International Journal of Nutrology
(Official Journal of the ABRAN - Brazilian Association of Nutrology)

DOI: 10.54448/ijn22207

CASE REPORT

False hyperparathyroids secondary to biotin prolonged use: a case report

Georgeanne Neves[1], Eduardo Carvalho Lira[2*]

[1] Municipal Service of the city of Paulista, Endocrinology department, Pernambuco, Brazil.
[2] Federal University of Pernambuco, Physiology and Pharmacology department, Pernambuco, Brazil.

Corresponding Author: Dr. Eduardo Carvalho Lira. Department of Physiology and Pharmacology, Center of Biological Sciences, Universidade Federal de Pernambuco. Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, PE, 50670-901. E-mail address: eduardo.clira2@ufpe.br
DOI: https://doi.org/10.54448/ijn22207
Received: 02-12-2022; Revised: 04-28-2022; Accepted: 05-14-2022; Published: 05-25-2022; IJN-id: e22207

Abstract

A 55-year-old overweight female patient with elevated parathyroid hormone (PTH) levels, without any other major plasma biochemical abnormalities, and with normal vitamin D and calcium levels. The condition was suggestive of normocalcemic primary hyperparathyroidism. Bone densitometry and 24-hour calciuria were normal. After extensive investigation and possible referral for exploratory surgery, the patient reported continued and chronic use of biotin supplementation (2.5 mg per day). Repeated tests after discontinuing the use of vitamin B7 showed normal PTH levels without surgical or pharmacological interventions. Biotin supplementation associated with the use of this vitamin as a component of biological assays for hormone dosage can generate incorrect laboratory results, which puts medical conduct at risk with inaccurate diagnoses and unnecessary procedures. Although, different substances or emotional conditions can interfere with hormone dosages, one must consider the use of food supplements such as biotin since it can also generate unreliable results. Discontinuation of the use of biotin, in addition to the assessment of serum biotin levels prior to hormone dosing is necessary for a correct evaluation of serum PTH levels. This study was analyzed and approved by the Research Ethics Committee, and obtaining the Informed Consent Form according to CNS/CONEP Resolution 466/12.

Keywords: Primary hyperparathyroidism. Parathyroid hormone. Biotin.

Introduction

Calcium, a divalent cation (Ca2+), is essential for different biological functions such as muscle contraction, blood clotting, neural transmission (synapses) and hormone secretion **[1-3]**. In addition, it is one of the most important signaling molecules for the cell, which is why calcium levels in the extracellular fluid (ECF) and intracellular fluid (ICF) are extremely well regulated by mechanisms involving organs such as bones, gastrointestinal tract (GIT), kidneys, and parathyroid glands **[2]**.

The total amount of calcium in the body ranges between 1000 and 1200g, of which 99% is associated with bone and 1% is distributed in the ICF and ECF **[2]**. Normal serum Ca2+ levels range between 8.5 and 10.5mg/dL (2.1 - 2.6mM), of which 4.6 - 5.3 mg/dL (50%) is free ionized Ca2+ (1.15 - 1.33mM), that is, the biologically active form that is maintained under strict hormonal control by vitamin D and parathormone (PTH) **[1,2]**.

The parathyroid glands originate around the 5th gestational week from the developing dorsal endoderm and the 3rd and 4th pharyngeal pouches. The upper parathyroid glands are formed from the 4th, while the lower parathyroid glands are derived from the 3rd pharyngeal pouch **[4]**. Usually, anatomical variations are more frequent in the inferior parathyroid, since during embryogenesis, these migrate caudally in the neck positioning themselves 1 cm from the intersection between the inferior thyroid artery and the recurrent laryngeal nerve **[5]**.

Also, PTH an 84 amino acid peptide synthesized by the main cells of the parathyroid gland, is the foremost minute to minute hormonal regulator of calcemia2 by acting directly on bone metabolism and kidneys and indirectly on the GIT **[3,6,7]**. PTH synthesis and secretion are regulated by the extracellular Ca2+

sensitive receptor (CaSR) found in the main cells of the parathyroid, so the primary stimulus for PTH synthesis and secretion is hypocalcemia **[8]**.

Primary hyperparathyroidism (PHPT) is the 3rd most common endocrinopathy and the leading cause of hypercalcemia. It is characterized by inappropriately high serum PTH levels arising from excessive secretion from one or more parathyroid glands **[9,10]**. It is most commonly seen in women, with the ratio of 3 to 4 women for every man diagnosed with PHPT **[10]**. The most frequent causes are: (a) adenoma located in a single gland is the most common form (75 - 85%); (b) multiple adenomas (2 glands: 2-12%; 3 glands:1-2% - 4; or more: <1-15%), and (c) parathyroid carcinoma which is a rare condition (~1%). From 4 to 16% of PHPT cases stem from ectopic parathyroid **[9,10]**.

