The Relationship between Hormones, Glucose, and Oxidative Damage Is Condition and Stress Dependent in a Free-Living Passerine Bird

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ABSTRACT

Physiological state is an emergent property of the interactions among physiological systems within an intricate network. Understanding the connections within this network is one of the goals in physiological ecology. Here, we studied the relationship between body condition, two neuroendocrine hormones (corticosterone and insulin-like growth factor 1 [IGF-1]) as physiological regulators, and two physiological systems related to resource metabolism (glucose) and oxidative balance (malondialdehyde). We measured these traits under baseline and stress-induced conditions in free-living house sparrows (*Passer domesticus*). We used path analysis to analyze different scenarios about the structure of the physiological network. Our data were most consistent with a model in which corticosterone was the major regulator under baseline conditions. This model shows that individuals in better condition have lower corticosterone levels; corticosterone and IGF-1 levels are positively associated; and oxidative damage is higher when levels of corticosterone, IGF-1, and glucose are elevated. After exposure to acute stress, these relationships were considerably reorganized. In response to acute stress, birds increased their corticosterone and glucose levels and decreased their IGF-1 levels. However, individuals in better condition increased their corticosterone levels more and better maintained their IGF-1 levels in response to acute stress. The acute stress–induced changes in corticosterone and IGF-1 levels were associated with an increase in glucose levels, which in turn was associated with a decrease in oxidative damage. We urge ecophysiologists to focus more on physiological networks, as the relationships between physiological traits are complex and dynamic during the organismal stress response.

Keywords: glucocorticoid, house sparrow, IGF-1, oxidative stress, restraint, phenotypic integration, somatotropic axis.

Introduction

Animals possess adaptations that permit phenotypic adjustments to prevailing and changing environmental conditions. Physiological traits are key in this flexible adjustment because they readily react to changes in extrinsic and intrinsic conditions and also have various downstream effects on other physiological traits, gene expression, behavior, and performance (Ricklefs and Wikelski 2002; Cohen et al. 2012). Animal physiology is composed of different systems that do not work in isolation but form an integrated physiological regulatory network (sensu Cohen et al. 2012). Hormones play a key regulatory role (called "integrators"; Martin et al. 2011; Cohen et al. 2012) in this physiological network because, through their pleiotropic effects, they can influence multiple physiological subnetworks, such as energy metabolism, oxidative balance, and the immune system (Cohen et al. 2012). Studying the relationships between these physiological systems in different contexts may therefore reveal which components are activated when organisms try to maintain homeostasis in the face of changing environmental conditions (Ouyang et al. 2016). The interaction between physiological systems ultimately leads to an integrated phenotype that will be the target of natural selection. Indeed, phenotypic integration, the study of complex patterns of covariation, will aid in understanding phenotypic evolution (Pigliucci 2003; Cox et al. 2016).

One of the traits that is kept under tight homeostatic control is glucose. Glucose is one of the main sources of energy in many organisms, yet it is also a putative causative agent of oxidative stress, which has detrimental effects on cellular function (Braun and Sweazea 2008). Several neuroendocrine hormones regulate glucose levels, and they may have both direct and indirect effects on oxidative balance. Birds are one of the most compelling candidates to assess these associations because, despite their high blood glucose levels, they have lower oxidative stress levels and are longer

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lived than size-matched mammals (coined the "bird paradox"; Costantini 2008). This paradox suggests that birds probably possess physiological adaptations to circumvent the damaging effects of hyperglycemia (Braun and Sweazea 2008).

Glucocorticoids (corticosterone in birds) and insulin-like growth factor 1 (IGF-1) are the endpoints of two separate neuroendocrine signaling pathways that play key regulatory roles in both glucose metabolism and life-history decisions (Dantzer and Swanson 2012; Hau et al. 2016). Corticosterone is produced by the hypothalamic-pituitary-adrenal (HPA) axis; it orchestrates a wide range of physiological and behavioral traits both under undisturbed (baseline) conditions and in the face of stressful stimuli (stress induced; Landys et al. 2006; Hau et al. 2016). Baseline levels of corticosterone maintain energy balance (including plasma glucose concentrations) in the face of predictable diel and seasonal rhythms (Landys et al. 2006; Hau et al. 2016). Moderate elevation of baseline corticosterone titers can enhance innate immune system activity or lower oxidative stress (Vágási et al. 2018). Elevated baseline corticosterone levels may also reflect recent chronic stress history, which is expected to covary with impaired physiological state (e.g., higher oxidative stress). Acute stress–induced increase in corticosterone concentrations promotes immediate survival by shutting down resource consumption processes, such as reproduction or immune system activity, while stimulating processes that increase energy availability, for example, mobilizing glucose from reserves (Sapolsky et al. 2000; Remage-Healey and Romero 2001; Romero 2004; Landys et al. 2006; Hau et al. 2016). High levels of corticosterone induced by acute stress may also cause oxidative stress, even after a short-term (e.g., 30-min) exposure to acute stressors (Costantini et al. 2007, 2011; Spiers et al. 2015; Marasco et al. 2017), but our understanding of how acute stress exposure affects oxidative damage is still scarce (for reactive oxygen metabolites, a marker of oxidative damage to lipids, see Costantini et al. 2007; Stier et al. 2019). Finally, the difference between baseline and acute stress–induced corticosterone levels (termed stress reactivity) shows considerable individual variation (Vitousek et al. 2014) and may be adaptively modulated according to life-history stage (Wingfield and Sapolsky 2003; Lendvai et al. 2007; Lendvai and Chastel 2008). However, our knowledge of how stress reactivity affects physiological state is limited.

