PERGAMON

Psychoneuroendocrinology 28 (2003) 121–137

PNEC

www.elsevier.com/locate/psyneuen

Heritability of cortisol levels: review and simultaneous analysis of twin studies

M. Bartels [a,*], M. Van den Berg [a], F. Sluyter [a,b], D.I. Boomsma [a], E.J.C. de Geus [a]

[a] *Department of Biological Psychology, Vrije Universiteit, Room 1F 57, van der Boechorststraat 1, 1081 BT, Amsterdam, The Netherlands*

[b] *MRC Social Genetic and Developmental Psychiatry (SGDP) Research Centre, Institute of Psychiatry, Kings College London, De Crespigny Park, London SE5 8AF, UK*

Received 5 June 2001; received in revised form 7 December 2001; accepted 11 December 2001

Abstract

Cortisol has a pivotal role in physical and mental health, but relatively few studies have paid attention to individual differences in cortisol levels and the etiology of these differences, in particular their possible genetic basis. In this article we review the existing literature on the heritability of cortisol levels. Most of the studies, which have been carried out in genetically informative samples, lack methodological consistency with regard to frequency and timing of sample collection. The circadian rhythm in cortisol levels was often not taken into account. A power analysis shows that none of these studies used adequate sample sizes to distinguish genetic from shared environmental influences as a cause for familial aggregation. Results of a simultaneous analysis of 5 comparable twin studies suggest a heritability of 62%. Hence, we conclude that, to understand the contribution of genetic and (shared) environmental influences to variation in basal cortisol levels, future studies should be designed more rigorously with strict collection and sampling protocols, sufficient sample size and repeated measures across multiple days.

Keywords: Genetics; Cortisol; Corticosteroid; Twins; Power analysis

* Corresponding author. Tel.: +31-20-4448812; fax: +31-20-4448832.
E-mail address: m.bartels@psy.vu.nl (M. Bartels).

0306-4530/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0306-4530(02)00003-3

Contents

1. Introduction

Cortisol is a steroid hormone secreted by the outer cortex of the adrenal gland. Its secretion is stimulated by ACTH (adrenocorticotrophic hormone), produced in the pituitary in response to corticotropin-releasing hormone (CRH), a product from the neurons in the paraventricular nucleus of the hypothalamus. In the characteristic diurnal rhythm of plasma cortisol level typically 10-15 well-defined pulses of variable amplitude are observed, with a morning maximum, declining levels throughout the daytime, a period of low concentrations generally centered around midnight, and an abrupt rise after the first few hours of sleep (Weitzman et al., 1971). This diurnal cycle is tied to the sleep-wake cycle and to the light-dark cycle (Spith-Schwalbe et al., 1993). Although both mechanisms are involved in the regulation of the HPA axis, the light-dark cycle is still the primary synchronizer of a basically endogenous rhythm originating in the suprachiasmatic nucleus of the hypothalamus (Fischman et al., 1988; Van Cauter, 1990; Boivin et al., 1996; Scheer and Buijs, 1999). The circadian rhythm is reversed in nocturnal species and disrupted by sleep deprivation, and changes in the sleep pattern (e.g. shift work and long distance travel). Further, plasma cortisol release is tightly regulated through negative feedback at the pituitary, hypothalamus and hippocampus (Kovacs et al., 1987; Jacobson and Sapolsky, 1991). Strength of this feedback signal strongly varies with time of day (Dorin et al., 1996; Huizinga et al., 1998; Young et al.,1998), contributing to the characteristic diurnal rhythm in plasma cortisol levels. After its release, the major proportion of cortisol binds to the plasma proteins corticosteroid binding globulin (CBG or transcortin) and albumin, which prevent the hormone from penetrating the membranes of their target cells. Only, about 3-5% of the total cortisol is the unbound, biologically active fraction.

Large individual differences exist in basal cortisol levels at all points of the circadian cortisol curve (Smyth et al., 1997). These individual differences in cortisol levels play a prominent role as an explanatory variable in studies on physical (Walker, 1996; Mantero and Boscaro, 1992; Pedersen and Hoffman-Goetz, 2000; Roy et al., 2001; Rosmond and Bjorntorp, 2000c) and mental health (Young et al., 2000; Pos-

ener et al., 2000; Goodyer et al., 2000). There are many sources of individual differences in cortisol levels, including negative feedback regulation through the corticoid receptors. In this regulation two receptor types can be distinguished: the mineralocorticoid (MR, or type-I) receptor and the glucocorticoid (GR, or type-II) receptor (Veldhuis et al., 1982; Reul and De Kloet, 1985). Because of its much higher affinity to cortisol, MRs are predominantly occupied under (nonstress) basal levels whereas during stress, when cortisol levels are much higher, GRs become increasingly occupied (Young et al., 1998). Individual differences in the number, affinity and efficiency of signaling cascades of these receptors will directly affect cortisol levels and biological activity. Further, individual differences may arise from the secretion of ACTH in response to CRH or the secretion of cortisol by the adrenal cortex in response to ACTH (Dorin et al., 1996; Posener et al., 1997; Beuschlein et al., 2001). Finally, basal cortisol levels are responsive to individual differences in the capacity of 11β-Hydroxy steroid dehydrogenase (11β-HSD), that causes the conversion of the biologically active cortisol to its inactive metabolite cortisone.

Ultimately, individual differences in all these mechanisms arise from two main factors: genetic and environmental influences. As for the latter, evidence suggests that early adverse experiences, like childhood abuse or parental separation, play a prominent role in development of mood and anxiety disorders and that corticotrophin-releasing hormone (CRH) systems may mediate this association (Mullen et al., 1996; Heim et al., 2000). Further evidence for this association has been assembled in animal models, where prenatal and early developmental stress, often related to parental rearing, have been shown to cause long-lasting or even permanent alteration of the HPA axis (Plotsky and Meaney, 1993; Levine, 1994). Not only early experiences, but also experiences later in life can influence HPA axis activity. For example, trauma survivors with posttraumatic stress disorder such as Vietnam veterans, holocaust survivors or victims of abuse are characterized by decreased urinary cortisol level as compared to healthy controls (see, among others, Yehuda et al, 1991, 1995, 2000). Accordingly, environmental challenges are important in the development of HPA axis disregulation and stress-related diseases. Still, this does not answer the question why similar stressors affect some individuals strongly, while others remain relatively untouched. These remaining individual differences point in the direction of genetic influences on variation in cortisol levels.

