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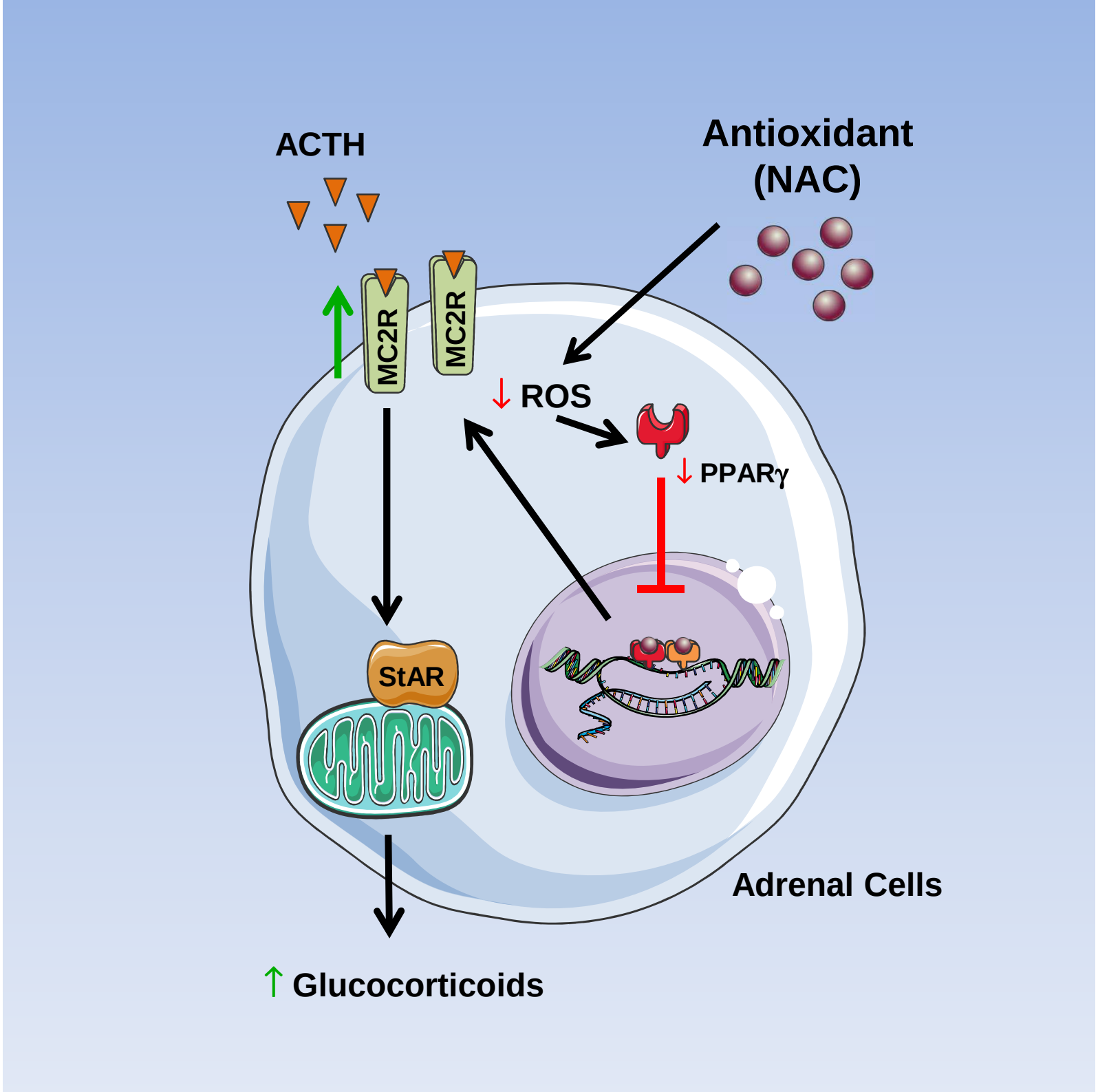
ACTH
Antioxidant
(NAC)
MC2R
MC2R
↓ ROS
↓ PPARγ
StAR
Adrenal Cells
↑ Glucocorticoids

Activation of PPARγ reduces N-acetyl-cysteine -induced hypercorticoidism by down-regulating MC2R expression into adrenal glands

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ABSTRACT

We previously demonstrated that oral supplementation with antioxidants induced hyperactivity of hypothalamus-pituitary-adrenal (HPA axis), attested by hypercorticoidism, through an up-regulation of adrenocorticotrophic hormone (ACTH) receptors (MC2R) in adrenal. This study analyzed the role of peroxisome proliferator-activated receptor (PPAR)-γ on HPA axis hyperactivity induced by N-acetyl-cysteine (NAC). Male Swiss-Webster mice were orally treated with NAC for 1, 3, 5, 10, 15, or 18 consecutive days. The PPAR-γ agonist rosiglitazone and/or antagonist GW9662 were daily-injected i.p. for 5 consecutive days, starting concomitantly with NAC treatment. Rosiglitazone treatment inhibited NAC-induced adrenal hypertrophy and hypercorticoidism. Rosiglitazone also significantly reversed the NAC-induced increase in the MC2R expression in adrenal, but not steroidogenic acute regulatory protein (StAR). NAC treatment reduces the expression of PPARγ in the adrenals, but rosiglitazone did not restore the expression of this cytoprotective gene. In addition, GW9662 blocked the ability of rosiglitazone to decrease plasma corticosterone levels in NAC-treated mice. In conclusion, our findings showed that antioxidant supplementation induced a state of hypercorticoidism through down-regulation of PPARγ expression in the adrenals, in a mechanism probably related to a down-regulation of ACTH receptor expression.

Keywords: Antioxidant; Glucocorticoids; HPA axis; PPAR-γ.

