3

Adverse effects

As previously mentioned, the use of steroids is impeded by two major problems. The first problem is loss of response to treatment. In some patients response to treatment reduces over time, requiring a higher dose to maintain the initial response. But a proportion of this group grows entirely refractory to treatment. The underlying cause is unclear and still subject to debate[1]. The second objection to GC treatment is its associations with a rather large number of severe adverse effects, particularly when using a systemic high dose. These adverse effects have been studied quite extensively, with the intention of discovering ways to reduce or even prevent them. With some of these adverse effects the underlying GC driven mechanism has been fully elucidated. Here we will discuss in broad detail which adverse effects are associated with GC treatment and what is known about the underlying mechanism.

GCs are synthetic analogues of a steroid hormone, which in the body serves a wide range of specific biological functions. Part of these functions is related to conditions such as stress or anxiety, which cause the levels of cortisol to increase[2]. In absence of stress, the level of cortisol fluctuates with a circadian rhythm, rising just before waking in the morning. This rise is believed to prepare people for the stress that is associated with everyday life[3].

Secretion of cortisol is self-limiting because the hormone binds receptors in the hypothalamus and anterior pituitary gland, reducing secretion of CRH (corticotropin releasing hormone) and ACTH (adrenocorticotropic hormone) and thereby inhibiting the stimulus that causes production of cortisol. High levels of synthetic GCs have a similar effect but due to a higher affinity for the GR and longer half-life, the effect can be much stronger. As a result, production of cortisol can be inhibited for as long as two months after stopping the treatment. However, this problem can be quite easily solved by slowly decreasing the dosage before stopping treatment.

Depending on the route of administration some adverse effects are more prominent than others. Topically applied GCs (such as skin crèmes or eye

droplets) will have mainly local side effects. Here we will focus primarily on side effects associated with systemic administration.

Adipogenesis

Next to the suppression of the hypothalamic-pituitary-adrenal function, long-term treatment with GCs can cause Cushing's syndrome. This syndrome results from Cushing's disease, a condition described by Harvey Cushing in 1932[4]. Overproduction of CRH or ACTH, for example due to an adenoma in the pituitary gland, results in elevated cortisol levels. Elevated levels have a profound effect on the human body and this effect is similar in patients who are treated with GCs. Striking is the change in appearance. Patients gain weight, particularly around the waist, back of neck (buffalo hump) and face (moonface). The cause for GC induced obesity is related to the biological role that GCs play in adipose tissue physiology. GCs can induce lipogenic genes (ACC, FAS) provided that insulin is present[5, 6], regulate lipolysis (ATGL, HSL, MGL)[7-9], adipose endocrine function (release of chemokines from the adipose tissue)[10] and restrain adipose tissue inflammation in obesity[11]. They are required for precursor cells to differentiate into adipocytes and necessary for keeping specific adipocyte related genes active to prevent isolated adipocytes or adipocyte tissue from dedifferentiation[12]. Interestingly, people suffering from obesity do not have increased blood cortisol levels but have higher local concentrations of cortisol in their adipose tissue. This is due to a higher activity of 11ß-hydroxysteroid dehydrogenase-1 (HSD1), which converts the inactive cortisone to cortisol[13]. In mice adipocyte specific overexpression of HSD1 leads to obesity with metabolic syndrome[14].

Apart from effects of GC treatment that relate to the adipose tissue, patients can experience an increased appetite[15]. The appetite in humans correlates with changes in adrenal steroid levels. Anorexia and weight loss for example is a typical symptom of Addison's disease, caused by adrenal insufficiency. It may also provide an answer on why people feel the need to eat under stress related conditions as stress increases cortisol levels. But how GCs affect appetite is unclear. On a molecular level GCs have been

found to elicit both orexigenic and anorexigenic effects. GCs increase neuropeptides that increase the appetite (NPY and AgRP) but on the other hand also inhibits signals that are associated with anorexia such as CRH.

Skin (thinning & striae)

Another symptom of Cushing's syndrome is the thinning of the skin and related to this, the appearance of striae. These symptoms are more common to topically applied steroids but also occur during systemic treatment. The skin adopts a paper like property, becoming fragile and easily damaged. Histological changes include a reduction of the epidermis[16], a decrease in keratinocyte size[17], reduction in the number of fibroblasts[18], rearrangement of the geometry of the dermal fibrous network[19], and a decrease in the size of both the cell layers and of the lipid lamellae that are in between the cell layers of the stratum corneum[20]. Because GCs reduce the production of collagen by fibroblasts[21], the skin becomes more rigid and less flexible. As a consequence the skin rips more easily resulting in stretch marks known as striae. These processes are affected in the skin because under normal conditions fibroblast proliferation, keratinocyte differentiation and collagen production are regulated through the local production of cortisol by HSD1[22, 23]. In has been shown in mice that the adverse effects of GC treatment on the skin can be countered by selective inhibition of HSD1[24].

Two of the most extensively studied adverse effects of GC treatment are loss of strength of bone and muscle[25]. The underlying mechanisms have been largely uncovered and may help prevent these side effects when GC treatment is compulsory.

Muscle (loss of strength)

Loss of muscle strength is a result of muscle atrophy. The breakdown of muscle is considered to be one of the major catabolic GC mechanisms[26]. GCs cause the thickness of the muscle fibers to reduce and the protein content of the myofibrils to decrease[27]. The decrease in protein content is the result of an increased breakdown and decreased synthesis. The

increased breakdown is the result of activation of the ubiquitin proteasome and lysosomal systems by increasing the expression of so called Atrogenes, genes that are involved in atrophy (FOXO, Atrogin-1, MuRF-1)[28]. The decreased protein synthesis results from a direct inhibition of the mTOR pathway by GCs[29]. The underlying cause of these changes in protein homeostasis may be the result of differences in expression of two growth factors which are produced in the muscle tissue; Insulin-like Growth Factor (IGF)-I[30], a muscle anabolic growth factor and Myostatin (Mstn)[31], a muscle catabolic growth factor. In animal models, muscle specific overexpression of IGF-I[32] or deletion of Mstn protect against GC induced muscle atrophy[33, 34]. GCs have been shown to inhibit IGF-I and to stimulate Mstn. The latter contains a GRE in the promoter region of the gene but more importantly, GCs stabilize Mstn mRNA[35].

Bone (loss of strength)

GCs can also greatly affect the health and quality of bones by suppression of osteoblast function and a reduction of bone mineral density[25]. But although high levels of GC can result in loss of bone, physiological concentrations of cortisol are important for the formation of bone mass. Tissue specific knockout of the GR in bone results in a reduction of the total bone mass[36]. And similarly, overexpression of HSD2 in osteoblasts reduces bone mass[37]. GC cause osteoblasts to produce Wnt proteins, which perform a paracrine function on neighboring mesenchymal stem cells[38]. The Wnt proteins induce Wnt signaling, which results in the accumulation of ß-catenin and the expression of transcription factor RUNX2 which together drive differentiation of stem cells into an osteoblastic lineage. Additionally, the Wnt proteins induce Mmp14 production in chondrocytes[39]. This metalloproteinase is important for the breakdown of extracellular matrix during the process of ossification. Failure to do so causes developmental anomalies such as delayed intramembranous ossification of the cranium as is apparent from HSD2 overexpressing mice.

But despite, or maybe because, of the role GCs play in bone formation, patients receiving high levels of systemic GCs can expect to lose bone, up to 12% in the first year of treatment and 2-3% in the years thereafter[40]. High levels of GCs cause an inhibitory effect on the both the function and differentiation of osteoblasts and induce apoptosis in this cell type[41, 42]. As a result bone formation is inhibited in a matter of hours after starting GC treatment. In contrast to physiological concentrations, high levels of GCs inhibit production of Wnt proteins by mature osteoblasts[43]. Consequently mesenchymal stem cells do not accumulate ß-catenin or increase production of RUNX2[44]. Moreover, GCs increase expression of glycogen synthase kinase 3ß (GSK-3ß), a protein that drives ß-catenin ubiquitinylation and proteasomal degradation and increases the expression of proteins that inhibit Wnt signaling[45]. High levels of GCs also inhibit another important regulator of osteoblast differentiation, bone morphogenetic protein 2 (BMP2). Expression is inhibited directly but also by increasing the expression of follistatin and Dan, two BMP-2 antagonists [46] and decreasing expression of IL-11[47] which stimulates expression of BMP-2. The decrease in number of osteoblasts also decreases the amount of proteins produced by these cells such as collagen and osteocalcin. Although only shown in vitro, GCs arrest osteoblast cell cycle progression in the G0, G1 (amongst others by decreasing expression of Cyclin D2 and increasing expression of p27kip1) and G2 phase (by decreasing expression of Cyclin A)[48]. And finally, GCs induce apoptosis in osteoblasts by increasing the expression of pro-apoptotic genes (Bim, Bak, P53)[49] and decreasing the expression of pro-survival genes (Bcl-XL)[48].

More than nighty percent of bone is made up of osteocytes. These cells are situated in oblong spaces called lacunae and are interconnected through small canals termed canaliculi. These canals serve as a way for cells to communicate, acquire nutrients and when necessary, the recruitment of osteoblasts. The fluid that is stored in this network of canals provides the bone with strength. Even in the case of minimal loss of bone, high levels of GCs reduce the number of osteoblasts which in turn causes fluid content and vascularity of the bone to decrease which leads to a reduction of bone strength.[50]. Furthermore osteocytes resolve cellular damage through

autophagy[51]. Prolonged treatment causes a pathological buildup of autophagosomes in the osteocytes[52]. Material secreted from these autophagosomes pollutes the microenvironment of the osteocyte and results in cell death.

Although GC treatment can also affect osteoclasts by extending their lifespan[53], the negative effect of GC treatment on bone quality relies mainly on osteoblasts and osteocytes. Protecting osteoclasts from GC treatment by cell specific overexpression of HSD2, had little effect on the quality of bone tissue[53]. This in contrast to the protection of osteoblasts (and osteocytes) from GCs in which case the quality of bones was maintained even in prolonged presence of high levels of GCs[41].

Glucose (diabetes)

Much as the name suggests GCs play an important role in the metabolism and uptake of glucose[54]. GCs antagonize the effect of insulin and can have a profound effect on blood glucose levels. Prolonged elevated blood glucose levels (hyperglycemia) resulting from GCs is commonly referred to as steroid induced diabetes mellitus[55]. This type of diabetes is in most cases transient in nature and the situation will return to normal after the GC treatment is stopped.

