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ORIGINAL ARTICLE

THE CALCIUM LOADING TEST IN PRIMARY HYPERPARATHYROIDISM – DOES IT PROVIDE USEFUL INFORMATION?

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Abstract. Objective: *to describe the results from intravenous calcium loads in patients with suspected PHPT.* ***Methods:*** *This was a retrospective cross-sectional study with inpatients' data review. Baseline serum calcium, phosphates, magnesium, creatinine, alkaline phosphatase, beta-crosslinks were recorded. The calcium loading had been performed after an overnight fasting. Calcium gluconate 0.25 mmol/kg body weight (10 mg/kg) dissolved in 0.9% Sodium Chloride was infused intravenously for 3 hours. Blood samples for total serum calcium, phosphates and iPTH were drawn before and up to 60 minutes after the calcium load. The intact parathyroid hormone (iPTH) and 25(OH)-vitamin D were determined by electro-hemi-luminescence (Elecsys, Roche Diagnostics). The PTH inhibition rate in % (PTH-IR), the calcium increment, the ratio R (∆PTH/∆Calcium) and the product P (calcium X PTH at test end) were calculated.* ***Results:*** *23 patients with PHPT had complete data from calcium loading tests. The PTH suppression was < 30% in 17.4% of the participants, 30% to 50% – in 21.7% and > 50% in the remaining 60.9%. One patient displayed PTH suppression > 75%. The Ratio R was < 4.0 in all but one patient. Product P was above 1100 mg/dl x pg/ml in 9 participants (39.1%). Sixteen patients (69.6%) had positive localization results from US or SPECT-CT. The level of PTH suppression was not related to any parameter except the volume of the suspected lesion.* ***Conclusion:*** *The calcium loading test was unable to differentiate the forms of PHPT. It did not add great value in the diagnostic work-up of PHPT.*

Key words: *primary hyperparathyroidism, laboratory findings, localization studies, calcium loading test*

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is a relatively common endocrine disorder. Approximately 1.0-1.5% of the postmenopausal women with osteoporosis might have some form of PHPT [1]. The prevalence of PHPT in the general population is estimated around 3-4/1000 [1, 2]. In a nationally representative Bulgarian study including 2032 individuals the prevalence of hyperparathyroidism (HPT) was 3.59%, and increasing with age – from 1.9% in the younger groups to 6.8% among individu-

als > 60 years [3]. The prevalence of the mildest form of PHPT, the normocalcemic PHPT (NPHPT), varies considerably between 0.1% and 8.9% [4]. The incidence of PHPT may reach 1 per 1,000 people (0.1%) [5]. A large-scale clinical study in Scotland demonstrated that the incidence of PHPT had been steadily increasing from 1997 to 2013 with a more obvious increase from 2004 to 2013 [6]. PHPT is also conferring increased risks for bone loss/fragility fractures as well as renal stone formation and impaired renal function [7].

The cornerstone in the diagnostic process of PHPT remains the inappropriately high serum parathyroid hormone (PTH) levels in relation to the calcium levels. There are a few other reasons for isolated elevation of PTH, such as vitamin D deficiency, disorders of the magnesium homeostasis, reduced glomerular filtration rate (eGFR) and many others [8]. The so-called calcium load test was primarily conceived for the distinction of patients with PHPT from healthy controls [9, 10]. The test was also used for differentiating autonomous secretion of PTH in PHPT from other secondary causes [11]. A number of studies have explored the test performance in different forms of PHPT such as adenoma or multi-glandular hyperplasia [10, 12]. The calcium load might be of significant help in cases of assumed PHPT with unsuccessful localization studies or with concomitant reduction in the GFR.

The purpose of this study was to describe the changes in serum calcium, phosphates and intact PTH (iPTH) during an intravenous calcium load in patients with assumed PHPT and therefore the clinical utility of this test.

MATERIALS AND METHODS

Design

This was a retrospective hospital chart review of routine clinical care (an observational cross-sectional study). All patients had given their informed consent for hospitalization and their data handling. All investigations including the calcium load were performed as part of routine clinical care with the patients' consent. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Institutional Review Board of the University Hospital gave consent for publication of the data included in this manuscript.

