Supplementary material

Glucocorticoid Receptor Alpha: Origins and Functions of the Master Regulator of Homeostatic Corrections in Health and Critical Illness.

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The Temporal Phases of GRα Function and the Potential Impact of Micronutrients on Its Regulation.

The temporal phases of glucocorticoid receptor alpha (GRα) function entail a series of complex molecular events and multiple control layers that unfold in sequential phases[1] (**Table S1**). These phases are crucial for their roles in stress response, immune modulation, and homeostatic adjustments. Various factors influence these phases, including hormone availability, chromatin accessibility, post-translational modifications, oxidative stress, and cellular signaling interactions.[2] Limited literature suggests that each phase is supported by specific micronutrients that may enhance GRα's function and protect it from damage, thus ensuring appropriate cellular responses during stress and recovery.

1. The *Initial Activation Phase* begins with the availability of cortisol, the primary ligand for GRα. The adrenal glands regulate cortisol production in response to stress, while circadian rhythms ensure the timely availability of hormones. Corticosteroid-binding globulin (CBG) and cortisol biosynthetic enzymes[3] facilitate cortisol's transport and availability, enabling GRα to recognize and bind to its target sites and activate the receptor's cellular functions. Essential nutrients supporting this phase include vitamins B1 and B6,[4,5] which are crucial for cortisol synthesis and regulation.[6,7] Vitamin B6 is necessary for the activity of liver glutamic-pyruvic transaminase, an enzyme linked to cortisol's gluconeogenic effects. Deficiency in vitamin B6 diminishes this enzyme's activity, affecting cortisol's function. L-ascorbic acid (L-AA), or vitamin C, is found in

high concentrations in the cortex of the adrenal glands, underscoring its essential role in cortisol synthesis. It participates in hydroxylation reactions, crucial steps in the cortisol synthesis pathway. Furthermore, vitamin C supports receptor function under oxidative conditions[8] and mitigates oxidative stress during cortisol production.[9] In patients with severe cardiac atherosclerotic disease, administering vitamins C, B1, and B6, along with cardiac glycosides, improved the adrenal cortex's glucocorticoid function.[4]

Sodium is essential for adrenal health, primarily because it affects aldosterone production. A sodium deficiency increases aldosterone secretion to maintain sodium balance while reducing the production of other adrenal hormones.[10] Hormonal fluctuations, particularly in cortisol levels, directly affect GRα activation. Cortisol levels naturally peak in the morning as part of the body's circadian rhythm, promoting GRα activation to prepare the body for daily activities.[6]

2. Once GRα is activated, the *Chromatin Remodeling and Gene Accessibility Phase* follows to make genes accessible for transcription. During this phase, chromatin remodeling processes—including histone acetylation—enable GRα to access specific genes within the DNA and regulate gene expression.[11,12] Key binding partners include chromatin remodeling complexes and histone acetyltransferases (HATs). Vitamins and minerals are crucial for chromatin remodeling and gene accessibility, acting as cofactors for enzymes and structural components of transcription factors, which influence the dynamic regulation of gene expression. Retinoic acid, the active form of vitamin A, impacts chromatin remodeling by binding to nuclear receptors that function as transcription factors. This interaction can cause changes in chromatin structure, thus influencing gene expression and cellular differentiation.[12]

Vitamin D interacts with the specific nuclear receptor, the Vitamin D Receptor (VDR), forming a VDR-RXR heterodimer that binds to the DNA's Vitamin D Response Elements (VDREs), affecting DNA and chromatin accessibility. Research shows vitamin D supplementation can impact steroid responsiveness.[13-15] The hormonally active form of vitamin D has been identified as a regulator of various enzymes involved

in the production of steroid hormones, thereby affecting both adrenal steroid hormones and sex hormones.[16,17] Notably, functional GR, specifically in T cells, is required for the VDR to signal appropriately to mediate the therapeutic effects of VitD.[17] Nutrients such as vitamin B9 provide methyl groups essential for DNA and histone modifications, and perinatal and postnatal supplementation with folic acid[18] has been shown to restore glucocorticoid receptor (GR) mRNA levels and activity in hypothalamic cells.[19] Vitamin C is essential for chromatin remodeling, acting as a cofactor for TET enzymes and JmjC histone demethylases that promote DNA demethylation and histone hydroxylation. These epigenetic modifications regulate gene expression by altering chromatin accessibility, thereby influencing cellular differentiation and proliferation.[20]

Zinc is essential for properly functioning glucocorticoid receptors (GRs) because of its role in zinc finger domains and zinc-binding proteins like metallothioneins. [21,22] Notably, zinc metallothioneins (Zn-MTs)—proteins that bind zinc—enhance glucocorticoid responsiveness by improving the receptor's DNA-binding and transcriptional activities. These interactions highlight zinc's critical role in modulating GR function and responsiveness.

3. In regulating GRα function, the *Early Post-Translational Modifications Phase* is a crucial stage where newly synthesized GRα proteins undergo various chemical changes that determine their functionality, stability, activity, and interactions with other proteins. During this phase, modifications such as phosphorylation, acetylation, and ubiquitination play a vital role in managing GRα function by influencing its stability, activity, and protein interactions. These changes are facilitated by specific enzymes that require cofactors like magnesium and zinc, highlighting the complexity and precision of cellular regulatory mechanisms. Kinases—such as mitogen-activated protein kinases (MAPKs)—and phosphatases act as key modifying enzymes. Magnesium and zinc are cofactors for numerous enzymes, including kinases and other proteins that impact GRα function. Zinc supplementation has beneficial anti-inflammatory and anti-oxidative effects in adults.[23] By orchestrating these post-translational modifications, cells can fine-tune GRα's role in gene expression, ensuring appropriate glucocorticoid responses under varying conditions.[6,24] Additionally,

vitamins B6, C, and E support overall cellular health and enzymatic activities, indirectly facilitating the post-translational modifications of GRα.

4. During the *Early Signaling and Interaction Phase*, GRα interacts with other receptors—including mineralocorticoid receptors (MR) and estrogen receptors (ER)—to modulate cellular responses to stress, metabolism, and inflammation. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) activate pathways like NF-κB and JAK-STAT that interact with GRα, influencing immune regulation. Additionally, Activator Protein-1 (AP-1) acts as a binding partner to modulate GRα's activity by regulating the transcription of pro-inflammatory and anti-inflammatory genes.

Magnesium is a cofactor for many enzymes and signaling proteins, including kinases that phosphorylate GRα, thus enhancing its ability to regulate gene expression. Adequate magnesium levels are crucial for proper receptor function and support signaling pathways that allow receptors like GRα to communicate with each other and other cellular proteins. Meanwhile, antioxidant vitamins C and E help reduce inflammation by alleviating oxidative stress, thereby preserving the integrity and functionality of GRα and its associated signaling molecules.

5. As signaling continues, the *Sustained Signaling Phase* focuses on protecting GRα from oxidative damage caused by stress. Oxidative stress can impair GRα's function and harm cellular components, making antioxidant defense mechanisms essential. Vitamins C and E are potent antioxidants, [8,25] neutralizing reactive oxygen species (ROS) and safeguarding against oxidative stress. Additionally, selenium, zinc, magnesium, and iron support mitochondrial function and enzymatic processes that help reduce oxidative damage by maintaining the integrity of cellular structures. While iron is crucial for mitochondrial function and various enzymatic activities, its levels must be carefully regulated to prevent the generation of harmful ROS through the Fenton reaction. Together, these antioxidants and minerals ensure the continued functionality of GRα, promoting cellular resilience and effective stress responses.

6. In the *Long-Term Regulation and Maintenance Phase*, the sustained activity of GRα is influenced by epigenetic modifications, interactions with commensal microbiota, and prolonged cortisol exposure. Epigenetic modifications can alter GRα gene expression; microbiota can influence immune responses and stress pathways affecting GRα, and prolonged cortisol exposure directly interacts with GRα, impacting its activity and sensitivity. Micronutrients, including vitamins and minerals, play a significant role in epigenetic regulation, affecting gene expression without modifying the DNA sequence. They serve as cofactors for enzymes involved in epigenetic modifications, such as DNA methylation and histone modification, essential for various biological processes and disease prevention. Key factors include DNA methyltransferases (DNMTs) and hepatic enzymes such as CYP3A4, 11β-HSD1, and 11β-HSD2. Vitamins B9 (folate) and B12 are essential for one-carbon metabolism, which provides methyl groups for DNA methylation. Proper functioning of this pathway is necessary for maintaining epigenetic marks that regulate genes involved in stress responses, including those encoding GRα. Microbiota-activated TLR pathways modulate immune responses and indirectly affect GRα through inflammatory cytokines. Hepatic enzymes metabolize glucocorticoids and control cortisol conversion, regulating GRα activation and tissue responsiveness. Together, these mechanisms ensure consistent GRα regulation, supporting cellular homeostasis and effective physiological responses.

Table S1. Phases of Glucocorticoid Receptor Alpha (GRα) Regulation: Key Factors, Binding Partners, Vitamins, and Micronutrients Supporting Each Phase

Temporal Phase	**Factors Affecting GRα Function**	**Binding Partners**	**Vitamins and Micronutrients Supporting GRα Function**

1. Initial Activation Phase	- Ligand availability (e.g., cortisol levels), - Adrenal gland function	- Corticosteroid-binding globulin (CBG), - Cortisol biosynthetic enzymes	- Vitamins: B1, B6, C, - Minerals: Sodium
2. Chromatin Remodeling and Gene Accessibility	- Chromatin accessibility - Histone modifications	- Chromatin remodeling complexes (e.g., SWI/SNF) - Histone acetyltransferases (HATs)	- Vitamins: B9, C, D - Minerals: Zinc
3. Early Post-translational Modifications	-Phosphorylation - Acetylation - Ubiquitination	- Kinases (e.g., MAPK), - Phosphatases	- Vitamins: B6, C, E - Minerals: Magnesium, Zinc
4. Early Signaling and Interaction Phase	- Receptor interactions (MR, ER), cytokine signaling (e.g., TNF-α, IL-6)	- Estrogen receptors (ER), - Mineralocorticoid receptors (MR), - NF-κB, AP-1	- Vitamins: C, E – - Minerals: Magnesium
5. Sustained Signaling Phase	- Prolonged ligand presence - Feedback inhibition mechanisms	- Coactivators and corepressors - Signal transduction molecules (e.g., MAPK, PI3K)	- Vitamins: C, E - Minerals: Selenium, Zinc, Iron
6. Long-term Regulation and Maintenance	- Epigenetic modifications, - Gut-brain axis signaling - Prolonged cortisol exposure	- DNA methyltransferases - Toll-like receptors - Hepatic enzymes (e.g., CYP3A4, 11β-HSD1, 11β-HSD2)	-Vitamins: B9, B12, D, E - Minerals: Zinc, Magnesium[7], Iron

Legend:

Regulatory processes refer to the complex biological mechanisms and signaling pathways that modulate the function of Glucocorticoid Receptor Alpha (GRα) throughout its various regulatory phases. These processes include hormone regulation (e.g., cortisol availability and synthesis), chromatin remodeling (which alters DNA accessibility for gene transcription), post-translational modifications (such as phosphorylation and ubiquitination), and stress response pathways. Together, these

mechanisms ensure that GRα can effectively mediate cellular responses to physiological and environmental change stimuli.

Binding partners are specific molecules or proteins that directly interact with Glucocorticoid Receptor Alpha (GRα) to modulate its activity and function. These partners include various enzymes (e.g., kinases and phosphatases that are involved in post-translational modifications), other receptors (such as estrogen receptors [ER] and mineralocorticoid receptors [MR]), transcription factors (like NF-κB and AP-1), and coactivators or corepressors (e.g., SRC-1, NCoR). These interactions are crucial for orchestrating precise cellular responses, regulating gene expression, and ensuring that GRα can effectively mediate its roles in processes such as immune modulation, metabolism, and stress adaptation.

