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Normocalcemic Hypoparathyroidism: Prevalence and Effect on Bone Status in Older Women. The OPUS Study.

Abbreviated title: normocalcemic hypoparathyroidism in older women.

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Abstract

Objective: There are no consistent data on the prevalence and bone status for normocalcemic hypoparathyroidism (NHYP) as defined by normal adjusted calcium and low PTH level. Our aim is to determine the prevalence and the bone metabolic profile of NHYP in older women assessing its evolution over the time. The second objective is to evaluate the prevalence of the other calcium metabolic disorders.

Design: The Osteoporosis and Ultrasound Study (OPUS) is a 6-yr prospective study of fracture-related factors.

Participants: a total of 2419 older women (ages 55-79) and 258 younger women (ages 30-40) participated. Complete follow-up data are available in 1416 subjects.

Measurements: After calculating the adjusted calcium according the James's formula, we identified the 'abnormal' calcium and PTH using Mahalanobis Distances and we allocated older women into different pathological categories using reference intervals from the healthy young women.

Results: We identified 57 subjects with NHYP (2.4%). These women have lower than expected bone turnover as assessed by bone alkaline phosphatase (-14.5%, 95% CI: -26.2 to -3.0, $p=0.007$), CTX (-66.3%, 95% CI: -74.0 to -56.4, $p<0.001$) and osteocalcin (-36.8%, 95% CI: -45.6 to -26.6, $p<0.001$). After 6 years, of the 35 NHYP subjects with follow-up data, none developed overt hypoparathyroidism and only 15 (0.6%) subjects had persistent evidence of NHYP. We also identified 86 subjects (3.6%) affected by hyperparathyroid hypercalcemia.

Conclusion: This is the first large population-based study that has investigated NHYP in older women. It is fairly common, not always persistent and is characterized by low bone turnover.

Background

Parathyroid hormone (PTH) is the major hormonal mediator of extracellular calcium homeostasis and regulates osteoclastic bone resorption, the renal excretion of calcium and synthesis of 1,25-dihydroxyvitamin D. Circulating PTH concentrations display a large inter-individual variability and this variance has been demonstrated to have a strong heritable component (1). However, the genetic factors governing serum PTH concentrations remain to be elucidated. PTH is an 84 amino-acid peptide, whose secretion by the parathyroid chief cells is regulated by the calcium-sensing receptor (CaSR), which is expressed at the parathyroid cell-surface. Mutations of the CaSR gene lead to inherited forms of hypercalcaemia and hypocalcaemia (2), and common coding region CaSR single nucleotide polymorphisms (SNPs) have been revealed as determinants of serum calcium concentrations (3). Cusano and colleagues recently reported the prevalence of normocalcemic hyperparathyroidism (NPHPT) and hypoparathyroidism (NHYP) in two unselected, non-referral community-dwelling populations identifying a prevalence of 0.4 - 3.1% and 1.1 - 1.9% respectively (4). NPHPT is characterized by normal calcium levels with high PTH in the absence of secondary causes of hyperparathyroidism (5) and it was officially recognized by the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism (6). These patients may develop a similar rate of low bone mineral density compared to subjects with primary hyperparathyroidism (PHPT) (7) and it seems that there is a trend toward a higher recurrence of NPHPT after parathyroidectomy (8).

While NPHPT is a well-documented diagnostic category, no consistent data are available for NHYP. The diagnosis of NHYP has been used for patients who develop hypocalcaemia in response to bisphosphonate therapy having had normal calcium values and low PTH levels prior to starting therapy; such patients were considered to have inadequate parathyroid gland reserve (9). We cannot yet be certain whether NHYP is a real diagnostic category. Indeed Cusano and colleagues identified 68 subjects with NHYP, none of whom developed overt hypoparathyroidism on follow-up and persistent disease was noted in 2 of 26 subjects that concluded the follow-up period (4).

It is possible that the prevalence of NHYP by Cusano and colleagues (4) may have underestimated the prevalence in the general population because they only studied men and young women. It is important to study older women as many parathyroid diseases such as PHPT have their peak incidence in the first decade after the menopause (10-12).

The primary end point of this study is to determine the prevalence and the bone metabolic profile of NHYP0 in our population assessing its evolution over the time. The secondary endpoint is to evaluate the prevalence of the other calcium metabolic disorders.

Materials and methods

Study design and population

We recruited 2419 older women (ages 55-79) and 258 younger women (ages 30-40) from 5 European cities (the OPUS study) (13). The OPUS study is a large population-based cohort study designed to determine quantitative ultrasound (QUS) performance in direct comparison with central DXA. The design of the Osteoporosis and Ultrasound (OPUS) study has previously been reported (13). In particular exclusion criteria were limited to disorders that precluded valid QUS measurements (i.e., bilateral fractures of the calcaneus, bilateral hip prostheses, disorder of the hand), general inability to undergo the specified exams, and cognitive limitations that preclude filling out self-administered questionnaires. Pregnant women were excluded because of potential risks associated with X-ray exposure. All investigations were conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local ethics committees and written informed consent was obtained from each subject.

