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THE EFFECTS OF MIGRATORY FLIGHT ON HEMATOLOGIC PARAMETERS IN NORTHERN BALD IBISES (*GERONTICUS EREMITA*)

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Abstract: Under the project of "Human-Led Migration," the authors had the unique opportunity to accompany hand-raised northern bald Iibises (NBIs; *Geronticus eremita*) during migration, which occurred in stages from Bavaria, Germany, to southern Tuscany, Italy. The aim of this study was to investigate the immediate effects of flight, with respect to flight duration, and the more delayed recovery effects on hematologic variables. A total of 31 birds were sampled. Blood samples were taken immediately before takeoff, after landing, and 1 day after the flight. Hematocrit was determined and blood smears were prepared to estimate the total white blood count (tWBC) with leukocyte concentrations (absolute [abs.]) and differential blood cell count (%). Postflight, significant decreases in hematocrit, tWBC, lymphocytes (abs., %), heterophils (abs.), eosinophils (abs., %), and monocytes (abs.) were observed. In contrast, heterophils (%), basophils (%), and the heterophil/lymphocyte (H/L) ratio increased significantly. With increasing flight duration, the H/L ratio increased further. One day postflight, there were still significant decreases in tWBC, lymphocytes (abs.), and eosinophils (abs., %) and significant increases in heterophils (%) and the H/L ratio. The hematocrit dropped even further. These data show that the decrease of tWBC is mainly caused by the lymphocyte fraction and that NBIs need more than 1 day to reverse the postflight changes in some hematologic values. Hematocrit changes postflight and on the recovery day are most likely to be explained by hemodynamics and the metabolic and hormonal changes caused by flight. The hematologic changes postflight in NBIs were largely consistent with those of other birds, but they differed from humans and mammals postexercise mainly in the levels of tWBC, heterophils (matching neutrophils in mammals), and lymphocytes.

Key words: Flight, *Geronticus eremita*, hematocrit, ibis, leukocytes, recover.

INTRODUCTION

In birds, only a few studies on the hematologic changes caused by flight have been published. One main problem is that under field conditions, it is difficult to obtain blood samples immediately before and after flight, especially from the same animal. The literature contains studies regarding hematologic changes caused by flight that were performed in wind tunnels with European starlings (*Sturnus vulgaris*), red knots (*Calidris canutus rufa*), or pigeons (*Columba livia*), which were transported to their release sites and were sampled after returning to their lofts.[5,34,43,46] In most of these studies, hematologic values were collected before flight from a control group of rested birds and postflight from another flown group.[5,43,46] Other studies describe hematologic changes caused by migration, for example in red knots, thrushes, and passerines caught at their migratory stopover sites.[12,34,47] In these studies, birds of different groups, but not individual animals, were compared. As the present study was performed within the framework of a feasibility study for the reintroduction of northern bald ibises (NBIs; *Geronticus eremita*), the authors had the opportunity to evaluate flight-induced changes within an individual bird during migration, which makes these data unique.

The NBI has been classified in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as critically endangered since 1994.[32] This species was formerly widespread in Europe, but it disappeared sub-

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stantially from the European continent over 400 yr, ago largely as a consequence of human impact.[39] Presently, only one wild breeding population consisting of a few hundred birds remains, located on the Atlantic coast of Morocco.[6] Until the 1950s, a migratory colony with more than 1,000 individuals existed in Birecik in southern Turkey. These birds wintered in Ethiopia.[30] During the 1960s through to the 1970s, the population decreased substantially, mainly due to intensive use of pesticides.[30] In 1990, the Birecik population was declared extinct.[3]

In their first year, young NBIs show an intrinsic motivation to leave their breeding region.[3,22,62] It is known that these birds require adult conspecifics to guide them to an appropriate wintering area, as the migration route is a social tradition passed on from generation to generation.[22,62] In 2002, a project group, the "Waldrappteam" ("Waldrapp" means NBI in German), was founded in Austria to reestablish a free-ranging and migrating population of the NBI in central Europe.[20] Since 2014, the Waldrappteam has led a European LIFE+ reintroduction project for the NBI.[21] The birds are encouraged to learn a migration route by "Human-Led Migration" (HLM). This setup offers an excellent opportunity to study the dynamics and function of formation flight as well as the physiology of migrating birds, as the tame birds are easy to handle and their flights are well controlled in terms of distance, velocity, and duration.[52,63]

The aims of the present study were to investigate the variation of hematologic variables in NBIs during migratory flight from the breeding site to a wintering site, to gain information regarding the immediate effects of flight and flight duration on variables in the peripheral blood, and to assess kinetics of recovery effects.