In response to high blood glucose levels, insulin is secreted by ß-cells in the pancreas. This causes the liver to increase conversion of glucose into glycogen through a process called glycogenesis. Insulin stimulates a Glut4 transporter mediated uptake of glucose by adipocytes and skeletal muscle cells. The effects of GCs on blood glucose levels are exactly opposite[56] and cause the ß-cells to dysfunction[57]. Although not all the steps in ß-cell insulin release have been elucidated, it is clear that the uptake of glucose by ß-cells causes an increase in ATP-ADP ratio which closes ATP sensitive potassium channels resulting in depolarization of the cellular membrane, leading to an influx of calcium and ultimately exocytosis of insulin filled granules[58]. GCs decrease glucose metabolism in ß-cells and thereby impair insulin release, disabling the body to respond to high glucose levels. In a similar fashion GC affects adipocytes and skeletal muscle cells,

decreasing glucose uptake in response to insulin release. Moreover, GCs affect insulin receptor (IR) signaling by decreasing expression of direct downstream signaling molecules such as insulin receptor substrate 1 (IRS1), phosphoinositide 3-kinase (PI3k) and protein kinase B (PKB)[59]. In skeletal muscle cells this effect is aided by an increase in proteolysis[56]. The resulting increased amino acid concentration has been reported to interfere with insulin signaling. The decrease of signaling molecules downstream of the IR causes cells to be less sensitive to insulin, decreasing glucose uptake. In adipocytes it has been reported that GCs increase lipolysis through downregulation of cyclic-nucleotide phosphodiesterase 3B[9]. An increase in lipolysis leads to elevated fatty acid plasma levels, which also negatively affects glucose uptake. And finally GCs have a direct effect on glycogen synthesis kinase 3 (GSK3) in skeletal muscle cells[60]. This leads to inactivation, causing a decrease in glucose conversion and thus blood glucose levels remain increased.

Blood pressure (hypertension)

Blood pressure is the cumulative result of blood volume, the viscosity of the blood and the resistance that is caused by vascular diameter and flexibility. This is regulated through the renin-angiotensin system and aldosterone release, which affects both vascular diameter and body fluid volume. Rise in blood pressure is a common side effect of GC treatment causing the cardiovascular associated mortality rate to increase fivefold[61]. In general it is assumed that the rise in blood pressure is the result of increased renal sodium and water reabsorption due to binding of GCs to the mineralocorticoid receptor (MR), also known as the aldosterone receptor[62]. Indeed GCs bind the MR just as easily as mineralocorticoids do. This off target effect of GC treatment will certainly play a role in GC induced hypertension. However, there are a number of observations that indicate that GCs induce hypertension primarily through activation of the GR. For example the glucocorticoid budesonide, commonly used for the treatment of asthma, can hardly bind the MR and yet, treatment induces hypertension[63]. More importantly, treatment with the GR antagonist RU486 protects against hypertension in patients suffering from Cushing

syndrome even though (despite the close similarity with the GR) the MR shows no affinity for RU486[64, 65]. And finally, blocking sodium and water retention through treatment with the MR antagonist spironolactone does not prevent GC induced hypertension[66].

In light of the above, GC induced hypertension is unlikely to be the result of activation of the MR but appears to be regulated through the GR. Long-term adjustment of blood pressure levels is regulated through the renin-angiotensin system in the kidneys. The kidneys release renin from their afferent arterioles. Renin hydrolyzes circulating angiotensinogen into the peptide angiotensin I. This peptide is then converted in the lungs by angiotensin-converting enzyme (ACE) into the potent vasoconstrictor angiotensin II. The GR is expressed throughout the kidneys but its role in the kidneys is unclear. Several studies suggested a functional role for the GR in the kidneys, in some cases even a role that may affect blood pressure primarily through an increase in sodium reabsorption[67-70]. However, experiments in animals have repeatedly shown that GCs induce natriuresis[71], making these results difficult to interpret. And perhaps the conclusion should be that GCs affect blood pressure mainly through effects on vascular smooth muscle cells. This would also fit with the relatively short time it takes for GC treatment to increase blood pressure. One study revealed that phenylephrine-induced vasoconstriction of isolated aortic rings is enhanced in a dose dependent manner by GC treatment[72]. This effect was blocked by pretreatment with L-NAME, an endothelial specific inhibitor of cGMP, suggesting that the effect might be dependent on endothelial cells. Indeed, in a recent study where the GR was conditionally knocked out in either smooth muscle cells or vascular endothelium, the latter appeared completely resistant to GC induced hypertension supporting a role for vascular endothelium[73]. And finally in vivo data obtained from a hypertension model in rats revealed that the effect of GCs on total peripheral pressure could be rescued by concomitant treatment with the vasodilator minoxidil, even though the GC induced hypertension remained unchanged[74]. This was explained by an increase in cardiac output. Considering the complexity of blood pressure regulation and the

many systems and cell types involved, further research is required to fully clarify this adverse effect.

Eye

Over time GC treatment increases the risk of developing glaucoma or cataract dose dependently [75]. Cataract is a clouding of the lens, which decreases vision. Although development of cataract as an adverse effect of GC treatment normally takes months or even years to develop, cases have been reported in which patients on a low dose developed cataract after only two months[76]. GCs induce a specific type of cataract, which is located in the center (pole) at back of the lens, termed posterior subcapsular cataract (PSC). This cataract is characterized by aberrant lens epithelial cells that have migrated from the lens equator to the pole. Although it has been challenged for a long time, recent studies have revealed the presence of a functional GR in these cells[77]. Treatment with GCs induces transcription of a number of genes of which GILZ and PAI-1[78] are most prominent. Although no functional data is available, both are involved in apoptosis, differentiation and migration. In GC induced cataract the proliferation and differentiation of lens epithelial cells is confounded. Normally the lens is replenished by new lens fibers through the differentiation of epithelial cells on the lens equator, into fiber cells lacking DNA and organelles. In PSC histological studies revealed the presence of semi-differentiated cells containing degenerating nuclei and few organelles on the posterior pole of the lens[79]. Another important mechanism in the etiology of PSC seems to be the reduction of lens glutathione (GSH)[80]. This molecule is the body's most potent antioxidant and protects macromolecules against reactive oxygen species and helps with H_2O_2 detoxification. Reduction of GSH is considered to be the main mechanism behind age related cataract and its role in PSC is similar. Experiments with in vitro cultured lenses showed that the effect of GC on GSH could be rescued with RU486[81], demonstrating involvement of the GR. The last and longest existing hypothesis on GC induced PSC is based on an interaction between steroids and lens proteins which eventually leads to conformational changes and oxidation of these proteins[82]. Study results

are often contradictory but its role cannot be denied and probably plays a part in PSC.

