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Original Article

Age-Related Cancellous Bone Loss in the Proximal Femur of Caucasian Females

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Abstract. The purpose of this investigation was to directly define the age-related intrafemoral variations in cancellous bone density, bone mineralization and rate of bone loss in a cadaveric population of Caucasian female femoral necks and trochanters. Forty-three Caucasian female femora were obtained and divided into premenopausal, postmenopausal and elderly age groups. The neck and trochanter were removed, and cores of cancellous bone were taken from the superior, middle and inferior regions; volume fraction and ash fraction were determined for each core. The cancellous bone volume fraction of the neck was significantly greater than that of the trochanter, as was that of the inferior region of the neck compared with the superior and middle regions at all age groups ($p < 0.05$). The mean neck/trochanter and neck inferior/superior volume fraction ratios did not change with age; however, the variance increased with age ($p < 0.001$). This increasing variability with age suggests that there may be a subpopulation of individuals within the elderly Caucasian population with a significantly different intrafemoral bone density distribution than was present prior to menopause. This study identified no mineralization changes with age in the cancellous bone of the proximal femur ($p > 0.05$). The influence of increased neck/trochanter and neck inferior/superior ratios on femoral neck integrity and fracture prediction is of interest and requires further investigation.

Keywords: Cancellous bone; Mineralization; Osteoporosis; Proximal femur; Volume fraction

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Introduction

Hip fractures remain a significant cause of morbidity and mortality in the elderly population. It has been documented that hip fracture incidence increases exponentially after the age of 50 years in Caucasian women [1–3]. It has also been demonstrated that trochanteric fractures become more common than cervical fractures after the age of 80 years for the same population [4–8]. Cancellous bone loss is believed to play a significant role in this increased incidence of hip fractures among the elderly.

Previous studies have indicated that cancellous bone loss begins in the early thirties and declines linearly with age [9–11]. However, other studies have suggested that there is an accelerated perimenopausal phase of cancellous bone loss that lasts 5–10 years [12,13]. Radiographic observations in the proximal femur suggested bone loss occurs first in the superior (tensile) aspect followed by loss in the inferior (compressive) portion of the proximal femur [14]. In contrast to these findings, Kawashima and Uhtoff [15] investigated the histologic pattern of age-related bone loss in the proximal femur and concluded that age-related bone loss in the femoral neck is not selective, but evenly distributed.

In addition to bone quantity, bone quality is also an issue under investigation in osteoporosis. Numerous studies have examined changes in bone mineralization associated with age and have been unable to identify age-related changes, although female cancellous bone in the proximal femur has not been investigated [16–19]. Parfitt [20] demonstrated an age-related decrease in overall mineralization of the cancellous bone of the ilium and forearm. Grynpas [21], however, has argued that bone mineralization typically increases with age due to decreased bone turnover. Age-related changes in proximal femur mineralization could affect vulnerability to proximal femur fractures, as mineralization has been linked to bone mechanical strength [22].

The objective of this investigation was to directly define the age-related intrafemoral variations in cancellous bone density and bone mineralization in a cadaveric population of Caucasian female femoral necks and trochanters. The hypothesis was that age-related changes in cancellous bone within the femoral neck are not uniform and specifically that there are individual variations in this population. The importance of this study is threefold. First, much of the work done to define age-related parameters in the proximal femur was previously performed with radiographic techniques, which cannot differentiate between cortical and cancellous bone. Thus, a clear understanding of the aging process of cancellous bone in the female proximal femur has not been established and the aging process is likely different from that in cortical bone. Second, it is vital that information concerning osteoporosis of the femur is not extrapolated from other skeletal sites, as it has been clearly shown that bone architecture varies according to skeletal location and that the femur is the best site for predicting femoral fractures [3,23]. Finally, recent literature has reported that the aging process is not uniform within the proximal femur, which may help explain the increased incidence of trochanteric fractures in elderly women [24,25]. This phenomenon, however, has not been described by direct measurements of cancellous bone in the Caucasian female proximal femur.