MATERIALS AND METHODS

Field study design and animals

The project was carried out within the framework of a feasibility study for the reintroduction of NBIs adhering to the IUCN reintroduction guidelines[33] during the HLM flights in 2008, 2009, and 2010. The fledglings were obtained under a license from the Vienna Zoo (Austria), the Zurich Zoo (Switzerland), the Prague Zoo (Czech Republic), the Konrad Lorenz Research Station (Austria), and the Game Park Rosegg (Austria). The animal experiment was under license from and approved by the Bundesministerium fuer Wissenschaft und Forschung, Referat fuer Tierversuchswesen und Gentechnik, Vienna, Austria (BMWF-66.006/0014-II/3b/2010). The hatchlings were hand-raised in Scharnstein/Upper Austria (2008) and at the Salzburg (2009) and Munich (2010) zoos. At the fledging stage, they were transferred to a training site near the city of Burghausen in Bavaria. In the fall they were guided along an assigned migrating route to a wintering area in southern Tuscany, Italy (World Wildlife Fund conservation area Laguna di Orbetello).[22] The HLM was performed using microlight aircraft (Paraplane Xcitor, Fresh Breeze, 30900 Wedemark/Bissendorf, Germany) in consecutive flights. The close relationships of the birds with their foster parents made the birds follow the aircraft with a foster parent sitting inside as a lure. The flight training began after the fledging stage, at the age of 6 to 7 wk, and continued until the start of the migration in August. The flights started in the morning between 7 and 8 AM. An accompanying ground team sought to reach the landing site before the birds arrived to enable immediate postlanding sampling. The takeoff and landing points were small airports or grasslands. The flight distances were planned in advance. Changing weather conditions or the birds' unwillingness to fly on some days caused repeated deviations from the daily flight plan.

In total, 39 clinically healthy birds (2008: 5 males and 7 females; 2009: 4 males and 7 females; 2010: 12 males and 4 females) aged 13 to 16 wk with body weights from 1.1 to 1.4 kg and estimated total blood volumes from 110 to 140 ml were included in the study. The total blood volume of a bird is approximately 10% of its body weight.[7] The health status was monitored by clinical, parasitologic, and microbiologic examinations. The birds were fed ad libitum three times a day. The diet was a mixture of minced rats, beef heart, and day-old chicks supplemented with insects, curds, crushed snail shells, and vitamins A, D_3, and E (Trigantol® solution, Bayer Austria, 1160 Vienna, Austria). The birds had free access to fresh drinking water and spent the nights in a transportable aviary ($6 \times 9 \times 3$ m; width $\times$ length $\times$ height).

In 2008, the birds were randomly divided into two groups and each group was sampled alternately, every second flight stage. In 2009 and 2010, all individuals of the group were sampled. The interval between the sampled flights was at least 4 days. The first blood sample (P1) was taken in the morning within 30 min before takeoff. The second blood sampling (P2) was conducted between 13

and 42 min after landing. The recovery-day sample (P3) was collected on the following day in the morning between 8 and 9 AM. In 2010 no recovery-day blood samples (P3) were taken.

Blood collection and analyses

All birds were familiar with being handled. For the blood collection they were restrained and the head was covered with a lightproof cloth hood. The blood samples were collected from the right jugular or basilic vein. To prevent blood clotting a 2.5-ml heparinized (Heparin Gilvasan, 5,000 IE/ml, Gilvasan Pharma Austria, 1190 Vienna, Austria) plastic syringe was used with a 25-gauge needle to draw 1.5 ml of blood. Immediately after blood collection, blood smears from each bird were prepared using the two-slide wedge technique at an angle of 45°,[54] and two microhematocrit tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey07417, USA) were filled and centrifuged at 10,000 *g* for 5 min to determine the hematocrit. The blood smears were stained with Hemacolor within a few days after the flight.[7,28] All blood smears were evaluated in random order by a veterinary pathologist (GS), trained in avian hematology, who was blinded to the samples in terms of individual and collection time. Cell morphology, presence of blood parasites, and all cell counts were assessed using an oil-immersion lens (×100). The microscope used was a Nikon ECLIPSE E200 (Optoteam Praezisionsinstrumente Vertriebsgesellschaft.m.b.H., 1230 Vienna, Austria) with an eyepiece diameter of 20 mm. The total white blood cell count (tWBC, cell count × 10^6/L) was estimated using a modified equation of Campbell and Ellis based on the average number of leukocytes in 20 (instead of 5) monolayer fields.[7] In individuals with hematocrit values outside the range of 40–50%, the samples were corrected with an extended equation.[7] For the differential blood cell count, 200 leukocytes in the monolayer field were counted and categorized into lymphocytes, monocytes, and heterophilic, eosinophilic, and basophilic granulocytes.[54] The absolute values (abs.) of leukocyte subpopulations was calculated from the estimates of total WBC (tWBC) and differential blood cell counts.