A powerful tool to unravel the genetic architecture of individual differences is to study genetically related individuals. Family studies might give a first impression of familial aggregation, but they can not distinguish between genetic and shared environmental effects. Similarities between family members may be created either by genetic relatedness or by sharing the same family environment, the so-called shared environment (C). A method that solves this problem, is the classical twin design. Monozygotic (MZ) twins derive from a single zygote and therefore two individuals of a MZ twin pair are genetically identical. Dizygotic (DZ) twins develop from two distinct zygotes and share on average 50% of their genes, like ‘ordinary’ brothers and sisters. Hence, the only possible way to explain the variation in cortisol levels between two members of a MZ twin pair are environmental effects that are not shared by those two: the so-called unique environmental influences (E). Con-

versely, the variation in cortisol levels between two members of a DZ twin pair could result from different genes and/or unique environmental influences. Accordingly, the difference in relatedness between MZ and DZ twin pairs (mostly expressed as correlation coefficients: r_{MZ} and r_{DZ}) gives information about the strength of the genetic and environmental influences on the trait under investigation (Martin et al., 1997). It further allows the separation of environmental influences into those of the environment shared by members of a family and those unique for each individual.

Twin and family studies constitute a powerful instrument, but surprisingly few attempts have been made to estimate the impact of genetic and environmental factors on the regulation of cortisol levels. The first and main purpose of this article was to review the existing studies, listed in Table 1, to obtain insight in the genetic and environmental influences on cortisol levels. Using PubMed and the search terms twin, cortisol, corticosteroid, heritability and family, 29 studies emerged. However, the studies by Norman et al. (1982; 1983a; 1983b; 1984) and Lopez Bernal et al. (1980) are based on neonates or twin pregnancies, which is beyond the scope of this paper. Further, several case studies (Mendlewicz et al., 1984; Milford et al., 1994; Li et al., 1997; Pinheiro et al., 1999) have been omitted, since no reliable heritability can be estimated based on one case. Likewise seven studies (Nurnberger et al., 1983; Linkowski et al., 1985; Schuckit et al., 1991; Karl and Schulte, 1994; Smyth et al., 1997; Walker et al., 1998; Yehuda et al., 2000) have been excluded because these are based on unrelated subjects or are family studies without any information on parent-offspring correlations, which gives no opportunity to estimate genetic parameters. A study by Schwartz et al. (1972) has been excluded because it is based on an ocular response to dexamethasone eye-drops. Finally, one study was published in Polish (Raczynska et al., 1978).

What immediately catches the eye in Table 1 is the huge variation in heritability estimates (0.0% to 84%, with a median of 52%). To explain this discrepancy, a secondary purpose of this paper was to scrutinize the methodological aspects of existing studies to select studies with comparable methodology for a simultaneous (or meta-) analysis of the MZ and DZ correlations reported in these studies. Three fundamental issues were addressed: how the samples were collected, when they were collected, and how they were analyzed in the laboratory.

1.1. Collection methods

Table 1 shows that different methods of collection –blood, saliva, urine - have been used over the years. In general, saliva collection is the most practical and stress-free method of cortisol collection in a large group of subjects (both adults and children). The reason why blood and urine sampling have been used more often, is probably historical as the development of the “Salivette” has taken place fairly recently and the knowledge about the use of saliva as a representative biological fluid has increased over the past years. Salivette samples are obtained by placing a small cotton swab into the mouth for two minutes after which it is stored in a closed plastic container. Because total serum cortisol may be altered by fluctuations in binding proteins, free serum cortisol is a better indication of adrenal activity. Salivary

Table 1
Overview of genetic studies conducted in a twin design

Study	*Subjects*	*Cortisol measurements*	*Results / direction of association*
Saliva cortisol sampling			
Young et al. (2000)	- 29 MZF[a]	- within 45 minutes after awakening - immediately before bedtime - during a two-week period DPC Coat-account Cortisol assay kits	- 40-45% of total variance in salivary cortisol shared by MZ twins
Wüst et al. (2000)	- 31 MZF - 21 MZM - 13 DZF - 13 DZM - 26 DOS	- 0, 30, 45, and 60 minutes after awakening at 0800h, 1500h and 2000h - over a two-day period - RIA	- significant genetic influence on the cortisol response to awakening - day-time cortisol profile is under influence of shared environmental factors - h^2 (mean increase) = 0.40[b] - h^2 (area under the curve)= 0.48
Kirschbaum et al. (1992)	- 7 MZF - 6 MZM - 11 DZ (12 F, 10 M)	- baseline - after ergometric exercise (ERG) - after psychological stress (PSY) - after CRH stimulation (CHR) - Between 1700-1830h - at a 10 min interval from –35 min (CRH) or –10 min (ERG, PSY) - through 90 minutes after stimulation onset - RIA	- genetic factors are involved in adrenocortical response to CRH stimulation, psychological stress and for the baseline condition, but not for physical exercise. - Strong intraindividual stability was found for females - MZ intraclass coefficients significant higher than DZ intraclass coefficients for baseline CRH, ERG and PSY and peak CRH - h^2 (baseline CRH) = 0.72 - h^2 (baseline ERG) = dominanceh^2 (baseline PSY) = 0.52 - h^2 (peak CRH) = 0.84
Plasma cortisol sampling			
Froelich et al. (2000)	- 23 MZM - 28 MZF - 16 DZM - 21 DZF	- baseline at 0750-0800h - 15, 60, 75, 120, 180, and 240 minutes after onset of drinking - RIA	- cortisol responses to alcohol showed no signs of heritability - basal cortisol levels (2 hours before drinking) showed no signs of heritability

(*continued on next page*)

Table 1 (*continued*)

