The association between bone turnover markers and kyphosis in community-dwelling older adults

Corinne R. McDaniels-Davidson f*, Donna Kritz-Silverstein a, Mei-Hua Huang b, Gail A. Laughlin a, Sarah Johnson c, Jouko Haapalahdi d, Diane L. Schneider e, Elizabeth Barrett-Connor a, Deborah M. Kado a

a Department of Family Medicine and Public Health, University of California, San Diego School of Medicine, San Diego, CA, United States
b Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, United States
c Department of Family Medicine and Public Health, University of California, San Diego School of Medicine, San Diego, CA, United States
d JouZeNet Consulting Limited, Kempele, Finland
e SPD Development Company Limited, Bedford, UK
f BoneHealth, La Jolla, CA, United States

Purpose: Hyperkyphosis, accentuated curvature of the thoracic spine, is often attributed to osteoporosis, yet its underlying pathophysiology is not well understood. Bone turnover markers (BTM) reflect the dynamic process of bone formation and resorption. This study examined the association between serum BTM levels and kyphosis in community-dwelling older adults.

Methods: Between 2003 and 2006, 760 men and women in the Rancho Bernardo Study age 60 and older had blood drawn and kyphosis measured. Fasting serum was assayed for N-telopeptide (NTX) and procollagen type 1 n-terminal propeptide (P1NP), markers of bone resorption and formation, respectively. Participants requiring two or more 1.7 cm blocks under their head to achieve a neutral supine position were classified as having accentuated kyphosis. Analyses were stratified by sex and use of estrogen therapy (ET). Odds of accentuated kyphosis were calculated for each standard deviation increase in log-transformed BTM.

Results: Mean age was 75 years. Overall, 51% of 341 non-ET using women, 41% of 111 ET-using women, and 75% of 308 men had accentuated kyphosis. In adjusted models, higher P1NP and NTX were associated with decreased odds of accentuated kyphosis in non-ET using women (P1NP: OR = 0.78 [95% CI, 0.58–0.92]; NTX: OR = 0.68 [95% CI, 0.54–0.86]), but not in men or ET-using women (p > 0.05).

Conclusions: The selective association of higher bone turnover with reduced odds of accentuated kyphosis in non-ET using women suggests that elevated BTM were associated with a lower likelihood of hyperkyphosis only in the low estrogen/high BTM environment characteristic of postmenopausal women who are not using ET.

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Bone remodeling or turnover is the dynamic process of bone formation and resorption that is carried out by osteoblasts and osteoclasts throughout the life span (Marieb, 2001). Previous studies have shown associations between elevated or unbalanced turnover, as indicated by various bone turnover markers (BTM), and bone disorders such as osteoporosis and fracture (Cauley et al., 2012; Gamero, 2009; Tamaki et al., 2013). For example, a prospective study of 522 postmenopausal women reported that higher BTM were associated with an increased risk of vertebral fracture among women who were at least five years past menopause (Tamaki et al., 2013). There are at least 15 different recognized biomarkers of bone turnover (Wheater et al., 2013), reflective of the bone metabolic processes formation and resorption. Although usually these processes are coupled, such that bone formation and bone resorption markers demonstrate parallel dynamics, a complete clinical picture is best obtained by measurement of both a resorption and a formation marker. A suitable bone formation marker for measurement in serum is P1NP, a cleavage product of Type 1 pro-collagen. There is low intra-individual variability of P1NP and a wide dynamic range in relation to clinical conditions. NTX, a cleavage product of Type 1 collagen, is an appropriate biomarker of bone resorption marker that is stable in serum; commercial assays provide measurements with the required precision.

Hyperkyphosis, accentuated curvature of the thoracic spine, has been estimated to affect up to 40% of community-dwelling older adults
and can be associated with poor health outcomes including impaired pulmonary function, increased falls and fractures, and mortality (Kado et al., 2003, 2004, 2007; Ryan and Fried, 1997). However, little is known about the mechanisms leading to hyperkyphosis. To date, there are no standard treatments available for people with accentuated kyphosis. Better understanding of the underlying pathophysiology of hyperkyphosis may help elucidate which types of interventions might be most promising to help individuals with hyperkyphosis.

To our knowledge, no studies have reported the association between bone turnover markers (BTM) and kyphosis. The purpose of this study is to examine the associations of accentuated kyphosis with serum collagen type 1 cross-linked N-telopeptide (NTX), a marker of bone resorption, and procollagen type 1 N-terminal propeptide (P1NP), a marker of bone formation, in a large sample of community-dwelling older men and women unscreened for osteoporosis or kyphosis.