Statistical analysis

The targets of the statistical analysis are the immediate preflight to postflight (P1–P2) and the preflight to 1-day postflight (P1–P3) changes concerning hematologic variables. Due to the inhomogeneous data structure and imbalanced study design of each individual year analysis of variance (ANOVA) and *t*-tests were applied. The data were normally distributed in accordance with the results of Kolmogorov-Smirnov. For each individual in 2008 and 2009, the mean was calculated for the hematologic variables and for the flight distance of all flights per bird. To screen the consistency of P1 in hematologic variables, the years were compared using ANOVA and the genders were compared using Student's *t*-test. With the paired *t*-test, differences associated with flight (P1–P2, P1–P3) were analyzed for all 3 yr cumulatively and for each individual year. Significant differences were corrected with a Bonferroni adjustment. The analysis for each individual year was performed to compare the tendencies of the changes between the years. Correlations between flight duration and hematologic effects were analyzed using the Pearson correlation test. Changes were considered significant, if $P < 0.05$. Additionally, the differences of P1–P2 and P1–P3 were calculated in percentages for all hematologic variables. All analyses were conducted using IBM SPSS v19 (SPSS Statistics 19, IBM, Armonk, New York 10504, USA).

RESULTS

Between years, the total flight distance of migration varied from 1,205 to 1,295 km due to differences in the itineraries. The flight altitudes were between 50 and 300 m above ground level and up to 1,500 m above sea level depending on geographic and meteorologic terms. The flight speeds (true air speed) ranged from 30 to 45 km/hr, with the variation resulting from the continuous adjustment of speed to match the flight behavior of the birds. The HLMs lasted 37 days (2008), 38 days (2009), and 26 days (2010) until arrival in Orbetello. Overall, 10/12 birds completed the journey in 2008, 11/11 in 2009, and 14/16 in 2010. The 2008 migration was completed in 13 flights, of which 10 were sampled. In the summer of 2009, constant rainfalls impeded the necessary flight training for 1 mo, hence only 3 of 17 flights could be sampled. The 2010 migration was completed in seven extended flights and the longest flight was sampled. Additional information regarding the birds, flights, and collected samples is summarized in Table 1. The sample-size numbers per individual varied, as not all of the projected blood samples could be successfully collected, and some blood smears had to be discarded due to poor quality. The cell morphology was similar in all birds; blood parasites could not be detected.

Table 1. Data from birds, flights, flight duration, collected samples, and usable samples for hematocrit (Hct) and white blood count (WBC).

Determining factor	2008	2009	2010
No. of birds	11	11	9
Gender of birds (female/male)	7/4	7/4	3/6
No. of consecutive flights	13	17	7
No. of sampled flights	10	3	1
No. of sampled flights per bird	4–5	1–3	1
Interval in days between flights	2–10	1–5	2–7
Interval in days between sampled flights	4–11	9, 10	—
Flight duration in minutes of sampled flights	66–205	75, 113, 148	335
No. of sample collection	156	87	18
No. of sample collection preflight	52	29	9
No. of sample collection postflight	52	29	9
No. of sample collection 1 day postflight	52	29	—
No. of usable samples (Hct/WBC)	116/129	72/70	14/13
No. of usable samples preflight (Hct/WBC)	39/41	29/29	6/6
No. of usable samples postflight (Hct/WBC)	39/49	25/23	8/7
No. of usable samples 1 day postflight (Hct/WBC)	38/39	18/18	—

Consistency of P1 in hematologic variables

The P1 values showed significant differences between the years in hematocrit ($P = 0.000$), heterophils (abs.: $P = 0.002$; %: $P = 0.001$), lymphocytes (abs.: $P = 0.013$; %: $P = 0.002$), eosinophils (%: $P = 0.039$), and the heterophil/lymphocyte ratio (H/L ratio: $P = 0.010$), see Table 2. There were no significant gender-specific differences found in the hematologic parameters for P1 between each year.

