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Calcium

Is Ionized Calcium Always Right and Total Calcium Always Wrong?

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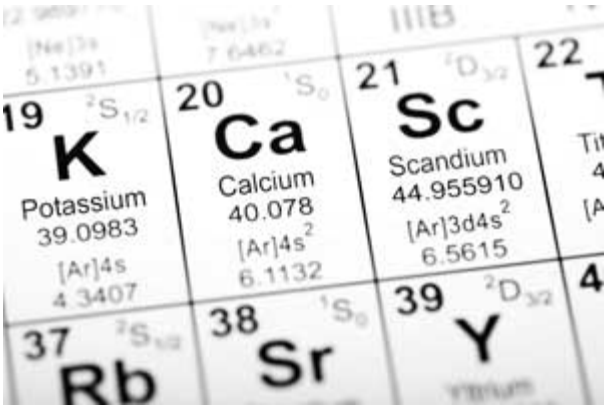

Since McLean and Hastings' pioneering studies on frog heart contraction in the early 1930s (1), ionized calcium has been regarded as the physiologically active form of calcium. Over the years, studies have shown that the movement of calcium ions into and out of cells is related to the most important physiologic functions, such as heart and smooth muscle contraction, hormone regulation, and intracellular second messenger signals received on the cell surface that are relayed to target molecules in the cytosol or nucleus.

However, monitoring patients' calcium status is not straightforward because both ionized and total calcium measurements are commonly available for clinical interpretation and each has advantages and disadvantages. Total calcium is more convenient to measure than ionized calcium, and therefore, more frequently measured by clinical laboratories. Total calcium includes ionized calcium plus physiologically inactive calcium bound to various anions, mainly albumin, as well as small anions such as bicarbonate, citrate, and lactate. Consequently, experts consider total calcium a less accurate measure of calcium status. When a patient's albumin or total protein (TP) levels are abnormal, the presumption is that total calcium becomes a flawed estimator of calcium status.

Nevertheless, total calcium has been, and will likely remain, the predominant type of calcium measured in clinical practice. Some clinicians are accustomed to using algorithms to correct total calcium to its presumed value at normal albumin and TP levels and to calculate ionized calcium concentration from total calcium values. Some laboratorians might say that clinicians are trying to force the "wrong" total calcium to agree with their "right" clinical judgement. This review will focus on the relationship between ionized calcium and total calcium and a rational approach to interpreting these results.

Calculating Corrected Calcium Values

In 1978, Ladenson and colleagues evaluated 13 published equations for correcting total calcium and concluded that none substantially improved agreement with ionized calcium compared to uncorrected total calcium (2). I thought this report would end the use of correction equations; however, that has not

proven to be the case. Some 26 years later in 2004, Dickerson and colleagues published an updated report and reached the same conclusion (3). Despite these reports, correcting total calcium results to mimic ionized calcium still persists in clinical practice, and reports of new equations still occasionally appear in the literature.

Clinical labs use several equations to calculate corrected calcium values. Some involve the patient's albumin level, while others use TP. A summary of these equations was published in 2004 (3). Some of them attempt to calculate ionized calcium concentration directly from total calcium and other parameters. A selection of four representative equations illustrates their remarkable diversity (Table 1).

Table 1
Examples of Equations Used to Calculate Calcium

1	**total calcium – 0.707 x (albumin – 3.4) = corrected calcium** This is a typical equation for calculating corrected total calcium using total calcium (mg/dL) and albumin (g/dL).
2	**total calcium / (0.6 + 0.05 x total protein) = corrected calcium** This is another typical equation for calculating corrected total calcium from total calcium (mg/dL) and total protein (g/dL).
3	**0.25 x [0.9 + (0.55 x total calcium) – (0.3 x albumin)] = ionized calcium** This equation calculates ionized calcium (mmol/L) from total calcium (mg/dL) and albumin (g/dL).
4	**total calcium – (0.00613 x total calcium x albumin) – (0.00244 x total calcium x globulin) – (0.0043 x total calcium x AG) – (0.00375 x total calcium x HCO3) = ionized calcium** This equation calculates ionized calcium (mmol/L) from six measurements: total calcium (mmol/L); albumin (g/L); globulin (total protein – albumin; g/L); anion gap (AG; mmol/L); and bicarbonate (HCO3; mmol/L).