A second adverse effect of GC treatment that affects the eye is GC induced glaucoma[83]. Glaucoma is an increase of intraocular pressure that may result in optic neuropathy if left untreated for too long. Changes in intraocular pressure are normal and pressure reflects the diurnal cortisol levels in the blood[84]. Adrenalectomized patients lack these diurnal changes in pressure supporting a role for cortisol in intraocular pressure. The pressure increases due to a decreased outflow of the aqueous humour[85], the fluid in the anterior chamber. It is believed that GCs increase outflow resistance by inhibiting degradation of extracellular matrix material in the trabecular meshwork[86]. This is the tissue that separates the anterior chamber from Schlemm's canal, the canal that serves to deliver the humour to the bloodstream. There is substantial evidence that supports the deposition of extracellular matrix[87-91] but the underlying mechanism remains to be elucidated.

The central nervous system

GCs play an important role in the central nervous system (CNS) both in function and in homeostasis. The elevated levels that result from GC treatment or Cushing's syndrome can have profound effects on the anatomy of the brain and increase the risk of developing psychiatric diseases, cognitive impairment, mood changes and sleep disturbances[92]. GCs can pass the blood brain barrier and exert their effect anywhere in the brain. This includes binding the GR in cerebral neurons and glial cells but also binding of MR in the limbic system[93]. Effects of GCs on the nervous system include non-genomic effects such as the rapid (>2 min) changes in electrical activity of neurons[94]. Long term exposure affects regenerative sprouting of axons that follows differentiation of hippocampal neurons[95], one of the two places in the CNS that is allowed to regenerate. It may lead to apoptosis, atrophy and cell death in the neurons of the hippocampus[96].

These effects on the CNS may have serious implications. The hippocampus is pivotal for processing and the storage of memories[97]. A striking example is why some distinct memories are long lasting and in great detail while most other memories are only vaguely remembered for a short time. The cause lies in the elevation of cortisol levels during moments of arousal, stress or excitement. This mechanism of memory consolidation is very likely evolved as means to ensure survival, retaining information more easily during moments of stress or trauma[98]. However, chronic stress or chronically elevated cortisol levels in Addison's disease or during treatment, can induce amnesia[99] but also cause problems with visual-spatial processing, conditional response, reasoning and verbal fluency[100]. This is also reflected in Cushing patients who learn at a slower pace, have a smaller short-term memory volume, memory contamination and false appraisal of task performance[101] suggesting extrahippocampal effects of glucocorticoids on memory impairment such as the amygdala and prefrontal cortex. Both are involved in connecting emotions to experience.

When considering the use of GCs, adverse effects on behavior or emotional state are rarely contemplated. And yet 75% of patients treated with GCs experience mood disturbance, which in 5% is quiet severe[102]. These effects have been described since GCs entered the clinic in the early fifties. And unlike osteoporosis, muscle atrophy or the thinning of skin, adverse effects on the CNS can take as little as one dose. Adverse effects on the CNS include a wide range of cognitive and emotional effects, including agitation, anxiety, apathy, auditory and visual hallucinations, delusions, depression, distractibility, disturbances of body image, emotional lability, hypomania, insomnia, intermittent memory impairment, mutism, perplexity, pressured speech, and sensory flooding[103]. In rare cases patients can develop serious psychotic disorders or even delirium[104] and dementia[105]. But even if there is no doubt that these adverse effects are associated with the use of GCs, it is difficult to predict which patients are at risk. Certainly patients with a predisposition to a certain effect are more susceptible. Often the effects are limited, because the treatment is short, and reversible.

Others

The list of adverse events that accompany GC treatment is long. Some are rare and have not been studied in great detail. Often the effects are reversible and the inconvenience for the patient very limited. However there are a few adverse effects that were not discussed here that can have major implications for the patient.

GC treatment is associated with an increased risk of cardiovascular diseases[106]. In particular ischemic heart disease, heart failure and atherosclerosis. The latter is believed to be the result of increased lipid levels in the blood but studies yielded contradictory results.

GCs increase the chance of adverse events in the gastrointestinal tract[107] including gastritis, ulcer formation, and gastrointestinal bleeding. The increase risk is small and causes most likely relate to suppression of the immune system. GCs also increase the risk of pancreatitis. Underlying cause is unclear.

And finally the chance of infection should be taken in account[108]. GCs are excellent anti-inflammatory drugs that reduce the ability of both the innate- and the adaptive- immune system to deal with invading pathogens. Common bacteria that under normal conditions pose no threat can become a real issue. Normal signs of infection can be absent due to suppression of inflammatory mediators such as cytokines.

