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Association between vitamin D status and long-term changes of lipid profile in healthy and younger adults

Master thesis in Human Movement Science, Department of Neuroscience, NTNU

Trondheim, Spring 2016

Abstract

Background

Dyslipidaemia is the condition of abnormal lipid levels and is considered a criterion in the pathogenesis of arteriosclerosis. Vitamin D is considered to be beneficial for overall health, however the prevalence of vitamin D deficiency is a global problem. Increasing evidence indicates that a higher vitamin D level may be beneficial in terms of a favourable lipid profile. Substantial evidence suggests a beneficial effect of a higher level of physical activity on lipid profile. Additionally a few studies have observed an association between vitamin D levels and levels of physical activity. The objective of this study was to investigate the association between vitamin D levels and the long-term changes in lipids during approximately an 11 years follow-up, in young adults, and to study if physical activity modifies the association.

Material and methods

We included subjects aged 19-55 years who had participated in both the HUNT2 (1995-1997) and HUNT3 (2006-2008) survey of the Nord-Trøndelag Health Study in Norway. After excluding persons with abnormal lipid levels at baseline (HUNT2), a total of 1820 persons (1074 women and 744 men) with sufficient measurements on vitamin D, lipids and physical activity. We used linear regression to compute coefficient for the mean change in lipids from HUNT2 to HUNT3, among categories of vitamin D. Logistic regression was used to compute odds ratios (ORs) as an estimate of relative risk of developing low density lipoprotein (LDL) dyslipidaemia from HUNT2 to HUNT3 associated with levels of vitamin D. Stratified analysis was preformed on physical inactive and physical active people separately.

Results

The results showed an association between serum 25-Hydroxyvitmain D (25(OH)D) levels of 61,4-156 nmol/L and the mean changes of high-density lipoprotein cholesterol (HDL-C) (-1.27; 95% CI= -2.52 - -0.03) from HUNT2 to HUNT3, compered to the reference group ($p<0.05$). Serum 25(OH)D levels of 43,8-61,3 nmol/L was significantly associated with mean change in the ratio of total cholesterol and HDL-C (TC-HDL) (-0.10; 95% CI= -0.19- -0.00), and 61,4-156 nmol/L (-0.12; 95% CI= -0.21- -0.02) compered with reference group ($p<0.05$).

Vitamin D as a continuous variable was significantly associated with mean change in TC-HDL ratio (-0.06; 95% CI= -0.11 - -0.01) from HUNT2 to HUNT3 ($p<0.05$). Stratified analyses showed no significant difference by formal tests of interaction between physical inactive group and physical active group. A large inverse association between serum 25(OH)D levels of 61,4-156 nmol/L and the new onset of LDL dyslipidaemia (OR= 0.75; 95% CI =0.57-0.97), was observed. No significant difference between the physical active and inactive groups was observed.

Conclusion

The results from this study indicate that a higher level of vitamin D is beneficial for a favourable change in lipids particularly in HDL, TC-HDL ratio and a reduced risk of LDL-dyslipidaemia, and levels of physical activity did not modify the association.

Sammendrag

Bakgrunn

Dyslipidemi er en tilstand med unormalt høye lipid verdier som ansees å være et kriteriet for å utvikle arteriosklerose. Vitamin D er ansett å være fordelaktig for den generelle helsen, men prevalensen av vitamin D mangel er et globalt problem. Økende bevis indikerer at en bedre vitamin D status kan ha en positiv effekt på lipid profilen. Betydelig bevis foreslår også at økt fysisk aktivitet har positiv effekt på lipid profilen. I tillegg har noen få studier observert en assosiasjon mellom vitamin D status og nivåer av fysisk aktivitet. Hensikten med denne studien var dermed å undersøke sammenhengen mellom nivåer av vitamin D og langvarige endringer i lipider over omtrent en 11 års periode, hos unge voksne, samt se på forskjellen mellom fysisk aktive og inaktive individer.

Materiale og metode

Forsøkspersoner fra Helseundersøkelsen i Nord-Trøndelag i alderen 19-55 år som deltok både i HUNT2 (195-1997) og i HUNT3 (2006-2008) ble inkludert. Personer med unormale nivåer av lipider ved baseline (HUNT2) ble ekskludert. Totalt 1820 forsøkspersoner (1074 kvinner og 744 menn) med tilstrekkelig data på vitamin D, lipider og fysisk aktivitet ble inkludert. Lineær regresjon ble brukt for å beregne koeffisienten av gjennomsnittlig endring i lipider fra HUNT2 til HUNT3, sammenlignet med nivåer av vitamin D. Logistisk regresjon ble brukt for beregne odds ratio (OR) som et estimat på relativ risiko for å utvikle low-density lipoprotein (LDL) dyslipidemi fra HUNT2 til HUNT3, sammenlignet med nivåer av vitamin D. Stratifiserte analyser ble utført på fysisk inaktive og fysisk aktive individer separat.

