Exploring the mechanistic link between corticosterone and insulin-like growth factor-1 in a wild passerine bird

Zsófia Tóth[1], Jenny Q. Ouyang[2], Ádám Z. Lendvai[1*]*

[1] Department of Evolutionary Zoology and Human Biology, University of Debrecen, 4032 Debrecen, Hungary

[2] Department of Biology, University of Nevada, Reno, Nevada 89557, USA

Running head: Mechanistic link between HPS and HPA-axes

*Corresponding authors: az.lendvai@gmail.com (Á.Z. Lendvai); hylidae91@gmail.com (Z. Tóth)

Abstract

Physiological regulators of life history trade-offs need to be responsive to sudden changes of resource availability. When homeostasis is challenged by unpredictable stressors, vertebrates respond through a set of physiological reactions, which can promote organismal survival. Glucocorticoids have been traditionally recognized as one of the main regulators of the physiological stress response, but the role of an evolutionarily more conserved pathway, the hypothalamic-pituitary-somatotropic (HPS) axis producing insulin-like growth factor-1 (IGF-1) has received much less attention. Although IGF-1 is known to affect several life history traits, little is known about its role in the physiological stress response and it has never been studied directly in adult wild animals. In this study, we combined field observations with a controlled experiment to investigate how circulating levels of IGF-1 change in response to stress and whether this change is due to concomitant change in glucocorticoids in a free-living songbird, the bearded reedling *Panurus biarmicus*. We showed that corticosterone levels significantly increased while IGF-1 levels significantly decreased during capture and handling stress. However, change in corticosterone levels were not related to change in IGF-1 levels. In a follow-up experiment, we used a non-invasive oral corticosterone manipulation. We found that experimentally elevated corticosterone levels did not affect IGF-1 levels. Our results are the first to highlight the circulating IGF-1 levels are responsive to stress independently from glucocorticoids and suggest that the HPS axis is an autonomous physiological pathway that needs to be recognised as an important regulator of life-history decisions.

Keywords:

glucocorticoid; IGF-1; stress response; Panurus biarmicus; HPS-axis; life-history trade-offs

Introduction

Resource allocation trade-offs are central to the evolution of life-histories. Physiological mediators of such trade-offs need to monitor resource availability and transmit a signal to relevant parts of the organism to adjust energy expenditure in face of environmental variation. Possible candidates of such key life-history regulatory mechanisms must therefore integrate information from both the external and internal environment and be responsive to changes in resource availability (Harshman and Zera, 2007). One way to investigate whether a given physiological mechanism could have such life-history regulatory functions is to assess whether it fulfils these requirements of information processing, integration and responsiveness. A useful framework to analyse these questions is when individuals are exposed to stressors. This approach is biologically and ecologically relevant because in order to successfully reproduce and survive, all organisms must be able to cope with unpredictable and predictable stressors. An organism can display a stress response when challenged by unpredictable, noxious changes in the environment, such as the attack of a predator, an infection, inclement weather, or food shortage (Hawlena and Schmitz, 2010; Kitaysky et al., 1999; Romero et al., 2000; Wingfield et al., 1998). Mounting an appropriate stress response requires a dramatic reorganization of resource allocation. Understanding this response provides great insight into life-history decisions and resource allocation mechanisms.

An important regulator of the stress response is the endocrine system, which plays a central role in regulating the adjustment of morphology, physiology and behaviour to deal with current conditions (Flatt and Heyland, 2011; Ricklefs and Wikelski, 2002; Wingfield and Boonstra, 2013). Hormones function as integrators that process information from the environment and orchestrate multiple processes simultaneously to maximise survival and reproduction under the given circumstances (Martin et al., 2011; Wingfield et al., 1998). Although some pathways have been clearly represented in the study of the stress response (e.g. HPA axis), other, more evolutionarily conserved pathways are understudied even though they can play a key role in the evolution of the endocrine system.

One of the most prominent pathways in the stress response is the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al., 2000). The cascade begins at the brain that perceives the stressor and launches a series of downstream hormonal changes. At the endpoint of this axis, the adrenal glands secrete increased amounts of glucocorticoids into the bloodstream, which stimulate energy production via gluconeogenesis. However, this pathway appeared during the evolution of vertebrates, by which period robust regulation of homeostasis and resource allocation had already evolved (Baker Michael E., 2003; Bijlsma and Loeschcke, 2005; Hawlena and Schmitz, 2010; Pauwels et al., 2005; Stoks, 2001). A more conserved endocrine pathway is the insulin/insulin-like signalling pathway (the

IIS pathway), which is present in all animals and regulates resource allocation and stress resistance (Broughton et al., 2005; Dantzer and Swanson, 2012; Harshman and Zera, 2007). In vertebrates, the IIS pathway is integrated into the Hypotalamic-Pituitary-Somatotropic (HPS) axis. As part of this hormonal cascade, GH stimulates the secretion of insulin-like growth factor-1 (IGF-1), primarily from the liver (Roith et al., 2001).

IGF-1 is an evolutionarily highly conserved nutrient sensing hormone that has pleiotropic effects influencing key life-history traits and major life-history trade-offs among growth, reproduction and lifespan (Dantzer and Swanson, 2012). IGF-1 is negatively related to lifespan (Holzenberger et al., 2003; Lewin et al., 2016), but has a positive effect on growth, sexual maturation and reproduction (Crain et al., 1995; Flatt et al., 2008; Lewin et al., 2016; Pine et al., 2006; Sparkman et al., 2009; Yakar et al., 1999). IGF-1 therefore may mediate the trade-off between longevity and reproduction because reduced IGF-1 signalling has been shown to increase lifespan and the expression of genes involved in stress resistance while increased IGF-1 signalling is necessary for reproduction (Dantzer and Swanson, 2012; Harshman and Zera, 2007; Holzenberger et al., 2003; Lewin et al., 2016).

Although the role of IGF-1 has been established in resource allocation, its role in coping with stressful situations and the crosstalk between the HPA and the HPS axes remain poorly understood. It has been shown that IGF-1 levels change under nutritional and handling stress. For instance, in response to 5 minutes of restraint, circulating IGF-1 levels decreased by 21% within 60 minutes in Yorkshire pigs *Sus crofa domesticus* and remained suppressed for up to 150 minutes (Farmer et al., 1991). A more recent study in pigs also found that restraint stress caused a drop in circulating IGF-1 levels and affected other components of the IIS pathway, suggesting that the IGF-system represents a physiologically relevant biomarker of stress response (Wirthgen et al., 2017). Similarly, IGF-1 levels significantly decreased due to short-term (15 min) and long term (24 hours) confinement stress in sunshine bass a hybrid of *Morone saxatilis* and *M. chrysops*, in Atlantic salmon *Salmo salar* and in rainbow trout *Oncorhynchus mykiss* (Davis and Peterson, 2006; Wilkinson et al., 2006).

