Calcium

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Contribution: Jessica Farebrother, Damon Parkington, Kerry Jones, Ann Prentice

Dr Jessica Farebrother Dr. sc. ETH Zurich, OpeN-Global Team
With:
Damon Parkington, Dr Kerry Jones PhD, OpeN-Global Expert Partners
NIHR BRC Nutritional Biomarker Laboratory, University of Cambridge, Cambridge UK
Website: http://www.mrc-epid.cam.ac.uk
Professor Ann Prentice OBE PhD, OpeN-Global Expert Partner and Steering Committee.

Contact: For questions on calcium assessment, please contact:
OpeN-Global team via the website at https://open-global.kcl.ac.uk/contact/

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Related nutrients/biomarkers: Magnesium, Phosphorus, Vitamin D, Vitamin K, Iron, Fluoride

Importance of calcium for health

Calcium is a fundamental nutrient that plays a central role in the skeleton and in a wide range of essential body functions, including nerve impulse transmission, vascular and muscular contraction, blood coagulation, hormone secretion, and intercellular adhesion (1, 2). It is most often associated with its role in bone mineral disorders such as osteoporosis and rickets.

Why measure population calcium status? Identifying populations, or sub-groups within populations, who are consuming or at risk of consuming inadequate calcium intakes is necessary to develop public health policy to reduce the incidence of osteoporosis.

Calcium homeostasis

Calcium is the fifth most abundant element in the human body (3); 99% is present in the skeleton and teeth. It is complexed with phosphorus in the molecule hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which provides strength for bones to support movement, and a reservoir with which to regulate blood calcium levels (3, 4) through liberation from bone. Otherwise, the only true source of calcium for humans is from the diet; it cannot be made in the body.

Calcium homeostasis is tightly regulated via three major transport systems: intestinal absorption, renal reabsorption and bone turnover (3, 4). Calcium homeostasis relies on an intricate mechanism involving parathyroid hormone (PTH), calcitonin, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), ionised calcium and the calcium sensing receptor (5). Extracellular calcium concentrations are retained at around 2.5 mmol/L, irrespective of bone calcium status and dietary intake. A diet deficient in calcium does not necessarily cause low blood calcium concentrations but instead results in the liberation of calcium from bone to maintain blood calcium homeostasis (6).

Calcium requirements

Calcium requirements are determined by the relationship between intestinal absorption, excretion (in urine, faeces, sweat, saliva, skin, hair and nails) and accretion for bone growth and development. In adults, a calcium balance is achieved when the rate of calcium absorption is equal to excretion, thereby preserving the skeleton from calcium depletion. In children and adolescents calcium is required to cover skeletal growth requirements until peak bone mass is achieved (7).

Sources of dietary calcium

Dairy products contribute over 50% of dietary calcium in some diets, and small fish such as sardines, provide a rich source especially if consumed whole (including the bones). Mineral-rich drinking waters may provide substantial contributions to calcium intakes in some areas (2). However, dietary phytic or oxalic acid content may negatively influence calcium absorption (8). Similarly, some plant foods, particularly green leafy vegetables and legumes also contribute to dietary calcium intakes, but their actual contribution to dietary calcium is impacted by impaired intestinal absorption if dietary oxalates (found in spinach, sweet potato, rhubarb, beans) or phytates (found in unleavened bread, raw beans, seeds, nuts, grains, soy isolates) are concurrently high (9). Calcium absorption from rice, a staple crop for many global populations, is particularly affected (8).

Globally, there is a general lack of data on population calcium intakes. A systematic review of dietary assessment (recall or food frequency questionnaires) data on global calcium intakes published in 2017 (10) found that 75% of the available data were not nationally representative, and 123 countries had no data (fitting review inclusion criteria) on population or population sub-group calcium intakes. However, this review does illustrate the variation in calcium intakes worldwide, in that some regions have higher intakes compared to others; largely due to the consumption of cow's milk and milk products.

Risk of calcium deficiency

Calcium deficiency can be caused by:

- *Low calcium intakes*, due to unavailability or avoidance of certain food groups, e.g. cow's milk and other dairy produce made from cow's milk;

- *Impaired intestinal calcium absorption* due to interactions between the calcium content of foods and dietary phytates or oxalates (see above);

- *Low calcium absorption*, due to increasing age , physiological disorders such as hypochlorhydria (11), or vitamin D receptor disorder in the gut (12);

- *Increased calcium excretion* due to elevated protein or sodium intakes (12).