The OPUS study became a longitudinal study when laboratory and other data were collected approximately 6 years later and complete follow-up data are available in 1416 subjects. Each subject had a first visit (between 1999-2001) and then 6 years later was invited to attend for a second visit (between 2005-2007). At the time of each study visit a modified version of the European Vertebral Osteoporosis Study (EVOS) risk factor questionnaire (14) was administered to each subject. From this we were able to collect medical and lifestyle information. Medical history of diseases and treatments was recorded. Non-fasting venous blood samples were collected from each subject between 12:00 and 15:00 into serum separating tubes. The blood was left to clot for 30 minutes at room temperature and centrifuged at 2500 g for 10 minutes. The serum was then collected and stored at -80°C until analysis. Second morning void urine samples were collected and stored at -20°C until analysis.

To estimate reference intervals for adjusted calcium and PTH, 107 young women were eligible after excluding those with low 25-hydroxyvitamin D (25OHD) (< 20 ng/ml), eGFR < 60 ml/min per 1.73m², T-score at lumbar spine or total hip equal to or less than -2.5 and those taking drugs or suffering

from diseases known to affect bone (Table 1). Both adjusted serum calcium and PTH were log₁₀ transformed prior to analysis.

Biochemical measurements

Blood samples were drawn to measure serum:

- Calcium and albumin. They were measured using the Cobas c701 (Roche Diagnostics, Germany) auto analyser in the Chemical Pathology laboratory, Sheffield Teaching Hospitals, UK. The manufacturer's reported inter assay precision is <2.0% for each test. We calculated adjusted calcium in all subjects (pre- and post-menopausal women were included in the analysis) based on the total calcium and albumin measurements according to James's formula (15) and (as recommended in that report) we excluded subjects with creatinine > 200 and/or albumin <20 g/L or 50 g/L > and/or total calcium >3 mmol/L. In particular, at baseline, 2638 women were included in the analysis and the local adjustment equation was expressed by the following relationship: adjusted Calcium = Total Calcium - (0.015 x albumin) + 0.699. After six years follow up (1652 women were included in the analysis), the local adjustment equation was expressed by the following relationship: adjusted Calcium = Total Calcium - (0.018 x albumin) + 1.581

- Collagen type 1 cross-linked C-telopeptide (CTX), intact procollagen type 1 N propeptide (PINP), bone alkaline phosphatase (bone ALP), 25OHD and PTHi. They were measured in serum using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, United Kingdom). 185 The inter assay coefficients of variation (CV) were 6.5%, 7.2%, 3.5%, 6.7% and 6.5% respectively.

- Creatinine. It was measured using the Cobas c 311 automated analyser (Roche Diagnostics, Germany). This was used to calculate the estimated glomerular filtration rate (eGFR) using the formula based on the modification of diet and renal disease (MDRD) (16)

We measured the samples from baseline and 6 years at the same time.

Bone densitometry

At both visits, bone mineral density (BMD) was performed using dual-energy X-ray absorptiometry (DXA) of the lumbar spine and the proximal femur in posteroanterior projection (Hologic QDR-4500; Hologic, Bedford, MA, USA in the Kiel, Paris, and Sheffield centers) or in anterior-posterior projection

(Lunar Expert devices; GE Lunar, Madison, WI, USA in the Aberdeen and Berlin centers).

Measurements were standardised and cross calibrated across centres.

Statistical analysis

To classify the abnormalities of calcium homeostasis, we used the following approach:

-We calculated adjusted calcium in all subjects based on the total calcium and albumin measurements (see above);

- Reference intervals were calculated for adjusted serum calcium and PTH using data from the healthy pre-menopausal women. Both adjusted serum calcium and PTH were log₁₀ transformed prior to analysis and the mean +/- 1.96SD calculated (Table 1).

-We allocated older women (baseline) into one of eight categories by using the ellipse defined by the Mahalanobis Distance Analysis (Figure 1) which measure how far each observation is from the center of a data cluster, taking into account the shape of the cluster. Observations are considered outliers if $MD^2 > \chi^2_{2;0.975} = 7.378$. We refer to 'high or low' if they are outside the ellipse and have values above or below those found in the young women. We refer to 'high or low normal' if they are outside the ellipse and have values above or below the mean found in the young women but within the reference interval.

- Normal. Anyone inside the ellipse, at baseline;
- Hyperparathyroid hypercalcemia (HH) that includes both PHPT and familial hypocalciuric hypercalcemia (FHH). Anyone outside the ellipse with high adjusted calcium and high or high normal PTH;
- NPHPT. Anyone outside the ellipse with normal adjusted calcium, high PTH, 25OHD > 20ng/ml and eGFR > 60 ml/min.
- Secondary hyperparathyroidism. Anyone outside the ellipse with low adjusted calcium and high-normal PTH, low adjusted calcium and high PTH, low-normal adjusted calcium and high PTH, high-normal adjusted calcium and high PTH with 25OHD < 20ng/ml or/and eGFR < 60 ml/min;
- Hypoparathyroidism. Anyone outside the ellipse with low adjusted calcium and low or low normal PTH;
- NHYPO. Anyone outside the ellipse with normal adjusted calcium and low PTH;

- Non-PTH hypercalcaemia. Anyone outside the ellipse with high adjusted calcium with low or low normal PTH;
- Missing data patients. Anyone with missing calcium, PTH, or albumin measurements.

We applied the same Mahalanobis Distance Analysis for the older women at follow up (figure 2). In this analysis subjects were included if they had measurements for PTH, calcium and albumin at baseline and follow-up (N = 1416).

Descriptive statistics

Characteristics of the subjects were summarized using frequencies and percentages for categorical variables. For continuous variables, mean and standard deviation were calculated. Characteristics were compared between groups using ANOVA. Post-hoc tests compared all groups to the Normal group using the Dunnett method. All measurements with a skewed distribution were log₁₀ transformed prior to analysis and differences between groups were back transformed and expressed as a percentage difference. We have performed a multiple regression analyses looking at the relationship between measurements at “baseline” and BMD T-score at baseline; measurements at baseline and change in BMD from baseline 238 to year 6. We have examined the change in PTH measurement from baseline to 6 years in the overall population using a paired t-test. The α -level was set at 0.05.

Results

Subject characteristics

The subject characteristics at baseline are shown in table 2a, table 2b.

Diagnostic categories according to calcium metabolism disorders at baseline

We have identified (Tables 3 and 4):

- 2063 subjects (85.3%) with no calcium abnormalities: normal adjusted calcium levels (2.4 mmol/L, 95% CI 2.39 - 2.41) with normal PTH (39 ng/L, 95% CI 38.4 – 39.7);
- 86 subjects (3.6%) affected by HH: high calcium levels (2.79 mmol/L, 95% CI 2.78 – 2.80) with elevated PTH (58.80 ng/L, 95% CI 52.6 – 65.8). At the second visit (after 6 years), of the 56 HH subjects with follow-up data, 47 (2%) subjects had persistent evidence of HH.

- 1 subject (0.1%) affected by NPHPT. At the second visit no subjects met the diagnostic criteria for the normocalcemic hyperparathyroidism.
- 69 subjects (2.8%) subjects affected by secondary hyperparathyroidism: low calcium (2.16 mmol/L, 95% CI 2.09 – 2.23) with high or high normal PTH (88.9 ng/L, 95% CI 75.8 – 104.3).
- 3 subjects (0.1%) affected by hypoparathyroidism;
- 57 subjects (2.4%) affected by NHYPO: normal calcium (2.39 mmol/L, 95% CI 2.36 – 2.42) with low PTH (10.1 ng/L, 95% CI 9.2 – 11.1).
- 12 subjects (0.5%) affected by non-PTH hypercalcaemia: high calcium (2.82 mmol/259 L, 95% CI 2.75 – 2.89) with low or low normal PTH (18.5 ng/L, 95% CI 16.2 – 21.3).

Normocalcemic hypoparathyroidism

There are statistically significant differences if we compare the NHYPO group with the normal group in 264 terms of BAP (difference = -15.4%, 95% CI: -26.2 to -3.0, p=0.007), CTX (difference = -66.3%, 95% CI: -74.0 to -56.4, p<0.001) and osteocalcin (difference = -36.8%, 95% CI: -45.6 to -26.6, p<0.001). Baseline BMD parameters and the lumbar spine and hip change over the time have not been shown any statistically significant differences compared to the normal group. At the second visit (after 6 years), of the 35 NHYPO subjects with follow-up data, none developed overt hypoparathyroidism and only 15 (0.6%) subjects had persistent evidence of NHYPO.

Comparisons between the different categories and normal subjects (Tables 3,4)

At baseline, no statistically significant differences have been found between the different groups compared to the normal subjects with regards to age, BMI and lumbar spine BMD. According to its definition, secondary hyperparathyroidism has been shown a statistically significant difference in terms of 25 (OH) vitamin D and eGFR compared to the normal group (respectively, difference = -15.9%, 95% CI: -27.2 to -2.8, p=0.011. Difference = -16.0%, 95% CI: -21.8 to -9.8, p<0.001). In subjects with HH and non-PTH hypercalcaemia there is a statistically significant reduction in terms of eGFR compared to the normal group (respectively, difference = -11.7%, 95% CI: -17.4 to -5.7, p<0.001; Difference = -17.0%, 95% CI: -30.3 to -1.2, p=0.024).

