β-Glucocerebrosidase Activity in the Stratum Corneum of House Sparrows following Acclimation to High and Low Humidity

Robert M. Cox[1,*]
Agustí Muñoz-Garcia[1]
Marianne S. Jurkowitz[2]
Joseph B. Williams[1]
[1]Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, Ohio 43210; [2]Department of Molecular and Cellular Biochemistry, Ohio State University, Columbus, Ohio 43210

Accepted 5/30/2007; Electronically Published 11/20/2007

ABSTRACT

Skin is an important avenue of water loss in terrestrial birds, so environmental conditions that necessitate water conservation should favor physiological mechanisms that reduce cutaneous water loss (CWL). Skin resistance to CWL is conferred by a barrier of lipid molecules located in the stratum corneum (SC), the outer layer of the epidermis. In mammals, SC barrier function depends on the conversion of cerebrosides to ceramides by the enzyme β-glucocerebrosidase (β-GlcCer'ase). Avian SC contains both cerebrosides and ceramides, suggesting that observed plasticity in CWL may be mediated by changes in β-GlcCer'ase activity and resultant SC lipid composition. We tested the hypothesis that changes in ambient humidity would alter β-GlcCer'ase activity by acclimating house sparrows (*Passer domesticus*) to either dry (6.5 g H_2O m^{-3} absolute humidity) or humid (31 g H_2O m^{-3}) conditions for 5 and 21 d at 30°C and then measuring β-GlcCer'ase activity from SC homogenates. Our results provide the first characterization of β-GlcCer'ase activity in any nonmammalian vertebrate. Relative to nonacclimated controls, both dry- and humid-acclimated sparrows had significantly elevated β-GlcCer'ase activity at 21 d postacclimation. Across individuals, we observed negative correlations between β-GlcCer'ase activity and both CWL and SC ceramide content. Although dry- and humid-acclimated sparrows did not differ in β-GlcCer'ase activity, these results are consistent with our findings that both humidity treatments caused a reduction in CWL and similar changes in SC lipid composition. Our results demonstrate physiological plasticity in CWL and provide tentative support for a role of β-GlcCer'ase in mediating this response.

* Corresponding author. Present address: Department of Biological Sciences, Dartmouth College, 401 Gilman Hall, Hanover, New Hampshire 03755; e-mail: robert.m.cox@dartmouth.edu.

Physiological and Biochemical Zoology 81(1):97–105. 2008. © 2007 by The University of Chicago. All rights reserved. 1522-2152/2008/8101-06146$15.00
DOI: 10.1086/522652

Introduction

Maintaining water homeostasis is a crucial physiological task, particularly in arid environments where ambient temperatures are high and humidity is low. Skin is an important avenue of water efflux in terrestrial birds, with cutaneous water loss (CWL) comprising as much as 50%–70% of total evaporative water loss at normothermic temperatures in many species (Bernstein 1971; Wolf and Walsberg 1996; Tieleman and Williams 2002; Muñoz-Garcia and Williams 2005). Comparisons of species from arid versus humid environments support the idea that desert birds have rates of total evaporative water loss lower than those of mesic counterparts (Williams 1996; Williams and Tieleman 2000), and data from larks indicate that this pattern is driven by underlying differences in CWL rather than respiratory water loss (RWL; Tieleman and Williams 1999, 2002; Tieleman et al. 1999). While these differences in CWL presumably reflect some degree of underlying genetic divergence, several species can also alter water loss in response to short-term humidity or temperature acclimation (Tieleman and Williams 2002; Haugen et al. 2003*a*; Tieleman et al. 2003). However, the cellular and molecular mechanisms that enable such physiological plasticity in CWL are not well understood.

In birds and mammals, skin resistance to CWL is conferred by a barrier consisting of lipid molecules arranged in an extracellular matrix located in the stratum corneum (SC), the outer layer of the epidermis (Menon et al. 1986*a*; Wertz 2000; Bouwstra et al. 2003; Coderch et al. 2003; Lillywhite 2006). The permeability of this barrier is a function of the total quantity of lipids and associated barrier thickness, the proportion of various lipid classes (mainly cholesterol, free fatty acids, ceramides, and cerebrosides), and the structural arrangement of lipid molecules, which, in turn, may depend on attributes such as polarity, saturation, and hydrocarbon chain length (Bouwstra et al. 2003; Coderch et al. 2003; Lillywhite 2004, 2006). Changes in lipid biosynthesis and metabolism may provide a mechanism for regulation of CWL in response to environmental demands on water homeostasis. Indeed, correlated differences in CWL and SC lipid composition in birds are associated with habitat aridity (Haugen et al. 2003*b*; Muñoz-Garcia and Williams 2005), thermal acclimation (Haugen et al. 2003*a*), and water deprivation (Menon et al. 1989), but the underlying mechanisms for these differences have not been investigated.

The functional significance of SC lipid composition with respect to CWL is best understood in mammals, in large part because of clinical research directed toward understanding human skin disorders. Gaucher disease is characterized by a deficiency in the enzyme β-glucocerebrosidase (β-GlcCer'ase), which converts cerebrosides to ceramides (Fig. 1). Ceramides are the primary lipid constituents of mammalian SC and are necessary for proper barrier function. In the absence of functional β-GlcCer'ase, mammalian SC is characterized by an abundance of cerebrosides, a reduction in ceramides, and impaired barrier function (Holleran et al. 1994*a*; Liu et al. 1998). Thus, β-GlcCer'ase is essential to the formation and maintenance of a competent lipid barrier to water loss in mammals (Glew et al. 1988; Holleran et al. 1992, 1993, 1994*a*, 1994*b*; Liu et al. 1998; Takagi et al. 1999).

