The Relationship between S-25(OH) Vitamin D Concentrations and Insulin Sensitivity in Subjects with Type 2 Diabetes

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Abstract

Background and aims: Several studies describe an association between vitamin D and glucose metabolism, however data are ambiguous. We aimed to study the relationship between serum levels of 25(OH) vitamin D and insulin action in subjects with type 2 diabetes and hypovitaminosis D.

Methods: Twenty-six subjects with type 2 diabetes and hypovitaminosis D, recruited from the DIVINE study (Diabetes Intervention Trial with Vitamin D in Subjects of Sub-Indian and Nordic Ethnicity), were included in the present analysis. Insulin action was measured as homeostasis model assessment of insulin resistance ($HOMA_{IR}$) and with the hyperinsulinemic euglycemic glucose clamp method.

Results: Subjects had a mean age of 57 years, a mean BMI of 31,1 kg/m^2 and a median S-25(OH) vitamin D of 39 nmol/l. S -25(OH) vitamin D did not correlate with measures of insulin action and was not significantly different between subjects of Nordic and South-Asian ethnicity.

Conclusions: S-25(OH) vitamin D did not correlate significantly with any measures of insulin action in subjects with hypovitaminosis D and type 2 diabetes. Possibly, a study including more subjects can detect such a relationship.

Acknowledgments

I chose to do a project work on the baseline data from the DIVINE-study as it was recommended by my tutor and because it is a highly interesting and current topic. I would like to thank my tutors Kåre I Birkeland[1] and Hanne L Gulseth[2] for instructing and guiding me and being near at hand at all times. I would also like to thank all subjects participating in this study.

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Table of Contents

1 Introduction

1.1 Type 2 diabetes

Type 2 diabetes is an increasing public health problem worldwide (1), and type 2 diabetes constitutes 85% to 95% of all diabetes in high-income countries and probably an even higher percentage in low- and middle income countries (2). It is estimated that 285 million people have diabetes in 2010. The prevalence of diabetes is estimated to almost double from year 2000 till 2030 in Norway and more than double in for example Pakistan, India and Bangladesh (3).

The complications associated with diabetes places a great burden on the individual and also on the society. Prolonged hyperglycemia is associated with changes in both the micro- and macrovasculature and causes damage in the eye, kidney and nerve tissue as well as accelerating atherosclerosis in larger arteries. Diabetes is the most common cause of blindness among subjects in working age and among the most common single causes of end stage renal failure worldwide. Diabetes is also the most common cause of non-traumatic lower limb amputation, as a consequence of neuropathy. Furthermore, people with diabetes have a significantly increased mortality from ischemic heart disease and stroke compared to an age- and sex-matched non-diabetic population (4). The total amount of deaths due to diabetes worldwide in 2010 is estimated to almost four million, or in other words, it is responsible for 6,8% of deaths in subjects between 20-79 years (5).

Today we know that type 2 diabetes is characterized by a variable degree of insulin resistance and relative insulin deficiency caused by hereditary disposition in combination with risk factors, such as overweight and physical inactivity. When insulin secretion no longer can keep blood glucose levels normal, diabetes results. The high blood glucose levels results from a combination of increased glucose production by the liver and delayed uptake of glucose in peripheral tissue, predominantly skeletal muscle (7). Inflammation is probably part of the pathogenesis of type 2

Textbox 1.

A link between diabetes and the pancreas was established in the 19th century when pancreas from dogs were removed and clinical signs characteristic of diabetes mellitus were observed; thirst, polyuria and wasting associated with glucosuria and hyperglycemia. Insulin was discovered 1921 at the University of Toronto and it was shown that extracts from the pancreas of dogs lowered the blood glucose concentrations in pancreatectomised dogs. (6)

diabetes as well (8).

As type 2 diabetes is a major health problem large efforts are made to understand its pathophysiology to be able to develop effective prevention and treatment of the disease. Recently, vitamin D has been introduced as a possible important factor.

1.2 Vitamin D

Vitamin D is derived from sterols in the skin when exposed to the ultraviolet rays in sunlight (9). It can also be obtained from food; fatty fish, eggs or vitamin D fortified foods (9;10). Vitamin D is hydroxylated first in the liver and then in the kidneys, to result in its active form, $1,25(OH)_2D_3$ (figure 1). This active form of vitamin D promotes intestinal absorption of calcium and phosphate, decreases renal excretion of calcium and phosphate and promotes bone calcification - thereby contributing to the feedback regulation of calcium and phosphate in extracellular fluid. Lack of vitamin D, leading to calcium or phosphate deficiency in extracellular fluid, therefore can cause rickets in children and osteomalacia in adults (9).

