Abstract of the Dissertation

Physical Activity and Genetics as Determinants of Limb Bone Structure

by

Ian Jacob Wallace

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The skeleton has the capacity to adjust its structure and strength throughout life in response to loads borne during physical activity. Typically, loading promotes bone formation, retards bone loss, and ultimately enhances structure and strength. Based on this observation, many anthropologists consider it possible to deduce the physical activity levels of ancient human populations by analyzing their skeletal remains. Populations with thick, strong bones are inferred to have been very physically active, while populations with slender, fragile bones are inferred to have been more sedentary.

Although the responsiveness of bone to applied loads is well documented, non-mechanical factors, particularly genetic background, also affect skeletal structure and strength, which may undermine the accuracy of anthropological inferences about the physical activity of past human populations. The goals of this dissertation were to clarify (1) the relative importance of genetics and physical activity in determining variation in limb bone morphology among populations and (2) the degree to which bone's response to loading varies among populations. To this end, an experimental approach was adopted using mice as a model organism.

The research consisted of three experiments. In the first experiment, mice were employed from a long-term artificial selection experiment for high levels of voluntary wheel running. Growing males from four replicate high runner (HR) lines and four

replicate non-selected control (C) lines were either allowed or denied wheel access for 2 months. Using μCT, femoral morphology was assessed at two cortical sites (mid-diaphysis, distal metaphysis) and one trabecular site (distal metaphysis). It was found that genetic differences between the linetypes (HR vs. C), between the replicate lines within linetype, and between individuals with and without the so-called 'mini-muscle' phenotype (caused by a Mendelian recessive gene that halves limb muscle mass) gave rise to significant variation in nearly all morphological indices examined. Wheel access also influenced femoral morphology, although the functional response did not generally result in enhanced structure. Exercise caused moderate periosteal enlargement, but relatively greater endocortical expansion, resulting in significantly thinner cortices and reduced bone quantity in the metaphysis. The magnitude of the response was independent of distance run. Mid-diaphyseal bone quantity and area moments, as well as trabecular morphology, were unaffected by exercise. These results underscore the strong influence of genetics on bone structure and the complexity by which mechanical stimuli may cause alterations in it.

In the second experiment, mice were used from two commercially available outbred stocks (Hsd:ICR, Crl:CD1) that have been reproductively isolated for >120 generations and that large-scale genetic analyses have shown to possess genetic architecture (variation) that is comparable to that of living human populations. Beginning shortly after weaning, females from each stock were either treated with a treadmill-running regimen for 1 month or served as sedentary controls. Home-cage activity of all animals was monitored during the experiment. Limb forces were recorded to verify that they were similar in the two stocks. At the end of the experiment, μCT was used to quantify cortical structure in femoral and tibial mid-diaphyses and trabecular structure in the distal femoral metaphysis and proximal tibial metaphysis. Mechanical testing was used to determine femoral and tibial diaphyseal strength. Among the Hsd:ICR mice, treadmill running led to significant improvements in femoral and tibial diaphyseal bone quantity, structural geometry, and mechanical strength, as well as enhanced trabecular bone morphology in the distal femur. In contrast, among the Crl:CD1 mice, the same running regimen had little effect on limb bone cortical and trabecular morphology, and led to significant reductions in femoral diaphyseal strength. Importantly, in neither stock

was body mass, muscle mass, or cage activity level significantly different between runners and sedentary controls. Given that most environmental variables were controlled in this study, the differential effects of exercise on the limb bones of Hsd:ICR and Crl:CD1 mice can reasonably be attributed to genetic differences between the stocks. These results suggest that the magnitude of the functional response can vary in the limb bones of individuals from different populations despite similar physical activity levels during life.

The third experiment investigated the possibility that genetic variation underlying limb bone structure is influenced by the physical activity levels of ancestral populations and might therefore have functional significance in an evolutionary context. In this experiment, 1-week-old mice were employed from the artificial selection experiment for high voluntary wheel running. Differences in limb bone structure at 1 week can be assumed to primarily reflect the effects of selective breeding rather than direct mechanical stimuli, given that the onset of locomotion in mice is shortly after day 7. It was hypothesized that if genetically influenced limb bone diaphyseal structure reflects the physical activity levels of members of a lineage, then selected animals would have enhanced femoral diaphyseal structure (measured using μCT) at 1 week of age compared to non-selected controls. The results provide strong support for this hypothesis and suggest that limb bone structure may not always only reflect the physical activity levels of particular fossil individuals, but may also convey an evolutionary signal providing information about human physical activity in the past.

In sum, the results of the research presented in this dissertation indicate that genetic background plays a significant role in determining variation in limb bone structure among populations and in the responsiveness of bone to loading. Ultimately, the results suggest that much prudence is necessary when using skeletal remains to gain information about the physical activity levels of human populations living in the past.