Serum Vitamin D Concentration Does Not Predict Insulin Action or Secretion in European Subjects With the Metabolic Syndrome

Hanne L. Gulseth, md[1,2]
Ingrid M.F. Gjelstad, msc[1,2]
Audrey C. Tierney, phd[3]
Julie A. Lovegrove, phd[4,5]
Catherine Defoort, phd[6]
Ellen E. Blaak, phd[7]
Jose Lopez-Miranda, md, phd[8]
Beata Kiec-Wilk, md[9]
Ulf Risérus, md, phd[10]
Helen M. Roche, phd[3]
Christian A. Drevon, md, phd[2]
Kåre I. Birkeland, md, phd[1]

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) indexes and intravenous glucose tolerance test to calculate acute insulin response, insulin sensitivity, and disposition index. Serum 25(OH)D was measured by high-performance liquid chromatography/mass spectrometry.

RESULTS — The $25(OH)D_3$ concentration was 57.1 ± 26.0 nmol/l (mean ± SD), and only 20% of the subjects had $25(OH)D_3$ levels ≥75 nmol/l. In multiple linear analyses, $25(OH)D_3$ concentrations were not associated with parameters of insulin action or secretion after adjustment for BMI and other covariates.

CONCLUSIONS — In a large sample of subjects with the metabolic syndrome, serum concentrations of $25(OH)D_3$ did not predict insulin action or secretion. Clear evidence that D vitamin status directly influences insulin secretion or action is still lacking.

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Low serum concentrations of 25-hydroxyvitamin D [25(OH)D] have 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 20–40 kg/m^2, recruited for the LIPGENE study (NCT00429195) performed in eight European countries in 2005 and 2006. All subjects had the metabolic syndrome defined by three or more slightly modified National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP)-III criteria (8): levels of fasting plasma glucose >5.5 mmol/l, triglycerides ≥1.5 mmol/l, HDL cholesterol <1.0 mmol/l (males) or <1.3 mmol/l (females), blood pressure ≥130/85 mmHg or on blood pressure–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 blood pressure measurements were recorded according to standard protocols. An insulin-modified IVGTT was performed as described earlier (10). Measures of insulin sensitivity (S_i) were obtained using the MINMOD Millennium 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 to 8 min. Disposition index was calculated as AIR × S_i. HOMA indexes (HOMA2, version 2.2.2 http://www.dtu.ox.ac.uk/index.php?maindoc=/homa) were used to assess insulin resistance (HOMA-IR) and β-cell function (HOMA-β) from fasting blood samples (12). Vitamin $25(OH)D_2$ and $25(OH)D_3$ were analyzed with high-per-

From the [1]Department of Clinical Endocrinology, Oslo University Hospital Aker and Faculty of Medicine, University of Oslo, Oslo, Norway; the [2]Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Olso, Norway; the [3]Nutrigenomics Research Group, UCD Conway Institute, University College Dublin, Dublin, Republic of Ireland; the [4]Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences, University of Reading, Reading, U.K.; the [5]Institute of Cardiovascular and Metabolic Research, University of Reading, Reading, U.K.; [6]Institut National de la Santé et de la Recherche Médicale (INSERM) 476, Human Nutrition and Lipids, University Méditerranée Aix-Marseille 2, Marseille, France; the [7]Department of Human Biology, School for Nutrition, Toxicology and Metabolism (NUTRIM), Maastricht University, Maastricht, the Netherlands; the [8]Lipids and Atherosclerosis Research Unit, Reina Sofía University Hospital, Maimonides Institute for Biomedical Research at Cordoba (IMIBIC), University of Cordoba, Ciber Phyisiopatology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Cordoba, Spain; the [9]Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland; and the [10]Department of Public Health and Caring Sciences/Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden.
Corresponding author: Hanne L. Gulseth, h.l.gulseth@medisin.uio.no.
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Table 1—*Adjusted regression coefficients of 25(OH) vitamin D_3 (nmol/l) with parameters of insulin action and secretion*

	Model 1*			Model 2†			Model 3‡		
	β	SE	*P*	β	SE	*P*	β	SE	*P*
S_i ($mU \cdot l^{-1} \cdot min^{-1}$)	0.005	0.003	0.17	0.003	0.003	0.60	0.002	0.003	0.69
AIR ($mU \cdot l^{-1} \cdot min^{-1}$)	−1.47	0.60	0.041	−1.26	0.60	0.078	−1.20	0.63	0.079
Disposition index	−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
HOMA-β (%)	−0.185	0.067	0.007	−0.128	0.066	0.063	−0.113	0.068	0.070

*Model 1: adjusted for age, sex, and geographic location. †Model 2: further adjusted for BMI. ‡Model 3: further adjusted for education, smoking, alcohol consumption, and use of vitamin supplements.

formance liquid chromatography/mass spectrometry. Only 15 subjects (3%) had measurable concentrations of 25(OH)D_2, mean 10.1 nmol/l, range 6.5–24.6 nmol/l. Including 25(OH)D_2 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_3 with S_i, AIR, disposition index, HOMA-β, 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 — Serum concentration of 25(OH)D_3 was 57.1 ± 26.0 nmol/l (mean ± SD), range 13.7–170.4 nmol/l. Only 91 (20%) subjects had levels ≥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_3 concentration (supplemental Table 1, available in an online-only appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-1692/DC1).

In unadjusted analyses, IVGTT-derived parameters did not differ across tertiles of 25(OH)D_3, whereas fasting insulin, HOMA-IR, and HOMA-β were significantly different (all $P < 0.015$), with higher values among subjects in the lower tertile of 25(OH)D_3 concentration (supplemental Table 2). Serum levels of 25(OH)D_3 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-β ($r = -0.15$, $P = 0.001$), but not with S_i ($r = 0.062$, $P = 0.21$) or disposition index ($r = -0.059$, $P = 0.24$). In a multivariate regression analysis including potential covariates (Table 1), serum 25(OH)D_3 concentration was a statistically significant predictor of HOMA-IR, HOMA-β, and AIR ($P < 0.05$) but not of S_i or disposition index when adjusting for sex, age, and geographic location. After adding BMI to the regression model, neither HOMA indexes nor AIR were significantly associated with 25(OH)D_3 (Table 1).

To further explore these relationships, we compared subjects with a severe biochemical vitamin D deficiency (<25 nmol/l, n = 20) to subjects with sufficient vitamin D status (≥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_3 concentrations. At variance with our findings, Chiu et al. (2) observed a positive association between vitamin D status and insulin sensitivity in 126 glucose-tolerant students investigated by hyperglycemic clamp, 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 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 modeling 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 centers across Europe and limiting the data sampling to 2 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, since 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_3 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.

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References

1. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. Diabetes 2008;57:2619–2625
2. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004;79:820–825
3. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007;30:980–986
4. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2007;92:2017–2029
5. Reis JP, von Mühlen D, Miller ER 3rd. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. Eur J Endocrinol 2008;159:41–48
6. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care 2005;28:2926–2932
7. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. Diabetes Care 2005;28:1228–1230
8. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–2497
9. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936–942
10. Steil GM, Volund A, Kahn SE, Bergman RN. Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model: suitability for use in population studies. Diabetes 1993;42:250–256
11. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol Ther 2003;5:1003–1015
12. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–1495
13. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–281
14. Lu L, Pan A, Hu FB, Franco OH, Li H, Li X, Yang X, Chen Y, Yu Z, Lin X. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes Care 2009;32:1278–1283
15. Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. J Nutr 2009;139:329–334