Journal of Biomechanics 41 (2008) 2868–2873

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Age-related changes in porosity and mineralization and in-service damage accumulation

Timothy L. Norman [a,*], Tara M. Little [b], Yener N. Yeni [c]

[a] Department of Engineering and Computer Science, Cedarville University, 251 N. Main Street, Cedarville, OH 45314, USA
[b] Technical Services Representative, Epic Systems Corporation, 5301 Tokay Blvd., Madison, WI 53711, USA
[c] Bone and Joint Center, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA

ARTICLE INFO

Article history:
Accepted 23 June 2008

Keywords:
Linear microcracks
Diffuse damage
Cortical bone
Mineralization
Porosity

ABSTRACT

It has been proposed that bone damageability (i.e. bone's susceptibility to formation of damage) increases with the elevation or suppression of bone turnover. Suppression of turnover via bisphosphonates increases local bone mineralization, which theoretically should increase the susceptibility of bone to microcrack formation. Elevation of bone turnover has also been proposed to increase bone microdamage through an increase in bone intracortical porosity and local stresses and strains. The goal of this paper was to investigate the above proposals, i.e., whether or not increases to mineral content and porosity increase bone in-service damageability. To do this, we measured in vivo diffuse damage area (Df.Dm.Ar, %) and microcrack density (Cr.Dn) (cracks/mm^2) in the same specimen from human cortical bone of the midshaft of the proximal femur obtained from cadavers with an age range of eight decades and examined their relationships with porosity, mineralization and age. Results of this study showed that Cr.Dn and Df.Dm.Ar increased with a decrease in bulk mineralization. This finding does not appear to support the proposal that damage accumulation increases with low bone turnover that results in increases mineralization. It was proposed however that the negative correlation between damage accumulation and mineralization may be attributed to highly mineralized regions of bone existing with under-mineralized regions resulting in an overall decrease in average bone mineralization. It was also found that microdamage accumulates with increasing porosity which does appear to support the proposal that elevated bone turnover that results in increased porosity can accelerate microdamage accumulation. Finally, it was shown that linear microcracks and Df.Dm.Ar accumulate with age differently, but because they correlate with each other, one may be the precursor for the other.

1. Introduction

Bone remodeling is a biological process that repairs bone damage by resorbing old bone and forming a new bone (osteoid) that is later mineralized. This process results in changes in bone porosity, mineralization and level of bone damage. Bone remodeling is a complex process consisting of three primary phases: activation (A), resorption (R) and formation (F). These phases occur throughout life and are the primary determinants of bone mechanical properties, resistance to fatigue damage and ability to function in a changing mechanical environment (Martin et al., 1998). These processes are location, magnitude and rate specific (Frost, 1964) so that changes in bone morphology can vary with skeletal location and over time. For example, an imbalance between the resorption and formation phases can result in an increase (resorption > formation) or decrease (formation > resorption) to bone porosity. The degree of mineral content in bone is also influenced by the rate of remodeling, and by rates of primary and secondary mineralization that occurs after bone formation. A consequence of bone remodeling is the turnover of bone tissue. The transient nature of bone turnover affects porosity and bone mineralization in ways that ultimately affect bone's susceptibility to damage.

It has been proposed that the accumulation of microscopic cracks in bone acts as a stimulus to bone remodeling (Frost, 1985) and serve as one of the major factors contributing to increased skeletal fragility (Freeman et al., 1974; Heaney, 1992; Sherman et al., 1993) and stress fractures (Giladi et al., 1991; Li et al., 1985; Matheson et al., 1987). Previous studies have shown that linear microcracks accumulate with age (Schaffler et al., 1995; Norman and Wang, 1997; Fazzalari et al., 1998; Zioupos, 2001b). However, damage does not appear to accumulate by the same rate in all individuals as they age (Burr, 2003), and this is likely due to the complex nature of bone turnover discussed previously. One proposal for the heterogeneity of damage accumulation is that damage accumulation may be related to the biological effects

* Corresponding author. Tel.: +1 937 766 3761; fax: +1 937 766 7689.
E-mail address: tnorman@cedarville.edu (T.L. Norman).

0021-9290/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2008.06.032

resulting from rate of bone turnover (Burr, 2003). Low bone turnover overall would result in increases in bone matrix mineral content at the microscopic level (Meunier and Boivin, 2000) due to secondary mineralization and these changes could cause bone to become more brittle rendering it more fragile and unable to sustain damage (Burr, 2003; Meunier and Boivin, 2000; Jepsen et al., 2001; Schaffler, 2003; Mashiba et al., 2000, 2001). The effects of elevated bone turnover may also accelerate microdamage accumulation. It has been suggested that damage accumulation results from an increase in remodeling-induced intracortical porosity, which would cause decreased stiffness and increased local stress and strain promoting the initiation of damage (Martin et al., 1980; Schaffler et al., 1990).

From the previously mentioned studies, it appears that bone damageability (i.e. bone's susceptibility to formation of damage) increases due to biological effects that occur with either the suppression or elevation of bone turnover and that the increases may be related in part to bone mineral content and porosity. If damage accumulation is associated with low bone turnover and subsequent increases in mineral content, damage accumulation should be related to bone mineral content in vivo. Similarly, if damage accumulation is associated with elevated bone turnover, damage should be related to in vivo porosity. However, a paradox exists in the current understanding of their relationships as elevated bone turnover could increase intracortical porosity and as proposed should increase damageability, while at the same time result in reduced mineral content that would, i.e. via previous proposals, lessen damageability. In addition, mineralization of mature bone has been shown in some studies to decrease or remain unchanged with age (Thompson, 1980; Yeni et al., 1998; Currey, 1979; Currey et al., 1996; Goldman et al., 2003) while microdamage has been shown to increase with age (Schaffler et al., 1995; Norman and Wang, 1997; Fazzalari et al., 1998; Zioupos, 2001a, b). Thus, it would appear that there may be competing biological processes affecting tissue quality and the outcome of elevation or suppression of intracortical remodeling on bone damage accumulation.

The incidence of linear microcracks and diffuse damage in human cortical bone from the midshaft of the proximal femur obtained from cadavers with an age range of eight decades at time of death was measured in this study. Porosity and mineralization were also measured on the same specimens and relationships with in vivo microcracks and diffuse damage area (Df.Dm.Ar) were examined. The hypothesis that damageability increases with increases to porosity and increases to mineralization was tested. Knowledge of the relationships between damage accumulation and bone's microstructure and mineralization may help in defining the role of the age-related effects of intracortical remodeling on damage accumulation processes.

2. Methods

Fifty-seven fresh human femurs were harvested from 31 male and 26 female cadavers. The male donors' ages ranged from 22 to 91 years (average age = 62.9 ± 19.3 years.) while female donors' ages ranged from 24 to 94 years (average age = 64.7 ± 19.9 years.). No attempt was made to control for donor disease state or prior medication other than donors with cancer or diabetes were not included in this study. The right femurs were cleaned of soft tissue, wrapped in gauze moistened with physiological saline and stored in a freezer at −15 °C until they could be prepared for damage evaluation. Once thawed, parallel cross-sectional cuts were made 1 cm apart at the proximal femur (1 cm distal from the base of the lesser trochanter). The bulk sections were stained with basic fuchsin (BF) according to published procedures (Frost, 1960; Burr and Stafford, 1990).

The stained bulk sections were cut into four quadrants (anterior, posterior, medial and lateral) with a metallurgical saw (Buehler, Lake Bluff, IL) with a diamond blade. The sections were then embedded in plastic; an 80 μm-thick transverse slice was removed from the center of each quadrant with a wire saw and mounted for examination using a brightfield and fluorescence microscope at a magnification of 125 ×. Artifactual damage due to cutting and machining did not stain. Five fields were randomly chosen from each slide for damage measurements yielding a total of 20 fields per bone. Microcracks were first identified and counted using brightfield and then viewed and counted using fluorescence. Diffuse damage and microcracks were simultaneously quantified from the same image. Results for microdamage density reported in this study were determined using fluorescent lighting. We found that the microdamage density found from fluorescence microscopy was approximately 2.5 times higher than microdamage density found using brightfield lighting.

The presence of microcracks were identified by linear type morphology, typically on the order of 30–100 μm in length (Frost, 1960; Schaffler et al., 1995; Norman and Wang, 1997; Zarrinkalam et al., 2005) having sharp borders with a halo of increased BF stain surrounding them (Burr and Stafford, 1990). In some cases, a macrocrack would be accompanied by secondary cracks emanating from them. Crack density parameter (Cr.Dn) was defined as the ratio of the total number of cracks (N.Cr) and the bone area (B.Ar) (Cr.Dn = N.Cr/B.Ar, N.Cr/mm^2) and was computed for each randomly chosen field and averaged per specimen.

Diffuse damage areas were identified as focal areas of diffuse staining with or without damage apparent within them (i.e. with or without the areas containing a network of small bright cracks surrounded with pooled blurry stained regions (Fig. 1)) (Schaffler et al., 1994; Zioupos and Currey, 1994; Fazzalari et al., 1998; Boyce et al., 1998). Those areas were subsequently circumscribed and the surface areas of circumscribed regions were measured. Diffuse damage area density parameter (Df.Dm.Ar) was defined as the ratio of the total damaged area (Dm.Ar) and bone area (B.Ar)(Df.Dm.Ar = Dm.Ar/B.Ar, mm^2/mm^2) and was computed for each randomly chosen field and averaged per specimen.

Porosity and mineral content were determined using methods from previous studies (Yeni et al., 1997, 1998). Briefly, transverse cross-sections were taken from the medial and lateral cortices from bone samples removed immediately adjacent (within ~1–2 cm) to bone used for damage evaluation. The cross-sections were polished, stained with hemotoxylin and eosin and viewed with a brightfield light microscope under 40 × magnification. Direct measurement of pore area (Haversian and Volkmann's Canals and resorption spaces) was made using an image analysis system (Optimus, Edmonds, WA) and an average taken for three fields per section. A remaining portion of each specimen was defatted in acetone by agitating overnight, dried in a vacuum (20 psi) oven with desiccant at 60 °C until weights became constant. Specimens were then ashed in a muffle furnace at 600 °C for determining mineral percentage calculated from the ratio of ash weight and dry weight.

