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Age-related reference intervals for bone turnover markers from an Australian reference population

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ABSTRACT

Background: This study was performed to establish age-related serum reference intervals for procollagen type I N-propeptide (P1NP) and type I collagen C-telopeptide (CTx) in the Australian population.
Methods: Fasting sera from 1143 males (mean age 60 years; range 20–97 years) and 1246 females (mean age 53 years; range 20–93 years) who participated in the Geelong Osteoporosis Study were analysed for CTx and P1NP using the automated Roche Modular Analytics E170 analyser.
Results: Optimal age-related reference intervals were based on the central 90% of the distribution. The male CTx reference interval was divided into three age groups. For men aged 25 to 40 years, the interval was 170–600 ng/L; 40 to 60 years, the interval was 130–600 ng/L; and for men aged greater 60 years the interval was 100–600 ng/L.
For P1NP the male reference interval was 15–80 μg/L for men aged between 25 to 70 years. In men greater than 70 years of age values were higher possibly due to increased bone turnover.
High values are frequently seen for both CTx and P1NP in males aged younger than 25 years. This is probably due to bone growth that is not completely finalised.
The female CTx reference interval was divided into four age groups. For women aged less than 30 years, the interval was 150–800 ng/L; 30–39 years, the interval was 100–700 ng/L; 40–49 years, the interval was 100–600 ng/L; and for women aged 50 years or more the interval was 100–700 ng/L.
The female P1NP reference interval was divided into four age groups. For women aged less than 30 years, the interval was 25–90 μg/L; 30–39 years, the interval was 15–80 μg/L; 40–49 years, the interval was 15–60 μg/L; and for women aged 50–69 years the interval was 15–75 μg/L. In women greater than 70 years of age values were higher possibly due to increased bone turnover.
Conclusion: Values obtained from this large study provide sound age-related reference intervals for serum P1NP and CTx values in the Australian population.

Introduction

Osteoporosis is a systemic skeletal disorder characterised by a reduced bone mass and deterioration of osseous microarchitecture, resulting in decreased bone strength and increased risk of fragility fractures, particularly of the spine, hip and wrist.

Osteoporosis currently affects approximately 10% of the Australian population and it is estimated that approximately 60% of women and 30% of men over the age of 60 will suffer from an osteoporotic fracture [1].

Monitoring acute changes in bone is difficult with the static information given by bone mass evaluation, usually carried out by means of bone mineral density (BMD) measurement. The annual changes in BMD, associated with age-related bone loss and therapeutic interventions are small and similar to the measurement error of the technique. Consequently the popularity of biochemical bone turnover markers (BTM) has grown. BTM have been shown in some studies to be significantly associated with subsequent bone loss [2,3]. In addition, an association has been found, though not consistently, between bone resorption markers and fracture risk [4]. Bone formation markers are generally not associated with fracture risk, except bone alkaline phosphatase, which has predicted fragility fractures in some cohorts [4–6].

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Table 1
Characteristics of the male cohort.

Age group (years)	n	CTx (ng/mL)	P1NP (μg/L)	Weight (kg)	Height (cm)	BMI (kg/m^2)
20–29	85	528 (453–676)	61 (45–78)	81.5 ± 19.2	179.9 ± 6.7	25 ± 5
30–39	114	361 (276–466)	41 (34–51)	84.9 ± 16.6	178.6 ± 7.3	27 ± 4
40–49	136	311 (228–399)	37 (29–47)	84.7 ± 12.7	176.5 ± 7.0	27 ± 4
50–59	196	303 (227–412)	36 (28–44)	86.7 ± 14.9	175.6 ± 6.2	28 ± 4
60–69	184	284 (209–398)	33 (24–43)	85.5 ± 13.7	173.8 ± 6.5	28 ± 4
70–79	230	315 (216–426)	35 (26–48)	82.0 ± 13.3	172.0 ± 6.5	28 ± 4
80+	198	339 (231–511)	37 (26–54)	75.8 ± 11.9	170.0 ± 6.6	26 ± 4

Values are mean ± standard deviation or median (inter-quartile range) as appropriate.

Procollagen type I N-propeptide (P1NP) and type I collagen C-telopeptide (CTx) have been identified as the most promising BTM by the Joint IOF (International Osteoporosis Foundation)–IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) Bone Marker Standards Working Group [7].