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Table S2 Role of Thiamin, Vitamin D, and Vitamin C in the three phases of homeostatic correction.

Homeostatic Phase	Thiamin	Vitamin D	Vitamin C
Reinforce innate immunity	-	Supports innate and adaptive immune system. ⇑ TLR coreceptor CD14. ⇑ antimicrobial peptides cathelicidin and LL-37. [26] ⇑ neutrophil recruitment, activation, and function. [27] ⇑ antibacterial activity. [28].	Supports neutrophil anti-bacterial function at hypoxic inflammatory sites.[29] ⇑ neutrophil and macrophage chemotaxis, phagocytic capacity, lysozyme activity for cell elimination, and bacterial killing.[29-31] Supports lymphocyte proliferation and differentiation.[29] ⇑ production of type I interferons (IFNs) for anti-viral immune responses against influenza virus infection. [32]
Bioenergetic supply	Essential for energy metabolism and carbohydrate breakdown/ATP production[33] Thiamin pyrophosphate is a cofactor for PDH, α-KGDC.[34]	⇑ mitochondrial number, morphology, physiology, and expression of key mitochondrial proteins, resulting in increased ATP synthesis.[35]	⇑ ATP synthesis.[36]
Vascular integrity	-	Modulate endothelial function (non-genomic up-regulation of eNOS gene expression) and vascular permeability (prevents the formation of intracellular endothelial gaps) via multiple genomic and extra-genomic pathways.[37] Protective effect on the alveolar capillary membrane.[38]	Improves endothelial permeability, microvascular and macrovascular function. [39] Preserves endothelial barrier integrity [40] in synergy with GC.[41] Cofactor for dopamine and vasopressin.[42] Down regulator of NET formation in sepsis.[31,43]
Repress inflammation	Exerts significant anti-inflammatory effects: (i) ⇓ activation of p38-MAPK, (ii) ⇓ degradation of Ik-Bα, and (iii) ⇑ activation and nuclear translocation of NF-kB, ⇓ expression of cytokines and chemokines, iNOS and COX-2. [44] ⇓ nuclear NF-kB/p65 protein level, ⇑ IL-10 synthesis – ⇓ synthesis of iNOS, COX-2, Hsp70, TNF-α, and IL-6. [45] Sinergy with glucocorticoids in inhibiting IL-6 transcription. [46]	⇑ GR concentration [47] and GC function. [48] ⇓ synthesis of TNF-α and IL-1β. [28] ⇑ GC-mediated MKP-1 ⇒ ⇓ p38 MAPK-mediated inflammatory genes. [48-50] ⇑ IκBα expression ⇒ ⇓ NF-kB. [51,52] GR represses Vitamin D inactivator CYP24A1.	Cofactor for GC synthesis. [53] Improves GR function. [54] Reverses oxidation of the GR. [55] GC facilitate Vit C cellular uptake ⇓ synthesis of TNF-α and IL-6. [56] ⇓ Ik-Bα degradation ⇒ ⇓ NF-kB activation and nuclear translocation. [57]

Repress oxidative stress	⇑ Transketolase a key enzyme for the pentose phosphate pathway and for the synthesis of NADPH with glutathione cycling, an important antioxidant pathway. [58]	VDR is a GR target for PGC-1α induction α. [59] Protective against ROS production [60] ⇑ Glutathione and glutamate formation ⇒ ⇓ ROS formation. [61]	General role: electron donation as one of the most potent antioxidants Suppress NADPH oxidase (NOX) pathway. [54] Prevents the depletion of other circulatory antioxidants, such as lipid-soluble vitamin E and glutathione. [40]
Resolve and restore anatomical function	-	Restore alveolar epithelial barrier, promoting the proliferation of type 2 epithelial cells, and inhibiting fibroproliferation	⇑ expression of pro-resolution and wound healing biomarkers, better matrix organization, and collagen deposition consistent with adaptive repair. [62] ⇑ neutrophils apoptosis and clearance from inflammatory sites. [63] ⇑ Collagen synthesis, recycles other antioxidants, improves wound healing

Legend: ⇑ = increase; ⇓ = decrease.

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