Table 1. Reference intervals for skeletal muscle, MNC, and plasma CoQ_{10} concentrations.

	CoQ_{10} concentration	Units
Skeletal muscle		
Observed range	140–580	pmol/mg of protein
Mean (SD)	241 (95)	pmol/mg of protein
MNCs		
Observed range	37–133	pmol/mg of protein
Mean (SD)	65 (24)	pmol/mg of protein
Plasma		
Observed range	227–1432	nmol/L
Mean (SD)	675 (315)	nmol/L

Reference intervals for skeletal muscle, MNCs, and plasma were established from the observed range of CoQ_{10} concentrations for these tissues (Table 1). The reference intervals for skeletal muscle and plasma were comparable to those reported by Artuch et al. *(16)* and Miles et al. *(17)* for skeletal muscle and plasma, respectively. To our knowledge, there have been no reference intervals for MNC CoQ_{10} reported by other laboratories. Age and sex had no significant influence on tissue CoQ_{10} concentrations in the reference population, allowing the effect of these variables to be excluded from the study (results not shown). By comparing the reference intervals, we found evidence of a CoQ_{10} deficiency in skeletal muscle (33 pmol/mg of protein) and MNCs (20 pmol/mg of protein) in the 47-year-old female patient with low skeletal muscle complex II-III activity.

The decreased CoQ_{10} status of MNCs and skeletal muscle from this patient suggested that a relationship might exist between the CoQ_{10} status of these tissues, and this prompted us to assess the relationship between skeletal muscle, MNC, and plasma CoQ_{10}. We found a close association between skeletal muscle and MNC CoQ_{10} concentrations in the 12 disease control patients and in the CoQ_{10}-deficient patient ($r = 0.89$; $P < 0.02$; n = 13). Exclusion of the CoQ_{10}-deficient patient from this correlation did not significantly alter this relationship ($r = 0.86$; $P < 0.02$; n = 12). We found no correlation between skeletal muscle and plasma CoQ_{10} concentrations ($r = 0.015$; n = 10) or between MNC and plasma CoQ_{10} concentrations ($r = 0.21$; n = 24).

In conclusion, we have synthesized a di-propoxy-CoQ_{10} IS that can be used in CoQ_{10} assessment in MNCs, skeletal muscle, and plasma, allowing precision and a good recovery. This IS enabled the establishment of reference intervals for the CoQ_{10} concentrations of skeletal muscle, MNCs, and plasma, which has facilitated the identification of a patient with a CoQ_{10} deficiency.

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Serum Tartrate-Resistant Acid Phosphatase 5b or Amino-Terminal Propeptide of Type I Procollagen for Monitoring Bisphosphonate Therapy in Postmenopausal Osteoporosis? *Matti J. Välimäki[1*] and Riitta Tähtelä[2]* ([1] Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; [2] Mehiläinen Oy Laboratoriopalvelut, Helsinki, Finland; * address correspondence to this author at: Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland; fax 358-9-47175798, e-mail matti.valimaki@hus.fi)

Bone markers to monitor the efficacy of antiresorptive therapy of osteoporosis are of great value to clinicians. Considerable decreases in markers can be seen within 3 to 6 months after the start of an efficient treatment, with considerable increases in bone mineral density (BMD)

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being observed in 1 to 2 years *(1–3)*. Decreases in marker concentrations reflect the patient's compliance to treatment and, particularly for bisphosphonate therapy, the intestinal absorption of the drug, which may be poor. By indicating that the treatment is efficacious, biomarkers may also encourage the patient to continue therapy *(4)*. Economic restrictions and the many bone turnover markers available make it challenging to choose the one that best serves these purposes and most reliably predicts fracture prevention. The most important consideration in practice may be to choose a marker that enables the clinician to make a clear distinction between responders and nonresponders to treatment.

The amino-terminal propeptide of type I procollagen (PINP) is liberated into the circulation during type I collagen formation, and its serum concentration reflects bone formation rate *(5)*. Because of the coupling between bone resorption and formation, PINP shows promise as a sensitive indicator of the efficacy of antiresorptive therapy *(6)*. Tartrate-resistant acid phosphatase (TRACP), an iron-containing 35-kDa enzyme, is produced in osteoclasts, beginning early in their development. TRACP has several known functions in the osteoclasts from which it is liberated into the circulation in active form *(7–9)*. In addition to osteoclastic isoform TRACP5b, human serum contains another, differently glycosylated isoform, TRACP5a. Secreted TRACP5b activity, which can be measured specifically with a novel immunoextraction method *(10)*, is believed to reflect bone resorption.