2. Materials and methods

2.1. Participants

Between 1972 and 1974, 6629 adult residents from Rancho Bernardo, a largely white, middle class community in southern California were enrolled in a cohort study of healthy aging. Between August 2003 and January 2006, 870 surviving, ambulatory participants attended a clinic visit designed to study osteoporosis and other age-related disorders. The study sample included 760 participants (308 men and 452 women) who remained after excluding 58 participants who were younger than age 60, 39 missing measures of kyphosis, 11 without stored serum for BTM assessment, and two lacking information on estrogen therapy (ET).

The University of California, San Diego Human Research Protections Program approved this research protocol; all participants gave written informed consent prior to participation.

2.2. Procedures

During the 2003–2006 research clinic visit, morning fasting blood samples were obtained by venipuncture by a clinic nurse and frozen for later analysis. Kyphotic status was assessed by a trained radiology technician by placing 1.7 cm blocks under each participant’s head. The number of blocks required to achieve a neutral position while lying supine on a flat surface was recorded; the greater the number of blocks required to achieve a neutral position, the more accentuated the angle of kyphosis. Details of this method of measuring kyphosis have been previously described (Kado et al., 2004).

Height and weight were also measured by a nurse using a calibrated stadiometer and balance beam scale with participants wearing light clothing and without shoes. Maximum waist girth was measured as an estimate of central obesity; body mass index (BMI; kg/m2) was calculated as an estimate of overall obesity.

Total hip bone mineral density (BMD; g/cm2) was measured using dual x-ray absorptiometry on a DXA Hologic 1000 (Waltham, MA), which was calibrated daily using a phantom with a precision error of 1.5% or less.

A self-administered survey was used to obtain information on smoking history (never/ever), alcohol intake (drinks per week), exercise ≥ 3 times per week (no/yes), education and history of physician-diagnosed comorbidities (stroke, diabetes, emphysema, chronic bronchitis, arthritis, Parkinson’s disease, and spine fracture). Information on current medication and supplement use, including ET in women, was obtained by a nurse who validated with medication containers and prescriptions brought to the clinic for that purpose.

In 2008, serum NTX (BCE/l) was measured at SPD Development Company Limited (Bedford, UK) using the Osteomark ELISA (Unipath Ltd, UK); serum P1NP (μg/L) was measured by (UniQ, Orion Diagnostica) at Orion Diagnostica Oy (Oulu, Finland). Intra- and interassay coefficients of variation ranged from 2–6%.

Education level was categorized into high school or less, some college, a college degree or more. Alcohol intake was dichotomized into heavy drinking (yes/no) using sex-specific criteria; men consuming 21 or more drinks per week (three or more drinks per day) and women consuming 14 or more drinks per week (two or more drinks per day) were considered heavy drinkers based on the USDA definition of moderate drinking as up to one drink per day for women and up to two drinks per day for men (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

2.3. Statistical analysis

All analyses were sex-specific due to differences between men and women in weight, height, and BMD; analyses of women were stratified by ET use due to the known effect of exogenous estrogen on bone turnover. Bivariate analyses to detect differences in covariates between those with normal versus accentuated kyphotic status were performed using age-adjusted logistic regression. Covariates with age adjusted \( p < 0.25 \) were included in saturated multivariate logistic regression models to examine the association between P1NP and NTX with kyphotic status (normal/accentuated) in each stratum. Covariates not significant at \( \alpha = 0.05 \) were removed from the saturated model. These variables were then placed back into the model one-by-one to assess for possible inclusion and/or confounding. Confounding was considered present if the effect size of the marker changed by a magnitude of 0.5 or more. Effect modification by use of osteoporosis medications was assessed by including an interaction term in the final model. Odds ratios from these logistic regression models represent the odds of accentuated kyphosis for each standard deviation increase in continuous variables.

Normal kyphotic status was defined as the use of 0 or 1 block to achieve a neutral supine position; those requiring 2 or more blocks were defined as having accentuated kyphotic status. This cut point was determined based on observed differences in physical function during exploratory analysis. NTX and P1NP levels were not normally distributed and were log transformed for all analyses; reported values are geometric means.

Statistical analyses were conducted using SPSS (version 19.0, SPSS Inc., Chicago, IL) and SAS (version 9.2, SAS Institute, Cary, NC). Statistical significance was defined as two-sided \( p < 0.05 \) for all tests.

