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ORIGINAL ARTICLE

Age, sex, and inflammatory markers predict chronic conditions, cardiac disease, and mortality among captive western lowland gorillas (*Gorilla gorilla gorilla*)

Ashley N. Edes[1,2,4] · **Colin M. Brand**[3]

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Abstract
In humans, inflammatory markers predict health risks. As great apes experience many similar conditions, measuring inflammation may provide valuable health information. We examined four serum inflammatory markers in zoo-housed gorillas ($n=48$): albumin, CRP, IL-6, and TNF-α. We first analyzed age- and sex-associated patterns, then used multimodel inference to evaluate models with age, sex, and inflammatory markers as predictors of all-cause morbidity, cardiac disease, and mortality. Older gorillas had lower albumin and higher IL-6, and males had higher albumin, lower CRP, and lower TNF-α. All-cause morbidity was best predicted by age, sex, and TNF-α, but the second model containing only age and sex was equivalent. Cardiac disease was best predicted by TNF-α alongside age and sex, with lower levels associated with increased risk. When outliers were removed, the model with TNF-α was second to the model containing only age and sex. Finally, mortality risk was best predicted by the model with only age and sex. Other models containing individual inflammatory markers were within top model sets for each health outcome. Our results indicate that age and sex are robust for predicting all-cause morbidity and mortality risk in gorillas; while models which include individual inflammatory markers also predict risk, they may not improve predictions over age and sex alone. However, given the prevalence of cardiac disease in great apes, these results suggest that TNF-α warrants further investigation. With their potential to provide valuable health information, data on inflammatory markers may contribute to the care and management of gorillas in human care.

Keywords Albumin · CRP · IL-6 · TNF-α · Great apes

Introduction

Similar to trends in some human populations, the leading cause of death in captive great apes is cardiac disease (Varki et al. 2009; McManamon and Lowenstine 2012; Lowenstine et al. 2016; Strong et al. 2016, 2018; Murphy et al. 2018). In North American zoos, approximately 45% of bonobos (*Pan paniscus*), 41% of gorillas (*Gorilla gorilla gorilla*), 38% of chimpanzees (*Pan troglodytes*), and 20% of orangutans (*Pongo* spp.) die from cardiac disease (Lowenstine et al. 2016). As in humans, males are more likely to develop cardiac disease than females; in gorillas, males are eight times as likely to die from the condition as females, and it is the cause of death for 70% of silverback gorillas aged 30 years or older (McManamon and Lowenstine 2012; Lowenstine et al. 2016; Strong et al. 2018). The etiology underlying the development of cardiac disease in great apes is currently unknown (Varki et al. 2009; McManamon and Lowenstine 2012; Lowenstine et al. 2016; Strong et al. 2016, 2018; Murphy et al. 2018).

In humans, coronary artery disease (CAD) is the most frequently diagnosed form of cardiac disease, and occurs when atherosclerotic plaques form within arterial walls (Libby et al. 2002; Libby 2006). Atherosclerosis is driven by chronic inflammation, which continues to play a role throughout disease progression (Libby et al. 2002; Libby

✉ Ashley N. Edes
aedes@stlzoo.org

1 Center for Species Survival, Smithsonian Conservation Biology Institution, Front Royal, VA, USA

2 Department of Anthropology, The Ohio State University, Columbus, OH, USA

3 Department of Anthropology, University of Oregon, Eugene, OR, USA

4 Department of Reproductive and Behavioral Sciences, Saint Louis Zoo, Saint Louis, MO, USA

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2006). However, while there have been diagnoses of CAD in gorillas (Murphy et al. 2011), severe atherosclerosis is rare in great apes (Varki et al. 2009; McManamon and Lowenstine 2012; Lowenstine et al. 2016). Great apes are most often diagnosed with fibrosing cardiomyopathy (FCM), which is characterized by increased fibrous connective tissue and decreased contractility and conductivity in the myocardium (McManamon and Lowenstine 2012; Lowenstine et al. 2016; Strong et al. 2018; Murphy et al. 2018). Even though severe atherosclerosis and CAD are uncommon, inflammation may still play a role in the development and progression of cardiac disease in gorillas. For example, myocarditis, also known as inflammatory cardiomyopathy, is inflammation of the heart muscle that can lead to heart failure and has been observed in some gorillas during necropsy (Murphy et al. 2011; McManamon and Lowenstine 2012; Strong et al. 2016, 2018). It has also been suggested that cardiac disease is rare in wild western lowland gorillas because they regularly consume *Aframomum* plants, which contain anti-inflammatory compounds that inhibit the expression of proinflammatory genes (Williamson et al. 1990; Doran et al. 2002; McManamon and Lowenstine 2012).

Although there is an ongoing effort to identify biomarkers that predict risk of heart disease in great apes, such as blood pressure, brain natriuretic peptide (BNP), leptin and adiposity, and lipid markers (Junge et al. 1998; Murphy et al. 2011, 2018; McManamon and Lowenstine 2012; Murray et al. 2019; Dennis et al. 2019; Edes et al. 2020a), thus far markers of inflammation have received little attention. We investigated the relationship between four serum inflammatory markers (Table 1) and health outcomes in zoo-housed western lowland gorillas. Albumin is a negative acute-phase protein, meaning that low levels indicate inflammation, which has been described as a "non-specific measure of underlying disease" (Glei et al. 2014) and a "very sensitive prognostic marker" of mortality risk (Grimm et al. 2009). C-reactive protein (CRP) is a major acute-phase protein in humans and nonhuman primates (Cray et al. 2009)

Table 1 Associations between inflammatory markers and age, sex, morbidity risk, and mortality risk in humans

Variable	Association	References
Older age	Alb ↓	(McPherson et al. 1978; Phillips et al. 1989; Cooper and Gardner 1989; Salive et al. 1992; Gomi et al. 2007)
	CRP ↑	(Yamada et al. 2001; Heikkilä et al. 2009; Puzianowska-Kuźnicka et al. 2016)
	IL-6 ↑	(Wei et al. 1992; Barton 1996; Myśliwska et al. 1998; Dobbs et al. 1999; Harris et al. 1999; Beharka et al. 2001; Kiecolt-Glaser et al. 2003; Costanzo et al. 2005; Friedman et al. 2005; Maggio et al. 2006; Heikkilä et al. 2009; Puzianowska-Kuźnicka et al. 2016)
	TNF-α ↑	(Paolisso et al. 1998; Dobbs et al. 1999; Bruunsgaard et al. 2000)
Male sex	Alb ↑	(McPherson et al. 1978; Manolio et al. 1992; Gomi et al. 2007; Grimm et al. 2009; Weaving et al. 2016)
	CRP –	No difference: (Tracy et al. 1997; Puzianowska-Kuźnicka et al. 2016); lower in men: (Ridker et al. 1998; Ford 1999; Khera et al. 2005; Lakoski et al. 2006); higher in men: (Yamada et al. 2001)
	IL-6 –	Lower in men: (Kiecolt-Glaser et al. 2003; Maggio et al. 2006; Puzianowska-Kuźnicka et al. 2016); higher in men: (Kiecolt-Glaser et al. 2005)
	TNF-α ↑	(Kiecolt-Glaser et al. 2005)
Morbidity risk (including cardiac disease)	Alb ↓	(Phillips et al. 1989; Himmelfarb and McMonagle 2001; Murata et al. 2004; Grimm et al. 2009; Glei et al. 2014)
	CRP ↑	(Amos et al. 1978; Otterness 1994; Ridker et al. 1998, 2000, 2001, 2002; Ford 1999; Harris et al. 1999; Koukkunen et al. 2001; Yamada et al. 2001; Libby et al. 2002; Pearson et al. 2003; Yeh and Willerson 2003; Don and Kaysen 2004; Willerson and Ridker 2004; Pai et al. 2004; Eckel et al. 2005; Alberti et al. 2006; Florez et al. 2006; Libby 2006; Emery et al. 2007; Higham et al. 2015; Tayefi et al. 2017; Tang et al. 2017)
	IL-6 ↑	(Harris et al. 1999; Lutgendorf et al. 2000; Libby et al. 2002; Costanzo et al. 2005; Eckel et al. 2005; Friedman et al. 2005; Glaser and Kiecolt-Glaser 2005; Libby 2006; Maggio et al. 2006; Kronfol 2007; Heikkilä et al. 2009; Hoffman et al. 2011; Golia et al. 2014; Grosse et al. 2016; Puzianowska-Kuźnicka et al. 2016)
	TNF-α ↑	(Maury and Teppo 1989; Levine et al. 1990; Odeh 1993; Azzawi and Hasleton 1999; Feldman et al. 2000; Koukkunen et al. 2001; Mann et al. 2002; Aker et al. 2003; Eckel et al. 2005; Wellen and Hotamisligil 2005; Alberti et al. 2006; Emery et al. 2007; Kronfol 2007; Golia et al. 2014; Tian et al. 2015; Urschel and Cicha 2015)
Mortality risk	Alb ↓	(Phillips et al. 1989; Don and Kaysen 2004; Grimm et al. 2009; Fischer et al. 2014)
	CRP ↑	(Mendall et al. 2000; Koukkunen et al. 2001; Don and Kaysen 2004; Puzianowska-Kuźnicka et al. 2016)
	IL-6 ↑	(Barton 1996; Harris et al. 1999; Koukkunen et al. 2001; Friedman et al. 2005; Hoffman et al. 2011; Puzianowska-Kuźnicka et al. 2016)
	TNF-α ↑	(Bruunsgaard et al. 2000; Koukkunen et al. 2001; Urschel and Cicha 2015)

