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What Is Hypercalcemia? The Importance of Fasting Samples

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Abstract
The differentiation between primary or tertiary (both hypercalcemic) and secondary (normocalcemic) hyperparathyroidism requires the identification of hypercalcemia. Calcium in the blood exists as bound, complexed and ionized fractions. Calcium sensors on parathyroid cells interact only with the ionized fraction (about 50% of the total calcium concentration). Many formulas using albumin, total protein or phosphate to correct or adjust total calcium to reflect the level of ionized calcium may be accurate only within a limited range. In addition, they can introduce errors based on inaccuracies in the measurement of these other metabolites. Clinical conditions, mainly those illnesses affecting acid-base balance, can alter the proportions of bound and free calcium. How and when the blood samples are drawn can alter the level of total calcium. Prolonged standing or prolonged venous stasis causes hemoconcentration, increasing the bound fraction. Preceding exercise can also affect blood calcium levels. Ingestion of calcium supplements or calcium-containing nutrients can cause transient elevations in blood calcium levels lasting several hours, leading to unnecessary further testing. Fasting total calcium levels may be sufficient for monitoring progress. However, for diagnostic purposes, fasting ionized calcium levels should be used. Therefore, for an isolated high total calcium level, we recommend obtaining a repeat fasting total and ionized calcium measurement before further investigations. Hypercalcemia may be diagnosed if there are persistent or frequent total or, preferably, ionized calcium levels >3 SD above the mean of the normal range or if there are progressively rising levels.

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The inclusion of calcium in common chemical screening panels has led to a frequent detection of levels above the normal range, leading to testing for causes of hypercalcemia and measurement of parathyroid hormone (PTH). Only about 1% of body calcium is extracellular, with most of the remaining 99% in bone. In blood, about 50% is bound to proteins, primarily albumin, while the remainder is ionized or 'free'. Some calcium exists in the blood as complexes with anions such as phosphate or sulfate [1]. Because calcium is the most important second messenger in the body [2], regulating metabolic processes in all tissues, the extracellular levels are tightly controlled by an intricate interplay of many factors, predominantly PTH, vitamin D, inorganic phosphate and fibroblast growth factor-23. The ionized fraction reacts with the calcium sensors on parathyroid cells, and PTH levels respond within minutes to any changes.

Elevated PTH may be due to a primary abnormality of the parathyroid gland(s), hyperplasia, adenoma or, rarely, carcinoma. In secondary hyperparathyroidism, the elevated PTH level is a response to decreased ionized calcium, even within the normal range. The most common causes are decreased renal function with increased phosphate retention, vitamin D deficiency or primary renal hypercalciuria. A rarer cause is mild to moderate magnesium deficiency. Severe magnesium deficiency impairs the release of PTH [3]. When all common known causes of secondary hyperparathyroidism have been excluded, it is simply called normocalcemic hyperparathyroidism [4]. Prolonged secondary hyperparathyroidism may lead to autonomous hyperfunction caused by parathyroid hyperplasia or even adenoma formation, so-called tertiary hyperparathyroidism. The differentiation of primary and tertiary (both hypercalcemic) hyperparathyroidism from secondary (normocalcemic) hyperparathyroidism requires the identification of hypercalcemia.

Measurement of Calcium

As stated by Larsson and Magnusson, 'the era has come for elimination of total serum calcium and albumin-corrected calcium in favor of ionized serum calcium in investigations of suspected calcium disturbance' [5]. The previously used color and biological reactions to measure ionized calcium have been replaced by ion-sensitive electrodes that are much more reliable and have analytical coefficients of variation comparable to those of total calcium measurements. Careful attention to technique is important; the process of collecting a sample for ionized calcium should be as 'anaerobic' as possible. Blood should be collected directly into a serum heparinized Vacutainer tube as those containing oxalate, citrate or ethylenediaminetetraacetic acid (EDTA) can decrease the calcium available for analysis due to these chemicals binding to it [6]. If a syringe is used, a premeasured quantity of dry heparin is preferable to manually coating the syringe with liquid heparin, which can dilute the sample and falsely decrease the measured ionized calcium. Even the kind of heparin can alter results as zinc heparin decreases pH and hence overestimates ionized calcium, while lithium heparin causes an underestimation [7, 8]. Incomplete filling of the blood sample tube should be avoided. Blood should be allowed to clot and should be separated by centrifugation, and the serum should be removed anaerobically with a spinal needle attached to a non-air-containing syringe. Samples should be stored at a temperature of 4°C and analyzed within 40 min. These measures are done to avoid carbon dioxide changing the blood pH, the major factor influencing ionized calcium binding to proteins [4, 5, 9]. Prolonged venous stasis and prolonged standing will increase total calcium levels by increasing the bound fraction [10]. Prolonged use of a tourniquet and clenching and unclenching of the fist to increase the suitability of veins

