Molecular regulation of urea cycle function by the liver glucocorticoid receptor

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ABSTRACT

Objective: One of the major side effects of glucocorticoid (GC) treatment is lean tissue wasting, indicating a prominent role in systemic amino acid metabolism. In order to uncover a novel aspect of GCs and their intracellular-receptor, the glucocorticoid receptor (GR), on metabolic control, we conducted amino acid and acylcarnitine profiling in human and mouse models of GC/GR gain- and loss-of-function.

Methods: Blood serum and tissue metabolite levels were determined in Human Addison’s disease (AD) patients as well as in mouse models of systemic and liver-specific GR loss-of-function (AAV-miR-GR) with or without dexamethasone (DEX) treatments. Body composition and neuromuscular and metabolic function tests were conducted \textit{in vivo} and \textit{ex vivo}, the latter using precision cut liver slices.

Results: A serum metabolite signature of impaired urea cycle function (i.e. higher [ARG]:[ORN + CIT]) was observed in human (CTRL: 0.45 ± 0.03, AD: 1.29 ± 0.04; p < 0.001) and mouse (AAV-miR-NC: 0.97 ± 0.13, AAV-miR-GR: 2.20 ± 0.19; p < 0.001) GC/GR loss-of-function, with similar patterns also observed in liver. Serum urea levels were consistently affected by GC/GR gain- (~−32%) and loss (−−33%) -of-function. Combined liver-specific GR loss-of-function with DEX treatment revealed a tissue-autonomous role for the GR to coordinate an upregulation of liver urea production rate \textit{in vivo} and \textit{ex vivo}, and prevent hyperammonaemia and associated neuromuscular dysfunction \textit{in vivo}. Liver mRNA expression profiling and GR-cistrome mining identified Arginase I (ARG1) a urea cycle gene targeted by the liver GR.

Conclusions: The liver GR controls systemic and liver urea cycle function by transcriptional regulation of ARG1 expression.

Keywords: Amino acid; Protein; Metabolism; Stress

1. INTRODUCTION

Glucocorticoid (GC) treatment remains an effective, and thus often prescribed, immunosuppressive therapy [1]. However, there are several negative side effects of GC treatment such as lean tissue wasting, osteoporosis, impaired wound healing, increased rates of infection and heightened markers of the metabolic syndrome such as hyperglycaemia and hypertriglyceridaemia [2–4]. Thus, there is a need for either targeted glucocorticoid therapies or identification of additional therapeutic agents/strategies limiting the unwanted side-effects of chronic GC treatment. Nonetheless, there is first a need to gain further insight into the biology of GC side effects in order to better devise such strategies.

While endogenous glucocorticoids signal through both the steroid-hormone receptors, namely the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) [2], a commonly prescribed glucocorticoid (aka Dexamethasone) is a potent GR agonist \textit{in vivo}, with much greater affinity for the GR versus MR [5]. In former studies, we and others have identified tissue-specific functions of the GR in controlling glucose [6–8], lipid [9,10], protein/amino acid [11,12], bile acid [13] and bone [14] metabolism as well as systemic energy homeostasis [13,15–17]. However, whether the liver GR contributes to other aspects of systemic GC action remains enigmatic. To reveal a novel aspect of liver GR function, we conducted amino acid and acylcarnitine profiling studies and uncovered an amino acid profile signature suggestive of altered urea cycle function. Ultimately, we demonstrated a novel role of the liver GR to activate the expression of the key urea cycle enzyme Arginase I, ultimately impacting endogenous amino-N disposal during DEX treatment, thereby affecting hyperammonaemia and associated neuromuscular dysfunction.

2. MATERIALS AND METHODS

2.1. Human experiments

Serum samples were obtained from patients with Addison’s disease after acute withdrawal from treatment and from carefully matched
control subjects, as outlined in detail [18]. In particular, the subset of samples analyzed were taken under fasting or 2 h after ingestion of an high-energy meal. Samples were stored at −80 °C until analysis and freeze-thawing was minimal. Human liver slice studies were conducted as described previously [13].