Materials and Methods

Forty-three human donor femora were obtained from young (premenopausal, $n = 15$, range 17–35 years old, mean 26 ± 6 years) and old Caucasian female cadavers. The old group was subdivided into two age groups: postmenopausal ($n = 15$, range 60–74 years old, mean 65 ± 4 years) and elderly ($n = 13$, range 76–92 years old, mean 85 ± 6 years). Women over the age of 60 years were divided into two groups (postmenopausal and elderly) because the incidence ratio of neck to intertrochanteric fractures changes in the elderly [4–8]. Only Caucasian femurs were used to eliminate the complications of inter-racial variations; in addition, this represents the ethnic group with the highest fracture risk [6,8]. Femurs from females in their fifties were excluded due to the variable time of onset and duration of menopause. Individuals with metabolic bone disease, on pharmaceutical regimens or with other pathologies that might affect the skeletal system were excluded from this study in accordance with standard bone banking procedures [26]. Other medical history was not available from these donors.

Each femur was stored in 70% ethyl alcohol for a minimum of 2 weeks, after which time it was manually cleaned of soft tissue. From the pair of femurs from each donor, one was randomly selected to eliminate potential left–right bias. There were a total of 25 left and 18 right femurs in this study. Using osteometric techniques described by Ruff and Hayes [27], each femur was measured and marked to identify three locations which were comparable between all specimens: the base of the head, the neck–trochanter junction (base of the neck), and through the base of the lesser and greater trochanters. The specimens were then cut at the specified locations using a band saw, providing two sections per femur: a neck section and a trochanter section (Fig. 1). The term 'location' in this paper will be used to identify the neck or trochanter sections of the proximal femur.

Cancellous bone specimens were obtained from the superior, middle and inferior aspects of each neck and trochanter segment using a coring drill bit with an inner diameter of 8 mm (Fig. 1). The specimens were drilled parallel to the cortical axis avoiding cortical bone within the specimens. There were six femoral neck sections that were too small in diameter to obtain all three regional core specimens. In these cases, only a superior and inferior specimen were available. The ends of each cylindrical specimen were ground by hand perpendicular to the long axis of the cylinder using fine grit sandpaper.

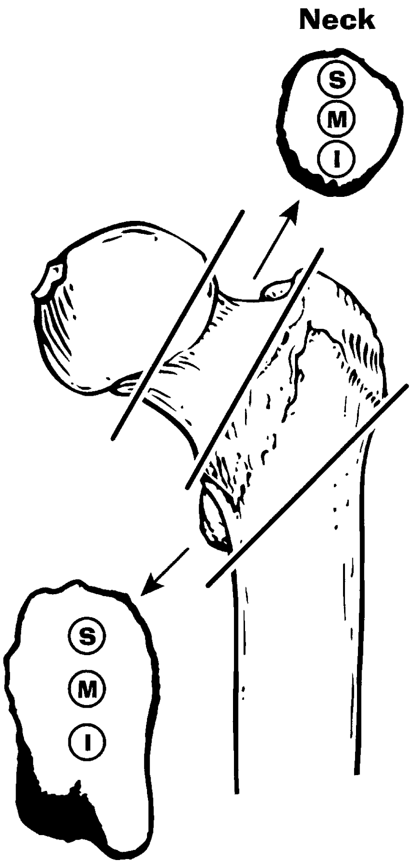

Fig. 1. The sectioning pattern used on the proximal femur to obtain the neck and trochanter segments, as well as the location of the superior (S), middle (M) and inferior (I) core sites within the cancellous bone of the neck and trochanter.

A total of 123 neck and 129 trochanter core specimens were obtained from the 43 femurs. The term 'region' in this paper will be used to describe the superior, middle and inferior cores removed from the neck and trochanter.

The specimens were defatted in chloroform for 14 days under negative pressure, after which they were placed in an oven at 80 °C for 24 h to drive off excess solvent. Bone marrow contents and other remaining debris were removed with a surgical water pick. Visual inspection using a dissecting microscope was performed to ensure that the marrow spaces were clear of debris. The specimens were placed in an oven at 80 °C for 24 h to remove excess water.

