Iliac crest histomorphometry and skeletal heterogeneity in men

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\textbf{A B S T R A C T}

\textbf{Purpose:} The cortical characteristics of the iliac crest in male have rarely been investigated with quantitative histomorphometry. Also it is still unknown how cortical microarchitecture may vary between the iliac crest and fractures related sites at the proximal femur. We studied the microarchitecture of both external and internal cortices within the iliac crest, and compared the results with femoral neck and subtrochanteric femoral shaft sites.

\textbf{Methods:} Undecalciﬁed histological sections of the iliac crest were obtained bicortically from cadavers ($n=20$, aged 18–82 years, males). They were cut (7 μm) and stained using modiﬁed Masson-Goldner stain. Histomorphometric parameters of cortical bone were analysed with low ($\times 50$) and high ($\times 100$) magniﬁcation, after identifying cortical bone boundaries using our previously validated method. Within cortical bone area, only complete osteons with typical concentric lamellae and cement line were selected and measured.

\textbf{Results:} At the iliac crest, the mean cortical width of external cortex was higher than at the internal cortex ($p<0.001$). Also, osteon structural parameters, e.g. mean osteonal perimeter, were higher in the external cortex ($p<0.05$). In both external and internal cortices, pore number per cortical bone area was higher in young subjects ($\leq 50$ years) ($p<0.05$) while mean pore perimeter was higher in the old subjects (>50 years) ($p<0.05$). Several cortical parameters (e.g. mean osteonal diameter and mean wall width) were the highest in the external cortex ($p<0.05$). The maximal osteonal diameter and mean wall width were the highest in the external cortex of the iliac crest ($p<0.05$), and the mean cortical width, osteon number per cortical area were the highest in the subtrochanteric femoral shaft ($p<0.05$). Some osteonal structural parameters (e.g. mean osteonal diameter) were signiﬁcantly positively correlated ($0.29 \leq R^2 \leq 0.45$, $p<0.05$) between the external iliac crest and the femoral neck.

\textbf{Conclusions:} This study reveals heterogeneity in cortical microarchitecture between the external and internal iliac crest cortices, as well as between the iliac crest, the femoral neck and the subtrochanteric femoral shaft. Standard iliac crest biopsy does not reﬂect accurately cortical microarchitecture of other skeletal sites.

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1. Introduction

It has been well established that age-related bone loss occurs throughout the skeleton and affects both cortical and cancellous bone in the normal population (Vedi et al., 1982; Zebaze et al., 2010). In humans, histomorphometric analysis of the underlying changes in bone remodelling and microarchitecture that may predispose to bone loss has been mostly carried out in the iliac crest (Podenphant et al., 1986; Recker and Barger-Lux, 2006). Although iliac crest may not be expressed in differences between the external and internal iliac crest cortices were demonstrated by studies on children (Schnitzler et al.,

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Chappard et al. found no significant differences for any cortical parameters between biopsies from the right and left iliac crests in the same individual (Chappard et al., 2008), while a difference in bone formation rate between the two cortices of the same iliac crest biopsy in women with osteoporosis was reported by Balena et al. (1992). Misof and co-workers indicated a difference in calcium content between the iliac cortices, and they also revealed that the bone mineralization density distribution (BMDD) in cortices of a transiliac biopsy generally correlates with the corresponding values in the trabecular compartment (Misof et al., 2014). However, few data is available for comparison of structural characteristics between the iliac crest cortices in healthy adults.

Studies analysing the skeletal microarchitecture enables better understanding of bone alterations due to aging and pathology (Amling et al., 1996). However, most of these studies are based on restricted sites of the skeleton, e.g. iliac crest, spine and proximal femur. Previous skeletal heterogeneity related studies have predominantly focused on the differences in structural and remodelling parameters of cancellous bone (Hildebrand et al., 1999; Lochmüller et al., 2008; Aaron et al., 2015), or the physical measurements of cortical composition (Boskey et al., 2016; Scerpella et al., 2016). Except for the cortical width (Dempster et al., 1993; Castillo et al., 2012), structural characteristics of the cortical bone have rarely been compared between different skeletal sites. Considering the fact that the cortical microarchitecture is complex and a considerable skeletal heterogeneity exists between the axial and appendicular subdivisions of the skeleton (Marcus et al., 2009), the analysis of cortical bone throughout the skeleton of the same individual is needed.

