**Title:** Costs and constraints of cellular Immune activity during development in blue monkeys

**Summary:** Life history theory predicts that, during development, investment in immunity must be balanced with the demands of growth. How, and at what timescales, this balance is negotiated is unclear. In this study, we examined the potential energetic costs and limitations to cellular immune activity during development, its trade-offs with growth, and the role of HPA axis activity in these relationships. We combined biomarker and socioenvironmental data on wild juvenile blue monkeys collected over 8 months. Rather than detract from energy balance (C-peptide) and growth of lean body mass (creatinine-SG residuals), cellular immune activity (neopterin) increased with energy balance and lean body mass at monthly timescales, suggesting an energetic constraint on cellular immunity. At shorter timescales, higher neopterin diminished subsequent growth. Constraints were weakly regulated by HPA activity during low energy states. Our results suggest that cellular immune activity is both costly and limited by body condition in wild developing primates.

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**Introduction**

Life history theory predicts variation and trade-offs in the amount of energy allocated to growth, reproduction, and maintenance throughout the life course [(McDade, 2003)](https://www.zotero.org/google-docs/?qxQy31). In recent decades, the field of evolutionary and eco-immunology has demonstrated that immune function and activity also come at a significant energetic cost, placing immunity among the pillars of life history theory [(Demas, 2004)](https://www.zotero.org/google-docs/?jv6umO). During development, physical growth is a high priority, however it may tradeoff with the simultaneous necessity to defend against pathogens. This trade-off between investment in growth and immune function may be particularly intense in resource-limited environments. Faltering growth and smaller adult body size have significant implications for adult health, including increased morbidity [(Min et al., 2021; Prendergast & Humphrey, 2014)](https://www.zotero.org/google-docs/?WsY0bk), mortality, and lower reproductive success [(Blanckenhorn, 2005; Hector & Nakagawa, 2012)](https://www.zotero.org/google-docs/?E7KC1Y), therefore successful growth has clear benefits. Less clear, however, is the extent to which immunity is prioritized relative to growth during development in wild animals inhabiting resource-limited environments, and whether low resource availability can pose an energetic constraint on immunity. Constraints on immunity imply susceptibility to infection, therefore potential tradeoffs between growth and immunity have broad implications for individual and population health.

The costs of immune activity can be high. In terms of metabolic rates, experimental stimulation of adaptive immunity increased resting metabolic rate substantially in mice [(20-30%, Demas et al., 1997)](https://www.zotero.org/google-docs/?FxuJ16) and house sparrows [(29%, Martin et al., 2003)](https://www.zotero.org/google-docs/?uD5xeF), and stimulation of innate immunity also led to a loss of body mass [(house sparrows, Bonneaud et al., 2003)](https://www.zotero.org/google-docs/?5v9Dxc). In humans, basal metabolic rate can increase 7-14% with acute infections [(Barr et al., 1922; Urlacher et al., 2018)](https://www.zotero.org/google-docs/?wVSnIN), while more severe infections can lead to increases up to 50% [(Urlacher et al., 2018)](https://www.zotero.org/google-docs/?roKSJf). Costs also include behavioral changes, such as less physical activity and increased sleep [(reviewed in Tizard, 2008)](https://www.zotero.org/google-docs/?8kRR0n). Such compensatory behavior represents an opportunity cost, as it appears to occur independently of energetic status and is adaptively regulated by cytokine signals in an effort to heal and maintain homeostasis [(Tizard, 2008)](https://www.zotero.org/google-docs/?Q0Pm4G).

During development, energetic investment in immune function can limit physiologically available energy and thereby compromise physical growth. Experimental stimulations of immune responses led to slower growth rates in juvenile barn swallows [(Saino et al., 1998)](https://www.zotero.org/google-docs/?UIP9TB) and magpies [(Soler et al., 2003)](https://www.zotero.org/google-docs/?fogrMg). Such tradeoffs may manifest at different timescales according to the type of immune activity. For example, high immune surveillance, as measured by non-acute concentrations of c-reactive protein (CRP, < 2mg/L), corresponded with slower growth in adolescent Gambian women over one year [(height velocity and lean mass deposition, Shattuck-Heidorn et al., 2017)](https://www.zotero.org/google-docs/?6DDp1u). However, among Shuar children in Amazonian Ecuador, acutely elevated CRP was associated with a 49% drop in growth over one week, but at longer intervals there were no associated changes in growth [(lower leg length, Urlacher et al., 2018)](https://www.zotero.org/google-docs/?4wfWl6). As life history theory predicts that tradeoffs result from limited resources, the occurrence of tradeoffs also likely depends on individual body condition and energetic reserves in adipose tissue and lean body mass. For example, although acute elevations of CRP compromised short-term growth in thinner Shuar children, those with more body fat had no reduction in growth [(Urlacher et al., 2018)](https://www.zotero.org/google-docs/?Yi4o2x), indicating that greater energetic reserves can minimize the need for tradeoffs.

