


# Determination of the Interday and Intraday Reliability of Serum Cartilage Oligomeric Matrix Protein in a Physically Active Population

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## Abstract

**Objective:** To determine the intraday and interday reliability of serum cartilage oligomeric matrix protein (sCOMP) in a physically active population with no history of lower extremity surgery. **Design:** A repeated-measures reliability study was employed to determine the intraday and interday reliability of sCOMP in a physically active cohort. A total of 23 subjects were recruited to the laboratory on 3 separate occasions for nonfasting serum collection. Subjects had no history of lower extremity surgery and were free from acute injury within the last 3 months. **Results:** Our results indicate strong reliability for both intraday intraclass correlation coefficient (ICC) (0.76) and interday ICC (0.74) sCOMP values. **Conclusion:** Our results demonstrate that following 30 minutes of inactivity, nonfasting serum samples remain stable over the course of 1 day and between 2 consecutive days in a healthy population with no history of lower extremity surgery. Future research studies are needed to further investigate the magnitude of change in this biomarker for patients with acute articular cartilage damage to determine its appropriateness for use in this population and for varying degrees of articular cartilage severity.

## Keywords

articular cartilage, biomarkers, knee injury

## Introduction

Serum cartilage oligomeric matrix protein (sCOMP) is a noncollagenous protein primarily identified in articular cartilage.<sup>1–3</sup> Elevated levels of this biomarker have been identified in patients suffering from knee osteoarthritis (OA), a chronic articular cartilage degradation disease, when compared to healthy controls.<sup>4–10</sup> In addition, elevations of this biomarker have been documented in patients who sustained acute knee ligament injury, such as anterior cruciate ligament (ACL) rupture.<sup>11–13</sup> The basic assumption is that these elevations are associated with articular cartilage damage, not ligament injury. However, documentation of concomitant injuries, such as bone bruise lesions and associated articular cartilage injury, was not reported in these studies.<sup>11–13</sup>

An estimated 80% of MRI-confirmed ACL ruptures have documented concomitant bone bruise lesions.<sup>14–17</sup> Often, these lesions are not associated with visible articular cartilage injury at the time of MRI and surgical intervention.<sup>18,19</sup> However, biopsies of the overlying articular cartilage indicate degeneration of the chondrocytes and substantial damage to the superficial matrix.<sup>20,21</sup> Therefore, sCOMP may

be a useful biomarker to better understand the articular cartilage damage that is associated with bone bruise lesions for patients who suffer acute knee injury.

In order to further investigate the utility of sCOMP as a biomarker for acute articular cartilage damage, the reliability of this measure must be documented. Determination of interday and intraday reliability measures is necessary to ensure this marker is clinically applicable for future research studies. The purpose of this study was to determine the interday and intraday reliability of sCOMP in a healthy, physically active cohort.

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## Methods

### Design

A repeated-measures reliability design was employed for this study. The independent variable was time (time 1 = T1, time 2 = T2, and time 3 = T3), and the dependent variable was sCOMP.

### Population

A sample of 23 physically active subjects (4 males and 19 females; age:  $27.2 \pm 3.8$  years; height:  $168.6 \pm 6.3$  cm; mass:  $150.6 \pm 31.4$  kg) volunteered to participate. Subjects were included if they had no history of lower extremity surgery and no history of lower extremity injury within the past 3 months. Subjects were considered physically active if they scored a 6 or higher on the NASA Physical Activity Status Scale.<sup>22</sup> A score of 6 represents heavy aerobic exercise in the form of either running 6 to 10 miles a week or walking 7 to 13 miles per week.<sup>22</sup> Subjects maintained their usual daily activities while participating, and hourly documentation logs were collected while the subjects were enrolled. This study was approved by the University of Kentucky Institutional Review Board, and approved informed consent was obtained from the subjects prior to participation in our study.

### Procedure

A total of 3 serum collections were performed on 2 separate days. Subjects reported for serum collection on 2 separate days and 3 different time points. To determine the intraday (within-day) reliability, subjects reported to the laboratory once in the morning (T1) and approximately 7 hours later on the same day (T2). To determine interday reliability (between days), subjects reported to the laboratory the morning after (24 hours) the first draw (T3).

Upon arrival, each subject was asked to remain seated at a table for 30 minutes prior to data collection based on the recommendations of Andersson *et al.*<sup>23</sup> and Mundermann *et al.*<sup>24</sup> Following 30 minutes of rest, a maximum of 10 mL of blood was collected from the antecubital vein in a red-top safety tube following standard operating procedures for venipuncture.

