Physiological and welfare consequences of transport, relocation, and acclimatization of chimpanzees (Pan troglodytes)

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\textbf{A B S T R A C T}

Manipulations of the environments of captive nonhuman primates often have welfare consequences to the animals, including behavioral effects, and for certain manipulations, physiological effects as well. The processes of transporting, relocating, and acclimatizing nonhuman primates across facilities represent manipulations that are likely to have welfare, behavioral, and physiological consequences to the relocated animals. Seventy-two chimpanzees were relocated from the Primate Foundation of Arizona (PFA) in Arizona to the Keeling Center (KCCMR) in Texas. Animals were transported for approximately 21 h in single cages in a USDA-approved, climate-controlled trailer. Chimpanzees were weighed, anesthetized, and blood samples were collected (1) immediately prior to departure from PFA, (2) immediately upon arrival at the KCCMR, and (3) at additional time point(s) between 3 and 12 weeks after arrival at the KCCMR. Chimpanzees were quarantined in familiar pairs or social groups for 60–90 days at the KCCMR. Blood samples were analyzed for hematological and clinical chemistry parameters and compared across time points. In addition, samples from a subset of animals were assayed for cell-mediated immune parameters. Comparisons of the data obtained just prior to transport, to the data obtained immediately upon arrival, revealed numerous statistically significant differences in hematological, clinical chemistry, and immunological parameters. Some of these were indicative of stress, and thus, changes in welfare state, although many remained within the published normal ranges for chimpanzees. Additional analyses showed that many of the clinical chemistry values collected 3–12 weeks after arrival at the KCCMR had returned to pre-transport values. In contrast, of the cell-mediated immune parameters that were affected by transport and relocation, few had returned to pre-transport levels 8 weeks after transport, and three of the four hematology variables analyzed had not returned to pre-transport levels 12 weeks after transport. Comparisons of body weights before and immediately after transport revealed that animals lost an average of 2.5 kg during the 21-h transport, a statistically significant reduction that some animals never regained. These results demonstrate that transport and relocation affect a variety of physiological parameters with potential welfare implications and that some of these effects last as long as 3 months. These findings have important implications for the welfare and use of recently transported nonhuman primates, especially chimpanzees, in biomedical research. In order to allow animals to adapt to their new surroundings and to prevent unwanted confounds from influencing experiments, sufficient time must be provided after transport for chimpanzees to acclimatize.

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1. Introduction

Nonhuman primates maintained in laboratory environments often experience a wide array of manipulations, such as weaning, single housing, and long distance transportation that can, at least transiently, affect both their welfare and their suitability as experimental subjects (Bloomsmith and Else, 2005; Bloomsmith et al., 2006; Capitanio et al., 2006; Tardif et al., 2006). While many welfare assessments have relied on changes in behavioral variables (Clay et al., 2009; Lutz and Novak, 2005; Schapiro, 2002; Schapiro et al., 2001), others have utilized a number of physiological measures (Boinski et al., 1999; Crockett et al., 1993; Lambeth et al., 2006; Schapiro et al., 1993). Specifically, among the most appropriate measures to use, are the physiological parameters that are likely to appear as dependent measures in experiments involving the nonhuman primate species (Lambeth et al., 2006; Schapiro et al., 2000, 2007; Schapiro, 2002). Such an assessment approach maximizes the relevance of the effects of the manipulation and its relation to welfare, and minimizes the potential confusion that may result from invoking procedurally problematic relationships between measures like cortisol and concepts like stress. If a management strategy affects natural killer (NK) cell activity, a measure of innate immunity, for a rhesus monkey that is going to be used in an investigation involving simian-human immunodeficiency virus (SHIV), it is critical to identify this effect (Hill et al., 2004). From the viewpoint of the specific study, it is not as important to relate the change in NK activity to cortisol levels or ‘stress’.

Nonhuman primates are frequently relocated prior to the beginning of an experimental study (Capitanio et al., 2006; Koban et al., 2010). Many of these relocations involve simple movements of animals from the holding/breeding/stock area at a facility to a new experimental room or building at the same facility (Capitanio and Lerche, 1998; Davenport et al., 2008; Kagira et al., 2007). However, some relocations involve more extensive interfacility transport episodes (Fernstrom et al., 2008; Honess et al., 2004; Kagira et al., 2007; Koban et al., 2010; Watson et al., 2005; Wolfensohn, 1997). These can be either within a continent, or across continents, as is the case for the importation of wild-caught or captive-bred monkeys from countries of origin in Africa, Asia, or South America for research conducted in Europe or North America (Honess et al., 2004; Koban et al., 2010; Malaga et al., 1991). Overall, relocations of nonhuman primates are of considerable interest from welfare and a number of other perspectives (Depoyster, 2003; Prescott and Jennings, 2004; Swallow et al., 2005).

There are relatively few published studies that have empirically analyzed the effects of transport and relocation on the welfare and utility of laboratory nonhuman primates. Koban et al. (2010), Kim et al. (2005), Honess et al. (2004), and Watson et al. (2005), have presented behavioral and/or physiological data on the effects of transport on cynomolagus macaques, rhesus macaques, and Garnett’s bushbabies, respectively. The responses of all three species were indicative of negative changes in welfare state as a function of the transportation episode. There are even fewer studies that have attempted to quantify the amount of time that nonhuman primates require to acclimatize to new environments and management procedures after they have been relocated; regardless of whether the relocation was to the next room or halfway around the world (Capitanio et al., 2006; Kagira et al., 2007; Obernier and Baldwin, 2006).

In contrast, there are a fair number of studies that have looked at the effects of transportation, relocation, and acclimatization on welfare, as assessed by both behavioral and physiological variables, in other laboratory and domestic animal species. A small sample of these studies focused on physiological variables have found changes in antibody levels in mice (Landi et al., 1982); blood glucose, cholesterol, and blood urea nitrogen in rats (van Ruiven et al., 1998); lymphocyte counts in dogs (Bergeron et al., 2002); white blood cell counts, body weights, and natural killer cell activity in pigs (McClone et al., 1993); and thyroid hormones in horses and calves (Fazio et al., 2001, 2009). We will examine similar variables for similar reasons in the current investigation.

