RESEARCH PAPER

Wounding Alters Blood Chemistry Parameters and Skin Mineralocorticoid Receptors in House Sparrows (*Passer Domesticus*)

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ABSTRACT

Because skin is an important physical barrier against pathogens, the ability to quickly and effectively heal wounds directly impacts an animal's health. The hormone corticosterone (CORT) has many complex effects on immune function and can slow wound healing. It has been suggested that CORT's role during wound healing may be to act as a "brake" on inflammation and cell proliferation. This project aimed to clarify the role of CORT in the healing process by quantifying concentrations of its two intracellular receptors, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), in the skin of wounded (n = 9) or unwounded (n = 8) house sparrows (*Passer domesticus*) using radioligand binding assays. We also quantified GR and MR in liver, spleen and pectoralis muscle, as well as several blood chemistry parameters, hypothesizing that wounding would alter whole-body energy use. One day post-wounding, wounded birds had higher blood glucose and lower aspartate aminotransferase (a marker indicating muscle damage or catabolism) compared to controls, which may be related to animals' changing metabolic needs in response to lymphocyte and macrophage recruitment at the wound site. Birds had significantly decreased MR, but not GR, in the skin of wounded legs compared to the skin of unwounded legs. There was also a trend towards lower MR in wounded skin compared to unwounded birds. Receptors in the three other tissues did not differ between groups. This study suggests that decreasing the skin's sensitivity to CORT immediately after wounding may be a necessary part of the normal healing process in wild birds. *J. Exp. Zool. 9999A: 1–9, 2015.* © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

The hormone corticosterone (CORT) plays a number of important physiological roles both at baseline levels and at the higher concentrations produced in response to environmental stressors (Sapolsky et al., 2000; Landys et al., 2006). CORT's effects on the immune system are particularly complex (reviewed in Beck et al. (2009); Spencer et al. (2001)); depending on the time frame of CORT elevation and the aspect of immunity examined, CORT can both enhance and suppress immune function (Martin, 2009). Because of this complexity, it has been suggested that it may be more useful to examine CORT's effects on specific immune

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components, rather than on "the immune system" generally (Spencer et al., 2001). One important component of immune function is wound healing ability. Skin is an important physical barrier against pathogens (Nelson et al., 2002), but in wild animals it can become damaged through fighting over mates or territory, predator attacks, and assaults by ticks, mites and other biting insects (Randall et al., '88; Lehmann, '93; Townsend et al., 2011). Therefore, an animal's health is directly impacted by its ability to heal wounds quickly and effectively, which is why this measure has become increasingly popular for assessing immune function (French et al., 2006; Boughton et al., 2011; Archie et al., 2012).

The healing process consists of a number of different stages (Martin, '97; Johnson, '60). Immediately after wounding, a clot of cross-linked platelets in a fibrin matrix forms. Then, inflammatory cells, fibroblasts and capillaries invade the clot to make a contractile tissue that draws the edges of the wound together. Quiescent epithelial cells also begin to proliferate and invade the wound in response to immune signals produced at the wound's edges, and these activated cells lay down new matrix in the wound gap. Finally, through continued collagen synthesis and catabolism, a scar forms. Elevated CORT induced either through a stress protocol or exogenous administration can slow this wound healing process (Hübner et al., '96; Padgett et al., '98; Rojas et al., 2002; Kinsey et al., 2003). CORT's effects on wound healing may seem maladaptive, but they fit well into the framework proposed by Sapolsky et al. (2000), where CORT's suppressive actions help to keep the response to a particular stressor (in this case, an immune challenge) from overshooting (Dantzer, 2004). Thus, CORT's role in wound healing may be to act as a kind of "brake" to keep the healing process from running amok. For instance, keloid scar tissue, which creates hugely overgrown masses that can cause pain and disfigurement, is CORT-insensitive (Russell et al.,'89, '95). Intriguingly, some epidermal cancer cells also contain higher-than-normal levels of the enzyme 11β hydroxysteroid dehydrogenase type 2 (11β–HSD2), which inactivates CORT, and lower-than-normal levels of the enzyme 11β hydroxysteroid dehydrogenase type 1 (11β–HSD1), which converts inactive metabolites to active CORT (Terao et al., 2013).