### Enzyme-Linked Immunosorbent Assay

Immediately after serum collection, the blood was placed on ice and transported for separation. After clotting for 30 minutes at room temperature, sera were separated in a refrigerated centrifuge at 4 °C at 2000g for 15 minutes, placed in labeled aliquots, and stored in a -80 °C freezer. Once all samples were collected, sCOMP concentrations were determined using a commercially available, competitive enzyme-linked immunosorbent assay (ELISA) kit (cat. no. COMP200,

**Table 1.** Serum Cartilage Oligomeric Matrix Intraclass Correlation Coefficients (ICCs), Standard Error of Measurements (SEMs), 95% Confidence Intervals (95% CIs), and Minimal Detectable Change (MDC) Values

|          | ICC  | SEM    | 95% CI    | MDC <sub>95%</sub><br>(ng/mL) |
|----------|------|--------|-----------|-------------------------------|
| Interday | 0.76 | 213.70 | 0.48-0.90 | 292.00                        |
| Intraday | 0.74 | 227.72 | 0.48-0.89 | 320.00                        |

Wieslab hCOMP quantitative kit, IBL Euro-Diagnostica, Malmo, Sweden). This ELISA kit utilized a rabbit polyclonal antiserum directed to human COMP, and the standard curve was determined using native human articular cartilage COMP (Wieslab hCOMP quantitative kit, IBL Euro-Diagnostica). Serum COMP values are expressed as ng/mL. The average intra-assay coefficient of variance (CV) of all controls was 2.8%, the average interassay CV of all controls was 2.1%, and the average CV of all samples was 2.5%.

### Statistical Analysis

Descriptive statistics (means  $\pm$  standard deviations) for each test session were calculated. Intraday and interday reliabilities were estimated using intraclass correlation coefficients (ICC<sub>2,1</sub>). A total of 23 participants' data were used to calculate intraday reliability (T1 v. T2). A total of 20 participants' data were used to calculate interday reliability (T1 v. T3). Standard error of measurement (SEM) was also calculated for each correlation. ICCs were interpreted as weak if they were less than 0.40, they were moderate if between 0.41 and 0.69, and values of more than 0.70 were interpreted as strong.<sup>25</sup> Within-day and between-day minimal detectable change (MDC) values were also calculated using the interday and intraday reliability and SEM data collected during each session. MDC values were calculated at the 95% confidence interval using the formula  $SEM * 1.96 * \sqrt{2}$ .<sup>26,27</sup> All statistical analyses were performed using PWAS software, version 18.0 (Somers, NY).

## Results

The mean sCOMP value for T1 was 1287.44 ( $\pm 205.70$ ) ng/mL, for T2 was 1292.90 ( $\pm 228.00$ ) ng/mL, and for T3 was 1270.15 ( $\pm 213.60$ ) ng/mL. ICCs, SEMs, 95% confidence intervals (CIs), and MDC values for each of the testing sessions are presented in **Table 1**. The ICCs for the interday (0.76) and intraday (0.74) were both interpreted as strong.

## Discussion

To our knowledge, we were the first to document the interday and intraday reliability of sCOMP in a healthy, physically active cohort with no history of lower extremity surgery or acute lower extremity injury within the last 3 months. The

results of our investigation indicate strong interday and intraday reliability for sCOMP in a young, physically active cohort. To provide a more comprehensive interpretation of our reliability values, we have compared our findings to those that have been previously published.

Several studies have reported temporal patterns for sCOMP. For example, Vilim *et al.*<sup>28</sup> conducted a study to verify their ELISA was sensitive to changes over time in sCOMP in multiple populations. As a subcomponent of their larger study, the authors reported that intersession variability was insignificant between days.<sup>28</sup> However, the specific variability of sCOMP was not reported as the data were presented in a line graph figure. Our interpretation of their data was that the variability of the 5 volunteers did not differ more than 1 µg/mL, a very small difference in sCOMP levels. Notably, no documentation of participant demographics (age, gender, medical history) was provided.<sup>28</sup> These results, in addition with our results, indicate sCOMP levels are stable and reliable between days in healthy populations.

