“The impact of vitamin D3 treatment on mitochondrial oxidative metabolism and neuroprotection in the rat hippocampus during long-term glucocorticosteroid exposure”
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Introduction: It is well known that one of the main stress response systems is the hypothalamic-pituitary-adrenal cortex (HPA) axis, which generates and modulates glucocorticosteroid (GC) levels in the body. Adverse environmental conditions may lead to overactivity of the HPA axis inducing hypersecretion of GC. The hippocampus, it seems, is particularly sensitive to the negative effects of GC exposure. High levels of GC affect its volume reduction as well as morphological, metabolic, and functional disturbance.

The main objective of my dissertation was to determine the effects of 28 days of vitamin D_3 supplementation at a dose of 600 IU/ kg/ day on mitochondrial oxidative metabolism and neuroprotection in the rat hippocampus during long-term exposure to glucocorticosteroids.

Materials and Methods: The study was performed with the approval of the Local Ethical Committee for Animal Research in Bydgoszcz, Poland (No. 10/2019). The conducted studies used two *in vivo* models of repeated exposure of animals to GC: exogenous, associated with the administration of the synthetic GC, dexamethasone (DEXA), and endogenous, through activation of the HPA axis induced by immersion in cold water. The studies presented in this dissertation were performed on Wistar rats, randomly divided into 6 groups: a control group receiving intraperitoneal 0.9% NaCl solution (n = 4-6); a group receiving intraperitoneal DEXA solution at a dose of 2 mg/ kg/day and supplemented with vitamin D_3 at a dose of 600 IU/ kg/day (n = 7-8); a group receiving intraperitoneal DEXA solution at a dose of 2 mg/ kg/day and supplemented with vegetable oil (placebo) (n = 6-7); a group subjected to cyclic cold exposure by immersion in 0-4ºC water (60 minutes per day), supplemented with vitamin D_3 (n = 8-9); a group subjected to cold water immersion and supplemented with placebo (n = 8-9); a group subjected to warm water immersion (34-36ºC) (n = 6). On the first and last day of the experiment, the animals were weighed, and blood was drawn from the tail vein. Plasma concentrations of corticosterone and vitamin D metabolites were determined by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) sequentially. After the animals were sacrificed, the hippocampus were isolated, weighed, and homogenized. The levels of specific GC and vitamin D binding receptors, as well as the levels of proteins associated with neuroprotection and mitochondrial energy metabolism (respiratory chain complex IV), were assessed by the Western blot. The

enzymatic activities of citrate synthase and cytochrome c oxidase were measured by kinetic methods. Then, the level of free radical damage to lipids and proteins in the hippocampus was assessed using ELISA and spectroscopic methods.

Results: The study showed that long-term exposure to GC, both synthetic and those associated with induction of HPA axis activity, causes a decrease in hippocampal mass. Vitamin D_3 supplementation had a protective effect on hippocampal mass with cyclic DEXA treatment in contrast to cold exposure. A partial modulation of glucocorticosteroid receptor (GR) levels, an increase in phosphorylated serine/threonine kinase (pAkt), the mature form of brain-derived neurotrophic factor (mBDNF), cytochrome c oxidase subunit II (COX II) and an increase in citrate synthase activity were observed.
In turn, long-term exposure to cold increased the level of corticosterone in the animals' plasma. The included vitamin D_3 supplementation only had a protective effect on mitochondrial oxygen metabolism and partially reduced the oxidative stress in the hippocampus.

Conclusions: Based on the results presented in this paper, it can be concluded that vitamin D_3 supplementation partially protects the hippocampus from the negative effects of GC by affecting mitochondrial energy metabolism and proteins associated with neuroprotection. The protective effects of vitamin D_3 are particularly focused on the neurobiological effects associated with DEXA pharmacotherapy.
It is interesting to note that vitamin D_3 supplementation also partially protected the hippocampus of animals exposed to cold stress from abnormalities in mitochondrial oxygen metabolism and oxidative stress-induced damage. The results of this study may contribute to a better understanding of the neurobiological mechanisms of vitamin D action under conditions of increased HPA axis activity, as well as during steroid therapy.

The research results obtained during this dissertation have been partially published in two original articles:

- Karnia, M.J.; **Korewo, D.**; Myślińska, D.; Ciepielewski, Z.M.; Puchalska, M.; Konieczna-Wolska, K.; Kowalski, K.; Kaczor, J.J. The Positive Impact of Vitamin D on Glucocorticoid-Dependent Skeletal Muscle Atrophy. Nutrients **2021**, 13, 936. https://doi.org/10.3390/nu13030936 (**IF = 4,8; MEiN = 140**).

- **Korewo-Labelle, D**.; Karnia, M.J.; Myślińska, D.; Kaczor, J.J. Supplementation with Vitamin D_3 Protects against Mitochondrial Dysfunction and Loss of BDNF-Mediated Akt Activity in the Hippocampus during Long-Term Dexamethasone Treatment in Rats. Int. J. Mol. Sci. **2023**, *24*, 13941. https://doi.org/10.3390/ijms241813941 (**IF = 4,9; MEiN = 140**)