Resultater

Resultater viste en signifikant sammenheng mellom serum 25-Hydroxyvitmain D (25(OH)D) nivåer på 61,4-156 nmol/L og en gjennomsnittlig endring i high-density lipoprotein cholesterol (HDL-C) (-1.27 95% CI= -2.52 - -0.03) fra HUNT2 til HUNT3, sammenlignet med referansegruppe ($p<0.05$). Serum 25(OH)D nivåer på 43,8-61,3 nmol/L viste en signifikant sammenheng mellom gjennomsnittlig endring i ratio av total kolesterol og HDL-C (TC-HDL) (-0.10; 95% CI= -0.19- -0.00), og 61,4-156 nmol/L (-0.12; 95% CI= -0.21- -0.02) sammenlignet med referansegruppe ($p<0.05$). Vitamin D som kontinuerlig variabel hadde en signifikant sammenheng med den gjennomsnittlige endringen i TC-HDL ratio (-0.06; 95% CI= -0.11 - -0.01) fra HUNT2 til HUNT3 ($p<0.05$). Formelle tester utført på interaksjon på

stratifiserte analyser viste ingen signifikant forskjell mellom fysisk inaktiv gruppe og fysisk aktiv gruppe. Det ble funnet en sterk omvendt sammenheng mellom serum 25(OH)D nivåer på 61,4-156 nmol/L og nye tilfeller av LDL-dyslipidemi (OR= 0.75; 95% CI =0.57-0.97). Ingen signifikante forskjeller mellom fysisk inaktive og fysisk aktive ble observert.

Konklusjon

Resultatene fra denne studien indikerer at et høyere nivå av vitamin D er fordelaktig for en gunstig endring i lipider, særlig med tanke på HDL-C, TC-HDL ratio, samt den reduserte risikoen for å utvikle LDL-dyslipidemi. I tillegg ser det ut til at denne sammenhengen ikke modifiseres av fysisk aktivitet.

Acknowledgment

This master thesis in Human Movement Science is based on the work carried out between August 2015 and June 2016 at the Department of Neuroscience, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway. The used data came from the second and third surveys of the Nord-Trøndelag Health (HUNT) Study. The HUNT study is a collaboration between the HUNT Research Centre (Faculty of Medicine, NTNU), the Nord- Trøndelag County Council and the Norwegian Institute of Public Health. A special thanks goes to the participants of HUNT, and to HUNT personnel for their contribution on objective biomarkers and responses on questioners. My sincere appreciation goes to my main supervisor Professor Xiao-Mei Mai, for quick, reliable and valuable feedback. And gratitude goes to my co-supervisor Tom Ivar Lund Nilsen for the epidemiologic guidance.

Table of Content

1 INTRODUCTION

Vitamin D is considered to be highly beneficial for overall health, and a sufficient vitamin D level is generally understood to be essential in the prevention of several diseases [1, 2]. Increasing evidence indicates an association between low vitamin D status and the risk of developing cardiovascular diseases (CVD) [3, 4]. Another acknowledged behavioural risk factor of developing CVD is physical inactivity (PA) [5]. PA is well recognised to have an overall positive effect on the health [6], and to have a preventive effect on the development of CVD in particular [7].

Vitamin D is a fat-soluble vitamin, which can be obtained from the sun in the skin, or retrieved from the diet and supplements. Serum 25-hydroxyvitamin D (25(OH) D level integrates sun exposure, dietary intake, supplement use and storage, and is therefore commonly used as a measurement when estimating vitamin D level in the blood [8]. Insufficient vitamin D level has become a worldwide problem [9], and a cross-sectional study preformed on Norwegian adults, showed a prevalence of vitamin D deficiency up to 40% [10].

Changes in lipids occur as a response to aging [11]. Dyslipidaemia however refers to a condition with increased concentration of low-density lipoproteins (LDL) levels, a decreased concentration of high-density lipoproteins (HDL) and increased triglyceride (TG) levels in the blood [12]. Dyslipidaemia is considered a criterion in the pathogeneses of atherosclerosis, hence an important risk factor in the development of CVD [13]. In the field of cholesterol-lowering treatment, the National Cholesterol Education Program (NCEP) has identified LDL-cholesterol (LDL-C) as the primary target, by which has been repeatedly demonstrated to increase CVD risk. HDL cholesterol (HDL-C) also shows a strong inverse association with prevalent CVD and is considered to have a protective effect on CVD. However the association between higher HDL-C levels and the reduced risk of CVD may be confounded by a healthier lifestyle [12, 14].

Several cross-sectional studies have found an association between higher vitamin D levels and a favourable lipid profile. The observed association are primarily made in regard to the ratio

of total cholesterol and HDL-C (TC-HDL) and TG levels [15, 16]. The few prospective studies preformed have also found an association between higher vitamin D levels and lower TG levels [17]. Skaaby et. al (2012) investigated the changes in lipids during a 5 years follow-up, and also found a significant decrease in TG. An additional significant decrease was observed in very low-density lipoproteins cholesterol (VLDL-C) associated with higher vitamin D levels [18]. Intervention studies investigating the association between vitamin D and changes in lipids are few, and the results are inconsistent [15]. Zitterman et. al (2009) found a significant decrease in TG after a randomised double blinded trial in overweight subjects, having a vitamin D supplementation group and a placebo group in a 12 months period [19]. However in most additional intervention studies the number of subjects are low, furthermore the results are inconsistent, and some studies even introduce a increase in TG and LDL-C levels after using vitamin D supplements [15]. Consequently the effects of vitamin D supplementation on lipids are uncertain, and moreover the literature and study designs lack to investigate the long-term and possible preventive effect of vitamin D on dyslipidaemia.