Whereas cerebrosides are present in only trace amounts in mammalian SC (Wertz 1992), the proportion of cerebrosides often equals or exceeds that of ceramides in avian SC (Menon et al. 1986*a*; Wertz et al. 1986; Muñoz-Garcia and Williams 2005). This condition would be pathological in terrestrial mammals (e.g., Liu et al. 1998), raising the question of how and why birds differ from mammals in SC lipid composition. In this study, we examine the mechanistic basis of this difference by developing an assay that provides the first characterization of β-GlcCer'ase activity in avian SC. Previous studies of β-GlcCer'ase have been conducted almost exclusively in the context of clinical research on humans or animal models for human disease. Here, we expand not only the taxonomic scope of these studies but also their ecological context by testing the hypothesis that β-GlcCer'ase mediates environmentally induced physiological plasticity in SC lipid composition and CWL.

Muñoz-Garcia and Williams (2005) reported that house sparrows (*Passer domesticus* L.) from the deserts of Saudi Arabia have lower CWL and greater quantities of ceramides and cerebrosides in their SC than do conspecifics from mesic Ohio. Among Ohio sparrows, CWL was negatively associated with proportions of both ceramides and cerebrosides. These authors hypothesized that changes in the activity of enzymes such as β-GlcCer'ase allow sparrows to adjust the relative proportions of ceramides and cerebrosides in the SC, thereby regulating CWL in response to environmental stimuli. Thus, desiccating conditions that favor water conservation should stimulate β-GlcCer'ase activity, leading to increased SC ceramide content and reduced CWL. In this study, we test this hypothesis by acclimating captive house sparrows to either high or low ambient humidity under thermoneutral conditions, measuring β-GlcCer'ase activity, and relating these data to changes in CWL and SC lipid composition. We predict that sparrows acclimated to low ambient humidity should increase β-GlcCer'ase activity, increase SC ceramide content, and reduce CWL relative to humid-acclimated sparrows and nonacclimated controls.

Material and Methods

Experimental Design

Details about animal collection, housing, acclimation to different humidity regimes, and respirometry methods are described by Muñoz-Garcia et al. (2008). Briefly, we captured adult house sparrows from Columbus, Ohio (40°00′N, 83°10′W) during the winter (January–March 2006), transported them to our laboratory, and measured their initial rates of CWL, RWL, and oxygen consumption. Sparrows were then randomly assigned to one of three treatment groups: nonacclimated ($n = 13$), humid-acclimated ($n = 15$), or dry-

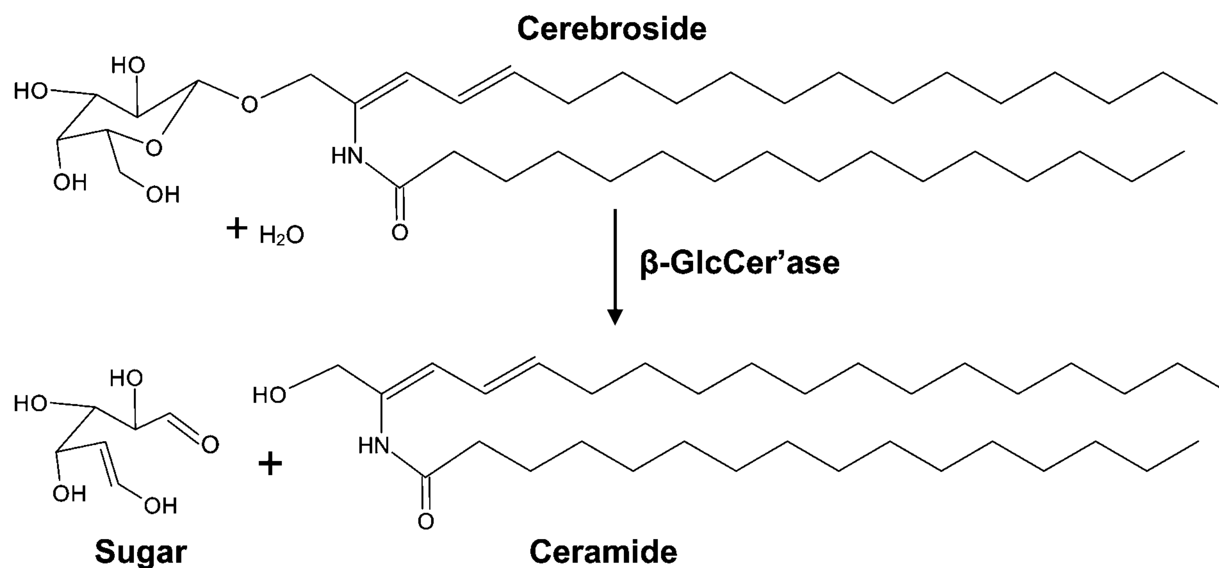

Figure 1. Cerebrosides are hydrolyzed by β-GlcCer'ase to form a sugar and a ceramide. Ceramides consist of a fatty acid and a sphingosine base and form the primary barrier to water diffusion in the mammalian stratum corneum.

acclimated ($n = 20$). Nonacclimated birds were immediately euthanized to obtain skin samples for enzyme assays. Humid- and dry-acclimated birds were held for either 5 d (short acclimation, $n = 6$ per treatment) or 21 d (long acclimation, $n = 9$ humid, 14 dry) in environmental chambers set to maintain dry (6.5 g H_2O m^{-3} absolute humidity) or humid (31 g H_2O m^{-3}) ambient conditions at a constant temperature of 30°C. At the end of the 21-d acclimation period, we measured whole-animal CWL, RWL, and oxygen consumption, after which we euthanized animals to obtain SC samples for enzyme assays. We also measured lipid content of the SC in most of these same animals ($n = 9$ nonacclimated, 9 humid, 14 dry), as described by Muñoz-Garcia et al. (2008). The Ohio State University Institutional Laboratory Animal Care and Use Committee approved all experimental procedures and animal facilities used in this study (protocol 2003A0072).

