

State-dependent behavior alters endocrine–energy relationship: implications for conservation and management

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Abstract. Glucocorticoids (GC) and triiodothyronine (T3) are two endocrine markers commonly used to quantify resource limitation, yet the relationships between these markers and the energetic state of animals has been studied primarily in small-bodied species in captivity. Free-ranging animals, however, adjust energy intake in accordance with their energy reserves, a behavior known as state-dependent foraging. Further, links between life-history strategies and metabolic allometries cause energy intake and energy reserves to be more strongly coupled in small animals relative to large animals. Because GC and T3 may reflect energy intake or energy reserves, state-dependent foraging and body size may cause endocrine–energy relationships to vary among taxa and environments. To extend the utility of endocrine markers to large-bodied, free-ranging animals, we evaluated how state-dependent foraging, energy reserves, and energy intake influenced fecal GC and fecal T3 concentrations in free-ranging moose (*Alces alces*). Compared with individuals possessing abundant energy reserves, individuals with few energy reserves had higher energy intake and high fecal T3 concentrations, thereby supporting state-dependent foraging. Although fecal GC did not vary strongly with energy reserves, individuals with higher fecal GC tended to have fewer energy reserves and substantially greater energy intake than those with low fecal GC. Consequently, individuals with greater energy intake had both high fecal T3 and high fecal GC concentrations, a pattern inconsistent with previous documentation from captive animal studies. We posit that a positive relationship between GC and T3 may be expected in animals exhibiting state-dependent foraging if GC is associated with increased foraging and energy intake. Thus, we recommend that additional investigations of GC– and T3–energy relationships be conducted in free-ranging animals across a diversity of body size and life-history strategies before these endocrine markers are applied broadly to wildlife conservation and management.

Key words: conservation physiology; endocrinology; energy intake; energy reserves; glucocorticoids; large herbivore; moose (*Alces alces*); nutrition; ruminant; state-dependent foraging behavior; stress; triiodothyronine.

INTRODUCTION

Resource consumption drives individual fitness and population dynamics across a diversity of vertebrates (O'Donoghue et al. 1997, Taylor et al. 2005, Falls et al. 2007, Parker et al. 2009, Cury et al. 2011, Monteith et al. 2014). Endocrine markers such as glucocorticoids (GC) and triiodothyronine (T3) are closely tied to energy balance (Danforth and Burger 1989, McEwen and Wingfield 2003), and thus provide a measure of resource limitation in animal populations. Both energy reserves (fat stores) and energy intake (forage) influence GC and T3 profiles (Dallman et al. 1999, Kitaysky et al. 1999, 2005, 2010, du Dot et al. 2009), making endocrinology a

useful lens for identifying the nutritional factors that affect population growth and a valuable tool for wildlife conservation and management (Wikelski and Cooke 2006).

The hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes are responsible for GC and T3 production. The conservation of these hormonal axes across vertebrate taxa (Denver 2009, Sower et al. 2009) suggests that GC and T3 might be interpreted as measures of energy balance, and thus resource limitation, across a multitude of taxonomic groups. When an animal experiences negative energy balance, declines in plasma glucose activate the hypothalamic-pituitary-adrenal axis and increase GC production (Dallman et al. 1999). Therefore, high levels of GC often indicate negative energy balance (i.e., low energy reserves or energy intake [Fig. 1A, B]; Kitaysky et al. 1999, du Dot et al. 2009). When an animal experiences positive energy