The total volume (V_t) of each specimen was determined by measuring the outer dimensions of each cylinder with a digital caliper ($V_t = \pi r^2 h$). The true cancellous bone volume was determined directly using micropycnometry, a helium displacement method based on Archimedes' principle. Each bone specimen was placed in a micropycnometer (Micropycnometer MPY-2, Quantachrome Co., Syosset, NY) and the volume of cancellous bone (V_b) was determined [28]. The volume fraction (VF) of cancellous bone was calculated according to the following equation: $VF = (V_b/V_t) \times 100$. In addition, a neck/trochanter (N/T) volume fraction ratio was determined for each femur by averaging the three regional core sections of the neck and trochanter for each donor. An inferior/superior (I/S) neck ratio was determined from the associated cores for each neck section.

The specimens were then dried at 80 °C for 72 h and quickly placed in a calcium carbonate desiccator where they were allowed to equilibrate to room temperature for 2–4 h. Specimens were weighed (W_d = dry weight), taking care to expose each specimen to room air for as little time as necessary, to minimize accumulation of ambient water. The specimens were ashed at 580 °C for 24 h, after which they were allowed to cool for 2–4 h in a desiccator. The specimens were reweighed (W_a = ash weight), and the ash fraction (AF) was determined: $AF = (W_a/W_d) \times 100$.

Statistical analysis of volume fraction and ash fraction was performed using a three-way general linear model analysis of variance, with age group (premenopausal, postmenopausal and elderly), location (neck, or trochanter) and region (superior, middle, inferior) as factors. Fisher's least significant difference post-hoc test was used for individual comparisons. The volume fraction data were transformed into a logarithmic scale to achieve normality for statistical analysis. A Kruskal–Wallis and Fisher test were performed on the neck/trochanter and inferior/superior ratio means and variances, respectively. Statistical significance was defined as $\alpha < 0.05$ for all tests.

Results

Volume Fraction Measurements

Cancellous bone volume fraction was significantly greater in the femoral neck than the trochanter at all ages ($p < 0.05$; Table 1). The average volume fraction in the neck was 22.0% ± 5.0%, 14.6% ± 4.3% and 11.9% ± 3.5% for the premenopausal, postmenopausal and elderly age groups, respectively (Table 1). There was a statistically significant difference in the volume fraction measurements between each age group in the neck ($p < 0.05$). The cancellous bone volume fraction was 46% lower in the elderly group compared with the premenopausal group. In the trochanter, the average volume fraction for each age group was as follows: premenopausal, 13.9% ± 3.9%; postmenopausal 8.8% ± 2.9%; and elderly, 6.1% ± 2.3% (Table 1). These volume fraction measurements were significantly different from each other at all ages ($p < 0.05$). The cancellous bone volume fraction was 56% lower in the elderly group compared with the premenopausal group.

When the regional volume fraction data were analyzed in the neck, a significant decrease in volume fraction was noted in the postmenopausal and elderly group with respect to the premenopausal group for each region ($p < 0.05$; Table 1, Fig. 2). In addition, the volume fraction in the inferior neck was statistically greater than in the superior or middle region for all age groups ($p < 0.05$). The superior and middle regional volume fraction measurements were not significantly different at any age group ($p > 0.05$). The inferior neck region demonstrated the smallest decline in volume fraction between the premenopausal and elderly age groups, with an overall decline of 21.4%. The superior and middle regions showed a greater overall decline in cancellous bone volume fraction between the premenopausal and elderly age groups, resulting in a 57.8% and 57.1% loss, respectively.

Table 1. Means and standard deviations for the volume fraction values (%), measured by location and age group

Age group	Location					
	Neck			Trochanter		
	Superior	Middle	Inferior	Superior	Middle	Inferior
Premenopausal	21.4 ± 6.5	20.5 ± 5.3	24.2 ± 4.9	14.6 ± 5.2	13.7 ± 4.2	13.6 ± 4.4
Postmenopausal	10.8 ± 3.5	13.0 ± 6.1	20.0 ± 6.5	9.5 ± 5.2	8.3 ± 4.2	9.1 ± 2.9
Elderly	9.0 ± 3.8	8.8 ± 2.6	19.0 ± 8.1	6.3 ± 3.2	5.8 ± 3.1	6.6 ± 2.8

