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Chronic psychological stress alters body weight and blood chemistry in European starlings (*Sturnus vulgaris*)

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ABSTRACT

One hallmark of chronic stress is a decrease in body weight that rebounds once chronic stress is alleviated. We applied chronic psychological stress by exposing European starlings (*Sturnus vulgaris*) to a previously validated chronic stress protocol (CSP) consisting of 4 different randomly applied stressors per day. Experimental design consisted of a 21 day CSP (CSP1), a 60 day recovery (R1), a second 14 day CSP (CSP2), and a second 30 day recovery (R2). Body weight decreased by approximately 5% during CSP1, but overshot to 5–10% above initial body weight during R1. To investigate underlying mechanisms, we periodically measured 12 biochemical analytes, including aspartate aminotransferase (AST), creatine kinase (CK), bile acids, total protein, albumin, globulin, glucose, uric acid, calcium (Ca^{++}), phosphorus (PHOS), potassium (K^{+}), and sodium (Na^{+}). AST and CK increased at the beginning of CSP1, suggesting muscle breakdown. Additionally, decreases in albumin and total protein paired with stable uric acid, but no associated change in glucose, suggested protein breakdown as a secondary energy source. Changes in blood parameters that occurred during CSP1 did not reverse during R1. During CSP2 and R2, weight loss and gain occurred in different proportions. CSP2 produced an approximate 15% decrease in body weight, but R2 resulted in only re-gaining 5% of this weight, although this was equivalent to the pre-CSP1 weight. In summary, protein metabolism appeared to mediate weight loss during chronic stress, but over-gaining weight was not a good indicator of recovery.

1. Introduction

The vertebrate stress response is activated when an animal perceives a stressor (noxious stimulus), which leads to the activation of the two components of the physiological stress response. The likely consequence of this acute stress response is to restore or maintain homeostasis and ultimately enhance the survival of the animal. The first component is the fight-or-flight response, which provides the animal with the necessary energy for a quick escape by increasing heart rate, blood pressure, and blood glucose levels (Cannon, 1929). The second component is the activation of the hypothalamic-pituitary-adrenal (HPA) axis that culminates in glucocorticoid release (Sapolsky et al., 2000). An increase in circulating glucocorticoids plays a number of suppressive, stimulative, and preparative roles that help bring the animal back to a homeostatic state (Sapolsky et al., 2000).

Both the fight-or-flight response and the HPA axis make more energy available to the muscle tissue by mobilizing glucose stores and inhibiting glucose storage (Siegel, 1980). Glucocorticoids accomplish this primarily through the stimulation of appetite, glycogenolysis, tissue gluconeogenesis (driven by glucagon and catcholamines), and hepatic gluconeogenesis (Sapolsky et al., 2000). Gluconeogenesis also occurs through the breakdown of muscle tissues — stimulated by high levels of glucocorticoids (Tomas et al., 1979). Additional energy is made available via the inhibition of glucose storage as glucocorticoids antagonize the effect of insulin (Thompson and Lippman, 1974; Strack et al., 1995). Further energy production may occur due to the breakdown of triglycerides to glycerol and non-esterified fatty acids later resulting in an increase of energy in the form of ATP (Norris, 2007). The stress pathway controls this reaction as well, with an increase in circulating glucocorticoids stimulating hydrolysis of triglycerides stored in adipocytes (Gregoire et al., 1991). Under conditions of acute stress, the release of glucocorticoids will end after stress has been relieved and homeostasis is restored (Sapolsky et al., 2000).

While acute stress is thought to be beneficial for the animal, chronic stress is detrimental and has been shown to suppress reproductive physiology and behavior, immune function, and somatic growth, as well as cause muscle wasting, neuronal cell death, and impaired metabolic function (Sapolsky et al., 2000; Wingfield and Romero, 2001). In addition, chronic activation of the HPA axis results in a negative energy balance (Michel and Cabanac, 1999; Bhatnagar et al., 2006; Harris et al., 2006). One type of chronic activation of the HPA axis may occur via chronic psychological stress. This is a prolonged period of stress during which an animal is exposed to a

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continuous or repeated psychological stressor without habituation (Dallman and Bhatnagar, 2001). Thus, the animal continues to perceive the stressors as stressful throughout the period. However, habituation can be very hard to predict because it depends on the interval between stressors and the intensity, duration, predictability and types of stressors used (McCarty et al., 1988). Physiologically, chronic psychological stress was originally thought to result in an increase in the rate of glucocorticoid production (Kant et al., 1983). However, past studies with European starlings (*Sturnus vulgaris*) have shown a reduction in baseline and stress-induced corticosterone (the primary glucocorticoid in birds) concentrations that disrupt the ability of the animal to mount an adequate stress response to an acute stressor (Rich and Romero, 2005; Cyr and Romero, 2007). Differences between the starling and earlier studies in laboratory rodents are thought to be due in large part to differences in the protocols of an induced chronic stress and in the study species.

Several studies have attempted to understand the avian stress response by using wild-caught starlings in controlled laboratory experiments (e.g. Remage-Healey and Romero, 2001; Nephew et al., 2003; Rich and Romero, 2005; Dickens et al., 2006; Cyr and Romero, 2007; Strochlic and Romero, 2008). One consistent pattern has been a significant decrease in weight during chronic psychological stress that is overcompensated for after the chronic stress is relieved (Rich and Romero, 2005; Cyr and Romero, 2007). Although triglyceride and glucose regulation differ during other energetically demanding periods in the life history of a bird, such as molt and migration (Jenni-Eiermann and Jenni, 1996; Remage-Healey and Romero, 2002), their regulation does not appear to change during chronic psychological stress (Cyr et al., 2007). This conclusion suggested that there is a different mechanism controlling weight change during chronic stress.

The present study investigated additional blood chemistry parameters that may play a mechanistic role in chronic stress-induced weight loss and subsequent recovery. We examined a variety of biochemical analytes used successfully in earlier studies (e.g. Jenni-Eiermann and Jenni, 1994; Rodriguez et al., 2005; Artacho et al., 2007a; Dietz et al., 2009) to determine which physiological parameters might be linked to weight change. Furthermore, we investigated how a second period of chronic stress would affect a bird that has stabilized its weight after a period of recovery (limited disturbances). We predicted that a similar pattern of loss and gain in weight would occur in a successive chronic stress period as occurred during the initial period of chronic stress. This prediction was based on the idea that the stabilization of weight reflected a full recovery of the bird and that a recovered bird would be no less or better prepared to respond to a subsequent chronic stress.

2. Materials and methods

2.1. Animals

Twelve adult wild, European starlings (*S. vulgaris*) were caught in mid January 2009 using mist nests in eastern Massachusetts and housed in an outdoor aviary at Tufts University (Medford, MA, USA). The starlings were selected for size (mean = 79 g) to accommodate the high volume of blood needed and thus included a higher male to female ratio (3 males:1 female). Past experiments have indicated that there are no sex differences in the stress response with non-breeding captive starlings (Romero and Remage-Healey, 2000) or other captive bird species (Marra et al., 1995; Breuner et al., 1999). For three weeks prior to the experiment, the birds were housed individually in a common room kept at a fixed light cycle of 15 L:9 D to mimic the mid June conditions outside at the time of the experiment. Starlings thus had approximately 5 months to adjust to captive conditions prior to collecting samples. Water and Purina Mills Start & Grow Sunfresh Recipe (18% protein) food were given *ad libitum*. Starlings were an ideal study system for this experiment as they were readily available, easily caught in the wild, and their physiological response to both acute and chronic stress had been extensively documented (e.g. Rich and Romero, 2005; Cyr et al., 2007; Cyr and Romero, 2008; Strochlic and Romero, 2008). All procedures adhered to AALAC guidelines and were approved by the Tufts University Institutional Animal Care and Use Committee.

2.2. Baseline (Day 0)

Birds were weighed and blood samples were taken as a baseline measurement before the start of the first chronic stress protocol (see below). Blood samples were taken over a two-day period with six birds being sampled on each day. Each blood sample consisted of puncturing the brachial wing vein and collecting 180–200 μL of blood in heparinized capillary tubes within 5 min of the experimenter entering the room.

2.3. Chronic stress protocol 1 (CSP1; Days 1–21)

Starlings were subjected to a 30-min stressor four times per day for 21 days. Stressor presentation was separated by at least 2 h and each specific stressor was used only once per day. The order and time of stressor presentation was chosen at random each day to avoid habituation. Five of the stressors were used as previously described by Rich and Romero (2005), including: restraint in an opaque bag, disturbance (e.g. cage tapping), crowding of groups of 6 birds per cage, loud radio set at approximately 100 dB, and rolling of all cages on a cart. In addition, the stressor of a novel human voice was added to the protocol as used by Cyr et al. (2007).

All birds were weighed at least once every three days between 12:00 h and 15:00 h. Blood samples were taken every four to five days as described previously. More-frequent blood samples were not possible due to the size of European starlings and the volume of blood required for the assays.

2.4. Recovery 1 (R1; Days 22–79)

No stressors were presented for 57 days. The room was only entered for daily food and water replacement, periodic mass measurement and blood sampling. During the first 21 days of recovery, mass measurement and blood sampling continued as described in CSP1. After 21 days, mass was measured once per week and blood was taken once every two weeks.

2.5. Chronic stress protocol 2 (CSP2; Days 80–93)

The same CSP as previously described was implemented over a 14-day period. During this protocol mass was measured once every three days. Blood was only sampled once at the end of the 14-day CSP.

2.6. Recovery 2 (R2; Days 94–127)

Once again disturbances to the birds were limited to daily food and water replacement, periodic mass measurements and blood sampling. Mass measurements continued every three days. However, only two blood samples were taken over this 33-day period.

2.7. Sample analyses

Blood chemistry parameters were measured using an Abaxis (Union City, CA, USA) Vetscan Classic analyzer (Model 200-1000) with an Avian/Reptilian Profile Plus rotor. These assays were originally designed to assay the clinical aspects of the avian/reptilian blood chemistry in routine and emergency cases and provide reliable values for the most clinically relevant biochemical analytes