



# Tumor markers

## Calcitonin

Analyte Information



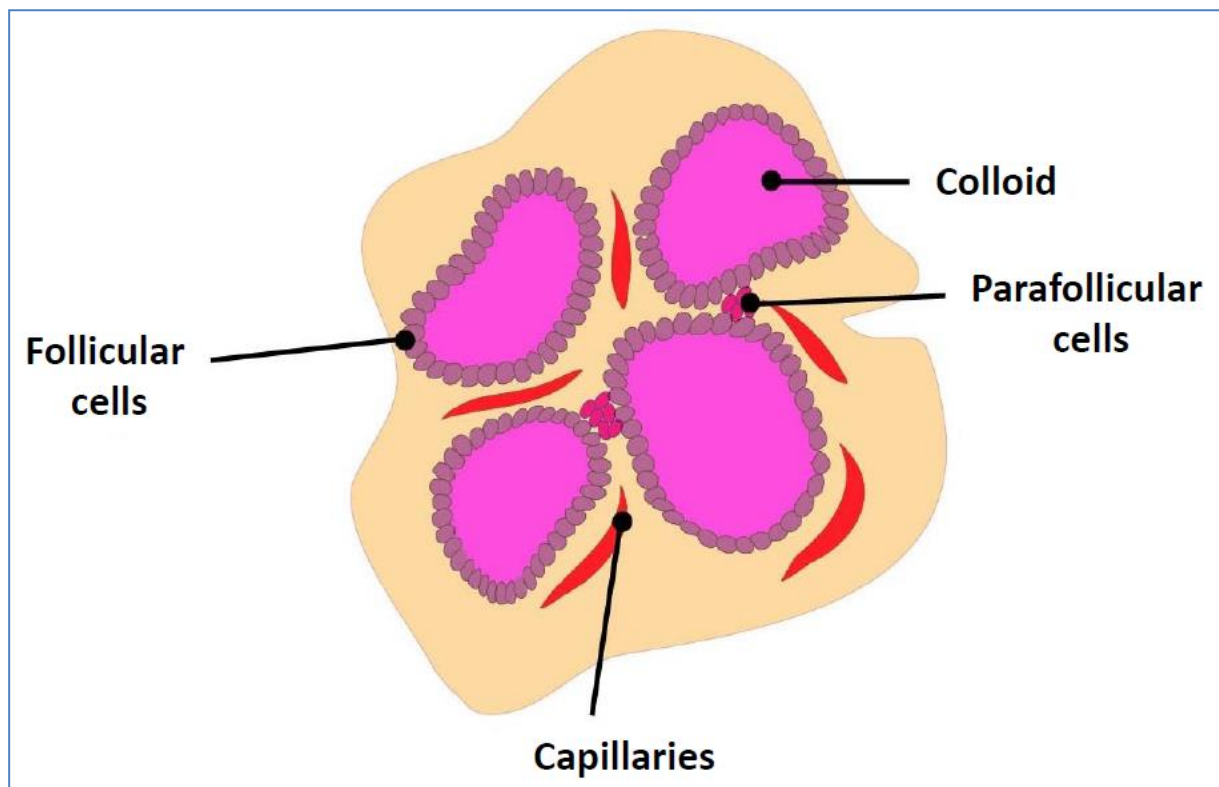


## Calcitonin

### Introduction

Calcitonin (CT) is secreted by the parafollicular cells (Fig.1) in the thyroid gland, and in ultimobranchial gland which is present in the neck region in many animals. In humans, the ultimobranchial gland is an embryological structure that gives rise to the calcitonin-producing cells (parafollicular cells or C cells) of the thyroid gland. Calcitonin has been found in a diversity of fish, reptiles, birds, and mammals, including humans. Species differences in amino acid sequences are small. Calcitonin belongs to the calcitonin-like protein family. It acts to reduce blood calcium ( $\text{Ca}^{2+}$ ), opposing the effects of parathyroid hormone (PTH). However, its precise physiological role in bone metabolism is not yet fully understood.

**Fig.1: Parafollicular cells in thyroid gland**





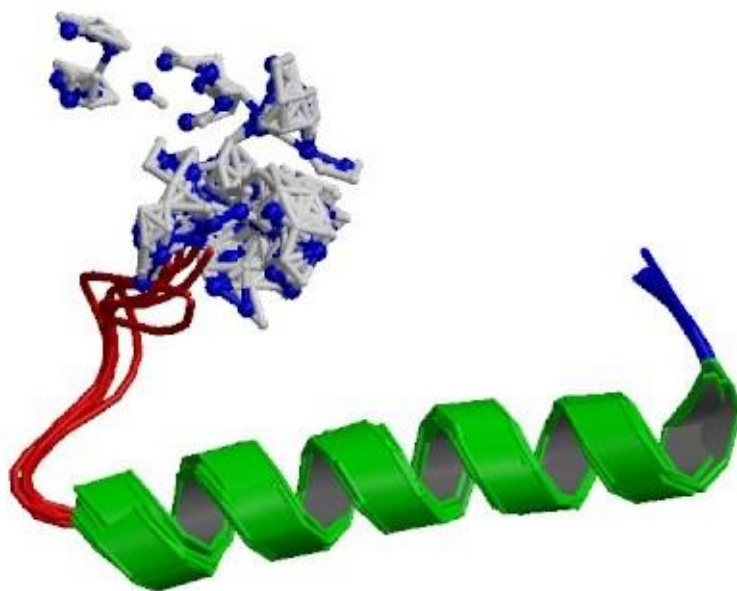
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## Metabolism

Calcitonin is a small linear polypeptide of 32 amino acids and a molecular weight of 3.5 kDa. In humans, calcitonin (Fig.2) is produced by the parafollicular cells in the thyroid gland. Calcitonin is formed by the proteolytic cleavage of a larger prepropeptide, which is the product of the CALC1 gene (CALCA). The CALC1 gene belongs to a superfamily of related protein hormone precursors including islet amyloid precursor protein, calcitonin gene related peptide, and the precursor of adrenomedullin. Calcitonin is cleaved from a prohormone that also contains two other peptides, katalcalcin and calcitonin gene related peptide (CGRP). These peptides are secreted in ratio 1:1 with calcitonin.

Biological half-life of calcitonin in the blood is 12 minutes.

**Fig.2: Calcitonin structure and amino acid sequence of 32 amino acids in human calcitonin molecule**



**Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro**

Source: Andreotti G, Méndez BL, Amodeo P, Morelli MA, Nakamuta H, Motta A. Structural determinants of salmon calcitonin bioactivity: the role of the Leu-based amphipathic alpha-helix. J Biol Chem. 2006 Aug 25;281(34):24193-203, 2006.



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The full spectrum of calcitonin action is not completely understood. The most prominent factor controlling calcitonin secretion is the extracellular concentration of  $\text{Ca}^{2+}$ . Elevated blood calcium levels strongly stimulate calcitonin secretion, and secretion is suppressed when calcium concentration falls below normal. A number of other hormones have been shown to stimulate calcitonin release in certain situations, and nervous controls also have been demonstrated.