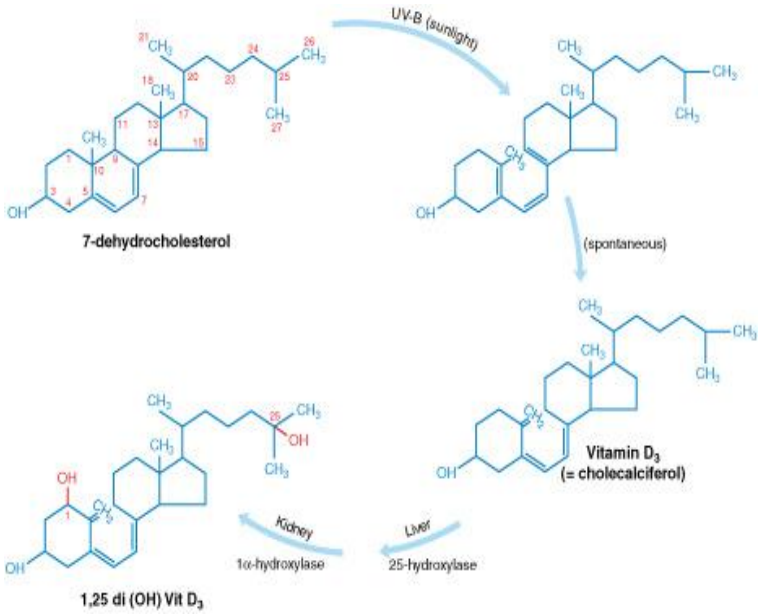

Figure 1. *Overview of vitamin D metabolism showing steps of hydroxylation. Illustration: R&D systems, www.rndsystems.com*

Aside from effects on bone metabolism, vitamin D may also have other important physiological effects. Many tissues, including the pancreas, expresses vitamin D receptors and also have the ability to convert 25(OH)D to its active form $1,25(OH)_2D$ by the expression of 25-hydroxyvitamin D-1-α-hydroxylase (11). This active form acts at the cell nucleus and at the cell membrane in over 30 tissues and organs. It has been increasingly documented that vitamin D has endocrine effects in the kidney and intestine, autocrine effects on cell differentiation,

proliferation and immune modulation and cell membrane effects in intestinal absorption of calcium.

Vitamin D deficiency is associated with increased cortical bone loss, increased bone turnover and increased levels of parathyroid hormone, predisposing to osteoporosis. Associations has also been made between low serum levels of 25(OH) vitamin D and cardiovascular disease, the development of autoimmune disease and certain cancers (colon- prostate- and breast cancers) (10). Epidemiological studies indicate that serum levels below 20 ng/ml (50 nmol/l)* 25(OH) vitamin D increases the risk of incidence of these cancer forms with 30-50% and also increases the mortality. Vitamin D-deficiency causes muscle weakness and adequate supplementation has been shown to reduce falls. A link to increased risk of depression and schizophrenia has also been made (12).

There is no absolute consensus for what a normal range of S-25(OH) vitamin D is but most experts now agree that deficiency is defined as levels < 20 ng/ml (50 nmol/l)* and that insufficiency can be considered as levels between 21-29 ng/ml (52-72 nmol/l)*. It is recognized that 30-50% of both the European and US population are vitamin D deficient or insufficient (13).

1.3 Vitamin D and glucose metabolism

Observational studies have shown an inverse association between S-25(OH) vitamin D status and the prevalence and/or incidence of diabetes, metabolic syndrome, hyperglycemia and insulin resistance (14-17). Pittas *et al.* found that a higher intake of vitamin D among U.S. female nurses, especially in form of supplements, was associated with a lower risk of diabetes (18). A seasonal variation has been reported, with a higher incidence of type 2 diabetes and poorer glycemic control in winter months and early spring, when levels of S-25(OH) vitamin D tend to be lower (19).

There is evidence that vitamin D plays a role in insulin secretion in animals, but results from studies in man are inconclusive. Likewise have studies regarding the effect of S- 25(OH) vitamin D on insulin sensitivity shown ambiguous results (20). There is a need for adequately powered randomized controlled trials exploring if supplementation to adequate serum levels

* Conversion factor to SI units: 2,496

of vitamin D actually improves glycemic control in already manifest disease and if supplementation is safe, acceptable and efficient enough to implement into clinical practice

1.4 Aim

The aim of this project work is to investigate the relationship between S-25(OH) vitamin D concentrations and insulin sensitivity in subjects with type 2 diabetes. To explore this, I used baseline data from the first 26 subjects included in an ongoing intervention study at the Department of Endocrinology, Oslo University Hospital, Aker. The DIVINE-study (Diabetes Intervention Trial with Vitamin D in Subjects of Sub-Indian and Nordic Ethnicity) is a randomized controlled trial with the primary objective to compare the change in insulin sensitivity, measured with euglycemic hyperinsulinemic clamp, in patients with hypovitaminosis D and type 2 diabetes treated for 26 weeks with high dose vitamin D or placebo.

2 Material and methods

2.1 Subjects

Baseline data from the first twenty-six subjects included in the DIVINE study was used in the present project. All subjects had type 2 diabetes, S-25(OH) vitamin D ≤ 50 nmol/l, were of Nordic or South-Asian ethnicity, above 18 years of age and able to communicate in Norwegian. Among the study exclusion criteria were untreated high blood pressure, liver or kidney disease, malignancy, hypercalcemia, a history of kidney stone disease, a history of cardiovascular event during the last six months, anemia or BMI >45kg/m^2. Women in reproductive age had to use adequate contraception. Subjects were recruited from advertisements, local general practitioners and the outpatient clinic at the Department of Endocrinology at Oslo University Hospital. All subjects had given their informed consent to participate before any study related procedures. The study was approved by the regional ethics committee and the Norwegian Medical Agency.

