J Vet Sci. 2022 Sep;23(5):e76
https://doi.org/10.4142/jvs.22112
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Original Article

Veterinary Nutrition and Metabolism

Check for updates

Dexamethasone enhances glucose uptake by SGLT1 and GLUT1 and boosts ATP generation through the PPP-TCA cycle in bovine neutrophils

Xinbo Wang [1,†], **Mingyu Tang** [1,†], **Yuming Zhang** [1,2,†], **Yansong Li** [1,†], **Jingdong Mao** [1,†], **Qinghua Deng** [1,2], **Shusen Li** [1], **Zhenwei Jia** [1], **Liyin Du** [1,2,*]

[1]College of Animal Science and Technology, Inner Mongolia Minzu University, Tongliao 028000, China
[2]Key Laboratory for Prevention and Control of Herbivorous Livestock Perinatal Diseases, Inner Mongolia Minzu University, Tongliao 028000, China

Received: Apr 23, 2022
Revised: Jul 18, 2022
Accepted: Jul 26, 2022
Published online: Aug 26, 2022

***Corresponding author:**
Liyin Du
College of Animal Science and Technology, Inner Mongolia Minzu University, No. 536 Huolinhe Street (West), Kerqin District, Tongliao 028000, China.
Email: duliyin2016@163.com
https://orcid.org/0000-0002-1513-0479

[†]Xinbo Wang, Mingyu Tang, Yuming Zhang, Yansong Li and Jingdong Mao equally contributed to this paper.

ABSTRACT

Background: Clinical dexamethasone (DEX) treatment or stress in bovines results in extensive physiological changes with prominent hyperglycemia and neutrophils dysfunction.
Objectives: To elucidate the effects of DEX treatment in vivo on cellular energy status and the underlying mechanism in circulating neutrophils.
Methods: We selected eight-month-old male bovines and injected DEX for 3 consecutive days (1 time/d). The levels of glucose, total protein (TP), total cholesterol (TC), and the proinflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in blood were examined, and we then detected glycogen and adenosine triphosphate (ATP) content, phosphofructosekinase-1 (PFK1) and glucose-6-phosphate dehydrogenase (G6PDH) activity, glucose transporter (GLUT)1, GLUT4, sodium/glucose cotransporter (SGLT)1 and citrate synthase (CS) protein expression and autophagy levels in circulating neutrophils.
Results: DEX injection markedly increased blood glucose, TP and TC levels, the Ca^{2+}/P^{5+} ratio and the neutrophil/lymphocyte ratio and significantly decreased blood IL-1β, IL-6 and TNF-α levels. Particularly in neutrophils, DEX injection inhibited p65-NFκB activation and elevated glycogen and ATP contents and SGLT1, GLUT1 and GR expression while inhibiting PFK1 activity, enhancing G6PDH activity and CS expression and lowering cell autophagy levels.
Conclusions: DEX induced neutrophils glucose uptake by enhancing SGLT1 and GLUT1 expression and the transformation of energy metabolism from glycolysis to pentose phosphate pathway (PPP)-tricarboxylic acid (TCA) cycle. This finding gives us a new perspective on deeper understanding of clinical anti-inflammatory effects of DEX on bovine.

Keywords: Neutrophils; Dexamethasone; Tricarboxylic Acid Cycle; Adenosine Triphosphate; Bovine

INTRODUCTION

Dexamethasone (DEX), one of the most effective synthetic glucocorticoids (GCs), is extensively used in human and veterinary practice for the treatment of a wide variety of

ORCID iDs

Xinbo Wang
https://orcid.org/0000-0001-9842-1855
Mingyu Tang
https://orcid.org/0000-0003-4423-3060
Yuming Zhang
https://orcid.org/0000-0003-4523-6618
Yansong Li
https://orcid.org/0000-0003-4041-9140
Jingdong Mao
https://orcid.org/0000-0003-4283-9253
Qinghua Deng
https://orcid.org/0000-0002-3659-0984
Shusen Li
https://orcid.org/0000-0002-6315-247X
Zhenwei Jia
https://orcid.org/0000-0002-3855-8603
Liyin Du
https://orcid.org/0000-0002-1513-0479

Author Contributions

Conceptualization: Du L; Formal analysis: Wang X; Investigation: Zhang Y; Methodology: Tang M, Li Y, Li S; Project administration: Deng Q; Resources: Jia Z; Software: Wang X, Tang M, Li Y, Li S; Supervision: Mao J; Validation: Du L; Visualization: Wang X, Zhang Y; Writing - original draft: Wang X; Writing - review & editing: Wang X, Du L.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This work was supported by National Natural Science Foundation of China (31760752; 31260626); Natural Science Foundation of Inner Mongolia Autonomous Region (2018LH03009; 2019LH03020); Young Scientific and Technological Talents in Inner Mongolia (Inner Mongolia, China, grant No. NJYT-20-B30)

metabolic diseases as well as stress and inflammatory diseases. Many clinical studies have verified that DEX treatment results in an observable disturbance of blood glucose, lipids, and mineral ions and affects the counts and function of peripheral blood leucocytes by altering glucose uptake and glycolysis through the glucocorticoid receptor (GR) [1-5], and decreases plasma tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels in lipopolysaccharide (LPS)-stimulated calves [6], especially, reduces the IL-1β, TNF-α, and IL-8 mRNA levels in equine and human neutrophils [7].

Neutrophils are well known as unusually glycogen-rich cells. ATP, as the direct energy source for resting neutrophils survival and for activated neutrophils functions, is largely derived from the glycolysis pathway, while the pentose phosphate pathway (PPP) also generates Adenosine Triphosphate (ATP) through the tricarboxylic acid cycle (TCA). Phosphofructosekinase-1 (PFK1) is the gatekeeper of glycolysis, and glucose-6-phosphate dehydrogenase (G6PDH) is the sole catalytic enzyme of the PPP. Regardless of the basal ATP expenditures for cell survival or the high ATP requirements for cell activation, the synergic or competitive action of PFK1 and G6PDH might be indispensable in response to hormonal signaling to fine-tune glycolytic flux to meet energy requirements [8]. Metabolomics analysis demonstrates that DEX significantly altered pathways of TCA cycle by binding to GR in pregnant women [9], hydrocortisone or other stress hormones increased glucose consumption in neutrophils [10,11], and rat neutrophils exposed to DEX *in vitro* present an increase in glucose consumption and a decrease in the activity and expression of G6PDH [12]. These data suggested that G6PDH activity is a matter of particular concern with respect to the effects of DEX on neutrophils metabolism were not entirely consistent although aforesaid data may not be entirely consistent.

Studies have demonstrated that the glucose utilized in neutrophils mainly depends on intracellular glycogen breakdown and glucose uptake from the environment. Glucose transporter (GLUT)1, the leading member of GLUTs in resting neutrophils under physiological circumstances, promotes glucose uptake, and it exhibits increased expression in activated neutrophils [13]. Sodium/glucose cotransporter 1 (SGLT1) can increase glucose uptake by collaborating with GLUTs in lymphocytes, even in an environment of very low extracellular glucose [14]. Although there is no evidence of DEX induced functional change of SGLT1 and GLUT1 and 4, and of resulted in the change of glycogen storage in neutrophils. A few studies have demonstrated that DEX can enhances GLUT1 expression [15] and injures the insulin-induced GLUT4 membrane translocation [16] in muscle cells, enhances SGLT1mRNA expression in intestine, skeletal muscle and liver of goats [17], meanwhile, increases glycogen content in myocardial cell [18] and hepatocyte [19]. In addition, GCs exposure in humans and animals initiates hyperglycemia and delays the apoptosis of circulating neutrophils [20,21]. DEX promotes cell apoptosis by enhancing the Bax/Bcl-2 ratio and autophagy via the upregulation of LC3 in lymphocytes [22,23], whereas correcting hyperglycemia with insulin can recover neutrophils function [24].

This study mainly evaluated the effect of DEX on the levels of glycogen and ATP; the activities of PFK1 and G6PDH; the expression of GLUT1, GLUT4, SGLT1 and citrate synthase (CS); and autophagy levels in circulating neutrophils. We sought to investigate the characteristics and intrinsic regulatory mechanism of energy status in circulating neutrophils in DEX-treated bovine.

MATERIALS AND METHODS

Animals

Five healthy eight-month-old male beef bovine were selected. Animals were placed in a temperature-controlled stable in individual tie-stalls with lights on for 16 h and lights off 8 h; animals were fed three times/d with the total mixed rations at 8:00 am, 14:00 pm and 20:00 pm and freely accessed to water from 8:00 am to 20:00 pm in the whole experiment. All animal experimental procedures were performed in accordance with relevant guidelines and regulations. The current study was conducted at Inner Mongolia Minzu University in Tongliao, China (no. SCXK-2020-0002).

Drug administration and blood collection

Drug administration and blood collection were performed before 8:00 am (namely before the first feed). The selected bovines were injected intravenously with 0.5 mg/kg DEX sodium phosphate (DEX) for 3 consecutive days (once a day) at 8:00 am. Blood was collected in a 50 mL centrifuge tube containing 0.1 mL heparin sodium (Gentihold) as an anticoagulant by jugular vein puncture. Blood collection occurred before the 1st DEX injection (named Pre-DEX), 24 h after the 1st, 2nd, and 3rd DEX injections (named DEX), and 3 d, 5 d, and 7 d after the 3rd DEX injection (named Post-DEX, and 24 h after the 3rd DEX injection was 1 d for Post-DEX). The collected blood was used for the following experiments.

Analyses of blood biochemical indices and proinflammatory cytokine levels

Part of each heparinized blood sample was used to analyze the number of white blood cells, and the levels of glucose, calcium (Ca^{2+}), phosphorus (P^{5+}), plasma total protein (TP) and plasma total cholesterol (TC) were detected by a Mindray BC-2800 Vet blood analyzer. The inflammatory cytokines IL-1β, IL-6 and TNF-α were detected by enzyme linked immunosorbent assay (ELISA).

Separation of neutrophils

The remaining portion of each heparinized blood sample was used to collect neutrophils, as detailed below. Heparinized blood samples were diluted with equal amounts of 1 × phosphate-buffered saline (PBS), placed on a Percoll (GE Healthcare, USA) separation solution, and centrifuged at 800 × g for 15 min. Plasma and lymphocytes were removed, and the samples were washed with PBS once. Then, red blood cell lysates were added and centrifuged at 800 × g for 8 min. The neutrophils were washed by 1 × PBS, and centrifuged at 800 × g for 10 min. The collected neutrophils were stored at −40°C for the following experiments.

Biochemical analyses

Biochemical analysis was used to detect G6PDH activity and ATP and glycogen contents in neutrophils. All biochemical tests were performed using commercial test kits (Solarbio, China) at 37°C in an automatic microplate reader (Multiskan Spectrum; Thermo Scientific, USA). Biochemical analyses were conducted in strict accordance with the instructions of the kit.

ELISA analyses

The PFK1 activity in the collected neutrophils and the levels of IL-1β, IL-6 and TNF-α in blood plasma were detected by ELISA kits (Solarbio). ELISA analysis was conducted in strict accordance with the instructions of the kit.

Western blotting analysis

TP was extracted from neutrophils with lysate buffer (Solarbio). The protein concentration was quantified using the BCA protein assay kit (Applygen, China). Then, the protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Immobilon). After blocking with 5% bovine serum albumin for 2 h, the membranes were blotted with 1:700 diluted primary antibodies against GR (Cell Signaling Technology, USA), NFκB (p65) (Cell Signaling Technology), p-NFκB (p-p65) (Cell Signaling Technology), CS (Abcam, USA), GLUT1 (Abcam), GLUT4 (Abcam, MA, USA), SGLT1 (Cell Signaling Technology), p62 (Abcam), LC3 (Cell Signaling Technology), β-actin (Absin, China), AMPK (Abcam), p-AMPK (PL Laboratories, Canada) at 4°C overnight. After that, the membrane was washed with TBST, and the membranes were incubated with secondary antibodies (Cell Signaling Technology) conjugated to horseradish peroxidase for 1 h at room temperature. The bands were visualized with an enhanced chemiluminescence system, and the gray densities were quantified with ImageJ software.

Statistical analysis

The gray values of the protein electrophoresis bands were analyzed by ImageJ software (National Institutes of Health, USA). The results were presented as the mean ± standard error of the mean and were analyzed using SPSS 19.0 software (SPSS, Inc., Chicago, IL). GraphPad Prism 8.0 was used for graph analysis. Analysis of variance was performed to evaluate the differences among the groups followed by a *t*-test. A p value lower than 0.05 was considered statistically significant, and a p value lower than 0.01 was considered highly significant.

RESULTS

Effects of DEX on the levels of glucose, TP, TC, Ca^{2+} and P^{5+} in peripheral blood

To investigate the effect of DEX injection on the blood biochemical indices, the levels of blood glucose, TP, TC, Ca^{2+} and P^{5+} were detected. The level of blood glucose (**Fig. 1A**) rapidly increased after the 1st injection ($p < 0.01$) and showed a gradual decline but was higher than that of Pre-DEX at the 3rd (1 d) injection ($p < 0.01$); then, it returned to the level of Pre-DEX at 3 d Post-DEX ($p > 0.05$). The level of TP (**Fig. 1B**) gradually increased from the 1st to 3rd DEX injection ($p < 0.01$) and gradually declined to that of Pre-DEX from 3 d to 7 d Post-DEX ($p > 0.05$). The level of TC (**Fig. 1C**) and the Ca^{2+}/P^{5+} ratio (**Fig. 1D**) showed a highly similar pattern to that of TP. In addition, triglycerides (TGs) were not increased and remained below 10 mg/dL (data not shown). From these results, we concluded that DEX resulted in a significant increase in blood glucose, TP and TC levels and the Ca^{2+}/P^{5+} ratio, but the corresponding levels or ratio and accumulation Post-DEX diminished to Pre-DEX levels, although there remained some differences in the rate of recovery. It is clear that the DEX-induced simultaneous occurrence of hyperglycemia and hyperlipidemia, together with DEX itself, are responsible for the change in the counts and function of immune cells.

Effects of DEX on neutrophils, lymphocytes and monocytes counts in peripheral blood

To determine the effects of DEX on changes in immune cell counts, neutrophils, lymphocytes and monocytes in peripheral blood were analyzed. Neutrophils (**Fig. 2A**) counts were increased in DEX compared to those of Pre-DEX ($p < 0.01$), rose to a maximum at the 2nd and 3rd (1 d) injections, and then gradually declined and dropped to that of Pre-DEX at 7 d Post-DEX ($p > 0.05$). Lymphocytes counts showed a reverse trend to that of

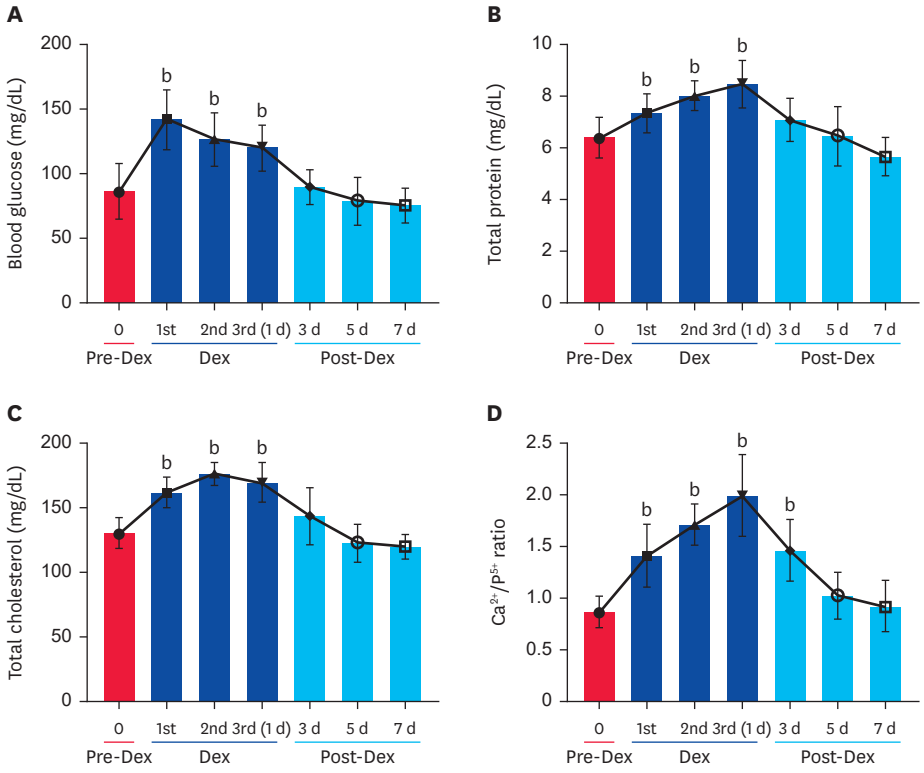

Fig. 1. Effects of DEX on the levels of glucose, TP, TC, Ca^{2+} and P^{5+} in blood. A Mindray BC-2800 Vet analyzer was used to analyze these biochemical indices. (A) Blood glucose. (B) TP. (C) TC. (D) Ca^{2+}/P^{5+} ratio. The results are shown as the mean ± standard error of the mean (n = 5). Differences between DEX and Post-DEX with Pre-DEX were analyzed using the *t*-test.
DEX, dexamethasone; TP, total protein; TC, total cholesterol; Ca^{2+}, calcium; P^{5+}, phosphorus.
$^{a}p < 0.05$; $^{b}p < 0.01$.

neutrophils (**Fig. 2B**), and the change in neutrophil/lymphocyte ratio (NLR) was similar to that of neutrophils counts (**Fig. 2C**), whereas monocytes counts remained steady ($p > 0.05$) (**Fig. 2D**). The results showed that DEX can significantly increase the NLR by increasing neutrophils counts while decreasing lymphocytes counts but has no effect on monocytes counts. Therefore, we inferred that the inflammatory response of these immune cells might be suppressed by DEX-induced hyperglycemia and hyperlipidemia together with DEX itself.

Effects of DEX on the levels of blood IL-1β, IL-6 and TNF-α and p65-NFκB activation in neutrophils

To determine whether DEX affected the immune response capacity of immune cells, we examined the levels of the proinflammatory cytokines IL-1β, IL-6 and TNF-α in blood and NFκB (p65) activation in neutrophils. The levels of IL-1β, IL-6 and TNF-α in DEX were significantly decreased compared to those in Pre-DEX ($p < 0.01$) and then rapidly returned to Pre-DEX levels in Post-DEX ($p > 0.05$) (**Fig. 3A-C**). The activation of NFκB (p65) in neutrophils in DEX-injection was significantly lower than that of Pre-DEX and then gradually returned to that of Pre-DEX in Post-DEX (**Fig. 3D and E**). GR expression rapidly increased in DEX-injection ($p < 0.01$) and declined gradually but was maintained at higher levels than that of Pre-DEX in Post-DEX ($p < 0.01$) (**Fig. 3D and F**). The results showed that DEX injection significantly reduced the release of IL-1β, IL-6, and TNF-α from immune cells, including neutrophils, lymphocytes and monocytes. Meanwhile, the inhibition of NFκB (p65)

https://doi.org/10.4142/jvs.22112

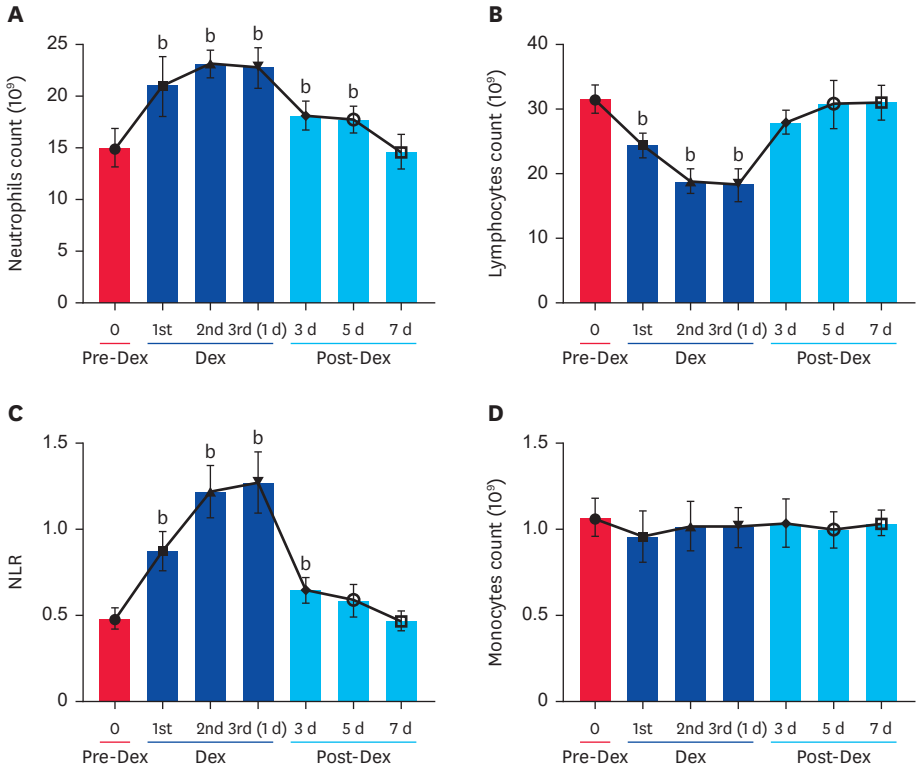

Fig. 2. Effects of DEX on neutrophils, lymphocytes, and monocytes counts in peripheral blood. A Mindray BC-2800 Vet analyzer was used to analyze (A) neutrophils, (B) lymphocytes, (C) the NLR, and (D) monocytes. The results are shown as the mean ± standard error of the mean (n = 5). Differences between DEX and Post-DEX with Pre-DEX were analyzed using the *t*-test.
DEX, dexamethasone; NLR, neutrophil/lymphocyte ratio.
[a]$p < 0.05$; [b]$p < 0.01$.

activation by DEX returned to the levels at Pre-DEX compared with those of IL-1β, IL-6 and TNF-α, and the enhanced GR expression by DEX required a longer time to return to the levels at Pre-DEX than those of IL-1β, IL-6, TNF-α and NFκB (p65) activation. Undoubtedly, these results indicated that DEX injection could intensively suppress the inflammatory response of immune cells through the specific receptor GR.

Effects of DEX on glucose uptake in neutrophils

We examined the expression of GLUT1, GLUT4, and SGLT1 to determine whether DEX injection affects glucose uptake. Expression levels of GLUT1 and SGLT1 in DEX-injection were sharply increased compared to Pre-DEX ($p < 0.01$) and then rapidly declined to preinjection levels in Post-DEX (**Fig. 4A-C**). By contrast, GLUT4 expression in DEX-injection was decreased ($p < 0.05$ or $p < 0.01$), but in Post-DEX, it rapidly increased to a significantly higher level than at Pre-DEX ($p < 0.05$ or $p < 0.01$) (**Fig. 4D and E**). The observed changes in GLUT1, SGLT1 and GLUT4 expression showed that neutrophils could clearly respond to DEX itself and to DEX-induced hyperglycemia through SGLT1 in concert with GLUT1 and GLUT4. Therefore, the above results indicated that DEX injection very likely changes cellular energy status and affects cell function (as shown in **Fig. 3**) through the synchronous upregulation of GLUT1 and SGLT1 and downregulation of GLUT4, restoring status to that of Pre-DEX with the waning of DEX activity and hyperglycemia in Post-DEX.

https://doi.org/10.4142/jvs.22112

Effects of DEX on glucose metabolism in neutrophils

We first measured glycogen and ATP contents to explore whether DEX affects cellular energy status. The glycogen and ATP contents synchronously and rapidly increased ($p < 0.01$) after DEX injection and then rapidly declined but were maintained at higher levels compared with Pre-DEX after DEX injection ($p < 0.01$) (**Fig. 5A and B**). Second, we examined PFK1 and G6PDH activity and CS expression to further evaluate the effects of DEX on the pathways of glucose catabolism closely related to glycogen reserve and ATP production. PFK1 activity rapidly decreased following DEX injection compared to preinjection ($p < 0.01$) and then rapidly increased to a higher level than that of Pre-DEX in Post-DEX ($p < 0.01$) (**Fig. 5C**); however, G6PDH activity rapidly increased following DEX injection compared with preinjection ($p < 0.01$) and then gradually declined in Post-DEX to below the level observed at Pre-DEX ($p < 0.05$) (**Fig. 5D**). CS expression showed a similar trend to G6PDH activity, with an even more marked increase at DEX injection (**Fig. 5E and F**). Finally, to confirm whether the increased ATP content is derived from other pathways, we measured cell autophagy. The results showed that AMPK activation was suppressed after DEX injection ($p < 0.01$) and gradually increased to Pre-DEX levels after DEX injection (**Fig. 5G and H**). The LC3II/LC3I ratio rapidly decreased after DEX injection ($p < 0.01$) and remained at lower levels, while p62 expression showed a contrasting response to that of LC3 at Post-DEX (**Fig. 5G, I, and J**). The results showed that the change in glycogen content was highly consistent with the levels of GLUT1 and SGLT1 expression but in contrast to the GLUT4 expression levels, indicating that the increased glycogen can be attributed to enhanced GLUT1 and SGLT1 expression, although DEX might affect glucose

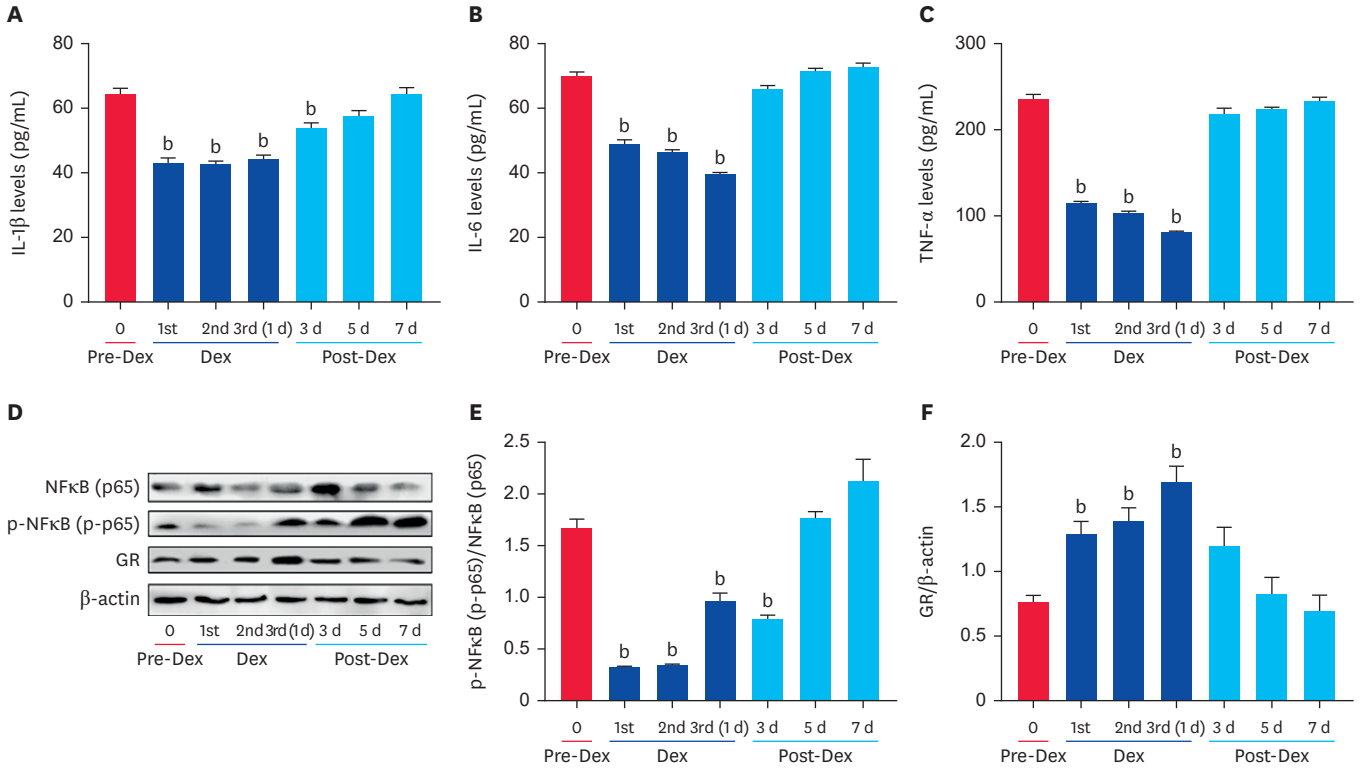

Fig. 3. Effects of DEX on the levels of IL-1β, IL-6 and TNF-α in blood and p65-NFκB activation and GR expression in neutrophils. (A) IL-1β, (B) IL-6 and (C) TNF-α were determined by enzyme linked immunosorbent assay. (A-C) The results are shown as the mean ± standard error of the mean (n = 5). (D, E) NFκB (p65) activation and (D, F) GR expression were detected by Western blotting, and β-actin was used as an internal control. (E, F) The results are shown as the mean ± standard error of the mean (n = 3). Differences were analyzed using the *t*-test.
DEX, dexamethasone; IL, interleukin; TNF, tumor necrosis factor; GR, glucocorticoid receptor.
[a]$p < 0.05$; [b]$p < 0.01$.

https://doi.org/10.4142/jvs.22112

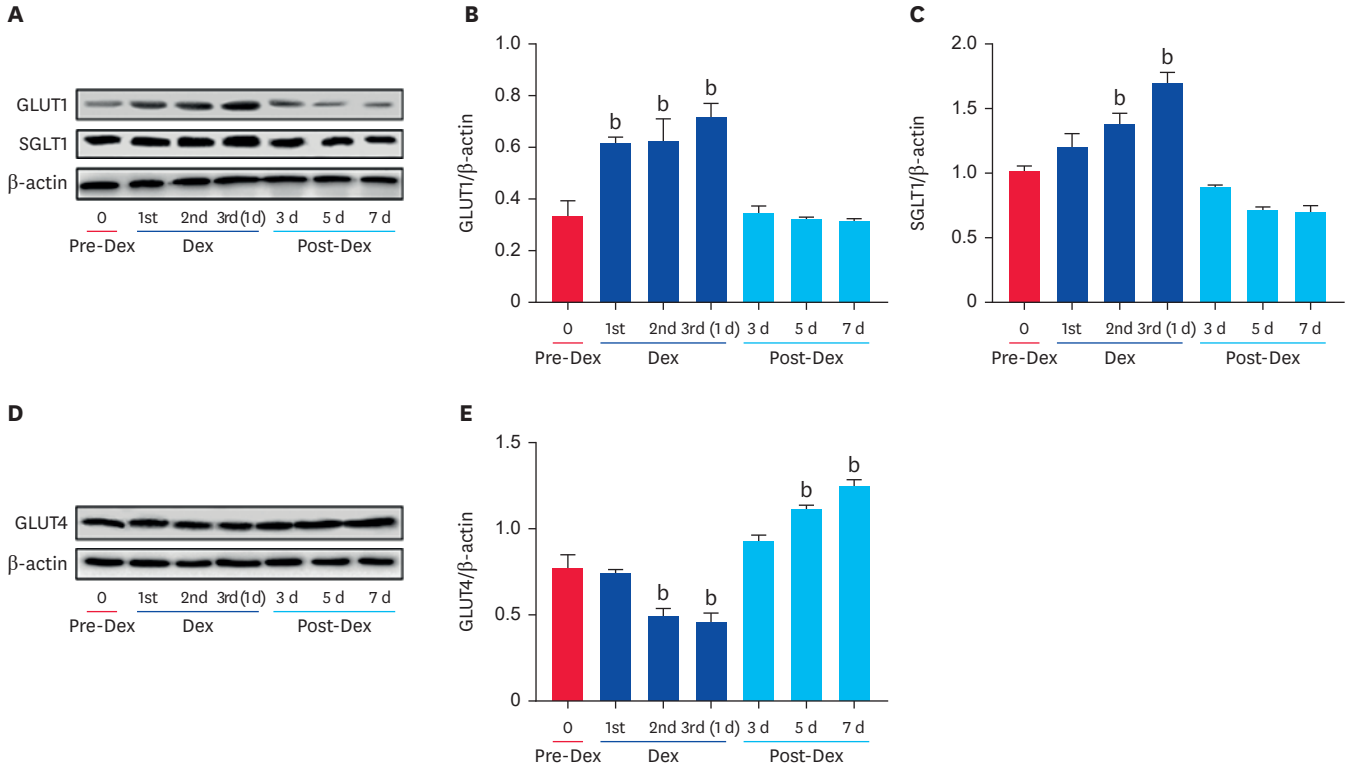

Fig. 4. DEX regulation of glucose uptake in neutrophils. Expression of (A, B) GLUT1, (A, C) SGLT1 and (D, E) GLUT4 was analyzed by Western blotting, and β-actin was used as an internal control. The results are shown as the mean ± standard error of the mean (n = 3). Differences were analyzed using the *t*-test.
DEX, dexamethasone; GLUT, glucose transporter; SGLT, sodium/glucose cotransporter.
[a]$p < 0.05$; [b]$p < 0.01$.

catabolism. However, we found that DEX significantly inhibited glycolysis by decreasing PFK1 activity but enhanced PPP-TCA by promoting G6PDH activity and CS expression, while the levels of cell autophagy were reduced. These results clearly demonstrated that the increase in ATP levels is dependent on the PPP-TCA pathway rather than the glycolysis-TCA pathway or autophagy, which support the hypothesis that the increased ATP levels induced by DEX injection result from the PPP-TCA pathway.

DISCUSSION

The major aim of this study was to investigate the characteristics of the systemically changed blood environment in DEX-injected bovines and clearly report the features of and the intrinsic relationship underlying glucose uptake and glucose utilization in circulating neutrophils. We found that DEX injection increased the levels of blood glucose, TP, and TC and the Ca^{2+}/P^{5+} ratio, and these indices returned rapidly to physiological levels following DEX withdrawal. This results were in agreement with earlier studies [20] reporting that DEX-treated chickens showed elevated levels of blood TP, TG and TC [25] and that Holstein heifers suffering road transportation stress had higher levels of plasma cortisol, nonesterified fatty acids, glucose and a higher Ca^{2+}/P^{5+} ratio [1]. Clearly, DEX injection-induced simultaneous hyperglycemia and hyperlipidemia, together with DEX itself, are responsible for the change in the number and function of immune cells. Our observations further showed that DEX injection increased the total leukocytes and neutrophils counts and the NLR ratio and decreased lymphocytes

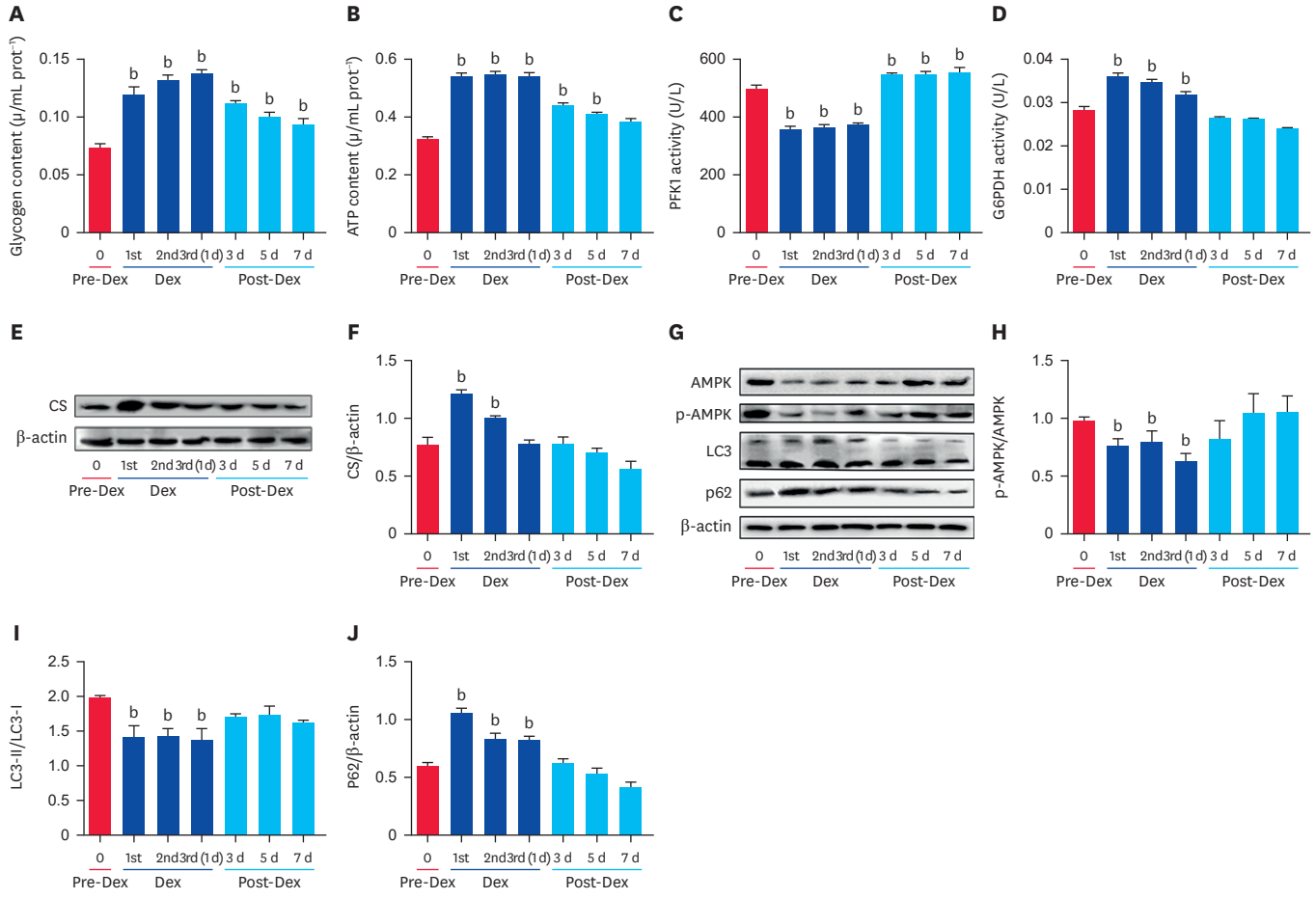

Fig. 5. DEX injection regulates intracellular glucose metabolism in neutrophils. (A) Glycogen content, (B) ATP content and (D) G6PDH activity were detected by biochemical methods. (C) PFK1 activity was detected by enzyme linked immunosorbent assay. (A-D) The results are shown as the mean ± standard error of the mean (n = 5). (E, F) CS, (G, H) AMPK, (G, I) LC3 and (G, J) p62 expression was detected by Western blotting, and β-actin was used as an internal control. (F, H, I, J) The results are shown as the mean ± standard error of the mean (n = 3). Differences were analyzed using the *t*-test.
DEX, dexamethasone; G6PDH, glucose-6-phosphate dehydrogenase; PFK, phosphofructosekinase; CS, citrate synthase; AMPK, AMP-activated protein kinase.
[a]$p < 0.05$; [b]$p < 0.01$.

counts, excluding unaffected monocytes counts. These results were consistent with previous clinical DEX treatment studies delineating that significantly increased circulating neutrophils counts (neutrophilia) mainly resulted from accelerated maturation and release from bone marrow into the bloodstream and impeded extravasation and migration as well as delayed apoptosis of cells [26]. Additionally, pharmacological concentrations of GCs have been shown to promote apoptosis of T lymphocytes [2,27] and increase the NLR [26]. Meanwhile, the unchanged monocytes count in our study might be due to the unaffected proliferation of cells by DEX [26].

Neutrophils, lymphocytes and monocytes are the primary sources of plasma proinflammatory cytokines. The inhibition of the immune response of these cells by GCs has been extensively described. DEX exposure ameliorated LPS-induced immunological injuries and decreased serum TNF-α and IL-1β levels in mice [28] and TNF-α and IL-6 levels in calves stimulated by LPS [6]. Our results were consistent with the above findings: DEX injection decreased IL-1β, IL-6 and TNF-α levels, which rapidly rose again to original levels after DEX withdrawal. Meanwhile, the suppressed NFκB (p65) activation in neutrophils following

DEX injection and its reactivation after DEX withdrawal were also highly consistent with the following findings: GCs directly act on NFκB (p65) or activator protein 1 by binding to GR and exert the anti-inflammatory actions and delayed apoptosis-related gene expression in neutrophils [29]. DEX mediates anti-inflammatory activity by reducing superoxide release and ROS levels [30-32] and reduces the mRNA levels of IL-1β, TNF-α, and IL-8 in equine and human neutrophils [7]. Although bovine monocyte function affected by GC exposure in vivo remains less documented, a few studies have found that DEX markedly inhibited phytohemagglutinin-upregulated mRNA expression of the proinflammatory cytokines interferon-γ, IL-2 and IL-4 in calf monocytes *in vitro* [33] and reversed LPS-stimulated higher levels of circulating TNF-α and IL-6 in calves [6]. A recent *in vitro* study also showed that DEX treatment reduced IL-6 and TNF-α production in healthy human monocytes [34]. Therefore, these findings suggested that DEX might directly inhibit TNF-α, IL-1β, and IL-6 production in bovine monocytes, which is partly responsible for the lower circling levels of these cytokines after DEX injection, notably, enhanced the anti-inflammatory function of bovine neutrophils by enhancing the expression of GR, down-regulating glycolysis, and inhibiting the expression of IL-1β, IL-6 and TNF-α and NFκB (p65) activation in this study.

The suppressed activation and delayed maturation of circulating neutrophils by DEX is certainly accompanied by the rebuilding of the cell energy state by regulating glucose uptake and utilization. Studies have verified that the glucose utilized in neutrophils mainly depends on intracellular glycogen breakdown and glucose absorbed from blood circulation by the GLUT system. GLUT1, but not GLUT4, has been considered the major transporter under physiological circumstances; GLUT1 can promote glucose uptake once translocated from the cytoplasm to the plasma membrane in resting neutrophils and shows increased expression in activated cells [13]. Although some data indicated that cellular net glucose uptake fully depends on SGLT1 in lymphocytes at low intracellular glucose concentrations [14,35], the expression and function of SGLT1 in neutrophils, especially in neutrophils exposed to GCs or DEX, is unclear. In our study, DEX injection concurrently enhanced the expression of GLUT1 and SGLT1 followed by a decline to preinjection levels, whereas GLUT4 expression decreased after DEX injection and then rose to higher levels than those of preinjection after DEX withdrawal. Furthermore, the up- or downregulation of the three transporters was accompanied by synchronous changes in glycogen content and ATP levels. This undoubtedly indicated that the promotion of glucose uptake by SGLT1 in concert with GLUT1 and GLUT4 guaranteed enough intracellular glucose to be used for the synchronous elevation of glycogen content and ATP levels in neutrophils under the complex blood environment induced by DEX injection.

In addition, our study also confirmed that DEX injection inhibited PFK1 activity while promoting G6PDH activity, which was also deeply involved in the observed elevated glycogen content and ATP levels excepting for the increased glucose uptake mediated by GLUTs. A recent report on human neutrophils claimed that the selective activation of PFK1 suppressed glycolytic flux through the PPP and reduced NOX2-dependent phagocytic oxidative burst [36]. This finding was consistent with the inhibition of PFK1 activity and promotion of G6PDH activity by DEX injection in our study. Beyond this question, it provided additional evidence suggesting that DEX favors an increase in glycogen content by inhibiting PFK1-mediated glycolysis instead of G6PDH-mediated PPP. DEX injection increased ATP raises another interesting question in whether enhanced PPP activation can lead to an increase in ATP under the context of glycolysis inhibition (decreased ATP production). Reports have shown that ATP is largely derived from glycolysis, while the PPP is mainly responsible for NADPH generation, and only 2% to 3% of total glucose is utilized through the PPP in resting neutrophils [37].

Our results showed that enhanced CS expression coincided with PPP activation and glycolysis inhibition. This finding encouraged us to conclude that the increase in ATP induced by DEX injection relies on the ribo-5-phosphate-glyceraldehyde-3-phosphate pathway instead of the fructose-1,6 diphosphate-glyceraldehyde-3-phosphate pathway to generate pyruvic acid, which then enters the TCA to generate ATP. It is clear then that DEX injection could boost ATP generation through the PPP-TCA pathway in bovine neutrophils, although neutrophils have very low TCA cycle activity.

Finally, we evaluated whether ATP generation is related to autophagy. Autophagy can supplement anabolic substrates and energy for cells under energy stress by degrading internal cellular components. Maintaining cellular energy homeostasis by autophagy is necessary for neutrophils survival because it can provide more ATP through different pathways in human neutrophils [38]. Although we did not find direct evidence that DEX affected neutrophils autophagy, GCs can suppress mitophagy by downregulating BNIP3-like (BNIP)/NIX via GR/PGC1α interactions [39] in hippocampal neurons, which are terminally differentiated cells. Circulating neutrophils, as highly differentiated terminal cells, show lower ATP levels in apoptotic cells [40], but exposure to GCs delays cell apoptosis [20]. In contrast to neutrophils, in proliferative cell types such as lymphocytes, GCs or DEX promotes cell apoptosis by enhancing the Bax/Bcl-2 ratio and autophagy by upregulating beclin1 and LC3 and downregulating p62 [22,23,27]. In this study, we found that DEX inhibited the activation of AMPK and LC3 and enhanced the expression of p62 and GR. These results indicated that DEX injection markedly inhibited autophagy through GR and that the increased ATP levels largely depended on PPP-TCA pathway activation rather than autophagy in bovine neutrophils.

In conclusion, DEX markedly affects blood glucose, TP and TC levels, the Ca^{2+}/P^{5+} ratio, and neutrophils and lymphocytes counts and inhibits the release of IL-1β, IL-6 and TNF-α from immune cells and NFκB (p65) activation in neutrophils. Of particular note is that the DEX-induced increase in glycogen content is likely dependent on the increase in glucose uptake by SGLT1 upregulation in concert with GLUT1 and the decrease in glucose consumption ascribed to glycolysis inhibition. Meanwhile, the synchronous increase in ATP levels and glycogen content is attributed to activation of the PPP-TCA pathway rather than autophagy in the circulating neutrophils of DEX-treated bovine. In conclusion, this study considered the DEX induced neutrophils glucose uptake and the transformation of energy metabolism from glycolysis to PPP-TCA cycle, providing a deeper understanding of clinical anti-inflammatory effects of DEX on bovine.

ACKNOWLEDGEMENTS

We thank the students of College of College of Animal Science and Technology of Inner Mongolia Minzu University who helped in this research and the support by the Natural Science Foundation of China, Natural Science Foundation of Inner Mongolia Autonomous Region and Young Scientific and Technological Talents in Inner Mongolia.

REFERENCES

1. Kang HJ, Lee IK, Piao MY, Kwak CW, Gu MJ, Yun CH, et al. Effects of road transportation on metabolic and immunological responses in Holstein heifers. Anim Sci J. 2017;88(1):140-148.
PUBMED | CROSSREF

2. MacDonald AS, Daloze P, Dandavino R, Jindal S, Bear L, Dossetor JB, et al. A randomized study of cyclosporine with and without prednisone in renal allograft recipients. Transplant Proc. 1987;19(1 Pt 3):1865-1866.
PUBMED

3. Wang Z, Malone MH, He H, McColl KS, Distelhorst CW. Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. J Biol Chem. 2003;278(26):23861-23867.
PUBMED | CROSSREF

4. Zhang L, Insel PA. The pro-apoptotic protein Bim is a convergence point for cAMP/protein kinase A- and glucocorticoid-promoted apoptosis of lymphoid cells. J Biol Chem. 2004;279(20):20858-20865.
PUBMED | CROSSREF

5. Medh RD, Webb MS, Miller AL, Johnson BH, Fofanov Y, Li T, et al. Gene expression profile of human lymphoid CEM cells sensitive and resistant to glucocorticoid-evoked apoptosis. Genomics. 2003;81(6):543-555.
PUBMED | CROSSREF

6. Plessers E, Watteyn A, Wyns H, Pardon B, De Backer P, Croubels S. Study of the immunomodulatory properties of gamithromycin and dexamethasone in a lipopolysaccharide inflammation model in calves. Res Vet Sci. 2015;103:218-223.
PUBMED | CROSSREF

7. Hirsch G, Lavoie-Lamoureux A, Beauchamp G, Lavoie JP. Neutrophils are not less sensitive than other blood leukocytes to the genomic effects of glucocorticoids. PLoS One. 2012;7(9):e44606.
PUBMED | CROSSREF

8. Kloos M, Brüser A, Kirchberger J, Schöneberg T, Sträter N. Crystal structure of human platelet phosphofructokinase-1 locked in an activated conformation. Biochem J. 2015;469(3):421-432.
PUBMED | CROSSREF

9. Corwin E, Dunlop AL, Fernandes J, Li S, Pearce B, Jones DP. Metabolites and metabolic pathways associated with glucocorticoid resistance in pregnant African-American women. Compr Psychoneuroendocrinol. 2020;1-2:100001.
PUBMED | CROSSREF

10. Ronchetti S, Migliorati G, Bruscoli S, Riccardi C. Defining the role of glucocorticoids in inflammation. Clin Sci (Lond). 2018;132(14):1529-1543.
PUBMED | CROSSREF

11. Maloney CG, Kutchera WA, Albertine KH, McIntyre TM, Prescott SM, Zimmerman GA. Inflammatory agonists induce cyclooxygenase type 2 expression by human neutrophils. J Immunol. 1998;160(3):1402-1410.
PUBMED

12. Garcia C, de Oliveira MC, Verlengia R, Curi R, Pithon-Curi TC. Effect of dexamethasone on neutrophil metabolism. Cell Biochem Funct. 2003;21(2):105-111.
PUBMED | CROSSREF

13. Maratou E, Dimitriadis G, Kollias A, Boutati E, Lambadiari V, Mitrou P, et al. Glucose transporter expression on the plasma membrane of resting and activated white blood cells. Eur J Clin Invest. 2007;37(4):282-290.
PUBMED | CROSSREF

14. Lang F, Singh Y, Salker MS, Ma K, Pandyra AA, Lang PA, et al. Glucose transport in lymphocytes. Pflugers Arch. 2020;472(9):1401-1406.
PUBMED | CROSSREF

15. Ewart HS, Somwar R, Klip A. Dexamethasone stimulates the expression of GLUT1 and GLUT4 proteins via different signalling pathways in L6 skeletal muscle cells. FEBS Lett. 1998;425(1):179-183.
PUBMED | CROSSREF

16. Weinstein SP, Wilson CM, Pritsker A, Cushman SW. Dexamethasone inhibits insulin-stimulated recruitment of GLUT4 to the cell surface in rat skeletal muscle. Metabolism. 1998;47(1):3-6.
PUBMED | CROSSREF

17. Niu L, Chen Q, Hua C, Geng Y, Cai L, Tao S, et al. Effects of chronic dexamethasone administration on hyperglycemia and insulin release in goats. J Anim Sci Biotechnol. 2018;9(1):26.
PUBMED | CROSSREF

18. Qi D, Pulinilkunnil T, An D, Ghosh S, Abrahani A, Pospisilik JA, et al. Single-dose dexamethasone induces whole-body insulin resistance and alters both cardiac fatty acid and carbohydrate metabolism. Diabetes. 2004;53(7):1790-1797.
PUBMED | CROSSREF

19. Spence JT, Koudelka AP. Pathway of glycogen synthesis from glucose in hepatocytes maintained in primary culture. J Biol Chem. 1985;260(3):1521-1526.
PUBMED | CROSSREF

20. Rose AJ, Herzig S. Metabolic control through glucocorticoid hormones: an update. Mol Cell Endocrinol. 2013;380(1-2):65-78.
PUBMED | CROSSREF

21. Parker CG, Cheung E. Metabolic control of teleost reproduction by leptin and its complements: understanding current insights from mammals. Gen Comp Endocrinol. 2020;292:113467.
PUBMED | CROSSREF

22. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;122(6):927-939.
PUBMED | CROSSREF

23. Criollo A, Maiuri MC, Tasdemir E, Vitale I, Fiebig AA, Andrews D, et al. Regulation of autophagy by the inositol trisphosphate receptor. Cell Death Differ. 2007;14(5):1029-1039.
PUBMED | CROSSREF

24. Fainsod-Levi T, Gershkovitz M, Völs S, Kumar S, Khawaled S, Sagiv JY, et al. Hyperglycemia impairs neutrophil mobilization leading to enhanced metastatic seeding. Cell Reports. 2017;21(9):2384-2392.
PUBMED | CROSSREF

25. Hu X, Li X, Xiao C, Kong L, Zhu Q, Song Z. Effects of dietary energy level on performance, plasma parameters, and central AMPK levels in stressed broilers. Front Vet Sci. 2021;8:681858.
PUBMED | CROSSREF

26. Vanacker N, Ollier S, Beaudoin F, Blouin R, Lacasse P. Effect of inhibiting the lactogenic signal at calving on milk production and metabolic and immune perturbations in dairy cows. J Dairy Sci. 2017;100(7):5782-5791.
PUBMED | CROSSREF

27. Swerdlow S, McColl K, Rong Y, Lam M, Gupta A, Distelhorst CW. Apoptosis inhibition by Bcl-2 gives way to autophagy in glucocorticoid-treated lymphocytes. Autophagy. 2008;4(5):612-620.
PUBMED | CROSSREF

28. Lu Y, Liu H, Bi Y, Yang H, Li Y, Wang J, et al. Glucocorticoid receptor promotes the function of myeloid-derived suppressor cells by suppressing HIF1α-dependent glycolysis. Cell Mol Immunol. 2018;15(6):618-629.
PUBMED | CROSSREF

29. Weber PS, Madsen-Bouterse SA, Rosa GJ, Sipkovsky S, Ren X, Almeida PE, et al. Analysis of the bovine neutrophil transcriptome during glucocorticoid treatment. Physiol Genomics. 2006;28(1):97-112.
PUBMED | CROSSREF

30. Ignacchiti MD, Sesti-Costa R, Marchi LF, Chedraoui-Silva S, Mantovani B. Effect of academic psychological stress in post-graduate students: the modulatory role of cortisol on superoxide release by neutrophils. Stress. 2011;14(3):290-300.
PUBMED | CROSSREF

31. Dandona P, Aljada A, Ghanim H, Mohanty P, Hamouda W, Al-Haddad W. Acute suppressive effect of hydrocortisone on p47 subunit of nicotinamide adenine dinucleotide phosphate oxidase. Metabolism. 2001;50(5):548-552.
PUBMED | CROSSREF

32. Dandona P, Mohanty P, Hamouda W, Aljada A, Kumbkarni Y, Garg R. Effect of dexamethasone on reactive oxygen species generation by leukocytes and plasma interleukin-10 concentrations: a pharmacodynamic study. Clin Pharmacol Ther. 1999;66(1):58-65.
PUBMED | CROSSREF

33. Maeda Y, Tanaka R, Ohtsuka H, Matsuda K, Tanabe T, Oikawa M. Comparison of the immunosuppressive effects of dexamethasone, flunixin meglumine and meloxicam on the *in vitro* response of calf peripheral blood mononuclear cells. J Vet Med Sci. 2011;73(7):957-960.
PUBMED | CROSSREF

34. de Almeida AR, Dantas AT, Pereira MC, Cordeiro MF, Gonçalves RS, de Melo Rêgo MJ, et al. Dexamethasone inhibits cytokine production in PBMC from systemic sclerosis patients. Inflammopharmacology. 2019;27(4):723-730.
PUBMED | CROSSREF

35. Wright EM, Turk E. The sodium/glucose cotransport family SLC5. Pflugers Arch. 2004;447(5):510-518.
PUBMED | CROSSREF

36. Amara N, Cooper MP, Voronkova MA, Webb BA, Lynch EM, Kollman JM, et al. Selective activation of PFKL suppresses the phagocytic oxidative burst. Cell. 2021;184(17):4480-4494.e15.
PUBMED | CROSSREF

37. Stjernholm RL, Manak RC. Carbohydrate metabolism in leukocytes. XIV. Regulation of pentose cycle activity and glycogen metabolism during phagocytosis. J Reticuloendothel Soc. 1970;8(6):550-560.
PUBMED

38. Riffelmacher T, Clarke A, Richter F, Stranks A, Pandey S, Danielli S, et al. Autophagy-dependent generation of free fatty acids is critical for normal neutrophil differentiation. Immunity. 2017;47(3):466-480.e5.
PUBMED | CROSSREF

39. Choi GE, Lee HJ, Chae CW, Cho JH, Jung YH, Kim JS, et al. BNIP3L/NIX-mediated mitophagy protects against glucocorticoid-induced synapse defects. Nat Commun. 2021;12(1):487.
PUBMED | CROSSREF

40. Yuyun X, Fan Y, Weiping W, Qing Y, Bingwei S. Metabolomic analysis of spontaneous neutrophil apoptosis reveals the potential involvement of glutathione depletion. Innate Immun. 2021;27(1):31-40.
PUBMED | CROSSREF