We compared serum PINP and TRACP5b as markers for distinguishing between responders and nonresponders to bisphosphonate treatment with alendronate or risedronate. The response was defined in terms of the least significant changes (LSC) in the markers. Sixty-nine postmenopausal women, 60 years of age or older, with osteoporosis (lumbar spine or total hip BMD T-scores −2.5 or lower, or both lumbar spine and total hip BMD T-scores −2.0 or lower) participated in the study, which was approved by the Ethics Committee of the Department of Medicine, Helsinki University Central Hospital. The study participants were a subgroup of a larger placebo-controlled trial in which changes in bone resorption and BMD were compared in patients receiving 70 mg of once-weekly alendronate and those receiving 5 mg of daily risedronate *(11)*.

In the present study, 20 women received placebo, 26 risedronate, and 23 alendronate. All patients maintained a daily calcium intake of 1000 mg/day, and those with a baseline serum 25-hydroxyvitamin D concentration <15 μg/L received supplementation of 400 IU/day of vitamin D. Blood was sampled before and after treatment at 1, 3, 6, and 12 months. Serum samples were kept frozen at −70 °C until assayed for intact PINP and TRACP5b. PINP was determined by a competitive RIA with reagents (Intact PINP RIA) from Orion Diagnostica. The detection limit of the assay was 2 μg/L, and its intra- and interassay CVs ranged from 2% to 6%. TRACP5b activity was measured by an immunoextraction method with reagents (BoneTRAP™) from Suomen Bioanalytiikka Oy. The limit of detection of this assay was 0.1 U/L, and the intra- and interassay CVs were ≤6%.

The long-term intraindividual variability of the markers ($CV_i = SD_i/mean_i$) was calculated for placebo group participants from 5 measurements at 0, 1, 3, 6, and 12 months. From these CV_is, the LSCs of the markers were calculated by the formula:

$$LSC = 1.96 \times \sqrt{2 \times (\sum CV_i^2)/n}$$

which gives the LSC with a 95% confidence interval *(12)*. The LSC for TRACP5b was 26.9% and for PINP was 28.3%. Responders with respect to changes in each marker were those who exhibited a decrease greater than or equal to the LSC. The change in a marker at each time point was expressed as percentage from the baseline value, using the formula:

$$[(\text{Actual value} - \text{baseline value})/\text{baseline value}] \times 100$$

The changes in the markers over time were analyzed with repeated-measures ANOVA (Proc MIXED), with treatment group, time, and interaction of group and time as factors in the model. The natural logarithm transformation was used to achieve a normality assumption. The Fisher exact *t*-test was used to compare the number of responders between the treatment groups at different time points. The measure of agreement between responders, defined by TRACP5b and PINP, was compared by McNemar test for the whole study population and for each treatment group. All tests were performed 2-sided, with a 0.05 significance level, using the SAS® System (Ver. 8.02 for Windows).

Serum TRACP5b and PINP concentrations decreased in both treatment groups, although in the risedronate group TRACP5b changes were of borderline significance at 6 months ($P = 0.091$) and 12 months ($P = 0.060$; Fig. 1). Over time, the alendronate group differed from the placebo group ($P < 0.0001$) and from the risedronate group ($P < 0.0001$) with respect to each marker, as did the risedronate group from the placebo group ($P = 0.001$ for TRAPC5b; $P = 0.011$ for PINP). In the whole study population (n = 69), the number of responders, based on the LSC, was higher at 1 month for TRACP5b than for PINP ($P = 0.002$; Table 1); from that time point onward, the number of responders was higher for PINP than for TRACP5b ($P < 0.0001$ to 0.008). The number of responders to risedronate identified by changes in PINP was significantly higher than the number identified by TRACP5b at 6 months ($P < 0.0001$) and at 12 months ($P = 0.008$), as was the case for the responders to alendronate at 12 months ($P = 0.031$). At 1 month, more women were identified as responders to alendronate by TRACP5b than PINP ($P < 0.0001$). At each time point, the number of responders was higher for alendronate than for risedronate ($P < 0.0001$ to 0.005).

In keeping with findings on urinary N-telopeptides of type 1 collagen in the primary study *(11)*, alendronate suppressed serum TRACP5b and PINP more efficiently than did risedronate. Accordingly, in terms of the LSC, the percentages of responders to risedronate and alendro-

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nate were 12%–19% and 65%–78%, respectively, as determined with TRACP5b, and up to 54% and 96%, respectively, as determined with PINP.