Immediate postflight changes in hematologic variables

Considering all 3 yr cumulatively, there was a highly significant change in P1–P2 in almost all variables (Table 3; Fig. 1A). Differences between absolute and relative values occurred in heterophils, basophils, and monocytes. Significant decreases were observed in hematocrit ($P = 0.002$; –4.6%), tWBC ($P = 0.000$; –31.7%) and the concentration of lymphocytes ($P = 0.000$; –41.4%), heterophils ($P = 0.008$; –9.8%), eosinophils ($P = 0.000$; –64.4%), and monocytes ($P = 0.015$; –13.17%). Regarding the differential blood count, a significant decrease was observed in lymphocytes ($P = 0.000$; –14.3%) and eosinophils ($P = 0.000$; –49.1%) and an increase in heterophils ($P = 0.000$; +32.6%), basophils ($P = 0.000$; +70.7%), and the H/L ratio ($P = 0.000$; +63.3%).

By comparing the changes in P1–P2 for each individual year, similar trends could be observed

Table 2. Mean, SD, and significance (*) of preflight hematologic values among the years 2008–2010. Means with different superscript letters refer to significant differences.

Analyte	*P*	2008 Mean ± SD	2009 Mean ± SD	2010 Mean ± SD
Hematocrit	0.000*	46.4 ± 1.9^{A}	51.2 ± 2.5^{B}	44.8 ± 2.6^{A}
Heterophils (%)	0.001*	32.7 ± 6.6	27.1 ± 3.1^{A}	38.2 ± 5.9^{B}
Eosinophils (%)	0.039*	8.7 ± 2.4^{A}	7.1 ± 1.4	6 ± 2.1^{B}
Basophils (%)	0.136	2.9 ± 0.8	2.6 ± 0.9	2 ± 0.6
Lymphocytes (%)	0.002*	54.4 ± 6^{A}	61.7 ± 3.3^{B}	52.8 ± 6.1^{A}
Monocytes (%)	0.138	1.4 ± 0.5	1.6 ± 0.5	1.1 ± 0.4
Total white blood count (10^6/L)	0.068	6,032.5 ± 451.8	6,590 ± 712.4	6,912.5 ± 1,195.9
Heterophils (10^6/L)	0.002*	1,964.1 ± 385.3^{A}	1,798.1 ± 346.7^{A}	2,637.7 ± 610.2^{B}
Eosinophils (10^6/L)	0.177	518.7 ± 151.3	467.5 ± 100.5	398.7 ± 100.2
Basophils (10^6/L)	0.329	171 ± 44.7	167 ± 49.5	136.8 ± 42.6
Lymphocytes (10^6/L)	0.013*	3,294.9 ± 468.6^{A}	4,058.2 ± 461.5^{B}	3,664.6 ± 826.5
Monocytes (10^6/L)	0.264	83.7 ± 27.6	99.2 ± 33.4	74.7 ± 30.9
Heterophil/lymphocyte ratio	0.010*	0.63 ± 0.24	0.45 ± 0.07^{A}	0.74 ± 0.21^{B}

Table 3. Mean, SD, and significance (*) of preflight–postflight and preflight–1 day postflight variations in hematologic values, which are expressed as percentage differences.

Analyte	Preflight–postflight			Preflight–1 day postflight		
	Mean	SD	*P*	Mean	SD	*P*
Hematocrit	−4.6	7.2	0.002*	−9.5	5	0.000*
Heterophils (%)	+32.6	16.9	0.000*	+7.9	12	0.005*
Eosinophils (%)	−49.1	21.2	0.000*	−18.6	20	0.000*
Basophils (%)	+70.7	79.3	0.000*	+6.6	32.8	0.831
Lymphocytes (%)	−14.3	9	0.000*	−1.4	6.6	0.334
Monocytes (%)	+24.2	64.6	0.278	+18.4	86.4	0.940
Total white blood count (10^6/L)	−31.7	10.7	0.000*	−8.1	12.2	0.005*
Heterophils (10^6/L)	−9.8	19.8	0.008*	−1	18.2	0.624
Eosinophils (10^6/L)	−64.4	16.4	0.000*	−25.6	19.9	0.000*
Basophils (10^6/L)	+15.2	53.8	0.438	−3.3	30.6	0.279
Lymphocytes (10^6/L)	−41.4	10	0.000*	−9.3	13.9	0.004*
Monocytes (10^6/L)	−13.2	52.4	0.015*	+10.3	80.4	0.490
Heterophil/lymphocyte ratio	+63.3	37.2	0.000*	+10.8	20.3	0.018*

in several areas (Table 4). Each year, tWBC ($P = 0.000$), lymphocytes (abs.: $P = 0.000$; %: $P < 0.02$), and eosinophils (abs.: $P < 0.01$; %: $P < 0.01$) decreased, and heterophils (%:$P < 0.01$) plus the H/L ratio ($P < 0.01$) increased significantly. A significant decrease in hematocrit ($P = 0.003$), heterophils (abs.: $P = 0.001$), and monocytes (abs.: $P = 0.009$) was found in 2009. A significant increase in monocytes (%: $P = 0.000$) was detected in 2008 and basophils (%: $P < 0.02$) in 2008 and 2009.