The prevalence depends on the population studied and the detection method used. In addition, 70 to 80% of patients are asymptomatic, with nonspecific complaints such as fatigue, depression and cognitive changes **[9,10]**. Usually, hypercalcemia is detected after a long period of abnormally high PTH values, which can generate important clinical changes such as renal diseases (nephrolithiasis and renal failure), bone diseases (bone pain and fibrous osteitis). In addition, severe hypercalcemic conditions can generate neuropsychiatric (psychosis) and neurological disorders **[4]**.

The first assays for PTH detection appeared in the 1960s and were based on the radioimmunoassay (RIA) technique for immunodetection of polyclonal anti-PTH antibodies produced from bovine PTH, human parathyroid adenoma extracts or human PTH fragments, whose results were difficult to interpret due to methodological limitations, especially the low sensitivity and specificity of the method **[2]**. Second-generation tests, also known as intact PTH dosage, use 2 antibodies, one to the N-terminal fragment (1-34) and one to the C-terminal fragment (35-84) of the PTH molecule **[11]**. Although, these second-generation tests, also known as intact PTH detection (iPTH) have allowed for reliable detection of serum plasma PTH levels, the use of cofactors for enzymatic reactions, such as biotin, still allows the interference of external factors such as dietary supplementation as a cause of artifacts capable of generating falsely elevated and/or reduced results.

In this regard, dietary supplementation with vitamin B7 has been associated with changes in the results of hormone detections that utilize enzyme-linked immunosorbent assays (ELISA), including total and free parathyroxine (T4), total and free tri-iodothyronine (T3), thyroid stimulating hormone (TSH), PTH, testosterone, estradiol, and different tumor markers **[6,12]**. The use of biotin in covalent bonds (biotinylation) with different molecules to form complexes is a widely used strategy for PTH detection by ELISA, which can generate interferences in laboratory results. Unfortunately, the assessment of biotin levels in patients with suspected hypo- or hyperparathyroidism is not a routine clinical practice, which can naturally lead to misinterpretation of the results and improper medical conduct.

To report the case of continued use of biotin as a possible cause of PHPT.

Methods

Study Design

The present study was elaborated according to the rules of CARE case report. Available in: https://www.care-statement.org/.

Ethical Aspects

This study was analyzed and approved by the Research Ethics Committee, and obtaining the Informed Consent Form according to CNS/CONEP Resolution 466/12.

Case Report

Patient Information, Clinical Findings, Timeline, Diagnostic Assessment, Therapeutic Intervention, and Follow-up

A female patient, J.T.S.B, 55 years old, married, living in Recife-PE, sought private outpatient endocrinology service on 06/21/2018 with the goal of losing weight, being submitted to anamnesis with the following personal history: obesity and thyroid nodule, this last one being previously discovered by the patient, but without a well-defined timeframe. On physical examination it was detected: thyroid with increased volume on the right, fibroelastic, painless and with a palpable nodule of approximately 02 cm, located in the upper pole of the left lobe of the gland. The patient had been menopausal for two years, without hormone replacement therapy, taking only vitamin D at a dose of 4,000 IU per day.

She denied the use of any other medication called into question in the anamnesis. No other alterations on physical examination. The first medical exam presented, previously requested by another professional, showed a 26-fold increase in serum PTH levels and normocalcemia (Table 1). Biochemical exams were repeated after 2 months with a similar pattern, elevated PTH levels (~14X) and normocalcemia (**Table 1**).

Cervical ultrasonography (USG) identified two nodules; one located in middle third of the right lobe with the following characteristics: 1.1 x 0.4cm nodule, solid, hypoechoic, with well-defined margins, lacking calcifications spots, peripheral vascularization, TR04, and the nodule in the upper pole of the left lobe with size of 1.6 x 0.9cm, solid, isoechoic, with well-defined margins, lacking calcifications spots, central and peripheral vascularization, TR03, without lymph node enlargement. With these findings, parathyroid scintigraphy was requested.

Table 1. Biochemical dosages throughout the clinical investigation.

