Exercise-Induced Changes in Mechanoresponsive Biomarkers with Increasing Walking Speed

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Introduction: Mechanical loading of articular cartilage can induce a broad cascade of biochemical changes both locally and systemically [1]. These biochemical changes may reflect changes in cartilage integrity and could be indicative of early osteoarthritis (OA) development [2]. Therefore, the goal of this study was to examine short-term biomarker responses to a 30-minute walk of varying intensities at physiologically relevant walking speeds. We characterized the response of four key biomarkers in healthy individuals. Cartilage oligomeric matrix protein (COMP), matrix metalloproteinase-3 (MMP-3), interlukin-6 (IL-6), and proteoglycan 4 (PRG4) were chosen given their relevance to articular cartilage health, their mechanoresponsive nature, and demonstrated changes in OA or joint-injured patients [3,4,5,6]. We hypothesized that the mechanoresponsive biomarkers would be transiently and dose-dependently elevated by increasing walking speeds.

Methods: *Study Design:* In this IRB approved study, seven healthy participants (4M/3F) age 18-40 with no history of OA were recruited. These individuals completed three separate visits. Visits began prior to 8am to minimize prior loading of the knee, and participants were asked to refrain from strenuous activity for the 24 hours prior to their visit. At each visit, participants rested in a supine position for 45 minutes, after which blood (Pre) was collected. Following this rest period, participants completed a 30-minute treadmill walk, immediately after which another blood sample was collected (Post). Participants then rested in a supine position for a 45 minutes recovery period, after which a third blood sample was collected (Recovery). At each visit, the treadmill speed was varied so that participants walked at a slow (Froude number of 0.15, average of 2.6 mph), moderate (Froude number of 0.25, average of 3.4 mph), or brisk (Froude number of 0.35, average of 3.9 mph) speed. Froude number was used to normalize each participant's walking speed to their leg length [7]. The order of each visit's walking speed was randomized. *Blood Processing:* All blood samples were allowed to clot for 30 minutes, after which serum was isolated by centrifugation at 3500rpm for 15 minutes. Serum was then stored at -80°C until analyzed. *Biomarker Analyses:* ELISA assays were used to determine COMP (BioVendor), MMP-3 (R&D Systems Quantikine), and PRG4 (Abbexa) concentrations following the manufacturer's protocols. IL-6 concentrations were assessed by a commercially available high sensitivity Simoa bead-based assay (Quanterix). Serum biomarker concentrations were normalized to the Pre concentrations for

*Figure 1. Immediately post exercise (Post), serum COMP was significantly elevated at the brisk walking speed compared to the slow and moderate walking speeds (A < B, p<0.05). COMP concentrations significantly decreased during the recovery window for all walking speeds (*p<0.05).*

each participant at each visit. A two-way repeated-measures ANOVA with Student-Newman-Keuls post hoc testing was performed to assess the effects and interactions of walking speed and timepoint on biomarker concentrations. Differences were considered statistically significant at p<0.05.

Results: There was a significant interaction between walking speed and timepoint for COMP (Figure 1) and MMP-3 (Figure 2). Increasing walking speed elicited a dose-dependent increase in both serum COMP and MMP-3 concentrations. Specifically, serum COMP concentrations for the brisk walking speed were significantly higher than to the two lower intensity walking speeds immediately post-exercise (43% versus 19% and 28%, p<0.05). Similarly, immediately after walking, serum MMP-3 was increased at the brisk walking speed compared to slow and moderate walking speeds (58% versus 17% and 26%, p < 0.05). Within the 45-minute recovery window, serum COMP and MMP-3 significantly decreased for all 3 walking intensities (p<0.05). There were no detectable differences observed for serum IL-6 or PRG4 concentrations (data not shown).

Discussion: Our findings reveal the mechanoresponsiveness of the serum biomarkers COMP and MMP-3 in response to differing intensities of walking speed in a healthy population. In this study, we varied the intensity of a single physiologically relevant activity of daily living in order to evaluate biomarker changes. Given the relevance of COMP and MMP-3 to the degenerative processes associated with OA development, these findings may serve as important baseline data for those at risk of disease. To this point, COMP is a structural protein in cartilage and MMP-3 is a catabolic mediator, both of which may serve as diagnostic biomarkers for early OA development [5]. Interestingly, while prior work has shown that serum IL-6 correlates with intensity of exercise, a minimum intensity threshold may need to be met in order to achieve a detectable effect for short-term exercise [8]. This may account for the lack of an observed change in serum IL-6 in this study, despite the high-sensitivity assay employed. The effect of acute exercise on PRG4 is far less documented but has been shown to increase due to a 10-km run. Thus, a higher intensity exercise or longer duration may be required to induce detectable changes in serum PRG4 concentrations [9]. Therefore, while further work is still needed, these biomarkers may serve as diagnostic tools when comparing the magnitude of response and recovery metrics in joints undergoing early OA changes.
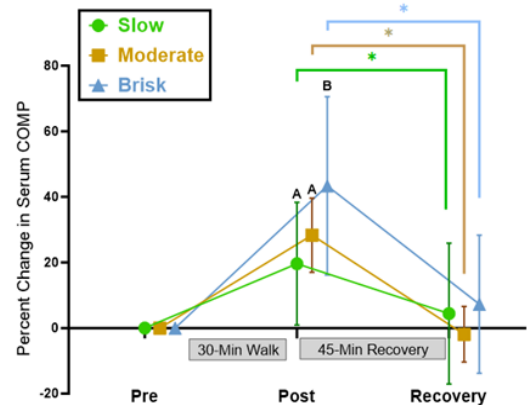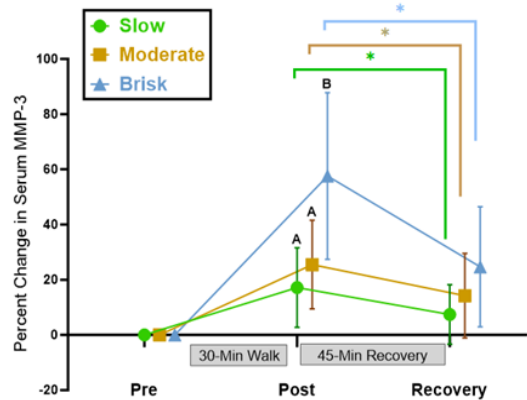

*Figure 2. Immediately post exercise (Post), serum MMP-3 was significantly elevated at the brisk walking speed compared to the slow and moderate walking speeds (A < B, p<0.05). MMP-3 concentrations significantly decreased during the recovery window for all walking speeds (*p<0.05).*

Significance: Our findings reveal the dose-dependent mechanoresponsiveness of the serum biomarkers COMP and MMP-3 in response to differing intensities of walking speed in a healthy population. Overall, these findings indicate that walking speed differentially regulates the turnover of OA-related biomarkers.

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