One-day-postflight changes in hematologic parameters and effects of flight duration

One day after flight, some hematologic variables still showed significant deviations from the initial hematologic status (Table 3; Fig. 1B). Differences between absolute and relative values occurred in heterophils and lymphocytes. A decrease was observed in the hematocrit ($P = 0.000$; −9.5%), the tWBC ($P = 0.005$; −8.1%), the concentration of lymphocytes ($P = 0.004$; −9.3%) and eosinophils ($P = 0.000$; −25.6%), and the percentage of eosinophils ($P = 0.00$; −18.6%). The H/L ratio ($P = 0.018$; +10.8%) and the percentage of heterophils ($P = 0.005$; +7.9%) were likewise still significantly increased.

When 2008 and 2009 were analyzed separately, similar significant trends were observed for P1–P3, with enduring decreases in the hematocrit ($P = 0.000$) and eosinophils (abs., %: $P < 0.02$). Differences from the overall trend for P1–P3 were observed through increases of heterophils (%:$P = 0.008$) and the H/L ratio ($P = 0.025$) in 2008 and decreases in tWBC ($P = 0.008$) and lymphocytes (abs.: $P = 0.017$) in 2009 (Table 4).

In response to increased flight lengths including sampled flights from all 3 yr, the H/L ratio ($P = 0.011$) and the percentage of heterophils ($P = 0.033$) increased significantly.

DISCUSSION

The present study is the first to analyze the influence of well-documented flights during migration on hematologic variables through paired blood samples obtained from defined individual birds.

Some preflight hematologic values of the examined NBIs differed significantly between the sampled years. Despite these differences, the WBC count was characterized by a lymphocytic hemogram. It is known that hematologic reference intervals in birds can be quite wide and, therefore, the observed differences among the years were expected and can be explained by biologic variance.[17,29,51]

The hematocrit of NBIs declined significantly due to the flights and declined even further 1 day after the flight. Postflight decreases in the hematocrit have also been reported in pigeons,[5] in red knots flying in wind tunnels for 10 hr, and in several migratory thrushes and bar-tailed godwits (*Limosa lapponica taymyrensis*).[34,40,47] It is suggested that during flight, the heart's load is reduced and the blood flow through the organs is improved by a lowered hematocrit.[5] Other assumptions are that with the onset of flight, hemodilution may be adaptive because it reduces blood viscosity and thereby the energy expenditure by the heart. Furthermore, during long flights it may be a sign of water conservation against the risk of dehydration, or may be adaptive to decreased oxygen

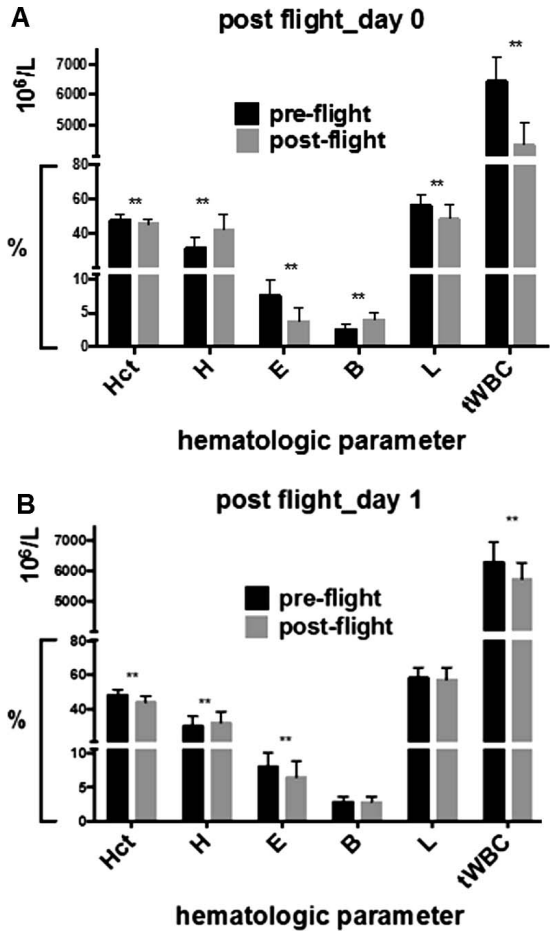

Figure 1. Effects of flight on hematocrit, total white blood count, and differential blood count. **A**. Immediately postflight. **B**. One day postflight. The black bars show the preflight values, and the gray bars the postflight values. Specification of significance using paired Student's *t*-test (**$P < 0.01$); equal distribution of the values has been tested; hematocrit (Hct); heterophils (H); eosinophils (E); basophils (B); lymphocytes (L); total white blood count (tWBC).