Preparation of Enzyme Homogenates

Animals were euthanized via cervical dislocation, and their feathers were plucked and skins removed. We then excised 4-cm^2 tissue samples from apteria on the dorsal and ventral surfaces of each animal. The remainder of the skin was used to assay lipid content of the SC (Muñoz-Garcia et al. 2008). Portions excised for our enzyme assays were pinned, dermis down, on filter paper that was impregnated with 0.5% trypsin in phosphate-buffered saline (PBS; pH 7.4; see Muñoz-Garcia et al. 2008) and were stored overnight at 4°C (Menon et al. 1989). After 24 h, we removed any remaining feathers, peeled off the SC, and manually homogenized it by using ground glass vials and pestles. Samples were homogenized in extraction buffer consisting of 300 μL PBS with 0.05% Tween 20, 0.1 mM phenylmethylsulfonyl fluoride, and 10 mg mL^{-1} sodium taurocholate, each included to improve extraction of soluble enzyme (Pentchev et al. 1973; Holleran et al. 1992). Samples were held on ice during homogenization and subsequent incubation for 30 min in extraction buffer. We then centrifuged samples at 10,000 g and used the supernatant for assays.

β-Glucosidase Assay

Our assay for β-glucosidase was modified from Holleran et al. (1992). Except where noted, assays were carried out in citrate-phosphate buffer (pH 5.6; McIlvaine 1921) containing 5 mM sodium taurocholate. We added 10 μL of enzyme sample (supernatant from tissue homogenates) to 40 μL of citrate-phosphate buffer and preheated this mixture to 37°C. We initiated reactions by adding 50 μL of citrate-phosphate buffer containing 10 mM 4-methylumbelliferyl-β-D-glucopyranoside (4MUG; Acros Organics). Reactions were run at 37°C for 60 min and were terminated by adding 1.25 mL of 0.2 M carbonate-bicarbonate buffer (pH 10.5; Delory 1945). We measured enzyme activity as the production of fluorescent 4-methylumbelliferone (4MU) from the 4MUG substrate by using an SLM 8100 DS spectrofluorometer (excitation $\lambda = 360$ nm, emission $\lambda = 450$ nm) calibrated with a standard dilution series of 0–300 nM 4MU (Sigma-Aldrich) in carbonate-bicarbonate buffer. We used a modified Bradford protein assay kit (Bio-Rad Laboratories) to determine the protein concentration of each sample on the basis of a bovine serum albumin standard. We report all measures of enzyme activity in units of product formed per minute per milligram protein.

We initially verified that production of 4MU was linear with time (0–120 min) beyond the duration of our assay (60 min) to ensure constant reaction velocity. We also diluted samples in PBS to verify that production of 4MU was linear over the range of protein concentrations obtained from SC homogenates. We confirmed linearity with time and enzyme concentration by using both sparrow SC homogenates and a purified solution of modified human β-GlcCer'ase (Ceradase alglucerase injection; Genzyme, Cambridge, MA). We varied 4MUG substrate concentration (0.01–5 mM) and estimated maximal velocity (V_{max}) and the substrate concentration yielding half-maximal velocity (K_M) by using nonlinear Michaelis-Menten curve fits implemented in GraphPad Prism (ver. 3.02; GraphPad Software, San Diego, CA). We tested for nonspecific product formation by assaying samples in the presence of the inhibitor conduritol B epoxide (CBE; Alexis Biochemicals; 0.4–4,000 μM) and used GraphPad Prism to fit sigmoid competitive binding curves to these data.

Formation of 4MU in our assay could reflect either specific β-GlcCer'ase or nonspecific β-glucosidase activity because both enzymes can occur in the epidermis and both hydrolyze the 4MUG substrate. However, only β-GlcCer'ase is involved in the conversion of cerebrosides to ceramides (Glew et al. 1988). Specific β-GlcCer'ase activity can be demonstrated unambiguously by using labeled glucocerebroside as a substrate or by including the selective β-GlcCer'ase inhibitor bromocondutitol B epoxide, but these compounds are impractical because of their restricted availability (Glew et al. 1988; Holleran et al. 1993). A practical solution is to include sodium taurocholate, which stimulates β-GlcCer'ase while inhibiting β-glucosidase (Peters et al. 1976; Glew et al. 1988; Holleran et al. 1992). Additionally, β-GlcCer'ase activity is typically maximal at pH values ranging from 4.8 to 5.8, whereas β-glucosidase activity is maximal in a lower pH range of 4.0 and below (Peters et al. 1976; Glew et al. 1988; Holleran et al. 1992). To determine whether 4MUG hydrolysis in our assay reflects specific β-GlcCer'ase activity, we conducted assays across a pH gradient (3.2–7.2) in the presence (5 mM) or absence of sodium taurocholate.

Statistical Analyses

Statistical analyses were conducted with SAS (ver. 8.2; SAS Institute, Cary, NC) and GraphPad Prism. All data were examined for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Bartlett's test) before analysis. Comparisons between nonacclimated and 21-d humid- or dry-acclimated groups were performed using one-way ANOVA with three treatment effects

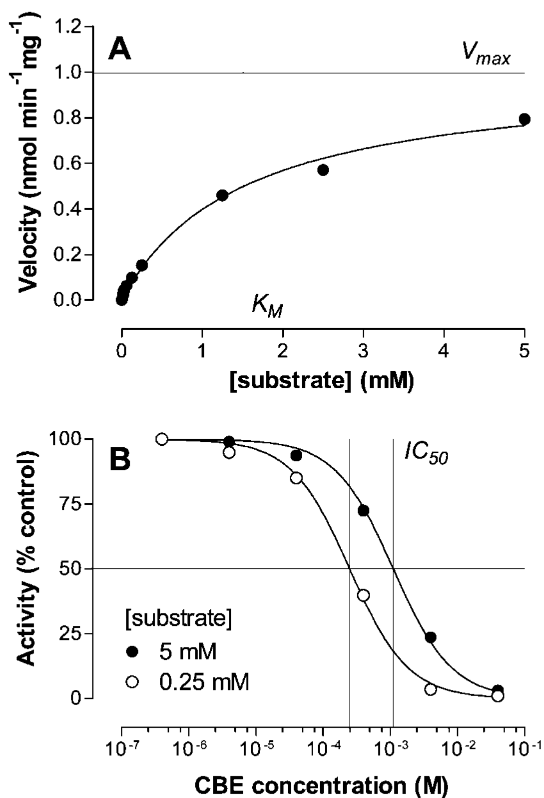

Figure 2. *A*, β-glucosidase velocity of sparrow stratum corneum homogenates, expressed as a function of substrate (4MUG) concentration. Dashed lines indicate maximal velocity (V_{max}) and the substrate concentration yielding half-maximal velocity (K_M). *B*, β-glucosidase activity as a function of inhibitor (CBE) concentration at two substrate concentrations. Values are expressed as percent activity relative to controls without CBE. Dashed lines indicate 50% inhibition of enzyme activity and associated CBE concentrations (IC_{50}). Values in each panel are means of triplicate determinations.