### **Physiological function of calcitonin**

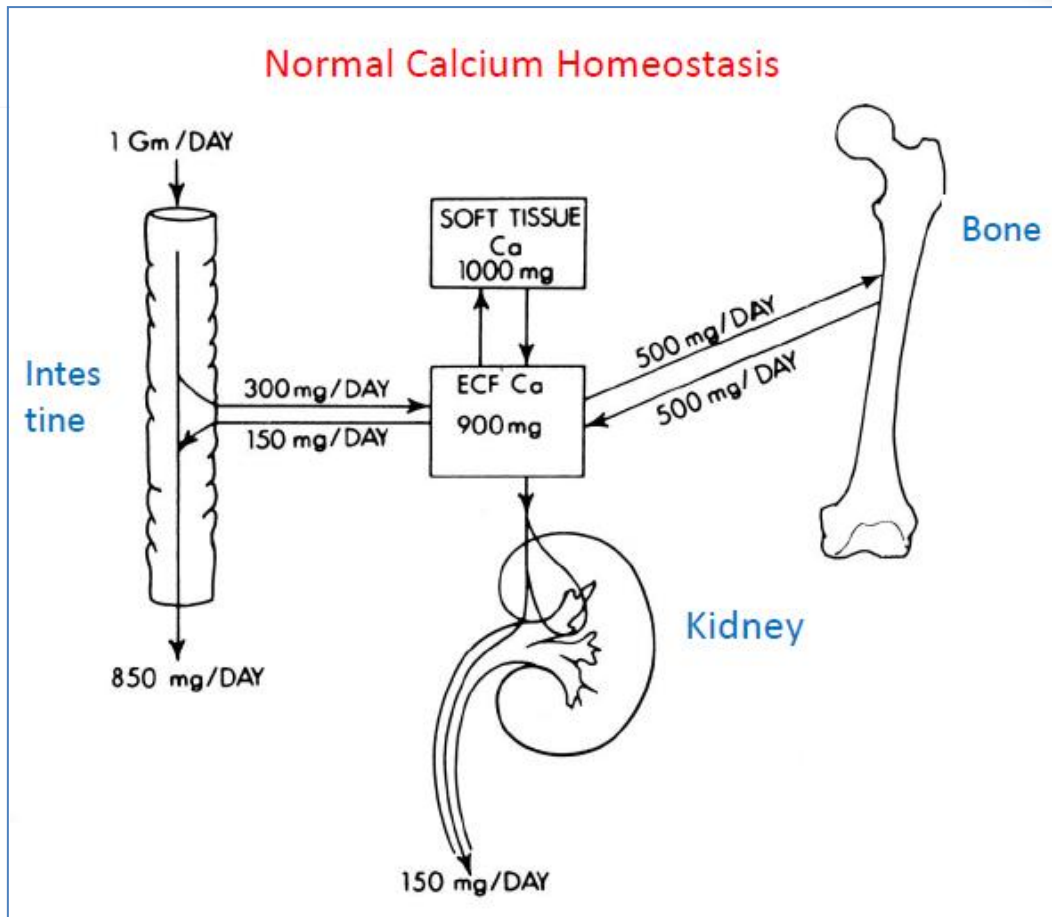
Calcitonin participates in calcium ( $\text{Ca}^{2+}$ ) and phosphorus metabolism (Fig.3). To be specific, calcitonin reduces blood  $\text{Ca}^{2+}$  levels in three ways:

- 1) Decreasing  $\text{Ca}^{2+}$  absorption by the intestines: calcitonin prevents postprandial hypercalcemia resulting from absorption of  $\text{Ca}^{2+}$  from food.
- 2) Decreasing osteoclast activity in bones: calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts, a cell type that "digests" bone matrix to release calcium and phosphorus into blood.
- 3) Decreasing  $\text{Ca}^{2+}$  and phosphate reabsorption by the kidney tubules:  $\text{Ca}^{2+}$  and phosphorus are prevented from being lost in urine by reabsorption in the kidney tubules. CT inhibits tubular reabsorption of these two ions, leading to increased rates of their loss in urine.

Binding of calcitonin to its receptor, which is primarily found on osteoclasts, inhibits releasing of  $\text{Ca}^{2+}$  into circulation.



**Fig.3: Physiological function of calcitonin**



ECF: Extra cellular fluid

In many ways, calcitonin has the opposite effects to parathyroid hormone (PTH). PTH is secreted by the parathyroid glands and it acts to increase the concentration of calcium  $\text{Ca}^{2+}$  in the blood. It does this by acting upon parathyroid hormone receptor in different parts of the body.

A large and diverse set of other effects has been attributed to calcitonin, but in many cases, these were seen in response to pharmacologic doses of the hormone, and their physiologic relevance is unclear.



Although the major source of calcitonin, are the parafollicular cells in the thyroid gland, calcitonin and CGRP have been also isolated from other organs, including the pituitary, suggesting that they may have other functions besides lowering plasma calcium.

