osteoblasts and incorporated into the bone matrix during bone formation and also released into the circulation from the matrix during bone resorption, is considered a marker of bone turnover. Its main advantages are tissue-specificity and relatively low within-individual variation, while its principal disadvantage concerns the instability of the intact molecule, which is rapidly degraded. The large N-terminal/midregion fragment (N-Mid, aa 1-43) appears to be considerably more stable.24 The C-terminal crosslinked telopeptide of type I collagen (CTX) is the bone resorption marker of choice, recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).25

β-CTX levels are indicative of the degree of the breakdown of mature type I collagen and are the preferred marker for monitoring the start of antiresorptive therapy. The main disadvantage stems from its large circadian variation, necessitating a morning fasting sample for accurate interpretation of results. The urine free deoxypyridinoline cross-links (Dpd), released when mature type I collagen is degraded, are tissue-specific and not influenced by the diet. The main disadvantage is the necessity of a 24-hour urine sample and the need to correct results for creatinine excretion.

As a general procedure, a blood sample was collected from each subject; the blood was allowed to clot and serum was separated by centrifugation, with the exception of HbA1c which was determined in whole blood. Testing was performed on the day of blood collection for routine biochemistry, HbA1c, CRP and hormonal measurements. Serum and 24-hour urine samples for 25-(OH)D and bone turnover biomarkers measurements were frozen at -20°C after collection and tested later, as assays for these parameters are performed on a weekly basis. All assays were performed according to the manufacturers’ instructions.

### Validation of the serum total 25-(OH)D assay used in our study

Prior to analysis, we confirmed that our assay actually measured the total amount of 25-(OH)D. For this purpose, we collected and pooled samples in which measured values were >30 ng/mL. One volume of saturated trichloroacetic acid (TCA) was added to four volumes of sample and the resulting mixture was vortexed and centrifuged. The addition of TCA causes protein precipitation and release of any bound (micro)molecules, including 25-(OH)D. The resulting supernatant was buffered with the 25-(OH)D assay buffer (200 μL supernatant plus 500