Interference of Nicotine Metabolites in Cotinine Determination by RIA, Piergiorgio Zuccaro,* Simona Pichini, Ilaria Allieri, Mirella Rosa, Manuela Pellegrini, and Roberta Pacifici (Clin. Biochem. Dept., Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Rome, Italy; *author for correspondence: fax ++ 39 6 4461961)

Cotinine (COT), a major metabolite of nicotine (NIC), has been used as a biomarker in many studies of active and passive smoking [1–4]. Methods of analysis for COT in biological fluids include gas chromatography, gas chromatography–mass spectrometry, HPLC, HPLC–mass spectrometry, and immunoassays [5–10]. In particular, the RIA developed for COT and NIC by Langone and Van Vunakis [11] was the method reported by the International Agency for Research on Cancer (IARC) for determination of these analytes in several biological matrices [12, 13].

In the description of the assay [11], no mention was made about cross-reactivity of anti-NIC and anti-COT antibodies with trans-3'-hydroxycotinine (THOC) and cotinine glucuronide (COT-G). Indeed, these two compounds were found to be the most abundant metabolites of NIC in urine of smokers [14]. Other studies, however, have investigated the cross-reactivity of the anti-COT antiserum with THOC and other metabolites in an ELISA [15].

The objective of this study was to investigate the cross-reactivity of the anti-COT and anti-NIC antisera with THOC and COT-G in the RIA [11–13]. We also used urine samples from active and passive smokers to compare data from the RIA with data obtained by HPLC.

The rabbit antiserum, (−)[3H]COT, and (−)[3H]NIC were supplied by H. Van Vunakis (Brandeis University, Waltham, MA). The detectable range of measurement from the calibration curve was 0.5–50 μg/L for NIC and 0.2–20 μg/L for COT. The cross-reactivities of the anti-COT and anti-NIC antibodies with NIC metabolites different from THOC and COT-G were reported as <5%. In addition, urine samples of smokers were analyzed for COT and THOC with an HPLC method described by Zuccaro et al. [16]. In brief, the method used reversed-phase chromatography with ultraviolet detection (254 nm) to measure NIC metabolites in urine samples that had undergone solid-phase extraction before chromatography. The limit of quantification with this method was 10 μg/L for NIC and 5 μg/L each for COT and THOC, and the assay showed good reproducibility, intra- and interday CVs being <4% for various concentrations of the analytes.

Calibrators for NIC, COT, THOC, and COT-G were prepared at several concentrations in Tris buffer (pH 7.4) and were used in the RIA to calculate inhibition curves by comparison with the calibration curves for COT and NIC. Duplicate or triplicate assays were performed on different days. The relative cross-reactivity was determined as calculated by Abraham [17]. In particular, the percent of cross-reaction was calculated from the percent ratio between the concentration (μg/L) of COT (or NIC) and that of the other metabolites required to displace 50% of the (−)[3H]COT or (−)[3H]NIC bound to the antisemur.

Stock solutions of NIC, COT, and THOC were prepared in methanol, stored at <0 °C, and used with appropriate dilutions for chromatographic analyses. We also obtained urine samples from 13 smokers and 6 smoke-exposed nonsmokers, stored these at −20 °C, and used aliquots of these samples for both RIA and HPLC analyses. In particular, aliquots used for the RIA were diluted (from 1:50 to 1:400) so as to fall within the range of measurement reported for the assay.

In addition, we added known quantities of COT and THOC to blank urines obtained from nonsmokers who had not been exposed to environmental tobacco smoke. We analyzed these samples by RIA and HPLC to verify the results obtained for the smokers and the smoke-exposed nonsmokers.

The relative cross-reactivities of NIC, COT, THOC, and COT-G with the anti-COT and anti-NIC antisera are reported in Table 1. Results obtained for NIC and COT confirmed the data in the literature [11]. Further, we observed no cross-reactivity of THOC and COT-G at increasing concentrations with anti-NIC antisemur, nor of COT-G with anti-COT antisemur. However, the cross-reactivity of THOC with anti-COT antisemur at 50% inhibition was 34%, high enough to lead to an overestimation of COT in the presence of THOC—as confirmed by the comparison between HPLC and RIA values of COT in blank urine samples with added COT and THOC and in samples from smokers and smoke-exposed nonsmokers (Table 2). Moreover, the HPLC and RIA values of blank samples supplemented with similar quantities of THOC and COT correlated highly: r = 0.98 (P = 0.0003) for RIA vs HPLC values for COT, and r = 0.99 (P = 0.0002) for RIA COT vs HPLC COT plus THOC. For samples from the smokers and the exposed nonsmokers, RIA vs HPLC values for COT (r = 0.8, P = 0.00002) and RIA COT vs HPLC COT plus THOC (r = 0.8, P = 0.00001) still correlated, even though the RIA results for high values of THOC differed from the true concentrations of COT and THOC (as measured by HPLC) (Table 2, subjects 7, 8, 10).

