Corticosterone Responses to Capture and Restraint in Emperor and Adelie Penguins in Antarctica

Authors: Cockrem, John F., Potter, Murray A., Barrett, D. Paul, and Candy, E. Jane

Source: Zoological Science, 25(3) : 291-298

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.25.291

ZOOLOGICAL SCIENCE **25**: 291–298 (2008)

Corticosterone Responses to Capture and Restraint in Emperor and Adelie Penguins in Antarctica

John F. Cockrem[1]*, Murray A. Potter[1,2], D. Paul Barrett[1,2] and E. Jane Candy[1]

[1]*Conservation Endocrinology Research Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand*
[2]*Institute of Natural Resources, Massey University, Palmerston North, New Zealand*

Birds respond to capture, handling and restraint with increased secretion of corticosterone, a glucocorticoid hormone that helps birds adjust to stressful situations. Hoods are reported to calm birds, but possible effects of hoods on corticosterone responses have not been reported for any bird. Corticosterone responses to restraint in Adelie penguins held by their legs with their head covered by a hood were markedly lower than responses of penguins restrained in a mesh bag inside a cardboard box (corticosterone at 30 min 15.69±1.72 cf. 28.32±2.75 ng/ml). The birds restrained by the two methods were sampled at the same location but in different years, so the differences in corticosterone responses cannot unequivocally be ascribed to an effect of hoods to reduce corticosterone responses. Corticosterone responses have been measured in some penguins, but not in the largest, the emperor penguin (*Aptenodytes forsteri*). The relationship between body mass and corticosterone responses to capture and restraint in penguins was examined in emperor penguins captured on sea ice in McMurdo Sound and Adelie penguins (*Pygoscelis adeliae*) captured at Cape Bird, Ross Island, Antarctica. Total integrated corticosterone responses were higher in the emperor than the Adelie penguins, but corrected integrated corticosterone responses, which represent the increase in corticosterone from initial concentrations and hence the corticosterone response to restraint, were the same. The results for the emperor and Adelie penguins, together with data from other penguin species, suggest that there is no relationship between the size of corticosterone responses and body mass in penguins.

Key words: corticosterone, perception, stressor, stress, penguin, Adelie, emperor, Antarctica

MINTRODUCTION

Corticosterone is a glucocorticoid hormone secreted from the avian adrenal gland. Corticosterone secretion increases when birds respond to stressors which can be classified as physical or emotional (Day et al., 1999; Cockrem, 2007), and changes in plasma corticosterone concentrations can reflect the degree of stress experienced by birds in different situations (Cockrem et al., 2004). Corticosterone responses to capture and restraint have been described for many species of free-living birds, with the great majority of these studies using a capture and handling protocol in which birds are held in cloth bags (Silverin, 1998). Birds have generally been caught in mist nets, and an initial blood sample collected within 3 min (Romero and Reed, 2005). Birds have then been held in cloth bags and further samples collected at intervals of 10 to 30 min until the bird is released 30 or 60 min after capture.

The protocol in which birds are caught in mist nets for the measurement of corticosterone responses has been useful for the comparison of corticosterone responses amongst species caught in this way (Romero, 2002). However, some bird species such as Antarctic penguins cannot be held within cloth bags (the penguins would become overheated), so other restraint methods must be used. The use of a hood or blindfold has been suggested as an aid to the restraint of animals (Fowler, 1995). A hood can calm raptors (White, 1990) and reduce struggling in broiler chickens (Jones and Satterlee, 1997). The cloth bags used to restrain small birds for the measurement of corticosterone responses may have some of the properties of hoods, but corticosterone responses have not been compared between birds in bags and birds restrained by other methods. The effect of a hood on the corticosterone response to restraint and hence the effectiveness of a hood to reduce the stress experienced by birds during restraint has also not been described. Indeed, there appears to be only one previous study of the effects of a hood on the glucocorticoid response to restraint in any animal. Cortisol was lower, although not significantly so, after 3 min of restraint in Brahman cows (*Bos taurus*) whose heads were covered by hoods compared with cows without hoods (Andrade et al., 2001). The first aim of the present study was therefore to compare corticosterone responses to different restraint methods, with Adelie penguins chosen as the study species.

* Corresponding author. Phone: +64-6-350-4483;
Fax : +64-6-350-5714;
E-mail : J.F.Cockrem@massey.ac.nz
doi:10.2108/zsj.25.291

It is not known whether corticosterone responses in birds are related to body mass. Penguins are larger than the passerine species in which the great majority of studies of corticosterone in free-living birds have been conducted (Silverin, 1998). Initial corticosterone concentrations have been measured in Adelie penguins (*Pygoscelis adeliae*; Vleck et al., 2000; Cockrem et al., 2006), and corticosterone responses to capture and restraint have been measured in Adelie (Cockrem et al., 2006), Magellanic (*Spheniscus magellanicus*; Hood et al., 1998; Walker et al., 2004; Walker et al., 2006), king (*Aptenodytes patagonicus*; Holberton et al., 1996) and gentoo (*Pygoscelis papua*; Holberton et al., 1996) penguins. The Adelie penguins were held in an open mesh bag within a cardboard box, whilst the restraint methods were not specified in the studies of Magellanic, king and gentoo penguins. Penguins and passerines have different life history patterns, so a comparison of corticosterone responses in these two groups would be of limited value for examining relationships between corticosterone and body mass. However, a comparison of corticosterone responses between the emperor penguin (*Aptenodytes forsteri*), the largest penguin species, and the much smaller Adelie penguin. which lives in the same environment, would be valuable for the examination of corticosterone responses in relation to body mass in birds. The second aim of the present study was to compare corticosterone responses of emperor and Adelie penguins restrained in the same manner. Emperor penguins were sampled on sea ice in McMurdo Sound, and Adelie penguins at Cape Bird on Ross Island, Antarctica.