IGF-1 in vertebrates is part of the evolutionarily conserved nutrient-sensing insulin/IGF-1-signaling (IIS) pathway (Flatt and Heyland 2011). When nutrients are plentiful, the hypothalamic-pituitary-somatotropic axis increases the production of IGF-1, which stimulates cell proliferation and reproduction (Kenyon 2011) and regulates glucose homeostasis (Clemmons 2004) while shutting down self-maintenance functions. Under poor nutrition or otherwise stressful conditions, IGF-1 signaling is reduced, which results in better stress resistance and extended life span in various model organisms (Kenyon 2010; Junnila et al. 2013; Tóth et al. 2018). The beneficial effects of reduced IGF-1 signaling are partly due to an increased resistance to oxidative stress, which would otherwise cause damage to cell components and thus trigger dysfunction and aging (Heck et al. 1999; Holzenberger et al. 2003; reviewed by Carter et al. 2002; Tatar et al. 2003; Bartke 2008; Kenyon 2010; Dantzer and Swanson 2012; Junnila et al. 2013). However, how IGF-1 fits into the physiological regulatory network (i.e., how it is related to corticosterone, glucose, and oxidative stress) has rarely been studied in wild animals. The only exception we are aware of is a study on pied flycatchers (*Ficedula albicollis*) in which nestlings responded to daily injection with IGF-1 by upregulating the activity of glutathione-peroxidase, an antioxidant enzyme (Lodjak and Mägi 2017). However, inferring oxidative stress state from measuring only antioxidants is not straightforward, because increased antioxidant levels might reflect either lack of oxidative stress or an upregulated antioxidant system in the face of oxidative stress (Monaghan et al. 2009). A good indicator of the degree of oxidative stress experienced by the organism is the amount of oxidative damage. However, it is still unknown whether circulating IGF-1 levels are related to oxidative damage in free-living animals.

We know little about the integrated physiological response to acute stress, including different hormonal integrators, energy availability, and oxidative state. In this study, we measured two hormones (corticosterone and IGF-1) that operate as physiological integrators, combined with glucose and oxidative damage to lipids (measured by malondialdehyde [MDA]), belonging to two different physiological subnetworks (resource metabolism and oxidative state, respectively). MDA is a reactive carbonyl compound that can induce mitochondrial dysfunction and can damage cellular macromolecules by forming adducts (reviewed in Vágási et al. 2019). Therefore, MDA is a marker highly relevant for fitness; indeed, it can indicate the costs of growth and reproduction (Metcalfe and Alonso-Alvarez 2010; Blount et al. 2016), and it can covary with longevity across birds (Vágási et al. 2019). These traits are condition dependent (von Schantz et al. 1999; Braun and Sweazea 2008; Bonier et al. 2009; Taylor et al. 2012) and are affected by acute stress, but the relationships between these physiological systems and how these relationships are affected by acute stress remain virtually unknown in any free-living animal. Using an approach that emphasizes competition among different complex models as opposed to null hypothesis testing, we use phenotypic integration to study the following specific questions: (1) How do these physiological traits relate to each other and to body condition under undisturbed baseline conditions? (2) Do the relationships among the physiological traits seen at baseline remain unchanged or rearrange after exposure to a standardized acute stressor? (3) Does the acute stress–induced change in physiological traits depend on stress reactivity (i.e., magnitude of change in corticosterone during a standardized restraint stress)? Using path analysis, we tested four possible scenarios with different levels of hierarchical organization at baseline and in response to standardized capture and restraint.

Methods

Field Methods

We captured 67 (31 females and 36 males) breeding house sparrows (*Passer domesticus*) at a cattle farm near Bălcaciu village, central Transylvania, Romania (46°11′28″N, 24°3′41″E),

on May 26 and 27, 2015. Each sparrow was sampled two times. The first sample (baseline) was taken as soon as possible after the bird hit the net; each baseline sample was taken within 3 min (mean ± SE handling time, 105 ± 4 s). Handling time was not related to the baseline corticosterone levels (Pearson correlation, $r = 0.289$, $P = 0.136$) or to any other physiological trait measured from baseline samples (all P values between 0.482 and 0.742). Therefore, these samples drawn within 3 min are considered representative of baseline levels (Romero and Reed 2005). Each bird was then restrained in a cloth bag and sampled once again after 30 min (acute stress–induced sample), when corticosterone levels are known to be at their peak in this species (Romero et al. 2006). Blood samples (~100 μL) were taken into heparinized capillaries by brachial venipuncture. Samples were stored in Eppendorf tubes in a cooled (~4°C), dark box until transported to the laboratory in less than 8 h, where they were centrifuged at 2,500 *g* for 5 min to separate the plasma and erythrocyte fractions. Plasma was frozen at −20°C until subsequent biochemical assays. Glucose was measured in situ by a drop of whole blood filled into a portable glucometer (Easy Touch GCU, Wellmed). The instrument has a reported accuracy of 3.2%–5.0% coefficient of variation (CV), and the measurements were validated by analyzing samples spiked with a known concentration of glucose and also by serial dilution of plasma samples. The obtained measurements were in high agreement with the expected values ($R^2 = 0.99$, $P < 0.001$). After collecting the stress-induced sample, we measured the body mass of the birds (Pesola spring balance, ±0.1 g), ringed them with a unique metal ring, and released them. None of the birds showed any signs of weakness or blood clotting when released. All applicable national and institutional guidelines for the care and use of animals were followed. Sampling was approved by the Romanian Academy of Sciences (permit 2257), and the study complied with the laws of Romania.