for venipuncture can increase the ionized calcium by 2 and 8%, respectively. Exercise may cause a transient decrease in ionized calcium with a concomitant rise in PTH level [11].

Autoanalyzers in clinical laboratories commonly measure total calcium by photometric measurement of the color intensity of the complex formed between calcium and the reagent *o*-cresolphthalein. Hyperbilirubinemia, whether caused by a hemolyzed sample or illness, can falsely decrease measured total calcium levels. In contrast, marked hyperlipidemia may result in a high calcium reading [12]. Paraproteinemias can cause pseudohypercalcemia (elevated total calcium due to increased bound calcium but normal ionized calcium and no symptoms or signs of hypercalcemia).

Estimation of Ionized Calcium from Total Calcium

The total calcium level is usually corrected or adjusted for changes in the fraction bound to proteins. Since the first attempt by McLean and Hastings [13] in 1935, there have been numerous formulas devised to correct or adjust the total calcium level to account for protein binding. The most frequently used is that proposed by Payne et al. [14] in 1973 to compensate for low albumin levels only: corrected calcium (mg/dl) = total calcium (mg/dl) + 0.8 × [4 – serum albumin (g/dl)]. This was adapted for SI units as: corrected calcium (mmol/l) = total calcium (mmol/l) + 0.02 [40 – albumin (g/l)] [15]. The formula of Payne et al. [14] may underestimate the ionized calcium level in patients with albumin levels higher than 40 g/l [16].

Many other formulas have been proposed. One for use in very elderly hospitalized patients applies total protein concentration [17]. Another, for use in patients with chronic kidney disease, allows for the increase in phosphate [18]. In a study of patients with chronic kidney disease not requiring dialysis, the deviation between simply assuming that half the calcium was bound and thus dividing the total calcium (mg/dl) by 8 gave a closer approximation to the measured ionized calcium (mmol/l) than any of the formulas [19]. However, a formula using total calcium, albumin, total protein and inorganic phosphate had a correlation with the ionized calcium level of 0.953 [20].

Further several formulas have been proposed for use in patients on dialysis. That proposed by Jain et al. [21] had a slightly better correlation with ionized calcium than the Orrell formula, the Clase formula and the conventional formula but still did not significantly outperform the total calcium level. All adjustments require accurate measurement of albumin and/or total protein levels.

Many studies have shown discrepancies between total or corrected total and ionized calcium levels [12]. In one study of 5,490 patients, there was discordance between total and ionized calcium in 12.6% of cases, which was worst with hypercalcemia (49%) and hypocalcemia (92%). Of 143 patients with histologically proven parathyroid disease, 24% had isolated ionized hypercalcemia at diagnosis [22].

Whether acute illness renders calcium measurement unreliable is still debatable [7, 23]. Acidosis causes a rise in the ionized fraction, while metabolic alkalosis can decrease the ionized calcium concentration as much as 0.36 mmol/l [5].

What Is a Normal Calcium Level?