Whether the decrease in IGF-1 levels is a direct consequence of exposure to stressors on the HPS axis or is due to the stress-induced activity of the HPA axis is controversial (Davis and Peterson, 2006; Dell et al., 1999; Unterman et al., 1993). On the one hand, it is well established that glucocorticoids initiate and orchestrate the emergency life-history stage within minutes to hours from the appearance of the stressor (Breuner et al., 1998; Lõhmus et al., 2006; Romero and Remage-Healey, 2000; Wingfield et al., 1998), and we also know that glucocorticoids are interconnected with the somatotropic axis (Dell et al., 1999). For instance, glucocorticoids play a role in the embryonic development of the HPS axis by affecting GH gene expression, IGF-1 transcription (Bossis and Porter, 2003; Dell et al., 1999; Reindl and Sheridan, 2012). Experimental studies have also shown

that exogenous glucocorticoids cause lower IGF-1 levels in rats (Gayan-Ramirez et al., 1999), chicken (Leili and Scanes, 1998) and fish (Kajimura et al., 2003; Peterson and Small, 2005). On the other hand, these effects seem to operate only at high glucocorticoid concentrations and/or at a prolonged exposure. For example, although Bossis and Porter (2003) found that glucocorticoids mediate the embryonic development of the HPS axis, hormone treatment for at least 8 hours was necessary to detect a significant increase in GH gene expression. Similarly, while a pharmacological increase in cortisol resulted in a decrease of IGF-1 levels in tilapia *Oreochromis mossambicus* 24-48h post injection (Kajimura et al., 2003), a more moderate, short-term elevation in cortisol did not affect IGF-1 levels in sunshine bass (Davis and Peterson, 2006).

While some of these laboratory, aquacultural and agricultural studies suggest a relationship between stress, glucocorticoids and IGF-1, our knowledge is still very limited about how stressors affect IGF-1 levels in free-living organisms. Importantly, we do not know how the HPA and HPS axes are linked mechanistically. Birds are particularly interesting model systems for studying the IIS, because their metabolism is faster yet their lifespan is longer than similar-sized mammals, and differences in how the IIS is regulated compared to the most studied mammalian models might provide a deeper understanding of the evolution of this pathway (Costantini, 2008; Dantzer and Swanson, 2012; Holmes and Ottinger, 2003). Although the IGF-system has been extensively studied in poultry (reviewed in McMurtry, 1998) almost nothing is known about its regulation in free-living birds (notable exceptions are Lodjak et al., 2017, 2016, 2014)

While interest in the ecological and evolutionary relevance of IGF-1 has recently increased (Lodjak et al., 2017, 2016, 2014; Palacios et al., 2012; Reding et al., 2016; Sparkman et al., 2010, 2009), to the best of our knowledge no study has directly tested the effects of stressors on the activity of the HPS axis or investigated the mechanistic link between the HPA and HPS axes in any free-living organisms. Therefore, we aimed at answering whether: (1) acute stress effects plasma levels of IGF-1 and whether (2) glucocorticoids directly affect circulating IGF-1 levels. First, we hypothesized that acute stress will affect the activity of the HPS axis; therefore, we predicted that circulating IGF-1 levels will decrease in response to short-term acute stress. Second, to investigate the crosstalk between the HPA and the HPS axes, we experimentally increased corticosterone levels non-invasively. We predicted that if the HPS axis receives direct input from glucocorticoids, then increased glucocorticoid levels will down-regulate IGF-1 levels. Alternatively, if the HPS axis responds to external stressors directly, then we predicted that short-term elevation of circulating glucocorticoids will not affect IGF-1 levels. To answer these questions, we studied free-living bearded reedlings *Panurus biarmicus*, a small (~14g), sexually dimorphic, resident songbird common throughout wetlands of Eurasia.

Material and methods

1. Study animal and field study

We captured 17 wintering free-living bearded reedlings *Panurus biarmicus* (Linnaeus, 1758) in Hungary at Virágoskúti-halastó (N 47.651873, E 21.358938) between September 2015 and January 2016. We caught the birds with continuously observed mist-nets (Gdynia, Poland - Ecotone) and subjected them to a standardized capture-handling-restraint protocol (Wingfield, 1994). The first blood sample (~100 µl) was taken as soon as the bird was removed from the net [mean handling time: 4:58 minutes, the handling time was not related to either corticosterone levels ($t = 1.276$, $p = 0.223$) or IGF-1 levels ($t = -0.785$ $p = 0.445$). The bird was then placed into an opaque cloth bag and a subsequent blood sample was taken 15 minutes after the initial capture. We chose to take the second blood sample after 15 minutes because we were interested in the short-term acute stress-response, and we wanted to minimize the possible downstream effects of corticosterone on IGF-1 levels.

2. Experimental study

Housing protocol

21 wintering bearded reedlings were captured with mist-nets in Hungary at Hortobágy-Halastó (N 47.621129, E 21.075788) between 18 of October and 16 of November in 2016. After capture, they were housed in an outdoor aviary at the Botanical Garden of the University of Debrecen where they were kept for 4 months and acclimated to captivity. Four weeks prior to the experiment, the birds were transferred to individual cages measuring 25×25×25 cm. All sides of the cages (except for the front) were made of a non-transparent board (OSB); therefore, birds were kept in visual but not acoustic isolation. Individual cages were separated by a non-transparent removable divider. Because bearded reedlings are very social, live in flocks throughout the year and maintain strong pair bonds, long-term individual separation may be perceived as stressful for the birds (as found in other social species, e.g. (Remage-Healey et al., 2003). To avoid additional stressors, before the experiment, birds were kept in pairs, by removing the divider between adjacent cages. Food (a mixture of apple, carrot, quark, cracked dried fish, dried *Gammarus* sp., cracked corn, cracked dry cat food, a commercial soft food mixture for birds and mealworms) and water were available *ad libitum* at all times.

Experimental design

Corticosterone levels were manipulated orally using a non-invasive technique described by Breuner et al. (1998), in which corticosterone dissolved in peanut oil was injected into mealworms *Tenebrio molitor*. We used a randomized block design with two doses of exogenous corticosterone and a control manipulation for each block.

