

Chronic Sleep Restriction Increases Late Adulthood Bone Fragility in a Rodent Model.

Mei Wang^{1,2}, Carol A. Everson¹, Jeffrey M. Toth^{1,2}

¹Medical College of Wisconsin, Milwaukee, WI, ²Marquette University, Milwaukee, WI
Email: meiwang@mcw.edu

Disclosures:

INTRODUCTION: More than 35% of adults in the U.S. experience chronic sleep restriction (SR) which is associated with multiple, incompletely understood adverse health outcomes. Chronic SR due to causes such as night shift work is increasingly recognized as a risk factor for osteopenia, osteoporosis, and bone fractures. Chronic SR in middle-aged to older adults imparts a similar risk of fracture as corticosteroid therapy in epidemiological studies. The biological effects of SR on bone health during adulthood are unknown and the implications for diseases of aging may be different than for immature bone. The objective of this study is to determine the effects of chronic SR exposure after attainment of skeletal maturity on biomechanical fragility of the femur using a well-validated rodent model.

METHODS: Under an IACUC-approved protocol, 16 Sprague-Dawley rats (8♀ 8♂, 11.4 mo.) were assigned to the SR group (4♀ 4♂) and to the ambulatory control (AC) group (4♀ 4♂). Rats were housed in Bergmann-Rechtschaffen apparatuses, which are large open-aided chambers that permit freedom of movement and normal behaviors. Animals under baseline conditions obtain normal sleep amounts (54-61% of total time), as shown by prior validation studies. After 7 days of baseline, SR was achieved by imposing brief and slow 6-second rotations of the housing platform which produced ambulation requirements at variable intervals incompatible with consolidated sleep. A validated 35% reduction in sleep was implemented, which is comparable to 5.2 hours of sleep per day in humans (1). Chronicity was produced by repeated exposure with 12-day cycles composed of 10 days of SR followed by 2 days of sleep *ad libitum* for 66 days until 13.6 months of age. This is theoretically equivalent to 7 human years of chronic sleep restriction. For the AC group, ambulation requirements were consolidated into periods that permitted more lengthy opportunities for sleep. After 66 days of repeated SR, all rats were sacrificed, one femur of each rat was isolated and stored at -80C until biomechanical testing. Flexural testing blinded to group assignment consisted of a three-point bending test (12-mm supporting span) on the femurs using a calibrated servo-hydraulic load frame (MTS Bionix, Eden Prairie, MN). Compressive force in the A/P direction was applied at a loading rate of 0.1 mm/sec to the mid-shaft of the femur until fracture occurred. The whole bone biomechanical properties of the femur, including ultimate force and deformations, stiffness, and work-to-fracture, were obtained from the force-deformation plots. Femoral geometry at the mid-shaft, including cross-sectional area, diameters, and cortex thickness was measured using a digital caliper before and after the three-point bending test. Intrinsic biomechanical properties, including the ultimate stress and strain, elastic modulus, and work to failure were derived based on the structural and geometric properties of the femur. The median and interquartile range (IQR) were calculated, and a two-tailed Mann-Whitney U-test was performed to compare the differences between SR and AC groups for each sex ($p < 0.05$).

RESULTS: Compared to the AC group, female rats in the SR group had a statistically significant reduction in ultimate force (30%, $p < 0.03$), work to failure (31%, $p < 0.03$), ultimate stress (34%, $p < 0.03$), and strain (38%, $p < 0.03$). Compared to the AC group, male rats in the SR group showed tendencies for reductions in all of these biomechanical parameters, but none of these reductions reached the level of statistical significance (Figure 1). A statistical trend of decreased ultimate deformation for SR rats compared to AC rats was observed in both sexes ($p = 0.086$, 31% reduction for both sexes). Morphologically, the average cortex thickness of the female SR group was significantly smaller than the female AC control (median 0.81 mm vs. 0.97 mm, $p < 0.03$); while a much smaller decrease was found in the male groups (median 1.00 mm vs. 1.05 mm, NS).

DISCUSSION: Results from this adult rodent model of chronic sleep restriction demonstrated that chronic sleep restriction had a marked impact on mechanical behaviors of the whole bone femur, causing a significant reduction in its bending strength and cortical thickness in the female group. These pilot data also suggest sex differences in the level of impact from sleep restriction that seems to be more burdensome in female animals. Further investigations are warranted to elucidate mechanisms affected by chronic sleep restriction that result in increased bone fragility.

SIGNIFICANCE/CLINICAL RELEVANCE: Epidemiologic studies of deficient sleep have reported observable bone health-related problems. The present study provides quantitative biomechanical evidence that exposure to chronic sleep restriction significantly increases late adulthood bone fragility.

REFERENCES: 1) Everson CA & Szabo A (2009) Am J Physiol Regul Integr Comp Physiol 297, R1430-1440.

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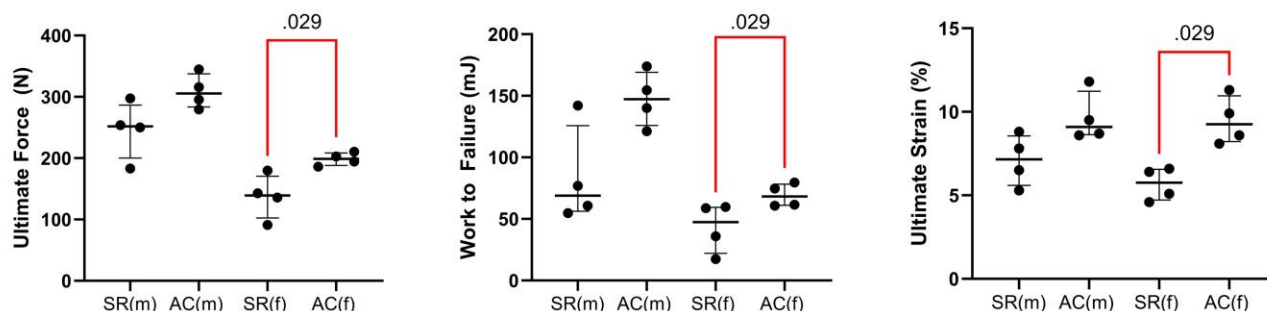


Figure 1. The ultimate force (left), work-to-failure (middle), and ultimate strain (right) of the SR vs. AC in male (m) and female (f) rat femur under three-point bending test (horizontal bar – median (IQR)).