2.2 Study procedures

DIVINE is a randomized, double blind, placebo controlled trial with the primary objective to compare the change from baseline insulin sensitivity, measured with euglycemic hyperinsulinemic clamp, in subjects with hypovitaminosis D and type 2 diabetes treated for

26 weeks with vitamin D or placebo. The main hypothesis is that vitamin D supplementation in patients with type 2 diabetes will improve insulin sensitivity and glucose metabolism.

At the screening visit S-25(OH) vitamin D, S-ionized calcium and S-PTH were measured and suitability according to inclusion and exclusion criteria was checked. After this subjects were enrolled into a 4 weeks lead in phase with the purpose to stabilize their diabetes treatment. At this time all patients were also give calcium supplementation 250 mg bid to be continued throughout the study period of 30 weeks (4 weeks lead in phase and 26 weeks of double – blind treatment). At visit 3 subjects were completely physically examined and measured body composition of, using DEXA. Height and weight were measured in light clothing, without shoes. At visit 4 a combined IVGTT and euglycemic hyperinsulinemic clamp with measurement of hepatic glucose production, using the stable isotope dilution method, was performed to measure insulin sensitivity and secretion at baseline. Subjects were informed to meet in the fasting state. No food, but water, was allowed after midnight the night before. Oral antihyperglycemic agents were withdrawn 48 hours and insulin 12 hours prior to the procedure and subject avoided strenuous physical exercise and alcohol the day before the investigation.

2.3 Biochemical measurements

S-25(OH) vitamin D is a valid marker of vitamin D status (13) and was measured at the Hormone Laboratory using the DiaSorin 25(OH) vitamin D radio immunoassay. The Hormone laboratory reports an intra-assay coefficient of variance of 6% for this analyze. The reference value for S-25(OH) vitamin D according to the Hormone Laboratory is 37-131 nmol/l (21), and according to Fürst 50-150 nmol/l (22). Glucose was measured locally with an YSI 2300 (Yellow Springs Instruments, Ohio, USA) using a glucose oxidase method. S-insulin concentrations were determined using a human immunometric assay kit (AutoDELFIA Insulin, Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland) on a 1235 automatic immunoassay system (Wallac 1235 AutoDELFIA, Wallac Oy, Turku, Finland). P-total cholesterol, P-HDL-cholesterol and P-triglycerides were determined with enzymatic colorimetric kits (Roche Diagnostics, Mannheim, Germany). P-LDL was calculated using the Friedwald formula. S-Ionized calcium was measured using Rapidlab 348 pH/Blood Gas

Analyzer and PTH was analyzed in serum with a non-competetive immunoluminometric assay (Immulite 2500 from Siemens Healthcare Diagnostics, Los Angeles, CA, USA).

2.4 Measurement of insulin sensitivity

2.4.1 Homeostasis model assessment of insulin resistance -$HOMA_{IR}$

Homeostasis model assessment of insulin resistance derives from a mathematical model of the glucose-insulin homeostatic system. This model can be used to calculate an index of insulin resistance in the fasting state from fasting P-glucose and fasting S-insulin (23). The HOMA2 calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php) was used here and the fasting samples of P-glucose (mmol/l) and S-insulin (pmol/l) were inserted. This calculator does not accept values of insulin above 400 pmol/l. For two of the subjects measured S-insulin exceeded this value and for the purpose of calculating $HOMA_{IR}$ they were set to 400 pmol/l.

2.4.2 IVGTT

A glucose bolus of 0,3g/kg body weight was given over one minute and then glucose-, insulin-, and c-peptide concentrations were measured at 0,2,4,6,8,10, 15 and 30 minutes. Insulin secretion in response to hyperglycemia can then be studied**,** as the purpose of the IVGTT is in DIVINE . An IVGTT also gives an opportunity to estimate the insulin sensitivity. The glucose concentration reaches a peak value and then starts to decline. Insulin, released in response to the increased glucose concentration, accelerates the decline – how much depends on the insulin level and its action. With the minimal model, a mathematical representation of the glucose-insulin relationship, the insulin sensitivity index (S_i) can be calculated (25). This index will not be used here.

2.4.3 Hyperinsulinemic euglycemic clamp

The glucose clamp technique in its euglycemic version is generally accepted as the gold standard for measurement of insulin action (25). Insulin (Actrapid, NovoNordisk, Denmark) was infused to create a stable level of hyperinsulinemia. First a priming dose was given, calculated from the patient's pre-clamp plasma glucose (those with a P-glucose above 13 mmol/l when the clamp started were given an extra bolus of insulin) then insulin was infused at a constant rate of 80 mU/m^2/min. Since a glucose bolus of 0,3g/kg body weight was given in the preceding IVGTT the P-glucose was monitored by measuring P-glucose every five

