General discussion

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1. RATIONALE BEHIND THE STUDIES IN THIS THESIS

The studies in this thesis are based on the hypothesis that variations in glucocorticoid hormone exposure have long-term consequences for health. This is clearly evident in Cushing's syndrome, in which the exposure to glucocorticoids is excessive, due to diseases associated with increased production of endogenous glucocorticoids, or due to treatment with systemic glucocorticoids such as prednisolone. Cushing's syndrome is rare, but is associated with a wide range of comorbidities (see Table 1) [1, 2]. Many of the signs and symptoms of Cushing's syndrome are common, however. Cushing's syndrome is usually accompanied by features of the metabolic syndrome: central obesity, insulin resistance, dyslipidemia and hypertension [2, 3]. Therefore, the question whether more subtle increases in glucocorticoid exposure may induce obesity and metabolic syndrome has been studied for decades [4]. However, in the past decades these questions remained unanswered mainly due to use of highly variable short-term cortisol measurements throughout the studies.

Within the general population, the exposure to glucocorticoids varies within much narrower ranges than in Cushing's syndrome. Most of the studies in this thesis are based on the concept that these smaller variations may influence cardiometabolic health. Three sources of glucocorticoid exposure variation can be distinguished (Table 1). First, the amount of glucocorticoids produced by the adrenal gland, the most important of which is cortisol, varies over time and between individuals [5]. Because cortisol levels are highly variable, time-point or short term measurements in urine, saliva and blood show a high amount of variation. In this thesis, glucocorticoid measurements in scalp hair are used, which represent cumulative glucocorticoid exposure over months of time [6]. Second, the glucocorticoid exposure in the general population is influenced by the widespread use of local glucocorticoid containing medication, such as inhaled glucocorticoids for asthma, nasal glucocorticoids for allergic rhinitis, and topical glucocorticoids for skin disorders. Although these agents are often assumed to only have localized effects, published research suggests that localized glucocorticoids may have systemic effects such as adrenal suppression and growth suppression in children [7, 8]. Third, glucocorticoid exposure is further influenced on the tissue level. The 11β-hydroxysteroid dehydrogenase (11β-HSD) system can inactivate cortisol to cortisone and vice versa, thereby regulating exposure to cortisol on a local level [9]. Furthermore, a variety of factors influence the sensitivity to glucocorticoid in both a constitutional as well as dynamic manner [10].

Table 1. a comparison between excessive and subtle increases in glucocorticoid exposure

Change in glucocorticoid exposure:	↑↑**Excessive (Cushing's syndrome)**	↑**Subtle**
Source of increased exposure	Endogenous Cushing's syndrome	Increased endogenous glucocorticoids
	Pituitary (i.e. Cushing's disease)	*Chronic and acute stress*
	Adrenal causes	*Psychopathology*
	Ectopic ACTH or CRH	*Disturbed sleep*
	Exogenous Cushing's syndrome	*Chronic inflammation*
	Systemic glucocorticoid therapy	*Carbohydrate rich food*
	Local glucocorticoids	Local glucocorticoid treatment
	(Excessive use, interactions)	*Inhaled*
		Nasal
		Topical
		Intra-articular injections
		Otological
		Ocular
		Intestinal
		Increased glucocorticoid sensitivity
		Genetic
		Acquired
Associated clinical features	Abdominal weight gain	Abdominal weight gain
	Dyslipidemia	Dyslipidemia
	Insulin resistance	Insulin resistance
	Hypertension	Hypertension
	Cardiovascular disease	Cardiovascular disease
	Facial plethora	
	Striae	
	Proximal muscle wasting	
	Dorsocervical and supraclavicular fat	
	Decreased growth velocity	
	Psychiatric symptoms	
	Osteoporosis	
	Easy bruising	