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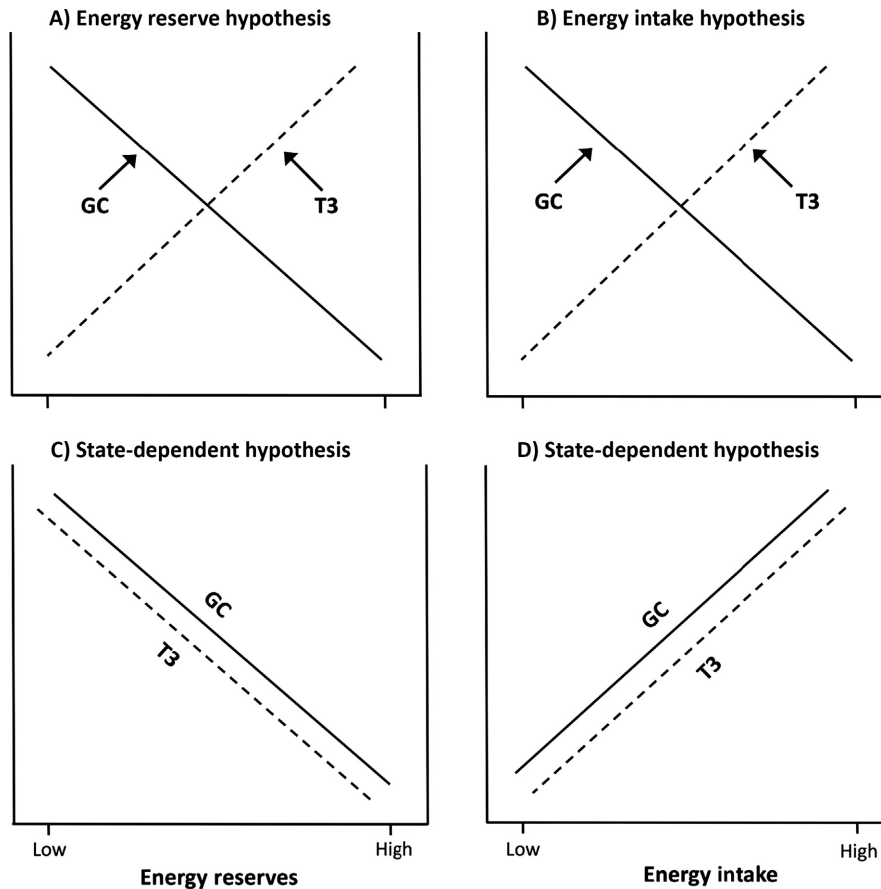


FIG. 1. Graphical comparison of (A and B) predictions associated with “classical” endocrine–energy relationships vs. (C and D) predictions of endocrine–energy relationships stemming from the state-dependent hypothesis. Although predictions of glucocorticoid (GC) and triiodothyronine (T3) profiles by themselves are common to multiple hypotheses, each hypothesis is defined by a unique combination of predicted GC and T3 profiles.

balance and plasma glucose is increased, the hypothalamic-pituitary-thyroid axis increases T3 production (Eales 1988). Consequently, high levels of T3 indicate positive energy balance (i.e., high energy reserves or energy intake [Fig. 1A, B]; Cherel et al. 1988b, Danforth and Burger 1989).

There is reason for skepticism regarding the extent to which GC– and T3–energy relationships can be generalized across taxa (for review see Bonier et al. 2009). For example, endocrine response to environmental stress varies among disparate life-history strategies (Boonstra 2013, Sheriff and Love 2013). Further, metabolic allometries cause energy intake and energy reserves to be more strongly coupled in taxa exhibiting “fast” life histories (typically small-bodied animals) compared to taxa exhibiting “slow” life histories (typically large-bodied animals; Lindstedt and Boyce 1985, Stearns 1989, Ricklefs and Wikelski 2002). Relationships between GC, T3, energy intake, and energy reserves are well documented in species with “fast” life histories, but usually only for one component of their energy budget (e.g., energy intake or energy reserves; Romero 2004, Dantzer et al.

2014), leading to uncertainty in whether GC and T3 reflect energy intake or energy reserves. Nevertheless, GC– and T3–energy relationships derived from small-bodied species are currently the only reference available for applying endocrine markers to large-bodied species (Wasser et al. 2011, Gobush et al. 2014). Therefore, if GC– and T3–energy relationships are to be broadly informative, it is critical to quantify their relationships across an array of life-history strategies (Crespi et al. 2013).

Our current understanding of GC– and T3–energy relationships is largely influenced by biomedical studies conducted in captivity (Eales 1988, Danforth and Burger 1989, Romero 2004, Dantzer et al. 2014). In captive studies of GC– and T3–energy relationships, researchers often control the quantity or quality of foods experimentally, and thus the amount of energy available for intake, which constrains an animal’s ability to adjust foraging in accord with energetic needs. In contrast, free-ranging animals often increase energy intake in response to negative energy balance, a phenomenon known as state-dependent foraging (Houston and McNamara 1999).

