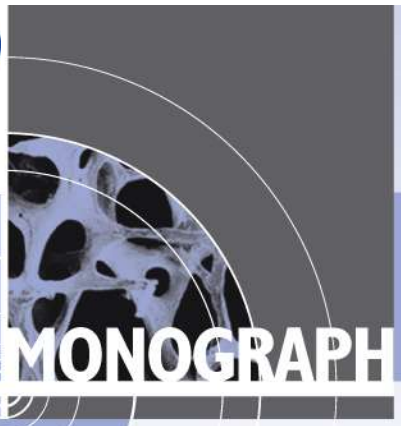
OSTEOmedical Group

CLINICAL & TECHNICAL MONOGRAPH



CICP

C-Terminal Propetide of Type-I-Collagen

ABSTRACT

There are many disorders and diseases in which the increased production of type I collagen is encountered. This increase can be detected by monitoring the produciton of a byproduct of type I collagen synthesis, the carboxyterminal propeptide, which is released stoichiometrically by enzymatic cleavage as the mature conformation of the collagen molecule is achieved.

A sandwich format enzyme immunoassay has been developed which captures the propeptide on a monoclonal antibody-coated microtiter stripwell. Bound propeptide is then detected using a polycolonal antibody followed by an alkaline phosphatase-conjugated secondary antibody and a substrate reaction. Intra-assay CV is less than 10% and inter-assay CV is less than 15%.

Preliminary pediatric ranges have been determined for boys and girls. From levels of >1000 ng/mL at time of birth there is a rapid decrease to a level of about 300 ng/mL throughout childhood. A peak of production of this analyte (to approximately 450 ng/mL) occurs at age 13-14, followed by a decrease to adult levels of about 100 ng/mL. Most children diagnosed as growth hormone deficient show an increase in CICP in response to recombinant human growth hormone.

When metastasis of cancer occurs to sites in bone and liver, production of type I collagen could be indicative of tumor activity at those sites. Groups of cancer patients with metastases to these tissues indeed show a greater proportion of individuals with elevated levels of C-terminal propeptide than those patients with metastases to other tissues.

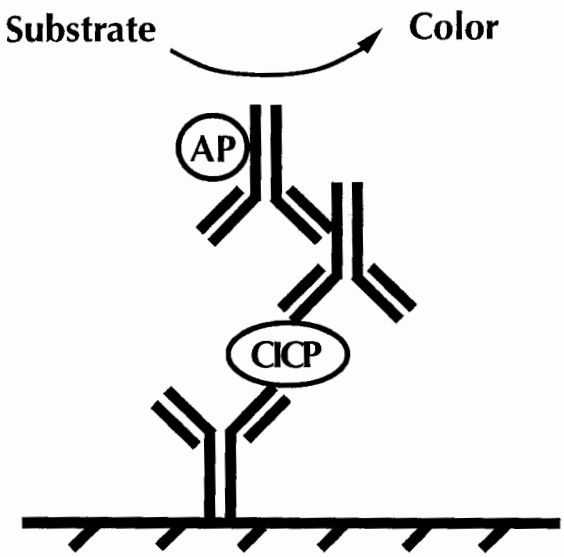
INTRODUCTION

There is substantial evidence that the measurement of collagen type I C-terminal propeptide (CICP) is a useful indicator of collagen production. The principal fibrous component of the extracellular matrix surrounding cells is type I collagen. The collagen molecules, which self-assemble into these fibers, are produced by osteoblasts and fibroblasts as larger precursor molecules called procollagen. Specific enzymes cleave the propeptides to release the mature molecule for incorporation into functional fibers. The propeptides released during this process are stoichiometrically representative of the number of collagen molecules produced.

Development of an immunoassay for detecting CICP was begun by culturing human fibroblasts in serum-containing medium. Serum-free medium conditioned by these cells was harvested, filtered, and concentrated. Collagenase digestion of this material released the propeptides, and the C-terminal propeptides were purified chromatographically. Following characterization, these molecules were used to immunize rabbits and mice. Polyclonal rabbit anitsera and murine monoclonal antibodies were obtained. The monoclonals have been used to isolate CICP from human serum.

