The role of glucocorticoid receptor signaling in metabolic disease: a matter of time and sex

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General introduction and outline

GENERAL INTRODUCTION AND OUTLINE

Glucocorticoids are steroid hormones that play an essential role in many physiological processes, including the stress response and the maintenance of homeostasis [1-3]. These hormones, primarily cortisol in humans and exclusively corticosterone in mice, are synthesized and secreted by the adrenal glands in response to various stimuli. A factor that strongly affects adrenal glucocorticoid secretion is the circadian rhythm. Glucocorticoid effects are achieved via different signaling pathways, predominantly through binding to the glucocorticoid receptor (GR). The GR is expressed in almost all cell types in the human body. Activation of GRs initiates a cascade of events that alters gene expression and thereby regulates immune responses, metabolism and many other processes [4-8].

Synthetic glucocorticoids are potent anti-inflammatory and immunosuppressive drugs that have been widely used for treating various medical conditions including inflammatory diseases [9-11]. However, chronic glucocorticoid exposure—whether from exogenous sources or prolonged increases in endogenous levels—can result in severe metabolic disturbances, including muscle mass loss, impaired glucose and lipid metabolism, and osteoporosis [12-14]. Understanding the molecular and endocrine effects of glucocorticoids is essential to design appropriate therapeutic strategies and to mitigate the adverse effects when these steroids are used for a long period of time.

In the work presented in thesis, I investigated several metabolic disturbances associated with glucocorticoids, their biological mechanisms, and whether glucocorticoid actions are sexually dimorphic - and if so, whether interactions with sex steroids play a role. In addition, I address the optimal time of treatment with glucocorticoids in relation to reduction of side effects while maintaining therapeutic effects.

1 Glucocorticoid receptor signaling

The hypothalamic-pituitary-adrenal (HPA) axis is the main endocrine system that regulates secretion of glucocorticoids by the adrenal gland. When the HPA-axis is stimulated, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released by the hypothalamic paraventricular nucleus (PVN) into a portal circulation system that connects the hypothalamus and the pituitary gland [15, 16]. Subsequently, CRH binds to the CRH-R1 receptor in the pituitary gland which leads to the release and secretion of the adrenocorticotrophic hormone (ACTH) into the systemic circulation. ACTH will

in turn increase the synthesis and secretion of cortisol and/or corticosterone from the adrenal glands [17]. At the basal non-stressed level, glucocorticoids are released from the adrenal glands in a circadian and ultradian rhythm. This release pattern is characterized by peak levels preceding and during the early active phase, which is in the morning in humans and at the beginning of the night in mice [18]. Next to the circadian variation, physical and psychological stress is an important stimulus of HPA-axis activation.

The HPA-axis is subject to negative feedback, as elevated circulating levels of glucocorticoids exert inhibition at the hypothalamic and pituitary level, suppressing the synthesis and release of CRH and ACTH respectively [19]. This regulatory mechanism is crucial since it helps in modulating the level of glucocorticoids in the body and balancing the stress response. Dysregulation of the secretion in the HPA-axis can lead to several health-related issues. For instance, long-term stress may lead to the sustained stimulation of the HPA-axis and the constant elevated levels of glucocorticoids in the bloodstream may cause anxiety and depression, immune dysfunction, as well as metabolic diseases [20].

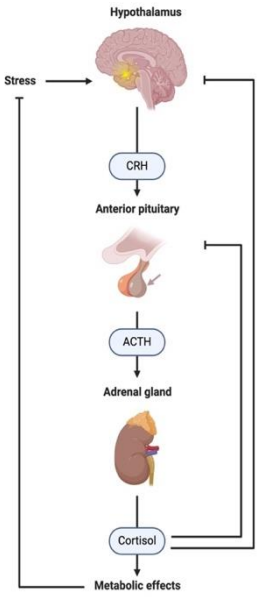

Fig. 1 Hypothalamic–pituitary–adrenal (HPA) axis. The neuroendocrine response to stress involves activation of the HPA axis, beginning with the release of corticotropin-releasing hormone (CRH) from the hypothalamus. CRH stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn triggers the adrenal glands to secrete glucocorticoids-cortisol in humans and corticosterone in rodents. Once in circulation, glucocorticoids exert both peripheral and central effects by binding to mineralocorticoid and/or glucocorticoid receptors in nearly all organs and tissues, including the brain. The hippocampus modulate hypothalamic activity, thereby regulating the HPA axis through feedback mechanisms.