The data were extracted from the electronic database of a tertiary endocrine clinic. The inpatients' hospital records for the last ten years were searched through with the keyword "primary hyperparathyroidism".

A total of 160 positive patient records were identified. Primary hyperparathyroidism (PHPT) was defined as either a combination of high albumin-corrected or ionized serum calcium and high parathyroid hormone (PTH), or as inappropriately high PTH in the presence of high normal calcium levels [1]. Other causes for elevated PTH were excluded including but not limited to: CKD grade 4 and 5, malabsorption syndromes, hypercalciuria due to other causes (loop diuretics, idiopathic, etc.), pseudohyperparathyroidism, severe hypothyroidism, recent application of high doses of denosumab or bisphosphonates [8]. Secondary causes for hyperparathyroidism were identified in 55 cases. Five cases had incomplete data and were not included in the analyses. Twenty-three of the remaining 100 patients had data from a venous calcium loading and were included. The primary reasons for the diagnostic calcium loading test had been primarily the presence of negative localization studies (in 10 of the 23 patients) and the need for confirmation of low PTH suppression rate especially in the presence of normocalcemia (an attempt to identify healthy individuals).

Laboratory and hormonal data

Total serum calcium was measured by a photometric assay and whenever possible by atomic absorptiometry – Ca-AAS (reference range 2.12-2.62 mmol/l). Albumin-corrected calcium was calculated by the formula:

Corrected calcium (mmol/l) = total serum calcium (mmol/l) + 0.8 x (mean albumin – patient's albumin in g/l), where mean albumin was 44 g/l (local lab reference range for albumin 35-52 g/l; and for corrected sCa: 2.10-2.60 mmol/l).

The calcium-phosphorus product was calculated. Ionized calcium (iCa+) was measured by atomic absorptiometry (reference range 1.1-1.3 mmol/l).

Intact PTH (iPTH) was measured by a second generation assay (Elecsys, Roche Diagnostics, Switzerland) and expressed in pmol/l (reference range 1.59-6.89 pmol/l). PTH values in pg/ml or ng/l were converted into SI units (pmol/l) by a factor of 9.43 (division). Serum levels of vitamin D were measured by electro-hemi-luminescent detection (ECLIA method) as 25(OH)D Total (Elecsys, Roche Diagnostics, Switzerland). Levels of 25(OH)D ≤ 25.0 nmol/l were interpreted as deficiency, between 25.0 and 50.0 nmol/l – as insufficiency, and above 50 nmol/l – as sufficiency. Optimal 25(OH)D levels were accepted for values above 75.0 nmol/l [13].

Serum creatinine was measured by a colorimetric method and the estimated glomerular filtration rate (eGFR) was calculated according to the MDRD-formula and expressed in ml/min/1.73 m². Urinary excretion of calcium and phosphate were measured from 24 h urinary samples in mmol/24 hr. Total alkaline phosphatase in IU/l and serum Beta-crosslinks in ng/ml (Elecsys, Roche Diagnostics, Switzerland) were also recorded.

The calcium loading test

The calcium loading test was performed after an overnight fasting and started between 8.00-9.00 a.m. on the second day of hospitalization. A total serum calcium ≥ 3.0 mmol/l on the day of admission was considered an exclusion criterion. On the first hospital day all patients were on a standardized diet devoid of dairy products. During the entire calcium loading test the patients remained in the supine position. Calcium gluconate 0.25 mmol/kg body weight (10 mg/kg) was dissolved in 500 ml 0.9% sodium chloride and infused intravenously over 3 hours. Blood samples for total serum calcium, phosphates and iPTH were drawn just before the infusion start and 4 hours later (up to 60 minutes after infusion end).

The Ratio R was defined as PTH percent decline/calcium percent increase. The Product P was calculated as serum calcium (in mg/dl) X PTH (in pg/ml) at study end (minimal PTH concentration X maximal calcium concentration during the test).

Imaging

The localization studies included neck ultrasound of all patients (using a linear 9-12 MHz transducer, including Doppler imaging) and single-photon emission computed tomography (SPECT-CT with 99mTc-MIBI) in selected cases. In cases of a visible parathyroid adenoma its volume was calculated by multiplying the three diameters (anterior-posterior, medial-lateral, proximal-distal) and dividing the product by a factor of 0.48 (formula for ellipsoids).