These antibodies have been used to develop a microtiter strip enzyme immunoassay for CICP, Prolagen-C®. Fluctuations in levels of this marker correlate to conditions where collagen production is known to be elevated. As collagen is so widespread in its distribution throughout the body, production of collagen, assessed by CICP levels, can be considered to be representative of overall growth.

PROLAGEN-C® ASSAY PROTOCOL



pNPP substrate; absorbance read at 405nm

Goat anti-rabbit IgG- alkaline phosphatase conjugate

Polyclonal rabbit anti-CICP antibody

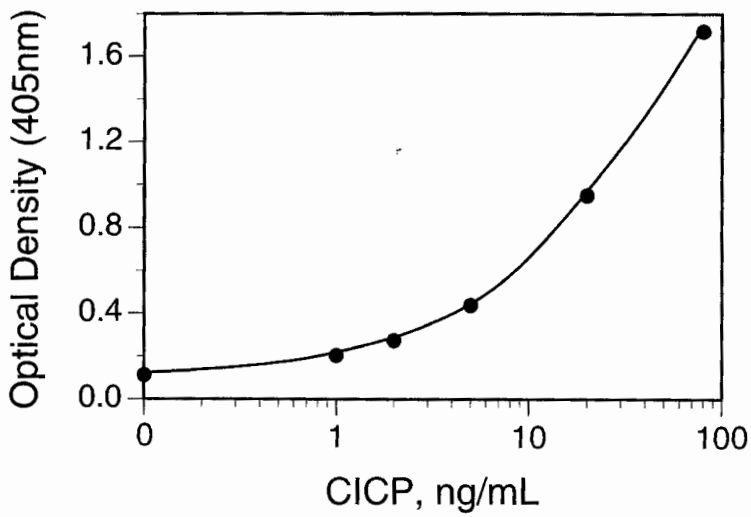
CICP from sample

Monoclonal mouse anti-CICP-coated microtiter well

- Dilute samples 1:12 in Assay Buffer
- Pipet 100 µL of standard, control, or diluted sample into each well
- Incubate 2 hours at room temperature then wash 3X
- Add 100 µL Rabbit Anti-CICP
- Incubate 45-50 minutes at room temperature then wash 3X
- Add 100 µL of reconstituted Enzyme Conjugate
- Incubate 45-50 minutes at room temperature then wash 3X
- Add 100 µL Working Substrate Solution (2 mg/mL p-NPP)
- Incubate 30-35 minutes at room temperature
- Add 50 µL Stop Solution (3N NaOH) and read at 405 nm

STANDARD CURVE

The six CICP standards are fit with a 4-parameter curve.



PRECISION

Within-run and between-run precision was determined by assaying 3 serum samples (within-run n=20, between-run n=9).

CICP ng/mL	Within-run CV %	Between-run CV %
80.8	6.8	7.0
98.1	5.5	7.2
296.7	6.6	5.0

SENSITIVITY

The minimum detectable limit of 0.2 ng/mL was determined by the upper 3SD limit in a zero standard precision study.

