No lipid lowering effect of vitamin D supplements found on statin users

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Abstract

Background

Tromsø endocrine research group has performed multiple RCTs with vitamin D supplements/placebo and effects on different outcomes. Compiling data from four of these studies I tested an hypothesis established by Kane et al. in 2013, which suggests that vitamin D supplements have a significant lipid lowering effect in statin users. Retesting hypotheses in general is vital to the scientific community. By using patient data from Tromsø endocrine research group I wanted to retest this new hypothesis because it has not been confirmed by other studies.

Method

To test the hypothesis I pooled subjects from four vitamin D intervention studies and split the subjects into statin users and non-statin users. I then performed independent samples t-test to see if vitamin D-supplements during 6-12 months had a lipid lowering effect compared to placebo. I performed correlations between baseline levels of vitamin D and lipids. To adjust for confounding factors in the correlations I used linear regression with age, sex and BMI as covariates.

Results

At baseline serum HDL, Apo-A and triglycerides correlated significantly with vitamin D ($p < 0.01$). After adjusting for confounders the baseline HDL levels correlated significantly with vitamin D ($p=0.001$). I found no significant results in the t-test or the linear regressions on changes in lipid levels based on vitamin D treatment.

Conclusions

I found no clear significant lipid-lowering effect of vitamin D supplementation compared to placebo in neither statin users nor non-statin users. After adjusting for confounders, serum HDL still remained significantly correlated with vitamin D at baseline.

1. Introduction

1.1. Vitamin D metabolism and function

Vitamin D is a lipid-soluble molecule that functions as both a vitamin and a steroid hormone. Vitamin D is obtained both through diet and through endogenous production in skin-tissue. The precursor to vitamin D is called 7-dehydrocholesterol and is produced in the liver (1). These molecules are deposited in the skin and are converted to 25-hydroxyvitamin-D3 (25(OH)D) when exposed to wavelengths of light between 270 and 300 nm (2). The fat soluble 25(OH)D is then transported in the blood stream carried by the carrier protein D-vitamin binding protein (DBP). Some of the 25(OH)D molecules are deposited in adipose tissues, some stay bound to DBP as an intermediate storage and some are converted to the active form of the vitamin 1,25-dihydroxyvitamin D3 (1,25 (OH)2D) in the kidneys. This activation may also occur in peripheral tissues (3). 1,25(OH)2D also binds to DBP, but about 1% of 1,25 (OH)2D and 25(OH)2D are free in the blood stream (4). According to the free hormone hypothesis (5), only the free fraction of a hormone has an signaling effect. There is an additional fraction of vitamin D called bioavailable vitamin D. This fraction consists of the free fraction of vitamin D and the vitamin D bound to albumin. Albumin binds between 10-15% of vitamin D in the blood stream (6). The theory of bioavailability claims that vitamin D binds so weakly to albumin that the vitamin D receptor (VDR) grabs 1,25(OH)2D from albumin during tissue perfusion. If this is the case, the albumin bound fraction is as available to VDR as the unbound fraction of 1.25(OH)2D (7). The free fraction of 1,25(OH)2D binds to vitamin D receptor (VDR) to produce different effects depending on which tissue the receptor is activated in. It is believed that vitamin D is mainly responsible for maintaining calcium homeostasis in

the blood. When a subject is vitamin D deficient, the secretion of parathyroid hormone (PTH) increases (8, 9). To increase calcium levels in blood, PTH increases calcium reabsorption from pre-urine in the kidneys (10), as well as bone turnover to free calcium (11) and absorption of calcium in bowels increases. This keeps the calcium-levels in the blood stream relatively stable, while the bone mineral density decreases. Vitamin D receptors are found in most tissues (12), but the full extent of the effects of vitamin D is not completely understood.

1.2. Vitamin D deficiency

The optimal level of vitamin D is under discussion in the scientific community. According to some sources 25(OH)D levels below 80 nmol/L is considered insufficient, while 25(OH)D levels below 25 nmol/L is considered deficient (13). Levels below 80 nmol/L is a risk factor of osteoporosis, while levels below 25 nmol/L is a strong risk factor for osteomalacia and rickets disease (14, 15). Osteomalacia is a defect in bone mineralization and development and symptoms include proximal muscle weakness and fragile bones. Rickets disease is the manifestation of osteomalacia in children, characterized by defects in bone formation, stunted growth, softening of the skull, skeletal deformities, dental problems, muscle weakness and tetanus. Fish oil has traditionally been given children to prevent rickets (16), and the disease has long been viewed as a condition of the past. In recent years rickets disease has become more and more prevalent, as vitamin D deficiency presents itself as an issue globally (17, 18).

1.3. Vitamin D deficiency risk factors

Vitamin D deficiency risk factors are often the result of religious, cultural and societal influences. The factors all relate to the two sources of vitamin: sunlight and diet. Approximately 10% of vitamin D is obtained through diet, and 90% are produced endogenously (19).

Vitamin D deficiency has become a common problem in geriatrics. Elderly people are less exposed to direct sunlight and have a reduced ability to produce vitamin D endogenously (20, 21). Young age has the latest years also come up as a risk factor because of lifestyle changes such as indoor activities, a sedentary lifestyle, lack of fish-oil supplements and poor diet (22-26).

Diet can be a risk factor depending on the amount of exposure to sunlight and skin tone. In the northern hemisphere vegans, and dark skinned individuals with a diet low in vitamin D is particularly at risk (27, 28).

Obesity has been linked to lower vitamin D levels. The leading theory is that the larger amount of adipose tissue causes the fat-soluble vitamin D to distribute over a larger volume and becomes diluted. This provides a lower amount of biologically available vitamin D (29).

Vitamin D deficiency is rampant in regions of the Middle East and some immigrant communities in the west. Primarily women are afflicted because of concealing clothing like the Burqa and staying indoors (30, 31).

Chinese people in urban smog-filled areas might see the same problems as the Londoners in the 1700-hundreds, where rickets disease first was described. The smog covers up the sun, and the lack of UV-exposure in city-dwellers can give rise to bone disease stemming from hypovitaminosis D (19).

All the aforementioned risk factors are becoming more prevalent. Additionally multiple potential negative health effects of vitamin D deficiency have been discovered. Because of this a surge of scientific effort in the vitamin D research field has been seen the last decades.

1.4. Vitamin D correlations and causality

Vitamin D has multiple known biological effects, besides calcium homeostasis (32). It has also been linked to, among other; immune activity (33), heart disease (34), diabetes (35) and mortality (36). However, it can be hard to differentiate between confounders and real causality since vitamin D seems to have a subtle effect on a lot of biological systems. The amount of variable interaction could be substantial in these correlations. The fact that vitamin D levels are higher in people that spend a lot of time outdoors and eat a lot of fatty fish, rich in omega-3 and vitamin D, can give false causality because of the beneficial life-style these people lead. There are some theories that mark vitamin D as a general anabolic hormone (37).

1.5. Cholesterol

Cholesterol is used as an umbrella-term to describe both the molecule and the lipoproteins that transport cholesterol molecules and triglycerides. The molecule cholesterol ($C_{27}H_{46}O$) is a vital building block in cells, and the precursor for steroid-

hormones, vitamin-D and bile salts (38). Cholesterol is also found as a structural component in the surface of the cell membrane (39), and can be acquired either through endogenous production in the liver or is gained through the diet. The lipoproteins that transport cholesterol and triglycerides are called HDL (high-density lipoprotein), LDL (low-density lipoprotein), VLDL (very low-density lipoprotein) and chylomicrons. All these lipoproteins are assembled from the apolipoproteins, Apo-A, -B, -C, -D, -E and –H, which contain multiple subclasses.

1.6. Chylomicrons

Chylomicrons transport dietary triglycerides and cholesterol from the intestines to the liver. The small intestine produces particles rich in triglycerides, called nascent chylomicrons. These particles are secreted to lymph and collects in the blood stream. By receiving apo CII and apo E from HDL, nascent chylomicrons are modified into mature chylomicrons. These chylomicrons bind to extracellular lipoprotein lipase in tissues via apo CII and split the triglycerides into two components, glycerol and free fatty acids. Glycerol is transported to the liver while the free fatty acids are transported into the tissue cells. This process leaves a drained chylomicron. This chylomicron again binds to HDL and returns apo CII. This new particle with greatly reduced triglyceride content and without apo CII is called a chylomicron remnant. A chylomicron remnant contains more cholesterol than triglyceride, and binds to receptors in hepatocytes to be absorbed (40).

1.7. VLDL

Triglycerides and cholesterol are also transported from the liver to peripheral tissues via VLDL, which has a similar function as chylomicrons. Immature versions are

produced in liver cells and receive apo CII and apo E from HDL in the blood stream. These mature VLDL particles then bind to lipoprotein lipases in peripheral tissue and transfers free fatty acids to the tissue cells. The particle that is now empty for triglycerides looses apo E and apo CII to HDL, and is later absorbed back into the liver cells or transformed into LDL via hepatic lipase (41).

1.8. LDL

LDL consists of apo B100 and cholesterol. LDL transports cholesterol from the liver to primarily muscle and adipose tissues. It delivers cholesterol to these tissues by binding to the LDL-receptor in the cell membranes and is absorbed by endocytosis. Excess LDL can either be absorbed by hepatocytes through the same mechanism or oxidized in the blood stream. This creates oxidized LDL-particles with potential to accumulate in the artery wall lining. Macrophages then absorb these fatty particles and turn into foam cells. This is the pathogenesis of atherosclerosis that can have many negative outcomes. Examples are thromboembolisms, aneurisms, and arterial auto dissection (42).

1.9. HDL

HDL, often called good cholesterol, consist of apo-A, apo-C and apo-E. The nascent form is synthesized in the liver and turns into mature HDL-particles after collecting cholesterol in peripheral tissues through the enzymatic activity of lechitin-cholesterol acyltransferase (LCAT). HDL functions as a supporting molecule by transferring apo-CII and apo-E to and from chylomicrons and VLDL, and as a transport lipoprotein from peripheral tissues to hepatic and steroid-producing cells(43).

