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Elevated neopterin levels in wild, healthy chimpanzees indicate constant investment in unspecific immune system

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Abstract

Background: Ecological immunology proposes that the optimal immune defence, and the costs coming with it, vary across environments. In environments with higher pathogen load, the immune system should experience greater challenges and, therefore, investment in maintaining it should be higher. The biomarker neopterin allows monitoring of innate immune responses, and is therefore an ideal tool to investigate the effects of ecological variables on the immune system. Here, we compared urinary neopterin levels of apparently healthy chimpanzees without acute symptoms of sickness across two environments: in captivity (22 zoos) and in the wild (two populations).

Results: Our results revealed that urinary neopterin levels were nearly twice as high in wild compared to captive chimpanzees, independent of chimpanzee subspecies.

Conclusion: We conclude that wild chimpanzees experience more frequent immune challenges in comparison to captive individuals. Therefore, wild individuals have to allocate more energy to immune function and away from reproduction and growth. Our data indicate that the generally delayed development of wild animals in comparison to captive individuals might not only be related to lower energy intake but might result from greater energy allocations to immune function. Finally, our data highlight the importance of understanding immune costs for accurate characterization of energy budgets in animals.

Keywords: Ecoimmunology, Immune response, Energy allocation, Costs, Captive and wild living

Background

Life-history theory (LHT) concepts show the demands and challenges that influence immune function in an ecological context. Increased energy allocation to immune function is an investment in maintenance and finally in survival, but leaves less energy for growth or reproduction [1–3]. Various assessments of energy allocation to the immune system suggest that maintenance and stimulation of immune functions are energetically expensive [1, 4, 5], thereby imposing a significant burden to organisms [2, 6, 7]. Therefore, life-history patterns among vertebrates have been shaped, among other factors, by general patterns of immunity and immune responses associated with environment-specific pathogens [5].

The mammalian immune system is divided into two major functional categories: innate (or constitutive) and adaptive (or acquired) immunity. Innate immunity consists of primary mechanisms providing the first line of disease defence. Although not fully developed in neonates, innate immunity develops faster than adaptive immunity. Such defences include both physical barriers and biochemical factors—e.g., anatomical and physiological barriers, inflammatory mediators, and cellular components [8]. Cellular components like macrophages are stimulated by interferon–γ and co-stimulated by tumour-necrosis factor and endotoxins. Interferon–γ is released by T-cells after antigen recognition; they release neopterin in a high and constant rate into body fluids. Therefore, neopterin is an ideal marker to monitor the

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degree of the innate immune response activation [9, 10]. So far, investigations of immune system responses or changes in immune functioning have been performed mainly invasively—e.g., in blood samples in humans, domestic animals, and wild living birds [11–14]. However, increases in neopterin concentrations in serum during the clinical course of infections are paralleled by the same patterns in urine [15], and the measurement of urinary neopterin levels in non-human primates with a commercial neopterin assay has been recently validated [16, 17].

In humans, many Indigenous people (for definition, please see Gracey & King [18]), experience low standards of health, which are associated with poverty, malnutrition, overcrowding, poor hygiene, and environmental contamination. They are exposed to a high rate of disease-causing infections. Moreover, infections are more frequently fatal in Indigenous people [18]. However, a comparison of human urinary neopterin levels, as an indicator of nonspecific immune system activation, indicates across studies that individuals living in environments with lower levels of pathogen prevalence indeed have lower levels of immune challenge, as indicated by lower urinary neopterin levels [15, 19]. Because the neopterin measurements were run with different methods (for example LC-MS in Fuchs et al. [15] and enzyme-immuno assay in Filteau et al. [19]), and in different laboratories [20], these comparisons must be interpreted with caution. Additionally, vaccination studies in humans indicate differences in natural immune background across human populations. One factor influencing the variation in immune responses to viral vaccines is genetic polymorphisms of the human leukocyte antigen system. However, in rural human populations, successful vaccination requires de-worming of patients prior to the vaccine's administration [21, 22], suggesting these populations have a constantly activated innate immune system preventing a specific immune response to the vaccination. Indirect evidence of potential health differences across human populations were shown in gut microbiome, which is vital for immune function, where differences in microbiome diversity were found between non-industrialized, rural communities from Africa and South America; and western industrialized populations [23].

Wild primates routinely sustain wounds and encounter infectious disease [24]. They face a diverse array of parasites and pathogens, which potentially reduce host survival and reproductive success [24–28]. However, wild animals have a long evolutionary history with a vast number of naturally occurring microorganisms, shaping their host-pathogen interaction [29]. In contrast, captive individuals are mainly exposed to pathogens of human origin to which they might not be well adapted. Those human pathogens are especially a threat to great ape species due to recent common ancestry [30]. Furthermore, captive animals may also face increased risk from unfamiliar infectious diseases of animal origin and may be more vulnerable to infection due to inadequate nutrition and environmental conditions [31, 32]. Studies comparing immune system components in the same species in different environments are rare and found contrasting results. First evidence for differences in immunity in wild and captive environments was found in a bird species. Some blood markers of immunity such as eosinophil concentrations were higher in wild birds, and some immune markers such as total leukocyte count were comparable in the two environments [33]. In contrast, in a recent study in serum of dolphins, free living individuals had elevated markers related the immunity such as lymphocytes, eosinophils and platelets compared to captive ones [34]. However, all makers measured in the study, whether related to endocrine system or immune system, were in general higher in wild compared to captive living individuals [34], and therefore results have to be interpreted with caution. Additionally, so far all studies used serum samples, which are impossible to collect in most wild living species. Therefore, one of our aims was to establish a marker that allows repeated non-invasive sampling to investigate the effect of immune energetic burden on life-history patterns.

To evaluate the impact of immune system challenges in different environments, we investigated patterns of urinary neopterin levels as a measure for the unspecific immune response in chimpanzees living in 22 zoos and in two natural environments. We examined potential differences in immune response, which might affect energy allocation and thereby influence life-history trade-offs. We predict that urinary neopterin levels are lower in zoo housed chimpanzees because wild living chimpanzees are exposed to a large number of microorganisms, while zoo chimpanzees are treated, vaccinated, and screened against a variety of pathogens [35]. Alternatively, captive chimpanzees may have higher urinary neopterin levels because they are exposed to a higher variety of human pathogens to which they are not adapted.

Urine samples were collected from chimpanzees housed in zoos and wild living chimpanzees coming from two field sites, one West African chimpanzee (*Pan troglodytes verus*) field site, and one East African chimpanzee (*Pan troglodytes schweinfurthii*) field site. This comparative approach controls for potentially unusual urinary neopterin levels due to higher pathogen loads at one field site than elsewhere. In addition, this approach controls for potential genetic differences between chimpanzee subspecies, since captive individuals belong to different chimpanzee subspecies or are sub-specific hybrids.

Results

Both mean and median urinary neopterin levels in both wild populations were nearly twice as high as the levels of captive chimpanzees (Table 1). The full/null model comparison ($\chi 2 = 28.608$, df = 8, $P < 0.001$) revealed that the environment was a significant predictor of urinary neopterin levels (Table 2), with significant lower urinary neopterin levels in captive chimpanzees in comparison to wild living individuals (captive vs. Sonso: Estimate = 0.558; SE = 0.122; $P < 0.001$; captive vs. Taï: Estimate = 0.555; SE = 0.112; $P < 0.001$; Fig. 1). However, urinary neopterin levels from Sonso were not different from those from Taï (Estimate 0.003; SE 0.118; $P = 0.978$) (Fig. 1), indicating that urinary neopterin levels of wild chimpanzees do not differ by subspecies or specific pathogens in one field site.

Discussion

The 43% higher urinary neopterin levels in wild chimpanzees most probably do not reflecting acute infections, but suggest a permanently challenged immune system faced with a high diversity of microorganisms and / or a higher disease burden. This assumption is supported by an even further increase in urinary neopterin levels in wild chimpanzees at Taï during a respiratory disease outbreak [36]. However, while there are non-infectious health conditions that lead to elevated neopterin levels such as sepsis, malignancies, autoimmune diseases, heart failure, and coronary artery diseases [37], we do not have any evidence that such conditions created the pattern observed in this study. Independently of the causes of higher neopterin levels in wild chimpanzees, constantly elevated urinary neopterin levels indicate a permanent energetic burden for wild chimpanzees, allocating energy into maintenance, and thereby away from other traits such as growth and reproduction [38, 39].

Developmental differences between wild and captive primates are well documented. For example, captive female chimpanzees reach menarche and give first birth at a younger age than chimpanzees in the wild [40]. Additionally, in wild great apes, tooth eruption is in some teeth two years later than in captivity [41, 42], but ranges overlap extensively [43]. These differences in the pace of development between wild and captive individuals were assumed to be related to constant energy abundance without seasonal variation and inter-individual variation in captive animals [43, 44]. However, differences might also exist in how much of the available energy needs to be allocated into immune function. For example, in humans living in developing countries, children with higher levels of immune activation have impaired growth [45].

Age effects on neopterin levels were found in a number of studies. In humans, children have higher urinary neopterin levels, declining with increasing age, which then increase again with senescence in adulthood. However, this effect might be mainly driven by age-related changes in creatinine clearance rate, since creatinine was used as a correction factor for urine concentration [15]. A pattern of increasing urinary neopterin levels with increasing senescence was found as well in Barbary macaques [46]. Such an effect might also be caused by an increase in chronic health problems with increasing age [47, 48]. However, age dependent effects are less pronounced than changes in clinical neopterin levels changes [15] and might therefore be difficult to find in populations experiencing constant immune challenge. For example, no age effect was found in two studies on urinary neopterin levels in wild chimpanzees [36, 49], and urinary neopterin levels were independent of sex and age as well in this study.

Table 1 Description of urinary neopterin levels (μmol/mol creatinine) in chimpanzees living in captivity ($N = 58$), at Taï ($N = 75$) and Sonso ($N = 45$)

	Captive	Taï	Sonso
Mean	203.2	345.6	302.9
Median	143.0	247.3	262.9
Stdev.	252.5	334.8	147.8

Table 2 Results of the full-null model comparison obtained by analysing urinary neopterin levels from captive and wild living chimpanzees with sex, age, day-time and storage time as fixed effect and animal ID as a random effect (SE = standard error, bold numbers indicate significance)

Term	Estimate	SE	DF	χ^2	*P*-value
Intercept	4.900	0.116			
Age at sample collection	−0.044	0.053	1	0.617	0.413
Sex	0.090	0.092	1	0.964	0.326
Environment	0.708	0.158	2	22.41	**< 0.001**
Day-time	0.085	0.062	1	1.852	0.174
Storage time	0.094	0.065	1	2.050	0.152

Conclusion

Our data support the idea that environmental gradients create varying selective pressures on immune systems that may result in different optimal life history strategies [50]. Individuals confronted with higher pathogen load or higher microorganism diversity and thereby with higher immune system activation, allocate energy into maintenance and less into other factors like growth and reproduction, which would allow captive individuals to grow faster and to reproduce earlier than wild living animals. Therefore, the measurement of immune system activation is essential for a more accurate characterization of energy budgets of animals and a better understanding

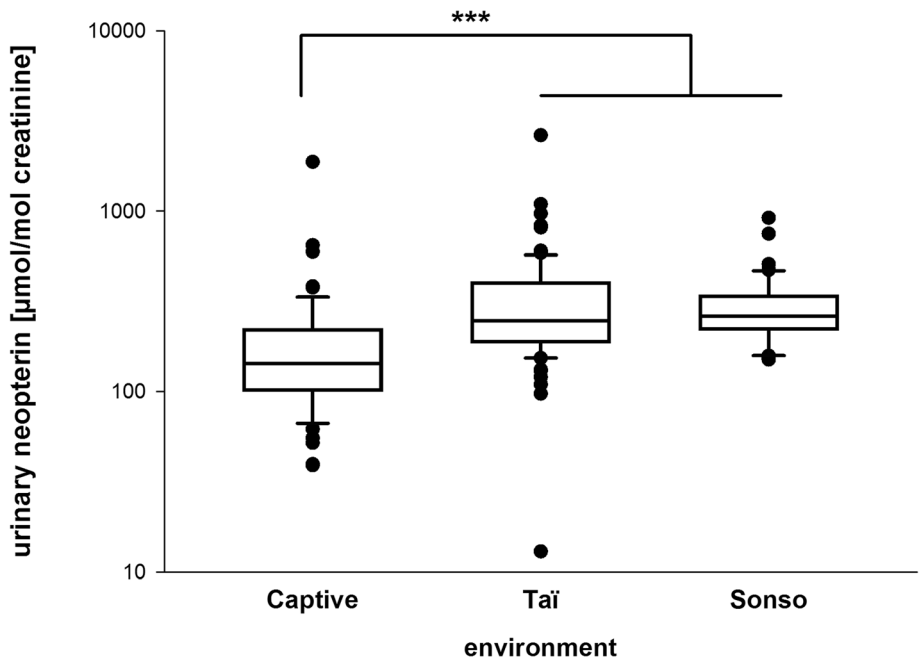

Fig. 1 Measures of urinary neopterin levels corrected for creatinine (µmol/mol) from chimpanzees living in captivity ($N = 58$ samples), Taï ($N = 75$ samples) and Sonso ($N = 45$ samples). Individuals: $N_{captivity} = 58$ (26 males, 32 females), $N_{Taï} = 29$ (15 males, 14 females), $N_{Sonso} = 29$ (12 males, 17 females). The y-axis is displayed on a log scale. The boxes illustrate the 25th and 75th percentiles, bars indicate medians, and circles indicate outliers

of the role of immunity in the evolution of life-history strategies [51].

Methods

We measured urinary neopterin levels in 178 samples of apparently healthy chimpanzees collected at random from two different environments (captive and wild). 'Apparently healthy' was defined as a chimpanzee not showing any current chronic or acute symptoms of sickness. The captive environment (22 zoos) was represented by 58 urine samples from 32 females and 26 males, collected between 2006 and 2014. Samples representing the wild environment were collected at two sites, communities of the Taï chimpanzee project in Taï National Park, Côte d'Ivoire (West Africa), and the Sonso community, Budongo Forest, Uganda (East Africa). In Taï, a number of acute diseases, including anthrax and respiratory infections, have been documented [52–54], which are absent in Sonso. From the Taï chimpanzees, samples of 14 females (35 samples, average of 2.5 samples per individual) and 15 males (40 samples, average 2.7 samples per individual) collected in 2009 were analysed. From the Sonso community, urine samples collected between 2009 and 2010 from 17 females (23 samples, average 1.4 samples per individual) and 12 males (22 samples, average 1.8 samples per individual) were used. The age range of the captive chimpanzees was between 4 and 53 years (median: 15 years), in Sonso individuals ranged between 11 and 50 years (median: 17 years), and in Taï between 2 and 45 years (median: 15 years). For 49 of the 58 captive chimpanzees the exact birthdate was available from the studbook. For three captive and three Taï chimpanzees, only the year and month of birth were known, and for these we set the day of birth to the 15th of the respective month. For the remaining individuals, only the year of birth was known, and in these cases, the day of birth was set to June 15th of the respective year (all Sonso chimpanzees, 15 Taï chimpanzees, and six captive chimpanzees). All urine samples were non-invasively collected in captivity and in the wild. For details on urine collection protocols, see Behringer et al. [55] for captive samples, Deschner et al. [56] for Taï samples, and Crockford et al. [57] for Sonso samples. After collection, the samples were frozen in the zoo or the field sites, and transported frozen to the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany.