LINEARITY

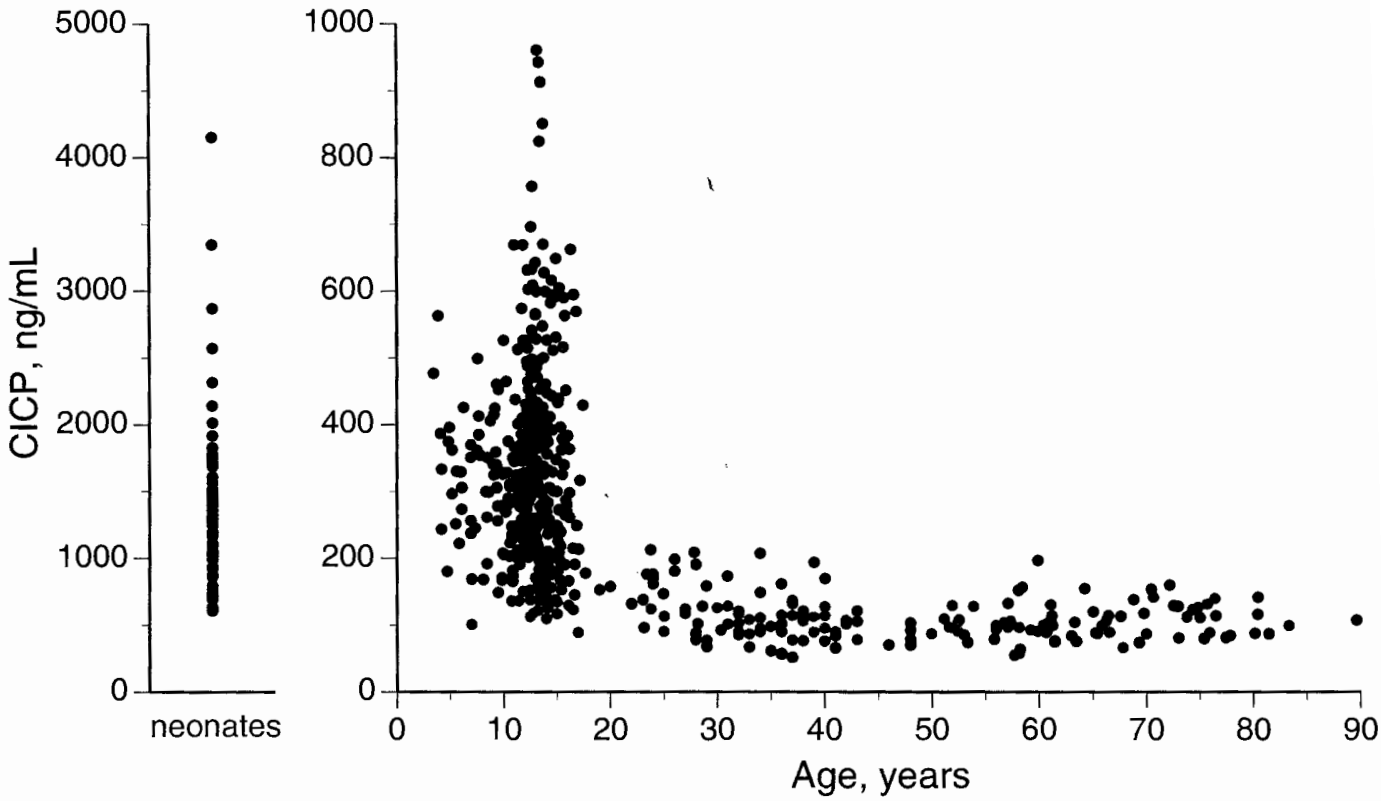
Sample	Dilution Factor	Observed ng/mL	Expected ng/mL	Recovery %
1	1:12	6.90	-	-
	1:24	3.50	3.45	101
	1:48	1.74	1.72	101
2	1:12	13.26	-	-
	1:24	6.56	6.63	99
	1:48	3.49	3.32	105
3	1:12	20.88	-	-
	1:24	10.43	10.44	100
	1:48	5.57	5.22	107

RECOVERY

Sample	Endogenous ng/mL	Added ng/mL	Observed ng/mL	Recovery %
1	9.09	13.24	22.28	100
		31.77	45.96	102
2	10.34	13.10	23.00	97
		32.71	43.05	96
3	12.43	13.24	22.28	100
		31.77	41.55	102

AGE DISTRIBUTION OF PROLAGEN-C® VALUES IN HEALTHY SUBJECTS

The graph below depicts the distribution of CICP values for individuals ranging in age from birth to 90 years. Highly elevated samples from neonates are shown on a separate scale. Noticeable features of this distribution include the elevation early in life, a peaking during the early teenage years and levels which change little throughout adult life.