Simple regression analysis of an average of all four quadrants of diffuse damage and microdamage versus age, porosity and mineralization was conducted. In addition, generalized linear models (least squares procedures using both discrete and continuous predictor variables) were used to explain the relationships between crack density and diffuse damage area and age, porosity and mineralization. An outlier analysis based on Jackknifed Mahalanobis Distances (Rousseeuw and Leroy, 1987) was performed using the full set of variables (age, porosity, mineralization, Cr.Dn and Df.Dm.Ar). Three potential outliers were identified. These corresponded to the highest values of Cr.Dn (a 83 year old male and a 92

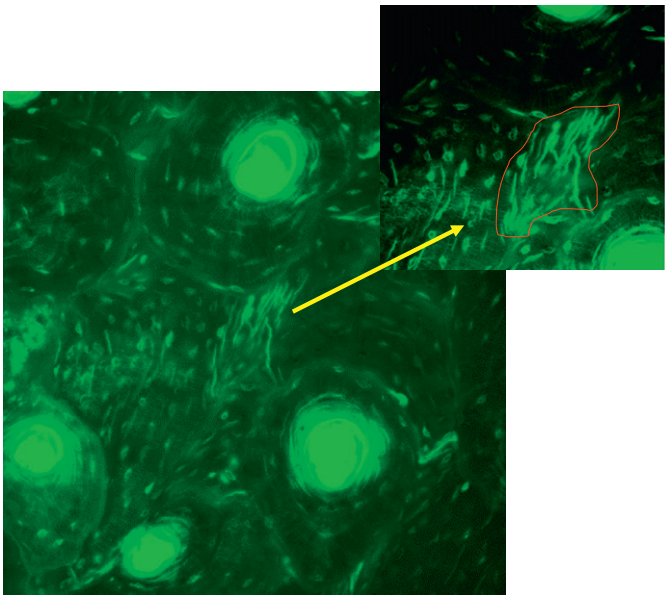

Fig. 1. A transverse section of bone viewed under a fluorescence microscope at 125 ×. The field width is approximately 400 μm. An enlarged portion of the figure (approximate width = 200 μm) shows a circumscribed damage area that contains diffuse staining, tiny cracks and linear microcracks.

year old Female) and one also corresponded to the maximum Df.Dm.Ar value (a 94 year old female). Statistical analysis was repeated with these points omitted. The statistical package JMP™ (SAS Institute, Cary, NC) was used to perform all analyses. Significance was set at $p<0.05$.

3. Results

The statistical analysis indicated no significant differences between male and female diffuse damage area ($p>0.88$) and microdamage density ($p>0.66$). Therefore, the male and female data were pooled. The results revealed that Cr.Dn increased with age ($p<0.0001$) (Fig. 2) whereas Df.Dm.Ar did not change with age ($p>0.26$; Fig. 3). The bulk mineral percentage negatively correlated with Cr.Dn ($p<0.03$) and Df.Dm.Ar ($p<0.01$) (Fig. 4) and porosity positively correlated with Cr.Dn ($p<0.01$) (Fig. 5). A fit of mineral percentage with age indicated that mineral content significantly ($p<0.0001$) decreased with age (Fig. 6). Porosity was found to significantly ($p<0.01$) increase with age. When mineral percentage and porosity were used together in a multivariate model (Model 1, Table 1), Cr.Dn significantly ($p<0.03$) increased with increasing porosity and decreased with increasing mineralization, the statistical significance of mineralization in the model having low confidence ($p<0.06$). However, when age was added to the model (Model 2, Table 1), mineral percentage and porosity were nonsignificant ($p>0.54$ and $p>0.27$, respectively) and therefore did not explain variability in Cr.Dn above that described by age alone. Mineral percentage was the only variable responsible for changes in Df.Dm.Ar ($p<0.01$) independent of age (Models 3 and 4, Table 1). In addition, Cr.Dn of pooled data was linearly related to Df.Dm.Ar ($p<0.03$) (Fig. 7). When Cr.Dn and Df.Dm.Ar outliers were omitted, p-values generally decreased and

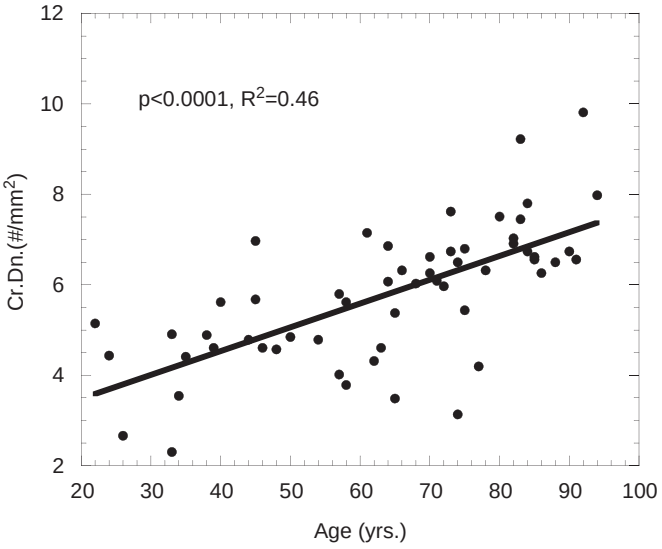

Fig. 2. Variation of Cr.Dn with age for male and female femurs. Average Cr.Dn was 5.78 #/mm^2 (SD = 1.51 #/mm^2). There is a significant ($p<0.0001$, $R^2=0.46$) trend for microdamage density to increase with increasing age. The data reported are an average of all four quadrants. With two outliers omitted ($n=55$), $p<0.0001$, $R^2=0.43$.