Severe hypocalcaemia (serum calcium <1.9 mmol/L) is a rare phenomenon that may cause cardiac arrhythmias leading to emergency hospital admission (13). It is not typically linked to dietary intakes (unless in the severely malnourished); instead the most likely causes are hypoparathyroidism, chronic kidney disease and vitamin D deficiency (13, 14), or malabsorption e.g. in coeliac disease and/or in particular, where malabsorption of vitamin D is concomitant (14).

In healthy populations, a lower calcium absorption will usually be met with a lower urinary calcium excretion, thereby maintaining calcium homeostasis. In the case of mild calcium deficiency, study results reporting on the health benefits of calcium supplementation that are often reported in the media, were found by a thorough review by the Institute of Medicine to be mixed and inconclusive (7).

Very low calcium intakes are also associated with non-infantile rickets, either alone or together with low, but not necessarily deficient, vitamin D status (15).

Calcium supplementation

Though calcium is an essential element in the diet, the use of supplements to maintain optimal intakes, and thereby prevent osteoporotic fractures, is controversial. Most studies show little evidence of a relationship between calcium intake, bone density or fracture prevention (16). Instead, calcium supplements in older people likely reduce bone loss through effects on bone remodelling rather than restitution of an underlying calcium deficiency (16).

Long-term calcium supplementation use may be associated with cardiovascular disease (17) and may increase the risk of non-oxalic kidney stones (16, 18).

Calcium excess

An excessive dietary intake of calcium is uncommon, although may rarely occur through excessive use of calcium containing supplements. "Milk-alkali" syndrome has been reported in response to high doses of calcium carbonate, with symptoms including renal failure, metabolic acidosis and precipitation of calcium salts in renal tissue (19). However, it has also been suggested that this phenomenon may equally be associated with the carbonate salt as the calcium intake itself (19). Under normal circumstances of calcium supplement use, calcium absorption rises slowly, negating the risk of hypercalcuria.

The principal causes of hypercalcaemia are hyperparathyroidism, malignancies especially with bone metastases, reduced renal excretion due to certain medications e.g. lithium or thiazide diuretics, and increased intestinal absorption of calcium secondary to increased vitamin D activity (20).

Human biomarkers of calcium status

Due to the complexity of calcium metabolism in the body and the tight homeostatic regulation of circulating calcium concentrations, **biochemical markers of calcium do not reflect calcium status.**

Clinical measurement of calcium in urine, serum or plasma is used in the diagnosis of parathyroid disease, bone disorders, chronic renal disease or defects in the uptake of calcium from the intestine. In disease states, calcium concentration may be either higher or lower than normal, with normal levels being highest in children and declining gradually through life.

Total calcium in serum/plasma or urine:

Serum calcium exists in three forms: i) free calcium ions, Ca^{2+}, at about 50% of the total, ii) protein bound calcium, at 45% of the total, and iii) complexed calcium, mainly with citrate, at about 5% of the total. The ionised calcium is physiologically most significant and indicates disturbances in calcium metabolism.

Total calcium can be measured in serum or lithium heparin plasma. Calcium cannot be measured in EDTA plasma and anticoagulants other than lithium heparin should be avoided because of complexing or precipitating the calcium. Urine calcium is also commonly measured.

Total calcium in serum/plasma or urine, is routinely measured using spectrophotometry on a clinical chemistry analyser; reagent kits are supplied ready to use and the most common methods are the o-Cresolphthalein Complexone method and the Arsenazo-III method.

Serum/plasma total calcium concentration is stable for 7 days at room temperature (15-25°C), about 22 days under refrigeration (2-8°C) and for longer than 1 year frozen at -20°C, provided no sample evaporation has occurred. Calcium was found to be stable in serum, for ≤3 freeze-thaw cycles at -20°C (21).

Because a large fraction of circulating calcium is bound to albumin, fluctuations in albumin concentration may have a profound effect on serum/plasma calcium concentration. Therefore, adjustment for albumin may be considered (22). Albumin is reduced in malnutrition and is also a negative acute phase marker (i.e. is reduced in infection), which can affect total calcium concentrations.

Acidification of urine samples is often recommended for the assessment of urinary calcium (and other minerals) to prevent precipitation and formation of calcium crystals. Whilst recommended, recent data suggest that acidification may not be essential in a clinical setting (23, 24). Acid may be added to the collection bottles or laboratory aliquots at a concentration of 10 ml/L of hydrochloric acid. Contamination needs to be carefully avoided by using new or acid-washed collection vessels.

