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"Nonspecific" Increases in Plasma Immunoreactive Calcitonin in Healthy Individuals: Discrimination from Medullary Thyroid Carcinoma by a New Extraction Technique

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Occasional seemingly healthy individuals have above-normal concentrations of calcitonin-like immunoreactivity in their plasma, which can lead to mistaken diagnosis of thyroidal or other cancer. We measured immunoreactive calcitonin (CT) before and after extracting the plasma on columns of silica (to improve sensitivity and specificity of the assay for monomeric calcitonin) in five "healthy high-CT" men (I), five patients with known medullary thyroid carcinoma (II), and 30 normal controls (III). Median (and range) values (pg/mL = ng/L) for whole-plasma immunoreactive CT in these groups were, respectively, 379 (157–526), 429 (174–563), and 33 (< 25–92). Dose–dilution curves for plasma samples from group I did not parallel the standard curve, in contrast to samples from the other two groups. Values for extractable CT from plasma from groups I and III, however, were indistinguishable, but remained significantly increased in group II. Infusions of Ca, 2 mg/kg body wt. in 5 min, produced the expected (normal) increases in extractable CT in group I. The occasional factor (or factors) in plasma of healthy persons that interferes in assays for CT is eliminated by the silica extraction method, and in this way such cases can be distinguished from cases of medullary thyroid carcinoma.

Additional Keyphrases: *cancer · thyroid status · misleading test result*

Measurement of calcitonin in serum or plasma by radioimmunoassay is widely used in diagnosis and management of the calcitonin-secreting tumor, medullary thyroid carcinoma (*1–3*). Some also recommend calcitonin measurement in the management of nonthyroidal cancers (*4*), because ectopic secretion of calcitonin-like materials accompanies certain tumors, particularly those of the lung (*5–8*). Thus, calcitonin will generally be assayed when the physician suspects that a calcitonin-secreting neoplasm is present.

During research in calcitonin physiology, our group has frequently taken plasma from healthy volunteers to serve as control specimens (e.g., *9–12*). Among such volunteers, we have encountered a few individuals whose values for plasma immunoreactive calcitonin were unexpectedly high, yet who lacked any other evidence of thyroidal or nonthyroidal neoplasms. Here we report immunochemical analyses of plasma from these persons that suggest the reacting material is not native calcitonin, and we offer a laboratory strategy to help discriminate nonspecific increases from those of medullary thyroid carcinoma.

Materials and Methods

Reagents and apparatus. The types and sources of all materials used in these studies have been described in detail elsewhere (*12, 13*).

Radioimmunoassay for calcitonin. The method, described extensively elsewhere (*9, 13*), is briefly as follows. It is a homologous radioimmunoassay involving use of antiserum G-1701, raised in a goat by injection of unconjugated synthetic human calcitonin (Ciba-Geigy Corp., Basle, Switzerland). The same calcitonin was used as standard and radioiodinated for tracer. The lower limit of detectability for calcitonin in unextracted human plasma averaged 20 to 25 pg/mL, and within- and between-assay coefficients of variation for appropriate internal reference plasmas were <15%.

Extraction and concentration of human plasma calcitonin. The extraction method, detailed in a recent publication (*12*), achieves virtually quantitative recoveries (≥ 90%) of monomeric calcitonin and minimizes recovery of higher-molecular-mass material with calcitonin-like immunoreactivity. In brief: 2–20 mL of plasma is passed through a pre-wetted disposable silica column (Silica Sep-Pak, Waters Associates, Milford, MA). The column is then washed with distilled water containing, per liter, 50 g of aprotinin (Trasylol; FBA Pharmaceuticals, New York, NY), and the wash solution is discarded. The calcitonin is eluted with methanol/water (70/30 by vol) and the eluate is dried in a vacuum centrifuge (Speed Vac Concentrator, Model SVC-200H; Savant Instruments, Inc., Hicksville, NY.).

We dissolved the residues in 0.5 mL of assay diluent buffer (*13*) and assayed them in triplicate at several dilutions. The interassay CV for extraction, concentration, and assay of a normal plasma pool was <12%.

Study subjects and procedures. During some 10 years of studies involving more than 200 normal volunteers, we encountered four individuals whose values for immunoreactive calcitonin in whole plasma exceeded the 3 SD limits for concurrently run normal specimens. Another patient was referred when plasma calcitonin, measured because of a long-past history of removal of a solitary pheochromocytoma, showed an above-normal value. These five men[3] form

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the primary study group, the "healthy, high-calcitonin" group (Table 1). None had historical, physical, or laboratory evidence to explain the supranormal values for plasma calcitonin.