Urinary neopterin analyses

For the measurement of urinary neopterin levels in chimpanzees, we used a commercial competitive neopterin ELISA (Neopterin ELISA, Ref. RE59321, IBL International GmbH, Hamburg, Germany), validated for the measurement of neopterin in urine of chimpanzees [16]. All urine samples were thawed, vortexed, and centrifuged. The captive chimpanzee urine was diluted 1:100 with the assay buffer of the supplier. Sonso samples were

diluted 1:200 or 1:400, and the urine of the Taï chimpanzees 1:400 or 1:600. The assays were performed following the instructions from the supplier and as described in Behringer et al. [16]. All samples, standards, and controls were measured in duplicate. To compensate for variation in volume and concentration of the collected urine, as well as to make the results comparable to human studies [19, 58–62], creatinine was measured, and results are expressed in μmol/mol creatinine.

Statistical analyses

We used a general linear mixed model (GLMM, [63]) to assess the impact of environment (test predictor with fixed effect) on the log transformed response variable urinary neopterin. The model was fitted in R [64] using the function "lmer" provided in the package "lme4" [65]. To control for the potential influence of day time, storage-time, age of the animal at sampling, and the sex of the animal, these parameters were included as control predictors with fixed effects. As a random effect, we included animal ID, and we included random slopes of age at sampling time, storage time, and day time within animal ID, to keep type I error rates at the nominal level of 5% [66, 67]. Age as well as the time of sample collection (to control for diurnal variation) was z-transformed to a mean of zero and a standard deviation of one to achieve comparable estimates [68].

The required normal distribution and homogeneity of residuals for the model were assessed by visual inspections of a histogram, a q-q plot of the residuals, and by plotting residuals against fitted values. All model assumptions were met. We assessed collinearity by determining variance inflation factors (VIF, [69]) using the function "vif" of the R-package "car" [70] based on a standard linear model excluding the random effects, which revealed no problems (maximum VIF: 2).

We investigated the significance of the fixed effect environment, by comparing the full model with a null model, excluding the predictor variable, but retaining time of sample collection, age and sex, and the random effect animal ID, as well as the random slopes component, using a likelihood ratio test [71]; (R function "anova"). To further explore the effect of each specific environment on urinary neopterin levels, we built two additional models, one compared the captive neopterin levels with the levels from Taï and Sonso, and the second model compared the levels of Sonso and captive chimpanzees with Taï, with the same fixed effects, random effect, and random slopes as in the full model. Significance for all tests was set at the $P = 0.05$ level.

Abbreviation

EAZA: European Association of Zoos and Aquaria; EEP: European Endangered Species Programmes; ELISA: Enzyme-linked Immunosorbent Assay; ESB: European Studbooks; GLMM: General linear mixed model; LC-MS: Liquid chromatography/mass spectrometry; LHT: Life-history theory; MPI-EVA: Max Planck Institute for Evolutionary Anthropology; VIF: Variance inflation factors

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

VB carried out the lab work and statistical analyses, VB and TD did data analysis and developed the study concept; TD, RMW, JMGS, CC, FHL and KZ collected data and/or provided essential materials; all authors (VB, JMGS, RMW, CC, KZ, FHL, TD) helped to draft the manuscript and approved the final version of the manuscript for submission.

Ethics approval and consent to participate

The protocol for urine sample collection in captive individuals was approved by the authorities of each zoo and supported by the coordinators of the EAZA Western Chimpanzee EEP and Robust Chimpanzee ESB (Frands Carlsen and Tom de Jongh). Permissions to conduct the research in Taï were granted by the Ministries of Research and Environment of Ivory Coast and Office Ivoirien des Parcs et Reserves. The Uganda Wildlife Authority, the Uganda National Council for Science and Technology, and the President's Office of Uganda gave permission to collecting samples in Sonso.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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