Bone metabolic parameters

BMD

At baseline, no statistically significant differences have been found between the different groups compared to normal subjects at the lumbar spine. Post-hoc testing has been demonstrated a statistically significant difference in the mean hip T-Score between the normal group and the secondary hyperparathyroidism group (difference = -0.47, 95% CI: -0.83 to -0.11, $p=0.004$). The lumbar spine and hip BMD change over the time (rate of bone loss per year from the baseline) did not show any statistically significant differences between abnormal calcaemia categories compared to the normal group. The baseline total hip BMD is inversely related to serum PTH ($R= -0.006$, $p<0.001$), age ($R= -0.058$, $p<0.001$). It is also positively related to BMI ($R= 0.105$, $p<0.001$). The change in total hip BMD is inversely related to serum calcium ($R= -27.567$, $p<0.001$) and age ($R= -0.425$, $p=0.029$) (Table 5).

Bone turnover markers (table 4)

CTX

We found a statistically significant difference between the normal group and the HH 294 PHPT (difference = 24.0%, 95% CI: 1.5 to 51.3, $p=0.0249$), secondary hyperparathyroidism (difference = 28.1%, 95% CI: 2.8 to 59.5, $p=0.020$) and non-PTH hypercalcaemia (difference = -54.6%, 95% CI: -73.2 to -23.3, $p=0.001$).

Osteocalcin

We found a statistically significant difference between the normal group and secondary hyperparathyroidism (difference = 35.6%, 95% CI: 18.0 to 55.9, $p<0.001$).

Bone ALP

We found a statistically significant difference between the normal group and non-PTH hypercalcaemia group (difference = -27.2%, 95% CI: -45.80 to -2.3, $p=0.023$).

PINP

There were no statistically significant differences when we compared the normal group to the other different categories.

Stability of PTH over time

In the overall population there is a statistically significant change in PTH measurement from baseline. The mean PTH measurement increased from 41.6 (SD = 21.1) at baseline to 45.5 (SD = 26.1) at six years (mean change = 3.9, 95% CI: 2.79 to 4.98, $P < 0.001$).

Discussion

This is the first study that has investigated the prevalence of NHYPO in a large cohort of postmenopausal women. Cusano *et al* have previously conducted a large trial to evaluate the epidemiology of these subclinical conditions in an unselected community-based sample of old men (The Osteoporotic Fractures in Men study, MrOS study) and young men with premenopausal women (Dallas Heart Study, DHS) revealing a prevalence respectively of 0.4% and 3.1% for NPHPT and of 1.1% and 1.9% respectively for NHYPO (4). At baseline we identified 57 subjects (2.4%) affected by NHYPO and just 1 subject (0.1%) affected by normal hyperparathyroidism after ruling out the main causes of secondary hyperparathyroidism (vitamin D deficiency, $eGFR < 60$ ml/min and only 6 NHYPO subjects were taking proton pump inhibitors). In our cohort the prevalence of NHYPO is higher in comparison with the previous study: this is probably due to the differences in gender and age between the three populations.

Even if the baseline cross-sectional data indicate the existence of the NHYPO as a new subclinical pathological category, the longitudinal data give rise to many doubts. Indeed at the 6 years visit, of the 35 NHYPO subjects with follow-up data, none of them developed overt hypoparathyroidism and only 15 (0.6%) out of 35 subjects had persistent evidence of NHYPO. This finding is in keeping with that reported by others; Cusano and colleagues identified 68 subjects with NHYPO, none of whom developed overt hypoparathyroidism on follow-up and persistent disease was noted in 2 of 26 subjects with follow-up (4).

A few studies have shown that bone turnover marker levels are frankly low or low-normal in patients with hypoparathyroidism compared to normal subjects (17-20) and these findings are consistent with histomorphometric analysis. In particular double-tetracycline labelling of bone biopsy specimens have demonstrated that dynamic skeletal indices are suppressed in hypoparathyroid patients (21). According to these previous data, NHYPO seems to be characterized by a "low bone turnover" without a significant BMD change over the time compared to the normal group. Indeed, we found a significant reduction in serum levels of CTX, BAP and osteocalcin compared to subjects with no impairment of calcium metabolism.

Once again these findings confirm the key role of PTH in the bone metabolism: a few studies have demonstrated that daily subcutaneous injections of PTH (1-84) result in a significant increase of bone formation and resorption markers (that were suppressed at baseline) in patients affected by hypoparathyroidism (22).