### Reference values

Calcitonin levels are slightly higher in healthy men than in healthy women, and they are weakly correlated with age, BMI (particularly in men) and smoking. The limited data available on young children suggest that calcitonin levels are relatively high during the first 6 months of life but that they decline progressively thereafter, reaching adult levels during the third year of life.

Age, pregnancy, lactation, and ingestion of food have been reported to influence calcitonin concentration in healthy individuals, but specific reference intervals have not been established.

Reference calcitonin levels are given in Tab.1. For each assay, the relevant reference values are shown in the appropriate Instructions for Use (IFU).

**Tab.1: Reference levels of Calcitonin**

<b>Specimen (serum)</b>	<b>Reference interval (pg/mL)</b>
<b>Unstimulated levels</b>	
<b>Cord blood</b>	<b>25 - 150</b>
<b>Newborn (1-7 days)</b>	<b>70 - 348</b>
<b>Child</b>	<b>&lt; 70</b>
<b>Adult</b>	<b>&lt; 150</b>
<b>Anthyroidal individuals</b>	<b>&lt; 0.5</b>



## Diagnostic utility

Calcitonin is used as a tumor marker for medullary thyroid cancer (MTC), in which high calcitonin levels are present and elevated levels after surgery may indicate recurrence. It may even be used on biopsy samples from suspicious lesions (e.g., lymph nodes that are swollen) to evaluate they are metastasis of the original cancer. Successful treatment of MTC depends on early detection; late detection confers a poor prognosis. Slight elevations in calcitonin with subsequent surgical exploration of the thyroid may allow the clinician to identify this lesion in its early, nonpalpable stage of development.



### Increased calcitonin levels are associated with:

- Medullary thyroid cancer
- C-cell hyperplasia
- Nonthyroidal small cell carcinoma and other nonthyroidal malignancies
- Acute and chronic renal failure
- Hypercalcemia
- Hypergastrinemia and other gastrointestinal disorders
- Pulmonary diseases

## Calcitonin measurement

The molecular heterogeneity of calcitonin is a factor that might affect the results of laboratory tests. Measurements of serum calcitonin concentration can vary widely because different assays exploit antibodies that recognize different epitopes of the hormone. Nonetheless, serum concentrations of mature calcitonin (i.e. in the absence of high molecular-weight precursors) can now be accurately quantified with two-site immunoassays. These tests combine monoclonal antibodies raised against the region unique to the mature form of calcitonin with other antibodies that recognize different portions of the molecule. Two-site immunoassays that incorporate radioisotopic, enzymatic or luminescent labeling are currently regarded as the most accurate means of measuring serum calcitonin levels. In healthy individuals, two-site immunoassays usually detect calcitonin levels of <10 pg/mL. A Hook effect should be suspected if low calcitonin levels are found in a patient with a large tumor burden.