Arrows indicate direction of association (e.g., increasing age is associated with decreasing albumin)

that can increase up to 1000-fold within hours of infection but tends to have little day-to-day variation (Willerson and Ridker 2004). Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are pleiotropic cytokines, having both pro- and anti-inflammatory roles as well as responding to acute and chronic stress (Lutgendorf et al. 2000; Costanzo et al. 2005; Kiecolt-Glaser et al. 2005). In humans, these four biomarkers have been well studied and are associated with the presence of cardiac disease and other chronic conditions such as arthritis, metabolic syndrome, insulin resistance and diabetes, hypertension, kidney disease, obesity, frailty, and cancer, as well as increased mortality risk (Table 1).

In contrast, in the available literature on gorillas, descriptive statistics for albumin were presented in a paper on hematologic and blood chemistry values from Yerkes National Primate Research Center (McClure et al. 1972) and hypoalbuminemia was associated with protein deficiency in a captive group in Gabon (Mundy et al. 1998), the ability to produce high levels of IL-6 and TNF-α has been documented (Ahne et al. 1996), and a recent paper showed that males housed in a bachelor group had significantly elevated salivary CRP following an agonistic interaction (Fuller and Allard 2018). To our knowledge, there are no other published reports presenting data on these individual biomarkers in gorillas, although some have been previously included in estimates of allostatic load alongside biomarkers from other somatic systems in this dataset (Edes et al. 2018, 2020b, 2021). However, allostatic load is not a strong predictor of all-cause morbidity and mortality, and estimates thus far do not predict cardiac disease (Edes et al. 2020b, 2021). Given these results, as well as the differences in methodologies used to calculate allostatic load and those used herein, we explored whether individual inflammatory markers would better predict health outcomes. As these biomarkers are underreported in this species, we first performed exploratory analyses on the potential age- and sex-related patterns of each inflammatory marker. We then evaluated how well age, sex, and inflammatory markers explained risk of poor health outcomes. Given the physiological similarities between humans and gorillas, we expected to observe comparable relationships between these markers of inflammation and age, sex, and poor health outcomes (Table 1). We predicted these inflammatory markers would be critical components of models predicting all-cause morbidity, cardiac disease, and mortality risk in gorillas.

Methods

Subjects

This research was conducted using banked serum samples collected during veterinary examinations and medical records from western lowland gorillas currently or previously housed at the Columbus Zoo and Aquarium, Louisville Zoo, and Omaha's Henry Doorly Zoo ($n = 63$). Data for 15 gorillas were excluded because their samples were obtained during immobilization for clinical events rather than routine exams. The subset of gorillas with samples collected during routine veterinary exams ($n = 48$) included 23 males ranging in age from 6 to 46 years ($\bar{x} = 21.4$, SD = 10.5) and 25 females aged 7–52 years ($\bar{x} = 21.9$, SD = 14.0).

Markers of inflammation and health data

Albumin is routinely measured during veterinary exams, so these values were available in zoo medical records. CRP, IL-6, and TNF-α were assayed from banked serum samples collected on the same date as the albumin measurement and cryopreserved at −70 °C. Samples were assayed by The Ohio State University Center for Clinical and Translational Science: Clinical Research Center (CRC). Samples were collected between 1991 and 2015, and assays were conducted in 2014 and 2016. While the variable and sometimes lengthy storage times are concerning, CRP (Doumatey et al. 2014) and both cytokines (Tworoger and Hankinson 2006) are stable when stored in ultralow freezers without multiple freeze–thaw cycles.

CRP, IL-6, and TNF-α were assayed using commercially available kits. CRP was assayed using solid-phase chemiluminescence immunometric assay on an Immulite 1000 (Siemens Healthcare Diagnostics Products Ltd.). Analytical sensitivity for CRP was 0.01 mg/L and functional sensitivity was 0.3 mg/L. Intra-assay variation for CRP was 3.1% and inter-assay variation was 7.3%. Solid-phase ELISA kits were used for IL-6 (HS600B, R and D Systems, Minnesota, USA) and TNF-α (HSTA00D, R&D Systems, Minneapolis, MN, USA). For IL-6, the minimum detectable dose was 0.039 pg/mL, intra-assay variation was < 7.4%, and inter-assay variation was < 7.8%. For TNF-α, the minimum detectable dose was 0.106 pg/mL, intra-assay variation was < 5.4%, and inter-assay variation was < 8.3%.

Data on any diagnosed chronic conditions were obtained from zoo medical records and included cardiac disease, osteoarthritis, hypothyroidism, neoplasia, and obesity. When applicable, age at death was also recorded. Chronic conditions and mortality were coded as dichotomous 0,1 variables (chronic conditions: 1 = presence; mortality: 1 = deceased).

Statistical analysis

First, we determined the effects of age and sex on circulating levels of inflammatory markers using linear regression for normally distributed biomarkers (albumin) and generalized linear models (GLMs) with a gamma distribution and a log-link function for positively skewed biomarkers (CRP, IL-6,

TNF-α). Four gorillas were missing values for albumin in their medical records, and there was insufficient serum to assay CRP for three gorillas. Gorillas with missing values were excluded from age and sex analyses involving those biomarkers. Analyses were conducted both with and without outliers. We determined outliers using Tukey's method for outlier detection (Tukey 1977). Zoo ID was not included as a random effect because this dataset did not meet the minimum number of levels/groups (five or six) required for robust estimates of variance (Harrison et al. 2018).

We used multimodel inference to determine whether inflammatory markers predict morbidity and mortality risk. As collinearity can make models difficult to interpret (Hair et al. 2010; Harrison et al. 2018), and physiological parameters often covary, we first assessed multicollinearity by estimating the variance inflation factor (VIF) between all biomarkers. Multicollinearity was not observed between any inflammatory markers (VIF < 4.0; Hair et al. 2010). Given that four and three individuals had missing albumin and CRP data, respectively, we replaced those missing values with the mean for each biomarker to use the full dataset and maximize power. To ensure that this imputation approach did not bias our analyses, we also imputed missing values using another donor-based approach: hot deck (Andridge and Little 2010). We generated five additional datasets where the missing values were imputed each time. We analyzed all six datasets using the following procedure and compared model ranks and other metrics across the datasets. We constructed six models using GLMs with a binomial error structure and a logit link: a baseline model including age and sex, as these may influence health outcomes; four models with age, sex, and one of the biomarkers; and a global model with age, sex, and all four biomarkers. While it is tempting to include all possible subsets of markers, this approach generally suffers from model overfitting (Burnham and Anderson 2002). We also did not examine interactions between predictors given our sample size. As with age and sex analyses, zoo ID was not included as a random effect. We then used multimodel inference to determine which models best explained variation in each health outcome (Symonds and Moussalli 2011; Harrison et al. 2018). Multimodel inference uses Akaike's information criterion (AIC and AICc, which is corrected for small sample sizes; Burnham and Anderson 2002; Symonds and Moussalli 2011) and model weights, which estimate the probability that a given model is the best of those included, to rank each model relative to one another. We present the top model set, which contains all models with ΔAICc ≤ 6 (Symonds and Moussalli 2011; Harrison et al. 2018). An evidence ratio (ER) also is presented for each model, which indicates the likelihood of the top model being the best model (e.g., an ER of 2.5 means the top model is 2.5 times as likely to be the best model as the model to which it is being compared; Symonds and Moussalli 2011). We also note how well these data fit each model using Nagelkerke's adjusted R^2.

All statistical analyses were conducted using R (v3.5.0, R Core Team 2018). Packages used included "VIM" (Kowarik and Templ 2016) to generate five additional datasets with missing values imputed via hot deck, "lme4" (Bates et al. 2015) to run GLMs, "usdm" (Naimi 2017) to assess multicollinearity between biomarkers, "MuMIN" (Bartoń 2019) to compare models using multimodel inference, and "rcompanion" (Mangiafico 2016) to estimate Nagelkerke's adjusted R^2. Scripts used in this analysis are available online (https://github.com/brandcm/Gorilla_Biomarkers).

Results

In this sample, 52.1% ($n = 25$) of gorillas were diagnosed with a chronic condition of some kind, including cardiac disease, and 27.0% ($n = 13$) had died since sample collection. Cardiac disease was diagnosed in 39.6% of gorillas ($n = 19$), while 16.7% ($n = 8$) had arthritis, 6.3% ($n = 3$) had obesity and/or hypothyroidism, 4.2% had hypertension ($n = 2$), and 2.1% had cancer ($n = 1$); 29.2% ($n = 14$) had just one condition while 14.6% ($n = 7$) had comorbidities. Males were more likely than females to present with a poor health outcome, with 69.6% ($n = 16$) having at least one chronic condition, 60.9% ($n = 14$) having cardiac disease, and 26.1% ($n = 6$) being deceased. Among females, 36% ($n = 9$) had one or more chronic conditions, 20% ($n = 5$) had cardiac disease, and 28% ($n = 7$) were deceased.

Ranges, means, and standard deviations for albumin, CRP, IL-6, and TNF-α are presented in Table 2. Age ($\beta \pm \text{SE} = -0.015 \pm 0.005$, $p = 0.004$) and sex ($\beta \pm \text{SE} = 0.376 \pm 0.123$, $p = 0.004$) had significant effects on albumin levels, which was higher in younger gorillas and in males (Fig. 1). When the single outlier for albumin was excluded, the association with age was no longer significant ($\beta \pm \text{SE} = -0.009 \pm 0.005$, $p = 0.061$). Age did not have a significant effect on CRP concentrations ($\beta \pm \text{SE} = -0.008 \pm 0.018$, $p = 0.646$), but sex did ($\beta \pm \text{SE} = -2.033 \pm 0.443$, $p < 0.0001$), with females having tenfold higher CRP (Fig. 2), a difference which remained significant even when outliers were excluded ($\beta \pm \text{SE} = -1.434 \pm 0.397$, $p = 0.0008$). Age was positively associated with IL-6 ($\beta \pm \text{SE} = 0.025 \pm 0.010$, $p = 0.016$), but the effect of sex was not significant ($\beta \pm \text{SE}$ 0.278 ± 0.247, $p = 0.267$; Fig. 3). When outliers were excluded, the association between age and IL-6 was no longer significant ($\beta \pm \text{SE} = 0.014 \pm 0.010$, $p = 0.187$). Finally, age did not have a significant effect on TNF-α levels ($\beta \pm \text{SE} = 0.011 \pm 0.011$, $p = 0.322$), but females had significantly higher TNF-α than males ($\beta \pm \text{SE} = -0.707 \pm 0.276$, $p = 0.014$; Fig. 4) even

Table 2 Descriptive statistics for albumin, C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) in a sample of zoo-housed western lowland gorillas

	All					Females only					Males only				
	n	$\bar{x}$	SD	Min	Max	*n*	$\bar{x}$	SD	Min	Max	*n*	$\bar{x}$	SD	Min	Max
All values															
Albumin (g/dl)	44	3.68	0.48	1.80	4.60	23	3.49	0.48	1.80	4.10	21	3.89	0.40	3.30	4.60
CRP (mg/dl)	45	13.70	24.82	0.30	115.60	23	24.28	31.14	0.30	115.60	22	2.69	5.05	0.30	22.90
IL-6 (pg/ml)	48	4.20	4.03	0.49	18.90	25	3.80	4.19	0.49	18.90	23	4.64	3.89	1.06	15.20
TNF-α (pg/ml)	48	0.51	0.59	0.01	3.83	25	0.68	0.76	0.06	3.83	23	0.33	0.21	0.01	0.98
Outliers excluded															
Albumin (g/dl)	43	3.73	0.39	3.00	4.60	22	3.57	0.31	3.00	4.10	No outliers				
CRP (mg/dl)	44	7.65	9.11	0.30	37.10	20	13.10	9.55	0.30	37.10	19	1.07	0.90	0.30	3.26
IL-6 (pg/ml)	45	3.43	2.67	0.49	10.89	22	2.49	1.71	0.49	6.84	21	3.72	2.52	1.06	9.30
TNF-α (pg/ml)	43	0.36	0.18	0.01	0.87	19	0.43	0.13	0.18	0.76	22	0.30	0.16	0.01	0.53

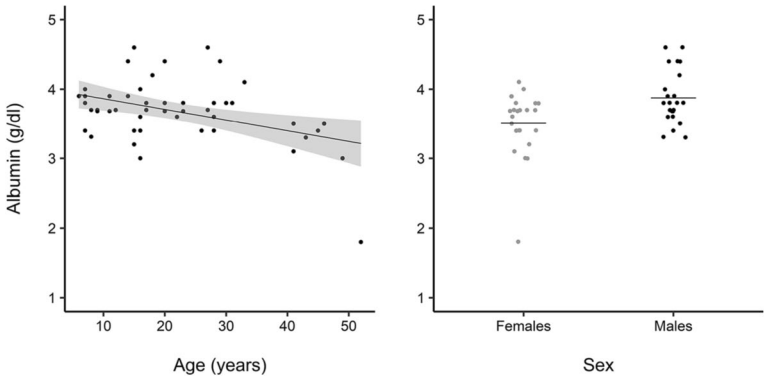

Fig. 1 Serum albumin (g/dl) in western lowland gorillas was significantly associated with age ($\beta \pm SE = -0.015 \pm 0.005$, $p = 0.004$) and sex ($\beta \pm SE = 0.358 \pm 0.113$, $p = 0.003$)

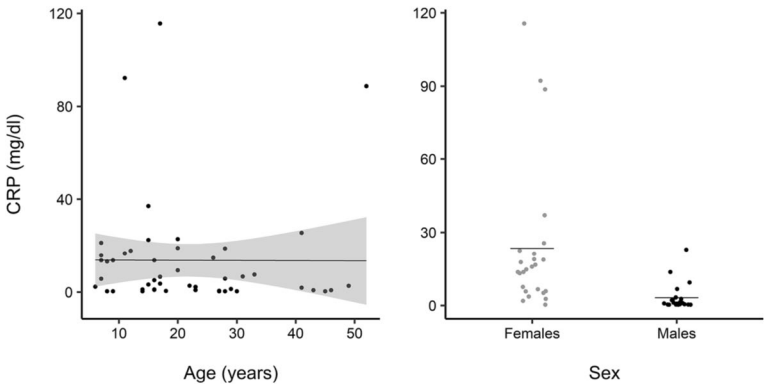

Fig. 2 Serum CRP (mg/dl) in western lowland gorillas was not significantly associated with age ($\beta \pm SE = -0.008 \pm 0.018$, $p = 0.646$), but was significantly associated with sex ($\beta \pm SE = -2.033 \pm 0.443$, $p < 0.0001$)

when five outliers were removed ($\beta \pm SE = -0.384 \pm 0.150$, $p = 0.015$).

We found no major differences in model ranks, ΔAIC values, or model weights between our dataset with missing values imputed with means and five datasets with missing values imputed via hot deck across all three conditions: all-cause morbidity risk, cardiac disease risk, and mortality risk (Supplemental File S1). Models that fit the data very well and very poorly were consistently ranked as such, while the ranks of some intermediate models shifted slightly. This demonstrates our results are robust regardless of the imputation method applied. Results presented below are from the dataset whose missing values for albumin and CRP were imputed with means for that biomarker.

Among the models for risk of all-cause morbidity (Table 3), the model containing age, sex, and TNF-α had the highest weight ($w_i = 0.340$), indicating a 34% chance it is the best model of those tested. However, given the similar weights and negligible difference in AICc, the second model containing age and sex only was equivalent to the

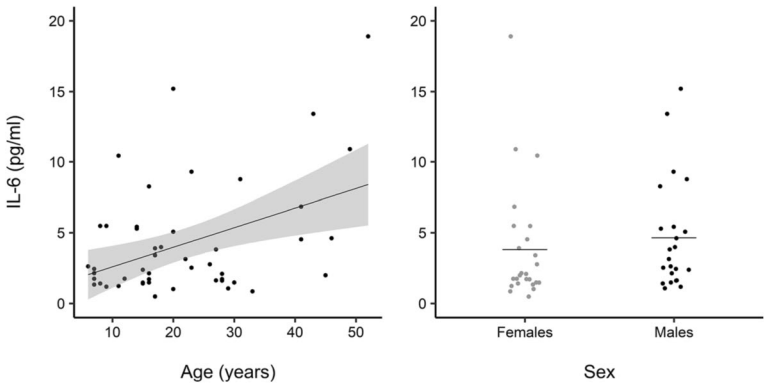

Fig. 3 Serum IL-6 (pg/ml) in western lowland gorillas was significantly associated with age ($\beta \pm SE = 0.025 \pm 0.010$, $p = 0.016$), but not with sex ($\beta \pm SE$ 0.278 ± 0.247, $p = 0.267$)

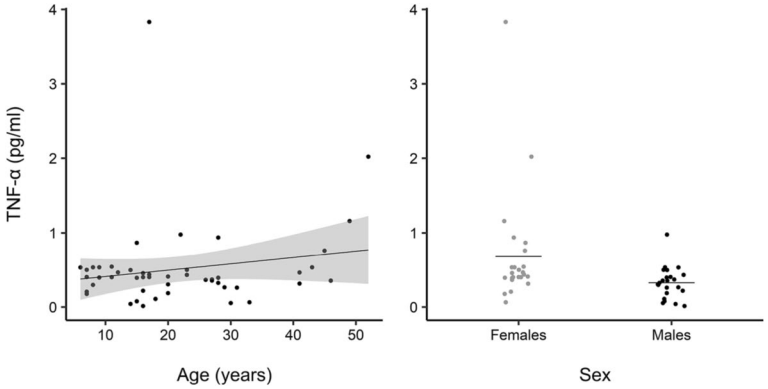

Fig. 4 Serum TNF-α (pg/ml) in western lowland gorillas was not significantly associated with age ($\beta \pm SE = 0.011 \pm 0.011$, $p = 0.322$), but was significantly associated with sex ($\beta \pm SE = -0.707 \pm 0.276$, $p = 0.014$)

Table 3 Results from multimodel inference on which models with age, sex, and inflammatory markers best explain the presence of chronic conditions in zoo-housed western lowland gorillas; models with $\Delta AICc \leq 6$ are within the top model set; italicized models are not contained within the top model set

Model	Standard estimate (β)						*K*	AICc	ΔAICc	w_i	ER	R^2
	Age	Sex (M)	Alb	CRP	IL-6	TNF-α						
Age + Sex + TNF-α	0.141	1.812				−1.838	4	50.6	0.00	0.340	–	0.535
Age + Sex	0.122	2.133					3	50.6	0.03	0.334	1.018	0.494
Age + Sex + Alb	0.125	2.018	0.370				4	52.9	2.29	0.108	3.148	0.496
Age + Sex + CRP	0.121	2.008		−0.007			4	52.9	2.30	0.107	3.178	0.496
Age + Sex + IL-6	0.123	2.151			−0.009		4	53.0	2.41	0.102	3.333	0.494
Age + Sex + All	*0.146*	*2.185*	*−0.750*	*0.014*	*−0.009*	*−2.847*	*7*	*57.8*	*7.19*	*0.009*	*38.89*	*0.547*

AICc Akaike's information criterion corrected for small sample size, *ΔAICc* difference between the model with the lowest AIC and the current model, *Alb* albumin, *CRP* C-reactive protein, *ER (evidence ratio)* weight of the model with the lowest AICc divided by the weight of the current model, *IL-6* interleukin-6, *K* number of variables included, R^2 *(coefficient of determination)* Nagelkerke's adjusted/pseudo-R^2, *TNF-α* tumor necrosis factor alpha, w_i (*Akaike's weight*) model probabilities

top model. Odds ratios indicated strong effects of age, with a 15% increase in risk of all-cause morbidity every year (OR = 1.151, 95% CI = 1.069–1.271), and sex (OR = 6.123, 95% CI = 1.286–35.542), with males six times as likely to develop a chronic condition as females. The rest of the top model set included each model with individual inflammatory markers but not the global model. Each model in the top model set explained approximately half of the variation in disease risk. These results did not change when outliers were removed from the dataset.

For cardiac disease risk (Table 4), the model containing sex, age, and TNF-α had the highest weight ($w_i = 0.847$), meaning there was an 85% chance it is the best model of those tested. Odds ratios again indicate strong effects of age, with cardiac disease risk increasing 9% every year (OR = 1.091, 95% CI = 1.021–1.180), and males were

Table 4 Results from multimodel inference on which models with age, sex, and inflammatory markers best explain the presence of cardiac disease in zoo-housed western lowland gorillas; models with ΔAICc ≤ 6 are within the top model set; italicized models are not contained within the top model set

Model	Standard estimate (β)						K	AICc	ΔAICc	w_i	ER	R^2
	Age	Sex (M)	Alb	CRP	IL-6	TNF-α						
All values retained ($n = 48$)												
Age + Sex + TNF-α	0.087	1.645				−4.341	4	51.6	0.00	0.847	–	0.504
Age + Sex + Alb	0.076	1.742	1.655				4	57.0	5.35	0.058	14.60	0.405
Age + Sex	*0.053*	*2.239*					*3*	*57.7*	*6.11*	*0.040*	*21.18*	*0.341*
Age + Sex + CRP	*0.068*	*1.950*			*−0.091*		*4*	*59.2*	*7.56*	*0.019*	*44.58*	*0.357*
Age + Sex + IL-6	*0.054*	*2.388*		*−0.018*			*4*	*59.3*	*7.71*	*0.018*	*47.06*	*0.360*
Age + Sex + All	*0.092*	*1.811*	*0.052*	*0.008*	*−0.031*	*−4.257*	*7*	*59.4*	*7.78*	*0.017*	*49.82*	*0.506*
Outliers for TNF-α excluded ($n = 43$)												
Age + Sex	0.087	2.104					3	50.4	0.00	0.357	-	0.407
Age + Sex + TNF-α	0.089	1.735				–	4	50.9	0.50	0.278	1.284	0.448
Age + Sex + Alb	0.089	1.838	0.900				4	52.2	1.79	0.146	2.445	0.421
Age + Sex + IL-6	0.088	2.146			−0.021		4	52.8	2.41	0.107	3.336	0.407
Age + Sex + CRP	0.087	2.140		0.003			4	52.8	2.43	0.106	3.368	0.407
Age + Sex + All	*0.093*	*1.835*	*0.202*	*0.006*	*−0.023*	*−3.007*	*7*	*59.0*	*8.56*	*0.005*	*71.40*	*0.449*

AICc Akaike's information criterion corrected for small sample size, *ΔAICc* difference between the model with the lowest AIC and the current model, *Alb* albumin, *CRP* C-reactive protein, *ER* (*evidence ratio*) weight of the model with the lowest AICc divided by the weight of the current model, *IL-6* interleukin-6, *K* number of variables included, R^2 *(coefficient of determination)* Nagelkerke's adjusted/pseudo-R^2, *TNF-α* tumor necrosis factor alpha, w_i (*Akaike's weight*) model probabilities

five times as likely to develop cardiac disease as females (OR = 5.183, 95% CI = 1.120–28.584). Only one other model was retained within the top model set; this model, which included age, sex, and albumin, was less than 6% likely to be the best model ($w_i = 0.058$). The top model explained 50% of the variation in cardiac disease risk, with the second model explaining approximately 40%. Based on visual examination of a plot showing TNF-α based on the presence or absence of diagnosed cardiac disease (Fig. 5), we determined that outliers might be driving the observed relationship. Five outliers were removed, including four females and one male (all had high TNF-α and were not diagnosed with cardiac disease). When outliers were removed, cardiac disease (Table 4) was best predicted by a top model containing only age and sex ($w_i = 0.357$), although the model with TNF-α was the second best ($w_i = 0.278$) and the difference in AICc was small. Older age (OR = 1.093, 95% CI = 1.023–1.183) and male sex (OR = 5.668, 95% CI = 1.195–32.792) remained strong predictors for cardiac disease risk. All models containing individual inflammatory markers were within the top model set and explained 41–42% of variation in cardiac disease risk when outliers were excluded.

For mortality risk (Table 5), the highest weight ($w_i = 0.389$) was observed for the model containing sex and age only. Odds ratios indicate that mortality risk was primarily driven by age, with a 6% increase in risk every year (OR = 1.062, 95% CI = 1.008–1.126), and that sex had little to no effect (OR = 0.978, 95% CI = 0.247–3.884). Each model containing one inflammatory marker was within the top model set. Overall, the top model set explained little variation in mortality risk (pseudo-R^2 values range from 0.147 to 0.168).

Discussion

As data for inflammatory markers in western lowland gorillas are sparse, we first analyzed whether albumin, CRP, IL-6, or TNF-α varied by age or sex. Albumin declined with age, and males had higher levels than females, which is consistent with patterns observed in humans (Table 1). However, the relationship between albumin and age may be driven by the presence of a single outlier, as the association was no longer significant when the outlier was removed, although the p value was just above the $\alpha = 0.05$ threshold. There was no

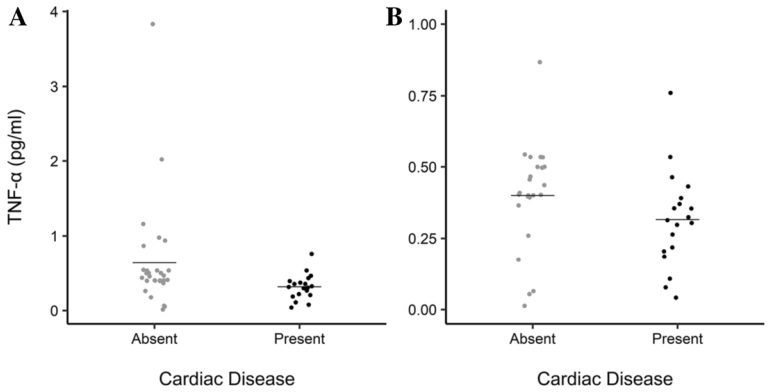

Fig. 5 Serum TNF-α in western lowland gorillas with and without cardiac disease when **A** all values are retained in the dataset, and **B** five outliers are excluded from the dataset

Table 5 Results from multimodel inference on which models with age, sex, and inflammatory markers best explain mortality risk in zoo-housed western lowland gorillas; models with ΔAICc ≤ 6 are within the top model set; italicized models are not contained within the top model set

Model	Standard estimate (β)						K	AICc	ΔAICc	w_i	ER	R^2
	Age	Sex (M)	Alb	CRP	IL-6	TNF-α						
Age + Sex	0.060	−0.023					3	57.5	0.00	0.389	–	0.147
Age + Sex + Alb	0.048	0.279	−0.832				4	59.1	1.61	0.174	2.24	0.168
Age + Sex + IL-6	0.050	−0.109			0.071		4	59.3	1.78	0.160	2.43	0.163
Age + Sex + CRP	0.060	−0.215		−0.011			4	59.5	2.03	0.141	2.76	0.156
Age + Sex + TNF-α	0.059	0.036				0.153	4	59.8	2.32	0.122	3.19	0.149
Age + Sex + All	*0.028*	*−0.406*	*−0.649*	*−0.051*	*0.107*	*1.282*	*7*	*64.2*	*6.73*	*0.013*	*29.9*	*0.239*

AICc Akaike's information criterion corrected for small sample size, *ΔAICc* difference between the model with the lowest AIC and the current model, *Alb* albumin, *CRP* C-reactive protein, *ER* (*evidence ratio*) weight of the model with the lowest AICc divided by the weight of the current model, *IL-6* interleukin-6, *K*number of variables included, R^2 *(coefficient of determination)* Nagelkerke's adjusted/pseudo-R^2, *TNF-α* tumor necrosis factor alpha, w_i (*Akaike's weight*) model probabilities

significant association between age and CRP in gorillas, but levels increase with age in humans (Table 1). Female gorillas had approximately tenfold higher CRP than males even when outliers were excluded. Reports on differences in CRP between men and women are inconsistent (Table 1). Fuller and Allard (2018) reported higher CRP in male gorillas housed in bachelor groups following agonistic interactions. A sex difference in CRP could reflect the increased likelihood for females to experience agonistic interactions given the social dynamics in mixed-sex gorilla troops (e.g., courtship aggression from males, conflict between non-related females; Stokes 2004; Leeds et al. 2015). Age and IL-6 were positively associated when all values were retained, which is consistent with reports in humans (Table 1), but not when outliers were excluded. There was no significant difference in IL-6 between male and female gorillas; as with CRP, reports on differences in IL-6 between men and women vary (Table 1). Finally, there was not a significant association between age and TNF-α in gorillas, despite reports of increasing levels with older age in humans (Table 1). Female gorillas had higher TNF-α than males, but the opposite pattern is observed in humans (Table 1).

We then evaluated how well age, sex, and inflammatory markers explained disease and mortality risk. Here, all-cause morbidity was best predicted by older age, male sex, and lower TNF-α, although the similar weight and small difference in AICc suggest that the second-best model, which included age and sex only, was equivalent to the top model. Similar results were observed for mortality risk, which was best predicted by the model containing age and sex alone. Contrary to our predictions, while models containing one inflammatory marker do predict risk of chronic disease and shortened lifespan in gorillas, these results suggest that age and sex alone are sufficient for predicting all-cause morbidity and mortality risk.

In contrast to all-cause morbidity and mortality, when all values were retained in the dataset cardiac disease was best predicted by older age, male sex, and lower TNF-α. This top model had an 85% chance of being the best model of those tested. Only one other model with age, sex, and albumin was also within the top model set, but there was a less than 6% chance that this model was the best of those tested. These results indicate that including TNF-α improves predictions over age and sex alone. However, it is possible these results were driven by the presence of outliers (Fig. 5). When outliers were removed, the top model contained only age and sex, although the model with TNF-α was second best, and the top model set contained all additional models with individual biomarkers. This top model had a 36% chance of being the best model of those tested. Regardless of whether outliers were retained or excluded, our data unexpectedly show an inverse relationship between risk of cardiac disease and TNF-α. As males are more likely to develop cardiac disease, the negative association observed here likely explains why we observed higher TNF-α in females. Evidence from humans and animal models demonstrates that *elevated* TNF-α plays an important role in cardiac disease and heart failure, including in relation to many symptoms which characterize FCM (e.g., reduced contractility, increased fibrosis, left ventricular hypertrophy, hypertension; Bozkurt et al. 1998; Patten et al. 2001; Bautista et al. 2005; Tian et al. 2015; Urschel and Cicha 2015). However, we are unaware of any reports of inverse associations between TNF-α and cardiac disease in humans or other species. Based on these results, TNF-α warrants further exploration as a biomarker for predicting and monitoring cardiac disease in this species, but additional research is needed to determine exactly how this inflammatory marker plays a role in gorillas or other great apes.

This report contributes to the limited research on how age and sex affect circulating levels of biomarkers of inflammation in western lowland gorillas, and, in turn, how those markers predict disease and mortality risk. While we tried to limit the likelihood of inflammation due to illness by only including samples collected during routine exams, it is possible some gorillas were experiencing undiagnosed infections. Additionally, our samples were collected from a mixture of individuals, with some at younger ages prior to any disease onset combined with older individuals who had already developed chronic conditions, which likely impacts the patterns we are seeing. Furthermore, biomarkers like cytokines often are pleiotropic, and their roles in other functions may confound our results. These limitations highlight the need to collect serial measures of biomarkers across the lifespan, which is critical to understanding levels that are healthy baselines versus transient but appropriate responses to stimuli versus dysregulated due to chronic illness, for which there is likely substantial inter-individual variation. Developing noninvasive assays and/or training animals to voluntarily participate in sample collection will aid these efforts, as ketamine, a common anesthetic agent and the one most commonly used for immobilization of gorillas in this study (either alone or in combination with other agents), has been shown to suppress the release of albumin (Bennett et al. 1992; Kim et al. 2005), CRP (Walker et al. 2015; Senapathi et al. 2016), and IL-6 and TNF-α (De Kock et al. 2013; Walker et al. 2015). This research would benefit from data on additional zoo-housed collections as well, as a larger sample size would allow for the inclusion of additional models and interaction terms, variables such as weight and blood pressure (when available), and random effects like facility. Analyses of other markers of inflammation, which may be better for predicting disease and mortality risk in this species, will further address the role inflammation plays in disease and mortality risk in gorillas.

In sum, models containing age and sex only are likely to best predict all-cause morbidity and mortality risk but adding TNF-α may improve predictions of cardiac disease in zoo-housed western lowland gorillas. Of the four inflammatory markers examined herein, only albumin is routinely measured in zoo-housed gorillas. Additional research is needed to confirm whether measuring inflammatory markers provides clinically meaningful information over age and sex alone. This concern is especially salient given that many animals need to be anesthetized for the collection of invasive samples, the limited quantity of previously collected samples for retrospective longitudinal analysis, and the potentially prohibitive costs of these assays. It also is possible that other markers of inflammation would better predict all-cause disease and mortality risk in gorillas. Similar research in other great apes will help us better understand how patterns of inflammation vary by age, sex, and disease and mortality risk across hominids.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study complied with The Ohio State University Animal Care and Use Committee (IACUC) protocols, was approved by each participating institution, and adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

References

Ahne W, Mayr A, Wiesner H (1996) Immunoreactive cytokines within primates. J Vet Med B 43:607–611

Aker S, Belosjorow S, Konietzka I et al (2003) Serum but not myocardial TNF-alpha concentration is increased in pacing-induced heart failure in rabbits. Am J Physiol Regul Integr Comp Physiol 285:R463–R469

Alberti KGMM, Zimmet PZ, Shaw J (2006) Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. Diabet Med 23:469–480

Amos RS, Crockson RA, Crockson AP et al (1978) Rheumatoid arthritis: C-reactive protein and erythrocyte sedimentation rate during initial treatment. Br Med J 1:1396

Andridge RR, Little RJA (2010) A review of hot deck imputation for survey non-response. Int Stat Rev 78:40–64

Azzawi M, Hasleton P (1999) Tumour necrosis factor alpha and the cardiovascular system: its role in cardiac allograft rejection and heart disease. Cardiovasc Res 43:850–859

Barton BE (1996) The biological effects of interleukin 6. Med Res Rev 16:87–109

Bartoń K (2019) MuMIn: multi-model inference. R package version 1.42.1. https://CRAN.R-project.org/package=MuMIn. Accessed 24 March 2020

Bates D, Maechler M, Bolker B, et al (2015) lme4: linear mixed-effects models using "Eigen" and S4. R package version 1.1-19. https://cran.r-project.org/package=lme4. Accessed 24 March 2020

Bautista LE, Vera LM, Arenas IA, Gamarra G (2005) Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-alpha) and essential hypertension. J Hum Hypertens 19:149–154

Beharka AA, Meydani M, Wu D et al (2001) Interleukin-6 production does not increase with age. J Gerontol A Biol Sci Med Sci 56A:B81–B88

Bennett JS, Gossett KA, McCarthy MP, Simpson ED (1992) Effects of ketamine hydrochloride on serum biochemical and hematologic variables in rhesus monkeys (*Macaca mulatta*). Vet Clin Pathol 21:15–18

Bozkurt B, Kribbs SB, Clubb FJ Jr et al (1998) Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation 97:1382–1391

Bruunsgaard H, Skinhøj P, Pedersen AN et al (2000) Ageing, tumor necrosis factor-alpha (TNF-α) and atherosclerosis. Clin Exp Immunol 121:255–260

Burnham KP, Anderson DR (eds) (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Cooper JK, Gardner C (1989) Effect of aging on serum albumin. J Am Geriatr Soc 37:1039–1042

Costanzo ES, Lutgendorf SK, Sood AK et al (2005) Psychosocial factors and interleukin-6 among women with advanced ovarian cancer. Cancer 104:305–313

Cray C, Zaias J, Altman NH (2009) Acute phase response in animals: a review. Comp Med 59:517–526

De Kock M, Loix S, Lavand'homme P (2013) Ketamine and peripheral inflammation. CNS Neurosci Ther 19:403–410

Dennis PM, Raghanti MA, Meindl RS et al (2019) Cardiac disease is linked to adiposity in male gorillas (Gorilla gorilla gorilla). PLoS ONE. https://doi.org/10.1371/journal.pone.0218763

Dobbs RJ, Charlett A, Purkiss AG et al (1999) Association of circulating TNF-a and IL-6 with ageing and parkinsonism. Acta Neurol Scand 100:34–41

Don BR, Kaysen G (2004) Serum albumin: relationship to inflammation and nutrition. Semin Dial 17:432–437

Doran DM, McNeilage A, Greer D et al (2002) Western lowland gorilla diet and resource availability: new evidence, cross-site comparisons, and reflections on indirect sampling methods. Am J Primatol 58:91–116

Doumatey AP, Zhou J, Adeyemo A, Rotimi C (2014) High-sensitivity C-reactive protein (Hs-CRP) remains highly stable in long-term archived human serum. Clin Biochem 47:315–318

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365:1415–1428
Edes AN, Wolfe BA, Crews DE (2018) The first multi-zoo application of an allostatic load index to western lowland gorillas (Gorilla gorilla gorilla). Gen Comp Endocrinol 266:135–149
Edes AN, Brown JL, Edwards KL (2020a) Testing lipid markers as predictors of all-cause morbidity, cardiac disease, and mortality risk in captive western lowland gorillas (*Gorilla gorilla gorilla*). Primate Biol 7:41–59
Edes AN, Edwards KL, Wolfe BA et al (2020b) Allostatic load indices with cholesterol and triglycerides predict disease and mortality risk in zoo-housed western lowland gorillas (Gorilla gorilla gorilla). Biomark Insights 15:1–13
Edes AN, Wolfe BA, Crews DE (2021) Testing a method to improve predictions of disease and mortality risk in western lowland gorillas (Gorilla gorilla gorilla) using allostatic load. Stress. https://doi.org/10.1080/10253890.2020.1748003
Emery P, Gabay C, Kraan M, Gomez-Reino J (2007) Evidence-based review of biologic markers as indicators of disease progression and remission in rheumatoid arthritis. Rheumatol Int 27:793–806
Feldman AM, Combes A, Wagner D et al (2000) The role of tumor necrosis factor in the pathophysiology of heart failure. J Am Coll Cardiol 35:537–544
Fischer K, Kettunen J, Würtz P et al (2014) Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med. https://doi.org/10.1371/journal.pmed.1001606
Florez H, Castillo-Florez S, Mendez A et al (2006) C-reactive protein is elevated in obese patients with the metabolic syndrome. Diabetes Res Clin Pract 71:92–100
Ford ES (1999) Body mass index, diabetes, and C-reactive protein among U.S adults. Diabetes Care 22:1971–1977
Friedman EM, Hayney MS, Love GD et al (2005) Social relationships, sleep quality, and interleukin-6 in aging women. Proc Natl Acad Sci 102:18757–18762
Fuller G, Allard S (2018) Preliminary data showing potential for salivary C-reactive protein as an indicator of welfare in western lowland gorillas (Gorilla gorilla gorilla). J Vet Behav 28:58–62
Glaser R, Kiecolt-Glaser JK (2005) Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 5:243–261
Glei DA, Goldman N, Rodríguez G, Weinstein M (2014) Beyond self-reports: changes in biomarkers as predictors of mortality. Popul Dev Rev 40:331–360
Golia E, Limongelli G, Natale F et al (2014) Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. Curr Atheroscler Rep 16:435
Gomi I, Fukushima H, Shiraki M et al (2007) Relationship between serum albumin level and aging in community-dwelling self-supported elderly population. J Nutr Sci Vitaminol 53:37–42
Grimm G, Haslacher H, Kampitsch T et al (2009) Sex differences in the association between albumin and all-cause and vascular mortality. Eur J Clin Invest 39:860–865
Grosse L, Ambrée O, Jörgens S et al (2016) Cytokine levels in major depression are related to childhood trauma but not to recent stressors. Psychoneuroendocrinology 73:24–31
Hair JF Jr, Black WC, Babin BJ, Anderson RE (2010) Multivariate data analysis, 7th edn. Pearson Education Limited, London
Harris TB, Ferucci L, Tracy RP et al (1999) Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 106:506–512
Harrison XA, Donaldson L, Correa-Cano ME et al (2018) A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ. https://doi.org/10.7717/peerj.4794
Heikkilä K, Harris R, Lowe G et al (2009) Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control 20:15–26
Higham JP, Kraus C, Stahl-Hennig C et al (2015) Evaluating non-invasive markers of nonhuman primate immune activation and inflammation. Am J Phys Anthropol 158:673–684
Himmelfarb J, McMonagle E (2001) Albumin is the major plasma protein target of oxidant stress in uremia. Kidney Int 60:358–363
Hoffman CL, Higham JP, Heistermann M et al (2011) Immune function and HPA axis activity in free-ranging rhesus macaques. Physiol Behav 104:507–514
Junge RE, Mezei LE, Muhlbauer M, Weber M (1998) Cardiovascular evaluation of lowland gorillas. J Am Vet Med Assoc 212:413–415
Khera A, McGuire DK, Murphy SA et al (2005) Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 46:464–469
Kiecolt-Glaser JK, Preacher KJ, MacCallum RC et al (2003) Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc Natl Acad Sci USA 100:9090–9095
Kiecolt-Glaser JK, Loving TJ, Stowell JR et al (2005) Hostile marital interactions, proinflammatory cytokine production, and wound healing. Arch Gen Psychiatry 62:1377–1384
Kim C-Y, Lee H-S, Han S-C et al (2005) Hematological and serum biochemical values in cynomolgus monkeys anesthetized with ketamine hydrochloride. J Med Primatol 34:96–100
Koukkunen H, Penttilä K, Kemppainen A et al (2001) C-reactive protein, fibrinogen, interleukin-6 and tumor necrosis factor- α in the prognostic classification of unstable angina pectoris. Ann Med 33:37–47
Kowarik A, Templ M (2016) Imputation with the R package VIM. J Stat Softw 74:1–16
Kronfol Z (2007) Behavioral effects of cytokines: a psychiatrist's perspective. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA (eds) Cytokines: stress and immunity, 2nd edn. CRC Press, Boca Raton, pp 1–16
Lakoski SG, Cushman M, Criqui M et al (2006) Gender and C-reactive protein: data from the multiethnic study of atherosclerosis (MESA) cohort. Am Heart J 152:593–598
Leeds A, Boyer D, Ross SR, Lukas KE (2015) The effects of group type and young silverbacks on wounding rates in western lowland gorilla (Gorilla gorilla gorilla) groups in North American zoos. Zoo Biol. https://doi.org/10.1002/zoo.21218
Levine B, Kalman J, Mayer L et al (1990) Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 323:236–241
Libby P (2006) Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 83:456S-460S
Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105:1135–1143
Lowenstine LJ, McManamon R, Terio KA (2016) Comparative pathology of aging great apes: bonobos, chimpanzees, gorillas, and orangutans. Vet Pathol 53:250–276
Lutgendorf SK, Anderson B, Sorosky JI et al (2000) Interleukin-6 and use of social support in gynecologic cancer patients. Int J Behav Med 7:127–142
Maggio M, Guralnik JM, Longo DL, Ferrucci L (2006) Interleukin-6 in aging and chronic disease: a magnificent pathway. J Gerontol A Biol Sci Med Sci 61:575–584
Mangiafico S (2016) rcompanion: Functions to support extension education program evaluation. R package version 2.3.25. https://cran.r-project.org/package=rcompanion
Mann DL, Deswal A, Bozkurt B, Torre-Amione G (2002) New therapeutics for chronic heart failure. Annu Rev Med 53:59–74

