

## **Serum Vitamin D concentration Does Not Predict Insulin Action or Secretion in European Subjects with the Metabolic Syndrome**

Running title: *Vitamin D and Insulin Action and Secretion*

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Submitted 30 September 2009 and accepted 30 December 2009.

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*Objective:* To investigate the relation between serum concentration of 25-hydroxyvitamin D [25(OH)D] and insulin action and secretion.

*Research Design and Methods:* In a cross-sectional study of 446 Pan-European subjects with the metabolic syndrome insulin action and secretion were assessed by homeostasis model assessment (HOMA) indices and intravenous glucose tolerance test to calculate acute insulin response, insulin sensitivity and disposition index. Serum 25(OH)D was measured by HPLC/MS.

*Results:* The mean (SD) 25(OH)D<sub>3</sub> concentration was 57.1 (26.0) nmol/l, and only 20% of the subjects had 25(OH)D<sub>3</sub> levels  $\geq 75$  nmol/l. In multiple linear analyses, 25(OH)D<sub>3</sub> concentrations were not associated with parameters of insulin action or secretion after adjustment for BMI and other covariates.

*Conclusion:* In a large sample of subjects with the metabolic syndrome, serum concentrations of 25(OH)D<sub>3</sub> did not predict insulin action or secretion. Clear evidence that D-vitamin status directly influences insulin secretion or action is still lacking.

Low serum concentrations of 25-hydroxyvitamin D [25(OH)D] has been linked to disturbances in glucose metabolism (1-3), development of type 2 diabetes (4) and increased risk of the metabolic syndrome (5-7). To explore the associations between serum concentrations of 25(OH)D and glucose metabolism, we evaluated the relationship between 25(OH)D status and insulin secretion and action estimated both by the homeostatic model assessment (HOMA) and intravenous glucose tolerance test (IVGTT), in a large sample of European subjects with the metabolic syndrome.

## RESEARCH DESIGN AND METHODS

Cross-sectional data were obtained from baseline assessment of 446 Caucasian subjects, aged 35-70 years, BMI 20-40kg/m<sup>2</sup> recruited for the LIPGENE study (NCT00429195) performed in eight European countries in 2005/2006. All subjects had the metabolic syndrome defined by  $\geq 3$  slightly modified NCEP ATP-III criteria (8); levels of fasting plasma glucose  $>5.5$  mmol/l, triglycerides  $\geq 1.5$  mmol/l, HDL-cholesterol  $<1.0$  mmol/l (males) or  $<1.3$  mmol/l (females), blood pressure (BP)  $\geq 130/85$  mmHg or on BP lowering medication, and waist circumference  $>102$  cm (males) or  $>88$  cm (females). The study was approved by local ethics committees at each center (Dublin, Reading, Oslo, Marseille, Maastricht, Cordoba, Krakow and Uppsala) and confirmed to the declaration of Helsinki. All participants gave written informed consent.

A questionnaire was used to assess the level of physical activity (9), smoking habits, alcohol consumption and demographic data. Anthropometric and BP measurements were recorded according to standard protocols. An insulin-modified IVGTT was performed as described earlier (10). Measures of insulin

sensitivity (Si) were obtained using the MINMOD Millenium Program (version 6.02, Richard N Bergman) (11). The acute insulin response to glucose (AIR) was defined as the incremental area under the curve from 0-8 minutes. Disposition index (DI) was calculated as AIR\*Si. HOMA indices (HOMA<sub>2</sub>, version 2.2.2 <http://www.dtu.ox.ac.uk/index.php?maindoc=/homa>) were used to assess insulin resistance (HOMA-IR) and  $\beta$ -cell function (HOMA  $\beta$ ) from fasting blood samples (12). Vitamin 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> were analyzed with HPLC/MS. Only 15 subjects (3%) had measurable concentrations of 25(OH)D<sub>2</sub>, mean 10.1 nmol/l, range 6.5-24.6 nmol/l. Including 25(OH)D<sub>2</sub> in the analyses did not influence the result. All examinations were performed in January/February to avoid seasonal variation. Correlations between parameters were calculated with Pearson's or Spearman's correlation coefficient as appropriate. Non-normally distributed data were transformed using logarithmic function. Multiple linear regression models were used to assess the relationship of 25(OH)D<sub>3</sub> with Si, AIR, DI, HOMA  $\beta$  and HOMA-IR, respectively. Statistical analyses were performed using SPSS® for Windows™ version 16.0. P-values  $<0.05$  (two-sided) were regarded as statistically significant.

## RESULTS

Mean (SD) serum concentration of 25(OH)D<sub>3</sub> was 57.1 (26.0) nmol/l, range 13.7-170.4 nmol/l. Only 91 (20%) had levels  $\geq 75$  nmol/l and a majority ( $n=227$ ) had biochemical vitamin D deficiency ( $<50$  nmol/l) (13). Subject characteristics are presented across tertiles of serum 25(OH)D<sub>3</sub> concentration (Online Appendix Table 1 which is available at <http://care.diabetesjournals.org>). In unadjusted analyses IVGTT-derived parameters did not differ across tertiles of 25(OH)D<sub>3</sub>, whereas fasting insulin, HOMA-