References

1. Barnes, P.J. and I.M. Adcock, *Glucocorticoid resistance in inflammatory diseases.* Lancet, 2009. **373**(9678): p. 1905-1917.
2. Myers, B., J.M. McKlveen, and J.P. Herman, *Glucocorticoid actions on synapses, circuits, and behavior: Implications for the energetics of stress.* Frontiers in Neuroendocrinology, 2014. **35**(2): p. 180-196.
3. Nicolaides, N.C., et al., *Circadian endocrine rhythms: the hypothalamic-pituitary-adrenal axis and its actions.* Steroids in Neuroendocrine Immunology and Therapy of Rheumatic Diseases Ii, 2014. **1318**: p. 71-80.

4. Cushing, H., *The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). .* 1932.
5. Lee, M.J., et al., *Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study.* American Journal of Physiology-Endocrinology and Metabolism, 2011. **300**(3): p. E571-E580.
6. Wang, Y.X., et al., *The human fatty acid synthase gene and de novo lipogenesis are coordinately regulated in human adipose tissue.* Journal of Nutrition, 2004. **134**(5): p. 1032-1038.
7. Campbell, J.E., et al., *Adipogenic and lipolytic effects of chronic glucocorticoid exposure.* American Journal of Physiology-Cell Physiology, 2011. **300**(1): p. C198-C209.
8. Kuo, T.Y., et al., *Genome-wide analysis of glucocorticoid receptor-binding sites in myotubes identifies gene networks modulating insulin signaling.* Proceedings of the National Academy of Sciences of the United States of America, 2012. **109**(28): p. 11160-11165.
9. Xu, C., et al., *Direct Effect of Glucocorticoids on Lipolysis in Adipocytes.* Molecular Endocrinology, 2009. **23**(8): p. 1161-1170.
10. Hoppmann, J., et al., *The balance between gluco- and mineralo-corticoid action critically determines inflammatory adipocyte responses.* Journal of Endocrinology, 2010. **204**(2): p. 153-164.
11. Patsouris, D., et al., *Glucocorticoids and Thiazolidinediones Interfere with Adipocyte-mediated Macrophage Chemotaxis and Recruitment.* Journal of Biological Chemistry, 2009. **284**(45): p. 31223-31235.
12. Yu, C.Y., et al., *Genome-Wide Analysis of Glucocorticoid Receptor Binding Regions in Adipocytes Reveal Gene Network Involved in Triglyceride Homeostasis.* PLoS One, 2010. **5**(12).
13. Kannisto, K., et al., *Overexpression of 11 beta-hydroxysteroid dehydrogenase-1 in adipose tissue is associated with acquired obesity and features of insulin resistance: Studies in young adult monozygotic twins.* Journal of Clinical Endocrinology & Metabolism, 2004. **89**(9): p. 4414-4421.
14. Masuzaki, H., et al., *A transgenic model of visceral obesity and the metabolic syndrome.* Science, 2001. **294**(5549): p. 2166-2170.
15. Bazhan, N. and D. Zelena, *Food-intake regulation during stress by the hypothalamo-pituitary-adrenal axis.* Brain Research Bulletin, 2013. **95**: p. 46-53.

16. Cossmann, M. and J. Welzel, *Evaluation of the atrophogenic potential of different glucocorticoids using optical coherence tomography, 20-MHz ultrasound and profilometry; a double-blind, placebo-controlled trial.* British Journal of Dermatology, 2006. **155**(4): p. 700-706.
17. Delforno, C., P.J.A. Holt, and R. Marks, *Corticosteroid Effect on Epidermal-Cell Size.* British Journal of Dermatology, 1978. **98**(6): p. 619-623.
18. Saarni, H., M. Tammi, and N.S. Doherty, *Decreased Hyaluronic-Acid Synthesis, a Sensitive Indicator of Cortisol Action on Fibroblast.* Journal of Pharmacy and Pharmacology, 1978. **30**(3): p. 200-201.
19. Lehmann, P.M., et al., *Corticosteroid Atrophy in Human-Skin - a Study by Light Scanning and Transmission Electron-Microscopy.* Clinical Research, 1983. **31**(2): p. A581-A581.
20. Sheu, H.M., et al., *Depletion of stratum corneum intercellular lipid lamellae and barrier function abnormalities after long-term topical corticosteroids.* British Journal of Dermatology, 1997. **136**(6): p. 884-890.
21. Kruse, N.J., et al., *Inhibitory Effects of Glucocorticoids on Collagen-Synthesis by Mouse Sponge Granulomas and Granuloma Fibroblasts in Culture.* Biochimica Et Biophysica Acta, 1978. **540**(1): p. 101-116.
22. Terao, M., et al., *11 beta-Hydroxysteroid Dehydrogenase 1 Specific Inhibitor Increased Dermal Collagen Content and Promotes Fibroblast Proliferation.* PLoS One, 2014. **9**(3).
23. Stojadinovic, O., et al., *Novel genomic effects of glucocorticoids in epidermal keratinocytes - Inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation.* Journal of Biological Chemistry, 2007. **282**(6): p. 4021-4034.
24. Tiganescu, A., et al., *11 beta-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects.* Journal of Clinical Investigation, 2013. **123**(7): p. 3051-3060.
25. Henneicke, H., et al., *Glucocorticoids and bone: local effects and systemic implications.* Trends in Endocrinology and Metabolism, 2014. **25**(4): p. 197-211.
26. Bonaldo, P. and M. Sandri, *Cellular and molecular mechanisms of muscle atrophy.* Disease Models & Mechanisms, 2013. **6**(1): p. 25-39.

27. Schakman, O., et al., *Glucocorticoid-induced skeletal muscle atrophy.* International Journal of Biochemistry & Cell Biology, 2013. **45**(10): p. 2163-2172.
28. Zheng, B., et al., *FOXO3a mediates signaling crosstalk that coordinates ubiquitin and atrogin-1/MAFbx expression during glucocorticoid-induced skeletal muscle atrophy.* Faseb Journal, 2010. **24**(8): p. 2660-2669.
29. Wang, H.M., et al., *Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1.* Journal of Biological Chemistry, 2006. **281**(51): p. 39128-39134.
30. Dardevet, D., et al., *Glucocorticoid effects on insulin- and IGF-I-regulated muscle protein metabolism during aging.* Journal of Endocrinology, 1998. **156**(1): p. 83-89.
31. Ma, K., et al., *Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression.* American Journal of Physiology-Endocrinology and Metabolism, 2003. **285**(2): p. E363-E371.
32. Schakman, O., et al., *Insulin-like growth factor-I gene transfer by electroporation prevents skeletal muscle atrophy in glucocorticoid-treated rats.* Endocrinology, 2005. **146**(4): p. 1789-1797.
33. Gilson, H., et al., *Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy.* Endocrinology, 2007. **148**(1): p. 452-460.
34. Grobet, L., et al., *Modulating skeletal muscle mass by postnatal, muscle-specific inactivation of the myostatin gene.* Genesis, 2003. **35**(4): p. 227-238.
35. Allen, D.L. and A.S. Loh, *Posttranscriptional mechanisms involving microRNA-27a and b contribute to fast-specific and glucocorticoid-mediated myostatin expression in skeletal muscle.* American Journal of Physiology-Cell Physiology, 2011. **300**(1): p. C124-C137.
36. Rauch, A., et al., *Glucocorticoids Suppress Bone Formation by Attenuating Osteoblast Differentiation via the Monomeric Glucocorticoid Receptor.* Cell Metabolism, 2010. **11**(6): p. 517-531.
37. Kalak, R., et al., *Endogenous glucocorticoid signalling in osteoblasts is necessary to maintain normal bone structure in mice.* Bone, 2009. **45**(1): p. 61-67.
38. Zhou, H., et al., *Osteoblasts directly control lineage commitment of mesenchymal progenitor cells through Wnt signaling.* Journal of Biological Chemistry, 2008. **283**(4): p. 1936-1945.

39. Zhou, H., et al., *Glucocorticoid-dependent Wnt signaling by mature osteoblasts is a key regulator of cranial skeletal development in mice.* Development, 2009. **136**(3): p. 427-436.
40. Locascio, V., et al., *Bone Loss in Response to Long-Term Glucocorticoid Therapy.* Bone and Mineral, 1990. **8**(1): p. 39-51.
41. O'Brien, C.A., et al., *Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength.* Endocrinology, 2004. **145**(4): p. 1835-1841.
42. Weinstein, R.S., et al., *Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids - Potential mechanisms of their deleterious effects on bone.* Journal of Clinical Investigation, 1998. **102**(2): p. 274-282.
43. Smith, E. and B. Frenkel, *Glucocorticoids inhibit the transcriptional activity of LEF/TCF in differentiating osteoblasts in a glycogen synthase kinase-3 beta-dependent and -independent manner.* Journal of Biological Chemistry, 2005. **280**(3): p. 2388-2394.
44. Mak, W., et al., *Biphasic Glucocorticoid-Dependent Regulation of Wnt Expression and Its Inhibitors in Mature Osteoblastic Cells.* Calcified Tissue International, 2009. **85**(6): p. 538-545.
45. Wang, F.S., et al., *Inhibition of glycogen synthase kinase-3 beta attenuates glucocorticoid-induced bone loss.* Life Sciences, 2009. **85**(19-20): p. 685-692.
46. Hayashi, K., et al., *BMP/Wnt antagonists are upregulated by dexamethasone in osteoblasts and reversed by alendronate and PTH: Potential therapeutic targets for glucocorticoid-induced osteoporosis.* Biochemical and Biophysical Research Communications, 2009. **379**(2): p. 261-266.
47. Rauch, A., et al., *An anti-inflammatory selective glucocorticoid receptor modulator preserves osteoblast differentiation.* Faseb Journal, 2011. **25**(4): p. 1323-1332.
48. Chang, J.K., et al., *Anti-inflammatory drugs suppress proliferation and induce apoptosis through altering expressions of cell cycle regulators and pro-apoptotic factors in cultured human osteoblasts.* Toxicology, 2009. **258**(2-3): p. 148-156.
49. Espina, B., et al., *Regulation of Bim in glucocorticoid-mediated osteoblast apoptosis.* Journal of Cellular Physiology, 2008. **215**(2): p. 488-496.
50. Weinstein, R.S., et al., *Intermittent Parathyroid Hormone Administration Counteracts the Adverse Effects of Glucocorticoids*

