Mediating factors in the association between anxious depression and cardiovascular disease risk

Of shriek never my soul be free
a pitch black sea, my pounding heart
By the father's message touched, I tremble with fear.
The noose I'd rather have around my neck
than to die under a damned man's hands!
No, rather death in the service of Hades!

Aeschylus

Mireille van den Berg

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Financial support by DEFACTO for the publication of this thesis is gratefully acknowledged.

ISBN 90-9016320-4
Printed by FEBO druk, Utrecht, the Netherlands
Lay-out Natascha Stroo, Mireille van den Berg
Cover Fred Koot

VRIJE UNIVERSITEIT

Mediating factors in the association between anxious depression and cardiovascular disease risk

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Psychologie en Pedagogiek
op dinsdag 3 december 2002 om 13.45 uur
in het auditorium van de universiteit,
De Boelelaan 1105

door

Mireille van den Berg

geboren te Amsterdam

promotor: prof.dr. D.I. Boomsma
copromotor: prof.dr. J.C.N. de Geus

Leescommissie: prof.dr. R.F.M. Vlietinck
prof.dr. L.J.P. van Doornen
prof.dr. W.A.M. Vollebergh
prof.dr. W.J.G. Hoogendijk
dr. J.H.M.Tulen
dr. A.L. Beem

Contents

Chapter 1

General introduction

In this thesis the pathways through which the association between depression and risk for cardiovascular disease is mediated are explored. In the current chapter factors which are in the mediating pathways and which are studied in the empirical chapters are introduced.

1.1 Anxious depression

According to the DSM-IV (APA, 2000; Diagnostic and Statistical Manual for psychiatric disorders) depression includes a variety of emotional, physiological, behavioural and cognitive symptoms. Depression can take several forms and not always the same symptoms are involved. It is rather a cluster of symptoms of which several must be apparent to diagnose depression. The most apparent symptoms are loss of interest or pleasure in activities that used to be satisfactory (anhedonia), fatigue and loss of energy. Symptoms have to continue for a period of at least two weeks and be present every day for most part of the day. Having a depression disrupts life greatly and causes intellectual problems by lack of concentration or lack of interest and social isolation. Furthermore it is a great burden on relationships, and may lead to loss of loved-ones and job-loss.

One of the most consistent findings in mental health research is that 25 to 30% of women and 10 to 15% of men will experience a depressive episode at least once in their lives (e.g. Leutwyler, 1995). This makes depression one of the worlds leading causes of disease. The disease burden of depression ranks world-wide after respiratory tract infections, diarrhoea and perinatal disorders, but ahead of cardiovascular disease according to a study of premature mortality and disability around the world (Murray and Lopez, 1997).

Anxiety is a prominent feature in depression (Kessler et al., 1994; Lewinsohn et al., 1997). The symptoms of generalised anxiety disorders (GAD) as mentioned in the DSM-IV overlap to some degree with those of depression. According to the DSM-IV criteria persons with GAD are often worried about things other people would not even consider. Because of their worries they cannot enjoy activities they would enjoy otherwise, they have problems concentrating, and are restless and irritable. Most of these symptoms also are found in depression. Furthermore there is great comorbidity between depression and GAD. Depression and GAD are thought to share a similar genotype that is non-specific in its impact on the expression of the disorder (Boomsma et al., 2000; Kendler et al., 1992; Kendler, 1996; Roy et al., 1995). According to Kendler et al (1992; 1996)

depression and GAD share a common element strongly related to neuroticism, a trait first proposed by Eysenck, which responds poorly to stress and is therefore bound to experience frequent and intense periods of distress and negative affect (Watson & Tellegen, 1985). The element shared by depression and GAD is for the purpose of this thesis called anxious depression (see for more information chapter 5). Anxious depression is a reliable measure for symptoms of depression and anxiety and reflects the vulnerability to clinical depression (Chapter 3).

Apart from being a mental burden, depression is also a major influence on both behavioural health-risk factors and physiological regulation. In many recent studies depression is associated with progressive cardiovascular disease, indicating that depression should be considered as a risk factor for cardiovascular disease (e.g. Ariyo et al., 2000; Ford et al., 1998; Penninx et al., 2001; Sesso et al., 1998). In addition to the possibility of depression being a risk factor for progressive cardiovascular disease, developing major depression after experiencing cardiovascular disease can have a negative effect on the prognosis. Suffering from cardiovascular disease is commonly accepted as a plausible cause for developing depression. In fact, depression is considered to be a normal reaction to cardiovascular disease: at least 30% of cardiovascular disease patients experience a minor or major form of depression (Frasure-Smith, Lespérance & Talajic, 1993, 1995; Lespérance, Frasure-Smith & Talajic 1996; Lespérance, Frasure-Smith, 2000). The high prevalence of depression in cardiovascular disease patients is alarming, since a number of clinical studies have concluded that depression has negative consequences for the prognosis of cardiovascular disease, doubling the risk of recurrence of cardiac events within 12 months (Carney et al., 1987) and increasing risk of mortality more than four-fold in the first 6 months after acute myocardial infarction (MI) after adjusting for other risk factors (Frasure-Smith, Lespérance & Talajic, 1995).

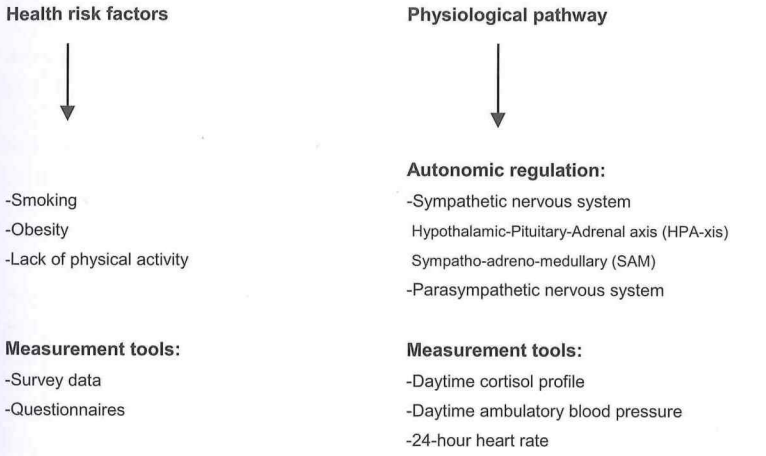
According to the stress diathesis model of mood disorders (Akiskal, 1995; Nemeroff, 1998) depression may result from an interplay between a genetic vulnerability to depression and adverse environmental factors, such as negative life events. From studies on the effects of early life trauma it was concluded that this may lead to biological and behavioural alterations (Dunn & Berridge, 1990; Kalin, 1990). These alterations include activation of the autonomic nervous system, for instance because sensitisation of the corticotrophin releasing factor (CRF) mechanisms in the central nervous system (Heim & Nemeroff, 1999) causes endocrine changes, including elevated cortisol levels and behavioural changes resembling symptoms of depression and anxiety (Nemeroff, 1999).

The pathway linking depression to the physiological reactions that have been associated with cardiovascular risk is largely unexplored, but could be related to the biological alterations proposed in the stress diathesis model of depression. In addition, depression is associated with a number of health risk behaviours also associated with cardiovascular risk, such as smoking.

1.2 A model for the association between anxious depression and CVD risk

Two possible pathways through which the factors possibly mediate the association between depression and cardiovascular disease risk factors are presented in figure 1.1. The first pathway concerns health risk behaviours that put a person at risk for developing cardiovascular disease. Examples of depression related health risk behaviours which possibly mediate cardiovascular disease risk are smoking and physical inactivity associated with obesity. The second pathway concerns autonomic regulation. An example is deregulation of the hypothalamic–pituitary-adrenocortical axis (HPA-axis) leading to higher cortisol levels. High cortisol levels are associated with depression (e.g. Holsboer, 2000; 2001) and hypertension (Kelly et al., 1998; Walker et al, 1998) which is considered to be a risk factor for cardiovascular disease (Kaplan, 1990). Individual differences in both health risk behaviours and physiological factors are influenced by genetic factors and may explain (part of) the genetic risk for cardiovascular disease.

Figure 1.1. Possible mediating factors in the association between anxious depression and cardiovascular disease.



In the present thesis the association between depression and the risk factors in the health risk pathway and the physiological pathway is assessed. In all chapters depression was measured

using both anxious depression derived from a composite factor score based on longitudinal survey data and DSM-IV diagnosis for clinical depression derived from a diagnostic interview.

1.3 Health risk factors in anxious depression

The model in figure 1.1 presents health risk factors as a mediator in the association between depression and cardiovascular disease. Three important health risk factors are considered: smoking, lack of physical activity and obesity. In the following paragraphs the role of these health risk factors in the association between depression and cardiovascular disease is discussed.

1.3.1 Smoking

Smoking and depression are among the most important and highly prevalent health risk factors in the industrialised countries (e.g. Mathers et al., 2001; Melse et al., 2000; Murray & Lopez, 1997). Importantly, there is consistent evidence for an association between smoking and depression. Evidence for this association comes from several lines of research. Both incidence rates and life time prevalence rates of smoking are higher in individuals with higher scores on depression scales (Anda et al., 1990; Glassman et al, 1990; Patton et al., 1996). Also, depressed patients and individuals with high depression scores are less likely to succeed in smoking cessation (Glassman et al., 1990). Furthermore, depression is related to a relapse in ex-smokers (Glassman et al., 1990; Shiffman, 1982). More evidence of the association between smoking and depression comes from studies that successfully used antidepressants, mainly Selective Serotonine Reuptake Inhibitors (SSRI's), as an aid to quit smoking (Berlin et al, 1995; Hitsman et al, 1999; Hughes et al, 2000; Hurt et al, 1998). The incidence of smoking in persons suffering from any kind of mental illness including depression is about 60% compared to 25% in the general population (Leonard et al., 2001). Smoking rate increases linearly with the severity of depression (Farrell et al, 1998; Kendler et al., 1993), and may therefore be most prominent in hospitalised patients (Acton et al., 2001; Hughes et al., 1986).

The dominant explanation for the association between smoking and depression is the self-medication hypothesis, which states that cigarette smoking is a form of self-medication against negative moods because of the actions of nicotine on central neuroregulators resulting in a relieve of dysphoric mood (Hughes, 1988; Pomerleau & Pomerleau, 1984). One of these neuroregulator mechanisms is the monoamine oxidase A and B systems. Chronic smoking has been shown to inhibit monoamine oxidase A and B (Fowler et al., 1996a; Fowler et al., 1996b). Inhibition of these enzymes can increase levels of among others dopamine in the brain, explaining the antidepressant actions of nicotine. In comparison: monoamine oxidase (MAO) inhibitors have for a long time been prescribed therapeutically to relieve depression.

An alternative explanation is that smoking and depression share a common aetiology. This common aetiology can be biological (e.g. genetic or neurochemical factors) or non-biological (e.g environmental factors). The possibility that depression and smoking share common genetic factors is assessed in a study by Kendler et al. (1993), in which a higher relative risk for smoking in life-time depression was found in monozygotic twins compared to dizygotic twins. Genetic model fitting indicated that the association between smoking and life-time depression could best be explained by a correlation between the genetic liability for smoking and the genetic liability for depression (Kendler et al., 1993). Alternatively, Roy et al. (2000) proposed that smoking and depression were associated through shared early negative factors, such as negative life-events (Roy et al., 2000) and showed that smokers were distinguished from non-smokers by greater exposure to aversive experiences in childhood.

Smoking is a classical risk factor for cardiovascular disease and may therefore be among the most important mediating factors in the association between depression and cardiovascular disease. Smoking stimulates the sympathetic branch of the autonomic nervous system (Cryer et al., 1976; Omvik, 1996; Oncken et al, 2001) resulting in higher cortisol and blood pressure levels. Furthermore smoking is associated with other risk factors for cardiovascular disease, such as higher risk for arteriosclerosis (Patel, Eggen & Strong, 1980) and thrombosis, i.e. by elevated white and red blood cell counts (Feher et al., 1990; Taylor, 1987), increased fibrinogen levels (Meade, Imerson & Stirling, 1987) and changes in platelet-vessel wall interaction (Lassila & Laustolia, 1992; Zieske et al. 1999). Since even after adjustment for smoking, depression is a risk factor for cardiovascular disease (Ariyo et al, 2000; Hippisley-Cox, Fielding & Pringle, 1998; Everson et al., 1998), it must be recognised that smoking among depressives can only increase their risk for cardiovascular disease even more. *Chapter 4* presents the results of a study on the relationship between depression, both trait depression and clinical depression, in both men and women. Information on life time smoking behaviour was available from the longitudinal survey data together with information on current smoking from the measurement day.

1.3.2 Physical activity and body weight

Body Mass Index (BMI), the weight in kilograms, divided by square height in meters, can be used as an index of obesity. Relative body weight, measured as BMI, is associated with major depression. In a recent study by Carpenter et al., (2000) increased BMI was associated with major depression in women. Among men lower BMI was associated with major depression. Among obese persons the incidence of life-time depression is higher than in a normal population (Britz et al., 2000; Specker et al., 1994). An explanation for the association between depression and obesity can be that depression is often associated with bad dietary habits, with an increased carbohydrate consumption, primarily due to an increase in sucrose consumption (Christensen & Somers, 1996). In addition, depression is associated with lower levels of physical activity, which also can lead to an

increase in BMI. Moreover, increase of activity level was associated with a decreased risk of depression (Alameda County study: Camacho et al., 1991; Milani, Lavie & Cassidi, 1996).

Increased body weight in general and specifically lack of physical activity are considered a major health risk factor possibly mediating cardiovascular disease risk according to the global burden of disease studies (Murray & Lopez, 1996). High BMI is positively associated with hypertension (Ernst et al., 1997; Garrison, Higgins & Kannel, 1996; Kannel, D'Agostino & Cobb, 1996). In this thesis BMI is used as a covariate in Chapter 6 and 7 when studying the association of depression with cortisol and blood pressure. Moreover, in Chapter 7 possible differences between depressed and non-depressed persons in physical activity during the measurement day were controlled for by selecting the blood pressure recordings during the sitting postures only.

1.4 Autonomic regulation

The physiologic pathway depicted in the model in figure 1.1, mainly concerns deregulation of the autonomic nervous system. In the current thesis it is hypothesised that in accordance with the stress diathesis model of depression there will be a deregulation of parasympathetic and sympathetic nervous system activity. This might be reflected in several psychophysiological parameters including cortisol and blood pressure levels. The sympathetic and the parasympathetic branches of the autonomic nervous system play a major role in the body's adjustments to normal demands in order to maintain metabolic homeostasis (see for a review: Lovallo & Thomas, 1990). They are essential in the regulation of the body's stress response. The perceived potential harm of a stressor activates a whole series of actions from the autonomic nervous system designed to avoid harm. Two primary systems are particularly involved in stress responsivity: the hypothalamic-pituitary-adrenal axis (HPA-axis), and the sympatho-adreno-medullary (SAM) system (Cannon, 1914; Lovallo, & Thomas, 2000; Selye, 1936). In psychophysiologic research cortisol is widely used as an index of HPA-axis reactivity, either bound or free in plasma, urine or saliva. SAM activation can be determined by variations in blood pressure and heart rate reactivity. In the study on which the present thesis is based, non-invasive techniques to obtain recordings of blood pressure and heart rate were used (De Geus & Van Doornen, 1996). In addition a non-invasive method for cortisol sampling by salivary sampling is used (Kirschbaum & Hellhammer, 1989, 1994). The collection of cortisol and blood pressure readings will be used to obtain information on differences in autonomic regulation between depressed and non-depressed persons. A short outline on cortisol collection and blood pressure, as far as relevant for the present thesis, will be given in the following section.

1.4.1 Index of HPA-axis reactivity: cortisol

The release of corticotrophin-releasing hormone (CRF), a product from the neurones in the paraventricular nucleus of the hypothalamus initiates the HPA-axis cascade. In the pituitary, CRF

stimulates the synthesis of pro-opiomelanocortin leading to the release of adrenocorticotrophic hormone (ACTH). In turn ACTH leads to synthesis and release of cortisol, a steroid hormone secreted by the outer cortex of the adrenal gland. Its secretion is regulated by negative feedback at the pituitary, hypothalamus and hippocampus (Jacobson & Sapolsky, 1991; Kovacs et al., 1987). Hypercortisolism, characterised by increased cortisol levels, can be caused by hypersensitivity to ACTH or CRF or by resistance of the hippocampus, hypothalamus or pituitary to the inhibiting effect of cortisol (Sapolsky et al., 2000). Hypersecretion at distinct levels of the HPA-axis (aberrations of CRF or ACTH) may also lead to an excess of circulating cortisol (Sapolskey et al., 2000).

One of the main functions of cortisol is to mobilise energy to prepare the body for a reaction to a stressor. It mobilises energy by increasing glucose levels, enhances the quality of the sensory systems and alters several bodily functions. Together with epinephrine, released by the medullar part of the adrenal glands, cortisol increases heart rate and blood pressure to supply the muscles with more blood (Sapolsky et al., 2000; Vander, Sherman & Luciano, 1998). Mobilisation of energy is also accomplished by shutting down systems that are at times of immediate stress less vital to the body, such as the digestive system and the reproductive system.

Once in the blood, cortisol is transported either freely or bound to corticosteroid-binding-globulin (CBG). Cortisol can enter all peripheral tissues and because of its chemical structure it can easily cross the blood-brain barrier and reach, among others, areas of the brain involved in blood pressure regulation (Wilson & Foster, 1992), such as the limbic system and hypothalamus. Glucocorticoid receptors are also present in the heart and in the vascular smooth muscle of the blood vessels and in the kidneys. Cortisol has therefore a direct influence on blood pressure (Kenyon & Fraser, 1992). Chronic exposure to stress leads to persistently elevated cortisol levels which may lead to various metabolic and cardiovascular effects (Brindley & Rolland, 1989). Mechanisms involved in chronic stress are also perceived in depression (Mizoguchi et al., 2001). Exposure to negative life-events and childhood abuse, which can be considered as chronic stress, is suggested to result in persistently increased activity of CRF and a sensitisation of the HPA-axis to stress (Coplan et al., 1996; Heim & Nemerof, 1999; 2001). This sensitisation to stress in early life is mentioned as a possible factor in the aetiology of depression (Mazure, 1994; Heim & Nemeroff, 1999).

Persistently elevated HPA-axis activity as a result of chronic stress is associated with familial predisposition to high blood pressure (Walker et al., 1998) and hypertension (Dötsch et al., 2001). Hypersecretion of cortisol is also related with indirect risk factors for cardiovascular disease, such as diabetes (Bjorntorp & Rosmond, 1999) and obesity (Mårin et al., 1992). In individuals with essential hypertension plasma and urine cortisol levels were elevated compared to those of matched healthy controls (Watt et al., 1992; Litchfield et al., 1998). Increased levels of cortisol

were also associated with other cardiovascular disease risk factors, such as high BMI and waist-to-hip ratio and decreased levels of high density lipids (Fraser et al., 1999).

Cortisol can be measured either in an invasive way, i.e. by drawing blood or at a non-invasive way, i.e. by collecting urine or saliva samples. The most prominent reason why in this thesis the non-invasive way is preferred to the invasive one is that drawing blood implies using a needle, a procedure which is a stressor by itself and results in increased cortisol levels (Grunberg & Singer, 1990). Although measures of urinary free cortisol correlate well with circulating blood levels (Trainer et al., 1993), they do, however, not directly reflect adrenal activity. Urinary cortisol levels do not provide information about a set period of time as in blood samples, where one can measure effects of a stressor on cortisol secretion within minutes. Urine collection has a number of complications in collection during 24-hour periods and is therefore less suitable for field measures of cortisol (Grunberg & Singer, 1990).

An alternative, which is also non-invasive and easy to use in an ambulatory design, is to measure cortisol in saliva (Dressendörfer et al., 1992; Kirschbaum and Hellhammer, 1994). Since cortisol is considered to enter saliva by passive diffusion or other means independent of an active transport mechanism, cortisol levels in saliva are unaffected by saliva flow rate and therefore represent the unbound active cortisol levels in the blood. Hence, there is a strong relationship between cortisol levels extracted from saliva and from blood (Aardal & Holm, 1995; Beerda et al, 1996; Bushong, Friend & Knabe, 2000; Kirschbaum & Hellhammer, 1994; Riad-Fahmy et al., 1982; Wedekind et al., 2000). About 95% of cortisol in plasma is bound to corticosteroid-binding globulin (CBG). The remaining 5% is circulating freely. One factor that needs to be controlled is that the characteristics of the proteins to which cortisol is bound, CBG and albumin, are not static, which may lead to cortisol measures that do not represent their true levels in the blood (e.g. Cooke, McIntosh & Murray-McIntosh, 1996). When measuring cortisol in saliva it is imperitive to assess factors that influence CBG and albumin, such as use of oral contraceptives and estrogen replacement therapy.

1.4.2 Anxious depression and cortisol

The HPA-axis seems to be one of the central biological pathways in the pathogenesis of depression. Deregulation of this axis has been proposed to causally contribute to the risk of developing depression (Holsboer, 2000; 2001). The link between HPA-axis deregulation and depression is supported by studies showing elevation of the basal cortisol level and dexamethasone-mediated negative feedback resistance (Evans et al., 1983; Holsboer et al., 1984; 1982a,1982b), increased cerebrospinal fluid levels of CRF (Charlton and Ferrier, 1989; Gold & Chrousos, 1985), and a blunted ACTH response to challenge with exogenous CRF (Linkowski et al., 1985; Plotsky et al., 1998). A number of studies on the association of basal cortisol levels with depression is presented in Table 1.1.

From Table 1.1 it is clear that, although many studies find hypercortisolism in depression, not all studies do. Studies measuring hypercortisolism, show increased interest in deviant circadian rhythms in addition to an elevation of basal cortisol level per se (Posener et al., 2000; Weber et al., 2000; Wong et al., 2000; Young et al., 2000). Population based samples seem to offer the least support for a link between cortisol and depression, whereas within patient populations, the relationship appears to be most solid in hospitalised patients with a relatively recent acute depressive episode (Maes et al., 1994). Chapter 6 of this thesis describes the relationship between daytime cortisol profiles in both trait depression and clinical depression with special attention to potential confounders, such as smoking. Salivary cortisol measures were used to determine the day-time cortisol profile on the measurement day.

Table 1.1. Selection of studies published after 1990 measuring basal cortisol levels in human subjects:

Hypercortisolism found in depressed persons compared to non-depressed persons

Author(s)	Year	Subjects	Methods	Results	Smoking as confounder
Goodyer et al.	2000 a	180 adolescents (73 boys, 107 girls) with high levels of life stress	Saliva cortisol sampled at 08.00h and 20.00h over 4 consecutive days	For both genders depression was predicted by higher cortisol levels over a one year interval	Not mentioned
Weber et al.	2000	25 depressed inpatients, 30 healthy controls	Plasma cortisol and cortisone sampling at 09.00, 11.00, 13.00, 20.00, 22.00, 01.00, 03.00, and 07.00h	Higher cortisol levels in the patients at all time points	Not mentioned
Wong et al.	2000	10 MD patients with melancholic features, 14 healthy controls	Continuous 30-hour plasma cortisol sampling	Mean cortisol levels were elevated in patients compared to controls (p<.02). ACTH levels were similar in both groups.	Not mentioned
Young et al.	2000	30 MZF pairs: 30 individuals with history of MD, 28 without	14 day period saliva cortisol sampling within 45 minutes after awakening, and immediately before bedtime	Trend towards higher cortisol in twins with history of MD (p=.056), 40-45% heritability	Mentioned as possible confounder.
Michael et al.	2000	44 MD of which 36 in- 8 outpatient, 35 remitted 41 normal controls	Four salivary samples at 8.00h and at 20.00h on two consecutive days	Cortisol was significantly higher in the saliva of depressed subjects than the other two groups on both time points (am, p<.01; pm, p<.01)	Smoking is taken into consideration as a confouder.
Scott & Dinan	1998	21 CFS patients, 10 melancholic depressives, and 15 healthy controls	24-hour urinary free cortisol	Patients with depression had significantly higher cortisol levels compared to healthy controls	Not mentioned
Catalán et al	1998	26 MD, 10 dysthymic depressives and 17 healthy controls	Plasma cortisol samples were obtained between 8.00h and 8.30h	Cortisol plasma concentrations were higher in MD and dysthymic depressives compared to controls.	Not mentioned
Deuschle et al.	1997	15 depressed patients, 22 matched controls	24-hour plasma cortisol was sampled every 30 minutes	Mean cortisol and ACTH levels increased and frequency of cortisol pulses higher during the night in patients	Not mentioned

				compared to controls.	
Steiger & Holsboer	1997	25 healthy subjects and 12 MD inpatients	Nocturnal secretion of plasma cortisol at a 20 minute interval between 22.00h and 07.00h	Significantly higher mean nocturnal cortisol levels in depressed patients compared to controls (p<.05).	Not mentioned
Rubin et al.	1996	35 inpatients with MD, 35 matched controls	Basal plasma cortisol: 30 minute interval sampling between 16.00h and 19.00h	Depressed patients had higher basal cortisol levels than their matched controls (p<.05)	Not mentioned
Gotthardt et al	1995	20 healthy subjects, 20 MD subjects under 30 or over 60 years of age	Basal plasma cortisol between 14.00 and 17.00h	Hypercortisolism in depressed subjects compared to healthy controls	Not mentioned
Kok et al.	1995	10 inpatients with MD, 10 matched controls	Plasma cortisol samples obtained at 8.ooh and 16.00h	Higher mean plasma cortisol levels at both times in depressed patients compared to controls	Not mentioned
Trestman et al	1995	28 depressed patients, 14 depressed patients in remission, 19 controls	Plasma cortisol at hourly interval between 10.00 and 18.00h	Acutely depressed patients had higher cortisol levels compared to healthy controls and patients during remission of depression	Cigarette smoking was not permitted on the testing day
Osran et al.	1993	10 male inpatients and 9 healthy male contols	Serum levels of cortisol sampled at 8.00h and 16.00h, plus 24-hour urinary cortisol samples	Hypercortisolism was found in depressed subjects (p<.001)	Not mentioned
Dahl et al.	1991	27 depressed, and 32 healthy adolescents	24-hour plasma cortisol levels, sampled at every 20 minutes	Higher cortisol levels in depressed adolescent before sleep onset, mainly explained by levels of suicidal depressed and the depressed inpatients.	Not mentioned

No hypercortisolism found in depressed compared to non-depressed

Author(s)	Year	Subjects	Methods	Results	Smoking as confounder
Goodyer et al.	2000 b	181 adolescents with elevated levels of life stress and 65 low-risk adolescents	Saliva DHEA and cortisol sampled at 08.00h and 20.00h over 4 consequetive days	Depression was not predicted by higher cortisol levels over a one year interval in either high or low risk groups	Not mentioned
Posener et al.	2000	11 psychotic MD, 38 nonpsychotic MD, and 33	24-hour plasma cortisol levels for every hour through iv.	Reduced cortisol and normal ACTH levels in non-psychotic MD compared with healthy subjects, and	Not mentioned

		healthy control subjects		increased ACTH and normal cortisol levels in psychotic depression compared with healthy subjects.	
De Bellis et al.	1996	38 children with MD, 28 healthy children	Plasma cortisol sampled every 20 minutes during the night	Lower cortisol levels until 4 hours after sleep onset, in depressed children. No significant differences	Not mentioned
Yehuda et al.	1996	15 PTSD patients, 14 MD, and 15 male controls	24-hour plasma cortisol measurement during bed-rest, sampled at 30 minute intervals	PTSD patients had lower levels, depressed patients had chaotic diumal pattern of release, no increase in levels compared to normals	Not mentioned
Maes, Calabrese, Meltzer,	1994	48 major depressed inpatients, 17 major depressed outpatients and 73 normal volunteers	basal 9:00h plasma cortisol and the integrated assessment of plasma morning cortisol secretion over 2 (AUC 120) hours	MD inpatients exhibit significantly higher values than healthy controls and major depressed outpatients. No significant difference between healthy controls and MD outpatients.	Not mentioned
Mazur	1994	4462 men from a random selection of Vietnam war veterans	Plasma cortisol drawn between 8.ooh and 10.00h before eating	No association between self reported symptoms of depression and cortisol levels	Not mentioned
Thompson et al.	1992	40 MD subjects, 40 healthy matched subjects	24-hour serum cortisol	No difference in basal cortisol levels between depressive and non-depressives	Not mentioned
Brändtstadter et al.	1991	Population sample of 767 adults age range of 35-65 years	Salivary cortisol: early morning sample (7.00-9.00h), afternoon sample (15.00-17.00h), and late evening sample (19.00-21.00h)	No correlation between negative affect and basal cortisol levels in women, in men cortisol levels are lower with higher negative affect	Not mentioned

MD=Major depression, PTSD=Post-traumatic Stress Disorder,AUC=area under the curve, DHEA= dehydroepiandrosterone

1.4.3 Blood Pressure

Blood pressure is one of the most frequently recorded physiological functions in psychosomatic research. It has evolved as a means to explore potential pathophysiological relationships between response patterns and cardiovascular disease risk factors such as hypertension (Brownley, Hurwitz & Schneiderman, 2000). According to the World Health Organisation (WHO) criteria, hypertensive status is defined as systolic blood pressure (SBP) > 160mm/HG and diastolic blood pressure (DBP) > 90 mm/HG. In young adults a DBP < 85 mm/HG is considered to be normal.

Blood pressure regulation is under control of both the sympathetic and the parasympathetic branch of the central nervous system. Through various feedback mechanisms, such as the baroreceptor reflex, involving both the autonomic and the central nervous system, blood pressure levels are maintained within a narrow range. With regard to all the factors involved in blood pressure regulation it is not possible to rely on a single physiological mechanism as an explanation for deviating blood pressure levels. Chronic deviations in blood pressure values and exaggerated reactivity to stressors do indicate however that the balance in the sympathetic and parasympathetic influence of blood pressure regulation is disrupted.

From the results of animal and human studies there is evidence that cardiac autonomic nervous system abnormalities, reflected by changes in sympathetic and parasympathetic activity, are predictive for future hypertension (Everson et al., 1998; Kaplan et al., 1991; Pike et al, 1997). Hypertension is thought to derive from a sympathetic-parasympathetic imbalance, with an emphasis on sympathetic overactivity (Julius, 1996). In a pathological model proposed by Julius (1993) prolonged elevations in sympathetic tone and increased sympathetic reactivity to stress promoted vascular hypertrophy and thus hypertension. In addition, sympathetic overregulation in hypertension has been shown to be associated with decreased parasympathetic activation or vagal tone (Julius, Pascual & London, 1971). Studies have shown that reduced vagal tone is not only associated with progression of focal coronary atherosclerosis (Huikuri et al., 1999), but also with (mild) hypertension (Vrijkotte et al., 2000).

1.4.4 Anxious depression and blood pressure

Although the association between risk for hypertension and personality traits has received a lot of attention in psychosomatic research (see for a historical review Gerin et al., 2000), there is relatively little research on the association between blood pressure levels and depression. There are some studies however that link depression with over-activation of the sympathetic nervous system and suppression of the parasympathetic nervous system which in turn is associated with risk for hypertension. Excessive activation of the sympathetic nervous system in depressives has been found in studies using electrodermal activity (EDA) as an index of sympathetic reactivity in both state and trait depression (Dawson, Schell & Catania, 1977; Iacono et al. 1983; Ward, Doerr & Storrie, 1983). Moreover, impaired vagal control is associated with depression (Carney,

Saunders, Freedland et al., 1987; 1995; Krittayaphong et al., 1997; Lehofer, Moser, Hoehn-Saric, 1997). In recent studies reduced vagal tone was found in subjects with high scores on the Beck Depression Inventory (Hughes & Stoney, 2000) and in patients with major depression melancholic type (Rechlin, Weis, Spitzer & Kaschka, 1994). Therefore in the design of the current thesis, blood pressure monitoring seems to be a valuable marker of autonomic regulation in depression.