IR and HOMA  $\beta$  were significantly different (all  $P < 0.015$ ), with higher values among subjects in the lower tertile of 25(OH)D<sub>3</sub> concentration (Online Appendix Table 2). Serum levels of 25(OH)D<sub>3</sub> correlated negatively with BMI ( $r = -0.28$ ,  $p < 0.001$ ), AIR ( $r = -0.11$ ,  $P = 0.033$ ), fasting insulin ( $r = -0.14$ ,  $P = 0.002$ ), HOMA-IR ( $r = -0.14$ ,  $P = 0.003$ ) and HOMA  $\beta$  ( $r = -0.15$ ,  $P = 0.001$ ), but not with Si ( $r = 0.062$ ,  $P = 0.21$ ) or DI ( $r = -0.059$ ,  $P = 0.24$ ). In a multivariate regression analysis including potential covariates (table 1), serum 25(OH)D<sub>3</sub> concentration was a statistically significant predictor of HOMA-IR, HOMA  $\beta$  and AIR ( $P < 0.05$ ) but not of Si or DI when adjusting for sex, age and geographic location. After adding BMI to the regression model, neither HOMA indices nor AIR were significantly associated with 25(OH)D<sub>3</sub> (Table 1).

To further explore these relationships we compared subjects with a severe biochemical vitamin D deficiency ( $< 25$  nmol/l,  $n = 20$ ) to those with sufficient vitamin D status ( $\geq 75$  nmol/l,  $n = 91$ ). Only BMI was significantly different between groups ( $P = 0.001$ ), whereas HOMA and IVGTT-parameters were not.

## CONCLUSIONS

We found no significant associations between IVGTT-derived parameters of insulin secretion and action and serum 25(OH)D<sub>3</sub> concentrations. At variance with our findings, Chui *et al* observed a positive association between vitamin D status and insulin sensitivity in 126 glucose-tolerant students investigated by hyperglycemic clamp (2), remaining significant also after adjustment for BMI. The reason for the different results between this study and ours might be the differences in populations or methods used to assess insulin sensitivity. In the former study, there were also an inverse relationships between first and second phase insulin secretion and serum 25(OH)D concentrations

that were not significant after adjusting for covariates, in accordance with our results.

A significant relationship between 25(OH)D and fasting insulin and HOMA-IR has been reported by others (1,14,15). The reason for the differences between these and our results may be that we investigated a more homogeneous group of subjects that all had the metabolic syndrome and hence some degree of insulin resistance. We speculate that vitamin D status may be more closely associated with hepatic insulin sensitivity reflected by fasting glucose and insulin levels, than with peripheral insulin sensitivity as measured by IVGTT. Thus, the link between vitamin D status and tissue specific insulin action requires further investigation.

Strengths of our study included the use of IVGTT with minimal modelling to assess insulin secretion and insulin action. This extends the knowledge from previous investigations that mostly were based on fasting blood samples. Furthermore, the inclusion of subjects from eight different centres across Europe, and limiting the data sampling to two months of the year also are advantageous. Limitations of the study were that we only investigated one ethnic group of individuals and that rather few had severe vitamin D deficiency. Also, as the presence of metabolic syndrome was an inclusion criterion for participation in the study, cross-sectional relationships may be attenuated in our population.

In conclusion, we found no correlations between vitamin 25(OH)D<sub>3</sub> and IVGTT-based estimates of insulin action and secretion in this large sample of subjects with the metabolic syndrome. Prospective and interventional studies using reliable techniques are needed to further elucidate the relation between 25(OH)D and insulin action and secretion.

## ACKNOWLEDGEMENTS

The study has been supported by LIPGENE - an EU 6th Framework Program Integrated Project (FOOD-CT-2003-505944); the Norwegian Foundation for Health and Rehabilitation; South-Eastern Norway Regional Health Authority; and Johan Throne Holst Foundation for Nutrition Research. CIBEROBN is an initiative of ISCIII Government of Spain. Parts of this study were presented in abstract form at the 69<sup>th</sup> annual meeting of the American Diabetes

Association, New Orleans, Louisiana June 5-9, 2009, at the 3<sup>rd</sup> International Congress on Prediabetes and the Metabolic Syndrome, Nice, France, April 1-4, 2009 and at the 45<sup>th</sup> annual meeting of the European Association for the Study of Diabetes, Vienna September 27<sup>th</sup> -October 1<sup>st</sup> 2009.

**Disclosure:** None of the authors had any financial or personal conflict of interest to disclose.

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Table 1. Adjusted regression coefficients of 25(OH) vitamin D<sub>3</sub> (nmol/l) with parameters of insulin action and secretion.

	Model 1 <sup>*</sup>			Model 2 <sup>†</sup>			Model 3 <sup>‡</sup>		
	β	SE	P	β	SE	P	β	SE	P
Si ((mU/l) <sup>-1</sup> min <sup>-1</sup> )	0.005	0.003	0.17	0.003	0.003	0.60	0.002	0.003	0.69
AIR (mU <sup>-1</sup> min <sup>-1</sup> )	-1.47	0.60	0.041	-1.26	0.60	0.078	-1.20	0.63	0.079
DI	-3.23	1.44	0.30	-3.65	1.45	0.17	-3.36	1.53	0.20
HOMA-IR	-0.004	0.002	0.016	-0.002	0.002	0.19	-0.002	0.002	0.24
								0.068	
HOMA β (%)	-0.185	0.067	0.007	-0.128	0.066	0.063	-0.113		0.070

Abbreviations: Si, insulin sensitivity index; AIR, acute insulin response; DI, disposition index; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA β, homeostasis model assessment of beta cell function.

<sup>\*</sup>Model 1: adjusted for age, sex and geographic location.

<sup>†</sup>Model 2: further adjusted for BMI.

<sup>‡</sup>Model 3: further adjusted for education, smoking, alcohol consumption and use of vitamin supplements.