The bioavailability of glucocorticoids is regulated by the balance between active and inactive forms. This process is regulated by two different enzymes that catalyze the turnover between the inactive (analogs of) cortisone or 11-dehydrocorticosterone and the active forms of cortisol or corticosterone. 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) positively affects cortisol

availability, by catalyzing the conversion of cortisone to cortisol, while 11β-HSD2 is responsible for the opposite reaction. 11β-HSD1 is predominantly expressed in metabolic tissues such as the liver and adipose tissue, locally amplifying intracellular glucocorticoid action. Its upregulation is often associated with metabolic dysregulation including insulin resistance, obesity, and dyslipidemia [21-23], emphasizing its role in metabolic homeostasis and the development of metabolic diseases. Conversely, 11β-HSD2 is expressed mainly in aldosterone target organs including the kidney and colon. It prevents cortisol or corticosterone from binding to mineralocorticoid receptors (MR), which allows selectivity for the mineralocorticoid hormone aldosterone. In this way 11β-HSD2 plays a role in the preservation of the levels of electrolytes and blood pressure. Gene mutations or inhibition of 11β-HSD2 are therefore associated with hypertension and abnormal electrolyte levels, demonstrating that 11β-HSD2 action is crucial in cardiovascular and renal functions [24, 25].

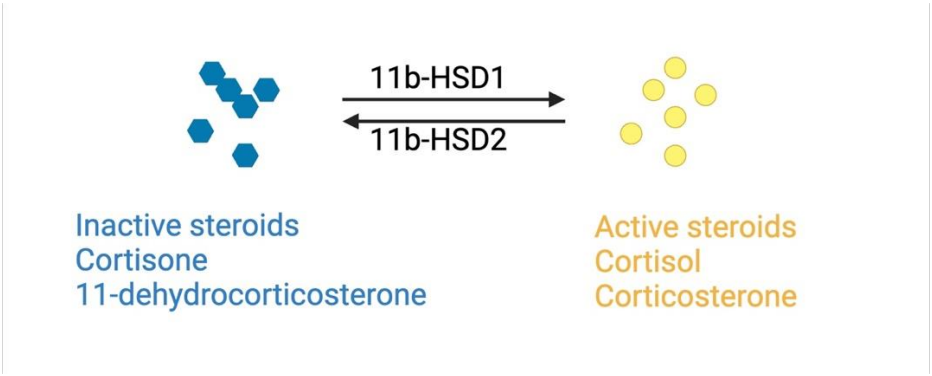

Fig. 2 Interconversion of Inactive and Active Glucocorticoids via 11β-HSD Enzymes. The balance between inactive and active glucocorticoids is regulated by the actions of 11β-HSD1 and 11β-HSD2. 11β-HSD1 converts inactive steroids, such as cortisone in humans and 11-dehydrocorticosterone in rodents, into their active forms, cortisol and corticosterone, respectively. 11β-HSD2 facilitates the reverse process, inactivating these active glucocorticoids to maintain proper signaling and prevent overstimulation.

Corticosterone-binding globulin (CBG) is a glycoprotein synthesized in the liver which modulates glucocorticoid activity. CBG can bind glucocorticoids, thereby limiting their availability in target tissues, and it plays a crucial role in the clearance of glucocorticoids from the circulation. Under basal conditions, 80% of circulating glucocorticoids is bound by CBG, around 15% to albumin and only 5% is available as the free fraction. During stress and inflammation, the concentration of glucocorticoids is increased and can saturate the binding capacity by CBG, which results in increased free glucocorticoids levels and enhanced anti-inflammatory effects [26].

Glucocorticoids can act via two types of receptors: the GR and - in cells that do not express 11β-HSD2 - the MR. These two receptor types are the members of the nuclear receptor (NR) family of intracellular receptors, which also contains the estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) [27, 28]. Many of these receptors influence various metabolic processes within different tissues. The MR is activated by the endogenous glucocorticoids, while synthetic glucocorticoids do not influence MR activity except at very high doses [29]. In contrast, the GR is activated by cortisol and corticosterone and by synthetic glucocorticoids alike [30]. Upon glucocorticoid binding, GR undergoes conformational changes and translocates into the nucleus where it binds to glucocorticoid response elements (GREs) in the DNA. GR DNA binding is influenced by tissue-specific chromatin accessibility and interactions with coregulators which help regulate transcription. GR signaling is further modulated by cellular variations in receptor isoforms, post-translational modifications, and interactions with other transcriptionally active proteins, which together shape the cell-specific response to glucocorticoid signaling across various tissues [31, 32].

GR is a modular protein comprising of several distinct domains: the N-terminal transactivation domain (NTD), the DNA-binding domain (DBD), the hinge region and the Ligand-Binding Domain (LBD), each contributing to receptor function. The NTD contains an activation function-1 (AF-1) for ligand-independent transcriptional activation [33, 34]. The GR target gene selection depends on the DBD of GR. It contains two zinc finger motifs that allow the receptor to bind to GREs within the DNA. This binding triggers other receptor domains to recruit coactivators, chromatin remodeling complexes and other transcription machinery to the promoter region of the target genes to regulate transcription [35, 36]. These interactions lead to histone modification and nucleosome remodeling, which in turn increases chromatin accessibility and transcriptional activation [37]. Conversely, GR can also repress gene expression by binding to negative GREs (nGREs) or by interacting with other transcription factors such as NF-κB and AP-1 [38, 39]. This repression often involves the recruitment of corepressors and histone deacetylases (HDACs), leading to chromatin condensation and decreased accessibility [40]. The DBD is necessary in the interaction with GREs to regulate the GR in the activation as well as suppression of genes in response to glucocorticoids. The LBD of the GR contains the ligand binding pocket of the receptor, and glucocorticoid binding to this pocket induces a structural (or conformational) change of the receptor. This conformational change forms activation function-2 (AF-2) that is required for transcriptional activation that occurs in the presence of ligand, via recruitment of coactivators and other transcription machinery [41]. Additionally, the LBD is crucial for receptor dimerization required for interaction with many GREs on the DNA [41].

2 Androgens, the HPG-axis and glucocorticoids

Androgens are a group of hormones primarily known for their role in male sexual development and function. Androgen production by the testes is regulated by the Hypothalamus-Pituitary-Gonadal (HPG)-axis [42]. Analogous to the HPA-axis, this process starts in the hypothalamus, which releases gonadotropin-releasing hormone (GnRH) in a pulsatile manner. GnRH stimulates the pituitary gland to secrete two key hormones: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH plays a crucial role in stimulating Leydig cells of the testes to secrete testosterone [43]. Similar to glucocorticoids, the levels of testosterone are also regulated through negative feedback loop in the HPG-axis.

Testosterone is mainly synthesized in the testes in men and in the adrenal glands of both men and women but in lesser amounts. It is involved in the development of the male reproductive tissues, secondary sexual characteristics and is essential to sexual health in both genders [44]. Similar to glucocorticoids, enzymatic modification of androgens is essential for this process. Dihydrotestosterone (DHT) is a potent steroid hormone with androgenic properties involved in several biological processes in human body. This sex hormone is derived from testosterone through the action of the enzyme 5-alpha reductase [45], and its physiological effects are therefore regulated by the expression of this enzyme. DHT exhibits a higher binding affinity to the AR and has increased biological activity in specific tissues that include prostate, skin, and hair follicles [46-48]. The pathways for androgen metabolism are not restricted to the conversion of testosterone to DHT. Aromatase is another essential enzyme which converts testosterone into estradiol, showing that the androgen and estrogen pathways are interrelated. This conversion is crucial in tissues such as adipose tissue, liver and the brain since (testosterone derived) estrogens via ERs regulate important (metabolic) processes in these tissues [49-51]. The equilibrium of these enzymatic conversions is extremely well maintained and loss of this balance has serious consequences for metabolic homeostasis. For instance, the activities of 5α-reductase and aromatase on androgens and estrogens affect muscle mass, body fat distribution, insulin sensitivity, and lipid metabolism [52-56], and dysregulation of these enzymes can lead to metabolic disorders.

