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

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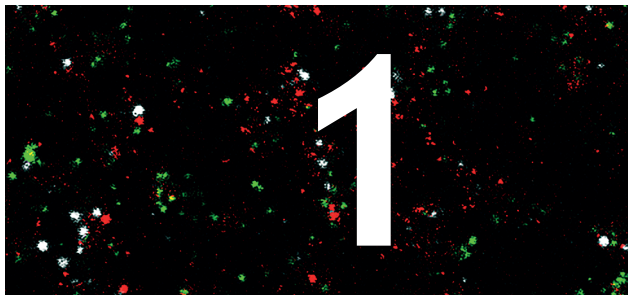

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General introduction and outline

"Stress in health and disease is medically, sociologically, and philosophically the most meaningful subject for humanity that I can think of" – Hans Selye

GENERAL INTRODUCTION

Stress, stress-related mood disorders and obesity are highly prevalent in modern society [1-3]. Stress is a reactive state that allows for adaptation to a changing environment. It may be that the rapid social and technological changes of recent decades, such as the emergence of social media and the growing 24-hour economy, has increased the number of demands on us and contributed to an increased stress load. However, our era is not unique in its high stress burden. People in past societies also faced many difficulties such as infant mortality, warfare and epidemic diseases. Reports already date back to 1860 in which stress, or the then-called 'American nervousness', was linked to the 'pressures of modern life' [4]. Nevertheless, stress clearly plays a major role in our modern society, illustrated by recent reports showing that 1 out of 5 in the Dutch population has ever suffered from a diagnosed depression (considered a stress-related disease) and 17% of Dutch employees from a burn-out [1, 2]. A parallel trend related to recent societal changes is the global rise of obesity. According to the World Health Organization, the global prevalence of obesity has nearly tripled between 1975 and 2016 [3]. In 2016, up to 40% of the global adult population were overweight [3]. Since obesity increases the susceptibility for other, more severe complications such as cardiovascular disease and non-alcoholic fatty liver disease, these conditions have also become increasingly prevalent [5-7]. Interestingly, stress also affects metabolism in many ways.

While each of these conditions has its own etiology, diseases also aggravate each other and, therefore, often coincide [8-10]. The 'disease triangle' in **Fig. 1** describes the bidirectional relationships between obesity, mood and stress [8]. According to this disease triangle, different disease states can form a vicious cycle by triggering and reinforcing each other. Although psychological and social factors contribute to these associations, in this thesis we focused on the(neuro-)biological mechanisms that connect mood, obesity and stress.

Good and bad stress

Any threat to the homeostasis of an organism can be perceived as a stressor and can initiate a stress response. Stressors can largely be divided in psychological (for example work pressure) and physical stressors (for example cold stress, physical activity, inflammation). The initial stress response typically is a fight, flight or fright reaction, which has the ultimate goal to restore or maintain the individual's homeostasis in response to an external threat. Whether a stressor falls within the ability to cope ('*eustress'*) or outside ('*distress'*) will depend on the predictability, controllability, intensity and duration of the stressor [11]. Eustress is often referred to as (and literally means) 'good stress', as it allows for adaptation and self-preservation. Distress on the other hand is (at least in part) maladaptive, and can increase the vulnerability to stress-related complications such as depression, anxiety and post-traumatic stress disorder [12-14].

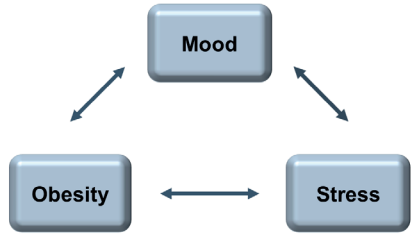

Figure 1: A disease triangle connects obesity, stress and (stress-related) mood disorders, such as depression and anxiety disorders.