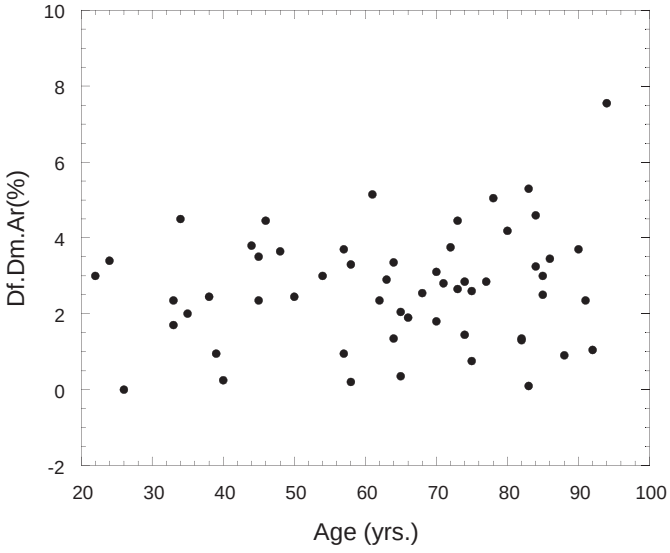

Fig. 3. Variation of percent Df.Dm.Ar with age for male and female femurs. Average Df.Dm.Ar was 2.78% (SD = 1.52%). The data reported are an average of all four quadrants.

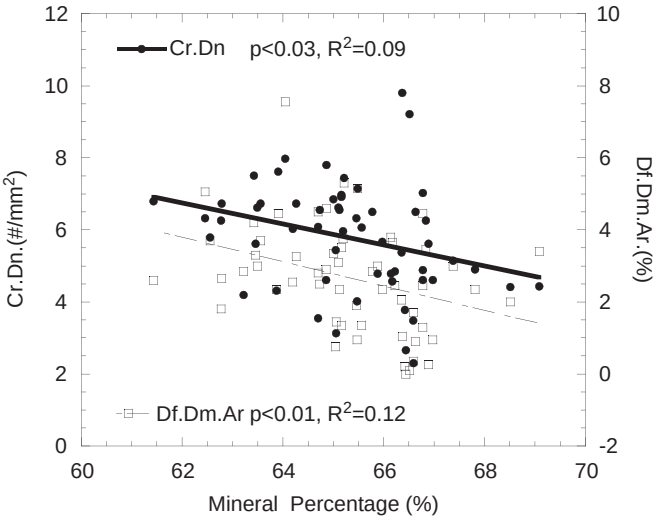

Fig. 4. Variation of Cr.Dn and Df.Dm.Ar with percent mineralization. Cr.Dn ($p<0.03$, $R^2=0.09$) and Df.Dm.Ar ($p<0.01$, $R^2=0.12$) significantly decrease with increasing mineral content. With two Cr.Dn outliers omitted ($n=55$), $p<0.001$, $R^2=0.18$. With one Df.Dm.Ar outlier omitted ($n=56$), $p<0.01$, $R^2=0.12$.

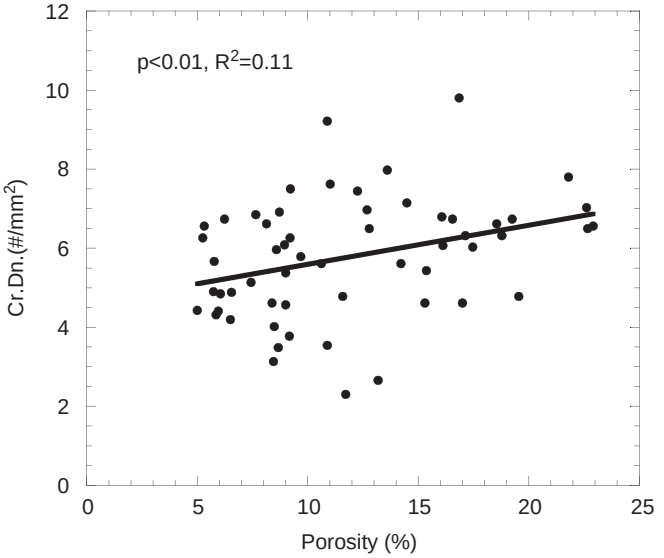

Fig. 5. Variation in Cr.Dn with porosity. Cr.Dn significantly ($p<0.01$, $R^2=0.11$) increases with increasing porosity. With two Cr.Dn outliers omitted ($n=55$), $p<0.01$, $R^2=0.11$.

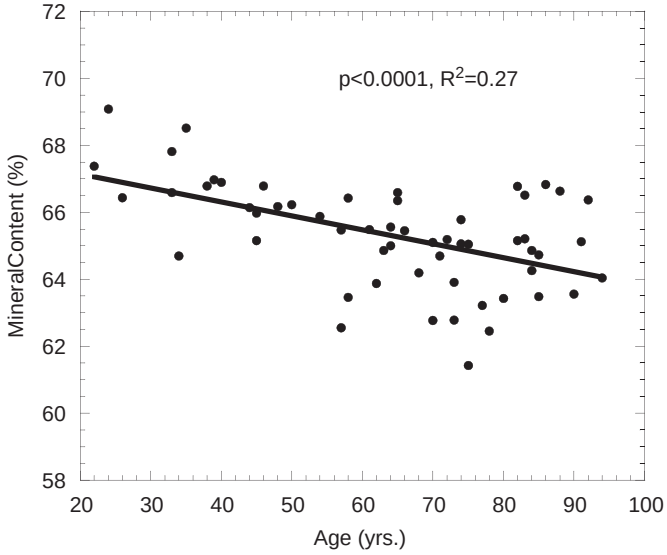

Fig. 6. Variation in average percent mineral content with age. Mineralization significantly ($p<0.0001$, $R^2=0.27$) decreases with increasing age.