The activation of the AR can induce genomic and non-genomic intracellular signaling. For genomic actions, testosterone and DHT diffuse through the cell membrane and bind to intracellular ARs which are present in the cytoplasm. In the nucleus, androgen-AR complexes bind to androgen response elements (AREs) of the regulatory regions of target genes [57]. Its genomic mechanisms of action are similar to those of GR. Expression of AR target genes in turn leads to the synthesis of proteins with various androgenic activities, to e.g. increase

muscle mass and to change the distribution of the body fat [58, 59]. Non-genomic actions involve rapid signaling pathways through membrane-associated AR and secondary messengers including the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway [60]. These pathways lead to rapid cellular responses that do not involve direct changes in gene expression, contribute to a variety of cellular effects, such as increased glucose uptake, enhanced muscle cell contraction, and immediate changes in cellular metabolism [59, 61]. The effects of androgens in adulthood are generally transient. For instance, muscle mass may reduce when androgen concentration is low, but typically restores upon androgen replacement [62]. However, prolonged and excessive exposure to androgens contribute to various deleterious effects as exemplified in patients with polycystic ovary syndrome (PCOS) [63, 64]. Intriguingly, these AR-driven metabolic disorders have several similarities with GR-driven effects, and therefore attenuating GR signaling may provide a novel strategy for some androgen-induced pathologies.

Several studies have shown interactions between glucocorticoids and androgens, *e.g.* with effects of glucocorticoid signaling on the HPG-axis. Elevated glucocorticoid levels as a response to stress inhibit reproductive function to prioritize self-preservation. Glucocorticoid excess suppresses the HPG-axis by inhibition of GnRH and testosterone secretion [65]. The inhibition of testosterone production by glucocorticoids was also found at the level of the testis. In the testis, GR is expressed in various interstitial cell types including Leydig cells, macrophages, fibroblasts, smooth muscle cells [66], and male reproductive accessory tissues including the epididymis and prostate are also GR-enriched [67]. Male patients with Cushing's syndrome, characterized by elevated cortisol levels, show a correlation between high cortisol levels and low plasma testosterone concentrations, illustrating a clinical condition in which glucocorticoids suppress androgen levels [68]. Administration of the synthetic glucocorticoid dexamethasone was shown to suppress testosterone levels [69]. Furthermore, glucocorticoids have been found to inhibit steroidogenesis in the testes, leading to a decrease in testosterone production [70].

In addition to their effects on testosterone levels, glucocorticoids also influence estrogen levels. The ovary, the primary source of estrogens in females, is regulated by glucocorticoids throughout a woman’s reproductive lifespan. Stress-related increases in glucocorticoids negatively affect fertility in women, compromising both ovarian function and uterine function. The GR is present in different ovarian cells including the follicles and corpus luteum and its expression is consistent throughout different stages of the reproductive cycle in rats [71]. Glucocorticoids inhibit LH-induced stimulation of steroidogenesis in ovarian cells, suppressing progesterone synthesis through direct effects on the

enzymes 3β hydroxysteroid dehydrogenase (3β-HSD) and 20α hydroxysteroid dehydrogenase (20α-HSD) [72, 73]. The ovary exhibits tissue-specific regulation of glucocorticoids, including the regulation of 11β-HSD expression during follicular maturation and ovulation [74]. These mechanisms regulate steroidogenesis, oocyte maturation, corpora lutea maintenance, and luteal regression [75, 76]. Although estrogen-glucocorticoid interactions are important, this thesis focuses primarily on androgen and glucocorticoid hormones.

3 Glucocorticoid receptor signaling in metabolic diseases

Metabolic diseases including obesity, type 2 diabetes, steatotic liver disease and cardiovascular diseases have become a global health burden. These conditions cause significant morbidity and mortality and are generally defined by a state of disrupted energy balance, insulin insensitivity and inflammation. Next to its profound effects on inflammatory and autoimmune diseases, GR also emerged as a critical player in the pathophysiology of these metabolic diseases due to its critical role in regulating metabolism, inflammation and the stress response.

GR signaling in various tissues is involved in the pathogenesis of metabolic diseases, including skeletal muscle, adipose tissue and liver [77-79]. However, these pathological effects via metabolic disturbances can differ between endogenous and exogenous glucocorticoids. The effects of synthetic glucocorticoids are often more pronounced due to their higher potency, longer half-life, and their administration may also disturb circadian regulation of endogenous glucocorticoids [80]. Endogenous glucocorticoids are tightly regulated by the body's feedback mechanisms, which (attempt to) mitigate prolonged exposure and its associated risks. In contrast, prolonged or overexposure to synthetic glucocorticoids can overwhelm these regulatory systems and lead to more severe side effects. Moreover, synthetic glucocorticoids are often administered in pharmacological doses that exceed physiological levels, further exacerbating their pathologic potential [80].