Our data on NHYPO raise questions on the appropriate management of this category. Probably the “wait and see theory” could be the most suitable. We don't have sufficient data for recommendations for these 345 subjects. Anyway if the patients take a medication that might induce hypocalcemia, such as an anti-resorptive drug (such as bisphosphonate, denosumab) or a loop-acting diuretic (such as furosemide) then monitoring of serum calcium is to be recommended. Therefore the potential choice of the treatment for subjects with NHYPO affected by osteoporosis is critical. Indeed powerful anti-resorptive drugs such as bisphosphonates and denosumab, could exacerbate the risk of adynamic bone disease by suppressing the bone turnover (23). Conversely, anabolic therapy could restore the physiological bone turnover (24) but we are not able to predict if patients with NHYPO will experience an improvement in BMD. Indeed, in contrast to the effect of PTH (1–84) treatment in patients with osteoporosis, PTH (1-84) replacement therapy causes a general decrease in BMD at the hip, lumbar spine and whole body (apart from the forearm) in subjects with hypoparathyroidism (22). Despite the significant changes in bone turnover, teriparatide did not provide any significant BMD improvement in hypoparathyroidism (20). In particular, after 3 years of twice daily PTH (1–34) treatment, BMD and bone mineral content (BMC) at the lumbar spine, femoral neck, and whole body maintained stable although there was a non-significant downward trend in the distal one-third radius BMD.

It was noteworthy that the prevalence of PHPT was so high. The biochemical abnormality of high serum calcium and high or high-normal PTH is also found in familial hypocalciuric hypercalcemia but we did not conduct any calcium excretion studies or gene testing to rule out it. Most our cases were mild and the mean PTH was only at the upper limit of the reference interval for PTH; this might explain why most bone turnover markers were normal. The diagnosis of hypercalcaemia was made on samples after the second visit so it did not influence clinical management during the follow-up period.

It was also surprising that our rate of NPHPT was lower than in the MrOS as many epidemiological studies have confirmed that hyperparathyroidism is less frequent in men(25, 26). Other large

retrospective studies have attempted to evaluate the epidemiology of NPHPT but most have not ruled out the main causes of secondary hyperparathyroidism (27-29). Probably the low prevalence of NPHPT in our population may be explained by the high rate of vitamin D insufficiency (52.3%, data not shown) and eGFR reduction (62.4 %, data not shown); the former due to geographical differences and that latter due to the higher mean age in comparison to Cusano's populations. Probably the current definition of NPHPT tends to underestimate the prevalence of this category. Indeed according the data published by Shibli-Rahhal (30), in our population only 10.7% (data not shown) of older women with 25OHD < 20 ng/ml had an elevated PTH.

However, circulating PTH concentrations display a large inter-individual variability: calcium intake, some drugs such as lithium, MgSO₄, diuretics etc. are able to affect the PTH serum levels. Moreover this variance has been demonstrated to have a strong heritable component (1) but the genetic factors governing serum PTH concentrations remain unknown. Indeed, although it is well documented that loss of function and gain of function mutations of the CaSR lead respectively to hypercalcemic and hypocalcemic disorders (31), no studies aimed to assess the status of CaSR in NPHPT and hypoparathyroidism subjects. Another interesting area of research could be the evaluation of the autoantibodies directed against the extracellular domain of the CaSR and NALP5 in subjects with NHYPO. Previous studies have identified the CaSR and NALP5 as parathyroid autoantibody targets in patients with autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy (32) or in subjects with autoimmune hypocalciuric hypercalcemia (33-35) but it is unclear whether these antibodies may also contribute to the variability in serum PTH concentrations observed in normocalcaemic individuals.

Our study has a few limitations. We didn't measure ionized calcium and fasting phosphate, we don't have any biochemical data between the first visit at baseline and the second visit at 6 years later, we have only a single value for each laboratory analyte at each time point, we lost a significant number of subjects over the course of the study and BMD analysis was performed by two different instruments. Moreover the prevalence of the different diagnostic categories might be affected by long-term PTH storage. Anyway we do not think that relatively high prevalence of NHYPO could be attributable to degradation of PTH during storage. Indeed it has been estimated by some authors that there is about a 1% increase in intact PTH every year in women, with an faster rise after the menopause (36,37); therefore the 9% increase we observed is likely to be age-related rather than resulting from minor degradation. Moreover if the low prevalence of SHPT and relatively high prevalence of NHYPO had been attributable to degradation of PTH during storage, we would have

expected also a low prevalence of hyperparathyroid hypercalcemia (PHPT and FHH). Instead we have a high prevalence of hyperparathyroid hypercalcemia.

In comparison to Cusano's findings, the prevalence of SHPT was quite low. This could be explained by the higher dietary calcium intake found in European countries compared to the US population. In one review, the mean calcium intake in the NHANES III study in older women 402 was 600 mg/day, considerable lower than the estimates for UK (800 mg), France (850 mg) and Germany (970 mg) (38). This is the first large population based study that has investigated the prevalence and the bone metabolic status of NPHPT in postmenopausal women: it is fairly common, not always persistent and is characterized by low bone turnover.

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Figure 1. Baseline data results for adjusted calcium and PTH. The ellipse was derived using Mahalanobis distances method to define normal (black) and abnormal (red) values. The horizontal and vertical lines indicate the geometric mean, and reference intervals described in Table 1. The pink rectangle identifies subjects with normocalcemic hypoparathyroidism.

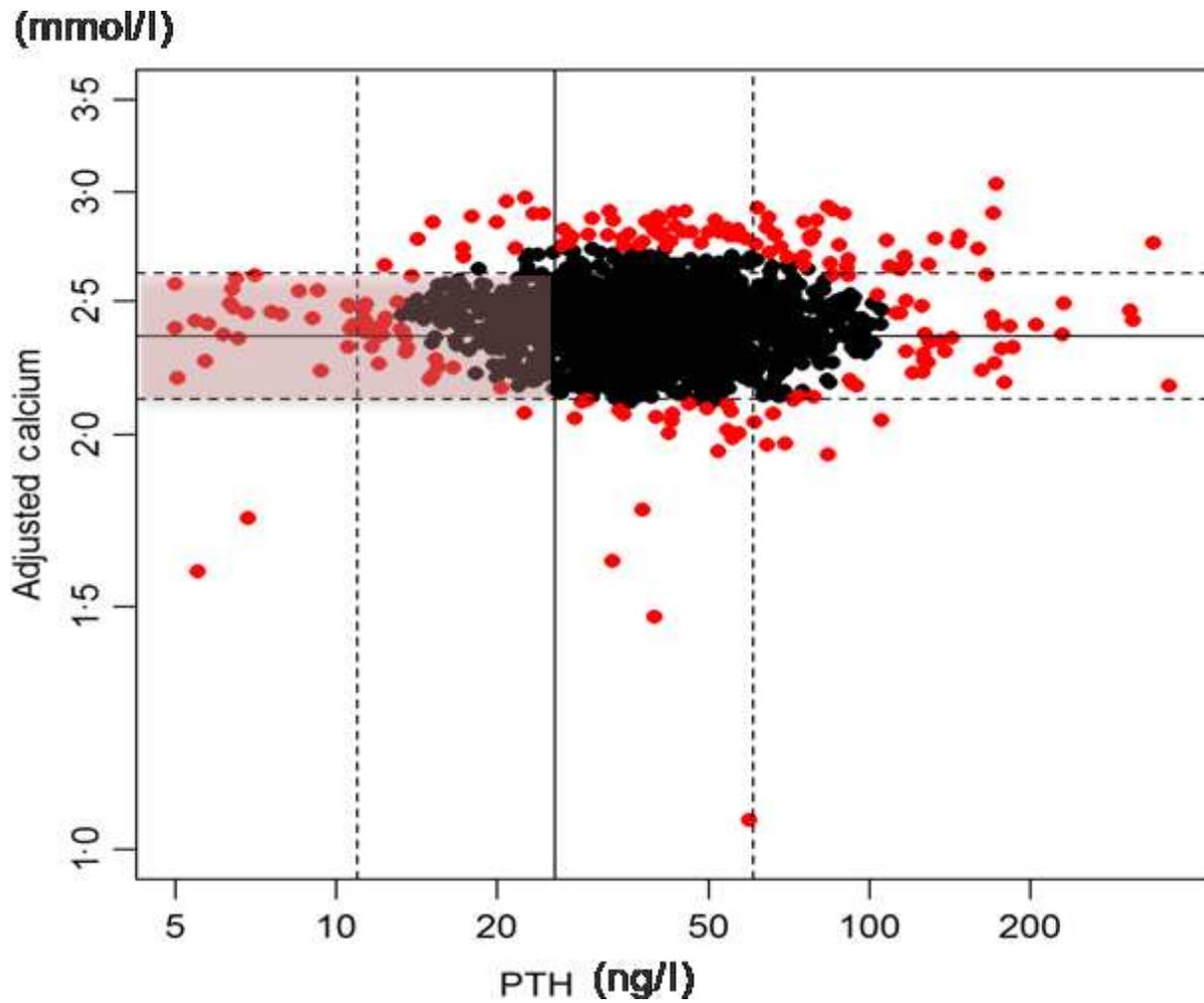


Figure 2. Follow-up results for adjusted calcium and PTH. The ellipse was derived using Mahalanobis distances method to define normal (black) and abnormal (red) values. The horizontal and vertical lines indicate the geometric mean, and reference intervals obtained from the healthy young women. The pink rectangle identifies subjects with normocalcemic hypoparathyroidism.