The day before experimental day, we removed the food from the birds 1.5 hour before sunset and the mobile dividers were inserted into the cages to keep the birds individually. The next morning (between 8:00 – 9:00, to avoid daily variation in hormone levels and to standardize the duration of the food removal), one mealworm was given to the birds through a small hole covered by a semi-transparent layer at the back of the cages, so that the birds could not see the experimenter, but we could observe the birds and record the time when they consumed the mealworm (mean time of digestion was 39 seconds). We paid particular attention to avoid any visual or acoustic contacts with the birds. 15 minutes after the bird consumed the mealworm, the bird was captured through a backdoor at the cages and a single blood sample (~70µl) was taken as soon as possible. After the blood sampling, the body mass (to the nearest 0.1g) of the sampled individuals was also recorded. The exact time when we caught the birds in the cages and also the time when blood sampling was completed was recorded. Treatments were carried out in blocks, so that 3 birds in a block got the mealworms subsequently (with approximately 1 minutes staggering). Two blocks were sampled in a morning. After the treatments, the birds received the usual ad libitum bird chow and fresh water and were left undisturbed for the rest of the day. After we processed all birds, the experiment was repeated one week later, in which each individual received a different treatment than in the first trial. The treatments for the second trial were randomized within blocks and birds were randomly assigned to the blocks.

Mealworm injection

Mealworms were injected with 20µl of peanut oil (VWR catalogue number: ACRO416855000) containing one of the following concentrations of corticosterone (Sigma catalogue number: NET399250UC): (1) control, no corticosterone; (2) low corticosterone, 0.2mg/ml (4µg corticosterone + 16µl peanut oil); and (3) 0.5mg/ml corticosterone concentration (10µg corticosterone + 10µl peanut oil). The chosen corticosterone concentrations were based on previous studies and were calculated as dose per body mass. In red-eyed vireos *Vireo olivaceus* (12-16g), plasma corticosterone concentrations were elevated with a 0.2mg/ml concentration corticosterone solution (which corresponds to 0.28µg corticosterone per 1g of body mass) (Lõhmus et al., 2006). In Gambel's white crowned sparrows *Zonotrichia leucophrys gambelii* (25-28g) a low (0.2mg/ml – 0.15µg/g) and a high

(1mg/ml – 0.7µg/g) dose were used, but the low dose did not elevate significantly the plasma corticosterone levels (Breuner et al., 1998). Nestling Zebra-finches *Taeniopygia guttata* (6-15 g) received a dose of 0.25mg/ml (1.19µg/g) corticosterone, which resulted in a significant increase in circulating corticosterone levels (Spencer and Verhulst, 2008). Therefore, our manipulations correspond to a dose of 0.25µg/g (low) and 0.62µg/g (high).

3. Blood sampling

We took the blood samples by puncturing the brachial vein with a 26G-needle (VWR catalogue number: 613-2014) and collecting blood in heparinized capillary tubes (VWR catalogue number: 609100275). Samples were centrifuged at 5000 rpm (approximately 2200g) and the plasma was removed with a Hamilton syringe. We divided the plasma into 2 different aliquots, one for IGF-1 (15µl) and one for corticosterone (15µl). We stored the samples at -20°C until assayed for corticosterone by radioimmunoassay (RIA) and assayed for IGF-1 by enzyme-linked immunosorbent assay (ELISA).

4. Hormone assays

Plasma IGF-1 levels were measured in duplicates by a commercial avian ELISA kit (catalogue: cIGF1ELISA, lot number D00035) from IBT GmbH, Germany (Intra assay CV: 3.88%). IGF-1 was extracted from plasma samples using an acidic extraction in accordance with the manufacturer's instructions. The final concentrations were determined colourimetrically measuring the absorbance at 450 nm using a Tecan F50 microplate reader. In this assay, we also included chicken *Gallus gallus* plasma samples as a reference, and the obtained IGF-1 concentrations for the chicken samples were at least an order of magnitude higher than what we had expected based on the literature (Ballard et al., 1990). Therefore, all concentrations were recalibrated using an international IGF-1 gold standard (WHO/NIBSC 02/254). IGF-1 concentrations of the recalibrated chicken reference samples were similar to published results and were in agreement with an in-house ELISA developed in our laboratory (Á. Z. Lendvai et al. unpublished data).

Total corticosterone from plasma samples was quantified through direct radioimmunoassay (Lendvai et al., 2011). We extracted the corticosterone from plasma using diethyl-ether, and extracts were reconstituted in phosphate-buffered saline. We let the samples incubate overnight at 4°C. We added ∼10K cpm of 3H-Cort (Perkin Elmer Company: Catalogue number: NET399250UC, lot number: B00025), antiserum (Sigma C8784-100ST, lot number: 092M4784) and phosphate-buffered saline. We let the solution sit overnight at 4°C. Dextran-coated charcoal was added to separate

corticosterone bound to antibodies. After centrifugation, the radioactivity of the bound fraction was counted in a liquid scintillation counter (QuantaSmart).

5. Statistical analyses

We analysed our data in R statistical environment, R version 3.3.2 (R Core Team, 2017). We fitted linear mixed models with function 'lmer' from package lme4 (Bates et al., 2014), and we used stepwise backward model selection to find the best fitting model.

To test the effects of handling stress on IGF-1 and corticosterone, we used generalized linear mixed model with individual as a random intercept. In the initial model, IGF-1 was the dependent variable, handling (baseline or stress induced) and its two-way interaction with body mass and sex were the explanatory variables, and the ring number was the random factor. The same initial model structure was used to model corticosterone levels. We also analysed the relationship between the baseline IGF-1 levels and baseline corticosterone levels with a linear model controlling for body mass. Next we calculated the stress-induced change in both corticosterone and IGF-1 levels (baseline values subtracted from the 15-minutes samples), and analysed whether the magnitude of change between these two hormones were related with a linear model. In this analysis, the dependent variable was the change in IGF-1 levels and the explanatory variable was the change in corticosterone levels, while controlling for body mass and sex.

The experimental data was analysed using linear mixed models. Here, we considered treatment as a three-level factor (high corticosterone, low corticosterone and control). In the initial model, IGF-1 or corticosterone was the dependent variable, treatment and its two-way interaction with sex and body mass were the explanatory variables. The treatment blocks and the weeks of the experiment were also included as fixed effects.

Results

Field samples

The 15 minute capture-restraint stress induced a significant increase in corticosterone levels ($t = 2.567$, $p = 0.017$, Fig.1a), and mass correlated positively with baseline corticosterone levels ($t = 3.619$, $p = 0.001$). Sex and the two-way interaction between stress and sex or body mass did not affect corticosterone levels (p's > 0.4). Handling stress caused a significant decrease in IGF-1 levels ($t = -5.732$, $p = 0.002$; Fig.1b), and IGF-1 levels were higher in males ($t = 3.635$, $p = 0.003$). Body mass was not related to baseline IGF-1 levels. Body mass and the two way interaction between stress and sex or body mass did not affect the IGF-1 levels (p's > 0.3).