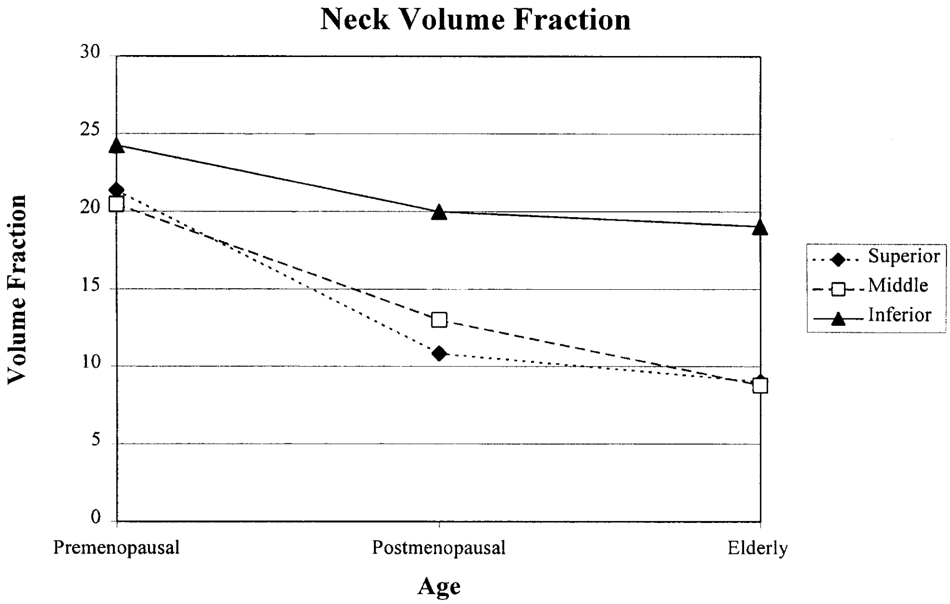

Fig. 2. Femoral neck volume fraction versus age group for the superior, middle and inferior regions.

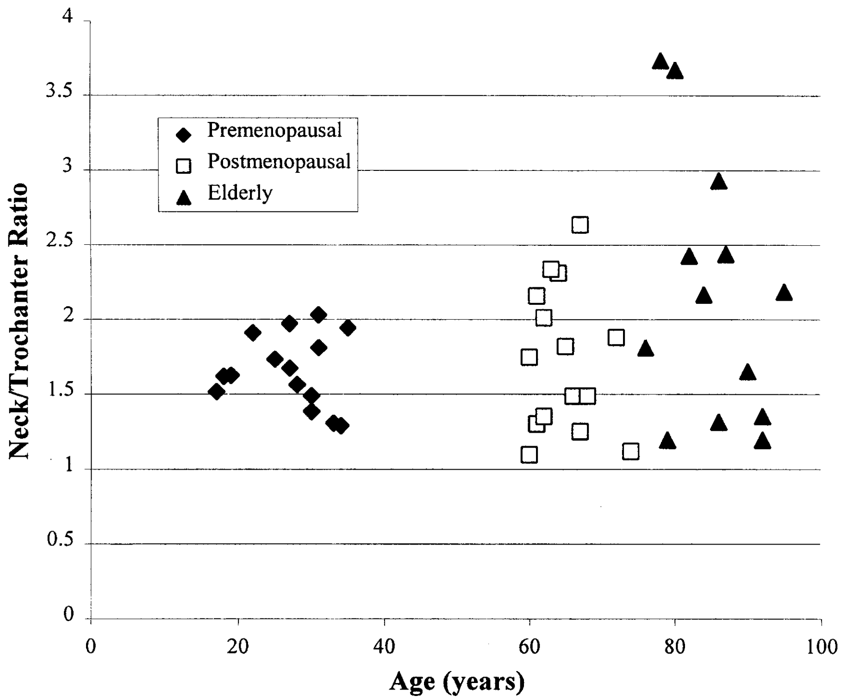

Fig. 3. Neck/trochanter volume fraction ratio versus age. Ratios are relatively uniform in premenopausal females but show a significant increase in variability after the menopause.

Regional analysis in the trochanter demonstrated no statistical difference between the superior, middle or inferior volume fractions at any age group (Table 1). When the anatomic regional volume fraction data were analyzed for the different age groups, a significant decline in volume fraction was noted in the postmenopausal and elderly age groups with respect to the premenopausal group ($p<0.05$). There was an overall decrease in cancellous bone volume fraction in the trochanter at the superior, middle and inferior regions by 56.7%, 57.5% and 51.2%, respectively.