In this study, cortical properties were compared between both cortices of the iliac crest, as well as to those reported earlier in the femoral neck and subtrochanteric femoral shaft of the same subject (Tong et al., 2015a; Tong et al., 2016). Both non-fracture (iliac crest) and fracture (proximal femur) skeletal sites were studied.

2. Materials and methods

2.1. Subjects

Iliac crests were obtained from 20 male cadavers (mean age, 47 ± 18.2 years, range 17–82 years) at Kuopio University Hospital, Kuopio, Finland. The subjects were divided into two sub-groups based on their age: young (≤ 50 years, n = 12), and old (> 50 years, n = 8) (Table 1). There was no previous history of medical conditions or use of drugs known to affect bone metabolism. Ethical approval for collection of samples was granted by the National Authority for Medicolegal Affairs (permission number: 5783/04/044/07).

2.2. Sample preparation

Iliac crest biopsies were taken bicortically from a standardized site located 2 cm below and posterior to the anterosuperior iliac spine (Tamminen et al., 2011) (Fig. 1). Samples were dehydrated in ethanol before being embedded in polymethylmethacrylate (PMMA) according to standard protocols (Raum, 2008). After embedding, 7-μm-thick sections were cut using a microtome (Reichert-Jung; Cambridge Instruments, Heidelberg, Germany) and stained with modified Masson Goldner trichrome stain. The entire section of the iliac crest was scanned using an auto-image scanner (Particle Analyzer; Carl Zeiss, Jena, Germany) to acquire a complete histological image (×50) for histomorphometric analysis (Fig. 1). An image program (GNU Image...
Manipulation Program, version 2.0) was utilized for delineation of different histological boundaries in the images (Tong et al., 2015b). The preparations for biopsies of femoral neck and subtrochanteric femoral shaft have been described earlier (Tong et al., 2015a; Tong et al., 2016) (Fig. 2).

2.3. Bone histomorphometry

Each histological image (×50) was separated into 5 zones: the external cortex area, the external endocortical bone area, the cancellous bone area, the internal endocortical bone area, the internal cortex area (Fig. 3). The cortex was identified based on the diameter and location of pores, as well as the structural size of the trabeculae, with respect to the "preliminary cortex boundary"; the endocortical bone area was identified as including the endocortical structures close to the cortex. Full details of the method to choose the respective area was presented in our earlier study (Tong et al., 2015b).

The histomorphometric analyses of cortical bone were conducted using Bioquant Osteo II (Bioquant Image Analysis, Nashville, TN, USA). The nomenclature, abbreviations, and parameters follow the recommendations by the American Society for Bone and Mineral Research (ASBMR) (Dempster et al., 2013). First, the samples were analysed with bright light microscopy using a magnification of ×50 in images (low-magnification measurements). Then, each cortex was evaluated under polarisation microscopy using a magnification of ×100 (high-magnification measurements). Both measurements covered the complete external and internal cortex. Measures of osteonal structure were limited to the complete osteons circumscribed by cement lines, since the age-associated changes in complete osteons are related to the skeletal fragility (Tong et al., 2015a). For cortical pore analysis, composite Haversian canals were identified as pores since the enlargement and clustering of the osteonal system have been suggested to predict the formation of cortical pores (Bell et al., 2000; Busse et al., 2010). The canals of complete osteons were evaluated separately as Haversian canal parameters.

Based on ×50 magnification imaging, the area and width parameters were determined. Cortical bone area [Ct.B.Ar. (mm²)] was measured as the tissue area between the absolute cortex boundary and the peristeme. Mean cortical width [Mean Ct.Wi. (mm)] was calculated as the average value of all (average 70 measurements/cortex) perpendicular widths between the absolute cortex boundary and the peristeme.

Based on ×100 magnification imaging, the osteon and cortical pore parameters were determined. The percentage of osteonal area and Haversian canal area per cortical area was calculated separately [On.Ar/Ct.Ar. (%)] [H.Ar/Ct.Ar. (%)]. The mean osteonal perimeter [On.Pm. (μm)], mean Haversian canal perimeter [H.Pm. (μm)], mean minimum osteonal diameter [Min.On.Dm. (μm)] and mean maximum osteonal diameter [Max.On.Dm. (μm)] were determined as the average value of all measured osteonal units. The mean wall width [WWi. (μm)] of osteons was calculated using all (average 12 measurements/osteon) perpendicular distances between the Haversian canal boundary and the outer edge of the complete osteon. Moreover, the cortical porosity [Ct.Po (%)] was calculated as the ratio of pore area divided by cortical area. The pore number and osteon number per cortical area (population density) [N.Po/Ct.Ar (#/mm²)] [N.On/Ct.Ar (#/mm²)] were also determined. The mean pore perimeter [Po.Pm (μm)] were determined as the average value of all measured pores.