Adding to the complexity of this picture, not only can circulating CORT titers potentially affect wound healing, there is also evidence in mammals for local CORT synthesis in skin (Taves et al., 2011), which can be induced by wounding (Vukelic et al., 2011). There are also two different receptors that could be responsible for CORT's effects on wound healing. Many immune tissues, including skin (Serres et al., '96; Farman et al., 2010; Lattin et al., 2012) have high concentrations of the two intracellular CORT receptors, the higher-affinity mineralocorticoid receptors (MR) and lower-affinity glucocorticoid receptors (GR). CORT exerts its effects by binding to specific receptors in different target tissues (Young and Crews, '95; Shaw and Kennedy, 2002; Ketterson et al., 2009), and MR and GR receptor number is highly correlated with the magnitude of CORT's response on downstream gene expression (Vanderbilt et al., '87). Therefore, if CORT's role in wound-healing is to help control the inflammation and cell proliferation involved in the healing process, one way to augment hormonal impacts on wounded skin is by increasing receptor concentrations. However, to our knowledge, only one study has examined changes in skin corticosteroid receptors with wounding (Tiganescu et al., 2014). This study examined GR and MR mRNA expression in laboratory mice, and found decreased MR expression two and four days post-wounding (Tiganescu et al., 2014). However, because GR and MR mRNA expression and protein expression may not be correlated (Medina et al., 2013), and because the CORT response can be altered by domestication for laboratory conditions (Kunzl and Sachser, '99; Gulevich et al., 2004), it is unclear whether GR and MR concentrations in the skin of a wild animal might change in response to wounding.

The spleen is another immune tissue with high GR and MR density (Lattin et al., 2012; Miller et al., '93). The spleen and skin interact remotely via delayed-type hypersensitivity reactions, where immune cells are reallocated from blood and lymphatic tissues, including spleen, to peripheral locations like skin (Dhabhar and McEwen, '99; Dhabhar, 2003). When elevated over short periods of time, CORT enhances delayed-type hypersensitivity reactions (Dhabhar and McEwen, '99; Dhabhar, 2009). Thus, if CORT is serving an anti-inflammatory role during wound healing, we hypothesized that spleen might decrease GR and/or MR density post-wounding to help reduce the skin's inflammatory response.

Several studies demonstrate that responding to an immune challenge can also increase an individual's daily energy expenditure and lead to metabolic changes (Demas et al., '97; Svensson et al., '98; Ots et al., 2001; Martin et al., 2008). Skin damage causes hyperglycemia and increases protein catabolism (Chioléro et al., '97; Atiyeh et al., 2008), which may be due to the need for amino acids and glucose by the immune cells involved in tissue repair (Deerenberg and Lochmiller, 2000) and for synthesis of acute phase proteins by the liver (Atiyeh et al., 2008). However, most studies showing metabolic changes in response to tissue damage were done in humans or lab animals with severe trauma, such as large burns (Kowal-Vern et al., '92; Atiyeh et al., 2008). It is less well known whether a small wound can cause similar metabolic changes in a wild animal species. CORT is one of the players involved in shifting energy balance away from protein synthesis and carbohydrate storage toward proteolysis and gluconeogenesis (Hasselgren, '99; Sapolsky et al., 2000); these roles are distinct from CORT's effects on inflammation. Therefore, we hypothesized that GR and MR concentrations may increase in muscle and liver after wounding as a way to enhance CORT's effects on these tissues specifically, to increase proteolysis in muscle and gluconeogenesis and acute phase protein synthesis in liver. In keeping with these kinds of metabolic changes, we also

predicted that wounded animals would show altered concentrations of plasma metabolites related to protein and carbohydrate metabolism.