3. Results

Mean age of the study sample was 75 years (range = 60–100). Prevalence of accentuated kyphotic status differed significantly between the three study groups \( (\chi^2 = 55.813, p < 0.001) \) but did not differ significantly between the non-ET using and ET-using women \( (\chi^2 = 3.610, p = 0.057) \); 51% of 341 non-ET using women, 41% of 111 ET-using women, and 75% of 308 men had accentuated kyphosis. Levels of both bone turnover biomarkers also differed significantly across the three study groups (ANOVA \( p < 0.001 \) for NTX and P1NP); NTX and P1NP levels were highest in non-ET using women (geometric means \( \text{GM} = 1.154 \) and 1.590, interquartile ranges \( \text{IQR} = 1.050–1.256 \) and 1.442–1.744), intermediate in men \( \text{GM} = 1.113 \) and 1.521, IQR = 1.015–1.208 and 1.397–1.648), and lowest in ET using women \( \text{GM} = 1.080 \) and 1.448, IQR = 0.993–1.146 and 1.280–1.603).

Age-adjusted comparisons of characteristics for each group by kyphotic status are shown in Table 1. NTX and P1NP were significantly lower in non-ET using women with accentuated kyphosis compared with their non-kyphotic counterparts, but did not differ by kyphotic status in men or ET using women. Men with accentuated kyphosis were significantly younger and weighed more than those with normal kyphotic status, but did not differ by mean height, BMI, total hip BMD, use of medications, or any behavioral or lifestyle characteristics. Non-ET using women with accentuated kyphosis weighed more, had higher
Among ET using women, no significant associations were found between BTM and kyphotic status. Higher BMI was independently associated with increased odds of accentuated kyphosis in women, regardless of ET use.

Table 3 shows the association of bone formation and resorption markers with kyphotic status in men. Neither NTX nor P1NP were associated with kyphotic status in men in either the age-adjusted or fully adjusted models. Lower age and higher weight were each independently associated with increased odds of accentuated kyphosis in men.

### 4. Discussion

Because hyperkyphosis in older persons is associated with several adverse outcomes and has no standard clinical treatment, it is important to investigate potential mechanisms for this disorder that might provide...
markers in female power athletes and postulated that the mechanical stress is able to trigger bone remodeling in humans (Marieb, 2001). Bennell (Bennell et al., 1997) observed elevated resorption (Weitzmann and Paci, 2006), which is likely due to survivor bias in the cohort. In our 2004 report (Kado et al., 2010), it was found that current smoking was associated with less kyphosis among women in SOF. However, 80% of the participants selected for the SOF kyphosis study had to have serial x-rays completed over a 15-year period of follow-up and survivor bias may explain the disparate findings.

This study has some limitations. Due to its cross-sectional nature, these results can be considered only suggestive, describing associations rather than causation. The blocks method may lack sensitivity and specificity, although inter-rater reliability was 0.85 at the Rancho Bernardo clinic. Nonetheless, this study might have benefited from a more precise measure of thoracic kyphosis, such as the Cobb angle, which was not calculated for most participants at this visit. The study might also have benefited from additional clinical measures such as fracture history in relation to the measurement of bone turnover markers, radiographic presence of degenerative disc disease and vertebral fractures as well as further information about type of estrogen use. Lastly, the generalizability of our results is limited to non-Hispanic white, ambulatory, community-dwelling older adults.

This study also has several strengths. Sample sizes of the men and non-ET using women were relatively large. Information was collected on a wide range of covariates that could have altered BTM, kyphotic status, or both. Additionally, bone turnover markers were assayed using state of the art methods performed in experienced laboratories.

Results of this study suggest that elevated BTM are associated with a lower likelihood of hyperkyphosis only in the low estrogen/high BTM environment that is characteristic of postmenopausal women who are not using ET. These findings should be validated in other cohorts. The effect of estrogen on the mechanisms leading to kyphosis warrant further research so that interventions can be developed to treat or prevent hyperkyphosis.

Conflict of interest

Dr. Sarah Johnson is a Senior Research Scientist at SPD Development Company Limited, the entity that performed the NTX assays.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Acknowledgments

The authors would like to acknowledge support from the following grants: NIH RO1 AR068288 and NIA AG07181. In addition, this study would not be possible without the dedication of the Rancho Bernardo Study participants.
References

Marieb, E.N., 2001. Human Anatomy & Physiology. fifth ed. Benjamin Cummings, San Francisco.

Cauley, J.A., Danielson, M.E., Greendale, G.A., Finkelstein, J.S., Chang, Y.F., Lo, J.C., Crandall, C.J, Neer, R.M., Ruppert, K., Meyn, L, Prairie, B.A., Sowers, M.R., 2012. Bone resorption and fracture across the menopausal transition: the study of women's health across the nation. Menopause (New York, NY) 19 (11), 1200–1207. http://dx.doi.org/10.1097/gme.0b013e31825ae176.

Garnero, P., 2009. Bone markers in osteoporosis. Curr. Osteoporos. Rep. 7 (3), 84–90.