MATERIALS AND METHODS

Study design

We caught emperor penguins in November 2003 close to the edge of sea ice in McMurdo Sound approximately 15 km west of Cape Royds (77°35'S, 166°10'E). Adelie penguins arriving at the northern colony at Cape Bird, Ross Island (77°13'S, 166°28'E) were caught on the beach in late November 2001 and early December 2003. The northern colony has approximately 40,000 pairs of nesting penguins (Taylor et al., 1990). The study was approved by the Massey University Animal Ethics Committee. Data from samples collected from Adelie penguins in 2001 have been used in a separate study of corticosterone in relation to fasting (Cockrem et al., 2006).

Sample collection

Emperor penguins

Emperor penguins do not nest in colonies like Adelie penguins, and it is logistically difficult to reach emperor penguins and to work with them in Antarctic conditions. It took more than one week of fieldwork to achieve a sample size of six emperor penguins. We travelled across the sea ice on snowmobiles, stopped 500 m from the ice edge and located groups of 1–4 non-breeding emperor penguins standing near the ice edge. Four people walked towards the ice edge until 50 m from the penguins. The birds did not respond to the presence of people at this distance. One person walked slowly towards a penguin until it began to walk away, usually when the approaching person was 3–4 m away. Some birds were then caught immediately whilst others were followed for up to 2 min. The catching process consisted of the person moving quickly towards the penguin so that the curved part of an aluminium crook 2 m in length could be placed around the body of the penguin to stop it from walking further. One or two other people who had been standing back then rapidly moved forward to capture the penguin and hold it around its body or by the feet. Birds initially held by their feet were then held around the body so they could stand upright. A brown canvas hood with a drawstring around the neck was placed over the head and bill of the bird and the drawstring tightened to lightly hold the hood in place. The hood was shaped so the end of the penguin's bill protruded and was not covered. A blood sample was collected from the ulnar flipper vein through a 23-ga needle into a 3-ml heparinised syringe. The bird was then restrained lying on its stomach with its legs held by the handler. Further blood samples were collected 15 and 30 min after capture, with the time of capture taken as the time when the penguin was caught with the crook. Blood samples were kept cool for up to 5 h before being centrifuged and plasma withdrawn and frozen at –20°C. The time from capture until collection of the blood sample was less than 3 min for all samples and there was no relationship between time taken to collect the sample and plasma corticosterone concentrations for the first samples (R^2=0.100, p=0.542). All birds were weighed after the last blood sample was collected (Salter balance±0.1 kg), and bill length, width and depth, and flipper length and width measured with Vernier callipers (±0.5 mm). Birds were then released at their capture site. Body condition indices were subsequently calculated using the formula condition index=body mass/flipper length^3.

Adelie penguins

Penguins arriving across the sea ice were caught on the beach at Cape Bird in 2001 and 2003. Birds were approached until they began to move away at a distance of 2–5 m. They were then caught in a hand net after a brief chase and carried a short distance to a sampling station. In 2001 a blood sample was immediately collected from the ulnar flipper vein through a 23-ga needle into a 3-ml heparinised syringe. Birds were then placed in a mesh bag and the bag placed in a cardboard box with an open top; this restraint method was termed the box method. In 2003 a brown canvas hood with a drawstring was placed over the head and bill of birds before the first blood sample was collected. The hood was similar to but smaller than the hood for emperor penguins. Birds fitted with a hood were then placed on their stomachs and held by their legs in the same way as the emperor penguins; this restraint method was termed the hood method. Further blood samples were collected from all birds 15 and 30 min after capture. Blood samples were kept cool for up to 2 h before being centrifuged and plasma withdrawn and frozen at –20°C. Measurements of body mass and beak and flipper size were then taken and the birds released. The time taken from capture to the collection of the blood sample was <3 min for the first sample in 2003 and <4 min in 2001 for all except one bird (4.50 min, plasma corticosterone 1.07 ng/ml). There was no relationship between time taken to collect the first sample and plasma corticosterone concentrations (R^2=0.003, p=0.805).

Penguins were weighed (Pesola balance±0.05 kg), and bill length, width and depth, and flipper length and width measured with Vernier callipers (±0.5 mm). A principal components analysis of six morphometric variables measured on 450 sexed Adelie penguins has been used by Dr C. M. Vleck (Iowa State University; pers. comm.) to derive a principal components score (PCA score) that differs significantly between known male and female Adelie penguins (0.809±0.092 and –0.916±0.097 respectively; p<0.0001). This score enables the likely sex of groups of birds to be predicted from mean scores. We calculated PCA scores for birds using Vleck's equation without toe length. Body condition indices were also calculated using the formula condition index=body mass/flipper length^3.