2.4. Comparison between the external and internal cortex of the iliac crest

The cortical characteristics were assessed in both cortices of the iliac crest. The following parameters were compared: Mean Ct.Wi, On.Ar/ Ct.Ar, On.Pm, Min.On.Dm, Max.On.Dm, W.Wi, N.On, N.On/Ct.Ar, H.Ar/ Ct.Ar, H.Pm, N.Po, N.Po/Ct.Ar, Po.Pm, Ct.Po.

2.5. Comparison of cortical characteristics in the iliac crest, femoral neck and subtrochanteric femoral shaft

Cortical characteristics of these skeletal sites were compared based on the similar histomorphometric procedure and identical criteria of histological boundary definition (Tong et al., 2015b). The external and internal cortex of the iliac crest were taken into the comparisons separately.
2.6. Statistical analysis

Shapiro–Wilk test was used to determine whether the data was normally distributed. At the iliac crest, variations of histomorphometric parameters between age groups (young and old) were evaluated by Mann-Whitney U test due to the non-normal distribution of data within the age groups. Analysis of covariance (general linear model) was used to adjust for subjects’ height, since the height was different between the age groups (p ≤ 0.011). The inter-cortical difference at the iliac crest was identified by Wilcoxon signed-rank test since the data within two cortices of the iliac crest was correlated but not normally distributed (Field, 2009). The correlation of histomorphometric parameters between the external and internal iliac crest cortices, as well as between the different skeletal sites (iliac crest, femoral neck and subtrochanteric femoral shaft) were assessed by Spearman’s correlation coefficient. Kruskal Wallis test followed by multiple comparison Mann-Whitney tests were used to compare histomorphometric parameters between these skeletal sites. The Bonferroni correction was applied. All analyses were performed using SPSS Statistical software (version 21, SPSS, Chicago, IL, USA). p-values ≤ 0.05 were considered to be statistically significant.

3. Results

3.1. Age association of cortical characteristics at the iliac crest

In both external and internal cortex, N.Po/Ct.Ar was found higher in the young subjects group (≤50 years) (p ≤ 0.05) while Po.Pm of old subjects group (>50 years) was higher (p ≤ 0.05). These differences also remained after adjusting for height in the external cortex. Additionally, several osteonal parameters (On.Pm, Min.On.Dm, Max.On.Dm, W.Wi and H.Pm) in the external cortex displayed increase with age after height adjustment (Table 2).

3.2. Comparison between the external and internal cortex of the iliac crest

The external cortex was thicker than the internal cortex (p ≤ 0.001). As for the osteonal parameters, On.Pm, Max.On.Dm, W.Wi and H.Pm were higher in the external cortex (p ≤ 0.05) (Table 3) (Fig. 3). Several cortical parameters in the iliac crest were positively correlating with each other: W.Wi, N.On/Ct.Ar, Max.On.Dm and On.Pm (0.24 ≤ R² ≤ 0.25, p ≤ 0.05) (Table 4).

3.3. Comparison of cortical characteristics in the iliac crest, femoral neck and subtrochanteric femoral shaft

In the analysis of osteonal parameters, Max.On.Dm and W.Wi were highest in the external iliac crest (p ≤ 0.05). On.Ar/Ct.Ar, On.Pm, Max.On.Dm, W.Wi, and H.Pm were lowest in the femoral neck (p ≤ 0.05) and N.On/Ct.Ar was highest in the femoral shaft (p ≤ 0.05). Min.On.Dm in the external iliac crest was higher than in the femoral neck (p ≤ 0.05), and On.Ar/Ct.Ar, On.Pm, Min.On.Dm in the femoral shaft were higher than in the internal iliac crest (p ≤ 0.05). As to the cortical pore parameters, N.Po/Ct.Ar was found higher in both cortices of the iliac crest than in the femoral neck and shaft (p ≤ 0.05) (Table 3) (Fig. 4).