State-dependent foraging is expected according to theory and has been empirically demonstrated across taxa (Arnold and Birrell 1977, Pettersson and Brönmark 1993, Skutelsky 1996, Gils et al. 2006, Hamel and Cote 2008). State-dependent foraging may alter GC- and T3-energy relationships compared with those documented in captive animals, especially in large-bodied animals where metabolic allometries cause energy reserves to respond to changes in energy intake much slower than in small-bodied species (Lindstedt and Boyce 1985). For example, captive animals with low energy reserves generally have high GC and low T3 levels (Bahnak et al. 1981, Kitaysky et al. 1999, Douyon and Scheingart 2002, Daminet et al. 2003, du Dot et al. 2009), but if GC and T3 reflect energy intake, large-bodied state-dependent foragers may instead exhibit high T3 because they increase energy intake when energy reserves are low (Fig. 1C). Accordingly, GC levels may rise in concert with T3 (Gobush et al. 2014), because increased GC is often associated with increased foraging activity and energy intake (Fig. 1C, D; Kitaysky et al. 2001, Wingfield and Kitaysky 2002).

To extend the utility of endocrine markers in wildlife ecology, we quantified energy intake, energy reserves, fecal GC, and fecal T3 in free-ranging moose (*Alces alces*). The large body size of moose (~300 kg in our study area) should cause their energy reserves to respond weakly to changes in energy intake over short time periods, and like other large herbivores, moose are likely to exhibit state-dependent foraging (Hamel and Cote 2008, Monteith et al. 2013). To evaluate moose endocrine–energy relationships we tested predictions stemming from three alternative hypotheses.

State-dependent hypothesis

If moose forage in a state-dependent manner, individuals with low energy reserves will have higher energy intake than individuals with greater energy reserves. Accordingly, GC and T3 will be greater in individuals with low energy reserves (Fig. 1C) because GC encourages energy intake and T3 production is expected to increase in response to increased energy intake (Fig. 1D).

Energy reserves hypothesis

Energy reserves determine GC and T3 profiles. This hypothesis predicts that T3 will be greater and GC to be lower in individuals with greater energy reserves (Fig. 1A).

Energy intake hypothesis

Current (past ~24 h) energy intake determines GC and T3 profiles. This hypothesis predicts T3 to be greater in animals with higher energy intake because increased energy intake should increase blood glucose. This hypothesis also predicts GC concentration to be lower in individuals with greater energy intake because

individuals should rely less on catabolism of energy reserves to reach energy homeostasis (Fig. 1B).

METHODS

Study area

We studied moose in the southern Greater Yellowstone Ecosystem of Wyoming, USA (42.8653° N, 110.0708° W) during mid-February in 2012 and 2013. The study area was characterized by deep snow (annual mean snowfall 160 cm) and cold temperatures (mean December–March temperature -10°C). Moose used riparian shrublands along the Green River and its primary tributaries: north and south Horse Creek, north and south Cottonwood Creek, and north and south Beaver Creek (~2,200 m in elevation). These riparian habitats were dominated by Booth's willow (*Salix boothii*), Geyer's willow (*Salix geyeriana*), and cottonwood (*Populus* spp.) adjacent to mixed coniferous (*Abies lasiocarpa*, *Picea engelmannii*, *Pinus contorta*, *Pseudotsuga menziesii*) forest, aspen (*Populus tremuloides*) forest, mixed conifer–aspen forest, and sagebrush (*Artemisia* spp.) steppe. Disturbance associated with human activity may represent a psychological stressor for wildlife and increase GC production (Creel et al. 2002). Although we did not monitor vehicle traffic or snowmobile activity in moose home ranges, the riparian habitats inhabited by moose during winter were located primarily on private ranch lands away from human activity (Oates 2016). During our study, no wolves (*Canis lupis*) existed within or near the home ranges of moose, bears (*Ursus americana* and *Ursus arctos*) were hibernating, and mountain lions (*Puma concolor*) were largely absent during our study (Oates 2016). The extremely low density of predators in our study area means that the potential influence of psychological stress caused by predation risk likely had little to no influence on GC levels (Creel et al. 2009).

Energy reserves, energy intake, and covariates

In February 2012 and February 2013, we captured 143 adult (>1 yr old) female moose using a net gun fired from a helicopter (Barrett et al. 1982, Krausman et al. 1985). To determine the energy reserves of each moose, we estimated percent ingesta-free body fat (%IFBFat). We used ultrasonography to determine the maximum depth of subcutaneous rump fat, and used a standardized protocol validated in other species to assign a body condition score (Stephenson et al. 1998, Cook et al. 2010). Whereas the depth of subcutaneous rump fat was used to estimate %IFBFat for moose with measurable fat, body condition scores were used to estimate %IFBFat for animals without subcutaneous fat based on the linear relationship between ingesta-free body fat and body condition score of moose with measurable rump fat (Cook et al. 2010). We collected fecal samples (10–12 pellets) via rectal palpation, which we immediately froze at -20°C until assayed

for fecal neutral detergent fiber (NDF), fecal nitrogen (N), fecal GC, and fecal T3 metabolite concentrations. All capture and handling methodologies were approved by the Institutional Animal Care and Use Committee at the University of Wyoming (Permit no. A-3216-01).