Of particular note is equation 4 that requires six laboratory measurements to calculate ionized calcium. I clearly recall my initial reaction to this equation when it was published in 1989 (4). I thought the researchers made a brave attempt to come up with an equation that included several variables related to calcium binding. On the other hand, I remember thinking that if the lab has to measure six analytes to calculate the desired one, maybe it's time to simply measure the desired one.

I also have reviewed several journal submissions over the past few years that have proposed new equations to correct total calcium values. While I have tried to be very generous in my reviews, most of these reports have ultimately been rejected. In one instance, the authors proposed a correction equation that would need yearly revalidation and did not consider evaluating patients being seen in endocrinology, hematology, oncology, or nephrology clinics. This would make the equation virtually useless because patients at these clinics are exactly the ones who are most likely to have abnormal calcium and/or protein levels, and who would have less access to an ionized calcium measurement.

What's Wrong with These Correction Equations?

There are several inherent problems with using equations to correct total calcium measurements. First, the equations either ignore or make assumptions about complex equilibria in vivo between calcium ions and anions of various sizes and affinities. In addition to albumin and protein, a patient's calcium equilibrium is sensitive to pH, bicarbonate, citrate, lactate, and phosphate concentrations. Furthermore, the correction equations are sensitive to the effects of analytical variations of calcium, albumin, protein, and any other analyte used in the calculation. This point was clearly illustrated in a report showing that the differences in albumin results using bromcresol green versus bromcresol purple methods accounted for significant differences in corrected calcium results (5).

Another reason that correction equations are problematic is that the "correctness" of any result depends on relevant reference ranges. Consequently, any imbalance between ionized and total calcium reference ranges changes the number of discrepant results. In addition, specimens containing decreased levels of both total calcium and albumin are relatively common. As described above, these specimens would appear to have normal ionized calcium or normal corrected calcium values according to the results of the correction equations. But such individuals often show decreased ionized calcium levels. In fact, corrected calcium values actually underestimate the prevalence of hypocalcemia (6). Finally, physicians often use calcium correction equations in a very selective manner, usually when they feel that the total calcium value is either in error or does not agree with their clinical judgement.

Why Not Only Measure Ionized Calcium?

Other factors also influence why total calcium continues to be a part of the clinical chemistry armamentarium. After a blood specimen is collected, the ionized calcium concentration changes even more than the total calcium concentration. Take, for example, anticoagulated blood specimens. Clearly, the strong calcium ion chelators such as citrate and EDTA radically alter the ionized calcium and affect the total calcium measurement. Ordinary heparin from lithium or sodium salts is an anion that also binds calcium ions. While this effect was formerly a significant concern for clinical analysis, the use of modern balanced or rapidly dissolving minimal heparin preparations in blood collection syringes has largely eliminated it, especially if the collection tube or syringe is filled to capacity.

Once collected, the pH of a blood sample also can decrease from cell metabolism or increase due to loss of CO2 if the specimen is exposed to air. Because pH affects the binding of calcium ions to albumin, ionized calcium values change inversely to pH, by approximately 0.05–0.06 mmol/L/0.1 pH change (7). Another little appreciated fact is that the clotting process affects pH in an unpredictable manner (8); therefore, even in serum with no anticoagulant, the ionized calcium concentration may be affected.

Finally, the analytical instruments used by clinical laboratories to measure ionized calcium are relatively limited in their test menu. The blood gas/electrolyte analyzers typically used in clinical labs to measure ionized calcium are based on electrochemical methods that are not well-suited to providing standard chemistry panels that include enzymes, proteins, bilirubin, lipids, and renal function tests. A few blood gas analyzers, however, do measure urea and creatinine on whole blood by electrochemical methods.

Clinical Usage of Ionized Calcium

Calcium levels are important, and even critical, in several clinical situations (9). For example, changes in blood electrolyte concentrations are common during major surgical operations involving the heart, liver, and abdomen. Clinicians often request ionized calcium tests during these procedures in order to monitor patients' blood levels and to decide if supplementation is necessary. The combined effects of giving patients heparin, citrate, bicarbonate, drugs, protein solutions, as well as alterations in blood pH and body temperature, can significantly affect concentrations of calcium in blood. Because these conditions frequently cause wide differences between total and ionized calcium concentrations, total calcium measurements are of little use in these circumstances.

