0.35 ± 1.00	0.00 ± 0.34	−0.21 ± 0.85	−0.11 ± 0.73	−0.03 ± 0.72
P for trend[a]		NS	NS	NS	NS	<0.002
Smokers						
Quartile 1 (< −10.3)	157	−0.41 ± 0.95	0.02 ± 0.31	−0.38 ± 0.85	−0.28 ± 0.81	0.11 ± 0.66
Quartile 2 (−10.2–0.87)	83	−0.27 ± 0.93	0.02 ± 0.31	−0.17 ± 0.80	−0.14 ± 0.92	−0.02 ± 0.61
Quartile 3 (0.88–12.0)	71	−0.30 ± 0.89	0.00 ± 0.31	−0.27 ± 0.77	−0.16 ± 0.78	0.04 ± 0.59
Quartile 4 (>12.0)	86	−0.34 ± 0.95	0.08 ± 0.33	−0.14 ± 0.92	−0.18 ± 0.85	−0.37 ± 1.33
P for trend[a]		NS	NS	NS	NS	<0.001

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, not significant; 25(OH)D, 25-hydroxyvitamin D; TAG, triacylglycerol; TC, total cholesterol.
[a]Linear regression with gender, age, corresponding serum lipid value from 1994 and Δ BMI as covariates; Δ Values are value in 2008 minus value in 1994.

Associations found in cross-sectional studies are no proof of a causal relationship. However, if such a causal relation exists, one would expect that changes in the serum 25(OH)D concentrations would be reflected in changes in serum lipid concentrations. In our study that was observed for TAG only, where an increase in the serum 25(OH)D concentrations from 1994 to 2008 was accompanied by a reduction in the serum TAG concentrations. Similarly, a nonsignificant reduction in serum TAG concentrations was observed during the summer months when the serum 25(OH)D concentrations are the highest. But again, this is no proof of causality, which has to come from intervention studies. So far only a few such intervention studies have been reported. Regarding serum TAG concentrations, which appear to be strongly associated with serum 25(OH)D, we have in our study of overweight and obese subjects found no effect on serum TAG of 20 000–40 000 IU vitamin D per week given for 1 year (Jorde *et al*., 2009); Heikkinen *et al*. (1997) found no effect by supplementation with 300 IU vitamin D for 3 years in 323 postmenopausal women; Andersen *et al*. (2009) found no change in 173 Pakistani subjects living in Denmark and given vitamin D in daily doses of 400 or 800 IU for 1 year; whereas Zittermann *et al*. (2009) in their study on 200 overweight subjects found a significant 13% decrease in the serum TAG in those given 3320 IU daily for 1 year. These discrepancies could be due to the doses of vitamin D given as well as differences between the study groups. Accordingly, the effect of different doses of vitamin D on serum TAG remains to be determined.

If there is a relation between serum 25(OH)D and lipid concentrations, this could be explained by several mechanisms. For TAG, one factor could be a vitamin-D-induced increase in calcium absorption leading to reduced formation of calcium-fatty soaps in the gut and thereby increased absorption of fat (Reid, 2004). At least in subjects with low serum 25(OH)D concentrations, supplementation with vitamin D leads to increased formation of $1,25(OH)_2D$, which again may increase lipogenesis and lipolysis (Zemel *et al*., 2000).

Owing to the unexpectedly high serum 25(OH)D concentrations in smokers than nonsmokers, for which we at present have no good explanation (Grimnes *et al*., 2010), the analyses were performed again according to smoking status and yielded similar results in nonsmokers and smokers. However, in the subjects using lipid-lowering drugs, the increase in serum cholesterol with increasing serum 25(OH)D quartiles was not observed. One possible explanation could be that in subjects with higher serum 25(OH)D concentrations statins are more effective, as it has been suggested that vitamin D and statins have synergistic effects on serum cholesterol concentrations (Schwartz, 2009). In the subjects with diabetes the relation between serum 25(OH)D and TAG was highly significant, whereas that was not observed regarding serum cholesterol, possibly because of the low number of subjects with diabetes studied.

Our study has some limitations. First of all we measured nonfasting lipid concentrations in both 1994 and 2008. Second, in the longitudinal study we had only two measurements of serum 25(OH)D and lipids, and ideally there should have been a larger series of measurements to evaluate the effects of changes over time. We did not measure apolipoprotein A-1, which has been reported to be associated with serum 25(OH)D (Auwerx *et al*., 1992; John *et al*., 2005), and would therefore have been of particular interest in this study. We did not have a detailed food frequency questionnaire for evaluation of vitamin D intake, nor did we have a measure of habitual solar exposure. However, the strengths of our study are that we included a large group of subjects and adjusted for relevant confounders, and in the longitudinal study that the observation time was long and similar in all subjects. Also, the fact that we found the expected relation between serum 25(OH)D and BMI at baseline (Snijder *et al*., 2005) adds external validity to the findings.

In conclusion, high serum 25(OH)D concentrations are associated with a favorable lipid profile.

Conflict of interest

The authors declare no conflict of interest.

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