Study	*Subjects*	*Cortisol measurements*	*Results / direction of association*
Pritchard et al. (1999)	- 7 MZM	- between 0730-0800h - after cycle-ergometer exercise - RIA	- observed changes in cortisol levels were correlated for both twins - no significant twin resembance for basal plasma cortisol levels
Inglis et al. (1999)	- 75 MZ - 71 DZ	- 09.00h, after 30 minutes of rest - 30 minutes after ACTH stimulation - RIA	- h^2 (basal plasma cortisol) = 0.46 - cortisol response to ACTH stimulation showed no signs of heritability
Pritchard et al. (1998)	- 12 MZM	- baseline levels and 24 hours after - termination of overfeeding between - 0730-8.00h after overnight fasting - RIA	- no genetic control of cortisol levels found in response to overfeeding
Linkowski et al. (1993)	- 11 MZM - 10 DZM	- every 15 minutes over a 25-hour period. - first hour is not considered - RIA	- genetic factors control absolute value of morning acrophase, timing nocturnal nadir and to some extent proportion of pulse variation - environmental factors contribute to morning acrophase - h^2 (pulsatility) = 0.74[a] - h^2 (morning acrophase) = 0.68 - h^2 (timing nocturnal nadir) = 0.48
Meikle et al. (1988)	- 20 MZM - 20 DZM	- 3 samples between 8 am and 9.30 am at 20 min. interval - levels of bound and unbound cortisol and DHEA-S - RIA	- heredity and environmental factors influence total bound and unbound cortisol levels - h^2 (total cortisol) = 0.51 - h^2 (unbound) = 0.58

Table 1 (*continued*)

Study	*Subjects*	*Cortisol measurements*	*Results / direction of association*
Nurnberger et al. (1982)	- 13 MZ - 3 DZ - 11 patients with bipolar affective disorder	- –35, -20, -5, +5, +15, +30, +45, +60 and +90 minutes and +3, +6, and +24 hours after amphetamine stimulation - timing stimulation between 0900h-10.45h - RIA	- no significant twin concordance in basal cortisol - no significant twin concordance in change in cortisol after amphetamine stimulation
Maxwell et al. (1969)	- 66 MZ - 76 DZ	- plasma 11-hydroxycorticosteroids were measured after determination of BP - both twins at the same time - fluorimetric method	- genetic factors contribute to variation of corticosteroid levels in females, not in males
Urine cortisol sampling			
Inglis et al. (1999)	- 75 MZ - 71 DZ	- collected in a 24-hour period. - corticosteroid metabolite excretion was measured - gas chromatography-mass spectrometry	- strong heritability for levels of cortisol metabolites in urine is found - h^2 (24-h tetrahydrodeoxycortisol) = 0.59

[a] MZM = monozygotic male, DZM= dizygotic male, MZF= monozygotic female, DZF= dizygotic female, DOS= dizygotic opposite sex.
[b] heritability (h^2) calculated on given MZ and DZ correlations.

cortisol measurements always reflect the biological active free form. Salivary free cortisol is approximately 70% of that of serum free cortisol because of conversion of cortisol to cortisone in the salivary glands. However, there is a strong relationship between cortisol levels extracted from saliva and from blood (Riad-Fahmy et al; 1982; Kirschbaum and Hellhammer 1994; Aardal and Holm 1995). In urine cortisol exists only in free form; secretion is dependent on serum levels, but also on renal glomular and tubular function. Both blood and saliva can provide information on the diurnal rhythm, while urine measures represent the cortisol production over a period of time. The latter is less informative, but, because it is a summary index, may show better reliability than blood samples. A possibility to get informative urine samples, taking the diurnal cycle into account, is by collecting at different times of the day instead of the commonly used 24-hour pooling method.

All three methods have their pro's and cons and it depends on the aim of the studies which method is more appropriate (Riad-Fahmy et al., 1982; Kirschbaum and Hellhammer, 1989, 1994; Trainer et al., 1993; Aardal and Holm 1995). Likewise, there is no theoretically optimal measure suitable for twin studies, because there too it depends on what exact cortisol phenotype is of interest. In the existing studies, significant genetic influences on baseline as well as stimulated cortisol levels have been found in saliva (Kirschbaum et al., 1992; Young et al., 2000; Wüst et al., 2000;), blood (Maxwell et al., 1969; Meikle et al., 1988; Linkowski et al. 1993; Inglis et al., 1999) and urine (Inglis et al., 1999). The difference in collection method may explain part of the difference in heritability estimates, but cannot explain all of it. For example, both the study by Froelich et al (2000) and Inglis et al. (1999) used plasma samples, collected in the morning, and analyzed by making use of a RIA. The two studies, nonetheless, find severely discrepant results with Froelich et al. showing no sign of heritability and Inglis et al. reporting a heritability estimate of 46%.

1.2. Methods of analysis

Levels of cortisol in plasma, urine and saliva can be estimated by commercial radioimmunoassay (RIA), high performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA). All three methods of analysis have their pro's and cons and it again depends on the aim of the studies which method is more appropriate (Liddle, 1960; Kuhn, 1989; Okumura et al, 1995). The commercial availability of sensitive and specific antisera for cortisol has made RIA the method of choice in most laboratories. However, the advantage of using an ELISA is that it does not require the use of any radioactive reagent, and therefore it is safer and more economical than standard RIAs. The disadvantage is that the sensitivity of an ELISA does not quite approach that available with standard RIA. HPLC might be more specific and accurate, but they are also considerably more time consuming, and require much more complicated instrumentation. There is again no theoretical advantage of any method in twin studies.

However, what is important is the handling of batch effects. Perhaps counter-intuitively, a random distribution of the samples of family members over different

batches is required to avoid confusing the genetic experiment. Like any family study, the classical twin design can provide evidence of familial aggregation of cortisol levels. In addition, the twin study exploits the difference in genetic similarity between two members of an MZ and of a DZ twin pair to differentiate between the two factors of familial aggregation: genetic influences and shared environmental influences. However, a third factor accounts for the observed variance in cortisol, the so-called unique environmental influences. These are influences unique to an individual, and include unpredictable measurement error due to the distance of the sample to the last CRF pulse but also predictable measurement error due to batch effects. If the within-family batch effects are removed, but between family batch effects are left to exist (because not all families can be run in a single batch), the extent of familial aggregation will be overestimated in MZ as well as DZ twin pairs. This overestimation will show up as influences of shared environmental factors. Random distribution over batches will force the batch effect to show up as a unique environmental effect, which is appropriate.