Several osteonal structural parameters (Min.On.Dm, Max.On.Dm and W.Wi) measured from the external iliac crest and the femoral neck were positively correlated (0.29 ≤ R² ≤ 0.45, p ≤ 0.05). Some other parameters (N.On/Ct.Ar, H.Ar/Ct.Ar, H.Pm and N.Po/Ct.Ar) of these two skeletal sites showed negative association, but only the correlation of N.Oh/Ct.Ar was statistically significant (R² = 0.23, p = 0.033). The osteonal parameters of On.Ar/Ct.Ar and Min.On.Dm measured from the external cortex of iliac crest and the subtrochanteric femoral shaft were found significantly positively correlated (0.30 ≤ R² ≤ 0.40, p ≤ 0.05) (Table 4). No significant correlations of the cortical parameters were detected between the internal cortex of iliac crest and the proximal femoral sites.
sider methodological difference in identifying cor-

tical pore size was lower, resulting in similar overall porosity in two age groups. In the present study, the canals of complete osteons were excluded from the cortical pore analysis, i.e. the composite Haversian canals of osteons (Riggs et al., 2008). However, the result of age associated increase in osteonal size (e.g. mean osteonal perimeter and mean Haversian canal perimeter) in the external cortex displayed increase with age. This seems inconsistent with the studies carried out by Schnitzler et al., who demonstrated the decline in osteonal diameter at the iliac crest cortex due to the slower bone turnover (resorption) with age (Schnitzler and Mesquita, 2006). However, the result of age associated increase in osteonal size revealed by present study became statistically significant only after the adjustment for differences in body height of the subjects. Since the understanding of the relationship between body height and osteonal geometry is limited (Brito et al., 2009), our finding may bring extra light on factors that indirectly controls changes in osteonal dimension.

4.2. Structural heterogeneity in iliac crest cortices

It has been suggested that a marked structural asymmetry between two cortices of growing ilium in children is mainly caused by the lateral modelling drift towards the external cortex (Schnitzler et al., 2009; Parfitt et al., 2000; Schnitzler and Mesquita, 2013). With diminishing growth, the modelling drift ceases after the mid-teens (Rauch et al., 2006; Schnitzler and Mesquita, 2013). As a result, in studies included growing individuals, properties of iliac cortices were usually recorded separately (Kulak and Dempster, 2010), while average values (esp. cortical width) of both inner and outer cortices were frequently utilized by studies on adults (Arlo et al., 2008; Ostertag et al., 2009). This raises an important question: will the asymmetry between cortices of the ilium still exist after adulthood? Mahato pointed out that the outer layers of the cortical bone in all segments (aged 40–60) of his study were thicker than the inner cortical layers (Mahato, 2011). This is in line with the finding in present study showing that the external cortex was from the measurements will reduce the likelihood of finding age associated increase in cortical porosity (Vedi et al., 2011).

In addition, we found that several parameters related to osteonal size (e.g. mean osteonal perimeter and mean Haversian canal perimeter) in the external cortex displayed increase with age. This seems inconsistent with the studies carried out by Schnitzler et al., who demonstrated the decline in osteonal diameter at the iliac crest cortex due to the slower bone turnover (resorption) with age (Schnitzler and Mesquita, 2006). However, the result of age associated increase in osteonal size revealed by present study became statistically significant only after the adjustment for differences in body height of the subjects. Since the understanding of the relationship between body height and osteonal geometry is limited (Brito et al., 2009), our finding may bring extra light on factors that indirectly controls changes in osteonal dimension.

### Table 4

| Parameters | External iliac crest | Internal iliac crest | Femoral neck | Femoral shaft | r | P |
|------------|----------------------|----------------------|--------------|--------------|---|---|
| On.Ar/Ct.Ar (%)a | 3.79 (0.38) | 4.30 (0.52) | 3.69 (0.20) | 5.91 (0.24) | 0.001 | 0.937 |
| W.Hi (μm)b | 78.4 (5.12) | 71.4 (4.60) | 62.1 (1.12) | 70.7 (1.0) | 0.17 | 0.474 |