Dysregulated GR signaling has a significant impact on whole body metabolism, contributing to different metabolic disturbances. Conditions of glucocorticoid deficiency (e.g. Addison’s disease) can be the result of several causes, including autoimmune disease, genetic defects in glucocorticoids production, or pituitary disease [81]. Symptoms associated with glucocorticoid deficiency include weight loss and low blood sugar levels. In contrast, patients with Cushing's syndrome with excessive cortisol production experience health issues such as central obesity, muscle loss, high blood sugar, fatty liver, high blood pressure, elevated cholesterol, weakened immune system, and insulin resistance [82]. In

patients with metabolic syndrome, elevated glucocorticoids levels are generally found and are often associated with hyperglycemia, insulin resistance and dyslipidemia [83-85]. However, obesity is not typically linked to high systemic glucocorticoid levels, but rather to an increase in local glucocorticoid effects that contribute to the development of metabolic syndrome [86].

One of the most concerning outcomes of chronic glucocorticoid exposure is muscle atrophy, a condition characterized by the loss of muscle mass and strength. This is particularly relevant in metabolic diseases where glucocorticoid levels are persistently high. Glucocorticoids stimulate the ubiquitin-proteasome pathway and the autophagy-lysosome system which degrades proteins from the skeletal muscles into amino acids [87, 88]. This catabolic effect is achieved through the upregulation of muscle-specific E3 ubiquitin ligases including muscle RING finger 1 (MuRF1) and atrogin-1 [89, 90]. As a result, glucocorticoids reduced muscle mass and function through this process of catabolism of muscle proteins, thereby resulting in muscle wasting and weakness [91]. GR signaling additionally impairs insulin signaling pathways in muscle tissue, leading to insulin resistance [92]. Glucocorticoids are involved in the activity of insulin receptor substrate (IRS) and phosphoinositide 3-kinase (PI3K)/Akt signaling which is crucial in muscle cells for glucose uptake and glycogen synthesis [87]. Decrease in the uptake and utilization of glucose in muscles contributes elevated blood glucose levels that lead to hyperglycemia in metabolic disorders.

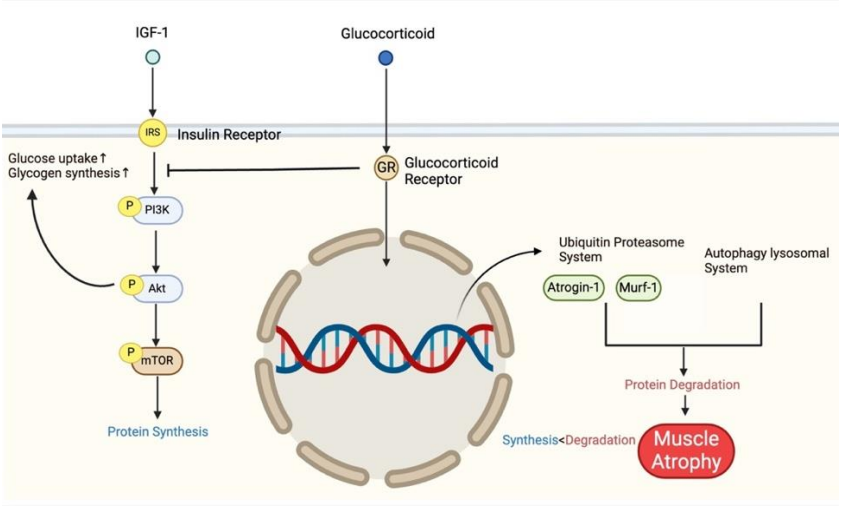

Fig. 3 Glucocorticoid-induced regulation of muscle protein synthesis and degradation pathways. Insulin-like growth factor 1 (IGF-1) signaling through the insulin receptor (IRS) activates the PI3K-Akt-mTOR pathway, promoting glucose uptake, glycogen synthesis, and protein synthesis. Conversely, glucocorticoids bind to the glucocorticoid receptor (GR) and modulate transcriptional activity in muscle cells, leading to the activation of catabolic

pathways, including the ubiquitin-proteasome system (UPS) and autophagy-lysosomal system. Key markers such as Atrogin-1 and Murf-1 facilitate protein degradation, tipping the balance towards muscle atrophy when protein degradation exceeds protein synthesis. The interplay between these anabolic and catabolic pathways highlights the impact of glucocorticoid signaling on muscle homeostasis.