minutes. When P-glucose had declined to between 6,0-5,5 mmol/l one started a variable glucose infusion (glucose 200 mg/ml, enriched with the tracer - 8mg/g glucose) with the aim to keep P-glucose as close to 5 mmol/l as possible. This was done by measuring P-glucose every five minutes and adjusting the glucose infusion rate empirically according to the result. For two of the subjects (one Nordic, one South-Asian) one was not able to reduce the P-glucose to 5,5-6,0 mmol/l, suggesting they were highly insulin resistant, and calculations of glucose infusion rate and insulin sensitivity index for those were therefore not done. The total clamp duration was set to 150 minutes and the glucose infusion rate was calculated from the last 30 minutes of stable euglycemia. S-insulin, P-glucose and glucose tracer were measured 30, 20 and 10 minutes before the end and at the end of the clamp. The glucose infusion rate (GIR, mmol/m^2/min) required to maintain constant euglycemia during the period of constant hyperinsulinemia provides a measure for the net effect of insulin on whole body glucose metabolism (26). After the infusions were switched of the subjects were asked to void and the urine volume was measured and urine samples were stored. Glucose lost via the urine was not taken into account in the present analyze of data.

Technically there are three major requirements to perform a glucose clamp. Two intravenous lines, for example one in each antecubital vein, must be kept patent throughout the study. One for infusions of glucose and insulin and one for sampling. The arm where samples were taken from was heated with a heating sleeve to 37°C to arterialize the blood taken for samples (25). The heating induces vasodilatation, arteriovenous anastomoses are opened, which reduces the blood transit time and thus also the arterio-venous difference in glucose concentration that would otherwise lead to a systematic underestimation of the actual P-glucose. Glucose would be over-infused and insulin sensitivity overestimated. The difference in arterial glucose concentration between individuals is also proportional to the insulin sensitivity and thus, theoretically, would lead to a greater overestimation in more insulin sensitive individuals (26). Second, pumps for infusion of glucose and insulin must be well-calibrated and must have sufficiently fine gears for one to adjust the glucose infusion rate to keep P-glucose as stable as possible. Third, samples for P-glucose must be analyzed quickly in order to be able to make necessary adjustments of the glucose infusion rate. Glucose must then, after the adjustment of the glucose infusion rate, again come to equilibrium with the extravascular, extracellular glucose compartment, before next sample

of P-glucose is drawn, not to overestimate the effect of the new glucose infusion rate (25;26).

Clamp data was standardized for the purpose of comparison. Insulin was administered per unit of body surface area to avoid administering to much insulin in obese individuals. Since muscle tissue is the principle site of glucose uptake, glucose infusion rate is best normalized by the fat-free mass, i.e. lean body mass. To account for differences in the clamp insulin levels one can correct for the clamp-insulin concentration (25).

An index of insulin sensitivity (ISI) can thus be expressed as GIR_{ffm}/I, where GIR_{ffm} is the glucose infusion rate normalized for fat free mass and I is the mean insulin concentration during the steady state period of the clamp. As dividing GIR_{ffm} with S-insulin gives a very small number, it was multiplied with 1000 to give a more suitable number.

2.4.4 The stable isotope dilution technique

The purpose of this method is to measure the endogen glucose production, where of the major part is of hepatic origin. The glucose utilized by the body during constant P-glucose equals the sum of glucose infused and endogen glucose produced. Normally an insulin infusion rate of 80 mU/min/m^2 would almost totally suppress the hepatic glucose production (HGP) but it has been demonstrated that insulin is less effective in suppressing HGP in subjects with type 2 diabetes (27). Since all the subjects in this material have diabetes one can not assume that HGP equals zero and therefore must measure it not to underestimate the glucose-utilization. The HGP rate also offers an idea of hepatic resistance to the suppressive actions of insulin, a higher rate of HGP indicating a higher degree of hepatic insulin resistance.

With the infusion of a tracer, in this case deuterium labeled glucose ([6,6-$^2H_2$]glucose), HGP can be calculated. A tracer behaves like the tracee i.e. glucose. In [6,6-$^2H_2$]glucose two hydrogen atoms on glucose is replaced by deuterium (hydrogen with two neutrons instead of one) and can therefore be differentiated from glucose by its higher molecular weight. When the amount of infused tracer and glucose into an estimated volume is known, one can calculate what the theoretical concentration of the tracer would be if infused glucose was the only source of P-glucose. If glucose in addition is endogenously produced, the measured tracer-concentration will be lower than it was calculated to be. The difference between the

calculated and the measured tracer-concentration gives the amount of endogenously produced glucose. Under non-steady-state conditions, as during the infusion of insulin and glucose, HGP is calculated using formulas such as Steel´s equation (28). However, in this analyze of data, tracer-concentrations were not available and HGP has therefore not been calculated.