None of the studies reviewed in Table 1 mention a random distribution over different batches. Hence, it is possible that samples of two members of a twin pair are analyzed in the same batch as a result of sample collection on the same day. Random distribution of the samples is the procedure to avoid correlated measurement errors, which are difficult to detect but could certainly influence the results.

1.3. Timing of sampling

The secretion of cortisol is a prominent part of the endocrine response to stress. Because of the complexity of the HPA-axis mechanism and the discrepancy in physiological background of basal cortisol levels in comparison to cortisol release in response to a stressor (psychological stress, chemical substance, exercise), we will focus on basal cortisol levels solely. Six of the 11 studies (Nurnberger et al., 1982; Kirschbaum, 1992; Pritchard et al., 1998; Froehlich et al., 2000; Inglis et al., 1999; Pritchard et al., 1999) focus on the cortisol response to a certain stimulus, but also took basal samples to determine baseline cortisol levels before application of the stressor. Unfortunately, because their basal sample only acted as baseline for computation of the reactivity levels, most completely ignored the timing of the basal sample in the circadian rhythm. Four out of these 6 studies did not find evidence for genetic influences on the basal levels. Only Inglis et al. (1999) and Kirschbaum et al. (1992), report significant genetic influences on basal cortisol.

Five of the 11 studies (Maxwell et al., 1969; Meikle et al., 1988; Linkowski et al., 1993; Young et al., 2000; Wüst et al., 2000), listed in Table 1, focus specifically on basal cortisol levels. These studies show an unfortunately large variation in frequency and the timing of the sampling across the measurement day. Some studies sampled twice or more a day, at fixed hours and over a longer period, whereas others sampled only once a day and not even at fixed times. Clearly, based on the knowledge of the circadian rhythm, the frequent sampling at fixed times is favored and except for those studies that used urinary sampling to measure 24 hour cortisol profiles, the other approaches introduce large between-subject variance due to time of sampling.

24-hour averages have the disadvantage, however, that they assume the cortisol level at all time points of the day to be influenced by the same genetic or environmental influences. This assumption need not hold, in the view of the complexity of the HPA system. It is entirely possible that different genes influence cortisol at different times of day.

In summary, the differences in the estimates for genetic influences could in part be due to collection methods, handling of batch effects, different time schedules for sample collection and different focus of studies (basal or reactivity). Apart from these differences in methodology, however, a major problem in most studies is the small sample size. The statistical power of quantitative genetic studies is influenced by the size of the effect (e.g. heritability), the sample size, the probability level (α) chosen, and the homogeneity of the sample (see among others, Neale and Cardon, 1992). Table 1 clearly shows that the number of twin pairs used in the different studies is rather low. This may be sufficient to demonstrate familial effects, but the statistical power to distinguish between genetic or shared environmental influences (environmental influences shared by different members of a twin pair or family) as the primary cause of familial aggregation may still be insufficient.

2. A simultaneous analysis

To deal with this problem of small sample sizes, we performed a simultaneous analysis on those five studies that used more or less comparable methodology to measure basal cortisol levels and which provided description of the sample size and MZ and DZ correlations. The studies used in the simultaneous analysis are described in Table 2.

What is immediately evident is that the MZ correlations of all studies are approximately 0.55, whereas the DZ correlations vary from 0.24 to 0.60. Based on the stable MZ correlation it was expected that basal cortisol levels are influenced by genetic factors with a maximum heritability of 60%. In absence of shared environmental influences the MZ correlation is equal to the heritability. However, MZ correlation alone cannot distinguish between genetic or shared environmental influences as the primary cause of familial aggregation. Making use of the difference in MZ and DZ correlation, a simultaneous analysis was used to disentangle these sources. Structural

Table 2
Descriptives of the studies used in the simultaneous analysis

Study	Sample	Sample Size	MZ & DZ Correlations
1. Wüst et al., 2000	saliva	52 MZ 52 DZ	rMZ: 0.58 rDZ: 0.38
2. Froehlich et al., 2000	plasma	51 MZ 37 DZ	rMZ: 0.57 rDZ: 0.49
3. Inglis et al., 1999	plasma	75 MZ 71 DZ	rMZ: 0.50 rDZ: 0.27
4. Linkowski et al., 1993	plasma	11 MZ 10 DZ	rMZ: 0.59 rDZ: 0.60
5. Meikle et al., 1988	plasma	20 MZ 20 DZ	rMZ: 0.50 rDZ: 0.24

equation modeling (Mx, see Neale et al., 1999) was used to fit, by maximum likelihood estimation, the observed twin correlations of all studies against different theoretical models (see Table 3). The full model allows for genetic (A), shared environmental (C), as well as unique environmental (E) influences on cortisol. More parsimonious models then leave out the genetic or environmental influences and test the loss of fit to the observed data by calculating the change in χ^2 ($\Delta\chi^2$) against the gain of degrees of freedom (Δdf). First, it was tested whether the five studies could be taken together to use all information to estimate the genetic and environmental influences (Model 2, 3, 4). Taking the various studies together did not result in a significant change of fit. Secondly, the significance of shared environmental influences was tested by dropping this factor from the model (model 5 and 6), which did not result in a significant change of χ^2. However, dropping genetic influences did significantly change the fit of the model (model 7 and 8).

3. Power-analysis

Based on the simultaneous analyses it can now be concluded that genetic factors are the major source of the familial aggregation. No evidence was found for shared environmental factors, but a major question remains whether statistical power, even with pooling of studies, was sufficient. A third and final purpose of this study, therefore, was to estimate the number of twin pairs required to obtain reliable estimates of heritability and shared environmental variance. A power analysis using Mx (Neale et al., 1999) was conducted to calculate the required sample sizes given heritability estimates of 5%, 15%, 25% 35%, 45% and 55%, so that rMZ=0.60 (Accordingly

Table 3
Model-fitting results of the simultaneous analysis based on twin correlations from 5 studies (see table II)

Model	χ^2	df	$\Delta\chi^2$	Δdf	compared to model	p	A	C
1. AC[a], all different[b]	0.009	0					0.00-0.49[c]	0.00-0.59
2. AC, A equal	1.05	4	1.04	4	1	n.s[d]	0.40	0.05-0.32
3. AC, C equal	3.16	4	3.07	4	1	n.s	0.31-0.65	0.13
4. AC, A equal, C equal	7.89	8	7.88	8	1	n.s.	0.37	0.17
5. A different, no C	5.36	5	5.35	5	1	n.s.	0.49-0.88	-
6. A equal, no C[e]	11.54	9	6.18	4	5	n.s.	0.62	-
7. C equal, no A[f]	16.30	9	13.14	5	3	0.02	-	0.45
8. C different, no A	11.69	5	11.68	5	1	0.04	-	0.37-0.59

[a] A= additive genetic factors, C= common (shared) environmental factors.
[b] different estimates for the separate studies.
[c] range of estimates for the different studies.
[d] n.s. = non-significant determination of fit.
[e] common environmental influences are dropped from the model.
[f] additive genetic influences are dropped from the model.

Table 4
Sample sizes needed to estimate genetic influences, common environmental influences and familial aggregation with a power of 0.80 and with simulated twin data (equal number of MZ and DZ)

Variance distribution	detection of genetic influences	detection of common environmental influences	detection of familial aggregation
h^2=0.05, c^2=0.55[a]	16087[b]	136	19
h^2=0.15, c^2=0.45	1984	215	21
h^2=0.25, c^2=0.35	786	374	22
h^2=0.35, c^2=0.25	437	769	24
h^2=0.45, c^2=0.15	286	2231	26
h^2=0.55, c^2=0.05	206	20878	28

[a] h^2 = heritability, c^2 = common environmental influences.
[b] total number of complete twin pairs (equally divided in MZ and DZ).

estimates of shared environmental influences are 55%, 45%, 35%, 25%, 15% and 5%, respectively). The assumption of rMZ=.60 was made based on the MZ correlations used for the simultaneous analyses.

The analyses were again based on a comparison of different models. To estimate the sample size to detect genetic influences a model with additive genetic (A), shared environmental (C) and unique environmental (E) factors was compared to a model with shared environmental and unique environmental factors only. Further, to estimate the sample size needed to detect the shared environmental influences, an ACE model was compared to an AE model. Finally, to estimate the sample size for detection of familial aggregation an ACE model was compared to a model with unique environmental influences only. Table 4 shows the required number of twin pairs on the basis of the power desired. As can be seen in Table 4, small effect, i.e. low heritabilities, are harder to detect and need far larger sample sizes than have been used in the existing studies.

4. Discussion

The first purpose of this paper was to critically examine the existing literature on the heritability of cortisol levels in twin and family studies. We found 11 studies that satisfied our search criteria; based on the search terms *twin, cortisol, corticosteroid, heritability and family*, no case studies, published in English, and genetically related subjects (see introduction). After careful inspection we concluded that these studies lack the methodological consistency required for a good comparison. A factor that makes it hard to compare the different studies is the multitude of cortisol measures used: basal cortisol, area under the curve, morning peak, nocturnal nadir and reactivity to acute stressors. These are clearly different phenotypes and need not be influenced by the same genes or environmental factors. The studies that focus on basal levels have used rather equivocal frequency and timing of sampling across the day. Also where the older studies sometimes neglected confounding factors such as physi-

cal exercise, smoking or type of depression, the more recent studies often rule out all possible individual differences by selecting non-smoking, same-sex subjects that use no oral contraceptives or other over the counter medications. This is good experimental practice, but makes it hard to compare them to the older studies. It is also uncertain how this selection affects the generalisability of the genetic architecture to that of the general population. Twin pairs with cortisol levels outside the expected range are mostly excluded, for instance, while it might be interesting to know why those twin pairs deviate.

The main problem plaguing existing studies is the relatively low number of twin pairs on whom cortisol was obtained. A power analysis revealed that none of the 11 studies examined consists of a large enough sample size to be able to separate genetic and environmental influences. A combined analysis of these studies estimated the heritability of 62% for basal cortisol levels with a combined sample size of 399 twin pairs (209 MZ and 190 DZ). According to Table 4, this simultaneous analysis has the power to separate familial influences into shared environmental and genetic influences. Disentangling the sources of familial aggregation is essential in understanding individual differences in basal cortisol levels. Previous studies point out that both genetic factors (see Table 1) and shared environmental factors, such as parenting styles, influence basal cortisol levels.

The obvious approach to increase power is to increase the sample size. The recent development of large Twin Registries all over the world (e.g. Boomsma, 1998) and of ambulatory sample collection methods will make it more feasible to measure on large numbers of subjects relatively easily in the future. Even so, the sample sizes shown in Table 4 are in some cases unrealistic high and beyond the scope of actual resources of time, money and practical attainability. Other remedies, therefore, must be considered. One might be the composition of the sample. Increase of power can be achieved by extending the twin design by adding siblings (Posthuma and Boomsma, 2000) or adding information from adoption studies (Schmitz et al., 1998). Most importantly, an increase in power can be achieved through multivariate analyses, i.e. by increasing the number of measurements through repeated measure designs. Provided that those repeated measurements correlate significantly with each other, this yields large gains in power (Schmitz et al., 1998).

Increasing the number of samples on a single measurement day is a first method to obtain repeated measures. However, in the view of the complexity of the HPA system, it is entirely possible that different genes influence cortisol at different times of day. It is valuable as such to assess the genetic architecture of cortisol level at different points of the diurnal curve, i.e. to understand the sources underlying individual variation in the morning peak level as well as in the evening. But if the samples across different time points are not (genetically) correlated increasing the number of samples on the same measurement day will do little to improve power to detect heritability. The optimal approach is to sample at *repeated time points* of the diurnal curve, on *repeated days* within the same subject as was done by Young et al. (2000). This still leaves error variance due to differences in day-to-day variation in wake-up times, but this can be dealt with by sampling at fixed time points relative to wake-up times.