GR signaling pathways also significantly influence the function of adipose tissues. Chronic activation of GR signaling can result in obesity and adipocyte hypertrophy [93]. This includes the differentiation of preadipocytes into adipocytes and the expansion of these cells due to increased lipid accumulation [93, 94]. The overexpression of key adipogenic transcription factors such as PPARγ and C/EBPα is driven by GR activation, and contributes to the expansion of adipose tissue mass, particularly in visceral fat depots [95]. The hypertrophic adipocytes become dysfunctional with reduced ability to store lipids, and with altered secretion of adipokines that in turn exacerbate the metabolic disturbances. The expanded visceral adipose tissue is metabolically active, secreting high levels pro-inflammatory cytokines, adipokines and free fatty acids, resulting in insulin resistance and cardiovascular disease [96, 97]. Moreover, overexpression of 11β-HSD1 in adipose tissues or liver is also associated with metabolic diseases. 11β-HSD1 increases local glucocorticoid levels and influences receptor activation in tissues, thereby affecting processes such as fatty acid metabolism and all other aspects mentioned above [98].

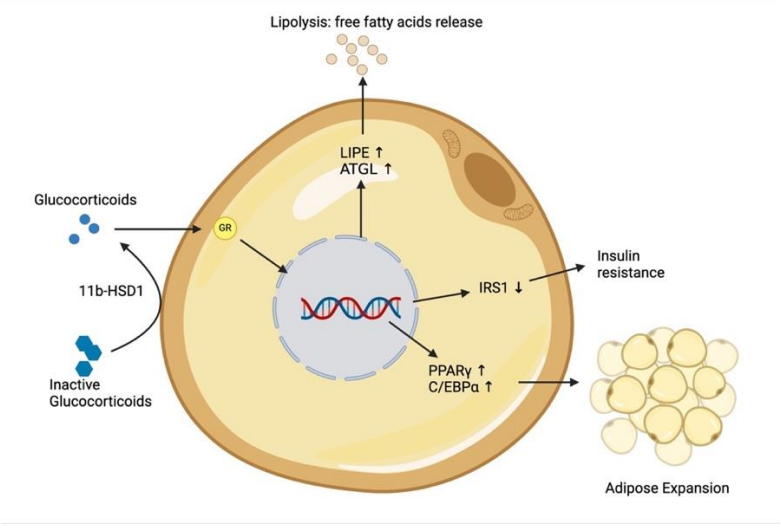

Fig. 4 Glucocorticoids signaling in Adipocytes. Glucocorticoids are activated locally within adipose tissue by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), converting inactive GCs into their active forms. Glucocorticoid activation of GRs leads to increased lipolysis, mediated by the upregulation of lipolytic enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (LIPE), resulting in the release of free fatty acids (FFAs) into circulation. This contributes to systemic metabolic changes. Concurrently, glucocorticoids

impair insulin signaling by reducing Insulin Receptor Substrate 1 (IRS1) activity, promoting insulin resistance. Additionally, Glucocorticoids enhance the expression of transcription factors, including peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPα), which drive adipocyte differentiation, hypertrophy, and hyperplasia. These combined processes contribute to adipose tissue remodeling and expansion, further exacerbating obesity-related metabolic dysfunctions.

The liver is a central organ in glucose homeostasis and GR signaling significantly affects hepatic glucose metabolism. Glucocorticoids increase gluconeogenesis through the upregulation of key enzymes expression in the hepatic gluconeogenic pathway such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [99]. In Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD), the chronic activation of GR signaling stimulates the hepatic gluconeogenesis and thus causes hyperglycemia and impaired glucose tolerance [100, 101]. In addition, prolonged activation of GR exacerbate hyperglycemia by disrupting glycogenolysis in states of fasting and stress [101]. Moreover, glucocorticoids affect lipid fluxes in the body, which may also contribute to obesity and metabolic disease [102, 103].

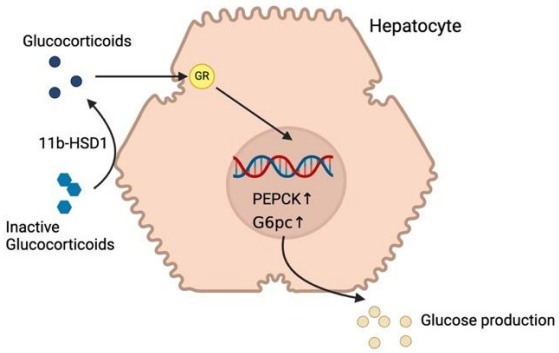

Fig. 5 Glucocorticoid-Regulated Hepatic Gluconeogenesis. Within the liver, inactive glucocorticoids are enzymatically converted into their active forms by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), thereby enhancing their local bioavailability. The GR signaling cascade upregulates the expression of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6pc), which are critical for the synthesis and release of glucose from non-carbohydrate substrates.