Between 2006 and 2009, 72 chimpanzees were relocated from the Primate Foundation of Arizona (PFA) in Mesa, Arizona, USA to the Michale E. Keeling Center for Comparative Medicine and Research (KCCMR) of The University of Texas MD Anderson Cancer Center in Bastrop, Texas, USA. This relocation episode provided an ideal opportunity to assess the welfare effects of a 21-h (1600 km) overland transportation episode. By weighing the animals and collecting anesthetized blood samples prior to shipment from PFA, immediately upon arrival at the KCCMR, and after various amounts of time spent at the KCCMR, it was possible to measure the effects of transport on welfare and to gain some insight into the amount of time required by chimpanzees to acclimatize (physiologically) to their new surroundings.

The data collected and analyzed were intended simply to begin to identify the potential welfare effects of transport and relocation, and to start to quantify aspects of the acclimatization process. The data were not intended to provide insights into the potential psychological and physiological mechanisms that might be driving the observed changes. In particular, serum cortisol was not selected as a dependent measure of interest, given the difficulties associated with its use as a measure of the effects of stressors in nonhuman primates, independent of the acute stress associated with the sampling process itself. As we have done in the past, we instead chose to study those dependent measures that are likely to be (1) affected by this type of manipulation, (2) useful in assessments of welfare, and (3) utilized in biomedical research projects (Lambeth et al., 2006; Schapiro et al., 2000, 2007; Schapiro, 2002). These included a select subset of clinical chemistry and hematology measures, as well as cell-mediated immune responses that are particularly useful in immunodeficiency virus investigations, such as proliferation responses, cytotoxic activity, and cytokine production (Nehete et al., 1995; Schapiro et al., 2000).

While transport and relocation were the independent variables of interest for this study, these are multidimensional manipulations that are comprised of many influential factors (i.e., anesthetization and recovery; time...
spent alone during transport; climate/management differences between the two facilities, etc.). Many of these factors are likely to contribute to the observed differences, however, it would be difficult to separate the effects of individual factors.

The present study was designed to (1) determine whether transportation and relocation significantly affected welfare-related physiological measures, including commonly collected clinical chemistry and hematology parameters, and less commonly collected, but potentially more revealing, cell-mediated immune measures; and (2) determine the amount of time required for affected dependent measures to return to baseline levels after animals were transported and relocated (acclimatization).

2. Materials, methods, and ethical approval

2.1. Subjects

Subjects included 72 common chimpanzees (Pan troglodytes) of both sexes, ranging in age from 11 to 47 years. All subjects were relocated to the KCCMR after residing at PFA for periods between 11 and more than 30 years. All animals were in good health at the time of transport and immediately upon arrival at the KCCMR. Neither pre-shipment nor post-shipment physical examinations identified any subjects with acute health problems.

2.2. Housing

Housing conditions were NOT identical at PFA and the KCCMR, however, both facilities maintain their chimpanzees in social groups; in enclosures that significantly exceed the proscribed minimums for area, volume, and complexity; and have active and effective husbandry, veterinary, and behavioral management programs. Three important differences between the facilities included (1) the use of a dry bedding husbandry system, (2) sexual segregation as a means of pregnancy prevention, and (3) the restriction of a small subset of animals to indoor-only housing on a rotating basis at PFA, compared to the KCCMR’s use of (1) a wet ‘wash-down’ husbandry system, (2) IUDs or hormonal implants as the preferred means of contraception, and (3) indoor–outdoor housing for all animals at all times. For recent, general descriptions of the PFA and KCCMR housing situations, see Fritz and Howell (2001) and Whiten et al. (2007), respectively.

2.3. Transport and relocation

Subjects traveled by USDA-approved, climate-controlled trailer from Mesa, Arizona to Bastrop, Texas in the USA. Driving time was approximately 21 h, with animals leaving PFA in the late afternoon and arriving at the KCCMR in the early afternoon of the next day. All chimpanzees were singly caged while in the trailer, were checked several times for general well-being, as well as for appropriate amounts of food and water, and were provided with enrichment during the journey. Between October 2006 and October 2009, 11 trips were required to move all 72 subjects. Animals were transported during periods of the year in which the weather was temperate in both Arizona and Texas; in March, April, October, or November. The trailer contained between five and eight animals per trip.

2.4. Sample collection

Animals were anesthetized with Telazol®, weighed, and blood was collected at PFA in the mid to late afternoon, just prior to boarding the trailer. Venipuncture from the cephalic vein was performed on anesthetized animals for CBC and clinical chemistry analyses at this point (the pre-transport time point) for all subjects. For some of the subjects (n = 24), additional tubes of blood were collected during the same anesthetic episode to be used for assays of cell-mediated immune responses. Immediately upon arrival at the KCCMR in the early afternoon, subjects were again anesthetized, weighed, and additional anesthetized blood samples were collected (the upon-arrival time point). The chimpanzees were placed in compatible pairs (comprised of former groupmates from PFA) in the KCCMR’s indoor–outdoor quarantine facilities. Chimpanzees were only singly housed (1) during the actual transport from Arizona to Texas and (2) during recovery from the initial anesthetic episode for their first few hours at the KCCMR. A maximum of 30 mL of blood was collected at each of the two time points, well below the maximum permissible limit for adult chimpanzees that weighed approximately 60 kg.

Additional weights and blood samples were collected from anesthetized subjects during the afternoon at various time points between 3 and 12 weeks after arrival (the post-arrival time points) at the Keeling Center. While we attempted to collect these samples at identical times (post-transport) for all subjects, this goal proved unattainable. Hence, many of the analyses presented below involve data collected from smaller subsets of the 72 transported animals. Each analysis presented below will specify the number of subjects from which data were included in the comparisons.