and Student-Newman-Keuls post hoc tests. We used *t*-tests to compare long versus short acclimation periods within humid and dry groups and also to compare humid versus dry groups within either acclimation period. We used Welch's *t*-tests to compare long and short acclimation periods because variances were significantly ($P < 0.05$) different between groups. We correlated individual measures of β-GlcCer'ase activity and CWL or SC lipid composition by using general linear models with treatment group included as a categorical effect. This allowed us to test for associations between enzyme activity and CWL or lipid composition after controlling for overall treatment effects on each variable. Because interactions with treatment were not significant ($P > 0.2$), we dropped them from our final statistical models.

Results

Assay Validation and Enzyme Characterization

When we used purified human β-GlcCer'ase, production of 4MUG increased linearly with reaction time (0–120 min; $r^2 = 0.967$) and sample concentration ($r^2 = 0.978$). This verified that our assay detects pure β-GlcCer'ase. In sparrow samples, formation of 4MUG was also linear with reaction time (0–120 min; $r^2 = 0.999$), indicating that reaction velocity is constant over our assay duration of 60 min. Formation of 4MUG was also linear with sample concentration (0–0.4 mg protein mL^{-1} sample; $r^2 = 0.988$), signifying that enzyme activity is proportional to protein concentration within the range of our experimental sample concentrations (0.13–0.42 mg mL^{-1}; mean ± SD = 0.23 ± 0.06 mg mL^{-1}).

Enzyme velocity increased curvilinearly with substrate concentration ($r^2 = 0.990$) with an apparent K_M of 1.51 ± 0.16 mM (Fig. 2*A*). Therefore, our substrate concentration for experimental samples (5 mM) was 3.3 times K_M, yielding enzyme activity at 80% V_{max}. Enzyme activity followed a negative sigmoid relationship with inhibitor (CBE) concentration (Fig. 2*B*). The estimated inhibitor concentration for half-maximal activity (IC_{50}) was 1.11 ± 0.07 mM at substrate concentration of 5 mM, with a reduction in IC_{50} at lower substrate concentrations (Fig. 2*B*).

In the absence of sodium taurocholate, enzyme activity decreased from maximal values at pH 3.2 to minimal values at pH 7.2 (Fig. 3). In the presence of 5 mM sodium taurocholate, enzyme activity was maximal at pH 4.0 (Fig. 3). At pH 5.6, enzyme activity doubled in the presence of sodium taurocholate (Fig. 3), a stimulatory effect characteristic of mammalian β-GlcCer'ase. Thus, although we observed maximal activity at low pH, we also found a clear stimulatory effect of taurocholate at pH values characteristic of the avian SC. To the extent that the characteristics of mammalian β-glucosidases are homologous in birds, these data suggest that our samples may have contained both β-glucosidase and β-GlcCer'ase but that our inclusion of taurocholate enabled us to preferentially assay β-GlcCer'ase at pH 5.6 in our experimental samples. However, the inclusion of a selective β-GlcCer'ase inhibitor, such as

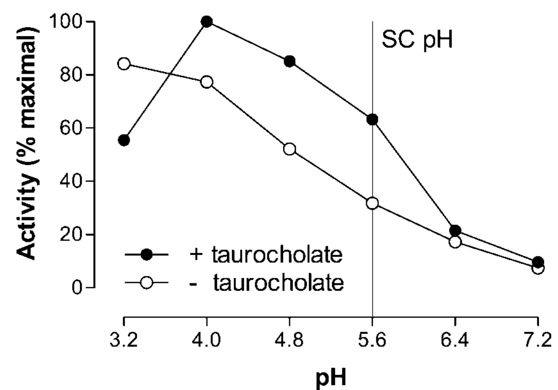

Figure 3. β-glucosidase activity of sparrow stratum corneum (SC) homogenates, expressed as a function of pH in the presence or absence of 0.5 mM sodium taurocholate. Values are means of triplicate determinations, expressed as percentage of maximal activity at pH = 4.0. Dashed line indicates the approximate pH of the SC, at which experimental samples were assayed.

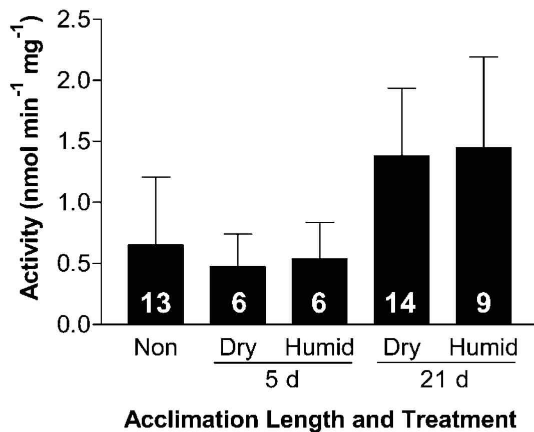

Figure 4. Mean (±1 SD) β-GlcCer'ase activity for nonacclimated and dry or humid groups after 5 or 21 d acclimation. Values for each individual are means from dorsal and ventral surfaces. Sample size is indicated at the base of each bar.

bromoconduritol B epoxide, would be necessary to verify this conclusion (Holleran et al. 1993). Hereafter, for simplicity, we refer to our measures as β-GlcCer'ase activity.

Treatment Effects on β-GlcCer'ase Activity

Measures of β-GlcCer'ase activity from dorsal and ventral SC samples of the same individuals were correlated ($r^2 = 0.80$; $P < 0.001$; data were $\log_{10}$ transformed for normality), with no systematic bias toward greater activity from either surface. Hereafter, we report results of analyses by using the mean of dorsal and ventral measures. The β-GlcCer'ase activity was low before acclimation or after only 5 d acclimation (Fig. 4). By contrast, β-GlcCer'ase activity was elevated after 21 d acclimation in both humid and dry groups (Fig. 4). Comparisons among humid, dry, and nonacclimated birds revealed a significant increase in β-GlcCer'ase activity after acclimation to either humidity regime ($F_{2,33} = 6.26$, $P = 0.005$; Fig. 4). Contrary to our predictions, we found no difference in β-GlcCer'ase activity between humid and dry treatments at either 5 d ($t = 0.38$; $P = 0.712$) or 21 d postacclimation ($t = 0.24$; $P = 0.817$). However, β-GlcCer'ase activity was greater at 21 d than at 5 d postacclimation within both humid (Welch's $t = 3.27$; $P = 0.008$) and dry groups (Welch's $t = 4.94$; $P < 0.001$).

β-GlcCer'ase and CWL

To investigate the functional consequences of β-GlcCer'ase activity, we examined among-individual correlations between enzyme activity and whole-animal rates of CWL. Data on CWL are reported in detail elsewhere (Muñoz-Garcia et al. 2008); we report only those data germane to β-GlcCer'ase activity. Across treatment groups, we found a negative relationship between β-GlcCer'ase activity and CWL ($F_{3,28} = 5.86$, $P = 0.022$; Fig. 5*A*). This correlation is driven by the low enzyme activity and high CWL of nonacclimated birds, as well as the weak negative relationship between CWL and β-GlcCer'ase activity within the humid-acclimated group ($r^2 = 0.43$; $P = 0.055$). CWL was not significantly correlated with β-GlcCer'ase activity within either the dry-acclimated or the nonacclimated groups ($P > 0.78$) or across groups once we removed variance attributable to treatment ($F_{3,28} = 2.85$, $P = 0.102$). Further, we found no relationship between β-GlcCer'ase activity and the

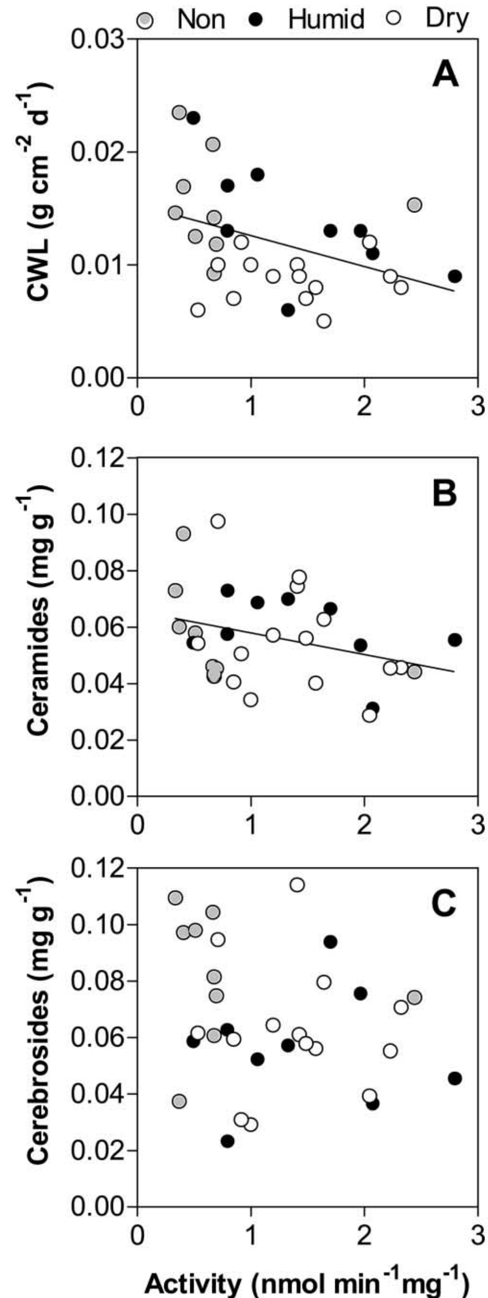

Figure 5. Cutaneous water loss (*A*) and stratum corneum concentration of ceramides (*B*) and cerebrosides (*C*), expressed as a function of β-GlcCer'ase activity for nonacclimated and 21-d humid- or dry-acclimated sparrows.

magnitude of change in CWL after acclimation in either the humid ($r^2 = 0.24$; $P = 0.186$) or the dry treatment ($r^2 = 0.05$; $P = 0.443$) or across the two groups combined ($F_{2,20} = 1.73$, $P = 0.376$; Fig. 6).

β-GlcCer'ase and Skin Lipids

We also examined among-individual correlations between β-GlcCer'ase activity and lipid content of the SC. These lipid data are reported in detail elsewhere (Muñoz-Garcia et al. 2008). After removing variance attributable to overall treatment effects, we found a weak negative relationship between β-GlcCer'ase activity and the total concentration of SC ceramides ($F_{3,28} = 4.37$, $P = 0.046$; Fig. 5*B*). We observed a similar trend after expressing ceramide concentration as a percentage of total SC lipid composition ($F_{3,28} = 3.69$, $P = 0.065$; percentages were arcsine transformed for analysis; Zar 1996). We also conducted separate analyses for each of the five major classes of ceramide molecules that could be distinguished on the basis of thin-layer chromatography (see Muñoz-Garcia et al. 2008). Although these classes differ in polarity and may therefore confer different permeability properties, we observed similarly weak negative relationships between SC lipid concentration and β-GlcCer'ase activity within each ceramide class ($0.03 < P < 0.2$). We did not find any correlations between β-GlcCer'ase activity and the concentration of cerebrosides within the SC (Fig. 5*C*), nor did we find a relationship between β-GlcCer'ase activity and the ratio of ceramides to cerebrosides in the SC ($P < 0.5$).

Discussion

Our results provide the first documentation of β-GlcCer'ase activity in the SC of any nonmammalian vertebrate. Overall, the specific activities that we observed (range 0.2–2.8 nmol min^{-1} mg^{-1}) are comparable to values reported for hydrolysis of 4MUG under similar conditions by tissue homogenate supernatants from mice (Holleran et al. 1992, 1993, 1994*a*, 1994*b*). The mean β-GlcCer'ase activity that we observed in nonacclimated sparrows (0.67 nmol min^{-1} mg^{-1}) is lower than typical values for homogenate supernatants from murine epidermis (2.60 nmol min^{-1} mg^{-1}) and SC (4.09 nmol min^{-1} mg^{-1}; Holleran et al. 1992). However, our data from acclimated sparrows demonstrate that comparable levels of β-GlcCer'ase activity are possible in avian SC. Unfortunately, it is difficult to assess the true magnitude of these differences between mammals and birds because of discrepancies in assay conditions. For example, we increased substrate concentration to 5 mM in order to substantially exceed our estimated K_M of 1.51 mM, whereas Holleran et al. (1992) assayed murine β-GlcCer'ase activity below their estimated K_M of 0.93 mM. Given that we obtained significantly lower estimates of β-GlcCer'ase activity at their preferred substrate concentration (data not shown), we believe it is reasonable to suggest that β-GlcCer'ase activity is greater in murine than in avian SC, even if the precise magnitude of this difference is uncertain. This difference may explain why cerebrosides, which occur in trace amounts in mammalian SC (Wertz 1992), are abundant in avian SC (Menon et al. 1986*a*; Wertz et al. 1986; Muñoz-Garcia and Williams 2005).

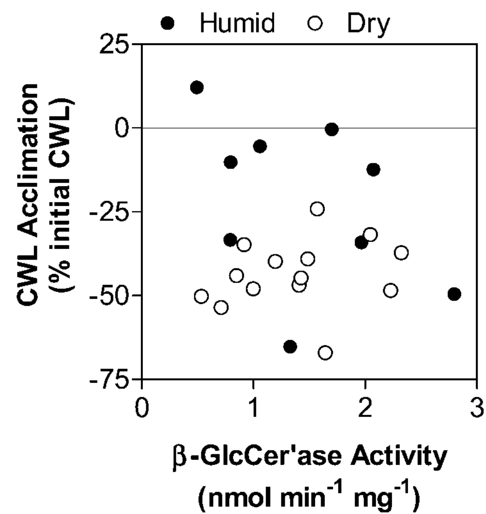

Figure 6. Acclimation response of cutaneous water loss (CWL) as a function of β-GlcCer'ase activity for humid or dry groups after acclimation for 21 d. CWL is expressed as percent change from initial measurements, such that negative values indicate a decrease in CWL. Percentages were arcsine transformed before analysis.

Given the role of β-GlcCer'ase in maintaining SC barrier function in mammals, we predicted that this enzyme is also involved in the regulation of skin resistance to CWL in birds. House sparrows exhibit geographic variation in CWL and SC lipid composition that is associated with habitat aridity (Muñoz-Garcia and Williams 2005) and can reduce whole-animal CWL by an average of 45% in response to short-term humidity acclimation (Muñoz-Garcia et al. 2008). In this study, we found that these same dry-acclimated sparrows exhibited a significant increase in β-GlcCer'ase activity relative to initial levels in nonacclimated birds (Fig. 4). However, contrary to our initial prediction, we found no difference in β-GlcCer'ase activity between humid- and dry-acclimated birds at either 5 d or 21 d postacclimation (Fig. 4). Although we predicted greater β-GlcCer'ase activity in dry-acclimated than in humid-acclimated sparrows, this prediction was based on the expectation that CWL would be reduced only in response to low humidity. Contrary to this expectation, we observed a significant decrease in CWL among humid-acclimated sparrows, although the average magnitude of this reduction (24%) was significantly less than in dry-acclimated birds (Muñoz-Garcia et al. 2008). Further, both dry- and humid-acclimated sparrows exhibited increases in the proportion of SC ceramides relative to nonacclimated birds (Muñoz-Garcia et al. 2008). This may indicate that thermal acclimation had an overriding effect on enzyme activity, SC lipid composition, and CWL.

Hoopoe larks (*Alaemon alaudipes*) acclimated to 35°C show reduced CWL and increased proportions of SC ceramides relative to those acclimated to 15°C (Haugen et al. 2003*a*). In this

study, we deliberately exposed both humid- and dry-acclimated sparrows to identical temperatures in the thermoneutral range (30°C; Hudson and Kimzey 1966) to minimize thermoregulatory demands on CWL. However, both groups still experienced a substantial change relative to ambient outdoor temperatures at their time of capture (−10° to 10°C). This suggests that, contrary to our initial assumptions, temperature may have an important effect on SC lipid composition, even at temperatures below extremes that favor CWL as an evaporative cooling mechanism. A more elaborate experimental design would be required to disentangle the separate effects of temperature and humidity on CWL acclimation. However, it is perhaps not surprising that we found increased *β*-GlcCer'ase activity in both humidity treatments, given that both treatments decreased CWL and increased SC ceramide content (Muñoz-Garcia et al. 2008). Thus, our overall treatment effects are consistent with the hypothesis that increased *β*-GlcCer'ase activity leads to increased SC ceramide content and reduced CWL, but the effects are ambiguous with respect to whether humidity, temperature, or their interaction mediates this acclimation.

To address the functional significance of *β*-GlcCer'ase activity, we examined correlations between *β*-GlcCer'ase activity, CWL, and SC lipid composition among individuals. We found a negative relationship between CWL and *β*-GlcCer'ase activity across treatments (Fig. 5*A*), but relationships within individual treatment groups were weak (humid group) or absent (nonacclimated and dry groups). Although we found a negative relationship between SC ceramide content and *β*-GlcCer'ase activity (Fig. 5*B*), this is contrary to the positive association predicted from the role of *β*-GlcCer'ase in ceramide formation. These results provide little support for a functional relationship between *β*-GlcCer'ase activity and either CWL or SC lipid composition, although several factors complicate this interpretation.