Biochemical Assays

Plasma corticosterone concentrations were measured by direct radioimmunoassay after extraction, using 3 mL of diethyl-ether (Lendvai et al. 2011). The extraction was reconstituted with phosphate-buffered saline. We used a commercial antiserum raised in rabbits against corticosterone-3-(O-carboxymethyl) oxime bovine serum albumin conjugate (C8784, Sigma-Aldrich). The reconstituted extracts were incubated for 48 h at 4°C with 100 μL of [3H]corticosterone (NET399250UC, Perkin Elmer) and antiserum. The total volume of the assay was 1 mL. The radioactively labeled corticosterone had an activity of ca. 10,000 dpm. Bound and free corticosterone were separated by adding 100 μL of dextran-coated charcoal (separation suspension: 10 g charcoal, 1 g dextran, 0.2 g commercial fat-free milk powder in 100 mL of distilled water). After centrifugation, 800 μL of the bound fraction was added to 6 mL of scintillation cocktail (Optima Gold, Perkin Elmer) and counted in a liquid scintillation counter (Tri-carb 2800TR, Perkin Elmer). The minimum detectable level of corticosterone was 3.70 pg per tube, and none of the samples fell below this limit. All samples were run in a single assay (intra-assay CV: 4.2%).

Plasma IGF-1 levels were measured in duplicates by a commercial enzyme-linked immunosorbent assay kit optimized for chicken (cIGF1ELISA, IBT). The assay was validated for house sparrows by testing serial dilutions of a pool of house sparrow plasma, which was parallel to the standard curve. IGF-1 was extracted from plasma samples using an acidic extraction in accordance with the manufacturer's instructions. The final concentrations were determined by colorimetrically measuring the absorbance at 450 nm (Tecan F50 microplate reader). The intra-assay CV was 3.28%, and the interassay CV was 9.8%.

MDA is a carbonyl compound that results from the peroxidative degeneration of membrane polyunsaturated fatty acids by reactive oxygen species, and thus, it is a widely used marker of oxidative stress (Del Rio et al. 2005). MDA concentration (μg/mL) was determined from 10 μL of plasma by high-performance liquid chromatography (HPLC Supelcosil LC-18 column and 5-μm particle size; Sigma-Aldrich) with UV detection at 254 nm (Jasco, UV-2075 Plus). MDA concentrations of each sample were within the standard range (sample range: 1.36–4.63 μg/mL; standard range: 0.091–5.84 μg/mL; standard curve: $R^2 > 0.99$). Although MDA was not measured in duplicate because of the small quantity of plasma, we have shown previously that our assay has high repeatability for samples of house sparrows (Bókony et al. 2014) and barn swallows (*Hirundo rustica*; Pap et al. 2018) and in a comparative study of more than 80 avian species (Vágási et al. 2016). Detailed MDA assay methodology can be found elsewhere (see Bókony et al. 2014). Although plasma triglyceride and MDA levels might correlate at least in certain species (Pérez-Rodríguez et al. 2015), this is not the case in house sparrows (Vágási et al. 2018).

Statistical Analysis

We used path analysis to investigate the relationships between physiological traits. Path analysis is a multivariate multiple regression method that is used to assess expected causal relationships, especially when there are multiple correlations between traits of interest and when both direct and indirect effects should be considered (Li 1975; Sinervo 1999; Lendvai and Chastel 2010). We calculated body condition as the scaled mass index (Peig and Green 2009) using body mass and tarsus length as an indicator of structural size. The values of all four physiological traits were log transformed before analyses to ensure that model requirements were met. We used a pairwise complete covariance matrix for the path analyses.

We considered body condition as an independent variable that may affect physiological traits but not vice versa (i.e., an exogenous variable). This was due to temporal constraints: while the circulating physiological traits represent the current state of the animals and may change drastically within minutes, body mass changes more slowly, and therefore, its current value is achieved during the period before the sampling began. We considered different a priori scenarios about how body condition may affect circulating physiological traits and how these in turn may be related to oxidative damage.

First, we analyzed traits at the baseline level in four different scenarios. These scenarios differed in how physiological traits conveyed information from body condition, that is, which traits were directly or indirectly related to body condition. In the first scenario, we postulated that body condition directly affects multiple interrelated physiological traits (corticosterone, IGF-1, and glucose) and these all affect MDA (fig. 1*a*). Under the second scenario, body condition affects the two integrators (corticosterone and IGF-1), these two have bidirectional relationships and both affect glucose, and all three circulating factors affect MDA (fig. 1*b*). In the third scenario, we postulated that IGF-1 acts as a central regulator, body condition affects IGF-1 directly, then IGF-1 regulates corticosterone and glucose, and all three traits affect MDA (fig. 1*c*). The fourth scenario was similar to the previous one, with corticosterone being the central regulator instead of IGF-1 (fig. 1*d*). To investigate the effects of body condition, we repeated these models by constraining the path coefficients originating from body condition to zero.

Next, we investigated how all four physiological traits were affected by acute stress and how the acute stress affected the relationship established in the previous step. We used repeated-measures linear mixed effects models (LMEs) to assess the reactivity of corticosterone, IGF-1, glucose, and MDA to restraint stress by setting these physiological traits separately as dependent variables, handling stress as a two-level fixed factor, and bird ID (ring number) as a random intercept. We also controlled for sex and body mass by including them in the model as additional explanatory variables.