We did not find a significant relationship between baseline IGF-1 and baseline corticosterone levels ($t = 0.786$, $p = 0.450$). The handling induced change in IGF-1 and in corticosterone ($t = 0.669$, $p = 0.520$) were also unrelated, even after controlling for body mass or sex p's > 0.9). One bird showed an unusual pattern in the corticosterone data, in which corticosterone decreased in response to handling stress. Removing this data point did not alter any of our conclusions.

Corticosterone manipulation

The body mass of the captive birds did not change during the two experimental weeks, and IGF-1 levels, corticosterone levels, block or sex were all unrelated to body mass (p's > 0.5). The experimental week or the treatment blocks did not affect corticosterone or IGF-1 levels (p's > 0.2).

Both low and high dose of treatment significantly increased corticosterone levels compared to the control treatment ($t = 3.15$, $p = 0.003$ in the low group and $t = 7.342$, $p < 0.001$ in the high corticosterone group, Fig.2a). Other effects, including sex, mass and the two-way interaction of treatment with sex and mass did not influence corticosterone levels (p's > 0.5). Although the manipulation was successful in creating differences in circulating corticosterone levels, the treatment did not affect IGF-1 levels ($t = 0.099$, $p = 0.921$ in the low group and $t = 0.28$, $p = 0.781$ in the high corticosterone group compared to the control group, Fig.2b). Furthermore, sex, mass and the two-way interaction of treatment with sex and mass did not affect IGF-1 levels during the experiment (p's > 0.1).

Discussion

We explored the mechanistic link between the HPA and HPS axes using a free-living songbird, resulting in two key findings. First, we found that in response to a standardized stressor, circulating IGF-1 levels decreased in free-living bearded reedlings within 15 minutes. To our knowledge, this is the first study that reports such an effect for any free-living species. Second, we found that experimentally elevated corticosterone levels did not result in a decrease in IGF-1 levels during the same time frame as we found in the field. This result suggests that the somatotropic axis may respond to environmental stimuli independently from the HPA axis and may be part of the adaptive physiological coping mechanisms used to maintain or restore homeostasis in stressful situations.

Increases in plasma corticosterone levels in response to stress have been previously reported in many species. Our data obtained in bearded reedlings in the field is consistent with these finding, showing that corticosterone levels increase in response to capture/restraint stress and suggest that the

birds perceived the procedure as stressful. Although many studies collect the stress-induced blood sample at 30 minutes (Buehler et al., 2008; Remage-Healey and Romero, 2001; Wingfield et al., 1992), we chose to reduce the restraint period to study the effects of short-term acute stress on IGF-1 levels, while minimizing the potential confounding downstream effects of corticosterone. The effects of glucocorticoids are mainly genomic, which typically act over an hour, but require at least 15 minutes (reviewed in Haller et al., 2008). Some short-term direct effects of corticosterone have been also demonstrated, such as effects on RNA synthesis (reviewed in Haller et al., 2008). Therefore, by choosing a shorter restraint period, we aimed at decreasing the time during which the organism may have been exposed to the physiological effects of elevated glucocorticoids (Buehler et al., 2008). Although the effects of stress on IGF-1 levels in free-living organisms have not been reported before, this finding is consistent with previous studies in captive animals (Davis and Peterson, 2006; Farmer et al., 1991; Wilkinson et al., 2006; Wirthgen et al., 2017). However, our findings do not support the conclusion of earlier avian studies (Lodjak et al., 2017, 2016, 2014), in which handling time was reported to be unrelated to IGF-1 levels, although in nestlings. Such effects may be age-specific. For example, gilthead seabream *Sparus aurata* differed in expression of IGF-1 and IGF-1R mRNA levels during ontogeny (Perrot et al., 1999). In a study on Korean native ogol chicken, circulating IGF-1 levels gradually increased during the post-hatching period (Yun et al., 2005). The stress-responsiveness of IGF-1 may also vary with development, and may explain why Lodjak et al (2014, 2016) did not find that IGF-1 was affected by handling. Furthermore, stress-responsiveness of IGF-1 may also be species-specific. For example, we also found that capture-restraint stress caused a decrease in IGF-1 levels in another free-living songbird, the house sparrow *Passer domesticus* (C. I. Vágási et al. unpublished data). Therefore, we suggest that further studies of IGF-1 levels should take into account the potentially confounding effect of additional stressors.

The decrease in IGF-1 under stress is consistent with the allostatic concept of stress response (McEwen and Wingfield, 2003). Under stressful situations, the organism has to re-establish homeostasis and in order to do so, it has to suppress energetically costly anabolic processes and reinforce those behavioural and physiological processes that promote immediate survival (Wingfield et al., 1998). IGF-1 is a prime regulator of anabolic processes and antagonistic of the catabolic effects of glucocorticoids, therefore the decrease of IGF-1 under acute stress is consistent with its role as one of the physiological mechanisms responsible for maintaining homeostasis. For instance, in a previous study in mice, IGF-1 levels decreased markedly in food restricted animals and the individuals started to lose weight (O'sullivan et al., 1989). Although, experimental IGF-1 administration during starvation reduced the rate of weight loss through the inhibition of the catabolic processes (O'sullivan et al., 1989). The sudden drop of IGF-1 levels in response to the stressor suggests that this

physiological change prepares the animal for the metabolic challenges faced by slowing down anabolic processes and permitting the catabolic effects of glucocorticoids.

In light of these results, we expected that higher glucocorticoid stress responses would be associated with the largest decrease in IGF-1 levels. However, despite the opposite direction of change in the two hormones, neither baseline levels nor the magnitude of corticosterone and IGF-1 stress responses were related in the field study at the individual level, birds with the strongest corticosterone increase were not the ones that decreased their IGF-1 levels the most, and vice versa. Furthermore, we did not find any relationship between those parameters if we controlled for body mass. In order to test the relationship between the two hormones more thoroughly, we carried out an experiment in which we manipulated corticosterone in a non-invasive manner.

Our dietary hormone treatment increased the circulating levels of corticosterone, while control birds did not show a marked increase in corticosterone levels over the course of the study. These results suggest that similar to previous studies (Breuner et al., 1998; Lõhmus et al., 2006; Spencer and Verhulst, 2008): our non-invasive hormone treatment was successful. Corticosterone concentrations after digesting the mealworm were significantly higher in both the low and the high dose group compared to the controls. Despite this rapid increase in corticosterone levels but in absence of a physical stressor, IGF-1 levels in our treated birds remained at the level of the controls, with minimal effect sizes; therefore, we can be confident that the treatment did not affect IGF-1 secretion. Our results are similar to those reported in the sunshine bass, in which confinement stress for 15 minutes resulted in a decrease in IGF-1 levels, but the dietary hormone treatment did not affect plasma IGF-1 concentrations (Davis and Peterson, 2006).