Critically ill patients in the intensive care unit (ICU), such as those with infections and sepsis, pancreatitis, burns, or major trauma, are highly susceptible to hypocalcemia. Because of its importance in maintaining cardiac output, arterial pressure, and systemic vascular resistance, adequate ionized

calcium concentrations are especially important. Patients with sepsis commonly have decreased blood ionized calcium concentrations, possibly related to the production of various cytokines that affect calcium regulation.

Critically ill patients, especially those with an infection, may have low or suppressed parathyroid hormone (PTH) levels related to increased levels of tumor necrosis factor, interleukin-6, and C-reactive protein. Total calcium measurements frequently have little meaning in these patients because serum protein concentrations are reduced, acid-base disturbances are common, and citrated-blood products may have been given. For patients who require calcium supplementation, however, measuring ionized calcium is helpful for guiding proper dosage.

For neonates, clinicians prefer measuring ionized calcium to follow calcium status. Neonatal hypocalcemia appears to be a normal occurrence during the first week of life and may act as a stimulus to induce the infant's parathyroid glands to become functional. Typically, calcium supplementation is not necessary or even beneficial; however, in preterm infants or neonates with severe or prolonged hypocalcemia, calcium supplementation may be necessary.

Is Total Calcium Always Wrong?

Before concluding that total calcium is an outdated laboratory pariah that should be banished to the laboratory scrap heap with other formerly glorious tests like lactate dehydrogenase and CK isoenzymes, allow me to provide a counterpoint view.

One interesting study monitored total calcium of both survivor and non-survivor ICU trauma patients (10). While both groups showed an expected decline in total calcium at day 2, after that, total calcium values returned to normal in survivors and remained low in non-survivors. Although ionized calcium was not measured, these results suggest that total calcium was an appropriate indicator of calcium status in trauma ICU patients.

For assessing PTH results in patients with hyperparathyroidism, hypoparathyroidism, malignancy, or renal disease, ionized calcium measurements paired with PTH measurements are preferred for interpreting the PTH result. But because specimens for PTH are often collected in clinics that may be located distant to the test site for PTH and ionized calcium, total calcium remains an acceptable alternative to ionized calcium for interpreting PTH results.

Where Does All This Leave Us?

Over the years, studies have shown that corrected total calcium is an unreliable estimator of patients' calcium status as assessed by ionized calcium (2,3). On average, corrected calcium results are not clearly better than just total calcium.

The problems surrounding total calcium values are augmented by the fact that studies comparing total calcium to ionized calcium make the assumption, albeit a reasonable one, that ionized calcium is always the "right" calcium result. Total calcium can therefore never be "right" when it disagrees with the ionized calcium value. A more objective assessment would be to blindly review cases of the patients having discrepancies between total and ionized calcium measurements. This can be a frustrating endeavor, because such case reviews often do not contain the type of "calcium-focused" workups that calcium aficionados would like to see.

In reports that have looked at the discrepancies between total and ionized calcium, a consistent finding is that the values agree with regard to reference range in about 75% of the cases. With the exception of samples collected from patients with high citrate levels from blood transfusions, it is rare to find total calcium and ionized calcium results that are widely discrepant. One example would be a patient who has an obviously elevated ionized calcium level while his total calcium is clearly decreased. This agreement between total and ionized calcium is even more frequent in healthy individuals and in many clinic patients with normal albumin/protein, fluid, and acid-base status. In the absence of gross mishandling, the main

collection error for total calcium is when the phlebotomist leaves the tourniquet in place for an excessively long period of time, thereby elevating total calcium by increasing the protein/albumin levels.

It seems reasonable to conclude that total calcium will peacefully coexist on lab menus with ionized calcium and that total calcium will remain a reliable screening test for calcium abnormalities. Total calcium measurements are relatively cheap, readily available, and more resistant to sample transportation variables. When the clinical stakes are high, however, such as when a clinician must decide whether a patient needs parathyroid surgery or whether a patient should receive calcium supplementation in a critical care situation, measuring ionized calcium is worth the extra cost and effort.

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