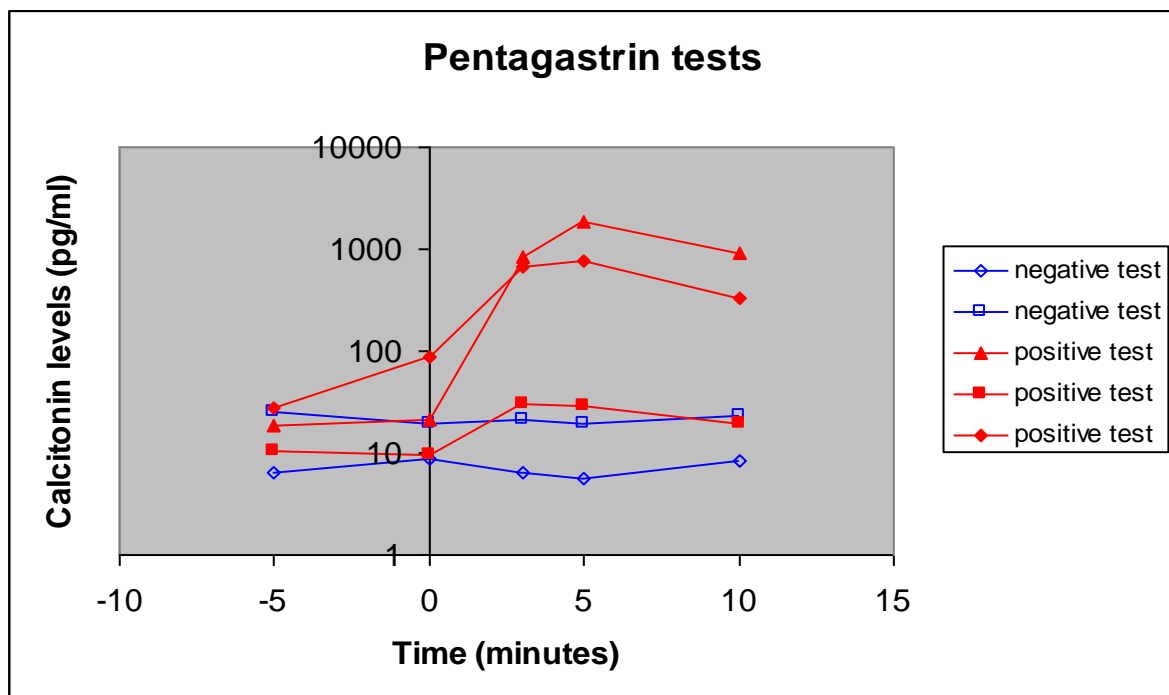


## Calcitonin secretion testing

The specificity of calcitonin testing increases with provocative testing.

### Pentagastrin test

The method most widely used to stimulate calcitonin secretion involves the slow intravenous administration of pentagastrin (0.5  $\mu\text{g}/\text{kg}$ ).



Serum calcitonin levels are measured before infusion and then at 3 min and 5 min after initiation of the infusion. Peak stimulated calcitonin levels  $<10$  pg/mL are detected in 80% of healthy individuals, whereas peak stimulated calcitonin levels  $<30$  pg/mL are detected in 95% of healthy individuals. Mean stimulated calcitonin levels are higher in men than in women. Stimulated levels  $>100$  pg/mL are suggestive of C-cell disease. For patients with MTC who exhibit elevated basal calcitonin levels, the peak observed after pentagastrin stimulation is usually 5-10 times higher than the basal level. By contrast, pentagastrin produces a more limited response (two-fold or less) in patients with other types of neuroendocrine tumors (e.g. gastroenteropancreatic).





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## **Other secretion tests**

Calcitonin secretion can also be provoked by a short intravenous calcium infusion. This approach provides a useful alternative to pentagastrin stimulation in countries where pentagastrin is unavailable. In addition, calcium stimulation can be combined with pentagastrin testing in order to enhance the sensitivity of the latter test. Both in healthy individuals and in patients with C-cell disease, serum calcitonin levels measured after a 30-sec infusion of calcium gluconate (2.5 mg/kg) are of a similar magnitude to those produced by pentagastrin administration.

## **Diagnostic utility – practical applications**

### **Medullary thyroid carcinoma (MTC)**

An elevated serum calcitonin level is a highly sensitive marker for MTC that can be used for screening, differential diagnosis, prognostic assessment, follow-up monitoring, and assessment of treatment response. The prognostic value of measuring calcitonin levels preoperatively, postoperatively, and during follow-up of patients with MTC is widely acknowledged. Furthermore, determination of calcitonin levels is also used to evaluate the response of MTC to novel forms of systemic treatment, such as tyrosine kinase inhibitors.

Routine measurement of serum calcitonin levels in patients with thyroid nodules has been investigated as a screening method for MTC and advocated by one European consensus group. Nevertheless, additional data are required to definitively support routine measurement of calcitonin levels in the initial work-up of patients with thyroid nodules, mainly because there is no convincing evidence that such testing actually reduces MTC-related mortality.

Preoperative calcitonin level (both basal and stimulated) in patients with thyroid nodules is a more sensitive predictor of MTC than is fine-needle aspiration cytology (FNAC).



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