Finally, we calculated the restraint stress–induced changes in all four physiological traits by subtracting the baseline value from the acute stress–induced value, both log transformed. We analyzed the change in the physiological traits instead of the acute stress–induced levels because we were specifically interested in how stress reactivity in different traits was related and because in some physiological traits the stress-induced levels may be either higher or lower than the baseline; therefore, stress-induced levels alone would not provide information about the change in these values.

Statistical analyses were carried out using the R statistical environment version 3.2.0 (R Core Team 2019) with the lme function of the R package nlme (Pinheiro et al. 2015) for the LMEs and the sem function of the R package sem (Fox et al. 2017) for path analyses. To test the fit of the path models to the data, we used a χ^2 test with root mean square error of approximation (RMSEA), which corrects χ^2 for model parsimony and small sample size. The RMSEA index shows good fit when it is close to zero (and the P value is not significant). Model selection was carried out using Akaike's information criteria corrected for small sample size (AICc value). To show the relationships between variables of interest, we provide standardized coefficients (i.e., partial regression parameters varying between -1 and 1). All data and the R code of the path analyses are provided online.

Results

Relationships at the Baseline Level

Our data were most consistent with corticosterone being a main regulator (i.e., scenario 4, fig. 1*d*). This model received the most support (table 1; fig. 2) and showed good fit to the data (RMSEA index = 0, $\chi^2 = 0.05$, $P = 0.997$). In this

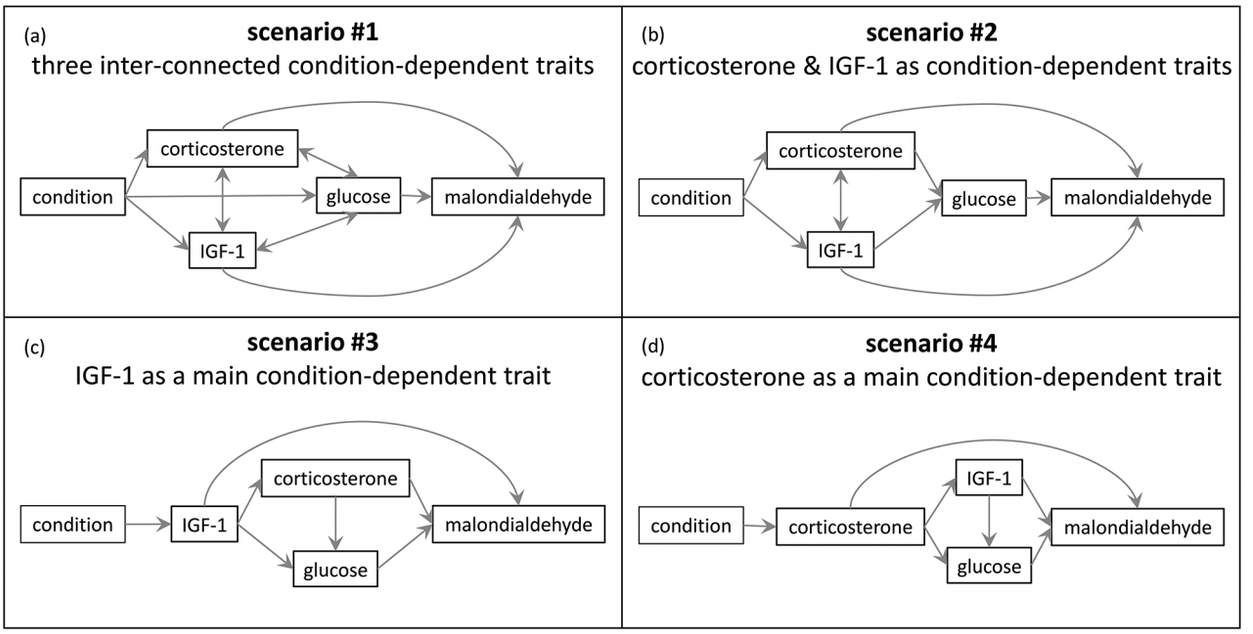

Figure 1. Path analysis scenarios defined a priori. One-headed arrows indicate cause-effect relationships, while two-headed arrows indicate bidirectional covariation. Physiological traits are represented by either their baseline levels (table 1) or their change from baseline to stress-induced levels (table 2). IGF-1 = insulin-like growth factor 1.

Table 1: Path analysis of the relationship between physiological traits at baseline level

Scenario	Description	RMSEA	χ^2	AICc	ΔAICc
4	Corticosterone as a main condition-dependent trait	.0	.1	10.8	.00
2	Corticosterone and IGF-1 as dual condition-dependent traits	.0	.0	13.04	2.2
1	Three interconnected condition-dependent traits	.0	.0	15.6	4.8
3	IGF-1 as a main condition-dependent trait	.4	20.8	31.5	20.7

Note. Scenarios are ranked by decreasing model fit (i.e., from lowest to highest AICc values [Akaike's information criteria corrected for small sample size]). Scenario 4, with corticosterone as a main condition-dependent trait, was best supported by the data, while other scenarios received less support (ΔAICc > 2). IGF-1 = insulin-like growth factor 1; RMSEA = root mean square error of approximation.

model, condition is negatively associated with corticosterone level (i.e., individuals in good condition have low baseline levels), while corticosterone and IGF-1 levels are positively related. The level of oxidative lipid damage (MDA) is positively associated with corticosterone, IGF-1, and glucose levels (fig. 2). The best-fitting model was followed by a less supported alternative model (i.e., with ΔAICc > 2; table 1), where corticosterone and IGF-1 were considered dual regulators (i.e., scenario 2, fig. 1*b*). The model in which body condition affected all three interconnected physiological traits (i.e., scenario 1, fig. 1*a*) and the model in which IGF-1 was considered a main regulator (i.e., scenario 3, fig. 1*c*) received much less support (table 1). Fixing any of the paths between condition and physiological traits to zero (i.e., creating the condition-independent equivalents of scenarios 1–4) dramatically decreased the model fit (ΔAICc > 21 in all cases).