Samples from University of Sheffield, Sheffield, UK; Hospital del Niño, Madrid, Spain; Hartford Hospital, Hartford, CT, USA

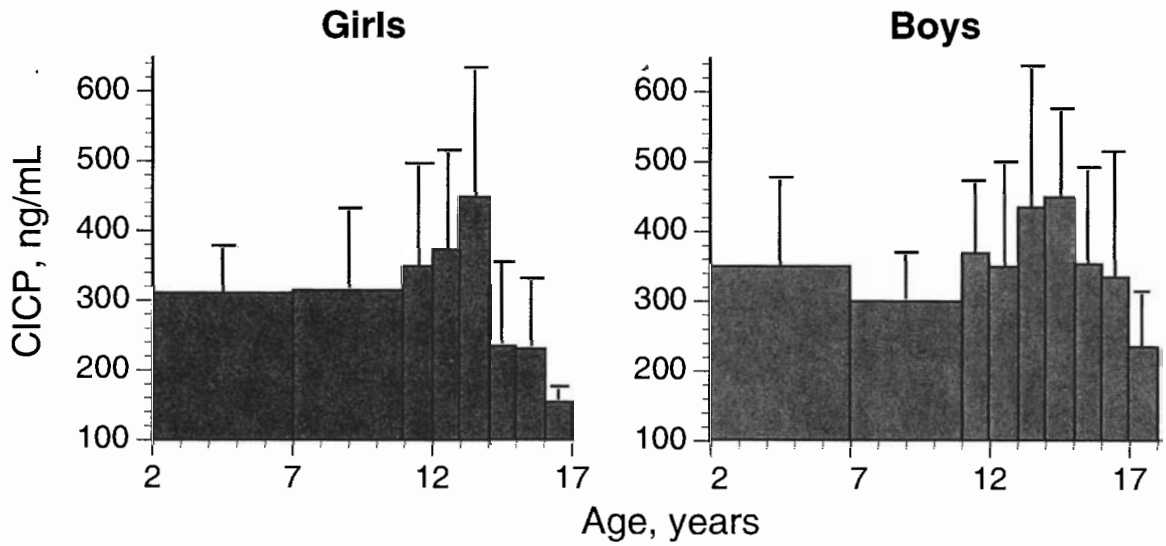
PROLAGEN-C® ADULT REFERENCE RANGES

Reference ranges were determined for premenopausal females and males over the age of 25. Subjects were healthy, with no bone, endocrine, or chronic disorders, and not taking any medication affecting bone metabolism. Nonparametric ranges were calculated due to the skewness of the distributions.

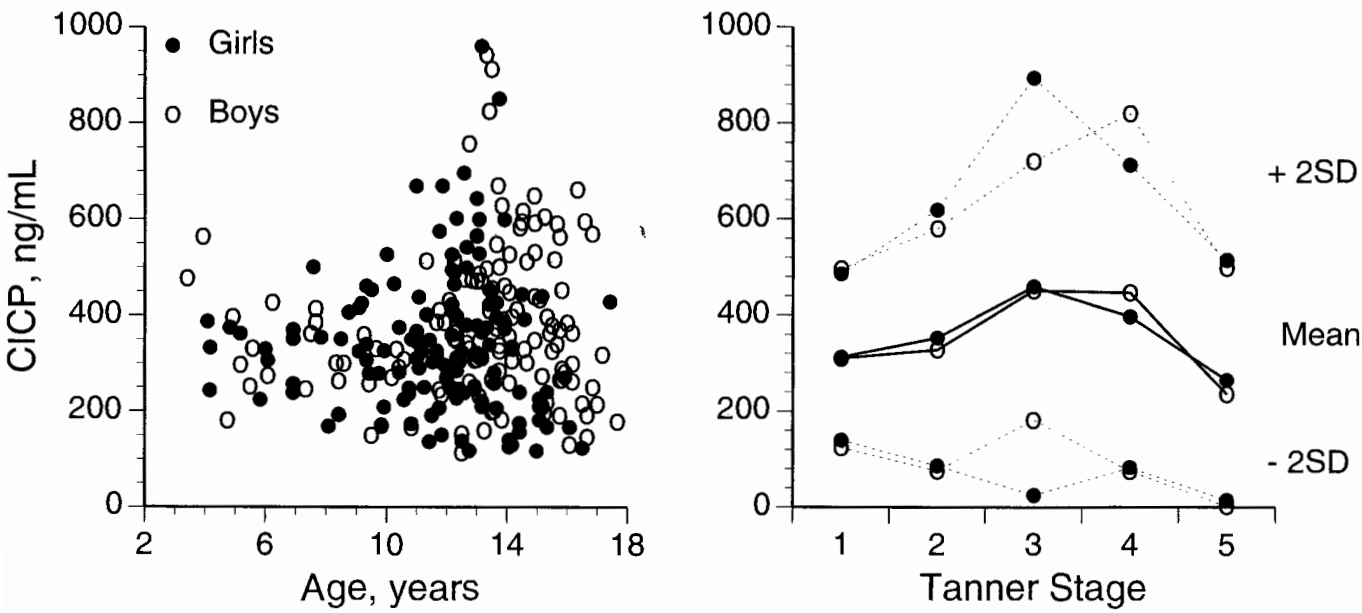
	N	Mean ng/mL	SD	Reference Interval 90% CI
Females	226	106	34	69 - 147
Males	53	112	37	76 - 163