First, CWL and SC lipid composition reflect the cumulative effects of lipid synthesis and metabolism throughout the acclimation period, whereas *β*-GlcCer'ase activity provides an instantaneous measure of this process. If sustained elevation in *β*-GlcCer'ase activity is not required to maintain increased skin resistance, then it may be unreasonable to expect instantaneous measures of *β*-GlcCer'ase activity to correlate strongly with CWL or SC lipid composition. For example, mammalian *β*-GlcCer'ase activity is maximal after disruption of the skin but returns to baseline levels once ceramide content of the SC has been restored (Holleran et al. 1994*b*). By analogy, avian *β*-GlcCer'ase activity may be elevated only when the lipid composition of the SC is insufficient for environmental demands on thermoregulation or water conservation, perhaps explaining the negative correlation between SC ceramide concentration and *β*-GlcCer'ase activity that we observed (Fig. 5*B*).

Second, *β*-GlcCer'ase activity is maximal in the basal layers of the SC but is also evident throughout the epidermis (Holleran et al. 1992; Takagi et al. 1999). On the basis of these data and our hypothesized functional model of avian SC (Muñoz-Garcia and Williams 2005), we assayed *β*-GlcCer'ase activity specifically in the SC rather than in the entire epidermis. However, our assumption that avian *β*-GlcCer'ase activity is primarily restricted to the SC remains to be verified (e.g., Takagi et al. 1999). We cannot discount the possibility that *β*-GlcCer'ase activity in underlying layers of the epidermis is also important for the regulation of SC lipid composition.

Third, ceramides are only one of several lipid classes that may contribute to skin permeability. Likewise, *β*-GlcCer'ase is only one of several putative enzymes, such as transferases and phospholipases, that regulate SC lipid composition (Coderch et al. 2003). In mammals, both free fatty acid and cholesterol composition of the SC can influence skin permeability (Brod 1991; Coderch et al. 2003). Comparisons of sparrows and larks from environments along a temperature-aridity gradient indicate that proportions of free fatty acids and cholesterol may also influence CWL (Haugen et al. 2003*b*; Muñoz-Garcia and Williams 2005). Further, short-term temperature or humidity acclimation increases the proportion of SC cholesterol in both house sparrows and hoopoe larks (Haugen et al. 2003*a*; Muñoz-Garcia et al. 2008). Interactions between the absolute quantity and relative proportions of these various lipid classes, and between various lipid molecules within each class, are likely to mediate CWL, and this complexity may confound simple attempts to correlate gross measures of enzyme activity, lipid proportions, and CWL.

Finally, lipid composition of the SC and associated skin resistance are not the only factors that determine CWL. Our rationale for focusing on structural changes to the lipid barrier of the SC is based on previous work demonstrating physiological plasticity of SC lipids in response to thermal acclimation (Haugen et al. 2003*a*) and natural variation in SC lipids with respect to habitat aridity (Haugen et al. 2003*b*; Muñoz-Garcia and Williams 2005). However, CWL is also influenced by the water vapor gradient between the skin and air. For example, heat-acclimated rock pigeons (*Columba livia*) increase CWL in response to heat stress as an adaptive cooling mechanism (Arieli et al. 2002). Although long-term heat acclimation induces structural modifications to the epidermis that presumably reduce its resistance to water diffusion (Peltonen et al. 1998), transient increases in CWL in response to heat stress are mediated primarily by changes in dermal blood flow and hydrostatic pressure within capillaries that increase water efflux from the capillaries to the epidermis (Arieli et al. 2002). However, we do not know the extent to which CWL in house sparrows is influenced by SC lipid composition, as opposed to other structural and physiological properties of the skin.

Our results demonstrate phenotypic plasticity in CWL of house sparrows; short-term laboratory acclimation responses are comparable to natural CWL differences between xeric and mesic populations. In addition to decreased CWL, acclimated sparrows exhibited increased SC *β*-GlcCer'ase activity and ceramide content, suggesting a physiological mechanism by which sparrows can adjust SC lipid composition and, ultimately, CWL. To directly address the importance of *β*-GlcCer'ase activity to avian SC function, it may be necessary to manipulate enzyme activity in vivo. This could be accomplished via topical appli-

cation of specific inhibitors (Holleran et al. 1993) and observation of the resultant effects on CWL and SC lipid composition. The interpretation of our results is also complicated by the similar responses that we observed in both humid- and dry-acclimated sparrows. These results indicate that ambient humidity per se may not be the primary environmental stimulus influencing SC physiology and CWL. On the basis of short-term physiological responses to thermal acclimation in other birds (e.g., Haugen et al. 2003*a*), we suspect that changes in ambient temperature may also promote changes in skin permeability and CWL.

Acknowledgments

We thank Dr. Walter Holleran and Dr. Yoshikazu Uchida for providing Ceradase and for helpful discussions regarding assay methodology. We also thank Warren Erdahl and Cliff Chapman for assistance with spectrofluorometry and other assay procedures and Jennifer Ro for critically reading this manuscript. This research was supported by a National Science Foundation grant (IBN-0212092) to J.B.W.

Literature Cited

Arieli Y., L. Peltonen, and E. Ophir. 2002. Cooling by cutaneous water evaporation in the heat-acclimated rock pigeon (*Columba livia*). Comp Biochem Physiol A 131:497–504.

Bernstein M.H. 1971. Cutaneous water loss in small birds. Condor 73:468–469.

Bouwstra J.A., P.L. Honeywell-Nguyen, G.S. Gooris, and M. Ponec. 2003. Structure of the skin barrier and its modulation by vesicular formations. Prog Lipid Res 42:1–36.

Brod J. 1991. Characterization and physiological role of epidermal lipids. J Investig Dermatol 30:84–90.

Coderch L., O. López, A. de la Maza, and J.L. Parra. 2003. Ceramides and skin function. Am J Clin Dermatol 4:107–129.