2.2. Mouse experiments
In all cases, mice were acclimated to the housing facility (12–12 h light–dark cycle, 22–24 °C) for at least one week prior to experimentation. Animal experiments were conducted according to local, national, and EU ethical guidelines and approved by local regulatory authorities. To examine the effects of long-term liver GR knockdown, C57 L/J male mice were administered 5 × 10^{11} viral genomes of non-speciﬁc negative control (NC miRNA) or GR-speciﬁc (GR miRNA) miRNA bearing adeno-associated virus as described in detail [13]. To examine the importance of GR dimerisation, 7–10 wk old homozygous glucocorticoid receptor dimerisation deficient (GR^{knockout}/GR^{knockout}) [19] and wild-type littermate mice were treated daily with 9x-Fluoro-16x-methylprednisolone (Dexamethasone, DEX; 1 mg/kg, D8893 Sigma—Aldrich, DEU) or Vehicle (VEH, 2% ethanol in isotonic saline) for 14 d [13]. Furthermore, 8 wk old C57 L/J male mice were also chronically treated with DEX as described [13].

To examine the effects of genetic hepatocyte-speciﬁc knockout of GR in mice GR ﬂoxed/ﬂoxed mice [20] were used. In particular, 7–9 wk old GR ﬂoxed/ﬂoxed male mice were administered 1.10^{11} ifu of control empty adeno-virus (Ad-NC) or an adeno-virus to transduce the expression of Cre-recombinase (Ad-CRE). Following 2 wk, mice were then administered DEX or VEH as above for 14 d. At d7, an intraperitoneal alanine tolerance test (ipATT) was conducted. At d10, the rotarod test was conducted and at d13 grip strength testing and body composition (ECHO-MRI; Echo Medical Systems, USA) were assessed. On d14, mice were sacriﬁced in the ad libitum fed state and blood and tissues were harvested.

2.3. In vivo experiments
To assess the capacity of the liver for urea production in vivo, we conducted an alanine tolerance test essentially as performed previously [21]. To this end, mice were fasted overnight and, after a basal blood sample was drawn, intraperitoneally injected with 1 mg/kg alanine solution in 0.9% saline. Additional blood samples were drawn at 1, 2 and 4 h after injection, and blood serum was subsequently analysed for urea concentration. Urea area under the curve was then calculated from 0 h.

Neuromuscular function was assessed using a grip strength tester (BIO-GS3, BIOSEB, Vitrolles Cedex, France) with the average of two measurements taken. The constant-speed rotarod assay to measure forelimb and hindlimb motor coordination and balance was performed using the rotarod apparatus (Ugo Basile, Biological Research Apparatus, Varese, Italy) as described [22]. After a training period, mice then received several trials at 35 rpm rotation speed with 5-min intervals between individual trials, and the mean latency to fall off the rotarod (for the two trials at each speed level) was recorded and used in subsequent analyses.

2.4. Ex vivo liver studies
These experiments were carried out on precision-cut liver slices as previously described [23]. In particular, liver slices were taken and prepared in UW solution (in absence of Insulin and Dexamethasone) and then pre-incubated in Williams media E containing 50 μg/mL gentamycin, 5% dialyzed FBS, 11 mM d-glucose, 0.3 mM pyruvate, 0.1 μM methyl-linoleate, and an amino acid mixture resembling the hepatic portal vein concentrations [24]. After 1 h, media was switched to that containing either vehicle (0.4% DMSO + 0.01% EtOH), dexamethasone (1 μM in 0.4% DMSO + 0.01% EtOH), RU486 (10 μM in 0.01% EtOH + 0.4% DMSO) and dexamethasone + RU486 (1 & 10 μM respectively in 0.4% DMSO + 0.01% EtOH) and incubated for 18 h after which media and liver samples were taken and snap frozen in LN2. To examine the role of Arginase I (ARG1) activity on DEX modulated urea cycle function, liver slices were prepared and treated with DEX as above with and without two structurally dissimilar ARG1 inhibitors, N-O-dimethylor-nor-l-arginine (L-NOHA; ALX-270-467, Enzo Life Sciences, DEU) and S-(2-Boronoethyl)-l-cysteine (BOC; ALX-270-345, Enzo Life Sciences, DEU), at concentrations (i.e. 100 μM) known to affect cellular urea cycle function [25]. Importantly, for all studies, media without liver sample was also incubated to serve as a metabolite assay control. Metabolite balance rates were calculated as media concentrations from liver slice incubations minus those from media without slice incubations, divided by the time of incubation (i.e. (slice media (M) − control media (M))/time (h)). Importantly, liver slice weights (4.2 ± 0.1 mg) did not differ between any of the conditions studied.

2.5. Recombinant viruses
Adenoviruses were produced according to the Life Technologies manual and titered via the serial dilution plaque-formation unit titration assay (Virapower Adenoviral Expression System). Adenoviruses were puriﬁed by the caesium chloride method and dialyzed against phosphate-buffered-saline containing 10% glycerol prior to animal injection.

For long-term inactivation of GR, recombinant adeno-associated viruses (AAV) encoding GR-speciﬁc miRNA were established and produced as outlined in detail by Rose et al. [13]. Importantly, using an hepatocyte-speciﬁc promoter [26] this method allows for hepatocyte restricted transduction/expression [13].

2.6. Metabolite measurements
Blood glucose levels were determined using an automatic glucose monitor (One Touch, Lifescan). In addition, commercially available kits were used to measure serum urea (Z5030016, Biochain, USA) and ammonia (K370-100, Biovision, USA) according to manufacturer’s instructions. All samples were loaded in order to ﬁt within the assay range of the reagents supplied. Amino acids and acyl-carnitines were determined in serum by electrospray ionization tandem mass spectrometry (ESI-MS/MS) according to a modiﬁed method as previously described [27], using a Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ion source and a Micromass Mass Lynx data system. For liver determinations, frozen pulverised liver was carefully weighed and then extracted in 10 volumes of ice cold PBS. Values obtained were then calculated to give μmol/g wet liver tissue weight.

2.7. RNA extraction and analysis
Total RNA was extracted from homogenized mouse liver or cell lysates using Qiazol reagent (Qiagen, Hilden, DEU). cDNA was prepared by reverse transcription using the M-MuLV enzyme and Oligo dT primer (Fermentas, St. Leon-Rot, DEU). cDNAs were ampliﬁed using assay-on-demand kits and an ABIPRISM 7700 Sequence detector (Applied Biosystems, Darmstadt, DEU). RNA expression data was normalized to levels of TATA-box binding protein (TBP) RNA. Particular product numbers for probe sets can be provided upon request.
2.8. Tissue protein extraction and analysis

For simple expression analyses from homogenates, protein was extracted from ~50 mg frozen liver powder in tissue lysis buffer [28]. To determine protein expression, total protein concentration was analyzed by BCA assay (23227, Fischer Scientific, DEU) and equal amounts of protein were loaded onto an SDS-polyacrylamide gel, subjected to electrophoresis and blotted onto nitrocellulose membrane. Western blot assays were performed using standard techniques with antibodies specific for the glucocorticoid receptor (GR; sc-1004, Santa Cruz Biotech., Heidelberg, DEU), Argenase I (ARG1; sc-20150, Santa Cruz Biotech., Heidelberg, DEU) or valosin-containing protein (Vcp; ab11433, Abcam, Cambridge, UK). For expression analyses, all western blots signals were within a linear range, with respect to protein loaded and antibodies used, as determined by preliminary experiments, and detected bands ran at the expected relative mobility (data not shown).

2.9. Liver GR DNA binding in vivo

Publically available sequencing data [29] were analyzed in silico in order to ascertain DEX-dependent GR-DNA binding regions. Accessible NCBI Gene Expression Omnibus GSE files for GR & CEBPB Chip-Seqs and DNAse Hypersensitivity were downloaded and uploaded to Ensembl Mouse Genome Browser NCBI m37. The browser was then configured to display Arginase 1 gene and promoter regions. In addition, accessible NCBI Gene Expression Omnibus GSE files for GR Chip-Seq were downloaded and mapped to the mouse mm10 genome by using Bowtie [30]. Subsequently, the mapped reads were analyzed with MACS2 software [31] to identify GR chips peaks. The input files were used as control. The wiggle files of GR chip and input control were uploaded to Integrative Genomics Viewer (IGV 2.3; [32]) to display Arginase 1 gene and promoter regions.

2.10. Statistical analysis

Statistical analyses were performed using t-tests, or 2-way analysis of variance (ANOVA) with or without repeated measures, where appropriate, with Holm–Sidak-adjusted post-tests. All analyses were carried out with SigmaPlot v.12 software (Systat Software GmbH, DEU).

3. RESULTS

Amino acid profiling reveals altered urea cycle function by the HPA-liver GR axis in human and mouse. To examine whether dysfunctional HPA axis and thus whole body glucocorticoid action affects novel aspects of metabolic function, we conducted amino acid/acylcarnitine profiling in Addison’s patients versus aged-matched controls. In the fasted state, analysis of the serum from Addison’s patients revealed higher amounts of tyrosine (TYR) and arginine (ARG), lower amounts of ornithine (ORN) and citrulline (CIT), and no change in any other amino acid (Figure 1A and Figure S1A) or acylcarnitine species (Figure S1B). In particular, there was a heightened ratio of serum [ARG]/[ORN] and [CIT] in Addison’s patients (1.29 ± 0.04 versus controls (0.45 ± 0.03; p < 0.001). Given that ARG, ORN and CIT are key metabolites within the urea cycle [33], we then examined serum urea, and we observed lower serum urea in Addison’s patients. Importantly, this pattern was observed whether we examined serum urea from individuals with Addison’s disease following acute treatment withdrawal and from matched healthy control individuals. Data are mean ± SEM; n = 9 per group. Difference between groups: *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 1: Serum amino acid profiling reveals a pattern of disturbed urea cycle function in Addison’s patients. Amino acid (A) arginine (ARG), ornithine (ORN) and citrulline (CIT) as well as urea (B) levels were measured from serum samples collected from individuals with Addison’s disease following acute treatment withdrawal and from matched healthy control individuals. Data are mean ± SEM; n = 9 per group. Difference between groups: *p < 0.05, **p < 0.01, ***p < 0.001.
(Figure S3C), but also dramatically reduced DEX-induced in vivo urea production rate, as judged by an alanine tolerance test (Figure 3D–E). Importantly, serum (Figure S3F) and liver (Figure S3G) amino acid profiling revealed that while DEX promoted lower ARG and higher ORN and CIT levels, these effects were blunted by liver-specific GR knockdown, highlighting a key role for the liver in mediating these changes. We then examined the potential functional consequences of GR loss-of-function in this study, and could demonstrate that the liver GR does not affect the reduction of body mass that typically occurs with DEX treatment in vivo (Figure 4A–B). In particular, ECHO-MRI revealed that DEX reduced lean mass (Figure 4C) but not fat mass (Figure S4A), with lower skeletal muscle (Figure S4B) and liver (Figure S4C) mass upon DEX treatment. We hypothesised that if the urea cycle regulation were dysfunctional, this would lead to hyperammonaemia upon DEX treatment, and, indeed, this was the case (Figure 4D). Given that hyperammonaemia typically results in a manifestation of neuromuscular dysfunction [34], we tested this possibility and could demonstrate that both forelimb grip strength (Figure 4E) as well as balance control (Figure 4F) were negatively affected by DEX treatment in liver GR knockout mice. Taken together, these data demonstrate that the liver GR coordinated effective amino acid disposal via tissue-autonomous upregulation of urea cycle function upon DEX-induced lean tissue wasting.

Liver Arginase I is a GR-target gene. Conceivably, the GR can confer function by either genomic or non-genomic mechanisms. However, since we could observe GR dimerization dependent effects of DEX treatment (Figure 2B), we hypothesised that GR-conferred function occurs most likely via transcriptional events, as GR dimerization-deficient mutants impair transcriptional effectiveness of the GR [19], particularly through canonical palindromic GR DNA binding motifs [35]. Hence, as there are only several key gene transcripts within the liver urea cycle [33], we performed a focused transcript analysis. In particular, the transcripts Cps1, Otc, Slc25a15, Ass1 and Asl were not affected by liver/hepatocyte GR knockdown, but liver Arg1 mRNA expression was <50% lower (Figure 5A). Importantly, we could confirm this result at the level of ARG1 protein expression (Figure 5B). Furthermore, although DEX induced liver Arg1 expression in vivo (Figure 5C) and ex vivo (Figure 5D–E), these effects were blocked by GR loss-of-function. A known [36] GR target gene Tyrosine amino-transferase (Tat) was also assessed, which confirmed the
The upregulation of ARG1 by the liver GR is likely of functional importance, as inhibition of ARG1 by two distinct inhibitors largely blunted the upregulation of liver urea production rate \textit{ex vivo} (Figure 5F). Lastly, to understand how the GR might regulate the \textit{Arg1} gene transcription, we examined pre-existing liver GR ChIP-seq data [29]. Importantly, we could demonstrate that DEX treatment \textit{in vivo} upregulated GR binding to a region within intron 1 of the \textit{Arg1} gene (Figure 5G), which contains a
consensus glucocorticoid regulatory element (data not shown) corresponding to DNAase hypersensitivity sites (DHS) and CCAAT/enhancer-binding protein β (CEBPβ) binding sites (Figure S5B). Of note, even though there were DHSs and CEBPβ binding sites farther upstream (Figure S5B), there were no consistent GR binding sites regulated by DEX in these regions (not shown).

4. DISCUSSION

In addition to our previously described effects on glucose [8], lipid [9,10], and bile acid [13] metabolism, here we demonstrate that the liver glucocorticoid receptor (GR) coordinates proper urea cycle function in mouse and human. In particular, the liver GR upregulates the expression of Arginase I (ARG1) during dexamethasone (DEX) treatment, aiding in the disposal of excess amino acid N yielded from lean tissue wasting and preventing accumulation of ammonia and associated pathophysiological side effects.

Although the increase in systemic urea cycle function by GR-agonism by DEX has been demonstrated previously in rodents [37,38] and humans [39], and although systemic pharmacological GR blockade blunts trauma-enhanced urea cycle capacity [40], the precise site(s) of control had not been identified. Moreover, it is feasible that the simple increase in hepatic amino acid delivery driven by amino acid release during GC-induced lean tissue wasting, which is mediated by the muscle GR [11,12], could potentially promote ureagenesis by substrate-driven flux [33]; i.e. increased liver amino-N supply. However, others have surmised that there is a specific control site in the liver [41], and in accordance with studies of perfused rodent liver [42], we demonstrated a tissue-specific role of the GR by both ex vivo liver studies as well as liver/hepatocyte specific GR loss-of-function studies in vivo. Furthermore, while others have hypothesised that the DEX driven activation of liver urea cycle function could control the effects on lean tissue wasting [38], we showed that these phenomena are actually disconnected. Of note, we also demonstrated that the loss of GR within the adult animal mediated this effect, thereby negating the argument that the effects that we observe could relate to altered liver-development by the GR, which could occur in conventional knockout mice or knockout mice driven by a promoter that drives expression during liver development.

Figure 4: Hyperammonaemia and dysfunctional neuromuscular function coincide with improper regulation of liver urea cycle function with liver GR loss in dexamethasone treatment. End-point (A) and delta (B) body mass and lean mass (G) in GR-floxed mice pre-treated with adenoviral constructs expressing (Ad-CRE) or not (Ad-NC) Cre-recombinase with (Dex) or without (Veh) chronic dexamethasone treatment (N = 6–7/group). Furthermore, serum ammonia (D) was measured and tests of neuromuscular function such as forelimb grip strength (E) and latency to fall during a rotarod test (F) were conducted. Data are mean ± SEM. Effect of Dex: *p < 0.05, **p < 0.01, ***p < 0.001. Effect of Ad-CRE: #p < 0.05, ##p < 0.01, ###p < 0.001.
Here we show that the main mechanism of action likely relates to the upregulation of a key transcript encoding a protein within the hepatic urea cycle, namely Arginase I. Indeed, the metabolic signature in the serum and liver that we observe with GR loss-of-function, i.e. higher arginine and lower ornithine/urea, are direct substrates and products of the ARG1 reaction[^33], fitting that this could be a key rate limiting event in vivo. In accordance, ARG1 inhibition largely blunted the DEX-induced upregulation of urea production rate by liver ex vivo. This is important, as it is thought that CPS1 and ASS1 urea cycle reactions are rate limiting under normal conditions[^33]. Nevertheless, it could be that under the particular condition of activation of the stress-response, and hence heightened HPA axis activity, the rise in GC-GR action and consequently whole body protein turnover and AA flux could shift the site of control to another step, in this case ARG1. In any case, that we observe increased serum arginine as well as accumulation of serum ammonia and consequent neuromuscular dysfunction, with the lack of upregulation of liver ARG1 by GR loss, is in accordance with other studies of ARG1 knockout mice[^43-45].

Regarding the molecular mechanism by which DEX/GR could upregulate liver urea cycle function, others have demonstrated that several of the urea cycle enzymes are indeed upregulated by GR-agonism in vivo[^38] and in vitro[^46]. Regarding ARG1, earlier studies using hepatoma cells have demonstrated a requirement for ongoing protein synthesis for the upregulation of Arg1 mRNA and have proposed that there is a two-step mechanism. The first step requires the synthesis of the transcription factor CCAAT/enhancer-binding protein b (CEBPb), which then binds to an enhancer region downstream of the promoter between exon 7 and 8[^47]. Indeed, CEBPb is required for DEX-induction of mouse hepatocytes in vitro, but not in vivo, suggesting a rather complicated mechanism[^48]. Regardless, we could demonstrate GR-dependent DEX-induction of Arg1 mRNA in mouse liver slices treated with dexamethasone, with and without ARG1 inhibitors N-hydroxy-nor-l-arginine (L-NOHA; 100 μM) and S-(2-Boronoethyl)-l-cysteine (BOC; 100 μM) ex vivo (N = 6-9/group). Glucocorticoid receptor (GR) ChIP-seq peaks around the Arg1 gene of liver of mice treated with (DEX) and without (VEH). Data are mean ± SEM. Effect of Dex: *p < 0.05, **p < 0.01, ***p < 0.001. Effect of knockdown/inhibitor: #p < 0.05, ##p < 0.01, ###p < 0.001.

Figure 5: Arginase I is a liver glucocorticoid receptor target gene coordinating proper regulation of urea cycle function upon glucocorticoid exposure. The liver mRNA expression (A) levels of carbamoyl-phosphate synthase 1 (Cps1), ornithine transcarbamylase (Otc), mitochondrial ornithine transporter (Slc25a15), arginosuccinate synthase 1 (Ass1), argininosuccinate lyase (Asl), and arginase 1 (Arg1) in mice pre-treated with adeno-associated virus to express a negative control miR (AAV-miR-NC) or GR-specific miRNA (AAV-miR-GR); n = 9/group. B: Liver arginase 1 protein (ARG1) expression in the same mice as in A. C: Liver ARG1 expression in the same mice as in Figures 3D-6 & 4 (N = 6-9/group). D: Liver Arg1 mRNA in mouse liver slices treated with dexamethasone, RU486 and corresponding vehicle treatments ex vivo (N = 4/group). E: Liver ARG1 mRNA in human liver slices treated as in D. Urea production rate of mouse liver slices treated with dexamethasone, with and without ARG1 inhibitors N-hydroxy-nor-l-arginine (L-NOHA; 100 μM) and S-(2-Boronoethyl)-l-cysteine (BOC; 100 μM) ex vivo (N = 4/group). F: Glucocorticoid receptor (GR) ChIP-seq peaks around the Arg1 gene of liver of mice treated with (DEX) and without (VEH). Data are mean ± SEM. Effect of Dex: *p < 0.05, **p < 0.01, ***p < 0.001. Effect of knockdown/inhibitor: #p < 0.05, ##p < 0.01, ###p < 0.001.

[^43] and [^45]: Taken
together, these data suggest that the activated GR directly binds the Arg1 gene, which perhaps together with CEBPβ [29] and steroid receptor coactivator 1 (SRC1) [51] is sufficient to enhance transcription rate, but do not rule out the possibility that other factors could be involved in vivo. Indeed, glucagon and dexamethasone have synergistic actions on Arg1 transcription [46], and glucocorticoids can increase glucagon levels either via direct [52] or indirect [53] mechanisms.

In addition to effects on the urea cycle metabolites, we consistently observed that the amino acid tyrosine and liver tyrosine aminotransferase (TAT) mRNA expression were affected by DEX and GR loss-of-function in vivo and ex vivo. Although TAT was already identified as a liver GR target gene [36], perhaps together with SRC1 [51] as well as other as-yet unidentified elements, we now demonstrate that transcriptional control through the liver GR modulates in vivo metabolic function of liver TAT, of which likely has physiological consequences in vivo [51].

Aside from chronic GC treatment, our results could extend to other conditions of chronic HPA-axis activation such as trauma/surgery, cachexia, type I diabetes, and perhaps obesity-driven type II diabetes [2,3]. Given that major mechanism contributing to diabetes is aberrant hepatic glucose production [54] and amino acids are potential gluconeogenic substrates [55], an enhanced amino acid turnover and urea cycle function may also contribute to impaired glucose control driven by high levels of GC. Indeed, here we observed a correlation between these two processes, and thus this a clear direction for further investigations. In summary, the liver GR controls urea cycle function, at least in part via transcriptional activation of the Arg1 gene, and thus contributes to amino acid homeostasis under conditions of glucocorticoid excess.

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SC, JS, AZ, OS, AM & AJR performed experiments and analysed/prepared samples. KVS & JGO conducted amino acid and acylcarnitine profiling of samples and edited the manuscript. JK & AP conducted the Addison’s patient experiments and edited the manuscript. XW and RdG conducted in silico analyses from publicly available datasets. SH provided infrastructure and funds and edited the manuscript. AJR designed and directed the research, analysed the data and wrote the manuscript.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2015.07.006.

REFERENCES

[1] Overman, R.A., Yeh, J.Y., Deal, C.L., 2013. Prevalence of oral glucocorticoid usage in the United States: a general population perspective. Arthritis Care & Research (Hoboken) 65(2):294–298.
[2] Rose, A.J., Vegiopoulos, A., Herzig, S., 2010. Role of glucocorticoids and the glucocorticoid receptor in metabolism: insights from genetic manipulations. The Journal of Steroid Biochemistry and Molecular Biology 122(1–3):10–20.
[3] Kleiman, A., Tuckermann, J.P., 2007. Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice. Molecular Cell Endocrinology 2751(1–2):98–108.
[4] Magomedova, L., Cummins, C.L., 2015. Glucocorticoids and metabolic control. Handbook of Experimental Pharmacology.
[5] Schimmer, B.P., Parker, K.L., 1996. Adrenocorticotropic hormone; adrenocorticoids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocorticosteroids. In: Hardman, J.G., Limbird, L.E. (Eds.), The pharmacological basis of therapeutics, 9th ed. New York: McGraw-Hill. p. 1459–85.
[6] Oghrer, C., Tronche, F., Kellendonk, C., Kohlmueller, D., Schulze, A., Schmid, W., et al., 2004. Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hypoglycaemia and ameliorates hyperglycaemia in streptozotocin-induced diabetes mellitus. Molecular Endocrinology 18(6):1346–1353.
[7] Reichardt, S.D., Foller, M., Rexhepaj, R., Pathare, G., Minnich, K., Tuckermann, J.P., et al., 2012. Glucocorticoids enhance intestinal glucose uptake via the dimerized glucocorticoid receptor in enterocytes. Endocrinology 153(4):1783–1794.
[8] Herzig, S., Long, F., Jhala, U.S., Hedrick, S., Quinm, R., Bauer, A., et al., 2001. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413(6852):179–183.
[9] de Guia, R.M., Rose, A.J., Sommerfeld, A., Seibert, O., Strzoda, D., Zota, A., et al., 2015. microRNA-379 couples glucocorticoid hormones to dysfunctional lipid homeostasis. The EMBO Journal 34(3):344–360.
[10] Lemptke, U., Krones-Herzig, A., Berriel Diaz, M., Narvekar, P., Ziegler, A., Vegiopoulos, A., et al., 2008. The glucocorticoid receptor controls hepatic lipid homeostasis during Hes1. Cell Metabolism 8(3):212–223.
[11] Watson, M.L., Baehr, L.M., Reichardt, H.M., Tuckermann, J.P., Bodine, S.C., Furlow, J.D., 2012. A cell-autonomous role for the glucocorticoid receptor in skeletal muscle atrophy induced by systemic glucocorticoid exposure. American Journal of Physiology. Endocrinology and Metabolism 302(10):E1210–E1220.
[12] Hu, Z., Wang, H., Lee, I.H., Du, J., Mitch, W.E., 2009. Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. The Journal of Clinical Investigation 119(10):3059–3069.
[13] Rose, A.J., Berriel Diaz, M., Reimann, A., Klement, J., Walcher, T., Krones-Herzig, A., et al., 2011. Molecular control of systemic bile acid homeostasis by the liver glucocorticoid receptor. Cell Metabolism 14(1):123–130.
[14] Rauch, A., Seitz, S., Baschant, U., Schilling, A.F., Illing, A., Stride, B., et al., 2010. Glucocorticoids suppress bone formation by attenuating osteoblast differentiation through the monomeric glucocorticoid receptor. Cell Metabolism 11(6):517–531.
[15] de Kloet, A.D., Krause, E.G., Solomon, M.B., Flak, J.N., Scott, K.A., Kim, D.H., et al., 2015. Adipocyte glucocorticoid receptors mediate fat-to-brain signaling. Psychoneuroendocrinology 56:110–119.
[16] Shimizu, N., Maruyama, T., Yoshikawa, N., Matsumiya, R., Ma, Y., Ito, N., et al., 2015. A muscle-liver-fat axis signalling axis is essential for central control of adaptive adipose remodelling. Nature Communications 6:6693.
[17] Kellendonk, C., Eiden, S., Kretz, O., Schuiz, G., Schmidt, I., Tronche, F., et al., 2002. Inactivation of the GR in the nervous system affects energy accumulation. Endocrinology 143(8):3333–3340.
[18] Klement, J., Hubold, C., Cords, H., Oltmanns, K.M., Hallenschmidt, M., Born, J., et al., 2010. High-calorie glucose-rich food attenuates neuroglycopenic symptoms in patients with Addison’s disease. The Journal of Clinical Endocrinology and Metabolism 95(2):522–528.
[19] Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., et al., 1998. DNA binding of the glucocorticoid receptor is not essential for survival. Cell 93(4):531–541.
[20] Tronche, F., Oghrer, C., Moniggl, R., Kellendonk, C., Reimann, A., Schwake, L., et al., 2004. Glucocorticoid receptor function in hepatocytes is essential to promote postnatal body growth. Genes & Development 18(5):492–497.