1.1.1 Consequences of increased long-term endogenous glucocorticoid exposure: studies with hair cortisol

Various studies have focused on the relationship between glucocorticoid levels in hair (in most cases, cortisol) and the cardiovascular risk profile. In chapter 2, we showed for the first time that obese patients had higher hair cortisol than non-obese controls. An association between body fatness and hair cortisol levels has been found in other cohorts as well, including in chapter 3 of this thesis, where a positive association between body

mass index and hair cortisol levels is described in a cohort of patients with structural heart disease. Other recent reports from our group, as well as others, show associations between long-term cortisol and adiposity in adults and children [11-15]. We can now place this evidence from hair glucocorticoid studies in the context of previous research using short-term and time point measurements of cortisol. Studies using measurements of cortisol in blood, saliva and urine indicate that obesity is associated with a blunted cortisol awakening response, a less sharp decline of cortisol secretion over the day, an increased 24-hour output of cortisol, and an exaggerated cortisol reactivity to acute stressors [4]. With the use of hair analysis, we now provide evidence that the end result of these alterations, is an increase in cumulative cortisol exposure.

In chapter 10, we found that hair glucocorticoids were positively associated with fasting glucose in a general population cohort. The association between increased hair glucocorticoids and diabetes mellitus has been shown in other studies as well [13, 16, 17]. Surprisingly, no independent associations between measures of adiposity (waist circumference, body mass index) and hair glucocorticoids were found in chapter 10.

Published research has shown that hair glucocorticoids are associated with metabolic syndrome presence in adults [11], and with cardiovascular disease presence in the elderly [18]. Together, these results strongly suggest that adiposity is associated with increased long-term cortisol exposure, and this may be responsible for part of the increased cardiovascular disease risk in obese individuals. However, no causal inferences can be drawn from these studies. Although it is theoretically likely that increased glucocorticoid exposure leads to obesity, the direction may be opposite as well. However, even if increased glucocorticoid production follows, rather than precedes obesity, it is still likely that this contributes to the maintenance of obesity.

Obesity is associated with increased psychosocial stress and a higher prevalence of psychopathology [19, 20], which may increase glucocorticoid production. In chapter 10 we observed that the recent occurrence of major stressful life events, such as the death of a family member, was associated with increased hair cortisol in a general population cohort. An association between life events and increased hair cortisol has been found in specific populations as well, including college students, elementary school girls, crack cocaine users, and patients with bipolar disorder [21-24]. In chapter 4, we found that hair cortisol is positively correlated with symptoms of depression and anxiety in a sample of patients with sarcoidosis. This adds to other research showing that depressive symptoms are related to increased long term cortisol [17, 25].

A recent study showed that within obese individuals, hair cortisol was markedly increased in those who experienced weight discrimination, indicating that a social stigma associated with obesity may be responsible for part of the increase in long-term cortisol [26]. Other factors associated with obesity, such as consumption of carbohydrate rich foods [27], chronic inflammation [28] and disturbed sleep patterns [29, 30] may increase glucocorticoids as well. In chapter 3, we found that in patients with structural heart disease, hair cortisol was higher in patients who experienced worse physical health. A negative appraisal of one's physical health may contribute to increased long-term glucocorticoid exposure in obesity as well, since obesity is associated with physical disabilities such as back problems and osteoarthritis [20]. Furthermore, obesity and metabolic syndrome are often accompanied by liver steatosis [31]. This may alter the metabolism of glucocorticoids, thereby increasing systemic exposure. Finally, part of the association between long-term glucocorticoids and cardiometabolic health may be part of a trait phenomenon, where a genetic predisposition to produce more glucocorticoids results in increased body fatness and associated metabolic derangements.

In Table 2, the clinical associations that have been found with hair glucocorticoids in this thesis and in other studies are summarized.