2.5. Dependent variables

All blood samples from all time points were analyzed in the clinical pathology laboratory at the KCCMR using the standard hematology and clinical chemistry procedures and panels employed for the chimpanzee colony (Ihrig et al., 2001; Lambeth et al., 2006). All standard clinical chemistry and CBC parameters were collected for all samples, but for the purposes of this report, only the subset of parameters that were thought to be of clinical/experimental relevance and that seemed likely to be affected by transport, relocation and acclimatization (Bergeron et al., 2002; Kagira et al., 2007; Kim et al., 2005; Landi et al., 1982; McGlone et al., 1993; Schapiro, 2002; van Ruiven et al., 1998) were analyzed. These included, for clinical chemistry, levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), cholesterol, creatinine, glucose, lactate dehydrogenase (LDH), potassium, total protein, and triglycerides. For hematology, lymphocytes (%), red blood cells (number),
segmented neutrophils (%), and white blood cells (number) were analyzed.

For those subjects for whom additional tubes of blood were collected for immunological analyses, the following assays were performed in the immunology research laboratory at the KCCMR according to previously published protocols (Dolbier et al., 2001; Nehete et al., 1995; Schapiro et al., 2000): natural killer cell activity (a measure of innate immunity; E:T from 12.5:1 to 100:1), proliferation responses to mitogens (measures of B-cell proliferation; phytohemagglutinin (PHA), pokeweed mitogen (PWM), lipopolysaccharide (LPS)); lymphocyte subset analyses (distributions of types of immune cells; CD3+, CD4+, CD8+, CD4+CD8+, CD16+, CD20+); cytokine production (measures of T-cell activation and immune reactivity: IL-2, IL-4, IL-10, IL-12, IFN-γ); and Elispot assays (measures of suppressor T-cell function; IFN-γ production to PHA, PWM, LPS, and concanavalin A (Con A)). These particular assays were chosen because they provide an assessment of a number of different types of immunological responses, and (1) are typically used in nonhuman primate immunodeficiency virus experiments (Nehete et al., 1995) and (2) have been shown to be potential indicators of changes in primate welfare in earlier studies of the effects of management practices, enrichment, dominance, and social housing (Lambeth et al., 2006; Schapiro et al., 2000, 2006; Schapiro, 2002).

Body weights of subjects (in kg) prior to transport were compared to body weights immediately upon arrival, and after 1 and 2 months of residence at the KCCMR.

2.6. Statistical analyses

Data were analyzed using parametric techniques, including primarily mixed-models and repeated-measures analysis of variance (ANOVA). To determine whether the transport and acclimatization process as a whole affected the dependent variables, mixed-models ANOVAs with sex as a between-subjects factor and time point as a within-subjects factor were performed. To specifically test whether the transport process affected the dependent variables, planned comparisons of the pre-transport time point to the ‘upon-arrival’ time point were performed. To determine whether subjects had acclimatized to their new environment, planned comparisons of the pre-transport time point to the ‘after-arrival’ time point(s) were performed. As mentioned above, data were not available for all subjects at all time points. Results were considered statistically significant if $P < 0.05$. Appropriate correction factors for multiple comparisons were employed when required.

2.7. Ethical approval

All procedures were conducted in accordance with all relevant guidelines and were approved by the UTMDACC IACUC (protocol # 07-92-03887, SJ Schapiro, PI and protocol # 08-02-10383, CR Abele, PI). The Primate Foundation of Arizona was fully accredited by AAALAC-International while the chimpanzees lived there and the KCCMR has been fully accredited by AAALAC-International since 1979.

3. Results

3.1. Clinical chemistry

3.1.1. Overall effect of transport, relocation, and acclimatization

Mixed-models ANOVA, with sex as a between-subjects factor and time point as a within-subjects factor were performed on all subjects for whom analyzable samples had been obtained at at least three time points (pre-transport, upon-arrival, and at least one after-arrival). Unless otherwise noted, $n = 52$ for all of the reported comparisons (see Table 1). These analyses revealed significant main effects of time point for glucose $[F(2,100) = 19.7, P < 0.001]$, total protein $[F(2,100) = 38.5, P < 0.001]$, ALT $[F(2,100) = 11.3, P < 0.001]$, ALK Phos $[F(2,100) = 8.3, P < 0.001]$, potassium $[F(2,100) = 37.0, P < 0.001]$, LDH $[F(2,100) = 27.6, P < 0.001]$, total protein $[F(2,100) = 38.5, P < 0.001]$, and creatinine $[F(2,100) = 33.0, P < 0.001]$. There were no statistically significant effects of sex, although there were significant time point by sex interaction effects for BUN $[F(2,58) = 4.1, P < 0.05, n = 31]$, potassium $[F(2,100) = 4.5, P < 0.05]$, and creatinine $[F(2,100) = 8.3, P < 0.001]$.

3.1.2. Effect of transport

Planned comparisons between the pre-transport and the upon-arrival time points revealed significant increases in glucose $[F(1,50) = 17.6, P < 0.001]$, total protein $[F(1,50) = 23.3, P < 0.001]$, ALT $[F(1,50) = 8.3, P < 0.001]$, ALK Phos $[F(1,50) = 6.8, P < 0.002]$, cholesterol $[F(1,50) = 12.8, P < 0.001]$, and creatinine $[F(1,50) = 15.0, P < 0.001]$, and significant decreases in potassium $[F(1,50) = 46.3, P < 0.001]$ and BUN $[F(1,29) = 6.0, P < 0.01, n = 31]$ for females, but not males. Comparisons resulting in statistically significant differences would be indicative of an effect of the transport process.

3.1.3. Process of acclimatization

Planned comparisons between the pre-transport and the after-arrival time points revealed significant increases in glucose $[F(1,50) = 9.2, P < 0.001]$ and cholesterol ($< 45$ days only) $[F(1,50) = 5.4, P < 0.01]$ and significant decreases in total protein ($< 45$ days only) $[F(1,50) = 7.1, P < 0.001]$, potassium $[F(1,50) = 45.7, P < 0.001]$, and creatinine ($< 45$ days only) $[F(1,50) = 7.4, P < 0.002]$. Comparisons resulting in differences that were NOT statistically significant would typically be indicative of a return of values to pre-transport levels, essentially acclimatization.

3.2. Hematology

3.2.1. Overall effect of transport, relocation, and acclimatization

Mixed-models ANOVA, with sex as a between-subjects factor and time point as a within-subjects factor were performed on all subjects for whom analyzable samples had been obtained at at least three time points (pre-transport, upon-arrival, and at least one after-arrival). Unless otherwise noted, $n = 60$ for all of the reported comparisons (see Table 2). These analyses revealed significant main effects of time point for white blood cells $[F(2,116) = 25.8, P < 0.001]$, red
Table 1
Chemistry data by time point (mean values ± 1 s.d.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Pre-transport</th>
<th>Upon arrival</th>
<th>≤45 days after arrival</th>
<th>Between 46 and 90 days after arrival</th>
<th>Typical values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>U/L</td>
<td>29.24 ± 12.03</td>
<td>47.11 ± 27.41</td>
<td>32.85 ± 20.16</td>
<td>29.77 ± 10.02</td>
<td>26.49–47.49</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALK Phos)</td>
<td>U/L</td>
<td>65.97 ± 40.21</td>
<td>67.66 ± 38.97</td>
<td>60.94 ± 18.54</td>
<td>64.06 ± 18.58</td>
<td>85.22–111.0</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN)</td>
<td>mg/dL</td>
<td>10.18</td>
<td>9.61</td>
<td>9.94</td>
<td>10.69</td>
<td>11.15–13.59</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN) females</td>
<td>mg/dL</td>
<td>9.67 ± 3.59</td>
<td>8.79 ± 4.01</td>
<td>9.24 ± 3.52</td>
<td>9.85 ± 4.40</td>
<td>11.15–12.15</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN) males</td>
<td>mg/dL</td>
<td>10.70 ± 3.47</td>
<td>12.77 ± 4.02</td>
<td>13.75 ± 5.20</td>
<td>13.70 ± 5.52</td>
<td>13.14–13.59</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg/dL</td>
<td>180.36 ± 38.99</td>
<td>193.36 ± 43.50</td>
<td>191.19 ± 38.91</td>
<td>191.40 ± 48.50</td>
<td>179.50–263.39</td>
</tr>
<tr>
<td>Creatinine all</td>
<td>mg/dL</td>
<td>1.03</td>
<td>1.11</td>
<td>0.96</td>
<td>1.00</td>
<td>0.86–1.20</td>
</tr>
<tr>
<td>Creatinine females</td>
<td>mg/dL</td>
<td>0.98 ± 0.27</td>
<td>1.03 ± 0.27</td>
<td>0.93 ± 0.27</td>
<td>0.97 ± 0.29</td>
<td>0.86–1.15</td>
</tr>
<tr>
<td>Creatinine males</td>
<td>mg/dL</td>
<td>1.16 ± 0.20</td>
<td>1.32 ± 0.36</td>
<td>1.07 ± 0.17</td>
<td>1.09 ± 0.22</td>
<td>1.00–1.20</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>79.61 ± 28.20</td>
<td>104.93 ± 19.92</td>
<td>90.58 ± 16.96</td>
<td>92.75 ± 19.18</td>
<td>83.60–121.72</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>U/L</td>
<td>443.43 ± 476.51</td>
<td>709.03 ± 808.38</td>
<td>330.64 ± 276.27</td>
<td>295.73 ± 122.31</td>
<td>272.72–401.70</td>
</tr>
<tr>
<td>Potassium all</td>
<td>mequiv./L</td>
<td>5.10</td>
<td>3.29</td>
<td>3.58</td>
<td>3.58</td>
<td>2.84–4.04</td>
</tr>
<tr>
<td>Potassium females</td>
<td>mequiv./L</td>
<td>5.20 ± 1.30</td>
<td>3.29 ± 0.61</td>
<td>3.50 ± 0.39</td>
<td>3.57 ± 0.42</td>
<td>2.84–3.72</td>
</tr>
<tr>
<td>Potassium males</td>
<td>mequiv./L</td>
<td>4.38 ± 1.11</td>
<td>3.40 ± 0.35</td>
<td>3.84 ± 0.38</td>
<td>3.74 ± 0.38</td>
<td>3.27–4.04</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/dL</td>
<td>7.48 ± 0.41</td>
<td>7.84 ± 0.46</td>
<td>7.29 ± 0.44</td>
<td>7.42 ± 0.53</td>
<td>7.20–7.80</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>mg/dL</td>
<td>140.53 ± 124.33</td>
<td>113.81 ± 78.76</td>
<td>124.54 ± 60.30</td>
<td>117.88 ± 50.60</td>
<td>69.84–119.50</td>
</tr>
</tbody>
</table>

* All n = 52, except for BUN where n = 31.
* All n = 48, except for BUN where n = 26.
* Adapted from Schapiro and Lambeth (2010), Herndon and Tiggès (2001), and Ihrig et al. (2001).
* P < 0.05 for the main effect of time point.
* P ≤ 0.05 for the comparison to the pre-transport value.
* P ≤ 0.05 for the sex × time point interaction effect.

3.2.2. Effect of transport
Planned comparisons between the pre-transport and the upon-arrival time points revealed significant increases in white blood cells [F(1,58) = 21.7, P ≤ 0.001], red blood cells [F(1,58) = 7.3, P ≤ 0.001; for males only], and segmented neutrophils [F(1,58) = 40.0, P ≤ 0.001], and a significant decrease in lymphocytes [F(1,58) = 41.2, P ≤ 0.001]; statistically significant differences again would be indicative of an effect of the transport process.

3.2.3. Process of acclimatization
Planned comparisons between the pre-transport and the after-arrival time points revealed a significant increase in segmented neutrophils [F(1,58) = 20.0, P ≤ 0.001], and significant decreases in red blood cells [F(1,58) = 28.6, P ≤ 0.001] and lymphocytes [F(1,58) = 22.9, P ≤ 0.001]; differences that were NOT statistically significant again would typically be indicative of a return of values to pre-transport levels, essentially acclimatization.

3.3. Immunology
3.3.1. Overall effect of transport, relocation, and acclimatization
Repeated-measures ANOVA, with time point as the within-subjects factor were performed on all subjects for whom analyzable samples had been obtained at four time points (pre-transport, upon-arrival, 1 month after arrival, and 2 months after arrival; see Table 3). These analyses revealed significant main effects of time point for IFNγ production stimulated with PHA [F(3,66) = 3.5, P ≤ 0.02, n = 23], IFNγ production stimulated with LPS [F(3,33) = 5.6,

Table 2
Hematology data by timepoint (mean values ± 1 s.d.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Pre-transport</th>
<th>Upon arrival</th>
<th>&lt;45 days after arrival</th>
<th>Between 45 and 90 days after arrival</th>
<th>Typical values</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>10³/µL</td>
<td>8.92 ± 3.65</td>
<td>12.10 ± 3.73</td>
<td>9.56 ± 4.07</td>
<td>9.81 ± 3.34</td>
<td>10.07–14.42</td>
</tr>
<tr>
<td>Red blood cells all</td>
<td>10⁹/µL</td>
<td>5.26</td>
<td>5.28</td>
<td>4.98</td>
<td>5.03</td>
<td>4.38–6.14</td>
</tr>
<tr>
<td>Red blood cells females</td>
<td>10⁹/µL</td>
<td>5.13 ± 0.42</td>
<td>5.10 ± 0.51</td>
<td>4.79 ± 0.39</td>
<td>4.96 ± 0.44</td>
<td>4.38–5.50</td>
</tr>
<tr>
<td>Red blood cells males</td>
<td>10⁹/µL</td>
<td>5.67 ± 0.25</td>
<td>5.96 ± 0.37</td>
<td>5.48 ± 0.36</td>
<td>5.42 ± 0.43</td>
<td>5.00–6.14</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>%</td>
<td>47.96 ± 19.99</td>
<td>71.45 ± 11.81</td>
<td>62.73 ± 15.61</td>
<td>62.52 ± 17.25</td>
<td>Not available</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>%</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
<td>3.61–9.51</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>10⁹/µL</td>
<td>46.18 ± 22.54</td>
<td>15.14 ± 11.55</td>
<td>32.00 ± 14.14</td>
<td>31.98 ± 15.24</td>
<td>Not available</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>10⁹/µL</td>
<td>3.06</td>
<td>2.48</td>
<td>2.52</td>
<td>2.42</td>
<td>3.88–6.39</td>
</tr>
</tbody>
</table>

* All n = 60, except lymphocyte number for which n = 11.
* All n = 46, except lymphocyte number for which n = 10.
* Adapted from Schapiro and Lambeth (2010).
* P ≤ 0.05 for the main effect of time point.
* P ≤ 0.05 for the comparison to the pre-transport value.
* P ≤ 0.05 for the sex × time point interaction effect.
Table 3

Immunology data by time point (mean values).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Units</th>
<th>Pre-transport</th>
<th>Upon arrival</th>
<th>One month after arrival</th>
<th>Two months after arrival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A</td>
<td>24</td>
<td>S.I.</td>
<td>3.44</td>
<td>4.80</td>
<td>5.18</td>
<td>3.16</td>
</tr>
<tr>
<td>PHA</td>
<td>24</td>
<td>S.I.</td>
<td>1.76</td>
<td>2.93</td>
<td>3.50</td>
<td>2.65</td>
</tr>
<tr>
<td>PWM</td>
<td>23</td>
<td>S.I.</td>
<td>2.14</td>
<td>2.69</td>
<td>2.48</td>
<td>1.62</td>
</tr>
<tr>
<td>IFN-γ to Con A</td>
<td>20</td>
<td>SFC/10^6 cells</td>
<td>475.55</td>
<td>692.65</td>
<td>649.55</td>
<td>559.75</td>
</tr>
<tr>
<td>IFN-γ to PHA</td>
<td>23</td>
<td>SFC/10^6 cells</td>
<td>562.17</td>
<td>916.7</td>
<td>561.13</td>
<td>587.39</td>
</tr>
<tr>
<td>IFN-γ to PWM</td>
<td>25</td>
<td>SFC/10^6 cells</td>
<td>2.14</td>
<td>2.69</td>
<td>2.48</td>
<td>1.62</td>
</tr>
<tr>
<td>IFN-γ to LPS</td>
<td>12</td>
<td>SFC/10^6 cells</td>
<td>145.08</td>
<td>160.50</td>
<td>243.67</td>
<td>77.50^b</td>
</tr>
<tr>
<td>NK (100:1)</td>
<td>6</td>
<td>% lysis</td>
<td>27.27</td>
<td>13.69</td>
<td>45.52</td>
<td>11.72</td>
</tr>
<tr>
<td>NK (50:1)^a</td>
<td>22</td>
<td>% lysis</td>
<td>26.64</td>
<td>13.04^a</td>
<td>19.95</td>
<td>12.77^b</td>
</tr>
<tr>
<td>NK (25:1)</td>
<td>11</td>
<td>% lysis</td>
<td>16.37</td>
<td>5.21</td>
<td>21.95</td>
<td>6.81</td>
</tr>
<tr>
<td>NK (12.5:1)</td>
<td>11</td>
<td>% lysis</td>
<td>10.47</td>
<td>3.35</td>
<td>15.72</td>
<td>3.72</td>
</tr>
<tr>
<td>CD3+^a</td>
<td>12</td>
<td>%</td>
<td>74.74</td>
<td>72.11</td>
<td>76.52</td>
<td>76.85</td>
</tr>
<tr>
<td>CD4+^a</td>
<td>11</td>
<td>%</td>
<td>35.06</td>
<td>35.90</td>
<td>41.89</td>
<td>40.22</td>
</tr>
<tr>
<td>CD4+CD8+</td>
<td>11</td>
<td>%</td>
<td>5.81</td>
<td>5.35</td>
<td>7.46</td>
<td>8.02</td>
</tr>
<tr>
<td>CD8+</td>
<td>11</td>
<td>%</td>
<td>37.58</td>
<td>34.35</td>
<td>33.58</td>
<td>33.67</td>
</tr>
<tr>
<td>CD16+</td>
<td>12</td>
<td>%</td>
<td>14.60</td>
<td>11.78</td>
<td>10.97</td>
<td>12.37</td>
</tr>
<tr>
<td>CD20+^a</td>
<td>11</td>
<td>%</td>
<td>9.18</td>
<td>14.91^b</td>
<td>8.93</td>
<td>8.09</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>21</td>
<td>pg/mL</td>
<td>8.07</td>
<td>7.80</td>
<td>7.21</td>
<td>–</td>
</tr>
<tr>
<td>TNF-α^a</td>
<td>21</td>
<td>pg/mL</td>
<td>13.60</td>
<td>11.70</td>
<td>9.74</td>
<td>–</td>
</tr>
<tr>
<td>IL-2</td>
<td>21</td>
<td>pg/mL</td>
<td>1.93</td>
<td>1.81</td>
<td>2.13</td>
<td>–</td>
</tr>
<tr>
<td>IL-4</td>
<td>21</td>
<td>pg/mL</td>
<td>7.16</td>
<td>9.44</td>
<td>5.88</td>
<td>–</td>
</tr>
<tr>
<td>IL-6</td>
<td>21</td>
<td>pg/mL</td>
<td>21.51</td>
<td>44.53</td>
<td>46.06</td>
<td>–</td>
</tr>
<tr>
<td>IL-10</td>
<td>21</td>
<td>pg/mL</td>
<td>5.20</td>
<td>12.69</td>
<td>12.45</td>
<td>–</td>
</tr>
<tr>
<td>IL-12</td>
<td>21</td>
<td>pg/mL</td>
<td>30.26</td>
<td>30.68</td>
<td>24.17</td>
<td>–</td>
</tr>
</tbody>
</table>