2.5 Statistical analyses

Normally distributed data are presented with mean and standard deviation and data not normally distributed are presented with median, 25th and 75th percentiles (interquartile range). Two- sample t-test was performed in Excel 2007 to compare subgroups for data considered to be normally distributed. For non-parametric data, independent samples Mann-Whitney U tests were used to compare groups. For testing significance of crosstabulated variables Chi-square test was applied. The tests were performed two sided and a p-value ≤ 0,05 was considered significant. In addition correlations were calculated using Spearman's rank correlation coefficient. The effect of levels of S-25(OH) vitamin D was also explore by comparing glucose parameters in two groups; one with S-25(OH) vitamin D above/equal to 40 nmol/l and one with S-25(OH) vitamin D below 40 nmol/l. We also explored the effects of ethnicity by comparing Nordic to South-Asian subjects. Non-parametric tests, correlations, chi-square, histograms and plots were done in PASW Statistics 18. For two of the Nordic subjects HbA1c, P-cholesterol, P-HDL, P-triglycerides and P-glucose are missing. P-LDL is missing in three Nordic subjects. S-ionized calcium is missing in one Nordic subject.

3 Results

A total of 26 subjects were included in this analysis. Two thirds of subjects were men. In the South-Asian group six were from Pakistan, three from Sri Lanka and one from India. Six included subjects were treated with diet only and twenty received antihyperglycemic medication (nine were on insulin, with or without oral antihyperglycemic agents, and eleven on oral antihyperglycemic agents only). Subject characteristics are described in table 1. BMI was > 25 kg/m² in all subjects, 14 subjects were overweight and 12 subjects were obese.

Table 1. Descriptive statistics and anthropometric measurements						
	All subjects *n=26*		Nordic *n=16*		South-Asian *n=10*	
	Mean	SD	Mean	SD	Mean	SD
Age	57	10	62	6	50	11
n* female (%)	8 (31)		5 (31)		3 (30)	
Height (cm)	170,8	8,4	172,5	8,8	168,0	7,4
Weight (kg)	90,4	13,8	96,7	12,5	80,2	9,2
BMI (kg/m²)	31,1	5,1	32,7	5,4	28,5	3,4
Diabetes duration (years)	9	6	10	7	9	5
SBP (mmHg)	131,8	15,4	133,3	15,6	129,4	15,6
DBP (mmHg)	87,6	7,6	87,5	6,6	87,8	9,4
Waist-to-hip ratio	1,04	0,06	1,04	0,07	1,03	0,06
Fat (%)	34,5	7,5	35,8	8,4	32,3	5,6
Lean body mass (kg)	55,7	8,3	58,4	8,4	51,4	6,2

* Expressed as number and percent

The Nordic subjects were older (p=0,01) and had higher body weight (p<0,01), BMI (p=0,02) and lean body mass (p=0,02) compared to South-Asians. Body height, known diabetes duration, blood pressure, waist-to-hip ratio and percent body fat did not differ significantly between the groups.

Table 2. Diabetes measurements and lipids in Nordic and South-Asian subjects						
	All subjects *n=24*		Nordic *n=14*		South-Asian *n=10*	
	Median	IQR	Median	IQR	Median	IQR
Fasting P-glucose (mmol/l)	10,6	8,3-11,9	10,4	8,4-11,5	11,1	8,6-12,6
HbA1c (%)	7,3	6,7-8,5	6,8	6,5-7,3	8,2	7,5-9,0
P-Cholesterol (mmol/l)	4,1	3,2-4,9	4,2	3,3-5,3	4,0	3,2-4,8
P-LDL (mmol/l)*	2,3	1,8-2,9	2,3	1,8-2,4	2,4	1,8-3,2
P-HDL (mmol/l)	0,9	0,8-1,2	1,0	0,82-1,34	0,9	0,8-1,1
P-Triglycerides (mmol/l)	1,3	0,9-1,9	1,5	1,1-1,9	1,1	0,9-1,7

* Only available in 13 Nordic subjects

There was no significant difference in fasting P-glucose, P-cholesterol, P-LDL, P-HDL or P-triglycerides between Nordic and South-Asian. HbA1c was significantly higher in the South-Asian group (p<0,01). Table 2 shows measures of glucose metabolism and lipids.

Table 3. Serum levels of 25-(OH) vitamin D, ionized calcium and PTH in Nordic and South-Asian subjects						
	All subjects *n=26*		Nordic *n=16*		South-Asian *n=10*	
	Median	IQR	Median	IQR	Median	IQR
S-25(OH) vitamin D (nmol/l)	39,0	29,0-44,8	40,0	28,0-45,5	35,0	29,8-41,5
S-Ionized calcium (mmol/l)*	1,27	1,25-1,29	1,26	1,24-1,29	1,28	1,26-1,31
P-PTH (pmol/l)	4,3	2,4-5,6	4,5	3,2-6,6	3,5	2,3-4,4

* Data missing for one Nordic subject.

All included subjects had vitamin D-deficiency – either moderate (S-25(OH) vitamin D 25-50 nmol/l, *n=22*) or severe (S-25(OH) vitamin D < 25 nmol/l , *n=5*) at the screening visit. S-ionized calcium was below 1,35 mmol/l in all subjects and S-PTH stretched from 1,2-11,4 pmol/l at the screening visit. Se Table 3.