needs as the body mass decreases.[34] The latter type of adaptation is also supported in bar-tailed godwits.[40] A further explanation regarding the lowered hematocrit in migratory birds is associated with a lower fat score[27,34] and the inability to sustain erythropoiesis,[34] and it is probably also associated with short-term food deprivation and malnutrition.[27,47]

Hematocrit changes in mammals after a bout of exercise show an opposing trend and correlate with the degree of exertion. In humans,[13,42,53] dogs, and horses,[31,38,55–57,61] the hematocrit has been shown to increase postexercise and decrease only after an extremely long endurance run of more than 1,000 km in humans and sled dogs.[15,18]

Hematocrit changes can be caused by the number and size of erythrocytes and/or by changes in the plasma volume. Avian erythrocytes have an ovoid shape and nucleus and tend to be larger and less deformable than mammalian erythrocytes, and thereby have more difficulties traveling through the capillaries.[60] A decrease in the hematocrit leads to a reduced viscosity and a tightened blood flow.[60] As a result, travel through the capillaries is facilitated. The decreased hematocrit postflight in the NBIs is most probably induced by the required adaptations of blood viscosity and peripheral blood flow during flight. This explanation is substantiated by the observations made in the year 2009, in which the birds had significantly higher initial hematocrit values than the birds of 2008 and 2010. Additionally, when the years were analyzed separately, only the birds of 2009 showed a significant decrease in the hematocrit postflight. This may have been a required adaptation to flight that was more essential in 2009 than in the other years, as due to bad weather conditions only a few training flights could be performed prior to HLM in 2009. The amount of sampling blood volume per flight was less than 0.3% of body weight, and should therefore not affect the hematocrit. Presumably, malnutrition, short-term food deprivation, severe intravascular hemolysis, and the inability to sustain erythropoiesis were not the reasons for the decreased hematocrit observed in the present study, as the NBIs were well nourished, the food deprivation lasted only for a few hours, the supernatant plasma did not show any red discoloration, and the lifespan of avian erythrocytes is longer than 1 day. Other factors, such as the levels of oxygen and carbon dioxide, oxygen tension, pH, metabolic products, extracellular ion concentration, vasoactive agents, circulating hormones, body mass, and erythrocyte size, may have influenced the hematocrit and/or the peripheral blood flow.[60] These variables were not evaluated in the present study, but according to the recently published additional data of the blood profile for NBIs of the year 2008, including plasma metabolites, enzymes, electrolytes, blood gases, and reactive oxygen compounds, no significant decrease in hematocrit was observed, although some of the mentioned blood variables were significantly altered postflight.[4] One of the mechanisms not mentioned so far could be sequestration of red blood cells (RBCs) into parenchymatous organs,

Table 4. Mean, SD, and significance (*) of preflight–postflight (years 2008–2010) and preflight–1 day postflight (years 2008 and 2009) variations in hematologic values, which are expressed as percentage differences.

	Pre-flight - post-flight			Pre-flight - 1 day post-flight		
Analyte	Mean	SD	*P*	Mean	SD	*P*
2008						
Hematocrit	−1.8	3.4	0.088	−6.9	4.2	0.000*
Heterophils (%)	+36.4	14.5	0.000*	+10.4	11.2	0.008*
Eosinophils (%)	−43.8	13.5	0.000*	−20.9	22.5	0.014*
Basophils (%)	+47.2	33	0.001*	+6.8	30.1	0.995
Lymphocytes (%)	−16.9	4.8	0.000*	−2.5	7.6	0.291
Monocytes (%)	+51.7	61.8	0.008*	+6.6	48.8	0.799
Total white blood count (10^6/L)	−23.6	9.2	0.000*	−3.9	11.2	0.238
Heterophils (10^6/L)	+3.5	17.7	0.627	+6	16.9	0.279
Eosinophils (10^6/L)	−56.2	10.9	0.000*	−22.8	24.6	0.013*
Basophils (10^6/L)	+10.9	24.8	0.379	+1.2	28.1	0.631
Lymphocytes (10^6/L)	−36	8.5	0.000*	−6.5	13.8	0.121
Monocytes (10^6/L)	+15.6	50.9	0.869	+3.2	49.7	0.534
Heterophil/lymphocyte ratio	+76.8	30.3	0.000*	+15.6	22	0.025*
2009						
Hematocrit	−9.3	7.3	0.003*	−12.4	4.2	0.000*
Heterophils (%)	+25.6	15.7	0.000*	+5.1	12.8	0.265
Eosinophils (%)	−40	17.3	0.000*	−16	17.6	0.012*
Basophils (%)	+74	86.9	0.017*	+6.4	37.1	0.741
Lymphocytes (%)	−8.2	7.2	0.006*	−0.2	5.4	0.885
Monocytes (%)	−6.4	59.5	0.339	+31.3	116.6	0.798
Total white blood count (10^6/L)	−37.6	9.2	0.000*	−12.8	12	0.008*
Heterophils (10^6/L)	−22.6	15.8	0.001*	−8.8	17	0.078
Eosinophils (10^6/L)	−62.5	13.8	0.000*	−28.6	13.6	0.000*
Basophils (10^6/L)	+8.5	59.6	0.810	−8.3	34	0.306
Lymphocytes (10^6/L)	−42.4	10.3	0.000*	−12.5	14	0.017*
Monocytes (10^6/L)	−40.3	46.6	0.009*	+18.2	107.1	0.689
Heterophil/lymphocyte ratio	+40.3	27.7	0.001*	+5.5	17.9	0.423
2010						
Hematocrit	−1.9	9	0.593	—	—	—
Heterophils (%)	+37.1	21.8	0.004*	—	—	—
Eosinophils (%)	−73.9	21.9	0.006*	—	—	—
Basophils (%)	+108.4	118.2	0.061	—	—	—
Lymphocytes (%)	−19.8	12.5	0.015*	—	—	—
Monocytes (%)	+24.9	64.7	0.533	—	—	—
Total white blood count (10^6/L)	−36.5	5.5	0.000*	—	—	—
Heterophils (10^6/L)	−12.9	15.4	0.086	—	—	—
Eosinophils (10^6/L)	−82.5	16.2	0.001*	—	—	—
Basophils (10^6/L)	+34	83	0.406	—	—	—
Lymphocytes (10^6/L)	−49.4	6.4	0.000*	—	—	—
Monocytes (10^6/L)	−20.6	42.7	0.201	—	—	—
Heterophil/lymphocyte ratio	+76.9	48.2	0.005*	—	—	—

such as spleen and liver, which could be investigated by radiolabeled RBCs.

The significantly lower tWBC in NBIs postflight was mainly caused by decreased circulating lymphocytes. Thrushes, like NBIs, undergo significant decreases in the tWBC and lymphocytes (abs.) due to migration. It is hypothesized that the low tWBC levels in migrating thrushes might indicate a stress-induced immunosuppression via depression of splenic function.[47] However, neither suppressed humoral nor cell-mediated immune responses were found in red knots after intensive flight exposure in a wind tunnel experiment.[26] There is evidence that in response to glucocorticoids, circulating lymphocytes adhere to the endothelial cells of blood vessel walls and transmigrate from the blood circulation into other tissues, such as the lymph nodes, spleen, bone marrow, and skin in rodents.[9,16,19] The bidirectional kinetics of lymphocytes, allowing them to travel

via bloodstream and lymphatics into lymphoid and nonlymphoid organs, are probably responsible for this phenomenon in NBIs. This suggestion is supported by the fact that preflight lymphocyte concentration is almost regained after 1 day of recovery. In contrast to the NBIs, heterophils (abs.) increased in Swainson's thrushes (*Catharus ustulatus*), but not in veery thrushes (*Catharus fuscescens*), eosinophils (abs.) stayed unaltered in both thrush species.[47] Surprisingly, no effects on the total leukocyte concentration or any type of leukocytes in European starlings flying in the wind tunnel have been reported.[46] These authors hypothesized that this was due to the highly variable leukocyte profiles in their study, a short flight time, stress-associated variability, and/or alterations by heparin.