Additional localization studies included fine-needle biopsy (FNAB) of the suspected parathyroid lesion with cytological analysis of the specimen and/or measurements of iPTH in the needle washout.

STATISTICAL ANALYSIS

All analyses were performed with the IBM SPSS 13.0 for Windows platform (SPSS Corp., Chicago, IL). Descriptive statistics and frequency analysis were performed. Missing data were not replaced. Most of the data were positively skewed, thus medians and quartile ranges were preferred. The Mann-Whitney and Wilcoxon tests were used for comparisons of independent and dependent samples. Correlations were assessed by the Spearman's Rho coefficient. The regression analysis included estimation of 7 possible curves. Statistical significance was set at $p < 0.05$.

RESULTS

Twenty-three female patients with suspected PHPT had complete data from calcium loading tests. Their mean age was 62.4 ± 9.8 years with the following age distribution: one patients was 32 years of age, 7 (30.4%) between 50 and 59, 9 (39.1%) between 60 and 69, and 6 (26.0%) were aged 70-76 years. The hyperparathyroidism was diagnosed by a median of 2.0 years before the calcium load (range 1 month to 8 years). The most common causes rising suspicion for PHPT were lab investigations for osteoporosis (16/23), followed by findings from neck ultrasound as part of the assessment for thyroid disease (3/23). Two patients had had prior operations for parathyroid adenoma, which were then assessed as unsuccessful (recurrence of high PTH).

Biochemical and hormonal data

The baseline biochemical and hormonal data of the patients on their first day in hospital are presented in Table 1.

Table 1. The baseline biochemical and hormonal data of the patients during the hospital stay for calcium loading

	Total sCa (mmol/l)	iCa++ (mmol/l)	sP (mmol/l)	sMg (mmol/l)	Corr. sCa (mmol/l)	ALP (IU/l)	iPTH (pmol/l)	25(OH)D nmol/l	eGFR (ml/min/1.73 m²)	uCa (mmol/day)	uP (mmol/day)
Mean	2.62	1.33	0.97	0.81	2.64	91.3	12.5	91.1	72.2	5.4	16.7
Minimum	2.29	1.12	0.64	0.66	2.20	40.0	7.3	29.0	36.6	0.9	7.1
25th percent	2.49	1.23	0.84	0.75	2.52	59.0	9.4	68.0	56.0	2.5	11.3
Median	2.60	1.35	0.91	0.84	2.63	96.0	11.0	81.0	74.8	5.6	14.2
75th percent	2.77	1.39	1.12	0.87	2.71	116.5	15.6	120.5	83.1	7.0	18.8
Maximum	2.91	1.56	1.22	0.94	2.90	127.0	23.3	190.0	132.0	15.7	49.3

Total serum calcium was ≤ 2.62 mmol/l in 56.5% of the patients (13/23), albumin-corrected calcium was ≤ 2.60 mmol/l in 47.8% (11/23) participants, and ionized calcium was ≤ 1.32 mmol/l in 43.5%.

Serum 25(OH)D was insufficient (25-50 nmol/l) in 3 participants (13.0%), sufficient (50-75 nmol/l) – in 5 participants (21.7%), and optimal (≥ 75 nmol/l) in the remaining 65.2%. Fifteen participants had been taking vitamin D supplementation (a mean of 1500 IU daily) prior to the hospitalization in an attempt to minimize the effect of vitamin D insufficiency on serum PTH. The estimated glomerular filtration rate (eGFR-MDRD, ml/min/1.73 m^2) was between 30 and 60 ml/min/1.73 m^2 in 34.8%, between 60 and 90 – in 52.2%, and ≥ 90 ml/min/1.73 m^2 – in the remaining 13.0%. 24hr urinary calcium was above 7.5 mmol/day in 5 participants (21.7%), while very low (<1.0 mmol/day) in 2 participants (8.7%). 24hr urinary phosphate excretion was elevated in 2 patients (8.7%) only.