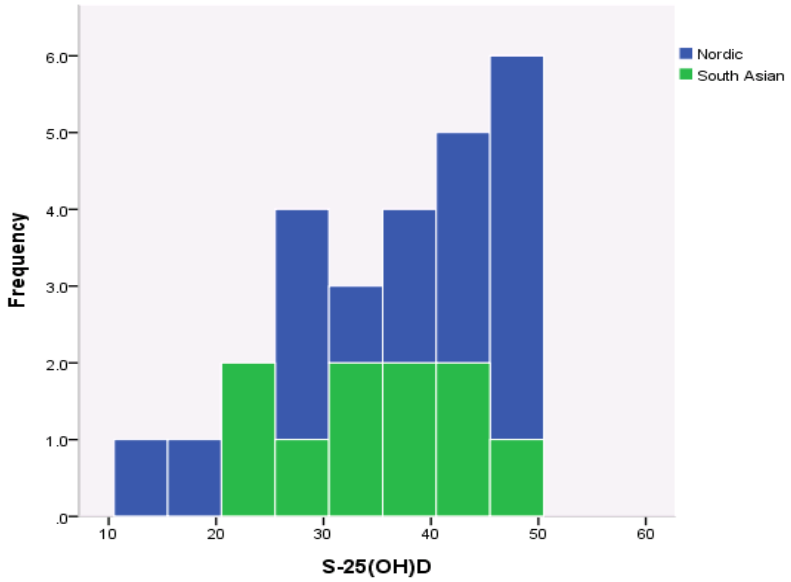

Figure 2. *Distribution of S-25(OH) vitamin D among Nordic and South-Asian subjects.*

No significant difference was found in S-25(OH) vitamin D, S-ionized calcium or S-PTH between the Nordic and South-Asian group. Distribution of S-25(OH) vitamin D is presented in a histogram (figure 2). S-25(OH) vitamin D was not significantly correlated with age, diabetes duration, blood pressure, fasting P-glucose, fasting S-insulin, HbA1c, anthropometric measures or P-lipids in any of the groups.

Serum levels of 25(OH) vitamin D did not show significant correlations to any of the insulin action measures performed (GIR: r=0,285 p=0,158, ISI: r=0,172 p=0,402, $HOMA_{IR}$: r=-0,064 p=0,756). Measures of insulin action separate for Nordic and South-Asian subjects are presented in table 4. Figure 3 shows a plot of the non-significant correlation between S-25(OH) vitamin D and insulin sensitivity index (ISI) separate for Nordic and South Asian subjects.

Table 4. Measures of insulin action in Nordic and South-Asian subjects

	All subjects *n=26*		Nordic *n=16*		South-Asian *n=10*	
	Median	IQR	Median	IQR	Median	IQR
$HOMA_{IR}$	1,7	1,1-3,0	2,1	1,1-3,4	1,6	1,0-2,0
GIR_{ffm} (mmol/kg/min)*	4,79	3,61-6,41	4,68	3,08-6,06	5,88	4,14-6,84
ISI*	4,97	2,43-6,09	4,22	0,94-5,43	5,25	4,60-6,77

* Data missing for one Nordic and one South-Asian subject

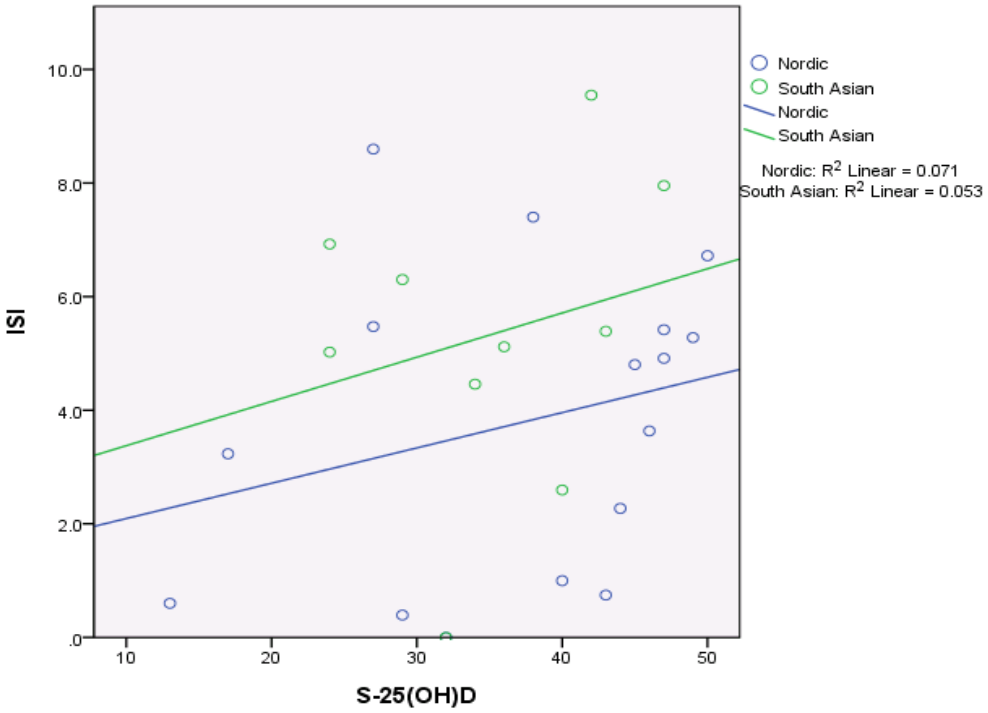

Figure 3. *Correlation between 25-(OH) vitamin D and ISI separate for Nordic and South-Asian subjects*