Radioimmunoassay of corticosterone

Corticosterone concentrations in plasma diluted in phosphate buffered saline with gelatine (PBSG) were measured by radioimmunoassay by the method of Cockrem et al. (2006). Plasma samples were initially spun for 10 min at 18 000×*g* to separate lipid from the plasma. The clear plasma from below the lipid layer was transferred

to another tube and diluted in PBSG for assay. Samples were assayed in duplicate. Ten microliters of diluted plasma was incubated for 2 h at room temperature (22–25°C) with iodinated corticosterone and antiserum from a Corticosterone Radioimmunoassay Kit (MP Biomedicals, USA). Precipitant solution (MP Biomedicals, USA) was added and each sample vortexed thoroughly, then 50 μl egg white (10 g/l dried egg white [Sigma] in PBSG) was added to increase adhesion of the pellet to the tube after centrifugation. The samples were incubated for 15 min at room temperature to separate bound and free corticosterone, then centrifuged for 15 min, and the supernatant aspirated, and the pellets were counted on a LKB Wallac 1261 Multigamma gamma counter.

This radioimmunoassay was previously validated by Cockrem et al. (2006) for the measurement of corticosterone in Adelie penguin plasma. Serial dilutions of emperor penguin plasma in PBSG were parallel to the corticosterone standard curve, and the recovery of corticosterone added to emperor penguin plasma was 102.8± 5.7%. The sensitivity of the corticosterone assay was the minimum

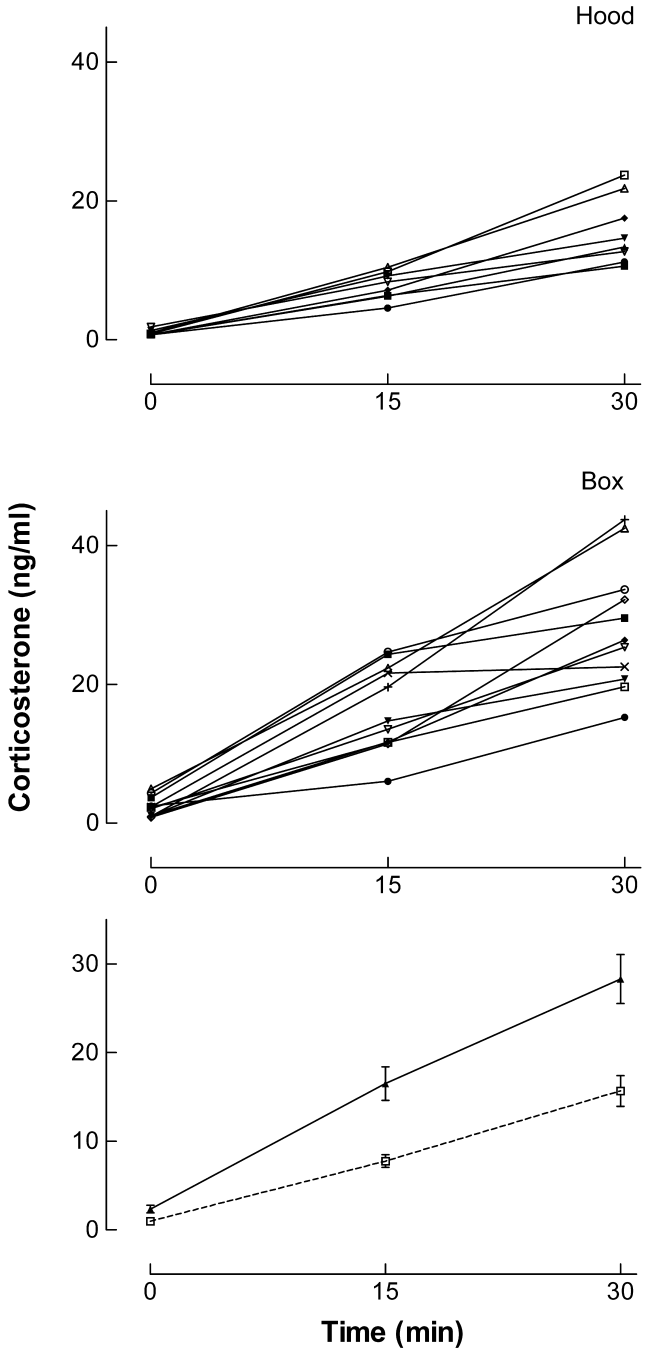

Fig. 1. Individual corticosterone responses in Adelie penguins restrained with a hood over their head or in boxes (upper and middle panels, respectively). Mean corticosterone responses in penguins restrained with hoods (---□---) or in boxes (—▲—) are shown in the lower panel. N=8 and 11.

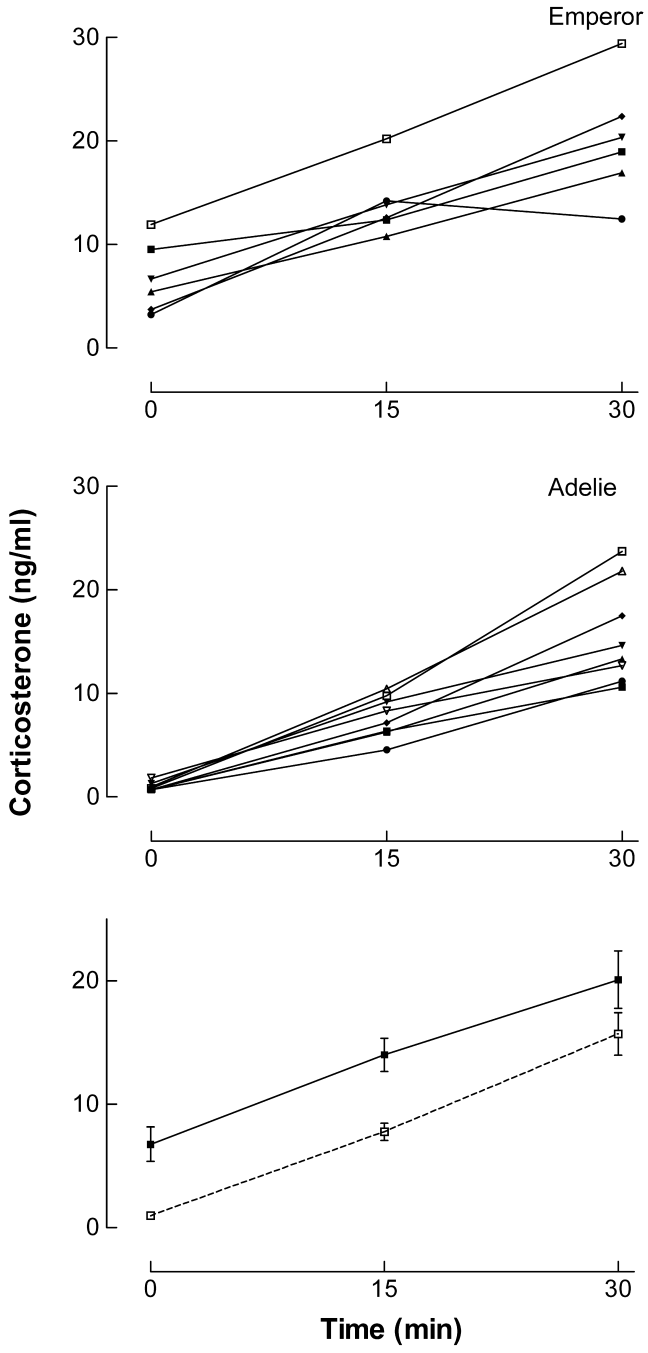

Fig. 2. Individual corticosterone responses in emperor and Adelie penguins restrained with a hood over their head (upper and middle panels, respectively). Mean corticosterone responses in the emperor (—■—) and Adelie penguins (---□---) are shown in the lower panel. N=6 and 8.

hormone level that could be consistently distinguished from zero. This was determined as the hormone concentration at the mean minus 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity, expressed as nanograms corticosterone per milliliter plasma, was 0.76 ng/ml. Solutions of corticosterone in PBSG at concentrations that gave approximately 80, 50 and 20% binding on the standard curve were used as low-, medium- and high-quality controls in every assay. The intra-assay coefficients of variation were 8.4%, 6.0%, and 7.2% and inter-assay coefficients of variation 7.8%, 8.4% and 11.5% for low-, medium- and high-quality controls.

Statistics

Plasma corticosterone concentrations and body masses were transformed to logarithms, and Levene's tests were performed on all variables to check for homogeneity of variances. Comparisons of body masses between emperor and Adelie penguins restrained with hoods, and comparisons of body masses, PCA scores and condition indices between Adelie penguins restrained by the hood method and arriving birds held in boxes were made using two-tailed T-tests. Changes in plasma corticosterone concentrations were compared between emperor and Adelie penguins restrained with hoods, and between Adelies restrained with hoods or in boxes using separate repeated-measures two-way ANOVAs. Time (0, 15 and 30 min) and group (emperor c.f. Adelie; Adelie restrained with hoods c.f. restrained in boxes) were the grouping factors. Post-hoc comparisons were made between times for each group and between groups for each time using univariate F tests (Neter et al., 1985; Systat Software, 2005). The areas under corticosterone response curves were determined in Prism (GraphPad Software Inc.) using the trapezoid rule and termed integrated corticosterone responses (Cockrem and Silverin, 2002a). The total area under the curve and the total area minus the area attributable to corticosterone concentrations at 0 min (corrected area) were calculated. Total and corrected integrated corticosterone responses were compared between emperor Adelie penguins restrained with hoods, and between Adelies restrained with hoods and in boxes, using two-tailed T-tests. Statistical analyses were performed using Systat (Systat Software). Data are presented as individual points or as mean±S.E.

RESULTS

Body mass, condition index and principal components scores

The mean body mass of the emperor penguins was 25.92±0.54 kg and their mean condition index 6.96±0.23 kg/$cm^3 \times 10^{-4}$. Body masses were similar in Adelie penguins restrained with hoods and in boxes (4.11±0.12 and 4.16±0.06 kg; T_{17}=–0.470, p=0.644). Condition indices and principal component scores did not differ between Adelie penguins restrained with hoods or in boxes (condition indices 6.34±0.19 and 6.85±0.24 kg/$cm^3 \times 10^{-4}$, T_{17}=–1.580, P=0.133; principal component scores 0.18±0.49 and –0.37±0.35, T_{17}=0.941, P=0.360). The absence of a difference in component scores between the two groups indicates that birds in both groups were likely to have been of the same sex.

Corticosterone responses in Adelie penguins restrained by two methods

Initial corticosterone concentrations at 0 min were low in Adelie penguins restrained after capture with hoods or in boxes. Corticosterone increased in birds restrained by both methods (Fig. 1), and reached lower concentrations at 30 min in birds restrained with hoods than those restrained in boxes (10.63–23.72 c.f. 15.23–43.72 ng/ml). A two-way repeated measures ANOVA showed that there were significant effects of restraint method and time on corticosterone,

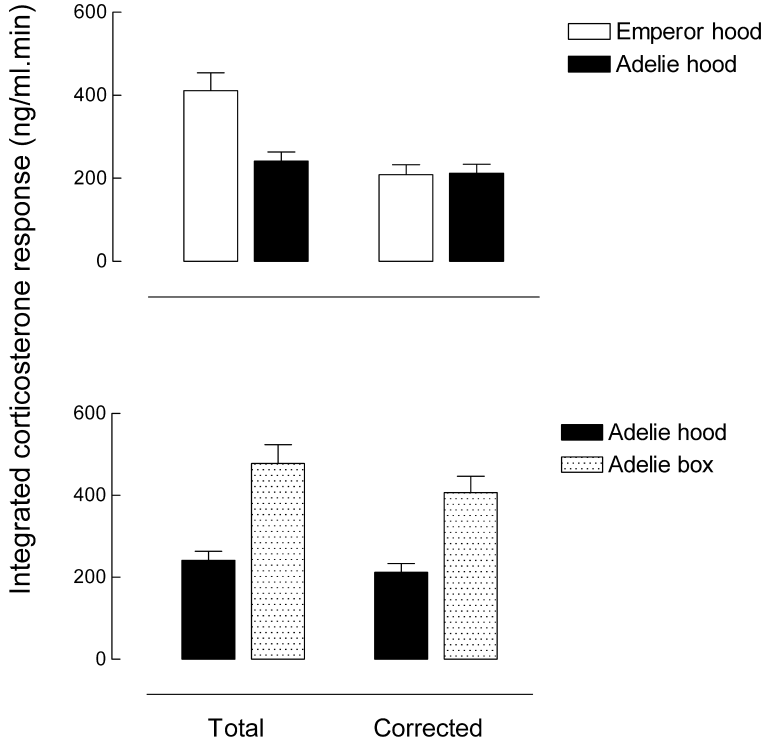

Fig. 3. Total and corrected integrated corticosterone responses in emperor and Adelie penguins restrained with a hood over their head (upper panel, N=6 and 8), and in the Adelie penguins restrained with a hood compared with penguins restrained in a box (lower panel, N=8 and 11).

and no significant interaction ($F_{1,17}$=22.816, p<0.001; $F_{2,34}$=322.799, p<0.001; $F_{2,34}$=0.454, p=0.369). Mean corticosterone concentrations were lower at 0, 15 and 30 min in birds restrained with hoods than birds in boxes (Fig. 1; $F_{1,17}$=10.213, p=0.005; $F_{1,17}$=16.596, p=0.001; $F_{1,17}$=15.428, p=0.001). Total and corrected integrated corticosterone responses were smaller in birds restrained with hoods than in boxes (Fig. 3 lower panel; T_{17}=–4.129, p=0.001 and T_{17}= –3.841, p=0.001).

Corticosterone responses in emperor and Adelie penguins

Initial plasma corticosterone concentrations varied from 3.24 to 11.93 ng/ml in emperor penguins whereas they were <2 ng/ml in Adelie penguins subsequently restrained by the same hood method. Corticosterone concentrations increased after capture in all birds (Fig. 2), reaching 12.47 to 29.42 ng/ml in emperor penguins and 10.63 to 23.72 ng/ml in Adelie penguins after 30 min. A two-way repeated measures ANOVA showed that there were significant effects of species and time on corticosterone, and a significant interaction ($F_{1,12}$=40.193, p<0.001; $F_{2,24}$=235.571, p<0.001; $F_{2,24}$=41.858, p<0.001). Mean corticosterone concentrations at 0 min in emperor and Adelie penguins were 6.76±1.39 and 0.97±0.15 ng/ml respectively. Mean corticosterone concentrations were higher in emperor than Adelie penguins at 0 and 15 min ($F_{1,12}$=66.706, p<0.001; $F_{1,12}$=19.198, p=0.001), but were not significantly higher at 30 min ($F_{1,12}$=2.569, p=0.135). The total integrated corticosterone response was greater in emperor than Adelie penguins, whereas the corrected integrated response (a measure of the amount of corticosterone secreted over 30 min minus the amount attributable to initial corticosterone concentrations) did not differ between species (Fig. 3 upper panel; T_{12}=3.783, p=0.003 and T_{12}=–0.111, p=0.913.

DISCUSSION

This is the first report of corticosterone responses in birds whose method of restraint included a hood covering their head. The mean corticosterone response of Adelie penguins restrained with a hood was less than the response of penguins placed in a mesh bag within an open cardboard box. This possible effect of hoods to reduce corticosterone responses must be confirmed in further studies. Corticosterone responses have not been described previously in emperor penguins. The magnitude of the corticosterone response did not differ between emperor and much smaller Adelie penguins restrained by the same method, so in these Antarctic penguins there was no apparent relationship between body mass and the size of corticosterone responses.

Restraint methods

Adelie penguins restrained with hoods over their heads were held by their legs with their ventral surface in contact with the ground. Although the birds struggled occasionally, they were mostly still during the 30 min restraint period. Adelie penguins restrained in mesh bags within boxes were not held by hand and could lie down or stand up and turn around within the boxes. These penguins had bouts of struggling lasting up to several minutes when they tried to escape from their boxes. Corticosterone responses were markedly lower in Adelie penguins restrained with hoods than in Adelie penguins restrained in boxes, with corticosterone at 30 min approximately twice as high in birds in boxes. The birds restrained by the two methods were sampled at the same location but in different years, so the differences in corticosterone responses cannot unequivocally be ascribed to an effect of hoods to reduce corticosterone responses. However, we have measured, in a different year from the current study, initial corticosterone and corticosterone responses in arriving Adelie penguins at three different colonies where the birds had walked across the sea ice for distances ranging from <0.5 km to more than 80 km. There was no effect on corticosterone of distance walked to the colony, and no difference between any of these results and those for arriving birds in the present study (unpubl. obs.). Whilst it is likely that hoods can reduce corticosterone responses in Adelie penguins, a further study in which birds are restrained with or without hoods is needed to confirm this suggestion.

Corticosterone has not previously been measured in any birds species during restraint that included covering of the head with a hood. The penguin data are consistent with a single report in mammals in which cortisol tended to be lower in cows whose head was covered by a hood during 3 min of restraint in a shute (Andrade et al., 2001). Broiler chickens struggle when they are shackled and suspended upside down during routine commercial operations, and Jones and Satterlee (1997) found that an opaque cotton hood covering the head with a drawstring around the neck reduced struggling in shackled chickens. They noted that the effect of the hood could have been due to a restriction of vision and the resulting inability of the birds to visually monitor their environment, to tactile properties of the hood, or to both factors. Two further studies of broilers (Jones et al., 1998a; Jones et al., 1998b) showed that struggling could be reduced to varying degrees by a reduction in ambient light intensity, a transparent nylon net hood, a drawstring around the neck, and by hanging a firm rubber surface next to a suspended bird so the rubber surface rested against the breast of the bird. It was concluded that reduced struggling in broilers with hoods was due to a combination of disruption of the birds' ability to check their environment for predators and for opportunities to escape, tactile stimulation that might simulate a continued predator encounter and promote tonic immobility (see Jones, 1986), and to reduced light intensity. These three factors could also have contributed to the likelihood that penguins held by their legs with their heads covered by a hood had lower corticosterone responses than penguins restrained in boxes. Penguins in boxes could see the sky through the open top of the boxes and hence could see an opportunity to escape, whereas penguins with hoods that were held by their legs could not see their environment and were physically prevented from escaping. Restraint within a cloth bag has been widely used in studies of corticosterone responses in smaller birds (Silverin, 1998). Birds restrained in cloth bags will experience disruption of their view of the environment, reduced light intensity and tactile stimulation to degrees that differ from the penguins with hoods. There have been no studies comparing corticosterone responses in birds restrained in bags and birds that

were physically restrained but able to see their surroundings, so the relative importance of factors that determine the magnitude of corticosterone responses of birds restrained in bags remain unknown.

It is widely assumed that birds experience capture and restraint as a form of predation (Silverin, 1998), although there are no data on corticosterone or cortisol responses of birds or mammals to actual predation events. Captive great tits (*Parus major*) had corticosterone responses when exposed to a stuffed owl in an aviary, whereas free-living great tits exposed to the same owl did not have corticosterone responses (Cockrem and Silverin, 2002b). Corticosterone responses in the captive but not the free-living great tits indicate that only the captive birds which could not move away from the predator perceived it to be a threat. Lower corticosterone responses in penguins held firmly with their heads covered compared with penguins in boxes show that the penguins with hoods perceived that situation as less threatening, even though their opportunity to escape was less than the penguins in boxes. A consideration of the degree of threat experienced by birds is useful for understanding the factors that influence their corticosterone responses in different situations.

Corticosterone responses and body mass

Plasma corticosterone has been measured in undisturbed emperor penguins during fasting (Robin et al., 1998), but corticosterone responses to capture and restraint have not previously been measured in this species. Initial corticosterone concentrations were higher in emperor than Adelie penguins. This may indicate that some emperor penguins initiated corticosterone responses whilst they were being followed across the sea ice towards a person who caught the birds, whereas the Adelie penguins were caught after brief chases. After the initial sample the rate of increase of plasma corticosterone concentrations in response to restraint was the same in the two species. Total integrated corticosterone responses were higher in the emperor than the Adelie penguins, but corrected integrated corticosterone responses, which represent the increase in corticosterone from initial concentrations and hence the corticosterone response to capture and restraint, were the same. Adelie penguins with a body mass of 4 kg and emperor penguins with a body mass of 26 kg thus had similar corticosterone responses. These two penguin species live in the same habitats and experience similar environmental conditions, so it is interesting that they have similar corticosterone responses. Corticosterone responses have been measured in other penguin species (Table 1), although the methods of restraint used for these other species were not specified. The results for the emperor and Adelie penguins, together with data from other species, suggest that there is no relationship between the size of corticosterone responses and body mass in penguins.

