Title

The components of variance and the critical difference in specific markers of bone turnover in healthy adult males & postmenopausal women

Authors

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Introduction

The study outlined here is part of an overall project, which aims to identify the optimum marker of bone resorption for both healthy young males and post-menopausal, non-osteoporotic women, and is as yet ongoing[1]. The purpose of this investigation was to quantify the components of variance in specific biochemical markers of bone turnover in healthy males and post-menopausal women i.e. total (CV_S), analytical (CV_A), within-subject biological (CV_I) and between-subject (CV_G) variances. Using CV_A and CV_I for each individual, the individual critical difference (CD_I) or least significant change ($P<0.05$) was calculated as previously described[2]. This value represents the minimal difference between two measurements of a biochemical marker that indicates a medically significant alteration of homeostasis (and is not due to normal biological and/or analytical variability alone). Given the CD of these markers the viability of using them in the assessment and/or monitoring of bone metabolism will be considered.

Methodology

With ethical approval and informed consent, 17 healthy males (28.2±1.0 years) and 13 healthy post-menopausal women (55.1±1.2 years) who were not on HRT participated in this study. Subjects did not suffer from a known clinical disorder of bone or calcium metabolism. All subjects were inactive, non-smokers and had not experienced a fracture or period of immobilisation in the six-month period prior to participation. Normal calcium intake was regulated by a prior 5 consecutive day dietary intake record (800mg/day for males and 1200mg/day) and alcohol consumption was not permitted for 3 days prior to and for the duration of the study. Blood (venepuncture, 8am-9am) samples were collected following an overnight fast (22h00) for 5 consecutive mornings. Serum was analysed for N-MID Osteocalcin (OC; ng/ml) and CrossLaps (ng/ml) as measured by electrochemiluminescence (ECL; *Roche Diagnostics*). Following the first morning void (FMV) sample, 24h urine and a spot mid-flow FMV sample were collected for 5 consecutive mornings. All samples were analysed for total dPyr by ELISAand creatinine by HPLC.

Results

Table 1 Summary of the components of variance and critical difference for serum and urinary markers of bone formation and resorption in healthy males (n=17) and post-menopausal females (n=13). Data is represented as mean ±SD.

Biochemical Marker	CV_S(%)	CV_A(%)	CV_I(%)	CD_I(%)	CV_G(%)
Males (n = 17)					
OC (mg / ml)	4.4 ± 1.6	1.6 ± 0.8	4.3 ± 1.7	9.5 ± 3.5	4.9
CrossLaps™ (ng/ml)	8.8 ± 3.8	2.2 ± 1.0	8.6 ± 3.8	19.0 ±7.9	9.8
24h dPyr (nmol/d)	29.3 ± 10.9	6.0 ± 2.3	28.9 ± 11.1	63.0 ± 23.5	32.1
FMV dPyr (nmol/d)	33.9 ± 15.5	5.9 ± 2.6	33.6 ± 15.5	72.8 ± 33.3	39.7
Creatinine (nmol/L)	32.9 ± 19.9	1.2 ± 0.5	37.3 ± 19.9	70.7 ± 42.7	37.1
Females (n=13)					
OC (mg/ml)	5.7 ± 4.6	1.0 ± 0.4	5.6 ± 4.6	12.3 ± 9.8	7.1
CrossLaps™ (ng/ml)	9.4 ± 3.9	2.8 ± 1.7	9.2 ± 3.9	20.3 ± 8.3	3.1
24h dPyr (nmol/d)	23.0 ± 9.5	5.8 ± 2.4	22.6 ± 9.6	49.4 ± 20.5	26.6
FMV dPyr (nmol/d)	27.0 ± 8.3	5.4 ± 1.9	26.7 ± 8.3	57.9 ± 17.9	27.6
Creatinine (nmol/L)	27.9 ± 20.0	1.1 ± 0.7	27.9 ± 20.0	56.0 ± 42.8	36.5

Conclusions

The CD_I of a biochemical marker is an objective index of the ability of that marker to detect a change in bone turnover and given that biochemical markers of bone turnover are generally used in monitoring intervention strategies, it would appear that the marker with the best potential to detect change would be preferred.

(1) As CV_A is common between subjects, CD_I is predominantly influenced by CV_I, which can vary greatly between subjects as evidenced by large SD values in Table 1.

(2) With respect to the serum markers, the mean intra-individual CV_I for OC is lower (4.5% and 5.6%) than that for CrossLaps™ (8.6% and 9.2%) which would suggest that the marker of bone formation (OC) is a more stable marker for both groups. The corresponding mean intra-individual CD_I is also lower (9.5% and 12.3%) for OC than for CrossLaps™ (19.0% and 20.3%).

(3) The mean intra-individual CV_I for the urinary measure of dPyr (marker of bone resorption) is 3.5 - 4 fold that of CrossLaps™ (serum marker of bone resorption) for the male subjects and 2.5 - 3 fold that of CrossLaps™ for the female subjects.

(4) The mean intra-individual CV_I was for dPyr lower in the 24h (nmol/day) urine sample (28.9% and 22.6) than in the mid-flow FMV (nM/mM Cr) urine sample (33.6% and 26.7%) for both groups. The greater biological variation in the latter measure may be attributed to the need to correct this data for creatinine output which varies itself (37.3% and 27.9%). The corresponding mean intra individual CD_I is also lower for the 24h sample (63.0% and 49.4%) than for the FMV (72.8% and 57.9%) sample.

References

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