Most of the studies on the association between psychological factors, like depression, and blood pressure have been conducted in a laboratory setting (e.g. Jonas et al., 1997; Everson, et al., 2000; Shin et al., 2001; Paterniti et al., 1999). Because there is evidence that ambulatory blood pressure measurements are more predictive of hypertension than laboratory based measurements (Clement, De Buyzere & Duperez, 1994; Khattar et al., 1999), ambulatory monitoring of blood pressure is now increasingly used in psychosomatic medicine (Schnall et al., 1998; Steptoe et al., 1995). Ambulatory monitoring may be particularly relevant in studying depression, because most major models of depression consider a high frequency or exaggerated amplitude of the responses to life stressors as an important part of the disorder (Nemeroff, 1999). Ambulatory monitoring also reduces the possible interference of stress reactivity to blood pressure measurements in a clinical or lab setting, known as 'white-coat hypertension' (Rickerby, 2002). Therefore in the current thesis ambulatory measurements will be used in order to assess the association between anxious depression and blood pressure levels.

Table 1.2 presents those studies on the association between depression and BP levels in which BP is measured ambulatory. Studies were included in Table 1.2 when the main focus of the study was the effect of depression or a comorbid trait, such as anxiety or negative affect, on blood pressure. Studies were not included when the focus was also on intermediating factors, not relevant to the current thesis, such as shift-work and exercise. All studies have been conducted in normotensive populations.

Table 1.2 selection of studies on the association between depression, anxiety or negative affect and ambulatory blood pressure measured in normotensive and non-clinical samples.

Author	Year	Participants (N, age, sex)	Psychological assessment	Measurement day	Summary of results	Confounders in statistical analyse
Friedman et al.	2001	283 men, employed, aged 30-60 years.	Depression: SCL-90-R. Anxiety: STAI and SCL-90-R*.	A workday.	typical No associations between BP and depression and anxiety were found.	Controlled for sex (male population), age and BMI. Not controlled for smoking and medication use.
Kario et al.	2001	126 men and 105 women, employed, aged 30-66 years. Mean age 46.0 (sd=8.9).	Depression and anxiety: SCL-90-R.	A workday.	typical Elevated SBP was associated with depression and anxiety in men and anxiety in women.	Controlled for sex, age, BMI, posture. Not controlled for smoking. Exclusion of medication users.
Shapiro et al.	2001	203 registered female nurses, premenopausal, 24-50 years. Mean age 37.7 (sd=6.6) years	Day-time mood states reported in a diary.	2 workdays, typical days.	typical 2 High ratings of negative mood were associated with a rise in BP.	Controlled for sex (female population). Not controlled for smoking, age and BMI. Exclusion of medication users and BMI > 30.
Carels et al.	2000	85 men and 77 women, employed, aged 25-45 years.	Day-time mood reported in a diary. Depression: BDI*. Anxiety: STAI*.	A workday.	typical Negative emotions were positively associated with higher SBP and DBP levels.	Controlled for posture. Not controlled for sex, age, smoking, and BMI. Exclusion of medication users.
Riese et al.	2000	159 registered female nurses, premenopausal. Mean age 35.9 (sd=8.5)	Depression: CES-D* Anxiety: STAI.	on a typical workand leisure day	High depression scores were associated with elevated DBP and SBP levels.	controlled for sex, age, posture, BMI, smoking Exclusion of medication users.
Räikkönen et al.	1999	50 men and 50 women, employed, managerial level, 30-45 years. Mean age men 36.5 (sd=4.7), women 37.1 (sd=4.1) years.	Day-time mood states reported in a diary. Anxiety: STAI.	two typical workand one typical leisure day.	Only anxiety was associated with higher SBP and DBP levels.	Controlled for sex, smoking. Not controlled for age and BMI. Exclusion of medication users and >20% overweight.
Ewart & Kolodner	1994	114 boys and 114 girls, mean age 14 (sd=0,5) years.	Trait anger, negative affect and affective communication were assessed.	A typical school day.	Negative affect was associated with elevated levels of SBP and DBP in boys.	Controlled for sex, age (narrow range), BMI. Not controlled for smoking, and medication use.
Southard et al.	1986	28 male adolescents, 13 to 18 years.	Mood states and perception of environment ratings.	A typical day.	Negative emotions and perceptions of the environment were associated with elevated SBP and DBP levels	Controlled for sex and age. Smoking and BMI were not controlled for

* SCL-90-R =Symptoms Checklist 90-Revised, STAI = Spielberger Trait Anxiety, BDI = Beck Depression Inventory, CES-D = Center for Epidemiological Studies-Depression questionnaire

Most studies mentioned in Table 1.2 find a relationship between trait depression, anxiety or negative affect and both diastolic and systolic blood pressure levels. Therefore it can be concluded from Table 1.2 that a person at risk of developing depression will also be at increased risk for hypertension. Chapter 7 of the thesis describes the effects of depression on ambulatory blood pressure.

1.5 Confounders

In order to determine the association between anxious depression and cortisol and blood pressure levels in a heterogeneous sample as used in the current thesis the most important factors to control for are age, and sex. In the following section the role of age and sex is discussed.

1.5.1 Age and sex

Depression is approximately twice as common in women than in men across diverse cultures (e.g. Blazer et al., 1994). However, the difference between men and women is not static but changes with age. For both men and women the incidence of depression rises with age (Palsson, Ostling & Skoog, 2001) and the difference in prevalence between men and women decreases (Der & Bebbington, 1987; Meltzer et al., 1995). Cardiovascular disease risk is also differently distributed over the sexes and changes with age. For both men and women cardiovascular disease is the leading cause of death in the industrialised countries, including The Netherlands. Over a third (35%) of all causes of death in The Netherlands are related to cardiovascular disease (CBS figures 2000). The prevalence of cardiovascular disease increases with advancing age, but also the known risk factors for cardiovascular disease increase with age (Province, Tishler & Rao, 1989) and are distributed differently over the sexes. Women have less risk for cardiovascular disease than men until the menopause, because estrogen is protective against the development of cardiovascular disease in women (Barrett-Connor & Bush, 1991). Other factors in cardiovascular disease risk are also age and sex dependent. A short outline of age and sex influences in the risk factors discussed in this chapter is presented.

For both men and women smoking initiation usually occurs in puberty and seldom later in life. A Dutch study on smoking initiation and smoking prevalence in The Netherlands shows that most people initiate smoking at age 13 or 14 and most smokers become regular smokers in the age-group of 10-19 years (DEFACTO, 2000). In the age-group of 20 to 34 years the number of current smokers stabilises and decreases thereafter. Generally speaking women smoke less than men (DEFACTO, 2000), but there is some evidence that men, when they are smokers have less trouble with quitting than women (e.g. Perkins et al, 1999, 2000). Smoking is a major risk factor for cardiovascular disease in both men and women, but smoking seems to negate the estrogen advantage seen in non-smoking pre-menopausal women, leading to equal risk for women and men of developing cardiovascular disease at the same age (Willet, Green, Stampfer et al., 1987).

The influence of age on cortisol levels is not much studied, but age seems to have no effect on cortisol (Carvalhaes-Neto et al., 2002). The influence of sex differences on cortisol is still unclear (Kirschbaum, Wust & Hellhammer, 1992), but it is suggested that there are sex differences in cortisol reactivity to psychological stress (Kirschbaum et al., 1999). It is often hypothesised that circulating estrogen levels in women might influence cortisol effects, but there is no hard evidence for this in humans (Huizenga, Koper, De Lange et al., 1998; Tersman, Collings & Eneroth, 1991; Trainer et al., 1998).

Age and sex dependent differences in blood pressure levels are better documented. Blood pressure levels change with age, part of the age effect on cardiovascular disease is mediated by the association of age with high blood pressure levels (Breslow, 1991), and will rise to hypertensive levels in men at an earlier age than in women (Price & Fowkes, 1997; Yong et al., 1993). In post-menopausal women risk of hypertension increases to the same level as in men (Stamler et al., 1976; August & Oparil, 1999).

1.6 In conclusion

As outlined in this chapter the association between anxious depression and cardiovascular disease will be investigated through assessment of the possible mediating factors involved: smoking, cortisol and blood pressure. Extra attention will be given to the confounding role of age, sex and BMI in the association between depression and cardiovascular disease.

Chapter 2

Research design & Participants

2.1 Introduction

The goal of this thesis was to investigate the relationship between anxious depression and risk factors for cardiovascular disease. Twin families selected for the purpose of a genome-wide search to localise Quantitative Trait Loci (QTL) involved in anxiety and depression (Boomsma et al., 2000) were asked to participate in ambulatory cardiovascular monitoring during a typical workday. This thesis reports on the results of questionnaire data on depression and smoking gathered in 1997, and on the results of ambulatory monitoring of cardiovascular and endocrine parameters in a sub-sample of twins and sibs.

Data collection for the present thesis can be summarised in 5 separate stages: (1) Collection of the survey data in 1997 in a follow-up of a longitudinal study on physical and mental health initiated in 1991 in twins, their parents and their siblings (Koopmans et al., 1997); (2) selection of families with sib pairs that were extremely discordant or concordant for anxious depression as assessed in the longitudinal survey study; (3) DNA collection for QTL mapping in all family members from the selected sample; (4) The CIDI interview which is a psychiatric diagnostic interview in offspring from the selected sample; (5) Ambulatory monitoring of endocrine and cardiovascular parameters in a subgroup of offspring from the selected sample.

Participants received extensive information by letters and an introductory brochure informing them of the study outline, its purpose and the procedures for the CIDI interview, DNA collection and for the ambulatory monitoring of cardiovascular and endocrine parameters. Participants could end their participation at any time they wished and filled out an informed consent. A copy of these letters and the brochure is included in the appendix (see appendix I to IV).

Data collected during ambulatory monitoring and in the first four waves of the longitudinal survey data will be combined to yield an assessment of risk factors for CVD. Table 2.1 provides an overview of all the available information on the current sample. This chapter will give a summary of the data collection by mailed surveys, the selection procedure for the QTL study based on longitudinal survey data, the final selection for ambulatory monitoring, and the measurement procedures for the physiological and psychological variables.

Table 2.1: Overview of variables measured in twins and their siblings participating in the ambulatory measurements and the longitudinal study

Personality/psychopathology	Assessment
Depression	BDI, YASR[a] CIDI[b], POMS[c]
Anxiety	YASR, STAI[a] CIDI[b]
Phobias	AFQ[a] CIDI[b]
Somatic anxiety	YASR, ABV[a]
Smoking	Questionnaire[a c]
Alcohol use	Questionnaire[a c]
Physical exercise	7-day recall[c]
Sports participation	Questionnaire[c]
24-hour activity pattern	Diary[c]
Medication	Questionnaire[c]
Family history of CVD	Questionnaire[c]
Occupational status	Questionnaire[a c]
Body Mass Index (BMI)	Body height[a] Body weight[a c]
Waist/Hip ratio (WHR)	Waist circumference[c] Hip circumference[c]
Heart Rate (continuous) Root Mean Square of the Successive Differences (RMSSD)	VU-AMS[c]
Pre ejection Period (PEP) Stroke Volume Index Respiratory Sinus Arrhythmia	VU-AMS[c]
Systolic Blood Pressure (SBP) Diastolic Blood Pressure (DBP)	Spacelabs[c]
Cortisol	Saliva[c]
DNA	Buccal swap[d]

[a] Questionnaire data from the longitudinal survey (1991, 1993, 1995 and 1997)
[b] Interview by telephone (April- September 1998)
[c] Ambulatory measurement day (1999 and 2000)
[d] By mail (1999)
BDI= Beck Depression inventory (Beck et al., 1961)
YASR= Young Adult Self-Report (Achenbach, 1990)
CIDI= Composite International Diagnostic Interview (Wittchen et al., 1991; 1994)
STAI= Spielberger Trait Anxiety (Spielberger et al., 1970)
AFQ= Anxiety and Phobia Questionnaire (Muris et al., 1997, 1999)
ABV= Amsterdamse Biografische Vragenlijst (Wilde, 1970)
VU-AMS= Ambulatory Measurement Device

2.2 Survey data collection

In a longitudinal study on physical and mental health and life style in young adult and adolescent twins, their parents and their siblings questionnaire data were collected on personality, general health and health behaviours. Data were collected by mailed surveys in 1991, 1993, 1995 and 1997. Table 2.2 lists the total number of individuals, twins, parents and siblings that participated in the four waves of the survey on which the selection for the current thesis was based and Table 2.3 gives the frequency with which they participated in the four waves.

Initially only twins and their parents were approached to participate in questionnaire research at the Netherlands Twin Register (NTR, see also http://www.psy.vu.nl/ntr/). Twin-families were recruited in 1990/1991 by asking city councils in The Netherlands for addresses of twins aged 13-22 years. 252 city counsels supplied 4036 addresses. In 1993 additional addresses were obtained for 1987 twin-families. Families that were willing to participate in the longitudinal study received a questionnaire booklet with each wave in 1991, 1993 and 1995. In 1995 two extra questionnaires were added which could be filled in by additional siblings when they were present in the family. A total number of 6529 individuals from 1697 families returned a complete questionnaire in 1991, of whom 3394 twins. In 1993 a total number of 7592 individuals from 1974 families returned a complete questionnaire, of whom 3884 twins. In 1995 a total of 8175 individuals from 1727 families returned a complete questionnaire, of whom 3408 twins and 1500 siblings.

In 1997 a number of adult twin pairs that volunteered for registration with the NTR were added to the study. Furthermore the twin-families that already participated were approached with a request for siblings in addition to the twins to participate in the survey. Parents were asked how many siblings of the twins would be willing to fill in a questionnaire and to provide, with their consent, their names and addresses. Of the families that were approached, 2773 supplied a form with this information. All twins and siblings who agreed to participate were sent a questionnaire booklet, a total of 7989 individuals (5546 twins and 2443 additional siblings) were included in the fourth wave of the survey. A completed questionnaire was received from 4585 individuals from 1965 families (3141 twins and 1444 siblings).

Table 2.2: Participation of twins and siblings for each wave of the questionnaire survey between 1991 and 1997.

Year	Number of families	Twins	Siblings
1991	1697	3394	-
1993	1974	3915	-
1995	1727	3408	1500
1997	1965	3141	1444

Table 2.3: Longitudinal participation: number of times (once, twice, thrice or four times) families and individuals participated in the study.

	Families	Twins	Siblings*
Once	1259 (38%)	2428 (38%)	1456 (66%)
Twice	687 (20%)	1448 (23%)	744 (34%)
Thrice	862 (26%)	1697 (26%)	-
Four times	536 (16%)	853 (13%)	-
Total	3344	6426	2200

*Siblings were invited to participate for the first time in 1995

2.3 Selection of sibpairs for the QTL study

Each survey collected information on lifestyle, including smoking, health, demographics and personality (Boomsma, Koopmans, Van Doornen, 1994; Koopmans, Slutske, Heath et al., 1999; Vink, Groot, Kerkhof, 2001). Table 2.4 lists the measures of personality that were collected in the first four surveys on which the selection for the current sample is based. The 13-item version of the Beck depression inventory (BDI; Beck et al., 1961) and the anxious depression symptom scale of the Young Adults Self Report (YASR; Achenbach, 1990) were used to assess depression. The Spielberger Trait Anxiety Inventory (STAI, Spielberger et al., 1970) and the neuroticism and somatic anxiety, also called neurotic somatic complaints (NSO), scales of the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970) were used to assess anxiety and neuroticism.

For the purpose of localising genes involved in anxiety and depression often referred to as Quantitative Trait Loci (QTL), the survey data were used to select the most informative families for a genome-wide search. According to a selection procedure developed by Eaves and Meyer (1994) and Risch and Zhang (1995; Dolan & Boomsma, 1998) this was done by selecting siblings for genotyping who formed extremely discordant or concordant sib pairs, (i.e. high/high, low/low, high/low, or low/high) according to their score on a quantitative scale for anxiety and depression (Boomsma & Dolan, 1998). For each individual that participated a factor score was computed. This factor score was based on a genetic multivariate analysis on the scores on the anxiety and depression scales included in the survey in 1991, 1993 and 1997 (see table 2.4). The survey data collected in 1995 were not used for calculating the factor score since in that year only the YASR was included for twins (and not for their siblings). Because women had higher scores than men and because a higher heritability was found in women than in men for all anxiety and depression scales involved, factor scores were computed separately for men and women. The higher heritability for anxiety and depression in women was explained by higher genetic variance in women. There was no evidence however for different genes influencing anxiety and depression in men and women. Similar results have been found in Australian and American twin studies (Kendler, Heath, Martin & Eaves, 1986; Kendler, Neale, Kessler et al, 1992; Kendler, Neale,

Kessler et al, 1993). The factor score represents the individual's value on the common genetic factor for depression and anxiety and can be interpreted as an estimate of an individual's genotypic value for anxious depression. The complete selection procedure is described in Boomsma et al. (2000, see apendix V).

Once a concordant or discordant sib pair was identified within a family, the whole family was asked to participate in the QTL study. All family members (parents and all twins and siblings who had at least once returned a questionnaire) were asked to supply a DNA sample. Selection was either within each measurement occasion (i.e. 1991, 1993 and 1997) or across occasions. In total, 2724 subjects (1007 parents and 1717 offspring) were approached and currently 1975 (643 parents and 1332 offspring) complied by returning a buccal swap for DNA isolation.

Table 2.4: Inventories used to compute the anxious depression factor score in twin-families

	1991	1993	1995	1997
Beck Depression Inventory (BDI)	-	X	-	X
ABV: Neuroticism	X	X	-	X
ABV: Somatic anxiety	X	X	-	X
Spielberger Trait Anxiety (STAI)	X	X	-	X
YASR: Young Adult Self Report (Anxious/Depressed)	X	-	X	X

1991: twins and parents,
1993: twins and parents,
1995: twins, parents and siblings (YASR only for twins)
1997: twins and siblings

2.4 CIDI interview

All selected siblings and twins were asked to participate in a telephone interview during which the sections on depression and anxiety from the Composite International Diagnostic Interview (CIDI) were administered (see appendix IV). The CIDI is a diagnostic psychiatric interview for the assessment of lifetime major depression according to the definitions of the Diagnostic Criteria for Research of ICD-10 (the tenth revision of the International statistical Classification of Diseases and Related Health Problems) and DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) (e.g. Wittchen et al., 1991; Wittchen, 1994; Peter and Andrews, 1995; Andrews & Peter, 1998; Peter et al., 1998). The following sections were administered: Demographics (section A); Social Phobia, Agoraphobia and Generalised Anxiety Disorder (D33 and further); Depression and Dysthymia (E); Mania Screen and Bipolar Affective Disorder (F) and Obsessive-compulsive disorder (K1-22). For the current thesis only the diagnostic criteria of the DSM-IV for depression were used.

The CIDI interview is designed for large sample sizes and can be administrated by lay persons because of the step by step structure of the interview. Ten experienced call-centre

employees received training at the official CIDI-training centre at the Academic Medical Centre in Amsterdam, the Netherlands. The selected siblings from the extreme scoring families were contacted by telephone for an appointment for the interview. The CIDI interview was held with 1253 individuals.

2.5 Smoking status

Participants were asked about their current smoking behaviour on the ambulatory measurement day (see: Ambulatory cardiovascular monitoring). This information was combined with the detailed information on smoking behaviour, available from the survey data, to determine smoking status. Participants were coded as smokers if they smoked on the ambulatory measurement day. They were coded ex-smokers if they had reported life-time smoking behaviour in the survey data, but were not currently smoking. Finally, non-smokers had reported never to have smoked, or only once or twice to try, and did not currently smoke. When possible, incomplete or inconsistent reports on smoking behaviour were checked using questionnaire data obtained in 2000.

2.6 Ambulatory cardiovascular monitoring

2.6.1 Selection for ambulatory cardiovascular monitoring

The sibpairs selected for the QTL-study were invited to participate in ambulatory measurement of cardiovascular parameters and daytime profile of cortisol. Table 2.5 represents participation on each ambulatory measured variable. A total number of 431 persons participated in the ambulatory cardiovascular measurements. Cortisol collection started when the cardiovascular measurements were already in progress, which explains that only 338 persons participated in cortisol collection. For each variable availability of CIDI data is presented.

Table 2.5 Number of persons participating in the ambulatory cardiovascular and endocrine measurements, with availability of CIDI data. For each measure the total n of participants is given followed by the n for each ambulatory measured variable.

	Total	Cortisol*	Blood Pressure	AMS	CIDI
Cortisol	338	338	314	314	278
Blood Pressure**	433	314	431	433	390
AMS-registration	433	314	431	433	390

*427 persons participated in the ambulatory cardiovascular measurements. Cortisol collection started when the cardiovascular measurements were already in progress, which explains that only 338 persons participated in cortisol collection.
**In two persons blood pressure registration failed.

Family members eligible for participation in ambulatory monitoring were contacted by telephone for further acquaintance with the study, and to make an appointment for monitoring. Individuals from the families with extreme sib pairs were approached regardless of sex, age, employment-status and physical state, i.e. there was no selection based on use of medication or current health status. Women were excluded from ambulatory monitoring if they were pregnant or lactating.

2.6.2 Procedure

Participants were informed about and invited for ambulatory monitoring by letter. Subsequently they were contacted by phone for an appointment on a by the subject self-selected, but representative day. Subjects were requested to refrain from intense physical activity both on the preceding and the ambulatory monitoring day. Before starting their normal daily routines subjects were visited at home for explanation and fitting of the ambulatory devices. Participants were instructed how to handle the ambulatory devices. They were shown how to turn the ambulatory BP-monitoring device off, for instance for safety during driving and how to turn it on again. Furthermore they were given an extra set of electrodes for the ambulatory heart-monitoring device and instructions on how to replace electrodes if necessary. In addition the participants were instructed how to use the salivettes for collection of cortisol. Further more, the participants were instructed how to fill in the activity diary (see appendix V). In this diary the participants had to give a chronological account of all activities performed, including a detailed description of bodily posture. Participants were prompted to fill in their diary during waking hours by an auditory tone every 30 minutes (±10 min.) which originated from the VU-AMS device (see: Ambulatory cardiovascular measures). A written instruction and telephone-support were available at all time during the registration. The next morning the device was collected by the researcher.

Before starting ambulatory measurements, information on the participants general health status, (i.e. use of medication, contraceptive use in women), health behaviours, such as smoking, alcohol consumption and physical activity was obtained by interview. Finally a family history of cardiovascular disease was obtained.

2.6.3 Ambulatory measurement devices

Ambulatory systolic and diastolic blood pressure values were obtained by means of a Spacelabs 90207 device (SpaceLabs Medical, Redmond, USA) with an arm-cuff on the non-dominant arm. Arm circumference was measured to choose the correct arm-cuff size. The arm-cuff was inflated automatically every 30 minutes during waking hours and the obtained values were not displayed on the SpaceLabs display. Before the cuff started to inflate the device gave an auditory two-tone-beep and subjects were instructed not to move and not to change their actual posture during the

measurement. There were no blood pressure recordings during sleep, participants were instructed to remove the SpaceLabs device before they went to bed (see appendix VI).

The VU-AMS device (version 4.6, VU-FPP, Amsterdam) was used for continuous 24-hour registration of the electrocardiogram and the impedance cardiogram. The protocol for the AMS-VU was obtained from previous ambulatory monitoring projects (Riese, 2000; Vrijkotte 2001). Reliability and validity aspects and recording methodology of the VU-AMS have been described previously (De Geus, Willemsen, Klaver & Van Doornen, 1995; De Geus & Van Doornen, 1996; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996; Vrijkotte, Riese & De Geus, 2001). More extensive descriptions of VU-AMS hardware, VU-AMS software, and VU-AMS manuals can be found on the VU-AMS homepage: http//www.psy.vu.nl/vu-ams. The ambulatory electrocardiogram (ECG) was obtained from disposable pre-gelled Ag-AgCl electrodes (ConMed, NY, USA) that were placed at the jugular notch of the sternum at four centimetres under the left nipple and at the right lateral side (ground). Each R-peak was detected with a level detector with automatic level adjustment (Thakor, 1983). From the R-peak time series an average value for heart rate (HR) and the root mean squared successive differences between two R-peak intervals (RMSSD) was obtained for each 30 seconds. ECG registrations continued during night-time sleep. The participants removed the VU-AMS device upon awakening the following morning.

2.6.4 Data reduction and labelling

Before analysing the data from the Spacelabs and the VU-AMS device a label data file is created. The information from the diary that is kept during the ambulatory measurement day is linked to the data files that contain the cardiovascular data. Labelling divides the measurement period in separate periods. For each period start and stop times are given as well as a set of codes and text labels describing the state of the subject during that period in terms of for instance intensity of physical activity (e.g. light, medium, heavy or very heavy physical activity), type of activity (e.g. engaged in desk work, having a social/professional conversation, watching television, eating or sleeping) and posture (e.g. sitting, walking, cycling or lying down). The aim of labelling is to classify all activities during the measurement day for each individual into a fixed number of activity categories with a fixed number of levels. The program designed to label the cardiovascular data is part of the software package that is provided with the VU-AMS, but the label files can also be used to label the blood pressure data gathered with the Spacelabs device.

All data (BP and AMS) were checked for artifactual readings, outliers, and frequency distributions in SPSS for windows (version 10.0., SPSS Inc). Previous recommendations for excluding artifactual readings and outliers from ambulatory BP recordings were followed (Berardi et al., 1992).

2.7 Cortisol collection

Salivary cortisol samples were collected at 6 time points using Salivette tubes (Sarstedt, Germany) with polyester swabs. After attachment of the ambulatory devices, the participants received instructions for the saliva sampling procedure (see appendix VII). Saliva collection was done according to the procedure described by Kirschbaum and Hellhammer (1989, 1994). Participants were instructed not to eat or drink and not to brush their teeth prior to the saliva collection, and to chew on the swab for 45 seconds. The first sample was collected in the presence of the researcher, insuring that the correct procedure for saliva sampling had been understood. The second sample was collected at 11.00h, the third at 15.00h, evening samples were collected at 20.00h and 22.30h. For the final sample the participants were instructed to collect it immediately after awakening, preferably while still in bed. The participants indicated the exact times of saliva sampling and indicated any missed samples.

The saliva samples were stored in a cool place for no more than 2 days, afterwards kept in –20° until delivery by courier to the assay laboratory in Düsseldorf every three months. Cortisol levels were measured employing a time-resolved immunoassay with fluorometric detection (DELFIA; Dressendörfer et al., 1992).

2.8 Research population

The present thesis reports on data obtained from the survey sent in 1997 and on the ambulatory cardiovascular and endocrine measurements. All participants gave written informed consent before entrance to the study. Table 2.6 presents the research populations for each study included in the thesis.

Table 2.6. Number of families and participants by sex for each study in the present thesis.

	Chapter	Participants	Families	Male	female
Survey data 1997	3 & 4	4447	1935	1788 (40.2%)	2659 (59.8%)
Cortisol	6	338	173	129 (38.2%)	209 (61.8%)
Blood pressure	7	431	218	157 (36.8%	270 (63.2%)

In Chapter four the association between smoking and depression is studied. In 1997 the survey included 7989 individuals (5546 twins and 2443 siblings). A total of 4584 persons send back a completed questionnaire. For the purpose of this study children below 15 years of age (n=137) were excluded after which 4447 from 1935 families (3076 twins and 1371 siblings) individuals were available for the analysis. Of these participants 1788 (40.2%) were male and 2659 (59.8%) were female. The age range was 15 to 83 years, with a mean of 26.4 (SD=10.14). Women in the sample had a mean age of 27.01 (SD=9.99) and men had a mean age of 26.40 (SD=10.14).

In Chapter six the association between cortisol levels and depression is studied. This study reports on 338 participants from 173 families that were randomly drawn from the total pool of selected families to participate in a 24-hour ambulatory assessment of cardiovascular parameters and salivary cortisol profile. Of these participants, 129 (38.2%) were male and 209 (61.8%) were female. The age range was 16 to 64 years, with a mean of 30.2 (SD=10.6).

Chapter seven reports on the association between blood pressure levels and depression. Data were obtained from 427 participants from 216 families who were randomly drawn from the families involved in the QTL study. Of these participants 157 (36.8%) were male and 270 (63.2%) were female. The age range was 15 to 63 years, with a mean of 30.32 (SD=10.05). Age and gender distribution did not differ from the total adolescent and adult population of the NTR.

Within each study the association between depression and the dependent variable is assessed first in the total sample without regard to family structure. Secondly the association between depression and the dependent variables is assessed in a between family design and finally in a within family design. By testing the association between depression and the dependent variables in a within family design, factors that vary between families, but not within families can be ruled out as an explanation for the association.

Chapter 3

The anxious depression factor score: associations with anxiety, depression and neuroticism measures and DSM-IV diagnosis of lifetime depression

Abstract

An anxious depression factor score, thought to reflect an individual's genetic vulnerability to anxiety and depression, was composed of five commonly used anxiety and depression scales. The present chapter clarifies the relationship of the factor score with these scales in a population sample (n=4447). To determine its validity, the anxious depression factor score was examined in relation to a clinical measure of lifetime depression according to DSM-IV criteria, as measured in a sub-sample that participated in the life-time version of the CIDI (n=1038).

There were strong positive associations between anxious depression and the separate scales indicating that the anxious depression factor score indeed reflected both anxious and depressive symptoms. In addition, a strong association was found between the anxious depression factor score and DSM-IV depression; individuals with an extremely high anxious depression score were at an increased risk of receiving a diagnosis of lifetime depression. Results were similar for men and women and did not change when controlling for age. In conclusion, the anxious depression factor score is a valid measure of depression, reflecting trait depression and vulnerability to clinical depression.

3.1 Introduction

High comorbidity between major depression and anxiety has been shown in both clinical and population studies (Kessler et al., 1996; Merikangas et al., 1996; Wittchen et al., 1994; 2000). This high comorbidity has led to the inclusion of mixed anxiety-depression as a separate diagnosis in the International Classification of Disease, 10th edition (ICD-10), while the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) is currently investigating inclusion of this diagnosis. The structural comorbidity of anxiety and depressive disorder, together with the fact that pharmacological treatment for depression is also successfully applied in comorbid anxiety (Nutt et al., 2000), suggests that the two disorders may share a common aetiology. This is supported by the results of twin studies which showed that the liability to major depression and generalised anxiety disorder was influenced by the same genetic factors (Kendler et al., 1992; Kendler, 1996; Roy et al., 1995). The expression of this genetic liability as depression or anxiety is then determined by the environmental challenges the person encounters during his life.

In a Dutch study of twin families, similar results were found: principal component analysis, in which personality questionnaires on a wide range of traits were combined, established three major components in the data of which the most important was characterised by high loadings of anxiety, neuroticism, and depression (Boomsma et al., 2000). The other two components were characterised by sensation seeking and by extraversion, trait-anger and type-A behaviour. The component of depression and anxiety was entered in a multivariate genetic analysis, applying the known difference in genetic relatedness of twins and siblings, to compute a genetic factor score (Boomsma et al., 1990). The results of this analysis showed that anxiety and depression had a high comorbidity and that the genetic covariance between the measures was due to a common genetic factor (Boomsma et al., 2000). Next, the factor score, called the anxious depression factor score, was used to select twin families for the purpose of a genome wide search for the genes (QTL) involved in anxiety and depression.

In this chapter the relationship between the anxious depression factor score and the anxiety, depression and neuroticism scales, of which the factor score was composed, is demonstrated. In addition, the anxious depression factor score is validated against a clinical measure for lifetime depression which was derived from the Composite International Diagnostic Interview (CIDI; Peters & Andrews, 1995; Wittchen, 1994). The anxious depression factor score is thought to represent the individual's vulnerability to both depression and anxiety and to reflect a normal distribution of anxious and depressive symptoms, with at the end of the distribution a clinical depression according to DSM-IV criteria. It was therefore hypothesised that:

I. The anxious depression factor score will be highly correlated with each of the anxiety, depression and neuroticism scales that were used to compose the factor score. A low anxious depression score will therefore be associated with low values, and a high anxious depression score will be associated with high values on the anxiety, depression and neuroticism questionnaires. This relationship will be similar for men and women and is not influenced by age.

II. The anxious depression factor score will reflect the risk of a DSM-IV diagnosis for depression. A low anxious depression score will be associated with a lower risk of a DSM-IV depression diagnosis, whereas a high anxious depression score will be associated with a higher risk of a DSM-IV depression diagnosis. No sex differences or age effects will be found.

3.2 Methods

3.2.1 Participants

Participants were ascertained from the Netherlands Twin Register (NTR). A total of 3344 NTR families with adolescent or adult twins and siblings participated in a longitudinal study of physical and mental health (Boomsma et al., 2000). The present study reports on survey data obtained in 1997. In that year the questionnaire was sent to 7989 individuals (5546 twins and 2443 siblings). A total of 4584 persons returned a completed questionnaire. Children below 15 years of age (n=137) were excluded after which 4447 individuals were available for the present analysis.

3.2.2 Anxious depression

To assess depression, the 13-item version of the Beck Depression Inventory (BDI; Beck et al., 1961) and the anxiety-depression symptom scale of the Young Adult Self-Report (YASR; Achenbach, 1990) were used. Anxiety was measured by the Spielberger Trait Anxiety Inventory (STAI, Spielberger et al., 1970). Furthermore, the Neuroticism and Somatic Anxiety scales of the 'Amsterdamse Biografische Vragenlijst' (ABV, Wilde, 1970) were included. These correlated values were combined in a genetic factor score according to multivariate genetic model fitting methods which specified additive genetic, common environmental and unique environmental sources of variation. The results of the multivariate genetic analysis were used to compute a genetic factor score for each individual in the study (Boomsma et al, 2000, this thesis). Because none of the anxiety and depression scales was normally distributed it was necessary to transform the data. A log transformation was conducted on the neuroticism (neu) and somatic anxiety scales (SoA) of the ABV, the STAI (anx) and the YASR anxious depression scale (Ydep). The depression data from the Beck Depression Inventory (BDI) data were transformed using arcsin transformations.

This resulted in the following transformations:

```
tneu=5*ln(neu)
tSoA=9*ln(Soa)
tanx=10*ln(anx)
tydep=12*ln(ydep)
if bdi>28:tbdi=10*sqrt(arsin(28/28))
if bdi<=28: tbdi=10*sqrt(arsin(bdi /28))
```

The genetic factor scores were after transformation calculated as a weighted sum of the scale values. Women had, compared to men, higher mean scores on all anxiety, depression and neuroticism scales. To correct for the sex differences, the mean values for men and women for each scale were subtracted from the individual observations. Next, the factor scores (FS) were computed separately for men and women. From the results of the NETSAD study, the following formula were obtained for men and women:

Men: FS1= 0.117*tneu+0.039*tSoA+0.144*tAnx+0.064*tYdep
FS2= 0.077*tneu+0.040*tSoA+0.130*tAnx+0.166*tbdi

Women: FS1= 0.117*tneu+0.066*tSoA+0.133*tAnx+0.053*tYdep
FS2= 0.086*tneu+0.077*tSoA+0.146*tAnx+0.062*tbdi

As can be seen, there are two formula for the factor scores. The first (FS1) includes the YASR anxious depression scale, the second (FS2) includes the BDI. In 1997, both scales were administered and two factor scores were computed. For the analyses reported in this paper, the average of FS1 and FS2 was used. There was a considerable difference for the loadings on the BDI and the STAI for men and women. The loadings represented the loading of the scale values on the genetic factor. For males there was a much larger contribution of the BDI to the construction of the genetic factor scores, whereas for women the contribution of somatic anxiety was larger than in men. As a result of the correction for sex differences, there were no differences in the anxious depression factor scores for men and women. For the purpose of this study persons with the 30% lowest mean anxious depression scores were classified as low anxious depressed, persons with the 30% highest mean anxious depression scores were classified as high anxious depressed. Remaining persons (40%) were classified as intermediate. This criterion was not as strict as the selection criterion used for the QTL analysis.

3.2.3 DSM-IV depression

All offspring of families selected for the QTL analysis was asked to participate in a telephone interview containing the sections on anxiety and depression from the World Health Organisation Composite International Diagnostic Interview (CIDI). The CIDI is a standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 and DSM-IV. The computer administered life-time version (2.1) was conducted by interviewers trained at the CIDI training centre at the Academic Medical Centre in Amsterdam, The Netherlands. The structure of the interview was such that when no symptoms of any kind were present, the duration of the interview was approximately ten minutes. For each reported symptom a series of structured questions regarding the exact nature, duration and severity of the symptoms were asked together with a number of questions regarding previous treatment and former diagnosis of the symptoms. Depending on the number of reported symptoms the interview could last from 15 minutes to an hour. The following sections were administered: Demographics (section A); Social Phobia, Agoraphobia and Generalised Anxiety Disorder (D33 and further); Depression and Dysthymia (E); Mania Screen and Bipolar Affective Disorder (F) and Obsessive-compulsive disorder (K1-22). For the purpose of this study, the diagnostic criteria of the DSM-IV were used to determine a clinical diagnosis for life-time depression. The interview placed participants diagnosed with DSM-IV major depression in one of seven classes: No depression, mild single depression, mild recurrent depression, moderate single depression, severe single depression, moderate recurrent depression and severe recurrent depression. Participants who had experienced a single or recurrent depressive episode at some point in their life, but were not currently depressed, and participants who were having a depressive episode at the time of the interview were diagnosed with major depression. No diagnosis for depression was assigned when episodes were directly related to a major life event (e.g. loss of family member) and in bipolar depression, evidenced by alternating manic and depressive phases.

3.2.4 Analyses

Analysis I. In analysis I a Pearson correlation coefficient was computed for each of the anxiety, depression and neuroticism scales (BDI depression, ABV neuroticism, ABV somatic anxiety, STAI anxiety and YASR anxious depression) with the anxious depression factor score. In addition, a one-way ANOVA procedure was used to assess the association between the anxious depression score (low, medium, high) and the scores on the scales. Because the values on the individual scales reflected sex differences whereas the anxious depression factor score did not, analyses were performed for the whole sample and for men and women separately.

Analysis II. In analysis II a Pearson correlation coefficient was computed for the factor score with the DSM-IV diagnosis (7 categories). Next, the DSM-IV diagnosis for depression was dichotomised

and a chi-square analysis was used to determine the association between anxious depression (low, medium, and high) and the dichotomised variable reflecting clinical depression (yes, no). Again, analyses were performed for the whole sample and for men and women separately.

3.3 Results

3.3.1 Descriptives

The results were reported over a total of 4447 individuals from 1935 families (3076 twins and 1371 siblings). Of these participants 1788 (40.2%) were men and 2659 (59.8%) were women. The age range was 15 to 83 years, with a mean age of 26.4 years (SD=10.14). Women in the sample had a mean age of 27.01 (SD=9.99) and men had a mean age of 26.40 (SD=10.14). For 90 individuals the anxious depression factor score was missing. Table 1 presents the mean scores for men and women on the BDI, ABV neuroticism and ABV somatic anxiety, STAI and the mixed anxiety depression scale of the YASR. Women had significantly higher scores on each scale compared to men.

Table 3.1 Mean (SD) scores on BDI, ABV (neuroticism, somatic anxiety), STAI, YASR (anxious depression) and anxious depression factor score for men and women separately.

	Men (n=1788)	Women (n=2659)	F	df	P
BDI	1.30 (2.33)	2.05 (3.00)	79.50	1	.000
ABV (Neu)	40.44 (21.14)	50.54 (24.40)	202.43	1	.000
ABV (SoA)	16.46 (4.56)	18.00 (5.41)	97.00	1	.000
STAI	30.46 (7.80)	33.68 (9.29)	145.23	1	.000
YASR (anxdep)	19.52 (3.50)	21.67 (4.74)	267.74	1	.000
Mean FSt4	0.62 (0.72)	0.00 (0.80)	.030	1	.863

BDI= Beck Depression Inventory, ABV (neu)= ABV neuroticism, ABV (SoA)= ABV somatic anxiety, STAI= Spielberger Trait Anxiety Inventory, YASR (Anxdep)= YASR anxious depression scale
Mean fst4= Mean factor score (1997)

Of the individuals who completed the questionnaire, 1038 individuals, 419 men and 619 women, participated in the CIDI interview. Of this group 160 (15.4%) received a diagnosis for life-time depression. In general, women were significantly more often diagnosed with life-time depression than men (19.9% vs. 8.8%, χ^2_1=23.36, p=.000). Both men and women with a DSM-IV diagnosis for depression were on average older (mean age in men 33.6 and women 30.1 years) than men and women without a DSM-IV diagnosis for depression (mean age in men 28.2 and women 27.8 years; F_1=9.19, p=.002). For 17 participants in the CIDI interview no factor score was computed, which left 1021 persons with both an anxious depression factor score and a DSM-IV diagnosis for depression.

3.3.2 Analysis I: anxious depression and the anxiety, depression and neuroticism scales

In analysis I, Pearson's correlation coefficients were computed for the anxious depression factor score with the scores on the anxiety, depression and neuroticism scales. Table 2 presents the correlations between the mean anxious depression factor score and the individual scales for the whole sample and for men and women separately. As can be seen, all scales correlated significantly high with the anxious depression factor score, and patterns are similar for men and women.

Table 3.2 Pearson correlation coefficients between the anxious depression factor score and the BDI, ABV, STAI, YASR scores for men and women.

	Correlations		
	All	Men	Women
BDI	.687	.702	.691
ABV (Neu)	.877	.882	.907
ABV (SoA)	.683	.638	.718
STAI	.901	.908	.921
YASR (anxdep)	.768	.762	.809

For all correlations p< .000
BDI= Beck Depression Inventory, ABV (neu)= ABV neuroticism, ABV (SoA)= ABV somatic anxiety, STAI= Spielberger Trait Anxiety Inventory, YASR (Anxdep)= YASR anxious depression scale, Mean fst4= Mean factorscore (1997)

Table 3.3 Distribution of scores on BDI, ABV, STAI, YASR for anxious depression scores (low, medium, high).

	Low	medium	high	F	Df	P
BDI	0.29	1.05	4.11	1043.86	2	.000
ABV(Neu)	24.71	42.76	73.00	3909.25	2	.000
ABV(SoA)	14.09	16.51	21.80	1208.00	2	.000
STAI	24.32	30.92	42.34	3824.38	2	.000
YASR(anxdep)	17.55	20.06	25.03	1783.00	2	.000
Mean FSt4*	-0.85	-0.58	0.94	9644.80	2	.000

BDI= Beck Depression Inventory, ABV (neu)= ABV neuroticism, ABV (SoA)= ABV somatic anxiety, STAI= Spielberger Trait Anxiety Inventory, YASR (Anxdep)= YASR anxious depression scale, Mean fst4= Mean factorscore (1997)

In addition, the association between low, medium and high anxious depression and the scale values was determined. Table 3 presents the mean values for each scale and the value of the mean factor score for the low, medium and high anxious depressed groups separately. The three groups differed from each other on each measure; scores on all scales increased when the level of anxious depression increased. Again, results were similar for men and women.

3.3.3 Analysis II: anxious depression and DSM-IV depression

For analysis II, a Pearson's correlation coefficient for the mean anxious depression factor score and the DSM-IV diagnosis for depression was computed. There was a significant correlation between DSM-IV depression and the anxious depression factor score (r=.330; p=.000). Figure 1

presents the relationship between anxious depression and the DSM-IV diagnosis categories for the whole population. The correlation between DSM-IV diagnosis and anxious depression factor score was similar when men and women were analysed separately (r=.337, p=.000 and r=.342, p=.000, respectively). Correction for age hardly influenced the results: in the whole population the partial correlation between anxious depression and DSM_IV was .335, p=.000. For men and women partial correlations were .336, p=.000 and .352, p=.000 respectively. In addition, the association between low, medium and high anxious depression scores and the dichotomised value for DSM-IV depression was determined. In accordance with previous results, persons with a high score for anxious depression were at higher risk of having developed clinical depression than persons with a low score for anxious depression (χ^2_2=88.23; p=.000). Again, results were similar for men and women (χ^2_2=36.98; p=.000 and χ^2_2=56.64; p=.000 respectively). No individuals with low anxious depression scores were diagnosed with moderate or severe recurrent depression.

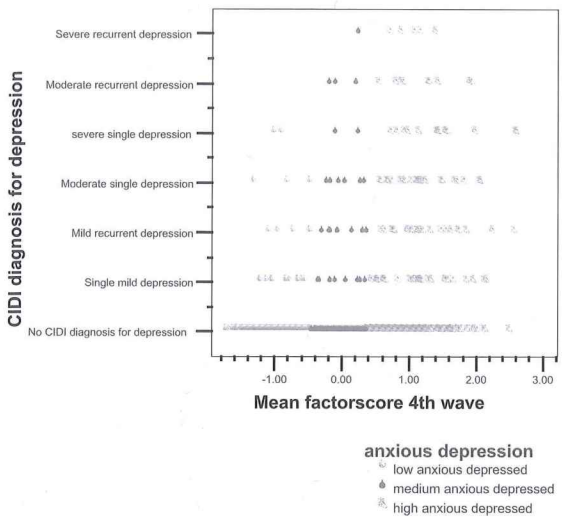
3.4 Discussion

In the present study, the relationship between the anxious depression factor score and the anxiety, depression and neuroticism scales used to compute the factor score was examined. In addition, the anxious depression factor score was validated against the clinical diagnosis of lifetime depression according to the diagnostic criteria of the DSM-IV.

3.4.1 Anxious depression and the anxiety, depression and neuroticism scales

In the first analysis, the relationship between the anxious depression factor score and scores on more commonly used anxiety, depression and neuroticism scales was assessed. It was hypothesised that the anxious depression factor score reflects vulnerability to both anxiety and depression and therefore will show a strong association with the scale scores. Indeed, results showed high positive correlations between the anxious depression factor scores and all scale scores, even after controlling for age. This indicates that anxious depression is a valid measure that combines both anxiety and depression in one score and supports the findings that anxiety and depression have a very high comorbidity and may share a common element, possibly a genetic predisposition (Kendler et al., 1992; Roy et al., 1995; Kendler, 1996). Relationships were similar for men and women, indicating the score may be a valid tool in the examination of gender differences in relations between anxiety/depression and other variables, e.g. smoking (see Chapter 4, this thesis).

Figure 3.1 The relationship of the anxious depression factor score with the DSM-IV diagnosis for depression derived from the CIDI interview.



3.4.2 Anxious depression and DSM-IV depression

In the second analysis the relationship between the anxious depression factor score and DSM-IV depression was assessed. It was hypothesised that persons with a high anxious depression factor score would be at increased risk of experiencing an episode of clinical depression according to the DSM-IV criteria. As expected, there was a strong positive association between the factor score for anxious depression and the DSM-IV diagnosis for depression, which remained after controlling for age. It can be concluded that in the current sample a DSM-IV diagnosis for depression is at the end of a normal distribution of depressive symptoms. This is in agreement with the results of other studies (e.g. Angst & Clayton, 1986; Judd et al., 2000; Kendler et al., 1993b; Solomon et al., 2001) which showed clinical depression to be the tail of the normal distribution of depressive symptoms rather than an entirely new process. In line with the previous results, no sex differences were

evident for the relationship between anxious depression and DSM-IV diagnosis of lifetime depression.

The CIDI interview rates depression on the basis of life time occurrence, whereas questionnaire data capture depression at one point in time. This would explain the fact that some persons received a diagnosis for clinical depression but had low factor scores for anxious depression. Nevertheless, the anxious depression factor score seems to be a valid measure of vulnerability to depression.

In conclusion, the anxious depression factor reflects increased levels of anxiety and depression. Furthermore, persons with extremely high anxious depression factor scores are at increased risk of a clinical diagnosis of lifetime depression. These associations are not influenced by age or sex. This indicates that the anxious depression factor score is a valid measure of depression, reflecting trait depression and vulnerability to clinical depression.

Chapter 4

Smoking and depression in a family-based population

Abstract

The current paper assesses the association between smoking status and anxious depression, using data from a family-based population study (n=4447) and from a sub-sample that participated in a psychiatric diagnostic interview to determine life-time depression according to the criteria of the DSM-IV (n=1038). Survey data on depression and anxiety were combined into a factor score, called anxious depression. The relationship between smoking status and anxious depression was examined in the whole population, in a between-family analysis comparing individuals with and without a family history for depression and in a within-family analysis of siblings discordant for depression.

Within the whole sample, a positive association between smoking and depression was found. Both depressed men and women were more often current smokers compared to non-depressed men and women but, interestingly, depressed women were less often ex-smokers than depressed men. Coming from a family high on anxious depression or with a history of depression increased a person's risk of smoking. In addition, non-depressed individuals with a family history of depression smoked more than non-depressed individuals without a family history of depression. However, sibling pairs discordant for depression did not differ in smoking behaviour.

In conclusion, both anxious depression and DSM-IV depression were positively associated with smoking. Women, more than men, may smoke to relieve depressive symptoms and smoking cessation therapy should therefore consider sex differences. Familial factors are implicated in the association between depression and smoking.

4.1 Introduction

Smoking and depression are among the most important and highly prevalent health risk factors in the industrialised countries (e.g. Mathers et al., 2001; Murray & Lopez, 1997; Melse et al., 2000). Importantly, smoking and depression have been shown to be associated. Incidence rates and life time prevalence rates of smoking are higher in individuals with higher scores on depression scales (Anda et al., 1990; Glassman et al, 1990; Patton et al., 1996). In addition, individuals with clinical depression or high trait depression are less successful in smoking cessation and more likely to suffer a relapse when having successfully quit smoking (Glassman et al., 1990; Shiffman, 1982). Also, tobacco use linearly increases with the severity of depression (Farrell et al, 1998), and may, therefore, be most prominent in clinically depressed patients (Acton et al., 2001; Hughes et al., 1986).

The dominant explanation for the association between smoking and depression is that cigarette smoking is a form of self-medication against negative moods. This is supported by the fact that nicotine has been shown to alter brain chemicals implied in depression (Berlin et al., 1995; Fowler et al., 1996a; Fowler et al., 1996b). However, an alternative explanation is that depression and smoking share a common aetiology and studies have shown evidence that smoking and depression are associated by common familial factors (Breslau et al., 1998; Kendler et al., 1993). The familial factor may consist of a genetic predisposition as proposed by Kendler et al. (1993) or environmental factors such as early negative life-events (Roy et al., 2000).

In both smoking and depression sex differences are evident; women experience more depression, especially anxious depression, than men (Silverstein, 2002) and are less likely to smoke than men (DEFACTO, 2000). Given these sex differences, the association between smoking and depression may be differently modulated in men and women. This has not been the focus of much research, although some information on the distribution of smoking patterns in depressed and non-depressed men and women may be gained from population studies on smoking and depression. Patton et al. (1996) showed that both men and women with high levels of psychiatric symptoms, including depressive symptoms, were more likely to become regular smokers, but risk ratios were higher in women than in men. However, other studies did not find a difference between male and female patterns of cigarette consumption in depression (Farrell et al., 2001; Hämäläinen et al., 2001).

Age is also an important factor in relation to first onset of depression as well as to smoking initiation. Studies have shown 15 to 24-year-olds to be most likely to have had a major depressive episode in the past month (Blazer et al., 1994). The lowest rates of depression are seen among 45 to 54-year-olds and among 55 to 70-year-olds (Newmann, 1989; Zisook & Downs, 1998) and an increase in depression rates is observed after age 70 (Murrell, Himmelfarb, & Wright, 1983). Dutch prevalence data for smoking showed that most people initiated smoking at age 13 or 14 years and

became regular smokers in the age-group of 10-19 years (DEFACTO, 2000). These data indicate that the prevalence of depression is highest in the same period in which experimenting with smoking can lead to regular smoking. It could very well be that depression is an important mediating factor in maintaining a regular smoking habit. Similar results have been found for anxiety (Brioni et al., 1993; Cheeta et al., 2001; Costall et al., 1989)

Anxiety and depressive disorder are thought to share a similar genotype that is non-specific and its expression entirely determined by the environment (Kendler et al., 1992; Kendler, 1996; Roy et al., 1995). In the Dutch twin and family study on anxiety and depression, referred to as the NETSAD study, high comorbidity of anxiety and depression was found in longitudinal survey data (Boomsma et al., 2000). A multivariate genetic analyses, applying the known difference in genetic relatedness of twins and siblings was used to compute a genetic factor score (Boomsma et al., 1990). This factor score represents the common element shared by anxiety, neuroticism and depression and can be used as an estimate of an individual's genotypic value for anxious depression as it optimizes the genetic contribution to the stability of anxiety and depression scores over time (Boomsma and Dolan, 1998; Boomsma et al., 2000; Dolan and Boomsma, 1998).

In the present study, data on anxious depression were used to examine the association between smoking and anxious depression, as indicated by the genetic factor score. In addition, in a sub-sample of persons that participated in a psychiatric diagnostic interview the association between smoking and clinical diagnosis for life-time depression was investigated. First, the association between depression and smoking was assessed in the whole sample without regard to family structure. The effect of sex and age on this relationship will be studied. Secondly, if there is a common aetiology for depression and smoking there should be an aggregation of smoking in the families in which siblings report more depressive symptoms. Therefore the association between depression and smoking status was assessed for individuals from families classified as concordant low or concordant high anxious depressed, as well as for individuals from families with and without a family history for clinical depression. Finally, if the common aetiology between depression and smoking is explained by familial factors, it is expected that within sibpairs discordant for depression, smoking will aggregate in the depressed siblings and not in the non-depressed siblings. By testing the association between depression and smoking in a family design we can rule out factors that vary between families. In summary we hypothesise that:

I. In the whole sample, depression will be associated with smoking behaviour. Persons with high levels of depression will be more often smokers than persons with low depression levels. In addition, persons with a diagnosis of lifetime depression will be more often smokers than persons without a diagnosis. The association between smoking and depression will independent from age or sex.
II. Between families, persons from families in which no low anxious depressed siblings are present will be more often smokers than persons from families in which no high anxious

depressed siblings are present. Furthermore, persons from families with a positive family history for clinical depression will be more often smokers than persons from families with a negative family history for clinical depression.

III. Within families, high anxious depressed siblings will be more often smokers than low anxious depressed siblings. Similarly, siblings with a diagnosis for lifetime depression will smoke more often than siblings without a diagnosis.

4.2 Methods

4.2.1 Participants

Participants were ascertained from the Netherlands Twin Register (NTR). A total of 3344 NTR families with adolescent or adult twins and siblings participated in a longitudinal study of physical and mental health (Boomsma et al., 2000). The present study reports on data obtained from the 1997 survey sent to 7989 individuals (5546 twins and 2443 siblings). A total of 4584 persons returned a completed questionnaire. Children below 15 years of age (n=137) were excluded after which 4447 individuals were available for the present study.

4.2.2 Anxious depression

To assess anxiety and depression, the 13-item version of the Beck Depression Inventory (Beck et al., 1961), the anxious/depression symptom scale of the Young Adult Self-Report (Achenbach, 1990), the Spielberger Trait Anxiety Inventory (Spielberger et al., 1970) and the Neuroticism and Somatic Anxiety scales of the Amsterdamse Biografische Vragenlijst (Wilde, 1970) were included in the questionnaire sent to the participants. These correlated values were combined in a genetic factor score applying the known difference in genetic relatedness of twins and siblings (Boomsma et al., 1990). Since women had, compared to men, higher mean scores on all anxiety and depression scales than men, the factor scores were computed separately for men and women on the sex-corrected data. As a result, there were no sex differences in the anxious depression factor scores. The factor score represents the individual's value on the common genetic factor for depression and anxiety and can be interpreted as an estimate of an individual's genotypic value for anxious depression. For a description of the factor score composition the reader is referred to Boomsma et al. (2000). For the purpose of this study, persons with the 30% lowest mean anxious depression scores were classified as low anxious depressed, persons with the 30% highest mean anxious depression scores were classified as high anxious depressed. Remaining persons (40%) were classified as intermediate.

4.2.3 DSM-IV depression

In addition to the assessment by questionnaire, a telephone interview containing the sections on anxiety and depression from the World Health Organisation Composite International Diagnostic Interview (CIDI) was administered. The CIDI is a standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 (the tenth revision of the international classification of diseases and related health problems) and DSM-IV (the fourth edition of the diagnostic and statistical manual for psychiatric disorders). The computer administered life-time version (2.1) was conducted by interviewers trained at the CIDI training centre at the Academic Medical Centre in Amsterdam, The Netherlands. For the purpose of this study the diagnostic criteria of the DSM-IV were used to determine a clinical diagnosis for life-time depression. Individuals could receive one of six diagnoses for DSM-IV major depression: mild single and mild recurrent depression, moderate single and severe single depression, moderate recurrent and severe recurrent depression. Participants received a diagnosis if they had experienced a depressive episode at some point in their life or were having a depressive episode at the time of the interview. No diagnosis for depression was assigned when episodes were directly related to a major life event (e.g. loss of family member) and in bipolar depression, evidenced by alternating manic and depressive phases.

4.2.4 Smoking status

The participants were asked about their past and current smoking behaviour. Participants were coded as smokers if they currently smoked. They were coded as ex-smokers if they had reported life-time smoking behaviour, but were currently not smoking. Finally, non-smokers had reported never to have smoked, or only once or twice to try, and did not currently smoke. When possible, incomplete or inconsistent reports on smoking behaviour were checked using questionnaire data obtained in 2000.

4.2.5 Analyses

Analysis I. To determine the association between smoking status (non-smoker, smoker, ex-smoker) and anxious depression (low, medium, high) a chi-square analysis was used. Sex and age were taken into account by running the analyses separately for sex and age groups. A similar procedure was used for clinical depression.

Analysis II. In the between-family analysis, chi-square analyses was used to examine the distribution of smoking status for persons from families classified as low anxious depressed in comparison to persons from families classified as high anxious depressed. Families were classified as low anxious depressed when there were no high anxious depressed siblings present and

families were classified as high anxious depressed when there were no low anxious depressed siblings present.

The same procedure was followed for persons coming from families with a positive or a negative family history for depression. Families had a positive family history for clinical depression when at least one sibling in the family received a diagnosis for life-time depression according to the DSM-IV criteria. A family had a negative family history for clinical depression when all siblings participated in the CIDI interview and none of them received a diagnosis for life-time depression according to the DSM-IV criteria. For both between-family analyses a chi-square analysis was used.

Analysis III. In the within-family analysis, discordant sibpairs for anxious depression were formed, i.e. one sibling was low anxious depressed and the other sibling was high anxious depressed. Similarly sibpairs discordant for clinical depression were formed i.e. one sibling had a DSM-IV diagnosis for depression and the other sibling had not. For both the anxious depression score as well as the DSM-IV diagnosis for depression the discordant sibpairs were, when possible matched on age and monozygotic twin pairs were excluded. Only same sex pairs were included in the analyses. To compare the smoking behaviour in the discordant siblings, a chi-square analysis was used.

4.3 Results

4.3.1 Descriptives

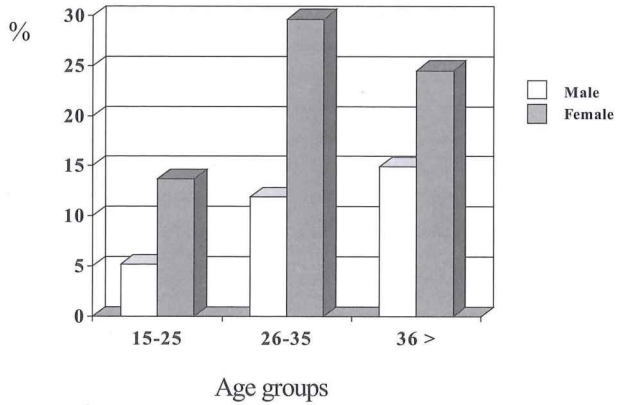
Table 1 presents an overview of the demographic data. The results are reported over a total of 4447 individuals from 1935 families (3076 twins and 1371 siblings). Of these participants 1788 (40.2%) were men and 2659 (59.8%) were women. The age range was 15 to 83 years, with a mean age of 26.8 years (SD=10.06). Women in the sample had a mean age of 27.01 (SD=9.99) and men had a mean age of 26.40 (SD=10.14). As most individuals were adolescents or young adults, 3 age cohorts were formed; less than 25 years, between 25 and 35 years, and older than 35 years.

Of the individuals who completed the questionnaire, 1038 individuals participated in the CIDI interview. Of this group 160 (15.4%) received a diagnosis for life-time depression. Figure 1 shows that women were significantly more often diagnosed with a history of clinical depression than men (χ^2_1=23.36, p=.000). The risk of a DSM-IV diagnosis for depression increased with age for both men (χ^2_2=9.00, p=.011) and women (χ^2_2=19.52, p=.000) and was least likely in the age-group of 15-25 years. Figure 1 presents the distribution of DSM-IV depression for the three age-groups in men and women. DSM-IV diagnosis for depression was more prevalent in women than in men in the age-groups 15 to 25 (χ^2_1=10.83; p=.001) and 26 to 35 (χ^2_1=11.18; p=.001). In the oldest age-group there was no difference between men and women.

Table 4.1 Demographic data by sex

	Men	Women
	N	N
Agegroup		
15-25	1132 (63.4%)	1580 (59.5%)
26-35	418 (23.3%)	653 (24.5%)
36 >	238 (13.3%)	426 (16.0%)
Anxious depression		
Low	513 (28.7%)	793 (29.8%)
Medium	743 (41.6%)	1001 (37.6%)
High	496 (27.7%)	811 (30.5%)
Missing	36 (2.0%)	54 (2.0%)
DSM-IV diagnosis		
No depression	382 (91.2%)	496 (80.1%)
Depression	37 (8.8%)	123 (19.9%)
Total	419	619
Smoke status		
Non-smoker	977 (54.6%)	1557 (58.6%)
Ex-smoker	536 (30.0%)	693 (26.1%)
Current-smoker	248 (13.9%)	386 (14.5%)
Missing	27 (1.5%)	23 (0.8%)

Figure 1. Distribution of a positive DSM-IV diagnosis for life-time depression in each age-group, by sex. Figure shows only percentage of men and women that received a DSM-IV diagnosis for life-time depression



For 50 persons (1.1%) no smoking status could be derived from the survey data. Smoking status for men and women is shown in Table 1. Men and women did not differ in their smoking behaviour. Figure 2a and 2b present the distribution of smoking status in each age-group by sex. The three age groups did differ in smoking behaviour (χ^2_4=455.06, p=.000). Overall, as age increased the number of non-smokers decreased and the number of current smokers and ex-smokers increased. Men below 25 years of age were more often smokers than women under 25 (χ^2_2=7.20; p=.027). In the age-group of 25 to 35 years women were more often current smokers and less often ex-smokers compared to men (χ^2_2=8.56; p=.014) whereas there was no difference in non-smokers between men and women. In de age group of 36 years and older there was no difference between men and women in smoking status.

Figure 2a. Distribution of smoking status in each age-group, for men only

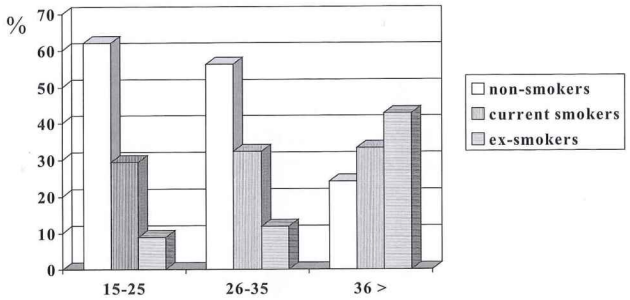
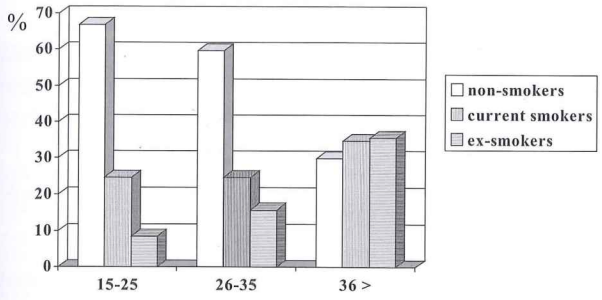


Figure 2b. Distribution of smoking status in each age-group, for women only



4.3.2 Analysis I: depression and smoking status in the whole sample

First, the relationship between smoking status and anxious depression was assessed in the whole sample. Table 2 presents the smoking status for anxious depression by sex. Both men (χ^2_4=16.07, p=.003) and women (χ^2_4=56.57, p=.000) smoked more when they had a high score for anxious depression. When examining the association for smoking and depression in the different age-groups, only the first age-group (15-25 years) showed a significant difference in smoking status between the low, medium and high anxious depressed (χ^2_4=73.40; p=.000). In this age group, high anxious depressed persons smoked more often than low anxious depressed.

Table 4.2 Smoking status by anxious depression for men and women separately.

	Men			Women		
	low	medium	high	low	Medium	high
non-smoker	306 (59.9%)	424 (57.7%)	234 (48.3%)	517 (65.4%)	610 (61.4%)	400 (49.8%)
Ex-smoker	69 (13.5%)	97 (13.2%)	79(16.3%)	99 (12.5%)	156 (15.7%)	119 (14.8%)
current smoker	136 (26.6%)	214 (29.1%)	172 (35.5%)	174 (22.0%)	227 (22.9%)	285 (35.4%)

Secondly, smoking status in relation to DSM-IV diagnosis for lifetime depression was examined. Table 3 presents the smoking status for DSM-IV diagnosis by sex. Persons with a DSM-IV diagnosis were more often current smokers and less often non-smokers compared to persons without an DSM-IV diagnosis for life-time depression in both men (χ^2_2=6.61; p=.037) and women (χ^2_2=12.42; p=.002). Within the age-groups there was no difference in smoking status between persons with and without a DSM-IV diagnosis for life-time depression.

Table 4.3 Smoking status by DSM-IV depression for men and women separately.

	Men		Women	
DSM-IV	no	Yes	No	Yes
non-smoker	210 (55.4%)	13 (37.1%)	302 (61.1%)	59 (48.0%)
ex-smoker	53 (14.2%)	10 (28.6%)	70 (14.2%)	14 (11.4%)
Current-smoker	116 (24.7%)	12 (34.3%)	122 (24.7%)	50 (40.7%)

4.3.3 Analysis II: between family analysis

In the between family analysis, individuals from families that were concordant high or low anxious depressed were compared. A number 659 families, consisting of 1518 persons, were classified as low anxious depressed and 651 families, consisting of 1462 persons, were classified as high anxious depressed. A number of 361 families, consisting of 607 persons were classified as medium anxious depressed as there were no low or high anxious depressed siblings present in the family. The distribution of smoking status for the low, medium and high anxious depressed families is presented in Table 4. The low and high anxious depressed families differed significantly in the distribution of smoking status (χ^2_4=43.84; p=.000). Low anxious depressed families consisted of more non-smokers and less current and ex-smokers than high anxious depressed families. The medium anxious depressed families fell between the low and high anxious depressed families.

Table 4.4 Smoking status in persons from low, medium and high anxious depressed families

	low*	medium*	High*
non-smokers	990 (65.2%)	864 (59.1%)	329 (54.2%)
ex-smokers	193 (12.7%)	224 (15.3%)	84 (13.9%)
Current smokers	335 (22.1%)	374 (25.6%)	194 (31.9%)

*Families were classified low anxious depressed when there were no high anxious depressed siblings present. Families were classified high anxious depressed when there were no low anxious depressed siblings present. Families in which both no low and no high anxious depressed siblings were present were classified as medium anxious depressed.

The distribution of smoking status was also assessed for families with a positive or a negative family history for DSM-IV life-time depression A number of 763 persons from 128 families received a positive family history for depression and a number of 468 persons from 177 families received a negative family history for depression. The remaining families could not be classified because not all family members had participated in the CIDI interview even though no DSM-IV diagnosis was

obtained by the participating members. The distribution of smoking status for the families with a positive and a negative family history for depression is presented in Table 5. Families with and without a family history of lifetime depression differed significantly in their smoking behaviour (χ^2_2==30.63; p=.000). Persons from families with a positive history of clinical depression were more likely to smoke or have smoked than persons from families without clinical depression. Moreover, further analyses showed depressed persons with a positive family history for depression were more often current smokers and less often ex-smoker compared to non-depressed persons with a positive family history for depression who, in turn, were more often smokers and ex-smokers than non-depressed persons with a negative family history for depression.

Table 4.5 Smoking status in persons with a positive and negative family history for depression

	positive family history*	negative family history*
non-smokers	378 (49.5%)	304 (64.9%)
ex-smokers	139 (18.2%)	46 (9.9%)
Current smokers	246 (32.3%)	118 (25.3%)

*In families with a positive family history at least one sibling which participated in the CIDI interview and received a DSM-IV diagnosis for life-time depression. In families with a negative family history for depression all siblings participated in the CIDI interview and none of them received a DSM-IV diagnosis for life-time depression.

4.3.4 Analysis III: within family analysis

Sibpairs were formed in which one sibling had an extremely low score for anxious depression and one sibling had an extremely high score for anxious depression. Of the 245 sibpairs discordant for anxious depression there were 145 pairs concordant for sex, 59 male and 86 female pairs. For one pair smoking status was not available. Results showed that the high anxious depressed siblings did not smoke more than the low anxious depressed siblings, nor did they report more life-time smoking.

From the total number of 160 persons that received a DSM-IV diagnosis for depression it was possible to form 103 discordant sibpairs (206 persons). The other 60 persons that received a DSM-IV diagnosis for depression were either the only participating family members, had no sibling without a DSM-IV diagnosis for depression or were an additional sibling to an already formed discordant sibpair. Of the total of 103 pairs, there were 74 same sex pairs, 18 male and 56 female pairs. Smoking status was available in all pairs. No association was found between smoking status and DSM-IV life-time depression. The siblings with a DSM-IV diagnosis for life-time depression did not smoke more often than the siblings without a DSM-IV diagnosis for life-time depression.

4.4 Discussion

In the current paper the association between depression and smoking status in relation to sex and age was assessed. To this aim, data from a large population study were assessed, including data from a sub-sample that participated in a psychiatric diagnostic interview (CIDI) to determine lifetime diagnosis of clinical depression.

As expected, both men and women with a high score on anxious depression were more often smokers than persons with low scores. Having a DSM-IV diagnosis of lifetime depression was also associated with an increased risk of smoking. Although the general pattern of the relationship between depression and smoking was similar for men and women, some differences emerged. Women were more often diagnosed with lifetime depression than men, especially in the younger age groups. As smoking initiation occurs in general in this age-group, there is a possibility that depression in 15 to 25 years-olds might contribute to becoming a regular smoker, in particular in women. Recognising and treating depression in this age-group may therefore be an important factor in preventing that young persons vulnerable for depression will develop a regular smoking habit. Future longitudinal studies may shed more light on this hypothesis. In addition, men with a diagnosis of life-time depression were often current smokers or ex-smokers, whereas women with a diagnosis of life-time depression were more often current smokers but less often ex-smokers. This further supports the suggestion that women, more than men, use smoking as a means to reduce depressive symptoms and are therefore less able to cease smoking. In a study by File, Fluck and Leahy (2001) nicotine administration reduced negative mood, such as stress and anxiety, in women whereas in men nicotine enhanced these symptoms. The authors suggested that because young women experience calming effects from nicotine when they are under stress, they will not give up smoking unless adequate alternative means of stress reduction are available. Indeed, women are less likely to succeed in smoking cessation without intervention than men (e.g. Gritz et al., 1998; Perkins, 2001; Ward et al., 1997) and have more trouble with smoking cessation via methods that do work in men such as nicotine replacement therapy (Perkins et al., 1999, 2001). This would suggest that there is a possibility that a therapeutic approach of smoking cessation, aimed at stress-reduction or mood enhancement would work better in women. Studies in which anti-depressive or anxiolytic medication was used to aid smoking cessation show support for this suggestion (Glassman et al., 1988, 1993).

In line with the hypothesis, persons from high anxious depressed families were more often current smokers or ex-smokers than persons from low anxious depressed families. Interestingly, persons who did not have a diagnosis of lifetime depression but were from families with a positive history of depression were more likely to smoke or be an ex-smoker than persons who had a negative family history for depression. This could indicate that coming from a family with a positive family history for depression increases vulnerability for smoking initiation. It is important to note, however, that the non-depressed siblings from families with a positive family history still had more

often a high anxious depression score than non-depressed siblings from families with a negative family history for depression.

The between-family results suggest that there is a familial factor causing depression and smoking to be associated. However, the nature of the familial factors is unclear, and it is possible that the results are caused by population stratification. For instance, social economic class differences may cause smoking and depression to cluster in families. This issue of population stratification may be resolved by examining smoking status of siblings who are discordant for depression. If there is a common aetiology related to factors within the family, then sibpairs discordant for anxious depression will also be discordant for smoking. However, the results of the present study did not support this hypothesis. Whether discordant for anxious depression levels or history of lifetime depression, siblings did not differ in smoking behaviour. It is possible that factors underlying both depression and smoking initiation contribute to the smoking status in families that are vulnerable for depression. A study by Fleming et al. (2002) reported that next to depression the family environment was one of the most important predictors of smoking initiation. This is in line with results of Boomsma et al. (1994) which showed a genetic factor to be involved in smoking initiation, but even a stronger shared environmental factor. In the present study, the higher prevalence of smoking in the families with depressed siblings may be caused by cultural transmission of smoking initiation by the depressed sibling to the non-depressed sib. As results showed, coming from a family in which there already is a depressed sibling, may be a risk-factor for smoking initiation. However, it is possible that the within family analysis is not be the most optimal design to examine an association between two variables that might have a common genetic or environmental aetiology. Siblings selected to be discordant for depression may resemble each other too much with respect to shared environmental factors to detect an association between depression and smoking (Posthuma et al., submitted; Witte, Gauderman and Thomas, 1999). The present results therefore suggest that there is a common aetiology for smoking and depression but cannot exclude alternative explanations.

In conclusion, anxious depression or life-time depression according to DSM-IV criteria was related to higher levels of smoking in both men and women. Depressed women were, however, more often current smokers and less ex-smokers than depressed men. This suggest that women, more than men, smoke to relieve depressive symptoms and smoking cessation therapy should therefore consider sex differences. Persons from families with a high anxious depression level or with a family history of lifetime depression were more often smokers than persons from families with low scores or without a family history of depression. This suggests an underlying familial factor in depression and smoking. No differences in smoking behaviour were found in sibpairs discordant for depression, limiting conclusions regarding the nature of the familial factors involved in the association between depression and smoking.

[1]Chapter 5

Netherlands Twin-family Study of Anxious Depression (NETSAD)

Abstract

In a longitudinal study of Dutch adolescent and young adult twins, their parents and their siblings, questionnaire data were collected on depression, anxiety and correlated personality traits, such as neuroticism. Data were collected by mailed surveys in 1991, 1993, 1995 and 1997. A total of 13,717 individuals from 3344 families were included in the study. To localize quantitative trait loci (QTLs) involved in anxiety and depression, the survey data were used to select the most informative families for a genome-wide search. For each subject a genetic factor score was computed, based on a genetic multivariate analysis of anxiety, depression, neuroticism and somatic-anxiety. A family was selected if at least 2 siblings (or DZ twins) had extreme factor scores. Both discordant (high-low) and concordant (high-high and low-low) pairs were included in the selected sample. Once an extreme sibling pair was selected, all family members (parents and additional siblings of the selected pair) who had at least once returned a questionnaire booklet were asked to provide a DNA sample. In total, 2724 individuals from 563 families (1007 parents and 1717 offspring) were approached and 1975 individuals from 479 families (643 parents and 1332 offspring) complied by returning a buccal swap for DNA isolation. All offspring from selected families were asked to participate in a psychiatric interview and in a 24-hour ambulatory assessment of cardiovascular parameters and cortisol. The interview consisted of the WHO-Composite International Diagnostic Interview and was administered to 1253 offspring. In this paper we describe the genetic-epidemiological analyses of the survey data on anxiety, somatic-anxiety, neuroticism and depression. We detail how these data were used to select families for the QTL study and discuss strategies that may help elucidate the molecular pathways leading from genes to anxious depression.

[1] This Chapter is based on the paper published in *Twin research* (2000) 3:323-334

5.1 Introduction

There is extensive evidence that a common gene, or a set of genes, underlies much of the genetic variation in anxiety and depression in humans (Jardine, Martin, & Henderson, 1984; Martin et al., 1988; Kendler et al., 1986; Kendler et al., 1992). For an analogous trait in mice, emotionality, several quantitative trait loci (QTLs) have been located on mouse chromosomes 1, 10, 12 and 15 (Flint et al., 1995; Mathis et al., 1995; Caldarone et al., 1997; Gershenfeld et al., 1997; Gershenfeld & Paul, 1997) and mapped to a 0.8 cM region on mouse chromosome 1 (Talbot et al., 1999) and a 1.4-3.2 cM region on mouse chromosome 15 (Turri et al., 1999). These findings in the mouse can be used as a starting point to search for the genes that underlie the genetic susceptibility for anxious depression in humans through linkage and association studies. Genetic association studies can be carried out with positional or functional candidate genes identified in the mouse. Likewise, linkage studies in humans may be carried out with positional markers from syntenic regions from the mouse. Because of the structural and functional homologies between human and animal genomes, and because pathways are often highly conserved, syntenic regions from animal research are primary candidates to be tested in human studies (Strachan & Read, 1999; Gershenfeld & Paul, 1998; Tarantino & Bucan, 2000).

In this paper we describe a large study of anxious depression in Dutch twin-families that has been going on since 1991. First, a summary is given of the genetic-epidemiological analyses of the twin-family data on anxiety, neuroticism, somatic-anxiety and depression. Next, we outline the selection strategies that were used to obtain a sub-sample of twin-families who are most informative for carrying out linkage and association studies to localize the genes that underlie the susceptibility for anxious depression. Information on the number of individuals currently available for gene-finding is provided. Finally, strategies are discussed that may help in elucidating the molecular pathways leading from DNA to depression, once a gene has been found.

Linkage approaches that have been developed to map quantitative trait loci for complex traits in humans, are often based on identification of marker alleles that are inherited identical-by-descent (IBD) in siblings. These methods suppose that if a DNA marker is cosegregating with a (quantitative) trait, then siblings whose trait values are more similar, are more likely to receive the same alleles identical-by-descent at a closely linked marker locus than siblings who resemble each other less for the trait (Haseman & Elston, 1972). However, even with the large numbers of highly polymorphic markers that are currently available, the power to detect loci that influence complex traits in humans is low (Blackwelder & Elston,1982). Because of the low power to detect QTLs in humans, we propose to use a combination of strategies for the collection and analysis of data to attain a higher power. Rather than assessing anxious depression as a dichotomy (i.e. affected / unaffected), indices of anxious depression and associated personality traits such as neuroticism are measured on quantitative scales. These correlated phenotypes are analysed with multivariate genetic models to establish to what extent they are influenced by a common set of genes. Based

on these results, the data are summarised into a genetic factor score for each subject, which gives an estimate of an individual's genotypic value for anxious depression. To select families for genotyping, the distribution of genetic factor scores is used to identify sibling pairs with extreme scores from both tails of the distribution. These sibling pairs, their parents and their additional siblings are genotyped to obtain an estimate of IBD status among the offspring (Kruglyak & Lander, 1995). The complete distribution of phenotypic and genotypic data within selected and unselected sibships is then analysed using a structural equation modeling approach (Eaves, Neale & Maes, 1996; Fulker & Cherny, 1996; Dolan & Boomsma, Neale, 1999). When available, longitudinal data are used to carry out the linkage analyses.

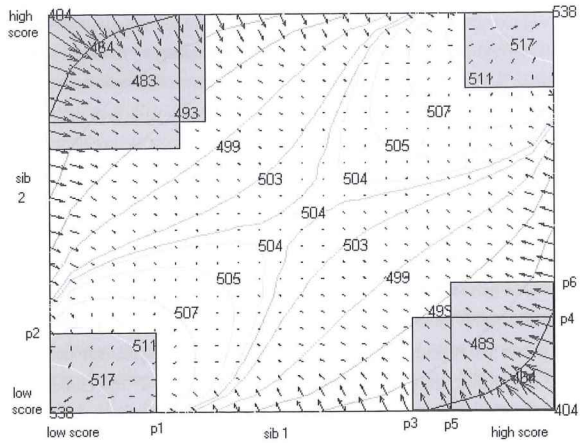
Eaves and Meyer (1994) and Risch and Zhang (1995) have recommended to select sib pairs for genotyping who score extreme (high/high, low/low, low/high or high/low) on a quantitative scale of interest. We have carried out extensive simulation studies (Dolan & Boomsma, 1998) to derive optimal selection percentages for linkage analysis of a QTL in sibpairs from random samples. From these simulations, the optimal criteria were to select concordant sibpairs whose members both have scores in the top 12% or in the bottom 12% of the phenotypic distribution and discordant sibpairs whose members have scores in the top 20% and the bottom 20% of the phenotypic distribution. Simulations suggested an 'asymmetrical' criterion for discordant sibling pairs (high scoring Ss from the upper 25% with low scoring Ss from the lower 20% of the distribution or vice versa). In Figure 1 the selection rules for our study are depicted graphically, based on a hypothetical bi-allelic, codominant QTL that explains 10% of the phenotypic variance; the residual background correlation between siblings is 0.25. Numbers in the plot represent the proportion (π) of alleles shared identical-by-descent (IBD/2) by siblings. Very discordant sibpairs have π values between 0.404 and 0.493; highly concordant low scoring sibpairs have π values between 0.511 and 0.538. Figure 1 shows the changes in π as a function of the phenotypic distributions of two siblings. The length of the arrows indicates the gradient of the change in π. The contour lines are lines of equal π. The gray areas represent the areas from the phenotypic distribution from which sib pairs were selected.

If the selected sibpairs with extreme scores had additional siblings with complete phenotypic information, these siblings were included in the QTL study. Larger sibships provide dramatic increases in power over size-2 sibships. Simulation studies (Dolan, Boomsma & Neale, 1999) indicate that, even in unselected families, a size-3 sibship is on average 3 times and a size-4 sibship 6 to 7 times as informative as a size 2-sibship.

Selection of extreme sibpairs was based on the individual genetic factor scores, because they optimally combine multiple trait information from genetically correlated phenotypes. The procedure to construct these factor scores has been described in detail (Boomsma, Molenaar & Orlebeke, 1990) and the application in linkage analyses has been shown to result in appreciable increases in

power (Martin, Boomsma & Machin, 1997; Boomsma & Dolan, 1998; Boomsma & Dolan CV, 2000).

Figure 5.1 Plots showing the changes in π (the proportion of marker alleles shared IBD) as a function of the sib 1 – sib 2 phenotypic scores, based on a codominant bi-allelic QTL that explains 10% of the phenotypic variance. The background correlation in siblings is 0.25 and the QTL allele frequency is 0.5. The length of the arrows indicates the gradient of the change in π. The contour lines, drawn at arbitrary intervals, are lines of equal π. The dark grey areas represent the parts of the distribution from which sibpairs were selected. These areas depend on the selection percentages p1 to p6. In the present study p1=p2=0.12 (concordant low - low), p3=0.75, p5=0.80, p4=0.20, and p6=0.25 (discordant low-high). Selection percentages for concordant high-high sibpairs were p1=p2=0.88 (i.e., 1-0.12). Selection percentages of the discordant high-low sibpairs were p3=0.25, (1-0.75), etc.



5.2 Methods

5.2.1 Participants

Families of adolescent and young-adult twins were recruited in 1990/1991 by asking city councils in The Netherlands for addresses of twins aged 13-22 years. There were 252 city councils that supplied 4036 addresses. In 1993 additional addresses were obtained for 1987 twin families. The new addresses included several of the larger cities in The Netherlands. In addition, a number of (mostly adult) twin pairs volunteered throughout the study period for registration with the Netherlands Twin Register (NTR) and were included in the 1997 and 2000 surveys.

Questionnaires on health and lifestyle were sent in 1991 to 2375 families (out of 4036) who had indicated that they were willing to participate in a survey study. Twins and both their parents received a 22-page booklet with personality and psychopathology inventories, and questions about health, demographic background and lifestyle. Completed questionnaires were obtained from 6529 individuals from 1697 families. There were 1471 complete families (father, mother and both twins), 167 families consisting of mother and both twins, 26 families consisting of father and both twins and 33 families in which only the twin pair returned a questionnaire.

A second booklet (18-pages) was sent in 1993 to 6023 families (including the 1987 new addresses and including all families who did not respond to the first request). Completed questionnaires were obtained from 7592 individuals from 1974 families; 959 families participated for the second time; 877 families came from the new addresses; 138 families were also contacted before in 1991 but had not responded at the time.

The number of complete families was 1727 (both parents and both twins), 200 families consisted of one parent and both children (176 mothers, 24 fathers), in the remaining 47 families there were 14 twin pairs and 33 other combinations of parents and offspring.

For the third wave of data collection, questionnaires (12-pages) were sent by the end of March 1995 to the 2712 families that participated in the first and/or second wave.

This time the data-collection included two questionnaires per family for siblings of the twins, if present. Questionnaires were returned by 8175 individuals from 1727 families

(3408 twins, 1500 siblings, 1577 fathers and 1690 mothers).

For the fourth wave all families in the NTR with twins aged 12 years or older were initially approached with a request to take part in the 1997 survey, even if they had not complied on previous occasions. Parents were asked how many additional siblings of twins would be willing to fill out a questionnaire and to provide the names and address of the siblings. Of the families that were approached, 2773 supplied a form with twin/sib information and these offspring were sent a 20-page questionnaire. A total of 7989 individuals (5546 twins and 2443 additional siblings), but not their parents, was included in the study at this occasion. For the first time, questionnaires were not sent to the parental address for all participants, but to individual twins and siblings. This

resulted in a much larger number of twins who returned the questionnaire without their cotwin. A completed questionnaire was received from 4585 individuals from 1965 families (3141 twins and 1444 siblings). There were 530 questionnaires from single twins. The number of participating siblings per family varied between 0 and 8. In 785 families one additional sibling, in 199 families two additional sibs and in 69 families three or more sibs sent back the survey.

A fifth (18-page) questionnaire has been distributed in May 2000 to 22,374 individuals (13,723 twins, 105 triplets, 2917 sibs and 5629 spouses/partners of twins over age 25) from 6914 families.

Selection of extreme sibling pairs for inclusion in the QTL study was based on the survey data collected in 1991, 1993 and 1997. In 1995 the YASR was included only in the survey of twins and not of their siblings. The fifth-wave data collection has just begun. Although the data collected in 1995 and 2000 could not be used for selection purposes, they will be used in the proposed linkage and association analyses, as the longitudinal information greatly enhances statistical power for QTL detection.

5.2.2 Instruments

Each survey collected abundant information on lifestyle including smoking and exercise status, alcohol use and abuse (Boomsma, 1994; Koopmans, Doornen & Boomsma, 1997), health, demographics, SES, religion (Boomsma, 1999), personality and psychopathology. Table 1 lists the measures of personality and psychopathology that were collected in each of the five surveys.

The 13-item version of the Beck Depression Inventory (BDI; Beck, 1961) and the anxious/depression symptom scale of the Young-Adult Self Report (YASR; Achenbach, 1990) were used to assess depression. The YASR consists of 7 additional scales, which measure different syndromes. Neuroticism, somatic-anxiety, extraversion, and test-attitude are part of the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970). The item content of the ABV neuroticism and extraversion scales is very similar to those from the Eysenck Personality Questionnaire. Dutch translations of the Cognitive Failures Questionnaire (CFQ; Broadbent, 1982), the Jenkins Activity Survey (JAS; Jenkins, Rosenman & Zyzanksi, 1974), the Spielberger State Anxiety Inventory (STAI; Spielberger, 1970) and the Trait Anger scale (Spielberger, 1983) were used to obtain measures of respectively every-day cognitive failures, type-A behaviour, which is thought to reflect coronary prone behaviour, anxiety and anger/hostility. The four dimensions of Sensation-Seeking behaviour were measured with the Zuckerman (1971) Sensation Seeking scales.

Table 5.1 Inventories used to assess Personality and Psychopathology in twin-families in NETSAD

	I	II	III	IV	V
Beck Depression Inventory	-	x	-	x	-
ABV: Neuroticism	x	x	-	x	X
ABV: Extraversion	x	x	-	x	X
ABV: Somatic anxiety	x	x	-	x	X
ABV: Test attitude (lie-scale)	x	x	-	x	X
Spielberger Trait Anxiety	x	x	-	x	X
Spielberger Trait Anger	x	X	-	-	-
Zuckerman Sensation Seeking	x	x	-	x	X
(Boredom Susceptibility, Disinhibition, Experience and Thrill and Adventure Seeking) Jenkins Activity Survey (Type-A behaviour)	x	-	-	-	-
Cognitive Failure Questionnaire	X	-	-	-	-
Phobia	-	-	-	X	X
YASR: Young Adult Self Report (Anxious/Depressed, Somatic Complaints, Withdrawn, Delinquent and Aggressive Behaviour, Social-, Thought-, and Attention Problems)	x	-	X	X	X
Burn-out	-	-	-	-	X
Traumatic life-events	-	-	-	-	x

I = 1991: twins and parents; II = 1993: twins and parents; III = 1995: twins, parents and siblings (YASR only for twins); IV = 1997: twins and siblings; V = 2000: twins, partners and siblings

5.2.3 Statistical analyses

Principal Components Analysis (PCA) of the combined 1991 and 1993 data on YASR- and BDI-depression, anxiety, anger, type-A behaviour, neuroticism, extraversion, somatic-anxiety, test-attitude and the Sensation Seeking scales established three major components in the data, which together explained around 55% of the variance. The first component, which explained 29% of the variance, was characterised by high loadings of anxiety, neuroticism, somatic-anxiety and both depression scales. The second component (17% of the variance) consisted mainly of the four Sensation Seeking scales and the third component (9% of the variance) of type-A behaviour, extraversion and trait anger. PCA of the data collected in 1997 almost exactly replicated these results. Three components were found that explained 65% of the variance. Variables loading on the first component (which explained 34% of the variance) again were anxiety, neuroticism, somatic-anxiety and YASR and BDI depression. These phenotypes were used to select families for the QTL study and are described in this paper.

5.2.4 Genetic Modeling

In order to establish the genetic architecture of the variables that clustered together phenotypically, multivariate genetic models were fitted to the twin data collected in 1991, 1993 and 1997. First, a full model (Choleski decomposition; Neale & Cardon, 1992) which specified additive genetic (A), Common environmental (C), and unique Environmental (E) sources of variation and covariation was evaluated. This model was fitted to the data with and without sex differences in the relative contributions of the genetic and environmental influences. Next, the significance of genetic and common environmental influences in explaining family resemblance was tested, by constraining each of these influences to have a zero contribution to family resemblance. Finally, the dimensionality of the genetic and environmental influences was explored by fitting a one-factor structure to genetic and environmental influences. These models test whether a common set of genes (or environments) influence all traits. Model fitting was carried out in Mx, using maximum-likelihood estimation (Neale, 1997). Testing of submodels was done by likelihood-ratio tests, by subtracting the chi-square for the more restricted model from the chi-square for the more general model.

5.2.5 Factor Scores

The results from the multivariate genetic analysis were used to compute a genetic factor score for each subject in the study, which represents the individual's value on the common genetic factor and can be interpreted as an estimate of an individual's genotypic value for anxious depression. For each individual a genetic factor score (**F**) was obtained according to:

$$\mathbf{F} = \mathbf{B}'\mathbf{P},$$

where ' denotes transpose, **B** is a (p x 1) vector of weights that is constant across subjects and depends on the loadings of the variables on the genetic factor and on their unique genetic variances. **P** (p x 1) is the vector of phenotypes of the individual (p is the number of variables measured on each subject). There are several estimators (which are perhaps more accurately described as predictors) of factor scores (Lawley & Maxwell, 1971; Saris, Pijper & Mulder, 1978). We used the regression method, which was first recommended by Thurstone (1935). The regression methods obtains the weight matrix **B** by minimizing the sum of squares of the difference between estimated and true factor scores. This method is equivalent to finding the linear regression of factor scores on phenotypes. Thompson (1951) has given a derivation for the weight matrix **B**. Equivalent estimators of unobserved random effects have been used in other contexts. In animal breeding the estimator is known as the best linear unbiased predictor (Robinson, 1991). In the construction of the genetic factor scores only phenotypic information from the individual subject was used, and not from other family members.

The weight matrix then is obtained as:

$$\mathbf{B}' = \lambda'\Sigma^{-1}$$

where the (p x 1) vector λ contains the factor loadings of the phenotypes on the common genetic factor and Σ (p x p) is the population covariance matrix.

All scales were transformed before genetic analyses were carried out, using a natural logarithm (Anxiety (20 items): 10ln(Anx); Neuroticism (30 items): 5ln(Neu); Somatic-anxiety (17 items): 9ln(SoA); YASR depression (16 items): 12ln(Ydep); or an arcsine transformation (BDI (13 items): (arcsine(BDI/max score)**0.5)*10). The arcsine transformation does little to render the distribution of the BDI data normal, but substantially reduces kurtosis.

5.2.6 DNA Collection

DNA was collected through the mail. A mouth swab procedure was used (Meulenbelt et al., 1995). Subjects received a test-kit with detailed instructions (photographs). They collected buccal swabs 3 times during the day and sent back their samples in a prepaid envelope. This procedure of DNA collection has been shown to be suitable for large-scale genotyping with markers in the Weber 8/8a set in our laboratory. DNA samples have recently (1999) also been tested successfully in the Marshfield laboratories.

5.2.7 Psychiatric Interview Data

All offspring from selected families were asked to participate in a telephone interview during which several sections from the WHO Composite International Diagnostic Interview (CIDI) were administered (Peters L, Andrews, 1995; Wittchen, 1994). The CIDI is a fully standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 and DSM-IV. We employed the computer-administered lifetime version (2.1) The following sections were administered: Demographics (section A); Social Phobia, Agoraphobia and Generalized Anxiety Disorder (D33 and further); Depression and Dysthymia (E); Mania Screen and Bipolar Affective Disorder (F) and Obsessive-compulsive disorder (K1-22).

5.2.8 Physiological parameters

Selected offspring were also invited to take part in ambulatory measurement of cortisol and cardiovascular parameters. The VU-AMS device (De Geus et al., 1995; Willemsen et al., 1996) is used for continuous 24-hour registration of the electrocardiogram and the impedance cardiogram. These registrations are used to derive indices of vagal tone (RSA: respiratory sinus arrthymia) and

cardiac sympathetic drive (PEP: pre-ejection period). This part of the study is still in progress. Subjects are visited at home during a working day and registration of physiological signals begins in the morning. Blood pressure is measured every 30 minutes during the day and salivary cortisol is measured 6 times during the 24-hour period.

5.3 Results

5.3.1 Descriptives

There were 13,717 subjects from 3344 families who took part in the study at least once. The subjects consisted of 6426 twins, 2200 siblings of twins and 5091 parents (2453 fathers and 2638 mothers). Table 2A lists the longitudinal response rates for these groups (note that parents could not participate more than 3 times and siblings not more than once or twice as their participation was requested for the first time in 1995). The results reported below are limited to the anxiety, depression, neuroticism and somatic-anxiety data collected in the twins and their siblings in 1991, 1993 and 1997, except when we report on the longitudinal correlations for these variables and also include the YASR-depression data collected in 1995.

In Table 3 average scores and standard deviations for anxiety, neuroticism, somatic-anxiety and depression are presented for males and females who participated in 1991, 1993 and 1997. The data are pooled over zygosities and over twins and siblings, since no effect was found on average scores of either zygosity or of being a twin. Women had higher scores than men for all variables (except age) listed in Table 3. The average age of the total sample in 1993 was almost equal to the average age in 1991, because roughly 50% of the sample in 1993 consisted of new families, with on average somewhat younger twins. In 1997, 36% of the subjects (twins and siblings) participated for the first time in the study; the average age of these who had participated at least once before was 21.9 years.

Cross-sectional and longitudinal correlations between variables are given in Tables 4A, 4B and 4C (the longitudinal correlations for depression in Table 4C also include the 1995 YASR-depression scores). Within each occasion, the correlations between age and the phenotypic scores were low and not statistically significant. Most cross-sectional and longitudinal correlations were somewhat higher in females. Cross-sectionally, somatic-anxiety showed the lowest correlations with the other scales. Correlations among the other scales varied between 0.5 and 0.77. The longitudinal correlations were between 0.4 and 0.5 for the longest interval (1991-1997) and around 0.6 for the 2-year intervals.

Table 5.2 Number of times families and individuals participated in the survey study (note that not all subjects could participate 4 times)

	N of families	N of individuals: Parents	twins	sibs
A: Longitudinal participation: for entire sample				
Once	1259 (38%)	1525 (30%)	2428 (38%)	1456 (66%)
Twice	687 (20%)	2110 (41%)	1448 (23%)	744 (34%)
Thrice	862 (26%)	1456 (29%)	1697 (26%)	-
Four times	536 (16%)	-	853 (13%)	-
B: Longitudinal participation for selected families who returned a buccal swap for DNA isolation				
Once	120 (24%)	86 (13%)	193 (23%)	246 (52%)
Twice	52 (10%)	221 (34%)	83 (10%)	230 (48%)
Thrice	142 (29%)	336 (52%)	261 (30%)	-
Four times	183 (37%)	-	319 (37%)	-

Table 5.3 Means and Standard Deviations for males and females for Anxiety, Neuroticism, Somatic-Anxiety and Depression (YASR and Beck Depression Inventory)

	1991		1993		1997	
	males	Females	males	Females	Males	Females
N	1545	1848	1733	2149	1848	2727
Anx	33.2 (7.7)	34.9 (8.3)	31.9 (7.4)	34.3 (8.7)	30.3 (7.7)	33.6 (9.3)
Neu	52.5 (21.7)	61.9 (23.1)	46.9 (21.6)	55.2 (23.8)	40.5 (21.1)	50.5 (24.4)
SoA	18.3(5.0)	19.6 (5.7)	17.9 (5.0)	19.1 (5.5)	16.5 (4.5)	18.0 (5.4)
Dep	19.9 (3.7)	21.5 (4.6)	-	-	19.5 (3.5)	21.6 (4.7)
BDI	-	-	1.2 (2.1)	1.7 (2.7)	1.3 (2.2)	2.0 (2.9)
Age	17.7 (2.3)	17.7 (2.3)	17.8 (3.1)	17.9 (3.1)	25.8 (10.3)	26.5 (10.1)

Table 5.4

	1991					1993					1997				
A: Cross-sectional Correlations among Anxiety, Neuroticism, Somatic-Anxiety and Depression (measured with YASR depression scale and BDI)	Anx	Neu	SoA	Dep	BDI	Anx	Neu	SoA	Dep	BDI	Anx	Neu	SoA	Dep	BDI
Anx	-	.67	.47	.65	-	-	.64	.43	-	.63	-	.70	.48	.69	.63
Neu	.72	-	.56	.62	-	.71	-	.60	-	.50	.77	-	.58	.65	.55
SoA	.48	.55	-	.46	-	.50	.59	-	-	.38	.55	.60	-	.45	.45
Dep	.73	.66	.44	-	-	-	-	-	-	-	.77	.74	.52	-	.60
BDI	-	-	-	-	-	.68	.55	.47	-	-	.70	.59	.52	.65	-

B: Longitudinal Correlations

	1991	1993	1997	1991	1993	1997	1991	1993	1997
1991	-	.60	.41	-	.58	.49	-	.47	.44
1993	.62	-	.51	.65	-	.61	.61	-	.40
1997	.44	.53	-	.50	.58	-	.43	.48	-

C: Longitudinal Correlations for YASR and BDI Depression scales

	Yde91	BDI93	Yde95	Yde97	BDI97
1991 YASR-Dep	-	.37	.45	.36	.33
1993 BDI	.44	-	.45	.41	.50
1995 YASR-Dep	.56	.49	-	.58	.42
1997 YASR-Dep	.47	.38	.65	-	.60
1997 BDI	.19	.33	.40	.65	-

Males upper diagonal, females lower diagonal

5.3.2 Genetic modeling

Table 5 gives the twin correlations for the 1991, 1993 and 1997 data for the variables that make up the anxious depression dimension. Correlations for all phenotypes were higher in monozygotic twins than in dizygotic twins. As is suggested by the correlations, the heritability of anxiety, depression, neuroticism and somatic-anxiety was around 50% in univariate genetic analyses. The best fitting models for these data indicated that familial resemblance must be attributed entirely to shared genes and not to shared environment. For all traits, a higher heritability in females than in males was found in univariate analyses. This higher heritability was explained by a higher genetic variance in females than in males. There was no evidence, however, that different genes influenced anxiety and depression in males and females. These results for the genetic architecture of anxiety, depression and neuroticism are very similar to results obtained in Australian (Kendler et al., 1986) and American twin studies (Kendler et al., 1992; Kendler, 1993).

Table 5.5 Twin correlations for Anxiety, Neuroticism, Somatic-Anxiety and Depression (measured with YASR depression scale and BDI);

	1991					1993					1997					
	N	Anx	Neu	SoA	Dep	N	Anx	Neu	SoA	BDI	N	Anx	Neu	SoA	BDI	Dep
MZM	273	.53	.45	.39	.41	327	.55	.48	.45	.43	196	.52	.51	.46	.48	.57
DZM	259	.26	.26	.15	.14	284	.32	.38	.33	.24	126	.16	.24	.17	.08	.07
MZF	365	.54	.57	.46	.51	457	.56	.61	.51	.55	328	.51	.54	.46	.36	.50
DZF	317	.27	.27	.21	.24	356	.37	.38	.31	.31	256	.27	.27	.21	.21	.32
DOS	483	.20	.23	.20	.23	543	.17	.22	.15	.16	288	.24	.17	.09	.12	.28

Goodness-of-fit chi-squared statistics for the multivariate genetic analyses of the twin data on anxiety, neuroticism, somatic-anxiety and depression data collected in 1991, 1993 and 1997 are summarised in Table 6 (for the 1997 data two series of analyses were carried out: once with YASR-depression and once with BDI-depression scales). These analyses showed very stable results across time: significant sex differences in parameter estimates and no contribution of common family environment to resemblance of family members. Only in the 1993 data set, leaving out the shared environmental variance led to a just-significant increase in chi-square (33.6, critical value is 31.41 for 20 degrees-of-freedom). This contribution of shared environment could not be explained by age. We explored if there was a difference between twin pairs who had participated before in 1991 and those who participated for the first time in 1993, but no differences in parameter estimates between the two groups were found. The amount of variance explained by shared environment, however, was very small and it was decided to use the same multivariate model without C in subsequent computation of factor scores for all measurement occasions. The genetic factor model fitted the data well (again with the possible exception of the 1993 data set). All the genetic covariances among measures could be attributed to a common genetic factor. The environmental covariance structure could not be explained by a common environmental factor.

Exploration of the environmental covariance matrix showed, that the failure of this model mainly was due to somatic-anxiety.

Table 5.6 Multivariate model fitting results for anxiety, neuroticism, somatic anxiety and depression (two series of analyses for 1997 data: with BDI and with YASR depression).

		1991		1993		1997 (B)		1997 (Y)	
	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p
ACE full M≠F	120	136.2	.15	146.5	.05	152.7	.02	154.9	.02
ACE full M=F	150	244.8	.00	243.1	.00	232.7	.00	272.1	.00
AE full M≠F	140	148.9	.29	180.1	.01	159.9	.12	162.4	.09
CE full M≠F	140	246.0	.00	282.1	.00	226.2	.00	228.8	.00
A factor M≠F	**144**	**157.1**	**.21**	**202.3**	**.00**	**167.7**	**.09**	**166.5**	**.10**
E factor M≠F	144	165.6	.10	215.5	.00	181.0	.00	175.4	.04

ACE full is full Choleski decomposition for Additive genetic, Common environmental and unique Environmental sources of variation; M≠F stands for sex differences in parameter estimates; factor models for A or E specify one common factor with specifics. Best model in bold.

Table 7 gives the percentages of variance for males and females explained by the common genetic factor and the specific genetic factors for each variable. The sum of these two percentages gives the total heritability each trait. Consistent with the univariate genetic analyses, these heritabilities are around 50%. More importantly, the largest part of the genetic variance in all phenotypes can be attributed to the common genetic factor. This is a very good starting point for the computation of genetic factor scores.

Table 5.7 Standardized heritability estimates (% of variance explained by common genetic factor and genetic specifics) for males and females in 1991, 1993 and 1997; based on multivariate genetic model in which genetic influences are modelled as a common factor and influences specific to each trait. For the 1997 data two series of analyses were carried out, once with BDI depression and once with YASR depression.

	Females 1991		Males 1991	
	G-factor	G-unique	G-factor	G-unique
Anx	0.48	0.04	0.41	0.10
Neu	0.44	0.12	0.38	0.07
SoA	0.28	0.15	0.23	0.16
Dep	0.40	0.09	0.32	0.11
	Females 1993		Males 1993	
	G-factor	G-unique	G-factor	G-unique
Anx	0.49	0.06	0.38	0.13
Neu	0.52	0.10	0.41	0.06
SoA	0.35	0.15	0.22	0.21
BDI	0.32	0.19	0.30	0.09
	Females 1997		Males-1997	
	G-factor	G-unique	G-factor	G-unique
Anx	0.48	0.06	0.39	0.05
Neu	0.44	0.12	0.34	0.09
SoA	0.29	0.15	0.21	0.11
BDI	0.34	0.08	0.35	0.10
	Females 1997		Males-1997	
	G-factor	G-unique	G-factor	G-unique
Anx	0.46	0.07	0.42	0.03
Neu	0.45	0.10	0.33	0.10
SoA	0.28	0.17	0.20	0.11
Dep	0.46	0.07	0.35	0.11

5.3.3 Computation of genetic factor scores

Genetic factor scores were calculated as a weighted sum of the observed phenotypes (after transformation) for each individual. Weights were estimated separately across occasions based on the multivariate genetic analyses presented above. Because the genetic factor model did not differ across occasions, the weights were averaged across time points, thus making the factor scores comparable across time. Factor scores were computed separately for males and females and also depended on whether the BDI or YASR depression scale was used in their construction. Using this approach the following formula obtained for males and females:

Males: **F** = 0.144 x Anx + 0.117 x Neu + 0.039 x SoA + 0.064 x Ydep
Males: **F** = 0.130 x Anx + 0.077 x Neu + 0.040 x SoA + 0.166 x BDI

Females: **F** = 0.133 x Anx + 0.117 x Neu + 0.066 x SoA + 0.053 x YDep
Females: **F** = 0.146 x Anx + 0.086 x Neu + 0.077 x SoA + 0.062 x BDI

For males, there is much larger contribution of the BDI to the construction of their genetic factor scores, whereas for females contribution of somatic-anxiety is relatively larger than in males. The contributions of anxiety and neuroticism are very similar in both sexes. Twin correlations for factor scores (averaged over across occasions) were 0.61 for MZM, 0.64 for MZF, 0.33 for DZM, 0.40 for DZF, and 0.28 for DOS twins.

5.3.4. Selection

The equations for the computation of genetic factor scores were applied to the twin data collected in 1991, 1993 and 1997 and the sibling data from 1997. The total number of families with at least 1 sibling pair with extreme genetic factor scores (concordant high-high, low-low or discordant high-low) was 563. These families were approached to participate in a search for QTLs influencing anxious depression. Selection was initially within each measurement occasion (i.e. 1991, 1993 and 1997) and additionally also on sibpairs who were extreme concordant or discordant across occasions. Once a family was selected, all family members (parents, all twins and siblings who had at least once returned a questionnaire) were asked to supply a DNA sample. This request included MZ twin pairs, in which one (or both) of the twins formed an extreme pair with an additional sibling. In total, 2724 subjects (1007 parents and 1717 offspring) were approached and currently 1975 (643 parents and 1332 offspring) complied by returning a buccal swap for DNA isolation. The average yield from buccal swaps was 25.8 micrograms of DNA (SD = 18.8). The offspring who were approached with the question to take part in the QTL study consisted of 374 MZ twins, 752 DZ twins and 591 sibs. The number of DNA samples returned by these 3 groups was 336, 520 and 476, respectively. The return rate of buccal swap samples did not differ significantly among those scoring in the high tail, the low tail or in the middle of the factor score distribution (67%, 70% and 74%, respectively, p = .12). Figure 2 gives the distribution of genetic factor scores (averaged over time) in the entire offspring sample, in the selected-offspring sample, and in the sample that returned a DNA sample. Factor scores in the entire sample showed a normal distribution (mean: 0.03, standard deviation: 0.71, skewness: 0.38 (0.03) and kurtosis: 0.0 (0.056)). There was a large effect of selection on the distribution of factor scores (mean: 0.04, SD: 0.88, skewness: 0.28 (.06) and kurtosis: -0.81 (.12) in the selected sample and mean: 0.02, SD: 0.83, skewness: 0.36 (.06) and kurtosis: -0.61 (.12) in the sample that returned a buccal swap). The effect of selection is evident in the significant kurtosis of the factor scores: in selected families many more offspring are in the tails of the distribution. The subjects in the middle of the distribution mainly are the additional twins and sibs from larger families, who were invited to participate with the extreme sibling pair in the family, because large sibships provide an increase in power over size-2 sibships (Dolan & Boomsma, 1999). For the families who returned a DNA sample, Table 8 present an overview of family size (between 1 and 10 offspring per family; except in the second row MZ twins are counted as 1 genotype) and of the number of families with extreme offspring (between 0 and 6 extremes

per family). The extreme individuals per family are grouped according to the concordant criterion (top or bottom 12% of the distribution) or discordant criterion (scores in the top 20% and bottom 25% or vice versa). For example, in the 43 families in which 4 offspring returned a DNA sample, there are 2, 3 or 4 extreme sibs according to the concordant criterion in respectively 18, 12 and 2 families. According to the discordant criterion the number of families with 2, 3 or 4 extreme sibs is 12, 22 and 7, respectively. As Table 8 shows, there were 379 families in which at least 2 siblings, who were not MZ twins, who returned material for DNA isolation. The average sibship size in these families was 2.8. Table 2B summarises the longitudinal response pattern of the subjects who returned a mouth swap for DNA isolation.

Figure 5.2 Distribution of genetic factor scores in the entire offspring sample (factor scores averaged over measurement occasions), in the entire selected offspring sample and in the offspring sample that participated in the QTL study.

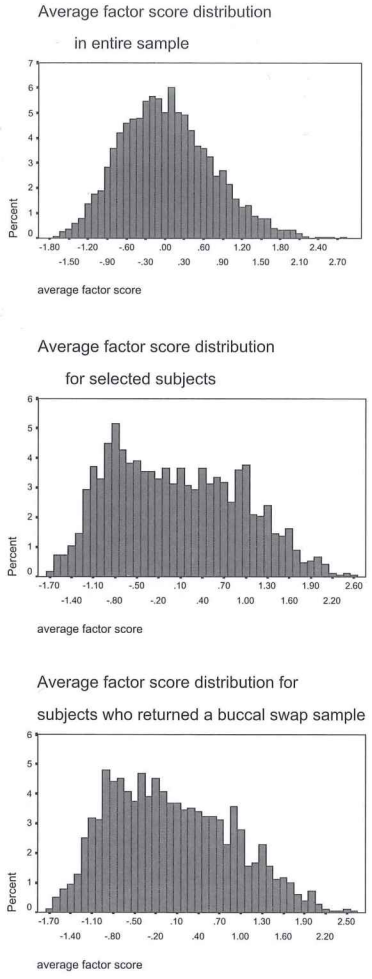


Table 5.8 Family composition for offspring who returned a DNA sample. Families are cross-classified by number of siblings (rows) per family and number of extreme siblings (columns) within a family (x / y refer to numbers of families for concordant (x) and discordant (y) criteria).

Family size (sibs)		Number of extreme Ss (according to concordant and to discordant criteria)						N of families	N of Ss	N of sibpairs
		0	1	2	3	4	>4			
1	Subject	28/16	35/47	-	-	-	-	63	63	-
2	Ss (MZ)	4/1	11/8	40/46	-	-	-	55	110	-
2	Ss	16/2	71/34	104/155	-	-	-	191	382	191
3	Ss	6/2	45/15	55/57	20/52	-	-	126	378	378
4	Ss	2/0	9/2	18/12	12/22	2/7	-	43	172	258
5	Ss	-	-	4/2	2/1	5/6	0/2	11	55	110
6	Ss	-	1/0	-	0/1	-	-	1	6	15
7	Ss	-	1/0	1/0	1/1	1/2	1/2	5	35	105
8	Ss	-	-	1/0	0/1	-	-	1	8	28
10	Ss	-	-	-	-	-	1/1	1	10	45
Total								497	1219	1130

Second row: families in which only a MZ pair returned a buccal swap. For another 113 MZ twin pairs with at least one additional sibling, only one twin is included in the Table. The total number of offspring for whom DNA is currently available thus is 1332.

All offspring from selected families were asked if they agreed to being interviewed on the telephone and if they wanted to participate in 24-hour registration of cardiovascular parameters and cortisol. The WHO-CIDI was administered to 1253 offspring. These data were used to obtain DSM-IV (single or recurrent) depression status. Participation in the CIDI interview was related to the factor scores distribution class (73% in the high, 78% in the low, and 69% in the middle of the distribution, p = .01). Of those who returned the buccal swap samples, 87% participated in the interview, as compared to 28% for those who did not return the samples. Currently, we have collected 24-hour profiles for cardiovascular parameters in 454 subjects and for cortisol in 328 subjects.

5.4 Discussion

The aim of our study is to detect the chromosomal regions harbouring the genetic polymorphisms responsible for variation in anxiety and depression. These traits place a large burden on the individual and on society, both in terms of the loss of quality of life and in health care costs. The genetic perspective does not reflect underestimation of the importance of environmental influences. Such influences are clearly demonstrated by our results: they explain about half of the variance in anxiety and depression at all time points. We found that genetic factors accounted for roughly 50% of the variance in anxiety, somatic-anxiety, depression, and neuroticism and that genetic influences accounted for most of the covariance between these traits. The genetic

covariance between measures was due to one common genetic factor. The largest part of the heritability in all anxious depression indices could be attributed to this common genetic factor (Table 6). These multivariate analyses confirmed the large overlap in the genes conveying susceptibility to anxiety, neuroticism, somatic-anxiety and depression, as previously reported by others (Jardin, Martin & Henderson, 1984; Kendler et al., 1992). This common set of genes appears to lead to a very stable genetic architecture for the traits we studied throughout the period from adolescence to adulthood: identical genetic factor structures were found in 1991, 1993, and 1997, with a substantial part of the subjects participating on all three occasions.

The results from the multivariate genetic analyses were applied to the computation of genetic factor scores for each individual in the study. The genetic factor score obtained by combining the various questionnaires is best described as "anxious depression" or more appropriately "genetic susceptibility for anxious depression". The factor scores are based on questionnaire instruments mostly geared towards a "normal" non-clinical population. The additional CIDI data strongly suggest that subjects at the extreme high end of the distribution of questionnaire scores are indeed at risk for depression. In short, the genetic factor scores look like a good starting point for linkage analyses to detect QTLs for anxious depression (Martin, Boomsma & Machin, 1997; Boomsma, 1996).

To increase the power of linkage analyses trying to map the actual genes conveying the genetic susceptibility for anxious depression, offspring with extreme genetic factor scores were selected from both tails of the quantitative distribution. Selection of families was based on the presence of at least 2 siblings in a family with extreme factor scores. Once an extreme pair was identified within a family, all family members
who had participated in the survey study (i.e. parents and additional sibs or twins) were asked to provide a DNA sample. Up until now we have collected 1975 DNA samples from 643 parents and 1332 offspring. In the offspring generation there are 168 MZ twin pairs (usually part of a larger family) who are not informative for traditional linkage analyses. They provide an opportunity, however, to hunt for genes that influence an individual's reactivity to environmental challenges (Martin, 2000) and are extremely valuable in association tests (Miller, 1998), as are parents and families with larger sibships (Abecasis, Cardon & Cookson, 2000).

Deviant functioning of the autonomic nervous system, both with regard to its resting tone and its responsiveness to psychological strain, often accompanies anxiety and depression. All three axes of the autonomic system, the sympathetic-medullary axis (SMA), the parasympathetic nervous system and the hypothalamic-pituitary-axis (HPA) have been implicated (Gold, Goodwin & Chrousos, 1988; Musselman, Evans & Nemeroff, 1998; Sapolsky, Romero & Munck, 2000). The association may reflect causality; disturbed HPA function in particular has been hypothesized as an etiological factor in depression (Nemeroff, 1996). To further increase the power of QTL detection we are collecting extensive data on autonomic nervous system functioning in the

selected subjects. Measures include non-invasive assessment of cardiac vagal and sympathetic drive and 24-hour cortisol profiles. Such endophenotypes can increase power for linkage in three ways: 1) they may be used as phenotypes for linkage instead of the factor scores; by being "closer" to the actual gene effects, some QTLs may explain more variance in the biological markers than in the more complex phenotype of depression; 2) they may be used in combination with the genetic factor scores to define a new multivariate phenotype that encompasses both questionnaire and psychophysiological data, thus providing a converging phenotype from multiple measurement levels; and 3) they may be used to refine the questionnaire data, e.g. a high score on anxious depression with high cortisol may reflect depression, whereas high anxious depression scores with low cortisol may reflect effects of chronic stress or post-traumatic stress disorder (Heim, Ehlert & Hellhammer, 2000). Such distinctions may be difficult to make on the basis of self-report only.

The added advantage of endophenotypes, such as cortisol over increasing power of linkage, is that they can help to elucidate the molecular pathways leading to anxious depression after a gene has been found. This also applies if other candidate genes should derive from parallel research by others or in animal models. In such future follow-up it is particularly important to have access to twin-family samples because the contributions of such candidate genes can be tested against the background of other genetic influences.

Chapter 6

Family study of daytime cortisol profile, anxious depression, and smoking behaviour

Abstract

This study tested the association between depression and daytime cortisol levels in a non-clinical population and a sub-population that was diagnosed according to the diagnostic criteria of the DSM-IV. Most previous studies documenting such a relationship have used clinical samples of depressed patients, often hospitalised. In our study, we contrasted high risk and low risk families as well as high and low scoring siblings within the same family. Since smoking is more prevalent in depressed subjects and nicotine has been shown to stimulate HPA-axis activity, all analyses were corrected for current smoking behaviour.

Repeated daytime salivary cortisol samples were obtained in 338 participants from 173 families. These families were selected from the 3344 twin families participating in the longitudinal Netherlands Twin-family Study of Anxious Depression (NETSAD). Selection was based on the criterion that at least 2 siblings in a family scored extremely high or extremely low on anxious depression. Those siblings could either be discordant (one scoring high and the other scoring low) or concordant (both scoring low or both scoring high) for anxious depression.

A significant association was found between both anxious depression and DSM-IV depression and smoking behaviour. Smokers showed an increase in daytime cortisol level, but a decrease was found in their cortisol level at awakening. Even after controlling for smoking behaviour, anxious depression was unrelated to cortisol level at any of six time points throughout the day, including the awakening peak. The association was lacking in the between-family as well as the within-family analyses.

In conclusion, the often-cited association between depression and hypercortisolism in patient samples is not reflected in an association between anxious depression and cortisol levels in a population-based sample.

6.1 Introduction

Multiple factors contribute to the onset of major depression. These factors include genetic vulnerability (e.g. Kendler et al., 1992; Kendler et al., 1993a; Kendler et al., 1999; Nurnberger and Gershon, 1981) and environmental factors such as adverse life-events and lack of social support (e.g. Heim et al., 1997a; Heim et al., 1997b; Heim & Nemeroff, 1999; Heim et al., 2000; Kaufman et al., 2000; Kendler et al., 1995). The hypothalamic-pituitary-adrenal axis (HPA-axis) seems to be one of the central biological pathways on which these factors converge, and deregulation of this axis has been proposed to causally contribute to the risk for depression (Holsboer, 2000; 2001). The link between HPA-axis deregulation and depression is supported by studies showing elevation of the basal cortisol level (e.g. Scott & Dinan, 1998; Young et al., 2000; Weber et al., 2000), dexamethasone-mediated negative feedback resistance (Evans et al., 1983; Holsboer et al., 1982a,1982b; Holsboer et al., 1984), increased cerebrospinal fluid levels of corticotrophin-releasing factor (CRF) (Charlton et al., 1987; Charlton and Ferrier, 1989; Gold & Chrousos, 1985), and a blunted adreno-corticotrophic hormone (ACTH) response to challenge with exogenous CRF (Kathol et al., 1989; Plotsky et al., 1998). Hypercortisolism has been studied the most, with recent studies showing increased interest in deviant circadian rhythms in addition to an elevation of basal cortisol level per se (Posener et al., 2000; Weber et al., 2000; Wong et al., 2000; Young et al., 2000a). To illustrate the diversity of methodology even in studies focussing on basal cortisol, tables 1a and 1b in Chapter 1 of this thesis display a selection of the many studies on cortisol levels in human subjects.

Depression is known to be a heritable affliction (Boomsma et al., 2000; Kendler, 1993, 2001; Kendler et al., 1986, 1992; Kendler & Prescott, 1999; McGuffin et al., 1991; Sullivan et al., 2000). Mounting evidence now also shows individual differences in daytime cortisol levels to be genetically determined (Bartels et al., 2001; Inglis et al., 1999; Kirschbaum et al., 1992; Linkowski et al., 1993; Meikle et al., 1988; Wüst et al., 2000; Young et al., 2000). This allows for the possibility that the association between cortisol and depression derives from an underlying genetic factor. This is put even more sharply in the dominant hypothesis in this field by Holsboer and co-workers that considers the genetic liability for depression to be conveyed in part by the genetic risk for a disturbed HPA-axis. In favour of this idea, the Munich Vulnerability study (Holsboer et al., 1995; Lauer et al., 1997; Krieg et al., 2001; Modell et al., 1998) compared unaffected first degree relatives of depressive index patients (i.e. healthy subjects at high familial risk for depression) to control subjects without affected family members (i.e. healthy subjects at low familial risk for depression) and to inpatients with major depression. Plasma cortisol response after a combined dexamethasone-CRF challenge was higher in depressed patients compared to the healthy low risk participants. Most importantly, however, the healthy high-risk participants also had higher cortisol levels compared to the healthy low-risk participants.

From table 1b from Chapter 1 of this thesis it is clear that, although many studies find hypercortisolism in depression, not all studies do. A number of population based samples have used questionnaires to assess depression as a continuous trait. A study by Brandtstädter et al. (1991), for instance, measured cortisol in a large population sample (n=767) and did not find the association, or even a reverse association, between hypercortisolism and various personality variables, including anxiety and depression scales. Also in a recent population based sample (n=437) by Strickland et al. (2002) no association was found between depression and cortisol. Even in patient samples the association is most evident in hospitalised patients with a relatively recent acute depressive episode, but not necessarily in outpatients (Maes et al., 1994). The absence of hypercortisolism in high trait depression and outpatients questions the idea that (familial) HPA-axis dysfunction is the biological cause of feelings of depression. Instead they suggest an alternative causality in which severe depression requiring hospitalisation, which may be regarded as a chronic stressor by itself, is the cause of the heightened level of cortisol (Maes et al., 1994).

One factor that severely hampers research on the (source of) association between cortisol and depression is the confounding role of smoking. Nicotine has been shown to stimulate HPA-axis activity, leading to an increase in ACTH and plasma corticosterone in rodents (Caggiula et al., 1998; Cam et al., 1979) and cortisol in humans (Del Arbol et al., 2000; Kirschbaum et al., 1992; Pomerleau and Pomerleau, 1990; Wilkins et al., 1982). In addition, it has consistently been demonstrated that cigarette smoking and depression are highly associated (Anda et al., 1990; Brown et al., 2000; Glassman et al., 1990; Kendler et al., 1993; Patton et al., 1996; Shiffman, 1982) and anti-depressants have been used successfully as an aid to quit smoking (Berlin et al., 1995; Hitsman et al., 1999; Hughes et al., 2000; Hurt et al., 1998). Tobacco dependence linearly increases with the severity of depressive illness, and may, therefore, be most prominent in hospitalised patients (Farrell et al, 1998). Only very few studies on the association between cortisol and depression have taken the higher incidence of smoking among depressives into account (see last column tables 1a and 1b, Chapter 1 of this thesis). For instance, although smoking and depression have been shown to derive from the same familial factors (Breslau et al., 1998; Kendler et al., 1993), which may well overlap with the familial risk for a disturbed HPA-axis, the Munich Vulnerability Study (Holsboer et al., 1995; Krieg et al., 2001; Lauer et al., 1997; Modell et al., 1998) did not explicitly control for an effect of smoking.

In the current study repeated saliva cortisol samples were obtained during a 24-hour period in 173 families. These families were selected from the 3344 twin families participating in the longitudinal Netherlands Twin-family Study of Anxious Depression (NETSAD) for the purpose of a whole genome scan for genes influencing anxious depression (Boomsma et al., 2000). Selection was based on the criterion that at least 2 siblings in a family scored extremely high or extremely low on anxious depression. Those siblings could either be discordant (one scoring high and the

6.1 Introduction

Multiple factors contribute to the onset of major depression. These factors include genetic vulnerability (e.g. Kendler et al., 1992; Kendler et al., 1993a; Kendler et al., 1999; Nurnberger and Gershon, 1981) and environmental factors such as adverse life-events and lack of social support (e.g. Heim et al., 1997a; Heim et al., 1997b; Heim & Nemeroff, 1999; Heim et al., 2000; Kaufman et al., 2000; Kendler et al., 1995). The hypothalamic-pituitary-adrenal axis (HPA-axis) seems to be one of the central biological pathways on which these factors converge, and deregulation of this axis has been proposed to causally contribute to the risk for depression (Holsboer, 2000; 2001). The link between HPA-axis deregulation and depression is supported by studies showing elevation of the basal cortisol level (e.g. Scott & Dinan, 1998; Young et al., 2000; Weber et al., 2000), dexamethasone-mediated negative feedback resistance (Evans et al., 1983; Holsboer et al., 1982a,1982b; Holsboer et al., 1984), increased cerebrospinal fluid levels of corticotrophin-releasing factor (CRF) (Charlton et al., 1987; Charlton and Ferrier, 1989; Gold & Chrousos, 1985), and a blunted adreno-corticotrophic hormone (ACTH) response to challenge with exogenous CRF (Kathol et al., 1989; Plotsky et al., 1998). Hypercortisolism has been studied the most, with recent studies showing increased interest in deviant circadian rhythms in addition to an elevation of basal cortisol level per se (Posener et al., 2000; Weber et al., 2000; Wong et al., 2000; Young et al., 2000a). To illustrate the diversity of methodology even in studies focussing on basal cortisol, tables 1a and 1b in Chapter 1 of this thesis display a selection of the many studies on cortisol levels in human subjects.

Depression is known to be a heritable affliction (Boomsma et al., 2000; Kendler, 1993, 2001; Kendler et al., 1986, 1992; Kendler & Prescott, 1999; McGuffin et al., 1991; Sullivan et al., 2000). Mounting evidence now also shows individual differences in daytime cortisol levels to be genetically determined (Bartels et al., 2001; Inglis et al., 1999; Kirschbaum et al., 1992; Linkowski et al., 1993; Meikle et al., 1988; Wüst et al., 2000; Young et al., 2000). This allows for the possibility that the association between cortisol and depression derives from an underlying genetic factor. This is put even more sharply in the dominant hypothesis in this field by Holsboer and co-workers that considers the genetic liability for depression to be conveyed in part by the genetic risk for a disturbed HPA-axis. In favour of this idea, the Munich Vulnerability study (Holsboer et al., 1995; Lauer et al., 1997; Krieg et al., 2001; Modell et al., 1998) compared unaffected first degree relatives of depressive index patients (i.e. healthy subjects at high familial risk for depression) to control subjects without affected family members (i.e. healthy subjects at low familial risk for depression) and to inpatients with major depression. Plasma cortisol response after a combined dexamethasone-CRF challenge was higher in depressed patients compared to the healthy low risk participants. Most importantly, however, the healthy high-risk participants also had higher cortisol levels compared to the healthy low-risk participants.

From table 1b from Chapter 1 of this thesis it is clear that, although many studies find hypercortisolism in depression, not all studies do. A number of population based samples have used questionnaires to assess depression as a continuous trait. A study by Brandtstädter et al. (1991), for instance, measured cortisol in a large population sample (n=767) and did not find the association, or even a reverse association, between hypercortisolism and various personality variables, including anxiety and depression scales. Also in a recent population based sample (n=437) by Strickland et al. (2002) no association was found between depression and cortisol. Even in patient samples the association is most evident in hospitalised patients with a relatively recent acute depressive episode, but not necessarily in outpatients (Maes et al., 1994). The absence of hypercortisolism in high trait depression and outpatients questions the idea that (familial) HPA-axis dysfunction is the biological cause of feelings of depression. Instead they suggest an alternative causality in which severe depression requiring hospitalisation, which may be regarded as a chronic stressor by itself, is the cause of the heightened level of cortisol (Maes et al., 1994).

One factor that severely hampers research on the (source of) association between cortisol and depression is the confounding role of smoking. Nicotine has been shown to stimulate HPA-axis activity, leading to an increase in ACTH and plasma corticosterone in rodents (Caggiula et al., 1998; Cam et al., 1979) and cortisol in humans (Del Arbol et al., 2000; Kirschbaum et al., 1992; Pomerleau and Pomerleau, 1990; Wilkins et al., 1982). In addition, it has consistently been demonstrated that cigarette smoking and depression are highly associated (Anda et al., 1990; Brown et al., 2000; Glassman et al., 1990; Kendler et al., 1993; Patton et al., 1996; Shiffman, 1982) and anti-depressants have been used successfully as an aid to quit smoking (Berlin et al., 1995; Hitsman et al., 1999; Hughes et al., 2000; Hurt et al., 1998). Tobacco dependence linearly increases with the severity of depressive illness, and may, therefore, be most prominent in hospitalised patients (Farrell et al, 1998). Only very few studies on the association between cortisol and depression have taken the higher incidence of smoking among depressives into account (see last column tables 1a and 1b, Chapter 1 of this thesis). For instance, although smoking and depression have been shown to derive from the same familial factors (Breslau et al., 1998; Kendler et al., 1993), which may well overlap with the familial risk for a disturbed HPA-axis, the Munich Vulnerability Study (Holsboer et al., 1995; Krieg et al., 2001; Lauer et al., 1997; Modell et al., 1998) did not explicitly control for an effect of smoking.

In the current study repeated saliva cortisol samples were obtained during a 24-hour period in 173 families. These families were selected from the 3344 twin families participating in the longitudinal Netherlands Twin-family Study of Anxious Depression (NETSAD) for the purpose of a whole genome scan for genes influencing anxious depression (Boomsma et al., 2000). Selection was based on the criterion that at least 2 siblings in a family scored extremely high or extremely low on anxious depression. Those siblings could either be discordant (one scoring high and the

other scoring low) or concordant (both scoring low or both scoring high) for anxious depression. In order to check the validity of the anxious depression factor score, a sub-sample of the participants were invited to participate in the Composite International Diagnostic Interview (CIDI) a diagnostic psychiatric interview for the assessment of lifetime major depression (e.g. Andrews & Peter, 1998; Peter et al., 1998; Peter and Andrews, 1995; Witchen et al., 1991; Wittchen, 1994). Unpublished results indicated that the anxious depression factor score indeed represented vulnerability for clinical depression, with clinical symptoms increasingly more likely at the high end of the trait value distribution (this thesis). Based on longitudinal questionnaire data participants were further categorised as smokers, ex-smokers or non-smokers.

Because twinning occurs in each region and at all socio-economic levels, ascertainment through a twin register yields a representative sample of the population at large. As a first aim, the association between cortisol and the trait of anxious depression will be tested on the entire sample, i.e. without regard to family structure. This approach replicates that of most existing studies, for instance, as those listed in table 1b from Chapter 1 of this thesis. If the association is dependent on smoking behaviour, adding smoking status as a factor should attenuate or remove this association.

A disadvantage of the population approach is that it may underestimate the association between anxious depression and cortisol. In a population sample, subjects may be included that come from a high-risk family background, yet are low in anxious depression. If the association derives from an underlying familial risk for hypercortisolism, as suggested by the work of Holsboer and co-workers (Holsboer et al., 1995; Krieg et al., 2001; Lauer et al., 1997; Modell et al., 1998), the cortisol levels in these subjects may be too high for the observed level of anxious depression. Vice versa, some subjects high in anxious depression may come from a low-risk background, and having low cortisol levels, again obscure the association. A between family design, in which participants are selected from high-risk families (no known siblings scoring low) and low-risk families (no known siblings scoring high) has more power than a population-based sample to detect the association. If the familial liability for hypercortisolism overlaps with that for anxious depression then we expect the members of high-risk families to have higher cortisol than the members of low-risk families. Because members of high risk families may also smoke more often, it should again be tested whether adding smoking status to the analysis attenuates or removes the association.

In spite of its greater power to detect an association, a between family design can also introduce stratification effects, e.g. if smoking, chronic stress and depression are more prevalent in certain families than in others, for instance, because of low socio-economic status (Baum et al., 1999; Marmot et al., 2001). This spurious association can be dealt with in a within family design in which high and low scoring subjects from the same family are compared. In such sib pairs, shared environment (like SES) cannot explain the differences in anxious depression which must instead derive from unique experience and differential segregation of parental genes. Under the more

stringent hypothesis of a common genetic liability for hypercortisolism and anxious depression, we expect siblings discordant for anxious depression to be symmetrically discordant for cortisol levels. Again, a joint (genetic) liability for anxious depression and smoking may, through the effects of smoking on cortisol, falsely induce an association between anxious depression and cortisol levels, and it must be explicitly tested whether the association upholds in siblings concordant for smoking behaviour.

In short it was hypothesised that when controlling for smoking status:

I. Higher daytime cortisol levels are more likely to be found in participants scoring extremely high on anxious depression than in participants scoring extremely low on anxious depression.

II. Between families, higher daytime cortisol levels are more likely to be found in families with multiple siblings scoring extremely high on anxious depression (high family risk) than in families with multiple siblings scoring extremely low on anxious depression (low family risk).

III. Within families, siblings extremely discordant for anxious depression are also discordant for daytime cortisol, such that the sibling with high anxious depression has the highest cortisol levels.

6.2 Methods

6.2.1 Participants

Participants were ascertained from the Netherlands Twin Register (NTR). 3344 NTR families with adult or adolescent twins and siblings participated in a longitudinal study on physical and mental health (e.g. Boomsma et al., 1994, 2000; Koopmans et al., 1996, 1997, 1999; Vink et al., 2001). Participants received a survey in 1991, 1993, 1995, and 1997. A total of 6426 twins and 2200 siblings of twins have participated in at least one of the first 4 waves of this study. From these, 1717 participants were selected to be included in a linkage study to localise quantitative trait loci involved in anxiety and depression. These participants came from families with twin or sibling pairs that were extremely discordant or concordant on a composite factor score for anxious depression, i.e. both sibs either scored very high or very low, or one scored very high and the other very low (Boomsma et al., 2000). Apart from the extreme pair, all other members of these families were also asked to participate, regardless of their anxious depression score (which could be intermediate).

The selection was first performed on the 1991 questionnaire data and repeated for the 1993 and 1997 questionnaire. These three waves yielded a final 1332 participants that returned buckle

swab for DNA isolation and were asked to participate in a psychiatric interview. This study reports on 338 participants from 173 families that were drawn from the total pool of selected families to participate in a 24-hour ambulatory assessment of cardiovascular parameters and salivary cortisol profile. Of these participants, 129 (38.2%) were male and 209 (61.8%) were female. The age range was 16 to 64 years, with a mean of 30.2 (SD=10.6). Age and gender distribution did not differ from the total adolescent and adult population of the NTR. All participants gave written informed consent before entrance to the study. The study was approved by the Ethics Committee of the Vrije Universiteit of Amsterdam.

6.2.2 Anxious depression

Rather than assessing anxious depression as a clinical dichotomy (i.e. affected/ unaffected), indices of anxious depression and associated personality traits such as neuroticism were measured on quantitative scales. To assess depression, the 13-item version of the Beck Depression Inventory (BDI; Beck et al., 1961) and the anxious/depression symptom scale of the Young Adult Self-Report (YASR; Achenbach, 1990) were used. Anxiety was measured by the Spielberger Trait Anxiety Inventory (STAI, Spielberger et al., 1970). Finally, the Neuroticism and Somatic anxiety scales of the Amsterdamse Biografische Vragenlijst (Wilde, 1970) were also used. These anxiety and depression scales were transformed to obtain normality, and corrected for sex differences by subtracting the group means for men and women separately. Multivariate genetic analysis, applying the known difference in genetic relatedness of twins and siblings (Boomsma et al, 1990) were used to compute a genetic factor score for men and women over all questionnaires at the three time points (1991, 1993, and 1997). This factor score represents the individuals value on the common genetic factor in all anxiety and depression scales. It can be interpreted as an estimate of an individual's genotypic value for anxious depression and optimises the genetic contribution to the stability of anxiety and depression scores over time (Boomsma and Dolan, 1998; Dolan and Boomsma, 1998).

For the purpose of this study, the mean factor score was computed over all factor scores available at each of the three time points. Cut-off points for this mean factor score were computed using data from all members of the original 3344 families such that participants below the 30% lowest factor scores were classified as low in anxious depression, participants with the 30% highest factor scores were classified as high in anxious depression. Remaining participants were classified as intermediate in anxious depression.

6.2.3 DSM-IV depression

In addition to the assessment by repeated questionnaires, 290 persons also participated in a telephone interview during which several sections from the WHO Composite International Diagnostic Interview (CIDI) were administered. The CIDI is a fully standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 and DSM-IV. The computer administered life-time version (2.1) was conducted by interviewers trained at the CIDI training centre at the Academic Medical Centre in Amsterdam, The Netherlands. For the purpose of the current study the diagnostic criteria of the DSM-IV were used. Depressive episodes directly related to a major life event (e.g. loss of family member) or bipolar depression, evidenced by alternating manic and depressive phases were diagnosed separately. According to the DSM-IV classification, participants who had experienced a single or recurrent depressive episodes (or had one now) were diagnosed as suffering from major depression.

6.2.4 Confounders

On the days of cortisol measurement, the participants were briefly interviewed on health behaviours and medication use. The latter was recoded into four categories: antidepressants, corticosteroids, anti-hypertensive/cardio-active medication, and analgesics/anti-inflammatory agents (NSAIDS). Participants were asked about their current smoking behaviour. This information was combined with the detailed information on smoking behaviour and nicotine dependence, available from previous survey data, to determine smoking status. Participants were coded smokers if they currently smoked. They were coded ex-smokers if they had reported life-time smoking behaviour in 1991, 1993, or 1997, but were not smoking now. Finally, non-smokers had indicated not to smoke on all questionnaires, and did not smoke now. A small category of 6 'chippers', participants smoking less than 2 cigarettes a day, was also coded as non-smoking.

6.2.5 24 hour physiological measurements

All participants underwent 24-hour ambulatory recording of heart rate variability and blood pressure using the SpaceLabs ABP (Spacelabs Medical, Redmond, USA) and the VU-AMS device (De Geus & van Doornen, 1996). In addition, salivary cortisol samples were taken at 6 time points using Salivette tubes (Sarstedt, Germany) with polyester swabs.

The participants were visited at home before they would start their normal daily routines. After fitting of the equipment, the waist hip ratio was assessed in cm. They then received instructions for the ambulatory cardiovascular recording and the saliva sampling procedure and a short

questionnaire on health and physical activity was administered. In addition the first sample was then collected in the presence of the researcher making sure that the correct procedure for saliva sampling had been understood. The second sample was collected at 11.00h, the third at 15.00h, the evening samples were collected at 20.00h and at 22.30h. For the final sample the participants were instructed to collect it immediately after awakening, preferably while still in bed. Saliva collection was done according to the procedure described by Kirschbaum and Hellhammer (1989, 1994), e.g. participants were instructed not to eat or drink and not to brush their teeth prior to the saliva collection, and to chew on the swab for 45 seconds. The saliva samples were stored in a cool place for no more than 2 days, afterwards kept in –20° until delivery by courier to the assay laboratory in Düsseldorf every three months. Cortisol levels were measured employing an time-resolved immunoassay with fluorometric detection (DELFIA; Dressendörfer et al., 1992). Participants kept a diary of their physical activities and social situations in which they also noted the time they went to bed and the time of awakening. The latter two were used to compute sleep duration. Sleep duration and time of awakening were considered possible confounds of (morning) cortisol levels. Subjects also noted the exact times of saliva sampling and indicated any missed samples. Based on previous studies we anticipated that participants would miss samples or collect at inappropriate times. Some samples are forgotten and sometimes sampling is considered too inconvenient, for instance when in the middle of a meeting or teaching in front of a classroom.

Participants were asked not to engage in heavy physical activity on the day before measurement. They were not restricted in the number of cigarettes they wanted to smoke on the days before or during the measurement day, but were asked to accurately report the number of cigarettes they had smoked afterwards in the diary.

6.2.6 Family structure

From the total of 173 families, there were 58 families in which only a single member participated. In 77 families 2 members participated, in 27 families 3 members participated, in 10 families 4 members participated, and 1 family had 5 participating members.

Table 1 displays all families and categorises them into concordant low, concordant high and discordant families based on the following rules: members from concordant low families had at least one member with low, but none with high anxious depression; concordant high families had at least one member with high, but none with low anxious depression; discordant families had at least one member with low and one member with high anxious depression. Note that in many families more siblings participated in the surveys than in the ambulatory measurement sessions. In some discordant families, therefore, only the low or only the high anxious depressed siblings provided cortisol data. These families did participate in the overall population analyses described below

under 'Analysis I', but were left out of the between-family analyses (Analysis II) and within family analysis (Analysis III) described below.

6.2.7 Statistical analysis

In Analysis I, a depression related difference in cortisol levels in the entire group was assessed to replicate the most common type of study in this field, i.e. that in samples of unrelated subjects. Repeated measurements MANOVA (SPSSwin version 10, GLM) was used with the 6 cortisol values as the "multivariate" repeated measure, Anxious Depression (High, Intermediate, Low), Smoking (current-, ex-, and non-smokers) and Sex as between subject factors. Complete data on all six time points was available for 241 participants.

For analysis II, the between-family analysis, only the concordant low or concordant high families were selected. Secondly, from these families the participants scoring intermediate on anxious depression were removed leaving only high and low scoring participants. To deal with the expected low sample size in some of the cells (even when no data would be missing), salivary cortisol levels were tested for each time point separately, rather than as repeated measurements. ANOVAs were performed for each time point with Anxious Depression (High, Low), Smoking (current-, ex-, and non-smokers) and Sex as between subject factors.

Finally, analysis III was made into a pure within-family analysis. Again intermediate scoring were discarded to contrast only the extremes, i.e. the low and high scoring participants. All possible extremely discordant pairs were selected from the families in the first column of table 2. Where more than one discordant pair could be formed within a single family, the pair that maximised the discordance in anxious depression[1] was selected. This resulted in 37 pairs, of which 23 same sex pairs, 7 in which the low anxious member was male and the high anxious female and 7 in which the low anxious member was female and the high anxious male. These pairs were scored for concordance for current smoking behaviour. Discordant pairs were split into high-is-a-smoker pairs, where only the high anxious depressed member smoked, and low-is-a-smoker pairs where only the low anxious depressed member smoked. In the five pairs that had ex-smokers, the ex-smoker was treated as a non-smoker. For each time point separately, ANOVA was used with Anxious Depression (High, Low) as a repeated measure and Smoking Concordance as a between subjects factor (concordant, discordant high-is-a-smoker, discordant low-is-a-smoker). Because sex of the pairs was balanced, sex was not entered as a separate factor in the analysis. Furthermore, the same procedure was conducted for the persons who received a DSM-IV diagnosis for depression, they were matched with a sibling that had no DSM-IV diagnosis for depression and that resembled them as much as possible with regard to age and sex. Monozygotic discordant pairs were avoided.

Before all analyses, age, medication use, oral contraceptives use, sleep duration, waist hip ratio were tested as possible confounds. Chi-square or ANOVA was used to test whether these behaviours were associated with anxious depression. Where appropriate these were added as a covariate to the ANOVAs testing for an effect of anxious depression and smoking on cortisol. Because waist-hip ratio or sleep duration could not be obtained reliably in all subjects, degrees of freedom occasionally differ across the results presented below.

Table 6.1 Composition of participating families by anxious depression of the offspring. High (H), intermediate (M) and low (L) anxious siblings with cortisol data are in boldface capitals. Unmeasured siblings, of which only the anxious depression score but no cortisol was available are in regular font (h, m, l).

Discordant Families		**Concordant Low Families**	**Concordant High Families**
At least one low scoring sib and at least one high scoring sib participated	No within family contrast between low or high scoring sibs could be made	Only low or intermediate scoring sibs in family	Only high or intermediate scoring sibs in family
HL **HhLm** **HL** **HHhL** **HL** **HHLm** **HL** **HHLM** **HL** **HLLL** **HL** **HLLLm** **HL** **HhLmm** **HL** **HhhLLlll** **HL** **HLlMm** **HLl** **HLMmm** **HLl** **HLMmmm** **HLL** **HhLLLlmmm** **HhL** m **HHL** **HLm** **HLm** **HLm** **HLm** **HLm** **HLM** **HLM** **HLM** **HLM** **HLM**	**Lh** **Hhhl** **Lh** **Hlmm** **hL** **Hllm** **Hl** **Hhll** **Hl** **HHlm** **Hl** **HHlm** **Hl** **hLlm** **Hll** **hLlm** **Hll** **hLLm** **LLh** **hLLM** **hLm** **hLMM** **hLm** **LLhm** **hLM** **Hhlmm** **hlM** **HHHHHl** **Hlm** **Hhhhhhlm** **Hlm** **Hlm** **Hlm** **Hlm** **HlM** HLm **Hhl** **Hhl** **Hhl** **HHl**	**L** **Llm** **L** **Llm** **Ll** **LLm** **Ll** **LLm** **LL** **LLm** **LL** **Lmm** **LL** **LMm** **LL** LMM **LL** **LlllM** **LL** **LllMm** **LL** **Llmm** **LL** **Llmm** **lM** **lMmm** **lM** **LLLL** **lM** **LLLLl** **LM** **LLLM** **LM** **LLLM** **LM** **LLMm** **LM** **LLMmm** **Ll** **Ll** **Ll** **LLl** **LLl** **LLL** **LLL** **lM**	**H** **HhM** **Hh** **HHm** **Hh** **HHm** **Hh** **HHm** **Hh** **HHM** **Hh** **HMm** **Hh** **Hmm** **HH** **HMm** **HH** **HMm** **HH** **HMm** **HH** **Hhhh** **HH** **HHhh** **HH** **HHHH** **Hm** **Hhhhm** **HM** **HHhm** **HM** **HHhm** **HM** **HHHm** **HM** **HhMm** **HM** **HHMM** **HHh** **HHh** **HHh** **HHH** **HHH** **HHH** **HHH** **Hhm**
N families: 36 **N subjects: 98**	**40** **55**	**46** **87**	**46** **90**
Used in ANALYSIS I			
		Used in ANALYSIS II	
Used in ANALYSIS III			

6.3 Results

6.3.1 Descriptives

Table 6.2a shows the main characteristics of the participants, separately for low, intermediate and high anxious depression groups. Table 6.2b presents the distribution of smoking status separately for low, intermediate and high anxious depression. The total female sample is larger than the male sample. The primary selection was for families with extreme concordant and discordant pairs. All siblings from these families were asked to participate even if they scored intermediately, but the intermediate group remained relatively small. The anxious depression factor score was obtained separately for men and women from the sex corrected neuroticism, anxiety and depression scales. Therefor the relative proportion of men and women in low, intermediate and high anxious groups is not significantly different. Of the 290 persons that participated in the CIDI interview a total of 34 persons, 8 males and 26 females, were diagnosed with life time major depression according to the diagnostic criteria of DSM-IV. Two participants that were taking antidepressants at the time of the measurement now had very low factor scores. They were not included in further analyses.

Table 6.2a Subject characteristics as a function of anxious depression. Means and standard deviations are given.

	Low Anxious Depression (N = 132)		Intermediate Depression (N = 59)	Anxious	High Anxious Depression (N = 147)	
	Female (N = 81)	Male (N=51)	Female (N = 40)	Male (N=19)	Female (N = 88)	Male (N=59)
Age (mean (SD) Sleep Duration	32.3(11.5) 8:03	29.4(8.5) 7:33	30.5 (12.4) 7:52	33.2 (12.3) 7:20	28.1 (9.0) 8:10	30.0 (10.9) 7:41
Waist to hip Ratio (mean and SD)	0.77 (0.06)	0.83 (0.06)	0.75 (0.05)	0.82 (0.06)	0.77 (0.08)	0.86 (0.10)
Oral contraceptives use	33 (41%)	--	18 (45%)	--	39 (44%)	--
Antidepressive medication	1 (1.2%)	0	3 (7.5%)	0	6 (6.8%)	0
Cardioactive and antihypertensive medication	2 (2.5%)	1 (2.0%)	1 (2.5%)	0	3 (3.4%)	6 (10%)
Corticosteroid medication	2 (2.5%)	2 (4.0%)	1 (2.5%)	0	3 (3.4%)	1 (1.6%)
Analgesics/ anti-inflammatory medication (NSAIDs)	13 (16.0%)	2 (4.0%)	11 (27.5%)	3 (16%)	11(12.5%)	13 (22.0%)

Table 6.2b Smoking status as a function of anxious depression.

	Low Anxious Depression (N = 132)		Intermediate Depression (N = 59)	Anxious	High Anxious Depression (N = 147)	
	Female (N = 81)	Male (N=51)	Female (N = 40)	Male (N=19)	Female (N = 88)	Male (N=59)
Smoking:						
Never	59 (72.8%)	36 (70.6%)	25 (62.5%)	10 (52.6%)	43(48.8%)	26 (45.6%)
Current	14 (17.4%)	9 (17.6%)	9 (22.5%)	5 (26.3%)	30(34.0%)	23 (40.4%)
Ex-smokers	8 (9.8%)	6 (11.8%)	6 (15.0%)	4 (21.1%)	15(17.0%)	8 (14.0%)

The sex by anxious depression ANOVA revealed no significant effects of anxious depression for age, sleep duration or waist hip ratio. Males were of the same age as women but reported to sleep significantly shorter than females ($F(1,318)=13.0$; $p<0.001$) and had larger waist hip ratio's ($F(1, 301)=54.5$; $p< 0.001$). Of these potential confounds, however, only age showed significant correlation to cortisol, at time 1 ($r = -0.15$, $p=0.01$) and time 5 ($r = 0.17$, $p=0.003$). Age was always entered as a covariate in analyses I to III.

The performed chi-square tests revealed no significant effects of anxious depression for oral contraceptives, corticosteroids or antidepressant use. Males high in anxious depression tended to take more anti-hypertensive or cardio-active medication ($\chi^2_2=4.47$, $p=0.08$) and took significantly more analgesics ($\chi^2_2=9.56$, $p=0.01$). The latter effects were not seen in females.

None of the various medications including oral contraceptives had a discernible effect on cortisol at any time point in either sex. However, because of their potential impact on the research questions, it was decided to repeat analyses I to III both with and without including the participants taking antidepressants or the participants taking corticosteroid medication. The re-analysis left the pattern of findings effectively unchanged.

6.3.2 Smoking and anxious depression

Cross-tabulating smoking with anxious depression in the entire sample showed the expected association between smoking and anxious depression to be nearly significant in male participants ($\chi^2_4=9.03$, $p=0.05$) but not in female participants ($\chi^2_4=7.9$, $p=0.09$). However, when participants were classified according to their family type, i.e. concordant high or concordant low anxious depression, the association became very robust in both sexes (χ^2_2-males=10.4, $p=0.003$; χ^2_2-females=10,3, $p=0.01$). Inspection of the standardised residuals revealed that the association showed a linear gradient, with smoking most common in the high anxious depression group and least common in the low anxious group. Intriguingly, with regard to their anxious depression scores, male ex-smokers were more similar to non-smokers, whereas in females ex-smokers were more similar to current smokers.

The contrast in robustness of the findings in the entire population versus that in selected concordant families suggests that the association may be less evident in discordant families. This was corroborated by the results from within-family comparison of siblings with high and low anxious depression. Here the association between smoking and anxious depression disappeared entirely. Out of the 37 pairs discordant for anxious depression, 20 were concordant for current smoking behaviour, in 9 pairs only the high scoring sibling smoked and in 8 pairs only the low scoring sibling smoked.

6.3.3 Cortisol, anxious depression, and smoking: whole sample

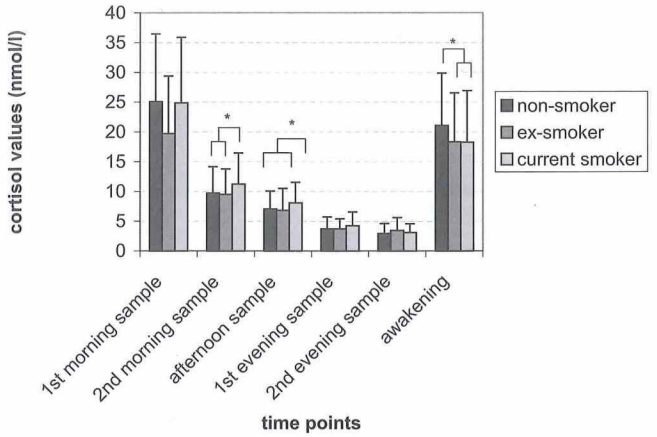
The 1st sample was taken in the morning at the participants' house, approximately 30 minutes before they would normally start their daily routine. Sample time was 8:32 am on average, but with large individual differences (06:45 – 10:34 am). The awakening sample was collected at the participants spontaneous wakening time, which was at 7:20 am on average for this population with a range of 6:00 to 9:05 am. All other samples were reported to have been taken close to the target time, with average sample times at 11:15 am, 03:15 pm, 08:11 pm and 11:30 pm.

Of the possible 2028 samples (six samples for each of the 338 participants) 116 (5.7%) samples were missing. Of the remaining 1912 samples 34 samples were deleted because they were much higher (>3 sd) than the population mean for that sample. These outliers appeared to occur at random, i.e., they occurred at all time points (not just morning peaks) and were not related to medication or smoking status, respectively. As in previous studies with salivary cortisol (Young et al., 2000, 2000b) only a low to modest correlation was found between the samples at various times points (lowest: $r_{awake-morning2} = 0.01$, highest: $r_{evening1\text{-}evening2} = 0.42$, mean: $r = 0.17$).

Repeated measurements ANOVA, taking age and sex into account, showed no effects of anxious depression on cortisol at any time point. This was true also for the effects of a DSM-IV diagnosis: life-time depression was not associated with higher cortisol levels The only effects to emerge on cortisol level were a significant effect of time point ($F(5,230)=17.8$, $p<0.0001$) and a trend towards an interaction of time point with smoking behaviour ($F(10,462)=1.75$, $p<0.06$). Figure 5.1 illustrates both effects by plotting the average daytime cortisol profiles for smokers, ex-smokers and non-smokers. The time points main effect simply reflects the well-known circadian rhythm in cortisol level, with cortisol rising sharply around awakening, peaking in the course of the morning and returning to its lowest levels in the afternoon and evening (Van Cauter et al., 1994, 1996). Post-hoc repetition of the analyses for each time point, contrasting smokers with non-smokers and ex-smokers with non-smokers, showed significant effects of smoking status for the second morning sample, the afternoon sample and the awakening sample (p's < 0.023). In the second morning and afternoon samples current smokers had significantly *higher* cortisol than non-smokers. The same trend was visible for ex-smokers, but due to the lower sample size for ex-

smokers this did not reach significance. At the awakening sample current smokers had significantly *lower* cortisol levels than non-smokers.

Figure 5.1 Daytime cortisol profile by smoking status: entire sample

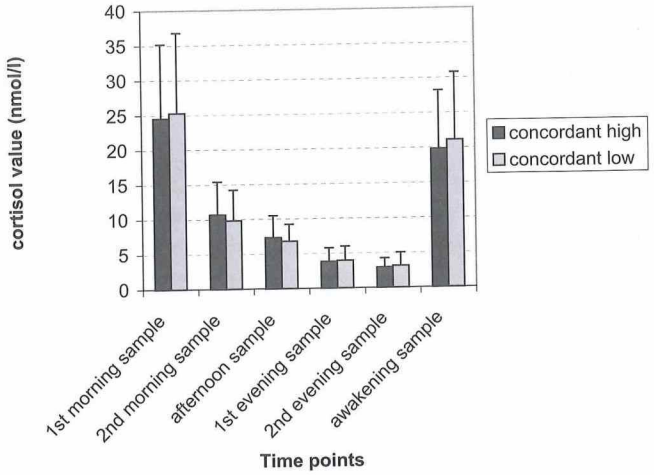


Repeating these analyses in the participants without antidepressant or corticosteroid medication did not change this pattern of results, other than that the effect of smoking status for the second morning sample was no longer significant.

6.3.4 Between-family analyses

Figure 5.2 shows the daytime cortisol profiles averaged over the families with siblings concordant low for anxious depression and the families with siblings concordant high for anxious depression. In contrast to our expectations no differences in cortisol level at any time point arose between the high and low risk families. The only effects to emerge from the ANOVA were for smoking status at time points 3, 4 and 6 confirming the findings above that smokers have higher afternoon/evening cortisol, but with a significantly lower awakening peak.

Figure 5.2 Daytime cortisol profile in siblings from high and low risk families

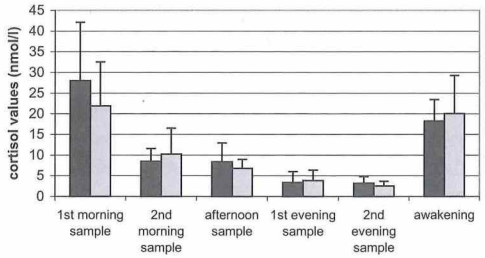


6.3.5 Within-family analyses

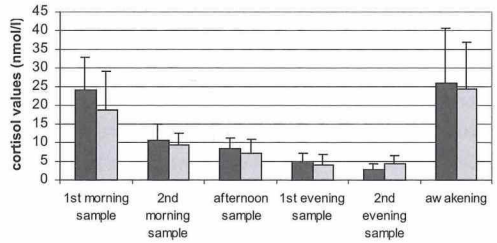
The final analysis contrasted the cortisol profile of the 37 extreme discordant sib pairs. This was done separately for pairs concordant for smoking, for pairs discordant for smoking with the high anxious depressed member a smoker, and for pairs discordant for smoking with the low anxious depressed member a smoker. Cortisol profiles are depicted in Figure 3. No evidence was found for a main effect of anxious depression on cortisol at any time point or an interaction between anxious depression and smoking. In short, no association between the trait of anxious depression and salivary cortisol levels arose in this within-family analysis, even when smoking was not controlled for. For the within family analysis on DSM-IV diagnosis for depression, not enough discordant sibpairs could be formed to perform the analysis on.

Figure 3: Within family comparison of a sibling high in anxious depression to a sibling low in anxious depression.
Low anxious depressed sib
High anxious depressed sib

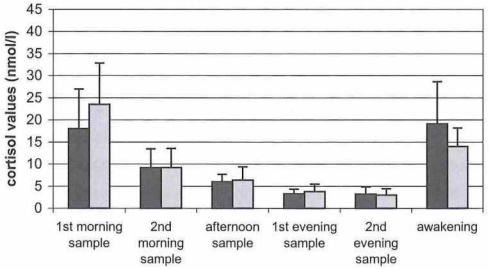
Siblings are concordant for smoking status.



The high anxious depressed sib smokes, the low anxious depressed sib does not smoke .



The low anxious depressed sib smokes, the high anxious depressed sib does not smoke.



6.4 Discussion

This study tested the association between anxious depression and daytime cortisol levels in a non-clinical population. Most previous studies documenting such a relationship have used clinical samples of depressed patients, often hospitalised (e.g. table 1a, chapter 1 of this thesis). Since ample evidence exists to show that clinical depression is preceded by high levels of trait anxiety and depression (e.g. Duggan et al., 1995; Kendler et al., 1999), we hypothesised that the association should also be apparent in the population at large. Since smoking is more prevalent in depression (Anda et al., 1990; Brown et al., 2000; Glassman et al, 1990; Kendler et al., 1993; Patton et al., 1996; Shiffman, 1982), and smoking has been shown to affect cortisol levels (Caggiula et al., 1998; Cam et al., 1979) we made sure to control our analyses for current smoking behaviour. Our results showed no evidence of an association between anxious depression and cortisol level at any of six time points throughout the day, including the awakening peak, which had recently been given most significance in stress-related disorders (Pruessner et al., 1997; Pruessner et al., 1999; Schmidt-Reinwald et al., 1999; Wüst et al., 2000). This 'null finding' was unrelated to the addition of smoking status to the analyses. No association between anxious depression and daytime cortisol levels was found either with or without controlling for current smoking behaviour.

Our findings were consistent with a recent study in women by Strickland et al (2002) that showed no effects of clinical depression on 09.00 and 23.00h cortisol samples. They were at odds however with many reports of a significant increase in basal cortisol levels in depressive patients and a large body of literature suggesting that a dysfunction in the HPA-axis contributes to -and possible causes- depressive disorder. The dominant hypothesis explaining the association between cortisol and clinical depression is that they are both influenced by an underlying genetic factor. In favour of this idea, The Munich vulnerability study (Holsboer et al., 1995; Krieg et al., 2001; Lauer et al., 1997 Modell et al., 1998) found disturbed HPA-functioning in the unaffected first degree relatives of depressive index patients (i.e. healthy subjects at high familial risk for depression). The authors concluded that part of the genetic risk for depression was conveyed by the genetic risk for a disturbed HPA-axis. The jump from a familial to a genetic explanation seems reasonable in view of the animal studies demonstrating that modification of the glucocorticoid receptor genes directly affects emotional behaviour (Gass et al., 2001; Holsboer, 1999; Linthorst, 2000). However, the alternative explanation for a familial effect is that, by selecting participants with a depressive family member, they may also have selected participants from families at increased risk for depression through shared environmental factors e.g. low SES or certain parenting styles. The healthy siblings from the high risk families all experienced a major life-event when one of their siblings suffered from depression. This fact alone can put high stress on a family.

To repeat and extend the family design of the Munich vulnerability study we used a between family comparison that contrasted daytime cortisol profiles from high risk families with those of low risk families. Risk level in these families was defined by the anxious depression scores of 2 to 7 siblings. To rule out a shared environmental explanation (like socio-economic status) we further contrasted daytime cortisol profiles of an extremely high anxious member with that of an extremely low anxious member within the same family. Neither comparison, however, yielded evidence for a disturbed HPA-axis in participants with high anxious depression, at least those disturbances that are reflected in daytime cortisol levels. Thus, our study adds to the existing discrepancy between positive findings in clinical samples (see table 1a, Chapter 1 of this thesis) versus null findings in population samples (Brandstädter et al., 1991; Mazur et al., 1994). There are a number of explanations for this discrepancy. High trait anxiety or high trait depression, a combination of which was indexed by our factor score for anxious depression may not reflect the underlying biological risk for clinical depression. More generally, high levels of anxious depression may not predict depressive disorder. There is a large amount of literature speaking against this. Many studies have testified that clinical depression is the tail of the normal distribution of neurotic personality rather than an entirely new process. (e.g. Angst & Clayton, 1986; Judd et al., 2000; Kendler et al., 1993b; Solomon et al., 2001). Our own data clearly show that the 12% of participants revealing evidence of a clinical episode in the CIDI interview mostly had high factor score, whereas those with a low factors score seldom had a clinical diagnosis of depression.

Even if a high factor score is a good predictor of clinical depression, it may still not be associated with hypercortisolism if the increase in cortisol levels only become apparent when depressive symptoms become severe enough to warrant (hospitalised) treatment. In line with a stress-diathesis model of depression, there may be an underlying biological defect in the HPA–axis in participants with anxious depression, but this will give rise to hypercortisolism only after exposure to the severe or chronic stressors that trigger the first (or a new) depressive episode. Before such an episode, the HPA defect may be accessible only through very specific testing, such as the combined dexamethasone-CRF test (Deuschle et al., 1998; Maes et al., 1992; Von Barleben et al., 1985). However, this attractive explanation must be weighted against the alternative that the hypercortisolism observed in clinical samples is a consequence of depression rather than a precursor or cause. Hospitalisation for depression or in general the depressive period itself may be the severe stressor that causes chronic stress-reactivity with high cortisol levels (Maes et al., 1994). In support of this explanation, Trestman et al. (1995) found that cortisol levels of depressive patients in their acute depressive episode were higher than when they were in remission. This is more in line with a "state" explanation of hypercortisolism, than to consider it a biological "trait". Also, long term administration of antidepressants, based on NA- or combined NA- and 5-HT re-uptake inhibition, suppresses the HPA-axis in parallel to an improvement in clinical condition (Barden et al., 1995; Beique et al., 1998; Holsboer & Barden, 1996; Okugawa et al.,

1999; Pepin et al.,1992; Sanchez and Hyttel, 1999). It is still unclear what the exact time courses of clinical improvement and HPA normalisation are, but such studies seem to indicate that hypercortisolism can reflect a state as much as a trait.

The present study tried to improve on previous studies in the field by taking smoking behaviour into account as a possible confounder of the association between anxious depression and daytime cortisol profile. Although it did not impact on the main finding the use of this strategy was vindicated on two accounts: smokers indeed had a different cortisol profile than non-smokers and familial risk for high anxious depression and smoking was shown to cluster. Previous studies in human subjects (Del Arbol et al., 2000; Kirschbaum et al., 1992; Pomerleau and Pomerleau, 1990; Wilkins et al., 1982) showed that nicotine intake increased cortisol levels. Animal studies confirmed a direct dose dependent stimulating effect of nicotine or nicotinergic agonists on the HPA-axis (Caggiula et al., 1998; Cam et al., 1979) The effect is in part mediated through brainstem noradrenergic structures that project to the paraventricular nucleus and stimulate the release of CRF from the parvocellular region (Matta et al, 1987, 1990, 1998) but other regions in the brain are involved as well (Hillhouse and Milton, 1989; Kasckow et al., 1999). Cortisol, in turn, decreases the sensitivity of the nACh-receptors, thus establishing tolerance for nicotine (Caggiula et al., 1998; Pauly et al., 1988) further enhancing nicotine intake.

Our data confirmed the increased basal cortisol level during the day in smokers, but found a significantly lower morning peak compared to non-smokers. This repeated a similar finding by Wust et al. (2000), although in that study, as in ours, only a small part of the variance in the morning peak was explained by smoking. At first sight this finding may appear counter-intuitive, but if sleep is considered a period of abstinence, it can be easily reconciled with studies that have shown that abstinence lowers cortisol to below a normal level (Frederick et al, 1998; Gilbert et al, 1999). Taken together, the daytime cortisol profile of smokers resembled the flattened cortisol profile found in 10-15% of participants (reviewed in Stone et al., 2001). Clearly, smoking should be taken into account in studies on daytime cortisol profile.

Smoking and depression were consistently found to correlate in many studies. Both incidence rates and life time prevalence rates were higher in individuals with higher scores on depression scales (Anda et al., 1990; Glassman et al, 1990; Patton et al., 1996). Also, depressed patients and individuals with high depression scores were less likely to succeed in smoking cessation (Glassman et al., 1990). Furthermore, depression was related to a relapse in ex-smokers (Glassman et al., 1990; Shiffman, 1982) and antidepressants had been used successfully as an aid to quit smoking (Berlin et al, 1995; Hitsman et al, 1999; Hughes et al, 2000; Hurt et al, 1998). The strongly increased risk of siblings in high risk families over those in low risk families in our study confirmed the conclusion from previous studies that the association derived from a shared familial factor (Breslau et al., 1998; Eaves & Eysenck, 1980; Kendler et al., 1993). Using bi-variate

genetic analyses in a female twin sample, Kendler et al. (1993) made a compelling case that this familial factor underlying co-morbidity of smoking and depression was genetic in nature.

One account of this common genetic factor holds that smoking functioned as "self-medication" for a genetic deficit in mood regulation (Hughes, 1988; Pomerleau and Pomerleau, 1984). Monoaminergic dysfunction remains a major explanation for anxiety and depressive disorders. Because chronic smoking inhibits monoamine oxidase A and B (Fowler et al., 1996a; Fowler et al., 1996b) yielding up to 40% lower levels of these enzymes, boosting (genetically) deficient monoaminergic transmission may explain part of the antidepressant actions of nicotine. Alternatively, the antidepressant effects may derive from the well-known increase in mesolimbic dopaminergic transmission induced by nicotine (e.g Fa et al., 2000; Nisell et al., 1994). Deficient dopaminergic neurotransmission was increasingly considered as a co-factor in depression, particularly its anhedonic aspects (Brown & Gershon, 1993; Diehl & Gershon, 1992). Interestingly, Breslau et al. (1998) hint at a history of early conduct disorders to be the underlying factor causing the association between smoking and depression. Thus, problem behaviours, smoking (and substance abuse), and depression may show a common genetic aetiology in dopamine deficiency.

6.5 Strengths & Limitations

The major strengths of this study were the between family and within family design which should have allowed detection of familial and more specifically genetic contributions to the association between cortisol and anxious depression. Second, possible confounding of the association by smoking behaviour was appropriately controlled for in all analyses. Thirdly, anxious depression and smoking behaviour were repeatedly assessed over time with validated tools (e.g. STAI, YASR, Beck & CIDI) and by using the mean factor score over time, we maximised assessment of the stable part of the trait of anxious depression. Finally, the number of participants in which daytime cortisol was measured was high in comparison to previous studies in the field, and six daytime samples were used to provide a reliable estimate of the complex diurnal cortisol profile. A possible limitation, however, was the selection procedure by which families were selected for inclusion in the study. For the purposes of future linkage analysis we selected families that had two concordant high, two concordant low or two discordant siblings for anxious depression (Boomsma et al, 2000; Risch & Zhang, 1995). It was possible that this selection somehow distorted the true association between anxious depression and daytime cortisol in the population at large. An alarming indication for such a selection effect was the absence of the usual comorbidity between depression and smoking behaviour in families selected to have siblings extremely discordant for anxious depression. The parents of such discordant families may be expected to be heterozygotes for the "anxious depression genes", such that half the offspring would be high on anxious depression, whereas the other half would be low on anxious depression. Familial composition in the left hand

column of table 1 largely agreed with this expectation. Under the assumption that the genes influencing anxious depression were also the genes influencing smoking, we should have found more smokers among the high anxious members of our pairs. This was not the case. However, if there were other genes influencing smoking than those that overlap with the genes for anxious depression less power was obtained to find co-morbidity in discordant siblings within the same family than in discordant individuals coming from different families (e.g. Witte, Gauderman and Thomas, 1999). A larger scale and unselected sample may be needed to resolve the contradiction in the between and within family results of the association between smoking and depression.

In conclusion, the often cited association between depression and hypercortisolism in patient groups was not paralleled by an association between anxious depression and cortisol levels in a population sample. This was true even in a design that optimised detection of a common familial factor influencing both traits.

Chapter 7

Ambulatory blood pressure and depression

Abstract

In the present study the association between depression and blood pressure levels was assessed in a sample of 160 male and 271 female adults. Trait depression was assessed by using longitudinal questionnaire data. Clinical depression was assessed by a psychiatric interview for life time depression (CIDI). Daytime ambulatory blood pressure measurements were obtained every half hour, and all analyses were carefully adjusted for time of day and posture. The association between life time depression and diastolic and systolic blood pressure was significant, whereas the association between trait depression and blood pressure was not. When the confounding influences of posture/physical activity on the day of measurement and of age, sex, BMI, smoking and medication status were taken into account, clinical depression was still associated with higher systolic blood pressure, and the association was found to be most pronounced in older women.

7.1 Introduction

There is growing evidence that depression is an important predictor of cardiovascular disease (Anda et al., 1993; Ariyo et al., 2000; Jonas & Mussolino, 2000; Kubzansky, Kawachi, Weiss, & Sparrow, 1998; Penninx et al., 2001; Wassertheil-Smoller et al., 1996). The prediction holds for cardiovascular morbidity in both men and women, but cardiovascular mortality is predicted by depression in men only (Ford, Mead, Chang et al, 1998; Ferketich, Schwartzbaum, Frid & Moeschberger, 2000; Hippisley-Cox, Fielding & Pringle 1998). A first factor that could mediate the prospective association of depression with cardiovascular disease is age, since the incidence of both depression and CVD increases with age in both sexes. Two other classical risk factors for cardiovascular disease, obesity (Brochu, Poehlman & Ades, 2000) and lack of physical activity (Haapanen-Niemi et al., 2000), both resulting in a higher BMI, have also shown high co-morbidity with depression. Finally, a consistent association has been reported between smoking and depression (Anda et al., 1990; Brown et al., 2000; Glassman et al., 1990; Glassman & Shapiro, 1998; Kendler et al., 1993; Patton et al., 1996; Shiffman, 1982). However, even after adjusting for these risk factors a significant association between depression and cardiovascular mortality and morbidity remains (Carney et al., 1987; Ford et al., 1998; Penninx et al., 2001). A depression-induced increase in blood pressure is a likely candidate to explain part of the remaining association between depression and cardiovascular disease.

The association between blood pressure and personality traits like type A and hostility traditionally received a lot of attention in psychosomatic research, but recently attention has shifted to depression as a major psychological factor in hypertension. Most of the studies on the association between psychological factors and blood pressure have been conducted in a laboratory setting (e.g. Jonas et al., 1997; Everson, et al., 2000; Shinn et al., 2001; Paterniti et al., 1999). Because there is evidence that ambulatory blood pressure measurements are more predictive of hypertension than laboratory based measurements (Clement, De Buyzere & Duperez, 1994; Khattar et al., 1999), ambulatory monitoring of BP is now increasingly used in psychosomatic medicine (Schnall et al., 1998; Steptoe et al., 1995). Ambulatory monitoring may be particularly relevant in studying depression, because most major models of depression consider a high frequency and/or exaggerated amplitude of the responses to life stressors as an important part of the disorder (Nemeroff, 1999).

Table 1.2 in Chapter 1 of this thesis presents a review of studies, published after 1990, based on a PubMed search using '(depression or negative affect)' AND '(blood pressure or hypertension)' AND 'ambulatory' as keywords. Studies were selected when they had as their main focus the association between ambulatory blood pressure levels and depression or anxiety. Anxiety was included because of its high comorbidity with depression. Studies with a different research focus such as job strain or exercise or social support were not included. Handling of the confounding

influences of age, BMI, sex, and smoking was examined for each study (last column). Most studies in Table 1.2 in Chapter 1 of this thesis reported a relationship between negative affect and diastolic as well as systolic blood pressure. However, the most recent and also largest study did not find this association (Friedman et al., 2001). In the studies in table 1.2 negative affect (depression or anxiety) was always assessed by questionnaire, and none used a clinical measure of depression. Also, where most studies did take sex and age into account, BMI and smoking were often not included as possible confounders.

The aim of the present study is to examine the association of ambulatory blood pressure and trait depression measured by questionnaire, as well as life time depression measured by clinical interview. Furthermore, the impact of age, sex, BMI, smoking on ambulatory blood pressure will be tested, and where appropriate, the association between ambulatory BP and depression will be controlled for these possible confounders. For trait depression, we used a composite "anxious depression" factor score computed from longitudinal survey data on depression and anxiety (Boomsma et al., 2000). For clinical depression, a DSM-IV diagnosis was derived from the Composite International Diagnostic Interview (CIDI) a diagnostic psychiatric interview for the assessment of life-time major depression (e.g. Wittchen, 1994; Peter and Andrews, 1995).

We hypothesized that:

I. High anxious depression factor scores, reflecting trait depression and increased risk for ever experiencing a depressive episode in life, would be associated with higher BP levels.

II. Having experienced a clinical depressive episode at some point in life according to DSM-IV criteria would be associated with higher BP levels.

7.2 Methods

7.2.1 Participants

Participants were ascertained from the Netherlands Twin Register (NTR). 3344 NTR families with adult or adolescent twins and siblings participated in a longitudinal study on physical and mental health (e.g. Koopmans et al., 1996, 1997, 1999; Vink et al., 2001; Boomsma et al., 1994, 2000). Participants received a questionnaire in 1991, 1993, 1995, and 1997. A total of 6426 twins and 2200 siblings of twins have participated in at least one of the first 4 waves of this study. From these, 1717 persons were selected to be included in a linkage study to localize quantitative trait loci (QTL) influencing anxious depression. Families were selected when there was at least one sibpair in the family extremely concordant or extremely discordant on a composite factor score for anxious depression, i.e. both sibs either scored very high or very low, or one scored very high and the other very low (Boomsma et al., 2000).

This study reports on data obtained from 431 (160 male and 271 female) participants from 218 families who were randomly selected from the families involved in the QTL study. The age range

was 15 to 70 years, with a mean age of 30.5 years (SD=10.4). Age and gender distribution did not differ from the total adolescent and adult population of the NTR.

7.2.2 Anxious depression

To assess depression, the 13-item version of the Beck Depression Inventory (BDI; Beck et al., 1961) and the anxious depression symptom scale of the Young Adult Self-Report (YASR; Achenbach, 1990) were used. Anxiety was measured by the Spielberger Trait Anxiety Inventory (STAI, Spielberger et al., 1970) and the neuroticism and somatic anxiety scales of the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970). The anxiety and depression scores over three time points (1991, 1993, 1997) were used to compute a genetic factor score. For the purpose of this study this genetic factor score will be named *the anxious depression score* which can be used as an estimate of an individual's genotypic value for *anxious depression* and which optimizes the genetic contribution to the stability of anxiety and depression scores over time (Boomsma and Dolan, 1998; Dolan and Boomsma, 1998).

For the purpose of this study, persons with the 30% lowest mean anxious depression factor scores were classified as low risk, persons with the 30% highest mean anxious depression factor scores were classified as high risk. Remaining persons were classified as intermediate. This cut-off was based on the mean anxious depression factor scores of the entire sample.

7.2.3 DSM-IV depression

In addition to the assessment by repeated questionnaires, 1290 participants selected for the QTL study, participated in a telephone interview during which several sections from the World Health Organisation Composite International Diagnostic Interview (CIDI) were administered. The CIDI is a standardised diagnostic interview designed for assessing mental disorders according to the definitions of the Diagnostic Criteria for Research of ICD-10 (the tenth revision of the International statistical Classification of Diseases and Related Health Problems) and DSM-IV. For the purpose of this study the DSM-IV criteria were used. The computer administered life-time version (2.1) was conducted by interviewers trained at the CIDI training centre at the Academic Medical Centre in Amsterdam, The Netherlands. Participants who had experienced a single or recurrent depressive episode at some point in their life or who were having a depressive episode at the time of the interview, were diagnosed with life-time depression. Persons who experienced a depressive episode directly related to a major life event (e.g. loss of family member) or had bipolar depression, evidenced by manic contingent on depressive phases, received no diagnosis for major depression.

7.2.4 Ambulatory measures, data handling and reduction

Procedure.

Participants were informed about, and invited for ambulatory monitoring by letter. Subsequently they were contacted by phone for an appointment on a self-selected, but representative day. Participants were requested to refrain from intense physical activity both on the preceding and the ambulatory monitoring day. Before starting their daily routines they were visited at home and the ambulatory BP (ABP) device was attached by the researcher. Basic operation of the monitor was explained (e.g how to turn off momentarily during car driving). A written instruction and telephone-support were available at all time during the registration. The subjects switched off the ABP device just before going to bed. The next morning the ABP device was picked-up by the researcher.

Ambulatory systolic and diastolic blood pressure values were obtained by means of a Spacelabs 90207 device (SpaceLabs Medical, Redmond, USA) with an arm-cuff on the non-dominant arm. Arm circumference was measured to choose the correct arm-cuff size. The arm-cuff was inflated automatically every 30 minutes and the obtained values were not displayed on the display of the blood pressure monitor. Before the cuff started to inflate the device gave an auditory two-tone-beep and participants were instructed to keep their arm as still as possible and not to change their actual posture during the measurement. Furthermore, there was a second auditory beep from a VU-AMS device (see www.psy.vu.nl\vu-ams for more information) alerting the participants to report on their activities and their posture every 30(±10) minutes as accurately as possible in a diary. The VU-AMS device also contains an accelerometer sensitive to changes in vertical acceleration. Using both the diary information and the accelerometer signal in an interactive graphical VU-AMS program, each blood pressure value was coded for posture and activity (de Geus & van Doornen, 1996).

All data were checked for artifacts, outliers and frequency distribution in SPSS (Version 10.0 for windows). Previous recommendations for excluding artifactual and outliers from ambulatory recordings were followed (after Bernardi et al., 1992); which resulted in rejection of values if the (1) 20 mmHG < pulse pressure (SBP-DBP), (2) 40 mmHG < DBP, (3) 70 mmHG < SBP < 190 mmHG. In this procedure 28 of the 11747 correctly obtained blood pressure readings were removed from the data set, resulting in an average of 27 readings per subject (rang 3-35). Data is aggregated over time of day resulting in four categories; a mean value for the morning (6:30 – 13:00), early afternoon (13:00 – 16:00), late afternoon (16:00-19:30) and, evening (19:30 – 00:00). Aggregation occurred for both the complete dataset, i.e. the BP recordings during all postures, and for a subset of BP recordings during sitting only.

7.2.5 General health and use of medication

On the day of ambulatory blood pressure registration, the participants were briefly interviewed on health behaviours and medication use. The latter was recoded into four categories: antidepressants, corticosteroids, anti-hypertensive/cardioactive medication, and analgesics/anti-inflammatory agents (NSAIDS). Use of oral contraceptives (OC) was treated as a separate confounder since it only concerns females.

7.2.6 Smoking status

On the measurement day participants were asked about their current smoking behaviour. This information was added to the detailed information on smoking behaviour available from previous survey data. Participants were coded as smokers if they currently smoked. They were coded as ex-smokers if they had reported life-time smoking behaviour in 1991, 1993, or 1997, but were not smoking on the measurement day. Finally, non-smokers had reported never to have smoked on all questionnaires, and did not smoke on the measurement day. Participants were not restricted in the number of cigarettes they wanted to smoke on the days before or during the measurement day, but were asked to accurately report the number of cigarettes they had smoked afterwards in the diary.

7.2.7 Statistical analysis

To test for differences in blood pressure levels in the different depression groups, anxious depression (low, medium, high) and DSM-IV diagnosis (yes, no) were used as between subjects factors in two separate repeated measurement MANOVAs (SPSSwin version 10, GLM). Repeated measurement variables were the morning, early afternoon, late afternoon and evening blood pressure values. The above analyses were repeated after selecting only those blood pressure registrations obtained during sitting postures. Next, possible confounding effects on blood pressure were tested for the categorical between subject factors sex, smoking status, and medication use, and the continuous covariates age, and BMI. Finally, the initial tests of effects of anxious depression (low, medium, high) and DSM-IV diagnosis (yes, no) were repeated, this time including all appropriate confounders.

All tests were performed for the dependent variables systolic and diastolic blood pressure separately.

7.3 Results

7.3.1 descriptions

In the population studied, 173 (40.1%) of the participants were classified as highly, and 177 (41.1%) as low anxious depressed according to the factor score obtained from the longitudinal questionnaire data. Of the 390 persons who additionally participated in the CIDI, 57 (14.6%) received a DSM-IV diagnosis for life-time depression. The association between anxious depression and DSM-IV depression was positive and highly significant ($\chi^2(2) = 28.3$, $p<.001$). In the low anxious depression group 4.8% were diagnosed as having suffered a depression according to the DSM-IV criteria, this was mostly a single mild episode. In the medium and high anxious depression group these percentages were 13.3% and 26.0%, respectively.

In Table 7.1a subject characteristics are given for the low, medium and high anxious depression groups and in Table 7.1b for subjects with and without a DSM-IV diagnosis for depression. Compared to the low and medium anxious depressed, the high anxious depressed smoked more and used more medication, but they did not differ on other characteristics. Compared to participants who did not receive a diagnosis for life-time depression, participants with DSM-IV depression were more often current smokers, used more medication, were on average older, and had a higher BMI. Five participants did not receive a diagnosis for life-time depression and/or had a low anxious depression score but nevertheless indicated having used antidepressants on the measurement day. They were excluded from further analysis.

Table 7.1a: Subject characteristics for the low, medium and high anxious depression groups.

	Anxious depression			
	Low (n=177)	Medium (n=81)	High (n=173)	p-value
Sex (%men)	36.7%	33.3%	39.3%	n.s.
Age	31.1 (10.2)	29.5(10.3)	30.4(10.8)	n.s.
BMI	23.1(3.1)	23.1(3.7)	23.4(4.0)	n.s.
Smoking status:				
Ex-smokers	13.6%	17.3%	23.1%	.000[#)]
Current smokers	16.9%	19.8%	33.5%	
Medication use:				
Anti-depressants	1.1%	2.5%	4.1%	n.s.
Anti-hypertensive/cardioactive	2.3%	1.2%	5.8%	n.s.
Analgesics/anti-inflammatory agents (NSAIDS)	13.1%	19.8%	23.3%	.047
Corticosteroids	2.8%	3.7%	3.5%	n.s.
Oral contraceptives [*)]	42.0%	46.3%	44.8%	n.s.

[*)] women only [#] chi-square over three categories of the smoking status

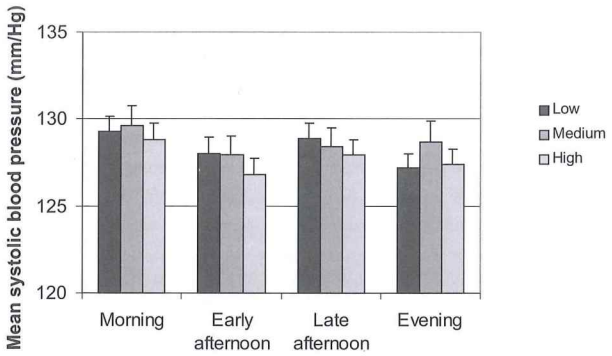
Table 7.1b: Subject characteristics of DSM-IV depressed and non-depressed groups.

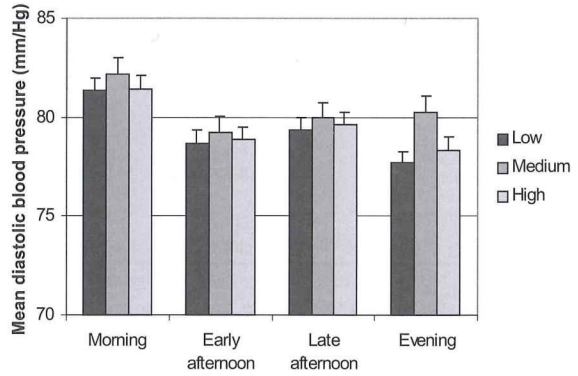
	DSM-IV depression		
	No DSM-IV depression (n=333)	DSM-IV depression (n=57)	p-value
Sex (%men)	39.3%	29.8%	n.s.
Age	29.6(9.9)	36.8(12.2)	.000
BMI	23.0(3.4)	24.1(4.8)	.029
Smoking status:			
Ex-smokers	18.3%	24.6%	.005[#)]
Current smokers	19.8%	35.1%	
Medication use:			
Anti-depressants	1.5%	7.0%	.011
anti-hypertensive/ cardioactive	3.0%	8.8%	.038
Analgesics/anti-inflammatory agents (NSAIDS)	16.3%	28.1%	.033
Corticosteroids	2.4%	8.8%	.014
Oral contraceptives [*)]	43.6%	32.5%	n.s.

[*)] women only, [#)] chi-square over three categories of the smoking status

From the remaining 426 persons valid blood pressure registrations on all four times of the day could be obtained for 412 persons. Figure 7.1 displays systolic and diastolic blood pressure levels as a function of time of day and anxious depression. Blood pressure was significantly affected by the time of day (SBP $F(3,407)=9.6$, $p<.001$; DBP $F(3,407)=46.7$, $p<.001$). Overall, blood pressure levels were highest in the morning and lowest in the evening, although relatively low blood pressure levels were also found in the early afternoon.

Figure 7.1 Time of day effects on systolic and diastolic blood pressure for the participants in the different anxious depression groups. Error bars represent standard errors.





No significant effects of anxious depression were apparent on any of the four time points. In contrast, persons who ever experienced a depressive episode according to the DSM-IV criteria had significantly higher systolic (F(1,372)=4.2 p=.04) and diastolic blood pressure (F(1,372)=5.2 p=.02) on all time points. Figure 7.2 displays the association of blood pressure with life time depression.

The above analyses were based on all available blood pressure data, including those obtained during physical activity. The effects of DSM-IV depression, therefore, could simply have reflected differential patterns of physical activity. For a second analysis we selected only those blood pressure registrations obtained during sitting postures and repeated the analysis. Because only 52% of the recordings was obtained during sitting, this analysis was more sensitive to data loss. Since no evidence was found for an interaction between time of day and anxious depression or DSM-IV depression, it was decided to use the mean of all available sitting blood pressure registrations, i.e. the time of day factor was dropped for all remaining analyses. For 418 persons average blood pressure values for sitting postures only were obtained (note that not all of these persons participated in the CIDI interview).

Using sitting blood pressure only, still no differences in blood pressure were found between the low, medium and high anxious depression groups. However, persons who ever experienced a depressive episode according to the DSM-IV criteria again had significantly higher systolic (F(1,367)=6.4, p=0.01) and diastolic (F(1,367)=6.0, p=0.02) blood pressure (see Table 7.2).

Table 7.2 Main effect DSM-IV depression on systolic and diastolic blood pressure without controlling for confounders. Means and standard deviations are presented.

	SBP (mm/Hg)	DBP (mm/Hg)
Anxious depression		
Low	127.1 (10.6)	78.5(7.7)
Medium	128.1 (9.3)	79.8(6.7)
High	127.2 (11.2)	78.8(8.5)
DSM-IV depression		
No	126.7 (10.0)	78.5(7.7)
Yes	130.6 (13.4)	81.3(9.1)

The next step was to test the effects of the four possible confounders sex, age, BMI, and smoking status on mean sitting blood pressure level. Men were found to have higher mean systolic blood pressure levels then women (F(1,394=38.6, p<.001, μ=132.0 versus 124.5 mmHg). Furthermore, age and smoking status had a significant interactive effect on SBP (F(2,394)=3.8, p=.02). Figure 7.3 graphically displays this interaction: systolic blood pressure levels increased with age in current smokers and ex-smokers, but it did not increase in non-smokers. For diastolic blood pressure, an interaction was found between age and sex (F(1,394)=6.3, p=.01). Figure 7.4

graphically displays this interaction: the increase in DBP with aging was more pronounced in men. In Figures 7.5a and 7.5b, the interaction effect of BMI and sex on SBP and DBP are depicted. Although they were only marginally significant, the effects of BMI on blood pressure appear to be more prominent in men than in women (SBP (F(1,394)=2.5, p=.11; DBP (F(1,394)=2.4, p=.12).

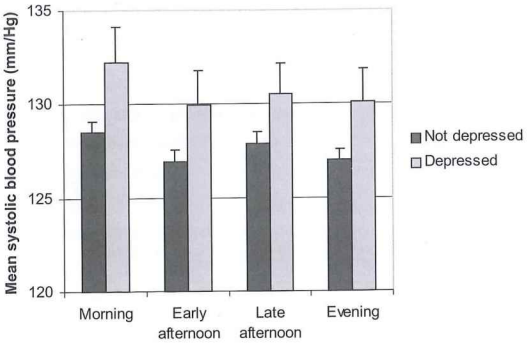
The effects of various medications and oral contraceptives on blood pressure were examined as a final possible source of confounding of the association of blood pressure with depression. The use of antidepressants, corticosteroids were not associated with blood pressure levels. The use of antihypertensive/cardioactive and analgestics/anti-inflammatory agents were both associated higher blood pressure levels (see Table 7.3), but in all instances this was simply due to older subjects using more medication than younger subjects: no significant medication effects were found when age was taken into account.

Table 7.3 Main effects medication use on systolic and diastolic blood pressure without controlling for confounders. Means and standard deviations are presented.

	SBP (mm/Hg)			DBP (mm/Hg)		
Medication use:	No	Yes	P	No	Yes	P
Anti-depressants	127.3(10.6)	131.4(12.5)	n.s.	78.9(7.9)	79.0 (2.7)	n.s.
Anti-hypertensive/ cardioactive	127.1(10.5)	136.2(10.5)	.002	78.7(7.7)	85.7 (9.5)	.001
Analgesics/anti-inflammatory agents	127.0(10.6)	129.0(10.6)	n.s.	78.4(7.8)	81.1 (8.0)	.009
Corticosteroids	127.5(10.7)	123.3(6.6)	n.s.	78.9(7.9)	77.8 (7.0)	n.s.
Oral contraceptives *)	124.2(11.3)	124.9(7.9)	n.s.	77.6(8.2)	79.1 (6.4)	n.s.