INTRODUCTION

Reactive oxygen species (ROS) are the initial species generated by oxygen reduction, including superoxide, hydroxyl radical, and hydrogen peroxide [1]. Under physiological conditions, endogenous antioxidant enzymes regulate the overproduction of ROS to prevent undesirable functional collateral damage [2]. The excessive production of ROS occurs when there is an imbalance between cellular antioxidant defense systems and the endogenous or exogenous pro-oxidant burden, leading to DNA cleavage, protein oxidation, and lipid peroxidation, ultimately resulting in cellular dysfunction and apoptosis [3,4]. Despite this, existent evidence highlights the beneficial role of superoxide and hydrogen peroxide in the maintenance of cellular redox homeostasis acting as signaling molecules in a physiological process. Accordingly, the release of reduced amounts of oxidants from the mitochondria or other sources can activate a defensive response that appears to protect the organism from subsequent higher stresses[5–7]**.** Thereat, the growth of indiscriminate consume of supplements with antioxidants to combat diseases associated with aging by the general people [8,9] can culminate in cellular stress affecting most diverse systems of the body.

Indeed, we previously showed that prolonged treatment with two different antioxidants, vitamin E and N-acetylcysteine (NAC), induced a rise in the circulating glucocorticoid levels in rats by a mechanism related to increased expression of both adrenocorticotrophic hormone (ACTH) receptor, known as MC2R, and steroidogenic acute regulatory protein (StAR) in the adrenal gland [10]. Peroxisome proliferator-activated receptor (PPAR)γ is an isoform of the PPAR subset of nuclear receptors, which activate the expression of their target genes by binding to peroxisome proliferator response elements (PPREs) [11]. The PPREs are found in genes that control the expression of endogenous antioxidant enzymes, including SOD, catalase, and heme oxygenase-1, and the activation of PPARγ induces a transactivation of these genes [12–14]**.** In addition, we demonstrated that the reduction of PPARγ expression in adrenals is directly associated with the high expression of MC2R in these glands as so as hypercorticoidism noted in animals with diabetes [15]. Thus, NAC-induced hyperactivation of the adrenal cortex could be associated with the interference of

signaling pathways mediated by PPARγ. In this study, we evaluated the contribution of the PPARγ to the NAC-induced hypercorticoidism in healthy mice, using a synthetic PPARγ agonist rosiglitazone.

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Co. (Saint Louis, MO, USA), rosiglitazone and GW9662 from Cayman Chemicals (Saint Louis, MO, USA), ethanol, methanol and xylene from Merck (Rio de Janeiro, Brazil) and sodium heparin from Roche (São Paulo, Brazil). All solutions were freshly prepared immediately before use.

Animals

In accordance with the guidelines of the Committee on Use of Laboratory Animals of Oswaldo Cruz Institute (CEUA-IOC/Fiocruz, license L-027/2016), male Swiss-Webster mice obtained from Oswaldo Cruz Foundation breeding colony were used. Mice were housed in groups of four in temperature-, humidity-, and light-controlled (12 h light: 12 h darkness cycle) colony room. Mice were given access *ad libitum* to food and water.

Treatments

Forty-two male mice were randomly assigned into 6 experimental groups as follows: control mice (n = 7); NAC-treated mice for 1 day (n = 7); NAC-treated mice for 3 days (n = 7); NAC-treated mice for 5 days (n = 7); NAC-treated mice for 10 days (n = 7); NAC-treated mice for 15 days (n = 7); NAC-treated mice for 18 days (n = 7). In another setting of experiments, 28 male mice were randomly divided into 4 experimental groups: control mice (n = 7); rosiglitazone-treated mice (n = 7); NAC-treated mice (n = 7); mice treated with NAC plus rosiglitazone (n = 7). In a third setting of experiments, 30 male mice were randomly assigned into 5 experimental groups as follows: control mice (n = 6); NAC-treated mice (n = 6); mice treated with NAC plus rosiglitazone (n = 6); mice

treated with NAC plus GW9662 (n = 6); mice treated with NAC plus rosiglitazone plus GW9662 (n = 6).

The mice were treated with antioxidant NAC (150 mg/kg body weight) [10] by gavage once a day, during 1, 3, 5, 10, 15, or 18 consecutive days. Control mice received an equal volume of vehicle (sterile saline 0.9%). In some experiments, the mice were treated concomitantly with NAC (150 mg/kg body weight), PPARγ agonist rosiglitazone (0.5 mg/kg, i.p.), and/or PPARγ antagonist GW9662 (0.5 mg/kg, i.p.) [16] daily for 5 consecutive days. Untreated mice received an equal volume of vehicle (DMSO 0.1%, i.p.). All analyzes were performed 24h after last treatment with NAC.

Determination of micro and macroscopic adrenal hypertrophy indexes

Adrenal glands were quickly removed from mice and cleaned of surrounding fat in ice. Instantly after dissection, the adrenal glands were fixed in Milloning fixative solution for 24h and, then, embedded in paraffin. Paraffin-embedded sections of 3 μm were deparaffinized with xylene, rehydrated by a graded series of ethanol washes, and stained with hematoxylin and eosin (H&E). The tissue sections were mounted in aqueous medium and images digitized via scanner microscope (Pannoramic SCAN150, 3D Histech, Budapest, Hungary) using a 20x objective lens. Images obtained from the *zona fasciculata* of the adrenal cortex were analyzed with Image Pro Plus 6.2 software (Media Cybernetics, Rockville, MD, USA) to determine the mean area of cells. We analyzed at least ten different fields from *zona fasciculata* of each adrenal gland section. To assess adrenal gland hypertrophy macroscopically, the ratio between adrenal weight and body weight was determined.

Evaluation of mRNA expression of MC2R, PPARχ, and StAR by real-time PCR

Total RNA was isolated from adrenal glands using TRI Reagent® and reverse-transcribed to cDNA using the RevertAid Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR was performed with the StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using a Mix (5x HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX); Solis BioDyne, Tartu, Estonia) according to the manufacturer's instructions. The amplification program included an initial activation step at 95ºC for 15min, followed by denaturation at 95ºC for 15s,

annealing between 59º-62ºC and finally elongation at 72ºC for 20s, for 40 cycles. Fluorescence was measured after each extension step, and the specificity of amplification was evaluated by melting curve analysis. The house-keeping gene GADPH was used as a control to normalize RNA samples. Relative gene expression levels were calculated using a standard curve for each gene. Amplification efficiencies were identical or similar between genes of interest and controls. Primers (Table 1) and probes were designed in our laboratory and purchased from Eurofins Genomics (Louisville, KY, USA) or Invitrogen (Carlsbad, CA, USA).

Table 1: Primer sequences used for RT-qPCR

Gene	Primer Sequence (5'-3')
GAPDH	Forward: GAAGGTCGGTGTGAACGGAT Reverse: CGTTGAATTTGCCGTGAGTGGA
MC2R	Forward: GACCTTCTGCCCAAATAACCCTT Reverse: CGGTTGCAGAAGAGCATCCTTT
PPARγ	Forward: AGACCACTCGCATTCCTTTGACAT Reverse: TCCCCACAGACTCGGCACTCAATG
StAR	Forward: TCACTTGGCTGCTCAGTATTGAC Reverse: GCGATAGGACCTGGTTGATGA

Corticosterone quantification

Mice were euthanized (ketamine 140 mg/Kg and xylazine 20 mg/Kg i.p.), during the nadir (08:00 h) of the circadian rhythm as described previously [17], and blood was immediately collected from abdominal aorta using heparin (40 U/ml) for corticosterone quantification. Plasma was obtained after sample centrifugation for 10 min at 1,000 × *g* and stored at -20°C until use. Plasma corticosterone levels were quantified by radioimmunoassay or ELISA, following manufacturer's

guidelines (MP Biomedicals, Solon, USA, and Cayman Chemicals, Ann Arbor, MI, USA, respectively).

Statistical analysis

Data are reported as mean ± Standard Error of the Mean (S.E.M.). The data were evaluated to ensure normal distribution and statistically analyzed by one-way ANOVA followed by a Student-Newman–Keuls post-hoc test. In the case of real-time PCR, the data were assessed by non-parametric test Kruskall-Wallis followed by U de Mann Whitney. Probability values (p) of 0.05 or less were considered significant.

RESULTS

NAC increases plasma corticosterone levels in Swiss-Webster mice

We observed that NAC induced an increase in the circulating levels of corticosterone when used for 3, 5, 10, 15, or 18 consecutive days compared to controls, however, a single administration of the antioxidant was unable to significantly alter circulating hormone levels (Figure 1). Based on these data, we chose the scheme of 5-day NAC treatment to evaluate the role of the nuclear receptor PPARγ on antioxidant-induced hypercorticoidism.

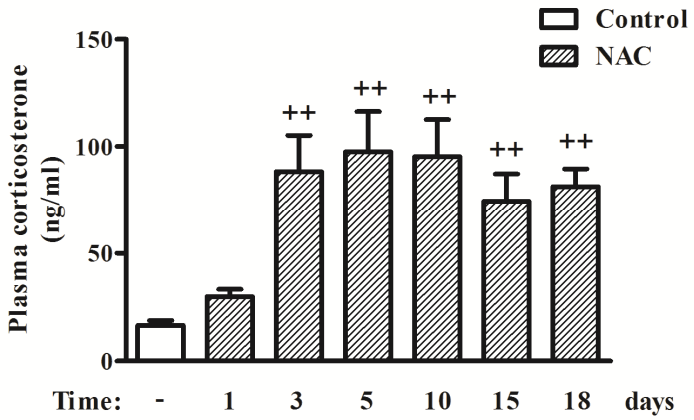

Figure 1: Kinetics of NAC-induced hypercorticoidism in mice. NAC (150 mg/kg, oral route) and was given daily for 1, 3, 5, 10, 15 or 18 consecutive days. Untreated animals received an equal amount of vehicle (0.9% saline). Data are expressed as the mean ± SEM. This result is a representative of two independent assays. $^{++}p < 0\ 01$ compared to control mice.

Rosiglitazone reduces adrenal hypertrophy and hypercorticoidism induced by NAC treatment

NAC-induced adrenal hypertrophy as evidenced by an increase in the mean area of cells in *zona fasciculata* of adrenal cortex (Figures 2C and 2E) and in the ratio between adrenal and body weight (Figure 2F) compared to untreated mice (Figures 2A, 2E, and 2F). Remarkably, all of these changes were clearly sensitive to rosiglitazone treatment (Figures 2D, 2E, and 2F), as evidenced by conditions in which the rosiglitazone did not alter these parameters in untreated mice (Figures 2B, 2E, and 2F). In addition, rosiglitazone significantly reduced the increase in plasma corticosterone levels induced by treatment with NAC, without modify the baseline levels of this hormone in the circulation (Figure 2G).

Rosiglitazone reduces adrenal overexpression of MC2R induced by the NAC treatment

We noted that mice treated with NAC increased the adrenal expression of both ACTH receptor (MC2R) (Figure 3A) and steroidogenic enzyme StAR (Figure 3A) in comparison to control mice. Rosiglitazone significantly reduced NAC-induced upregulation of MC2R expression without interfering with StAR expression (Figure 3A and 3B, respectively), under conditions where the baseline levels of these receptors remained unaltered (Figure 3).

Rosiglitazone reduces hypercorticoidism induced by NAC treatment through PPARγ activation

Treatment with NAC reduced PPARγ expression in adrenal glands compared to untreated mice. It is of interest note that rosiglitazone did not enhance the PPARγ RNAm expression in both untreated and NAC-treated mice (Figure 4A). Likewise, the pre-treatment with PPARγ antagonist GW9662 blocked

the inhibitory properties of rosiglitazone on NAC-induced hypercorticoidism, indicating that PPARγ negatively regulates corticosterone production. Furthermore, the treatment with GW9662 did not alter NAC-induced upregulation in plasma corticosterone levels in this model (Figure 4B).

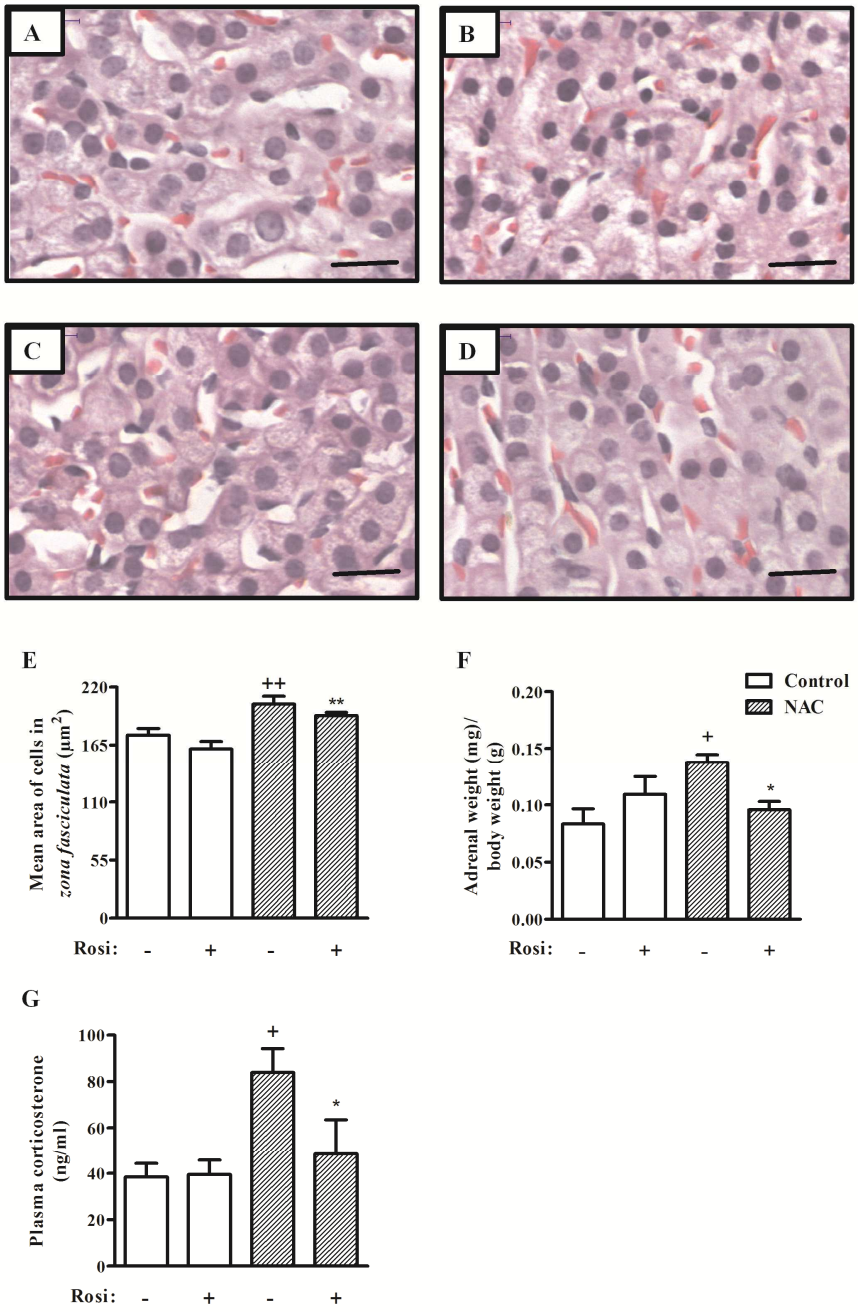

Figure 2: Rosiglitazone reduces adrenal hypertrophy and plasma corticosterone levels observed in NAC-treated mice. Mice were treated concomitantly with NAC (150 mg/kg, oral route) and rosiglitazone (0.5 mg/kg, i.p.) once daily for 5 consecutive days. Control animals were treated daily with rosiglitazone for 5 consecutive days. Untreated animals received an equal amount of vehicle (DMSO 0.05 %, i.p.), and analyses were performed 24 hours after treatments. Representative photomicrographs of *zona fasciculata* of adrenal glands stained with Hematoxylin & Eosin of naive

mice (A), rosiglitazone treated mice (B), NAC treated mice (C) and NAC plus rosiglitazone treated mice (D). (E) Quantification of the mean area of *zona fasciculata* cells. (F) The ratio between adrenal and body weight. (G) Plasma quantification of corticosterone levels. Data are expressed as the mean ± SEM. $^{+}P<0.05$ compared to control mice. $^{++}P<0.01$ compared to control mice. $^{*}P<0.05$ compared to NAC-treated mice. $^{**}P<0.01$ compared to NAC-treated mice. Scale bar = 50 μm. NAC = N-acetylcysteine. Rosi = Rosiglitazone.

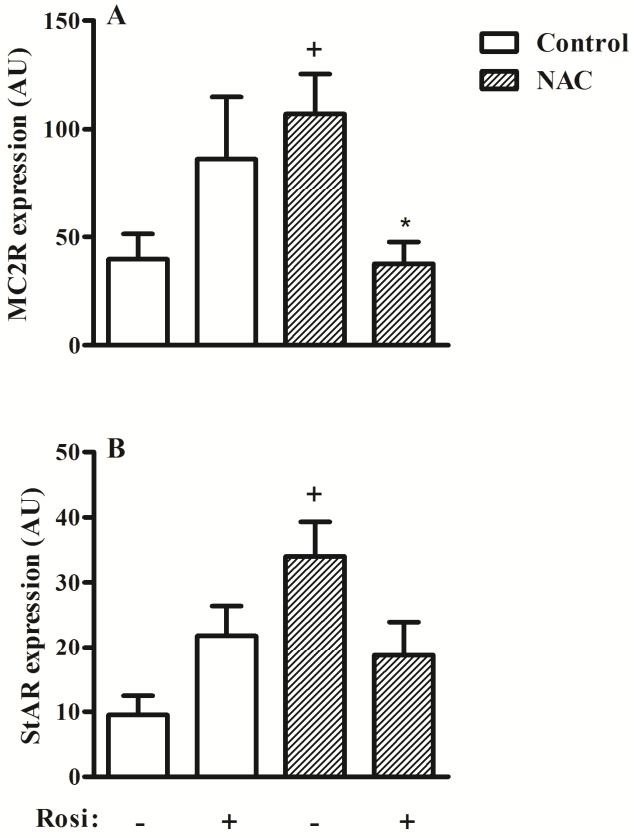

Figure 3: Rosiglitazone reduces MC2R expression but not StAR in the adrenals of NAC-treated mice. Mice were treated concomitantly with NAC (150 mg/kg, oral route) and rosiglitazone (0.5 mg/kg, i.p.) once daily for 5 consecutive days. Control animals were treated daily with rosiglitazone for 5 consecutive days. Untreated animals received an equal amount of vehicle (DMSO 0.05 %, i.p.), and analyses were performed 24 hours after treatments. MC2R (A) and StAR (B) gene expression in adrenals of mice measured by qPCR. Data are expressed as the mean ± SEM. $^{+}P<0.05$ compared to control mice. $^{*}P<0.05$ compared to NAC-treated mice. NAC = N-acetylcysteine. Rosi = Rosiglitazone.

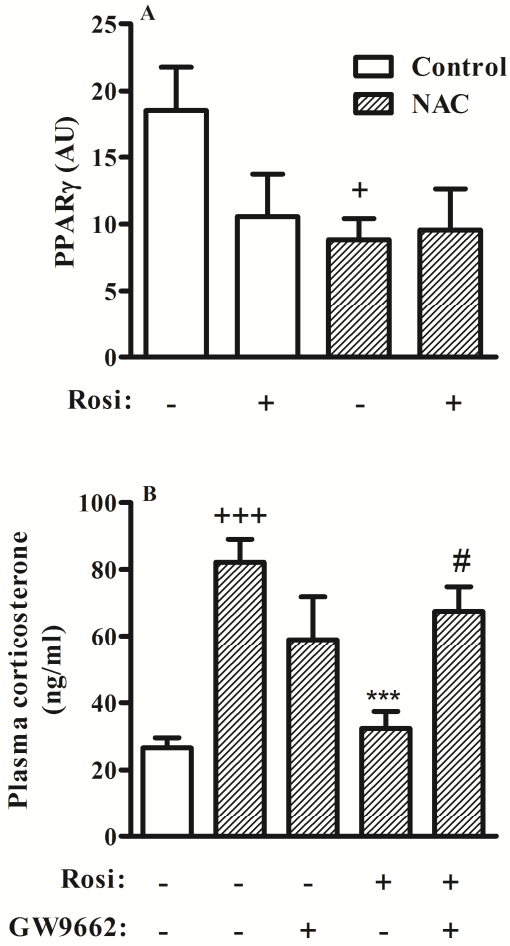

Figure 4: Blockade of PPARγ impaired rosiglitazone-mediated reduction of plasma corticosterone levels in NAC-treated mice. Mice were treated concomitantly with NAC (150 mg/kg, oral route), rosiglitazone (0.5 mg/kg, i.p.), and/or GW9662 (0.5 mg/kg, i.p.) once daily for 5 consecutive days. Untreated animals received an equal amount of vehicle (DMSO 0.1 %, i.p.), and analyses were performed 24 hours after treatments. (A) PPARγ gene expression in adrenals of mice measured by qPCR. (B) Plasma quantification of corticosterone levels. Data are expressed as the mean ± SEM from. $^{+}P<0.05$ compared to control mice. $^{+++}P<0.001$ compared to control mice. $^{***}P<0.001$ compared to NAC-treated mice. $^{\#}P<0.05$ compared to NAC plus rosiglitazone-treated mice. NAC = N-acetylcysteine. Rosi = Rosiglitazone.

DISCUSSION

This study investigated the role of PPARγ on the NAC treatment-induced hypercorticoidism. We found that the antioxidant NAC increased plasma levels of corticosterone in mice even in a short time treatment. Furthermore, we showed that the PPARγ agonist rosiglitazone reversed the hypercorticoidism and the adrenal hypertrophy caused by NAC treatment. Rosiglitazone also decreased the local expression of MC2R. The reduction in plasma corticosterone levels provoked by rosiglitazone was blocked by GW9662, a PPARγ antagonist, regardless the level of PPARγ expression. Our findings indicate that lower expression of PPARγ in adrenals of NAC-treated mice might account for the hypercorticoidism observed in these animals, along with up-regulation of ACTH receptor expression.

Currently, many people consume antioxidant supplements regularly to avoid developing diseases associated with aging [18,19]. Nevertheless, several clinical trials testing benefits and harms of dietary supplementation with antioxidants found that these drugs have been unable to show helpful effects and pointed that they seemed to induce an augmentation in all-cause mortality [20–23]. We previously demonstrated that the prolonged treatment with two different antioxidants, NAC and vitamin E, which act through distinct mechanisms of action increased plasma corticosterone levels in rats [10], however, the effects of acute use of these drugs on steroidogenesis are unknown. We performed here a treatment kinetics in Swiss-Webster mice and demonstrated that NAC rises the circulating levels of corticosterone after three consecutive days of treatment, remaining high at all subsequent times analyzed. Here, we noted that NAC treatment was able to induce the production of the primary stress hormone in an animal species other than the rat, suggesting that this phenomenon may occur in a larger spectrum of species, including human beings. Furthermore, we demonstrated in this work that even an acute treatment with NAC can increase glucocorticoid production by mice, which makes the indiscriminate use of antioxidant dietary supplementation even more risky.

It is currently known that ROS, including superoxide and hydrogen peroxide, generated by normal cell metabolism can act as intracellular signaling being crucial for the maintenance of cellular homeostasis [6,7,24]. In addition, exogenous antioxidants are able to reduce the expression and/or activity of endogenous antioxidant enzymes [25,26] by down-regulation of transcription factors

engaged in crosstalk for cytoprotection [10]. PPARγ is expressed in both murine and human adrenals[15,27,28], but also in cell lines[29], and its activation attenuated cortisol levels in patients with Cushing disease [30], as well as played a significant role in conferring cytoprotection against endogenous oxidative stress [12–14]. Therefore, it could then be hypothesized that NAC could decreasing PPARγ expression and/or activation in adrenal glands, provoking an enhancement of the steroidogenic pathway. The fact that the rosiglitazone inhibited NAC-induced hypercorticoidism in parallel to a reduction of adrenal micro and macro hypertrophy, reinforce this hypothesis. Moreover, this idea is consistent with data showing that rosiglitazone treatment inhibited both adrenal hypertrophy and hypercorticoidism in diabetic animals [15].

We previously showed that antioxidant-induced hypercorticoidism occurred in parallel with an increase in the expression of the ACTH receptor MC2R and the steroidogenic enzyme StAR in the adrenals [10]. Here, we showed that rosiglitazone significantly inhibited NAC-induced overexpression of MC2R in adrenals, but not StAR. The reestablishment of MC2R expression by rosiglitazone may explain the reduction of plasma corticosterone levels induced by NAC, considering that the MC2R signaling pathway is essential to induce steroidogenesis and provoke subsequent adrenal hypertrophy and glucocorticoid secretion [31].

We showed in this work that NAC treatment reduced the expression of PPARγ in adrenal glands, however, treatment with rosiglitazone did not interfere with this effect of NAC. Despite that PPARγ activation usually induces its own gene transcription, including in adrenal glands [15,32], rosiglitazone could increase PPARγ activity without up-regulating protein amounts, especially considering the short period of drug administration [33]. Nevertheless, Since rosiglitazone did not increase PPARγ expression in the adrenal glands of antioxidant-treated mice, one possible explanation would be that this thiazolidinedione could inhibit NAC-induced steroidogenesis through receptor-independent effects [34,35].

To understand whether a decrease in the circulating levels of corticosterone by rosiglitazone is PPARγ-dependent in NAC-treated mice, we performed experiments using a PPARγ antagonist GW9662. Here, we showed that the effect of rosiglitazone in reducing NAC treatment-induced

hypercorticoidism was completely abolished in mice treated simultaneously with GW9662, suggesting that this effect is dependent on the PPARγ activation by rosiglitazone. Although we did not observe that rosiglitazone increased the expression of PPARγ in the adrenal glands of NAC-treated mice, the fact that the PPARγ antagonist prevents the effect of rosiglitazone on NAC-induced hypercorticoidism indicates that rosiglitazone is acting by activating this receptor. In addition, the fact that the lower expression of PPARγ induced by NAC is related directly to the higher expression of MC2R in the adrenals is in line with what was shown by us earlier in diabetic rats [15]. Thus, these findings also suggest that in adrenal glands, PPARγ is a transcription factor that induces MC2R gene transrepression.

In summary, our results indicate that NAC-induced hypercorticoidism in mice is related to the loss of PPARγ cytoprotective capacity. This effect seems be caused by a reduced PPARγ mRNA transcription, which could lead to a subsequent transactivation of the ACTH receptor (MC2R). In addition, the activation of PPARγ with rosiglitazone normalizes the dysregulation in adrenal steroidogenesis induced by NAC treatment.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHORSHIP

RDV: acquisition of data; analysis and interpretation of data; final approval of the version to be submitted. ASC: acquisition of data; analysis and interpretation of data; final approval of the version to be submitted. NSM: acquisition of data; analysis and interpretation of data; final approval of the version to be submitted. FBG: acquisition of data; analysis and interpretation of data; final approval of the version to be submitted. MFP: acquisition of data; analysis and interpretation of data; final approval of the version to be submitted. ARP: revising the article critically for important intellectual content; analysis and interpretation of data; final approval of the version to be submitted. PMRS:

revising the article critically for important intellectual content; final approval of the version to be submitted. MAM: revising the article critically for important intellectual content; final approval of the version to be submitted. VFC: the conception and design of the study; revising the article critically for important intellectual content; analysis and interpretation of data; final approval of the version to be submitted.

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REFERENCES

[1] H.J. Forman, O. Augusto, R. Brigelius-Flohe, P.A. Dennery, B. Kalyanaraman, H. Ischiropoulos, G.E. Mann, R. Radi, L.J. Roberts, J. Vina, K.J.A. Davies, Even free radicals should follow some rules: A Guide to free radical research terminology and methodology, Free Radic. Biol. Med. 78 (2015) 233–235. https://doi.org/10.1016/j.freeradbiomed.2014.10.504.

[2] L. Hu, Y. Zhang, W. Miao, T. Cheng, Reactive Oxygen Species and Nrf2: Functional and Transcriptional Regulators of Hematopoiesis, Oxid. Med. Cell. Longev. 2019 (2019). https://doi.org/10.1155/2019/5153268.

[3] H. Xie, S. Hou, J. Jiang, M. Sekutowicz, J. Kelly, B.J. Bacskai, Rapid cell death is preceded by amyloid plaque-mediated oxidative stress, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 7904–7909. https://doi.org/10.1073/pnas.1217938110.

[4] J. Lee, Y. Kim, T. Liu, Y.J. Hwang, S.J. Hyeon, H. Im, K. Lee, V.E. Alvarez, A.C. McKee,

S.J. Um, M. Hur, I. Mook-Jung, N.W. Kowall, H. Ryu, SIRT3 deregulation is linked to mitochondrial dysfunction in Alzheimer's disease, Aging Cell. 17 (2018) 1–12. https://doi.org/10.1111/acel.12679.

[5] D.R. Gough, T.G. Cotter, Hydrogen peroxide: A Jekyll and Hyde signalling molecule, Cell Death Dis. 2 (2011) e213-8. https://doi.org/10.1038/cddis.2011.96.

[6] J. Yun, T. Finkel, Mitohormesis, Cell Metab. 19 (2014) 757–766. https://doi.org/10.1016/j.cmet.2014.01.011.

[7] C.S. Cox, S.E. McKay, M.A. Holmbeck, B.E. Christian, A.C. Scortea, A.J. Tsay, L.E. Newman, G.S. Shadel, Mitohormesis in Mice via Sustained Basal Activation of Mitochondrial and Antioxidant Signaling, Cell Metab. 28 (2018) 776-786.e5. https://doi.org/10.1016/j.cmet.2018.07.011.

[8] D. Fusco, G. Colloca, M.R. Lo Monaco, M. Cesari, Effects of antioxidant supplementation on the aging process., Clin. Interv. Aging. 2 (2007) 377–387.

[9] A.F. Chen, D.D. Chen, A. Daiber, F.M. Faraci, H. Li, C.M. Rembold, I. Laher, Free radical biology of the cardiovascular system, Clin. Sci. 123 (2012) 73–91. https://doi.org/10.1042/CS20110562.

[10] J.P. Prevatto, R.C. Torres, B.L. Diaz, P.M.R.E. Silva, M.A. Martins, V.F. Carvalho, Antioxidant Treatment Induces Hyperactivation of the HPA Axis by Upregulating ACTH Receptor in the Adrenal and Downregulating Glucocorticoid Receptors in the Pituitary, Oxid. Med. Cell. Longev. 2017 (2017). https://doi.org/10.1155/2017/4156361.

[11] A.R. Silva, C.F. Gonçalves-de-Albuquerque, A.R. Pérez, V. de F. Carvalho, Immune-endocrine interactions related to a high risk of infections in chronic metabolic diseases: The role of PPAR gamma, Eur. J. Pharmacol. 854 (2019) 272–281. https://doi.org/10.1016/j.ejphar.2019.04.008.

[12] A. Von Knethen, H. Neb, V. Morbitzer, M.V. Schmidt, A.M. Kuhn, L. Kuchler, B. Brüne, PPARγ stabilizes HO-1 mRNA in monocytes/macrophages which affects IFN-β expression, Free Radic. Biol. Med. 51 (2011) 396–405. https://doi.org/10.1016/j.freeradbiomed.2011.04.033.

[13] N.K.H. Khoo, S. Hebbar, W. Zhao, S.A. Moore, F.E. Domann, M.E. Robbins, Differential activation of catalase expression and activity by PPAR agonists: Implications for astrocyte protection in anti-glioma therapy, Redox Biol. 1 (2013) 70–79. https://doi.org/10.1016/j.redox.2012.12.006.

[14] T.G. Araújo, A.G. Oliveira, J.F. Vecina, R.M. Marin, E.S. Franco, M.J. Abdalla Saad, M.B. de Sousa Maia, Treatment with Parkinsonia aculeata combats insulin resistance-induced oxidative stress through the increase in PPARγ/CuZn-SOD axis expression in diet-induced obesity mice, Mol. Cell. Biochem. 419 (2016) 93–101. https://doi.org/10.1007/s11010-016-2753-7.

[15] R.C. Torres, N.S. Magalhães, P.M.R. e Silva, M.A. Martins, V.F. Carvalho, Activation of PPAR-γ reduces HPA axis activity in diabetic rats by up-regulating PI3K expression, Exp. Mol. Pathol. 101 (2016) 290–301. https://doi.org/10.1016/j.yexmp.2016.10.002.

[16] R.C. Torres, M.M. Batista, A.H. Pons, A.R. Silva, R.S.B. Cordeiro, M.A. Martins, P.M.R. E Silva, V.F. Carvalho, Activation of PPARγ by restores mast cell numbers and reactivity in alloxan-diabetic rats by reducing the systemic glucocorticoid levels, Eur. J. Pharmacol. 691 (2012) 261–267. https://doi.org/10.1016/j.ejphar.2012.06.010.

[17] O. Chan, K. Inouye, M. Vranic, S.G. Matthews, Hyperactivation of the hypothalamo-pituitary-adrenocortical axis in streptozotocin-diabetes is associated with reduced stress responsiveness and decreased pituitary and adrenal sensitivity, Endocrinology. 143 (2002) 1761–1768. https://doi.org/10.1210/endo.143.5.8809.

[18] F.H. Sarkar, Nutraceuticals and Cancer, Springer Netherlands, Dordrecht, 2012. https://doi.org/10.1007/978-94-007-2630-7.

[19] S.-K. Myung, H.J. Yang, Efficacy of Vitamin and Antioxidant Supplements in Prevention of Esophageal Cancer: Meta-analysis of Randomized Controlled Trials, J. Cancer Prev. 18 (2013) 135–143. https://doi.org/10.15430/jcp.2013.18.2.135.

[20] E.R. Miller Iii, R. Pastor-Barriuso, D. Dalal, R.A. Riemersma, L.J. Appel, E. Guallar, Meta-Analysis: High-Dosage Vitamin E Supplementation May Increase All-Cause Mortality Background: Experimental models and observational studies, Ann. Intern. Med. 142 (2005) 37–46.

[21] G. Bjelakovic, D. Nikolova, L.L. Gluud, R.G. Simonetti, C. Gluud, Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases, Cochrane Database Syst. Rev. (2012). https://doi.org/10.1002/14651858.cd007176.pub2.

[22] H. Macpherson, A. Pipingas, M.P. Pase, Multivitamin-multimineral supplementation and mortality: A meta-analysis of randomized controlled trials, Am. J. Clin. Nutr. 97 (2013) 437–444. https://doi.org/10.3945/ajcn.112.049304.

[23] J.R. Evans, J.G. Lawrenson, Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration, Cochrane Database Syst. Rev. 2017 (2017). https://doi.org/10.1002/14651858.CD000254.pub4.

[24] M. Ristow, Unraveling the truth about antioxidants, Nat. Med. 20 (2014) 709–711. https://doi.org/10.1038/nm.3624.

[25] K.M.P. Pires, M. Lanzetti, C.R. Rueff-Barroso, P. Castro, A. Abrahão, V.L.G. Koatz, S.S. Valença, L.C. Porto, Oxidative damage in alveolar macrophages exposed to cigarette smoke extract and participation of nitric oxide in redox balance, Toxicol. Vitr. 26 (2012) 791–798. https://doi.org/10.1016/j.tiv.2012.05.011.

[26] J.K. Kim, J. Park, T.H. Ryu, M. Nili, Effect of N-acetyl-l-cysteine on Saccharomyces cerevisiae irradiated with gamma-rays, Chemosphere. 92 (2013) 512–516.

https://doi.org/10.1016/j.chemosphere.2013.02.035.

[27] J.B. Ewaschuk, J.W. Walker, H. Diaz, K.L. Madsen, Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions, J. Nutr. 136 (2006) 1483–1487. https://doi.org/10.3945/jn.109.106377.is.

[28] A. Uruno, K. Matsuda, N. Noguchi, T. Yoshikawa, M. Kudo, F. Satoh, W.E. Rainey, X.-G. Hui, J. -i. Akahira, Y. Nakamura, H. Sasano, H. Okamoto, S. Ito, A. Sugawara, Peroxisome proliferator-activated receptor- suppresses CYP11B2 expression and aldosterone production, J. Mol. Endocrinol. 46 (2011) 37–49. https://doi.org/10.1677/JME-10-0088.

[29] M.J. Betz, I. Shapiro, M. Fassnacht, S. Hahner, M. Reincke, F. Beuschlein, Peroxisome Proliferator-Activated Receptor-γ Agonists Suppress Adrenocortical Tumor Cell Proliferation and Induce Differentiation, J. Clin. Endocrinol. Metab. 90 (2005) 3886–3896. https://doi.org/10.1210/jc.2004-1267.

[30] B. Ambrosi, C. Dall'Asta, S. Cannavò, R. Libè, T. Vigo, P. Epaminonda, I. Chiodini, S. Ferrero, F. Trimarchi, M. Arosio, P. Beck-Peccoz, Effects of chronic administration of PPAR-γ ligand rosiglitazone in Cushing's disease, Eur. J. Endocrinol. 151 (2004) 173–178. https://doi.org/10.1530/eje.0.1510173.

[31] S.Y. Park, J.J. Walker, N.W. Johnson, Z. Zhao, S.L. Lightman, F. Spiga, Constant light disrupts the circadian rhythm of steroidogenic proteins in the rat adrenal gland, Mol. Cell. Endocrinol. 371 (2013) 114–123. https://doi.org/10.1016/j.mce.2012.11.010.

[32] S. Goyal, S. Arora, T.K. Bhatt, P. Das, A. Sharma, S. Kumari, D.S. Arya, Modulation of PPAR-γ by telmisartan protects the heart against myocardial infarction in experimental diabetes, Chem. Biol. Interact. 185 (2010) 271–280. https://doi.org/10.1016/j.cbi.2010.03.030.

[33] C. Martinez Calejman, J.M. Di Gruccio, M.E. Mercau, E.M. Repetto, F. Astort, R. Sanchez, M. Pandolfi, G. Berg, L. Schreier, P. Arias, C.B. Cymeryng, Insulin sensitization with a

peroxisome proliferator-activated receptor γ agonist prevents adrenocortical lipid infiltration and secretory changes induced by a high-sucrose diet., J. Endocrinol. 214 (2012) 267–76. https://doi.org/10.1530/JOE-12-0193.

[34] F. Biscetti, G. Straface, V. Arena, E. Stigliano, G. Pecorini, P. Rizzo, G. De Angelis, L. Iuliano, G. Ghirlanda, A. Flex, Pioglitazone enhances collateral blood flow in ischemic hindlimb of diabetic mice through an Akt-dependent VEGF-mediated mechanism, regardless of PPARγ stimulation, Cardiovasc. Diabetol. 8 (2009) 49. https://doi.org/10.1186/1475-2840-8-49.

[35] D.S. Coutinho, E.A. Anjos-Valotta, C.V.M.F. do Nascimento, A.L.A. Pires, M.H. Napimoga, V.F. Carvalho, R.C. Torres, P.M.R. e Silva, M.A. Martins, 15-deoxy-delta-12,14-Prostaglandin J2 inhibits lung inflammation and remodeling in distinct murine models of Asthma, Front. Immunol. 8 (2017) 1–13. https://doi.org/10.3389/fimmu.2017.00740.

Highlights

- Oral supplementation with N-acetyl-cysteine (NAC) induces hypercorticoidism.
- NAC induces hypercorticoidism by reduction of PPARγ expression in adrenals.
- PPARγ activation reduces NAC-induced ACTH receptor expression in adrenals.