^a P ≤ 0.05 for the main effect of time point.
^b P ≤ 0.05 for the comparison to the pre-transport value.

3.3.2. Effect of transport
Planned comparisons between the pre-transport and the upon-arrival time points revealed significant increases in IFN-γ production stimulated with PHA [F(1,22) = 44.4, P ≤ 0.05] and CD20+ counts [F(1,10) = 23.7, P ≤ 0.001], and significant decreases in NK activity at an E:T of 50:1 [F(1,21) = 7.5, P ≤ 0.02] and CD3+ counts [F(1,11) = 4.8, P ≤ 0.051].

3.3.3. Process of acclimatization
Planned comparisons between the pre-transport and the after arrival time points revealed significant decreases in IFN-γ production stimulated with LPS (2-month time point) [F(1,11) = 6.5, P ≤ 0.03] and NK activity (50:1; 2-month time point) [F(1,21) = 13.2, P ≤ 0.002], and a significant increase in CD4+ counts (1-month time point) [F(1,10) = 15.4, P ≤ 0.003].

3.4. Body weight

3.4.1. Overall effect of transport, relocation, and acclimatization
Mixed-models ANOVA, with sex as a between-subjects factor and time point as a within-subjects factor were performed on subjects for whom weights had been obtained at four time points (pre-transport, upon-arrival, 1 month after arrival, and 2 months after arrival; n = 45, see Table 4). These analyses revealed a significant main effect of time point [F(3,129) = 15.9, P ≤ 0.001], but no significant main effect of sex, and no significant sex × time point interaction for these 45 subjects.

3.4.2. Effect of transport
A planned comparison between the pre-transport and the upon-arrival time points revealed a significant decrease in weight [F(1,44) = 108.6, P ≤ 0.001] indicative of an effect of the transport process.

3.4.3. Process of acclimatization
Planned comparisons between the pre-transport and the after arrival time points revealed significant differences in body weight both after 1 month [F(1,44) = 14.9, P ≤ 0.001] and 2 months [F(1,44) = 13.4, P ≤ 0.001]. Both of these comparisons are indicative of body weights that did not return to pre-transport levels.

3.4.4. Other weight-related findings
Analyses of the body weight data from all transported subjects using unpaired t-tests, revealed that males were significantly heavier than females at all time points (see Table 4 for t-test values) and both sexes lost weight during the 21 h of transport [t(65) = 17.7, P < 0.001]. The weight of males after 1 and 2 months at the KCCMR did not differ from their pre-transport weights, while females were still significantly lighter than their pre-transport weights, 1 month [t(39) = 4.3, P < 0.001] and 2 months [t(31) = 4.8, P < 0.001] after arrival at the KCCMR.

4. Discussion
The data demonstrate that the processes of being transported from one facility and relocated to another affect chimpanzee welfare, as indicated by changes in a variety...
of physiological parameters that have been used to assess welfare in other species and situations (Kagira et al., 2007; Kim et al., 2005; Lambeth et al., 2006; Landi et al., 1982; McGlone et al., 1993; van Ruiven et al., 1998), including cell-mediated immune responses (Schapiro et al., 2000, 2006). Others have previously studied the effects of transport, relocation, and/or acclimatization on nonhuman primates (Capitano and Lerche, 1998; Davenport et al., 2008; Fernstrom et al., 2008; Honess et al., 2004; Kagira et al., 2007; Kim et al., 2005; Ross et al., 2011; Schaffner and Smith, 2005; Watson et al., 2005), however most of these studies were conducted on species other than chimpanzees and focused on limited sets of behavioral or physiological responses. In fact, there are very few published reports of transportation, relocation, and acclimatization effects on physiological parameters in nonhuman primates (decreased lymphocytes in cynomolgus macaques (Kim et al., 2005) and changes in hematological parameters for recently trapped vervet monkeys (Kagira et al., 2007) are among the only physiological findings published). The dependent measures chosen for analysis in the present study were selected specifically because we knew that they (1) had been affected by other management manipulations with welfare implications (and were therefore likely to be affected by transport and relocation (Schapiro, 2002; Schapiro et al., 2000), providing insight into the welfare of the transported subjects) and (2) were measures that are typically assessed in biomedical research projects (Nehete et al., 1995).