The normal range for total calcium, about 8.6–10.2 mg/dl (2.15–2.54 mmol/l), varies somewhat between laboratories depending on the measurement methodology. By definition, the normal range includes only the range between 2 standard deviations (SD) above and below the mean. This includes 95% of the normal population, leaving 2.5% with higher and

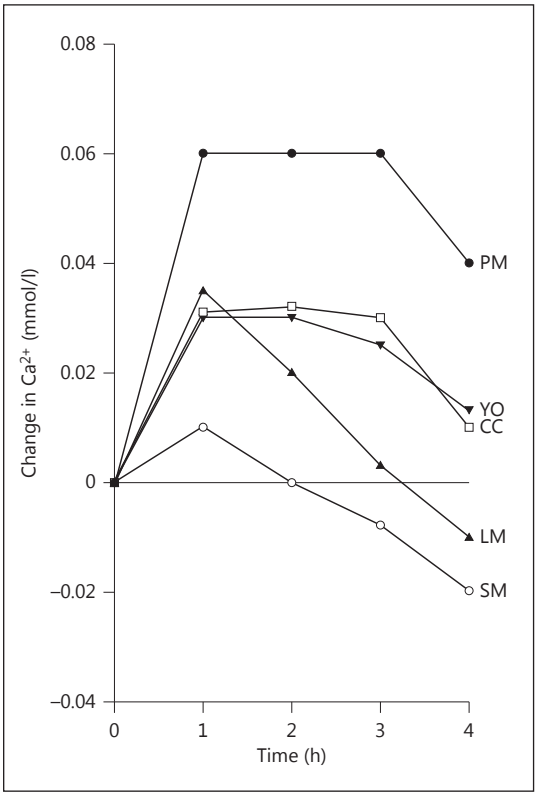

Fig. 1. Changes in serum ionized calcium concentration after administration of soybean imitation milk (SM), or after 500 mg of elemental calcium intake as calcium citrate-enriched powdered milk (PM), yogurt (YO), liquid milk (LM) or a calcium carbonate pill (CC). Compared with baseline, the following results could be found: (1) calcium citrate-enriched PM induced the highest significant increase in ionized calcium concentration ($p < 0.001$); (2) YO and CC induced a significant but lower increase in ionized calcium ($p < 0.05$), and (3) LM induced a significant increase in ionized calcium, but only during the first hour ($p < 0.05$), and then tended to remain elevated at t2 ($p < 0.1$) and to decrease to baseline from t3 to t4 (p = n.s.). Comparison of the area under the curve (AUC) for ionized calcium showed that: (1) after PM intake, the ionized calcium AUC was 51% larger than that after CC intake ($p < 0.01$), 54% larger than that after YO intake ($p < 0.01$) and 70% larger than that after LM intake ($p < 0.001$); (2) after YO/CC intake, the ionized calcium AUC was 37% larger than that after LM intake ($p < 0.05$) [from 25; reproduced with the permission of Springer London].

2.5% with lower values. Using 3 SD above or below the mean would include 99% of the population. This would expand the above range to 8.2–10.6 mg/dl (2.05–2.65 mmol/l). Additionally, even though levels may be within the normal range for the population, an individual's set point may be anywhere within this range, and changes, even within the range, may be reflected by changes in PTH levels.

For the above reasons, hypercalcemia has often been defined as an increase in total serum calcium of at least 1 mg/ml (0.25 mmol/l) above the normal range [2], which leaves a 'gray zone' above the upper limit of the normal range.

Temporary Hypercalcemia

There have been numerous studies investigating the effect of ingestion of various dairy foods and calcium supplements on increasing serum and urine calcium and suppressing PTH levels. The ingestion of five different calcium supplements all caused a rise in serum calcium, with calcium carbonate and citrate showing the greatest effect on decreasing PTH levels, calcium gluconolactate having a delayed effect and calcium pidolate and ossein-hydroxyapatite complex causing no detectable PTH suppression [24]. The amount and duration of increase depend on both the type of supplement and the dose. For calcium carbonate, the rise in ionized calcium 3 h after ingestion of 500 mg averaged 0.3 mmol (about 2.5%) [25]; 600 mg caused an increase in total calcium of about 4.4% [26], while after ingestion of 1,000 mg, serum calcium increased by 6.4–8.1% [27]. In a study comparing 1,200 mg calcium as skim milk powder fortified with calcium carbonate or milk calcium, serum calcium after the preparation containing the calcium carbonate remained higher between 2 and 8 h [28]. The

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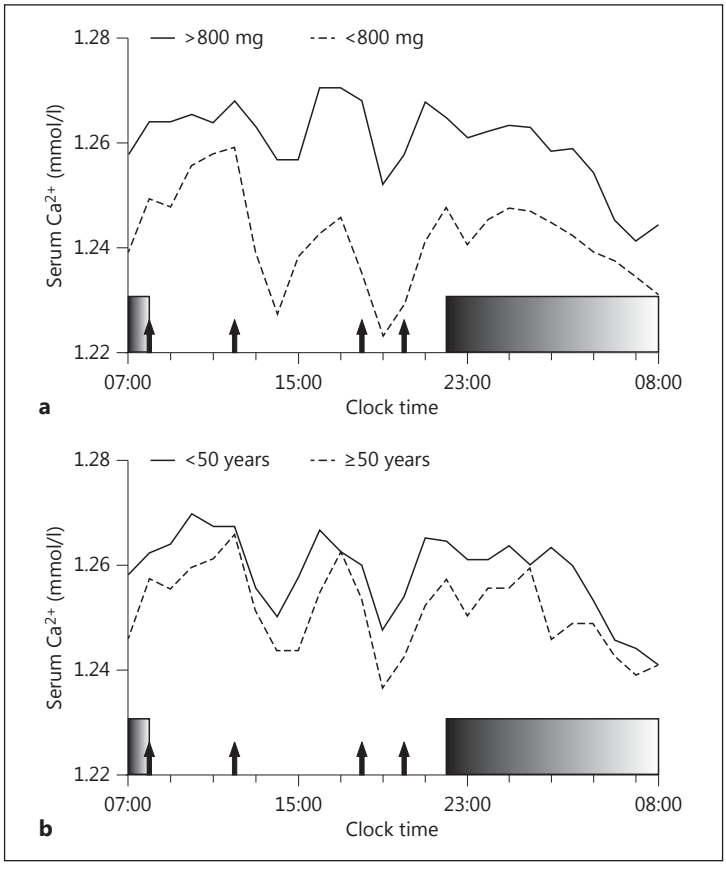

Fig. 2. a Serum iCa rhythm in women consuming more than 800 mg calcium on the day of the study (n = 15) and in women consuming less than 800 mg calcium (n = 10). **b** Mean serum iCa levels in the menopausal women (≥50 years; n = 10) and in the remaining premenopausal subjects (<50 years; n = 15). Arrows indicate the times of meals, and the stippled horizontal bars indicate the period of recumbency [from 32; reproduced with the permission of the Endocrine Society].

concomitant ingestion of calcium and phosphate as tricalcium phosphate causes an increase in serum phosphate without affecting the changes in serum calcium or PTH [29, 30]. There were quantitative differences between the effectiveness of different supplements. Generally, the greatest increases in serum calcium were seen after bone meal powder [23], calcium citrate or calcium carbonate. Even with the consumption of dairy products, yogurt and milk showed different effects (fig. 1) [23].

The inclusion of calcium in a mixed meal had similar effects on increasing serum calcium, whether the standard breakfast contained calcium carbonate, milk or orange juice fortified with calcium citrate malate [31]. In addition, a study on the circadian rhythm of PTH showed definite increases in serum ionized calcium levels after meals (fig. 2) [32]. Therefore, for any investigation of abnormalities in calcium metabolism, blood samples should be drawn in the fasting state.

Conclusion and Recommendations

Interpretation of blood calcium concentration requires care in the timing of sample collection in relation to calcium intake and exercise, the method of collecting the sample and the assay procedure. Fasting total calcium levels may be sufficient for monitoring progress. However, for diagnostic purposes, fasting ionized calcium levels are preferable. Ingestion of calcium supplements or calcium-containing nutrients can cause transient elevations in blood calcium levels lasting several hours, leading to unnecessary further testing. Therefore, for an

isolated high total calcium level, we recommend obtaining a repeat fasting total and ionized calcium measurement before further investigation. Hypercalcemia may be diagnosed by persistent or frequent total or, preferably, ionized levels >3 SD above the mean and may be suspected if there are progressively rising levels.

Disclosure Statement

The authors have nothing to disclose.

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