While immune activity is costly, it can also be energetically constrained. Several branches of the immune system are dependent on adequate caloric and nutrient intake and energy stores for proper function [(Nelson et al., 2002; Vogel et al., 2024)](https://www.zotero.org/google-docs/?6mutkg). Fasting under experimental conditions suppresses T-cell mediated immunity [(humans, Castaneda et al., 1995; Mongolian gerbils, Xu & Wang, 2010)](https://www.zotero.org/google-docs/?LpIuFb), humoral immune function [(Siberian hamsters, Zysling & Demas, 2007)](https://www.zotero.org/google-docs/?4CG0Tk), and circulating concentrations of interferon-γ [(rats, Jolly, 2004)](https://www.zotero.org/google-docs/?qYO5kT). In humans, malnutrition is the most common cause of immunodeficiency worldwide [(Chandra, 1996)](https://www.zotero.org/google-docs/?TALeqw), where malnourished vs. well-nourished children are more susceptible to higher rates of diarrhea, respiratory infections, measles, and malaria [(Schaible & Kaufmann, 2007)](https://www.zotero.org/google-docs/?xF8FTb). Immunocompetence also varies in response to natural seasonal fluctuations in body fat stores in several seasonally breeding rodents [(Bartness et al., 2002)](https://www.zotero.org/google-docs/?XnOsvX). Further, although survival in the face of pathogens is of great importance to fitness, immune function can be deprioritized and subjected to the same tradeoffs as other life history pillars. For example, domesticated chickens selected for faster growth demonstrated weaker cellular and humoral immune responses [(van der Most et al., 2011)](https://www.zotero.org/google-docs/?tUmaAU).

Several potential mechanisms mediate energetic constraints on the allocation of energy to immune function. Individuals may experience a direct lack of fuel for immune cells, such as glucose [(rats, Lysle et al., 1988; mice, E. S. Miller et al., 1994)](https://www.zotero.org/google-docs/?X7A3Ds) or free fatty acids [(Pond, 1996)](https://www.zotero.org/google-docs/?SdcCfE). Such metabolic stress, however, can result in a general stress response, with the release of glucocorticoids (GCs) mediating the immunosuppressive effects of low energy. Indeed, concentrations of GCs rise in response to experimental fasting (mongolian gerbils, xu wang) [(Mongolian gerbils, Xu & Wang, 2010)](https://www.zotero.org/google-docs/?Y0mh18) and in naturally occurring periods of low energy balance [(blue monkeys, Thompson et al., 2020)](https://www.zotero.org/google-docs/?7XkIc2). Lastly, depletion of body fat reduces circulating concentrations of leptin, a hormone similar in structure to IL-2 and similarly critical for sustaining cell-mediated immunity [(McDade, 2003)](https://www.zotero.org/google-docs/?uiTxMD). Though difficult to disentangle, understanding the mechanisms of energy-regulated immunomodulation is important for predicting circumstances in which immune function may be constrained and, more broadly, helps elucidate how life history trade-offs may be regulated [(e.g., Ellison, 2017)](https://www.zotero.org/google-docs/?pbxQAH).

Inadequate energy intake can lower cellular immune activity specifically, relative to humoral immunity [(Long & Nanthakumar, 2004)](https://www.zotero.org/google-docs/?qmzBoT). The cellular arm of the adaptive immune response involves the development and mobilization of T helper type 1 cells (Th1) and their recruitment of monocyte-derived macrophages. This activity corresponds with inflammation, a particularly costly aspect of the immune response that generally requires processes to repair collateral damage to self [(Weavers et al., 2019)](https://www.zotero.org/google-docs/?BJH3sG). In mice, lower circulating leptin concentrations during fasting led to a decrease in Th1 cytokines and a predominance of Th2, whereas refeeding restored Th1 and suppressed Th2 cytokines [(Lord et al., 1998)](https://www.zotero.org/google-docs/?j67yF0). Although T cell profiles require invasive sampling to quantify, cellular immune activity can be reliably measured non-invasively. The biomarker neopterin is a useful non-invasive marker of cellular immune activity, as well as general inflammatory activity of macrophages, as it is produced by type I macrophages typically after their activation by IFNy produced by Th1 cells [(Murr et al., 2002)](https://www.zotero.org/google-docs/?oOravV). Neopterin has been validated to increase in response to acute infection in macaques [(Higham et al., 2015)](https://www.zotero.org/google-docs/?tlsLFK) and great apes [(bonobos, Behringer et al., 2017; chimpanzees, Thompson González et al., 2020)](https://www.zotero.org/google-docs/?zjBe8D). It is excreted in urine and is highly stable when stored in the absence of light at low temperatures, making it an ideal marker of cellular immune activity in wild primate populations [(Heistermann & Higham, 2015)](https://www.zotero.org/google-docs/?ijpDog).

Energy balance and bodily condition are challenging to quantify in wild primates: the gold standard for measuring each requires capture or controls that are not feasible in most wild populations (e.g., doubly-labeled water method). Nevertheless, the status of each can be approximated non-invasively. Energy balance, or the difference between energy intake and expenditure, can be well-approximated with C-peptide of insulin [(Emery Thompson, 2016; Girard-Buttoz et al., 2011; Higham et al., 2011)](https://www.zotero.org/google-docs/?zIO1yi). C-peptide is excreted in urine and produced in a 1:1 ratio with insulin, providing an integrated signal of energy balance to the brain. C-peptide has been validated in several primate species, shown to correspond with body mass [(bonobos, Deschner et al., 2008; macaques, Girard-Buttoz et al., 2011)](https://www.zotero.org/google-docs/?LHCfsJ), feeding rates and mass gain and loss ([macaques, Girard-Buttoz et al., 2011](https://www.zotero.org/google-docs/?nF2frf)), and food availability [(chimpanzees, Emery Thompson et al., 2009; orangutans, Emery Thompson & Knott, 2008; gorillas, Grueter et al., 2014; blue monkeys, Thompson et al., 2020)](https://www.zotero.org/google-docs/?oS6s4W). Body condition typically refers to the availability of energetic reserves, namely body fat and lean muscle [(Schulte-Hostedde et al., 2005)](https://www.zotero.org/google-docs/?NWDFQi). To date, no non-invasive methods have been validated to measure body fat, however approximation of lean body mass is possible with a combination of urinary creatinine and specific gravity [(Emery Thompson et al., 2012)](https://www.zotero.org/google-docs/?EsaR8K). Although lipids in adipose tissue are typically metabolized prior to protein in lean muscle, lean muscle still constitutes an energetic reserve [(O’Connell et al., 2021; Schulte-Hostedde et al., 2005)](https://www.zotero.org/google-docs/?RYbxYY). Creatinine content of urine that exceeds expected values based on urine’s specific gravity represents relatively higher muscle mass, and lower muscle mass if creatinine falls below expected values.