Stress Reactivity of Physiological Traits

As expected, restraint stress resulted in a significant increase in corticosterone levels ($F_{1, 25} = 191.79$, $P < 0.001$; fig. 3*a*). Restraint stress also caused a significant increase in glucose levels ($F_{1, 24} = 40.65$, $P < 0.001$; fig. 3*c*) and a decrease in circulating IGF-1 levels ($F_{1, 30} = 4.31$, $P = 0.046$; fig. 3*b*). Controlling for sex or body mass in these models did not change the conclusion that acute stress affects corticosterone, glucose, and IGF-1 (sex: $F_{1, 28} = 2.70$, $P = 0.112$; body mass: $F_{1, 28} = 0.20$, $P = 0.670$). Although mean MDA levels did not differ significantly between baseline and acute stress–induced levels ($F_{1, 30} = 0.35$, $P = 0.558$, fig. 3*d*), many individuals showed substantial changes between the two sampling points (ranging from −41% to +56%; average change being +1%, with 48% of the birds increasing, 52% decreasing), resulting in an overall lack of difference between the sampling points. As above, controlling for sex or body mass did not affect this relationship (sex: $F_{1, 28} = 2.41, P = 0.131$; body mass: $F_{1, 28} = 0.23, P = 0.635$).

Effects of Acute Stress on the Physiological Network

To investigate how acute stress affects the relationship between the physiological traits, we first calculated the acute stress–induced change in each physiological trait (i.e., from the stress-induced value we subtracted the corresponding baseline value; the difference was denoted by the symbol Δ). The initial model (scenario 1) did not fit the data well ($\chi^2 = 4.53$, $P < 0.001$, RMSEA index = 0.30 [90% confidence interval (CI): 0.07–0.60]). The other three scenarios (2–4) had even worse fit (table 2). This lack of fit was caused by the direction of the paths going from Δcorticosterone, ΔIGF-1, and Δglucose toward ΔMDA. Allowing for bidirectional variation along these paths immediately decreased AICc values by at least 8.3 (scenarios 1.1, 2.1, 3.1, and 4.1; table 2). We thus used this path structure to test the hypotheses of Δcorticosterone and ΔIGF-1 being dual regulators (scenario 2.1) and either Δcorticosterone or ΔIGF-1 being the main regulator alone (scenarios 3.1 and 4.1, respectively). Among those models, none of them was close to the current top model (scenario 1.1; ΔAICc > 3.5). Therefore, in subsequent

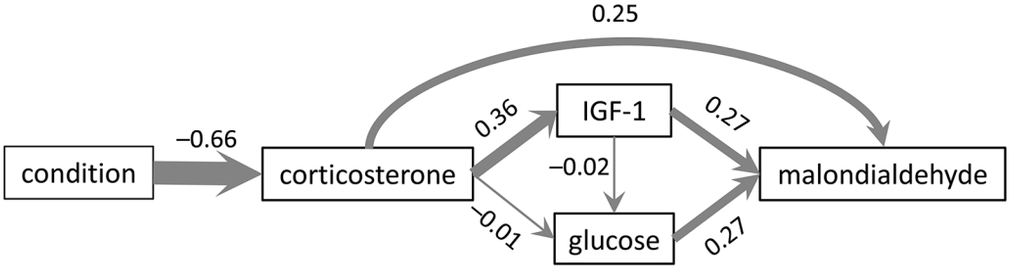

Figure 2. Scenario 4 with corticosterone as a main condition-dependent trait proved to be the best-supported model for baseline measures. One-headed arrows indicate cause-effect relationships; arrow width is proportional to standardized partial regression coefficients (numbers above the arrows). IGF-1 = insulin-like growth factor 1.

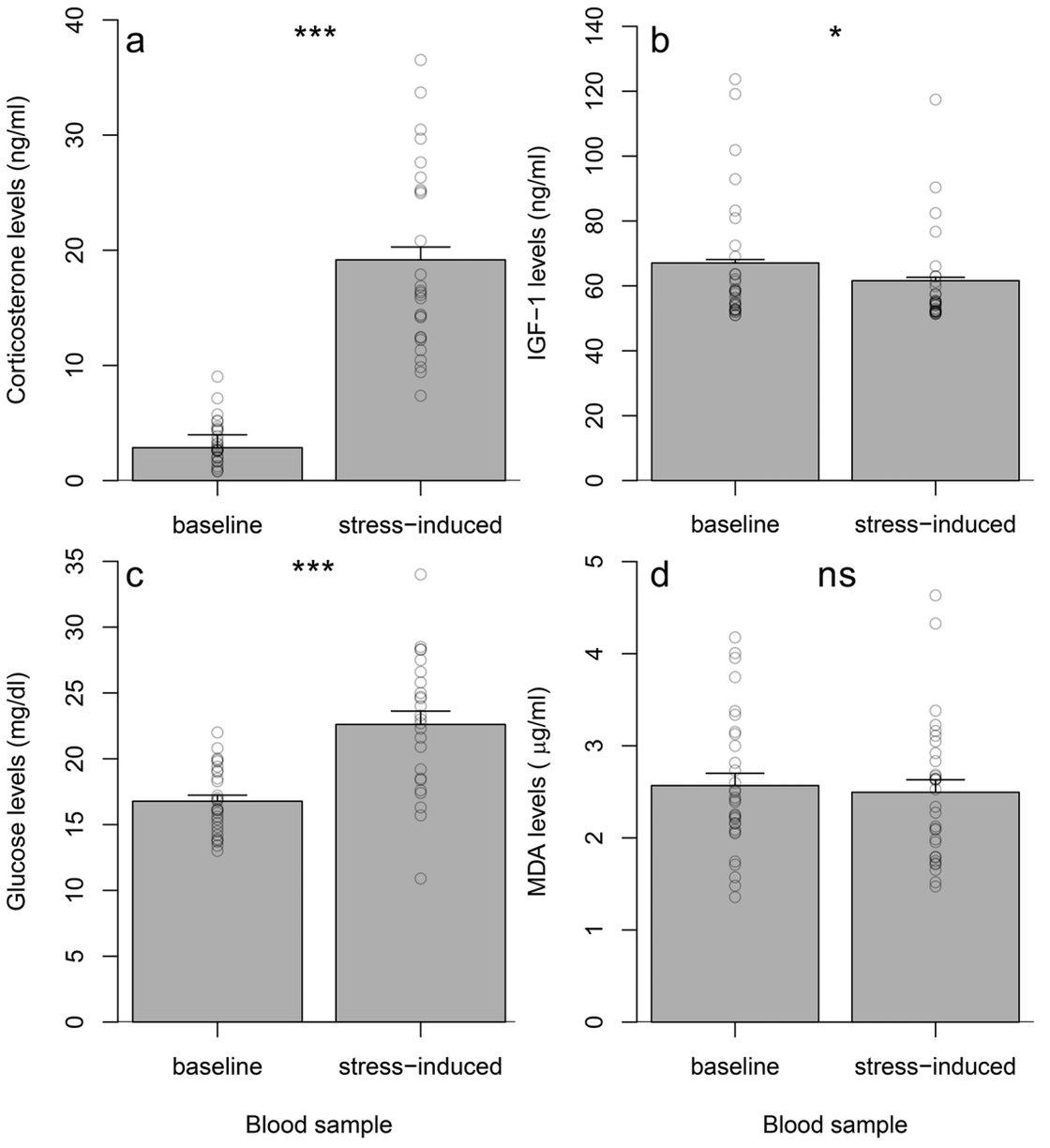

Figure 3. Stress sensitivity of physiological traits. Corticosterone (*a*) and glucose (*c*) levels increased, insulin-like growth factor 1 (IGF-1; *b*) levels decreased, and malondialdehyde (MDA; *d*) levels did not change between baseline samples (within 3 min) and stress-induced samples (after 30 min of restraint stress). Means + 1 SE are plotted, dots denote the scatter of the raw values, and asterisks indicate significance level (***$P < 0.001$, *$P < 0.05$, ns = nonsignificant).

steps, we modified the model structure of only the top model. Removing paths between changes in hormone and MDA levels increased model fit even further (scenario 1.2; table 2). Finally, removing the bidirectional path between ΔIGF-1 and Δcorticosterone and removing the path between condition and Δglucose improved model fit substantially (scenario 1.3; table 2). This final model (fig. 4) had a good fit to the data (RMSEA index = 0, $\chi^2 = 3.39$, $P = 0.639$). In this model, condition is positively associated with both Δcorticosterone and ΔIGF-1, Δglucose is positively correlated with Δcorticosterone and ΔIGF-1, while change in oxidative damage to lipids (ΔMDA) is negatively correlated with Δglucose (fig. 4). Similarly to the baseline case, removing condition dependence from the models decreased the model fit considerably under all scenarios (ΔAICc > 15).

Discussion

We looked at the relationships among four physiological traits belonging to different systems within the physiological network,

Table 2: Path scenarios of the relationships between the stress-induced change (Δ) values of physiological traits

Scenario	Description	RMSEA	χ^2	AICc	ΔAICc
1.3	Final simplified model (fig. 4)	.0	3.4	10.7	.0
1.2	Three condition-dependent traits, with only Δglucose having a bidirectional path to Δmalondialdehyde	.05	3.3	14.5	3.8
1.1	Bidirectional paths between Δmalondialdehyde and the three traits	.0	.0	16.2	5.5
2.1	ΔCorticosterone and ΔIGF-1 as dual condition-dependent traits with bidirectional paths to Δmalondialdehyde	.23	6.2	19.7	9.0
1	Three interconnected condition-dependent traits (Δ values)	.3	4.5	20.7	10.0
2	ΔCorticosterone and ΔIGF-1 as dual condition-dependent traits	.31	9.8	23.3	12.6
3.1	ΔIGF-1 as a main condition-dependent trait with bidirectional paths to Δmalondialdehyde	.28	12.4	23.5	12.8
4.1	ΔCorticosterone as a main condition-dependent trait with bidirectional paths to Δmalondialdehyde	.31	14.8	26.0	15.3
3	ΔIGF-1 as a main condition-dependent trait	.32	15.1	26.2	15.5
4	ΔCorticosterone as a main condition-dependent trait	.34	16.8	27.9	17.2

Note. AICc = Akaike's information criteria corrected for small sample size; IGF-1 = insulin-like growth factor 1; RMSEA = root mean square error of approximation.

both at baseline conditions and after exposure to a standardized acute stressor, and we assessed whether body condition influences these relationships. We found that condition was strongly related to most of the physiological traits and that the relationships between physiological traits differed markedly between the baseline and the acute stress–induced state.