From more than 75 patients with known medullary thyroid carcinoma (*2*), we selected five with stable, low-grade metastatic disease, whose values for immunoreactive calcitonin in unextracted plasma approximated those for the healthy, high-calcitonin group.

Finally, we studied 30 healthy male volunteers over the same time period as the above.

Table 1. Characteristics of Five Apparently Healthy Men with Above-Normal Concentrations of Immunoreactive Calcitonin in Plasma

Age, yr[a]	Calcitonin, pg/mL[b]	Clinical presentation
40	526	Normal volunteer 1976; degenerative joint disease knees and spine; many visits for low back pain (laborer); 7.5-yr followup
41	417	Normal volunteer 1976; old duodenal ulcer; occasional migraine headache; 4 yr later, onset of symptomatic esophageal reflux, with diaphragmatic hernia, deformed antrum but no ulcer, and normal serum gastrin, 7.5-yr followup
44	368	Solitary pheochromocytoma removed 1970 with remission of hypertension; possible alcoholism; calcitonin measured incidentally 1982, found above normal; no clinical or biochemical evidence other disease; 1.5-yr followup
29	238	Normal volunteer 1982; participant in many other research studies; no other clinical or chemical abnormalities; 1-yr followup
34	157	Physician, normal volunteer 1981; low back pain, degenerative joint disease lumbosacral spine; 2.5-yr followup

[a] When discovered to have above-normal concentrations of calcitonin.
[b] Concentration in plasma after fasting. Absolute range in 30 male normal controls, <25–92 (median 33) pg/mL.

All subjects had venous blood sampled, after an overnight fast, into chilled heparinized tubes. Plasma was separated within 1 h and stored at −20 °C until extracted and assayed. In addition, most study subjects underwent a short intravenous infusion of calcium (2 mg of elemental Ca per kilogram of body weight, as the gluconate salt, infused at a constant rate over 5 min) to stimulate calcitonin secretion (*12*). Plasma was collected before and at the end of the 5-min infusion, and again 5 min later.

Calcitonin values were tested for normality of distribution by the Wilk–Shapiro statistic before and after log transfusion. Group comparisons were by non-paired *t*-testing with statistical significance accepted at $p < 0.01$.

Results

Basal plasma calcitonin. Figure 1 depicts immunoreactive calcitonin concentrations in unextracted plasma and silica extracts of plasma. Neither set of calcitonin values was normally distributed, but each was normalized by log transformation. The group medians (and absolute ranges) for calcitonin in unextracted plasma were as follows: normal controls, 33 (< 25–92) pg/mL, medullary thyroid carcinoma, 429 (174–563) pg/mL; and healthy, high-calcitonin subjects, 379 (157–526)[4] pg/mL. Values for calcitonin in unextracted plasma were indistinguishable in the medullary thyroid carcinoma and healthy, high-calcitonin groups; both significantly exceeded normal.

The median value for extractable calcitonin in plasma from the normal controls was 7.3 (range 3.2–19.4) pg/mL. That for patients with medullary thyroid carcinoma remained significantly above normal—237 (range 36.0–311) pg/mL—whereas that for the healthy, high-calcitonin group was indistinguishable from normal 9.0 (range 6.1–21.3) pg/mL. For the pre-calcium infusion samples, the mean percentage (and standard error) of whole-plasma immunoreactivity that was extracted by passage through a silica column, reflecting the proportion of total immunoreactivity consisting of monomeric calcitonin (*12*), was: normals, 24.5% (2.4%); medullary thyroid carcinoma, 37.3% (9.3%);

[4] Values of unextracted plasma calcitonin for this group were based on additions to the assay of undiluted plasma.