Physical growth is a cornerstone of the developmental period, as is the development of immunity to novel pathogens, and so we expect that competing investments in immunity and growth are likely to arise during this life stage. Nevertheless, the tradeoffs between these investments likely depend on species life history dynamics and physical environments. The juvenile period of development, defined as when individuals are no longer dependent on parental care for survival yet not reproductively mature [(Pereira & Fairbanks, 2003)](https://www.zotero.org/google-docs/?y9HuAn), is prolonged in primates relative to other mammals of similar body size [(Pagel & Harvey, 2003)](https://www.zotero.org/google-docs/?eBhWbo). A prominent adaptive explanation of this trend is that slow growth buffers the risk of energy shortfalls in seasonal and unpredictable environments (Janson Van Schaik 2003). Blue monkeys (*Cercopithecus mitis*) demonstrate a particularly prolonged juvenile period even among primates, ranging from 1.5 to 10 years old (unpublished data), and a slow life history overall [(Cords & Chowdhury, 2010)](https://www.zotero.org/google-docs/?cmCbj3). Given their suggested slow rate of growth, we expect that juvenile blue monkeys are able to withstand and recover from energetic shocks to growth. Nevertheless, juvenile blue monkeys do experience energetic shortfalls, as their hypothalamic-pituitary-adrenal (HPA) axis activity is attuned to energy balance such that glucocorticoids increase during low energy states [(Thompson et al., 2020)](https://www.zotero.org/google-docs/?N5yyqy). As such, slow growth may be an adaptive response to low resource availability in their environment. We expect to see limitations to investment in either immunity or growth, depending on which is more highly prioritized.

In this study, we aim to examine the energetic costs and constraints associated with cellular immunity during juvenile development in blue monkeys. Our study had two main aims, to study the: **1) Energetic costs of immune activity**; and the **2) Energetic constraints on immune activity**. If investment in cellular immune activity is highly prioritized at the cost of growth, we expect that higher concentrations of urinary neopterin will correspond with lower C-peptide and smaller changes in lean body mass over time. We further expect the relationship between immune activity and growth to vary at different timescales, such that acute investment in immunity, such as in response to acute infections, will compromise short-term vs. long-term growth in lean muscle. Alternatively, if investment in immune activity is energetically constrained, we expect urinary neopterin to increase with energy balance and lean body mass. Lastly, in the event of constraints, we aimed to examine whether immunity is limited by low energy balance itself or via the immunosuppressive effects of glucocorticoids.

**Methods**

*Ethical statement*

*Study Site*

The wild study population resides in the Isecheno area of the Kakamega Forest, Kenya. Data were collected on 41 juveniles (20 females and 21 males, mean age 4.5 ± 1.7 yrs) for 8 months from Aug 2015 to March 2016. Subjects lived in 3 neighboring social groups (mean group sizes: 37-65). Subjects were individually identifiable by their natural physical variation and their ages were known from precise, long-term demographic records on the study population [(Cords, 2012)](https://www.zotero.org/google-docs/?mDqWiI). A team of 4 observers, including author NTG, collected all biomarker and behavioral samples after a training period of 2-months to ensure reliable identification of all subjects.

*Behavioral data collection*

Observers in the field (including NTG) conducted 20 minute focal follows, during which they recorded a subject’s activity at 1 minute intervals (e.g. resting, feeding, locomoting). Follows occurred between 07:30 and 17:00, and focal subjects were targeted for even progression in focal sampling over the month and during morning, midday, and afternoon periods. In total, observers collected 1591 h of activity budget data, with an average of 39 ± 3.1 hours per subject.

*Urine and fecal sample collection and preparation*

Biological samples were collected between the hours of 07:30 and 17:00, *ad libitum* after excretion by identified subjects. Additional sampling targeted subjects to ensure that each was sampled approximately once every 2 weeks. Urine samples that were uncontaminated by dirt, feces, or urine from other animals were pipetted from leaves or caught on a plastic sheet and transferred to a 1.5 ml polypropylene tube. Fecal samples that were uncontaminated with urine were fully homogenized with a clean stick upon collection and approximately 1 g of feces were transferred into a 1.5-15 ml plastic tube. Both types of samples were stored on icepacks and in the dark until transferred to a -20ºC solar freezer within 4 hours of collection. Samples remained frozen until they were shipped on ice to New York University for further processing. In total, observers collected 620 urine and 627 fecal samples, with each subject sampled on average 2 ± 0.8 urine and 2 ± 0.7 fecal samples per month.

NTG extracted glucocorticoid metabolites from feces at NYU following the protocols of Heistermann 1995 and Palme 2013. For full details of the extraction procedure see Thompson et al. 2020. NTG initially thawed urine samples to assay for creatinine by diluting urine samples 1:10 and following a standard Jaffe reaction, and then aliquoted remaining sample for urinary C-peptide and neopterin assays. Sample specific gravity (SG) was also measured using an AtagoTM handheld refractometer.

*Enzyme immunoassays*

NTG and JPH assayed samples for urinary neopterin with Tecan IBLTM ELISA kits (product RE59321) at New York University from Jan 2017 – May 2018. Samples were diluted 1:10 - 1:80 in ELISA kit buffer and assayed following manufacturer protocols. Serial dilutions demonstrated parallelism with the standard curve. The average intra-assay coefficient of variation (CV) for neopterin was 7.9% (N = 561 samples), and inter-assay CVs of 6.7% and 17% for high and low quality controls (N = 16 plates). Because of varying and limited urine sample volume, not all urine samples were able to be assayed for neopterin. The resulting coverage was 1.91 ± 0.75 neopterin samples per subject/month.

