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Low circulating vitamin D levels are associated with increased arterial stiffness in prediabetic subjects identified according to HbA_{1c}

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ABSTRACT

Background and aims: We investigated serum -hydroxyvitamin D levels [25(OH)D] and their correlation with early markers of cardiovascular disease in subjects with pre-diabetes. We particularly focused on individuals identified only by glycated hemoglobin A_{1c} (HbA_{1c} 5.7–6.4%) according to the American Diabetes Association criteria but who were normotolerant (NT) after oral glucose tolerance test (OGTT) and had normal fasting glucose (NFG).

Methods: 25(OH)D levels, HbA_{1c}, OGTT, arterial stiffness and intima-media thickness (IMT) were evaluated in 286 subjects without history of diabetes. Subjects were stratified into four groups: controls with HbA_{1c} <5.7%, NFG and NT; prediabetic patients with pre-diabetes according to only HbA_{1c} (HbA_{1c} 5.7–6.4% and NFG/NT); subjects with impaired fasting glucose and impaired glucose tolerance (IFG/IGT); new onset type 2 diabetes ($HbA_{1c} \geq 6.5\%$).

Results: Subjects with NFG/NT and HbA_{1c} 5.7–6.4% (n = 83) showed lower 25(OH)D levels compared with controls (n = 80) (21.7 [15.8–31.1] vs 23.1 [17.1–29.7] ng/mL, $P = 0.009$); these values were similar to those of the IFG/IGT group and were higher but not significantly different from subjects with new onset type 2 diabetes. After multiple regression analyses, only HbA_{1c} and BMI were independently associated with 25(OH)D levels. Age, HbA_{1c} and 25(OH)D were the major determinants of Augmentation Index. No independent association between 25(OH)D and IMT was found.

Conclusions: Subjects with pre-diabetes (HbA_{1c} 5.7–6.4% and NFG/NT) had significantly reduced 25(OH)D levels compared with controls. Reduction of 25(OH)D levels is inversely associated with arterial stiffness independently of classical risk factors and inflammatory markers. Based on these data, subjects with NFG and NT are not a homogeneous population of patients, and they present different cardiovascular and glycometabolic risks. Our data suggest considering HbA_{1c} as a reliable marker of cardiovascular and metabolic risk independent of fasting and post-load glycemia.

Pre-diabetes is prevalent in the general population, and those affected are at high risk of progression to overt diabetes and cardiovascular disease [1,2].

In 2011, the American Diabetes Association (ADA) recognized a new method to identify prediabetic subjects in addition to impaired fasting glucose (IFG) and impaired glucose tolerance (IGT): a glycated hemoglobin (HbA_{1c}) value between 5.7 and 6.4% [1].

The clinical relevance of HbA_{1c} and its agreement with fasting plasma glucose and 2-h glucose post-OGTT for the diagnosis of pre-diabetes remain controversial. In a recent study, we analyzed the cardiovascular risk profile in subjects with pre-diabetes identified according to HbA_{1c} (5.7–6.4%), normal fasting glycemia and normal glucose tolerance (NFG/NT) after an oral glucose tolerance test (OGTT). These subjects would not have been classified as prediabetic on the basis of fasting or post-OGTT values.

We found that these subjects with pre-diabetes identified by only HbA_{1c} presented with increased arterial stiffness and carotid intima-media thickness (IMT) in a similar manner to those with new onset type 2 diabetes [3].

The pathogenic mechanism leading to vascular damage in this

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population is unknown. To further characterize the cardiovascular risk profile of these subjects, we decided to study their vitamin D levels.

Growing evidence indicates that suboptimal vitamin D levels play a role in the development of various clinical conditions, including cardiovascular disease [4–6]. A regulatory role for vitamin D in the cardiovascular system has been clearly demonstrated in animal studies examining activation of the systemic and local cardiac renin-angiotensin system. A study in knockout mice confirmed that the absence of vitamin D receptor activation leads to tonic up-regulation of the renin-angiotensin system, with the development of hypertension and left ventricular hypertrophy [7,8].

A strong association of vitamin D deficiency with increased all-cause and cardiovascular mortality has been described in the general population [9]. Moreover, suboptimal vitamin D status appears to be involved in impaired glucose homeostasis, insulin resistance and obesity, and this condition may predispose people to type 2 diabetes. Clinical cross-sectional studies have shown a significant inverse relationship between HbA_{1c} and serum 25(OH)D levels in diabetic subjects [10]; furthermore, other studies have observed low serum 25(OH)D levels in individuals with pre-diabetes identified according to ADA recommendations (IFG, IGT and HbA_{1c} 5.7–6.4%) [2]. However, recent literature has indicated that the association between 25(OH)D with cardiovascular and metabolic disease could be weaker in special populations [11,12]. Then, the exact role of vitamin D in these diseases needs to be clarified in randomized clinical trials.

The purpose of this study was to evaluate the levels of vitamin D in patients with pre-diabetes, particularly in those identified by HbA_{1c} with NFG and NT after OGTT, and whether these values are associated with alterations of arterial stiffness and IMT, early markers of atherosclerosis.

1. Research design and methods

1.1. Study subjects

Subjects (n = 286, age range of 18–65 years) with no previous diagnosis of diabetes were consecutively recruited from patients attending our University Hospital for diabetes and cardiovascular risk evaluation during the winter months (November–March). The exclusion criteria were the following: a previous history of diabetes; previous history of overt cardiovascular events (atrial fibrillation, stroke, ischemic heart disease, chronic obstructive peripheral arteriopathy, or heart failure); primary hyperparathyroidism; clinical evidence of advanced liver or renal disease; anemia or hemoglobinopathies; use of medications known to affect glucose metabolism; positivity for antibodies to hepatitis C virus or hepatitis B surface antigen; chronic gastrointestinal diseases associated with malabsorption or chronic pancreatitis; rheumatic diseases; and/or recent history of acute illness, malignant disease, and drug or alcohol abuse. All patients were Caucasian and underwent a physical examination and review of their clinical history and alcohol consumption. Smoking status was assessed in all the patients. During the visit we ask the number of cigarettes and years smoked in order to obtain a categorical variable (active smokers or nonsmokers). Those who reported smoking cigarettes regularly during the year before the exam were considered active smokers. None were taking calcium or vitamin D supplements.

Body weight and height were measured, and BMI was calculated as weight (kg)/[height (m)]^2. Waist circumference was measured in a standing position at the level of the umbilicus. Blood pressure (BP) was measured with a calibrated sphygmomanometer after the subject had rested in the supine position for 10 min. Venous blood samples were drawn from the antecubital vein on the morning after an overnight fast. All subjects underwent a 75-g OGTT with 0-, 30-, 60-, 90- and 120-min sampling for plasma and insulin as previously described [13]. Glucose tolerance status was definited on the basis of OGTT according to ADA recommendations [1].

The study was approved by the local ethics committee. Informed consent was obtained from each participant.

1.2. Biochemical analyses

Plasma glucose, serum total cholesterol, triglycerides, HDL cholesterol, and hs-CRP were measured using available enzymatic methods as previously described [14]. LDL cholesterol concentrations were estimated using the Friedewald formula. Serum calcium and phosphorus levels were measured in all subjects.

Serum 25(OH)D was measured using a chemiluminescent microparticle immunoassay; the interassay and intra-assay coefficient of variation were $\leq$10% (ARCHITECT; ABBOTT, Wiesbaden, Germany). PTH was measured using a chemiluminescent assay (PTH LIAISON N-TACT; DiaSorin, Saluggia [VC]). Estimated glomerular filtration rate (e-GFR) was calculated with the Cockcroft–Gault formula.

HbA_{1c} was measured via high-performance liquid chromatography using a National Glycohemoglobin Standardization Program and standardized to the Diabetes Control and Complications Trial (DCCT) assay reference [15]. Chromatography was performed using a certified automated analyzer (HPLC; HLC-723G7 hemoglobin HPLC analyzer; Tosoh Corp.) (normal range 4.25–5.9% [23–41 mmol/mol]; intra- and inter-assay CVs were 1.7% and 2.6%, respectively).

1.3. Pulse wave analysis

Evaluation of arterial stiffness was performed with the patients in fasting status and explicitly expressed the recommendation to avoid coffee intake in the morning of the procedure. All measurements were made from the right radial artery by applanation tonometry using a Millar tonometer (SPC-301; Millar Instruments, Houston, TX) [16]. The measurements were performed by a single investigator with the subject in the supine position. The data were collected directly with a desktop computer and processed with SphygmoCorCvMS (AtCor Medical, Sydney, Australia). The aortic waveform in pulse wave analysis was subjected to analysis for the calculation of the aortic Aug and AugI (calculated by dividing augmentation by pulse pressure). Pulse pressure was calculated as the difference between the systolic and diastolic BPs.

1.4. Carotid ultrasound examinations

Ultrasound scans were performed using a high-resolution B-mode ultrasound system (MyLab 50 XVision; Esaote Biomedica SpA, Florence, Italy) equipped with a 7.5-MHz linear array transducer. All ultrasound examinations were performed by a single physician who was blinded to the clinical and laboratory characteristics of the patients. Scans were performed and measurements were conducted at a total of six plaque-free sites 1 cm proximal to the carotid bulb. The obtained values were averaged and are presented as the means of the IMT of the common carotid artery. All measurements were obtained in diastole, assessed as the phase in which the lumen diameter is at its smallest and the IMT is at its largest.

1.5. Statistical analyses

We based the power calculation on previous studies examining