These results suggest that the HPA and HPS axes are not linked downstream at the endpoint of these hormonal cascades, but the crosstalk between these pathways happens at the hypothalamic-pituitary level. We argue that this is the reason behind the discrepancy between the conclusions of studies using physiological and pharmacological doses (see above). Dexamethasone, a powerful glucocorticoid agonist, which is known for having a strong negative feedback on the HPA axis has also been shown to decrease plasma IGF-1 concentration in chicken (Leili and Scanes, 1998), which supports the notion that integration of the HPA and HPS axes is operating at higher regulatory levels.

According to a recent study performed on great tit nestlings, the relationship between corticosterone and IGF-1 varies with the nutritional condition of the individuals. Lodjak et al. (2016) found that pre-fledging plasma IGF-1 levels of nestlings in good condition (from broods that were experimentally reduced) were positively related to feather corticosterone (an integrated measure of corticosterone over several days during the development), whereas the association between IGF-1 and feather corticosterone levels was negative in nestlings with lower nutritional condition (from enlarged broods). In control broods however, there were no association between the two hormones.

In our captive study, the birds had *ad libitum* food availability and they were all in good condition; therefore, one possible explanation for the absence of the relationship between IGF-1 and corticosterone may be that birds in good condition can afford to tolerate higher glucocorticoid concentrations without decreasing IGF-1 levels. In line with this possibility, biomedical studies have shown that IGF-1 diminishes the protein catabolic effects of glucocorticoids only in normally fed but not in starved subjects (Botfield et al., 1997).

These results raise the possibility that the interaction between IGF-1 and corticosterone may modulate the adaptive response of organisms in stressful situations. Investigations of the relationship between glucocorticoids and fitness remain equivocal, with some studies showing positive, negative and also no relationship (reviewed in Bonier et al., 2009). The lack of a relationship has been suggested to be a result of the flexibility and environmentally context dependent nature of glucocorticoids. Our results showing that IGF-1 levels are responsive to stress independently from glucocorticoids suggest that the HPS axis is an autonomous physiological cascade that may be also involved in the mediation of life history decisions and affect fitness components (Dantzer and Swanson, 2012; Harshman and Zera, 2007; Lewin et al., 2016). IGF-1 is an evolutionary ancient regulatory hormone with a primary role to provide an organism-wide internal signal about resource availability, and may alter the function of glucocorticoids. If overall resource availability is high (as was the case in our captive study), then IGF-1 levels can act as a physiological buffer against the adverse effects of increased corticosterone levels. However, when the central nervous system receives input from the environment challenging the organism, it may require the reallocation of resources, which is reflected in decreased IGF-1 levels. Circulating IGF-1 levels can be an important biological indicator of individual internal state and a useful parameter to investigate in the study of life-history decisions. This physiological mechanism warrants more attention in vertebrate evolutionary ecology if we are to understand the evolution of life-histories.

Competing interests

The authors declare no competing or financial interests.

Authors' contributions

ZT, AZL and JQO conceived and designed the study, ZT and AZL collected the data, ZT and AZL performed the laboratory and statistical analysis, ZT wrote the first draft, ZT, AZL and JQO revised the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data for this paper will be deposited in Dryad.

References

Baker Michael E., 2003. Evolution of adrenal and sex steroid action in vertebrates: a ligand-based mechanism for complexity. BioEssays 25, 396–400. https://doi.org/10.1002/bies.10252

Ballard, F.J., Johnson, R.J., Owens, P.C., Francis, G.L., Upton, F.M., McMurtry, J.P., Wallace, J.C., 1990. Chicken insulin-like growth factor-I: Amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. Gen. Comp. Endocrinol. 79, 459–468. https://doi.org/10.1016/0016-6480(90)90076-X

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models using lme4. *J. Stat. Soft*, Foundation of Open Access Statistics. 67, 1-48. https://doi.org/10.18637/jss.v067.i01

Bijlsma, R., Loeschcke, V., 2005. Environmental stress, adaptation and evolution: an overview. J. Evol. Biol. 18, 744–749. https://doi.org/10.1111/j.1420-9101.2005.00962.x

Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? Trends Ecol. Evol. 24, 634–642. https://doi.org/10.1016/j.tree.2009.04.013

Bossis, I., Porter, T.E., 2003. Evaluation of glucocorticoid-induced growth hormone gene expression in chicken embryonic pituitary cells using a novel in situ mRNA quantitation method. Mol. Cell. Endocrinol. 201, 13–23. https://doi.org/10.1016/S0303-7207(03)00006-6

Botfield, C., Ross, R.J.M., Hinds, C.J., 1997. The role of IGFs in catabolism. Baillières Clin. Endocrinol. Metab., Energy Metabolism in Trauma 11, 679–697. https://doi.org/10.1016/S0950-351X(97)80958-5

Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive Corticosterone Treatment Rapidly Increases Activity in Gambel's White-Crowned Sparrows (Zonotrichia leucophrys gambelii). Gen. Comp. Endocrinol. 111, 386–394. https://doi.org/10.1006/gcen.1998.7128

Broughton, S.J., Piper, M.D.W., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D.J., Leevers, S.J., Partridge, L., 2005. Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. U. S. A. 102, 3105–3110. https://doi.org/10.1073/pnas.0405775102

Buehler, D.M., Bhola, N., Barjaktarov, D., Goymann, W., Schwabl, I., Tieleman, B.I., Piersma, T., 2008. Constitutive Immune Function Responds More Slowly to Handling Stress than Corticosterone in a Shorebird. Physiol. Biochem. Zool. 81, 673–681. https://doi.org/10.1086/588591

Costantini, D., 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecol. Lett. 11, 1238–1251. https://doi.org/10.1111/j.1461-0248.2008.01246.x

Crain, D.A., Bolten, A.B., Bjorndal, K.A., Guillerte Jr., L.J., Gross, T.S., 1995. Size-Dependent, Sex-Dependent, and Seasonal Changes in Insulin-like Growth Factor I in the Loggerhead Sea Turtle (Caretta caretta). Gen. Comp. Endocrinol. 98, 219–226. https://doi.org/10.1006/gcen.1995.1063

Dantzer, B., Swanson, E.M., 2012. Mediation of vertebrate life histories via insulin-like growth factor-1. Biol. Rev. 87, 414–429. https://doi.org/10.1111/j.1469-185X.2011.00204.x

Davis, K.B., Peterson, B.C., 2006. The effect of temperature, stress, and cortisol on plasma IGF-I and IGFBPs in sunshine bass. Gen. Comp. Endocrinol. 149, 219–225. https://doi.org/10.1016/j.ygcen.2006.05.009

Dell, G., Ward, A., Shokrai, A., Madej, A., Engström, W., 1999. Regulation of the IGF System by Glucocortidoids. Zoolog. Sci. 16, 377–385. https://doi.org/10.2108/zsj.16.377

Farmer, C., Dubreuil, P., Couture, Y., Brazeau, P., Petitclerc, D., 1991. Hormonal changes following an acute stress in control and somatostatin-immunized pigs. Domest. Anim. Endocrinol. 8, 527–536. https://doi.org/10.1016/0739-7240(91)90022-C

Flatt, T., Heyland, A., 2011. Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs. OUP Oxford.