PEDIATRIC PROLAGEN-C® VALUES

Pediatric levels of CICP are shown below by chronological age for boys and girls. The histogram bars represent the mean for each age range, bars are 1SD. Levels for boys and girls are similar at each age. Collagen production in girls appears to peak and drop off toward adult levels a little earlier than in boys.

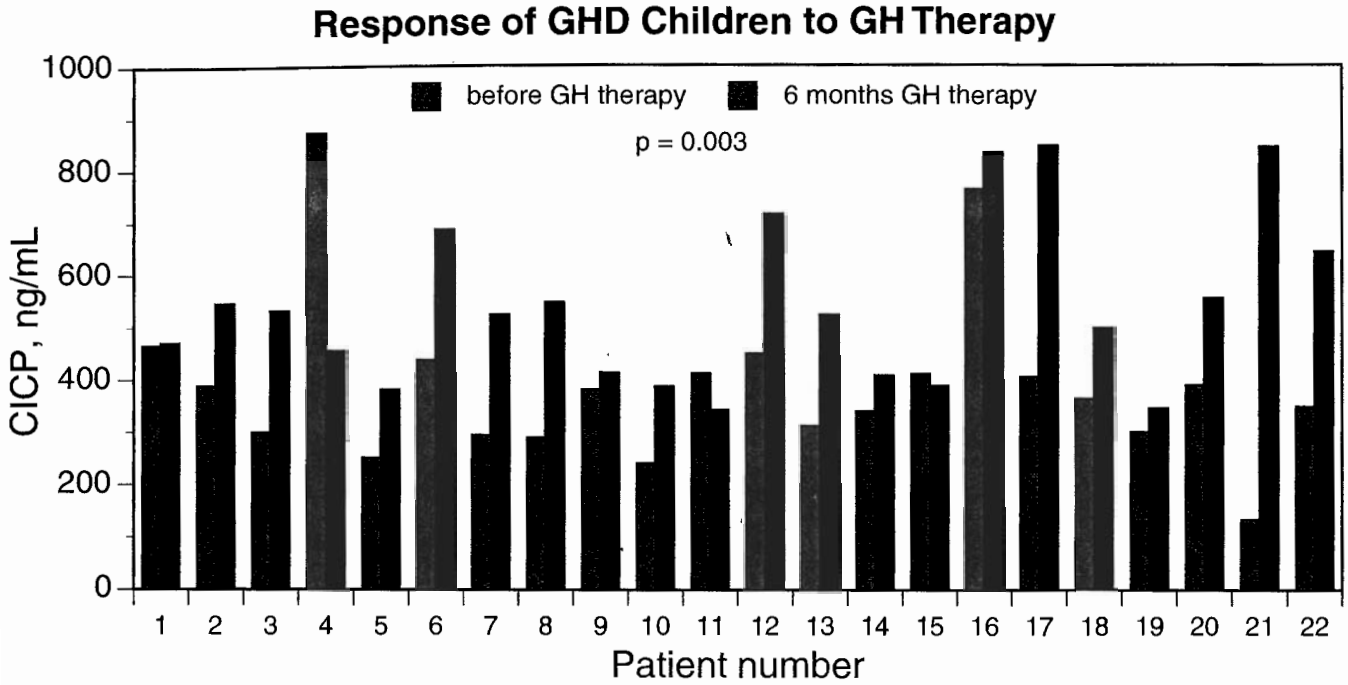


Pediatric levels of CICP are shown below both by age and Tanner stage of pubertal development for boys and girls. The peaking of CICP level is again apparent at mid-puberty (Tanner stage 3).