Urinary calcium is stable for 5 days at room temperature (15-25°C), for 5 weeks under refrigeration (2-8 °C) and for 6 months frozen at temperatures up to -20°C (25). Long term storage for 15 y at -22°C did not affect calcium concentrations in urine (26).

Ionised (free) Calcium

It is possible to measure ionised (free) calcium only. Measurements of ionised calcium are considered more clinically relevant than total calcium, this is because it is the physiologically active form and unlike total calcium it is not affected by fluctuations in albumin concentration (22).

Ionised calcium in whole blood is measured by ion-selective electrode on specialised electrolyte or blood gas chemistry analysers (e.g. from Novamedical or Radiometer). In a clinical setting, it is more common to measure ionised calcium using a blood gas analyser at the point of care. Ionised calcium can be measured in heparinised whole blood. Blood should be drawn anaerobically, mixed per protocol, placed on ice and measured with 30 min. Changes in pH have a significant effect on ionised calcium and samples should be measured soon after collection. As the collection protocol for ionised calcium is very restrictive and does not allow for the use of stored samples it is often more practical to measure total calcium.

Other biomarkers of calcium status:

Parathyroid hormone (PTH) and 1,25$(OH)_2$D are elevated when there is increased calcium demand or when calcium intakes are low (27). Calcitonin also has a role in maintaining serum calcium (28).

Bone density measurements (e.g. by dual X-ray absorptiometry, computerized tomography or quantitative ultrasound) may be used as long-term indicators of the adequacy of calcium intake, and are often combined with dietary assessment of calcium intake (assessed via questionnaire) (16).

Bone turnover markers (e.g. bone-specific alkaline phosphatase or osteocalcin) may also provide insight into relative calcium requirements. Metabolic adaptations to pregnancy and lactation will affect interpretation of calcium and bone-related biomarkers (29).

Several further novel metabolites from urinary metabonomics analysis of calcium-deficient and normal rats have been proposed as biomarkers of calcium deficiency (30) but these have yet to be validated as indicators of calcium status of use for human population assessment.

SOPs:

Total serum or urinary calcium is most-commonly measured using commercial clinical chemistry analysers (e.g. Siemens, Beckmann, Abbott etc.)

Flame photometry, atomic absorption spectroscopy (AAS) or ICP-MS may also be used. AAS and ICP-MS have high levels of precision and have been both been used as reference methods.

ICP-MS: https://www.nist.gov/sites/default/files/documents/srm/SP260-162-2.pdf

Standard reference materials

Standard Reference Materials (SRM) are available from the National Institute of Standards and Technology (NIST): 956c Electrolytes in Frozen Human Serum

See https://www.nist.gov/sites/default/files/documents/srm/SP260-162-2.pdf p 2-3.
To order SRM from NIST: https://www-s.nist.gov/srmors/

Quality control material is also available from commercial suppliers of kits.

Accreditation schemes

Several clinical accreditation schemes include calcium and ionised calcium:

NEQAS provides External Quality Assessment/Proficiency Testing for all major aspects of clinical laboratory testing. Calcium in human serum
https://ukneqas.org.uk/programmes/result/?programme=clinical-chemistry

and urine https://ukneqas.org.uk/programmes/result/?programme=urine-chemistries

Randox RIQAS General Clinical Chemistry EQA programme is designed to monitor the performance of up to 52 parameters https://www.randox.com/clinical-chemistry-eqa/
Total and ionised calcium.

WEQAS, one of the largest External Quality Assessment providers in the UK, with over 50 years of experience in delivering global Quality Assurance Programmes in Laboratory Medicine:
http://www.weqas.com/services/eqa/urine-chemistry/

For further details on laboratory accreditation, see the dedicated OpeN-Global page on Quality control and lab accreditation; https://open-global.kcl.ac.uk/accreditation/

Technical assistance

For questions on methods of calcium assessment or for technical assistance, please contact the OpeN-Global team at https://open-global.kcl.ac.uk/contact/

Useful links

Institute of Medicine, National Academies Press: Dietary Reference Intakes for calcium and vitamin D (2011): https://www.nap.edu/catalog/13050/dietary-reference-intakes-for-calcium-and-vitamin-d
Associated Report Brief: http://www.nationalacademies.org/hmd/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Report-Brief.aspx

WHO “Keeping fit for life: meeting the nutritional needs of older persons”:
http://apps.who.int/iris/bitstream/handle/10665/42515/9241562102.pdf?sequence=1

International Osteoporosis Foundation: https://www.iofbonehealth.org

NIST: https://www.nist.gov/sites/default/files/documents/srm/SP260-162-2.pdf page 2-1

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