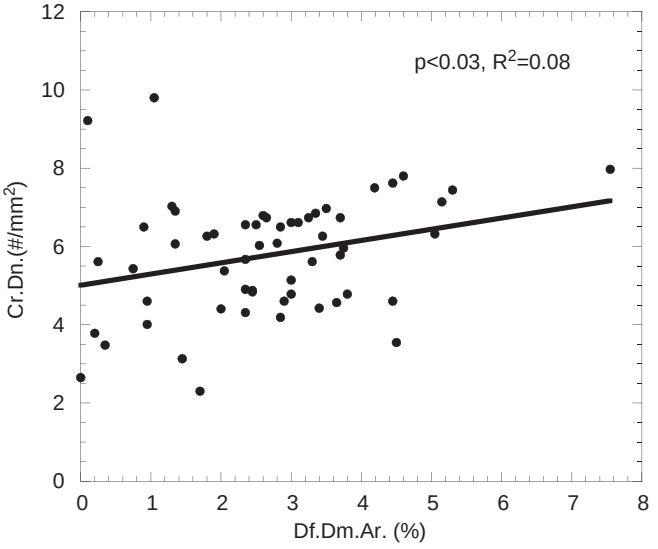

Fig. 7. Variation of Cr.Dn with Df.Dm.Ar. Cr.Dn significantly ($p<0.03$, $R^2=0.08$) increases with increasing Df.Dm.Ar. With three damage outliers omitted ($n=54$), $p<0.001$, $R^2=0.19$.

Table 1
Multivariable models for Cr.Dn and Df.Dm.Ar

Model/variable	Term	Estimate	Partial R^2/R^2_{adj}	p-value
Model 1			0.17/0.14	<0.0066
	Intercept	20.43		<0.0148
Cr.Dn	% Min	−0.2398	0.07	<0.0559
	% Por	0.08520	0.09	<0.0270
Model 2			0.47/0.44	<0.0001
	Intercept	−2.530		<0.7451
Cr.Dn	% Min	0.06990	0.01	<0.5409
	% Por	0.03453	0.02	<0.2781
	Age	0.05227	0.36	<0.0001
Model 3			0.12/0.09	<0.0282
	Intercept	24.49		<0.0044
Df.Dm.Ar	% Min	−0.3348	0.12	<0.0096
	% Por	0.004613	0.00	<0.9039
Model 4			0.13/0.08	<0.0661
	Intercept	26.33		<0.0100
Df.Dm.Ar	% Min	−0.3596	0.10	<0.0160
	% Por	0.008665	0.00	<0.8298
	Age	−0.004180	0.00	<0.7302

Note that R^2_{adj} is the R^2 adjusted for degrees of freedom for the entire model.

R^2 values increased, however, the omission of the outliers did not change the significance or lack of significance in any case.

4. Discussion

As far as we are aware, this study is the first to report in vivo microdamage density and diffuse damage area collected simultaneously from the same human femoral bone as a function of age using fluorescence microscopy. Results show that there is a significant trend for crack density to increase with increasing age whereas no correlation of diffuse damage of the pooled data with age was found over eight decades. The increase in crack density with age agrees with previous studies that microdamage in cortical and trabecular bone (Schaffler et al., 1995; Norman and Wang, 1997; Mori et al., 1997; Fazzalari et al., 1998; Vashishth et al., 1997). Although the light microscopy-based studies could have missed a significant number of microcracks, it is reassuring that the fluorescence and light microscopy-based results agree. The current finding that the pooled Df.Dm.Ar does not increase with age in femoral cortical bone is consistent with a previous report that diffuse damage area does not change with age in vertebral trabecular bone (Vashishth et al., 2000). A relationship between diffuse damage area and age has not been previously established for cortical bone of the femur, although the anterior cortex of tibiae obtained from young donors contained more diffuse damage than bones from old donors (Diab and Vashishth, 2007).

Results of this study also show how porosity and average mineralization of femoral cortical bone from the femur change with age. Cortical bone specimens taken from the proximal femur were found to become less mineralized in vivo with age. This finding agrees with work of Goldman et al. (2003), who found a decrease in the overall degree of mineralization with adult age using femoral midshafts. Our results also agree with the data presented previously for human bone, which indicated a downward trend in bone mineral with age after approximately 30 years of age (Currey, 1979). In another study, the ash content of bone increased in younger bones but there was no regular trend in 25 year olds and upwards (Currey et al., 1996), similar to results of Zioupos (2001a) and McCalden et al. (1993). Intracortical porosity was found to increase with age, a finding consistent with previous studies (Martin et al., 1980; Yeni et al., 1997).