Evidence on the preventive effect of physical activity on dyslipidaemia is substantial, and it is especially endurance training that has shown to be beneficial in terms of a favourable lipid profile [20]. Interestingly Zittermann et. al (2000) found a higher serum 25(OH)D levels in more physically active subjects [21]. Furthermore Scragg and Camargo (2008) observed an association between outdoor activity and a higher serum 25(OH)D [22]. However due to the limitation of without adjustment for sun exposure, it is uncertain that PA has independent effect on raising the serum 25(OH)D levels. Nevertheless this raises the question of PA as a possible modifier in the association between vitamin D and lipids.

Thus the objective of this prospective population-based study was to investigate the association between vitamin D levels and the long-term changes in lipids during approximately an 11 years follow-up, in young adults, and to study if the association differs in physical active versus inactive individuals.

2 MATERIAL AND METHODS

2.1 Study Population

Helseundersøkelsen i Nord-Trøndelag (HUNT) is a large health study based on the population of Nord-Trøndelag County, who are located at latitude 64° North in the middle of Norway. The HUNT2 study was conducted in 1995-1997 and had a number of 65 237 participants. A number of 37 059 subjects participated in both HUNT2 and HUNT3, and the HUNT 3 study was conducted in 2006-2008 [23]. The participants completed a clinical examination and answered a comprehensive questioner regarding health related factors and lifestyle. The clinical examination included standardised anthropometric measurements and a non-fasting venous blood sample [24].

The current cohort was established containing participants from HUNT2 and HUNT3. There were 25 616 participants in the age 19-55 years at HUNT2 who also participated in the HUNT 3 study. A random sample of 5723 participants had sufficient vitamin D measurements, and 3605 had complete information on exposure and outcome measurements. NCEP considers LDL-C as the primary target for cholesterol lowering therapy and prevention of CVD. The LDL-C measurement required an indirect calculation, using an equation including all other lipids, and according to NCEP the calculated LDL level was accurate, if the TG level was below 400 mg/dl [14]. Thus to investigate the possible preventive effect of vitamin D on lipids, the subjects with normal lipid levels defined by normal LDL level, i.e. LDL-C <130 mg/dl and TG <400 mg/dl at baseline were included in the study [14]. This left a number of 1820 subjects who were included in the analysis sample.

2.3 Vitamin D

Vitamin D was measured as serum 25(OH) D levels in the serum, using LIASON 25-OH Vitamin D TOTAL (DiaSorin, Saluggia, Italy), a fully automated antibody-based chemiluminescence assay. Analyses were performed with vitamin D as exposure variable and categorised into groups of cut off points and tertiles. The following cut off points used in the current study are widely used to classify serum 25(OH) D; <50nmol/L as deficient, ≥50-75nmol/L as insufficient and ≥75nmol/L as sufficient [25, 26]. The cut off points in tertiles were as follows; the 1st 10-43,7 nmol/L serum 25(OH) D (N=607), mean 33,5 std 7,4, the 2nd 43,8-61,3 nmol/L serum 25(OH) D (N=609), mean 52,3 std 5,1 and the 3rd 61,4-156 nmol/L

serum 25(OH) D (N=604), mean 79,0 std 14,5. Additional analyses were conducted with vitamin D as continuous variable.

2.4 Lipids

Lipid measurements contained total cholesterol (TC), HDL-C and TG. LDL-C was indirectly calculated according to the Firdewald equation; TC – HDL-C – TG/5 [27] and TC-HDL ratio was calculated by TC/HDL [14]. Baseline lipids measurements were compered with the follow up measurements, and changes were calculated. The levels of TC, LDL-C, TC-HDL ratio and TG are assumed to increase due to aging [11], and therefore the changes were calculated by HUNT3 levels – HUNT2 levels. The HDL-C levels are consequently expected to decrease by age and the change was calculated by HUNT2 – HUNT3 levels. New onset of cases with LDL-dyslipidaemia during follow up was defined as TG<400 mg/dl and LDL-C >130mg/dl or TG>400mg/dl.

2.5 Physical Activity

Leisure time PA at baseline was defined by answers collected from the HUNT2 questionnaire. Participants were asked to report how many hours of hard or light PA they had been conducted in an average week in past year. Light PA was defined by not being sweat and/or out of breath, while hard PA was defined by being sweat and/or out of breath. The response options was 0, <1, 1-2 and ≥3 hours a week for each category. Initially PA was defined as a combined variable of four groups. The inactive group reported no activity, the low activity group reported less than 3h light and no hard activity, the moderate group reported more than 3h light and no hard, or less than 1h hard with any light activity and finally the high activity group reported more than 1h hard with any light activity. However the groups were merged to make two comparable sized groups. The physical active people were classified as more than one-hour hard activity combined with any amount of light activity per week, while the rest was classified as physical inactive. The physical active group was the closest to fulfil the Norwegian Institute of Public Health recommendations of 30 minutes of moderate PA every day.