Tamaki, J., Iki, M., Kadowaki, E., Sato, Y., Chiba, Y., Akiba, T., Matsumoto, T., Nishino, H., Kaganiniori, S., Kagawa, Y., Yoneshima, H., 2013. Biochemical markers for bone turnover predict risk of vertebral fractures in postmenopausal women over 10 years: the Japanese Population-based Osteoporosis (JPOS) Cohort Study. Osteoporos. Int. J. Estab. Result Coop. Betw. Europ. Found. Osteoporos. Natl. Osteoporos. Found. U.S.A. 24 (3), 887–897. http://dx.doi.org/10.1007/s00198-012-2106-7.

Wheater, G., Elshahaly, M., Tuck, S.P., Datta, H.K., van Laar, J.M., 2013. The clinical utility of bone marker measurements in osteoporosis. J. Transl. Med. 11 (201).http://dx.doi.org/10.1186/1479-5876-11-201.

Kado, D.M., Duong, T., Stone, K.L., Ensrud, K.E., Nevitt, M.C., Greendale, G.A., Cummings, S.R., 2003. Incident vertebral fractures and mortality in older women: a prospective study. Osteoporos. Int. J. Estab. Re. Coop. Betw. Europ. Found. Osteoporos. Natl. Osteoporos. Found. U.S.A. 14 (7), 589–594. http://dx.doi.org/10.1007/s00198-003-1412-5.

Kado, D.M., Huang, M.H., Karlamangla, A.S., Barrett-Connor, E., Greendale, G.A., 2004. Hyperkyphotic posture predicts mortality in older community-dwelling men and women: a prospective study. J. Am. Geriatr. Soc. 52 (10), 1662–1667. http://dx.doi.org/10.1111/j.1532-5415.2004.52458.x.

Kado, D.M., Huang, M.H., Nguyen, C.B., Barrett-Connor, E., Greendale, G.A., 2007. Hyperkyphotic posture and risk of injurious falls in older persons: the Rancho Bernardo Study. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 62 (6), 652–657.

Ryan, S.D., Fried, L.P., 1997. The impact of kyphosis on daily functioning. J. Am. Geriatr. Soc. 45 (12), 1479–1486.

U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010. Dietary Guidelines for Americans, 2010. seventh ed. U.S. Government Printing Office, Washington, CD.

Manolagas, S.C., 2000. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. 21 (2), 115–137. http://dx.doi.org/10.1210/edrv.21.2.0395.

Weitzmann, M.N., Paci, R., 2006. Estrogen deficiency and bone loss: an inflammatory tale. J. Clin. Invest. 116 (5), 1186–1194. http://dx.doi.org/10.1172/jci28550.

Bennell, K.L., Malcolm, S.A., Khan, K.M., Thomas, S.A., Reid, S.J., Brukner, P.D., Ebeling, P.R., Wark, J.D., 1997. Bone mass and bone turnover in power athletes, endurance athletes, and controls: a 12-month longitudinal study. Bone 20 (5), 477–484.

Ebbesen, E.N., Thomsen, J.S., Beck-Nielsen, H., Nepper-Rasmussen, H.J., Moselkilde, L., 1999. Age- and gender-related differences in vertebral bone mass, density, and strength. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 14 (8), 1394–1403. http://dx.doi.org/10.1002/jbmr.1999.14.8.1394.

Kado, D.M., Laughlin, G., Cauley, J., Orwell, E., Barrett-Connor, E., Cawthon, P., 2013a. Sex steroid hormones and kyphosis in older men: the MoOS Study. Paper Presented at the ASBMR Annual Meeting, Baltimore, MD, October 4–7.

Woods, G., Huang, M.H., Fink, H., McDaniels-Davidson, C., Cawthon, P., Kado, D.M., 2014. Self-reported estrogen use, kyphosis, and kyphosis progression in older women: the study of osteoporotic fractures. Paper Presented at the ASBMR Annual Meeting, Houston, TX, September 12–15.

Kado, D.M., Huang, M.H., Karlamangla, A.S., Cawthon, P., Katzman, W., Hillier, T.A., Ensrud, K., Cummings, S.R., 2013b. Factors associated with kyphosis progression in older women: 15 years’ experience in the study of osteoporotic fractures. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 28 (1), 179–187. http://dx.doi.org/10.1002/jbmr.1728.

Lang, T., Streeter, T., Cawthon, P., Baldwin, K., Taaffe, D.R., Harris, T.R., 2010. Sarcopenia: etiology, clinical consequences, intervention, and assessment. Osteoporos. Int. J. Estab. Res. Coop. Betw. Europ. Found. Osteoporos. Natl. Osteoporos. Found. U.S.A. 21 (4), 543–539. http://dx.doi.org/10.1007/s00198-009-1059-y.