Cr.Dn increased with decreasing mineralization and with increasing porosity. Because increased porosity and decreased mineralization may be expected in the case of elevated bone turnover (Jowsey, 1960), this finding is consistent with the idea that increased bone turnover promotes damage accumulation through increased porosity (Schaffler et al., 1990). However, it may not be consistent with the current thinking that as bone becomes more mineralized (thus more brittle) it becomes more susceptible to form damage (Jepsen et al., 2001) due to decreases in the amount of plastic deformation that can occur before failure (Currey, 1969; Burr, 2003). It may be consistent if we consider the possibility that decreased mineralization could effectively increase strain (due to decreased material stiffness) which could cause greater local strain driving the greater damage formation, a notion that has not been previously explored. It should be noted that mineral content and porosity did not fully account for

changes in Cr.Dn described by aging. Therefore, other factors beyond those measured macroscopically in this study may be responsible for age-related increases in damage accumulation. It is also noted that this study's measurement of mineralization does not account for the possible age-related increases in fraction of hypermineralized bone (Boyce and Bloebaum, 1993; Simmons et al., 1991).

Accumulation of microdamage could also result from suppression of bone turnover due to an overall lack of repair or impairment in targeted remodeling. Decreased bone turnover due to treatment results in an inability to repair damage (Burr, 2003). Earlier work by Mashiba et al. (2000, 2001) showed a two- to six-fold increase in damage accumulation in cortical bone of ribs and in trabecular bone of the vertebrae after bone remodeling is suppressed for 12 months using bisphosphonates in dogs. The increase in damage accumulation corresponded with an increase in mineralization (and trabecular bone volume), although no direct cause and effect relationship was established. A lack of available repair mechanisms would result in elevated damage accumulation independent of mineral content. Simmons et al. (1991) suggested that failure to repair bone is not because remodeling is necessarily slower, but perhaps because the crack regions are not targeted for repair. They further suggested that an increase in the highest density fraction of mineral with aging may represent a pool of bone mineral that is less accessible to remodeling, which they suggested could be interstitial bone, thus supporting the idea that there exists damaged bone that is inaccessible or not targeted for remodeling.

Mineralization heterogeneity may have a role in defining the relationship between microdamage and overall mineralization. Clearly, there are significantly more cracks in interstitial regions of bone (Schaffler et al., 1995; Norman and Wang, 1997) that may be a result of increased mineralization in those regions as described by Wasserman et al. (2005). Wasserman et al. (2005) tested the hypothesis that microcracks initiate within more mineralized regions of bone. Using femurs from male donors, mineralization was assessed globally and in the vicinity of cracks by Raman microspectroscopy. Their data revealed that mean mineralization of the damaged loci was significantly greater than the overall mineralization for each donor. They called this highly mineralized region the "brittle volume." According to the authors, results suggest that initiation and colocalization of microcracks may be driven by local composition of bone. It is possible that although damage accumulates in hypermineralized regions, bone that has more highly mineralized regions also has under-mineralized regions resulting in a lower average mineralization. Accordingly, even though microdamage is positively related to high mineralized regions it may appear that it is negatively related to average mineralization.

The differences found between how Df.Dm.Ar and Cr.Dn change with age, porosity and mineralization suggest that the two forms of microdamage are somewhat different in human femoral bone. Recent studies suggest that young bone develops fatigue damage differently than old bone (Wank et al., 2001; Diab et al., 2005). During in vitro fatigue loading (Diab et al., 2005) and also in vivo (Diab and Vashishth, 2007), younger bone forms diffuse damage whereas older bone formed linear microcracks. They concluded that the propensity of aging human bone to form more linear microcracks than diffuse damage may be a significant contributor to bone quality and age-related fragility. The results of the current study are consistent with previous work, which show that young and old bone develop damage differently; in the current study, the number of linear microcracks accumulate with age, whereas the area of diffuse damage (averaged over all cortices) is sustained at a constant level throughout life. The relationship between diffuse damage and age may depend on the anatomic location of the tissue and the type of loading it experiences in vivo for the femur as suggested by the work on the tibia (Diab and Vashishth, 2007). Thus, it is shown in this study that diffuse damage and linear cracks are different, because they do not correlate with age the same way; however, they are also associated because they correlate with each other.

One possible explanation for the positive correlation between Cr.Dn and Df.Dm.Ar is that microdamage begins at the ultrastructural level; therefore, one type of damage is a precursor of the other. Even though the mechanics and origin of microcrack initiation are not clear, it is reasonable that microcracks can result from coalescence of the smaller, submicroscopic cracks that occur in a damage process zone. Vashishth et al. (1997) demonstrated a toughening behavior due to a "fracture process zone", a region with increased localized crack density. Such localized regions occur in areas of high stress concentration, e.g. notches, voids and other microstructural features, where cracks tend to initiate. By examining the fracture process zone in a fracture mechanics experiment, Parsamian and Norman (2001) were able to demonstrate early material property changes prior to the identification of microdamage that were attributed to the accumulation of diffuse damage, a mechanism proposed for stiffness degradation in early stages of fatigue by Burr et al. (1998) and Schaffler et al. (1996). Microscopic observation in the current study showed linear and tiny microcracks in a damage process zone (Fig. 1). However, this does not preclude that cracks also initiate at the microscopic level as well.

Another explanation for the positive correlation between Cr.Dn and Df.Dm.Ar is that they correlate due to the coexistence of different factors that independently favor each type of damage. One pair of such factors is the loading mode; tensile strains promote diffuse damage and compressive strains promote linear cracks (Boyce et al., 1998). The correlation of the two types of damage could be explained by two modes being equally present. Although different loading modes promote distinctly different damage morphologies in vitro, the evidence is that bone contains both types of in vivo damage in cortices subjected to tensile loads as well as in cortices subjected to compressive loads (Diab and Vashishth, 2007).

