INTRODUCTION Despite the ideal location of the osteocytes to sense the local environment and thereby influence bone remodeling, the functions of osteocytes in bone remain hotly debated. Previous studies have shown that osteocytes are capable of osteolytic and osteoplastics activities, however, the current trend is to regard osteocytes as sensory cells which are capable of (i) detecting the local mechanical and biochemical environments and (ii) conveying signals to each other and to other bone cells thereby influencing bone remodeling response. In this study histomorphometrical examination of male and female femoral mid-diaphyseal cortical bone was conducted to determine if bone’s remodeling response, indicated by tissue porosity and accumulation of damage, is reflected by the osteocyte lacunar density. Based on previous studies (1,2), we hypothesized that osteocyte lacunar density would decrease with age and be correlated to the age-related accumulation of microdamage.

METHODS Previously prepared histological sections from 16 males (Ages 16-73) and 9 females (Ages 28-63) were used. All individuals died suddenly and had no prior history of bone disease. Preparation of these sections included bulk staining of a 3-cm long femoral mid-diaphyseal cylinder in 1% basic fuchsin based in ascending series of ethanol followed by sectioning (3 sections per donor) and polishing of undecalcified cross-sections to 100 µm thickness (1). For morphometric analyses and quantification of osteocyte lacunae, blue light epifluorescence was used as it penetrates only the first few microns of the relatively thick sections and osteocyte lacunae could be readily identified due to the fluorescence of basic fuchsin present at the lacunar edges and canalicular processes. Osteocyte lacunae were counted in three sections per donor using a standard point counting stereological technique at 125X magnification. Output included the total number of osteocyte lacunae per bone area and % porosity (includes haversian canals and resorption spaces). For statistical analyses, non-parametric tests were used as the osteocyte lacunar density was not normally distributed in both genders. The relationships between osteocyte lacunar density and porosity with age and between osteocyte lacunar density and previously published microcrack data (1) were analyzed for gender difference using multiple regression models. The coefficient of variation for the osteocyte lacunar density, calculated by dividing the standard deviation by mean, was tested for correlation with age.

RESULTS The osteocyte lacunar density decreased exponentially with age in both males ($r^2=0.91; p<0.001$) and females ($r^2=0.52; p=0.04$) and was not different by gender ($p=0.53$) (Fig 1a). The coefficient of variation of the osteocyte lacunar density significantly increased with age in males ($p=0.008$) but not in females ($p=0.46$). The accumulation of microcracks and porosity did not correlate with the osteocyte lacunar density until a threshold decline in osteocyte lacunar density (approx. 600 osteocyte lacunae/mm²) was reached. After that threshold, however, sudden increases in microcrack accumulation (Fig.1b) and tissue porosity (not shown) were clearly seen. Relationships between osteocyte lacunar density and microcrack/porosity were not significantly different between males and females ($p=0.30/p=0.77$).

DISCUSSION Mechanotransduction or the conversion of a physical force into a cellular response is considered to be a fundamental mechanism by which the osteocyte network senses the local environment and affects remodeling by modulating the osteoblasts and osteoclasts (3,4). The results of this study support the sensory role of osteocytes in human cortical bone as a decline in osteocyte lacunar density predicts the accumulation of microcracks and increase in porosity with age. In this study osteocyte lacunae were regarded as a quantitative measure of the osteocyte network. Previous studies (5,6) have shown that not all lacunae contain osteocytes and that the percent of empty lacunae increases with age. It is, therefore, reasonable to consider that the age-related decline in osteocyte density is more pronounced than the age-related decline in lacunar density shown here. Age-related decline in the osteocyte lacunar density with increased porosity with consistency from an in vitro study (7) and the notion that, with age, osteocyte rich bone is preferentially resorbed and selectively repopulated with osteocytes. Such a remodeling process will lead to clustering of osteocytes which, in turn, will result in increased heterogeneity in spatial organization of osteocytes as shown by the age-related increase in the coefficient of variation of osteocyte lacunar density with age.

Based on the results of this study, it is not possible to determine whether the accumulation of microdamage and increased porosity cause an impaired osteocyte network or vice versa. In either case, however, the damaged and porous bone do accumulate with age which suggests that an impaired osteocyte network is indeed associated with the failure of bone to repair itself. More significantly it appears that a certain number of osteocytes are essential for an ‘operational’ network. There are many reasons why a minimum number of osteocytes may be essential. The well defined spatial organization of osteocytes relies on the fine network of canaliculi for transport of metabolites. Furthermore, osteocytes may form a neural network (8) organized in series (i.e.in hierarchical order) or parallel or both. Such networks are common in biological systems and recent studies have shown that the adaptation properties of these networks are a consequence of their connectivity (9).


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