P1NP is a propeptide that is released during the processing of type I procollagen into collagen. It is regarded as a bone formation marker, is synthesised by osteoblasts and correlates with histomorphometric parameters of bone formation [8,9]. By contrast, CTx arises from collagen degradation and correlates with histomorphometric parameters of bone resorption [8,9].

Reference intervals for women, particularly post-menopausal have been widely published [10–15]. However there is an absence of this type of data in men. The aim of this study was to establish age-related serum reference intervals for P1NP and CTx values in the Australian population as determined by automated methods.

Materials and methods

Study population

The specimens used in this study were obtained from an age-stratified random sample of participants from the Geelong Osteoporosis Study. This study consists of a large, strictly random, community dwelling population-based cohort. All subjects provided written consent and were recruited from an area surrounding Geelong, called the Barwon Statistical Division. This region is comparable to the Australian population in age distribution and socio-economic indicators consisting of urban and rural communities. Subjects were not screened for bone or metabolic diseases, nor were they screened for use of drugs that affect bone turnover. Details of recruitment and exclusion criteria of the study sample have been published elsewhere [16].

The male samples (n = 1143; mean age 60 years; range 20–97 years) were collected from 2003–2008. There were originally 1540 male subjects however 397 were excluded as no fasting serum sample was available. Characteristics of the male cohort are shown in Table 1.

The female samples used in this study (n = 1246; mean age 53 years; range 20–93 years) were collected from 1993–1997. There were originally 1494 female subjects however 248 were excluded as no fasting serum sample was available. Characteristics of the female cohort are shown in Table 2.

Serum samples were collected from both cohorts after an overnight fast between 07:30 and 11:45. They were then centrifuged for 10 min at 3000 *g* approximately 1–3 h after collection. The samples were then aliquoted and stored frozen at −80 °C until analysis.

Ethics approval was received from the Barwon Health Human Research Ethics Committee.

Biochemical markers

All samples were analysed in 2008 for CTx and P1NP using the automated Roche Modular Analytics E170 analyser (Roche Diagnostics, Mannheim, Germany).

Both biochemical markers were measured at the same time using a single lot of each assay reagent, according to the manufacturer's protocol and following the laboratory quality control procedures.

The serum CTx limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L (n = 10).

Serum P1NP inter-assay CVs were 4.9% at 73 μg/L, 2.6% at 392 μg/L, and 2.1% at 768 μg/L (n = 10) with a limit of detection of 5 μg/L.

Statistical analysis

Statistical analysis of data was performed using SAS software version 9.1 (SAS Institute Inc, Cary, NC, USA). CTx and P1NP were initially assessed for normality and found to have distributions that were skewed to the right. Thus these variables underwent logarithmic transformation before statistical analyses.

The geometric mean (95% confidence interval), as well as the 2.5th, 5th, 95th and 97.5th percentiles were calculated for participants separated into age groups by decades. Optimal age-related reference intervals were based on the central 90% of the distribution. Characteristics of female and male cohorts were summarised using mean ± standard deviation, median (inter-quartile range) or proportions as appropriate.

Results

Male cohort

In the male cohort the median serum P1NP and CTx values were 37 μg/L (IQR 27–49 μg/L) and 328 ng/L (IQR 235–459 ng/L) respectively. Neither of the BTM were distributed normally, but skewed towards the right. After log-transformation, data for both variables showed a Gaussian distribution. The ranges of distributions were from 5 to 726 μg/L for P1NP and 18 to 1810 ng/L for CTx.

There was no seasonal variation seen.

When the data were plotted against age by decade a U-shaped relationship was seen for CTx (Fig. 1). CTx declined until ages 40–50 years

Table 2
Characteristics of the female cohort.

Age group (years)	n	CTx (ng/mL)	P1NP (μg/L)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Post-menopausal (%)	Oral contraceptive use (%)
20–29	166	379 (291–554)	45 (35–61)	65.7 ± 13.3	163.8 ± 5.8	24 ± 5	0	53
30–39	215	264 (184–384)	31 (24–46)	69.2 ± 16.3	163.3 ± 6.1	26 ± 6	2	28
40–49	209	246 (160–341)	29 (23–37)	71.8 ± 15.4	162.8 ± 6.0	27 ± 6	20	12
50–59	176	331 (196–479)	34 (24–51)	71.3 ± 13.6	161.1 ± 5.9	27 ± 5	82	2
60–69	176	343 (204–484)	38 (26–48)	71.7 ± 14.3	159.5 ± 6.1	28 ± 6	100	0
70–79	151	407 (281–596)	39 (28–53)	66.7 ± 12.9	156.8 ± 5.8	27 ± 5	100	0
80+	153	507 (372–630)	49 (36–63)	62.1 ± 10.8	155.0 ± 6.0	26 ± 4	100	0

Values are mean ± standard deviation, median (inter-quartile range) or proportions.

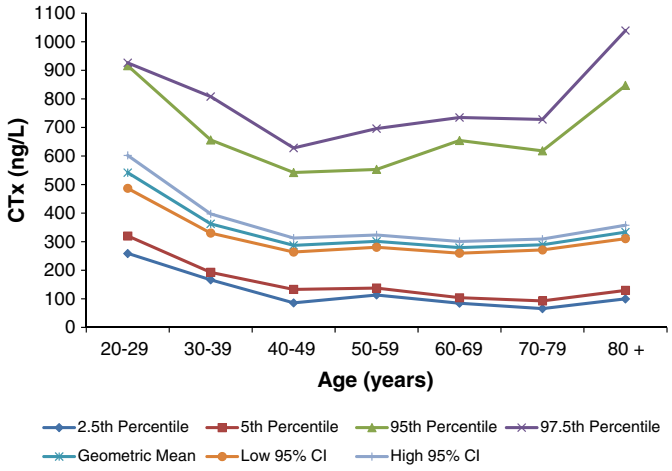

Fig. 1. Age-related distribution of CTx values in the male cohort.

and then gradually increased until age 70 years, after which it increased sharply.

P1NP values also showed a similar U-shaped distribution (Fig. 2). P1NP was relatively higher for men in their 20s and then fell and remained stable until age 80 years, after which the values increased sharply.

Within the 20–29 year age group CTx and P1NP values decreased with increasing age (data not shown).

Reference intervals were determined using the central 90% of the distribution. These are shown in Table 3.

If we were to base the reference intervals on the 2.5th and 97.5th percentile the male CTx reference interval would have been divided into two age groups. For men aged 25 to 40 years, the interval would be 150–800 ng/L; and for men aged greater than 40 years the range would be 100–700 ng/L.

The P1NP male reference interval would have been 10–80 μg/L for men aged between 25 and 70 years.

Female cohort

The female cohort had a median serum P1NP value of 37 μg/L (IQR 26–51 μg/L) and a median CTx value of 338 ng/L (IQR 212–499 ng/L). The values were skewed towards the right and required log-transformation to achieve a Gaussian distribution. The distribution of these results ranged from 5 to 495 μg/L for P1NP and 10 to 2863 ng/L for CTx.

Table 3
Suggested male and female reference intervals for CTx and P1NP.

Gender	Age group (years)	Suggested CTx reference interval (ng/L)	Suggested P1NP reference interval (μg/L)
Male	25–40	170–600	
	40–60	130–600	
	>60	100–600	
	25–70		15–80
Female	<30	150–800	25–90
	30–39	100–700	15–80
	40–49	100–600	15–60
	>50	100–700	
	50–69		15–75

When we investigated the bone resorption marker for seasonal variation, we found lower CTx values in autumn compared to higher values in spring. By contrast, the bone formation marker (P1NP) values showed no seasonal variation.

CTx initially decreased between the ages of 20–49 years after which the values started to rise (Fig. 3). P1NP showed a similar pattern where values decreased between the ages of 20–49 years and then started to increase (Fig. 4).

When separated according to menopausal status, the pre-menopausal women had a median serum P1NP value of 33 μg/L (IQR 25–48 μg/L) and a median CTx value of 289 ng/L (IQR 191–406 ng/L). The post-menopausal group had a serum P1NP value of 40 μg/L (IQR 28–53 μg/L) and a median CTx value of 399 ng/L (IQR 241–546 ng/L).

The reference intervals were determined using the central 90% of the distribution. These are shown in Table 3.

If we were to base the reference intervals on the 2.5th and 97.5th percentile the female CTx reference interval would have been divided into four age groups. For women aged less than 30 years, the interval would be 100–900 ng/L; 30–39 years, the interval would be 50–900 ng/L; 40–49 years, the interval would be 50–700 ng/L; and for women aged 50 years or more the interval would be 50–900 ng/L.

The female P1NP reference interval would have also been divided into four age groups. For women aged less than 30 years, the interval would be 20–100 μg/L; 30–39 years, the interval would be 10–100 μg/L; 40–49 years, the interval would be 10–70 μg/L; and for women aged 50–69 years the interval would be 10–85 μg/L.

Discussion

As new automated techniques to determine BTM are becoming increasingly common, it is essential that relevant normal reference intervals be established. Typically assays do not completely agree on

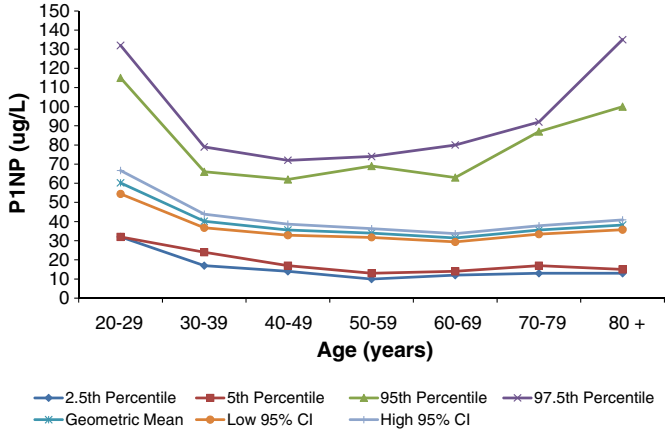

Fig. 2. Age-related distribution of P1NP values in the male cohort.

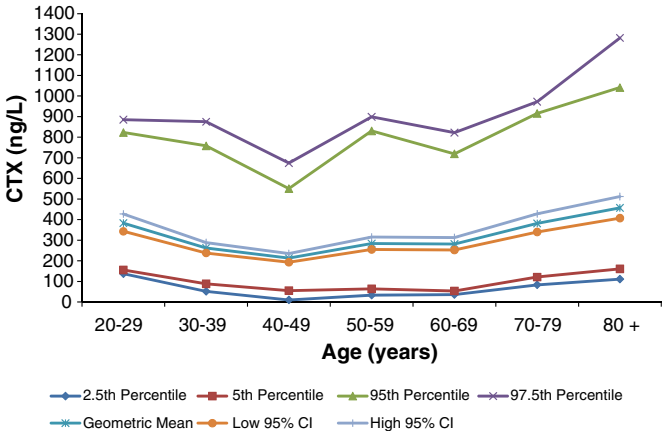

Fig. 3. Age-related distribution of CTx values in the female cohort.

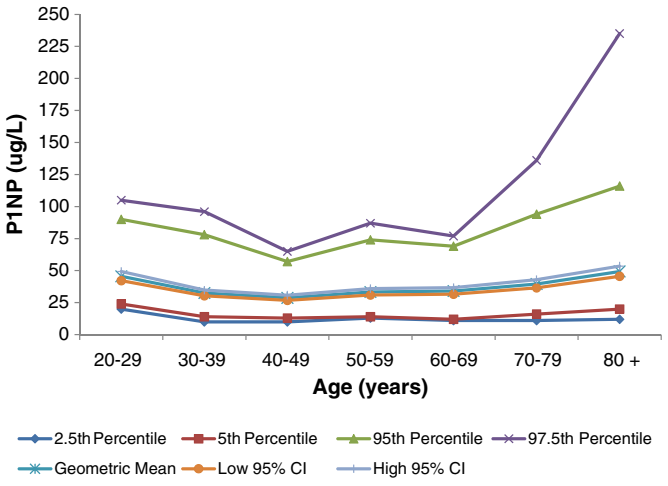

Fig. 4. Age-related distribution of P1NP values in the female cohort.

different platforms and differences of up to 15–20% have been seen. We have studied the serum levels of P1NP and CTx determined by the Elecsys E170 analyser (Roche Diagnostics) in a large well characterised cohort of 1143 Australian men and 1246 Australian women who were randomly selected from an electoral register and are thus representative of the underlying population.

Literature shows that P1NP values assessed by automated methods appear to be similar in Caucasian men in different countries whereas CTx values appear to differ across different populations (Table 4). In this study, the male CTx reference interval was divided into three age groups. Whilst the upper end of the reference interval stayed stable, the lower end decreased with age. These findings are important as CTx values less than 150–200 ng/L have been used to identify patients that need to cease bisphosphonate use due to the risk for developing osteonecrosis of the jaw after tooth extraction [19]. Based on our data a part of the "normal" population will have CTx values below 200 ng/L.

The male 20–29 year age group was further analysed and it could be seen that CTx and P1NP values decrease with increasing age during this decade. This is likely the result of consolidation of bone formation.

Bone histomorphometry studies have shown that bone resorption, bone formation and cancellous bone volume (BV/TV) decrease with age [20,21]. Generally BV/TV increases progressively, albeit slightly until the third decade of life, and decreases thereafter. In males there is a continuous loss of bone volume.

Wishart et al. found that BTM in men show their lowest levels between 50–60 years [22]. Similarly we find a slow decrease in median CTx levels until they stabilise after age 40–49 years.

In elderly men, age-related increase, stability or decrease of bone resorption markers have been described [22–24]. Bone resorption does increase in elderly men, but this increase is slight, progressive and starts after the age of 60. This increase was seen in our study but is sometimes not detected in cohorts containing few men aged 60 and over.

Bone formation markers such as P1NP remain roughly stable between 40 and 60 years, independent of the marker studied. Over 60 years, they remain stable or increase slightly in cohorts that include very old men [23]. This suggests that, in elderly men, slightly increased bone resorption is not matched by a parallel increase in bone formation and this imbalance might be expected to result in a net bone loss.

As in men, literature shows that P1NP values assessed by automated methods appear to be alike in Caucasian women in different countries whereas CTx values vary across different populations (Table 5).

Table 4
Literature review of male P1NP and CTx values in different populations.

First author	Year published	Journal	Age (years)	Sex	Population (n)	Subjects	Origin	P1NP method	P1NP mean (μg/L)	P1NP reference interval (μg/L)	CTx method	CTx mean (ng/L)	CTx reference interval (ng/L)
Bauer [3]	2009	J Bone Miner Res	>65	Male	933	General population	USA	Roche Auto-analyser	38.7 ± 23.9		Roche Auto-analyser	400 ± 210	
Chen [10]	2006	J Bone Miner Res	65–101	Male	238	Frail	Australia	Roche E170		34.2–85.4	Roche E170	388 ± 218	160–390
Olmos [17]	2010	Clinica Chimica Acta	≥50	Male	660	Healthy	Spain	Elecsys 2010	37.1 ± 16.7	15–78	Elecsys 2010	300 ± 171	69–760
MCE study [18]	2000		>30	Male	639	Healthy		Elecsys Auto-analyser			Elecsys Auto-analyser	30–50 years; 300 ± 142 50–70 years; 304 ± 200 >70 years; 394 ± 230	

Table 5
Literature review of female P1NP and CTx values in different populations.

First author	Year published	Journal	Age (years)	Sex	Population (n)	Subjects	Origin	P1NP method	P1NP mean (μg/L)	P1NP reference interval (μg/L)	CTx method	CTx mean (ng/L)	CTx reference interval (ng/L)
Chen [10]	2006	J Bone Miner Res	65–101	Female	826	Frail	Australia	Roche E170		47.4–99.6	Roche E170		200–470
De Papp [11]	2007	Bone	28–45	Female	181	Healthy	USA	Manual Orion assay	39.5 ± 16.6	16–83	Roche Auto-analyser	280 ± 135	94–659
Glover [12]	2008	Bone	30–45	Female	193	Healthy pre-menopausal		Elecsys Auto-analyser	35.9 ± 16.4	16.2–60.9	Elecsys Auto-analyser	300 ± 150	100–620
Glover [13]	2009	J Bone Miner Res	30–39	Female	637	Healthy pre-menopausal	France, Belgium, UK, USA	Roche Modular Auto-analyser		16.3–78.2	Roche Modular Auto-analyser		114–628
Martinez [14]	2009	Clinica Chimica Acta	44–93	Female	1080	Healthy	Spain	Elecsys 2010	47.7 ± 10.9	19–100	Elecsys 2010	387 ± 197	112–1018
Lenora [15]	2007	Osteoporos Int	75	Female	579	General population	Sweden				Roche Elecsys	312 ± 186	

Among females, bone histomorphometry studies have shown that there is a trend toward reduction in BV/TV after the age of 50, probably corresponding to the onset of menopause [20]. In women, mineral apposition rate, wall thickness and osteoid thickness also decline significantly with age [25]. As a novel finding we observed that bone resorption markers decrease continuously after the second decade of life until menopause, when bone turnover increases.