In this project, we aimed to clarify whether a relatively minor wound could cause changes in metabolic markers and CORT receptors in house sparrows (*Passer domesticus*), a common wild bird species used in many physiological studies (Martin et al., 2003; Sweazea and Braun, 2006; Khalilieh et al., 2012). We chose to examine GR and MR 24 hr after wounding because many inflammatory cytokines show maximal levels 15–24 hr after wounding (Beer et al., 2000); therefore, CORT's role in suppressing inflammation and altering metabolism may be especially important during this time period. We specifically predicted that, compared to unwounded controls, wounded birds would have increased GR and/or MR in skin, muscle and liver, and decreased GR and/or MR in spleen. We also predicted wounded birds would have increased glucose, and increased aspartate aminotransferase and creatine kinase, both of which can potentially indicate muscle damage or catabolism (Fudge, 2000; Harr, 2002). Furthermore, we predicted the response of GR and MR to wounding would be localized, so although receptors would be upregulated in skin directly surrounding the wound site, there would be no changes in receptors in other skin regions in wounded animals.

METHODS

Study Species, Experimental Design and Wounding

House sparrows were caught in Medford and Arlington, MA, USA from 4 February to 7 March 2013 using seed-baited Potter traps and mist nets at bird feeders. Upon capture, birds were housed together in an outdoor aviary with ad libitum access to mixed seed, grit and water, multiple perches and a small artificial tree for cover. All procedures were performed according to Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines, and all protocols were approved by the Tufts University Animal Care and Use Committee.

To examine the effects of wounding on CORT receptors, we compared skin, spleen, liver, and pectoralis muscle GR and MR density in a wounded group of sparrows ($n = 9$; 4 females, 5 males) to a non-wounded control group ($n = 8$; 4 females, 4 males). In wounded birds, we assayed skin from both wounded and unwounded legs. We also compared blood chemistry parameters in wounded and non-wounded animals; however, receptor assays require pre-administration of mitotane (see "Mitotane Treatment," below), and we were concerned that mitotane could affect blood metabolites, especially glucose (Breuner et al., 2000). Therefore, we also included a control group of wounded animals that did not receive mitotane ($n = 6$; 3 females, 3 males). On 27 March 2013 (20 days after the last animals were brought to the outdoor aviary), we administered a small superficial wound to either the left or right thigh of birds using a 4 mm biopsy punch (Miltex 33–34, York, PA) following the methods of French et al. (2006) and Carter et al. (2013). The leg for wounding was randomly chosen. Prior to wounding we anesthetized birds using isoflurane.

Blood Chemistry Data Analysis

For blood chemistry analyses, we used an Abaxis VetScan Classic analyzer (Model 200–1000, Union City, CA, USA) with Avian/Reptilian Plus Rotors to quantify eight different analytes that can indicate metabolic changes and/or pathology: aspartate aminotransferase, creatine kinase, uric acid, glucose, phosphorus, calcium ions, total protein, and sodium ions. These rotors are also supposed to give measures of bile acids, potassium ions, albumin, and globulin. However, bile acid concentrations in house sparrow blood were usually below the machine's dynamic range (< 35 μmol/L), and potassium ions usually above the machine's dynamic range (>8.5 mmol/L), making them undetectable. The VetScan uses the bromcreosol green dye-binding method to assess albumin concentrations, and measured total protein and albumin to calculate globulin concentrations. However, the bromcreosol green dye-binding method is generally considered unreliable in birds (Lumeij, '97; Cray et al., 2011). Therefore, albumin and globulin are also not included in any analyses.

Blood chemistry parameters were assessed ~24 hr after wounding for wounded animals, on the morning of the day animals were euthanized for tissue collection. For all VetScan samples, 100 ul of whole blood was drawn from the brachial vein using heparinized microcapillary tubes within 14 min of entering animal rooms, and kept on ice until they were used in pre-programmed assays within 4 hr of collection.

Mitotane Treatment and Radioligand Binding Assays

All birds except for the mitotane control group were given two injections of mitotane ~36 and ~24 hr before euthanasia. Mitotane prevents secretion of endogenous CORT, which can otherwise interfere with radioligand binding assays (Breuner et al., 2000; Lattin et al., 2012). Mitotane (ortho, para-DDD; 180 mg/kg body weight) was dissolved in peanut oil and injected into pectoralis muscle. Mitotane control animals received an injection of peanut oil only.

To measure the success of mitotane treatment, after sampling for blood chemistry parameters animals were restrained in cloth bags for 30 min and blood samples of ~30 ul taken from the brachial vein using heparinized microcapillary tubes. Birds were also weighed at this time using Pesola spring scales to assess body mass. Whole blood was kept on ice until centrifuged 2–4 hr later; we then pulled off and froze plasma.

Radioimmunoassays were done following Wingfield et al. ('92), using antibody B3–163 (Esoterix, Calabasas Hills, CA, USA). All samples were run in the same assay, and individual samples adjusted based on recoveries. Average recovery was 70%, detectability was 1 ng CORT/ml plasma, and the intra-assay

coefficient of variation was 1.3%. Mitotane successfully reduced stress-induced CORT below the detectable limit of our assay for all animals except for one individual in the control group that had plasma CORT concentrations of 1.6 ng/ml, compared to ~20–30 ng/ml for house sparrows not treated with mitotane (Romero et al., 2006).

For animals used in receptor assays (n = 9 wounded, 8 controls), we euthanized animals ~36 hr after their first mitotane injection (and ~24 hr after wounding, for wounded birds). Birds were deeply anesthetized using intramuscular injections of ketamine (~80 mg/kg body weight) and xylazine (~20 mg/kg body weight), at species-appropriate doses (Muresan et al., 2008). We then transcardially perfused animals with cold heparinized saline, and extracted liver, the left pectoralis muscle, spleen, and leg skin from both wounded and unwounded legs (for wounded birds) or from a randomly-chosen leg (for unwounded birds) and flash-froze all tissue using dry ice. Leg skin was plucked of feathers before freezing. We always collected tissues in the same order; time to extract all tissues was ≤ 10 min (mean time ± SD: 7.7 min ± 1.1 min). Tissues were stored at −80 °C until assayed.

We quantified GR and MR concentrations in tissue using radioligand binding assays following Breuner and Orchinik (2001) and Lattin et al. (2012). Briefly, on the day of assay, tissues were homogenized and spun in an ultracentrifuge. Supernatant was incubated with 10 nM [^{3}H]CORT and either: 1) buffer, to measure total binding; 2) excess unlabeled CORT, to measure non-specific binding; 3) excess RU486 (mifepristone), which only binds GR. After subtracting nonspecific binding, MR binding can be calculated directly from test tubes containing RU486; GR binding can be calculated by subtracting MR binding from total binding. Based on affinity estimates from previously-published saturation experiments (Lattin et al., 2012), mass action predicts that 10 nM [^{3}H]CORT should occupy > 95% of MR and ~63% of GR. Each point sample was run in triplicate, and samples incubated at optimized temperatures and times for each tissue (Lattin et al., 2012). Binding in individual samples was standardized per mg protein using Bradford assays with bovine serum albumin standards.

Statistics

For skin GR and MR and blood chemistry statistical analyses, we used SAS 9.3 (SAS Institute, Cary, NC, USA); for spleen, muscle and liver GR and MR and tissue mass analyses, we used JMP 11 (SAS Institute). Where appropriate, we tested for normality and equal variance using Ryan-Joiner's, Levene's and Bartlett's tests, and used Tukey's LSD test for post-hoc analyses. Raw data were used in analyses unless otherwise indicated. Statistical significance was recognized at $\alpha < 0.05$.