In summary, understanding the genetic architecture of basal cortisol level awaits studies with large twin samples that measure cortisol repeatedly at fixed time points from the awakening time, and do so on repeated days. In parallel, twin studies must be aware of the rapid progress in animal research. A number of candidate genes with respect to basal cortisol levels have emerged, like those that affect corticotrophin-releasing hormone (CRH) or ACTH synthesis (and also the production of their receptors) and those that code for mineralocorticoid (MR, or type-I receptor) and the glucocorticoid (GR, or type-II receptor). Polymorphism(s) in the latter gene have already been associated with various aspects of cortisol metabolism such as varying basal cortisol levels (Rosmond et al., 2000a; Rosmond et al., 2000b) and differences in sensitivity to glucocorticoids (Huizinga et al., 1998). One of the huge advantages of a twin sample is that such observed genes (candidate genes) and unobserved genes (estimates of heritability through the MZ-DZ comparison) can be simultaneously tested.

Acknowledgements

M. Bartels was financially supported by The Netherlands Organization for Scientific Research (575-25-012).

F. Sluyter was supported by a PULS Grant (48.001) from the Earth and Life Sciences Foundation (ALW), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

References

Aardal, E., Holm, A.C., 1995. Cortisol in saliva--reference ranges and relation to cortisol in serum. Eur. J. Clin. Chem. Clin. Biochem 33 (12), 927–932.

Beuschlein, F., Fassnacht, M., Klink, A., Allolio, B., Reincke, M., 2001. ACTH-receptor expression, regulation and role in adrenocortial tumor formation. Eur. J. Endocrinol. 144 (3), 199–206.

Boivin, D.B., Duffy, J.F., Kronauer, R.E., Czeisler, C.A., 1996. Dose-response relationship for resetting of human circadian clock by ligth. Nature 379, 540–542.

Boomsma, D.I., 1998. Twin registers in Europe: an overview. Twin. Res. 1, 34–51.

Dorin, R.I., Ferries, L.M., Roberts, B., Qualls, C.R., Veldhuis, J.D., Lisansky, E.J, 1996. Assessment of stimulated and spontaneous adrenocorticotropin secretory dynamics identifies distinct components of cortisol feedback inhibition in healthy humans. J. Clin. Endocrinol. Metab. 81 (11), 3883–3891.

Fischman, A.J., Kastin, A.J., Graf, M.V., Moldow, R.L., 1988. Constant ligth and dark affect the circadian rhythm of the hypothalamic-pituitary-adrenal axis. Neuroendocrinology 47, 309–316.

Froehlich, J.C., Zink, R.W., Li, T.K., Christian, J.C., 2000. Analysis of heritability of hormonal responses to alcohol in twins: beta-endorphin as a potential biomarker of genetic risk for alcoholism. Alcohol Clin. Exp. Res. 24, 265–277.

Goodyer, I.M., Herbert, J., Tamplin, A., Altham, P.M., 2000. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. Br. J. Psychiatry 177, 499–504.

Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., Miller, A.H., Nemeroff, C.B., 2000. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. Jama 284 (5), 592–597.

Huizenga, N.A., Koper, J.W., De Lange, P., Pols, H.A., Stolk, R.P., Burger, H., Grobbee, D.E., Brink-

mann, A.O., De Jong, F.H., Lamberts, S.W., 1998. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J. Clin. Endocrinol. Metab. 83, 144–151.

Inglis, G.C., Ingram, M.C., Holloway, C.D., Swan, L., Birnie, D., Hillis, W.S., Davies, E., Fraser, R., Connell, J.M., 1999. Familial pattern of corticosteroids and their metabolism in adult human subjects--the Scottish Adult Twin Study. J. Clin. Endocrinol. Metab. 84 (11), 4132–4137.

Jacobson, L., Sapolsky, R., 1991. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev. 12, 118–134.

Karl, M., Schulte, H.M., 1994. Familiäre Glucocorticoidresistenz als Differentialdiagnose des Hypercortisolismus. Klinische und molekulargenetische Aspekte. [Familial glucocorticoid resistance as the differential diagnosis of hypercortisolism. Clinical and molecular genetic aspects]. Dtsch Med Wochenschr 119, 74–79.

Kirschbaum, C., Hellhammer, D.H., 1994. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19 (4), 313–333.

Kirschbaum, C., Wüst, S., Faig, H.G., Hellhammer, D.H., 1992. Heritability of cortisol responses to human corticotrophin-releasing hormone, ergometry, and psychological stress in humans. J. Clin. Endocrinol. Metab. 75 (6), 1526–1530.

Kirschbaum, C., Hellhammer, D.H., 1989. Salivary Cortisol in psychobiological research: An overview. Neuropsychobiology 22, 150–169.

Kovacs, G.L., Fekete, M., Szabo, G., Telegdy, G., 1987. Action of the ACTH-corticosteroid axis on the central nervous system. Front. Horm. Res. 15, 79–127.

Kuhn, C., 1989. Adrenocortical and gonadal steroids in behavioral cardiovascular medicine. In: Schneiderman, N., Weis, S.M., Kaufman, P.G. (Eds.), Handbook of Research Methods on Cardiovascular Behavioral Medicine. Plenum, New York, pp. 185–204.

Levine, S., 1994. The ontogeny of the HPA axis: the influence of maternal factors. Ann. N. Y. Acad. Sci. 746, 275–288.

Li, A., Li, K.X., Marui, S., Krozowski, Z.S., Batista, M.C., Whorwood, C.B., Arnhold, I.J., Shackleton, C.H., Mendonca, B.B., Stewart, P.M., 1997. Apparent mineralocorticoid excess in a Brazilian kindred: hypertension in the heterozygote state. J. Hypertens. 15, 1397–1402.

Liddle, G., 1960. Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. J. Clin. Endocrinol. Metab. 20, 1539–1560.