In our study P1NP was not significantly different between pre- and post-menopausal women.

Previously, menopause has been shown to be associated with an acceleration in bone turnover, which is paralleled by a 50–100% increase in both bone formation and bone resorption markers [26–28] and consistent with the higher CTx values in post-menopausal women compared with pre-menopausal women we found. In addition, bone turnover continues to increase even during late menopause [29]. In this study we find that older post-menopausal women (over 70 years) have evidence of greater bone turnover as shown by the higher values of the central 90% of the CTx and P1NP distributions compared with the decade before. However, in this study we cannot exclude that the increased serum CTx values we saw in elderly post-menopausal women was not the result of decreased clearance as we did not measure renal function.

There is no simple explanation for the differences seen in CTx reference intervals between studies whilst the P1NP reference intervals agree. It is possible that bone resorption markers may be more influenced by the particular characteristics and exposures of the participants. Some of these differences could be accounted for by the particular characteristics of the participants included in each study such as age, weight, smoking habits, and physical activity.

The significant intra-individual and inter-assay variability of BTM represents a significant problem in their clinical application. Variability of collection as well as diurnal variability have been described and lead to wider reference intervals and less diagnostic certainty. It is known that circadian variation is greater for bone resorption than bone formation. For example serum CTx exhibits a circadian rhythm with an amplitude of 80% of the 24 hour mean [30]. The amplitude of the variation of bone formation markers ranges from 5% to 30% [31,32]. Food intake, hormonal changes, and renal function are also other important interfering factors.

In this study, the samples were collected throughout the year however in the male cohort the CTx and P1NP values did not show seasonal variation. There was no seasonal variation in P1NP values in women. Conversely the CTx and 25-hydroxyvitamin D3 data did show seasonal variation as previously reported [33]. In the female cohort 25-hydroxyvitamin D3 levels decreased in winter whilst the CTx values increased. The observed acceleration of bone turnover during winter may at least in part be due to subclinical vitamin D deficiency and secondary hyperparathyroidism. In studies that have exhibited a significant seasonal variation in BTM, bone turnover tends to be lower in summer than in winter and is related to seasonal changes in the vitamin D-Parathyroid Hormone (PTH) axis [34]. It has also been suggested that lower physical outdoor activities in winter may lead to a net increase in bone resorption [35]. Other studies have shown no wintertime increase in bone turnover despite a significant seasonal decrease in vitamin D levels [36,37].

The strengths of this study include the large sample size, and that the participants were well characterised and representative of the Australian population. All the samples were obtained at the same time of day in a fasting state so that factors leading to biological variability could be minimised.

However, this study has some limitations. One source of potential error relates to the age of the samples. When assayed the specimens from the female cohort were up to 15 years old whilst the male cohort specimens were up to 5 years old. The serum samples used were stored at −80 °C and did not undergo multiple freeze–thaw cycles. Studies have shown that CTx is stable for at least 3 years in serum samples frozen at or below −20 °C [38–40]. Others have

demonstrated that P1NP is stable in serum when stored frozen for at least 12 months at −80 °C [39,41].

As we did not measure PTH we were unable to exclude subjects with abnormal PTH values and some may have been included in this study. However by excluding such subjects results would then not be able to be extrapolated to the general population. Caucasian participants comprised >99% of subjects and thus, this data cannot be extrapolated to other ethnicities. We recommend that reference intervals be established for different ethnic groups.

Conclusion

In this study we report reference intervals for two BTM from a well characterised population of Australian men and women spanning a wide range of ages, using an automated technique. For serum P1NP these values were similar to those previously described.

In women serum CTx levels were similar to those from studies using pre-menopausal women but quite different from those focussing on post-menopausal women.

The male serum CTx data was different to those previously reported.

These differences emphasise the importance of establishing reference intervals for different populations, and the interest of carrying out comparisons.

We believe that our reference intervals can serve as useful standards for bone turnover in the adult population in Australia.

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