The neck/trochanter volume fraction ratio when all anatomic regions were averaged together was 1.7 ± 0.2, 1.7 ± 0.5 and 2.2 ± 0.9 for the premenopausal, postmenopausal and elderly groups, respectively. Although the ratio increased with age, no statistically

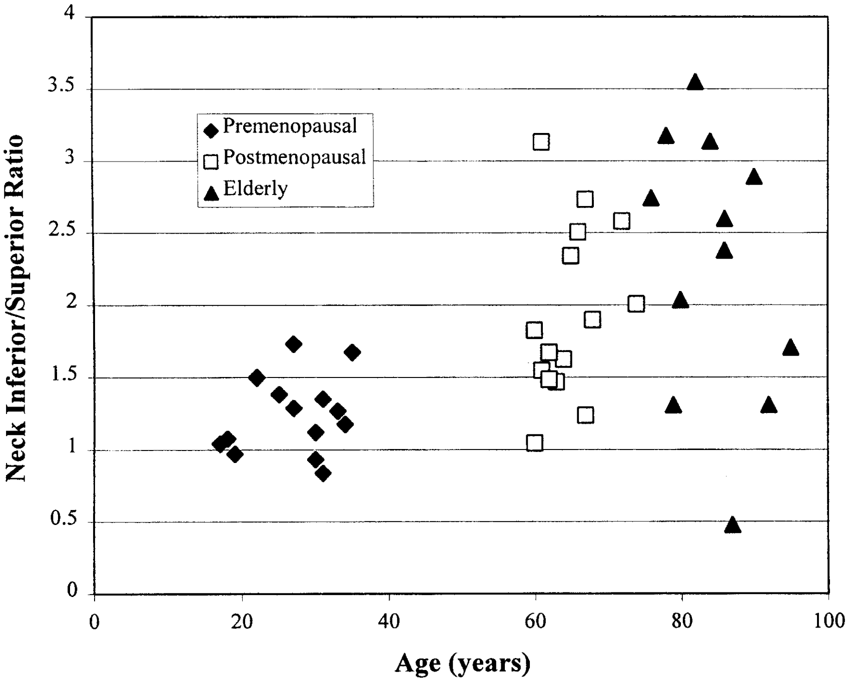

Fig. 4. Femoral neck inferior/superior volume fraction ratio versus age. Ratios are relatively uniform in premenopausal females but show a significant increase in variability after the menopause.

significant differences were detected ($p < 0.05$). A statistical difference was, however, identified when the neck/trochanter variances were analyzed, with a significant increase in variance with age ($p < 0.001$; Fig. 3). Similar results were found for the mean neck inferior/superior ratios, which where 1.2 ± 0.3, 1.9 ± 0.6 and 2.3 ± 0.9 for the premenopausal, postmenopausal and elderly age groups, respectively. There was no statistically significant difference in the mean neck inferior/superior ratio among the different age groups ($p > 0.05$); however, the variance of the elderly group was significantly larger than that of the premenopausal group ($p < 0.001$; Fig. 4).

Ash Fraction Measurements

There were no statistically significant differences in ash fraction among the three age groups ($p > 0.05$; Table 2). Similarly, there were no statistically significant differences in ash fraction between the neck and trochanter ($p > 0.05$; Table 2). A statistically significant difference in ash fraction was identified between the superior and inferior regions of the trochanter at all age groups, with the inferior region containing more mineral ($p < 0.05$). The mineral content of the superior region of the trochanter averaged over all age groups was 65.7% and that of the inferior region was 66.5%, representing a mineral content difference of only 0.8 percentage points.

Discussion

This investigation has demonstrated that the distribution of age-related cancellous bone loss within the female proximal femur is not uniform in this cadaveric population. The most convincing evidence for this was the changes in the neck/trochanter and neck inferior/superior ratios with age. Although there was no statistically significant increase in the mean neck/

Table 2. Means and standard deviations for the ash fraction values (%) measured by location and age group