1.10. Statins

According to Norwegian guidelines, statins are the primary pharmaceutical choice to reduce cholesterol levels in the blood (44). Statins as a group are defined as HMG-CoA reductase inhibitors. They bind to HMG-CoA reductase in the liver and block the pathway for the cholesterol substrate HMG-CoA(45). This causes the endogenous cholesterol to drop and quickly reduces blood levels of total cholesterol and LDL cholesterol. Lowered concentration of cholesterol in hepatocytes causes an increased expression of LDL-receptors (46) and increases cholesterol clearance from the blood. This is the effect that ultimately has the largest lipid-lowering effect in the long term.

1.11. Kanes study

A 12-week randomized controlled trial conducted in 2013 by Kane et al (47) examined the effect of vitamin D supplements versus placebo on lipid lowering in statin users. They examined 49 participants who were selected on the background of vitamin 25(OH)D < 25 ng/mL (62.5 nmol/L). 19 of these subjects were using statins, 11 of the statin users were using atorvastatin, while the remaining 8 used simvastatin. The reason they wanted to examine this was the inverse relationship between vitamin D and heart disease earlier reported (36, 48-50). A potentiating effect by vitamin D on statins could potentially be an explanation for these findings. 26 subjects received vitamin D and 23 subjects received placebo. The participants receiving vitamin D got doses to achieve levels above 25 ng/mL. The doses (IU/d) were titrated according to their 25(OH)D serum level response during the intervention. By the end of the study 15 subjects were using 1000 IU/d, 10 used 2000 IU/d and 1 subject got 3000 IU/d. In their analyses they compared both total 25(OH)D and free 25(OH)D to lipid lowering effects in statin users and non statin users during the 12-week period.

They found no correlation between 25(OH)D and lipid lowering. However they found an inverse relationship between free 25(OH)D and LDL and total cholesterol at baseline. They also found a significant total cholesterol and LDL lowering in vitamin D deficient statin users receiving supplements. No significant lipid lowering effects were found in non-statin users or statin users receiving placebo.

1.12. Study objectives

In this study I wanted to examine the results from Kane et al. to see if I can reproduce them. This is important since the results of one study are not enough to conclude a hypothesis as true. The more thoroughly a hypothesis is examined, the more power one has in falsifying or confirming it. This is an essential concept in the scientific theory. When someone presents a positive result, other scientists should try to objectively refute their theory. If this proves impossible, the theory gains strength. The strongest theories are known as facts, and acts as a foundation for further research and knowledge. The aim of this study was therefore to test the possible interaction between vitamin D and lipid levels in statin users in a large Norwegian population.

Some statin-vitamin D interactions have been postulated. Theories include direct interaction with statins, inhibition of cholesterol uptake in the bowels or inhibition of endogenous cholesterol synthesis. One study reported HMG-CoA reductase inhibitory effect by vitamin D in vitro on cultured human skin fibroblasts, transformed human liver cells and mouse peritoneal macrophages (51).

2. Method

2.1. The work process

I am a part of the endocrine research group in Tromsø, which I joined it as a part of my PhD-education in 2012. My research councilor, Professor Rolf Jorde, suggested this project when I turned to him for the 5. year project during the spring 2014. Shortly after I decided on the project, we met and discussed the project objectives and I received the SPSS worksheet containing the data for the assignment. Since the spring of 2014 he has provided me with literature that could prove relevant to the work I would be doing. I wrote this paper alone, with the help of my councilor, Professor Jorde.

I started working on the project March 2015 and initially used my time reading through the literature on statins and lipid metabolism/physiology. I also searched through other relevant literature on vitamin D and added it to the endnote library.

After finishing the reference library I started writing the introduction. When I got to the method and results part of the paper I met with Professor Jorde to clarify what analyses we would use, and what data to present. After the meeting I used the statistical tool SPSS to finish the analyses and wrote the method, results and discussion, which was controlled by Professor Jorde. Lastly I wrote the abstract. I sent the paper to Professor Jorde for correction and, we exchanged the paper twice with suggested changes and corrections before I submitted the paper to the exam committee. The assignment was concluded in accordance with the councilor contract.

After getting a failing grade I discussed the paper and the feedback from the exam committee with Professor Jorde. I made a list of corrections and followed it, before submitting the paper to Professor Jorde for feedback. After another round of corrections and additions I re-submitted the finished paper to the exam committee.

2.2. Subjects

The data I used is compiled from four double blind, randomized controlled trials (RCT) (Table 1) conducted in Tromsø by the endocrine research-group. These four studies were chosen since data on statin use (yes/no) was available, together with information on relevant confounders like age, gender, serum 25(OH)D level and BMI. The 938 subjects were gathered from the following studies;

Study		Osteoporosis	Depression	Clamp	Overweight
Sex	Male	0	100	53	128
	Female	275	128	52	204
Treatment	Vit-D	134	119	52	221
	Placebo	139	109	55	111
Statins	Yes	31	20	5	13
	No	242	208	100	319

Table 1. This table shows the distribution of patient sex, study affiliation, vitamin D supplementation and statin treatment.

(1) 332 participants from "No improvements in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year" by Jorde et al. (52) (the overweight study). In this study they examined the relationship

between vitamin D and cardio-vascular risk factors. They found no beneficial effect of vitamin D supplementation on glucose tolerance, blood pressure or serum lipids.