Linkowski, P., Mendlewicz, J., Leclercq, R., Brasseur, M., Hubain, P., Golstein, J., Copinschi, G., Van Cauter, E., 1985. The 24-hour profile of adrenocorticotropin and cortisol in major depressive illness. J ClinEndocrinol Metab 61 (3), 429–438.

Linkowski, P., Van Onderbergen, A., Kerkhofs, M., Bosson, D., Mendlewicz, J., Van Cauter, E., 1993. Twin study of the 24-h cortisol profile: evidence for genetic control of the human circadian clock. Am. J. Physiol. 264 (2(Pt 1)), E173–E181.

Lopez Bernal, A., Anderson, A.B., Parry, D.M., Turnbull, A.C., 1980. Evidence that fetal membranes are not involved in cortisol metabolism: study of dichorionic twin pregnancies. Am. J. Obstet. Gynecol. 138 (8), 1168–1172.

Mantero, F., Boscaro, M., 1992. Glucocorticoid-dependent hypertension. J. Steroid Biochem. Mol. Biol. 43 (5), 409–413.

Martin, N., Boomsma, D.I., Machin, G.A., 1997. A twin-pronged attack on complex traits. Nat. Genet. 17, 387–391.

Maxwell, J.D., Boyle, L.A., Greig, W.R., Buchanan, W.W., 1969. Plasma Corticosteroids in Healthy Twin Pairs. J. Med. Genet. 6, 294–297.

Meikle, A.W., Stringham, J.D., Woodward, M.G., Bishop, D.T., 1988. Heritability of variation of plasma cortisol levels. Metabolism 37 (6), 514–517.

Mendlewicz, J., Hoffmann, G., Kerkhofs, M., Linkowski, P., 1984. Electroencephalogram and neuroendocrine parameters in pubertal and adolescent depressed children. A case report study. J Affect Disord 6 (3-4), 265–272.

Milford, D.V., Shackleton, C.H.L., Stewart, P.M., 1994. Mineralocorticoid hypertension and congenital deficiency of 11 β-hydroxysteroid dehydrogenase in a family with the syndrome of 'apparent' mineralocorticoid excess. Clin. Endocrinol. 43, 241–246.

Mullen, P.E., Martin, J.L., Anderson, J.C., Romans, S.E., Herbison, G.P., 1996. The long-term impact of the physical, emotional, and sexual abuse of children: a community study. Child. Abuse. Negl. 20, 7–21.

Neale, M.C., Cardon, L.R., 1992. Methodology for Genetic Studies of Twins and Families. NATO ASI Series, Series D: Behavioral and Social Sciences, Vol. 67. Kluwer Academic Publishers, Dordrecht/Boston/London.

Neale, M.C., Boker, S.M., Xie, G., Maes, H.H., 1999. Mx: Statistical Modeling. Box 126 MCV, Richmond, VA 23298: Department of Psychiatry. 5th Edition.

Norman, R.J., Joubert, S.M., 1982. Fetal adrenal gland maturation in growth-retarded twin pregnancies. Acta Genet. Med. Gemellol.(Roma) 31 (3-4), 59–62.

Norman, R.J., Deppe, W.M., Coutts, P.C., Marivate, M., Joubert, S.M., 1983a. Twin pregnancy as a model for studies in fetal cortisol concentrations in labour: relation to prostaglandins, prolactin and ACTH. Br. J. Obstet. Gynaecol. 90 (11), 1033–1039.

Norman, R.J., Joubert, S.M., Marivate, M., 1983b. Amniotic fluid phospholipids and glucocorticoids in multiple pregnancy. Br. J. Obstet. Gynaecol. 90 (1), 51–55.

Norman, R.J., Maharaj, C., Adhikari, M., Joubert, S.M., 1984. Adrenal sensitivity to adrenocorticotrophin in twin neonates--effect of birth order and growth retardation. S. Afr. Med. J. 66 (3), 90–92.

Nurnberger, J.I., Gershon, E.S., Simmons, S., Ebert, M., Kessler, L.R., Dibble, E.D., Jimerson, S.S., Brown, G.M., Gold, P., Jimerson, D.C., Guroff, J.J., Storch, F.I., 1982. Behavioral, biochemical and neuroendocrine responses to amphetamine in normal twins and 'well-state' bipolar patients. Psychoneuroendocrinology 7, 163–176.

Nurnberger, J.I., Jimerson, D.C., Simmons-Alling, S., Tamminga, C., Nadi, N.S., Lawrence, D., Sitaram, N., Gillin, J.C., Gershon, E.S., 1983. Behavioral, physiological, and neuroendocrine responses to arecoline in normal twins and "well state" bipolar patients. Psychiatry Res. 9 (3), 191–200.

Okumura, T., Nakajima, Y., Takamatsu, T., Matsuoka, M., 1995. Column-switching high-performance liquid chromatographic system with a laser-induced fluorimetric detector for direct automated assay of salivary cortisol. J. Chromatog. B: Biomed. Applications 670, 11–20.

Pedersen, B.K., Hoffman-Goetz, L., 2000. Exercise and the immune system: regulation, integration, and adaptation. Physiol. Rev. 80 (3), 1055–1081.

Pinheiro, M.M., Liberman, B., Salgado, L.R., Goldman, J., Nery, M., Cukiert, A., 1999. Identical twins discordant for Cushing's disease: case report. Arq. Neuropsiquiatr. 57, 686–688.

Plotsky, P.M., Meany, M.J., 1993. Early, postnatal experience alters hypothalamic corticotrophin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Mol. Brain Res 18, 195–200.

Posener, J.A., DeBattista, C., Williams, G.H., Chmura Kraemer, H., Kalehzan, B.M., Schatzberg, A.F., 2000. 24-Hour monitoring of cortisol and corticotrophin secretion in psychotic and nonpsychotic major depression. Arch. Gen. Psychiatry. 57 (8), 755–760.

Posener, J.A., Schildkraut, J.J., Williams, G.H., Schatzberg, A.F., 1997. Cortisol feedback effects on plasma corticotropin levels in healthy subjects. Psychoneuroendocrinology 22 (3), 169–176.

Posthuma, D., Boomsma, D.I., 2000. A note on the statistical power in extended twin designs. Behav. Genet. 30 (2), 147–158.