2.6 Other factors

Potentially confounding factors and other important variables were collected in HUNT2. These factors were categorised as follows: age (19-29, 30-39, 40-49, 50-55), sex (female/male), body mass index (BMI) (normal: 18,5-24,9 kg/m^2, overweight: 25-29,9 kg/m^2, obese: ≥30 kg/m^2), education years (<10, 10-12, ≥13), social benefits (no recipient, recipient), economic difficulties the past year (never, yes), alcohol consumption (abstainer or less than monthly, 1-4 times/moth, ≥5 times/month), and smoking habits (never smoked daily, ex smoker daily, current smoker daily). A variable expressing family history of CVD was defined and included if myocardial infraction had occurred in the family. Vitamin D blood sample season was defined by the period of the collected blood sample and classified according to Norwegian Meteorological Institute [28].

2.7 Statistics

All the statistical analyses were completed using the statistical software STATA for Windows, version 13.1 (StataCorp LP, College Station, Texas, USA). Linear regression was used to compute the coefficient for the mean change in lipids from HUNT2 to HUNT3, among vitamin D categories. Logistic regression was used to compute odds ratios (ORs) as an estimate of relative risk of developing LDL-dyslipidaemia during follow-up associated with different levels of vitamin D. Further stratified analysis were conducted in physical inactive and physical active people separately.

2.8 Ethics

The Norwegian Regional Committee for Ethics in Medical Research (REK) approved the study. All participants signed a written informed consent at participation of HUNT2 and HUNT3.

3 RESULTS

Baseline characteristics in Table 1 show the differences between the analysis sample (n = 1820), the sample with complete data on vitamin D and lipid levels (n = 3605) and the random cohort (n = 5723). Despite no major differences between the groups, the participants

in the analysis sample were overall younger and healthier compered with the complete cohort sample and the random sample.

The baseline characteristics distributed according to vitamin D status in cut off points are presented in Table 2. Notably the differences between the groups in BMI showed a higher prevalence of obesity in adults with serum 25(OH) D levels <50nmol/L compered with adults with 25(OH) D at 50-75 and ≥75nmol/L, additionally the differences between the groups in smoking habits showed that participants with serum 25(OH) D levels <50nmol/L tend to have more current smokers.

Results on the association between vitamin D status and changes in lipids are presented in Table 3. Results showed a significant association between vitamin D in the 3rd tertile and the mean changes of HDL-C -1.27 (95% CI= -2.52 - -0.03) from HUNT2 to HUNT3, compered with the reference group ($p<0.05$). The vitamin D in cut off points showed a tendency of an association between serum 25(OH) D level >75nmol/L and mean change in HDL-C -1.30 (95% CI= -2.72-0.13) from HUNT2 to HUNT3, compered with reference group ($p<0.1$). Vitamin D as continuous variable showed a tendency to be associated with mean change in HDL-C -0.56 (95% CI= -1.17-0.05) from HUNT2 to HUNT3 ($p<0.1$). Vitamin D in the 2nd tertile was significantly associated with mean change in TC-HDL ratio -0.10 (95% CI= -0.19--0.00), and additional significant association was seen in the 3rd tertile -0.12 (95% CI= -0.21--0.02) compered with reference group ($p<0.05$). The vitamin D in cut off point showed a tendency of an association between serum 25(OH) D >75nmol/L and the mean change in TC-HDL ratio -0.10 (95% CI= -0.22 – 0.01) from HUNT2 to HUNT3 compered to the reference group ($p<0.1$). Vitamin D as a continuous variable was significant associated with mean change in TC-HDL ratio -0.06 (95% CI= -0.11 - -0.01) from HUNT2 to HUNT3 ($p<0.05$). A clear dose-response association was displayed between vitamin D and mean change in LDL-C, however not statistical significant.

Stratified analyses presented in Table 4 suggest a stronger association between vitamin D and mean change in TC-HDL ratio in the physical inactive group compered to the physical active group. However no significant difference between the two groups by formal tests of interaction was shown. Table 5 showed a large inverse association between vitamin D in the 3rd tertile and the new onset of LDL dyslipidaemia with ORs of 0.75 (95% CI= 0.57-0.97)

respectively. Furthermore there was no significant difference between the physical active and inactive groups.

Table 1: Baseline characteristics

	Random Sample (N=5723)		Complete Cohort (N=3605)		Analysis Sample (N=1820)	
	N	%	N	%	N	%
Age, years						
19-29	888	15,5	636	17,6	456	25,0
30-39	1736	30,3	1151	31,9	657	36,1
40-49	2227	38,9	1350	37,4	570	31,3
50-55	872	15,2	468	13,0	137	7,5
Sex						
Female	3135	54,8	1846	51,2	1076	59,0
Male	2588	45,2	1759	48,8	744	41,0
Vitamin D						
<50	3001	52,4	1828	50,7	831	45,6
≥50-75	1984	34,7	1281	35,5	682	37,5
≥75	738	12,9	496	13,8	307	16,9
Physical activity						
Inactive	236	4,1	181	5,0	78	4,2
Low	938	16,4	730	20,2	359	19,7
Moderate	1622	28,4	1265	35,1	614	33,7
High	1835	32,0	1429	39,6	769	42,2
Unknown	1092	19,0	0	0	0	0
Blood sample Vit D season						
Winter	1739	30,4	1188	33,0	597	32,8
Spring	1381	24,1	718	19,9	341	18,7
Summer	697	12,2	349	9,7	205	11,3
Autumn	1904	33,3	1350	37,4	677	37,2
Unknown	2	0,03	0	0	0	0
Body mass index						
Normal	2624	45,9	1654	45,9	1011	55,5
Overweight	2414	42,1	1541	42,7	653	36,0
Obesity	673	11,8	401	11,1	153	8,4
Unknown	12	0,2	9	0,2	3	0,1
Education, years						
<10	1105	19,3	614	17,0	214	11,7
10-12	3967	53,6	1965	54,6	1032	56,7
≥13	1496	26,1	1005	27,9	564	31,0
Unknown	55	1,0	21	0,6	10	0,6
Social benefits						
Nonrecipient	3643	63,7	2426	67,3	1199	65,8
Recipient	995	17,3	591	16,4	301	16,5
Unknown	1085	19,0	588	16,3	320	17,5
Economic difficulties in the past year						
Never	3341	58,4	2189	60,7	1077	59,2
Yes	1562	27,3	985	27,3	501	27,5
Unknown	820	14,3	431	12,0	242	13,3
Alcohol consumption						
Abstainer or less than monthly	1555	27,2	943	26,2	456	25,0
1-4 times/month	3204	56,0	2074	57,5	1083	59,5
≥5 times/month	782	13,7	514	14,3	247	13,6
Unknown	182	3,2	74	2,0	34	1,9
Smoking						
Never smoked daily	2621	45,8	1720	47,7	963	52,9
Ex smoker daily	1461	25,5	912	25,3	416	22,9
Current smoker daily	1599	27,9	948	26,3	424	23,0
Unknown	42	0,7	25	0,7	17	0,9

Table 2: Distribution of baseline characteristics according to vitamin D in analysis sample (N=1820)

Vitamin D	<50	≥50-75	≥75
Sex			
Female	57,04	62,76	56,68
Male	42,96	37,24	43,32
Age, years			
19-29	27,20	21,11	28,01
30-39	33,69	37,98	38,44
40-49	32,13	33,14	25,08
50-55	6,98	7,77	8,47
Body mass index			
Normal	48,86	59,53	64,82
Overweight	38,51	35,04	30,62
Obesity	12,39	5,43	4,23
Unknown	0,24	0	0,33
Education, years			
<10	13,72	10,85	8,47
10-12	56,68	54,69	61,24
≥13	29,00	33,87	29,97
Unknown	0,60	0,59	0,33
Social benefits			
Nonrecipient	63,66	66,86	69,71
Recipient	16,85	16,86	14,98
Unknown	19,49	16,28	15,31
Economic difficulties in the past year			
Never	57,52	59,97	61,89
Yes	27,20	28,01	27,36
Unknown	15,28	12,02	10,75
Alcohol consumption			
Abstainer or less than monthly	28,04	23,02	21,50
1-4 times/month	57,64	59,38	64,82
≥5 times/month	13,00	14,96	12,05
Unknown	1,32	2,64	1,63
Smoking			
Never smoked daily	48,73	55,52	61,54
Ex smoker daily	23,27	23,42	21,74
Current smoker daily	28,00	21,06	16,72
Blood sample Vit D season			
Winter	47,89	23,02	13,68
Spring	20,22	20,09	11,73
Summer	5,54	12,90	23,13
Autumn	26,35	43,99	51,47

**values in percentages*

Table 3: Association between vitamin D status and changes in lipids HUNT2 – HUNT3

		Total Cholesterol	
	Crude Mean	Crude Coef (95% CI)	Adjusted Coef (95% CI)
Vit D cut off			
<50	16.38	Reference group	Reference group
50-74,9	17.20	0.82 (–2.07 – 3.71)	–0.74 (–3.76–2.28)
>75	17.72	1.34 (–2.39 – 5.08)	–0.19 (–4.20–3.81)
Vit D tertile			
1st	16.37	Reference group	Reference group
2nd	16.38	0.01 (–3.20 – 3.22)	–2.04 (–5.34–1.26)
3rd	18.00	1.63 (–1.59 – 4.85)	–0.42 (–3.91–3.08)
Vit D continuous			
(+25 units)		0.56 (–0.99 – 2.12)	–0.26 (–1.97–1.45)
		HDL – Cholesterol	
Vit D cut off			
<50	1.98	Reference group	Reference group
50-74,9	1.83	–0.15 (–1.18 – 0.87)	–0.10 (–1.18–0.98)
>75	0.81	–1.17 (–2.50 – 0.15)	–1.30 (–2.72–0.13)
Vit D tertile			
1st	2.22	Reference group	Reference group
2nd	1.95	–0.27 (–1.40 – 0.87)	–0.32 (–1.50–0.86)
3rd	1.00	–1.22 (–2.36 – –0.08)	–1.28 (–2.52– –0.03)
Vit D continuous			
(+25 units)		–0.50 (–1.05 – 0.05)	–0.56 (–1.17–0.05)
		Ratio Total Cholesterol/HDL - Cholesterol	
Vit D cut off			
<50	0.45	Reference group	Reference group
50-74,9	0.43	–0.02 (–0.10 – 0.06)	–0.02 (–0.11–0.06)
>75	0.37	–0.08 (–0.18 – 0.03)	–0.10 (–0.22–0.01)
Vit D tertile			
1st	0.48	Reference group	Reference group
2nd	0.41	–0.07 (–0.17 – 0.01)	–0.10 (–0.19– –0.00)
3rd	0.39	–0.08 (–018 – –0.00)	–0.12 (–0.21– –0.02)
Vit D continuous			
(+25 units)		–0.05 (–0.09 – –0.00)	–0.06 (–0.11– –0.01)
		Triglycerides	
Vit D cut off			
<50	7.05	Reference group	Reference group
50-74,9	11.62	4.57 (–2.86 – 12.02)	3.71 (–4.20–11.63)
>75	13.48	6.43 (–3.19 – 16.05)	5.34 (–5.14–15.81)
Vit D tertile			
1st	8.67	Reference group	Reference group
2nd	6.31	–2.36 (–10.61 – 5.90)	–4.45 (–13.08–4.18)
3rd	14.59	5.92 (–2.34 – 14.20)	4.25 (–4.89–13.38)
Vit D continuous			
(+25 units)		2.10 (–1.91 – 6.10)	1.29 (–3.18–5.76)
		LDL – Cholesterol	
Vit D cut off			
<50	16.95	Reference group	Reference group
50-74,9	16.70	–0.25 (–2.78 – 2.29)	–1.58 (–4.23–1.08)
>75	15.84	–1.11 (–4.38 – 2.16)	–2.56 (–6.07–0.96)
Vit D tertile			
1st	16.85	Reference group	Reference group
2nd	17.07	0.22 (–2.60 – 3.02)	–1.47 (–4.37–1.43)
3rd	16.08	–0.77 (–3.59 – 2.04)	–2.54 (–5.60–0.53)
Vit D continuous			
(+25 units)		–0.36 (–1.72 – 1.00)	–0.04 (–2.58–0.42)

Abbreviations: CI, confidence interval. Crude mean values in mg/dl

Table 4: Stratified analysis

	Physical Active (N=769)		Physical Inactive (N=1051)	
	Crude Coef (95% CI)	Adjusted Coef (95% CI)	Crude Coef (95% CI)	Adjusted Coef (95% CI)
Total Cholesterol				
Vit D cut off				
<50	Reference group	Reference group	Reference group	Reference group
50-74,9	2.04 (–2.35–6.43)	1.51 (–3.14–6.17)	–0.33 (–4.21–3.55)	–1.30 (–5.29–2.69)
>75	1.99 (–3.16–715)	–0.16 (–5.80–5.48)	0.17 (–5.40–5.74)	0.02 (–5.82–5.85)
Vit D tertile				
1st	Reference group	Reference group	Reference group	Reference group
2nd	0.76 (–4.25–5.78)	–1.29 (–6.51–3.94)	–0.70 (–4.93–3.53)	–2.45 (–6.76–1.85)
3rd	2.65 (–2.24–7.53)	0.43 (–4.97–5.83)	0.39 (–3.40–4.77)	–0.42 (–5.06–4.22)
Vit D continuous				
(+ 25 units)	0.75 (–1.46–2,97)	–0.25 (–2.74–2.23)	0.06 (–2.16–2.27)	–0.23 (–2.60–2.14)
HDL – Cholesterol				
Vit D cut off				
<50	Reference group	Reference group	Reference group	Reference group
50-74,9	0.67 (–0.87–2.20)	0.52 (–1.12–2.16)	–0.81 (–2.19–0.57)	–0.57 (–2.01–0.87)
>75	–1.42 (–3.22–0.38)	–1.53 (–3.51–0.46)	–0.80 (–2.79–1.18)	–0.97 (–3.08–1.14)
Vit D tertile				
1st	Reference group	Reference group	Reference group	Reference group
2nd	1.31 (–0.44–3.06)	1.20 (–0.64–3.03)	–1.36 (–2.87–0.15)	–1.21 (–2.77–0.34)
3rd	–0.87 (–2.57–0.83)	–1.11 (–3.01–0.79)	–1.33 (–2.89–0.23)	–1.11 (–2.79–0.57)
Vit D continuous				
(+ 25 units)	–0.48 (–1.25–0.30)	–0.58 (–1.46–0.30)	–0.589 (–1.379–0.201)	–0.56 (1.41–3.00)
Ratio Total Cholesterol/HDL Cholesterol				
Vit D cut off				
<50	Reference group	Reference group	Reference group	Reference group
50-74,9	0.05 (–0.07–0.17)	0.063 (–0.067–0.193)	–0.08 (–0.19–0.03)	–0.06 (–0.18–0.05)
>75	–0.06 (–0.21–0.08)	–0.094 (–0.251–0.064)	–0.11 (–0.26–0.05)	–0.10 (–0.27–0.06)
Vit D tertile				
1st	Reference group	Reference group	Reference group	Reference group
2nd	0.01 (–0.13–0.15)	–0.00 (–0.15–0.14)	–0.14 (–0.26—0.02)	–0.15 (–0.27—0.03)
3rd	–0.04 (–0.18–0.10)	–0.07 (–0.22–0.08)	–0.14 (–0.26—0.02)	–0.12 (–0.25–0.01)
Vit D continuous				
(+ 25 units)	–0.04 (–0.10–0.03)	–0.05 (–0.12–0.02)	–0.07 (–0.132—0.01)	–0.07 (–0.13–0.00)
Triglycerides				
Vit D cut off				
<50	Reference group	Reference group	Reference group	Reference group
50-74,9	5.64 (–5.78–17.05)	5.106 (–7.201–17.413)	3.17 (–6.74–13.77)	3.30 (–7.18–13.78)
>75	12.13 (–1.27–25.53)	10.249 (–4.667–25.165)	–2.27 (–16.48–11.94)	–1.10 (–16.42–14.22)
Vit D tertile				
1st	Reference group	Reference group	Reference group	Reference group
2nd	–3.21 (–16.25–9.83)	–6.14 (–19.95–7.67)	–2.66 (–13.46–8.14)	–3.45 (–14.75–7.86)
3rd	7.39 (–5.32–20.09)	4.31 (–9.96–18.58)	2.73 (–8.47–13.92)	3.41 (–8.78–15.60)
Vit D continuous				
(+ 25 units)	3,41 (–2.37–9.18)	2.22 (–4.36–8.80)	–0.16 (–5.81–5,50)	0.20 (–6.02–6.42)
LDL – Cholesterol				
Vit D cut off				
<50	Reference group	Reference group	Reference group	Reference group
50-74,9	1.58 (–2.31–5.48)	1.01 (–3.13–5.15)	–1.77 (–5.14–1.60)	–2.53 (–6.01–0.95)
>75	–1.86 (–6.43–2.72)	–3.74 (–8.76–1.28)	–0.18 (–5.01–4.65)	–0.73 (–5.82–4.36)
Vit D tertile				
1st	Reference group	Reference group	Reference group	Reference group
2nd	2.72 (–1.74–7.16)	1.14 (–3.52–5.80)	–1.53 (–5.21–2.14)	–2.98 (–6.73–0.78)
3rd	0.30 (–4.04–4.64)	–1.54 (–6.36–3.28)	–1.49 (–5.29–2.32)	–2.21 (–6.26–1.84)
Vit D continuous				
(+ 25 units)	–0.41 (–2.38–1.57)	–1.28 (–3.49–0.94)	–0.50 (–2.43–1.42)	–0.83 (–2.89–1.24)

Abbreviations: CI, confidence interval

Table 5: Odds ratio of an unfavourable lipid level associated with vitamin D

				Total sample (N=1820)	
	N	New Cases	%	Crude OR (95% CI)	Adjusted OR (95% CI)
Vit D cut off					
<50	831	306	48.3	Reference group	Reference group
50-74,9	682	227	35.9	0.86 (0.69–1.06)	0.82 (0.65–1.03)
>75	307	100	15.8	0.83 (0.63–1.09)	0.87 (0.64–1.18)
Vit D tertile					
1st				Reference group	Reference group
2nd				0.89 (0.70–1.12)	0.83 (0.65–1.07)
3rd				0.78 (0.62–0.99)	0.75 (0.57–0.98)
				Physical Active (N= 769)	**(N=767)**
Vit D cut off					
<50	287	101	39.4	Reference group	Reference group
50-74,9	309	100	39.1	0.88 (0.63–1.24)	0.89 (0.61–1.30)
>75	173	55	21.5	0.86 (0.58–1.28)	0.81 (0.51–1.27)
Vit D tertile					
1st				Reference group	Reference group
2nd				0.82 (0.56–1.21)	0.76 (0.50–1.16)
3rd				0.81 (0.56–1.18)	0.76 (0.49–1.18)
				Physical Inactive (N=1051)	
Vit D cut off					
<50	544	205	54.4	Reference group	Reference group
50-74,9	373	127	33.7	0.85 (0.65–1.13)	0.83 (0.61–1.11)
>75	134	45	11.9	0.84 (0.56–1.25)	0.89 (0.58–1.39)
Vit D tertile					
1st				Reference group	Reference group
2nd				0.95 (0.71–1.28)	0.89 (0.64–1.22)
3rd				0.76 (0.56–1.05)	0.75 (0.53–1.06)

Abbreviations: CI, confidence interval; OR, odds ratio

4 DISCUSSION

4.1 Main findings

This prospective study showed that a higher level of serum 25(OH) D in the blood was associated with a reduced risk of developing LDL-dyslipidaemia, a less reduction in HDL-C, and a less increase in TC-HDL ratio. Furthermore, the associations did not differ significantly in physical active and inactive adults.

4.2 Comparison with existing literature

These findings are partially consistent with previous studies. Some prospective studies have observed the association between a higher vitamin D level and a decrease in TG [17, 18], respectively. This is consistent with several cross-sectional studies [15]. However due to the exclusion of subjects with TG>400mg/dl at baseline and the inclusion of young population, this association was not made in our study.

New findings in our study are the association between vitamin D and the new onset of LDL-dyslipidaemia. The method used to make this observation have, to my knowledge, not previous been conducted. This is an important observation, and according to NCEP is LDL-C considered the primary target for cholesterol lowering therapy for the prevention of CVD [14]. This is important information that strengthens the assertion of a higher vitamin D level as a preventive factor in the development of CVD. The observed dose response relationship between levels of vitamin D and change in LDL-C during the 11-year follow-up additionally support this suggestion. The association between vitamin D and a less reduction in HDL-C has previous been observed in prospective studies investigating the effect of vitamin D supplementations [29, 30]. Epidemiological evidence connects low HDL-C levels to increased risk of developing CVD [14]. This suggests an additional beneficial effect of vitamin D in regard to the prevention of CVD. However the association between high HDL-C level and reduced CVD risk may be confounded by other healthy lifestyle factors [14], which may limit any true association between vitamin D and the less reduction in HDL-C levels.

The lack in influence by PA on the association between vitamin D and lipids gives reason to question the assertion of PA as a modifier. Furthermore, PA was included as a potential confounder in the main analysis, and the adjusted model still showed a significant association

between vitamin D in the 3rd tertile and changes in HDL-C. Moreover there was no difference between active vs. inactive group. This suggests that the effect of vitamin D on lipids is independent of PA.

4.3 Possible mechanisms

The mechanisms by which vitamin D may influence lipid levels are uncertain and not well documented. However some suggested mechanisms might be that vitamin D by itself or by suppressing the secretion of parathyroid hormone (PTH) [19] to increase lipolysis [31], and thereby increase the breakdown of lipids. Other suggestions are the increase in calcium levels caused by elevated vitamin D levels, which may lead to a reduction in TG levels [18]. However the conclusion made by Cho et. al. (2005) indicate that an increase in calcium levels inhibits hypertriglyceridemia [32]. Hence due to the exclusion criteria in the current study the condition of hypertriglyceridemia would not be an issue.

4.4 Strengths and limitations

The strengths of the current study include a long-term follow-up with objective biomarkers of exposure and outcome variables, and the detailed information on several relevant confounders. The numerous different analysis preformed strengthens the results, and the inclusion criteria made it possible to investigate the preventive effect of vitamin D status on lipid levels in a healthy adult population. The different ways of categorizing vitamin D levels, as well as the studied continuous variable, which in general showed similar results and strengthened our findings.

Limitations of our study, which may inflict the true association, involve the lack of measurements on cholesterol lowering medication. This limitation may have produced an overestimate in the protective effect of vitamin D associated with prevalent LDL-dyslipidaemia. Dietary and vitamin D supplementation may have the potential to affect both vitamin D status and lipid levels [33, 34]. Thus the lack of measurements on dietary and vitamin D supplementation may be the cause of an overestimation or underestimation of the protective effect of vitamin D. To determine a lager effect of vitamin D, sample size should be larger. The self-reported confounders might be inaccurate du to the subjective manner of the questioner in HUNT. PA was also self-reported in our study, and results from a comprehensive review that compared direct and self-reported PA measures, showed that self-

reported PA measures were both higher and lower compered to direct measures [35]. This indicates that self-reported PA may be unreliable. Outcome lipid measurements came from a non-fasting test, however according to NCEP the indirect calculation of LDL-C requires a 9- to 12-hour fast. Observations in an intervention study preformed by Craig et. al. (2000), showed that the mean levels of TG and calculated LDL-C did not change substantially after a period of fasting in an intervention study [36], which indicates that fasting time prior to testing of lipids may not be necessary [37]. Limitations due to the narrow population age in our study prevent the possibility of generalizing the results to the youngest or oldest subpopulations. Additionally our participants were mainly Caucasians and therefore the generalizability to more ethnically diverse populations is reduced.

4.5 CONCLUSION

In healthy and younger adults we found that a higher level of vitamin D was beneficial for a favourable change in lipids particularly in HDL, TC-HDL ratio and a reduced risk of LDL dyslipidaemia, PA did not modify the association.

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