Spleen, liver and pectoralis receptor concentrations, total body mass and tissue mass analyses involved simple one-way ANOVAs comparing wounded and control animals. We compared GR and MR in leg skin surrounding wounds to both: 1) skin from one of the legs of an unwounded bird, 2) skin from wounded birds from the opposite (non-wounded) leg. For this analysis, we used a mixed procedures ANOVA in SAS. Skin GR and MR concentrations were log-transformed to better meet assumptions of normality and equal variances. We accounted for multiple observations from a single bird (wounded and unwounded legs) by including bird ID as a random variable in the model. We used a MANOVA (SAS proc glm) to compare differences in blood chemistry parameters among treatments (wounded birds that received mitotane, unwounded birds that received mitotane, and wounded birds that did not receive mitotane). The model included aspartate aminotransferase, creatine kinase, uric acid, glucose, calcium, phosphorus, total protein, and sodium as independent variables.

RESULTS

Wounded and non-wounded birds did not show differences in GR or MR density in liver (GR: $P = 0.55$; MR: $P = 0.17$; Fig 1a), pectoralis muscle (GR: $P = 0.39$; MR: $P = 0.77$; Fig 1b) or spleen (GR: $P = 0.27$; MR: $P = 0.68$; Fig 1c). There were also no differences in total body mass, spleen mass or pectoralis mass between wounded and unwounded birds (Table 1), although there was a trend ($P = 0.072$) for the livers of wounded birds to be larger than those of control animals.

In skin, GR density did not differ significantly among groups (Fig 2; $P = 0.25$). In contrast, MR density differed significantly among groups ($F_{2,8} = 5.33$; $P = 0.034$). Tukey's post hoc comparisons revealed that in wounded birds, MR density from skin in the wounded leg was 81% lower than tissue from the control (unwounded) leg ($P = 0.040$), and there was a tendency for tissue from the wounded leg to have 82% lower MR concentrations than tissue from legs of control (unwounded) birds ($P = 0.078$).

MANOVA results indicated that there were differences in blood chemistry parameters among treatments ($F_{16,24} = 2.36$; $P = 0.028$). Analysis of individual ANOVAs including contrast statements indicated that wounded birds, regardless of mitotane treatment, had 21–31% higher glucose concentrations than non-wounded birds (Fig 3a; $F_{1,19} = 4.83$; $P = 0.020$). In addition, birds that were not wounded had 117–128% higher aspartate aminotransferase levels than both wounded birds that received mitotane injections and wounded birds that did not receive mitotane injections (Fig 3b; $F_{1,19} = 3.99$; $P = 0.036$). No other analyte differed significantly among treatments (Table 2).

DISCUSSION

Our results demonstrate that even a relatively small wound can affect CORT receptor concentrations in skin surrounding the wound site 24 hr later. However, contrary to our hypothesis, wounding did not cause skin CORT receptor density to increase. Instead, we saw a decrease in MR skin receptors. A decrease in skin GR also appears to have occurred, but modest sample sizes

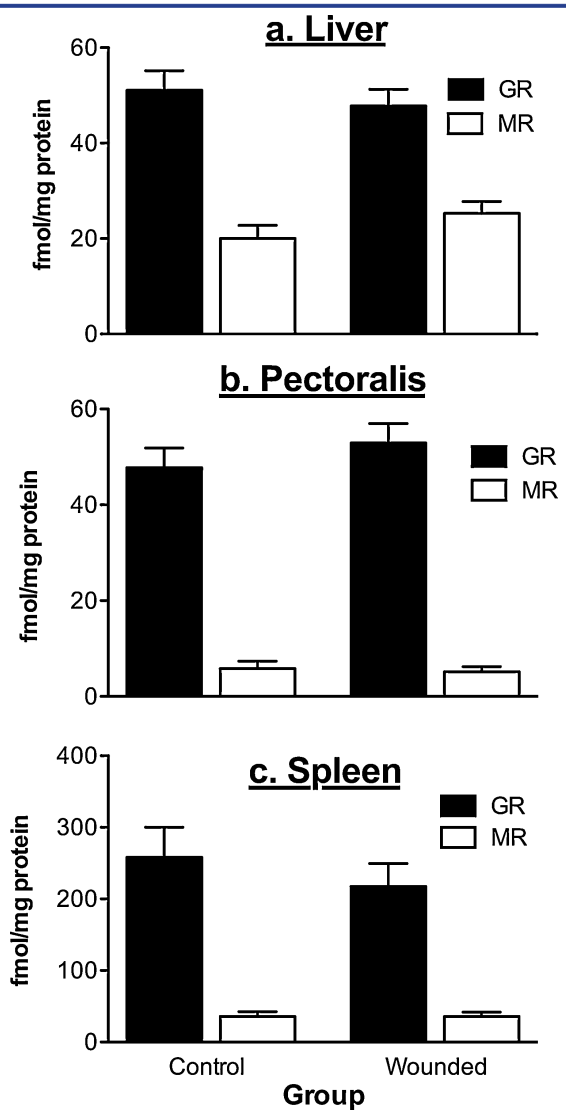

Figure 1. Concentrations of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) did not differ in liver (a, top graph), pectoralis muscle (b, middle graph) or spleen (c, bottom graph) between house sparrows (*Passer domesticus*) that received a 4 mm superficial flank wound ~24 hr before sacrifice ($n = 9$; 4 females, 5 males) and a non-wounded control group ($n = 8$; 4 females, 4 males). Receptor concentrations were quantified using radioligand binding assays, and standardized by protein concentration. Wounded and control groups were compared using one-way ANOVAs. Data are depicted as means ± SEM.

combined with large individual variation may have prevented achieving statistical significance. This suggests that if CORT helps suppress inflammation during wound healing in house sparrows, it does not do so by increasing tissue sensitivity to CORT at the wound site.

There are two possible interpretations of these data. First, it is possible that wounded animals selectively *increase* inflammation at the wound site during this early period of wound healing. Decreasing sensitivity to CORT by reducing receptors at the wound site may thus be an important part of a localized pro-inflammatory response. The second possibility is that animals take advantage of CORT's anti-inflammatory properties via other hormonal mechanisms at the wound site, including transitory increases in circulating CORT concentrations and/or local CORT production in response to wounding, or increased expression or activity of the enzyme 11β-HSD1, which converts inactive 11-keto metabolites to active CORT. There is currently evidence that all three of these mechanisms may occur in mammalian species (Spencer et al., 2001; Vukelic et al., 2011; Tiganescu et al., 2014).

It has been proposed that having two separate receptor types with differential affinity for CORT could lead to a two-tiered response, with the effects of baseline CORT mediated primarily via higher-affinity MR, and the effects of stress-induced CORT titers occurring through binding to both GR and MR (Landys et al., 2006). However, this hypothesis is based on studies of MR and GR in brain (de Kloet et al., '98), and the respective roles of MR and GR are less clear in an organ like skin, especially given the potential for local synthesis of CORT. There is also some debate as to whether skin MR may actually be a target for the mineralocorticoid hormone aldosterone (Kenouch et al., '94). Generally, however, because most epidermal cell types contain low 11β-HSD2 (meaning CORT is not inactivated), and MR preferentially binds to CORT over aldosterone, skin MR is thought to be a target for CORT (Farman et al., 2010).

Only a few mammalian studies have examined the specific role of MR in skin. For example, laboratory mice overexpressing MR in basal keratinocytes during development had epidermal abnormalities, such as missing eyelids, that were apparently due to increased apoptosis of skin cells (Farman et al., 2010). In addition, decreased mRNA for MR (but not GR) was also found in mouse skin two and four days after wounding (Tiganescu et al., 2014), suggesting reducing skin MR in response to wounding could be a conserved response across different vertebrate taxa, and that it is somehow necessary to normal wound healing (perhaps related to MR's apoptotic role, or again, to a pro-inflammatory response at the wound site only). Future studies should further investigate the role of MR in both healthy and damaged epidermal tissue.

Also contrary to our predictions, we did not see increased GR or MR in the pectoralis muscles or livers of wounded birds, despite the fact that immune challenges often require increased gluconeogenesis and protein mobilization from muscle tissue and CORT is involved in these processes (Lochmiller and Deerenberg, 2000; Sapolsky et al., 2000; Landys et al., 2006). However, we only examined receptors 24 hr after wounding, and it is possible that there could be receptor changes in these tissues at later time points. We did see evidence that wounding induced

Table 1. Total body mass, and the mass of three different organs, in wounded and unwounded house sparrows (*Passer domesticus*).

	Mean mass wounded birds (± SD)	Mean mass unwounded birds (± SD)	F ratio (df)	*P*-value
Liver	0.85 (± 0.15) g	0.71 (± 0.06) g	3.75 (15)	0.072
Spleen	24 (± 11) mg	27 (± 10) mg	0.44 (15)	0.52
Pectoralis	2.0 ± (0.1) g	1.9 (± 0.2) g	0.75 (15)	0.40
Body mass	27.5 (± 1.7) g	26.8 (± 1.5) g	0.89 (15)	0.36

See Fig. 1 caption for more details. Mean mass values show raw data, but statistics for liver, spleen and pectoralis were run on tissue mass as a fraction of total body mass to correct for potential part-whole correlations (Christians, '99). All samples were compared using one-way ANOVA.

metabolic changes, however, as whole-blood glucose concentrations were higher, and aspartate aminotransferase concentrations were lower, in wounded birds compared to controls. Tissue damage in mammals from burns or wounds can result in glucose elevations both by increasing hepatic glucose production and causing insulin resistance in peripheral tissues (Wolfe et al., '79 ; Lochmiller and Deerenberg, 2000; Atiyeh et al., 2008). Fewer studies have examined changes in other blood chemistry parameters with wounding; although elevated aspartate aminotransferase can indicate muscle damage, we actually saw a decrease in this measure, which may reflect altered liver function

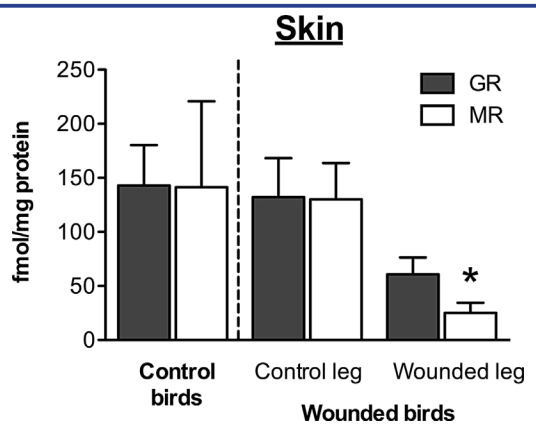

Figure 2. Skin concentrations of glucocorticoid receptors (GR) did not differ between wounded and control house sparrows (*Passer domesticus*), or between the wounded and unwounded legs of wounded animals. However, mineralocorticoid receptor (MR) concentrations did differ among groups (Mixed procedures ANOVA: $P = 0.034$). Post-hoc analysis revealed the MR density was lower in the skin surrounding the wound site of wounded animals than in the skin of their unwounded leg, indicated by * (Tukey's LSD: $P = 0.040$). There was also a trend for the skin surrounding the wound site to have lower MR concentrations than the leg skin of an unwounded animal (Tukey's LSD: $P = 0.078$). Data are depicted as means ± SEM; see Fig. 1 caption for more information.

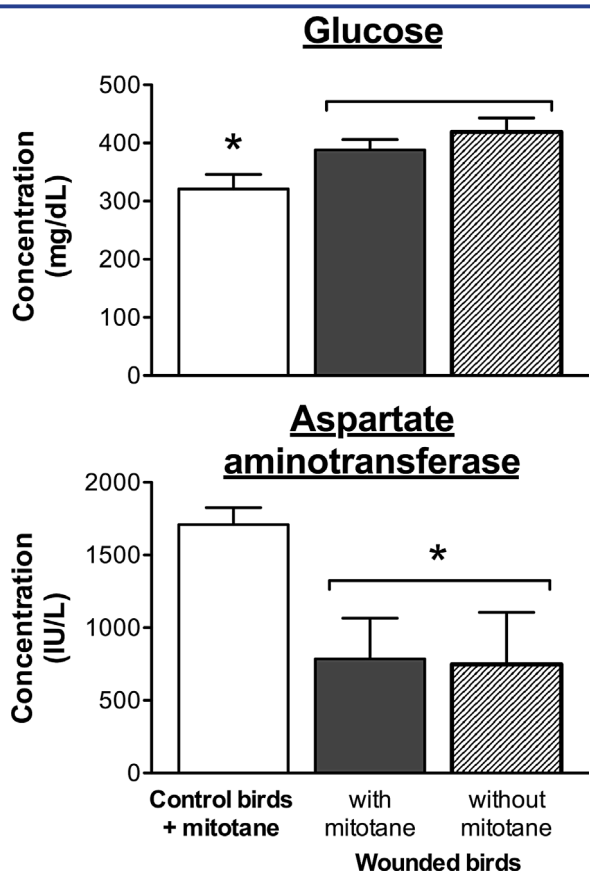

Figure 3. Whole blood concentrations of glucose were higher (a; top graph) and whole blood concentrations of aspartate aminotransferase were lower (b; bottom graph) in house sparrows (*Passer domesticus*) that received 4 mm superficial flank wounds with (n = 9) or without (n = 6) mitotane compared to animals that received mitotane but were not wounded (n = 8). Wounds were administered ~24 h before measuring blood chemistry analytes. Values were determined using Avian/Reptilian Plus rotors on a portable VetScan machine, and are presented as mean ± SEM. * indicates significant differences between unwounded birds and both groups of wounded birds ($P < 0.05$; see text for details of statistical analyses).

Table 2. Blood chemistry parameters in house sparrows (*Passer domesticus*) that received small superficial leg wounds and injections of mitotane (n = 9), wounded animals that did not receive mitotane (n = 6), and animals that received mitotane but were not wounded (n=8).

Parameter (units)	Wounded animals (mitotane)	Wounded animals (no mitotane)	Unwounded animals (mitotane)	F ratio (DF)	*P*-value
Creatine kinase (IU/L)	3637 ± 704	2177 ± 815	1627 ± 594	2.34 (19)	0.123
Uric acid (mg/dL)	12.3 ± 1.2	11.0 ± 1.2	13.1 ± 1.4	0.64 (19)	0.540
Phosphorus (mg/dL)	6.3 ± 0.7	6.3 ± 1.3	6.6 ± 0.6	0.05 (19)	0.955
Calcium ions (mg/dL)	8.5 ± 0.2	8.9 ± 0.3	8.5 ± 0.1	1.06 (19)	0.368
Total protein (g/dL)	3.2 ± 0.2	3.6 ± 0.3	3.4 ± 0.3	0.67 (19)	0.522
Sodium ions (mmol/L)	155.2 ± 0.7	144.0 ± 8.9	155.4 ± 1.6	2.02 (19)	0.160

Values were determined using avian/reptilian plus rotors on a portable vetscan machine, and are presented as mean ± SEM.

(Awerman and Romero, 2010; Harr, 2002). Thus, elevated glucose and reduced aspartate aminotransferase may be useful indicators of metabolic shifts in wild bird species induced by immune activation.

In conclusion, we showed that a small leg wound altered blood chemistry parameters and MR in skin surrounding the wound site in house sparrows. However, we saw no difference between wounded birds and controls in GR or MR concentrations in liver, muscle, or another immune tissue, the spleen. These results emphasize the heterogeneity of CORT's effects on different tissues, even to the point where different regions of the same tissue type (as in skin) may respond differently to the same hormonal signal because of regional differences in GR or MR density. Localized CORT production in response to tissue damage or other noxious stimuli, and local increases in the expression or activity of enzymes like 11β-HSD1 could further increase the heterogeneity of CORT's effects on skin. The ability to be regulated at so many different levels (e.g., hormone release, enzyme activity, receptor expression, etc.) helps illustrate how CORT can be such a flexible and powerful mediator of immune function.

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