1Significantly different as compared to the external iliac crest. 2Significantly different as compared to the internal iliac crest. 3Significantly different as compared to the external iliac crest and the subtrochanteric femoral crest. 4Significantly different as compared to the external iliac crest and the femoral neck. 5Significantly different as compared to both the external and internal iliac crest. P was obtained with Kruskal-Wallis test. Significances (p ≤ 0.05) were highlighted in bold.
significantly thicker than the internal cortex. The asymmetry in cortical thickness may be related to the difference in the magnitude of biomechanical loading. Since more abundant muscles have been suggested to attach on the periosteal surface of external iliac cortex (Rauch et al., 2006; Rauch et al., 2007), producing higher biomechanical stimulation which may increase the periosteal bone cell recruitment, bone formation, and ultimately the cortical thickness (Balena et al., 1992; van Oers et al., 2008). In addition, we also find that several osteonal structural parameters (e.g. On.Pm and H.Pm) were significantly higher in the external cortex. This can be translated into the structural features that the external cortex has much larger complete osteons (both in perimeter and wall width) and larger Haversian canals than the internal cortex. The combination of these features may be expected to confer greater bone strength in external iliac crest. Larger osteonal systems are able to derive greater strength from both greater wall thickness and greater osteonal diameter, leading to a biomechanical advantage on the bone structure (Bostrom et al., 2000).

4.3. Cortical heterogeneity in iliac crest, femoral neck and subtrochanteric femoral shaft

In present study, we compared the cortical microarchitecture at the “hot spots” of both axial and appendicular skeleton. As expected, the mean cortical width was found significantly higher in the femoral neck and subtrochanteric femoral shaft compared to the iliac crest. Cortices of peripheral skeleton were thicker than that of central skeleton (Marcus et al., 2009). This may explain another finding: namely that cortical pore population density was significantly higher in the iliac crest than in the femoral neck and shaft, since relatively narrow cortical area may favour the finding of higher density. On the other hand, cortical porosity may change as a result of variation in number or size of cortical pores (Chappard et al., 2013). In the present study, the difference in cortical porosity between skeletal sites was non-significant. Also because iliac crest generally has more pores in same-sized cortex, it could be speculated that the cortical pores in femoral neck and shaft tended to be larger.

Moreover, the osteonal parameters were found highly variable between different skeletal sites. In general the osteonal heterogeneity were: (1) osteons in the iliac cortices were much longer than in the femoral neck and shaft; (2) as compared to the internal iliac crest and the femoral neck, osteons in the subtrochanteric femoral shaft were larger both in size and population density. These new findings indicated higher ratio of complete osteonal area in cortical area at the external iliac crest and subtrochanteric femoral shaft. The relationship between the osteonal size and mechanical stimuli has been described by many studies (Skedros et al., 2013; Bernhard et al., 2013), but their results are conflicting. It is still unclear whether the compressive strain or tension may increase the osteonal size. Although it is challenging to compare the magnitude of mechanical effects between the ilium, femoral neck and shaft (Rudman et al., 2006; Brown et al., 1998), we believe the heterogeneity in osteonal structure and distribution could be related to local predominance of a specific loading mode (Skedros et al., 2012). Specifically, high osteon population density might be a response to increased loading resulting in higher toughness (Yeni et al., 1997). Also because larger osteonal dimension may confer additional bone strength (Bostrom et al., 2000), the subtrochanteric cortex which had highest osteon population density and larger-sized osteonal system in the current study is therefore stronger than the cortices of the iliac crest and the femoral neck. This is consistent with Donnelly et al.’s finding that compared to the cortices of the greater trochanter or iliac crest, the subtrochanteric cortex had a 20% greater mineral/matrix ratio which strengthens and stiffens the subtrochanteric femur (Donnelly et al., 2012).

In addition, the present study revealed a significant correlation between the external iliac crest and the femoral neck in osteonal characteristics, although there were differences in the structure and distribution of osteons. The relationship showed a similar variation of osteonal size (both in length and width), as well as the inverse variation trend of osteon population density. However, we still cannot conclude that the external iliac cortical bone can be of use in estimating the osteonal properties of the femoral neck.

To our knowledge, the present study was the first to investigate the cortical microarchitectural heterogeneity across multiple skeletal sites in men. One limitation of this study is that we characterized the cortical bone of healthy subjects, thus the heterogeneity may not be generalized to patients with metabolic bone diseases such as the osteoporosis. Moreover, the secular changes in cortical dimensions and architecture cannot be excluded, as this was a cross-sectional study. Further, the
relatively low number of subjects decreases the statistical power to assess age related changes.

5. Conclusion

This study indicates that the histomorphometric parameters of the iliac crest cortices differ between two age groups, and the cortical microarchitecture is highly variable between different skeletal regions. The structural asymmetry between cortices of the ilium remains after childhood. Due to higher osteon population density, the subtrochanteric femoral shaft could be stronger than the iliac crest and the femoral neck. These findings extend the limited reference data available on cortical microarchitecture in adult iliac crest and provide innovative understanding for skeletal heterogeneity.

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