Flatt, T., Min, K.-J., D'Alterio, C., Villa-Cuesta, E., Cumbers, J., Lehmann, R., Jones, D.L., Tatar, M., 2008. Drosophila germ-line modulation of insulin signaling and lifespan. Proc. Natl. Acad. Sci. 105, 6368–6373. https://doi.org/10.1073/pnas.0709128105

Gayan-Ramirez, G., Vanderhoydonc, F., Verhoeven, G., Decramer, M., 1999. Acute Treatment with Corticosteroids Decreases IGF-1 and IGF-2 Expression in the Rat Diaphragm and Gastrocnemius. Am. J. Respir. Crit. Care Med. 159, 283–289. https://doi.org/10.1164/ajrccm.159.1.9803021

Haller, J., Mikics, É., Makara, G.B., 2008. The effects of non-genomic glucocorticoid mechanisms on bodily functions and the central neural system. A critical evaluation of findings. Front. Neuroendocrinol., Rapid Actions of Steroid Hormones 29, 273–291. https://doi.org/10.1016/j.yfrne.2007.10.004

Harshman, L.G., Zera, A.J., 2007. The cost of reproduction: the devil in the details. Trends Ecol. Evol. 22, 80–86. https://doi.org/10.1016/j.tree.2006.10.008

Hawlena, D., Schmitz, O.J., 2010. Physiological Stress as a Fundamental Mechanism Linking Predation to Ecosystem Functioning. Am. Nat. 176, 537–556. https://doi.org/10.1086/656495

Holmes, D.J., Ottinger, M.A., 2003. Birds as long-lived animal models for the study of aging. Exp. Gerontol., Model Systems used in Aging Research 38, 1365–1375. https://doi.org/10.1016/j.exger.2003.10.018

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Géloën, A., Even, P.C., Cervera, P., Le Bouc, Y., 2003. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421, 182–187. https://doi.org/10.1038/nature01298

Kajimura, S., Hirano, T., Visitacion, N., Moriyama, S., Aida, K., Grau, E.G., 2003. Dual mode of cortisol action on GH/IGF-I/IGF binding proteins in the tilapia, Oreochromis mossambicus. J. Endocrinol. 178, 91–99. https://doi.org/10.1677/joe.0.1780091

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Funct. Ecol. 13, 577–584. https://doi.org/10.1046/j.1365-2435.1999.00352.x

Leili, S., Scanes, C.G., 1998. The Effects of Glucocorticoids (Dexamethasone) on Insulin-Like Growth Factor-I, IGF-Binding Proteins, and Growth in Chickens. Proc. Soc. Exp. Biol. Med. 218, 329–333. https://doi.org/10.3181/00379727-218-44300

Lendvai, Á.Z., Bókony, V., Chastel, O., 2011. Coping with novelty and stress in free-living house sparrows. J. Exp. Biol. 214, 821–828. https://doi.org/10.1242/jeb.047712

Lewin, N., Swanson, E.M., Williams, B.L., Holekamp, K.E., 2016. Juvenile concentrations of IGF-1 predict life-history trade-offs in a wild mammal. Funct. Ecol. 31, 894-902. https://doi.org/10.1111/1365-2435.12808

Lodjak, J., Mägi, M., Sild, E., Mänd, R., 2017. Causal link between insulin-like growth factor 1 and growth in nestlings of a wild passerine bird. Funct. Ecol. 31, 184–191. https://doi.org/10.1111/1365-2435.12679

Lodjak, J., Mägi, M., Tilgar, V., 2014. Insulin-like growth factor 1 and growth rate in nestlings of a wild passerine bird. Funct. Ecol. 28, 159–166. https://doi.org/10.1111/1365-2435.12164

Lodjak, J., Tilgar, V., Mägi, M., 2016. Does the interaction between glucocorticoids and insulin-like growth factor 1 predict nestling fitness in a wild passerine? Gen. Comp. Endocrinol. 225, 149–154. https://doi.org/10.1016/j.ygcen.2015.10.016

Lõhmus, M., Sundström, L.F., Moore, F.R., 2006. Non-invasive corticosterone treatment changes foraging intensity in red-eyed vireos Vireo olivaceus. J. Avian Biol. 37, 523–526. https://doi.org/10.1111/j.0908-8857.2006.03733.x

Martin, L.B., Liebl, A.L., Trotter, J.H., Richards, C.L., McCoy, K., McCoy, M.W., 2011. Integrator Networks: Illuminating the Black Box Linking Genotype and Phenotype. Integr. Comp. Biol. 51, 514–527. https://doi.org/10.1093/icb/icr049

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7

McMurtry, J.P., 1998. Nutritional and Developmental Roles of Insulin-like Growth Factors in Poultry. J. Nutr. 128, 302–305. https://doi.org/10.1093/jn/128.2.302S

O'sullivan, U., Gluckman, P.D., Breier, B.H., Woodall, S., Siddiqui, R.A., McCUTCHEON, S.N., 1989. Insulin-like growth factor-1 (igf-1) in mice reduces weight loss during starvation. Endocrinology 125, 2793–2794. https://doi.org/10.1210/endo-125-5-2793

Palacios, M.G., Sparkman, A.M., Bronikowski, A.M., 2012. Corticosterone and pace of life in two life-history ecotypes of the garter snake Thamnophis elegans. Gen. Comp. Endocrinol. 175, 443–448. https://doi.org/10.1016/j.ygcen.2011.11.042

Pauwels, K., Stoks, R., De Meester, L., 2005. Coping with predator stress: interclonal differences in induction of heat-shock proteins in the water flea Daphnia magna. J. Evol. Biol. 18, 867–872. https://doi.org/10.1111/j.1420-9101.2005.00890.x

Perrot, V., Moiseeva, E.B., Gozes, Y., Chan, S.J., Ingleton, P., Funkenstein, B., 1999. Ontogeny of the Insulin-like Growth Factor System (IGF-I, IGF-II, and IGF-1R) in Gilthead Seabream (Sparus aurata): Expression and Cellular Localization. Gen. Comp. Endocrinol. 116, 445–460. https://doi.org/10.1006/gcen.1999.7337

Peterson, B.C., Small, B.C., 2005. Effects of exogenous cortisol on the GH/IGF-I/IGFBP network in channel catfish. Domest. Anim. Endocrinol. 28, 391–404. https://doi.org/10.1016/j.domaniend.2005.01.003