NTG assayed C-peptide of insulin in urine samples (uCP) using a Merck MilliporeTM RIA kit at Rutgers University. Samples were diluted 1:2 to 1:20 in RIA kit buffer prior to assay based on sample specific gravity (SG) and assayed following manufacturer’s protocols. Average inter-assay CV was 4.4% (N = 612) and inter-assay CVs were 3.92% and 3.78% for high and low quality controls (N = 9 batches). For further details of uCP assays seeThompson et al. [(2020)](https://www.zotero.org/google-docs/?9PVCcy). uCP and neopterin concentrations were corrected for SG following Miller et al. [(2004)](https://www.zotero.org/google-docs/?i8kSoh), where a given marker concentration was multiplied by a factor of the average sample SG divided by the given sample’s SG. Samples with SG < 1.003 were removed as too dilute (n=1), i.e., approaching the SG of pure water itself.

NTG assayed fecal samples for the immunoreactive fecal glucocorticoid (fGC) metabolite 11β-hydroxyetiocholanolone at the German Primate Center, Göttingen, Germany. Samples were diluted 1:80 or 1:800 and assayed according to the detailed protocol in Heisterman et al 2004. For full details surrounding the validity of the enzyme-linked immunoassay for fGCs see Thompson et al. [(2020)](https://www.zotero.org/google-docs/?Xqjscw). Intra- and inter-assay CVs were evaluated by replicated high and low quality controls run in each assay, with intra-assay CVs of 3.9% and 6% for high and low quality controls (N = 20 plates) and inter-assay CVs of 8.9% and 11.9% (N = 42 wells).

*Data analysis*

Given circadian rhythms in physiological activity, we evaluated the effects of the time of sample collection on uCP, neopterin, and fGC concentrations using a generalized linear mixed effects model (lme4 v 1.1-31, lmerTest v 3.1-3) with a gamma error distribution and log link. Marker concentrations were regressed against hours from midnight with random slopes and intercepts by subject ID. Time of day was negatively correlated with uCP, and so uCP concentrations were expressed as residuals from predicted values, derived from population average slopes and intercepts. These time adjusted residuals were made positive by adding to them the absolute value of the minimum time-adjusted residual and an infinitesimal value of 0.0001 to ensure values > 0 for log transformation.

We calculated estimated lean body mass (ELBM) as residuals of observed to expected creatinine concentrations for a given SG, according to the formula presented in Emery Thompson (2012, 2020 PTRSB) and validated in multiple primate species (e.g. orangutans, O’Connell 2021, chimpanzees, Emery Thompson et al 2012). Residuals of observed creatinine values were calculated relative to predicted creatinine values, derived from a linear regression of creatinine ng/ml predicted by linear and quadratic terms of SG (minus 1). Raw creatinine concentrations were unrelated to physical activities (i.e., time spent resting, feeding, locomoting, each p > 0.05). Residual creatinine increased with age in both individual samples (ß age = 0.04 ± 0.008, p <0.0001) and as monthly average residuals (ß age = 0.04 ± 0.009, p <0.0001; Figure 1A), and was unrelated to subject sex (sample: ß sex = 0.003 ± 0.03, p > 0.05: monthly average: ß sex = 0.004 ± 0.03 p > 0.05). Creatinine residuals also positively corresponded with uCP at the sample (ß ELBMsample = 0.013 ± 0.005, p < 0.05) and monthly average levels (ß ELBMmonth = 0.025 ± 0.01, p < 0.05; Figure 1B). These results suggest that our calculation of ELBM represented an accurate estimate of lean body mass during development in this species.

[Figure 1A & B – validation relationships of age and energy balance with estimated lean body mass]

To represent subjects’ general physiological state, urinary neopterin, uCP, fGCs, and ELBM were averaged per subject by month for several analyses (Tables 1 & 2). This time frame further allowed pairing between urinary markers, fecal markers, and monthly activity budgets. To examine longer-term effects of immune activity on growth, we calculated monthly change in lean body mass (∆ELBMmonth = month2 – month1). To assess short-term effects of immune activity on growth, we calculated change in lean body mass by subtracting ELBM of given sample at t1 from ELBM of the subsequent sample at t2 (∆ELBMsample = t2 – t1, interval of varying lengths). Total averages and standard deviations in biomarker concentrations (neopterin, uCP, lean body mass, and fGCs) are available in supplementary info (Table S1).

As dominance rank can influence priority of access to energy-rich foods (Foerster et al., 2011) and is socially inherited from mothers in this species [(Donabedian & Cords, 2021; Klass & Cords, 2015)](https://www.zotero.org/google-docs/?VDa72v), we included subject maternal dominance rank as a predictor (Tables 1 & 2). Ranks were calculated using the I&SI method in DomiCalc by author MC [(Schmid & de Vries, 2013)](https://www.zotero.org/google-docs/?8ssp58) and were based on clear winner-loser interactions using either data collected during the study period if mothers were still alive or during the mother’s last year of life. Maternal rank was expressed as the proportion of group females that a mother outranked, ranging from 0 to 1. Subject age was calculated at the mid-date of the month. Subjects’ times spent resting, feeding, and locomoting were calculated as the proportion of 1 min point samples of total observation time per month that subjects were engaged in that behavior.

Lastly, to statistically isolate the influence of body condition on investments in cellular immune function we aimed to control for seasonal and social variables that are likely to contribute to immune marker variation resulting from pathogen exposure. Monthly rainfall was calculated as a sum of local daily rainfall records in mm, collected by the Kenya Forest Service. Individuals’ total number of social partners was extracted from focal data and included unique partners with whom individuals rested in proximity, sat in contact, played, and groomed.