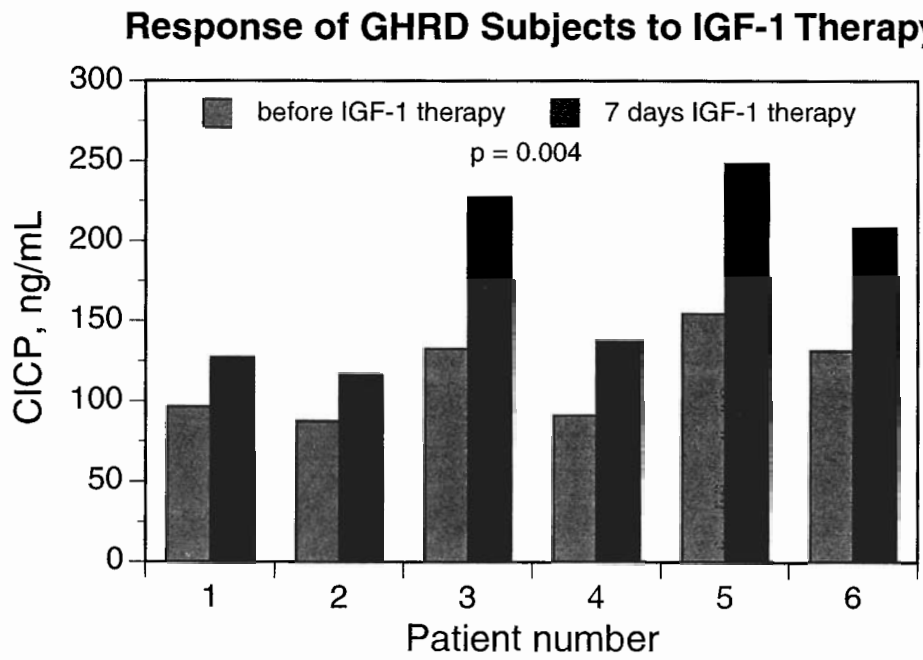


PROLAGEN-C® RESPONSES TO GROWTH THERAPIES

Growth hormone-deficient (GHD) children administered rhGH were largely seen to increase production of collagen. The average increase seen among these patients was 57% (range -50% to +440%).

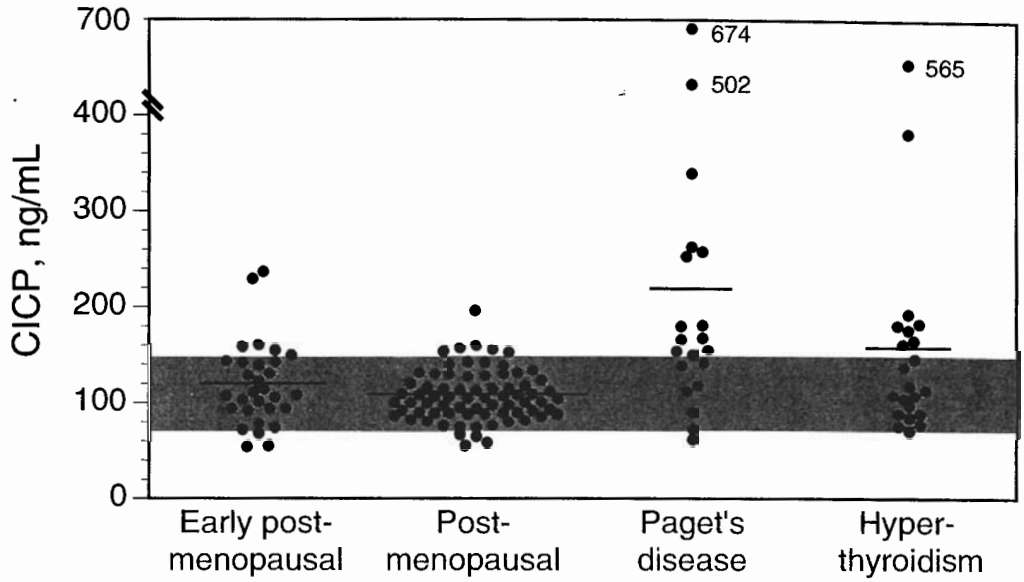


Growth hormone receptor deficient (GHRD) subjects administered IGF-1 therapy also showed an increase in collagen production.