Pine, M.D., Hiney, J.K., Dearth, R.K., Bratton, G.R., Dees, W.L., 2006. IGF-1 administration to prepubertal female rats can overcome delayed puberty caused by maternal Pb exposure. Reprod. Toxicol. 21, 104–109. https://doi.org/10.1016/j.reprotox.2005.07.003

R Core Team, 2017. R: The R Project for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Reding, D.M., Addis, E.A., Palacios, M.G., Schwartz, T.S., Bronikowski, A.M., 2016. Insulin-like signaling (IIS) responses to temperature, genetic background, and growth variation in garter snakes with divergent life histories. Gen. Comp. Endocrinol. 233, 88–99. https://doi.org/10.1016/j.ygcen.2016.05.018

Reindl, K.M., Sheridan, M.A., 2012. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 231–245. https://doi.org/10.1016/j.cbpa.2012.08.003

Remage-Healey, L., Adkins-Regan, E., Romero, L.M., 2003. Behavioral and adrenocortical responses to mate separation and reunion in the zebra finch. Horm. Behav. 43, 108–114. https://doi.org/10.1016/S0018-506X(02)00012-0

Remage-Healey, L., Romero, L.M., 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 281, R994–R1003.

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. TRENDS in Ecology and Evolution 17, 462–468. https://doi.org/10.1016/S0169-5347(02)02578-8

Roith, D.L., Scavo, L., Butler, A., 2001. What is the role of circulating IGF-I? Trends Endocrinol. Metab. 12, 48–52. https://doi.org/10.1016/S1043-2760(00)00349-0

Romero, L.M., Reed, J.M., Wingfield, J.C., 2000. Effects of Weather on Corticosterone Responses in Wild Free-Living Passerine Birds. Gen. Comp. Endocrinol. 118, 113–122. https://doi.org/10.1006/gcen.1999.7446

Romero, L.M., Remage-Healey, L., 2000. Daily and Seasonal Variation in Response to Stress in Captive Starlings (Sturnus vulgaris): Corticosterone. Gen. Comp. Endocrinol. 119, 52–59. https://doi.org/10.1006/gcen.2000.7491

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. Endocr. Rev. 21, 55–89. https://doi.org/10.1210/edrv.21.1.0389

Sparkman, A.M., Byars, D., Ford, N.B., Bronikowski, A.M., 2010. The role of insulin-like growth factor-1 (IGF-1) in growth and reproduction in female brown house snakes (Lamprophis fuliginosus). Gen. Comp. Endocrinol. 168, 408–414. https://doi.org/10.1016/j.ygcen.2010.05.006

Sparkman, A.M., Vleck, C.M., Bronikowski, A.M., 2009. Evolutionary ecology of endocrine-mediated life-history variation in the garter snake Thamnophis elegans. Ecology 90, 720–728. https://doi.org/10.1890/08-0850.1

Spencer, K.A., Verhulst, S., 2008. Post-natal exposure to corticosterone affects standard metabolic rate in the zebra finch (Taeniopygia guttata). Gen. Comp. Endocrinol. 159, 250–256. https://doi.org/10.1016/j.ygcen.2008.09.007

Stoks, R., 2001. Food stress and predator-induced stress shape developmental performance in a damselfly. Oecologia 127, 222–229. https://doi.org/10.1007/s004420000595

Unterman, T.G., Jentel, J.J., Oehler, D.T., Lacson, R.G., Hofert, J.F., 1993. Effects of glucocorticoids on circulating levels and hepatic expression of insulin-like growth factor (IGF)-binding proteins and IGF-I in the adrenalectomized streptozotocin-diabetic rat. Endocrinology 133, 2531–2539. https://doi.org/10.1210/endo.133.6.7694841

Wilkinson, R.J., Porter, M., Woolcott, H., Longland, R., Carragher, J.F., 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 145, 214–224. https://doi.org/10.1016/j.cbpa.2006.06.010

Wingfield, J.C., 1994. Modulation of the adrenocortical response to stress in birds. Perspect. Comp. Endocrinol. 520–528.

Wingfield, J.C., Boonstra, R., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct. Ecol. 27, 37–44. https://doi.org/10.1111/1365-2435.12039

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological Bases of Hormone—Behavior Interactions: The "Emergency Life History Stage." Am. Zool. 38, 191–206. https://doi.org/10.1093/icb/38.1.191

Wingfield, J.C., Vleck, C.M., Moore, M.C., 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. J. Exp. Zool. 264, 419–428. https://doi.org/10.1002/jez.1402640407

Wirthgen, E., Kunze, M., Goumon, S., Walz, C., Höflich, C., Spitschak, M., Brenmoehl, J., Kanitz, E., Tuchscherer, M., Otten, W., Gimsa, U., Schön, P., Manteuffel, C., Tuchscherer, A., Pfuhl, R., Metges, C.C., Stabenow, B., Erdmann, S., Schluricke, K., Faucitano, L., Hoeflich, A., 2017. Interference of stress with the somatotropic axis in pigs – lights on new biomarkers. Sci. Rep. 7, 12055. https://doi.org/10.1038/s41598-017-11521-5

Yakar, S., Liu, J.L., Stannard, B., Butler, A., Accili, D., Sauer, B., LeRoith, D., 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. U. S. A. 96, 7324–7329.

Yun, J.S., Seo, D.S., Kim, W.K., Ko, Y., 2005. Expression and relationship of the insulin-like growth factor system with posthatch growth in the Korean Native Ogol chicken. Poult. Sci. 84, 83–90. https://doi.org/10.1093/ps/84.1.83

Figure 1: Capture handling stress causes (a) a significant increase in circulating corticosterone levels (n = 16) and (b) a significant decrease in circulating IGF-1 levels (n = 17) in free-living bearded reedlings (*Panurus biarmicus*). Squares denote the individual IGF-1 and corticosterone concentrations, and lines between the squares connect the baseline and stress-induced samples from the same individual. The dots and arrows beside the individual points represent the mean and standard error values of corticosterone (a) or IGF-1 (b) levels.