Choosing bone markers to use in clinical practice is highly dependent on the antiresorptive treatment used. If a highly efficacious suppressant of bone turnover such as alendronate is used, several markers may be used to distinguish between responders and nonresponders. In the alendronate-treated patients in this study, TRACP5b identified responders after 1 month of treatment, but over time more responders (up to 96%) were identified by PINP, although differences between the markers remained small and statistically nonsignificant. The results were different for risedronate, the less suppressive effect of which was poorly indicated by TRACP5b but much better by PINP from 6 months onward. According to LSC, however, only 54% or fewer of patients treated with risedronate appeared to be responders. We came to a similar conclusion in our earlier study *(13)* of patients treated with clodronate for 2 years: 79% were responders by PINP, 34% by TRACP5b, and 40% by urine N-telopeptides of type 1 collagen when the LSC values of 32% (28% in the present study), 27% (27%), and 55%, respectively, were used.

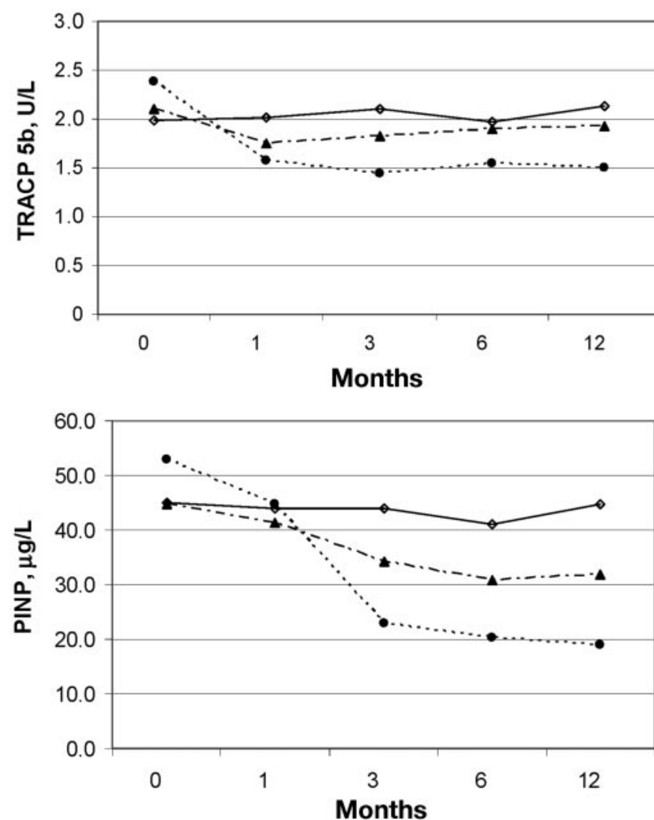

Fig. 1. Mean serum concentrations of TRACP5b and PINP in the study groups.

Over time, the alendronate group (●) differed from the placebo group (◇) and the risedronate group (▲) with respect to each marker (P <0.0001), and the risedronate group from the placebo group (P = 0.001 for TRACP5b and P = 0.0011 for PINP). In the alendronate group, changes from baseline for both markers were significant at each time point (P <0.0001). In the risedronate group, this was the case for PINP (P = 0.003 to <0.0001) and for TRACP5b at 1 (P <0.0001) and 3 months (P = 0.045).

Table 1. Responders to different treatments according to changes in TRACP5b and PINP at different time points.

Treatment	Time point, months	TRACP5b, n (%)	PINP, n (%)	*P*
All (n = 69)	1	21 (30.4)	6 (8.7)	0.002
	3	21 (30.9)[a]	29 (42.7)[a]	0.008
	6	23 (33.3)	36 (52.2)	0.004
	12	21 (30.4)	37 (53.6)	<0.0001
Placebo (n = 20)	1	2 (10.0)	1 (5.0)	
	3	0	1 (5.0)	
	6	2 (10.0)	1 (5.0)	
	12	0	1 (5.0)	
Risedronate (n = 26)	1	4 (15.4)	2 (7.7)	0.69
	3	4 (15.4)	7 (26.9)	0.25
	6	3 (11.5)	14 (53.9)	<0.0001
	12	5 (19.2)	13 (50.0)	0.008
Alendronate (n = 23)	1	15 (65.2)[b]	3 (13.0)	<0.0001
	3	17 (77.3)[a,b]	21 (95.5)[a,b]	0.13
	6	18 (78.3)[b]	21 (91.3)[c]	0.38
	12	16 (69.6)[b]	22 (95.7)[b]	0.031

[a] One sample lacking.

[b,c] Significant difference between numbers of responders to risedronate and alendronate according to changes in each marker at different time points: [b] P <0.0001; [c] P = 0.005.

