INTRODUCTION:
Conventional techniques used to image microdamage in cortical bone require the preparation of many histologic sections which is inherently invasive, destructive, two-dimensional, and tedious [1]. These limitations inhibit evaluation of the effects of microdamage on whole bone strength and prohibit detection of microdamage in vivo. Therefore, micro-computed tomography (micro-CT) has been investigated for the detection of microdamage using iodinated [1], lead sulfide [2,3], and barium sulfate (BaSO₄) [4,5] contrast agents. Damage accumulation ahead of a notch in bovine cortical bone beams loaded in cyclic four-point bending was stained in vitro by precipitation of BaSO₄ and imaged using micro-CT [4]. The objective of this study was to detect the presence and location of fatigue microdamage and/or propagating cracks within a uniformly stressed volume of human cortical bone using micro-CT with a BaSO₄ contrast agent.

METHODS:
Twenty specimens were sectioned from the femoral cortex of three adult men (63 ± 1.7 years of age), turned down to a 2.5 mm diameter by 5 mm gauge length using a CNC mini-lathe, and randomly divided into an unloaded control group and loaded group. Specimens were wrapped in gauze, hydrated in PBS, and stored at -20°C in airtight containers during interim periods.

Specimens were tested in load-controlled (R = 0) cyclic uniaxial tension at 2 Hz on an electromagnetic test instrument (Bose Electroforce 3300) while hydrated with a water drip at ambient temperature until a 10% reduction in secant modulus. Specimens were preloaded at 60 MPa for 20 cycles to measure the initial secant modulus and the fatigue load was normalized to an initial maximum strain of 6400 ± 300 microstrain.

Specimens from both groups were stained with BaSO₄ by immersion in solutions of equal parts 0.5 M NaCl₂, buffered saline, and acetone solution for three days, followed by equal parts 0.5 M NaSO₄, buffered saline, and acetone for three days, and rinsed with de-ionized water to remove surface bound particles or ions. The entire gauge length of each specimen was imaged by micro-CT (μCT-80, Scanco Medical) at 10 μm resolution, 70 kVp voltage, 113 μA current, and 200 ms integration time with slices taken perpendicular to the longitudinal axis of the specimen. Images were thresholded to determine the total bone volume (BV) and BaSO₄ stained volume (SV).

RESULTS:
Non-specific BaSO₄ staining was observed on the free surface and scattered within some Haversian canals in unloaded control specimens (Figs. 1a & 2a). All specimens loaded in cyclic uniaxial tension also exhibited at least one distinct region of concentrated BaSO₄ stain which appeared characteristic of fatigue damage and/or propagating cracks (Figs. 1b,c & 2b). The ratio of the thresholded BaSO₄ stain volume to bone volume (SV/BV) was significantly greater for the loaded group compared to the control group (p < 0.05, t-test) (Fig. 3). There was no correlation between the number of loading cycles and SV/BV (p = 0.7).

DISCUSSION:
Micro-CT was able to detect a relatively small volume of fatigue microdamage within a uniformly stressed volume of human cortical bone using a BaSO₄ contrast agent. Note that the micro-CT scanner (10 μm resolution) could not detect damage without the use of a contrast agent. In a previous study using similar staining and imaging methods for notched specimens loaded in cyclic four-point bending, distinct regions of bright voxels around the notch tip or along propagating cracks were verified to be due to the presence of precipitated BaSO₄ by backscattered electron imaging and energy dispersive spectroscopy [4].

The technique demonstrated in this study is not without limitations. Variability within both experimental groups can be partially attributed to non-specific staining within void space and on free surfaces (Fig. 1). Staining by BaSO₄ precipitation is also limited to in vitro studies since the staining solutions are not biocompatible.

The specimens in this study will be sectioned and imaged using backscattered SEM to clarify whether the concentrated BaSO₄ stain in loaded specimens was due to propagating cracks, accumulated microcracks, and/or diffuse damage. This technique will also be validated against a second loaded group stained and imaged using conventional histological techniques.

REFERENCES: