

Do glucocorticoids indicate stress? Meta-analysis reveals a tight association between glucocorticoids and metabolic rate

Reviewed Preprint

Revised by authors after peer review.

[About eLife's process](#)

Reviewed preprint version 2

September 27, 2023 (this version)

Reviewed preprint version 1

June 12, 2023

Posted to bioRxiv

April 19, 2023

Sent for peer review

April 17, 2023

Blanca Jimeno , Simon Verhulst

Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo 12, 13005 Ciudad Real, Spain • Instituto Pirenaico de Ecología (IPE), CSIC, Avda. Nuestra Señora de la Victoria, 16, Jaca, Spain • University of Groningen, the Netherlands

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

Abstract

Glucocorticoid (GC) variation has long been thought to reflect variation in organismal ‘stress’, but associations between GCs and Darwinian fitness components are diverse in magnitude, direction, and highly context-dependent. This paradox reveals our poor understanding of the causes of GC variation, contrasting with the detailed knowledge of the functional consequences of GC variation. Amongst an array of effects in many physiological systems, GCs orchestrate energy availability to anticipate and recover from predictable and unpredictable environmental fluctuations and challenges. Although this is mechanistically well-known, the extent to which GC levels are quantitatively explained by energy metabolism is unresolved. We investigated this association through meta-analysis, selecting studies of endotherms in which (1) an experiment was performed that affected metabolic rate and (2) metabolic rate and GC-levels were measured simultaneously. We found that an increase in metabolic rate was associated with an increase in GC levels in 20 out of 21 studies (32 out of 35 effect sizes). More importantly, there was a strong positive correlation between the increases in metabolic rate and GCs ($p=0.003$). This pattern was similar in birds and mammals, and independent of the nature of the experimental treatment. We conclude that metabolic rate is a major driver of GC variation within individuals. Stressors often affect metabolic rate, leading us to question whether GC levels provide information on ‘stress’ beyond the stressor’s effect on metabolic rate.

eLife assessment

This work presents a **fundamental** meta-analysis on the causes of glucocorticoid variations in birds and mammals. It provides **convincing** evidence that an increase in metabolic rates increases glucocorticoid concentrations. The work will be of broad interest to animal physiologists.

The accepted - but often overlooked - metabolic role of glucocorticoids

Glucocorticoid hormones (GCs; e.g. cortisol, corticosterone) were identified by Hans Selye (1907-1982) as the key molecular mediators of the ‘stress reaction’, and named in reference to their capacity to increase glucose in blood. Selye highlighted the fundamental role of GCs in the ‘general adaptation syndrome’ - i.e. “*the physiological mechanisms that help to raise resistance to damage as such, irrespective of the specific nature of the damaging agents*” (Selye 1950 [\[1\]](#)). In this view, GCs play a role in ‘adapting’ to the challenge, by triggering mechanisms that help the organism return to, or maintain, homeostasis after an environmental challenge. Indeed, towards the later stages of his career, Selye’s definition of stress was “*the nonspecific response of the body on any demand on it*” (Selye 1976 [\[2\]](#)), making the point that very different stimuli (i.e. ‘stressors’) triggered the same response. However, in the literature this perspective has changed over time, and ‘stress’ and, by extension, GCs, have been widely linked to negative outcomes (but see Koolhaas *et al.* 2011 [\[3\]](#); Herman 2022 [\[4\]](#), MacDougall-Shackleton *et al.* 2019 [\[5\]](#)). Consequently, researchers in fields from biomedicine to conservation physiology and animal husbandry have focused on GCs to find proxies of ‘physiological and/or psychological stress’ to evaluate physical and/or welfare status. Indeed, GCs have predominated over other traits throughout the stress physiology literature, to the point of being referred to as vertebrate ‘stress hormones’ (Madliger *et al.* 2015 [\[6\]](#); Madliger & Love 2014 [\[7\]](#); McCormick & Romero 2017 [\[8\]](#)). While several authors have argued against this simplified view of GC regulation during the past decades (e.g. Koolhaas *et al.*, 2011 [\[3\]](#); MacDougall-Shackleton *et al.* 2019 [\[5\]](#); Herman 2022 [\[4\]](#); Romero 2004 [\[9\]](#); Landys *et al.* 2006 [\[10\]](#); Bonier *et al.* 2009 [\[11\]](#)), such association prevails, in the sense that publications still abound in which GC levels are assumed to provide information on organismal stress.

The focus on GCs to measure organismal stress can be understood from their role as key mediators of organismal responses to challenges, triggering a cascade of effects on many physiological systems (Koolhaas *et al.*, 2011 [\[3\]](#); Sapolsky, Romero & Munck, 2000 [\[12\]](#); Deviche *et al.* 2017 [\[13\]](#); Zimmer *et al.* 2019 [\[14\]](#); Zimmer *et al.* 2020 [\[15\]](#)). Furthermore, acute increases in circulating GCs are a defining aspect of the so-called “stress response” (Koolhaas *et al.*, 2011 [\[3\]](#); Sapolsky *et al.* 2000 [\[12\]](#)). Consequently, higher GC levels circulating in plasma or deposited in keratinized tissues (i.e. hair or feathers) have traditionally been interpreted as an indication of homeostatic unbalance, poor condition, implicating low fitness prospects (reviewed in Schoenle *et al.* 2021 [\[16\]](#), Romero & Fairhurst 2016 [\[17\]](#)). However, the latter assumption is at best poorly supported by the literature, with associations between GCs and fitness (survival / reproduction) being diverse in direction and often non-existent (Schoenle *et al.* 2021 [\[16\]](#); Zimmer *et al.* 2022 [\[18\]](#); Busch & Hayward 2009 [\[19\]](#); Bonier *et al.* 2009 [\[11\]](#); Petrullo *et al.* 2022 [\[20\]](#)). This inconsistency raises the question what alternative inference can be made from GC-variation. The urgency of this question is further underlined by the observation that GCs also increase in response to experiences we would not normally qualify as stress; for example, sexual activity induces a GC increase in humans, horses and rodents (Siciliani 2000 [\[21\]](#); Colborn *et al.* 1991 [\[22\]](#); Buwalda *et al.* 2012 [\[23\]](#)). We suggest that this question can potentially be resolved through a better understanding of physiological mechanisms and environmental factors driving GC variation.

Selye (1976) [\[2\]](#) emphasised that homeostatic challenges of any kind lead to increases in body demands, loosely defined as “*the rate at which we live at any one moment*”. Activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis, represents a primary hormonal response to homeostatic challenges that, through the release of GCs, mobilises the resources needed to fuel the current or anticipated rise in energy expenditure (current and anticipatory responses) or recover from an immediate threat that induced an unanticipated increase in energy expenditure (reactive response) (Herman *et al.* 2016 [\[24\]](#), McEwen & Wingfield 2003 [\[25\]](#)). In this context, GCs are involved in the metabolism of most types of energy reserves, modulating glucose, fat and protein metabolism

in liver, skeletal muscle, and other target tissues (Box 1). Allusions to the energetic role of GCs and their tight link to energy expenditure are common in physiological and ecological studies, especially those using approaches which underline the adaptive function of GC responses (McEwen & Wingfield 2003 [↗](#); Landys *et al.* 2006 [↗](#); Romero *et al.* 2009 [↗](#); Deviche *et al.* 2017 [↗](#)), but the extent to which GC variation can be quantitatively explained as facilitator of variation in energy expenditure has rarely been addressed (but see Jimeno *et al.* 2018 [↗](#), Malkoc *et al.* 2021 [↗](#) supplementary information). We here schematically review the role of GCs in energy metabolism (Box 1), and investigate this link quantitatively through meta-analysis.

Revisiting the associations between GCs and metabolic rate. A meta-analytic approach

Although previous evidence supports the link between energy expenditure and GC secretion (e.g. Koolhaas *et al.* 2011 [↗](#); Sapolsky *et al.* 2000 [↗](#); Beerling *et al.* 2011 [↗](#); Buwalda *et al.* 2012 [↗](#); Jimeno *et al.* 2018 [↗](#); Malkoc *et al.* 2021 [↗](#) supplementary information), the qualitative importance of both this association and the underlying processes to explain GC variation remains unexplored; this is however fundamental towards accurately interpreting GC variation. We here test whether changes in energetic demands are associated with variation in GC levels. Specifically, we (i) use a meta-analytic approach to test whether experimental manipulations leading to increases in metabolic rate in endotherms also lead to an increase in GCs (qualitative approach). We included only experimentally-induced increases of energy expenditure to avoid potential masking effects of anticipatory responses or delayed effects of GCs. Because metabolic rate and GCs can fluctuate rapidly, we targeted metabolic rate and GC measurements taken simultaneously or when animals could be assumed to be in the same physiological state (e.g. within the same day and experimental treatment). We further investigated (ii) whether the magnitude of the experimentally induced changes in metabolic rate and GCs were correlated (quantitative approach) through meta-regression. Our predictions are that (i) increases in metabolic rates are associated with increases in plasma GC concentrations, (ii) that changes in GCs are proportional to induced changes in metabolic rate, and (iii) that the association between increases in metabolic rate and GCs is independent of the treatment used to increase the metabolic rate.

BOX 1:

Glucocorticoids and energy metabolism

We here consider GC regulation from the perspective of their role in fuelling metabolic rate. When metabolic rate is low, for example during periods of inactivity, circulating GCs are maintained at low levels and glucose release from fuel stores is released in the blood stream at a low rate matching the modest metabolic needs. (*permissive* actions; Sapolsky *et al.* 2000 [↗](#); **Fig. 1** [↗](#)). An increase in metabolic rate can be anticipated or unanticipated (GCs will exert *preparative* or *stimulating* actions, respectively; Sapolsky *et al.* 2000 [↗](#); **Fig. 1** [↗](#)), and acute or gradual. Unanticipated but gradual increases in metabolic rate will occur for example when thermoregulatory costs unexpectedly increase.

In both gradual and acute increases in metabolic rate, a main role of GCs is to increase circulating glucose at a rate matching the metabolic requirements through diverse mechanisms. Decreasing plasma glucose levels trigger a series of hormonal changes that promote a switch in energy usage. Together with a decrease of insulin (in mammals, less so in birds), GCs are released into the circulation (Andrews & Walker 1999 [↗](#); Rosmond & Bjorntorp 2000) reducing anabolic insulin actions (Vegiopoulos & Hertzog 2007). Blood glucose level then increases, both by mobilization from existing stores, and by inhibition of further storage. GCs also inhibit glucose uptake and glycogen synthesis in the liver, redirecting resources to gluconeogenesis and glycogenolysis, along with glucagon and catecholamines as part of the most immediate acute response. Catecholamines act quickly and increase within seconds to induce the release of energy needed to fuel the

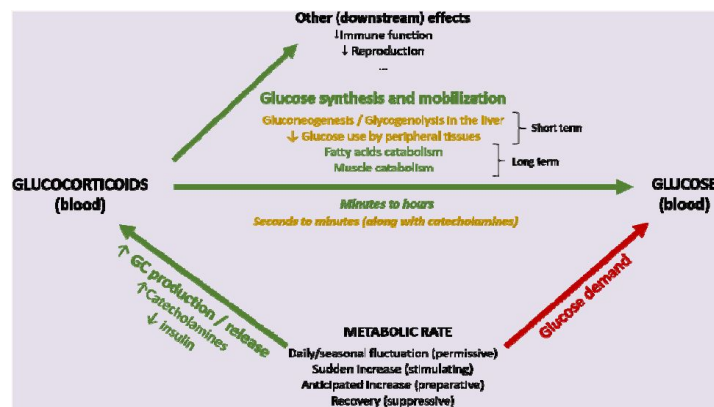


Fig. 1

Schematic representation of the association between metabolic rate and plasma levels of glucocorticoids and glucose. Green arrows represent increasing effects whereas red arrows represent reducing effects

response (Herman *et al.* 2016 [↗](#); Romero & Beattie 2021 [↗](#); Sapolsky *et al.* 2000 [↗](#)). The GC response lags in time –as GCs are produced *de novo* at the adrenal and take minutes to be secreted– and lasts substantially longer, depending on active (feedback signalling) and passive (GC degradation) processes (Herman *et al.*, 2016 [↗](#); **Fig. 1** [↗](#)), enhancing and prolonging the increase in blood glucose (Nonogaki 2000 [↗](#); Romero & Beattie 2021 [↗](#)), or recovering energy stores after a brief burst of activity. In addition, inhibition of peripheral glucose transport and utilization in response to GCs increases the availability for other tissues, such as the brain (reviewed in Sapolsky *et al.* 2000 [↗](#); Herman *et al.* 2016 [↗](#)). GCs also act in other substrates, further increasing lipolysis by inducing hormone-sensitive lipase (Slavin *et al.* 1994 [↗](#)), and reducing lipoprotein lipase activity in peripheral fat depots. They also promote pre-adipocyte differentiation, pro-lipogenic pathway activity, and cellular hypertrophy in central fat (Vegiopoulos & Hertzog 2007) as well as decreased thermogenesis in brown adipose tissue (Soumano *et al.* 2000 [↗](#)). In various muscle types, GCs suppress protein synthesis while promoting protein degradation and amino acid export. When the energetic and substrate requirements of the organism are further increased (e.g. during fasting or illness), muscle tissue (40% of total body mass) becomes a rich source of amino acids, which can be mobilized as substrates for energy generation, gluconeogenesis and protein synthesis (Kuo *et al.* 2013 [↗](#)).

Given the existing evidence on the metabolic role of GCs, and the different time scales associated to the kind of response (anticipatory vs perceived) as well as to the hormones and substrates' physiological actions (see above), GC concentrations cannot be expected to always reflect the 'immediate' energy expenditure. However, we would expect changes in energetic demands to always require a GC response/input to meet the derived metabolic needs (**Fig. 1** [↗](#)). This prediction of a strong association between GCs and metabolic rate, however, is not well researched, and does not necessarily imply that one trait necessarily affects the other *per se*, as their interplay is likely to be shaped by the environmental or physiological context. Note further that we make no distinction between baseline and stress-induced GC-levels, and thereby in effect assume these to be points in a continuum from a metabolic perspective; a perspective supported by the monotonic effects of GCs on glucose uptake and fat depletion (Kattwinkel & Munck 1966 [↗](#); Dallman *et al.* 1993 [↗](#)). Additionally, although we consider GCs to be regulated to meet energetic demands, we are aware GCs have many complex downstream effects at both baseline and stress-induced levels, besides energy-mobilization (**Fig. 1** [↗](#)).

Methods

Literature search

We reviewed the literature to identify empirical studies reporting measurements of both metabolic rate and plasma GCs. We compiled studies that met all following criteria: (1) Including an experimental manipulation of any kind leading to increases in metabolic rate which was quantified (i.e. both significant and non-significant increases). Among these, we also included those studies reporting heart rate as a metabolic measure, as heart rate and metabolic rate are strongly correlated (Bevan *et al.* 1994 [↗](#); Bevan *et al.* 1995 [↗](#); Butler *et al.* 2004 [↗](#); Word *et al.* 2022 [↗](#)). (2) Including measurements of natural GC concentrations in plasma (i.e. not exogenous or chemically induced –e.g. with ACTH or CRH). (3) measurements of GCs and metabolism had to be on the same individuals and measured in the same physiological state. The latter condition excludes, for example, studies with daily energy expenditure measurements combined with GCs measured at one time-point. Finally, we only included studies on endotherms (birds and mammals), because metabolic regulation differs strongly between endotherms and ectotherms.

We conducted a database search (Web of Science, 20/07/2021) to identify candidate studies, using the following two combinations of search terms: “energy expenditure” AND (glucocorticoid OR cortisol OR corticosterone) and “metabolic rate” AND (glucocorticoid OR cortisol OR corticosterone).

After the search, we consecutively selected articles after a) abstract review, b) full text review and c) data availability for effect size calculations. Using this approach, we identified a total of 14 studies that met all our criteria (see **Table S1** [for additional information on the number of studies obtained on each of the search steps](#)). We also systematically checked the reference list of these 14 papers, which yielded an additional 7 papers. Thus, we included a total of 21 papers in our analyses, of which 12 were on birds, and 9 on mammals. Nine of the 22 papers included more than one experimental treatment, yielding a total of 35 effect sizes. For each of these studies, we extracted information on study species or metabolic and GC variables reported, among others (Table S2). Additionally, we recorded variation related to the experimental design, the variables that were quantified, and the type of treatment used: a) Before / after design: whether the experimental manipulation included a time effect (i.e. individuals served as their own control, being measured before and after the experimental manipulation); b) Experiment / control design: Whether the experiment accounted for within-individual variation (i.e. all individuals went through all experimental treatments); c) Whether metabolic rate or heart rate was the metabolic variable; and d) The type of treatment that induced an increase in metabolic rate (see below) (Table S2).

Effect size calculations

To estimate effect sizes of metabolism and GCs, we used the web-based effect size calculator Practical Meta-Analysis Effect Size Calculator (www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php (<http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php>) [\(Lipsey & Wilson 2000 and Nakagawa & Cuthill 2007\)](#)). We calculated standardized mean-difference effect sizes (Cohen's D), which we computed from means and standard deviations (19 studies) or t-test (2 studies). When metrics were presented graphically only, we extracted data from the figure(s) using the GetData Graph Digitizer software (<http://getdata-graph-digitizer.com/>) [\(Lipsey & Wilson 2000 and Nakagawa & Cuthill 2007\)](#). See Table S3 for details on data extraction and effect size calculations.

For each study, we compared the mean metabolic rate and level of plasma GCs of individuals in the treatment group(s) to that of individuals in the control group. For studies in which treatment was confounded with time, because pre-treatment measurements were used as control and compared with measurements during treatment, the pre-treatment measure was used as control when calculating effect sizes in studies where there was a single treatment. When studies with a before-after design included more than one experimental treatment, the treatment yielding the lowest metabolism was taken as control for the effect size calculations. Thus, confounding time with treatment was avoided whenever possible.

Statistical analyses

We conducted all meta-analyses using the *rma.mv* function from the *metafor* package (Viechtbauer 2010 [\(Viechtbauer 2010\)](#)), implemented in R (version 4.0.1, R Core Team 2020 [\(R Core Team 2020\)](#)). Standard errors were used for the weigh factor. All models contained a random intercept for study identity to account for inclusion of multiple experimental treatments or groups from the same study. Most species were used in a single study, and we therefore did not include species as a random effect in addition to study identity. The number of species was however insufficient to reliably estimate phylogenetic effects, we therefore limited the analysis in this respect with a comparison between birds and mammals (see below). The dependent variable was either the metabolic rate or the GC effect size. One model was fitted with the metabolic rate effect size as a dependent variable, to estimate the average effect on metabolic rate across all studies in the analyses. All other models had the GC effect size as dependent variable, and metabolic rate effect size as a moderator. Distribution of metabolic rate effect sizes was skewed which was resolved by ln-transformation, which yielded a better fit when compared to a model using the linear term (evaluated using AIC, see results for details). Our first GC model contained only the metabolic rate effect size as a fixed independent variable. This model provides a qualitative test of whether GC levels increase when metabolic rate is increased and

tests prediction (i) by providing an estimate of the intercept, which represents the average GC effect size because we mean centered the ln-transformed metabolic rate effect size (Schielzeth 2010). The same model tests prediction (ii) whether the GC effect increases with an increasing metabolic rate effect size, which will be expressed in a significant regression coefficient of the metabolic rate effect size.

Following the model with which we tested our main predictions, we ran additional models to test for the effects on GC effect sizes of (a) taxa (birds vs. mammals), two design effects, namely (b) before / after effect, and (c) experiment / control effect, (d) metabolic variable measured (metabolic rate or heart rate), (e) type of treatment (categorized in climate, psychological, or other). This last factor tests our prediction iii. We included these variables as modulators in the analysis, as well as the two-way interactions of these factors with the metabolic rate effect size. All factors were coded as 1 (bird / no before – after effect / no experiment-control effect / metabolic rate) or 2 (mammal / before-after effect / experiment-control effect / heart rate), and then mean-centered. Treatment type was categorized as 1 (climate), 2 (psychological), or 3 (others). We compared models with vs. without these additional variables using Akaike's Information Criterion, with correction for small sample sizes (AICc, Akaike, 1974) for which a change in AICc of 2 is considered significant (Burnham, Anderson & Huyvaert 2011). Models within $\Delta\text{AICc} < 2$ were considered best fitting models, and we further explored the effects of the main predictors when present in these top models. To rule out publication bias effects (i.e. regression test for funnel plot asymmetry), we included a weighing variable (square root of the sample size) as moderator in the models, as Egger's test is not a reliable test of funnel plot asymmetry in multilevel models. Variable effects and results remained quantitatively very similar and qualitatively unchanged. Furthermore, because many labs contributed multiple studies, we tested the effect of including Lab (N=16) as random factor in the models. However, this variable had a negligible effect on the models, and we therefore excluded it from the final models.

Results

Among the studies selected for inclusion in the analysis, the treatment effect size on metabolic rate (MR) was on average 1.85 ± 0.87 (Fig. S1). In accordance with prediction, effects on GCs were positive in the majority of cases (32/35; Fig. 2), and consequently the overall average effect size deviated significantly from zero, with the average GC effect size estimated at 0.73 ± 0.11 (Table 1). There was a strong association between MR effect sizes and GC effect sizes (Table 1, Fig. 3), thus confirming prediction ii. It is further worth noting that the residual heterogeneity did not exceed the level expected by chance (Table 1). MR Cohen's D was Ln-transformed (see methods) to normalize the distribution (Fig. S2), and AICc of models including Ln MR were significantly lower when compared to models including untransformed MR Cohen's D (AICc = 70.58 vs 74.97, respectively).

The association between MR and GC effect sizes remained statistically significant when adding Taxa, before / after, experiment / control effect, metabolic variable or treatment type one by one to the model. Furthermore, none of these variables had a significant effect on GC effect size, nor did the association between MR and GC effect sizes depend on those factors (i.e. interactions between these variables and MR effect sizes were always non-significant; Table 2, S4). The latter result confirms prediction iii. Given that none of these effects significantly improved the model, the final model when removing all factors was the one including MR effect size as only predictor of GC effect size (Table 1). Despite these modulators being non-significant, the associations were in the expected directions, with studies including within-individual variation (i.e. experiment / control effect), and not including a before / after effect reporting higher GC effect sizes (Table S4, Fig. S3).

	Estimate	s.e.	Z	P	95% C.I..
Intercept	0.72	0.11	6.40	<0.0001	0.50-0.95
MR effect size (ln)	0.31	0.10	2.94	0.003	0.10-0.51
Variance components: Study.ID (Sigma^2)– Estimate = 0.00, sqrt = 0.00, n= 21					
Residual heterogeneity: QE(df = 33) = 28.40, p = 0.70					
Test of moderators: QM(df = 1) = 8.64, p= 0.003					

Table 1.

Meta regression model testing the association between metabolic rate (MR) effect sizes and glucocorticoid effect sizes.

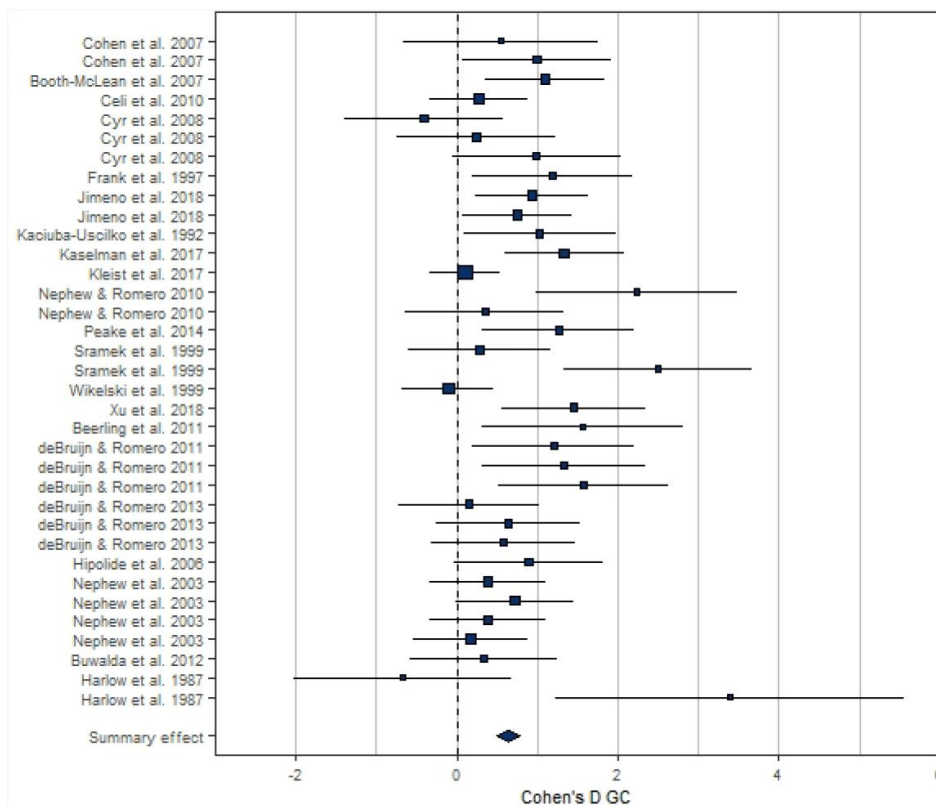


Fig. 2

Forest plot showing the glucocorticoid (GC) effect sizes (Cohen's D ± 95% CI) associated to experimental manipulations of metabolic rate, grouped by treatment group and study. Area of squares is proportional to the experiment sample size (1/s.e.).

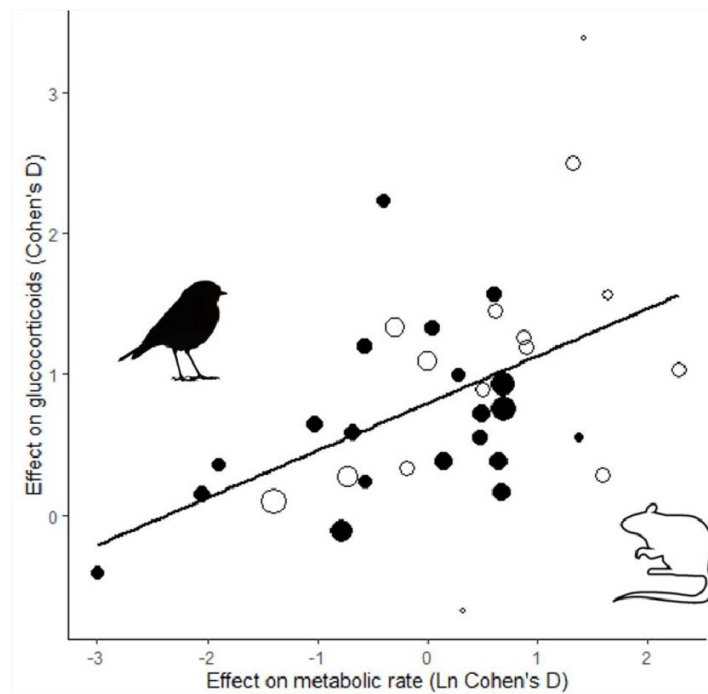


Fig 3.

Glucocorticoid effect size (Cohen's D) increases with increasing metabolic rate effect size similarly in studies of mammals (open circles) and birds (closed circles). Area of dots is proportional to the experiment sample size (i.e. square root of the number of individuals in which GCs were measured).

Variable	P
MR effect size (ln)	0.003
Taxa	0.63
Before / after	0.49
Experiment / control	0.68
Metabolic variable	0.94
Treatment type	
Treat. 2	0.93
Treat. 3	0.60

Table 2.

Table showing the main effects of all variables considered (Metabolic Rate, Taxa, Time effect, Within-individual variation, Metabolic variable, and Treatment Type) to modulate glucocorticoid effect sizes across studies. Full models are shown in [Table S4](#).

Finding a consistent functional interpretation of GC variation has proven challenging, and to this end we presented a simplified framework focusing on the interplay between energy metabolism and GCs (Box 1). Based on this framework, we made three predictions that we tested through a meta-analysis of studies in endotherms in which metabolic rate was manipulated and GCs were measured at the same time. The analysis confirmed our predictions, showing that experimental manipulations that increased metabolic rate induced a proportional increase in GCs (Fig.3), and our interpretation of this effect is that GC secretion facilitated increases in MR. This association indicates that fluctuations in energy turnover are a key factor driving variation in GC levels. From this perspective, the many downstream effects of GCs (e.g. down regulation of immune function and reproduction; [McEwen & Wingfield 2003](#); [Sapolsky et al. 2000](#)) may be interpreted as allocation adjustments to the metabolic level at which organisms operate. Specifically, within-individual blood GC variation signals the metabolic rate at which the organism is functioning to all systems in the body. In this light, downstream effects of GCs can be interpreted as evolved responses to metabolic rate fluctuations, reallocating resources in the face of shifting demands on the whole organism level.

The effect of metabolic rate on GC levels was independent of the type of manipulation used to increase metabolic rate, confirming our third prediction. Note however that confirmation of this prediction relied on the absence of a significant effect, and absence of evidence is not evidence of absence. However, the residual heterogeneity of our final model did not deviate from a level expected due to sampling variance, providing additional support for our third prediction.

Note that in the context of our analysis we made no distinction between ‘baseline’ and ‘stress-induced GC-levels (Box 1). Firstly, because these concepts are not operationally well defined – baseline GC-levels are usually no better defined than ‘not stress-induced’. Secondly, when considering the facilitation of metabolic rate as primary driver of GC regulation, there does not appear a need to invoke different classes of GC-levels instead of the more parsimonious treatment as continuum. This is not to say that this also applies to the functional consequences of GC-level variation: it is well known that receptor types differ in sensitivity to GCs ([Landys et al. 2006](#); [Sapolsky et al. 2000](#); [Romero 2004](#)), thereby potentially generating step functions in the response to an increase in GC-levels.

We restricted the meta-analysis to experimental studies, and expect the association between MR and GCs to be less evident in a more natural context. Associations between GCs and MR will be most evident when animals are maintained at different but stable levels of metabolic rate, because then the rate at which tissues are fuelled is likely to be in equilibrium with the metabolic needs. While equilibrium conditions can be created in laboratory studies, conditions will usually be more variable in the wild. When metabolic rate fluctuates, e.g. due to short term variation in activity, GC variation will track metabolic rate fluctuations, but with a time lag (Box 1), thus adding complexity to the MR / GC association and its detectability. Furthermore, experiments yield estimates of associations within the average individual in the study, while data collected in a natural context usually rely on variation between individuals (but see [Malkoc et al. 2022](#)). Associations between individuals will be less strong than associations within individuals due to individual variation in GC levels and GC reactivity (e.g. [Liu et al. 1997](#); [Weaver et al. 2004](#); [Yehuda et al. 2014](#); [Taff et al 2018](#), 2022). Between subject variation is likely to be even larger on an interspecific level, and in line with this expectation the comparative evidence on the MR / GC association is mixed. While a strong positive MR / GC correlation was reported for mammals ([Haase et al 2016](#)), [Francis et al \(2018\)](#) found no consistent GC / MR association in birds and other tetrapod taxa. However, recent comparative analyses showed avian GC variation to be positively correlated with estimated thermoregulatory costs ([Rubalcaba & Jimeno 2022a](#)), and GC variation in lizards to be positively correlated with body temperature, which directly influences metabolic rate in ectotherms ([Rubalcaba & Jimeno 2022b](#)). The contrast between the findings may be due to the

MR and GC data not always being collected on individuals in a comparable state. We emphasize therefore the importance of measuring metabolic rate and GCs when animals are in the same state, preferably by measuring both variables at the same time.

GCs increased in the studies included in our meta-analysis in response to an induced increase in MR, but GCs can also increase in response to an *anticipated* increase in MR (Box 1). For example, the early morning GC increase in humans, known as the ‘cortisol awakening response’ (Fries et al 2009 [\[1\]](#)) can be interpreted as preparation for an increase in MR – indeed human “early birds” show higher GC-levels than “night owls” in the hour after awakening (Kudielka et al 2006 [\[2\]](#)). Likewise, GC levels increase in athletes preceding competition (van Paridon et al 2017 [\[3\]](#)), although separating effects of psychological stress from anticipated metabolic needs is difficult in this context. Experiments in which animals are trained to anticipate an increase in MR to investigate whether this generates an anticipatory increase in GCs would be an interesting additional test of the framework laid out in Box 1.

Given that GC levels are often assumed to provide information on organismal ‘stress’ and welfare, the question arises whether the observed pattern can be the consequence of effects on psychological stress instead of metabolic rate. This question arises because manipulations of energy expenditure are always ‘indirect’, in that an external treatment is used to induce an increase in metabolic rate, as opposed to a direct manipulation of metabolic rate, and this leaves room for other factors to cause the observed effects. While we acknowledge that it is not possible to demonstrate conclusively that a process is not happening, we consider it unlikely that ‘stress effects’ explain our findings. Firstly, because the way metabolic rate was manipulated varied widely between studies but manipulation type had no discernible effect on the MR / GC association. This was also the conclusion of one of the studies included in our meta-analysis, designed specifically to compare MR / GC associations between two MR increasing treatments, ambient temperature and noise as psychological stressor (Jimeno et al 2018 [\[4\]](#)). Secondly, the finding that the GC increase was proportional to the increase in MR can only be explained by psychological stress when the induced psychological stress was proportional to the induced MR. Thirdly, the pattern is consistent with what is known of the functional consequences of GC variation in relation to metabolic needs (Box 1). Lastly, diverse non-injurious psychological stressors increase metabolic rate in humans (Sawai et al. 2007 [\[5\]](#), Balanos et al. 2010 [\[6\]](#), Carroll et al. 2009 [\[7\]](#)), mammals (Harris et al. 2006 [\[8\]](#); mild and unpredictable chronic stress; García-Díaz et al. 2007 [\[9\]](#)) and birds (Jimeno et al. 2018 [\[4\]](#)), explaining why GCs generally increase in response to a stressor. We conclude therefore that while a causal link between MR and GCs is not the only possible explanation of our findings, we argue it to be the most parsimonious explanation. Direct manipulations of MR could confirm or reject this explanation, and may for example be achieved using thyroid hormones, which have been shown to affect MR (Moreno et al. 2002 [\[10\]](#), Kim 2008 [\[11\]](#)).

We selected studies in which experimental treatments affected MR, leading us to conclude that the most parsimonious explanation of our finding is that GC levels were causally related to MR. Suppose however that instead we reported a correlation between MR and GCs, using for example unmanipulated individuals. The question would then be justified whether changes in GCs affected MR or vice versa. Direct effects of GCs could be studied using pharmacological manipulations. However, while many studies show that GC administration induces a cascade of effects, when the function of GCs is to *facilitate* a level of MR, as opposed to *regulate* variation in MR, we do not anticipate such manipulations to induce an increase in MR (Box 1). On the other hand, when MR is experimentally increased in conjunction with pharmacological manipulations that *suppress* the expected GC-increase (an experiment that to our best knowledge has not yet been done), we would predict that the increase in MR can be maintained less well compared to the same MR treatment in the absence of the pharmaceutical manipulation. This result, we would interpret to demonstrate that maintaining a particular level of MR may be dependent on GCs as facilitator, but it would be misleading to interpret this pattern to indicate that GCs regulate MR, as is sometimes proposed. Additionally, it would be informative to investigate whether energy turnover immediately before

blood sampling is a predictor of GC levels, as we would predict on the basis of the interpretation of our findings. Increasing the use of devices and techniques that monitor energy expenditure or its proxies (e.g. accelerometers) may be a way to increase our understanding of the generality of the GC-MR association.

Authors that assumed GC levels to be a proxy of physiological stress have struggled with the interpretation of findings such as the mixed results with respect to fitness consequences of GC-variation. Our findings offer a way to interpret such variation: GCs are regulated with respect to their role in facilitating energy metabolism, and we encourage researchers to approach and interpret findings from this perspective. For example, a positive association between GCs and reproductive success may indicate that individuals that are able to sustain high metabolic rates attain higher fitness (e.g. Bauch *et al.*, 2016 [↗](#)), while a negative association indicates the opposite effect (e.g. Ouyang *et al.* 2013 [↗](#); see Atema *et al.*, 2021 [↗](#), for a more general discussion of this specific contrast). Given that GCs have many other downstream effects (Sapolsky *et al.* 2000 [↗](#)), for example suppressing immune function (Cain & Cidlowski 2017 [↗](#)), and growth (Allen 1996 [↗](#)), this may seem an overly simplistic approach. However, in this framework, downstream effects of GCs may be understood as responses to a system level readout of the current level of energy metabolism, with high levels affecting the allocation of energy to different energy demanding processes. In this view, the link between GCs and energy demanding processes is asymmetric, in the sense that GCs affect energy allocation to e.g. growth, but there is no direct feedback from growth to GC levels. In conclusion, whereas GCs are widely seen as ‘stress hormones’, we offer a different interpretation and question whether GC variation reveals any physiological stress beyond fluctuations in energy expenditure.

Funding

BJ was funded by grant FJC2019-039748-I of MCIN/AEI /10.130 39/501100011033.

Supplementary information

Table S2: Table with the information extracted from each study included in the meta analysis (excel sheet available).

Table S3: Effect size calculations. The document Includes one sheet per study with the data extracted, the part of the article it was extracted from, and the effect size calculations and results further included in the meta analysis and Table S2 (excel sheets available).

“energy expenditure” AND (glucocorticoid OR cortisol OR corticosterone) (20/07/2021)			
Total	After abstract	After text	After effect size calculations
707	108	43	9
“metabolic rate” AND (glucocorticoid OR cortisol OR corticosterone) (20/07/2021)			
Total	After abstract	After text	After effect size calculations
447	71 (64*)	28	5
Selected papers’ reference check			
Total	After abstract	After text	After effect size calculations
(excl. previously included)			
20	15	9	7

Text step requires paper selection criteria to be met. Effect size calculation step requires enough data reported to calculate effect sizes by experimental treatment.

Main reasons for not including articles after abstract / text step: i) Experimental treatment had no effect on metabolic rate, ii) chemical or drug induction of metabolic rate increase, iii) GCs were measured in other tissues than plasma, iv) no experimental manipulation of metabolic rate.

**Number of studies excluding those overlapping with previous search, which were not further considered.*

Table S1.

Study selection steps and number of studies found

(a)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.70	0.12	6.00	<0.0001	0.47-0.93
MR effect size (ln)	0.29	0.11	2.70	0.007	0.08-0.50
Taxa (mammal)	0.12	0.24	0.49	0.63	-0.35-0.58
MR : Taxa	0.17	0.22	0.80	0.42	-0.25-0.60
Variance components: Study.ID (Sigma^2)– Estimate = 0.00, sqrt = 0.00, n= 21					
Residual heterogeneity: QE(df = 31) = 27.44, p = 0.65					
Test of moderators: QM(df = 3) = 9.60, p= 0.022					
(b)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.73	0.11	6.42	<0.0001	0.51-0.95
MR effect size (ln)	0.31	0.11	2.95	0.003	0.10-0.52
Before / after effect (yes)	-0.26	0.38	-0.70	0.49	-0.99-0.48
MR : Time effect	0.02	0.31	0.05	0.96	-0.59-0.62
Variance components: Study.ID (Sigma^2)– Estimate = 0.00, sqrt = 0.00, n= 21					
Residual heterogeneity: QE(df = 31) = 27.91, p = 0.62					
Test of moderators: QM(df = 3) = 9.13, p= 0.028					
(c)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.73	0.11	6.51	<0.0001	0.51-0.96
MR effect size (ln)	0.31	0.10	2.97	0.003	0.11-0.51
Exp./ control effect (yes)	0.05	0.31	0.16	0.87	-0.56-0.65
MR : Exp. / control effect	-0.06	0.30	-0.22	0.83	-0.64-0.51
Variance components: Study.ID (Sigma^2)– Estimate = 0.00, sqrt = 0.00, n= 21					
Residual heterogeneity: QE(df = 31) = 28.18, p = 0.61					
Test of moderators: QM(df = 3) = 8.94, p= 0.03					
(d)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.73	0.12	6.11	<0.0001	0.50-0.97
MR effect size (ln)	0.33	0.11	2.97	0.003	0.11-0.54
Met. variable (HR)	0.14	0.24	0.57	0.57	-0.33-0.60
MR : Met. variable	-0.02	0.22	-0.07	0.94	-0.45-0.42
Variance components: Study.ID (Sigma^2)– Estimate = 0.01, sqrt = 0.09, n= 21					
Residual heterogeneity: QE(df = 31) = 28.09, p = 0.62					
Test of moderators: QM(df = 3) = 8.67, p= 0.022					
(e)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.78	0.21	3.79	0.0002	0.38-1.19
MR effect size (ln)	0.27	0.19	1.39	0.166	-0.11-0.64
Treat. Type 2	-0.03	0.27	-0.09	0.93	-0.56-0.51
Treat. Type 3	-0.16	0.31	-0.52	0.60	-0.78-0.45
MR : Treat. Type 2	0.05	0.25	0.22	0.83	-0.44-0.55
MR : Treat. Type 3	0.08	0.29	0.27	0.79	-0.48-0.64
Variance components: Study.ID (Sigma^2)– Estimate = 0.02, sqrt = 0.14, n= 21					
Residual heterogeneity: QE(df = 29) = 27.99, p = 0.52					
Test of moderators: QM(df = 5) = 8.86, p= 0.115					

Table S4.

Meta regression model (quantitative approach) testing the effect of (a) Taxa, (b) Before / after effect, (c) Experiment / control effect, (d) Use of Metabolic Rate or Heart Rate as metabolic variable and (e) Treatment type, on the association between metabolic rate (MR) and glucocorticoid effect sizes across studies.

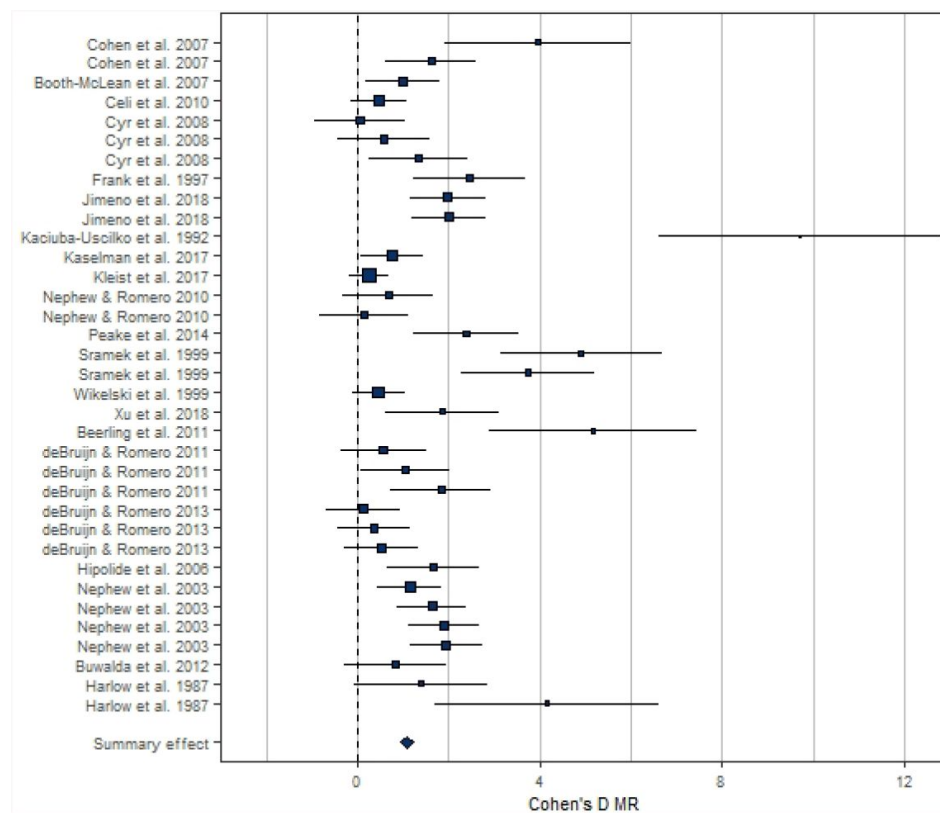


Fig S1

Forest plot showing the metabolic rate (MR) effect sizes (Cohen's $D \pm 95\%$ CI) associated to experimental manipulations of metabolic rate, grouped by treatment group and study. Area of squares is proportional to the experiment sample size (1/s.e.).

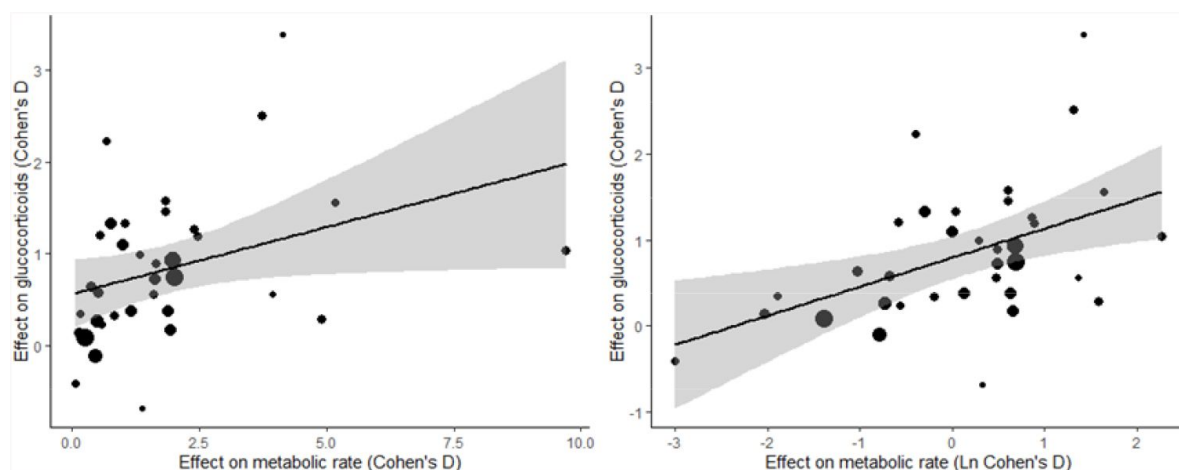


Fig S2.

Relationship between metabolic rate and glucocorticoid effect sizes (Cohen's D) across studies. Panels show the association without ln-transforming metabolic rate effect sizes (left panel) and when ln-transforming them (right panel). Size of dots is proportional to the experiment sample size (i.e. square root of the number of individuals in which glucocorticoids were measured). Shaded areas represent 95% C.I. Note that the number of data points in the graph is higher than the number of studies, as some of the studies included multiple experimental treatments (Study ID was included as random factor in statistical analyses).

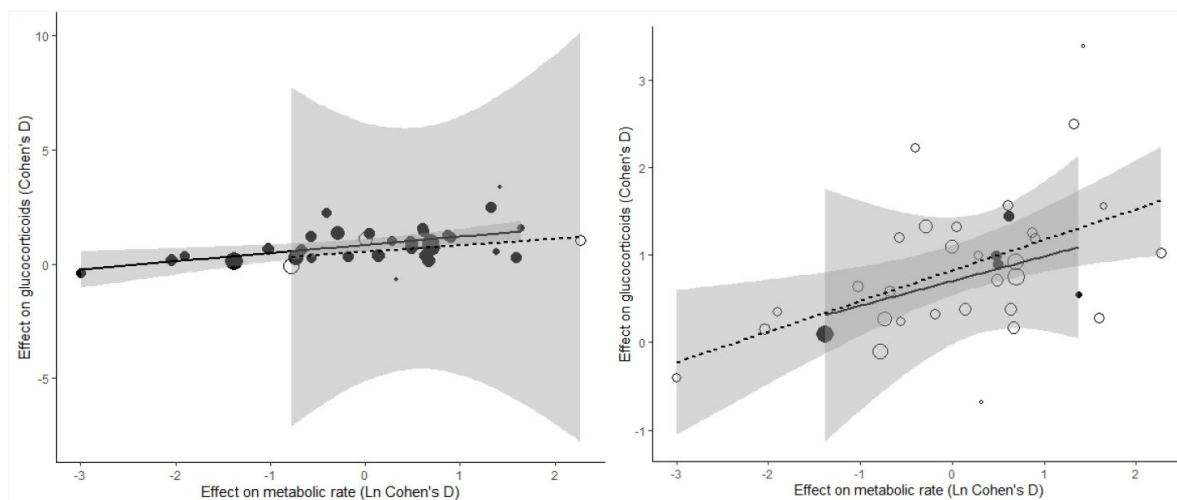


Fig S3.

Relationship between metabolic rate and glucocorticoid effect sizes (Cohen's D) across studies as a function of a) Before / after effect (open circles and dashed line for studies including a time effect; closed circles and continuous line for studies not including a time effect; see methods) and b) Experiment / control effect (open circles and dashed line for studies including within-individual variation; closed circles and continuous line for studies not including within-individual variation). Size of dots is proportional to the experiment sample size (i.e. square root of the number of individuals in which glucocorticoids were measured). Shaded areas represent 95% C.I. Note that the number of data points in the graph is higher than the number of studies, as some of the studies included multiple experimental treatments (Study ID was included as random factor in statistical analyses). Before / after effect and Experiment / control effect results should be interpreted with caution, as most studies do include within individual variation and do not include time effect.

References

References marked with an asterisk were included in the meta-analysis but not otherwise cited in the paper, Akaike H. (1974) **A New Look at the Statistical Model Identification** *IEEE Transactions on Automatic Control* **19**:6–723 <https://doi.org/10.1109/TAC.1974.1100705>

Allen D. B. (1996) **Growth suppression by glucocorticoid therapy** *Endocrinology and metabolism clinics of North America* **25**:9–717

Andrews R. C., Walker B. R. (1999) **Glucocorticoids and insulin resistance: old hormones, new targets** *Clinical Science* **96**:3–523 <https://doi.org/10.1042/CS0960513>

Atema E., van Noordwijk A. J., Verhulst S. (2021) **Telomere dynamics in relation to experimentally increased locomotion costs and fitness in great tits** *Molecular Ecology*

Balanos G. M., Phillips A. C., Frenneaux M. P., McIntyre D., Lykidis C., Griffin H. S., Carroll D. (2010) **Metabolically exaggerated cardiac reactions to acute psychological stress: the effects of resting blood pressure status and possible underlying mechanisms** *Biological Psychology* **85**:4–111

Bauch C., Riechert J., Verhulst S., Becker P. H. (2016) **Telomere length reflects reproductive effort indicated by corticosterone levels in a long-lived seabird** *Molecular Ecology* **25**:785–5794

Beerling W., Koolhaas J. M., Ahnaou A., Bouwknecht J. A., de Boer S. F., Meerlo P., Drinkenburg W. H. I. M. (2011) **Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing behavioral activity** *Physiology & behavior* **103**:412–420

Bevan R. M., Woakes A. J., Butler P. J., Boyd I. L. (1994) **The use of heart rate to estimate oxygen consumption of free-ranging black-browed albatrosses *Diomedea melanophrys*** *The Journal of experimental biology* **193**:19–137

Bevan R. M., Speakman J. R., Butler P. J. (1995) **Daily energy expenditure of tufted ducks: a comparison between indirect calorimetry, doubly labelled water and heart rate** *Functional Ecology* :40–47

Bonier F., Martin P. R., Moore I. T., Wingfield J. C. (2009) **Do baseline glucocorticoids predict fitness?** *Trends in ecology & Evolution* **24**:34–642

* Booth-Mclean M. E., Schwartzkopf-Genswein K. S., Brown F. A., Holmes C. L., Schaefer A. L., McAllister T. A., Mears G. J. (2007) **Physiological and behavioural responses to short-haul transport by stock trailer in finished steers** *Canadian journal of animal science* **87**:291–297

* de Bruijn R., Romero L. M. (2011) **Behavioral and physiological responses of wild-caught European starlings (*Sturnus vulgaris*) to a minor, rapid change in ambient temperature** *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **160**:260–266

* de Bruijn R., Romero L. M. (2013) **Artificial rain and cold wind act as stressors to captive molting and non-molting European starlings (*Sturnus vulgaris*)** *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **164**:512–519

- Burnham K. P., Anderson D. R., Huyvaert K. P. (2011) **AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons** *Behavioral Ecology and Sociobiology* **65**:23–35 <https://doi.org/10.1007/S00265-010-1029-6/FIGURES/3>
- Busch D. S., Hayward L. S. (2009) **Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables** *Biological Conservation* **142**:2844–2853
- Burton T., Killen S. S., Armstrong J. D., Metcalfe N. B. (2011) **What causes intraspecific variation in resting metabolic rate and what are its ecological consequences?** *Proceedings of the Royal Society B: Biological Sciences* **278**:3465–3473
- Butler P. J., Green J. A., Boyd I. L., Speakman J. R. (2004) **Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods** *Functional ecology* **18**:8–183
- Buwalda B., Scholte J., de Boer S. F., Coppens C. M., Koolhaas J. M. (2012) **The acute glucocorticoid stress response does not differentiate between rewarding and aversive social stimuli in rats** *Hormones and behavior* **61**:218–226
- Cain D. W., Cidlowski J. A. (2017) **Immune regulation by corticosteroids** *Nat Rev Immunol* **17**:233–247
- Carroll D., Phillips A. C., Balanos G. M. (2009) **Metabolically exaggerated cardiac reactions to acute psychological stress revisited** *Psychophysiology* **46**:0–275
- * Celi F. S., Brychta R. J., Linderman J. D., Butler P. W., Alberobello A. T., Smith S., Chen K. Y. (2010) **Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults** *European journal of endocrinology/European Federation of Endocrine Societies* **163**
- * Cohen A., Hau M., Wikelski M. (2008) **Stress, metabolism, and antioxidants in two wild passerine bird species** *Physiological and Biochemical Zoology* **81**:463–472
- Colborn D. R., Thompson Jr D. L., Roth T. L., Capehart J. S., White K. L. (1991) **Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings** *Journal of Animal Science* **69**:56–2562
- * Cyr N. E., Wikelski M., Romero L. M. (2008) **Increased energy expenditure but decreased stress responsiveness during molt** *Physiological and Biochemical Zoology* **81**:452–462
- Dallman M. F., Strack A. M., Akana S. F., Bradbury M. J., Hanson E. S., Scribner K. A., Smith M. (1993) **Feast and famine: critical role of glucocorticoids with insulin in daily energy flow** *Frontiers in neuroendocrinology* **14**:3–347
- Deviche P., Bittner S., Gao S., Valle S. (2017) **Roles and mechanistic bases of glucocorticoid regulation of avian reproduction** *Integrative and comparative biology* **57**:84–1193
- Francis C. D., Donald J. W., Fuxjager M. J., Goymann W., Hau M., Husak J. F., Downs C. J. (2018) **Metabolic scaling of stress hormones in vertebrates** *Integrative and comparative biology* **58**:9–738

- * Frank S. M., Higgins M. S., Fleisher L. A., Sitzmann J. V., Raff H., Breslow M. J. (1997) **Adrenergic, respiratory, and cardiovascular effects of core cooling in humans** *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **272**:R557–R562
- Fries E., Dettenborn L., Kirschbaum C. (2009) **The cortisol awakening response (CAR): facts and future directions** *International journal of Psychophysiology* **72**:67–73
- García-Díaz D. F., Campion J., Milagro F. I., Lomba A., Marzo F., Martínez J. A. (2007) **Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats** *Journal of physiology and biochemistry* **63**:7–346
- Haase C. G., Long A. K., Gillooly J. F. (2016) **Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals** *Biology letters* **12**
- Harris R. B., Palmondon J., Leshin S., Flatt W. P., Richard D. (2006) **Chronic disruption of body weight but not of stress peptides or receptors in rats exposed to repeated restraint stress** *Hormones and behavior* **49**:5–625
- * Harlow H. J., Thorne E. T., Williams E. S., Belden E. L., Gern W. A. (1987) **Adrenal responsiveness in domestic sheep (Ovis aries) to acute and chronic stressors as predicted by remote monitoring of cardiac frequency** *Canadian journal of zoology* **65**:2021–2027
- Herman J. P., McKlveen J. M., Ghosal S., Kopp B., Wulsin A., Makinson R., Myers B. (2016) **Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response** *In Comprehensive Physiology* **6**:603–621 <https://doi.org/10.1002/cphy.c150015>
- Herman J. P. (2022) **The neuroendocrinology of stress: Glucocorticoid signaling mechanisms** *Psychoneuroendocrinology*
- * Hipolide D. C., Suchecki D., de Carvalho Pinto A. P., Chiconelli Faria E., Tufik S., Luz J. (2006) **Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats** *Journal of neuroendocrinology* **18**:231–238
- Jessop T. S., Lane M. L., Teasdale L., Stuart-Fox D., Wilson R. S., Careau V., Moore I. T. (2016) **Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates** *The American Naturalist* **188**:342–356
- Jimeno B., Hau M., Verhulst S. (2018) **Corticosterone levels reflect variation in metabolic rate, independent of 'stress'** *Scientific Reports* **8** <https://doi.org/10.1038/s41598-018-31258-z>
- Kattwinkel J., Munck A. (1966) **Activities in vitro of glucocorticoids and related steroids on glucose uptake by rat thymus cell suspensions** *Endocrinology* **79**:387–390
- Kim B. (2008) **Thyroid hormone as a determinant of energy expenditure and the basal metabolic rate** *Thyroid* **18**:141–144
- Kudielka B. M., Federenko I. S., Hellhammer D. H., Wüst S. (2006) **Morningness and eveningness: the free cortisol rise after awakening in "early birds" and "night owls"** *Biological psychology* **72**:141–146

- * Kaciuba-Uscilko H., Kruk B., Szczypaczewska M., Opaszowski B., Stupnicka E., Bicz B., Nazar K. (1992) **Metabolic, body temperature and hormonal responses to repeated periods of prolonged cycleergometer exercise in men** *European journal of applied physiology and occupational physiology* **64**:26–31
- * Keselman B., Vergara M., Nyberg S., Nystrom F. H. (2017) **A randomized cross-over study of the acute effects of running 5 km on glucose, insulin, metabolic rate, cortisol and Troponin T** *PloS one* **12**
- * Kleist B., Wahrburg U., Stehle P., Schomaker R., Greiwing A., Stoffel-Wagner B., Egert S. (2017) **Moderate walking enhances the effects of an energy-restricted diet on fat mass loss and serum insulin in overweight and obese adults in a 12-week randomized controlled trial** *The Journal of nutrition* **147**:1875–1884
- Koolhaas J. M., Bartolomucci A., Buwalda B., de Boer S. F., Flügge G., Korte S. M., Fuchs E. (2011) **Stress revisited: a critical evaluation of the stress concept** *Neuroscience & Biobehavioral Reviews* **35**:1291–1301
- Kuo T., Harris C. A., Wang J. C. (2013) **Metabolic functions of glucocorticoid receptor in skeletal muscle** *Molecular and cellular endocrinology* **380**:79–88
- Landys M. M., Ramenofsky M., Wingfield J. C. (2006) **Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes** *General and comparative endocrinology* **148**:132–149
- Lipsey M. W., Wilson D. B. (2001) **Practical meta-analysis**
- Liu D., Diorio J., Tannenbaum B., Caldji C., Francis D., Freedman A., Meaney M.J. (1997) **Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress** *Science* **277**:1659–1662 <https://doi.org/10.1126/science.277.5332.1659>
- MacDougall-Shackleton S. A., Bonier F., Romero L. M., Moore I. T. (2019) **Glucocorticoids and “Stress” Are Not Synonymous** *Integrative Organismal Biology* **1** <https://doi.org/10.1093/IOB/OBZ017>
- Madliger C. L., Love O. P. (2014) **The need for a predictive, context-dependent approach to the application of stress hormones in conservation** *Conservation Biology* **28**:283–287
- Madliger C. L., Semeniuk C. A. D., Harris C. M., Love O. P. (2015) **Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker** *Biological Conservation* **192**:409–417 <https://doi.org/10.1016/j.biocon.2015.10.021>
- Malkoc K., Casagrande S., Hau M. (2021) **Inferring whole-organism metabolic rate from red blood cells in birds** *Frontiers in Physiology* **12**
- Malkoc K., Montesana L., Casagrande S., Hau M. (2022) **Quantifying glucocorticoid plasticity using reaction norm approaches: There still is so much to discover!** *Integrative and Comparative Biology* **62**:58–70
- McCormick S. D., Romero L. M. (2017) **Conservation Endocrinology** *BioScience* **67**:429–442 <https://doi.org/10.1093/BIOSCI/BIX026>

- McEwen B. S., Wingfield J. C. (2003) **The concept of allostasis in biology and biomedicine** *Hormones and behavior* **43**:2–15
- Moreno M., Lombardi A., Beneduce L., Silvestri E., Pinna G., Goglia F., Lanni A. (2002) **Are the effects of T3 on resting metabolic rate in euthyroid rats entirely caused by T3 itself?** *Endocrinology* **143**:504–510
- Nakagawa S., Cuthill I. C. (2007) **Effect size, confidence interval and statistical significance: a practical guide for biologists** *Biological Reviews* **82**:591–605 <https://doi.org/10.1111/J.1469-185X.2007.00027.X>
- * Nephew B. C., Romero L. M. (2003) **Behavioral, physiological, and endocrine responses of starlings to acute increases in density** *Hormones and Behavior* **44**:222–232
- * Nephew B. C., Kahn S. A., Romero L. M. (2003) **Heart rate and behavior are regulated independently of corticosterone following diverse acute stressors** *General and comparative endocrinology* **133**:173–180
- Nonogaki K. (2000) **New insights into sympathetic regulation of glucose and fat metabolism** *Diabetologia* **43**:533–549
- Ouyang J. Q., Sharp P., Quetting M., Hau M. (2013) **Endocrine phenotype, reproductive success and survival in the great tit, *Parus major*** *Journal of Evolutionary Biology* **26**:1988–1998
- van Paridon K. N., Timmis M. A., Nevison C. M., Bristow M. (2017) **The anticipatory stress response to sport competition; a systematic review with meta-analysis of cortisol reactivity** *BMJ open sport & exercise medicine* **3**
- Patterson S. H., Hahn T. P., Cornelius J. M., Breuner C. W. (2014) **Natural selection and glucocorticoid physiology** *Journal of Evolutionary Biology* **27**:259–274 <https://doi.org/10.1111/jeb.12286>
- * Peake J. M., Tan S. J., Markworth J. F., Broadbent J. A., Skinner T. L., Cameron-Smith D. (2014) **Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise** *American Journal of Physiology-Endocrinology and Metabolism* **307**:E539–E552
- Petrullo L., Delaney D., Boutin S., McAdam A. G., Lane J. E., Boonstra R., Dantzer B. (2022) **The glucocorticoid response to environmental change is not specific to agents of natural selection in wild red squirrels** *Hormones and Behavior* **146**
- R Core Team (2020) **R: A language and environment for statistical computing** *R Foundation for Statistical Computing*
- Romero L. M. (2004) **Physiological stress in ecology: lessons from biomedical research** *Trends in ecology & evolution* **19**:249–255
- Romero L. M., Dickens M. J., Cyr N. E. (2009) **The reactive scope model—a new model integrating homeostasis, allostasis, and stress** *Hormones and behavior* **55**:375–389
- Romero L. M., Beattie U. K. (2021) **Common myths of glucocorticoid function in ecology and conservation** *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* <https://doi.org/10.1002/JEZ.2459>

- Romero L. M., Fairhurst G. D. (2016) **Measuring corticosterone in feathers: strengths, limitations, and suggestions for the future** *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **202**:112–122
- Rosmond R. A., Björntorp P. (2000) **The hypothalamic–pituitary–adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke** *Journal of internal medicine* **247**:188–197
- Rubalcaba J. G., Jimeno B. (2022) **Biophysical models unravel associations between glucocorticoids and thermoregulatory costs across avian species** *Functional Ecology* **36**:64–72
- Rubalcaba J. G., Jimeno B. (2022) **Body temperature and activity patterns modulate glucocorticoid levels across lizard species. A macrophysiological approach** *Frontiers in Ecology and Evolution* **10**
- Samra J. S., Clark M. L., Humphreys S. M., MacDonald I. A., Bannister P. A., Frayn K. N. (1998) **Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue** *The Journal of Clinical Endocrinology & Metabolism* **83**:626–631
- Sapolsky R. M., Romero L. M., Munck A. U. (2000) **How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions** *Endocrine reviews* **21**:55–89
- Sawai A., Ohshige K., Yamasue K., Hayashi T., Tochikubo O. (2007) **Influence of mental stress on cardiovascular function as evaluated by changes in energy expenditure** *Hypertension Research* **30**:1019–1027
- Schielzeth H. (2010) **Simple means to improve the interpretability of regression coefficients** *Methods in Ecology and Evolution* **1**:103–113 <https://doi.org/10.1111/j.2041-210X.2010.00012.X>
- Schoenle L. A., Zimmer C., Miller E. T., Vitousek M. N. (2021) **Does variation in glucocorticoid concentrations predict fitness? A phylogenetic meta-analysis** *General and Comparative Endocrinology* **300** <https://doi.org/10.1016/j.ygcen.2020.113611>
- Selye H. (1950) **Stress and the general adaptation syndrome** *British medical journal* **1**
- Selye H. (1976) **Stress without distress** *In Psychopathology of human adaptation* :137–146
- Siciliani J. L. (2000) **“The Effect of Sexual Activity on Secretory Immunoglobulin a and Cortisol Levels in Married Couples”** *Dissertation. University of Tennessee Knoxville*
- Slavin B. G., Ong J. M., Kern P. A. (1994) **Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes** *Journal of lipid research* **35**:1535–1541
- Soumano K., Desbiens S., Rabelo R., Bakopanos E., Camirand A., Silva J. E. (2000) **Glucocorticoids inhibit the transcriptional response of the uncoupling protein-1 gene to adrenergic stimulation in a brown adipose cell line** *Molecular and cellular endocrinology* **165**:7–15
- * Šrámek P., imecková M., Janský L., Šavlíková J., Vybíral S. (2000) **Human physiological responses to immersion into water of different temperatures** *European journal of applied physiology* **81**:436–442