Pritchard, J., Despres, J.P., Gagnon, J., Tchernof, A., Nadeau, A., Tremblay, A., Bouchard, C., 1998. Plasma adrenal, gonadal, and conjugated steroids before and after long-term overfeeding in identical twins. J. Clin. Endocrinol. Metab. 83, 3277–3284.

Pritchard, J., Despres, J.P., Gagnon, J., Tchernof, A., Nadeau, A., Tremblay, A., Bouchard, C., 1999. Plasma adrenal, gonadal, and conjugated steroids following long-term exercise-induced negative energy balance in identical twins. Metabolism 48, 1120–1127.

Raczynska, B., Lukaszewska, J., Sklad, M., 1978. Plasma cortisol level in monozygotic and dizygotic twins. Endokrynol Pol 29 (1), 7–10.

Reul, J.M.H.M., De Kloet, E.R., 1985. Two receptor systems for corticosterone in the rat brain: Microdistribution and differential occupation. Endocrinology 117, 2505–2511.

Riad Fahmy, D., Read, G.F., Walker, R.F., Griffiths, K., 1982. Steroids in saliva for assessing endocrine function. Endocr. Rev. 3 (4), 367–395.

Rosmond, R., Chagnon, Y.C., Chagnon, M., Perusse, L., Bouchard, C., Bjorntorp, P., 2000a. A polymor-

phism of the 5'-flanking region of the glucocorticoid receptor gene locus is associated with basal cortisol secretion in men. Metabolism 49, 1197–1199.
Rosmond, R., Chagnon, Y.C., Holm, G., Chagnon, M., Perusse, L., Lindell, K., Carlsson, B., Bouchard, C., Bjorntorp, P., 2000b. A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. Obes. 8, 211–218.
Rosmond, R., Bjorntorp, P., 2000c. The hypothalamic-pituitary-adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. J. Intern. Med. 246 (2), 188–197.
Roy, M.P., Kirschbaum, C., Steptoe, A., 2001. Psychological cardiovascular and metabolic correlates of individual differences in cortisol stress recovery in young men. Psychoneuroendocrinology 26 (4), 375–391.
Scheer, F.A.J.L., Buijs, R.M., 1999. Ligth Affects Morning Salivary Cortisol in Humans. J Clin Endocrinol Metab 84 (9), 3395–3398.
Schmitz, S., Cherny, S.S., Fulker, D.W., 1998. Increase in power through multivariate analyses. Behav. Genet. 28, 357–364.
Schuckit, M.A., Hauger, R.L., Monteiro, M.G., Irwin, M., Duthie, L.A., Mahler, H.I., 1991. Response of three hormones to diazepam challenge in sons of alcoholics and controls. Alcohol. Clin. Exp. Res. 15, 537–542.
Schwartz, J.T., Reuling, F.H., Feinleib, M., Garrison, R.J., Collie, D.J., 1972. Twin heritability study of the effect of corticosteroids on intraocular pressure. J. Med. Genet. 9 (2), 137–143.
Smyth, J.M., Ockenfels, M.C., Gorin, A.A., Catley, D., Porter, L.S., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1997. Individual differences in the diurnal cycle of cortisol. Psychoneuroendocrinology 22 (2), 89–105.
Spith-Schwalbe, E., Uthgenannt, D., Voget, D., Kern, W., Born, J., Fehm, H.-L., 1993. Corticotropin-releasing hormone-induced adrenocorticotropin and cortisol secretion depends on sleep and wakefulness. J. Clin. Endocrinol. Metab. 77, 1170–1173.
Trainer, P.J., McHardy, K.C., Harvey, R.D., Reid, I.W., 1993. Urinary free cortisol in the assessment of hydrocortisone replacement therapy. Horm. Metab. Res. 25, 117–120.
Van Cauter, E., 1990. Diurnal and ultradian rhythms in human endocrine function: a minireview. Horm. Res. 34 (2), 45–53.
Veldhuis, H.D., Van Koppen, C., Van Ittersum, M., De Kloet, E.R., 1982. Specificity of the adrenal steroid receptor system in the rat hippocampus. Endocrinology 110, 2044–2049.
Walker, B.R., 1996. Abnormal glucocorticoid activity in subjects with risk for cardiovascular disease. Endocr. Res. 22 (4), 701–708.
Walker, B.R., Phillips, D.I., Noon, J.P., Panarelli, M., Andrew, R., Edwards, H.V., Holton, D.W., Seckl, J.R., Webb, D.J., Watt, G.C., 1998. Increased glucocorticoid activity in men with cardiovascular risk factors. Hypertension 31, 891–895.
Weitzman, E.D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T.F., Hellman, L., 1971. Twenty-four Hour Pattern of the Episodic Secretion of Cortisol in Normal Subjects. J. Clin. Endocrinol. Metab. 33, 14–22.
Wüst, S., Federenko, I., Hellhammer, D.H., Kirschbaum, C., 2000. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinology 25, 707–720.
Yehuda, R., Bierer, L.M., Schmeidler, J., Aferiat, D.H., Breslau, I., Dolan, S., 2000. Low cortisol and risk for PTSD in adult offspring of holocaust survivors. Am. J. Psychiatry. 157, 1252–1259.
Yehuda, R., Kahana, B., Schmeidler, J., Southwick, S.M., Wilson, S., Giller, E.L., 1995. Impact of cumulative lifetime trauma and recent stress on current posttraumatic stress disorder symptoms in holocaust survivors. Am. J. Psychiatry. 152, 1815–1818.
Yehuda, R., Giller, E.L., Southwick, S.M., Lowy, M.T., Mason, J.W., 1991. Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. Biol. Psychiatry. 30, 1031–1048.
Young, E.A., Aggen, S.H., Prescott, C.A., Kendler, K.S., 2000. Similarity in saliva cortisol measures in monozygotic twins and the influence of past major depression. Biol. Psychiatry 48, 70–74.
Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J., Akil, H., 1998. The role of mineralocorticoid receptors in hypothalamic-pituitary-adrenal axis regulation in humans. J. Clin. Endocrinol. Metab. 83 (9), 3339–3345.