The sympathoadrenal system and the hypothalamus-pituitary-adrenal-axis

The stress response in the body is mediated by two hormonal systems, the rapid sympathoadrenal system with epinephrine (also known as adrenalin) as end product, and the slower, more thorough hypothalamus-pituitary-adrenal (HPA)-axis with glucocorticoids as end product. The sympathoadrenal nervous system modulates signal transmission between neurons in the brain and the peripheral sympathetic nervous system. In the brain, signal transmission is mediated by neurotransmitter norepinephrine that is released from the locus coeruleus (**Fig. 2A**) [15]. By binding to α-adrenoreceptors, norepinephrine increases arousal and affects emotions and memory. Norepinephrine also stimulates sympathetic outflow from the brain to *peripheral* tissues, which are all tissues outside the brain. In the adrenals, norepinephrine triggers the release of epinephrine. Epinephrine binds to peripheral α- and ß-adrenoreceptors and, together with norepinephrine, coordinates a body-wide sympathetic response [15]. Peripheral actions of norepinephrine and epinephrine are aimed to support the fight/flight response, and include increased heart rate, redistribution of blood to active organs, glucose mobilization and dilation of pupils and lung bronchi (**Fig. 2A**) [15].

The HPA-axis mediates other, complementary aspects of the stress response, which will be the focus of this thesis. The HPA-axis controls the release of glucocorticoids at multiple levels. In the brain, the hypothalamic paraventricular nucleus releases corticotropin releasing hormone (CRH) which stimulates the pituitary to release adrenocorticotrophic hormone (ACTH) into the blood circulation (**Fig. 2B**). ACTH induces the secretion of glucocorticoids from the adrenal glands (**Fig. 2B**). Glucocorticoids are released in both stressed and non-stressed conditions. In non-stressed conditions, glucocorticoids synchronize organs to the body's internal clock ('*circadian rhythmicity*'), which is essential for the proper functioning of peripheral tissues and the brain. As part of this circadian rhythmicity, glucocorticoid levels peak before, and fall after the active period of the 24 hour day-night cycle [16].

Glucocorticoids, such as cortisol and corticosterone, mediate their effects via binding to the *high-affinity* mineralocorticoid receptor (MR) and the *lower affinity* glucocorti-

coid receptor (GR) [17]. Affinity is a measure of the strength of attraction between a receptor and its *ligand,* which is the compound that binds the receptor (in this case: the glucocorticoid hormone) and thereby regulates the extent of receptor activation. Given that MR has a high-affinity for glucocorticoids, the MR is already bound at relatively low glucocorticoid levels. In contrast, the low-affinity GR requires higher glucocorticoid levels for its activation. Consequently, MR is important during the initiation phase of the stress response during which stressors are appraised and a coping style is chosen [18]. GRs are involved in the termination phase of the stress response and GRs in hypothalamus and pituitary mediate two negative feedback loops that restore glucocorticoid levels to normal (**Fig. 2B).**

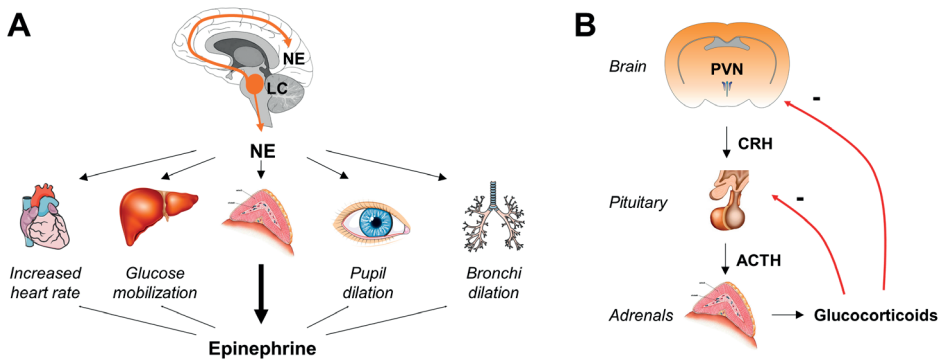

Figure 2: The stress response is mediated by the sympathoadrenal system and the HPA-axis. A) In response to stress, the locus coeruleus (LC) secretes neurotransmitter norepinephrine (NE) that acts on the brain and increases sympathetic outflow to peripheral tissues. In the adrenals, NE stimulates epinephrine release. NE and epinephrine work together to coordinate the fight/flight/fright reaction by acting on multiple tissues, including the heart, liver, eyes and lungs. B) Glucocorticoid release is under control of hypothalamus-pituitary adrenal (HPA)-axis activity. In the brain, the paraventricular nucleus of the hypothalamus (PVN) releases corticotropin releasing hormone (CRH), which stimulates adrenocorticotropin hormone (ACTH) secretion from the pituitary. ACTH induces the secretion of glucocorticoids from the adrenals. Glucocorticoids suppress HPA-axis activity by activating two negative feedback loops (*red arrows*) at the level of the pituitary and PVN.

The hormonal basis of fear: CRH, glucocorticoid and the amygdala

One of the psychological aspects of stress is the activation of the fear system. The core of the fear circuitry resides in the amygdala, which is the 'emotion center' of the brain. Since glucocorticoid levels only peak 15 to 30 minutes after the start of the stress response, CRH in the amygdala mediates the acute effects of fear independent of HPA-axis activation [19]. While the 'overall' effect of amygdaloid CRH is to increase fear (*'anxiogenesis'*), the CRH receptor system is more complex, with two receptors and more than one ligand [20]. CRH is the primary ligand for CRH receptor 1 (CRHR1) which mostly increases fear (*'anxiogenic'*). The closely related urocortin peptides are primarily ligands for the CRH receptor 2 (CRHR2), which reduces fear levels *('anxiolysis'*). Via amygdaloid projections to other regions in the brain, CRH also mediates other acute

aspects of the stress response, such as increased sympathetic outflow and freezing behavior.

During the later stages of the stress response, glucocorticoids also become important for fear regulation. Glucocorticoids potentiate the fear response by increasing CRH expression in the amygdala [21]. Glucocorticoids additionally promote the acquisition and long-term consolidation of (fear) memories, so that an individual is better prepared (or 'primed') for future stressors [22]. As part of the termination phase of stress, glucocorticoids support the extinction of fear [23]. Although these effects are adaptive on the short term, in particular the 'priming' glucocorticoid effects can become maladaptive when stressors are too severe, chronic, or out of context. In this way glucocorticoids can contribute to the development of anxiety disorders, depression and post-traumatic-stress disorder [9].

GR and MR are nuclear receptors that cooperate or oppose each other

Although rapid actions of GR and MR have been described that occur within minutes after receptor binding (*'non-genomic'*) [24], GR and MR are best understood as ligand-activated transcription factors belonging to the nuclear receptor family. When activated by a hormone ligand, the ligand-receptor complex translocates from the cytoplasm to the nucleus of a cell. Here it recruits coregulator proteins that influence DNA accessibility and gene transcription [25]. GR and MR transactivate genes by direct binding of their DNA binding domain to accessible glucocorticoid response elements (GRE) in the DNA. Since the DNA binding domains of GR and MR are highly similar, GR and MR recognize the same GRE and therefore share a large number of target genes [26]. Despite the high similarity, GR and MR also have unique binding sites in the genome that may depend on MR-specific coregulators and other transcription factors that bind nearby GREs [27-29]. GR can also transrepress gene transcription, which either involves the binding to *negative* GREs, or is mediated via indirect mechanisms like tethering to other transcription factors [30, 31].

The GR is widely expressed in the body, while the MR is expressed in a more restricted, tissue-specific manner. At the functional level, GR and MR are classically known to oppose each other in the brain [32]. These opposing actions have formed the basis of the GR:MR balance theory, which states that an imbalance of GR- and MR-mediated actions may compromise initiation and/or management of the stress response and may ultimately disrupt neuro-endocrine and behavioral functioning [33, 34]. However, recent work underlines that GR and MR can also act synergistically, and that GR-MR interactions may also occur in peripheral tissues [35, 36].

Glucocorticoids increase nutrient fluxes

Metabolic effects of glucocorticoids help to support the body's metabolic needs in response to (metabolic) stressors such as (heavy) physical activity or during a fight/flight response. Glucocorticoids promote the overall flux of nutrients through the body by promoting the build-up and the breakdown of nutrients [37]. In this way, metabolic flexibility is promoted during stress, and nutrient stores are replenished during stress recovery. However, overexposure to glucocorticoids, for example due to prolonged use of the glucocorticoid-like drug prednisolone, leads to adverse metabolic effects, such as obesity, elevated blood glucose and insulin levels (*'hyperglycemia'* and *'hyperinsulinemia'*), non-alcoholic fatty liver disease and increased blood pressure (*'hypertension'*) [38]. These symptoms are highly similar to the cluster of metabolic disorders linked to obesity, referred to as the *'metabolic syndrome'*, including hyperglycemia, hyperinsulinemia, hypertension and abnormal triglyceride and cholesterol levels (*'dyslipidemia'*). The high similarity between the metabolic syndrome and glucocorticoid effects underlies the hypothesis that inhibition of glucocorticoid signaling during obesity may improve the metabolic profile [8].

Effects of glucocorticoids on the liver, white adipose tissue and muscle

The liver is central to metabolism, as it is the first organ to receive the nutrient-rich portal blood from the intestines, and is the distribution center for lipids and glucose. The liver is the only site where new glucose can be produced (*'de novo gluconeogenesis'*), which is essential to keep blood glucose levels constant [39]. The liver, along with muscle tissue, is also the major storage site of glucose in the form of glycogen. Glucocorticoids raise blood glucose levels by stimulating both gluconeogenesis and the breakdown of glycogen stores (*'glycogenolysis'*) in the liver (**Fig. 3**) [40]. To this end, glucocorticoids increase expression of respective enzymes, and increase substrate for *de novo* gluconeogenesis by promoting the breakdown of proteins (*'proteolysis'*) in muscle.

With regard to lipid metabolism, the liver receives dietary triglycerides from the intestines and non-dietary free fatty acids from the white adipose tissue. In white adipose tissue, lipids are stored as triglycerides. When necessary, triglycerides are broken down (*'lipolysis'*) in white adipocytes and released as free fatty acids into the circulation [41]. In the liver, triglycerides and fatty acids are taken up by transporters. Fatty acids are converted back to triglycerides, which are either stored in the liver or packaged as very-low-density-lipoprotein particles. These particles are secreted into the circulation to provide triglycerides to the rest of the body [42]. A healthy liver does not store many triglycerides, but when exposed to a high lipid and/or glucose load, it will serve as an 'ectopic' storage site [43]. This increased hepatic lipid storage, or *liver steatosis,* is the first step in the development of non-alcoholic fatty liver disease. Glucocorticoids

increase the *influx* of lipids in the liver by increasing the hepatic expression of lipid transporters, and by increasing lipid flow towards the liver by stimulating lipolysis in the white adipose tissue (**Fig. 3**) [37]. Simultaneously, glucocorticoids stimulate *efflux* of lipids by upregulating expression of genes involved in lipoprotein production (**Fig. 3**). Glucocorticoids also allow for replenishment of lipid stores by stimulating the production of new lipids (*'de novo lipogenesis'*) [37]. While glucocorticoids promote both the build-up and breakdown of energy stores, the net effect of chronic glucocorticoid exposure on the liver is to increase lipid storage, which ultimately leads to development of non-alcoholic fatty liver disease [44].

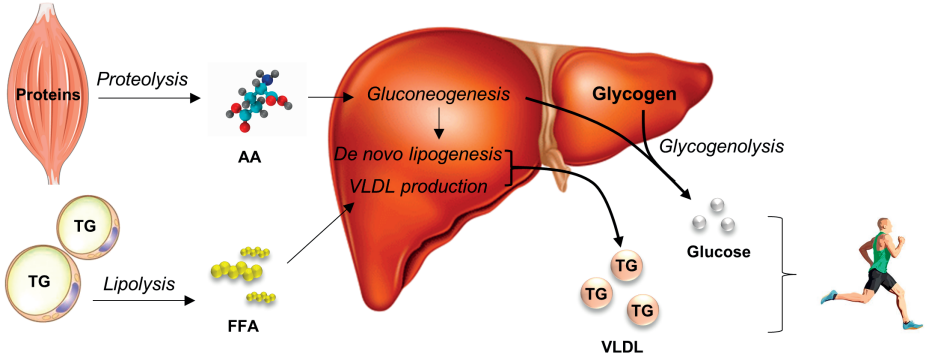

Figure 3: Summary of metabolic effects of glucocorticoids. Glucocorticoids stimulate the metabolic processes depicted in *italics*. In the muscle, glucocorticoids catabolize proteins to amino acids (AA) which serve as substrate for gluconeogenesis in the liver. Glucose is either used as substrate for *de novo* lipogenesis, or directly released into the circulation to provide the rest of the body with energy. In the white adipose tissue, glucocorticoids break down stored triglycerides (TG) into free fatty acids (FFA). FFA are taken up by the liver and converted back to TG. Glucocorticoids increase expression of lipid transporters and *de novo* lipogenesis enzymes, which increases the TG available for very-low-density lipoprotein (VLDL) production. The VLDL particles released into the circulation supply the rest of the body with TG.

Effects of glucocorticoids on brown adipose tissue

In contrast to white adipocytes, brown adipocytes do not store triglycerides but rather utilize triglycerides to produce heat (*'thermogenesis'*). The majority of brown adipocytes can be found in the brown adipose tissue, but there is also a inducible brown adipocyte population (*'beige/brite adipocytes'*) in the white adipose tissue. These beige/brite adipocytes are derived from white adipocytes that underwent the process of *'browning'* [45]. In response to cold, brown adipose tissue is activated by norepinephrine released from sympathetic nerve terminals [46]. Norepinephrine rapidly induces lipolysis of the small lipid droplets present in brown adipocytes, which raises intracellular free fatty acids levels. In the mitochondria, these fatty acids are oxidized for thermogenesis for which uncoupling protein 1 (UCP1) is essential. Without UCP1, the energy obtained from fatty acid oxidation is chemically stored as ATP, which can later

be used for other cellular processes. However, in the presence of activated UCP1, the energy is not stored, but rather released directly as heat.

The effect of glucocorticoids on brown adipose tissue is complex and not completely understood. Classically, glucocorticoids are believed to inhibit activation of the tissue, especially in chronic treatment settings [47]. Glucocorticoid suppress UCP1 expression and replenish lipid stores by increasing lipid transporters in brown adipose tissue, and increasing lipid flow towards the tissue. However, it is debatable whether the reduced UCP1 expression and increased lipid storage really reflect a reduced thermogenic capacity [47]. It is also unclear whether the GRs and MRs in brown adipose tissue mediate these processes [48-50]. Effects of glucocorticoids additionally depend on treatment duration, as in acute treatment regimens, glucocorticoid can also increase brown fat activation [51].

Pharmacokinetics: pre-receptor metabolism

When hormones or drugs reach the bloodstream, the eventual effect of a compound strongly depends on various *pharmacodynamic* factors (how does the compound affect the body), and *pharmacokinetic* factors (what the body does to the compound in terms of distribution and excretion). Body processes that affect (local) ligand concentrations include compound conversion enzymes, drug efflux pumps, tissue-barriers and enzymes in the liver and kidney that break down the molecules [52]. In addition, the mode of administration strongly determines the dynamics of compound concentrations in the blood [52]. For example, intravenously administered compounds tend to induce higher peak compound concentrations in the blood, but disappear more rapidly from the blood than orally administered compounds [53]. One tissue barrier that is especially relevant is the blood-brain-barrier, which selectively controls the entrance of compounds into the brain. Due to this blood-brain-barrier, the amount of compound 'seen' by the brain and other, peripheral tissues can differ substantially. This is especially relevant for drugs such as dexamethasone, which is actively pumped out of the brain by drug efflux pump p-glycoprotein [54]. In adipose tissues similar pumps are present, which may limit the access of particular steroid hormones to these tissues [55].

Apart from adrenal secretion and uptake by tissues, local glucocorticoid concentrations are determined by 11ß-hydroxysteroid dehydrogenase type 1 (11ßHSD1) and 11ß-hydroxysteroid dehydrogenase type 2 (11ßHSD2) activity. 11ßHSD1 amplifies the local glucocorticoid signal by converting inactive glucocorticoids (e.g. cortis*one*) to their active form, while 11ßHSD2 inactivates glucocorticoids [56]. In some tissues, the 11ßHSD1-induced increase of glucocorticoid levels is essential for proper GR activation [57, 58]. 11ßHSD2 is especially relevant for the MRs expressed in the tubular cells of the kidney. Aldosterone is a steroid hormone that acts on MRs in these tubular cells to regulate the salt and water balance in the body. Given that glucocorticoids are more

abundant than aldosterone, aldosterone is normally outcompeted in the binding of MR by glucocorticoids. By co-expressing MR and 11ßHSD2, tubular cells make the MR available for aldosterone-binding, as inactivated glucocorticoids cannot bind MR.

Pharmacodynamics: ligand types

The effect of a compound on receptor activation depends on the type of ligand. Various ligand types can best be discriminated by their relative *efficacy* and *potency* [52]. The efficacy is the maximum effect that can be achieved with an *agonist* (**Fig. 4A**). The potency depends on the affinity of the ligand for its receptor, and reflects the concentration of compound that is needed to produce a given effect. Potency is quantified by the *EC50*, which is the concentration of compound that causes 50% of the maximum effect. A more *potent agonist* thus has a lower EC50 (**Fig. 4A**). A *partial agonist* activates a receptor at a lower efficacy compared to a full agonist. An *antagonist* also binds the receptor but has no efficacy. Consequently, in presence of both agonist and antagonist, antagonists inhibit agonist action by competing for the same receptor binding site (**Fig. 4B**). The *IC50* describes the potency of an antagonist and is the amount of antagonist needed to inhibit receptor action by 50% (at a given agonist concentration). A *selective receptor modulator* has mixed agonist and antagonist properties. While a full agonist of a steroid receptor recruits the complete set of coregulators, a selective receptor modulator recruits a subset of coregulators [59]. Since coregulators differentially influence transcription of genes, a selective receptor modulator stimulates expression of certain genes but not others. These dual actions makes selective receptor modulation

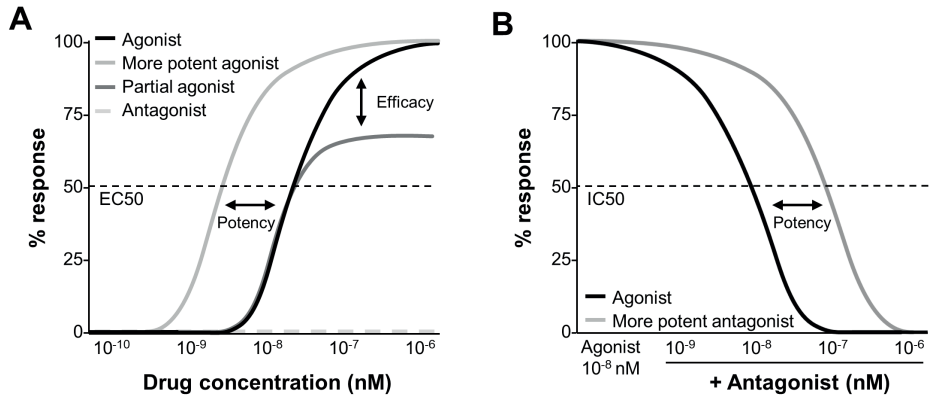

Figure 4: Effects of various ligands on receptor activation. A) Effects of ligands measured in 'agonist mode': ligands are titrated at increasing doses and receptor activation (%response) is measured. Efficacy is determined by the maximum effect that a drug produces. The EC50 describes the potency of an agonist, and is the amount of agonist needed to cause 50% of the maximum effect. B) Effects of ligands measured in 'antagonist mode': Antagonists are titrated at increasing doses in the presence of constant agonist levels. The IC50 describes the potency of an antagonist, and is the amount of antagonist needed to inhibit receptor action by 50%.

an attractive treatment strategy, as it has the potential to separate desired therapeutic outcome from undesired side-effects.

THESIS OUTLINE

In this thesis, we investigated the relation between obesity, the HPA-axis and mood. We aimed to identify metabolic factors that affect fear and their sites of action in the brain (**chapter 2**). We explored novel pharmacological treatment strategies that selectively target glucocorticoid signaling to alleviate diet-induced, or glucocorticoid-induced adverse metabolic effects (**chapter 3-6**). Finally, we investigated whether absolute GR and MR mRNA levels and GR:MR balance can predict the expression of shared target genes at both the cellular and tissue level (**chapter 7**).