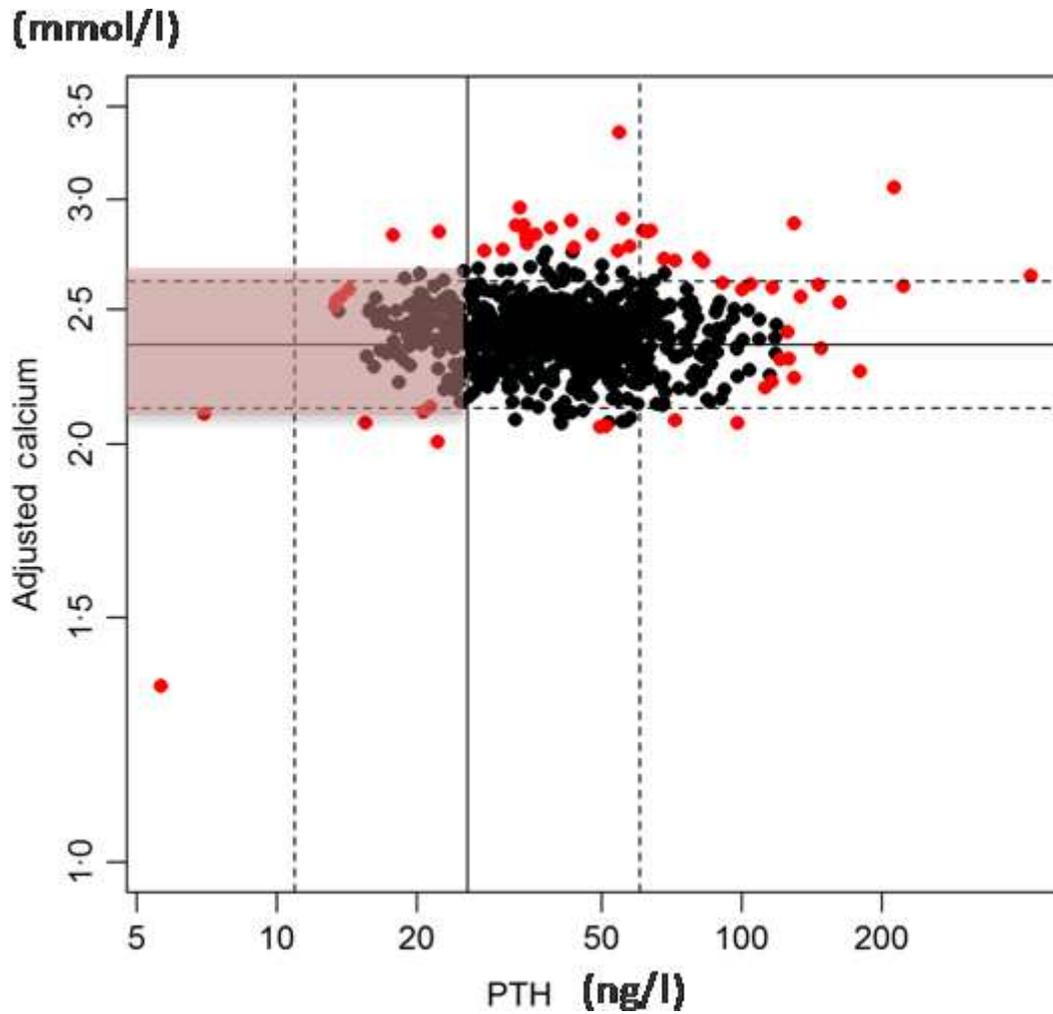


Table 1. Reference Intervals for Adjusted Calcium and PTH Healthy Young Women.

	Geometric Mean	Reference Range	95% CI of lower limit of reference range	95% CI of upper limit of reference range	Mean (SD) \log_{10}
Adjusted Serum Calcium (mmol/L) (N = 107)	2.359	2.123 – 2.620	2.086 – 2.160	2.575 – 2.667	0.373 (0.023)
PTH (ng/L) (N=107)	25.707	10.937 – 60.419	9.493 – 12.602	52.441 – 69.612	1.410 (0.189)

Table 2a. Baseline characteristics of the older and younger women in the OPUS study

	Premenopausal			Postmenopausal		
	N	Mean	SD	N	Mean	SD
Age (years)	463	31.3	5.5	2419	67.1	7.1
Height (cm)	463	166.0	6.7	2418	160.3	6.3
Weight (kg)	463	66.3	13.0	2418	68.7	12.3
BMI (kg/m²)	463	24.0	4.4	2418	26.7	4.5
Lumbar Spine BMD T-Score	462	0.09	1.12	2372	-0.94	1.51
Total Hip BMD T-Score	462	0.27	1.01	2396	-0.64	1.16
CTX (ng/mL)	423	0.25	0.17	2287	0.36	0.27
PINP (ng/mL)	435	39.7	20.6	2260	42.5	22.0
Bone ALP (ng/mL)	441	11.5	5.1	2313	15.1	6.4
Osteocalcin (ng/mL)	441	21.2	8.0	2303	24.5	13.9
25 (OH) Vitamin D (ng/mL)	440	25.4	12.6	2311	21.3	10.3
eGFR (mL/minute/1.73m²)	439	86.1	12.3	2317	57.5	13.5
Calcium (mmol/L)	440	2.37	0.13	2320	2.39	0.16

Table 2b. Baseline characteristics for normal and NHYO subjects

	Age (years)		BMI (Kg/m ²)		Spine T-Score		Hip T-Score	
	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)
Normal	2063	67.0 (66.7, 67.3)	2062	26.6 (26.5, 26.8)	2024	-0.95 (-1.01, -0.88)	2043	-0.64 (-0.69, 0.59)
Normocalcaemic Hypoparathyroidism	57	66.3 (64.6, 67.9)	57	26.2 (25.1, 27.3)	57	-0.64 (-1.07, 0.21)	57	-0.47 (-0.78, -0.16)
P-Value ^a	ns		ns		ns		ns	

^a P-Value from ANOVA testing for an overall difference in means

Table 3. Baseline calcium metabolism characteristics for each category.

	Adjusted Calcium (mmol/L)		PTH (ng/L)		25(OH)D (ng/ml)		eGFR (ml/min)	
	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)
Normal	2063	2.40 (2.39, 2.41)	2063	39.0 (38.4, 39.7)	2058	19.2 (18.9, 19.6)	2060	56.7 (56.1, 57.2)
Primary Hyperparathyroidism	86	2.79*** (2.78, 2.80)	86	58.8*** (52.6, 65.8)	85	17.9 (16.1, 19.9)	85	50.0*** (47.8, 52.2)
Secondary Hyperparathyroidism	69	2.16*** (2.09, 2.23)	69	88.9*** (75.8, 104.3)	69	16.2* (14.1, 18.6)	69	47.6*** (44.1, 51.3)
Normocalcaemic Hypoparathyroidism	57	2.39 (2.36, 2.42)	57	10.1*** (9.2, 11.1)	57	20.7 (18.0, 23.8)	57	53.0 (50.3, 55.9)
Non-PTH Hypercalcaemia	12	2.82*** (2.75, 2.89)	12	18.6*** (16.2, 21.3)	12	19.9 (15.1, 26.2)	12	47.0* (40.8, 54.2)
P-Value ^a	<0.001		<0.001		0.015		<0.001	

^aP-Value from ANOVA testing for an overall difference in means

Post-hoc testing for a difference in mean from the Normal group: * P-Value <0.050, ** P-Value < 0.010, *** P-Value < 0.001

Table 4. Baseline bone turnover markers for each category.

	PINP (ng/mL)		BAP (ng/mL)		CTX (ng/mL)		OC (ng/mL)	
	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)
Normal	2013	36.7 (35.7, 37.6)	2061	14.0 (13.7, 14.2)	2046	0.29 (0.28, 0.30)	2032	22.1 (21.7, 22.5)
Primary Hyperparathyroidism	85	41.0 (35.9, 46.9)	86	14.8 (13.4, 16.3)	86	0.35* (0.30, 0.41)	85	24.2 (21.1, 27.7)
Secondary Hyperparathyroidism	69	39.8 (33.9, 46.7)	69	14.7 (12.8, 17.0)	67	0.36* (0.29, 0.45)	63	30.0*** (25.4, 35.5)
Normocalcaemic Hypoparathyroidism	55	31.0 (26.6, 36.3)	57	11.8** (10.5, 13.3)	50	0.10*** (0.07, 0.13)	57	14.0*** (12.3, 16.0)
Non-PTH Hypercalcaemia	11	30.6 (20.7, 45.1)	12	10.2* (6.1, 16.8)	12	0.13*** (0.07, 0.24)	12	16.3 (13.3, 20.1)
P-Value ^a	0.048		<0.001		<0.001		<0.001	

^aP-Value from ANOVA testing for an overall difference in means

Post-hoc testing for a difference in mean from the Normal group: * P-Value <0.050, ** P-Value < 0.010, *** P-Value < 0.001

Table 5a. Multiple regression analysis – do baseline measurements predict baseline BMD?

$$\text{Hip T-Score at Baseline} = \text{Intercept} + \text{Calcium} + \text{PTH} + \text{Age} + \text{BMI}$$

	Coefficient	95% CI	P-Value
Adjusted Calcium	0.277	(0.017, 0.537)	0.037
PTH	-0.006	(-0.007, -0.004)	<0.001
Age	-0.058	(-0.063, -0.052)	<0.001
BMI	0.105	(0.096, 0.114)	<0.001

Values are per unit increase

Table 5b. Multiple regression analysis – do baseline measurements predict change in BMD?

Change in Hip BMD = Intercept + Baseline BMD + Calcium + PTH + Age + BMI

	Coefficient	95% CI	P-Value
Baseline Hip BMD	-0.095	(-0.116, -0.075)	<0.001
Adjusted Calcium	-27.567	(-43.318, -11.815)	0.001
PTH	-0.081	(-0.195, 0.033)	0.164
Age	-0.425	(-0.806, -0.044)	0.029
BMI	0.455	(-0.140, 1.049)	0.134