Laboratory Parameters	Reference Values	06/22/2018	08/04/2018	10/02/2018	11/07/2018	09/21/2019	07/18/2020
PTH	10 – 65pg/mL	1.710 g/mL	902pg/mL	1.900pg/mL	30pg/mL	38pg/mL	25pg/mL
Calcemia	8.6-10.3 mg/dL	9.4mg/dL	9.3mg/dL	9.3mg/dL	9.9mg/dL	9.6mg/dL	9.1mg/dL
Albumin	3.5-5.2mg/dL	3.9g/dL	3.7g/dL	3.7g/dL	3.9g/dL	4.0g/dL	3.9g/dL
Phosphorus	2.5-4.5mg/dL	3.5mg/dL	3.6mg/dL	3.4mg/dL	3.8mg/dL	3.1mg/dL	3.5mg/dL
Magnesium	1.6-2.6mg/dL	1.8mg/dL	1.9mg/dL	1.8mg/dL	1.9mg/dL	1.9mg/dL	1.9mg/dL
25-OH-D	>20ng/mL	35.8ng/mL	33.8ng/mL	31.5ng/mL	39ng/mL	27ng/mL	35ng/mL
Osteocalcin	11-46ng/mL	19ng/mL	19ng/mL	17ng/mL	21.1ng/mL	19.8ng/mL	27.4ng/mL
CTX	<0.650ng/mL	0.503ng/mL	0.472ng/mL	0.650ng/mL	0.310ng/mL	0.442ng/mL	0.628ng/mL
24-hour Calciuria			128.9mg/L			192mg/L	136mg/L
P1NP							81.2ng/mL

Parathyroid hormone Hydroxy-vitamin D25OHD); C-Telopeptide (CTX) procollagen type 1 amino-terminal propeptide

Scintigraphy of the parathyroid glands with 99mTc-Sestamibi showed two areas with an affinity for the tracer in a nodular image posterior to the right lobe and in the inferior pole of the left lobe of the thyroid, suggesting primary thyroid lesion or intrathyroidal hyperfunctioning parathyroid glands. She also presented bone densitometry of the lumbar spine and proximal femur with a diagnosis of osteopenia and total abdominal USG within the normal range.

Because of the chronically high values of PTH, measured in two different laboratories, associated with scintigraphy alteration suggestive of PHPT, it was decided for the patient to repeat the PTH test, evaluating this time the intact molecule, the fine-needle aspiration of the thyroid nodules with an investigation of PTH and thyroglobulin (TG) in the aspirate from the puncture and bone densitometry of the distal radius (33%).

After 4 months, PTH levels persisted high (29X, **Table 1**). Cytology revealed adenomatous goiter in both nodules, positivity for TG, and a negative result for PTH in the aspirate by fine-needle aspiration technique, verified by this exam, which establishes that the nodules originate from the thyroid and not from the parathyroid (**Table 2**). Bone densitometry of the distal radius (33%) yielded normal results.

Table 2. Fine needle aspiration of thyroid nodules.

Laboratory Parameters	Right lobe puncture aspirate	Left lobe puncture aspirate
PTH	<3.0pg/mL	<3.0pg/mL
Thyroglobulin	500ng/mL	81.77ng/mL
Cytopathology of thyroid nodules	adenomatous goiter	adenomatous goiter

The eligibility criteria for parathyroidectomy (**Table 3**) were not met, so the patient remained being monitored as an outpatient.

After detailed investigation and the absence of clinical elements that would justify the pharmacotherapeutic and/or surgical conduct, during

the consultation for body mass evaluation and dietary guidance, the patient reported the use of vitamin B7 (biotin) supplementation of 2.5 mg per day, by recommendation of the dermatologist in the treatment of hair loss and nail strengthening for at least a year. After 15 days of suspension of biotin use, the exams showed normal PTH values (**Table 1**). One and two years after discontinuing the use of biotin, the serum levels of PTH remained within normal limits, as well as the other parameters evaluated (**Table 1**).

Table 3. Criteria for election for surgical removal of the parathyroid (parathyroidectomy).

Symptomatic	Asymptomatic
Nephrolithiasis	less than 50 years of age;
Cystic fibrous osteitis	Serum calcium 1mg above the upper limit;
	Creatinine clearance less than 60mL/min/1.73m^2;
	Osteoporosis at any site assessed in bone desintometry;
	24-hour calciuria above 400mg/24h.

Discussion

Primary hyperparathyroidism (PHPT) is a relatively common endocrine disorder that alters calcium metabolism resulting from hypersecretion of PTH by one or more parathyroid glands **[13]**. The estimated incidence in countries such as the United States of America and the United Kingdom is 27 to 30 patients per 100,000 people per year. The highest prevalence is in women over 50 years of age **[14]**. A Brazilian study showed a ratio of cases in women of 7.1:1 compared with the diagnosis in men **[15]**.

Classic hyperparathyroidism is diagnosed in patients with cystic fibrous osteitis, renal lithiasis, and acute neuropsychiatric syndrome induced by severe hypercalcemia **[15]**. Although the leading cause of hypercalcemia, 90% of patients are asymptomatic **[1,15]**. Furthermore, 0.4 to 6% of asymptomatic PHPT cases are normocalcemic, although they can become hypercalcemic with disease progression **[16]**.