Alkaline phosphatase was normal in all participants, while the beta-CTX was above the premenopausal range in all but two participants.

The calcium loading test

The baseline and post-load data (total serum calcium, serum phosphate and iPTH) are presented in Table 2.

The changes in total serum calcium and iPTH during the test on the individual level are displayed in Fig. 1 a and 1 b.

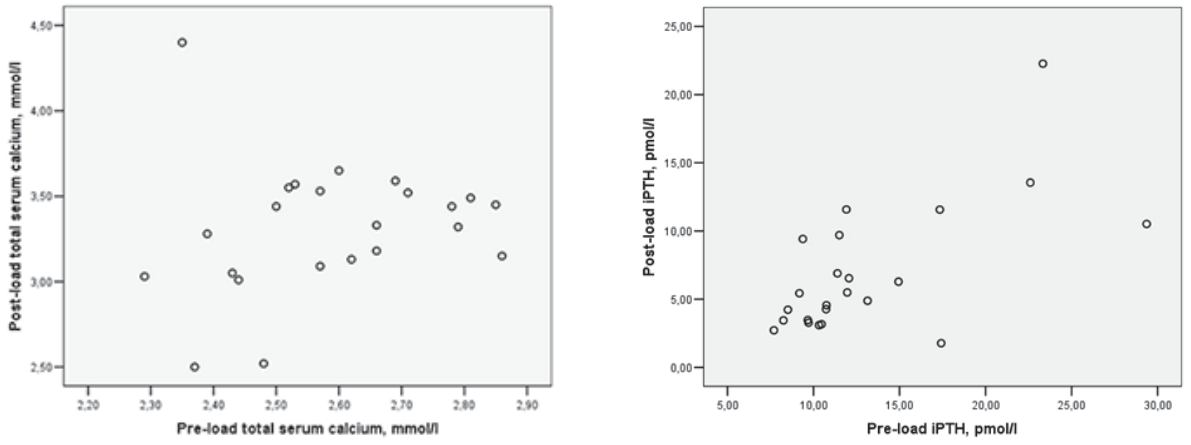
Post loading total serum calcium reached values between 3.0 and 3.4 mmol/l in 14 patients (60.9%) and values ≥ 3.5 mmol/l in 7 patients (30.4%). Post-load iPTH was below the upper reference range of 6.9 pmol/l in 16 patients (69.6%) and in the lower normal range (< 4.0 pmol/l) – in 7 patients (30.4%). The mean increment in total serum calcium and the mean decline in iPTH expressed in percentages from the baseline value are summarized in Table 3.

Table 2. The baseline and post-load data are presented as total serum calcium, serum phosphate and iPTH

Variables of interest	Mean	Standard deviation	P-value for the difference pre/post-load
Baseline total sCa (mmol/l)	2.59	0.17	< 0.001
Post-load total sCa (mmol/l)	3.31	0.39	
Baseline serum P (mmol/l)	0.97	0.18	0.007
Post-load serum P (mmol/l)	1.15	0.29	
Baseline serum iPTH (pmol/l)	13.19	5.48	<0.001
Post-load serum iPTH (pmol/l)	6.88	4.71	

The iPTH decrease was below 30% from baseline in 4 patients (17.4%), between 30% and 50% – in 5 patients (21.7%) and above 50% in the remaining 60.9%. One patient only had above 75% PTH suppression. The Ratio R was < 4.0 in all but one patient. Product P was above 1100 mg/dl x pg/ml in 9 out of 23 participants (39.1%) – 3 patients with a PTH decline < 30% (75% of them), and in 2 of the 5 patients with PTH decline between 30% and 50% (40% of them). Therefore three patients only (10.3%) displayed the combination of < 30% PTH suppression + Ratio R < 4 + Product P > 1100.