1.1.2 Systemic effects of local glucocorticoid treatment

In chapter 8 we investigated the prevalence of potentially weight-contributing factors in a cohort of 404 obese patients. The most common contributing factor reported was the use of potentially weight contributing drugs. Interestingly, the most common group of drugs reported by patients was formed by glucocorticoid containing medications. Over the past few decades, local glucocorticoids have emerged in the treatment of many disorders. More than two million individuals in the Netherlands currently use local glucocorticoid therapy, such as inhaled glucocorticoids for asthma and chronic obstructive pulmonary disease (COPD), nasal glucocorticoids for allergic rhinitis (i.e. hay fever), and topical glucocorticoids for skin disorders such as eczema and psoriasis (GIP databank, Health Council of the Netherlands, https://www.gipdatabank.nl/).

Little is known about the effects of local glucocorticoids on tissues other than their target tissues. Although it is often assumed that local glucocorticoid therapy has little significant adverse effects in normal use, evidence to the contrary exists. In all doses and administration form of glucocorticoids, adrenocortical suppression can be observed [7], a clear indication of systemic effects. In chapter 10, we found that in a general population cohort, use of local glucocorticoids is associated with lower cortisone in scalp hair. This is further evidence that normal use of local glucocorticoids may result in a slight adrenocortical suppression. This is likely due to the fact that the exogenous glucocor-

Table 2. clinical associations with hair glucocorticoids described in this thesis and other published literature

	Increased hair glucocorticoids	**Decreased hair glucocorticoids**
Somatic health factors	Cushing's syndrome (Ch. 5, [32, 33])	Use of local glucocorticoids (Ch. 10, [34])
	Hydrocortisone use [35-38]	
	Obesity and increased body fatness (Ch. 2, Ch 3, [11-15])	
	Metabolic syndrome [11]	
	Diabetes mellitus and increased glucose (Ch. 10, [13, 16, 17])	
	Cardiovascular disease [18, 39]	
	Alcohol use [16, 18, 40]	
	Endometriosis [41]	
	Epilepsy [42]	
Chronic and acute stressors	Intensive aerobic exercise [43]	Childhood maltreatment [44]
	Life events (Ch. 10, [21-24])	
	Unemployment [45]	
	Shift work [30]	
	Severe chronic pain [46]	
	Impaired subjective physical health (Ch. 3)	
	Living in areas with less natural environment [47]	
Psychopathology	Posttraumatic stress disorder* [48]	Posttraumatic stress disorder* [49-51]
	Depressive symptoms and major depressive disorder (Ch. 4, [17, 25])	Generalized anxiety disorder [52]
	Bipolar disorder [53, 54]	Panic disorder** [53]

*Posttraumatic stress disorder has been associated with both increased and decreased hair cortisol concentrations (depending the type of traumatic event, characteristics of the patient sample examined, and the timespan between the trauma and assessment), when compared to controls.**Shown in bipolar disorder patients.

ticoid increases negative feedback on the pituitary level, which causes a decrease in ACTH production, leading to a lower cortisol production [55]. The systemic effects of local glucocorticoids likely depend on the dose [7], rate of absorption, and metabolism of the exogenous glucocorticoid [56].

Local glucocorticoids are often prescribed for chronic conditions, such as asthma, hay fever and psoriasis. Therefore, patients will often use these agents for repeated short courses, or chronically use them for years, potentially even decades. Very little is known about the long-term effects of chronic use, but it is theoretically likely that a chronic slight increase in glucocorticoid exposure will lead to increased weight and components

of the metabolic syndrome [2]. In chapter 9, we compared the use of glucocorticoids between a cohort of patients visiting an outpatient obesity clinic and two non-obese population based cohorts. We found that obese patients has significant higher use of glucocorticoids of all administration forms. In chapter 11, we studied the associations of glucocorticoid use with metabolic syndrome and body mass index in a large population based cohort. In women, use of systemic or local glucocorticoids was associated with presence of metabolic syndrome and higher body mass index. Furthermore, use of commonly prescribed inhaled glucocorticoids was associated with higher body mass index in both sexes. Although prospective research is needed to further investigate this, the results in chapter 9, 10 and 11 indicate that local glucocorticoid therapy should be considered as a potential causative factor in the onset of obesity and associated cardiometabolic traits.

1.1.3 Increased sensitivity to glucocorticoids and the metabolic syndrome

The actions of glucocorticoids do not only depend on the level of circulating hormones, but is regulated locally as well. After cortisol diffuses into the tissue, it can be inactivated into cortisone by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2. Cortisone can be activated back into cortisol by 11β-HSD type 1. At the cellular level, cortisol works by binding to nuclear receptors: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Sensitivity of the GR to cortisol varies between individuals, and is dynamically altered as well [10]. Part of the sensitivity is determined by functional variations in the GR, which are associated with increased or decreased sensitivity to glucocorticoids, and differences in in vitro activity of GR transactivation and transrepression. Four of these variations have been described, and these have been associated with subtle changes in body composition and metabolic syndrome components ([57], for an overview, see Figure 5, Chapter 1).

However, the clinical relevance of these variations has not fully been established. Therefore, in chapter 7, we investigated function GR variations in relationship to the presence of metabolic syndrome in a large population-based cohort. We found that the N363S polymorphism, which is associated with increased sensitivity to glucocorticoids, increases the prevalence of metabolic syndrome, but only in young adult males, and in individuals of low education status. This indicates that glucocorticoid sensitivity may increase the odds of metabolic syndrome in interaction with the environment. The age and sex-dependent increase seems to indicate that N363S influences *when,* rather than *if,* men develop metabolic syndrome. This may have clinical consequences, because it would subject affected men to a deleterious cardiovascular risk profile starting from a younger age, possibly resulting in an increased risk of cardiovascular disease later in life. In chapter 7, no overall effect of functional GR variations on metabolic syndrome

presence were found. Several reasons could explain this lack of association. First, the change in GR sensitivity that is caused by functional GR variations may be so minor, that it does not result in a change in metabolic syndrome prevalence. Second, the change in sensitivity may be counteracted by several regulatory mechanisms, such as the HPA axis which would theoretically be expected to orchestrate a lower glucocorticoid production when there is a genetically determined increased glucocorticoid sensitivity [5]. Local conversion of cortisol into cortisone by the 11β HSD system may influence cortisol exposure at the tissue level [9]. Furthermore, the sensitivity of the GR to glucocorticoids is dynamically influenced by other factors than genetic variations in the GR, such expression of GR and associated proteins, and local cytokine production [10].

1.2 Hair cortisol for the diagnosis of Cushing's syndrome

In chapter 5, we present the largest study to date investigating hair cortisol as a diagnostic test for endogenous Cushing's syndrome. In this study, we compared hair cortisol in 43 patients with known Cushing's syndrome, 35 patients who were initially suspected of Cushing's but in whom the diagnosis could be excluded, and 171 healthy controls. We found that for the diagnosis of Cushing's syndrome, a single measurement of hair cortisol yielded a sensitivity of 93%, a specificity of 90% when compared to healthy controls, and 91% when compared to patient controls. Although independent validation is warranted, this diagnostic accuracy compares well to the current first-line screening tests (late-night salivary cortisol [LNSC], the 1 mg dexamethasone suppression test [DST], and 24-hour urinary free cortisol [UFC]) [58]. An important advantage of hair cortisol compared to the current screening tests, is that hair sampling can be done in any setting with very little burden to the patient. Furthermore, hair analysis is not limited by patient adherence to collection instructions (as with UFC of LNSC) or to medication intake (as with DST).

One of the main advantages of hair measurements is the possibility to create retrospective timelines of cortisol exposure, from months up to years back in time, depending of the length of the hair. In published research, several cases of Cushing's syndrome have been presented in which the timelines of hair cortisol concentrations corresponded with disease course. Creating timelines can be especially helpful in cases of suspected cyclic Cushing's, where cortisol production is highly variable and may cause many false-negative test results in the classical time-point and short-term measurements of cortisol [32, 33]. In chapter 5, we made use of the possibility to create retrospective timelines with hair cortisol in two patients with recent onset ectopic Cushing's syndrome. We retrospectively observed increased cortisol levels in hair, months before the onset of clinical signs and symptoms of Cushing's syndrome. These cases illustrated that hair cortisol timelines can be used to study the onset, the duration, and natural history of Cushing's syndrome.

2. TECHNICAL ASPECTS OF HAIR GLUCOCORTICOID MEASUREMENTS

2.1 Do hair glucocorticoids truly represent long-term glucocorticoid exposure?

Hair cortisol measurements have been used for several years under the assumption that cortisol is continuously built into the hair through passive diffusion from the bloodstream, and therefore represent long-term exposure to cortisol. It is not exactly known how cortisol enters the hair shaft. Additional contributions to the steroid hormone content in hair may derive from sebum and sweat [6].

Evidence that hair cortisol truly represents long-term cortisol levels can be derived from two sources. Indirect evidence comes from the associations with high hair cortisol levels found in epidemiological studies, including the studies described in this thesis. Much of this evidence is in line with tissue effects of cortisol, including hair cortisol increases in Cushing's syndrome, abdominal obesity, metabolic syndrome, depression, and cardiovascular disease (see Table 2). Direct evidence comes from the comparison between hair cortisol and cortisol measurements in other matrices. Short *et al*. compared the cortisol levels in the proximal 1 cm of scalp hair (representative of the month before sample collection) with integrated daily salivary cortisol, based on three daily saliva samples which were collected for a 30-day period. In a total of 17 participants, hair cortisol and 30-day integrated salivary cortisol correlated with a Pearson's r of 0.61. In contrast, integrated weekly urinary free cortisol did not significantly correlate with hair cortisol [59]. Because salivary cortisol represents freely circulating cortisol levels [60], this provides strong evidence that hair cortisol is representative of freely circulating systemic cortisol levels.

In the past a controversial in vitro study was published, showing that hair follicles display a functional equivalent of the hypothalamus-pituitary-adrenal (HPA) axis (for an overview of the HPA axis, see Chapter 1). Most components of the steroid synthesizing system have been shown in hair follicles and in skin [61, 62]. However, Ito *et al.* found that but CRH stimulated cortisol secretion in hair follicles. Furthermore, evidence of negative feedback was shown, since CRH was downregulated in response to cortisol [62]. The findings by Ito *et al.* have never been replicated, and since this study was performed *in vitro*, it is currently unclear to what extent local production of glucocorticoid in the hair follicle contribute to hair glucocorticoid content. RNA expression data may provide additional insight, however. It can be shown that gene expression of steroidogenic enzymes is extremely low in the skin, compared to in the adrenal gland, where RNA expression is around a thousand fold higher [63]. This makes it very unlikely that hair follicles in the skin provide a substantial amount of glucocorticoids to the hair *in vivo*, compared to adrenal production of glucocorticoids. Combined with the clinical associa-

tions found with hair glucocorticoid levels, which are overwhelmingly in line with tissue effects of glucocorticoids in different organ systems, and the strong correlation between hair cortisol and 30-day integrated salivary cortisol, we are therefore confident that hair glucocorticoids indeed represent long-term systemic exposure to glucocorticoids.

2.2 Confounders of hair glucocorticoid measurements

In chapter 10, we investigated the influence on several confounders on hair glucocorticoid levels in a population-based cohort. We found that hair cortisol and cortisone increase with age, are higher in men, in subjects with black or brown hair color, and decrease with higher hair washing frequency (also see Table 3). These findings are in line results from other cohorts. In the larger published studies, hair glucocorticoids also increased with age [11, 13, 16]. Furthermore, children have been found to have significantly lower hair cortisol than adults [64]. Several published studies report higher hair glucocorticoids in males than in females [13, 16-18], but in other studies no sex difference was observed [11, 12, 65]. In two studies carried out in older adults, men had higher hair cortisol, indicating that the sex difference in hair cortisol becomes more pronounced later in life [16, 18]. It is currently unknown whether the age and sex differences in hair cortisol levels represent a true biological factor, indicative of increased long-term glucocorticoid exposure in older age and in men. Alternatively, these observations may represent structural differences in hair which influence hair glucocorticoid measurements. The associations between ethnicity and hair glucocorticoids have largely been unexplored, and need further study.

Table 3. overview of demographic and confounding factors that (potentially) affect hair cortisol concentrations.

Factor:	Significance for hair glucocorticoid levels:
Age	Increase with age (Ch. 4, [11, 13, 16, 64])
Sex	Higher in men (Ch. 4, [13, 16-18]
Natural hair color	Higher in dark hair color (Ch. 4, [13])
Hair treatment	Inconsistent results; may decrease hair glucocorticoids (Ch. 4, [11-13, 17, 43, 64-69]
Hair washing frequency	Slightly lower with higher hair washing frequency (Ch. 4, [11, 13])
Sweating on the scalp	Does not appear to acutely increase hair glucocorticoids, but may cause increases over time (Ch. 4, [71])
Season	Conflicting results [13, 17]
Sunlight exposure	Decreased hair glucocorticoids (Ch. 6, [72])

Hair treatments such as hair dyeing, permanent curling or straightening have been reported to decrease hair cortisol [11, 17, 65, 66], although other studies, including the study we present in chapter 10, did not find this [12, 13, 43, 64, 67-69]. Possibly, the influ-

ence of hair treatment may be dependent on the hair sample work-up and analytical method used. Furthermore, the amount of cortisone decreased with higher hair washing frequency in large studies [11, 13], in line with our findings in chapter 10. Dark hair color has been associated with increased hair cortisol and cortisone in another study from our group [13]. Furthermore, hair cortisol levels can be partly predicted by using a polygenetic score for hair pigmentation variants [70]. Possibly, the structural differences between light and dark hair influence hair glucocorticoid measurements.

In chapter 10, for the first time we described a relationship between the number of days of sweating on the scalp a person reports, and higher hair glucocorticoids. The influence of sweating on hair glucocorticoid levels has been debated in literature [71, 73]. The most comprehensive experimental study investigating this association concluded that single short-term occurrences of sweat-inducing interventions such as sauna bathing and treadmill running did not increase hair cortisol levels [71]. Our results indicate that repeated sweating on the scalp over an extended period of time may increase the glucocorticoid content of hair. A definite conclusion regarding the influence of scalp sweating cannot be reached based on the results from chapter 10 however, since sweating was not objectively assessed, but estimated by questionnaire.

In chapter 6, we studied the influence of natural sunlight on hair glucocorticoids. The rationale behind this study was that hair glucocorticoids gradually decrease along the shaft of the hair, from proximal to distal; the so-called "wash-out" effect. We found that hair glucocorticoids were decreased not only after exposure to a high amount of artificial UV radiation, but also after 40 hours of natural sunlight exposure. UV radiations has been shown to induce degradation of glucocorticoids [74]. However, crosslinking between glucocorticoids and the hair matrix could also decrease the extractable amounts of glucocorticoids in hair. An influence of sunlight on hair glucocorticoids has since been confirmed in another study [72]. These results indicate that sunlight exposure should be considered as a potential confounder in studies investigating hair glucocorticoids. Although this may not play a role in every study using hair glucocorticoids, it is of particular relevance in studies where an exposure variable of interest is associated with the time spent outside, and therefore, the exposure to natural sunlight.

Because hair analysis is still relatively novel in endocrinology, there is understandable concern about the magnitude of the influence of various confounders. It is important to realize that not just hair analysis, but all measurements of glucocorticoids are subject to confounders that emerge from the matrix in which they are measured (for an overview, see Table 4). Examples include the diurnal rhythm, hormonal contraception and acute stress for blood, kidney function for urine, and gingival bleeding for saliva measure-

Table 4. matrix-specific limitations of cortisol measurements

Matrix in which cortisol is measured:	**Matrix-specific limitations:**
Blood	Acute variations: stress, diurnal rhythm
Time-point measurement of total circulating cortisol	Increased with higher CBG levels (hormonal contraception)
	For DST: false-positives due to CYP3A4 inducing drugs (anti-epileptics)
	For DST: dependence on adequate dexamethasone intake
Saliva	Acute variations: stress, diurnal rhythm
Time-point measurement, represents freely circulating cortisol	Blunted or absent diurnal rhythm in depression, shift workers and critical illness
	False elevations due to 11β-HSD 2 inhibition (liquorice, tobacco)
	Contamination with blood (gingival trauma, toothbrushing)
Urine	Day-to-day variation in cortisol production
Short-term cortisol output (usually 24 hours)	Highly depended on adequate sampling by patients
	False increases in polydipsia
	False decreases in renal impairment
Hair	Hair-related confounders (hair treatment and color)
Long-term exposure (months)	Wash-out effect (potentially due to UV exposure)

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; CBG, corticosteroid binding globulin; CYP3A4, cytochrome P450 3A4; DST, dexamethasone suppression test; UV, ultraviolet.

ments. For instance, urinary cortisol is highly dependent on adherence to collection instructions [2], since many patients may find it cumbersome to collect all their urine for 24 hours.

Overall, the magnitude of the effects of hair glucocorticoid confounders is relatively minor. A recent study by Staufenbiel *et al.* showed that by taking into account all significant confounders of hair glucocorticoids, six to seven percent of the variance in hair glucocorticoid levels can be explained [13].

3. CONCLUSIONS AND FUTURE DIRECTIONS: IS HAIR THE NEW BLOOD?

The studies in this thesis deal with the consequences of long-term glucocorticoid exposure. As is summarized in the conceptual overview in Figure 1, we found long-term increases in endogenous glucocorticoids, and the use of local glucocorticoids to be associated with obesity – and they may therefore be important contributors to the obesity epidemic seen today. In interaction with local conversion of glucocorticoids by the 11β-HSD system, glucocorticoid sensitivity, and individual differences in the absorption and metabolism of exogenous glucocorticoids, this may lead to disturbances in the cardiometabolic profile, and cardiovascular disease.

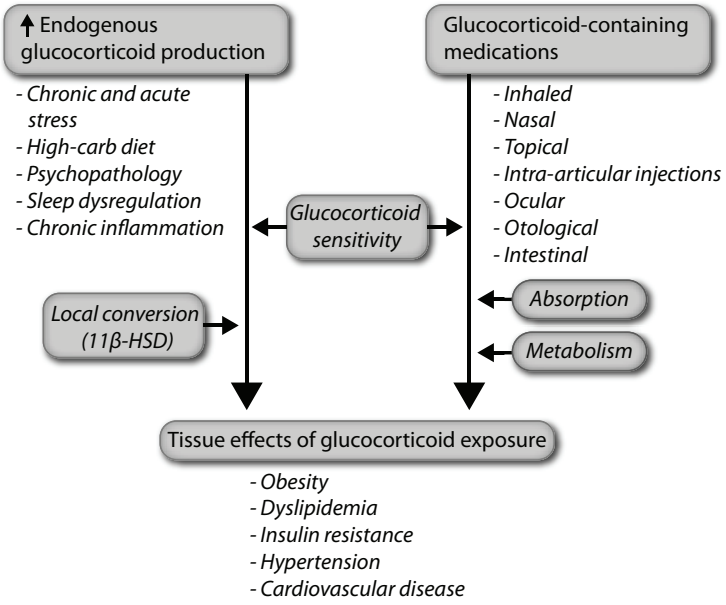

Figure 1. conceptual overview

Scalp hair analysis is a promising and rapidly developing additional tool to evaluate HPA axis activity. In observational research, the association between increased hair glucocorticoids and an adverse cardiometabolic risk profile appears to be the most consistent clinical finding. All published studies which investigated this association thus far are cross-sectional, and therefore no conclusions about causal inferences can be made. Because an extreme glucocorticoid excess (i.e. Cushing's syndrome) is strongly associated with metabolic syndrome components (central adiposity, insulin resistance, dyslipidemia, hypertension) [2], an increased long-term cortisol exposure within the normal range is theoretically likely to have a causative role in increasing cardiovascular risk. However, the possibility of reverse causality cannot be discarded. For instance, chronic stress and increased psychopathology associated with obesity [75], or altered steroid metabolism could lead to increased cortisol levels. Therefore, there is a clear need for longitudinal studies examining the influence of long-term cortisol exposure on cardiovascular risk. The same applies to the relationship between the use of local glucocorticoid therapy and obesity, where prospective studies are warranted as well. This research may pave the way for clinically relevant cut-off points for cortisol in cardiovascular risk stratification, and new interventions to treat obesity and metabolic syndrome by decreasing the exposure to glucocorticoids. Depending on the cause of increased glucocorticoid exposure in an individuals, these interventions may include cessation of local glucocorticoid therapy, behavioral strategies which may decrease glucocorticoid exposure, but also pharmacological strategies including selective glucocorticoid recep-

tor modulators and agents that target the local conversion of glucocorticoids by the 11β-HSD system [76, 77].

Another important future venue is to study the genomic contributions to hair glucocorticoids. For plasma cortisol, a genome-wide association study (GWAS) only showed associations with loci associated with cortisol binding globulin. It is unsure whether these genetic variations influence the free or bioavailable cortisol. A GWAS for hair glucocorticoids may help to further understand the genetic influences on glucocorticoid exposure, especially because hair analysis deliver a long-term cumulative measure and appears to represent free cortisol. This could also facilitate Mendelian randomization studies, which could help to understand the causal relationships between long-term glucocorticoid exposure, cardiometabolic disturbances and cardiovascular disease risk.

For clinical use in the diagnosis of Cushing's syndrome, one important practical advantage of hair cortisol is that sampling can easily be performed in any setting, even at home and sent by mail to an expertise center. Furthermore, hair analysis is not dependent on patient adherence to sampling instructions (as with 24 hour urine collections or late night salivary cortisol) or medication intake (as in dexamethasone suppression testing). The ability to create retrospective timelines creates the opportunity to investigate patterns of cortisol exposure for months up to years back in time, with the collection of a single hair sample. For hair cortisol to become a fully established method in clinical practice, findings concerning Cushing's syndrome need to be replicated. An important next step in the development of hair analysis for the diagnosis of Cushing's syndrome, is the use of steroid analysis using liquid chromatography – tandem mass spectrometry (LC-MS/MS). Through increased specificity of steroid hormone detection, and the possibility to measure multiple adrenal steroids, diagnostic accuracy may be increased.

Measurement of hair glucocorticoids creates the unique opportunity to study the effects of interventions on long-term glucocorticoid exposure, due to the possibility of creating retrospective timelines by dividing hair samples into segments. Randomized controlled trials could easily incorporate this method, because depending on the length of the hair, a single hair sample may be used to evaluate changes in cortisol production over multiple months. In chapter 3, we observed no difference in between patient who underwent a mindfulness based stress reduction intervention, compared to patients who were assigned to the control group, although the whole study population on average showed a decrease in HCC during the study. Future studies will show if, and which behavioral and medical interventions are capable of modulating long-term cortisol exposure as measured using hair analysis. Evidence of the observational studies in this

thesis indicate that a decrease may be associated with health benefits, especially in cardiometabolic health.

In conclusion, the studies in this thesis highlight the potential of scalp hair measurements, which have provided researchers and clinicians with the never-before possibility to study the long-term exposure to glucocorticoids in a straightforward manner. This has already contributed to a greater understanding of the association between glucocorticoid exposure and the cardiometabolic risk profile, and has given clinicians the possibility to retrospectively assess long-term cortisol levels in Cushing's syndrome. Future research using hair glucocorticoids may help to further understand the etiology behind obesity and cardiovascular disease risk, and open new roads towards individualized treatment of the cardiometabolic risk profile.

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