(2) 228 participants from "Effects of vitamin D supplement on depression scores in people with low levels of serum 25-hydroxyvitamin D: nested case-control study and randomized clinical trial." by Kjærgaard et al. (53)(the depression study). In this study they examined the effect of vitamin D supplementation on depression (Beck Depression Inventory) in overweight and obese subjects. They found a possible improvement in depression symptoms in the subjects with very low serum 25(OH)D levels receiving vitamin D, suggesting a causal relationship between vitamin D and depression.

(3) 273 participants from "The effects of high dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass – a randomized controlled 1 – year trial" by Grimnes et al. (54) (the osteoporosis study). Grimnes et al. examined the effect of high dose (6500 IU/d) vitamin D supplementation versus the recommended daily intake (800 IU/d) on bone mass density and bone turnover in postmenopausal women. There was no difference in BMD between the groups. High dose vitamin D supplements were also less efficient in reducing bone turnover than the recommended daily dose.

(4) 105 participants from "Vitamin D, Insulin Secretion, Sensitivity, and Lipids. Results from a Case-Control study and a Randomized Controlled Trial Using Hyperglycemic Clamp Technique" by Grimnes et al. (55) (the clamp study). In this study they examined the effect of vitamin D supplementation on insulin sensitivity

and lipid-profiles in vitamin D deficient subjects. They found no beneficial effects of vitamin D supplementation on lipid-profiles and insulin sensitivity.

In all four studies the subjects received either vitamin D3 supplements or placebo. The supplements were given as either 20.000 or 40.000 I.U. per week for six months (depression study and clamp study) and one year (the overweight and osteoporosis study). I did not differentiate between the doses and intervention periods since I expected that if there was an effect of vitamin D on serum lipids, that would be evident with both doses in question (which were significantly larger than the Norwegian recommended daily doses (56)), as well as after both 6 and 12 months. I used the 6 months values in the two studies that lasted 6 months, and the 12 months values in the two studies that lasted 12 months as end of study values.

The method I chose for this assignment is a compiled randomized controlled trial based on individual person data. Accordingly, I pooled all the subjects together and analyzed them as one combined RCT with adjustment for study group. Ideally, a formal meta-analysis based on individual patient data should have been performed, but that was beyond my level of statistical knowledge. Since the hypothesis was that vitamin D had a lipid lowering effect in statin users only, I split the subjects in statin users and non-statin users and analyzed these two groups separately regarding effects of vitamin D.

The regional ethics committee (REK) approved all the four projects. The patient-data I have used for this project is anonymous.

2.3. Statistics

I performed a bivariate two-tailed Pearson correlation between the lipids and serum 25(OH)D in all 938 subjects combined to get an overview of the correlations at the baseline. I also executed a linear regression in all subjects combined on the baseline lipids and vitamin D with adjustment for confounding factors (study, age, sex and BMI). For the independent sample t-test I needed the delta value (change) in lipid levels. I calculated these using the values at 6 or 12 months with vitamin D or placebo minus values at baseline (before start of vitamin D supplementation or placebo). I conducted an independent sample t-test to compare the changes in lipid levels between those who received vitamin D versus those who received placebo (statin-users and non-statin users analyzed separately). I also did this comparison using linear regression with adjustment for study, age, sex and BMI. The statin users were not split according to type of statin as that information was not available to me.

Descriptive data are presented as means and standard deviations (SD). Normal distribution was tested by visually evaluating histograms for skewedness and kurtosis.

3. Results

3.1. Descriptive statistics

Table 2 shows the baseline values in the four studies. As expected, there were obvious differences between these studies in age, BMI, serum 25(OH)D levels and gender due to different inclusion criteria. In the linear regression model these differences were therefore adjusted for.

In Table 3 the same baseline characteristics are shown in relation to statin use in the vitamin D and placebo groups. The statin and non-statin users differed regarding age, whereas the vitamin D and placebo groups were similar, as expected.

	All studies N=939	Osteoporosis study N=273	Depression study N=228	Clamp study N=105	Overweight study N=333
BMI	29.1(5.56)	24.7(3.33)	27.7(4.11)	26.5(3.10)	34.6(3.86)
Age	54.2(11.3)	63.1(7.15)	51.8(10.2)	52.0(9.36)	49.2(11.2)
Vit 25(OH)D baseline	55.9(20.9)	71.0(22.6)	47.5(15.6)	40.3(12.8)	54.1(16.8)
Cholesterol	5.56(1.00)	5.65(0.92)	5.64(1.05)	5.58(1.06)	5.41(1.00)
Triglycerides	1.40(0.84)	1.11(0.51)	1.59(0.91)	1.23(0.83)	1.57(0.93)
LDL	3.65(0.93)	3.53(0.85)	3.57(0.95)	3.51(0.97)	3.86(0.95)
HDL	1.57(0.48)	1.88(0.48)	1.47(0.46)	1.51(0.41)	1.40(0.37)
Apo-A	1.56(0.29)	1.75(0.26)	1.55(0.29)	1.53(0.28)	1.43(0.25)
Apo-B	0.98(0.24)	0.96(0.22)	0.97(0.25)	0.94(0.25)	1.03(0.23)
Sex (men %)	30	0	44	50	38

Table 2. The table shows descriptive statistics of variable means with standard deviations in parenthesis. These are baseline values. The vertical columns show the means of the combined studies data and the means from the individual studies.