The correlation between ΔPTH and Δ sCa was characterized by a Spearman's Rho of 0.607 (p = 0.002). Their relationship was further explored in regression analysis, as shown in Table 4 (regression coefficients in detail).



a pre- and post-load total serum calcium in mmol/l

b pre- and post-load iPTH in pmol/l

Fig. 1. The pre- and post-load values of total serum calcium (a) and iPTH (b) are displayed as on the individual level

Table 3. The mean increase in total serum calcium and the mean decline in iPTH are displayed as percentages from the baseline value

Difference versus baseline, %	PTH decline (ΔPTH, %)	Calcium Increase (Δ sCa, %)	Ratio R* (ΔPTH / Δ sCa, %)	Product P** (mg/dl x pg/ml)
Minimum	-0.43	1.6	-0.02	201.5
25th percentile	39.4	19.5	1.57	463.2
Median	57.6	25.2	1.78	649.0
75th percentile	64.2	37.4	2.76	833.3
Maximum	89.8	87.2	4.51	2110.1

* Ratio R – the PTH decline divided by the calcium increase, both expressed in percentages from baseline.

** Product P – multiplication of sCa (in mg/dl) and iPTH (in pg/ml) after the calcium load.

Table 4. The model summary from the regression analysis (ΔPTH upon Δ sCa as the independent variable) is shown

Best models	R Square	F (degrees of freedom)	Sig. (p)
Linear	0.240	6.648	0.018
Logarithmic	0.347	11.183	0.003
Quadratic	0.332	4.961	0.018

Imaging (localization studies)

The neck ultrasound (US) located single lesions suspicious for parathyroid adenoma in 13 patients (56.5%). The suspected lesion was behind or below the left inferior pole of the thyroid gland in 4 cases (17.4%), behind the middle left portion – in 1 case (4.3%), at the upper left pole – in 1 case, at the right upper pole – in 1 case, behind the middle right lobe – in 1 case, and behind or below the right inferior pole – in 5 cases (21.7%). The median volume of the suspected lesion was 0.24 cm^3 (range 0.1-5.78 cm^3). The lesion volume was < 0.25 cm^3 in 7 subjects. The volume of the suspected parathyroid lesion did not correlate with total / ionized serum calcium or PTH/ PTH decline. The neck US had revealed thyroid nodules in addition in 6 patients (26.1%) and autoimmune thyroid disease was present in 9 subjects (39.1%).

Fine-needle aspiration biopsy with needle-washout and cytological examination was performed in 9 of the patients with clearly identified suspicious lesions. Parathyroid cells were identified in 4 of the 9 cases, insufficient smears were found in 2 cases and thyroid cells (Bethesda class II) – in 3 of the 9 cases.

The US localization studies were negative in 10 cases (43.5%). Nuclear imaging (SPECT-CT with 99mTc-MIBI) was performed in a total of 14 cases, 5 of which had no lesion on US. In 3 of them SPECT-CT was also negative (3/5) while increased local radionuclide uptake was found in the remaining 2 cases (2/5).

Sub-analysis of factors related to the PTH inhibition rate (rate of PTH decline)

The baseline eGFR affected the magnitude of PTH decline. The PTH inhibition was 51.8 ± 16.2% in subjects with eGFR ≥ 60 ml/min/1.72 m^2 and 39.5 ± 32% in subjects with eGFR < 60 ml/min/1.72 m^2 (p = 0.017). The vitamin D status (25-OH-D < or ≥ 50 nmol/l) did not affect the PTH decline.

Analyzing patients with a PTH decline ≥ 50% versus < 50%, no significant differences in the baseline values of serum and urinary calcium and phosphate, serum magnesium and iPTH were noted. Both subgroups did not differ in age or duration of known PHPT, however there was a tendency for greater PTH decline in smaller lesions on US – 58.2% in lesions < 0.25 cm^3, compared to 47.1% in larger lesions (p = 0.078). No significant differences in the US or SPECT-CT findings could otherwise be identified. Findings indicative of possible parathyroid lesions on US or SPECT-CT were documented in 75% (3/4) in patients with PTH inhibition < 30%, in 60% (3/5) in those with 30-50% inhibition and in 76.9% (10/13) of those with 50.1-75.0% inhibition. The only patient with a PTH inhibition > 75% had no proven parathyroid localization.

The calcium load was unable to differentiate between the normocalcemic (N = 7) and hypercalcemic (N = 16) subjects. The median PTH decline was 58.0% in normocalcemic participants and 52.2% in the hypercalcemic ones. The median calcium increments were 32.2% versus 24.0%, while the Ratio R – 1.60 versus 1.90 respectively. The Mann-Whitney test did not show significant inter-group differences (p > 0.05).