Manolio TA, Burke GL, Savage PJ et al (1992) Sex- and race-related differences in liver-associated serum chemistry tests in young adults in the CARDIA study. Clin Chem 38:1853–1859
Maury CPJ, Teppo A-M (1989) Circulating tumor necrosis factor-α (cachectin). J Intern Med 225:333–336
McClure HM, Keeling ME, Guilloud NB (1972) Hematologic and blood chemistry data for the gorilla (Gorilla gorilla). Folia Primatol 18:300–316
McManamon R, Lowenstine L (2012) Cardiovascular disease in great apes. In: Miller RE, Fowler ME (eds) Fowler's zoo and wild animal medicine: current therapy. Elsevier, Saint Louis, pp 408–415
McPherson K, Healy MJR, Flynn FV et al (1978) The effect of age, sex and other factors on blood chemistry in health. Clin Chim Acta 84:373–397
Mendall MA, Strachan DP, Butland BK et al (2000) C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. Eur Heart J 21:1584–1590
Mundy NI, Ancrenaz M, Wickings EJ, Lunn PG (1998) Protein deficiency in a colony of western lowland gorillas (Gorilla g. gorilla). J Zoo Wildl Med 29:261–268
Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 168:28–40
Murphy HW, Dennis P, Devlin W et al (2011) Echocardiographic parameters of captive western lowland gorillas (Gorilla gorilla gorilla). J Zoo Wildl Med 42:572–579
Murphy HW, Danforth MD, Clyde VL (2018) The great ape heart project. Int Zoo Yb 52:103–112
Murray S, Kishbaugh JC, Hayek L-AC et al (2019) Diagnosing cardiovascular disease in western lowland gorillas (Gorilla gorilla gorilla) with brain natriuretic peptide. PLoS ONE. https://doi.org/10.1371/journal.pone.0214101
Myśliwska J, Bryl E, Foerster J, Myśliwski A (1998) Increase of interleukin 6 and decrease of interleukin 2 production during the ageing process are influenced by the health status. Mech Ageing Dev 100:313–328
Naimi B (2017) usdm: uncertainty analysis for species distribution models. R package version 1.1-18. https://cran.r-project.org/package=usdm. Accessed 5 March 2020
Odeh M (1993) Tumor necrosis factor-α as a myocardial depressant substance. Int J Cardiol 42:231–238
Otterness IG (1994) The value of C-reactive protein measurement in rheumatoid arthritis. Semin Arthritis Rheum 24:91–104
Pai JK, Pischon T, Ma J et al (2004) Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 351:2599–2610
Paolisso G, Rizzo MR, Mazziotti G et al (1998) Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. Am J Physiol 275:E294–E299
Patten M, Krämer E, Bünemann J et al (2001) Endotoxin and cytokines alter contractile protein expression in cardiac myocytes in vivo. Pflügers Arch Eur J Physiol 442:920–927
Pearson TA, Mensah GA, Alexander RW et al (2003) Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107:499–511
Phillips A, Shaper AG, Whincup PH (1989) Association between serum albumin and mortality from cardiovascular disease, cancer, and other causes. Lancet 334:1434–1436
Puzianowska-Kuźnicka M, Owczarz M, Wieczorowska-Tobis K et al (2016) Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun Ageing 13:1–12
R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Ridker PM, Buring JE, Shih J et al (1998) Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 98:731–733
Ridker PM, Hennekens CH, Buring JE, Rifai N (2000) C-Reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843
Ridker PM, Stampfer MJ, Rifai N (2001) Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. J Am Med Assoc 285:2481–2485
Ridker PM, Rifai N, Rose L et al (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 347:1557–1565
Salive ME, Cornoni-Huntley J, Phillips CL et al (1992) Serum albumin in older persons: relationship with age and health status. J Clin Epidemiol 45:213–221
Senapathi TGA, Widnyana IMG, Wiryana M et al (2016) Effectiveness of low-dose intravenous ketamine to attenuate stress response in patients undergoing emergency cesarean section with spinal anesthesia. J Pain Res 9:689–692
Stokes EJ (2004) Within-group social relationships among females and adult males in wild western lowland gorillas (Gorilla gorilla gorilla). Am J Primatol 64:233–246
Strong VJ, Grindlay D, Redrobe S et al (2016) A systematic review of the literature relating to captive great ape morbidity and mortality. J Zoo Wildl Med 47:697–710
Strong VJ, Martin M, Redrobe S et al (2018) A retrospective review of great ape cardiovascular disease epidemiology and pathology. Int Zoo Yb 52:113–125
Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav Ecol Sociobiol 65:13–21
Tang Y, Fung E, Xu A, Lan H-Y (2017) C-reactive protein and ageing. Clin Exp Pharmacol Physiol 44(Suppl 1):9–14
Tayefi M, Shafiee M, Kazemi-Bajestani SMR et al (2017) Depression and anxiety both associate with serum level of hs-CRP: a gender-stratified analysis in a population-based study. Psychoneuroendocrinology 81:63–69
Tian M, Yuan Y-C, Li J-Y et al (2015) Tumor necrosis factor-α and its role as a mediator in myocardial infarction: a brief review. Chronic Dis Transl Med 1:18–26
Tracy RP, Psaty BM, Macy E et al (1997) Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. Arterioscler Thromb Vasc Biol 17:2167–2176
Tukey JW (1977) Exploratory data analysis. Addison-Wesley, Reading
Tworoger SS, Hankinson SE (2006) Collection, processing, and storage of biological samples in epidemiological studies: sex hormones, carotenoids, inflammatory markers, and proteomics as examples. CEBP 15:1578–1581
Urschel K, Cicha I (2015) TNF-α in the cardiovascular system: from physiology to therapy. Int J Interferon Cytokine Mediator Res 7:9–25
Varki N, Anderson D, Herndon JG et al (2009) Heart disease is common in humans and chimpanzees, but is caused by different pathological processes. Evol Appl 2:101–112
Walker AJ, Foley BM, Sutor SL et al (2015) Peripheral proinflammatory markers associated with ketamine response in a preclinical model of antidepressant-resistance. Behav Brain Res 293:198–202
Weaving G, Batstone GF, Jones RG (2016) Age and sex variation in serum albumin concentration: an observational study. Ann Clin Biochem 53:106–111

Wei J, Xu H, Davies JL, Hemmings GP (1992) Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci 51:1953–1956

Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119

Willerson JT, Ridker PM (2004) Inflammation as a cardiovascular risk factor. Circulation 109:II–2

Williamson EA, Tutin CEG, Rogers ME, Fernandez M (1990) Composition of the diet of lowland gorillas at Lopé in Gabon. Am J Primatol 21:265–277

Yamada S, Gotoh T, Nakashima Y et al (2001) Distribution of serum C-reactive protein and its association with atherosclerotic risk factors in a Japanese population: Jichi Medical School Cohort Study. Am J Epidemiol 153:1183–1190

Yeh ETH, Willerson JT (2003) Coming of age of C-reactive protein: using inflammation markers in cardiology. Circulation 107:370–372