In contrast to NBIs postflight, the tWBC and the concentration of neutrophils (matching heterophils in birds) increased and the concentration of lymphocytes changed more or less nonuniformly in humans and other mammals following endurance-based exercise.[8,10,13,15,36,42,53,55,59]

The changes in the differential blood count postflight in NBIs, including the H/L ratio (matching neutrophil/lymphocyte [N/L] ratio in mammals), coincide largely with the results of comparable studies in birds, humans, and mammals, except for the smaller leukocyte subpopulations (eosinophils, monocytes, and basophils). Postflight a similarly significant increase in heterophils (%) and a decrease in eosinophils (%) was reported in pigeons, as in NBIs. In contrast to NBIs, pigeons exhibited an additional decrease in monocytes (%) postflight.[43] As in NBIs, significant increases in heterophils (%) and basophils (%) and a decrease in lymphocytes (%) were observed in red knots postmigration. Contrary to the present study, monocytes (%) decreased and eosinophils (%) remained unaltered in red knots postmigration.[12]

Like NBI heterophils, neutrophils (%) increased and lymphocytes (%) decreased after endurance running in humans and in horses.[42,53,55] In humans, lymphocytes (%) increased after a half marathon and decreased after a 100-km run.[42,53] In humans, the decrease of lymphocytes (%) seems to be dependent on the length of the run and thus on exercise duration. Similar to NBIs, a decrease of eosinophils (%) was reported in humans after a half marathon and horses.[42,55] Irregularities found in the percentages and concentrations of eosinophils, basophils, and monocytes among species postflight or after endurance-based exercise indicate an inconsistent reaction in these types of blood cells to physical effort, or are attributable to error-proneness inherent to technical/manual and further statistical evaluations.

Regarding the H/L ratio or the N/L ratio, a significant increase was reported in pigeons[43] and migratory birds (red knots, thrushes) postflight as well as in humans and horses after endurance running.[8,11,12,47] In vertebrates, the elevation in the H/L ratio or the N/L ratio is a reliable indicator of stress, and presumably the adrenal and leukocyte responses to stress are closely linked.[14] In case of eosinophils it is suspected that a reduction may be associated with stress reaction.[14] In birds, leukocyte variables other than H/L ratio may vary between species and different stressors.[1,14,45,48,58] The mechanism behind the changes in leukocyte count and distribution during and after exercise is partially attributed to neuroendocrinologic factors, such as catecholamines (adrenalin, noradrenalin) and glucocorticoids (cortisol, corticosterone) in humans and horses.[36,44,50,55,59]. In humans, a transient lymphocytosis during exercise is attributed to catecholamines, and a delayed lymphocytopenia and neutrophilia to glucocorticoids.[49] Chickens also exhibit an elevated H/L ratio in response to elevated corticosterone levels.[24] In pigeons, an elevated corticosterone concentration was documented postflight.[25] Moreover, long migratory bar-tailed godwits exhibited higher corticosterone concentrations after arriving on a migratory stopover site compared to during the refueling period, and corticosterone concentrations during refueling were positively correlated with size-corrected body mass, suggesting a corticosterone elevation when birds prepare to reinitiate flight.[41] However, studies in migratory red knots suggest that corticosterone concentrations are not affected by long-distance flight.[26,35] In migratory and nonmigratory birds, the possible effect of neuroendocrine factors remains to be determined in the context of leukocyte counts postflight.

One day postflight, the hematocrit continued to decrease in NBIs, as has been reported in humans and horses following endurance-based exercise.[13,37,42,55] In humans, this phenomenon seems to be the result of an increased plasma volume 1 day postexercise, which is presumably caused by an influx of plasma proteins and/or by an increased plasma albumin content.[2,23] Thus, the further decrease of the hematocrit 1 day postflight in NBIs could be the effect of a fluid shift between the intracellular and the extracellular compartment caused by metabolic changes.

Further long-term 1-day-postflight effects in NBIs included persistent decreases in tWBC, lymphocytes (abs.), and eosinophils (abs. and %), and increases in H/L ratio and heterophils (%). Although these variables were still significantly altered, they approximated the preflight values.

In conclusion, the prolonged exertion of migratory flight appears to affect the hematologic variables of NBIs by decreasing the hematocrit and the tWBC and increasing the H/L ratio. The decreases in hematocrit postflight and 1 day postflight were most likely a result of hemodynamic, metabolic, and hormonal changes caused by flight, but the mechanism behind the decreases could not be clarified. It was proven that the decrease of tWBC is mainly caused by the lymphocyte fraction. Furthermore, NBIs require more than 1 day to recover from the postflight changes in some leukocyte variables. Hematologic changes postflight in NBIs largely agree with those of other birds but differ from mammals and humans following endurance-based exercise mainly in the levels of tWBC; heterophils and neutrophils, respectively; and lymphocytes. Due to the difficulty in obtaining blood samples from the same individual immediately preflight and postflight, there are only a few comparable studies on postflight hematologic parameters in birds. To clarify and decipher the physiologic causes of the hematologic changes observed in birds postflight, further specific studies will be necessary.

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