DISCUSSION

In this study we analyzed data from the calcium loading test in 23 patients with biochemically proven PHPT. We observed different degrees of PTH sup-

pression – < 30% in 17.4%, 30% to 50% – in 21.7% and > 50% in the remaining 60.9%. One patient only displayed PTH suppression > 75%. The Ratio R was < 4.0 in all but one patient. Product P was above 1100 mg/dl x pg/ml in 9 out of 23 participants (39.1%). The level of PTH suppression was not related to the results from the imaging studies except for a tendency for the volume of the suspected lesion. Therefore, the calcium loading test did not help us to confirm or exclude the presence of PHPT. In addition, it was unable to differentiate between the normocalcemic and hypercalcemic subjects.

The calcium loading was initially conceived to differentiate PHPT characterized by very low suppression rates of PTH from other causes for secondary hyperparathyroidism with high susceptibility to suppression [14-17]. Some authors suggested a threshold of 30% PTH inhibition differentiating PHPT from SHPT/healthy controls [17], while others used a 50% threshold [9, 15, 18]. Those earlier studies revealed a whole spectrum of responsiveness and formulated the hypothesis that absolute autonomy of the parathyroid glands from serum calcium was quite unusual [16]. More recent studies tried to make a more subtle differentiation [12, 19-21]. In the study by Titon et al. the PHPT patients exhibited a PTH inhibition of 64% while the healthy controls – of 73% [19]. The authors overcame the possible overlap by suggesting a PTH value > 14 ng/ml at the end of the test as accurately defining patients with PHPT [19]. Other authors defined combinations of values to identify the PHPT patients [20, 21]. Zhao et al. developed a combination of serum calcium > 2.43 mmol/l, PTH inhibition rate < 73% and Ratio R (ΔPTH/Δ sCa) < 1.27 as a reliable indicator for mild PHPT [21]. Hagag et al. applied an oral calcium test and suggested a PTH decline < 30% in combination of Ratio R < 4.0 as typical for parathyroid adenomas. They also registered a PTH inhibition of > 60% in cases of parathyroid hyperplasia [20]. In a recent work a PTH inhibition rate < 50% was indicative of adenoma, 50-75% – of normocalcemic PHPT, and > 75% – of absence of PHPT [12].

In our study we observed the whole spectrum of PTH inhibition rate without any obvious reason for the different biological responses. Moreover, we had positive localization findings in 16 patients (13 from US and 3 additionally from SPECT-CT). However the PTH inhibition rate was not related to any useful clinical or laboratory variables. In fact, more recent molecular studies in PHPT have suggested different patterns of normal and abnormal parathyroid growth [22]. A major difference is inherent to the underlying parathyroid pathology – abnormal calcium-PTH set-point control or abnormal cell cycle control. The origin of the pathologic cells – monoclonal versus polyclonal growth – makes also an important difference. All these underlying pathologies might explain the variability in the clinical and laboratory picture. However they cannot be detected by the calcium loading. In addition, the clinical decisions for surgery or active observation in PHPT are based on factors other than the degree of PTH suppression or results from calcium loading [23].

Strengths and limitations

The major strength of this study is that it is based on contemporary data creating a complete picture of the biological responses to calcium loading in PHPT.

Our study has three major limitations. The first is the lack of histological verification. Data from parathyroid surgery were present in a minority of patients (4 of 23) preventing us from differentiating parathyroid adenoma and hyperplasia from absence of morphological substrate. Secondly, no healthy controls were available for comparison. It must be however kept in mind that ethical approval and patient's informed consent would be difficult to obtain in healthy controls.

CONCLUSION

We registered a great variety of parathyroid secretion responses to calcium loading in patients with biochemically proven PHPT. The different degrees of parathyroid autonomy could not be directly related to specific pathologic processes or forms. The calcium loading test is quite unable to differentiate PHPT from secondary causes for elevated PTH or to determine otherwise healthy individuals. We feel that this test is not of great help in the contemporary diagnostic work-up of PHPT.

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