In addition, Vilim *et al.*<sup>28</sup> also reported significant differences in time point 1 (morning fasting sample) and time point 2 (2 hours after lunch) in 4 of the 20 subjects who participated in their research study. For the remaining 16 subjects in their research study, no variation in the serum samples between the time points was identified.<sup>28</sup> It was concluded that the only differences noted for the 4 individuals were related to food consumption, and it was recommended that for future clinical research, serum draws should be performed as fasting, morning collection.<sup>28</sup> However, the results of our study indicate strong intraday reliability, where the subjects were allowed to eat a nonregimented meal prior to each of the testing sessions (breakfast and lunch).

Diurnal variation of sCOMP has been documented in patients with OA and rheumatoid arthritis during a 24-hour observational study.<sup>29,30</sup> The results of a 24-hour observational study where patients with OA and rheumatoid arthritis had serum collected every 4 hours indicated no significant changes in sCOMP levels throughout the waking hours.<sup>29</sup> For patients with OA, the results of the investigation revealed no significant changes in sCOMP between 09:00 and 21:00.<sup>29</sup> However, the results of the study did reveal a significant decrease in sCOMP levels at 05:00 ( $P < 0.03$ ).<sup>29</sup> For patients with rheumatoid arthritis, no significant changes were observed between 08:00 and 20:00.<sup>29</sup> In addition, a significant decrease in sCOMP was noted at 04:00 ( $P < 0.001$ ).<sup>29</sup> The results of this study show that sCOMP levels remain similar during the waking hours, indicating sCOMP levels can be collected throughout the day for either clinical and/or research purposes.<sup>29</sup> Therefore, based on the results of this study and our study, the variation in sCOMP across the day is insubstantial.

A study conducted by Kong *et al.*<sup>30</sup> reported diurnal variation in sCOMP in a cohort of patients with OA. The results of their clinical laboratory investigation revealed significant differences in sCOMP levels measured between the time before arising from bed (T0) and 1 hour after rising from bed

(T1,  $P < 0.001$ ), between T0 and 4 hours after arising from bed (T2,  $P < 0.001$ ), and between T0 and 12 hours of daily activity (T3,  $P < 0.01$ ).<sup>30</sup> It must be noted the authors reported they did not allow the participants to remain seated for more than 30 minutes at a time during study participation, and subjects were encouraged to engage in activities of daily living throughout the study duration.<sup>30</sup> Based on the recommendations of Andersson *et al.*<sup>23</sup> and Mundermann *et al.*,<sup>24</sup> 30 minutes of rest prior to serum collection is necessary to ensure elevations in sCOMP levels are not due to any moderate exercise, such as walking to the data collection site. However, it must be noted that the results reported by Kong *et al.*<sup>30</sup> would have not been privy to the effect of exercise on sCOMP levels as their study published at the same time as the results of the Mundermann *et al.*<sup>24</sup> and Andersson *et al.*<sup>23</sup> studies, which indicated exercise and activities of daily living have a direct effect on sCOMP levels. Our methods required the subjects to remain seated for 30 minutes prior to serum collection. We believe the addition of this resting time is necessary in order to provide the most valid results of sCOMP levels.

We did not limit the amount of physical activity each of the subjects engaged in while participating in this study. Also, our subjects' meal times were not regulated, as the subjects were encouraged to engage in their normal activities of daily living while participating in our research study. Therefore, our results indicate this biomarker is highly reliable even when the subjects are not in a research laboratory environment where exercise, meal times, and sleeping habits are regulated.<sup>29,30</sup>

Our study is not without limitations. In order to be included in our research investigation, the subjects had to have no history of lower extremity surgery and no current acute lower extremity injury. Subjects were not given a physical examination or radiographic examination prior to study inclusion. Therefore, we were unable to verify each of the subjects were completely free of joint damage, such as OA. However, these were relatively young and active participants who reported no history of serious knee joint injury. Based on this, it is unlikely that substantial knee cartilage degradation had occurred in any of our participants.

## Conclusion

Our results indicate strong interday and intraday reliability for sCOMP values in a healthy, physically active cohort with no history of lower extremity surgery. The strong reliability of this measure demonstrates serum samples can be collected throughout the day for future clinical research studies.<sup>29</sup> The importance of these findings is that sCOMP levels are stable in an uninjured sample, and measurable increases are likely due to injury and not interday or intraday fluctuation. Our MDC<sub>95%</sub> values suggest that a change in sCOMP levels of 290 to 320 ng/mL in an injured population represents a meaningful change that exceeds the variability associated

with the measure. Future research studies are needed to further investigate the magnitude of change in this biomarker for patients with acute articular cartilage damage to determine its appropriateness for use in this population and for varying degrees of articular cartilage severity.

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### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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