Relationships at the Baseline Level

Our data were most consistent with the model in which corticosterone was the main physiological regulator affecting other elements in the physiological network (fig. 2). In this scenario, birds in superior body condition have low baseline corticosterone titers, supporting the corticosterone-fitness hypothesis (Bonier et al. 2009). Our model also showed a positive association between baseline corticosterone and IGF-1 levels, indicating that birds with lower corticosterone levels also have lower IGF-1 levels. However, the models in which we postulated a direct link between condition and baseline IGF-1 (scenarios 2 and 3, fig. 1) were less supported by our path analyses. Blood glucose was not related either directly or indirectly to body condition, suggesting that baseline glucose levels are under strong homeostatic control and do not reflect variation in nutritional state or the availability of energetic reserves (Fokidis et al. 2011).

Most importantly, baseline levels of corticosterone, IGF-1, and glucose were all positively and approximately equally related to the amount of oxidative lipid damage. These findings corroborate the observation that at increased activity of IGF-1 signaling, higher levels of glucocorticoids and blood glucose may cause oxidative stress (Holzenberger et al. 2003; Braun and Sweazea 2008; Costantini et al. 2011). Although the evidence for corticosterone-induced oxidative stress is mixed, a meta-analysis showed that experimental administration of high doses of glucocorticoids can induce oxidative stress (Costantini et al. 2011). Our results suggest that moderate differences between individuals in their baseline corticosterone levels may already have consequences for oxidative damage (but see Ouyang et al. 2016).

Enhanced activity of the IIS pathway (and, consequently, higher IGF-1 levels) is also thought to trigger oxidative stress (reviewed by Carter et al. 2002; Tatar et al. 2003; Bartke 2008; Kenyon 2010; Dantzer and Swanson 2012; Junnila et al. 2013). For instance, female mice with reduced IIS activity as a result of a heterozygous mutation for the IGF-1 receptor are more resistant to oxidative stress than wild-type mice (Holzenberger

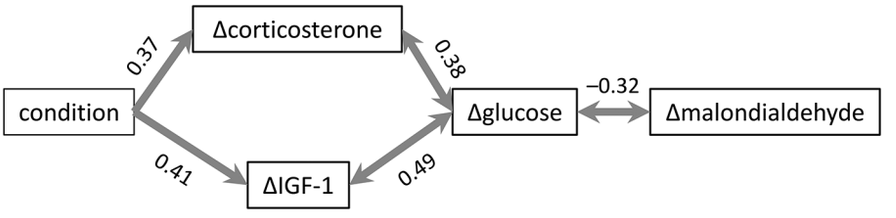

Figure 4. Scenario 1.3 is the best-supported model of stress-induced change measures (Δ values). One-headed arrows indicate cause-effect relationships, while two-headed arrows indicate bidirectional covariation; arrow width is proportional to standardized partial regression coefficients (numbers above the arrows). IGF-1 = insulin-like growth factor 1.

et al. 2003). To date, only one study has looked at the connection between oxidative stress and IGF-1 in wild animals, and it found that injections of IGF-1 caused an increase in antioxidant enzymes (Lodjak and Mägi 2017). However, an increase in antioxidant levels reflects either a lack of oxidative stress or an upregulated antioxidant system during oxidative stress. Our results would support the latter, in that IGF-1 and oxidative damage are positively linked.

High blood glucose levels can trigger an increased production of reactive oxygen species and thus can cause oxidative stress (Braun and Sweazea 2008; Holmes and Martin 2009). However, a connection between blood glucose levels and oxidative damage has not been assessed in free-living animals. Our results suggest that individuals with high blood glucose concentrations may suffer more oxidative damage to lipids. The oxidative cost of hyperglycemia potentially keeps baseline glucose within narrow margins, and glucose levels are augmented only if the emergency life-history stage is activated to survive a truly stressful situation.

Stress Reactivity of Physiological Traits

The rise of circulating glucocorticoid levels and glucose levels is a well-documented stress response of vertebrates. However, the stress reactivity of IGF-1 is far less explored. Given the pleiotropic effects of both glucocorticoid hormones and IGF-1, knowing the interaction between the HPA and the somatotropic axes can help us understand how animals regulate their physiology in order to maximize fitness in the face of stressors. In a study on pied flycatcher nestlings, the sensitivity of IGF-1 levels to handling stress (i.e., time until the blood sample was drawn) was negligible if handling time was short, within 4 min (Lodjak et al. 2017). However, we found a significant decrease in IGF-1 levels when exposure to acute stress is long enough to activate the HPA-mediated stress response (30 min in this study). This finding is consistent with our recent study in another free-living songbird species, the bearded reedling (*Panurus biarmicus*), in which a 15-min restraint stress resulted in a decrease in circulating IGF-1 levels (Tóth et al. 2018). Restraint stress spanning from 5 min to 24 h caused a decrease in IGF-1 levels in adult fish (Davis and Peterson 2006; Wilkinson et al. 2006) and pigs (Wirthgen et al. 2017). Similarly, IGF-1 decreased after 180 min or 24 h of confinement in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), respectively (Wilkinson et al. 2006). In contrast, IGF-1 stayed relatively stable up to 34 min in some passerine birds (Lodjak et al. 2018). These results suggest that the stress sensitivity of IGF-1 may vary across species and/or life-history stages and therefore should be accounted for in future studies involving free-living nonmodel organisms in which sampling time usually exceeds 3 min.