For ruminants, dietary N and its fecal proxy are measures of protein and energy intake (Van Soest 1994, Hodgman et al. 1996, Leslie et al. 2008). Further, NDF of forage and its fecal proxy provide a measure of digestible energy and an additional measure of protein availability (Van Soest 1994, Brown et al. 1995, Hodgman et al. 1996). Under high-protein–high-energy diets, fecal NDF is reduced relative to low-protein–high-energy diets (Brown et al. 1995), likely because increased protein can increase gut microbe production and enhance fiber digestion. Therefore, the interaction between fecal NDF and fecal N may be a better measure of energy intake compared to either metric alone. Additionally, increased NDF increases digestion time, thereby reducing forage intake (Mubanga et al. 1985, Church 1988, Allen 1996, Meyer et al. 2010). Moreover, small changes in diet quality can lead to large changes in energy intake over both short and long time scales (i.e., the “multiplier effect”; White 1983). Because increased NDF reduces both digestible energy and forage intake and this can lead to meaningful changes in energy intake, we consider the inverse of fecal NDF (NDF^{-1}) a proxy for energy intake.

Lab analyses

Fecal GC and fecal T3 analyses were conducted by the Center for Conservation Biology (University of Washington, Seattle, Washington, USA). Six pellets from each fecal sample were chosen at random and freeze-dried for 24–48 h in a Labconco Freeze-Dry system (Labconco Corp., Kansas City, Missouri, USA) at -50°C , then thoroughly homogenized into a fine powder. Approximately 0.1 g dry mass from each sample was used to control for mass-induced bias in metabolite concentration, thereby reducing the potential effect of inter-sample variation in fecal bulk caused by dietary fiber (Millsbaugh and Washburn 2003, Page and Underwood 2006, Goymann 2012). A pulse-vortex double extraction with 15 mL 70% ethanol was performed, and extracts were stored at -20°C until assayed. Radioimmunoassays were performed on ethanol extracts at previously validated dilutions for GC (Wasser et al. 2000) and T3 (Wasser et al. 2010) using MP Biomedicals’ 125-I corticosterone kit and 125-I Total T3 kit, respectively. The cross-reactivity between corticosterone and progesterone is 0.02% for MP Biomedicals’ 125-I kit. All hormone extractions were performed in duplicate for each assay, and only those with intra-assay variation (% CV) below 10% were accepted.

Fecal NDF and fecal N analyses were performed by the Washington State Habitat Lab (Washington State University, Pullman, Washington, USA). Fecal samples were oven-dried at 55°C , ground in a Wiley Mill, passed

through a 1.0-mm screen and homogenized. Fecal NDF was analyzed with an Ankom Fiber Analyzer (Ankom Technology, Fairport, New York, USA) following standard preparation procedures (Van Soest et al. 1970, Komarek 1993). The Dumas method of combustion (Association Official Analytical Chemists; Etheridge et al. 1998, Marvier et al. 2004) was used to determine fecal N using a Truspec CN analyzer (LECO, St. Joseph, Michigan, USA). We report fecal NDF and fecal N on a percent dry matter basis.

Statistical analyses

Percent ingesta-free body fat of the 143 individuals ranged from 0.7% to 10.5%. We stratified individuals into one of 19 1% body-fat strata to ensure that we sampled the entire range of energy reserves. We then chose at random five individuals within each of the first nine strata and all three individuals present within the 9.5–10.5% body fat strata ($n = 48$) to assess endocrine–energy relationships. We used linear regression and calculated Pearson’s correlation coefficient (r) to examine the effects of energy reserves on energy intake, and the effects of energy reserves and energy intake on fecal GC and fecal T3 profiles. We assessed the potential confounding effects of dietary fiber, age, and pregnancy on endocrine–energy relationships derived from fecal samples prior to characterizing the effects of energy intake and energy reserves on fecal hormone concentrations (Appendix S1). Shapiro–Wilk tests of normality (Royston 1982) were performed on the distribution of residuals to ensure model assumptions were met. All analyses were performed using program R (R Core Team 2014).