In summary, results of this study showed that Cr.Dn and Df.Dm.Ar increased with a decrease in bulk mineralization. This finding does not appear to support the proposal that damage accumulation increases with low bone turnover that results in increased mineralization. It was proposed however that the negative correlation between damage accumulation and mineralization may be attributed to highly mineralized regions of bone existing with under-mineralized regions, resulting in an overall decrease in average bone mineralization. It was also found that microdamage accumulates with increasing porosity which does appear to support the proposal that elevated bone turnover that results in increased porosity can accelerate microdamage accumulation. Finally, it was shown that linear microcracks and diffuse damage area accumulate with age differently, but because they correlate with each other, one may be the precursor for the other.

Conflict of interest

The authors have no financial relationships with other people or organizations that could inappropriately influence or bias their work.

Acknowledgments

The authors would like to thank Suzanne Smith, Department of Orthopaedics at West Virginia University, for histological

preparation. The microdamage measurements were made at West Virginia University, Department of Anatomy Image Analysis Laboratory.

References

Boyce, T.M., Bloebaum, R.D., 1993. Cortical aging differences and fracture implications for the human femoral neck. Bone 14 (5), 769–778.

Boyce, T.D., Fyhrie, D.P., Glotkowski, M.C., Radin, E.L., Schaffler, M.B., 1998. Damage type and strain mode associations in human compact bone bending fatigue. Journal of Orthopaedic Research 16, 322–329.

Burr, D.B., 2003. Microdamage and bone strength. Osteoporosis International 14 (Suppl. 5), S67–S72.

Burr, D.B., Stafford, T., 1990. Validity of the Bulk-staining technique to separate artifactual from *in-vivo* bone microdamage. Clinical Orthopaedics and Related Research 260, 305–308.

Burr, D.B., Turner, C.H., Naick, P., Forwood, M.R., Ambrosius, W., Hasan, M.S., Pidaparti, R., 1998. Does microdamage accumulation affect the mechanical properties of bone? Journal of Biomechanics 31, 337–345.

Currey, J.D., 1969. The mechanical consequences of variation in the mineral content of bone. Journal of Biomechanics 2, 1–11.

Currey, J.D., 1979. Changes in the impact energy absorption of bone with age. Journal of Biomechanics 12, 459–469.

Currey, J.D., Brear, K., Zioupos, P., 1996. The effects of ageing and changes in mineral content in degrading the toughness of human femora. Journal of Biomechanics 12 (2), 257–260.

Diab, T., Vashishth, D., 2007. Morphology, localization and accumulation of in vivo microdamage in human cortical bone. Bone 40, 612–618.

Diab, T., Sit, S., Kim, D., Rho, J., Vashishth, D., 2005. Age-dependent fatigue behavior of human cortical bone. European Journal of Morphology 45 (1/2), 53–59.

Fazzalari, N.L., Forwood, M.R., Smith, K., Mathey, B.A., Herreen, P., 1998. Assessment of cancellous bone quality in severe osteoarthrosis: bone mineral density, mechanics, and microdamage. Bone 22, 381–388.

Freeman, M.A.R., Todd, R.C., Pirie, C.J., 1974. The role of fatigue in the pathogenesis of senile femoral neck fractures. Journal of Bone and Joint Surgery 56B, 698.

Frost, H.M., 1960. Presence of microscopic cracks in vivo in bone. Henry Ford Hospital Bulletin 8, 25–35.

Frost, H.M., 1964. Mathematical Elements of Lamellar Bone Remodeling. Charles C. Thomas, Springfield, IL.

Frost, H.M., 1985. Bone microdamage: factors that impair its repair. In: Uhtoff, H.D. (Ed.), Current Concepts of Bone Fragility. Springer, Berlin, pp. 123–148.

Giladi, M., Milgrom, C., Simkin, A., Danon, Y., 1991. Stress fractures: identifiable risk factors. American Journal of Sports Medicine 19 (6), 647–652.

Goldman, H.M., Bromage, T.G., Boyde, A., Thomas, C.D.L., Clement, J.G., 2003. Intrapopulation variability in mineralization density at the human femoral mid-shaft. Journal of Anatomy 203, 243–255.

Heaney, R.P., 1992. The natural history of vertebral osteoporosis. Is low bone mass an Epiphenomenon? Bone 13 (Suppl. 2), S23–S26.

Jepsen, K.J., Pennington, D.E., Lee, Y.L., Warman, M., Nadeau, J., 2001. Bone brittleness varies with genetic background in A/J and C57BL/6 inbred mice. Journal of Bone and Mineral Research 16, 1854–1862.

Jowsey, J., 1960. Age changes in human bone. Clinical Orthopaedics and Related Research, 210–218.

Li, G., Zhang, S., Chen, H., Wang, A., 1985. Radiographic and histologic analyses of stress fracture in rabbit tibias. American Journal of Sports Medicine 13 (5), 285–294.

Martin, R.B., Pickett, J.C., Zinaich, S., 1980. Studies of skeletal remodeling in aging men. Clinical Orthopaedic and Related Research 149, 268–282.

Martin, R.B., Burr, D.B., Sharkey, N.A., 1998. Skeletal Tissue Mechanics. Springer, New York.

Mashiba, T., Hirano, T., Turner, C.H., Forward, M.R., Johnson, C.C., Burr, D.B., 2000. Suppressed bone turnoverby bisphosphonates increases microdamage accumulation and reduces some biomechanical properties in dog rib. Journal of Bone and Mineral Research 15 (4), 613–620.