In the recent years, COT has been used in epidemiological screenings as a biomarker to discriminate between active and passive smokers and to quantify the exposure

<table>
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<th>Compound</th>
<th>Anti-COT antiserum</th>
<th>Anti-NIC antiserum</th>
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<tr>
<td>(−)NIC</td>
<td>0.05</td>
<td>100 (13.5)</td>
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<tr>
<td>(−)COT</td>
<td>100 (3.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(±)THOC</td>
<td>34.0 (10)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>COT-G</td>
<td>&lt;0.01</td>
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* Shown in parentheses is the concentration (μg/L) of compound required to produce 50% inhibition in the respective assay.
to environmental tobacco smoke [2]. Discrimination between active and passive smokers is reasonably easy, the mean concentrations of COT in the former group being as much as 10 times those in the latter. Much more difficult is differentiation between light smokers and highly exposed passive smokers, or between exposed passive smokers and nonexposed nonsmokers; in these subjects the COT values are low and may overlap, approaching cutoff values. For this situation, accuracy and precision of the measurements need to be improved, e.g., by adjusting urinary COT for the urinary creatinine concentration [18] or by using a more sensitive and specific method of detection such as isotope dilution liquid chromatography–tandem mass spectrometry [19] or a monoclonal antibody immunoassay tested for cross-reactivity with THOC.

Indeed, although cross-reactivity of THOC for COT antiserum might be useful from an epidemiological point of view (by indirectly increasing the sensitivity of the COT RIA), it represents an analytical bias, which should be indicated in reports of the characteristics of the assay. In any case, one must take into account the method of COT analysis when choosing the cutoff value to discriminate between exposed and nonexposed nonsmokers, to be able to correctly compare results obtained with methods based on different principles.

Finally, in the present study, we determined the cross-reactivity of THOC by assaysing its racemic form, whereas the naturally occurring metabolite is (−)-THOC; cross-reactivity of the natural metabolite with anti-COT antiserum, therefore, which is stereospecific [11], can be higher than values reported here.

<table>
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<th>Table 2. COT and THOC concentrations (μg/L) measured by HPLC and RIA in supplemented urine and in urine from smokers and exposed nonsmokers.</th>
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<tr>
<td>Samples with added COT/THOC (μg/L)</td>
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<td>------------------------------------</td>
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<td>40/250</td>
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<td>2000/4000</td>
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<tr>
<td>Smokers</td>
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<tr>
<td>Exposed nonsmokers</td>
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References

Avoidance of False Positives in PCR-Based mRNA Differential Display During Investigation of Nonstandardized Tissues or Cells, Susanne Mohr, Paul Cullen, Roland Schmidt, Andrea Cignarella, and Gerd Assmann

Although not yet a part of clinical chemical practice, analyzing patterns of differential gene expression in tissues such as cancers will likely become a routine method in coming years. The technique of PCR-based differential display developed by Liang and Pardee [1] has become increasingly popular during the last 4 years and is a considerable advance on conventional methods used to identify differentially expressed genes, such as differential hybridization and subtractive library construction. The advantages of differential display are the low RNA requirement and high versatility. However, serious problems remain, one of the most important being the high rate of false positives. Several methods have been described to deal with this problem, including the simultaneous display of PCR products from two uninduced and two induced lines with the requirement that the patterns from the pairs of uninduced or induced lines agree [2]; the running of duplicate, identical samples from each RNA preparation side by side [3, 4]; the display of PCR products from uninduced and induced lines over a time course of induction [2]; and the repeating of experiments for those lanes in which putative candidate bands were identified [5].

We describe here a method for effectively eliminating false positives and spurious true positives (i.e., genes that really are regulated but whose regulation is not a result of the manipulation under investigation). This method is particularly useful when investigating gene expression in nonstandardized material such as fresh human or animal tissue or cells.