RESULTS

Fecal NDF^{-1} and fecal N were not strongly correlated ($r = 0.21$), so we considered fecal NDF^{-1} and fecal N to be independent predictors of energy intake. Energy reserves were weakly and negatively correlated with energy intake as indexed by fecal NDF^{-1} (Fig. 2A; $r = -0.22$, $P = 0.13$) and fecal N (Fig. 2B; $r = -0.35$, $P = 0.09$), but energy reserves were strongly and negatively correlated with an interaction between fecal NDF^{-1} and fecal N (Fig. 2C; $r = -0.38$, $P < 0.01$), indicating that individuals with low energy reserves had greater energy intake (i.e., foraged in a state-dependent manner).

Fecal GC and fecal T3 were best described by a single measure of energy intake, fecal NDF^{-1} (Appendix S1: Table S1), indicating that these endocrine markers are more responsive to energy intake than energy reserves (%IFBFat) in moose. Both fecal GC (Fig. 3B; $r = 0.56$, $P < 0.001$) and fecal T3 (Fig. 3D; $r = 0.36$, $P = 0.01$) were substantially higher in individuals with greater energy intake than those with low energy intake. Fecal T3 concentrations were related negatively to energy reserves (Fig. 3C; $r = -0.27$, $P = 0.05$), whereas fecal GC was related weakly to energy reserves (Fig. 3A,

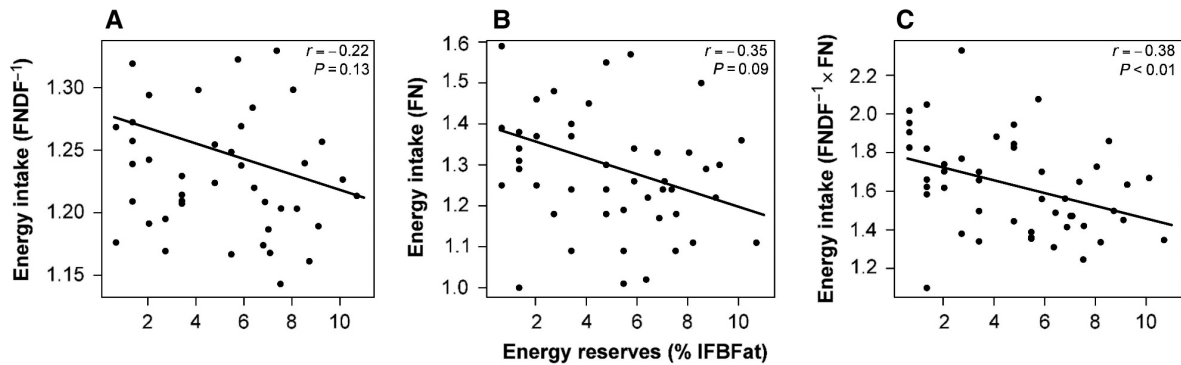


FIG. 2. Relationship between energy reserves (%IFBFat) and three metrics of energy intake for free-ranging moose in the southern Greater Yellowstone Ecosystem, Wyoming, USA during winter: (A) fecal neutral detergent fiber (FNDF^{-1}), (B) fecal N (FN), and (C) $\text{FNDF}^{-1} \times \text{FN}$ (solid lines illustrate fitted regression line). Negative correlation coefficients indicate state-dependent foraging.

$r = -0.13$, $P = 0.25$). Fecal GC and fecal T3 were strongly and positively related (Fig. 4; $r = 0.55$, $P < 0.0001$). In summary, all models possessed slope coefficients consistent with state-dependent foraging, with the slope coefficients of three out of four models in

the opposite direction of those reported for captive, small-bodied animals (compare Figs. 1, 3).