Mashiba, T., Turner, C.H., Hirano, T., Forward, M.R., Johnson, C.C., Burr, D.B., 2001. Effects of suppressed bone turnover by bisphosphonates on microdamage accumulation and biomechanical properties in clinically relevant skeletal sites in beagles. Bone 28 (5), 524–531.

Matheson, G.O., Clement, D.B., Mckenzie, D.C., Taunton, J.F., Lloyd-Smith, D.R., Macintyre, J.G., 1987. Stress fracture in athletes: a study of 320 cases. American Journal of Sports Medicine 15 (1), 46–59.

McCalden, R.W., McGeough, J.A., Barker, M.B., Court-Brown, C.M., 1993. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. Journal of Bone Joint Surgery American 75, 1193–1205.

Meunier, P.J., Boivin, G., 2000. Influence of changes in bone remodeling rate in the degree of mineralization of bone: therapeutic implications. Journal Bone Mineral Research 15, 818–825.

Mori, S., Harruff, R., Ambrosius, W., Burr, D.B., 1997. Trabecular bone volume and microdamage accumulation in the femoral heads of women with and without femoral neck fractures. Bone 21, 521–526.

Norman, T.L., Wang, Z., 1997. Microdamage of human cortical bone: incidence and morphology in long bones. Bone 20 (4), 375–379.

Parsamian, G.P., Norman, T.L., 2001. Diffuse damage accumulation in the fracture process zone of human cortical bone specimens and its influence on fracture toughness. Journal of Materials Science: Materials in Medicine 12, 779–783.

Rousseeuw, P.J., Leroy, A.M., 1987. Robust Regression and Outlier Detection. Wiley, New York.

Schaffler, M.B., 2003. Role of bone turnover in microdamage. Osteoporosis International 14 (Suppl 5), S73–S80.

Schaffler, M.B., Radin, E.L., Burr, D.B., 1990. Long-term fatigue behavior of compact bone at low strain magnitude and rate. Bone 11, 321–326.

Schaffler, M.B., Pitchford, W.C., Choi, K., Riddle, J.M., 1994. Examination of compact bone microdamage using back-scattered electron microscopy. Bone 15, 483–488.

Schaffler, M.B., Choi, K., Milgrom, C., 1995. Aging and matrix microdamage accumulation in human compact bone. Bone 17 (6), 521–525.

Schaffler, M.B., Boyce, T.M., Fyhrie, D.P., 1996. Tissue and matrix failure modes in human compact bone during tensile fatigue. Transactions of Orthopaedic Research Society 21, 57.

Sherman, S., Heaney, R.P., Parfitt, A.M., Hadley, E.C., Dutta, C., (Eds.), 1993. NIA workshop on aging and bone quality. Calcified Tissue International 53(Supplment 1), pp. S1–S180

Simmons, E.D., Pritzker, K.P.H., Grynpas, M.D., 1991. Age-related changes in the human femoral cortex. Journal of Orthopaedic Research 9, 155–167.

Thompson, D.D., 1980. Age changes in bone mineralization, cortical thickness and Haversian canal areal. Calcified Tissue International 31 (1), 5–11.

Vashishth, D., Behiri, J.C., Bonfield, W., 1997. Crack growth resistance in cortical bone: concept of microcrack toughening. Journal of Biomechanics 30, 763–769.

Vashishth, D., Koontz, J., Qiu, S.J., Lundin-Cannon, D., Yeni, Y.N., Schaffler, M.B., Fyhrie, D.P., 2000. In vivo diffuse damage in human vertebral trabecular bone. Bone 2 (2), 147–152.

Wank, R., Jepsen, K.J., Nasser, P., Schaffler, M.B., 2001. Aged rat bone fatigues in a fundamentally different manner from yonder adult bone. 47 Annual Meeting, Orthopaedic Research Society, San Francisco, CA, February 25–28, 0111:2001.

Wasserman, N., Yerramshetty, J., Akkus, O., 2005. Microcracks colocalize within highly mineralized regions of cortical bone tissue. European Journal of Morphology 42 (1/2), 43–51.

Yeni, Y.N., Brown, C.U., Wang, Z., Norman, T.L., 1997. Influence of bone morphology on fracture toughness of the human femur and tibia. Bone 21 (5), 453–459.

Yeni, Y.N., Brown, C.U., Norman, T.L., 1998. Influence of bone composition and apparent density on fracture toughness of the human femur and tibia. Bone 22 (1), 79–84.

Zarrinkalam, K.H., Kuliwaba, J.S., Martin, R.B., Wallwork, M.A.B., Fazzalari, N.L., 2005. New insights into the propagation of fatigue damage in cortical bone using confocal microscopy and chelating flurochromes. European Journal of Morphology 42 (1/2), 81–90.

Zioupos, P., 2001a. Ageing human bone: factors affecting the biomechanical properties and the role of collagen. Journal of Biomaterials Applications 15, 187–229.

Zioupos, P., 2001b. Accumulation of in-vivo fatigue microdamage and its relation to biomechanical properties in ageing human cortical bone. Journal of Microscopy 201, 270–278.

Zioupos, P., Currey, J.D., 1994. The extent of microcracking and the morphology of microcracks in damaged bone. Journal Material Science 29, 978–986.