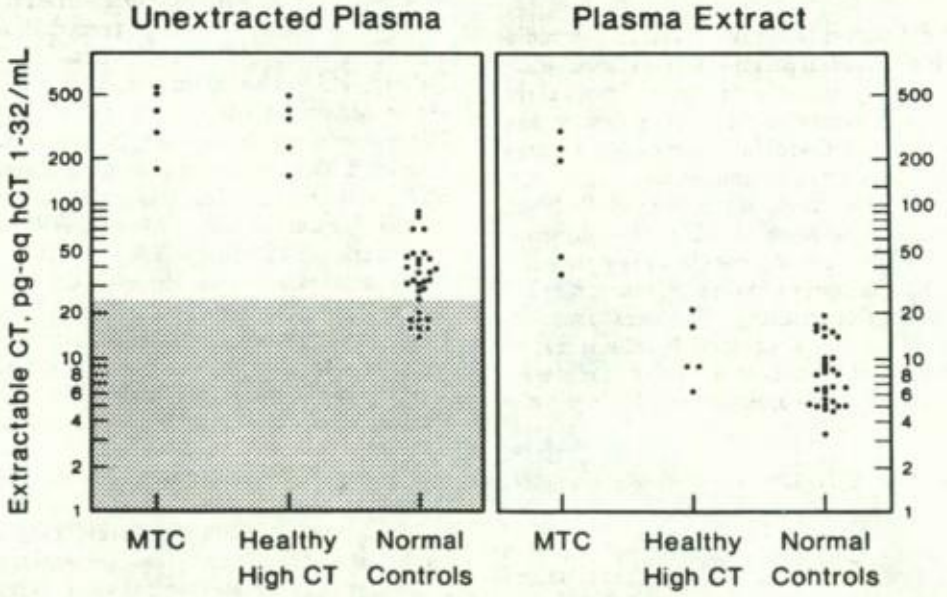

Fig. 1. Immunoreactive calcitonin (iCT) in whole (unextracted) plasma (*left panel*), and silica-extractable *CT* (*right panel*) in patients having medullary thyroid carcinoma (MTC), seemingly healthy men with supranormal whole-plasma CT (healthy high CT), and healthy men (controls)
The *shaded area* (left panel) indicates values below the assay detection limit. Note log scales for CT values

and healthy, high-calcitonin, 3.8% (0.8%) ($p < 0.001$ vs either the normal group or the cancer group).

Dose–dilution curves for plasma from both normal subjects (*9*) and patients with medullary thyroid carcinoma (*14*) paralleled the standard curve. In contrast, dose–dilution curves for unextracted plasma from the healthy, high-calcitonin group uniformly deviated from the standard curve; examples are shown in Figure 2. Dose–dilution curves for extracts of plasma from this group were indistinguishable from those of authentic hormone (Figure 2).

Response to calcium infusion. All five members of the healthy, high-calcitonin group had normal responses to induced hypercalcemia when assessed by the extraction technique (Figure 3).

Discussion

Radioimmunoassay of calcitonin is widely used to detect and aid in post-surgical management of the calcitonin-secreting neoplasm, medullary thyroid carcinoma (*1–3*). In the proper context—i.e., family screening and postoperative testing—such testing has been highly-effective, with a very low false-negative rate (*2*) and an acceptably low false-positive rate (*1, 2, 15, 16*). Nonetheless, there are problems with calcitonin assay that we have discussed extensively elsewhere (*13, 17*). Some groups, on the strength of above-normal values for immunoreactive calcitonin in plasma, have done thyroid gland explorations and removals that revealed no thyroid abnormalities (*15, 16*). We found no thyroid gland disease besides Hashimoto's thyroiditis in two female members of a family having multiple endocrine neoplasia, type 2; their values for whole-plasma calcitonin were high pre-operatively but were unchanged after total thyroidectomy (unpublished results).

Although there are hints in the literature (*15, 16*), there are no systematic studies of these relatively rare persons who seem to be healthy but have clearly above-normal values for immunoreactive calcitonin in plasma. Ordinarily, healthy individuals would not have the test done, but because we have made calcitonin measurements in a large number of normal volunteers, we have found four such persons over a 10-year period, and one other, accidentally found, has been referred to us. Our immunochemical studies suggest that none of these men had above-normal concentrations of authentic calcitonin in their plasma. First, their plasmas yielded dose–dilution curves that were not parallel to those of standard calcitonin. Second, the fraction of their whole-plasma calcitonin-like immunoreactivity that was silica-extractable was very low compared with plasma from normal persons and those having medullary thyroid carcinoma. Third, the absolute values for extractable calcitonin in plasma were normal in the healthy, high-calcitonin group, but remained high in samples from the cancer patients. Finally, the responses of the extractable calcitonin to intravenous calcium were normal in the healthy, high-calcitonin group.

These "healthy, high-calcitonin" men have each undergone detailed, specific evaluations by their personal physicians without any findings to indicate sporadic or familial medullary thyroid carcinoma, multiple endocrine neoplasia, other neoplasms, or any of the other various conditions reportedly associated with increased plasma calcitonin (*18*). Under careful scrutiny, none has developed relevant medical problems during follow-up now extending to seven years. Thus, on clinical grounds as well as the immunochemical data, there is reason to believe that the high calcitonin values are of no significance to their health. Of course, only continued observation of the patients will assure us that they do not have some underlying illness that first manifested itself in the calcitonin measurements.