REFERENCES

1. 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.
2. 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.
3. *Noncommunicable diseases country profiles 2018*. 2018, Geneva: World Health Organization.
4. Jackson, M., *The stress of life: a modern complaint?* Lancet, 2014. **383**(9914): p. 300-1.
5. Mitra, S., A. De, and A. Chowdhury, *Epidemiology of non-alcoholic and alcoholic fatty liver diseases*. Transl Gastroenterol Hepatol, 2020. **5**: p. 16.
6. Zheng, Y., S.H. Ley, and F.B. Hu, *Global aetiology and epidemiology of type 2 diabetes mellitus and its complications*. Nat Rev Endocrinol, 2018. **14**(2): p. 88-98.
7. Ruff, C.T. and E. Braunwald, *The evolving epidemiology of acute coronary syndromes*. Nat Rev Cardiol, 2011. **8**(3): p. 140-7.
8. van Rossum, E.F., *Obesity and cortisol: New perspectives on an old theme*. Obesity (Silver Spring), 2017. **25**(3): p. 500-501.
9. McEwen, B.S., *Glucocorticoids, depression, and mood disorders: structural remodeling in the brain*. Metabolism, 2005. **54**(5 Suppl 1): p. 20-3.
10. Penninx, B. and S.M.M. Lange, *Metabolic syndrome in psychiatric patients: overview, mechanisms, and implications*. Dialogues Clin Neurosci, 2018. **20**(1): p. 63-73.
11. Selye, H., *[Stress without distress]*. Brux Med, 1976. **56**(5): p. 205-10.
12. Hammen, C., *Stress and depression*. Annu Rev Clin Psychol, 2005. **1**: p. 293-319.
13. Pitman, R.K., et al., *Biological studies of post-traumatic stress disorder*. Nat Rev Neurosci, 2012. **13**(11): p. 769-87.
14. Hassell, J.E., Jr., et al., *The Impact of Stressor Exposure and Glucocorticoids on Anxiety and Fear*. Curr Top Behav Neurosci, 2019. **43**: p. 271-321.
15. Chrousos, G.P., *Stress and disorders of the stress system*. Nat Rev Endocrinol, 2009. **5**(7): p. 374-81.
16. Lightman, S.L. and B.L. Conway-Campbell, *The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration*. Nat Rev Neurosci, 2010. **11**(10): p. 710-8.
17. Herman, J.P., et al., *Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response*. Compr Physiol, 2016. **6**(2): p. 603-21.
18. De Kloet, E.R., et al., *Brain corticosteroid receptor balance in health and disease*. Endocr Rev, 1998. **19**(3): p. 269-301.
19. Cook, C.J., *Glucocorticoid feedback increases the sensitivity of the limbic system to stress*. Physiol Behav, 2002. **75**(4): p. 455-64.
20. Deussing, J.M. and A. Chen, *The Corticotropin-Releasing Factor Family: Physiology of the Stress Response*. Physiol Rev, 2018. **98**(4): p. 2225-2286.
21. Korte, S.M., *Corticosteroids in relation to fear, anxiety and psychopathology*. Neurosci Biobehav Rev, 2001. **25**(2): p. 117-42.
22. Oitzl, M.S. and E.R. de Kloet, *Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning*. Behav Neurosci, 1992. **106**(1): p. 62-71.

23. 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.
24. Groeneweg, F.L., et al., *Rapid non-genomic effects of corticosteroids and their role in the central stress response*. J Endocrinol, 2011. **209**(2): p. 153-67.
25. Koning, A., et al., *Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective*. J Endocr Soc, 2019. **3**(10): p. 1917-1930.
26. Ueda, K., et al., *Genome-wide analysis of murine renal distal convoluted tubular cells for the target genes of mineralocorticoid receptor.* Biochem Biophys Res Commun, 2014. **445**(1): p. 132-7.
27. 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.
28. 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.
29. Yang, J., et al., *Use of phage display to identify novel mineralocorticoid receptor-interacting proteins*. Mol Endocrinol, 2014. **28**(9): p. 1571-84.
30. Surjit, M., et al., *Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor.* Cell, 2011. **145**(2): p. 224-41.
31. Hudson, W.H., C. Youn, and E.A. Ortlund, *The structural basis of direct glucocorticoid-mediated transrepression*. Nat Struct Mol Biol, 2013. **20**(1): p. 53-8.
32. Joels, M. and E.R. De Kloet, *Coordinative mineralocorticoid and glucocorticoid receptor-mediated control of responses to serotonin in rat hippocampus*. Neuroendocrinology, 1992. **55**(3): p. 344-50.
33. De Kloet, E.R., et al., *Plasticity and function of brain corticosteroid receptors during aging*. Acta Endocrinol (Copenh), 1991. **125 Suppl 1**: p. 65-72.
34. Harris, A.P., et al., *Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour.* Psychoneuroendocrinology, 2013. **38**(5): p. 648-58.
35. Stienstra, C.M. and M. Joels, *Effect of corticosteroid treatment in vitro on adrenalectomy-induced impairment of synaptic transmission in the rat dentate gyrus*. J Neuroendocrinol, 2000. **12**(3): p. 199-205.
36. 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.
37. Macfarlane, D.P., S. Forbes, and B.R. Walker, *Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome*. J Endocrinol, 2008. **197**(2): p. 189-204.
38. Judd, L.L., et al., *Adverse consequences of glucocorticoid medication: psychological, cognitive, and behavioral effects*. Am J Psychiatry, 2014. **171**(10): p. 1045-51.
39. Petersen, M.C., D.F. Vatner, and G.I. Shulman, *Regulation of hepatic glucose metabolism in health and disease*. Nat Rev Endocrinol, 2017. **13**(10): p. 572-587.
40. Andrews, R.C. and B.R. Walker, *Glucocorticoids and insulin resistance: old hormones, new targets*. Clin Sci (Lond), 1999. **96**(5): p. 513-23.
41. Kajimura, S., *Adipose tissue in 2016: Advances in the understanding of adipose tissue biology.* Nat Rev Endocrinol, 2017. **13**(2): p. 69-70.
42. Illingworth, D.R., *Lipoprotein metabolism*. Am J Kidney Dis, 1993. **22**(1): p. 90-7.
43. Friedman, S.L., et al., *Mechanisms of NAFLD development and therapeutic strategies*. Nat Med, 2018. **24**(7): p. 908-922.

44. Tarantino, G. and C. Finelli, *Pathogenesis of hepatic steatosis: the link between hypercortisolism and non-alcoholic fatty liver disease*. World J Gastroenterol, 2013. **19**(40): p. 6735-43.
45. Bartelt, A. and J. Heeren, *Adipose tissue browning and metabolic health*. Nat Rev Endocrinol, 2014. **10**(1): p. 24-36.
46. Hoeke, G., et al., *Role of Brown Fat in Lipoprotein Metabolism and Atherosclerosis.* Circ Res, 2016. **118**(1): p. 173-82.
47. 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.
48. Glantschnig, C., et al., *The glucocorticoid receptor in brown adipocytes is dispensable for control of energy homeostasis.* EMBO Rep, 2019. **20**(11): p. e48552.
49. Feraco, A., et al., *Minor role of mature adipocyte mineralocorticoid receptor in high fat induced obesity.* J Endocrinol, 2018.
50. Hayakawa, T., et al., *Impact of MR on mature adipocytes in high-fat/high-sucrose diet-induced obesity.* J Endocrinol, 2018. **239**(1): p. 63-71.
51. Ramage, L.E., et al., *Glucocorticoids Acutely Increase Brown Adipose Tissue Activity in Humans, Revealing Species-Specific Differences in UCP-1 Regulation.* Cell Metab, 2016. **24**(1): p. 130-41.
52. Rang HP, D.M., Ritter JM, Flower RJ, Henderson G, *Rang and Dale's pharmacology*. 2012, Elsevier Churchill Livingstone.
53. Charmandari, E., et al., *Bioavailability of oral hydrocortisone in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.* J Endocrinol, 2001. **169**(1): p. 65-70.
54. 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.
55. 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.
56. Lakshmi, V. and C. Monder, *Evidence for independent 11-oxidase and 11-reductase activities of 11 beta-hydroxysteroid dehydrogenase: enzyme latency, phase transitions, and lipid requirements*. Endocrinology, 1985. **116**(2): p. 552-60.
57. Morton, N.M., et al., *Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice*. Diabetes, 2004. **53**(4): p. 931-8.
58. Yau, J.L., et al., *Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knock-out mice*. J Neurosci, 2007. **27**(39): p. 10487-96.
59. Meijer, O.C., L.L. Koorneef, and J. Kroon, *Glucocorticoid receptor modulators*. Ann Endocrinol (Paris), 2018. **79**(3): p. 107-111.