The outpatient dosage of PTH implemented from the 1970s onwards, allowed the diagnosis of PTH in asymptomatic patients, which generated a 4- to 5-fold increase in the number of diagnosed cases **[17]**. In these patients, hypercalcemia and PTH levels are mildly elevated. There is no doubt that outpatient screening of serum calcium and PTH levels are strategic for well-guided clinical practice. However, it is necessary to correlate the clinical signs and symptoms with the laboratory findings to avoid erroneous conduct, especially the possibility of aggravating hypercalcemia and/or the unnecessary surgical removal of the parathyroid gland.

It has been reported that the use of vitamin B7 as a dietary supplement causes inaccuracies in the serum dosage of different hormones, including PTH **[12]**. In a 32-year-old male patient, although he had no clinical signs of thyrotoxicosis, the biochemical findings strongly confirmed the diagnosis of hyperthyroidism **[18]**. Some methodologies for hormone dosage use biotinylated monoclonal antibodies, which can generate interferences and falsely elevated and/or reduced results. In this reported case, the patient was using 100mg of biotin daily. The repetition of the hormone test by a biotinylated antibody-free method confirmed the hypothesis of laboratory artifact, mischaracterizing the diagnosis of Grave's disease.

In this context, Waghray et al **[6]** reported abnormally reduced PTH levels in patients using 1,500 to 5,000μg of biotin per day and with the clinical diagnosis typical of PHPT. After discontinuing the use of vitamin supplementation, laboratory results showed elevated PTH levels consistent with the patient's clinical condition.

The recommended daily intake of biotin is 30μg/day for men and non-lactating women. It is a water-soluble molecule widely found in different foods such as egg yolk, cereals, and vegetables and is essential as a coenzyme for different enzymatic reactions such as glycolysis and neoglycogenesis **[19]**. Severe vitamin B7 deficiency causes orofacial dermatitis, conjunctivitis, alopecia, ataxia, seizures, skin infections, and severe skin dryness **[19]**.

Naturally, biotin supplementation, besides being recommended for patients with partial gastrectomy, epilepsies, pregnant women, and patients with intestinal malabsorption syndromes, is also widely used in the treatment of hair and nail loss and weight loss **[6,19]**. In addition, biotin binds to a variety of molecules without changing their chemical properties, a characteristic that makes it an excellent molecule to be used in various biochemical assays, including serum dosage of hormones, including PTH **[18]**.

For this reason, biotin levels can interfere with 2 types of biochemical assays. In the first, sandwich ELISA, two antibodies (AB) are used to bind to the analyte. The capture AB adsorbed on the plate is biotinylated. Beads of streptavidin (biotin-specific ligand) bind to the biotinylated AB, which generates fluorescence for detection of the analyte. Thus, when there are high levels of biotin in the plasma, there is a saturation of the binding to the streptavidin granules

and a reduction in the fluorescence signal, which generates a falsely reduced result **[18]**.

The other method that can be influenced by serum biotin levels is the competition ELISA which is based on the use of biotinylated antibodies that bind to the analyte and streptavidin on the surface of the plate but should compete with another molecule that is chemically similar to the analyte that can be quantified by fluorescence. If the concentration of the analyte is high in the sample, fewer AB will be bound to the labeled competitor, which generates a weak signal. Thus, if there are high concentrations of biotin in the sample, it will eagerly bind to streptavidin on the plate, which causes less AB binding, which generates a weak signal and falsely elevated results **[12,18]**.

The use of vitamin supplements, prescribed or not, by health professionals is underrated, however, it should become a concern during anamnesis. Prescribed supplements would not be endocrine disruptors or toxic agents, however, one must consider aspects such as abusive, prolonged, and non-prescribed use **[12]**. In this sense, it is necessary to know the dose of biotin used and discontinue it at least 24h before the examination depending on the guidelines of the manufacturer of the ELISA kit for PTH. In addition, one should carefully correlate the laboratory findings with the patient's clinical condition to avoid unnecessary diagnoses or procedures that subject the patient to intense emotional and physical stress.

Conclusion

This patient presented a clinical profile conflicting with laboratory tests that indicated a normocalcemic PTH. After a thorough investigation, it was realized that the reason for the elevated serum PTH levels was prolonged biotin supplementation. Thus, the investigation of the use of biotin before hormone dosage as well as the joint measurement of serum levels of biotin and PTH are important for accurate evaluation and correct diagnosis of primary hyperparathyroidism.

Acknowledgement

Not applicable.

Ethics approval

This study was analyzed and approved by the Research Ethics Committee, and obtaining the Informed Consent Form according to CNS/CONEP Resolution 466/12.

Informed consent

The patient signed the consent form.

Funding

Not applicable.

Data sharing statement

No additional data are available.

Conflict of interest

The authors declare no conflict of interest.

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