on Osteoblast and Osteocyte Viability, Bone Formation, and Strength in Mice. Endocrinology, 2010. **151**(6): p. 2641-2649.
51. Xia, X.C., et al., *Glucocorticoid-Induced Autophagy in Osteocytes.* Journal of Bone and Mineral Research, 2010. **25**(11): p. 2479-2488.
52. Jia, J.J., et al., *Glucocorticoid dose determines osteocyte cell fate.* Faseb Journal, 2011. **25**(10): p. 3366-3376.
53. Jia, D., et al., *Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density.* Endocrinology, 2006. **147**(12): p. 5592-5599.
54. Rose, A.J. and S. Herzig, *Metabolic control through glucocorticoid hormones: An update.* Molecular and Cellular Endocrinology, 2013. **380**(1-2): p. 65-78.
55. Mazziotti, G., C. Gazzaruso, and A. Giustina, *Diabetes in Cushing syndrome: basic and clinical aspects.* Trends in Endocrinology and Metabolism, 2011. **22**(12): p. 499-506.
56. Pivonello, R., et al., *Pathophysiology of Diabetes Mellitus in Cushing's Syndrome.* Neuroendocrinology, 2010. **92**: p. 77-81.
57. van Raalte, D.H., D.M. Ouwens, and M. Diamant, *Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options?* European Journal of Clinical Investigation, 2009. **39**(2): p. 81-93.
58. Seino, S., T. Shibasaki, and K. Minami, *Pancreatic beta-cell signaling: toward better understanding of diabetes and its treatment.* Proceedings of the Japan Academy Series B-Physical and Biological Sciences, 2010. **86**(6): p. 563-577.
59. Folli, F., M.J.A. Saad, and C.R. Kahn, *Insulin receptor/IRS-1/PI 3-kinase signaling system in corticosteroid-induced insulin resistance.* Acta Diabetologica, 1996. **33**(3): p. 185-192.
60. Verhees, K.J.P., et al., *Glycogen synthase kinase-3 beta is required for the induction of skeletal muscle atrophy.* American Journal of Physiology-Cell Physiology, 2011. **301**(5): p. C995-C1007.
61. Etxabe, J. and J.A. Vazquez, *Morbidity and Mortality in Cushings-Disease - an Epidemiologic Approach.* Clinical Endocrinology, 1994. **40**(4): p. 479-484.
62. Cicala, M.V. and F. Mantero, *Hypertension in Cushing's Syndrome: From Pathogenesis to Treatment.* Neuroendocrinology, 2010. **92**: p. 44-49.
63. De Wachter, E., et al., *Rapidly developing Cushing syndrome in a 4-year-old patient during combined treatment with itraconazole and*

inhaled budesonide. European Journal of Pediatrics, 2003. **162**(7-8): p. 488-489.
64. Bertagna, X., et al., *Pituitary-Adrenal Response to the Antiglucocorticoid Action of Ru-486 in Cushings-Syndrome.* Journal of Clinical Endocrinology & Metabolism, 1986. **63**(3): p. 639-643.
65. Nieman, L.K., et al., *Successful Treatment of Cushings-Syndrome with the Glucocorticoid Antagonist Ru-486.* Journal of Clinical Endocrinology & Metabolism, 1985. **61**(3): p. 536-540.
66. Mangos, G.J., et al., *Glucocorticoids and the kidney.* Nephrology, 2003. **8**(6): p. 267-273.
67. Freiberg, J.M., J.L. Kinsella, and B. Sacktor, *Glucocorticoid Effects on Na+-H+ Exchange and Na+-Phosphate (Pi) and Na+-Glucose Co-Transports in Isolated Renal Brush-Border Membrane-Vesicles (Bb).* Federation Proceedings, 1982. **41**(5): p. 1494-1494.
68. Kinsella, J., T. Cujdik, and B. Sacktor, *Na+-H+ Exchange Activity in Renal Brush-Border Membrane-Vesicles in Response to Metabolic-Acidosis - the Role of Glucocorticoids.* Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 1984. **81**(2): p. 630-634.
69. Rodriguez, H.J., et al., *Regulation of Renal Na+-K+-Atpase in the Rat by Adrenal-Steroids.* American Journal of Physiology, 1981. **241**(2): p. F186-F195.
70. Welbourne, T.C., *Glucocorticoid Control of Ammoniagenesis in the Proximal Tubule.* Seminars in Nephrology, 1990. **10**(4): p. 339-349.
71. Goodwin, J.E., J.H. Zhang, and D.S. Geller, *A critical role for vascular smooth muscle in acute glucocorticoid-induced hypertension.* Journal of the American Society of Nephrology, 2008. **19**(7): p. 1291-1299.
72. Molnar, G.A., et al., *Glucocorticoid-related signaling effects in vascular smooth muscle cells.* Journal of Hypertension, 2008. **26**: p. S5-S6.
73. Goodwin, J.E., et al., *Knockout of the vascular endothelial glucocorticoid receptor abrogates dexamethasone-induced hypertension.* Journal of Hypertension, 2011. **29**(7): p. 1347-1356.
74. Ong, S.L.H., et al., *Hemodynamics of dexamethasone-induced hypertension in the rat.* Hypertension Research, 2009. **32**(10): p. 889-894.

75. Carnahan, M.C. and D.A. Goldstein, *Ocular complications of topical, peri-ocular, and systemic corticosteroids.* Current Opinion in Ophthalmology, 2000. **11**(6): p. 478-83.
76. Urban, R.C., Jr. and E. Cotlier, *Corticosteroid-induced cataracts.* Survey of Ophthalmology, 1986. **31**(2): p. 102-10.
77. James, E.R., et al., *Presence of a transcriptionally active glucocorticoid receptor alpha in lens epithelial cells.* Invest Ophthalmol Vis Sci, 2003. **44**(12): p. 5269-76.
78. Gupta, V., et al., *Global gene profiling reveals novel glucocorticoid induced changes in gene expression of human lens epithelial cells.* Molecular Vision, 2005. **11**(121-22): p. 1018-1040.
79. Greiner, J.V. and L.T. Chylack, *Posterior Subcapsular Cataracts - Histopathologic Study of Steroid-Associated Cataracts.* Archives of Ophthalmology, 1979. **97**(1): p. 135-144.
80. Spector, A., *Oxidative Stress-Induced Cataract - Mechanism of Action.* Faseb Journal, 1995. **9**(12): p. 1173-1182.
81. Dickerson, J.E., E. Dotzel, and A.F. Clark, *Steroid-induced cataract: New perspectives from in vitro and lens culture studies.* Experimental Eye Research, 1997. **65**(4): p. 507-516.
82. Bucala, R., et al., *Glucocorticoid Lens Protein Adducts in Experimentally Induced Steroid Cataracts.* Experimental Eye Research, 1985. **40**(6): p. 853-863.
83. Kersey, J.P. and D.C. Broadway, *Corticosteroid-induced glaucoma: a review of the literature.* Eye, 2006. **20**(4): p. 407-416.
84. Smith, C.L., *"Corticosteroid glaucoma" a summary and review of the literaure.* American Journal of the Medical Sciences, 1966. **252**(2): p. 239-44.
85. Renfro, L. and J.S. Snow, *Ocular effects of topical and systemic steroids.* Dermatol Clin, 1992. **10**(3): p. 505-12.
86. Wordinger, R.J. and A.F. Clark, *Effects of glucocorticoids on the trabecular meshwork: towards a better understanding of glaucoma.* Prog Retin Eye Res, 1999. **18**(5): p. 629-67.
87. McCarty, G.R. and B. Schwartz, *Increased concentration of glucocorticoid receptors in rabbit iris--ciliary body compared to rabbit liver.* Invest Ophthalmol Vis Sci, 1982. **23**(4): p. 525-8.
88. Tripathi, B.J., R.C. Tripathi, and H.H. Swift, *Hydrocortisone-induced DNA endoreplication in human trabecular cells in vitro.* Experimental Eye Research, 1989. **49**(2): p. 259-70.

89. Weinreb, R.N., M.D. Mitchell, and J.R. Polansky, *Prostaglandin production by human trabecular cells: in vitro inhibition by dexamethasone.* Invest Ophthalmol Vis Sci, 1983. **24**(12): p. 1541-5.
90. Wilson, K., et al., *Dexamethasone induced ultrastructural changes in cultured human trabecular meshwork cells.* Curr Eye Res, 1993. **12**(9): p. 783-93.
91. Yue, B.Y., *The extracellular matrix and its modulation in the trabecular meshwork.* Survey of Ophthalmology, 1996. **40**(5): p. 379-90.
92. Bourdeau, I., et al., *Cognitive function and cerebral assessment in patients who have Cushing's syndrome.* Endocrinol Metab Clin North Am, 2005. **34**(2): p. 357-69, ix.
93. De Kloet, E.R., et al., *Brain corticosteroid receptor balance in health and disease.* Endocrine Reviews, 1998. **19**(3): p. 269-301.
94. Samarasinghe, R.A., et al., *Nongenomic glucocorticoid receptor action regulates gap junction intercellular communication and neural progenitor cell proliferation.* Proc Natl Acad Sci U S A, 2011. **108**(40): p. 16657-62.
95. Scheff, S.W. and C.W. Cotman, *Chronic glucocorticoid therapy alters axon sprouting in the hippocampal dentate gyrus.* Exp Neurol, 1982. **76**(3): p. 644-54.
96. Crochemore, C., et al., *Direct targeting of hippocampal neurons for apoptosis by glucocorticoids is reversible by mineralocorticoid receptor activation.* Mol Psychiatry, 2005. **10**(8): p. 790-8.
97. Bannerman, D.M., et al., *Regional dissociations within the hippocampus--memory and anxiety.* Neurosci Biobehav Rev, 2004. **28**(3): p. 273-83.
98. Reul, J.M., *Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways.* Front Psychiatry, 2014. **5**: p. 5.
99. Nagaraja, N., et al., *Glucocorticoid mechanisms may contribute to ECT-induced retrograde amnesia.* Psychopharmacology (Berl), 2007. **190**(1): p. 73-80.
100. Arnsten, A.F., *Stress signalling pathways that impair prefrontal cortex structure and function.* Nat Rev Neurosci, 2009. **10**(6): p. 410-22.
101. Leon-Carrion, J., et al., *A clinical profile of memory impairment in humans due to endogenous glucocorticoid excess.* Clin Endocrinol (Oxf), 2009. **70**(2): p. 192-200.

102. Sirois, F., *Steroid psychosis: a review.* Gen Hosp Psychiatry, 2003. **25**(1): p. 27-33.
103. Hall, R.C., et al., *Presentation of the steroid psychoses.* J Nerv Ment Dis, 1979. **167**(4): p. 229-36.
104. Lewis, D.A. and R.E. Smith, *Steroid-induced psychiatric syndromes. A report of 14 cases and a review of the literature.* J Affect Disord, 1983. **5**(4): p. 319-32.
105. Wolkowitz, O.M., et al., *The "steroid dementia syndrome": an unrecognized complication of glucocorticoid treatment.* Ann N Y Acad Sci, 2004. **1032**: p. 191-4.
106. Walker, B.R., *Glucocorticoids and cardiovascular disease.* European Journal of Endocrinology, 2007. **157**(5): p. 545-59.
107. Rogler, G., *Gastrointestinal and liver adverse effects of drugs used for treating IBD.* Best Pract Res Clin Gastroenterol, 2010. **24**(2): p. 157-65.
108. Stuck, A.E., C.E. Minder, and F.J. Frey, *Risk of infectious complications in patients taking glucocorticosteroids.* Rev Infect Dis, 1989. **11**(6): p. 954-63.