Our validation of the effects of dietary fiber, pregnancy, and age on fecal hormone concentrations indicate that pregnancy and age (Appendix S1: Table S1), but not

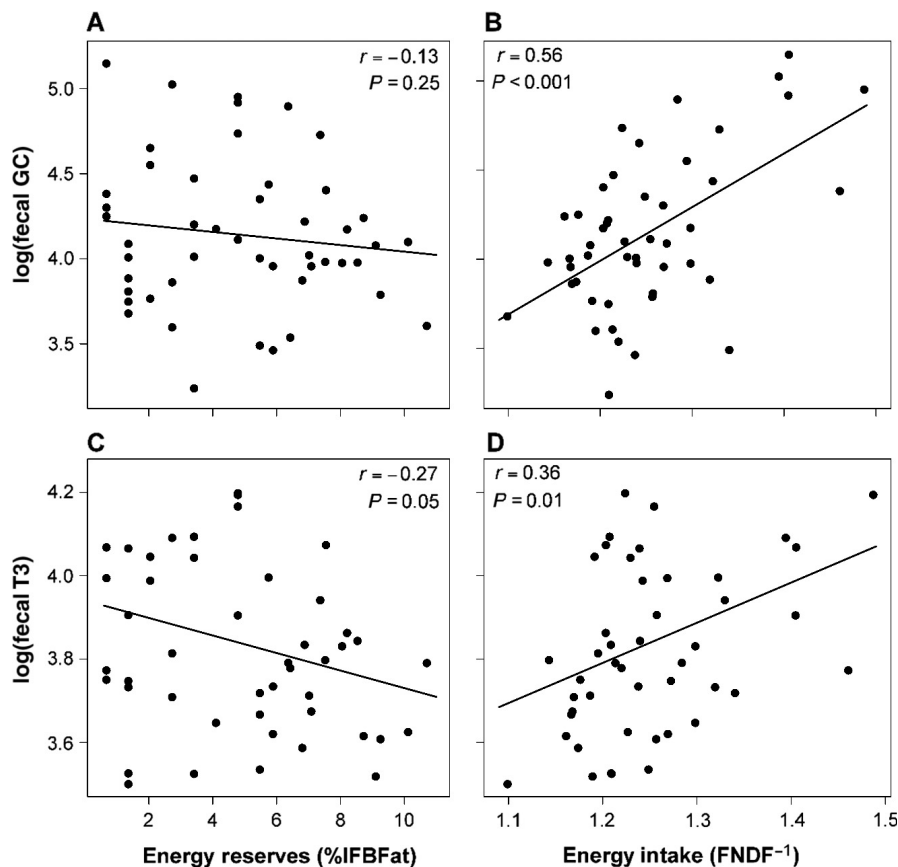


FIG. 3. The relationships between fecal glucocorticoid (GC) and fecal triiodothyronine (T3) metabolites (measured as ng/g) and varying levels of energy reserves (%IFBFat) and energy intake (FNDF^{-1}) in free-ranging moose during winter in the southern Greater Yellowstone Ecosystem, Wyoming, USA (solid lines illustrate fitted regression line). Correlation coefficients support the state-dependent hypothesis (Fig. 1C, D).

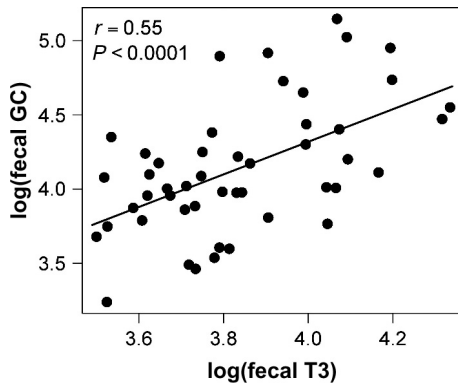


FIG. 4. The relationship between fecal glucocorticoid (GC) and triiodothyronine (T3; both measured as ng/g) in free-ranging moose during winter in the southern Greater Yellowstone Ecosystem, Wyoming, USA (solid lines illustrate fitted regression line). A positive correlation between high stress levels (GC) and high energy intake (T3) indicates state-dependent foraging.

dietary fiber (fecal NDF; Appendix S1: Fig. S1), influenced fecal hormone concentrations (Appendix S1). Controlling for the effects of dietary fiber on fecal hormone concentration did not change either the slope or the intercept of endocrine–energy relationships (Appendix S1: Fig. S1; ANCOVA, all $P > 0.5$). Age was included in top models (i.e., within 2 AIC_c [Akaike information criterion corrected for small sample size]) for fecal T3, but explained only 1% additional variation beyond the effects of energy intake and energy reserves (%IFBFat; Appendix S1: Table S1). Both age and pregnancy were included in top models for fecal GC and explained an additional 6% variation. Neither age nor pregnancy weakened or altered the directional effect of energy intake and energy reserves on fecal T3 and fecal GC concentrations.