Because the extent to which bone turnover should be suppressed by antiresorptive drugs to achieve the optimum balance between safety and efficacy is not known, the choice of bone markers for use in clinical practice is challenging. In risedronate-treated patients, the prevention of vertebral fractures was not improved by more advanced suppression of bone turnover *(14)*, but this was not the case in alendronate-treated patients *(15)*. Risk of adynamic bone disease, however, must be kept in mind when using efficacious bisphosphonates *(16)*. We used the LSC values to compare the 2 markers. This method is statistically correct, but if sufficient fracture reduction and safety are achieved with only mild-to-moderate suppression of bone turnover, then changes in markers less than the LSC may be the aim of treatment, and LSC comparison would not be useful.

TRACP5b was inferior to PINP in revealing responders to treatment with risedronate or alendronate, although it detected the response to alendronate within 1 month. This quick response is not very useful in clinical practice, however, because measurements of bone markers are typically repeated after 3 to 6 months of treatment. In addition, measurement of PINP instead of TRACP5b is indicated for monitoring treatment with less efficacious suppressants of bone turnover.

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Variation with Time in Components of Variance for Measurements of Therapeutic Drugs, *John F. Wilson[1]* and Kathleen Barnett[2]* ([1] Department of Pharmacology, Therapeutics and Toxicology, Wales College of Medicine, Cardiff University, Cardiff, Wales, United Kingdom; [2] Cardiff Bioanalytical Services Ltd., Cardiff, Wales, United Kingdom; * address correspondence to this author at: Cardiff Bioanalytical Services Ltd., 16 Mount Stuart Square, Cardiff CF10 5DP, Wales, United Kingdom; fax 44-29-2048-9003, e-mail wilsonjf@cardiff.ac.uk)

Analyses for therapeutic drugs are commonly made with automated, high-throughput, multichannel instruments that require minimal operator intervention. Performance monitoring of these assays is accomplished by means of internal and external (proficiency testing) quality-control procedures. Proficiency test schemes have demonstrated that the within-laboratory sources of variation are more important than between-laboratory sources *(1–3)*, and the College of American Pathologists laboratory improvement program showed that the within-laboratory variance doubled for samples measured 4 months apart compared with measurements made at the same time *(2)*. The present study was designed to track this decrease in within-laboratory precision over time to provide insights into the possible sources of imprecision in routine clinical measurements of therapeutic drugs in serum.

A lyophilized proficiency test sample was prepared by adding midtherapeutic concentrations of 14 drugs to 5.9 L of human serum (Scipac Ltd.). Drug concentrations were as follows: phenytoin, 15.2 mg/L; phenobarbital, 30.3 mg/L; primidone, 7.1 mg/L; carbamazepine, 7.7 mg/L; carbamazepine 10,11-epoxide, 1.9 mg/L; ethosuximide, 68.1 mg/L; valproate, 76.8 mg/L; clonazepam, 42.0 μg/L; lamotrigine, 3.1 mg/L; theophylline, 15.0 mg/L; caffeine, 7.8 mg/L; digoxin, 1.2 μg/L; gentamicin, 2.7 mg/L; and lithium 0.76, mmol/L. The CV of dispensing of test sample aliquots by weight was 0.08%. We distributed 5 differently coded aliquots of the proficiency test sample, on 4 occasions, for analysis by members of the United Kingdom National External Quality Assessment scheme for drug assays. The scheme has an international membership of mostly hospital- or clinic-based sites, with 60% of their participants from the United Kingdom, 30% from Europe, and 10% outside Europe. A pair of samples was sent for analysis 1 month, and 3 single samples were sent at intervals to permit comparisons between pairs of measurements on the same sample, measured 0 to 6 months apart. The 5 samples formed part of the routine circulation of materials, and their identity was blinded to scheme participants. Laboratories reported the measured drug concentrations for their available range of drug assays and, for each measurement, the analytical technique used.

Drug measurements for all techniques combined were screened individually for the 5 sample distributions to reject those >3 SD from the sample mean *(4)*. The percentage of outliers for each technique was as follows: HPLC, 4.1%; Abbott TDx, 1.9%; Abbott AxSYM, 0.6%; Roche fluorescence polarization immunoassay (FPIA), 1.9%; Roche kinetic interaction of microparticles (KIMS) immunoassay, 1.5%; Roche Tina-quant, 1.8%; Beckman turbidimetric assay, 2.6%; Bayer chemiluminescent assay, 0.9%; cloned enzyme donor immunoassay (CEDIA), 4.3%; Olympus, 3.5%; Vitros, 2.8% (see footnotes to Table 1 for manufacturers of assays used). Data were taken for analysis when a laboratory had reported nonrejected data for a drug for all 5 samples assayed by the same technique. Data for each analytical technique were analyzed independently, thereby excluding the known variation attributable to differences in accuracy among techniques *(3)*. Data for a technique were analyzed when data for a drug

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