Delory G.E. 1945. A sodium carbonate-bicarbonate buffer for alkaline phosphotases. Biochemistry 39:245.

Glew R.H., A. Basu, K.L. LaMarco, and E.M. Prence. 1988. Mammalian glucocerebrosidase: implications for Gaucher's disease. Lab Invest 58:5–25.

Haugen M.J., B.I. Tieleman, and J.B. Williams. 2003*a*. Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum. J Exp Biol 206:3581–3588.

Haugen M.J., J.B. Williams, P. Wertz, and B.I. Tieleman. 2003*b*. Lipids of the stratum corneum vary with cutaneous water loss among larks along a temperature-moisture gradient. Physiol Biochem Zool 76:907–917.

Holleran W.M., E.I. Ginns, G.K. Menon, J.U. Grundmann, M. Fartasch, C.E. McKinney, P.M. Elias, and E. Sidransky. 1994*a*. Consequences of beta-glucocerebrosidase deficiency in the epidermis: ultrastructure and permeability barrier alterations in Gaucher disease. J Clin Invest 93:1756–1764.

Holleran W.M., Y. Takagi, G. Imokawa, S.M. Jackson, J.M. Lee, and P.M. Elias. 1992. β-Glucocerebrosidase activity in murine epidermis: characterization and localization in relation to differentiation. J Lipid Res 33:1201–1209.

Holleran W.M., Y. Takagi, G.K. Menon, S.M. Jackson, J.M. Lee, K.R. Feingold, and P.M. Elias. 1994*b*. Permeability barrier requirements regulate epidermal β-glucocerebrosidase. J Lipid Res 35:905–912.

Holleran W.M., Y. Takagi, G.K. Menon, G. Legler, K.R. Feingold, and P.M. Elias. 1993. Processing of epidermal glucosylceramides is required for optimal mammalian cutaneous permeability barrier function. J Clin Invest 91:1656–1664.

Hudson J.W. and S.L. Kimzey. 1966. Temperature regulation and metabolic rhythms in populations of the house sparrow, *Passer domesticus*. Comp Biochem Physiol A 17:203–217.

Lillywhite H.B. 2004. Plasticity of the water barrier in vertebrate integument. Int Congr Ser 1275:283–290.

———. 2006. Water relations of tetrapod integument. J Exp Biol 209:202–226.

Liu Y., K. Suzuki, J.D. Reed, A. Grinberg, H. Westphal, A. Hoffman, T. Döring, K. Sandhoff, and R.L. Proia. 1998. Mice with type 2 and 3 Gaucher disease point mutations generated by a single insertion mutagenesis procedure (SIMP). Proc Natl Acad Sci USA 95:2503–2508.

McIlvaine T.C. 1921. A buffer solution for calorimetric comparison. J Biol Chem 49:183–186.

Menon G.K, L.F. Baptista, B.E. Brown, and P.M. Elias. 1989. Avian epidermal differentiation. II. Adaptive response of permeability barrier to water deprivation and replenishment. Tissue Cell 21:83–92.

Menon G.K., B.E. Brown, and P.M. Elias. 1986*a*. Avian epidermal differentiation: role of lipids in permeability barrier formation. Tissue Cell 18:71–82.

Muñoz-Garcia A. and J.B. Williams. 2005. Cutaneous water loss and lipids of the stratum corneum in house sparrows *Passer domesticus* from arid and mesic environments. J Exp Biol 208:3689–3700.

Muñoz-Garcia A., R. Cox, and J.B. Williams. 2008. Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum in house sparrows (*Passer domesticus*) following acclimation to high and low humidity. Physiol Biochem Zool 81:87–96.

Peltonen L., Y. Arieli, A. Pyörnilä, and J. Marder. 1998. Adaptive changes in the epidermal structure of the heat-acclimated rock pigeon (*Columba livia*): a comparative electron microscopy study. J Morphol 235:17–29.

Pentchev P.G., R.O. Brady, S.R. Hibbert, A.E. Gal, and D. Shapiro. 1973. Isolation and characterization of glucocerebrosidase from human placental tissue. J Biol Chem 248:5256–5261.

Peters S.P., P. Coyle, and R.H. Glew. 1976. Differentiation of β-glucocerebrosidase from β-glucosidase in human tissues using sodium taurocholate. Arch Biochem Biophys 175:569–582.

Takagi Y., E. Kriehuber, G. Imokawa, P.M. Elias, and W.M. Holleran. 1999. *β*-Glucocerebrosidase activity in mammalian stratum corneum. J Lipid Res 40:861–869.

Tieleman B.I. and J.B. Williams. 1999. The role of hyperthermia in the water economy of desert birds. Physiol Biochem Zool 72:87–100.

———. 2002. Cutaneous and respiratory water loss in larks from arid and mesic environments. Physiol Biochem Zool 75:590–599.

Tieleman B.I., J.B. Williams, M.E. Buschur, and C.R. Brown. 2003. Phenotypic variation of larks along an aridity gradient: are desert birds more flexible? Ecology 84:1800–1815.

Tieleman B.I., J.B. Williams, G. Michaeli, and B. Pinshow. 1999. The role of nasal passages in the water economy of crested larks and desert larks. Physiol Biochem Zool 72:219–226.

Wertz P.W. 1992. Epidermal lipids. Semin Dermatol 11:106–113.

———. 2000. Lipids and barrier function of the skin. Acta Dermato-Venereol Suppl 208:7–11.

Wertz P.W., P.M. Stover, W. Abraham, and D.T. Downing. 1986. Lipids of the chicken epidermis. J Lipid Res 27:427–435.

Williams J.B. 1996. A phylogenetic perspective of evaporative water loss in birds. Auk 113:457–472.

Williams J.B. and B.I. Tieleman. 2000. Flexibility in basal metabolism and evaporative water loss in hoopoe larks from the Arabian desert. J Exp Biol 203:153–159.

Wolf B.O. and G.E. Walsberg. 1996. Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. J Exp Biol 199:451–457.

Zar J.H. 1996. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ.