Stress, obesity and mood disorders: towards breaking a vicious cycle

Koorneef, L.L.

8

Discussion and Future Perspectives

DISCUSSION AND FUTURE PERSPECTIVES

In this thesis, we explored the relation between obesity, stress and mood (**Fig. 1**). We identified new metabolic factors that likely affect fear and discovered new sites of action in the brain for known fear-regulating metabolic factors (**chapter 2**). We applied novel treatment strategies that target the glucocorticoid receptor (GR) and/or the mineralocorticoid receptor (MR) to alleviate glucocorticoid-induced, or diet-induced metabolic disease (**chapter 3-6**). We demonstrated that a full and specific antagonist for GR, as well as a selective GR modulator reduced diet-induced obesity (**chapter 4-5**). The selective GR modulator also had striking lipid-lowering effects in the liver (**chapter 5**). In chapter 6, we explored the metabolic effects of MR reactivation during dexamethasone treatment. We showed that corticosterone add-on treatment reduced a subset of the metabolic effects of dexamethasone, while exacerbating others (**chapter 6**). Finally, we concluded that in the hippocampus of the brain, mRNA levels of MR but not GR reflect receptor activity at the tissue level, but not at the cellular level (**chapter 7**).

Specific receptor targeting: not a trivial thing

MR and GR mediate different glucocorticoid effects, but neither ligand binding nor signaling mechanisms are fully specific [1]. GR and MR have a similar protein structure that consists of a ligand binding domain (LBD), a DNA-binding domain (DBD) and activation function 1 (AF1) and 2 (AF2)-domains for coregulator binding [2]. The LBDs of GR and MR are highly similar which allows binding of cortisol and corticosterone, although MR has a 10-fold higher affinity for these glucocorticoids as compared to GR [3, 4]. Aldosterone is a selective ligand for the MR under physiological conditions, but can bind to GR at supraphysiological levels [3]. The AF2-domain is part of the highly similar LBD, and therefore GR and MR recruit many overlapping coregulators at this domain [5]. In contrast, the AF1–domain is located in the 'intrinsically unstructured' N-terminal transactivation domain of which neither length or sequence is comparable between GR and MR [6, 7]. Therefore, coregulators that interact with the AF1-domain are believed to be important for receptor-specific actions [8, 9]. Given that the DBDs of GR and MR are 96% identical, both receptors have the glucocorticoid response element (GRE) as their dominant binding motif in the brain and thus share a considerable number of binding sites and target genes [10, 11]. GR and MR bind to the palindromic GRE as homodimers, but also as heterodimers and higher order complexes [11, 12]. GR may be the only steroid receptor to also bind to so called 'negative GREs' to repress gene transcription. In the hippocampus, a degree of specificity for MR over GR binding seems to be conferred by a *NeuroD* binding motif near the GRE [10]. Other mechanisms for receptor specificity include the regulation of ligand bio-availability, the more restricted

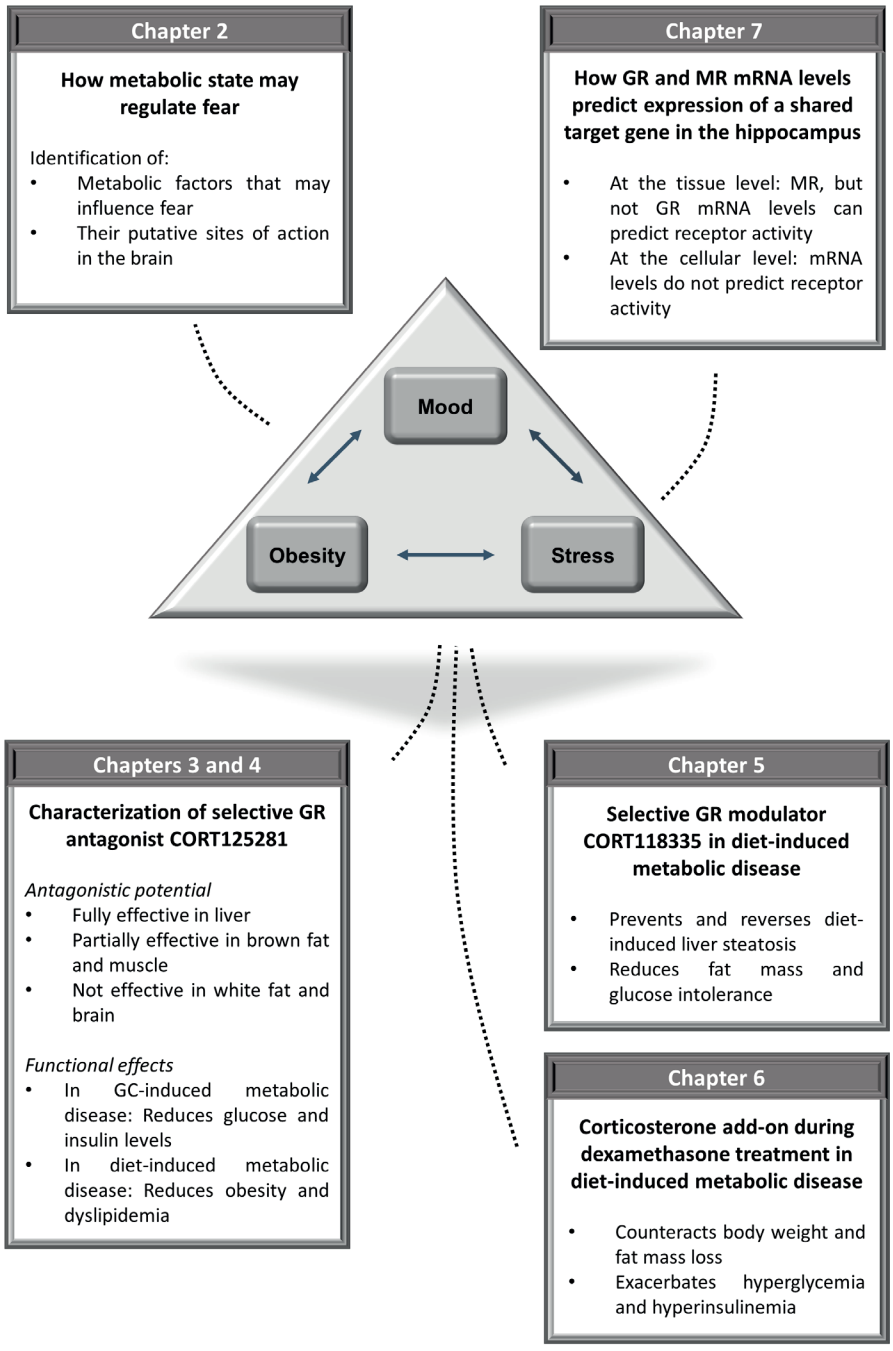

Figure 1: Thesis summary. A disease triangle connects obesity, stress and (stress-related) mood disorders, such as depression and anxiety disorders. The chapters of this thesis focused on different aspects of this disease triangle. The main findings of each chapter are shown in the figure. GR = glucocorticoid receptor, MR = mineralocorticoid receptor, GC = glucocorticoid.

expression pattern of MR, the different receptor binding affinities and the recruitment of MR- and GR-specific coregulators [3, 5, 9, 13].

Given that the signaling pathways and structures of GR and MR are highly similar, it is a major challenge to selectively target one of the receptors without affecting the other. A complicating factor is that other members of the nuclear receptor family, in particular the androgen and progesterone receptor, have a similar protein structure of which the DBD and, to a lesser extent, the LBD is highly conserved [14, 15]. The similar LBDs complicate the design of a receptor-specific ligand and thus many GR and MR ligands show cross-reactivity with other (cortico)steroid receptors. Commonly used GR antagonist RU486/Mifepristone, for example, has potent progesterone receptor and androgen receptor activities while MR antagonist spironolactone also inhibits progesterone and androgen receptor signaling (**chapter 4**, [16, 17]). Interestingly, CORT125281 is one of the first GR antagonists that does not show cross-reactivity for any of the steroid receptors (**chapter 4**), which makes the compound attractive for both scientific and clinical purposes. In contrast to many GR antagonists, synthetic GR agonists typically do not exhibit cross-reactivity for the androgen and progesterone receptor, but they vary in their level of MR activation [18]. For example, prednisolone has similar affinity for GR and MR, while beclomethasone and dexamethasone have a much higher affinity for GR than for MR [18].

Another example of a mixed receptor binding ligand is CORT118335, which combines selective GR modulation with MR antagonism [18]. CORT118335 has strong lipid-lowering effects in the liver which we explained by selective GR modulation (**chapter 5**). However, it is possible that MR antagonism also played a role in this, with the effect depending on the tissue and cell type expressing MR. MR is hardly expressed in hepatocytes, so it is unlikely that MR played a direct role in these cells [19]. However, MR is expressed in other cell types present in the liver, such as immune and endothelial cells [19]. MR antagonism in hepatic immune resident cells may reduce hepatic inflammation [20]. In hepatic endothelial cells, MR antagonism might reduce the uptake of fatty acids, as was previously found in cardiomyocytes treated with an MR antagonist [21]. In addition to these cell-specific effects of MR in the liver, liver steatosis can also be affected by extra-hepatic tissues that express MR, such as the adipose tissues. Absence of adipose MR expression indeed reduces lipid accumulation in the liver [22]. Interestingly, CORT118335 was found to influence adipocyte functioning through its actions on MR, as it counteracted the differentiation of cultured white pre-adipocytes induced by aldosterone [23]. Therefore, the dual actions of CORT118335 on GR and MR is highly relevant, and perhaps even essential to its effects.

Even with a *selective* synthetic GR ligand, the signaling pathway of MR can be affected by endocrine effects that affect MR occupancy. Dexamethasone strongly suppresses the hypothalamus-pituitary-adrenal (HPA)-axis, halting endogenous glucocorticoid produc-

tion. Since dexamethasone has a low affinity for the MR, the resulting low endogenous glucocorticoid levels deplete the MR of its ligand [24, 25]. This resulting MR hypoactivation can have major implications for metabolism (**chapter 6**) and mental wellbeing [26]. Conversely, selective *inhibition* of GR might lead to hyperactivated MRs. GR antagonists such as RU486 raise glucocorticoid levels by interfering with GR-mediated negative feedback on the HPA-axis [27, 28]. However, it is uncertain whether increased hormone levels will lead to more MR activation. In rodents, brain MRs are already about 80% occupied at basal (trough) levels of glucocorticoids, so an additional 20% may not have a biologically relevant effect [4, 29]. In addition, receptor levels, and not ligand levels, can be limiting for MR signaling (**chapter 7**). On the other hand, recent data showed that two classically 'MR-saturating' doses of corticosterone induced differential binding of MR in the hippocampus [10]. Likewise, the expression of an apparently MR-specific target gene was induced by novelty stress [30]. These data show that ligand levels can be of importance to the level of MR activation. Interestingly, GR antagonist CORT125281 did not disinhibit the HPA-axis, but did have a tendency to reduce peak corticosterone levels after novelty stress (**chapter 3,** [31]). Thus, CORT125281 seems to inhibit GR signaling while leaving MR occupancy essentially undisturbed.

Selective targeting of MR can also influence the occupancy of GRs. MRs in the hippocampus regulate the basal tone of the HPA-axis by inhibiting its activity [32]. Therefore, MR antagonists can raise glucocorticoid levels by disinhibiting the HPA-axis [32, 33]. Since ligand levels are mostly the limiting factor for GR signaling, elevated glucocorticoid levels are likely to be accompanied by increased GR activation (**chapter 7**, [3]).

In addition to inducing endocrine effects, selective MR or GR ligands can influence each other's signaling pathways through genomic interactions. Receptor crosstalk can occur through epigenomic mechanisms such as 'dynamic assisted loading', where at shared binding sites one receptor augments the DNA binding of the other receptor by increasing DNA accessibility [34, 35]. Other crosstalk mechanisms include the regulation of expression or activity of coregulators, transcription factors (such as NFκB and AP1) or other up- or downstream signaling partners [36]. Given the large number of shared binding sites and target genes, GR and MR can also functionally compete or cooperate for binding sites as homodimers, or bind these sites as heterodimers or higher order complexes [11, 12]. Depending on the cellular context, genomic interactions between GR and MR may eventually lead to functional synergy or antagonism (**chapter 7**, [37]).

In a more general sense, nuclear receptors have extensive bidirectional cross talk with MR and GR. Known examples include the receptors for androgens, progesterone and estrogens, and the peroxisome proliferator-activated receptors (PPAR) [3, 38-42]. These crosstalk mechanisms again include regulation of ligand availability as well as genomic interactions [36, 43]. For example, androgens can influence the bio-availability of glucocorticoids by regulating expression of 11ß-hydroxysteroid dehydrogenase 1 type

1 [38]. At the genomic level, the androgen receptor and the GR may form heterodimers that bind to shared DNA binding sites [39]. PPARα is able to antagonize GR-driven gene expression by tethering to GR, which prevents the physical recruitment of GR to the genome [41]. Conversely, GR induces PPARα expression to initiate PPARα-driven gene programs [44]. Because of this receptor crosstalk, both beneficial and disadvantageous actions of a compound can depend on the endocrine context. One of these endocrine contexts is sex, which importantly determines the metabolic outcome of glucocorticoid treatment [45-47]. While male mice develop hyperglycemia and hyperinsulinemia upon glucocorticoid exposure, these effects are milder or not observed in females [46, 47]. Similarly, males but not females develop hyperlipidemia and adiposity upon glucocorticoid treatment [46, 47]. Androgens contribute to these sex differences, because effects were lost in castrated males, while dihydrotestosterone triggered 'male' glucocorticoid effects in females [47]. In this light, it is important to investigate the efficacy of CORT125281, CORT118335 and corticosterone add-on treatments in females as well.

In conclusion, direct and indirect crosstalk between GR, MR and other steroid receptors is an important consideration when targeting GR or MR. Transgenic mouse models can be used to experimentally exclude the contribution of cross-reactivity with other steroid receptors. Inducible GR and MR knockout models, with temporal and/or spatial control over the gene deletion, are preferred over constitutive knock-out models because these models have a lower risk of compensatory mechanisms and are not postnatally lethal [48, 49]. The involvement of MR during corticosterone add-on treatment would therefore ideally be demonstrated with a kidney-sparing, or adipocyte-specific inducible MR knockout model (**chapter 6**, [50]).

Cell autonomous or systemic effects of novel GR ligands?

The metabolic system involves many organs, endocrine and cellular systems (**chapter 2**). CORT118335 and CORT125281 have pronounced metabolic effects on liver (**Chapter 4-5**) and brown fat (**chapter 4**), but it is unclear whether these effects were mediated by corticosteroid receptors in those tissues, or by receptors in other organs. Some of the systemic effects induced by CORT11835 can contribute to the decreased lipid accumulation in the liver, such as the reduced food intake and improved glucose tolerance. Lower caloric intake reduces fat mass expansion, which in turn lowers the fatty acid flux towards the liver [51]. Since insulin inhibits lipolysis in white adipose tissue, improved insulin sensitivity can further decrease the fatty acid flux towards the liver [51]. GR activity in adipose tissues can influence these processes and thus the development of liver steatosis. The absence of adipose GRs reduces glucose intolerance and liver steatosis induced by glucocorticoids and, albeit less consistently, by high-fat diets [31, 52-56].

CORT118335 may also reduce liver steatosis by lowering cholesterol levels in the liver [57-59]. Part of this cholesterol-lowering effect may be due to decreased cholesterol synthesis, because CORT118335 reduced the expression of the rate-limiting enzyme of cholesterol synthesis (**chapter 5**). Simultaneously, CORT118335 increased very-low-density lipoprotein production, which likely resulted in an efflux of cholesterol from the liver. Cholesterol can also be converted into bile acids and bile acid excretion thereby is the main route of cholesterol elimination. A fraction of these excreted bile acids is reabsorbed by the intestines and returned to the liver. Glucocorticoids are known to increase this 'enterohepatic recycling' of bile acids by regulating the expression of bile acid transporters in the liver and intestine [60-62]. An indication that CORT118335 influences bile acid metabolism is the reduced hepatic expression of the farnesoid X receptor, a master regulator of bile acid metabolism (**chapter 5**). However, functional studies are needed to pinpoint the mechanisms by which CORT118335 affects cholesterol and/or bile acid metabolism in the liver.

Indirect evidence supports a liver autonomous effect of CORT118335. First, most other GR selective modulators and antagonists (RU486, CORT125281 and CORT108297) induce a comparable reduction of food intake, fat mass and glucose intolerance as CORT118335, but the liver lipid lowering-effects are unique to CORT118335 (**Chapter 4**, [63]). However, this finding may also be related to the smaller xenobiotic response induced by CORT118335, compared to CORT125281 and RU486 (data not shown). Second, hepatic GRs control processes similar to those affected by CORT118335 treatment. This includes lipoprotein production and fatty acid transport in the liver, but also regulation of fat mass and glucose tolerance [55, 64, 65].

GRs in liver may well mediate most effects of CORT118335, but even then multiple cell types will be affected. Lipoprotein production takes place in hepatocytes, while fatty acid uptake depends on endothelial cells [66, 67]. Moreover, hepatic inflammation aggravates liver steatosis, and so modulation of hepatic immune resident cells may contribute to the liver steatosis-reducing effects of CORT118335 [68]. The putative effects of CORT118335 on bile acid metabolism may point towards a role for GRs in cholangiocytes (**chapter 5**, [69]). To unravel systemic and cell-autonomous effects of CORT118335, cell type-specific GR and/or MR knock-out mice could be considered. A first approach could involve the deletion of GR in both hepatocytes and/or endothelial cells to investigate the effects of selective GR modulation on lipid metabolism in the liver [55, 70]**.** Other approaches could focus on the involvement of other organs, including the adipose tissues and the beta-cells in the pancreas [50, 54, 71].

It is unknown whether CORT125281 stimulated brown fat activity by directly acting on GRs in brown adipocytes. CORT125281 is capable of directly influencing GR signaling in brown fat, as it blocked the corticosterone-induced expression of some classical GR target genes in this tissue (**chapter 3**). Glucocorticoids suppress the transcription of

an essential gene for brown fat functioning, uncoupling protein 1 (UCP1). CORT125281 prevented the suppression of *Ucp1* by glucocorticoids in cultured brown adipocytes, supporting a brown adipocyte-autonomous effect (**chapter 4**). However, there is much debate on the *in vivo* involvement of brown fat GRs in regulating tissue activity [53, 55, 72, 73]. Supporting a role for brown fat GRs, the absence of GR in brown and white adipose tissue protects mice against glucocorticoid-induced lipid accumulation in brown fat. It also reduces the induction of lipogenic genes in this tissue [53, 55]. Arguing against a role for brown fat GRs, a recent study showed that brown adipocyte-specific deletion of GR did not influence brown adipose tissue activity [73]. Rather than acting directly on brown fat GRs, CORT125281 may induce systemic effects that indirectly activate brown adipose tissue. High-fat diet flattens the diurnal rhythm of corticosterone, which may impair brown fat functioning (**chapter 4**, [74, 75]). Interestingly, CORT125281 restored this diet-disrupted corticosterone rhythm, which may have improved brown fat function (**chapter 4**). To elucidate the role of GRs in brown adipose tissue, metabolic effects of CORT125281 could be investigated in mice in which the GR is deleted in brown adipocytes specifically [73].

The most striking example of antagonist effects that are not cell-autonomous is the CORT125281-induced reduction of fat mass (**chapter 4**). We established that CORT125281 is not able to antagonize the induction of any of the classical GR target genes by corticosterone in white adipose tissue (**chapter 3**). Therefore, CORT125281 likely affects the lipid flux in other tissues which causes a net efflux of lipids from white adipose tissue**.** Interestingly, absence of GR in adipocytes protects against obesity after high-fat diet [31, 56]. Thus, GR antagonism that does not involve white adipocytes, and specific adipose GR knockout can have a similar outcome.

Although several organ systems have been investigated in this thesis, unexamined organ systems may have contributed to (metabolic) effects of our manipulations. GR modulating drugs may interfere with the intestinal absorption which is known to be controlled by enterocyte GRs [76-78]. Treatment strategies in this thesis may also have influenced skeletal muscle or the insulin-releasing beta cells of the pancreas [79, 80]. Skeletal muscle strongly contributes to energy expenditure and influences insulin sensitivity by releasing myokines and by taking up nutrients from the blood through insulin-dependent and independent pathways [81-84]. CORT118335 treatment reduced lean mass, which is probably explained by its effects on protein metabolism in skeletal muscle (**chapter 5**, [85-87]). Glucocorticoids are known for their catabolic effects on skeletal muscle, so the effect of CORT118335 on lean mass likely reflects its agonistic properties on the GR [87]. Amino acids derived from skeletal muscle not only serve as fuel and substrate for de novo gluconeogenesis, but can also have other (signaling) functions [88]. Glucocorticoid-elevated amino acids include arginine, which regulates insulin release, and alanine, which stimulates both insulin and glucagon release [89].

The effects of CORT118335 on glucose metabolism could thus be mediated via amino acids derived from skeletal muscle, which is subject for future investigation.

High-fat diet affects HPA-axis activity and glucocorticoid signaling

In this thesis, metabolic effects of GR and MR ligands have primarily been investigated under diet-induced obese conditions, in view of the potential clinical applications of targeting glucocorticoid signaling in subjects with metabolic syndrome. However, actions of glucocorticoids are highly context-dependent, and so the observed effects of GR/MR manipulating drugs may have depended on the high-fat diet context. For example, a high-fat diet can affect HPA-axis activity and thereby the basal tone of glucocorticoid signaling against which an antagonist competes. High-fat diet was previously shown to flatten glucocorticoid rhythmicity and to alter corticotropin-releasing hormone and GR expression in the hypothalamus (**chapter 4,** [74]). Metabolic factors may act on nutrient sensors in the hypothalamus including the PPARs and sucrose sensors which in turn influence HPA-axis activity and rhythmicity. [90, 91]. Metabolic factors may also act on the adrenals to directly influence glucocorticoid secretion, which has been described for leptin [92]. GR is likely involved in the diet-induced disturbances of corticosterone rhythm, since GR antagonist CORT125281 restored corticosterone rhythmicity in obese mice, but did not affect rhythmicity in lean mice (**chapter 3-4**). However, we observed that diet-induced disturbances of HPA-axis activity are highly variable, as corticosterone rhythm was flattened in the CORT125281 experiment (**chapter 4**) while the effects were minimal in another, unpublished 3-week experiment.

Metabolic state can also affect glucocorticoid signaling through crosstalk with the signaling pathways of other metabolic factors, such as insulin, leptin and free fatty acids [40, 93, 94]. Fatty acids and their derivatives activate the PPARs, which activate a cascade of metabolic pathways that serve adaptation to a changing metabolic status [95]. GR crosstalk with PPARγ may be especially important for the metabolic effects of dexamethasone and corticosterone add-on treatment. The lipid redistribution induced by dexamethasone seemed to be largely driven by increased lipolysis in adipose tissues, likely mediated by increased activity of the rate-limiting lipolysis enzyme adipose triglyceride lipase (*Atgl*). Corticosterone add-on counteracted this lipid redistribution as well as the induction of *Atgl* expression (**chapter 6**). Interestingly, *Atgl* is also under direct transcriptional control of PPARγ, which strongly potentiates the *Atgl* induction by dexamethasone in cultured white adipocytes [96]. Thus metabolic status determines the level of PPARγ activation, which in turn may determine effects of dexamethasone on lipolysis and, probably, lipid redistribution. Therefore, it would be worthwhile to investigate the effects of dexamethasone and corticosterone add-on treatment in lean mice as well.

The influence of metabolic state on glucocorticoid signaling is also illustrated by the different effects of CORT125281 and CORT118335 in lean and obese rodents. CORT125281 reduced fat mass in obese mice but not in lean mice (**Chapter 4**). CORT118335 increased plasma triglyceride levels (likely reflecting VLDL levels) in obese rats only, which was not caused by a different hepatic lipid content [97]. Crosstalk with signaling pathways of insulin and PPARs may be involved in this, as both factors control lipoprotein production [98, 99]. However, not all metabolic effects of glucocorticoids depend on the metabolic state, as glucocorticoids invariably increase plasma fatty acid and triglyceride levels and hepatic lipid accumulation in both lean and obese rodents [100, 101]. Accordingly, CORT125281 reduced plasma fatty acid and triglyceride levels in both obese and lean mice (**Chapter 4**).

Interactions with other components of the disease triangle

Obesity, stress and mood can be conceptualized in a disease triangle (**Fig. 1).** While the above discussion focused on the relation between stress and obesity, stress and obesity can also influence the emotional brain. Glucocorticoids increase fear and promote the consolidation of fear memories by binding to receptors in the brain and are implicated in the pathogenesis of depression [102-104]. Since metabolic status influences fear behavior and depression, glucocorticoids can also indirectly affect mood through their metabolic actions (**chapter 2**, [105-107]). Accordingly, CORT125281, CORT118335 and corticosterone add-on may also influence fear and mood via direct and indirect mechanisms.

It is unlikely that CORT125281 has direct central effects, given that it neither reaches the brain nor has substantial effects on HPA-axis activity. However, CORT125281 could indirectly influence mood by changing the metabolic status (**chapter 2**). For example, CORT125281 decreases plasma free fatty acid levels, which may affect fatty acid receptors expressed in monoaminergic brain regions (**chapter 2**). Next to restoring corticosterone rhythmicity, CORT125281 also improved food intake rhythm that is disturbed upon high fat-diet feeding (data not shown). CORT125281 thus affects feeding behavior, suggesting the involvement of central factors that regulate food intake. Some of these factors, such as neuropeptide Y and α-melanocyte stimulating hormone, are known to affect the fear response as well (**chapter 2**, [105, 108, 109])

Similarly, CORT118335 may indirectly influence mood through factors that regulate feeding behavior, as the compound reduced food intake (**chapter 5**). CORT118335 also has known direct central effects. CORT118335 mainly has antagonistic actions on GR and MR in the brain [18]. For example, it blocks the strengthening of memory consolidation that occurs after glucocorticoid treatment. However, it does not affect depressive-like behavior [18, 110]. Although effects on fear regulation have never been investigated, GR and MR antagonism would result in anxiolysis and anxiogenesis respectively, making

the ultimate effect on fear difficult to predict [103, 111]. CORT118335 is also effective in counteracting addictive behavior and currently is in trial as potential drug to reduce alcohol craving in alcohol addicts [112, 113].

Corticosterone add-on treatment likely influences mood directly. In fact, first evidence for a possible metabolic benefit of corticosterone add-on was provided by studies showing that cortisol add-on reduced emotional and psychological side effects induced by dexamethasone [26]. The rationale to reactivate MR with corticosterone add-on is that MR activity in the brain reduces anxiety, improves sleep and protects from depression [111]. In line with these expected effects of MR reactivation, cortisol add-on treatment reduced emotional symptoms and sleep problems induced by dexamethasone in pediatric leukemia patients [26]. While effects on depressive and anxiety-related symptoms were not included in this study, these will be measured in an ongoing clinical trial in brain tumor patients [114].

Next to direct central effects, we propose an alternative mechanism for the efficacy of corticosterone add-on during dexamethasone treatment. Corticosterone add-on may counteract the hypocorticoid state of the brain caused by low-to-moderate dose dexamethasone. This central hypocorticism is caused because dexamethasone does not reach the brain at a moderate dose [115]. However, dexamethasone still fully suppresses HPA-axis activity at this dose, likely by acting on pituitary GRs [116]. The strongly reduced endogenous glucocorticoid levels in combination with the poor brain penetrance of dexamethasone likely leads to central hypocorticism [116]. This theory is supported by reduced expression of GR and MR target genes in the brain after moderate dose dexamethasone treatment (**chapter 6,** [116]). While the phenomenon of central hypocorticism after dexamethasone has previously been described, the effects on metabolism and fear have not been extensively investigated [116]. We show that corticosterone, which easily penetrates the blood-brain-barrier, counteracted the dexamethasone-induced reduction of GR target gene expression in the brain, likely by restoring brain corticosteroid levels (**chapter 6**). Therefore, part of the effects of corticosterone add-on may be attributed to the reversal of dexamethasone-induced central hypocorticism, next to direct effects on brain corticosteroid receptors and changes in metabolic status.

General outlook

In this thesis, we pharmacologically targeted glucocorticoid signaling to reduce diet-induced and glucocorticoid-induced metabolic effects. While it is not surprising that GR antagonism counteracts glucocorticoid-induced metabolic effects (**chapter 3**), it is remarkable that GR antagonism also improves metabolic features of obese mice without overt hypercorticism (**chapter 4**). This suggests that the HPA-axis does not need to be severely deregulated for GR antagonists to be effective in metabolic disease. This find-

ing also supports that deregulated glucocorticoid signaling contributes to the adverse effects of a high-fat diet [117]. From a translational perspective, GR antagonism should thus not only be reserved for patients with hypercortisolism, but can be applied for a broader range of diseases in which glucocorticoid signaling is deregulated, including (aspects of) the metabolic syndrome.

Conclusions of **chapter 2** were mostly based on the mRNA levels of receptors, which were used to identify brain region that are regulated by metabolic factors. However, in **chapter 7**, we have observed that mRNA levels of receptors do not necessarily reflect receptor activity. It would be useful to decipher to what extent receptor mRNA levels must differ in order to be biologically relevant. In **chapter 7** mRNA levels of receptors were used for a *quantitative* assessment of receptor activity, in **chapter 2** we aimed for a *qualitative* outcome. For qualitative assessments, it appears that mRNA levels sufficiently differentiate to identify expression patterns of receptors. In chapter 1, we used these expression patterns to measure 'enrichment' of metabolic receptors in the fear circuitry to identify receptors involved in fear. A remarkable amount of these identified receptors were already known to affect fear, supporting the validity of the approach. For quantitative purposes, mRNA levels should be interpreted with more caution, as we and others have shown that mRNA levels do not necessarily translate to receptor activity (**chapter 7**, [118]). Multiple factors eventually determine receptor activity, including the type of receptor, ligand levels and the cellular context. Nevertheless, mRNA levels can reflect the activity of some receptors, especially when receptor expression levels are limiting for signaling, as is the case with MR (**chapter 7**).

It remains of interest whether corticosterone add-on treatment is also of benefit for other commonly used glucocorticoid-like drugs that have similar properties as dexamethasone. This includes drugs with a higher affinity for GR than for MR and with strong HPA-axis suppressing activities, such as beclomethasone [119, 120]. The potential of a drug to induce central hypocorticism can additionally be considered, which can be estimated by the degree of P-glycoprotein-mediated efflux from the brain [121]. Since the HPA-axis needs to be suppressed for corticosterone add-on to be effective, corticosterone is likely only beneficial in combination with GR agonists that are systemically administered. Because synthetic GR agonists are used for immunosuppression and MR acts pro-inflammatory, it should always be monitored whether the add-on treatment interferes with the treatment efficacy of the GR agonist.

For all treatment strategies in this thesis, the question is how the preclinical findings translate from rodents to humans (**chapter 3-6**). Depending on treatment dose and regimen, dexamethasone can induce a severe, almost cachexic loss of fat mass in rodents, but this effect is not observed in humans (**chapter 6,** [100, 122-124]). We found that corticosterone add-on counteracted this loss of fat mass in mice. Although this effect is beneficial in rodents, the question is whether this is also the case in

humans, especially since dexamethasone does not reduce fat mass in this species [125]. Another factor to consider is that corticosterone has slightly different properties than cortisol. Corticosterone but not cortisol is selectively exported of adipose tissues in humans and corticosterone penetrates the blood-brain-barrier more easily than cortisol [115, 126, 127]. Therefore, corticosterone may more effectively reduce the central side effects of dexamethasone in humans than cortisol, while inducing fewer metabolic side effects. So far, there is only one clinical trial that investigated metabolic effects of cortisol add-on, which showed no effect on body weight and waist-hip ratio [26]. However, this study used a very young study population and did not stratify for obesity. Therefore, a dedicated study is needed to elucidate the metabolic effects of cortisol and corticosterone add-on treatment in humans.

Inter-species differences are also relevant to the therapeutic effects of CORT118335. The ability of a drug to reduce liver steatosis can differ significantly between mice and humans [128]. Part of this translational gap is related to the fact that some drugs reduce fat mass and glucose intolerance in rodents, but not in humans. The question whether CORT118335 can still reduce liver steatosis in humans is currently being investigated in a phase II trial [129]. Furthermore, the fat mass-reducing effects of CORT118335 are further explored in trials aimed to reduce metabolic side effects of drugs for psychosis, bipolar disease and schizophrenia [129-132].

The development of liver steatosis, likely related to liver toxicity, is the most important adverse effect of CORT125281 in our studies in mice. The question is whether CORT125281 induces similar effects in humans. It is possible that it induces less or no liver toxicity in humans, because this has previously been observed with RU486 which activates the same xenobiotic pathway as CORT125281 in mice, but not in humans (**chapter 4**, [133-136]). CORT125281 has been tested in a phase 1 clinical trial, and its therapeutic potential as GR antagonist is currently further investigated in patients with metastatic castration-resistant prostate cancer [137, 138].

For all tested therapeutic approaches, the eventual treatment response will vary between patients. Factors that could influence treatment response include sex, subjective and objective measures of perceived stress, metabolic profile, diet and psychological factors such as history of mental illness [45, 139]. In addition, treatment response may be influenced by the MR and/or GR haplotype that a patient carries [139-145]. Various GR and MR variants exist in the general population that are associated with altered receptor sensitivity and a different risk of developing metabolic or psychiatric diseases [139-144]. It is possible that MR haplotypes differ in their affinity for glucocorticoid-like drugs, which may be relevant to the action of CORT118335 and corticosterone add-on. In addition, certain MR haplotypes are associated with increased receptor sensitivity, which may protect against the effects of dexamethasone that are related to MR

hypoactivation [146]. Consequently, these subjects may benefit less, or not at all of corticosterone add-on treatment.

This thesis adds to the understanding of two compounds that are currently investigated in phase II trials (CORT118335, CORT125281), and one that is prescribed to millions of patients every year (dexamethasone) [147]. We shed light on the biological processes linking stress to obesity and obesity to fear. We showed that targeting glucocorticoid signaling successfully reduces obesity and associated complications. These therapeutic strategies could be a step towards breaking the vicious circle of obesity, stress and mood disorders, which could ultimately affect the lives of many, given the high prevalence of these conditions in modern society [148-150].

REFERENCES

1. Koning, A., et al., *Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective*. J Endocr Soc, 2019. **3**(10): p. 1917-1930.
2. Rastinejad, F., et al., *Understanding nuclear receptor form and function using structural biology*. J Mol Endocrinol, 2013. **51**(3): p. T1-T21.
3. Rupprecht, R., et al., *Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands*. Eur J Pharmacol, 1993. **247**(2): p. 145-54.
4. De Kloet, E.R., et al., *Relative binding affinity of steroids for the corticosterone receptor system in rat hippocampus*. J Steroid Biochem, 1984. **21**(2): p. 173-8.
5. Broekema, M.F., et al., *Profiling of 3696 Nuclear Receptor-Coregulator Interactions: A Resource for Biological and Clinical Discovery*. Endocrinology, 2018. **159**(6): p. 2397-2407.
6. Helsen, C. and F. Claessens, *Looking at nuclear receptors from a new angle*. Mol Cell Endocrinol, 2014. **382**(1): p. 97-106.
7. Fischer, K., et al., *Conformation of the mineralocorticoid receptor N-terminal domain: evidence for induced and stable structure*. Mol Endocrinol, 2010. **24**(10): p. 1935-48.
8. Pascual-Le Tallec, L., et al., *The elongation factor ELL (eleven-nineteen lysine-rich leukemia) is a selective coregulator for steroid receptor functions*. Mol Endocrinol, 2005. **19**(5): p. 1158-69.
9. Pascual-Le Tallec, L. and M. Lombes, *The mineralocorticoid receptor: a journey exploring its diversity and specificity of action*. Mol Endocrinol, 2005. **19**(9): p. 2211-21.
10. van Weert, L., et al., *NeuroD Factors Discriminate Mineralocorticoid From Glucocorticoid Receptor DNA Binding in the Male Rat Brain*. Endocrinology, 2017. **158**(5): p. 1511-1522.
11. Mifsud, K.R. and J.M. Reul, *Acute stress enhances heterodimerization and binding of corticosteroid receptors at glucocorticoid target genes in the hippocampus*. Proc Natl Acad Sci U S A, 2016. **113**(40): p. 11336-11341.
12. Pooley, J.R., et al., *Beyond the heterodimer model for mineralocorticoid and glucocorticoid receptor interactions in nuclei and at DNA*. PLoS One, 2020. **15**(1): p. e0227520.
13. Edwards, C.R., et al., *Localisation of 11 beta-hydroxysteroid dehydrogenase--tissue specific protector of the mineralocorticoid receptor*. Lancet, 1988. **2**(8618): p. 986-9.
14. Kumar, R. and G. Litwack, *Structural and functional relationships of the steroid hormone receptors' N-terminal transactivation domain*. Steroids, 2009. **74**(12): p. 877-83.
15. Schwabe, J.W. and S.A. Teichmann, *Nuclear receptors: the evolution of diversity*. Sci STKE, 2004. **2004**(217): p. pe4.
16. Bourgeois, S., M. Pfahl, and E.E. Baulieu, *DNA binding properties of glucocorticosteroid receptors bound to the steroid antagonist RU-486*. EMBO J, 1984. **3**(4): p. 751-5.
17. Struthers, A., H. Krum, and G.H. Williams, *A comparison of the aldosterone-blocking agents eplerenone and spironolactone*. Clin Cardiol, 2008. **31**(4): p. 153-8.
18. Atucha, E., et al., *A Mixed Glucocorticoid/Mineralocorticoid Selective Modulator With Dominant Antagonism in the Male Rat Brain*. Endocrinology, 2015. **156**(11): p. 4105-14.
19. Aizarani, N., et al., *A human liver cell atlas reveals heterogeneity and epithelial progenitors*. Nature, 2019. **572**(7768): p. 199-204.
20. Bene, N.C., et al., *Mineralocorticoid receptors in immune cells: emerging role in cardiovascular disease*. Steroids, 2014. **91**: p. 38-45.
21. Ramirez, E., et al., *Eplerenone attenuated cardiac steatosis, apoptosis and diastolic dysfunction in experimental type-II diabetes*. Cardiovasc Diabetol, 2013. **12**: p. 172.

22. Ferguson, D., et al., *Role of Mineralocorticoid Receptor in Adipogenesis and Obesity in Male Mice*. Endocrinology, 2020. **161**(2).
23. Mammi, C., et al., *A novel combined glucocorticoid-mineralocorticoid receptor selective modulator markedly prevents weight gain and fat mass expansion in mice fed a high-fat diet*. Int J Obes (Lond), 2016. **40**(6): p. 964-72.
24. Meijer, O.C. and E.R. de Kloet, *A Refill for the Brain Mineralocorticoid Receptor: The Benefit of Cortisol Add-On to Dexamethasone Therapy.* Endocrinology, 2017. **158**(3): p. 448-454.
25. Funder, J.W., *The Potential of ACTH in the Genesis of Primary Aldosteronism*. Front Endocrinol (Lausanne), 2016. **7**: p. 40.
26. Warris, L.T., et al., *Hydrocortisone as an Intervention for Dexamethasone-Induced Adverse Effects in Pediatric Patients With Acute Lymphoblastic Leukemia: Results of a Double-Blind, Randomized Controlled Trial*. J Clin Oncol, 2016. **34**(19): p. 2287-93.
27. Gaillard, R.C., et al., *RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day.* Proc Natl Acad Sci U S A, 1984. **81**(12): p. 3879-82.
28. De Kloet, E.R., et al., *Antiglucocorticoid RU 38486 attenuates retention of a behaviour and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites*. Neuroendocrinology, 1988. **47**(2): p. 109-15.
29. Reul, J.M., F.R. van den Bosch, and E.R. de Kloet, *Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: functional implications*. J Endocrinol, 1987. **115**(3): p. 459-67.
30. van Weert, L., et al., *Identification of mineralocorticoid receptor target genes in the mouse hippocampus*. J Neuroendocrinol, 2019. **31**(8): p. e12735.
31. de Kloet, A.D., et al., *Adipocyte glucocorticoid receptors mediate fat-to-brain signaling*. Psychoneuroendocrinology, 2015. **56**: p. 110-9.
32. De Kloet, E.R. and J.M. Reul, *Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems*. Psychoneuroendocrinology, 1987. **12**(2): p. 83-105.
33. Otte, C., et al., *Modulation of the mineralocorticoid receptor as add-on treatment in depression: a randomized, double-blind, placebo-controlled proof-of-concept study.* J Psychiatr Res, 2010. **44**(6): p. 339-46.
34. Rivers, C.A., et al., *Glucocorticoid Receptor-Tethered Mineralocorticoid Receptors Increase Glucocorticoid-Induced Transcriptional Responses*. Endocrinology, 2019. **160**(5): p. 1044-1056.
35. Groeneweg, F.L., et al., *Quantitation of glucocorticoid receptor DNA-binding dynamics by single-molecule microscopy and FRAP.* PLoS One, 2014. **9**(3): p. e90532.
36. Ratman, D., et al., *How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering*. Mol Cell Endocrinol, 2013. **380**(1-2): p. 41-54.
37. Joels, M., *Corticosteroid effects in the brain: U-shape it*. Trends Pharmacol Sci, 2006. **27**(5): p. 244-50.
38. Spaanderman, D.C.E., et al., *Androgens modulate glucocorticoid receptor activity in adipose tissue and liver.* J Endocrinol, 2018.
39. Chen, S., et al., *Androgen and glucocorticoid receptor heterodimer formation. A possible mechanism for mutual inhibition of transcriptional activity.* J Biol Chem, 1997. **272**(22): p. 14087-92.

40. Bougarne, N., et al., *Mechanisms Underlying the Functional Cooperation Between PPARalpha and GRalpha to Attenuate Inflammatory Responses*. Front Immunol, 2019. **10**: p. 1769.
41. Bougarne, N., et al., *PPARalpha blocks glucocorticoid receptor alpha-mediated transactivation but cooperates with the activated glucocorticoid receptor alpha for transrepression on NF-kappaB*. Proc Natl Acad Sci U S A, 2009. **106**(18): p. 7397-402.
42. Miranda, T.B., et al., *Reprogramming the chromatin landscape: interplay of the estrogen and glucocorticoid receptors at the genomic level*. Cancer Res, 2013. **73**(16): p. 5130-9.
43. De Bosscher, K., et al., *Nuclear receptor crosstalk - defining the mechanisms for therapeutic innovation*. Nat Rev Endocrinol, 2020. **16**(7): p. 363-377.
44. Goldstein, I., et al., *Transcription factor assisted loading and enhancer dynamics dictate the hepatic fasting response*. Genome Res, 2017. **27**(3): p. 427-439.
45. Kroon, J., A.M. Pereira, and O.C. Meijer, *Glucocorticoid Sexual Dimorphism in Metabolism: Dissecting the Role of Sex Hormones*. Trends Endocrinol Metab, 2020. **31**(5): p. 357-367.
46. Kaikaew, K., et al., *Sex Difference in Corticosterone-Induced Insulin Resistance in Mice*. Endocrinology, 2019. **160**(10): p. 2367-2387.
47. Gasparini, S.J., et al., *Androgens sensitise mice to glucocorticoid-induced insulin resistance and fat accumulation*. Diabetologia, 2019. **62**(8): p. 1463-1477.
48. Berger, S., et al., *Mineralocorticoid receptor knockout mice: pathophysiology of Na+ metabolism*. Proc Natl Acad Sci U S A, 1998. **95**(16): p. 9424-9.
49. Tronche, F., et al., *Genetic dissection of glucocorticoid receptor function in mice*. Curr Opin Genet Dev, 1998. **8**(5): p. 532-8.
50. Feraco, A., et al., *Minor role of mature adipocyte mineralocorticoid receptor in high fat induced obesity*. J Endocrinol, 2018.
51. Byrne, C.D. and G. Targher, *NAFLD: a multisystem disease*. J Hepatol, 2015. **62**(1 Suppl): p. S47-64.
52. Desarzens, S. and N. Faresse, *Adipocyte glucocorticoid receptor has a minor contribution in adipose tissue growth*. J Endocrinol, 2016. **230**(1): p. 1-11.
53. Shen, Y., et al., *Adipocyte glucocorticoid receptor is important in lipolysis and insulin resistance due to exogenous steroids, but not insulin resistance caused by high fat feeding*. Mol Metab, 2017. **6**(10): p. 1150-1160.
54. Dalle, H., et al., *Adipocyte Glucocorticoid Receptor Deficiency Promotes Adipose Tissue Expandability and Improves the Metabolic Profile Under Corticosterone Exposure*. Diabetes, 2019. **68**(2): p. 305-317.
55. Bose, S.K., I. Hutson, and C.A. Harris, *Hepatic Glucocorticoid Receptor Plays a Greater Role Than Adipose GR in Metabolic Syndrome Despite Renal Compensation*. Endocrinology, 2016. **157**(12): p. 4943-4960.
56. Mueller, K.M., et al., *Adipocyte Glucocorticoid Receptor Deficiency Attenuates Aging- and HFD-Induced Obesity and Impairs the Feeding-Fasting Transition*. Diabetes, 2017. **66**(2): p. 272-286.
57. Ioannou, G.N., *The Role of Cholesterol in the Pathogenesis of NASH*. Trends Endocrinol Metab, 2016. **27**(2): p. 84-95.
58. Carr, R.M. and A.E. Reid, *FXR agonists as therapeutic agents for non-alcoholic fatty liver disease*. Curr Atheroscler Rep, 2015. **17**(4): p. 500.
59. Kerr, T.A. and N.O. Davidson, *Cholesterol and nonalcoholic fatty liver disease: renewed focus on an old villain*. Hepatology, 2012. **56**(5): p. 1995-8.

60. Xiao, Y., et al., *Glucocorticoid treatment alters systemic bile acid homeostasis by regulating the biosynthesis and transport of bile salts.* Dig Liver Dis, 2016. **48**(7): p. 771-9.
61. Tang, W., M. Norlin, and K. Wikvall, *Glucocorticoid receptor-mediated upregulation of human CYP27A1, a potential anti-atherogenic enzyme.* Biochim Biophys Acta, 2008. **1781**(11-12): p. 718-23.
62. Rose, A.J., et al., *Molecular control of systemic bile acid homeostasis by the liver glucocorticoid receptor.* Cell Metab, 2011. **14**(1): p. 123-30.
63. van den Heuvel, J.K., et al., *Identification of a selective glucocorticoid receptor modulator that prevents both diet-induced obesity and inflammation.* Br J Pharmacol, 2016. **173**(11): p. 1793-804.
64. Quagliarini, F., et al., *Cistromic Reprogramming of the Diurnal Glucocorticoid Hormone Response by High-Fat Diet.* Mol Cell, 2019. **76**(4): p. 531-545 e5.
65. Lemke, U., et al., *The glucocorticoid receptor controls hepatic dyslipidemia through Hes1.* Cell Metab, 2008. **8**(3): p. 212-23.
66. Raabe, M., et al., *Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice.* J Clin Invest, 1999. **103**(9): p. 1287-98.
67. Su, X. and N.A. Abumrad, *Cellular fatty acid uptake: a pathway under construction.* Trends Endocrinol Metab, 2009. **20**(2): p. 72-7.
68. Schuster, S., et al., *Triggering and resolution of inflammation in NASH.* Nat Rev Gastroenterol Hepatol, 2018. **15**(6): p. 349-364.
69. Wang, H., et al., *FXR modulators for enterohepatic and metabolic diseases.* Expert Opin Ther Pat, 2018. **28**(11): p. 765-782.
70. Goodwin, J.E., et al., *Knockout of the vascular endothelial glucocorticoid receptor abrogates dexamethasone-induced hypertension.* J Hypertens, 2011. **29**(7): p. 1347-56.
71. Gesina, E., et al., *Dissecting the role of glucocorticoids on pancreas development.* Diabetes, 2004. **53**(9): p. 2322-9.
72. Luijten, I.H.N., B. Cannon, and J. Nedergaard, *Glucocorticoids and Brown Adipose Tissue: Do glucocorticoids really inhibit thermogenesis?* Mol Aspects Med, 2019. **68**: p. 42-59.
73. Glantschnig, C., et al., *The glucocorticoid receptor in brown adipocytes is dispensable for control of energy homeostasis.* EMBO Rep, 2019. **20**(11): p. e48552.
74. Auvinen, H.E., et al., *The effects of high fat diet on the basal activity of the hypothalamus-pituitary-adrenal axis in mice.* J Endocrinol, 2012. **214**(2): p. 191-7.
75. Kroon, J., et al., *A physiological glucocorticoid rhythm is an important regulator of brown adipose tissue function.* Mol Metab, 2021. **47**: p. 101179.
76. Reichardt, S.D., et al., *Glucocorticoids enhance intestinal glucose uptake via the dimerized glucocorticoid receptor in enterocytes.* Endocrinology, 2012. **153**(4): p. 1783-94.
77. Thiesen, A., et al., *Locally and systemically active glucocorticosteroids modify intestinal absorption of sugars in rats.* J Appl Physiol (1985), 2003. **94**(2): p. 583-90.
78. Thiesen, A., et al., *Locally and systemically active glucocorticosteroids modify intestinal absorption of lipids in rats.* Lipids, 2002. **37**(2): p. 159-66.
79. Delaunay, F., et al., *Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids.* J Clin Invest, 1997. **100**(8): p. 2094-8.
80. Rafacho, A., et al., *Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes.* J Endocrinol, 2014. **223**(3): p. R49-62.

81. Hughes, V.A., et al., *Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance*. Am J Physiol, 1993. **264**(6 Pt 1): p. E855-62.
82. Westerblad, H., J.D. Bruton, and A. Katz, *Skeletal muscle: energy metabolism, fiber types, fatigue and adaptability.* Exp Cell Res, 2010. **316**(18): p. 3093-9.
83. Morales, P.E., J.L. Bucarey, and A. Espinosa, *Muscle Lipid Metabolism: Role of Lipid Droplets and Perilipins*. J Diabetes Res, 2017. **2017**: p. 1789395.
84. Eckardt, K., et al., *Myokines in insulin resistance and type 2 diabetes*. Diabetologia, 2014. **57**(6): p. 1087-99.
85. Bordag, N., et al., *Glucocorticoid (dexamethasone)-induced metabolome changes in healthy males suggest prediction of response and side effects*. Sci Rep, 2015. **5**: p. 15954.
86. Betheil, J.J., M. Feigelson, and P. Feigelson, *The differential effects of glucocorticoid on tissue and plasma amino acid levels*. Biochim Biophys Acta, 1965. **104**(1): p. 92-7.
87. Schakman, O., et al., *Glucocorticoid-induced skeletal muscle atrophy.* Int J Biochem Cell Biol, 2013. **45**(10): p. 2163-72.
88. Kalhan, S.C., *Fatty acids, insulin resistance, and protein metabolism*. J Clin Endocrinol Metab, 2009. **94**(8): p. 2725-7.
89. Wise, J.K., R. Hendler, and P. Felig, *Influence of glucocorticoids on glucagon secretion and plasma amino acid concentrations in man*. J Clin Invest, 1973. **52**(11): p. 2774-82.
90. Oh, Y.T., et al., *A Fall in plasma free fatty acid (FFA) level activates the hypothalamic-pituitary-adrenal axis independent of plasma glucose: evidence for brain sensing of circulating FFA*. Endocrinology, 2012. **153**(8): p. 3587-92.
91. Laugero, K.D., et al., *Sucrose ingestion normalizes central expression of corticotropin-releasing-factor messenger ribonucleic acid and energy balance in adrenalectomized rats: a glucocorticoid-metabolic-brain axis?* Endocrinology, 2001. **142**(7): p. 2796-804.
92. Malendowicz, L.K., et al., *Leptin and the regulation of the hypothalamic-pituitary-adrenal axis*. Int Rev Cytol, 2007. **263**: p. 63-102.
93. Kuo, T., et al., *Regulation of Glucose Homeostasis by Glucocorticoids*. Adv Exp Med Biol, 2015. **872**: p. 99-126.
94. Harris, R.B., *Leptin--much more than a satiety signal*. Annu Rev Nutr, 2000. **20**: p. 45-75.
95. Dubois, V., et al., *Distinct but complementary contributions of PPAR isotypes to energy homeostasis*. J Clin Invest, 2017. **127**(4): p. 1202-1214.
96. Hasan, A.U., et al., *PPARgamma activation mitigates glucocorticoid receptor-induced excessive lipolysis in adipocytes via homeostatic crosstalk*. J Cell Biochem, 2018. **119**(6): p. 4627-4635.
97. Nguyen, E.T., et al., *Effects of combined glucocorticoid/mineralocorticoid receptor modulation (CORT118335) on energy balance, adiposity, and lipid metabolism in male rats*. Am J Physiol Endocrinol Metab, 2019. **317**(2): p. E337-E349.
98. Ameen, C., et al., *Activation of peroxisome proliferator-activated receptor alpha increases the expression and activity of microsomal triglyceride transfer protein in the liver.* J Biol Chem, 2005. **280**(2): p. 1224-9.
99. Haas, M.E., A.D. Attie, and S.B. Biddinger, *The regulation of ApoB metabolism by insulin*. Trends Endocrinol Metab, 2013. **24**(8): p. 391-7.
100. Harvey, I., et al., *Glucocorticoid-Induced Metabolic Disturbances Are Exacerbated in Obese Male Mice*. Endocrinology, 2018. **159**(6): p. 2275-2287.
101. Shpilberg, Y., et al., *A rodent model of rapid-onset diabetes induced by glucocorticoids and high-fat feeding*. Dis Model Mech, 2012. **5**(5): p. 671-80.

102. de Quervain, D., L. Schwabe, and B. Roozendaal, *Stress, glucocorticoids and memory: implications for treating fear-related disorders*. Nat Rev Neurosci, 2017. **18**(1): p. 7-19.
103. Korte, S.M., *Corticosteroids in relation to fear, anxiety and psychopathology*. Neurosci Biobehav Rev, 2001. **25**(2): p. 117-42.
104. McEwen, B.S., *Glucocorticoids, depression, and mood disorders: structural remodeling in the brain*. Metabolism, 2005. **54**(5 Suppl 1): p. 20-3.
105. Verma, D., et al., *Hunger Promotes Fear Extinction by Activation of an Amygdala Microcircuit*. Neuropsychopharmacology, 2016. **41**(2): p. 431-9.
106. Rajan, T.M. and V. Menon, *Psychiatric disorders and obesity: A review of association studies*. J Postgrad Med, 2017. **63**(3): p. 182-190.
107. Milaneschi, Y., et al., *Depression and obesity: evidence of shared biological mechanisms*. Mol Psychiatry, 2019. **24**(1): p. 18-33.
108. Wu, G., et al., *Central functions of neuropeptide Y in mood and anxiety disorders*. Expert Opin Ther Targets, 2011. **15**(11): p. 1317-31.
109. Kokare, D.M., et al., *Interaction between neuropeptide Y and alpha-melanocyte stimulating hormone in amygdala regulates anxiety in rats*. Brain Res, 2005. **1043**(1-2): p. 107-14.
110. Nguyen, E.T., et al., *Differential effects of imipramine and CORT118335 (Glucocorticoid receptor modulator/mineralocorticoid receptor antagonist) on brain-endocrine stress responses and depression-like behavior in female rats*. Behav Brain Res, 2018. **336**: p. 99-110.
111. de Kloet, E.R., R.H. Derijk, and O.C. Meijer, *Therapy Insight: is there an imbalanced response of mineralocorticoid and glucocorticoid receptors in depression?* Nat Clin Pract Endocrinol Metab, 2007. **3**(2): p. 168-79.
112. McGinn, M.A., et al., *Glucocorticoid receptor modulators decrease alcohol self-administration in male rats*. Neuropharmacology, 2021: p. 108510.
113. *Medication Development for Protracted Abstinence in Alcoholism: CORT118335 Versus Placebo*. Available from: https://ClinicalTrials.gov/show/NCT04466215.
114. *Hydrocortisone as co-treatment to prevent neuropsychiatric adverse effects of dexamethasone.*; Available from: https://www.trialregister.nl/trial/6726.
115. Karssen, A.M., et al., *Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain*. Endocrinology, 2001. **142**(6): p. 2686-94.
116. Karssen, A.M., et al., *Low doses of dexamethasone can produce a hypocorticosteroid state in the brain*. Endocrinology, 2005. **146**(12): p. 5587-95.
117. van Rossum, E.F., *Obesity and cortisol: New perspectives on an old theme*. Obesity (Silver Spring), 2017. **25**(3): p. 500-501.
118. Liu, Y., A. Beyer, and R. Aebersold, *On the Dependency of Cellular Protein Levels on mRNA Abundance*. Cell, 2016. **165**(3): p. 535-50.
119. Kwda, A., et al., *Effect of long term inhaled corticosteroid therapy on adrenal suppression, growth and bone health in children with asthma*. BMC Pediatr, 2019. **19**(1): p. 411.
120. Grossmann, C., et al., *Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties*. Eur J Endocrinol, 2004. **151**(3): p. 397-406.
121. Crowe, A. and A.M. Tan, *Oral and inhaled corticosteroids: differences in P-glycoprotein (ABCB1) mediated efflux*. Toxicol Appl Pharmacol, 2012. **260**(3): p. 294-302.

122. Bonisch, C., et al., *Dexamethasone treatment alters insulin, leptin, and adiponectin levels in male mice as observed in DIO but does not lead to alterations of metabolic phenotypes in the offspring*. Mamm Genome, 2016. **27**(1-2): p. 17-28.
123. Aru, M., et al., *Changes in Body Composition of Old Rats at Different Time Points After Dexamethasone Administration*. Curr Aging Sci, 2019. **11**(4): p. 255-260.
124. Palacios, E., et al., *Effect of lipectomy and long-term dexamethasone on visceral fat and metabolic variables in rats*. Metabolism, 1995. **44**(12): p. 1631-8.
125. Sarcev, T., et al., *[Influence of dexamethasone on appetite and body weight in lung cancer patients]*. Med Pregl, 2008. **61**(11-12): p. 571-5.
126. Raubenheimer, P.J., et al., *The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback*. Clin Endocrinol (Oxf), 2006. **65**(1): p. 22-6.
127. Nixon, M., et al., *ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy*. Sci Transl Med, 2016. **8**(352): p. 352ra109.
128. Hunter, H., et al., *Weight loss, insulin resistance, and study design confound results in a meta-analysis of animal models of fatty liver*. Elife, 2020. **9**.
129. *Study Evaluating the Safety, Efficacy, and Pharmacokinetics of Miricorilant in Patients With Presumed Nonalcoholic Steatohepatitis (NASH)*. Available from: https://ClinicalTrials.gov/show/NCT03823703.
130. *The Effect of CORT118335 on Olanzapine-Induced Weight Gain*. Available from: https://ClinicalTrials.gov/show/NCT03877562.
131. *This Phase 2, Double Blind, Placebo-controlled, Randomized Study is to Assess the Safety and Efficacy of Miricorilant in Obese Adult With Schizophrenia or Bipolar Disorder Treated With Antipsychotic Medications*. Available from: https://ClinicalTrials.gov/show/NCT03818256.
132. *Study Evaluating the Safety, Efficacy, and Pharmacokinetics of Miricorilant in Obese Adult Patients With Schizophrenia While Taking Antipsychotic Medications (GRATITUDE II)*. Available from: https://ClinicalTrials.gov/show/NCT04524403.
133. Spitz, I.M., et al., *Management of patients receiving long-term treatment with mifepristone*. Fertil Steril, 2005. **84**(6): p. 1719-26.
134. *Mifepristone*, in *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. 2012: Bethesda (MD).
135. Xiao, Y., et al., *Thirty-day rat toxicity study reveals reversible liver toxicity of mifepristone (RU486) and metapristone*. Toxicol Mech Methods, 2016. **26**(1): p. 36-45.
136. Pal'chikova, N.A., et al., *Effects Mifepristone on Aminotransferase Activities in the Liver in Rats with Streptozotocin-Induced Diabetes Mellitus*. Bull Exp Biol Med, 2018. **165**(4): p. 474-477.
137. *CORT125281 Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD)*. Available from: https://ClinicalTrials.gov/show/NCT03335956.
138. *Study to Evaluate CORT125281 in Combination With Enzalutamide in Patients With mCRPC*. Available from: https://ClinicalTrials.gov/show/NCT03437941.
139. Klok, M.D., et al., *A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females*. Transl Psychiatry, 2011. **1**: p. e62.
140. de Kloet, E.R., et al., *Stress and Depression: a Crucial Role of the Mineralocorticoid Receptor*. J Neuroendocrinol, 2016. **28**(8).

141. Koper, J.W., E.F. van Rossum, and E.L. van den Akker, *Glucocorticoid receptor polymorphisms and haplotypes and their expression in health and disease*. Steroids, 2014. **92**: p. 62-73.
142. Wester, V.L., et al., *Glucocorticoid receptor haplotype and metabolic syndrome: the Lifelines cohort study.* Eur J Endocrinol, 2016. **175**(6): p. 645-651.
143. Endedijk, H.M., et al., *The Role of Stress and Mineralocorticoid Receptor Haplotypes in the Development of Symptoms of Depression and Anxiety During Adolescence*. Front Psychiatry, 2020. **11**: p. 367.
144. Vinkers, C.H., et al., *Mineralocorticoid receptor haplotypes sex-dependently moderate depression susceptibility following childhood maltreatment*. Psychoneuroendocrinology, 2015. **54**: p. 90-102.
145. Santen, R.J., et al., *Glucocorticoid Receptor Mutations and Hypersensitivity to Endogenous and Exogenous Glucocorticoids.* J Clin Endocrinol Metab, 2018. **103**(10): p. 3630-3639.
146. van Leeuwen, N., et al., *Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and transactivation: implication for the stress response*. Psychoneuroendocrinology, 2011. **36**(5): p. 699-709.
147. *Medical Expenditure Panel Survey (MEPS)*. 2018, Rockeville, MD.: Agency for Healthcare Research and Quality.
148. de Graaf, R., M. Ten Have, and S. van Dorsselaer, *The Netherlands Mental Health Survey and Incidence Study-2 (NEMESIS-2): design and methods.* Int J Methods Psychiatr Res, 2010. **19**(3): p. 125-41.
149. Hooftman W.E. Mars G.M.J., J.B., de Vroome E.M.M., Janssen B.J.M., Pleijers A.J.S.F. Ramaekers M. M. M. J. and S.N.J. van den Bossche, *Nationale Enquête Arbeidsomstandigheden 2018: Methodologie en globale resultaten*. 2019, Leiden, Heerlen: TNO ; CBS.
150. *Noncommunicable diseases country profiles 2018*. 2018, Geneva: World Health Organization.