Supplementary Appendix

Materials and methods

Exclusion criteria

Exclusion criteria included the following: i) known history of rickets, osteopenia and osteoporosis; ii) history of disorders known to affect bone mineral density (BMD) and bone metabolism such as collagen metabolism disorders, chronic kidney disease, chronic liver disease, malabsorption syndromes, Crohn's disease, inflammatory bowel disease, Cushing's syndrome, defects in the growth hormone (GH)-insulin-like growth factor 1 (IGF-1) axis, thyrotoxicosis and hyperthyroidism, hyperparathyroidism, hypogonadism, hematologic disorders; iii) presence of autoimmune comorbidities and/or inflammatory disorders such as celiac disease (the latter defined as anti-tissue transglutaminase antibody positivity); iv) history or recent diagnosis of malignancy; v) presence of an allograft and/or use of corticosteroids, anticonvulsants or immunosuppressive drugs. The aforementioned exclusion criteria were selected to avoid the influence of confounding factors on dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) measurements.

Biochemical and hormonal assessment: blood assays

Serum levels of calcium, phosphate and 25-hydroxyvitamin D [25(OH)D] and plasma levels of intact parathyroid hormone (PTH) through the intact PTH assay (which detects both 1-84 PTH and 7-84 PTH fragments) were also measured. Serum calcium (reference range: 2.1-2.5 mmol/L), serum phosphate (reference range: 0.9-1.8 mmol/L), serum total alkaline phosphatase (ALP; reference range: males, 40-129 U/L; females, 35-104 U/L) and fasting plasma glucose (FPG) were measured using standard colorimetric and enzymatic methods via Cobas C 501 analyzer with reagents supplied by Roche Diagnostics GmbH (Mannheim, Germany). Inter-assay coefficient of variation (CV) was 0.9% at serum calcium levels of 2.5 mmol/L, 1.4% at serum phosphate levels of 1.5 mmol/L, 2.1% at serum total ALP levels of 82 U/L, and 1.1% at FPG levels of 95.5 mg/dL. Glycated hemoglobin (HbA1c) was measured by capillary electrophoresis through the Capillarys 2 Flex Piercing analyzer supplied by Sebia (Lisses, France). The CV range for the HbA1c assay was 1.1%-2.6% at a HbA1c value of 5.0%. Fasting plasma C-peptide (FCP) levels (reference range: 370-1470 pmol/L) and intact PTH (reference range: 15-65 pg/mL), and serum levels of total 25(OH)D (reference range: ≥30 ng/mL, indicative of vitamin D sufficiency), osteocalcin (reference

range: premenopausal females, 11-43 ng/mL; males, 14-42 ng/mL) and Beta-CrossLaps (Beta-CTx; reference range: ≤18 years of age, 0.10-0.58 ng/mL) were measured via a Cobas E 601 analyzer using competitive and sandwich immunological methods, with reagents supplied by Roche Diagnostics GmbH (Mannheim, Germany). The inter-assay CV for C-peptide assay was 1.6% at a FCP level of 615 pmol/L, whereas the inter-assay CV for 25(OH)D was 4.9% at a 25(OH)D level of 24.9 ng/mL. Inter-assay CV for plasma intact PTH and serum osteocalcin were 2.7% at a plasma intact PTH level of 35.9 pmol/L and 2% at a serum osteocalcin level of 34.5 ng/mL, respectively. Inter-assay CV for serum Beta-CTx was 1.8% at a serum Beta-CTx level of 0.5 ng/mL. Islet autoantibody positivity was assessed by measuring insulin autoantibodies (IAAs), glutamic acid decarboxylase autoantibodies (GADA), islet tyrosine phosphatase-like protein IA2-antibodies (IA-2A), or zinc transporter 8 autoantibodies (ZnT8A) through radioimmunoassay methodology. Single islet autoantibody positivity was defined as the positivity for only one of the measured autoantibodies, whereas multiple islet autoantibody positivity was defined as the positivity for two or more islet autoantibodies.

Bone diagnostic tools

Bone densitometry: lumbar spine DXA analysis

The CV for Z-score values (indicative of the intra-individual precision of the measurements) was 1.1% at a lumbar spine BMD value of 1.0%-1.2%.

Phalangeal QUS measurements

The calculated CV for QUS measurements was equal to 0.64%. We calculated the main QUS parameters measured by the phalangeal QUS devices, namely:

- Amplitude-dependent speed of sound (AD-SoS, expressed in meters per second or m/s): it reflects the amplitude-dependent velocity with a threshold of 2 mV [1]. AD-SoS is a partly amplitude-dependent QUS variable deriving from the measurement of the interval between the start time of the transmitted signal and the time the signal received achieves the predetermined minimum amplitude value of 2 mV for the first time [2,3]. AD-SoS reflects the ultrasound velocity inside the bone.
- Bone transmission time (BTT, expressed in microseconds or ms): it is the difference between the time when the first peak of the signal received reaches its maximum level and the time that would be measured if no bone but only soft tissue is present between the two transducers; thus, BTT, unlike AD-SoS, reflects the bone properties independent of the confounding effect of ultrasound attenuation and soft tissue bias

[1,2,4], but it depends only on bone properties and it has been proven comparable to DXA parameters in the prediction of fracture risk in men [5].

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