Given the central role of GR in metabolic diseases, intervention of GR signaling could be a potential therapeutic approach. Furthermore, novel and targetable biochemical pathways can be discovered by understanding the tissue-specific effects of GR and the molecular mechanisms behind its interactions with other metabolic regulators.

3.1 Androgen and glucocorticoid signaling crosstalk in metabolic tissues and sexual dimorphism of glucocorticoid effects

Sexually dimorphic effects of glucocorticoids have been observed in metabolic processes including inflammation and glucose metabolism. Males and females exhibit sex differences in transcriptional regulation in response to glucocorticoid treatment, involving differential regulation of signaling pathways such as apoptosis in thymocytes [104] and circadian rhythm of skeletal muscle, liver, adipose tissues, kidney [105-108]. In addition, chronic glucocorticoid exposure-induced metabolic alterations differ between sexes, in which male mice show increased blood glucose levels, insulin resistance, insulinemia, adiposity and hyperlipidemia as compared to female mice [109]. These findings suggest that males are more susceptible to the adverse metabolic effects of glucocorticoid exposure.

In skeletal muscle, previous studies suggest steroid hormone interaction between androgens and glucocorticoids. Dexamethasone treatment decreased muscle weight in male rats, which was prevented by concurrent administration of testosterone [110, 111]. Androgen administration thus protects against glucocorticoid-induced muscle atrophy, and this is likely mediated via downregulation of muscle specific ubiquitin ligases atrogin-1 and Murf1, which are known to be involved in glucocorticoid-induced protein degradation and muscle wasting [112]. These findings suggest direct crosstalk between glucocorticoids and androgens in skeletal muscle. In this tissue, the two steroids tend to have opposite (anabolic and catabolic) effects.

In white adipose tissue and liver, glucocorticoid-induced gene expression is in part dependent on AR signaling [113]. This suggests that AR activity can – in contrast to effects in skeletal muscle - increase GR-induced transcription in various peripheral tissues and this is possibly related to metabolic outcomes. In male but not in female mice, chronic exposure to glucocorticoids inhibits thermogenic activity in brown adipose tissue. [79, 114], indicating a sexual dimorphism that is possible related to differences in androgen and/or estrogen signaling. Excess corticosterone leads to lipid accumulation and a white adipose tissue-like appearance of brown adipose tissue in male mice, which is reversed by orchiectomy and restored with DHT administration [79]. Furthermore, DHT treatment potentiates GR signaling in brown adipose tissue in intact male mice [79]. In contrast, female mice are inherently more resistant to glucocorticoid-induced effects and exhibit lipid accumulation in brown adipose tissue following AR activation with DHT [115]. Altogether, many metabolic effects of glucocorticoids, including insulin resistance, seem to be androgen-dependent in mice.

Glucocorticoids and androgens exhibit different interactions in various tissues via different potential mechanisms. This crosstalk may involve competitive binding to shared response elements and possible coordination in the process of transcription. GR DNA binding is dependent on chromatin pre-accessibility [116], but can be influenced by AR-mediated chromatin opening [117]. In addition, various modulatory coactivators and the chaperone protein FKBP51 can affect GR signaling and are also associated with AR signaling, contributing to the complex crosstalk between glucocorticoids and androgens [118]. Other mechanisms potentially involve a negative androgen response element (nARE) in the GR promoter, overlapping cistromes of GRs and ARs, and potential cooperative transcriptional regulation through assisted loading [119]. This interference can result in mutual repression or modulation of target gene expression, affecting metabolic pathways regulated by both receptors. Besides direct interaction, AR activity can induce 11β-HSD1, influencing the local balance of GR and AR activation [120]. In addition, cytochrome P450 Enzymes (CYPs) are involved in the metabolism of both glucocorticoids and androgens. Regulation of CYP enzymes by GR and AR can affect the clearance and activity of these hormones, influencing their overall effects on metabolism [121].

The main focus of this thesis is crosstalk between glucocorticoids and androgens, but it should be noted that estrogens can also interact with glucocorticoids at the endocrine and molecular level. Estrogen signaling can contribute to the sexually dimorphic effects of glucocorticoids, and molecular interactions between these hormone systems play a crucial role in shaping metabolic processes and inflammatory responses.