DISCUSSION

Endocrine markers are an attractive tool for assessing resource limitation and informing conservation and management decisions because they offer a method for quantifying energetic state and can be non-invasively obtained. Moose exhibited endocrine–energy relationships that contrast with those of studies on captive and small-bodied animals (Figs. 1, 3, 4). In extrapolating from studies on captive animals, researchers often have made two assumptions about free-ranging animals: GC is related negatively to both energy reserves and energy intake, and T3 is related positively to both energy reserves and energy intake (Romero 2004, Welcker et al. 2009, Hayward et al. 2011, Wasser et al. 2011, Boonstra 2013, Gobush et al. 2014). These assumptions are upheld in some study systems, such as marine iguanas (*Amblyrhynchus cristatus*; Romero and Wikelski 2001) and Black-legged Kittiwakes (*Rissa tridactyla*; Kitaysky et al. 2010), but were not supported here for a large-bodied, state-dependent forager (i.e., moose). Therefore, we believe that assumptions regarding endocrine–energy

relationships deserve scrutiny when applied to taxa that exhibit state-dependent foraging and whose energy reserves do not respond quickly to changes in energy intake (e.g., large, free-ranging mammals).

Most research indicates that GC and T3 primarily reflect energy intake (Eales 1988, Kitaysky et al. 2007), although because energy reserves quickly respond to changes in energy intake for species with high mass-specific metabolic rates (“fast” life histories), some studies have also related endocrine markers to energy reserves (Cherel et al. 1988b, Kitaysky et al. 1999, Daminet et al. 2003). The response of energy reserves to changes in energy intake of species possessing relatively low mass-specific metabolic rates (“slow” life histories), however, are slow, which may allow for a clearer understanding of whether GC and T3 reflect energy intake or energy reserves. The relationship between fecal T3 and energy intake in moose was much stronger than the relationship between fecal T3 and energy reserves (Figs. 3C, D; Appendix S1: Table S1), indicating that energy intake, and not energy reserves, more strongly controls expression of T3. These results support those of Hayden et al. (1993) who found that T3 levels in cattle (*Bos taurus*) increase rapidly with increased energy intake. In contrast with previous reports, fecal T3 was negatively related to energy reserves (Fig. 3D; Danforth et al. 1979, Burger et al. 1980, Danforth 1984, Cherel et al. 1988a, b, Eales 1988, Danforth and Burger 1989), which we suggest occurred because moose with few energy reserves had higher energy intake than moose with high energy reserves (Fig. 2). Although fecal GC was not related strongly to energy reserves (Fig. 3A), individuals with high energy intake possessed higher levels of fecal GC than those with low energy intake (Fig. 3B)—a pattern also in contradiction with previous reports (Kitaysky et al. 1999, 2007, du Dot et al. 2009). We suggest that state-dependent foraging is the most likely explanation for these conflicting patterns (Figs. 1–3). Since state-dependent foraging is common among free-ranging animals, we recommend considering this behavior in future interpretations and applications of GC– and T3–energy relationships.

Glucocorticoid production has been suggested to influence behavior and has been linked to state-dependent foraging through the idea of an “emergency life-history stage” (Wingfield et al. 1998). Animals experiencing an energy crisis (i.e., negative energy balance) enter an emergency life-history stage wherein behavior (foraging) and physiology (hormone production) are altered to regain energy balance. Glucocorticoids have been proposed to act as an anti-stress hormone rather than a stress hormone because the emergency life-history stage is adaptive (Wingfield and Kitaysky 2002, Boonstra 2013). In line with this notion, evidence indicates that increased GC resulting from reduced energy reserves or energy intake influences behaviors such as locomotor activity (Breuner et al. 1998, Lynn et al. 2003) and foraging rate (Kitaysky et al. 2001, Angelier et al. 2008). Although the relationship between energy reserves and fecal GC was not

statistically significant, moose with low energy reserves generally exhibited higher levels of fecal GC than those with high energy reserves (Fig. 3A), and individuals with high fecal GC had higher energy intake than those with low fecal GC (Fig. 3B), which supports the state-dependent hypothesis and the notion that GC response in wild vertebrates is adaptive rather than pathological.

Triiodothyronine profiles also may reflect foraging effort, and may therefore be useful in understanding state-dependent foraging. When energy reserves are depleted and energy intake is insufficient during fasting (e.g., breeding or molting in the wild, starvation in captivity), animals fall into negative energy balance and T3 declines to reduce energy consumption (Danforth 1984, Cherel et al. 1988a, b). Most free-ranging animals, however, are expected to be state-dependent foragers and alter foraging behavior when energetic reserves diminish (Houston and McNamara 1999). Increased foraging and locomotor activity increases field metabolic rate, which can be highly correlated with basal metabolic rate (Birt-Friesen et al. 1989). Although not confirmatory evidence, basal metabolic rate and the metabolic rate of many specific tissues is highly correlated with T3 production (Zheng et al. 2014). Thus, T3 may increase in concert with GC because GC encourages foraging activity and energy intake (Kitaysky et al. 2001). Supporting this notion, fecal GC was strongly and positively correlated with fecal T3 in moose (Fig. 4), a relationship also reported in free-ranging Hawaiian monk seals (*Monachus schauinslandi*; Gobush et al. 2014). Therefore, a positive relationship between GC and T3 may be expected in free-ranging animals if GC is associated with increased foraging and animals increase foraging when energy reserves are low (i.e., forage in a state-dependent manner).

We assessed the effect of dietary fiber on fecal hormone concentrations because dietary fiber can both dilute or concentrate levels of fecal hormones relative to serum hormones (Goymann 2012). Further, we characterized the effects of age and pregnancy in moose before evaluating energy–endocrine relationships based on fecal hormones because these factors influence fecal GC independent of energy intake and energy reserves in red squirrels (*Tamiasciurus hudsonicus*; Dantzer et al. 2010) and elk (*Cervus elaphus*; Creel et al. 2002; see Appendix S1 for further discussion). Age and pregnancy influenced fecal GC and fecal T3 concentrations in a similar fashion as reported for red squirrels and elk; the endocrine response of younger individuals was more sensitive to low levels of energy intake and energy reserves than the endocrine response of older individuals (Appendix S1: Table S1). Similar to a previous report in another large herbivore (cattle; Rabiee et al. 2002), dietary fiber had no measurable effect on fecal hormone concentration in moose (Appendix S1). We suspect that our findings, and those previously reported for large herbivores, differ from the dilutive effects of dietary fiber discussed by Goymann (2012) for monogastric organisms, such as European stonechats (*Saxicola torquatus*)

and chimpanzees (*Pan troglodytes*), because the digestive physiology of the rumen differs markedly from monogastric guts. For example, increased dietary fiber should reduce intake, reduce rate of digesta flow from rumen, and reduce fecal output, resulting in increased digesta transit time for ruminants (Gregory et al. 1985, Mertens 1987, Van Soest 1994, Allen 1996, Morrow et al. 2002). In contrast, increased fiber decreases digesta transit time in monogastric fermenters (Wasser et al. 1993, Goymann 2005). We suggest that the effects of fiber on fecal-based endocrine–energy relationships may differ across taxa, especially monogastric and ruminant fermenters (Millsbaugh and Washburn 2004). We do acknowledge, however, that future experimental approaches to validating the relationship between fecal GC, fecal T3, and potentially confounding covariates are warranted. Accounting for such confounds in fecal assays and other non-invasive techniques is critical to ensure accurate application of endocrine markers.

Understanding how energy intake and energy reserves influence endocrine markers is critical if these markers are to be used to identify factors limiting population growth and make conservation and management decisions regarding wild populations. Had we assumed GC– and T3–energy relationships derived from captive animals translated well to free-ranging moose, we would have mischaracterized the nutritional condition of moose in our study. This result carries important implications for the management and conservation of both harvestable species and species of conservation concern. The nutritional condition (energy reserves) of large herbivores underpins individual life-history characteristics, which in turn determine population dynamics, especially in the absence of strong top-down forcing (Eberhardt 2002, Monteith et al. 2014). Hence, harvest quotas for large herbivores are often set to maintain populations near nutritional carrying capacity (i.e., the number of animals the landscape can energetically and nutritionally support). For species of conservation need, which tend to be cryptic or rare, endocrine markers often represent one of few approaches available to managers and scientists for assessing resource limitation (Millsbaugh and Washburn 2004, Wikelski and Cooke 2006). Therefore, it is critical that endocrine–energy relationships are broadly understood, and not simply assumed, so that endocrine markers can be implemented across taxa and environments without misleading inference regarding conservation and management. By demonstrating how endocrine–energy relationships can be altered from previous expectations through the foraging behavior and physiology of a free-ranging, large-bodied species, our study represents an important step towards a broader understanding of endocrine–energy relationships, and thus more accurate application of endocrine markers.

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