- Taff C. C., Zimmer C., Ryan T. A., van Oordt D. C., Aborn D. A., Ardia D. R., Vitousek M. N. (2022) **Individual variation in natural or manipulated corticosterone does not covary with circulating glucose in a wild bird** *Journal of Experimental Biology* **225**
- Taff C. C., Schoenle L. A., Vitousek M. N. (2018) **The repeatability of glucocorticoids: a review and metaanalysis** *General and Comparative Endocrinology* **260**:136–145
- Vegiopoulos A., Herzig S. (2007) **Glucocorticoids, metabolism and metabolic diseases** *Molecular and cellular endocrinology* **275**:43–61
- Versteegh M. A., Helm B., Dingemanse N. J., Tieleman B. I. (2008) **Repeatability and individual correlates of basal metabolic rate and total evaporative water loss in birds: a case study in European stonechats** *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **150**:452–457
- Viechtbauer W. (2010) **Conducting Meta-Analyses in R with the metafor Package** *Journal of Statistical Software* **36**:1–48 <https://doi.org/10.18637/JSS.V036.I03>
- Weaver I.C.G., Cervoni N., Champagne F.A., D'Alessio A.C., Sharma S., Seckl J.R., Meaney M.J. (2004) **Epigenetic programming by maternal behavior** *Nat. Neurosci* **7**:847–854
- * Wikelski M., Lynn S., Breuner J. C., Wingfield J. C., Kenagy G. J. (1999) **Energy metabolism, testosterone and corticosterone in white-crowned sparrows** *Journal of Comparative Physiology A* **185**:463–470
- Word K.R., Austin S.H., Wingfield JC (2022) **Allostatic Load in Gambel's White Crowned Sparrow, *Zonotrichia leucophrys gambelii*: Relationships With Glucocorticoids** *Frontiers in Ecology and Evolution* **10**
- * Xu X. M., Chi Q. S., Cao J., Zhao Z. J. (2018) **The effect of aggression I: the increases of metabolic cost and mobilization of fat reserves in male striped hamsters** *Hormones and Behavior* **98**:55–62
- Yehuda R., Daskalakis N.P., Lehrner A., Desarnaud F., Bader H.N., Makotkine I., Meaney M.J. (2014) **Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in holocaust survivor offspring** *American Journal of Psychiatry* **171**:872–880
- Zimmer C., Taff C. C., Ardia D. R., Ryan T. A., Winkler D. W., Vitousek M. N. (2019) **On again, off again: acute stress response and negative feedback together predict resilience to experimental challenges** *Functional Ecology* **33**:619–628
- Zimmer C., Taff C. C., Ardia D. R., Rose A. P., Aborn D. A., Johnson L. S., Vitousek M. N. (2020) **Environmental unpredictability shapes glucocorticoid regulation across populations of tree swallows** *Scientific reports* **10**:1–13
- Zimmer C., Woods H. A., Martin L. B. (2022) **Information theory in vertebrate stress physiology** *Trends in Endocrinology & Metabolism* **33**:8–17

Article and author information

Blanca Jimeno

Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo 12, 13005 Ciudad Real, Spain, Instituto Pirenaico de Ecología (IPE), CSIC, Avda. Nuestra Señora de la Victoria, 16, Jaca, Spain

For correspondence: bjimenorev@gmail.com

ORCID iD: [0000-0003-3040-0163](https://orcid.org/0000-0003-3040-0163)

Simon Verhulst

University of Groningen, the Netherlands

Copyright

© 2023, Blanca Jimeno & Simon Verhulst

This article is distributed under the terms of the [Creative Commons Attribution License \(https://creativecommons.org/licenses/by/4.0/\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use and redistribution provided that the original author and source are credited.

Editors

Reviewing Editor

Yuuki Watanabe

Graduate University for Advanced Studies, SOKENDAI, Japan

Senior Editor

George Perry

Pennsylvania State University, United States of America

Reviewer #1 (Public Review):

Authors performed a meta-analysis of GC concentrations and metabolic rates in birds and mammals. They found close associations for all studies showing a positive association between these two traits. As GCs have been viewed with close links to "stress," authors suggest that this overlooks the importance of metabolism and perhaps GC variation does not relate to "stress" per se but an increase in metabolism instead.

This is an important meta-analysis, as most researchers acknowledge the link between GCs and metabolism, metabolism is often overlooked in studies. The field of conservation physiology is especially focused on GCs being a "stress" hormone, which overlooks the importance of GCs in mediating energy balance, i.e., an animal that has high GC concentrations may not be doing that poorly compared to an animal with low GC concentrations, it might just be expending more energy, e.g., caring for young. The results, with overwhelming directionality and strong effect sizes, support the link for a positive association with these two variables.

My main concern lies in that most of the studies come from a few labs, therefore there may be limited data to test this relationship. I would include lab as a random effect to see how strong this effect might be. Furthermore, I would like to see a test of the directionality of the two variables. Authors suggest that changes in metabolism affect GC levels but likely changes in GC levels would affect metabolism. Why not look into studies that have altered GC levels experimentally and see the effect on metabolism? Based on the close link, authors suggest

that GCs may not play a role outside of "stress" beyond the stressor's effect on metabolic rate. However, if they were to investigate manipulations of GCs on metabolic rate, the link may or may not be there, which would be interesting to look at. I firmly believe that GCs are tightly linked to metabolism; however, I also think that GCs have a range of effects outside of metabolism as well, depending on the course and strength of the stressor.

This work helps in the thinking that GCs are not the same as a "stress" hormone or labelling hormones with only one function. As hormones are naturally pleiotropic, the view of any one hormone being X is overly simplistic.

Reviewer #2 (Public Review):

Where this study is interesting is that the authors do a meta-analysis of studies in which metabolic rate was experimentally manipulated and both this rate and glucocorticoid levels were simultaneously measured. Unsurprisingly, there are relatively few such studies and many are from a single lab. More studies are needed. While the results of the analysis are compelling, they are not surprising. That said, this work is important.

Author Response

The following is the authors' response to the original reviews.

Reviewer #1 (Public Review):

The authors performed a meta-analysis of GC concentrations and metabolic rates in birds and mammals. They found close associations for all studies showing a positive association between these two traits. As GCs have been viewed with close links to "stress," authors suggest that this overlooks the importance of metabolism and perhaps GC variation does not relate to "stress" per se but an increase in metabolism instead.

This is an important meta-analysis, as most researchers acknowledge the link between GCs and metabolism, metabolism is often overlooked in studies. The field of conservation physiology is especially focused on GCs being a "stress" hormone, which overlooks the importance of GCs in mediating energy balance, i.e., an animal that has high GC concentrations may not be doing that poorly compared to an animal with low GC concentrations, it might just be expending more energy, e.g., caring for young. The results, with overwhelming directionality and strong effect sizes, support the link for a positive association with these two variables.

My main concern lies in that most of the studies come from a few labs, therefore there may be limited data to test this relationship. I would include lab as a random effect to see how strong this effect might be.

We think this is a good point, and we ran the main models included in the manuscript including Lab as random effect (N= 35 experiments, 21 studies, 16 labs). This did not affect the results, leading to negligible changes in the model parameters (alternative model tables are shown in Author response table 1 and 2). In the revised version of the manuscript we mention that we tested the effect of Lab but did not keep this variable in the models (lines 183-185)

Author response table 1.

Meta regression model testing the association between metabolic rate (MR) effect sizes and glucocorticoid effect sizes.

	Estimate	s.e.	Z	P	95% C.I..
Intercept	0.76	0.11	7.20	<0.0001	0.55-0.96
MR effect size (ln)	0.32	0.10	2.97	0.003	0.11-0.52
Variance components: outer factor: Lab (N=16), inner factor: Study.ID (N=21). tau^2= Estimate = 0.02, sqrt = 0.13					
Residual heterogeneity: QE(df = 33) = 28.26, p = 0.70					
Test of moderators: QM(df = 1) = 8.83, p= 0.003					

Author response table 2.

Meta regression model (quantitative approach) testing the effect of (a) Taxa, (b) Before / after effect, (c) Experiment / control effect, (d) Use of Metabolic Rate or Heart Rate as metabolic variable and (e) Treatment type, on the association between metabolic rate (MR) and glucocorticoid effect sizes across studies.

(a)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.72	0.11	6.30	<0.0001	0.50-0.95
MR effect size (ln)	0.30	0.11	2.75	0.006	0.09-0.52
Taxa (mammal)	0.07	0.23	0.33	0.74	-0.38-0.54
MR : Taxa	0.16	0.22	0.71	0.48	-0.28-0.59
Variance components: outer factor: Lab (N=16), inner factor: Study.ID (N=21). tau^2- Estimate = 0.00, sqrt = 0.09					
Residual heterogeneity: QE(df = 31) = 27.47, p = 0.65					
Test of moderators: QM(df = 3) = 9.53, p= 0.023					
(b)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.77	0.10	7.69	<0.0001	0.57-0.96
MR effect size (ln)	0.32	0.11	3.00	0.002	0.11-0.54
Before / after effect (yes)	-0.30	0.38	-0.79	0.43	-1.06-0.45
MR : Time effect	-0.00	0.31	0.05	0.96	-0.59-0.62
Variance components: Lab (N=16), inner factor: Study.ID (N=21). tau^2 - Estimate = 0.03, sqrt = 0.16					
Residual heterogeneity: QE(df = 31) = 27.72, p = 0.64					
Test of moderators: QM(df = 3) = 9.39, p= 0.024					
(c)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.76	0.10	7.51	<0.0001	0.56-0.96
MR effect size (ln)	0.32	0.11	2.97	0.003	0.11-0.52
Exp./ control effect (yes)	0.08	0.31	0.25	0.80	-0.53-0.69
MR : Exp. / control effect	-0.05	0.32	-0.00	0.99	-0.62-0.62
Variance components: Lab (N=16), inner factor: Study.ID (N=21). tau^2- Estimate = 0.02, sqrt = 0.15					
Residual heterogeneity: QE(df = 31) = 28.18, p = 0.61					
Test of moderators: QM(df = 3) = 8.88, p= 0.030					
(d)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.76	0.11	7.12	<0.0001	0.55-0.97
MR effect size (ln)	0.34	0.11	3.00	0.003	0.12-0.56
Met. variable (HR)	0.15	0.22	0.68	0.49	-0.28-0.59
MR : Met. variable	0.01	0.23	0.06	0.96	-0.43-0.46
Variance components: outer factor: Lab (N=16), inner factor: Study.ID (N=21). tau^2- Estimate = 0.03, sqrt = 0.16					
Residual heterogeneity: QE(df = 31) = 28.03, p = 0.62					
Test of moderators: QM(df = 3) = 9.21, p= 0.027					
(e)	Estimate	s.e.	Z	P	95% C.I.
Intercept	0.82	0.20	4.09	<0.0001	0.43-1.21
MR effect size (ln)	0.26	0.20	1.30	0.19	-0.13-0.64
Treat. Type 2	0.01	0.29	0.02	0.98	-0.57-0.58
Treat. Type 3	-0.20	0.32	-0.62	0.53	-0.81-0.42
MR : Treat. Type 2	0.09	0.27	0.34	0.74	-0.44-0.62
MR : Treat. Type 3	0.08	0.29	0.28	0.78	-0.49-0.66
Variance components: Lab (N=16), inner factor: Study.ID (N=21). tau^2- Estimate = 0.04, sqrt = 0.20					
Residual heterogeneity: QE(df = 29) = 27.77, p = 0.53					
Test of moderators: QM(df = 5) = 9.32, p= 0.097					

Furthermore, I would like to see a test of the directionality of the two variables. Authors suggest that changes in metabolism affect GC levels but likely changes in GC levels would affect metabolism. Why not look into studies that have altered GC levels experimentally and see the effect on metabolism? Based on the close link, authors suggest that GCs may not play a role outside of "stress" beyond the stressor's effect on metabolic rate. However, if they were to investigate manipulations of GCs on metabolic rate, the link may or may not be there, which would be interesting to look at. I firmly believe that GCs are tightly linked to metabolism; however, I also think that GCs have a range of effects outside of metabolism as well, depending on the course and strength of the stressor.