	GIR_{ffm}	ISI	$HOMA_{IR}$	WHR	BMI
GIR_{ffm}		r= 0,923 p<0,01	r= -0,591 p<0,01	r= -0,395 p=0,05	r= -0,272 p=0,179
ISI	r= 0,923 p<0,01		r= -0,582 p<0,01	r= -0,446 p=0,02	r= -0,300 p=0,14
$HOMA_{IR}$	r= -0,591 p<0,01	r= -0,582 p<0,01		r= 0,283 p=0,161	r= 0,389 p=0,05
WHR	r= -0,395 p=0,05	r= -0,446 p=0,02	r= 0,283 p=0,161		r= 0,516 p<0,01
BMI	r= -0,272 p=0,179	r= -0,300 p=0,14	r= 0,389 p=0,05	r= 0,516 p<0,01	

Figure 4. *Data correlating significantly with measures of insulin action. Significant correlations are marked out.*

Table 6. Characteristics of subjects based on S-25(OH)D (cutoff value: 40 nmol/l)

	S-25(OH)D <40 nmol/l n=13	S-25(OH)D ≥ 40 nmol/l n=13
n (%)	13 (50)	13 (50)
sex (% female)	2 (15)	6 (46)
Nordic (%)	7 (54)	9 (69)
Age (years)*	56 ± 11	58 ± 10
Weight (kg)*	88,8 ± 11,0	91,9 ± 16,5
BMI (kg/m2)*	29,8 ± 4,2	32,3 ± 5,7
Waist-to-hip ratio*	1,06 ± 0,07	1,01 ± 0,05
Body fat (%)*	31,9 ± 7,1	37,0 ± 7,4
Lean body mass (kg)*	57,2 ± 7,8	54,1 ± 8,8
Diabetes duration (years)*	10 ± 6	9 ± 6
HbA1c (%)¤**	7,3 (6,7-8,0)	7,3 (6,7-8,5)

*Data expressed as mean ± SD

** Data expressed as median (IQR)

¤ Data missing for two subjects in the S-25(OH)D ≥ 40 nmol/l group

The waist-to-hip ratio was significantly higher in subjects with S-25(OH)D < 40 nmol/l (p=0,02), the other parameters did not differ. Table 6 demonstrates baseline characteristics for subjects divided according to S-25(OH) vitamin D with a cut off value of 40 nmol/l.

Table 7. Measures of insulin action in subjects above and below 40 nmol/l S-25(OH)D				
	S-25(OH)D <40 nmol/l *n=13*		S-25(OH)D ≥40 nmol/l *n=13*	
	Median	IQR	Median	IQR
$HOMA_{IR}$	1,7	1,1-3,0	2,1	0,9-2,8
GIR_{ffm} (mmol/kg/min)*	4,55	1,75-6,09	4,87	3,70-6,52
ISI*	5,02	0,60-6,30	4,91	2,60-5,42

* Data missing for one Nordic and one South Asian subject

There was no significant difference in $HOMA_{IR}$, GIR_{ffm} or ISI between those with S-25(OH) vitamin D <40 nmol/l and those with S-25(OH) vitamin D ≥40 nmol/l. See table 6.

Diabetic late complications was found in 10 of the patients. 6 out of 13 in the S-25(OH) vitamin D <40-group had complications (three with retinopathy, one with neuro- and nephropathy, one with diabetes foot complications and one not specified) and 4 out of 13 in the S-25(OH) vitamin D ≥40-group (one with cardio-vascular, one with neuropathy and two not specified) (figure 5). Five with complications were Nordic and five were South-Asian.

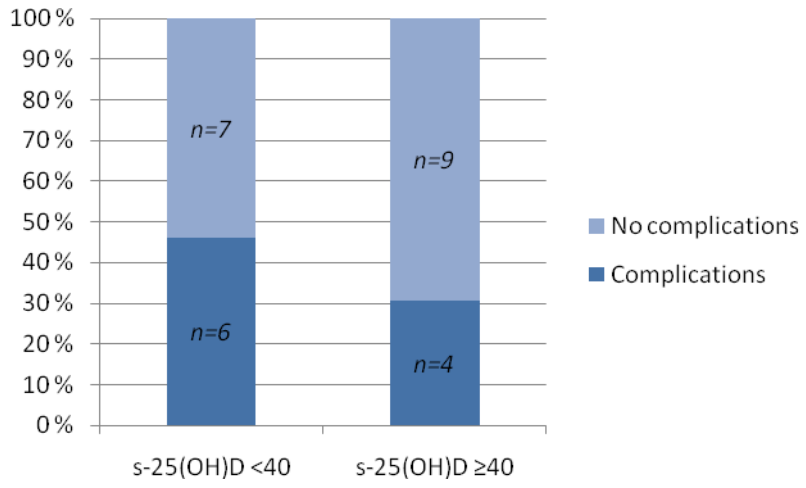

***Figure 5**. Prevalence of diabetic late complications according to S-25(OH) vitamin D status*

4 Discussion

The main finding in the present study was that S-25(OH) vitamin D did not correlate significantly with any of the measurements of insulin action in the 26 included subjects of Nordic and South Asian ethnicity with type 2 diabetes. Furthermore, vitamin-D levels did not correlate to fasting S-glucose, fasting S-insulin or HbA1c. No significant difference in insulin

action between groups divided according to S-25(OH) vitamin D above or below 40 nmol/l was found. Our findings is in accordance with several previously reported studies (29;30).

