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# Preliminary data showing potential for salivary C-reactive protein as an indicator of welfare in western lowland gorillas (*Gorilla gorilla gorilla*)

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ABSTRACT

C-reactive protein (CRP) is an acute phase protein often used as a biomarker for inflammation related to acute trauma or chronic illness. Animal studies showing elevations in CRP following events such as road transport and moving to new housing suggest that CRP fluctuations may indicate how behavioral stress affects animal welfare. As part of a study about behavioral opportunities, salivary CRP was measured in three zoo-housed western lowland gorillas (*Gorilla gorilla gorilla*) to assess potential antiinflammatory effects of a foraging manipulation. Although the foraging conditions did not significantly affect CRP concentrations, an agonistic encounter—typical of the posturing that occurs among adolescent male gorillas—resulted in very minor injuries to one gorilla. Concentrations of salivary CRP increased over seventeen-fold for this gorilla, although his wounds were superficial and did not require veterinary treatment. Although animal care staff did not observe any wounds on the other two gorillas, CRP concentrations approximately doubled for both of them after this event, exceeding more than two standard deviations above their respective baselines. C-reactive protein was also correlated among individuals across the study period and with fecal glucocorticoid metabolite concentrations. These data provide first validation of CRP as a measure of the acute-phase response to injury in a gorilla and suggest that CRP may also fluctuate in response to social stressors, such as agonistic encounters. Salivary CRP may be a useful biomarker for several states that can contribute to negative welfare, including injury and social stress. These preliminary data should encourage additional investigations of CRP as a novel indicator of gorilla welfare.



## Introduction

C-reactive protein (CRP) is one of several hundred proteins involved in the acute phase response of the innate immune system. Functions of CRP include marking foreign organisms for complement or phagocytosis and regulating cytokine production (Murata et al., 2004; Cray et al., 2009). Concentrations of CRP rise dramatically after physical trauma in a variety of species (equids, suids, Murata et al., 2004; rhesus macaques, *Macaca mulatta*, Krogh et al., 2014; domestic dogs, Christensen et al., 2015).

Human studies consistently link elevated CRP to disease states involving chronic, low-grade inflammation, such as obesity and cardiovascular disease (Danesh et al., 1998; Goodson et al., 2014). Cardiac disease is the primary source of mortality for great apes in zoos, including western lowland gorillas (*Gorilla gorilla gorilla*) (Lowenstine et al., 2016). Obesity, inactivity, and diet are all considered likely contributors to gorilla cardiac disease, and dietary changes have previously shown positive effects on noninflammatory biomarkers for obesity and insulin resistance in zoo-housed gorillas (Less et al., 2014). However, there do not appear to be any published reports examining CRP in gorillas.

A study was undertaken to evaluate the effects of a foraging manipulation on behavior and physiology in western lowland gorillas at the Detroit Zoo, using salivary CRP to track potential changes in inflammation (Fuller et al., 2018). Although there were no significant changes in CRP related to the foraging conditions, an

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agonistic event during the study resulted in some very minor injuries for 1 gorilla. In other species, CRP responds to behavioral, as well as physical, challenges to homeostasis. For example, CRP increases after road transport and during adjustment to new housing in pigs (Pineiro et al., 2007; Salamano et al., 2008). These trends led Murata (2007) to propose that CRP may be an indicator of animal welfare. To explore these connections, we used data surrounding this agonistic encounter to examine how levels of CRP in gorillas fluctuate in response to physical and social stressors. The preliminary data reported here raise interesting hypotheses about relationships between CRP and social stress, as well as the potential value of CRP as a novel indicator of gorilla welfare.

## Methods

The subjects were a bachelor group of 3 gorillas who were captive-born, paternal half-siblings: Chipua (Chip), 18 years; Pendeka (Pende), 17 years; and Kongo-Mbeli (Kongo), 16 years. The gorillas occupied an indoor habitat for the duration of this study. They were fed a standard zoo diet for gorillas including a low-starch primate biscuit, leafy greens, vegetables, limited fruit, regular foraging material, and various items offered as enrichment. For further details, including the schedule for the foraging manipulation and observational data collection methods, see Fuller et al. (2018).

Animal care staff who have worked with these gorillas for over a decade assessed dominance through general observations. Staff considered individuals' dominance based on preferential access to food and space, as well as by the order of progression when shifting between habitat areas. A previous study in a small group of gorillas confirmed that these methods are consistent with dominance as assessed through formal observations of dyadic interactions (Peel et al., 2005). Although Chip was dominant several years ago, statuses have shifted as the gorillas age, and animal care staff now consider Kongo the most dominant. Pende and Chip's statuses relative to one another are more fluid, with Chip generally being the least dominant. Although the hierarchy is fairly stable at this point, dominance challenges sometimes occur. On February 5, 2015, animal care staff observed an agonistic interaction involving Chip and Kongo. Pende remained nearby but did not participate. Chip received 2 cuts on his side and 1 near his shoulder blades. The injuries were mild and no medical intervention was necessary. Staff did not observe any injuries on Kongo or Pende at this time, nor for any of the gorillas for the remainder of the study.

Animal care staff collected saliva samples each morning between 0730 and 1000 hours from January 4 to March 5, 2015. The gorillas had previously been trained for voluntary collection of saliva samples using positive reinforcement for chewing on an absorbent swab (Salimetrics Oral Swab, Salimetrics LLC, State College, PA). Samples were immediately labeled and stored at −20°C. A total of 58 saliva samples each were collected from Chip and Kongo, and 59 from Pende.

For analysis, samples were thawed and centrifuged for 20 minutes at 2500× g at 4°C. Concentrations of CRP in unextracted saliva were measured using an enzyme-immunoassay kit (#1-3302, Salimetrics LLC, State College). Serial dilutions ($n = 7$) of pooled gorilla samples showed parallel displacement to the standard curve (ANCOVA: $F_{1,12}$=1.28, $P = 0.28$). Recovery values for pooled samples spiked with high controls (1962.36 ± 490.59 pg/mL) were 105% and 116% for low controls (193.29 ± 77.32 pg/mL). All samples were analyzed in duplicate at a 1:10 dilution on randomly assigned plates. Intraassay coefficients of variation averaged below 10%, and the interassay coefficient of variation was below 15%.

Staff also collected daily fecal samples for analysis of fecal glucocorticoid metabolites (FGMs). Briefly, samples were lyophilized and extracted in methanol. Concentrations of FGM were measured using an enzyme-immunoassay kit (#K003, Arbor Assays, Ann Arbor, MI) validated for gorillas using standard tests of parallelism and recovery. In addition, the assay was validated biologically for stress using a routine veterinary immobilization, which confirmed a 24-hour lag time for fecal hormone excretion. Full methods for FGM analyses are available in Fuller et al. (2018).

Data were analyzed using SPSS version 22 (IBM Corporation, Armonk, NY). Nonnormally distributed hormone and behavioral data were compared using nonparametric Spearman correlations. All means ± standard deviation (SD) are reported. Baseline hormone concentrations for each gorilla were calculated using an iterative process of averaging all samples, removing values more than 2 SD above or below the mean, then recalculating the mean, and repeating this process until no values fell outside 2 SD. Comparisons of peak CRP samples to baselines were made using both the percent increase and the fold method (Romero, 2004). Daily values more than 2 SD above individual baselines were considered significant spikes in CRP activity.

## Results

Two days after the agonistic interaction, Chip's salivary CRP concentrations increased 17-fold to a peak of 1,621.31% above a baseline of 1.83 ± 0.24 ng/mL. Chip's CRP returned to baseline levels 14 days after the incident (Figure 1a). For Kongo, CRP increased 90.72%, or nearly twofold, from a baseline of 1.94 ± 0.33 ng/mL on the day after the incident and remained slightly elevated for the next 4 days. For Pende, CRP rose steadily after the incident, peaking 3 days later at a twofold or 124.14% increase from his baseline of 1.45 ± 0.30 ng/mL, returning to baseline levels 2 days later.

There were 2 other periods when all 3 gorillas had significantly elevated CRP concentrations. For one of these, CRP spiked for Chip and Kongo on the same day, then for Pende on the following day. On the second occasion, CRP first spiked for Pende. Next, CRP spiked for Chip 2 days later, for Kongo the next day, and spiked again for Chip the day after that. For the full study period, daily CRP concentrations were correlated between Chip and Kongo ($\rho = 0.41$, $N = 58$, $P = 0.001$), Chip and Pende ($\rho = 0.32$, $N = 58$, $P = 0.016$), and Pende and Kongo ($\rho = 0.40$, $N = 58$, $P = 0.002$). Excluding the 14 days after Chip's injury, CRP remained significantly correlated between Chip and Kongo ($\rho = 0.34$, $N = 44$, $P = 0.025$) and approached significance for Pende and Kongo ($\rho = 0.26$, $N = 44$, $P = 0.089$). The correlation between Chip and Pende was no longer significant ($\rho = 0.22$, $N = 44$, $P = 0.160$).

For all samples from the group, there was a positive correlation between daily FGM and CRP concentrations (Spearman's $\rho = 0.22$, $N = 115$, $P = 0.02$). This relationship was not significant when tested for Chip ($\rho = 0.20$, $N = 23$, $P = 0.37$) or Pende ($\rho = -0.26$, $N = 36$, $P = 0.13$) alone, but the correlation between Kongo's FGM and CRP concentrations trended toward significance ($\rho = 0.23$, $N = 56$, $P = 0.09$). When samples from the 14 days after Chip was wounded were excluded from analysis, the correlation between FGM and CRP for the group was no longer significant ($\rho = 0.14$, $N = 88$, $P = 0.20$). For all samples from the group, there was also no significant correlation between daily rates of agonistic behavior and CRP concentrations ($\rho = -0.03$, $N = 119$, $P = 0.71$).

## Discussion

All 3 gorillas in this study exhibited significant increases in salivary CRP after 1 individual received minor wounds in an