	Statin users		Non-statin users	
	Vitamin D N=38	**Placebo N= 31**	**Vitamin D N=374**	**Placebo N=495**
BMI	28.4 (6.60)	28.5 (5.46)	29.7 (5.45)	28.5 (5.60)
Age	78.1 (7.50)	60.9 (8.82)	52.5 (11.6)	55.1 (10.8)
Vit 25(OH)D	63.9 (22.7)	67.1(26.3)	55.3 (20.4)	54.9 (20.4)
Cholesterol	5.18 (0.81)	4.73 (0.95)	5.59 (1.02)	5.63 (0.96)
Triglycerides	1.42 (0.83)	1.52 (0.82)	1.43 (0.89)	1.35 (0.78)
LDL	3.20 (0.79)	2.77 (0.79)	3.72 (0.93)	3.69 (0.91)
HDL	1.66 (0.52)	1.56 (0.44)	1.55 (0.46)	1.59 (0.50)
Apo-A	1.69 (0.31)	1.59 (0.29)	1.54 (0.29)	1.57 (0.30)
Apo-B	0.91 (0.21)	0.82 (0.19)	0.99 (0.24)	0.99 (0.24)
Sex (men %)	16	24	33	28

Table 3. Descriptive statistics presented as means with standard deviation in parenthesis. Sex is presented as the percentage of men in the selected groups. The table shows those who received vitamin D supplementation and placebo in non-statin and statin users. These statistics represents the participants from the four studies pooled together.

3.2. Normal distribution

The variables had a good normal distribution with a generally low skewedness and kurtosis. Histograms were examined and evaluated visually. The normal distribution of total cholesterol is presented as an example (Figure 1). An exception was the triglyceride baseline variable with a skewedness of 1.98 (Figure 2). This was corrected using a log 10 – transformation in SPSS. The resulting variable had a skewedness of 0.38 (Figure 3). This is the variable that will be used from this point in the analyses.

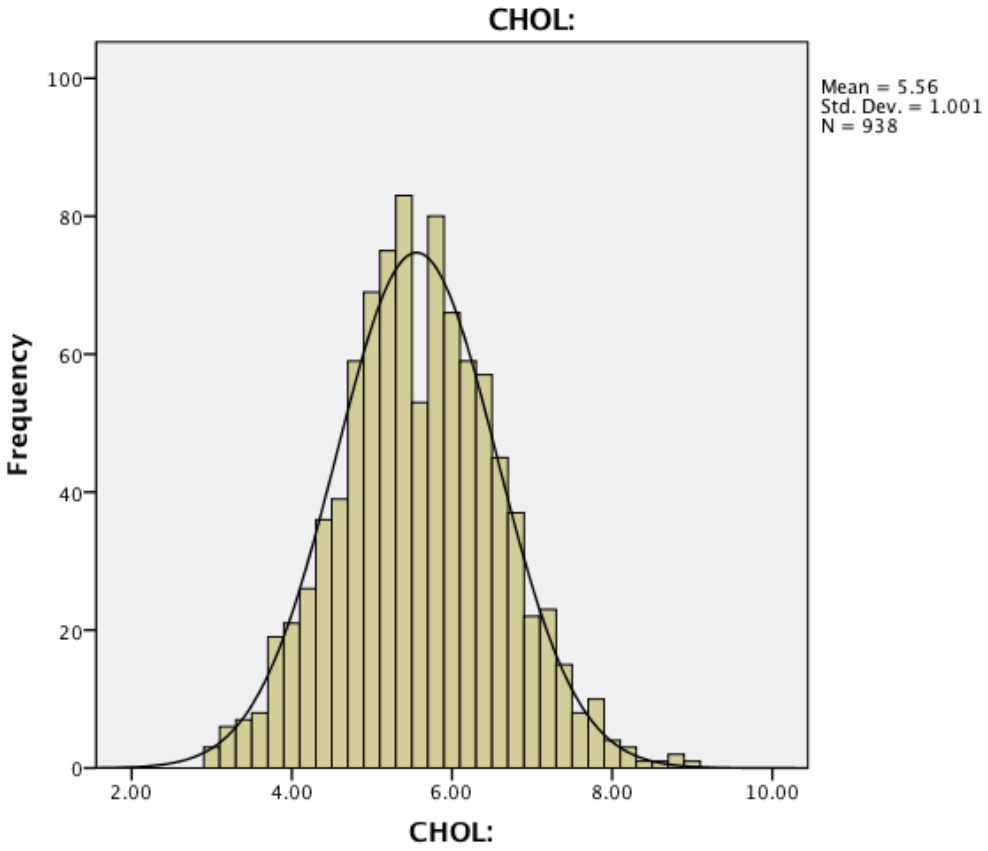

Figure 1. This figure shows the normal distribution of cholesterol, and is used as an example of an acceptable normal distribution for statistical purposes.