The directionality of the two variables is indeed a question of interest – we show that changes in metabolic rate affect GCs, but does the reverse also happen? In the schematic model we propose in Box 1, we propose that the effect is uni-directional, i.e. metabolic rate affects GC-levels, but GCs have no direct effect on metabolic rate. We note that there may however be an indirect effect, in that in the absence of a GC-response to an increase in metabolic rate the organism would after some time no longer be able to fuel the metabolic rate. Because we anticipate that more readers may raise this question, we have added the following paragraph to the discussion:

“We selected studies in which experimental treatments affected MR, leading us to conclude that the most parsimonious explanation of our finding is that GC levels were causally related to MR. Suppose however that instead we reported a correlation between MR and GCs, using for example unmanipulated individuals. The question would then be justified whether changes in GCs affected MR or vice versa. Direct effects of GCs could be studied using pharmacological manipulations. However, while many studies show that GC administration induces a cascade of effects, when the function of GCs is to facilitate a level of MR, as opposed to regulate variation in MR, we do not anticipate such manipulations to induce an increase in MR (Box 1). On the other hand, when MR is experimentally increased in conjunction with pharmacological manipulations that suppress the expected GC-increase (an experiment that to our best knowledge has not yet been done), we would predict that the increase in MR can be maintained less well compared to the same MR treatment in the absence of the pharmaceutical manipulation. This result, we would interpret to demonstrate that maintaining a particular level of MR may be dependent on GCs as facilitator, but it would be misleading to interpret this pattern to indicate that GCs regulate MR, as is sometimes proposed. Additionally, it would be informative to investigate whether energy turnover immediately before blood sampling is a predictor of GC levels, as we would predict on the basis of the interpretation of our findings. Increasing the use of devices and techniques that monitor energy expenditure or its proxies (e.g. accelerometers) may be a way to increase our understanding of the generality of the GC-MR association. “

We based our hypotheses and searching criteria on the assumption that GCs induce physiological processes to help the organism facilitate energetic demands. Pharmacologically induced increases in GCs would lead to physiological responses and associations that we consider not comparable to the ones reported in this work, as we base our hypotheses on natural (i.e. non pharmacologically induced) GC and MR variation. This said, with exogenous GC administration, we may expect GC cascade effects, but not necessarily an increase in MR. Here - and acknowledging that the link between GCs and metabolic rate may entail complex steps - we predict that GC administration may lead to an increase in blood glucose and may affect energy allocation at a tissue-specific level. However, such increase may have no effect on whole-organism energy expenditure, unless energy expenditure is limited by glucose availability. We however acknowledge that it would be interesting to investigate the kind of associations between MR, GCs and other physiological variables (e.g. glucose) that appear when inducing an increase in GCs, as these would broaden our understanding of the mechanistic processes underlying these associations.

We show that variation in GC levels was explained by variation in MR, independent of the stimulus that caused the increase in MR. We propose that the most parsimonious interpretation of our findings is that GC variation is an indicator of variation in MR, independent of the cause of variation in MR. We do not intend to prove causality when making predictions on the co-dependency of metabolic rate and GCs. In fact, our predictions do not imply that one trait necessarily affects the other per se, as these interplay is likely to be shaped by the environmental or physiological context (Box 1). Thus, the specific mechanisms underlying how changes in metabolic rate induce changes in GCs - or the other way around -

need to be investigated. One step to tackle this in upcoming research would indeed be studying the effects of exogenous GCs on metabolic rate.

In the manuscript, we clarify that GCs have a variety of cascade effects besides metabolism (Box 1). On the basis of our results, however, we suggest that many of the downstream effects of GCs may be interpreted as allocation adjustments to the metabolic level at which organisms operate (lines 235236), but we do acknowledge that these cascade effects are complex and affects many systems besides metabolism.

This work helps in the thinking that GCs are not the same as a "stress" hormone or labelling hormones with only one function. As hormones are naturally pleiotropic, the view of any one hormone being X is overly simplistic.

We fully agree, but stress that we focus on how GCs are regulated, which may be less complex than its pleiotropic functions. Indeed, we consider that the many functions of GCs have potentially clouded the question as to how GCs are regulated.

Reviewer #2 (Public Review):

Where this study is interesting is that the authors do a meta-analysis of studies in which metabolic rate was experimentally manipulated and both this rate and glucocorticoid levels were simultaneously measured. Unsurprisingly, there are relatively few such studies and many are from the lab of Michael Romero. While the results of the analysis are compelling, they are not surprising. That said, this work is important.

It is worth noting that in this analysis, the majority of the studies, if not all, are dealing with variation in baseline levels of glucocorticoids. That means the hormone is mostly acting metabolically at these lower levels and not as a stress response hormone as it does when levels are much higher. This difference is probably due to differences in receptors being activated. This could be discussed.

As mentioned in Box 1, within our hypothesis framework we make no distinction between baseline and stress-induced GC-levels, and thereby in effect assume these to be points in a continuum from a metabolic perspective. Our results support this view, as our sample includes baseline- and stressinduced –range GC values, and these are not distinguishable (Fig. 3). We do however recognize that we did not return to this issue in the Discussion, while the same issue may well occur to many readers familiar with the literature. We therefore added the following paragraph to the discussion:

“ Note that in the context of our analysis we made no distinction between ‘baseline’ and ‘stressinduced GC-levels (Box 1). Firstly, because these concepts are not operationally well defined – baseline GC-levels are usually no better defined than ‘not stress-induced’. Secondly, when considering the facilitation of metabolic rate as primary driver of GC regulation, there does not appear a need to invoke different classes of GC-levels instead of the more parsimonious treatment as continuum. This is not to say that this also applies to the functional consequences of GC-level variation: it is well known that receptor types differ in sensitivity to GCs (Landys et al. 2006; Sapolsky et al. 2000; Romero 2004), thereby potentially generating step functions in the response to an increase in GC-levels.”

We note further that to our best knowledge there are no standard or established thresholds that allow us to separate GC levels into “baseline” and “stress-induced”, and in any case these concentration ranges differ strongly among species and experimental set-ups (e.g. captive vs. free-living individuals). Consequently, many of the studies included in our work report what would typically be interpreted as “stress-induced” levels, and thus within the range of those reported by standardized stress protocols (e.g. levels above 20-30 ng/ml for corticosterone in

bird species, Cohen et al. 2007, Jimeno et al. 2018; levels between 150-300 ng/ml in captive rats, Buwalda et al. 2012, Beerling et al. 2011; levels 2-10 times above baseline in humans, Sramek et al. 1999). We also want to note that we work with effect sizes, i.e. not GC levels, and that GC measurement units differ among studies. Mean GC values by study in the original units are shown in Table S3.

Reviewer #1 (Recommendations For The Authors):

L26: why is the causality in this direction? Not that I don't think that metabolic rate drives GC variation but the meta-analyses here could suggest the opposite direction as well? That GC phenotype could limit or promote metabolic activity? (In terms of the natural variation studies and not the experimental ones)

See our detailed response above, on the directionality of the association and the hypotheses underlying our searching criteria and the paragraph on this topic added to the discussion.

L27: again, I am not sure the meta-analyses can lead to this question. Although there is a tight link between GC and metabolic rate, there is still variation around that is unexplained.

See our detailed response above, on the directionality of the association and the hypotheses underlying our searching criteria and the paragraph on this topic added to the discussion.

L45: I think there is plenty of literature in the field that would say that GCs are linked to metabolism and don't define GCs as synonymous with stress. See MacDougall and others that you cite later in the paragraph: "GCs and stress are not synonymous." I think maybe shifting the strong language at the beginning might help with your argument later on.

We do not disagree, but two considerations made us retain the 'strong language'. Firstly, while many authors mention links between GCs and metabolic rate, as we read the literature, the quantitative importance of this link to understand GC variation is underestimated in our view. Secondly, the literature is rife with articles that clearly do not consider metabolic rate variation as a driver of the GC variation they observe.

Box 1: on the diagram the link between GCs and learning is problematic as there are plenty of studies that show a negative effect on learning with GC exposure. It usually depends on the time course of GCs and learning outcomes.

We agree with the referee's point. Learning was deleted from the diagram to avoid confusion.

The diagram also suggests that GCs in the blood decreases insulin. For Aves that are rather insulin insensitive, the evidence that GCs affect insulin concentrations are very limited, even in the poultry literature.

Indeed, and we now mention in box 1 that GC effects on insulin are primarily found in mammals, and less so in birds.

Box 1 at the end also makes a point about GCs having complex downstream effects at baseline and stress-induced levels, besides energy mobilization but the abstract seems to indicate that there are limited effects of GCs outside of metabolism. Hence why I also advocate being careful about the wording in the abstract.

The related abstract sentence has been rewritten to avoid this inconsistency (lines 17-18)

| L107: "being or not significant" meaning significant or not? The wording is awkward

We reworded the sentence for clarity. We included studies reporting both significant and nonsignificant increases in metabolic rate.

| L110: why not look at whether experimental increases in GCs also induce increases in metabolic rate, i.e., the directionality of the two variables. (point 2)

See our detailed response above, on the directionality of the association and the hypotheses underlying our searching criteria and the paragraph on this topic added to the discussion.

| The studies, although there are ~30, are overlapping in terms of labs, i.e., a lot of them came from the same lab. Did you think to include lab as a random effect to see if there are effects of one or two labs doing work that strengthened the results?

We think this is a good point, and we ran the main models included in the manuscript including Lab as random effect (N= 35 experiments, 21 studies, 16 labs). Including Lab as random factor did not affect the results, leading to negligible changes in the model parameters. We provide tables with the model results in our previous response. In the text we now mention that we tested the effect of Lab but did not keep this variable in the models (lines 183-185)

| L314: I think it depends on the time course and intensity of the stressor. I firmly believe that outside of metabolic demands, high levels of GCs chronically or the inability to mount a proper stress response is indicative of pathology or something outside of metabolism.

Whether the association between GCs and MR holds under a context of 'chronic stress' (i.e. understood as chronically elevated GCs) remains to be tested. We note, however, that chronically high levels of metabolic rate may potentially have pathological effects.

Reviewer #2 (Recommendations For The Authors):

| I find the title a bit misleading. The conclusion from the study is that glucocorticoid levels can reflect metabolic rate, not that glucocorticoid levels do not indicate stress. Remember, stress can certainly affect metabolic rate.

We see the point but note that other drivers of variation in metabolic rate also increase GCs, as we show in our analysis, and hence we propose that GC variation always indicate variation metabolic rate, and only stress when stress is the cause of the increase in metabolic rate.