Although not significant, we observed correlation coefficients associating GIR_{ffm}, ISI and $HOMA_{IR}$ to S-25(OH) vitamin D in such a way that a larger study maybe would have found that insulin action is significantly correlated with higher levels of S-25(OH) vitamin D, as also have been reported in some previous studies (14;31;32). One study also reported improved insulin action after supplementation of vitamin D (33). We found some indications of a variable strength in relationship between insulin sensitivity and S-25(OH) vitamin D levels in Nordic versus South-Asian, although our groups were far too small to assess this in a proper way. The NHANES III reported different associations between S-25(OH) vitamin D and $HOMA_{IR}$ in different ethnic groups – a significant inverse correlation in Mexican American and inverse, but not significant in Non-Hispanic white but a slightly positive, non-significant correlation in Non-Hispanic black. They suggested a possibility of an ethnically related threshold effect of vitamin D with a decreased sensitivity of vitamin D among Non-Hispanic black (14).

When subjects were divided into groups according to their S-25(OH) vitamin D level, only waist-to-hip ratio was found to be significantly different between the groups - with a higher waist-to-hip ratio in subjects below 40 nmol/l 25(OH) vitamin D. Furthermore, correlations between S-25(OH) vitamin D and waist-to-hip ratio were negative, but not significant, in all subjects together, in Nordic and South-Asian (p=0,06, 0,09 and 0,21 respectively). In contrast S-25(OH) vitamin D was positively correlated (not significant) with body fat percent and slightly positively correlated with BMI. This might indicate a specific relation between abdominal obesity and vitamin D deficiency, and not obesity per se, in accordance with one study finding a strong association between S-25(OH) vitamin D and abdominal obesity even after adjustment for obesity (15). Other studies have though reported a negative correlation between S-25(OH) vitamin D and BMI (29;32). In addition a significant inverse correlation between GIR_{ffm}, ISI and waist-to-hip ratio was found – showing a relation between insulin resistance and abdominal obesity.

Many mechanisms have been proposed as a link between S-25(OH) vitamin D and diabetes. Bland *et al.* showed that pancreatic cells contain 25-hydroxyvitamin D_3-1α-hydroxylase – the

enzyme converting vitamin D to its active form $1,25(OH)_2D$ - and vitamin D receptors and that $1,25(OH)_2D$ evoked a rise in $[Ca^{2+}]_i$ in insulin-secreting cells from mouse (11). Maestro *et al*. demonstrated that the human insulin gene promoter has a vitamin D responsive element and that vitamin D increases the activity of the human insulin promoter (34). Pittas *et al.* suggested an indirect effect of vitamin D via regulation of extracellular calcium and calcium flux through β-cells, as insulin secretion is a calcium-dependent process. The same review also proposed that vitamin D, through modulation of the generation and effects of cytokines, may improve insulin sensitivity (35). One study, on the contrary, suggested that the association between S-25(OH) vitamin D and insulin resistance simply might be the result of increased body size (32).

The strength of the present study was in particular that the euglycemic hyperinsulinemic glucose clamp method was used to assess the insulin sensitivity. This is considered to be the gold standard of measuring insulin sensitivity. In addition, the studied group of individuals was clearly defined by inclusion- and exclusion criteria. All subjects had vitamin D-deficiency and both Nordic and South-Asian were included. Only 26 subjects had so far been included and were available for this analyze of baseline data. Dividing those into subgroups according to S-25(OH) vitamin D level or ethnicity gave small numbers of subjects in each group. Particularly the South Asian group was small with only 10 subjects. Thus the statistical power to detect relationships was moderate. Also, as all the included subjects had moderate or severe vitamin D-deficiency at baseline the range of 25-(OH) vitamin D was fairly small which limits the power further. Furthermore, as this is a study of data at one point of time – the causative nature of the associations can not be established.

5 Conclusion

There was no significant difference in insulin sensitivity between groups divided according to levels of S-25(OH) vitamin D and no significant correlations between S-25(OH) vitamin D and $HOMA_{IR}$, GIR_{ffm} or ISI were found. In the group with less than 40 nmol/l 25(OH) vitamin D, waist-to-hip ratio was significantly higher, which could advert abdominal obesity as a risk factor of vitamin D-deficiency or vice versa. As waist-to-hip ratio also was found to significantly correlate with insulin sensitivity expressed as GIR_{ffm} or ISI in all subjects collectively one could propose abdominal obesity to be a common risk factor of vitamin D-

deficiency and insulin resistance. A non-significant positive correlation between S-25(OH) vitamin D and insulin sensitivity in this study may indicate a possible relationship that needs to be studied in a larger population.

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