Age group	Location					
	Neck			Trochanter		
	Superior	Middle	Inferior	Superior	Middle	Inferior
Premenopausal	66.0 ± 0.8	66.1 ± 0.9	65.9 ± 1.4	65.6 ± 1.2	66.4 ± 0.9	66.5 ± 0.8
Postmenopausal	66.0 ± 1.2	66.3 ± 1.1	66.6 ± 0.9	65.3 ± 4.3	66.5 ± 1.5	66.8 ± 1.4
Elderly	65.4 ± 1.9	65.6 ± 2.5	66.5 ± 1.2	65.9 ± 1.5	65.5 ± 1.5	66.9 ± 1.7

trochanter or neck inferior/superior ratio with age, the variance increased significantly with age in both these parameters ($p < 0.001$). Examination of Figs 3 and 4 demonstrates the tight grouping of these ratios prior to menopause, followed by a widening distribution postmenopausally. It is also of merit to note that this widening distribution is primarily due to the neck/trochanter and neck inferior/superior ratios increasing rather than decreasing in certain individuals within the study population. This increasing variability with age suggests that there may be a subpopulation of individuals within the elderly Caucasian population with a significantly different intrafemoral bone density distribution than was present prior to menopause. The cancellous bone neck/trochanter volume fraction ratio corresponds to the report by Greenspan et al. [25], which found that trochanteric fractures were independently associated with low trochanteric or high femoral neck dual-energy X-ray absorptiometry bone mineral density (BMD) values.

There have been no clinical studies to support the significance of the increased inferior/superior neck cancellous bone density ratio. However, BMD measurements for individuals with an elevated neck inferior/superior ratio would be misleading, as current radiology techniques provide an averaged value for the entire neck and would not detect regional heterogeneity. It is likely that the absolute BMD of the superior region would be a useful clinical measurement, as this region is believed to be under the greatest stress during a fall as well as the initiating site of neck fractures [29].

The increased variation in the neck/trochanter and neck inferior/superior ratios corresponds with the preferential bone loss in the femoral neck reported in the literature [14]. It is possible that preferential bone loss does occur in the proximal femur; however, not as a phenomenon that occurs across the entire population, but rather to specific individuals with unidentified predisposing factors. Individuals in the population who do not demonstrate preferential bone loss, as well as current measurements with radiologic methods, may explain the numerous studies that have reported a linear rate of bone loss of the proximal femur with age [9–11]. In addition, this may also explain the findings of Kawashima and Uhtoff [15], who reported no preferential bone loss between anatomic regions in the femoral neck.

Although there were differences measured in the distribution of cancellous bone density in the proximal femur, there were some trends that were consistent across all age groups. The femoral neck maintained a significantly greater cancellous volume fraction than the trochanter at all ages ($p < 0.05$). Likewise, the volume fraction of the inferior region in the neck was significantly greater than that of the superior or middle regions at all ages ($p < 0.05$; Fig. 2). The anatomic regions within the trochanter remained statistically similar for each age group.

There were no statistically significant differences in mineralization of cancellous bone in the female proximal femur among the different age groups or between regions within the neck ($p > 0.05$). Although previous studies have also found no change in mineralization with age, the current study is the first to specifically investigate human female cancellous bone of the proximal femur [16–19]. This provides reassurance that the bone loss associated with osteoporosis does not change the gross composition of the cancellous bone. Although it has been reported that microscopic hypermineralization occurs with age in cortical bone [30,31], widening the range of mineralization within aging bone, the data from this study demonstrated that this phenomenon does not influence the gross mineralization characteristics of Caucasian female proximal femur cancellous bone [18,21]. The results of this study do not support the reduced mineralization with aging reported in the cancellous bone of the ilium and forearm, providing increasing evidence for the heterogeneity of bone changes throughout the skeletal system [20].

The limitations of the current investigation lie in the relatively small sample size in each group as well as the cross-sectional nature of the study population. Regional proximal femur cancellous bone density changes were not measured in patients over time, which would have provided stronger evidence for preferential bone loss and changing distribution of cancellous bone volume with age. In addition, it is possible that the cross-sectional population used in this study may have introduced a selection bias in the results.

Cancellous bone plays a significant role in proximal femur integrity [32,33]. The preferred method to determine proximal femoral strength is a measurement of bone mineral density, which accounts for up to 90% of its strength [34,35]. This study has demonstrated that bone density varies significantly within anatomic regions in the proximal femur, and suggests that certain individuals within the female population may have some predisposition to losing bone at specific regions. This influence on femoral strength and potential improvement in fracture prediction requires additional investigation.

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