We use PCR-based differential display to investigate gene regulation in human foam cells produced by loading macrophages for 48 h with 80 \( \mu \)g/mL acetlyated (Ac) or oxidized (Ox) low-density lipoprotein (LDL). Human peripheral blood monocytes are isolated from volunteers by a combination of cell separation and countercurrent elutriation as previously described [6]. Aliquots of the fractions are examined for their purity in a FACScan (Becton Dickinson, San Jose, CA). Fractions containing >95% monocytes are pooled and the cells are plated at a density of \( 10^6 \) cells/L in 35-mm cell culture dishes (Falcon, Heidelberg, Germany) in RPMI 1640 (Gibco, Eggenstein, Germany) and incubated at 37 °C in a humidified incubator (5% CO₂). After 1 h, the monocytes adhere to the surface of the dishes, and nonadherent cells are removed by washing. The monocytes are then cultured for 14 days in RPMI 1640 supplemented with 200 mL/L human serum obtained from healthy volunteers. Total cellular RNA from unloaded (control) macrophages and from macrophages that have been loaded with cholesterol by using either AcLDL or OxLDL is isolated by means of a standard procedure using guanidinium isothiocyanate [7], and differential display is performed with a commercially available kit (Display Systems, Los Angeles, CA) and a standard protocol using AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT) and \( a^{-[35S]}dATP \) (Amersham, Middlesex, UK).

To identify spurious changes in the band pattern (false positives), we perform all experiments (including controls) twice, i.e., we prepare RNA from two different batches of macrophages isolated separately from different volunteers and loaded with different preparations of modified lipoproteins. We then load the corresponding amplified cDNA products from both differential display PCR reactions in adjacent lanes on a 6% denaturing polyacylamide sequencing gel 53 cm long. After 3 h, the gel is carefully transferred from the glass plates of the gel chamber to blotting paper and thoroughly dried at 80 °C for 1 h by using a vacuum gel-drier (Bio-Rad, Munich, Germany). This last step ensures that the dried gel is completely flat, because it is difficult, if not impossible, to achieve perfect alignment of a gel that has buckled.

After electrophoresis, the gels are exposed to Kodak XAR-5 film for 48 h. Achieving exact alignment of the dried differential display gel with the exposed x-ray film is difficult, but we eliminate this problem by using TrackerTape™ (Amersham), a phosphorescent tape that can be written on and then used to label the dried gel and hence the corresponding x-ray film. We mark the TrackerTape with suitable symbols and stick it onto opposite corners of the gel (Fig. 1) before exposure. By using the symbols written on the tape, we can exactly align the developed film with the gel. To mark the differentially

Fig. 1. Typical 6% denaturing polyacrylamide gel of a differential display reaction showing TrackerTape at three corners, adorned with appropriate symbols.

Exact alignment of the film with the flat dried gel is achieved by exactly lining up symbols on opposite corners of the film (arrows) with the original drawings on the TrackerTape.
exposed bands on the dried sequencing gel, we punch through the four corners of each band of interest in the film with a pointed scalpel.

A representative differential display pattern from a typical double experiment is shown in Fig. 2. The amplification products of the RNA preparations from two different batches of control cells are shown in lanes 1 (donor 1) and 2 (donor 2), and the products of the PCR using the same primers on RNA preparations from the same two different batches of cells in the loaded state are shown in lanes 3 (donor 1) and 4 (donor 2) from AcLDL-loaded cells and lanes 5 (donor 1) and 6 (donor 2) from OxLDL-loaded cells. Typically, 120–150 bands are seen per lane.

Most bands are present in all six lanes, which provides an important check on the reaction in general: Gross differences between the lanes are likely to represent a fundamental problem with the reaction (e.g., sample mix-up). Arrow A indicates a band that, although almost absent in all four of the loaded cell preparations (lanes 3–6), is present in only one of the unloaded cell preparations (lane 1). This band is therefore a false positive (for downregulation of the mRNA in question) but would have been classified as a true positive if the experiment had not been performed in duplicate, i.e., if only cells from donor 2 had been used as control.