3.2 The role of circadian glucocorticoid signaling in metabolic health

The daily oscillation of glucocorticoids is controlled by the central clock and the adrenal clock. In the suprachiasmatic nucleus of the hypothalamus, the central clock controls the circadian rhythm by regulating the activity of HPA-axis and the sympathetic innervation of the adrenal gland [122]. This regulation concerns the release of CRH and ACTH in response to environmental stimuli [123]. In addition, the adrenal gland also has an intrinsic clock that controls the steroid production and its response to ACTH. This peripheral clock is synchronized by the central clock and forms part of the regulation of this rhythm by controlling the adrenal's capability to secrete glucocorticoids [124]. This regulation is crucial for optimizing physiological processes and behavior at the right time of day [125]. The circadian rhythm of adrenal glucocorticoids is an important 'zeitgeber' mechanism for many cells in the body, and has significant implications for human health and disease.

The circadian secretion of glucocorticoids plays a vital role in regulating energy balance by increasing glucocorticoids levels before the active period [126]. Imbalances in glucocorticoid rhythms are associated with metabolic disorders like obesity, diabetes, dyslipidemia, and atherosclerosis [127]. Pathological excess or glucocorticoid insufficiency can lead to symptoms affecting metabolic functions, but loss of rhythmicity is often intrinsic to these situations, and disrupted circadian glucocorticoid rhythms are also linked to metabolic disorders [128]. The circadian aspect may well play a role in the onset or progression of conditions like obesity, type 2 diabetes, dyslipidemia, and atherosclerosis.

The strategies of chronotherapy in medicine have gained attention in the recent years, with studies demonstrating that the timing of medication administration may influence therapeutic outcomes [129, 130]. Recent findings suggest that whether or not the timing of glucocorticoid administration aligns with body's endogenous circadian rhythms may significantly influence their metabolic effects [130, 131]. In the clinic, morning compared to evening administration of glucocorticoids, when given in a pattern consistent with the endogenous rhythm of cortisol, improved glycemic control and reduced insulin resistance [132, 133]. The GR itself is also subjected to circadian regulation with variations in its expression and responsiveness during the day. Administration glucocorticoids when endogenous glucocorticoids levels are high can potentially minimize the negative effects including insulin insensitivity and dyslipidemia development caused by the prolonged exposure. However, the underlying mechanisms and clinical implications of these findings remain to be fully elucidated [134].

OUTLINE OF THIS THESIS

In this thesis we investigated how sex (hormones) and time can influence the functional and transcriptional response of glucocorticoid signaling, with a particular focus on metabolic processes in different peripheral tissues and under different pathological conditions.

In chapter 2, we investigated the potential sex differences in the effects of chronic corticosterone exposure and synthetic glucocorticoid treatment on muscle atrophy and dysfunction in mice. This revealed robust sex differences in muscle function and transcriptome in response to glucocorticoid exposure. Increased corticosterone exposure reduced grip strength specifically in female mice, while muscle mass decreased in both sexes. On skeletal muscle transcriptome, we observed that male mice exhibited more pronounced transcriptional variations in response to corticosterone treatment compared to

female mice. Altogether these findings help to outline the influence of sex on the skeletal muscle response to glucocorticoids.

In chapter 3, we evaluated whether the timing of synthetic glucocorticoid treatment affects the development of (metabolic) side effects. We found that out-of-phase but not in-phase treatment of synthetic glucocorticoid betamethasone induced insulin resistance and hyperinsulinemia. In the context of glucose metabolism, in-phase treatment generally caused less side effects compared to the out-of-phase treatment. The time of treatment in relation to the circadian variation in endogenous glucocorticoid levels should be considered when measuring glucocorticoid response.

In chapter 4, we investigated the role of GR signaling in the metabolic symptoms of polycystic ovary syndrome (PCOS) using a mouse model of prolonged DHT exposure. We observed that *Nr3c1* (GR) and *Hsd11b1* mRNA expression were upregulated various tissues of DHT-treated mice, suggesting a deregulated GR signaling. We evaluated the importance of GR signaling by performing treatment with a selective GR antagonist and found that this alleviated DHT-induced hyperglycemia and restored glucose tolerance. Given the similarities in metabolic symptoms between PCOS and excess glucocorticoid exposure, our results suggest that GR signaling may contribute to the metabolic symptoms observed in PCOS, but further research is required to determine the relevance of these findings to humans. To conclude, results of these studies and indications for human applications are discussed in chapter 5.

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