There are considerably more data available on the effects of transport, relocation, and acclimatization in other animal species. These data are in general agreement with ours, identifying alterations in welfare state for mice, rats, dogs, and pigs as a function of transport, relocation, and/or acclimatization as suggested by changes in blood glucose, cholesterol, and blood urea nitrogen (van Ruiven et al., 1998); lymphocyte counts (Bergeron et al., 2002); and white blood cell counts, body weights, and natural killer cell activity (Dalin et al., 1993; McGlone et al., 1993).

The data from the present study provide some insight into the time that it takes chimpanzees to acclimatize to their new surroundings. Some standard clinical chemistry values appeared to return to pre-transport levels by about 6 weeks after arrival, while others did not. Some of the cell-mediated immune responses that were affected by transport and relocation had not returned to pre-transport levels, even 8 weeks after arrival. And three of the four hematological variables that had been affected by transport had still not returned to pre-transport levels 12 weeks after arrival. Both the welfare and research implications of the data should be clear; chimpanzees are still affected by the transport process weeks after transport and relocation, and probably should not be considered acclimatized, nor should they serve as subjects in studies that use the measured parameters as dependent variables, until they have had adequate time to adjust to their new conditions. This may require at least 6 weeks for studies that will assess some chemistry responses. More than 8 weeks may be necessary for studies that will measure cell-mediated immunity and a period longer than 12 weeks may be required for studies that use hematology parameters. The current data set however, does not address how much longer than 8 or 12 weeks might be necessary for cell-mediated and hematological responses, respectively, to return to pre-transport levels. The empirical descriptive data included in this study should be useful in anticipating and managing the welfare- and research-related effects of other transport and relocation scenarios.

Many of the changes observed in physiological parameters upon arrival at the KCCMR can be related to the process of transportation. Transport involves multiple factors that can be considered to potentially negatively affect animals’ welfare, including anesthesia; loading and unloading; separation from familiar social partners and environments; novel noises, smells, and vibrations; and relocation to a new and unfamiliar environment (Fazio and Ferlazzo, 2003; Wolfensohn, 1997). Negative welfare implications of these events have been assessed via serum levels of cortisol and other physiological measures in several species (Bergeron et al., 2002; Fazio et al., 2001; Kim et al., 2005; McGlone et al., 1993; Schaffner and Smith, 2005; Watson et al., 2005), but not in chimpanzees. However, serum cortisol potentially only provides information concerning very short term stress responses. For the purposes of this study, and for the purposes of many studies that evaluate the effects of a management manipulation on primate welfare, such short term responses may not be the most illustrative. The handling of animals, the loss of consciousness from anesthesia, and the recovery process associated with the attainment of serum samples are all factors that may acutely affect cortisol levels (Deckardt et al., 2007; Gil et al., 2007; Wall et al., 1985; Whitten et al., 1998) and therefore diminish the usefulness of serum cortisol as a measure of a broad, multifaceted concept like welfare. Because an increase in serum cortisol is seen within minutes after an animal perceives a stressor (such as the presence of a technician that will administer

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean body weight by time point in kg (± s.d.), including data from 45 subjects that were weighed at all four time points, plus any other weights that were obtained from transported animals.</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Body weight – subjects with weights at 4 time points (n = 45)$^{a}$</td>
</tr>
<tr>
<td>Body weight – females only</td>
</tr>
<tr>
<td>Body weight – males only</td>
</tr>
<tr>
<td>r-Test values for comparison of males and females</td>
</tr>
</tbody>
</table>

$^{a}$ $P < 0.05$ for the effect of time point (n = 45).
$^{b}$ $P < 0.05$ for the comparison to the pre-transport value.
$^{a}$ $P < 0.05$ for the comparison of females to males.
that cortisol data were not collected for this study. Fecal cortisol levels might have been useful as non-invasive measurements of acclimatization (Watson et al., 2005), however, due to large amounts of variability in fecal cortisol readings (Carlsson et al., 2007; Millsapgh and Washburn, 2003; Paramastri et al., 2007; Pihl and Hau, 2003), we chose not to collect such data.

This study focused on the welfare consequences of transportation and relocation as assessed by a variety of parameters that are also likely to be dependent measures in biomedical investigations, rather than on cortisol as a measure of ‘stress’ caused by transportation and relocation. One of the focal points of this project was welfare, a bigger concept than stress (as simply measured by cortisol). While a cortisol response indicative of stress is clearly a component of welfare, the broader concept is more relevant for this special issue. The observed changes in hematology, chemistry, and immunology do not depict a unidirectional pattern of effects on welfare (all improvements or all decrements to welfare); any time multiple measures of a multifaceted concept (like welfare) are assessed, a mixed pattern of effects is likely to be discovered (Lambeth et al., 2006; Schapiro et al., 2000), with some findings easy to interpret and others much more difficult to understand. At this point, it may be more important to identify factors that influence/change welfare, setting the framework for future studies to attempt to improve our understanding of the mechanisms of these changes.

A few of the hematological and clinical chemistry values collected at the different time points fell outside the published normal ranges for captive chimpanzees (Herndon and Tigges, 2001; Ihrig et al., 2001; Schapiro and Lambeth, 2010; Videan et al., 2008), however, the majority fell within these published normal ranges. Such findings necessitate an exploration of the biological meaning of these statistically significant results. While many of the changes observed as a function of transport, relocation, and acclimatization were statistically significant, few of them resulted in abnormal values that might have been of clinical significance. The published normal ranges of clinical chemistry and hematological values for chimpanzees have been established using several populations of chimpanzees that differed in terms of their age–sex composition and their health status. Additionally, the samples collected to generate such normal ranges are typically obtained from anesthetized subjects that have been affected by the acute stress associated with the process of anesthetization. Lambeth et al. (2006) have demonstrated that something as simple as the technique used to administer anesthesia (present for injection versus darting) can significantly affect a variety of stress-sensitive, hematological and clinical chemistry parameters. While comparisons with previously published normal ranges can be meaningful for assessing the general health of a population, within-subjects comparisons, utilizing individuals’ own baseline values (repeated-measures analyses), provide deeper insights when evaluating the potential welfare effects of specific manipulations on dependent variables that are subject to high levels of interindividual variability. This may be especially true when values approach the extremes of these wide ranges.

Significant changes as a function of transport or acclimatization may have few clinical implications in healthy animals, yet they may have numerous research implications. In the absence of any other type of manipulation, a change from a value at one extreme of the normal range to a value at the other extreme may have no observable health consequences for the animal. However, such a change could have considerable consequences for certain types of experimental investigations (for instance, changes in red blood cell counts in investigations of malaria (Barasa et al., 2010) or changes in CD4+ counts in immunodeficiency virus investigations (Nehete et al., 1995)). Published assessments of the welfare effects of a number of psychological manipulations have identified statistically significant differences in behavior as a function of these manipulations, but the biological significance of the observed behavioral changes may be more difficult to determine. Similarly, many of these changes are unlikely to represent deviations from the biological ‘norms’ for these behaviors, especially since there are few published species–typical ranges for many of these behaviors. For instance, in one study, female chimpanzees used fixed enrichment for 3.03% of observation time, whereas males used the same enrichment only 2.68% of observation time, a statistically significant difference (Videan et al., 2005) with potentially limited biological meaning.

Currently, there are no published norms for chimpanzee cell-mediated immune responses, so little can be said about the relationships among the clinical, statistical, or experimental significance of the recorded changes in these parameters.

Weight loss following transportation is a common finding for primates and several other species (Malaga et al., 1991; McGlone et al., 1993; Obernier and Baldwin, 2006). The most parsimonious explanation for the significant loss of body weight by the chimpanzees during the 21-h transport process revolves around the potential dehydration of the animals. While the chimpanzees had access to ample amounts of water and other liquids during the drive from Arizona to Texas, the quantity of fluid actually consumed by the subjects while traveling is unknown. It seems reasonably likely that some of the short term physiological changes described above (e.g., increased cholesterol levels and WBC counts immediately upon arrival) could have been related to the possibility that the subjects did not drink much during the journey, and therefore, became slightly dehydrated. However, the maintenance of statistically significant physiological differences, 3–12 weeks after transport, when animals had access to, and had taken advantage of, many hydration opportunities, diminishes the explanatory power of dehydration as a predominant factor in both the observed short term and long term differences in physiological parameters and body weights.

Among the 45 subjects for whom four different body weight measurements were available, weights did not return to pre-transport levels, even 8 weeks after arrival at the Keeling Center. Increased exercise opportunities and decreased access to high-calorie primate chow at the Keeling Center compared to PFA are likely to account for this overall weight loss. In fact, many of the PFA animals arrived at the Keeling Center just as a weight management program
was being implemented for the chimpanzees in an attempt to reduce the number of obese animals (Lambeth et al., in prep.). When we examined the weights of all transported subjects (using unpaired t-tests), it became obvious that males weighed more than females at all time points and that males regained the weight they lost during transport by the end of the first month at the KCCMR. On the other hand, females had not regained the weight after 2 months. Males and females were quarantined and treated identically during their first few months at the KCCMR, so there is no clear explanation for this sex difference in weight recovery.

Many attempts were made to minimize the disruptive effects to animal welfare of the transition from PFA to the Keeling Center. In addition to assembling ‘quarantine pairs’ that included animals that were compatible and familiar with one another from their time at PFA, the same familiar caregiver from PFA that placed the animals in the trailer was at the Keeling Center when the animals arrived to help with their removal from the trailer. Newly arrived animals were housed in pairs with former groupmates during quarantine at the KCCMR with visual and auditory access to chimpanzees that had already made the trip from Arizona to Texas, and for the first few days, were cared for by their caregiver from PFA. A small number of newly arrived animals displayed some abnormal behavior patterns at the KCCMR, but virtually all of these behavioral problems resolved within the first 2 weeks in Texas (Lambeth et al., in prep.).

Developing a better understanding of the effects of transport and relocation on the behavior and physiology of captive nonhuman primates should be one long term goal of any captive management and research program. The more that is understood about the effects of such manipulations on the welfare, health, and suitability of subjects for participation in research protocols, the better the refinements to management procedures can be. The development of more refined management techniques will result in enhanced welfare for the animals (Laule and Whittaker, 2007; Perlman et al., 2010; Schapiro and Lambeth, 2007; Schapiro et al., 2005; Veeder et al., 2009), enhanced abilities to directly test experimental hypotheses, and may ultimately result in important reductions in the number of primate subjects required to effectively test hypotheses.

Future studies will examine smaller-scale movements of chimpanzees within the various housing settings of the KCCMR. We have additional data (Koban et al., 2010; Williams et al., 2010) from reasonably sized samples of transported and relocated squirrel monkeys, owl monkeys, and cynomolgus monkeys that also demonstrate that transport and relocation result in statistically significant changes in a variety of hematological, clinical chemistry, and immunological parameters for these species, although the specific measures affected differ by species.

5. Conclusion

Transport and relocation of chimpanzees resulted in statistically significant changes in a variety of hematological, clinical chemistry, and cell-mediated immune parameters that are likely to be indicators of changes in welfare. Body weights were also affected by transport and relocation. While some parameters no longer differed significantly from pre-transport levels after 3–12 weeks of residence at the new facility, others still differed 8–12 weeks after arrival. Changes in certain biochemical parameters, like blood glucose levels, may be indicative of transient negative effects on well-being, while changes in some of the cell-mediated immune responses may be indicative of longer term negative consequences to the animals’ welfare. The data suggest that transportation and relocation affect animal welfare, and that chimpanzees need to be provided with sufficient periods after relocation to properly acclimate to their new conditions. This would be especially important for animals that might be involved in studies that include the assessment of hematological variables and some of the cell-mediated immune responses that had yet to return to normal at the end of the present study.

Conflict of interest

The author and co-authors of this manuscript have no conflict of interest, real or perceived.

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References