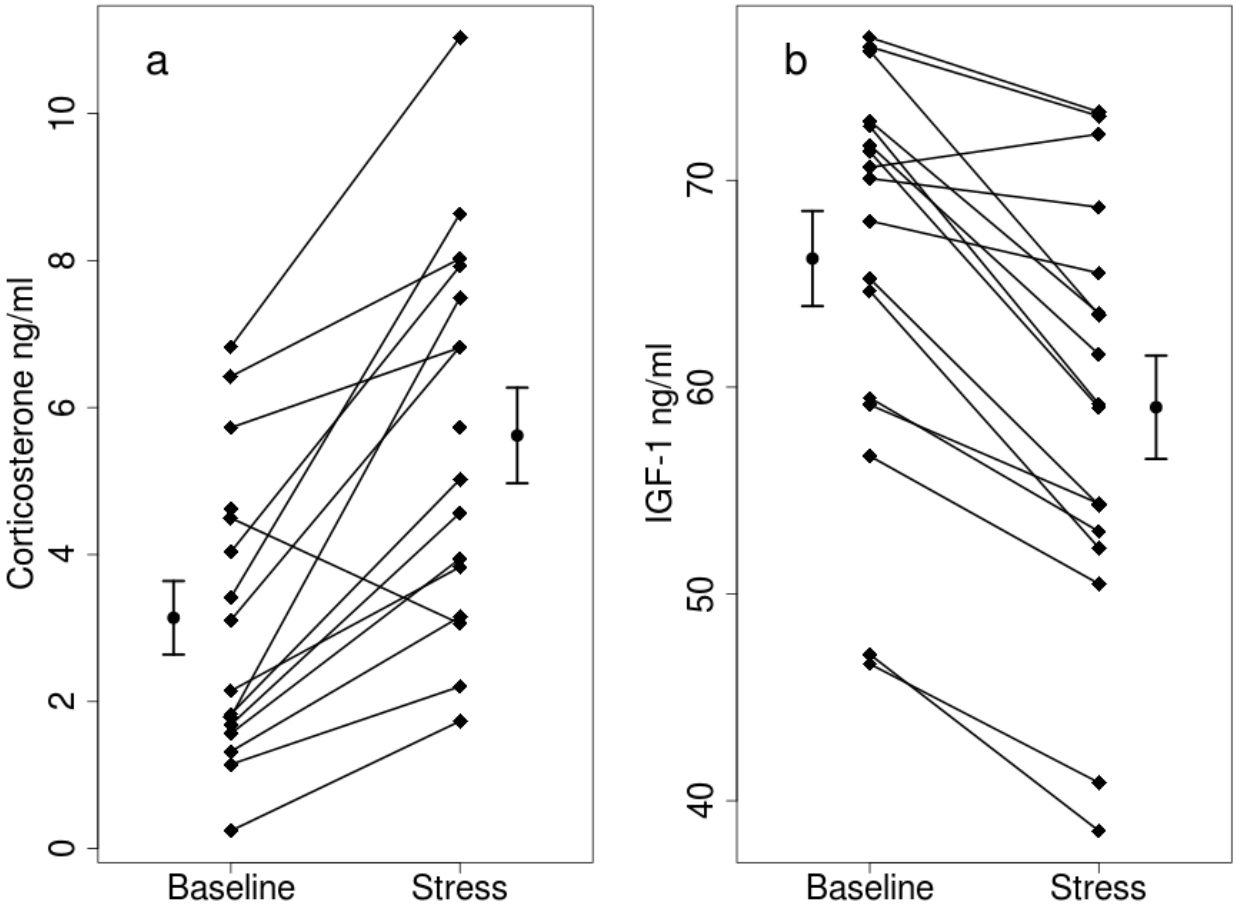
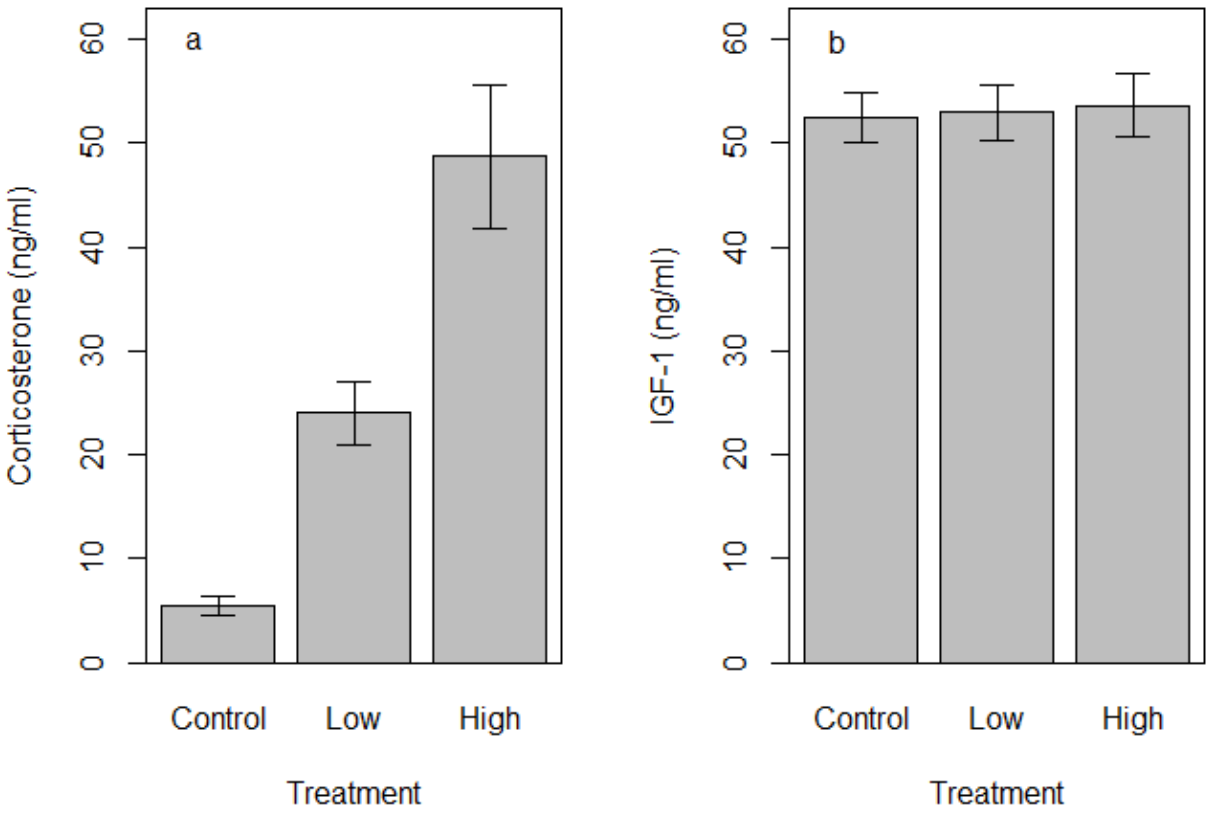
Figure 2: Effects of dietary corticosterone treatment on circulating (a) corticosterone (n = 42) and (b) IGF-1 levels (n = 42). Corticosterone levels were significantly higher in the low corticosterone and the high corticosterone group compared with the control group, although the IGF-1 levels did not differ between the treatment groups.