We are aware of no studies that have looked at MDA within individuals after a standardized stress exposure. Reactive oxygen metabolites, another marker of oxidative damage to lipids, show no clear within-individual stress response pattern after 30 min of restraint stress, as either it does not change systematically after stress exposure (Costantini et al. 2007) or it decreases (Stier et al. 2019), depending on the studied species. Our marker of lipid oxidative damage (i.e., MDA) also did not change systematically after 30 min of restraint stress. This result is not confounded by large changes in MDA as a result of the standardized restraint stress exposure, as the stress-induced values are within a normal range and are comparable with our previous measures of MDA in house sparrows (Bókony et al. 2014; Pap et al. 2014, 2015; Vágási et al. 2018).

Effects of Acute Stress on the Physiological Network

The magnitude of the physiological stress response was strongly condition dependent. Similarly to the baseline case, removing the connection between body condition and the physiological traits was rejected in all models. However, in contrast with the baseline case, in which body condition was directly related to corticosterone alone, the magnitude of acute stress–induced changes in both corticosterone and IGF-1 was condition dependent. The direction of these relationships between condition and hormones was contrasting: birds in good condition increased corticosterone the most (i.e., showed highest stress reactivity) and decreased IGF-1 the least (i.e., maintained their IGF-1 levels). Currently, the relative importance of glucocorticoids and IGF-1 in regulating behavioral and physiological processes in stressful situations is unknown, but we found that condition was equally related to the acute stress–induced changes in both hormones. Therefore, future studies investigating how wild animals cope with environmental challenges may benefit from including the somatotropic axis into their analyses.

Although condition predicted the magnitude of changes in both corticosterone and IGF-1, the changes in the two hormones were not related to each other. Although it may seem contradictory, this result is consistent with earlier results. Exogenously administered glucocorticoids lower IGF-1 levels in fish (Kajimura et al. 2003; Peterson and Small 2005), chicken (Leili and Scanes 1998), and rats (Gayan-Ramirez et al. 1999), but only at pharmacologically high-dose treatments and/or when glucocorticoid treatment was applied for a prolonged period (Davis and Peterson 2006). In contrast, IGF-1 remains stable in response to treatment with corticosterone at physiological doses in bearded reedlings (Tóth et al. 2018). The low sensitivity of IGF-1 to glucocorticoid treatment in earlier studies, together with our current finding that within-individual changes in corticosterone and IGF-1 were not correlated, suggests that the somatotropic axis represents a separate stress response pathway and may alter or modulate the behavioral and physiological effects of the glucocorticoid stress response (Wirthgen et al. 2017).

We found that circulating glucose levels rose the most in birds with a higher acute stress–induced increase in corticosterone and a weaker acute stress–induced decrease in IGF-1. The effect of corticosterone is expected, as the main function of this hormone is to cease glucose uptake and block glucose-consuming processes (e.g., growth and reproduction), on the one hand, and to stimulate glucose mobilization and neogenesis, on the other hand (Romero et al. 2009). How the maintenance of IGF-1 under acute

stress is related to higher increase in glucose is less clear. Our models strongly preferred a bidirectional relationship between change in IGF-1 and change in glucose. Hence, it is possible that the high glucose levels constrained the drop of IGF-1 under acute stress because hyperglycemia may stimulate an increased production of IGF-1 that may help to restore glucose homeostasis (Venkateswaran et al. 2007). However, IGF-1 seems ineffective in stimulating glucose uptake in birds (Braun and Sweazea 2008).

Finally, in contrast with the positive association between glucose and oxidative damage at baseline, a higher increase in glucose levels under acute stress was associated with a drop in oxidative damage levels. This unexpected result can be explained by tracing this glucose-oxidative damage relationship back to body condition. Sparrows in better body condition had hormone changes under acute stress that led to a higher increase in glucose levels. These same birds might also be able to better maintain their oxidative homeostasis, given that the antioxidant machinery is costly. From the perspective of oxidative stress, individuals with increased oxidative damage during exposure to acute stress showed a blunted glucose stress response. One probable explanation is that an oxidative stress state, especially in poor-condition individuals, prevents an increase in glucose levels to avoid further potential damage. This explanation is supported by the fact that our model did not fit the data when variation in hormone and glucose reactivity explained changes in oxidative damage, while the reverse causation improved model fit substantially.

To summarize, we investigated the relationships between the glucocorticoid and somatotropic hormones at both baseline and acute stress–induced conditions and asked how these hormones are related to the availability of energy (glucose) and the levels of oxidative damage. Our study provided three key results. We found that (1) the oxidative damage to lipids, a trait of high fitness relevance, is dependent on the circulating baseline levels of corticosterone, IGF-1, and glucose; (2) IGF-1 is responsive to capture and restraint stress, but this sensitivity is not related to the glucocorticoid stress response; and (3) the relationships between physiological traits seen at baseline are considerably reorganized by stress exposure to acute stress. These findings suggest that the physiological network is dynamically reset in stressful conditions, with IGF-1 emerging parallel to corticosterone as an alternative pathway for individuals to respond to stressful situations. We recommend considering the effects of handling stress on IGF-1 and to consider the somatotropic axis in life-history studies, as it is related to oxidative physiology in addition to growth, survival, and reproduction.

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