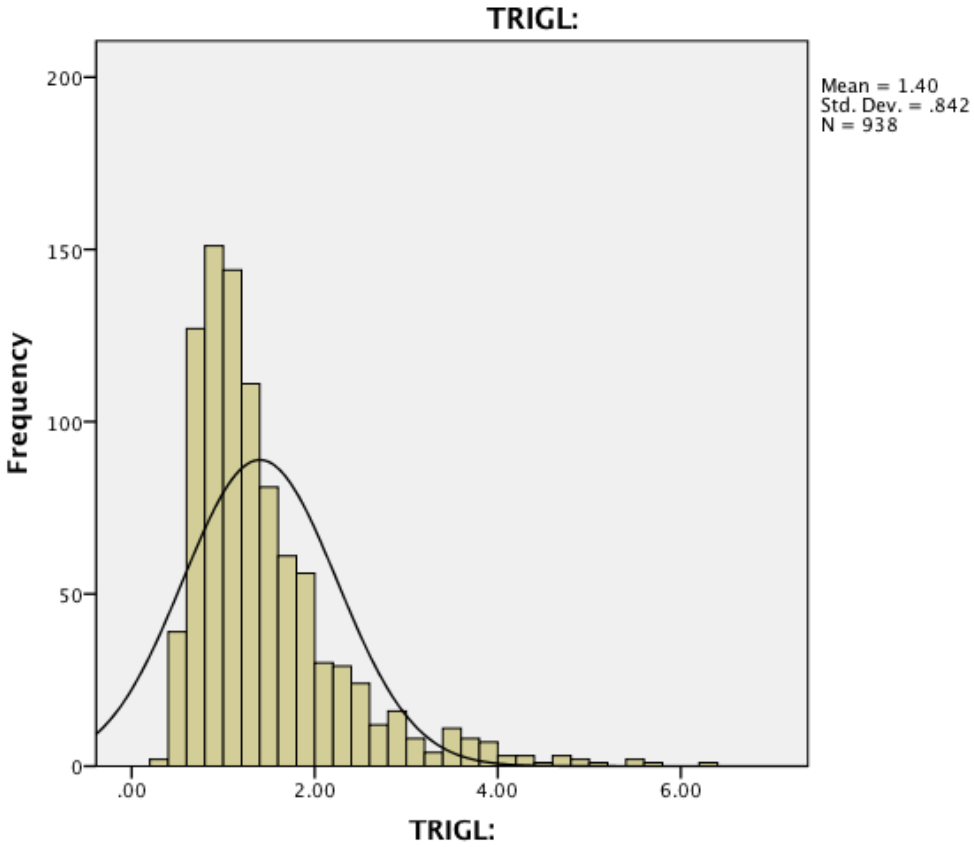

Figure 2. The skewedness in the normal distribution of triglycerides could invalidate the results, and the data have to be logarithmically transformed to be usable in the final analysis.

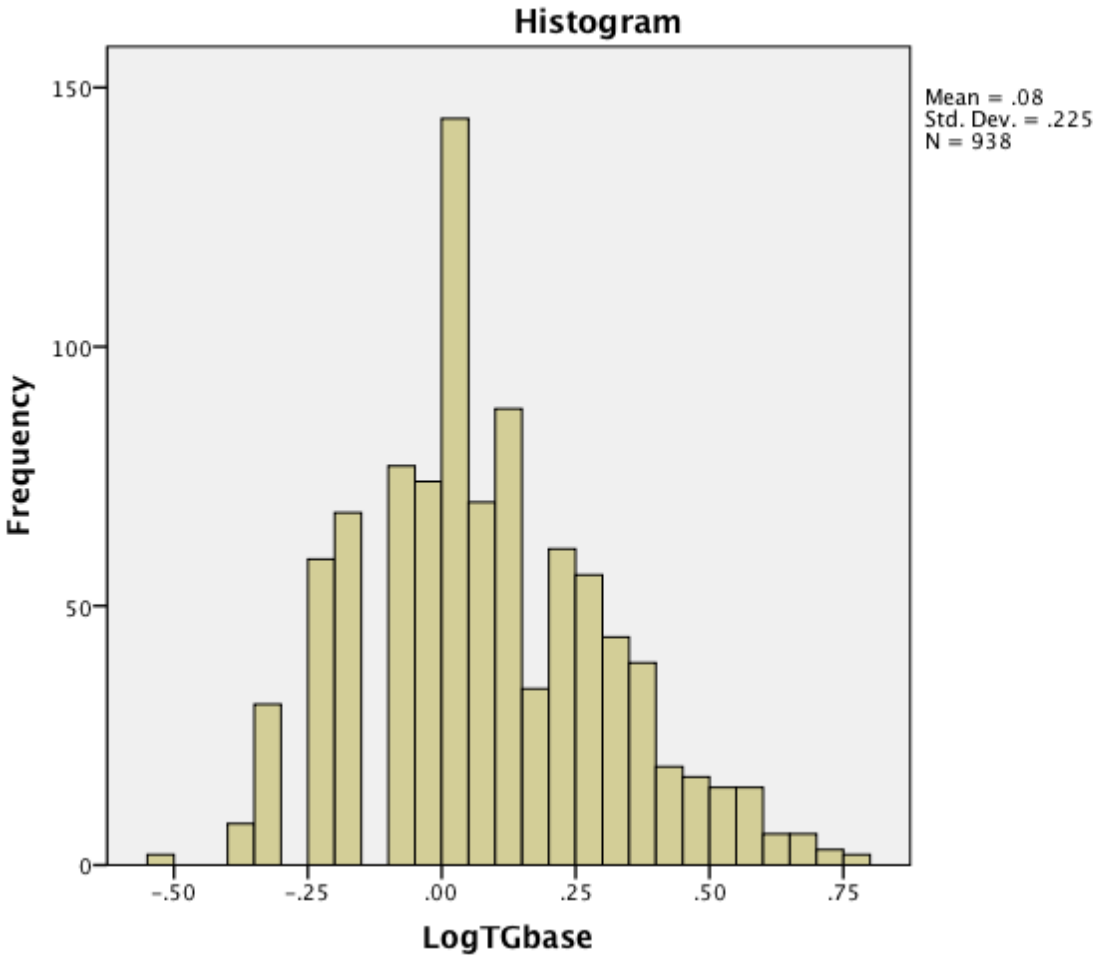

Figure 3. This figure shows the triglycerides after logarithmic transformation. As we can see the normal distribution is now at a more acceptable level.

3.3. Correlations at baseline

When all the 938 subjects were combined, serum 25(OH)D correlated positively with HDL and APO-A at a significant level ($p < 0.00$), and had an inverse correlation with triglycerides ($p < 0.00$) (Table 4). However, these results are not adjusted for confounders or interactions. The correlations at baseline levels adjusted for age, gender and BMI showed a statistically significant relationship between vitamin D and HDL only ($p < 0.00$) (Table 5).

	Vit-D	Cholesterol	LDL	HDL	APOA	APOB	TriGly
Vit-D Pearson's correlation coefficient:	1	0.03	-0.02	0.28	0.25	-0.04	-0.15
Significance:		0.34	0.63	0.00	0.00	0.28	0.00

Table 4. Bivariate correlation 2-tailed. Vitamin 25(OH)D and lipids at baseline level. The unadjusted correlation shows statistically significant (p>0.01) correlation between vitamin D and HDL, APOA and triglycerides.

	Cholesterol	LDL	HDL	APOA	APOB	TriGly
Beta-coefficient:	-0.03	0.01	0.10	0.05	-0.00	-0.06
Significance:	0.44	0.70	0.00	0.07	0.97	0.09

Table 5. Linear regression adjusted for statin treatment, study, sex, age and BMI. Only HDL has a statistically significant (p>0.01) correlation with vitamin D. Variables examined are at baseline levels.

3.4. Comparison between the vitamin D and placebo groups regarding change in serum lipids

When using the independent sample t-test there were no statistically significant difference in change (end of study value minus baseline value) in serum lipids between the vitamin D group and placebo group in statin users (Table 6). In the non-statin user group only triglyceride values were affected significantly in the subjects receiving vitamin D compared to those receiving placebo (Table 7). Because of potential interaction with other variables, the analyses were also performed with linear regression with adjustment for sex, age, BMI and study. However, this did not reveal any significant differences between the vitamin D and placebo group regarding change in lipids (Table 6, table 7). However the change in triglycerides remained statistically significant after adjustment.

Statin users		
	t-test	Linear regression
Cholesterol	0.14 (0.19) p= 0.480	p= 0.400
Triglycerides	0.19 (0.17) p= 0.202	p= 0.116
LDL	-0.03 (0.16) p= 0.851	p= 0.956
HDL	0.03 (0.05) p= 0.537	p= 0.428
Apo-A	-0.01 (0.04) p= 0.826	p= 0.979
Apo-B	-0.00 (0.04) p= 0.950	p= 0.910

Table 6. The table shows significance in difference in lipid lowering effect of placebo versus vitamin D in non-statin users at the end of the intervention. The table shows mean change, standard deviation in parenthesis and p-values for the independent samples t-test, and p-values for linear regression adjusting for age, sex, BMI and study affiliation. No statistically significant difference was determined.

Non-statin users		
	t-test	Regression
Cholesterol	0.02 (0.04) p= 0.644	p= 0.245
Triglycerides	0.08 (0.04) p= 0.04	p= 0.046
LDL	-0.01 (0.04) p= 0.834	p= 0.658
HDL	-0.00 (0.01) p= 0.938	p= 0.732
Apo-A	0.00 (0.01) p= 0.910	p= 0.508
Apo-B	0.01 (0.01) p= 0.428	p= 0.312

Table 7. The table shows significance in difference in lipid lowering effect of placebo versus vitamin D in non-statin users at the end of the intervention. The table shows mean change, standard deviation in parenthesis and p-values for the independent samples t-test, and p-values for linear regression adjusting for age, sex, BMI and study affiliation. Only the change triglycerides showed statistical significance with a p-value of 0.04.

4. Discussion

Except for HDL-cholesterol I found no correlation between total serum 25(OH)D and serum lipid levels. This matches the results from Kane et al (47) who found no correlation between total 25(OH)D and lipids. Furthermore, Kane et al found a significant decrease in LDL and total cholesterol in statin users that received vitamin D supplements, compared to statin users receiving placebo. I did not detect any significant effect of vitamin D versus placebo on lipids in neither statin users nor non-statin users. The exception was an increased triglyceride lowering effect of vitamin D supplementation in non-statin users.

The difference can be the result of multiple factors. The population of Kanes study was selected on the background of insufficient vitamin D levels and given vitamin D

to reach normal levels. One could argue that the potential effect of vitamin D supplements on lipids could only apply to those with very low vitamin D levels. However, the mean vitamin 25(OH)D levels at baseline in our study was 55.9±20.9 nmol/L, which some still define as vitamin D insufficiency, and at risk of osteoporosis. If lipid-lowering effects of vitamin D is the main cause of the correlation between high vitamin D levels and cardiac health, one should expect the correlation to be more apparent.

The significant lowering of triglycerides in non-statin users receiving vitamin D supplements were unexpected, and is probably best explained as a randomly occurring false positive (type I error). The positive correlation between vitamin D and HDL is probably due to a lifestyle confounder. People who eat more foods containing vitamin D and spends more time outdoors would probably have both higher HDL and vitamin D levels. Unfortunately I did not have any lifestyle or diet variables I could factor into the adjusted correlation.

A general problem in research is the publication bias. This is a problem I think is quite applicable to studies such as this. The concept of publication-bias refers to the trend where journals only publicize studies with positive results. Inherent in all testing, including studies, are the uncertainty of false positives and false negatives. This means that if causality between two subjects are examined enough times there are bound to occur positive results, even if there is no real causality between subjects. Because of publication bias these false positives are often the only results published. When scientists then try to re-test these hypotheses and get negative results, it poses a

challenge for them to get it published since journals attract more readers with positive than with negative study outcomes (57).

The main strength of this study is that compared to the study by Kane et al. (47), who included only 49 subjects of whom 19 were statin users, our group of 938 subjects with 69 statin users is relatively large.

However, there are also a number of weaknesses in the present study. First of all, the subjects included were complied from four separate studies that differed considerably regarding age, gender BMI and serum 25(OH)D level at inclusion. Accordingly, the correct analysis would be to perform a meta-analysis, and since individual subject data were available, a formal, individual patient data meta-analysis should have been performed. However, that is at present beyond my statistical competence. In spite of this, I feel that my analysis did not mask a significant effect of vitamin D supplementation in our subjects; the lipid changes in the vitamin D and placebo groups were almost identical, and when analyzing the four studies separately, no effect or trend were seen in any of studies (data not shown). It should also be pointed out that effects on lipids was a predefined objective in only two of the four studies, and the compiling of the four studies was not preplanned. Therefore, the optimal would be a new study with factorial design with inclusion of subjects with high lipid levels and randomize to statin alone, vitamin D alone, statin and vitamin D combined, and placebo.

Furthermore, the result cannot be generalized to the general population since the subjects included were highly selected; in particular young subjects were not included

as most of the subjects were middle age or elderly. Nor did I include type of statin (like atorvastatin or simvastatin) as a covariate, and in theory, the lipid lowering effect of vitamin D in statin users could be linked to a specific statin and not be a statin group effect.

The effects of vitamin D on health outcomes are most likely related to dose given, too little has no effect, whereas too much could be harmful. So far there is no agreement on what the optimal doses of vitamin D or serum levels of 25(OH)D are, and since we gave higher doses than those given by Kane et al (47), our study doses not rule out efficacy of lower doses in this regard. We also had a considerably longer intervention period than Kane et al. (12-weeks) which could have resulted in adaption and waning of an initial effect.

Bioavailable and free 25(OH)D (58) adjusted for genetic polymorphisms would be useful to strengthen the results. Based on the results from a previous study we conduced in Tromsø, these variables may give a more accurate picture of correlations between vitamin D and its suggested effects (59). Vitamin D binding protein has six different phenotypes. These can be determined using a single nucleotide polymorphism analyses to determine genotype, and then convert the genotype to the phenotype. These six variants of DBP have different binding coefficients to vitamin D. This means that the free fraction of vitamin D will be different in subjects with different phenotypes of DBP and the same total 25(OH)D levels. Accordingly, there may be sub-group effects that were missed in our study as I did not have free serum 25(OH)D levels calculated or measured.

Currently there are two main theories on the possible mechanism of action vitamin D could have for lowering serum cholesterol. Either vitamin D inhibits the absorption of exogenous cholesterol, or it inhibits the endogenous synthesis of cholesterol. Theoretically vitamin D or one of its metabolites could also have a direct interaction with the statin molecules themselves. One study found an inhibitory effect on HMG-CoA reductase on human skin cells in vitro (60). The problem with in vitro studies is that the concentration of substrate (vitamin D in this case) is non-physiological (higher than sustainable in the human body), and the results are not always translatable to in vivo experiments. As of now, there has not been published any in vivo laboratory studies (animal-studies) on the possible lipid lowering effects of vitamin D. Kane et al. argues, reasonably, that an effect on HMG-CoA reductase is unlikely since a lipid lowering effect is not seen in non-statin users. Accordingly, the reason for a lipid lowering effect by vitamin D in statin users (if present) remains to be determined

4.1 Conclusion

In conclusion, I have not been able to confirm the observation of a lipid lowering effect by vitamin D in statin users as described by Kane et al. (47). However, since my study has a number of weaknesses together with the great clinical potential such a lipid lowering effect could have, there is obviously a need for more studies on vitamin D, statins and lipids.

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