Corticosterone responses have been measured in many other species of birds that are smaller than penguins. These studies have mostly involved restraint of birds in cloth bags, so comparisons of responses can be made between birds of different sizes restrained by the same method. Mean plasma corticosterone concentrations after 30 min of restraint in cloth bags were 10–90 ng/ml in bush warblers (*Cettia diphone*; body mass 15–20 g; Wada and Shimizu, 2004), 12–120 ng/ml in one study and 45 ng/ml in another study of white-crowned sparrows (*Zonotrichia leucophrys*; 25–30 g; Astheimer et al., 1994; Breuner et al., 2003); 20–30 ng/ml in Florida scrub jays (*Aphelocoma coerulescens*; 70 g; Schoech et al., 1997); 20–80 ng/ml in black-legged kittiwakes (*Rissa tridactyla*; 400 g; Kitaysky et al., 1999) and 50–90 ng/ml in grey faced petrels (*Pterodroma macroptera gouldi*; 500–600 g; Adams et al., 2005). There does not

Table 1. Corticosterone responses to restraint in penguins.

Species	Corticosterone (ng/ml)		Restraint method	Body mass (kg)	Reference
	0 min	30 min			
Adelie	1.0±0.2	15.7±1.7	Held by legs with head covered by hood; 15 min blood sample	4–6	This study
Adelie	2.4±0.4	28.3±2.8	Open mesh bag in cardboard box; 15 min blood sample		Arriving birds, Cockrem et al. (2006)
Adelie	1.1±0.2	28.7±4.3	Open mesh bag in cardboard box; 15 min blood sample		Incubating birds, Cockrem et al. (2006)
Emperor	6.8±1.3	20.1±2.3	Held by legs with head covered by hood; 15 min blood sample	20–40	This study
Gentoo	10.2±2.3	34.7±3.5	Not specified; 5 and 10 min blood samples	5–7	Holberton et al. (1996)
King	6.0±1.4	21.4±2.7	Not specified; 5 and 10 min blood samples	10–15	Holberton et al. (1996)
Magellanic	~4	30–35	Not specified; birds weighed, measured and foot tag inserted between first and second sample (second sample at 25 min)	3–5	Hood et al. (1998)
Magellanic	<5	~30	Not specified; 10 min blood sample		Walker et al. (2004)
Magellanic	<5	~30	Not specified; 10 min blood sample		Walker et al. (2006)

appear to be any relationship between body mass and the size of corticosterone responses to restraint in birds in general.

Capture methods

The moment of capture for a flying bird is when it hits a mist net, is caught underneath a cannon net or enters a trap, and corticosterone generally begins to increase within 2 to 3 min of capture (Romero and Reed, 2005). Penguins must be approached on the ground before they can be caught, so in contrast to flying birds penguins might initiate corticosterone stress responses before they are captured. Initial plasma corticosterone concentrations were higher in the emperor penguins than in Adelie penguins caught on a beach or picked up from nests. The emperor penguins were caught on the flat sea ice of McMurdo Sound. A person walked towards the penguin, so the penguin could see the approaching person. Some penguins were caught after brief chases, and others were followed for up to 2 min and guided towards a second person who then caught the bird. The time between the penguin first moving away from the approaching person and capture varied from 1 to 3 min. Initial corticosterone concentrations in the emperor penguins were 3.24 to 11.93 ng/ml, with the highest concentration in a bird that was followed for longest before capture. The capture times were recorded only to the nearest minute owing to the field conditions, so a regression of corticosterone on capture time could not be performed. Adelie penguins restrained after capture with hoods were caught when they were walking within 20 m of people on the beach at Cape Bird in 2003. These penguins were caught after brief chases of <0.5 min, whereas Adelie penguins caught in 2001 after they had just arrived on the beach after walking across sea ice were approached from distances of 20–50 m and caught after chases of up to 2 min. Initial corticosterone concentrations were <2 ng/ml in the penguins caught after very brief chases in 2003, whereas they were up to 4.9 ng/ml in the arriving penguins in 2001. Initial corticosterone concentrations in Adelie penguins may be elevated in birds of low body mass (Cockrem et al., 2006), but the absence of differences in body mass and condition index between the two groups of penguins indicates that differences between groups in corticosterone concentrations were not related to body mass. Principal component scores for body size also indicate that these groups of birds were of the same sex, which is consistent with these birds being caught at times when females were returning to the colony to relieve their mates at the end of the first incubation spell. The data from the Adelie and emperor penguins suggest that penguins can initiate corticosterone responses when approached or chased before they are actually captured.

ACKNOWLEDGMENTS

This research was supported by Antarctica New Zealand, the Institute of Veterinary, Animal and Biomedical Sciences, and the Massey University Research Fund.

REFERENCES

Adams NJ, Cockrem JF, Taylor GA, Candy EJ, Bridges J (2005) Corticosterone responses of grey-faced petrels (*Pterodroma macroptera gouldi*) are higher during incubation than during other breeding stages. Physiol Biochem Zool 78: 69–77

Andrade O, Orihuela A, Solano J, Galina CS (2001) Some effects of repeated handling and the use of a mask on stress responses in Zebu cattle during restraint. Appl Anim Behav Sci 71: 175–181

Astheimer LB, Buttemer WA, Wingfield JC (1994) Gender and seasonal differences in the adrenocortical response to ACTH challenge in an arctic passerine, *Zonotrichia leucophrys gambelli*. Gen Comp Endocrinol 94: 33–43

Breuner CW, Orchinik M, Hahn TP, Meddle SL, Moore IT, Owen-Ashley NT, Sperry TS, Wingfield JC (2003) Differential mechanisms for regulation of the stress response across latitudinal gradients. Am J Physiol Regul Integr Comp Physiol 285: R594–R600

Cockrem JF (2007) Corticosterone stress responses and avian personalities. J Ornithol (Suppl 2): 147

Cockrem JF, Adams DC, Bennett EJ, Candy EJ, Henare SJ, Hawke EJ, Potter MA (2004) Endocrinology and the conservation of New Zealand birds. In "Experimental Approaches to Conservation Biology" Ed by MS Gordon, SM Bartol, University of California Press, Los Angeles, pp 101–121

Cockrem JF, Potter MA, Candy EJ (2006) Corticosterone in relation to body mass in Adelie penguins (*Pygoscelis adeliae*) affected by unusual sea ice conditions at Ross Island, Antarctica. Gen Comp Endocrinol 149: 244–252

Cockrem JF, Silverin B (2002a) Variation within and between birds in corticosterone responses of great tits (*Parus major*). Gen Comp Endocrinol 125: 197–206

Cockrem JF, Silverin B (2002b) Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). Gen Comp Endocrinol 125: 248–255

Day TA, Buller KM, Xu Y, Dayas CV, Crane JW (1999) Separation of neural pathways mediating HPA axis responses to emotional and physical stressors. In "Control Mechanisms of Stress and Emotion: Neuroendocrine-Based Studies" Ed by H Yamashita, JW Funder, JG Verbalis, Y Ueta, Elsevier, Amsterdam, pp 213–221

Fowler ME (1995) Restraint and Handling of Wild and Domestic Animals. 2nd ed, Iowa State University Press, Ames, Iowa

Holberton RL, Helmuth B, Wingfield JC (1996) The corticosterone stress response in gentoo and king penguins during the nonfasting period. Condor 98: 850–854

Hood LC, Boersma PD, Wingfield JC (1998) The adrenocortical response to stress in incubating Magellanic penguins (*Spheniscus magellanicus*). Auk 115: 76–84

Jones RB (1986) The tonic immobility of the domestic fowl: a review. World Poult Sci 42: 82–96

Jones RB, Satterlee DG (1997) Restricted visual input reduces struggling in shackled broiler chickens. Appl Anim Behav Sci 52: 109–117

Jones RB, Hagedorn TK, Satterlee DG (1998a) Adoption of immobility by shackled broiler chickens: effects of light intensity and diverse hooding devices. Appl Anim Behav Sci 55: 327–335

Jones RB, Satterlee DG, Cadd GG (1998b) Struggling responses of broiler chickens shackled in groups on a moving line: effects of light intensity, hoods, and 'curtains'. Appl Anim Behav Sci 58: 341–352

Kitaysky AS, Wingfield JC, Piatt JF (1999) Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Funct Ecol 13: 577–584

Neter J, Wasserman W, Kutner MH (1985) Applied Liner Statistical Models. 2nd ed, Irwin, Homewood, Illinois

Robin JP, Boucontet L, Chillet P, Groscolas R (1998) Behavioral changes in fasting emperor penguins: evidence for a 'refeeding signal' linked to a metabolic shift. Am J Physiol 274: R746–R753

Romero LM (2002) Seasonal changes in plasma glucocorticoid con-

centrations in free-living vertebrates. Gen Comp Endocrinol 128: 1–24

Romero LM, Reed JM (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp Biochem Physiol A Mol Integr Physiol 140: 73–79

Schoech SJ, Mumme RL, Wingfield JC (1997) Corticosterone, reproductive status, and body mass in a cooperative breeder, the Florida scrub-jay (*Aphelocoma coerulescens*). Physiol Zool 70: 68–73

Silverin B (1998) Stress responses in birds. Poult Avian Biol Rev 9: 153–168

Systat Software (2005) Systat 11 Statistics. Systat Software, Point Richmond, CA

Taylor RH, Wilson PR, Thomas BW (1990) Status and trends of Adelie penguin populations in the Ross Sea region. Polar Rec 26: 293–304

Vleck CM, Vertalino N, Vleck D, Bucher TL (2000) Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adelie penguins. Condor 102: 392–400

Wada M, Shimizu T (2004) Seasonal changes in adrenocortical responses to acute stress in polygynous male bush warblers (*Cettia diphone*). Gen Comp Endocrinol 135: 193–200

Walker BG, Boersma PD, Wingfield JC (2004) Physiological condition in Magellanic penguins: does it matter if you have to walk a long way to your nest? Condor 106: 696–701

Walker BG, Boersma PD, Wingfield JC (2006) Habituation of adult Magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. Conserv Biol 20: 146–154

White J (1990) Raptor restraint. J Assoc Avian Vet 4: 91–92

(Received July 8, 2007 / Accepted December 13, 2007)