PROLAGEN-C® IN POSTMENOPAUSAL WOMEN AND METABOLIC BONE DISEASES

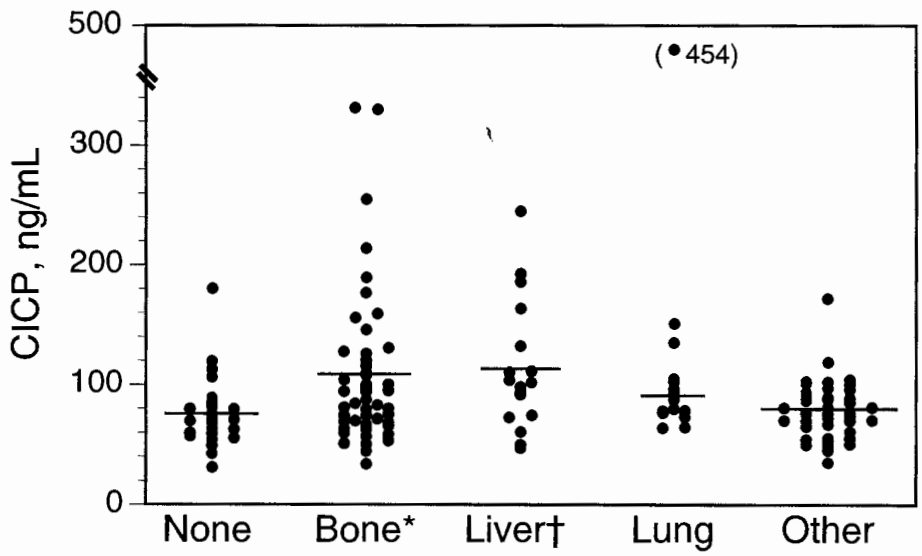
Some women early in the menopause exhibited CICP levels elevated above the normal values for premenopausal women (shaded area). Mean CICP values for subjects with Paget's disease of bone and hyperthyroidism were significantly elevated (p = 0.0001).



Samples from INSERM, Lyon, France; University of Sheffield, Sheffield, UK; Mayo Clinic, Rochester, MN.

PROLAGEN-C® IN CANCER WITH AND WITHOUT METASTASES

CICP levels in plasma samples from cancer subjects with metastases to bone, liver, lung, and other tissues were compared to those with no metastases. Significant elevations were seen in the groups with bone and liver metastases but not metastases to other tissues.



compared to no metastases, *p<0.05, †p<0.01

CONCLUSIONS

- We have developed the Prolagen-C® EIA, a reliable assay for the C-terminal propeptide of type I collagen (CICP), which is indicative of the level of type I collagen production.

- The normal distribution of CICP values has been examined for individuals from birth to old age. A fairly restricted reference range exists for adults, but levels in pediatric patients, while substantially elevated, vary widely.

- When expressed in relation to pubertal development (Tanner stage), levels of CICP are seen to peak at mid-puberty (approximately 50% elevation) and then drop toward normal adult levels. The variability of CICP levels in peripubertal children probably represents the different phases of growth activity which can be seen in all categories of age or development.

- The increased collagen production observed in GHD children treated with rhGH and GHRD adults treated with IGF-1 suggests a utility for this marker in monitoring the progress of growth stimulating therapy.

- Elevated CICP levels were observed in subjects with Paget's disease and hyperthyroidism, diseases which are characterized by high bone turnover. These data suggest the assay may be useful in metabolic bone disease management.

- The increased collagen production observed in some cancer patients exhibiting metastases to bone and liver sites, but not to other tissues, possibly suggests increased bone turnover in bone and fibrotic activity in liver.

Presented at the 16th Annual Meeting of the American Society for Bone and Mineral Research, September 9-13, 1994, Kansas City, Missouri. Abstract: *J.Bone Miner.Res.* 1994;9(Suppl.1):S349.