Serum 25-Hydroxyvitamin D and Adipose Tissue Vitamin D Receptor Gene Expression: Relationship With Obesity and Type 2 Diabetes

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Context: The relationship between 25-hydroxyvitamin D [25(OH)D] and obesity and type 2 diabetes is not completely understood. Vitamin D receptor (VDR) expression in adipose tissue (AT) is related to obesity and might be regulated by 1,25-dihydroxyvitamin D3 [1,25(OH)2D3].

Objective: To analyze serum 25(OH)D and VDR gene expression in AT according to body mass index (BMI) and glycemic status and the effect of 1,25(OH)2D3 on AT according to BMI.

Design and Patients: Two cohorts were studied: 1) 118 subjects classified according to their BMI (lean, overweight, obese, or morbidly obese [MO]) and their glycemic status (normoglycemic [NG] and prediabetic and diabetic [P&D]); and 2) 30 obese subjects (BMI > 30 kg/m²) classified as NG and P&D.

VDR gene expression was analyzed during preadipocyte differentiation and in vitro stimulation with 1,25(OH)2D3 of AT explants from donors with different BMI values.

Setting: University Hospital.

Main Outcome Measures: Serum 25(OH)D, parathyroid hormone (PTH), and AT VDR gene expression.

Results: 25(OH)D levels were lower in P&D than NG subjects, significantly so in the lean and MO groups (P < .05). 25(OH)D levels correlated negatively with homeostasis model of assessment for insulin resistance (HOMA-IR) (r = -0.200; P = .032) and glucose (r = -0.295; P = .001), but not with BMI. VDR gene expression was higher in MO than in the other BMI groups (P < .05). 1,25(OH)2D3 increased VDR gene expression in AT from obese patients (P < .05) but not from lean subjects.

Conclusions: 25(OH)D levels are diminished in P&D compared to NG subjects, independently of BMI, and are closely related to glucose metabolism variables, suggesting that vitamin D deficiency is associated more with carbohydrate metabolism than with obesity. Moreover, AT has a different response to 1,25(OH)2D3 depending on the degree of obesity. (J Clin Endocrinol Metab 100: E0000–E0000, 2015)
Vitamin D (VD) deficiency has been associated with obesity and diabetes (1–4), although some have found no clear relationship between these variables (5, 6). Most studies that have so far examined 25-hydroxyvitamin D [25(OH)D] levels according to body mass index (BMI) failed to consider whether the participants were or were not diabetic (3, 7), which is noteworthy because most obese patients have altered glucose metabolism (8). This should be considered to discern whether VD deficiency is related to obesity by itself or whether it is a consequence of altered carbohydrate metabolism.

25(OH)D is hydroxylated to produce 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$), the biologically active form (9). 1,25(OH)$_2$D$_3$ interacts with the VD receptor (VDR), which acts as a transcription factor (9).

VDR is highly expressed in preadipocytes from obese subjects (10), but it is unknown whether its expression during adipogenesis differs depending on BMI, and although total adipose tissue (AT) gene expression has been related to obesity (10, 11), its possible relation with diabetes has not yet been studied. The relationship between VDR in AT and obesity might be mediated by VD, since previous studies suggested a regulation of VDR gene expression and adipogenesis by 1,25(OH)$_2$D$_3$ (11, 12) but did not analyze whether the effect of 1,25(OH)$_2$D$_3$ on AT differs depending on the degree of obesity.

Thus, the aim of this study was to analyze serum 25(OH)D and VDR gene expression in AT according to a range of BMI values and the glycemic status of the participants. Additionally, we studied the effect of 1,25(OH)$_2$D$_3$ on AT explants according to the degree of obesity.

Subjects and Methods

Subjects

Cohort 1 comprised 118 participants recruited at the University Hospital (Malaga, Spain) classified according to their BMI as morbidly obese (MO; BMI > 40 kg/m$^2$), obese (BMI = 30–40 kg/m$^2$), overweight (BMI = 25–30 kg/m$^2$) or lean (BMI < 25 kg/m$^2$) (13) and to their glycemic profile as normoglycemic (NG) (fasting glucose levels < 100 mg/dL, and homeostasis model of assessment for insulin resistance [HOMA-IR; described below] < 3.5) or prediabetic and diabetic (P&D; fasting glucose levels > 100 mg/dL) (14). Cohort 2 comprised 30 obese patients (BMI = 33.5–58.4 kg/m$^2$) recruited at the Hospital Universitari Dr Josep Trueta (Girona, Spain). The participants gave written informed consent, and the study was reviewed and approved by the Ethics and Research Committee (see Supplemental Data).

Before surgery and after an overnight fast, blood samples were obtained, and serum and plasma were separated for biochemical determinations. Visceral AT (VAT), used to study gene expression and perform AT explant cultures, was obtained during bariatric surgery in the MO patients or during hiatal hernia surgery or cholecystectomy in the lean, overweight, or obese subjects from cohort 1. For cohort 2, VAT and sc AT (SAT), used to study gene expression, were obtained during elective surgical procedures (cholecystectomy, abdominal hernia, or gastric bypass). The AT samples were washed in physiological saline, immediately frozen in liquid nitrogen, and maintained at −80°C until analysis.

Laboratory measurements

Plasma glucose, cholesterol, triglycerides, and high-density lipoprotein cholesterol were measured in a Dimension autoanalyzer (Dade Behring Inc) by enzymatic methods (Randox Laboratories Ltd); insulin was measured by RIA (BioSource International); and leptin and adiponectin were measured by ELISA (DSL and DRG Diagnostics, respectively). Low-density lipoprotein cholesterol was calculated from the Friedewald equation (13), and the HOMA-IR was calculated as follows: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 (8, 13). Serum 25(OH)D and PTH levels from cohort 1 were determined by ELISA (Immundiagnostik and DRG Diagnostics, respectively) and for cohort 2 by electrochemiluminescence immunoassay (Modular Analytics E170; Roche Diagnostics).

Human preadipocyte differentiation

Visceral and sc human preadipocytes isolated from both obese (BMI > 30 kg/m$^2$) and lean (BMI < 25 kg/m$^2$) donors were purchased from Zen-Bio Inc and cultured (~40 000 cells/cm$^2$) as described in the Supplemental Data.

AT explant culture

VAT from three NG healthy MO donors and three NG healthy lean donors was cut into 5- to 10-mg pieces and treated as described in detail in the Supplemental Data.

RNA isolation and real-time quantitative PCR

Total RNA isolation and cDNA synthesis were performed as described (13). Gene expression was assessed by real-time PCR using an Applied Biosystems 7500 Fast Real-Time PCR (see Supplemental Data).

Statistical analysis

The sample size was determined with the ENE 3.0 statistical program (GlaxoSmithKline). To detect differences for 25(OH)D concentrations of 25%, at least 12 subjects per group were required for the study (α = 0.05; power = 0.8). Comparisons between the study groups were made with ANOVA and Duncan’s post hoc tests in cohort 1. Student’s t test was used for comparisons between the groups in cohort 2 and for in vitro analysis. Pearson’s (cohort 1) or Spearman’s (cohort 2) correlation analyses were used to study variable associations. Statistical significance was set at P < .05. Analyses were performed with SPSS 15.0 (SPSS Iberica).

Results

The biochemical and anthropometric characteristics of each study group are summarized in Table 1.

Serum 25(OH)D levels were significantly higher in NG than in P&D in lean and MO subjects. Significant differ-
toward a negative correlation between serum 25(OH)D levels positively with BMI (r = 0.146; P = .001) and tended to correlate with insulin levels (r = 0.463; P = .015).

In vitro experiments showed that VDR gene expression was significantly higher in visceral preadipocytes from obese subjects than from lean subjects during the differentiation process, but no differences were found during sc preadipocyte differentiation (Figure 1E and F).

Moreover, 1,25(OH)2D3 stimulation significantly increased VDR gene expression in AT explants from MO subjects. However, no effect on VDR mRNA levels was observed in AT explants from lean donors (Figure 1D).

**Discussion**

The results of this study show that low 25(OH)D levels are associated with diabetes, independently of BMI. We also found that AT VDR gene expression is higher in MO patients compared to subjects with a lower BMI and explored for the first time its relationship with glucose metabolism. Our results also suggest that AT from MO subjects has a different response to 1,25(OH)2D3 compared to AT from lean subjects.

Recent years have seen increasing studies concerning the association between 25(OH)D levels and both obesity and diabetes, with these conditions found to lead to lower 25(OH)D levels (2–4). Nevertheless, the results are sometimes contradictory, and the role of VD in the development of obesity is still not completely understood (1, 5). A number of confounding factors, such as ethnicity, nutritional...
status, or sun exposure, have been proposed to be involved in these inconsistent results (15). Furthermore, obesity is a major risk factor for diabetes (16). In fact, obesity together with VD insufficiency interact synergistically to influence the risk of insulin resistance (2, 16, 17). Despite this, most studies dealing with BMI and VD compared healthy lean subjects with obese patients displaying an impaired glucose metabolism or omitted data about this aspect, which makes it difficult to discern whether the low 25(OH)D levels were really associated with obesity or rather with glycemic status (3, 7).

The major strength of this study is that it is the first cross-sectional study to compare serum 25(OH)D simultaneously between a wide range of BMI values taking into account whether the subjects were P&D or NG. This approach, avoiding the interaction between obesity and diabetes, showed that the P&D patients had the lowest serum 25(OH)D levels, and most interestingly, there were no differences between BMI groups with the same glycemic status. Our results agree with previous studies finding an independent association between VD deficiency and diabetes or prediabetes after adjusting for BMI (4, 16, 18). This association is supported by biological evidence showing that VD influences pancreatic β-cell function directly by binding to VDR or indirectly by the role of VD in regulating extracellular calcium level and calcium flux through the β-cell (4).

Recent studies have shown a role for AT in VD metabolism. Reciprocally, VD has multiple effects on the physiology of adipocytes and AT (9–11, 19). Animal studies support this hypothesis that VD and VDR might be involved in the development of obesity and diabetes (19).

Human studies have shown higher mRNA VDR levels and lower mRNA levels of genes involved in lipid processing in the AT of MO patients compared to lean subjects (11, 13). In agreement with these studies, we found an inverse relationship between VDR and genes involved in AT physiology. Furthermore, we studied for the first time whether VDR gene expression in AT was related to diabetes, finding a trend toward a higher VDR gene expression in P&D compared to NG subjects.

In vitro studies have shown that 1,25(OH)2D3 influences VDR gene expression and adipogenesis (14). However, no previous studies have analyzed whether the effect of 1,25(OH)2D3 on AT differs according to BMI. Our results showed that VDR gene expression during adipogenesis is higher in visceral preadipocytes from obese than from lean subjects, which agrees with the higher VDR mRNA levels in AT from MO subjects. Furthermore, we demonstrated that the previously described up-regulation of AT VDR expression induced by 1,25(OH)2D3 (10, 12) only happens in obesity, with no response in lean subjects. This agrees with an intervention study with nonobese healthy subjects showing that VD supplementation had no effect on the expression of genes related to fat metabolism in AT (20).

Further studies will be necessary to understand the physiological consequences of the different AT response to 1,25(OH)2D3, depending on the degree of obesity and its relevance in clinical practice, as well as to confirm the role of VDR in diabetes.

**Figure 1.** A and B, Serum 25(OH)D (A) and PTH (B) levels in the study groups from cohort 1. C, VDR mRNA expression in the study groups from cohort 1. D, VDR mRNA expression in visceral AT explants cultured with a range of 1,25(OH)2D3 concentrations or vehicle (control) from morbidly obese subjects (MO) and lean subjects. E and F, VDR mRNA expression in sc (E) and visceral (F) human preadipocytes during adipogenic differentiation. Values are expressed as means ± SD. White circles, BMI < 25 kg/m²; black squares, BMI > 30 kg/m². Groups not sharing any superscript letters are significantly different (P < .05) according to ANOVA (Duncan’s post hoc test). *, P < .05 between different BMI. #, P < .05 compared to control.
A limitation of this study was that P&D patients were classified in the same group. This was due to the notable difficulty in finding lean diabetic subjects. Moreover, the cross-sectional nature of the study did not allow establishing a temporal association or causality.

In conclusion, 25(OH)D levels showed a close relationship with variables related to glucose metabolism, suggesting that VD deficiency is associated more with carbohydrate metabolism than with obesity. Additionally, AT VDR gene expression might also be related to glucose metabolism disorders, and AT has a different response to 1,25(OH)_{2}D_{3} depending on the degree of obesity.

**Acknowledgments**

The authors thank all the subjects for their collaboration and Fundación Pública Andaluza para la Investigación de Málaga en Biomedicina y Salud (FIMABIS). We also gratefully acknowledge the help of Ian Johnstone for his expertise in preparing this manuscript, and Juan Alcaide-Torres, Instituto de Investigación Biomédica de Málaga, for his technical contribution.

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This study was supported by Centros de Investigación Biomédica En Red (CB06/03/0018) of the Instituto de Salud Carlos III (ISCIII) and Grants PI11/01661, PI08/1655, and PI12/02355 from the ISCIII, Madrid, Spain. M.C.-P. was the recipient of FPU (Formación de Profesorado Universitario) Grant AP2009-4537 from the Education Ministry, Madrid, Spain. L.G.-S. was supported by “Miguel Servet Type I” (CP13/00188), and F.C. by “Miguel Servet Type II” program (CP13/00023) from the ISCIII, Madrid, Spain. M.M.-G. was the recipient of the Nicolas Monarde program from the Servicio Andaluza de Salud, Junta de Andalucía, Spain (C-0029-2014).

Disclosure Summary: The authors have nothing to disclose.

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