We conclude that a small part of the healthy population has a circulating factor or group of factors that can interfere in radioimmunoassay of plasma for calcitonin. This factor is not monomeric calcitonin, although our data give no further insight into its nature. The artifactitiously high plasma calcitonin values thus produced could cause erroneous diagnoses of malignancy and lead to unnecessary surgery (*15, 16*). The simple technique we have devised for silica extraction of calcitonin from plasma allows distinction of "healthy, high-calcitonin" plasmas from those of patients with medul-

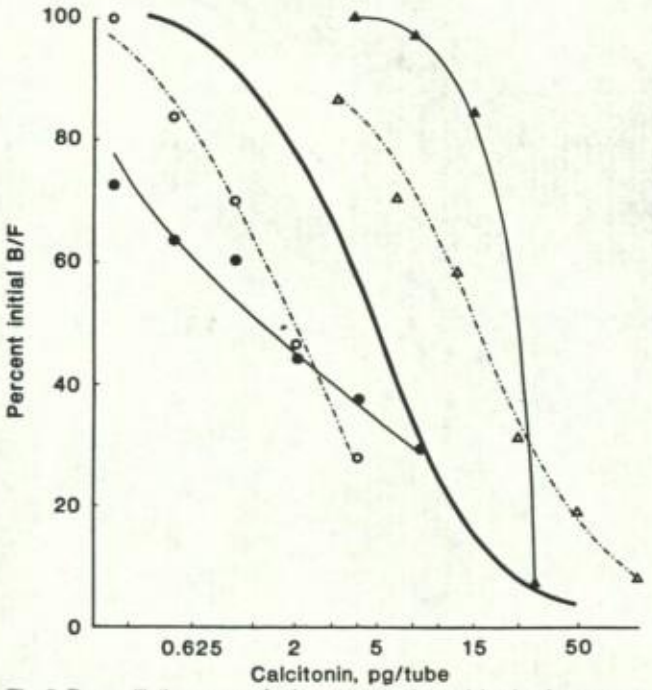

Fig. 2. Dose–dilution curves for immunoreactive calcitonin of unextracted plasma (*closed symbols, thin solid lines*) and silica extracts of plasma (*open symbols, dashed lines*) compared with authentic calcitonin standard (*heavy solid line*)

Samples came from two seemingly healthy men with increased calcitonin-like immunoreactivity in unextracted plasma. Note nonparallelism of unextracted plasma curves with each other and with the standard; plasma extracts gave fully parallel dilution curves

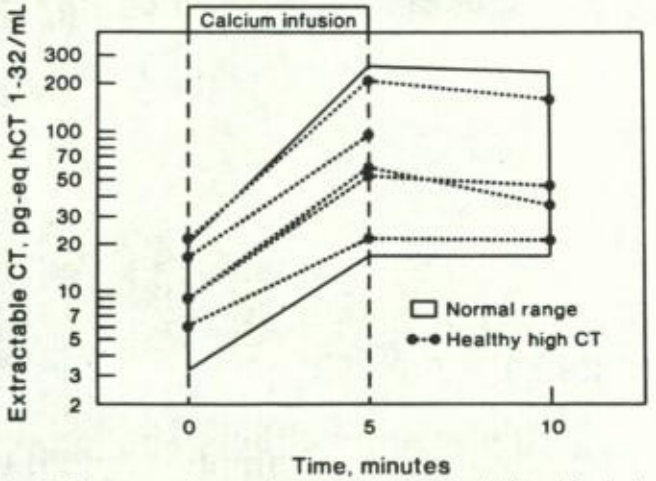

Fig. 3. Response of concentrations of silica-extractable calcitonin to infusion of Ca (2 mg of elemental Ca per kg body wt. over 5 min) in seemingly healthy men with above-normal calcitonin-like immunoreactivity in unextracted plasma

The *boxed area* ("normal range") indicates the absolute limits of responses for 30 normal male controls. The patients' responses were clearly normal

lary thyroid carcinoma. This may be accomplished on a single plasma sample, but including the response to a calcitonin secretagogue probably adds further diagnostic certainty. We do not yet know if this technique also differentiates seeming ectopic calcitonin secretion by nonthyroid tumors (*7*). The relationship of the circulating calcitonin-like factor to heterogeneous forms of immunoreactive calcitonin found in other studies (*14, 19*) is unknown and deserves further study.

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