*Statistical analysis*

1. **Energetic costs of immune activity:** To evaluate the potential energetic costs of cellular immune activity, we constructed 3 linear mixed effects regression models using the lme4 (v 1.1-31) and lmerTest (v 3.1-3) packages (Table 1). All analyses were run in R version 4.2.1. Models 1 and 2 evaluated costs at broad levels of energy balance and growth. These included subjects’ monthly average concentrations of uCP and monthly growth (month2-month1 average ELBM) as responses, with subject age, sex, maternal rank, and monthly average neopterin concentrations as fixed effects. All models include random effects for subject ID and month to capture additional seasonal influences on biomarkers. We then examined the short term effects of immune activity on growth, using ∆ELBMsample as an outcome and sample neopterin concentration at t1 , age, and maternal rank as fixed effects (Model 3). Additionally, these models included time interval between samples at t1 and t2 (mean ± sd, 14 ± 11 days) as an independent fixed effect and in interaction with neopterin, as we expected the influence of acute concentrations in neopterin to wane with greater time between sampling. To further evaluate if juvenile behavior responded to the energetic demands of immune activity in a compensatory way, we evaluated monthly time spent resting, feeding, and locomoting as responses to average neopterin, age, sex, and maternal rank (models 4-6).

Table 1. Generalized linear mixed model structures examining **Aim 1: Energetic costs of immune activity in n = 41 subjects.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Costs Model\*** | **Response** | **Predictors** | **n** |
| 1. Energy Balance | log2 uCPmonth | Age + Sex + Maternal Rank + log2 Neopterinmonth | 293 subject months |
| 2. Monthly growth | ∆ELBMmonth | Age + Sex + Maternal Rank + log2 Neopterinmonth | 252 subject monthly ∆s |
| 3. Short-term growth | ∆ELBMsample t2-t1 | Age + Sex + Maternal Rank + log2 Neopterint1 + intervalt2-t1+ log2 Neopterint1  : intervalt2-t1 | 520 subject sample ∆s |
| 4-6. Compensatory behavior | Monthly time resting, feeding, or locomoting | Age + Sex + Maternal Rank + log2 Neopterinmonth | 293 subject months |

\*All models include subject ID and month as random effects.

1. **Energetic constraints on immune activity:** we constructed a model where energy balance and lean body mass predicted neopterin concentrations (Model 7). Here, we further controlled for total monthly rainfall (mm) and a subject’s number of social partners in the same month, as potential proxies of pathogen exposure [(Altizer et al., 2006; Nunn et al., 2015; Page et al., 2017)](https://www.zotero.org/google-docs/?TFvNUy). Given the strong positive effects of energy balance on neopterin in this model, we further explored whether low energy balance may restrict neopterin production via its inverse relationship with anti-inflammatory glucocorticoids. We constructed mediation models that included sub-models of the total effect of energy balance on neopterin (submodel a), the effect of energy balance on the potential mediator fGCs (submodel b) and the direct effect of energy balance on neopterin, controlling for fGCs (submodel c). We used the mediation package in R, which takes submodels b and c as arguments, to evaluate the proportion of the total effect of energy balance on neopterin is accounted for by the relationship of energy balance with fGCs and fGCs’ subsequent relationship with neopterin.

In all models, average and raw sample concentrations of uCP and neopterin were scaled by log base 2, to bring them onto similar scales of other predictors and to easily interpret their effect on the response. This means that a doubling of a predictor value increases the response by ß. For parsimony, we also maintained variable values on a log2 scale as responses to normalize their distributions. Where models including all individuals indicated significant costs or constraints on immune activity, we further examined the estimated coefficients of key predictor variables within individuals to evaluate the consistency of the directionality of their effects.

Table 2. Generalized linear mixed model structures examining **Aim 2: Energetic constraints on immune activity in n = 41 subjects.**

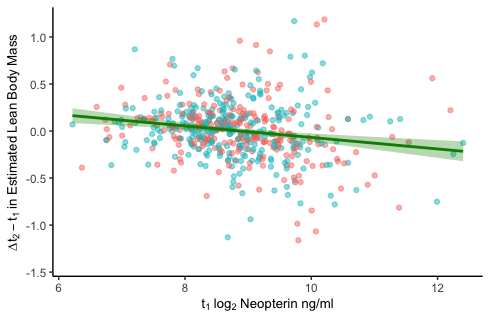
|  |  |  |  |
| --- | --- | --- | --- |
| **Constraints Model\*** | **Response** | **Predictors** | **n** |
| 7. Constraints on immune activity\*\* | log2 Neopterinmonth | Age + Sex + Maternal Rank + log2 uCPmonth + ELBMmonth + Rainfallmonth + N social partnersmonth | 293 subject months |
| *fGC mediation of constraints:* |  |  | 288 subject months |
| 8a. Total effect | log2 Neopterinmonth | Age + Sex + log2 uCPmonth |  |
| 8b. Effect on mediator | log2 fGCsmonth | Age + Sex + log2 uCPmonth |  |
| 8c. Direct effect | log2 Neopterinmonth | Age + Sex + log2 uCPmonth + fGCmonth |  |

\*All models include subject ID as a random effect. \*\*Also includes month as a random effect.

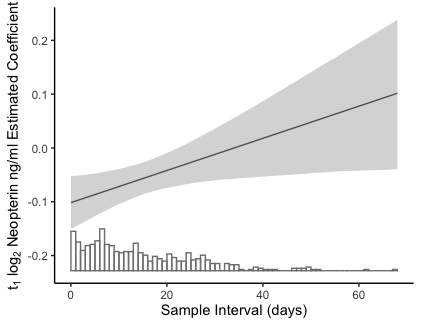
**Results**

1. *Energetic costs of immune activity*

Counter to predictions of the energetic costs and prioritization of immune activity, we found no evidence that higher monthly concentrations of neopterin predicted lower monthly growth and had a positive relationship with monthly energy balance (uCP, Table 1). Nevertheless, at shorter time scales, higher neopterin concentrations at sample t1 resulted in smaller subsequent changes in ELBM at t2 (Table 3, Fig. 1). Consistent with an acute effect of immune activity on growth, the relationship between sample neopterin and short-term growth faded as the interval between sampling times became longer (Fig. 2). The relationship between neopterin and short-term growth, alongside an absence of neopterin’s influence on monthly growth, suggests compensation in growth after acute rises in inflammation subside. The negative relationship between short term changes in body mass and neopterin concentrations was present in most within-individual analyses, 26 of 41, where the mean slope and its mean standard error were -0.15 ± 0.27. Lastly, in line with sickness behavior, higher concentrations of monthly neopterin corresponded with increased time spent resting (ß = 0.014 ± 0.005, p = 0.003, Table S2). Neopterin concentration had no relationship with monthly time spent feeding or locomoting.



**Figure 1.** Decrease of short-term growth (estimated lean body mass at sample t2-t1) with cellular immune activity (neopterin concentrations at sample t1) using LMM, n = 520. Points indicate subject-sample values for males (blue) and females (red).



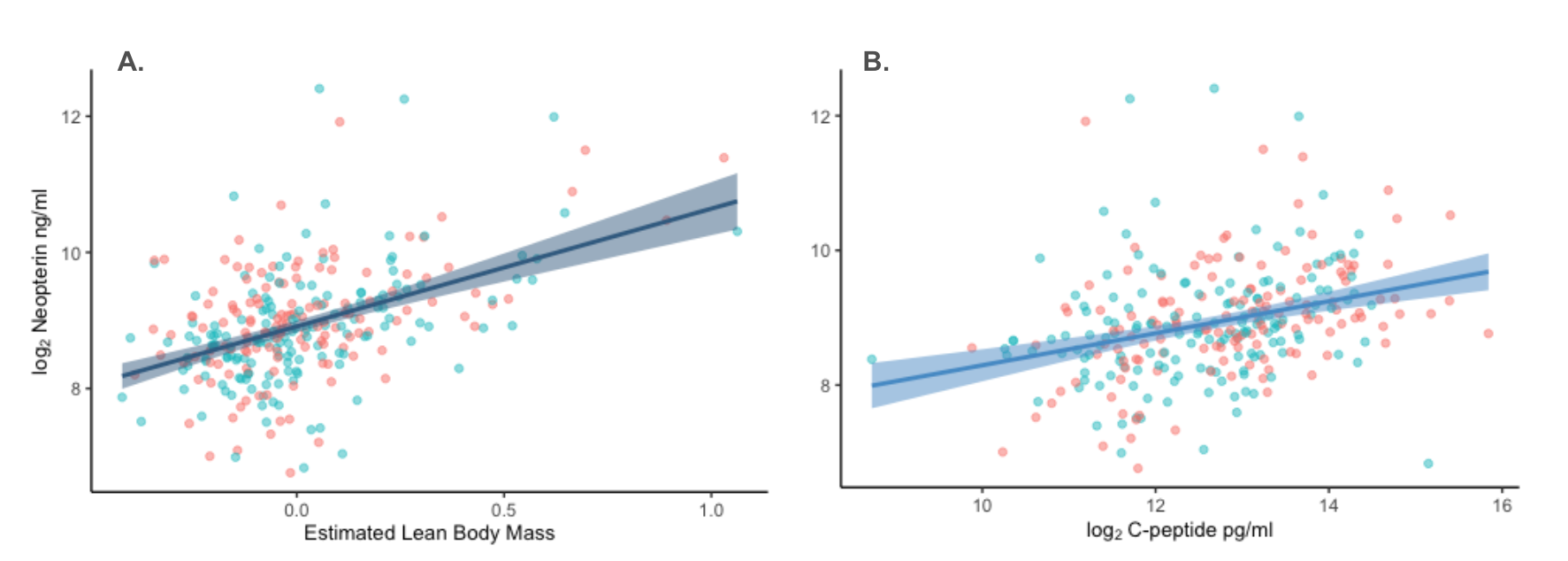
**Figure 2.** Change in model estimated coefficient (LMM) of sample t1 neopterin concentrations on subsequent short-term growth (sample t2-t1 ELBM) as the interval of t1-t2 increases. Bar rug represents frequency of intervals of a given length. The negative effect of neopterin on subsequent growth fades as the sample interval becomes longer.

Table 3. Costs of immune activity: results from LMM.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Response** | **Predictor** | **Estimate** | **SE** | **CI** | **p\_value** |
| Avg. C-peptide | Intercept | 9.532 | 0.708 | [8.14,10.92] | <0.001 |
| n = 293 | Sex | -0.302 | 0.155 | [-0.61,0] | 0.059 |
|  | Age | 0.088 | 0.047 | [0,0.18] | 0.07 |
|  | Maternal Rank | -0.062 | 0.251 | [-0.55,0.43] | 0.806 |
|  | **log2 avg Neopterin** | 0.328 | 0.075 | [0.18,0.48] | **<0.001** |
| ∆ ELBMmonth2 - 1 | Intercept | 0.617 | 0.237 | [0.15,1.08] | 0.01 |
| n = 252 | Age | 0.008 | 0.009 | [-0.01,0.03] | 0.372 |
|  | Sex | -0.023 | 0.031 | [-0.08,0.04] | 0.444 |
|  | Maternal Rank | 0.009 | 0.049 | [-0.09,0.1] | 0.846 |
|  | log2 avg. Neopterin | -0.011 | 0.021 | [-0.05,0.03] | 0.587 |
|  | **log2 avg. C-peptide** | -0.040 | 0.015 | [-0.07,-0.01] | **0.01** |
| ∆ ELBMsample t2-t1 | Intercept | 1.027 | 0.247 | [0.54,1.51] | <0.001 |
| n = 520 | Age | 0.011 | 0.008 | [0,0.03] | 0.195 |
|  | Sex | -0.005 | 0.027 | [-0.06,0.05] | 0.862 |
|  | Maternal Rank | 0.011 | 0.043 | [-0.07,0.1] | 0.802 |
|  | **log2 Neopterin**t1 | -0.102 | 0.025 | [-0.15,-0.05] | **<0.001** |
|  | log2 C-peptidet1 | -0.019 | 0.016 | [-0.05,0.01] | 0.248 |
|  | **Sample interval**t2-t1 | -0.033 | 0.013 | [-0.06,-0.01] | **0.012\*** |
|  | **log2 Neo**t1 **x sample interval** | 0.003 | 0.001 | [0,0.01] | **0.023\*** |
|  | log2 CPt1 x sample interval | 0.001 | 0.001 | [0,0] | 0.27 |

1. *Energetic constraints on immune activity*

We found that both energy balance and estimated lean body mass had strong positive relationships with neopterin, independent of subject age, sex, maternal rank, and potential pathogen exposure (Figs 3A & B, Table 4). None of these control variables demonstrated a clear relationship with average neopterin concentrations. The positive relationship between neopterin and energy balance, and neopterin and lean body mass, were present in most within individual analyses, 30 and 33 of 41 respectively, where the mean slopes and their mean standard errors were 0.16 ± 0.33 and 1.8 ± 1.8. Mediation analysis revealed that most of the total effect of uCP on neopterin was accounted for by their direct relationship. Only 5% (95% CI 0.1-14%) of variation in average neopterin concentrations was accounted for by glucocorticoids, suggesting a weak mediating role of the anti-inflammatory effects of GC secretion during states of low energy balance (Table S3).



**Figure 3.**  A)Increase in cellular immune activity (neopterin ng/ml) with estimated lean body mass (monthly average Creatinine-SG residual). B) Increase in cellular immune activity with energy balance (monthly average C-peptide ng/ml). Results using LMM, n = 293. Points indicate subject-month values for males (blue) and females (red).

**Table 4.** Constraints and drivers of cellular immune activity: results of LMM, n = 293 subject months.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Response** | **Predictor** | **Estimate** | **SE** | **CI** | **p\_value** |
| Avg. Neopterin | Intercept | 7.144 | 0.641 | [5.89,8.4] | <0.001 |
|  | Sex | -0.041 | 0.099 | [-0.23,0.15] | 0.678 |
|  | Age | 0.001 | 0.032 | [-0.06,0.06] | 0.975 |
|  | Maternal Rank | 0.051 | 0.158 | [-0.26,0.36] | 0.748 |
|  | **ELBM** | 1.447 | 0.239 | [0.98,1.91] | **<0.001** |
|  | **log2 C-peptide** | 0.14 | 0.042 | [0.06,0.22] | **0.001** |
|  | Monthly rainfall | -0.016 | 0.031 | [-0.08,0.05] | 0.630 |
|  | N social partners | 0.007 | 0.01 | [-0.01,0.03] | 0.476 |

**Discussion**

In this study, we aimed to evaluate the energetics of cellular immune activity during development in a wild primate, considering both its potential energetic costs and constraints. Evidence of the costs of cellular immunity was limited to short term growth: elevated concentrations of neopterin were unrelated to growth from month to month, however they corresponded with less growth over shorter time intervals. Juveniles with higher neopterin rested more but did not change time spent feeding or locomoting. In addition to its cost in relation to short-term growth, cellular immune activity appeared to be limited by individual body condition: both estimated lean body mass (creatine-SG residual) and energy balance (urinary C-peptide) corresponded positively with neopterin concentrations, independently of any individual attribute, or environmental and social factors. This apparent energetic constraint on cellular immunity was only weakly mediated by anti-inflammatory effects of glucocorticoids that increased in low energy conditions. Overall, these energetic dynamics of cellular immune activity suggest that cellular immunity is not generally prioritized over growth during development in blue monkeys and can in fact be energetically constrained. We discuss the energetic costs and constraints on immune activity in detail and in the context of current literature.

Cellular immune activity, as measured by concentrations of urinary neopterin, did not influence changes in estimated lean body mass from month to month in juvenile blue monkeys, however, it did acutely inhibit growth. Increased resting did not account for lower short-term growth, as creatinine excretion, which served as the basis of lean body mass estimates, was unrelated to physical activity. The timescale of neopterin’s influence on growth in lean body mass suggests that developmental growth was able to compensate quickly for short term setbacks. Despite a general gap in animal physiological literature on the time scale of immune costs to developmental growth, these results do align with literature demonstrating that growth is highly prioritized during development. In chickens selected for enhanced immune function, there was little observed trade-off with growth [(van der Most et al., 2011)](https://www.zotero.org/google-docs/?vEYWZ1). In human forager-horticulturalists, inflammatory immune activity also showed trade-offs in childhood height velocity over 1-week intervals, however did not affect longer-term changes in height [(Urlacher et al., 2018)](https://www.zotero.org/google-docs/?F7a4m2).

However costly, cellular immune activity also appeared constrained by body condition in juvenile blue monkeys. Lower energy balance and lean body mass both corresponded with lower neopterin levels. This relationship was independent of seasonal and social variables that represent potential pathogen exposure, i.e. monthly rainfall and an individual’s number of social partners, which were unrelated to neopterin concentrations. Although seasonal effects on immune function are abundant in the animal literature, evidence for a direct effect of energetic constraints is rare. For example, prairie voles decrease body mass and IgG production during short-day photoperiods [(Nelson et al., 1996)](https://www.zotero.org/google-docs/?upw0lW), however these effects are likely driven by seasonal reproductive regression and corresponding changes in androgens [(Dark et al., 1983; Nelson et al., 1996)](https://www.zotero.org/google-docs/?jviaz8). Other animals, such as Siberian hamsters, have demonstrated a direct effect of energy on immunity, such that experimental reductions in circulating energy availability directly reduced humoral immune function [(Zysling & Demas, 2007)](https://www.zotero.org/google-docs/?3AUYkA). Similarly, in healthy and non-obese human adults, caloric restriction lowers total white blood cell and lymphocyte counts [(Meydani et al., 2016)](https://www.zotero.org/google-docs/?V9Orlp).

An alternative explanation for the positive relationship observed between immune activity and energy balance could involve the consumption of foods that are energetically dense yet found in areas that pose high infection risk, such as anthropogenic food sources [(Becker et al., 2015)](https://www.zotero.org/google-docs/?XfU8Ew). Although better nutrition from calorically dense foods may augment healthy immune function, initial exposures to anthropogenic pathogens nevertheless heighten immune activity. Heightened cellular immune activity after exposures could be particularly likely for young individuals that are encountering many pathogens for the first time. In this study’s population, the home ranges of multiple study groups overlap with a forest station and tree nursery that contains a large oil palm with high fat fruits [(Takahashi et al., 2019)](https://www.zotero.org/google-docs/?MtGTRt). When feeding in this area, animals may consume abundant calories, however they are exposed to anthropogenic disturbance and potential one-on-one interactions with humans. Future analyses could analyze whether the relationship of immune activity and energy balance is indirectly driven, in part, by the differential use of habitats and the nutritional content of diets consumed therein.

The relationship of physical condition and cellular immune activity in juvenile blue monkeys was not substantially mediated by the immunosuppressive effects of elevated glucocorticoids during low energy states, similar to results found for Siberian hamsters [(Zysling & Demas, 2007)](https://www.zotero.org/google-docs/?mL5A2l). This evidence is consistent with the hypothesis that low energy, in the form of low glucose, may directly constrain immune activity, however other mediating factors could be at play. For example, fasting leads to lower levels of circulating leptin in rodents (Lord 1998; Xu Wang 2010), which is essential for maintenance of cell-mediated immunity (McDade; 2003). The mediating role of adipose tissue and leptin have yet to be evaluated in the study population.

It is possible that energetic limitations on cellular immune activity are a particular risk for developing individuals, given the high priority given to physical growth during development. During adulthood, a primary competing demand to immunity and somatic maintenance is reproduction, which can be delayed or terminated [(Emery Thompson, 2013)](https://www.zotero.org/google-docs/?kJMG5V) with arguably fewer impacts to lifetime fitness than stunted growth [(Blanckenhorn, 2005; Prendergast & Humphrey, 2014)](https://www.zotero.org/google-docs/?7HbHo0) or the costs associated with compensatory growth [(Hector & Nakagawa, 2012; Lin et al., 2022)](https://www.zotero.org/google-docs/?VweCTn). Further, juveniles may be particularly susceptible to the negative consequences resulting from energetic constraints on immune activity because of their immunological naïveté [(Altizer et al., 2006)](https://www.zotero.org/google-docs/?ltNffs) and their corresponding need to sustain higher WBC counts than adults [(Nunn et al., 2009)](https://www.zotero.org/google-docs/?8j8b4Z). For this reason, a population or social group with a greater number of juveniles may overall become more susceptible to disease spread during food scarcity [(Altizer et al., 2006)](https://www.zotero.org/google-docs/?GJZAmY).

**Limitations of the study**

Our study is limited by the difficulty of differentiating immune activity and immune function *per se* in an observational study. Juveniles’ true infection status or pathogen exposure was unknown, therefore low concentrations of markers for immune activity could indicate a relatively healthy state, free of infection, or an immunosuppressed state, which would respond insufficiently to infection. Independent markers of infection status (e.g., experimentally induced infection, antigen-specific antibodies) and immune function (e.g., challenge tests), would allow for a more precise evaluation of the costs of activity and energetic limitations on function. Nevertheless, two aspects of this study bolster our interpretations. First, monthly average markers of immune activity provide a more integrative measure of investment in immune activity than single samples. Second, we control for environmental and social dimensions of pathogen exposure in analyzing the energetic constraints of immune activity (Tables 2 & 4). Together, these methods and controls allow us a degree of confidence that individuals in poorer body condition, i.e. with lower body mass for a given age and energy balance, face energetic limitations to cellular immune activity and function.

The mechanisms of energetic constraints on immunity during development in blue monkeys are still unclear. Our findings suggest that glucocorticoids are not a primary mediator responsible for lower neopterin with lower energy balance, however we do not rule out their role entirely. Measures of urinary glucocorticoids, rather than fecal metabolites, would allow for finer temporal pairings between markers of HPA axis activity and urinary markers of energy balance and immune activity, to potentially reveal stronger associations and immunosuppression. Further, future research to develop non-invasive techniques of measuring adipose tissue and circulating leptin will aid in determining whether depleted fat stores are critical to limiting cellular immunity in wild mammal populations.

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**Author contributions:**

NTG designed and performed the research. NTG and LF analysed data and wrote the initial draft. JH and EV supported laboratory analyses. MC contributed long-term demographic data, maintained and provided access to the fieldsite. All authors contributed to edits and gave final approval for publication.

**Declaration of Interest:**

The authors declare no competing interests.

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