Arrow B indicates a band that is present in both the unloaded (lane 1) and loaded (lanes 3 and 5) cells from donor 1 but absent in both the unloaded (lane 2) and loaded (lanes 4 and 6) cells from donor 2 (spurious true positive). In our system, we have found that about one quarter of all differentially expressed genes are of this type, i.e., they are differentially expressed from cell preparation to preparation, but not in response to the manipulation under investigation (in our case, loading of the cells with AcLDL or OxLDL). The importance of this phenomenon is that these genes are not false positives because they are truly differentially expressed. Thus, if our experiment had been performed with unloaded cells from donor 1 and loaded cells from donor 2 (or vice versa), we would have come to the incorrect conclusion that expression of the band shown is related to cholesterol-loading of the cells. Furthermore, because this is a truly differentially regulated gene, we would have confirmed this incorrect result by using Northern analysis.

Arrow C indicates a band that is present in both of the control preparations (lanes 1 and 2) and absent in both of the preparations from the loaded cells (lanes 3–6, true positive). We cut out such bands for further analysis. In our experience, it is usually not worthwhile isolating bands from the lower 1/3 to 1/2 (lower ~20 cm) of the gel because these are often short 3′ cDNAs that are difficult to characterize because 3′ ends of known genes are often absent from the genetic databases.

Performing all experiments in duplicate using different cell donors and different sets of reagents has three advantages: (a) The incidence of false positives is greatly reduced by allowing identification of genes that are consistently regulated by the manipulation under study. (b) It avoids the isolation of genes that are truly differentially regulated but whose regulation is not related to the manipulation under study but to other factors specific to the individuals under study. (This is particularly important when experiments are performed with nonstandardized systems such as freshly isolated human or animal cells or tissues. We have not pursued genes that are differentially regulated between various cell types and we are not aware of the reasons for the differential regulation. Possible causes include differences in cell isolation or cell culture conditions or innate differences in the response of the cells to experimental circumstances.) (c) Our approach allows reliable identification of genes that are differentially expressed in a quantitative fashion without being completely absent in any lane. Such quantitatively regulated genes can be difficult to identify using the conventional single preparation approach because often a quantitative difference in band strength cannot be distinguished from loading differences between lanes, even when the strength on nonregulated bands is taken into account.

Although duplicate performance of experiments may seem time consuming, our experience has shown that the extra effort expended at this stage is more than repaid by...
reducing unnecessary cloning, sequencing, and Northern blotting later in the process.

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References

Specificity of Salivary Thiocyanate as Marker of Cigarette Smoking Is Not Affected by Alimentary Sources, Laurence M. Galanti (Clinical Laboratory, Cliniques Universitaires de Mont-Godinne, B-5530 Yvoir, Belgium; fax 32 81 42 3204)

Thiocyanate is an end product of detoxification of hydrogen cyanide present in cigarette smoke. Its excretion in urine, saliva, and sweat can thus provide a useful marker of exposure in smokers and nonsmokers [1–4]. Salivary tests have the advantages of easy, noninvasive sampling and of good stability, even in nondemanding storage conditions [5]. However, thiocyanate and other cyanogens are also found in many foods including cabbage, broccoli, almond, and horseradish. Consequently, their ingestion can be supposed to artifactually increase thiocyanate excretion, thereby affecting the specificity of the test [6].

To evaluate the importance of this alimentary source of thiocyanate, we selected 65 subjects from the screening of 221 consecutive subjects in a smoking cessation program and from a healthy population of adult volunteers. Ages ranged from 18 to 72 years (median 48 years). All subjects were asked to fill in a questionnaire on their smoking habits and on their alimentary intakes during the 3 weeks preceding sampling. The criteria of selection were as follows: smokers (>10 cigarettes/day) who did not ingest cyanogenic food during the last 3 weeks, ex-smokers for >3 weeks, and nonsmokers. Salivary thiocyanate concentration was measured in duplicate by the method of Densen et al. [7]. On the basis of the questionnaire, three groups were compared: Group A included 30 nonsmokers or ex-smokers who had ingested food known to contain cyanogens during the last 3 weeks. Among those subjects, 10 had ingested such food at more than three meals, and three subjects at more than five (up to eight) meals. Group B (n = 20) included smokers, and group C (n = 15) included nonsmokers or ex-smokers who had avoided these foods. The study was in agreement with the guidelines approved by the ethics committee at our institution.

Salivary thiocyanate concentrations were similar in groups A and C (mean ± SD 101 ± 51 vs 92 ± 90 mg/L; not significant) but were significantly higher in group B (413 ± 172 mg/L; P < 0.01 vs groups A and B). Considering a cutoff value of 230 mg/L to separate smokers from non- or ex-smokers, all but two subjects (one in group B and one in group C) were correctly classified (Fig. 1), which corresponds to a specificity of 95% and a specificity of 98%. In addition, no trend toward higher salivary thiocyanate concentrations was observed in group A subjects, whose ingestion of food containing cyanogens was the most important (more than three meals: 97 ± 57 mg/L).

Among the biological markers of the exposure to tobacco smoke, thiocyanate is widely used in epidemiological studies [8–12]. Thiocyanate is a metabolite of cyanide and the end product of the detoxification of cyanide
compounds by the catalytic action of a mitochondrial enzyme called rhodanase. Hydrogen cyanide and organic cyanides are respectively present in the gaseous and in the particulate phases of tobacco smoke and are absorbed mainly at the pulmonary level. These cyanide compounds are the main source of exogenous thiocyanate in smokers. Saliva tests are the most stable and sensitive procedure for obtaining thiocyanate measures of smoking exposure [13]. The half-life of salivary thiocyanate is 10 to 14 days, allowing the evaluation of exposure to the toxic components of tobacco smoke during the preceding 3 weeks. Besides cabbage, broccoli, almond, and horseradish, cyanide compounds are also present in manioc, wood millet grass, and corn; ingestion of these foods could theoretically increase the thiocyanate concentration independently of smoking exposure. Although no study to date has determined whether or not this alimentary supply may significantly affect the specificity of salivary thiocyanate concentrations as a marker of smoking exposure, alimentary contamination is generally considered, on a theoretical basis, a possible cause of a false-positive test.

Our data show that, in non- and ex-smokers, the concentration of thiocyanate in saliva is not affected by alimentary sources and that the specificity of salivary thiocyanate concentrations to identify smokers is not affected by alimentation. This suggests that, despite the presence of cyanogens in some foods, the usual alimentary supply is too low to significantly affect the diagnostic value of the test. Consequently, the results of this test may be interpreted independently of any dietetic consideration.

References


Measurement of Maternal Folate Status and Risk of Neural Tube Defects, Gregory S. Makowski* and Sidney M. Hopfer (Div. of Clin. Chem., Dept. of Lab. Med., Univ. of Connecticut School of Med., Farmington, CT 06030; *address for correspondence: Dept. of Lab. Med., Univ. of Connecticut Health Center, MC-2235, 263 Farmington Ave., Farmington, CT 06030-2235; fax 806-679-2154, e-mail makowski@nso1.uchc.edu)

Good evidence exists that increased maternal plasma/red blood cell folate concentrations is associated with fewer neural tube defects in newborns [1, 2]. This finding has led to the suggestion that women pre- and periconception maintain folate adequacy—the concentration of which remains controversial [3–5]. Equally important yet generally overlooked is the variety of analytical methods used for folate measurement. For example, review of data obtained from ~1400 clinical laboratories participating in the 1995 College of American Pathologists (CAP) analytical survey [6] yields striking differences in plasma folate measurement. In three challenges during 1995 (K-A, K-B, and K-C), laboratories were asked to determine folate concentration in nine plasma samples (red blood cell folate was not measured). Survey data were grouped by methodology and statistical analysis was performed. Only results obtained from at least 20 laboratories reporting were considered for this study (>90% data).

Large differences in mean plasma folate concentration were observed when a manual radioisotopic immunnoassay was compared with an automated fluorescent method (Fig. 1A). Of the nine samples evaluated during this survey period, the most discrepant (comparison of mean) plasma folate results were seen in six samples analyzed by these two methods throughout the concentration range tested (marked with an asterisk, Fig. 1A). Differences in the nine folate results obtained by these methods ranged from 144% to 264% and placed first [6], second [11], or third [2] in degree of discrepancy when compared with all methods cited in the survey (Table 1). Comparison of results for mean plasma folate concentration obtained with the manual vs automated method revealed considerable bias (Fig. 1B) (e.g., an automated plasma folate concentration of 5 μg/L (adequate) would be considered marginal or inadequate (2.5 μg/L) by manual method). Average CV was not, however, substantially different for the automated and manual method: 9.6% (range 4.7–12.2%) and 10.5% (range 8.2–16.6%), respectively. Also notable is the trend in the number of laboratories using these methods (Fig. 1C). The manual RIA method was most cited in K-A, whereas the automated method was most cited in K-C.

The shift in plasma folate methodology may reflect decreased reliance on radioisotopes in general as well as
marketplace vagaries. It is reasonable that the dramatic changes in the healthcare industry have prompted the clinical laboratory to choose automated cost-saving methods. Although the advantages of analytical precision and accuracy can certainly be debated [7–9], it is evident that clinicians must be aware of these seldom-explored issues as they affect patient care. Establishment of method-dependent reference intervals will minimize this type of discrepancy. Future resolutions may require improved assay standardization and use of an international reference material.

References


Bone alkaline phosphatase (b-ALP; EC 3.1.3.1) is a tetrameric glycoprotein found on the cell surface of osteoblasts. Previous studies have shown that both b-ALP [1] and total ALP [2] can be considered markers of bone formation. Because postmenopausal osteoporosis in women and senile osteoporosis in both sexes are global epidemiological problems, biochemical markers of bone turnover have become important clinical tests. Such markers are used to identify subjects with rapid bone turnover (and thus bone loss) [3] and to monitor the therapeutic efficacy of various drugs [4]. These two purposes mandate separate reference ranges for different sexes and age groups. To evaluate the age-related changes of these two markers in Chinese men and women, we measured both total ALP and b-ALP of healthy Chinese men (n = 156, ages 20–90 years) and women (n = 385, ages 40–70 years; 118 premenopausal and 267 postmenopausal), all of whom underwent a careful screening and showed no liver or bone disease.

Total serum ALP activity was analyzed with a Hitachi-7450 automated analyzer and diphenylcresol phosphate as the substrate (Hitachi, Tokyo, Japan). The procedure has a long-term (over 1 year) imprecision
(CV) of 5%. β-ALP was measured with a commercial kit (Alkphase-B™; Metra Biosystems, Los Angeles, CA) that utilized a monoclonal anti-β-ALP antibody coated on a microtiter plate to capture β-ALP in the samples; the enzyme activity of the captured β-ALP was then detected by using p-nitrophenyl phosphate substrate [5]. The intra- and interassay CVs in our laboratory were 8% and 11%, respectively, for β-ALP at 25 U/L. We then adjusted the reading of total ALP (Hitachi ALP) to the reading obtained on the microtiter plate and with p-nitrophenyl phosphate substrate (plate ALP), according to a published method [6]; in our laboratory, Hitachi ALP = 2.93 plate ALP + 12.57 (r = 0.97, P < 0.0001). We then derived the nonbone fraction of ALP by subtracting β-ALP from plate ALP activity.

The results (Fig. 1) showed increased β-ALP activity in women and a borderline decrease of β-ALP in men (P = 0.015) with aging. In contrast, the nonbone form (assumed to be largely the liver form) of ALP showed the same trends of increase in both men and women, but the slope of the regression line was slightly steeper for the men’s results. The total ALP activity showed a borderline increase (P = 0.088) in men and a significant (P = 0.0001) increase in women with increasing age. The proposed age-specific reference intervals (central 95% percentiles) for men and women, premenopausal and postmenopausal, are given in Table 1.

Our findings suggested that bone formation rates—as reflected by β-ALP, a specific bone formation marker—decreased rather than increased in elderly men. This is compatible with the findings of decreases in markers of bone formation in the histomorphometry of bone in older men [7] and with previous reports of decreased bone formation markers including β-ALP [8] and osteocalcin [7, 8] in elderly men. Our results were also compatible with a previous study of elderly or postmenopausal women, which showed increased turnover rate of bone in both histology markers [7] and biochemical bone markers [2, 3].

The increase of β-ALP after menopause in women has been explained by removal of the inhibitory effects of estrogen on turnover rate [9]. At present, the relation

![Fig. 1. Linear regression of total ALP (plate method; see text), β-ALP, and nonbone ALP (in U/L) vs age in healthy Chinese men and women. (●) Premenopausal; (○) postmenopausal women. The two r values at the left upper corners of the panels for women are for pre- and postmenopausal women separately.](image)
between the decrease of b-ALP and testosterone concentrations in elderly men is controversial [10]. Other bone markers, including osteocalcin and carboxyl-terminal propeptide of type I procollagen, have been shown to correlate poorly with testosterone concentrations in elderly men [8, 10], and increased nonbone ALP may in part represent subclinical liver diseases or a decreased clearance of ALP in elderly men and women.

In conclusion, because total ALP and b-ALP showed in men opposite trends of age-related changes, our results suggest that total ALP is not a good marker of bone turnover, especially for men.

### References