*) women only.

Figure 2 Time of day effects on systolic and diastolic blood pressure for the participants with and without a DSM-IV depression diagnosis. Error bars represent standard errors.



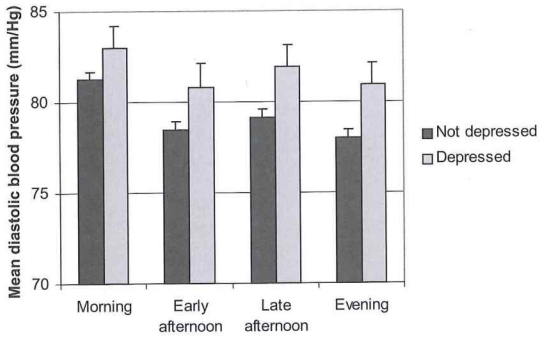


Figure 7.3 Correlation between age and systolic blood pressure. Effects as modulated by smoking status are indicated by separate regression lines for the non-smokers, current smokers and ex-smokers.

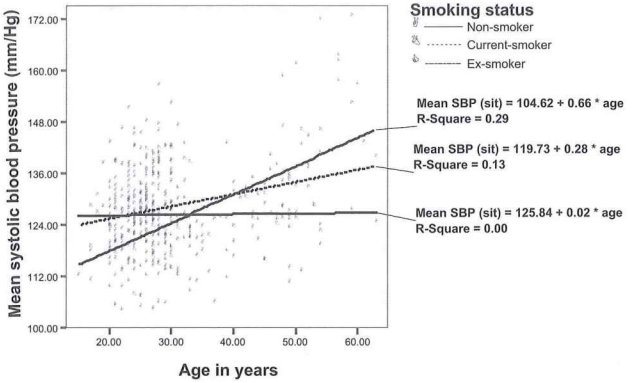


Figure 7.4 Correlation between age and diastolic blood pressure. Effects as modulated by sex are indicated by separate regression lines for men and women.

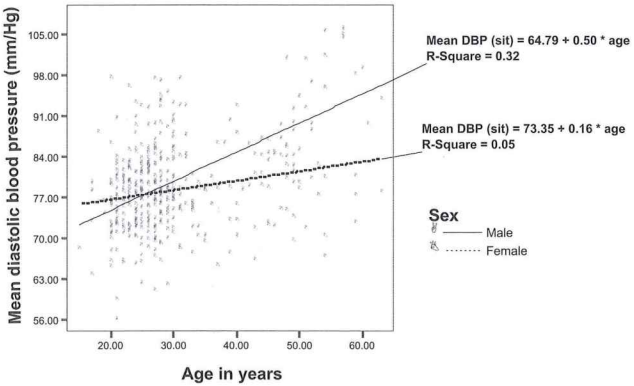
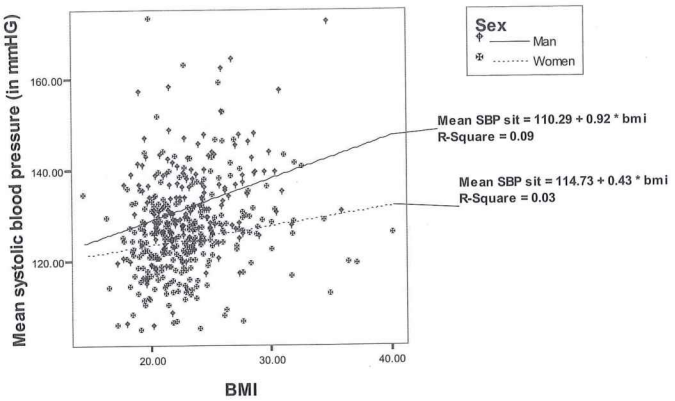
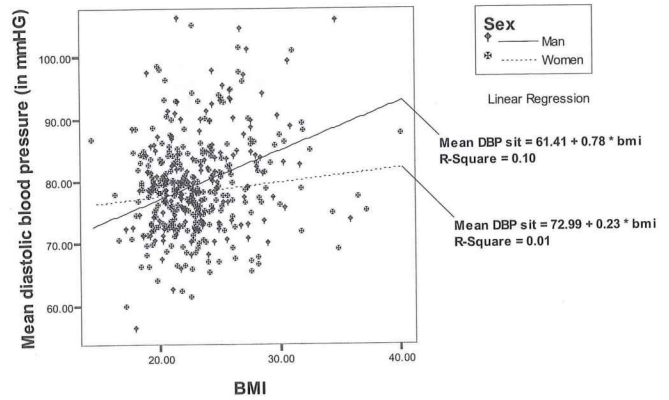


Figure 7.5 A: Correlation between body mass index and systolic blood pressure. Effects as modulated by sex are indicated by separate regression lines for men and women.
B: Correlation between body mass index and diastolic blood pressure. Effects of sex are indicated by separate regression lines for men and women.





Based on the pattern of findings above, we repeated the analyses of the associations of trait and clinical depression with blood pressure taking all the established confounding into account. First, only sitting blood pressure measurements were used. Secondly, sex, age, BMI were entered as covariates, and smoking status was used as an additional factor. Because aging effects on blood pressure in ex-smokers resembled those in smokers much more than those in non-smokers, smoking status was reduced to two categories, smoker or ex-smoker versus non-smokers.

When confounding was taken into account, still no differences in systolic or diastolic blood pressure were found between the low, medium and high anxious depression groups. For clinical depression, the main effect on diastolic blood pressure disappeared, but the effect on systolic blood pressure was only attenuated ($F(1,153)=3.3$, $p=.07$). Moreover, a significant interaction emerged for systolic blood pressure involving sex, age, and DSM-IV depression ($F(1,351)=4.6$, $p=.03$). As illustrated in figure 6, higher systolic blood pressure was found in depressed older women than in depressed young women. A similar interaction between age and life time depression was not found in men. Findings for neither trait depression nor clinical depression were changed meaningfully when the analyses were repeated after removing the 12 persons using anti-hypertensive medication.

Figure 7.6 Correlation between age and systolic blood pressure for men and women separately. Effects of clinical DSM-IV depression are indicated by separate regression lines for subjects with and without a life time depression.

7.4 Discussion

In the current study the association between depression and ambulatory blood pressure was assessed. Without controlling for the relevant confounders age, sex, BMI, and smoking, clinical DSM-IV depression was significantly associated with higher diastolic and systolic blood pressure levels. After controlling for these confounders the effect on systolic blood pressure was still significant. The effect was most pronounced in older women, but -to a lesser degree- also present in younger women and in men. Analysis on trait levels of anxious depression, which reflect an individual's vulnerability to clinical depression (see also chapter 3, this thesis), revealed no significant link between depression and high diastolic or systolic blood pressure.

Our findings support the conclusion of the bulk of the previous studies on ambulatory blood pressure and depression as listed in table 1.2: depression is associated with high blood pressure throughout the day. In contrast to these previous studies, however, we only found effects of clinical depression and not of trait depression. Moreover, the inclusion of relevant confounders in our study revealed that the association is not as straightforward as it has been presented before.

Only few studies to date had included smoking as a possible confounder in the association between depression and blood pressure. This is surprising because smoking increases blood pressure level (Oncken et al., 2001; Kannel & Higgins, 1990; Sleight, 1993; Kochar & Bindra,1996) and it is now well established that depressed persons are more often smokers than non-depressed persons (Glassman et al, 1990; Anda et al., 1990; Patton et al., 1996; see also chapter 4, this thesis). Both associations were confirmed in this sample: persons with a DSM-IV diagnosis for clinical depression were more often smokers, and smokers had significantly higher blood pressure. We additionally showed that ex-smokers had higher blood pressure, but this was largely due to their older age and the fact that most ex-smokers were male.

Apart from smoking, high BMI should also be considered a potential confounder of the association between depression and blood pressure. BMI is well recognized as a strong risk factor in hypertension (Ishibashi, 1993; Sinaiko, Gomea-Marin & Prineas, 1997). BMI is also associated with depression: among obese persons the incidence of life-time depression is higher than in a normal population (Britz et al., 2000; Specker et al., 1994). A recent study by Carpenter et al. (2000) suggest that the BMI-depression association is modulated by sex. In women major depression was associated with a higher BMI, whereas depressed men in contrast had a lower BMI. To complicate matters, we found that blood pressure increased relatively more in overweight males than it did in overweight females. Taken together, these findings point to the necessity of including sex, BMI and their interaction as possible confounders.

Most of the studies in Table 1.2 carefully controlled for use of cardio-active medication, except for Friedman et al. (2001). Ewart and Kolodner (1994) also did not record medication, but this study was conducted in high school students, with little probability of medication use. In the current study, we recorded all ongoing medication use and tested its possible influence on blood pressure.

As in most other studies, cardio-active medication (usually anti-hypertensives) was more prevalent in subjects with high blood pressure, but we found this effect to be completely confounded with age. More analgesics and anti-inflammatory medication were taken by subjects with higher blood pressure, but again this association disappeared when controlled for age.

Two final risk factors for hypertension, age and sex, have a well-known effect on depression and as such have been taken into account in virtually all studies to date. The prevalence of clinical depression in the current sample increased with age, which is in correspondence with results from other studies (e.g. Palsson, Ostling & Skoog, 2001). Although the sex difference did not reach significance ($p=0.10$), analysis in the larger sample of which these subjects were drawn clearly confirmed a larger prevalence of clinical depression in women.

Ambulatory blood pressure was associated with clinical depression, even after inclusion of confounders, but it was not associated with trait depression, with or without confounders. It is well known that clinical depression is predicted by pre-morbid high levels of trait depression (e.g. Kendler et al, 1993; Solomon et al., 2001). Indeed in this sample, and in the population from which it is drawn (Van den Berg et al., this thesis), a strong association is found between the factor score used to define trait depression and clinical depression as obtained from the CIDI interview. What could explain the discrepant effects of trait and clinical depression on blood pressure? An obvious route to explaining this discrepancy is to focus on the differences rather the similarities of these constructs. The anxious depression factor score reflects an individual's vulnerability to clinical depression, based on genetic susceptibility or early negative life-events (Nemeroff, 1999; Kendler et al, 1995). This vulnerability will also be high in subjects with lifetime depression, but these may, in addition, have been exposed to the required environmental triggers in adulthood. Virtually all models of depression consider exposure to chronic stress a major candidate for such environmental effects.

The following pathophysiological pathway, therefore, can be considered to explain the association between clinical depression and elevated blood pressure levels. First, exposure to an initial chronic stressor may independently, and more or less in parallel, cause both depression and high blood pressure, for instance, through an impact on monoaminergic neurotransmission in both central and autonomic nervous system. The idea that chronic stress can cause elevated blood pressure through increased sympathetic and reduced parasympathetic drive is widespread (Vrijkotte et al., 2000; Julius, & Jamerson, 1994; Oparil et al., 1992, Porges, 1992, 1995). Likewise, chronic stress is thought to play a central role in the disregulation of the central "CRF stress" system (Reul & Holsboer, 2002). Because an episode of clinical depression itself should be regarded as a major stressor, this in turn will further induce elevated blood pressure.

If the exposure to stress is indeed the critical factor that explains why self-reported life time depression but not trait depression or anxiety affects blood pressure, we can further speculate on the source of the interaction between sex, age and depression. As can be gauged from figure 6,

systolic blood pressure in women was responsive to clinical depression mostly from the age 40-45 onward. This is the typical age at which the menopause starts and the natural protection of estrogens against effects of repeated autonomic nervous system activation is lost (Mercuro et al., 2000; McKinlay, Brambilla, & Posner, 1992).

The above hypothesis still leaves unexplained why other studies did find an association between blood pressure and questionnaire based trait depression. Part of this may be attributable to incomplete control of confounders in these studies, but another explanation is the higher average age and lower variance of age in the samples studied in some of these previous studies (see table 1) compared to our sample. Questionnaires like the BDI, the CES-D or the SCL-90 have been shown to predict clinical depression quite well (e.g. Arnau et al., 2001; Lustman et al., 1997; Steer, Rissmiller & Beck, 2000), and their correlation with life time depression may increase in older samples. The prevalence of clinical depression increases with age as was shown for the current sample (this thesis, chapter 3). The high trait depression groups in previous studies, therefore, may have included a substantial number of persons who are vulnerable for clinical depression and would have been diagnosed with life time depression had the CIDI been administered.

In conclusion, this largest ambulatory blood pressure monitoring study to date has confirmed the existence of a significant association between blood pressure and depression. This relation, however, was not as straightforward as reported in previous studies. A diagnosis of life time depression, but not a high anxious depression score, was associated with elevated blood pressure. This association was attenuated by controlling for smoking and BMI, and modulated by age and sex.

Chapter 8

Summary & general discussion

8.1 Introduction

The present thesis examined the association between depression and risk factors for cardiovascular disease, smoking, cortisol, and blood pressure in a large population sample ascertained from the Netherlands Twin Register (NTR). Depression was measured in two ways: 1) as an anxious depression factor score based on longitudinal survey data, and 2) as incidence of life-time clinical depression based on a psychiatric diagnostic interview. Smoking was assessed by means of survey data in 4584 participants. During a representative day salivary cortisol samples and ambulatory blood pressure measurements were obtained in 431 persons. This chapter discusses the results for each measured parameter and attempts to formulate a general conclusion from the results of the preceding chapters. In conclusion some recommendations for future research will be made.

8.2 Validity of the anxious depression score

An anxious depression factor score was computed for the purpose of identification of the genes involved in anxiety and depression in future. This score was based on the multivariate analysis of anxiety, neuroticism, and depression in twins and their siblings and represents the genetic vulnerability to anxious depression. The analyses in the current thesis were based on this score. To ensure that the anxious depression factor score indeed represented vulnerability to anxiety and depression, the anxious depression factor score was validated against a clinical measure of depression obtained from the Composite International Diagnostic Interview (CIDI). Validation of anxious depression against DSM-IV depression in the sample that participated in the 1997 survey is presented in Chapter 3. Findings confirmed that the anxious depression factor score reflected the vulnerability to clinical depression. A strong association between the anxious depression factor score and the categories of the DSM-IV diagnosis for depression was found. In general it appeared that high anxious depression was associated with high risk of a DSM-IV depression diagnosis. Especially the diagnosis of recurrent depression and severe depression was more prevalent in the persons with high factor scores.

Based on the results of the validation study it can be concluded that the composite factor score, combining both anxiety and depression scales, indeed represents the vulnerability to clinical depression and can be used as a reliable measure of trait depression.

8.3 Smoking status

In Chapter 4 the association between smoking and depression was assessed in a family based population. The assessment of smoking status was based on both longitudinal survey data and on interview data obtained on the measurement day. Smoking status derived from this data categorised the participants into three groups: non-smokers, ex-smokers and current smokers. First the association between smoking and depression was assessed in the whole sample and in addition to control for possible common aetiologic factors underlying the association, in a between family and within family design. In the whole sample, both men and women that had either a high score on anxious depression or a clinical depression were more often smokers than non-depressed persons. The association between depression and smoking appeared to be extra strong in the youngest age cohort (15 to 25 years). Since smoking initiation occurs in this age cohort, it is possible that depression is a mediating factor in smoking initiation. Women who were diagnosed with DSM-IV depression appeared to be more often current smokers and less often ex-smokers than men diagnosed with DSM-IV depression. This supports the suggestion that women, more than men use smoking as a means to reduce depressive symptoms and are therefore less able to cease smoking. Non-depressed persons from families with a positive family history were also more likely to be current smokers or ex-smokers compared to non-depressed persons from families with a negative family history for depression. Even though these persons had themselves a higher mean anxious depression score than persons from a negative family history, this could indicate that having a positive family history for depression increases vulnerability to smoking initiation. In sum, smoking status proved to be strongly associated with both anxious depression and clinical depression, particularly in the youngest cohort in the study. Therefore, smoking is a likely mediating factor in the association between depression and cardiovascular risk.

Apart from a strong association between smoking and depression, smoking was also found to be associated with both cortisol and ambulatory blood pressure levels. The stimulating effects of smoking on both cortisol (Wilkins et al., 1982; Pomerleau and Pomerleau, 1990; Kirschbaum et al., 1992; Del Arbol et al., 2000) and blood pressure (e.g. Oncken et al., 2001; Kannel & Higgins, 1990; Sleight, 1993; Kochar & Bindra, 1996) were also found in this thesis. More specific, in the assessment of cortisol levels in Chapter 6 it appeared that current smokers had higher cortisol levels during the day, i.e. on the second morning sample and the afternoon sample, but lower cortisol levels at awakening. Furthermore, in the assessment of ambulatory blood pressure (Chapter 7) levels it was found that smokers and ex-smokers had higher blood pressure than non-smokers. It is therefore surprising that smoking status was not always considered as a major confounder in earlier studies that assessed the association between depression and cortisol and depression and blood pressure.

8.4 Cortisol

In the current thesis, no association between daytime cortisol levels and depression, both trait and clinical, was found. Noteworthy is that there are only a few studies with a similar sample size as reported on in the current thesis (n=338). Only the studies by Brandtstadter et al. (1991, n=767), Strickland et al. (2002, n=343) and Mazur et al. (1994, n=4462) provided data on larger samples and found no association between depression and cortisol levels.

A major strength of the design used in Chapter 6 was the availability of both trait levels of anxious depression, which reflect an individual's vulnerability to clinical depression and clinical depression. In addition to the assessment of an association between depression and cortisol levels in the whole sample, a similar assessment within and between families was performed. This way aetiologic mechanisms related to familial factors could be accounted for. Most of the earlier studies reviewed in Chapter 6 reported cortisol levels of in-patient samples that had a history of major depression, or they reported trait depression derived from personality questionnaires administered on the measurement day. But none of the studies reviewed in Table 1.1 in Chapter 1 of this thesis provided such a complete assessment of both trait and clinical depression as in the present thesis. Furthermore, based on the strong association found in the literature between smoking and cortisol levels and smoking and depression special attention was given to the confounding role of smoking. It was rather remarkable that only a few studies from the review in Table 1.1 mentioned cigarette smoking as a confounder.

Cortisol was measured in saliva. This method is relatively new to the field of cortisol studies (Bartels et al., in press). It was decided mainly because saliva collection is, as opposed to collection of blood samples, not an invasive method and therefore not a stressor. Furthermore, it is easy to perform by the participants themselves, and therefore does not require professionals to handle needles. Finally saliva cortisol sampling can be done in any setting and is therefore ideal for ambulatory measurements. From the results in Chapter 6 it is clear that saliva sampling provides the expected circadian variance in the cortisol profile. The participants in the study had no trouble to comply with the cortisol sampling protocol during their daily activities, illustrating that this strategy is useful for cortisol sampling in large populations.

However, no association between depression and cortisol was found in the current study despite the large sample size, control for possible confounders and valid assessment of cortisol and trait and clinical depression.

8.5 Blood pressure

In Chapter 7 the association between depression and blood pressure was assessed. Analysis on trait levels of anxious depression revealed no significant association between depression and high blood pressure. The association between life-time depression and ambulatory blood pressure, however, appeared to be significant. Both diastolic blood pressure and systolic blood pressure

levels were higher in persons who had experienced a life-time clinical depression compared to persons who never had experienced such an episode. When the confounding influences of posture/physical activity on the day of measurement and of age, sex, BMI, smoking and medication status were taken into account, clinical depression was still associated with higher systolic blood pressure, but the association was found to be most pronounced in older women.

Our findings confirm findings of previous studies on the positive association between depression and ambulatory blood pressure. However, compared to other studies our study adds to the literature on two important points. 1) In this study a major advantage was the assessment of both trait depression and clinical depression. The results showed that not the vulnerability for developing a clinical depression is associated with higher blood pressure levels but only having experienced a clinical depression itself. 2) In this study possible influencing factors on which depressed persons differ from non-depressed were strictly controlled for. Because depressed persons have repeatedly been found to be less physical active during the day, controlling for posture and activity effects on blood pressure during the measurement day is appropriate in an ambulatory measurement design. Therefore, only blood pressure registrations obtained during sitting postures were included in the final analyses in Chapter 7. Second, since both depression and blood pressure are know to be associated with smoking status, age, sex, BMI and medication use, these confounders were taken into account in the current study as well. Inclusion of confounders in the analyses in fact revealed that the effects of clinical depression on ambulatory blood pressure were not as straight forward as had been reported before in previous studies. Unfortunately inclusion of all these confounders also meant that the within and between family design as used in chapter 4 (smoking) and 6 (cortisol) could not be performed on the blood pressure data: even though the number of participants in ambulatory monitoring was quit large, within or between family analyses required more families when controlling for so many confounders is necessary.

In sum, this largest ambulatory blood pressure monitoring study to date has confirmed the existence of a significant association between blood pressure and depression. This relation, however, was not as straight forward as reported in previous studies. A diagnosis of life time depression, but not a high anxious depression score, was associated with elevated blood pressure. This association was attenuated by controlling for smoking and BMI, and modulated by age and sex.

8.6 Synthesis

In the present thesis, an a priori decision was made to measure possible risk of cardiovascular disease in depression in a normotensive, non-clinical population. Although life-time clinical depression was determined, there was no division in acute or remitted depression. The rationale for this approach was that if according to the stress diathesis model of depression, depression was associated with biological alterations, these would also be present in remitted depression and to a

lesser extent in persons at risk for a clinical depression who had not already experienced a depressive episode. Participants were ascertained from Dutch twin families. Because twinning occurs in each region and at all socio-economic levels, ascertainment through a twin register yields a representative sample of the population at large. It is therefore assumed that the results from the present thesis can be generalised to the population at large.

In the present thesis depression was associated with a number of health risk factors. Persons with a DSM-IV diagnosis for depression differed from non-depressed persons in that they had higher BMI and were more often current or ex-smokers. Persons with a DSM-IV depression were in general older than persons who were not diagnosed with depression and possibly in relation to that DSM-IV depression was associated with more medication use in general. High anxious depressed persons, who were at risk for a clinical depression, were more often current or ex-smokers than low anxious depressed persons. From the results on the health risk behaviours it can be concluded that clinical depression is associated with an unfavourable risk profile for cardiovascular disease.

The results from the analysis on depression and salivary cortisol and depression and ambulatory blood pressure were less unambiguous. The hypothesis was that there would be increased sympathetic and reduced parasympathetic drive in depressed persons which would in time result in elevated cortisol and blood pressure levels. No evidence of higher cortisol levels was found for either anxious depression or clinical depression. This does not mean, however, that there is no disturbance on the CNS-CRF pathway, since cortisol is an end product of a chain of events regulated by various feedback mechanisms. One important reason why the present study did not find the association between depression and cortisol could be that cortisol was measured in a population sample. Other population sample studies did not find the expected relationship (e.g. Brandtstadter et al., 1991; Mazur et al, 1994; Strickland et al, 2002). It could be that a clinical diagnosis of depression is required for elevated cortisol levels. However, no association was found between depression and cortisol levels in DSM-IV depression either. An alternative explanation for the lack of evidence for an association between depression and cortisol is that it is only found in acute depression as opposed to remitted depression. Support for this possibility is found in a study by Trestman et al. (1995). The explanation for higher cortisol levels in acute depression is that experiencing depression or even hospitalization may be a stressor in itself that causes chronic stress-reactivity resulting in high cortisol levels (Maes et al., 1994). Results from the blood pressure analyses showed no effects on anxious depression but elevated blood pressure in clinical depression. After controlling for the relevant confounders, age, sex, BMI, smoking, the effect appeared to be attenuated and significant only for systolic blood pressure in older women, and -to a lesser degree- in younger women and in men.

Since elevated blood pressure can be seen as the end product of autonomic deregulation it is possible that this is the underlying mechanism explaining the elevated blood pressure levels in this study. Depression has been associated with sympathetic overactivation (Dawson, Schell &

Catania, 1977; Iacono et al. 1983; Ward, Doerr & Storrie, 1983)and reduced parasympathetic activity (Carney et al., 1995; Krittayaphong et al., 1997; Watkins & Grossman, 1999). Autonomic deregulation may account for the higher systolic blood pressure levels found in older women who were diagnosed with DSM-IV depression. To reliably answer this question it is necessary to have recordings of heart rate, impedance cardiogram and respiration. From these two variables two reliable measures of sympathetic and vagal tone can be derived, namely: pre-ejection period (PEP), respiratory sinus arrhythmia (RSA) and the root mean square of the successive differences (rMSSD). Recordings of heart rate and respiration were made and these data will become available in the near future.

8.7 Future research

This study is the first in a large project in which it is attempted to identify the genes that are involved in anxiety and depression. Furthermore the goal of the project is also to identify endophenotypes, or biological markers of anxiety and depression, which can enhance the knowledge on the mechanisms underlying anxiety and depression. The follow-up on the ambulatory measurement started one year ago and will yield a larger sample of twins and siblings and consequently more complete families. Also more complete monozygotic and dizygotic twin pairs will be included. The benefit of more complete families and more complete twin pairs is that it will from now on be possible to include genetic analysis into the research design. This will reveal more information on the possibility that there are common genetic factors underlying the association between depression and cardiovascular disease risk.

Finally an interesting point in the within family analysis has to be made with regard to the unaffected sibling of the discordant sib pair. It is often assumed in within family analyses in which a trait (e.g. smoking) is compared between the index sib and the unaffected control sib that any resemblance between the siblings would reflect familial factors not related to depression. However, as found in Chapter 4 the non-depressed siblings from families with a positive family history for depression had more often a high anxious depression score than the non-depressed siblings from the families with a negative family history for depression. Moreover it must not be forgotten that the unaffected siblings of families with a positive history of depression experienced a negative life event when one of their siblings experienced a clinical depression. This fact alone can put high stress on a family. It is therefore recommended that for further within family analysis not a first degree unaffected relative is compared to the index sibling, but a second degree unaffected relative, i.e. a cousin.

Hoofdstuk 9

Nederlandstalige samenvatting

9.1 Inleiding

In dit proefschrift werd de relatie tussen depressie en de kans op hart- en vaatziekten onderzocht in een grote groep mensen uit het Nederlands Tweelingen Register (NTR). Depressie werd gemeten op twee manieren: ten eerste werd een factorscore voor angstige depressie gebruikt die was gebaseerd op longitudinale vragenlijstdata met betrekking tot angst en depressie. Ten tweede werd de incidentie van life-time depressie volgens de diagnostische criteria van de DSM-IV gebruikt. Variabelen die werden gemeten als mogelijke mediators voor de relatie tussen depressie en de kans op hart- en vaatziekten waren de rookstatus, cortisol en bloeddruk. De rookstatus werd bepaald met behulp van longitudinale vragenlijstdata en een vragenlijst die werd afgenomen op de ambulante meetdag. Bloeddruk- en cortisolwaarden werden ambulant gemeten gedurende een representatieve (werk)dag. In deze Nederlandstalige samenvatting worden de resultaten voor elke gemeten variabele besproken en wordt ingegaan op de algemene conclusie. Tenslotte worden er enkele aanbevelingen voor toekomstig onderzoek gedaan.

9.2 De validiteit van de factorscore voor angstige depressie

Met als doel uiteindelijk de genen te identificeren die een rol spelen bij angst en depressie, werd een factorscore berekend die de mate van genetische kwetsbaarheid voor angstige depressie weergeeft. Deze factorscore was gebaseerd op de multivariate analyse van angst, neuroticisme en depressie bij tweelingen en hun broers en zussen. Alle analyses in dit proefschrift zijn gebaseerd op deze factorscore. Om er zeker van te zijn dat de factorscore voor angstige depressie inderdaad de kwetsbaarheid voor angst en depressie weergeeft, is deze gevalideerd tegen een klinische maat voor depressie, verkregen met de 'Composite International Diagnostic Interview' (CIDI), een psychiatrisch diagnostisch interview. De in Hoofdstuk 3 gegeven resultaten van deze validatiestudie zijn uitgevoerd met de gegevens van de vierde vragenlijst uit het longitudinale onderzoek, verzonden in 1997, waaraan 4584 personen meededen. Uit de resultaten bleek een sterk verband tussen de factorscore voor angstige depressie en het hebben van een DSM-IV diagnose voor life-time depressie: een hoge factorscore voor angstige depressie werd geassocieerd met een groter risico voor klinische depressie. Met name de diagnose voor herhaalde episodes en episodes van een ernstige aard kwam meer voor bij mensen die een hoge factorscore hadden voor angstige depressie.

Gebaseerd op de resultaten van de validatiestudie kan worden geconstateerd dat de factorscore die is opgebouwd uit de scores van verschillende angst- en depressievragenlijsten, inderdaad de kwetsbaarheid voor klinische depressie weergeeft en daarom kan worden gebruikt als een betrouwbare maat voor angstige depressie.

9.3 Rookstatus

In Hoofdstuk 4 werd het verband tussen de rookstatus en depressie onderzocht in een onderzoekspopulatie bestaande uit tweelingenfamilies. Met de rookstatus van een persoon wordt bedoeld of een persoon een roker of een ex-roker is of nog nooit in zijn leven heeft gerookt. Het vaststellen van de rookstatus gebeurde op basis van de gegevens uit het longitudinale vragenlijstonderzoek en voor de mensen die hadden meegedaan aan de ambulante cardiovasculaire metingen op basis van de gegevens verzameld tijdens de meetdag. Als eerste werd de relatie tussen roken en depressie onderzocht in de gehele onderzoekspopulatie onafhankelijk van de familieverbanden. Uit de resultaten bleek dat zowel mannen als vrouwen, wanneer ze een hoge score hadden voor depressie of een klinische diagnose voor life-time depressie, vaker rookten dan personen die nooit depressief waren geweest. Het verband tussen depressie en roken was het sterkst in de leeftijdsgroep van vijftien tot en met vijfentwintig jaar. Aangezien dit over het algemeen de leeftijdsgroep is waarbinnen ook begonnen wordt met roken, bestaat er een mogelijkheid dat depressie een belangrijke factor is bij het beginnen met roken. Vrouwen met een life-time diagnose voor klinische depressie bleken vaker rokers te zijn en minder vaak ex-rokers dan mannen met een life-time diagnose voor klinische depressie. Dit bevestigt de theorie dat vrouwen, meer dan mannen, het roken van sigaretten gebruiken als een middel om hun depressieve symptomen te verminderen en daarom dus minder goed in staat zullen zijn om te stoppen met roken.

Vervolgens werden families vergeleken waarbinnen alle kinderen een lage factorscore voor angstige depressie hadden met families waarbinnen alle kinderen een hoge factorscore voor angstige depressiviteit hadden. Daarnaast werden families waarvan één of meer kinderen een klinische depressie hadden (gehad) (positieve familiehistorie) vergeleken met families waarvan geen enkel kind een klinische depressie had (gehad) (negatieve familiehistorie). Zoals verwacht waren personen uit families met overwegend een hoge factorscore voor angstige depressie vaker rokers of ex-rokers dan personen uit families met overwegend een lage factorscore voor angstige depressie. Niet-depressieve personen uit families met een positieve familiehistorie voor depressie waren vaker rokers of ex-rokers in vergelijking met niet-depressieve personen uit families met een negatieve familiehistorie voor depressie. De gemiddelde factorscore van personen uit families met een positieve familiehistorie was hoger dan die voor personen uit families met een negatieve familiehistorie, toch kan dit een aanwijzing zijn dat een positieve familiehistorie voor depressie een rol speelt in het beginnen met roken.

Ten slotte werden binnen families broers en zussen vergeleken, waarvan de één een hoge factorscore voor angstige depressie had en de ander een lage, of waarvan er één een klinische depressie had (gehad) en de ander niet. Er bleek geen verschil te zijn in rookstatus tussen broers en zussen die discordant waren voor angstige depressie. De broers en zussen die discordant waren voor angstige depressie, leken dus met betrekking tot roken vaak wel op elkaar.

Samengevat: de rookstatus van een persoon blijkt sterk gerelateerd te zijn aan diens factorscore voor angstige depressie en aan de life-time diagnose voor depressie, met name in het jongste cohort uit deze studie. Het is daarom mogelijk dat roken een belangrijke factor is in de relatie tussen depressie en de kans op hart- en vaatziekten.

9.4 Cortisol

Cortisol werd in het speeksel gemeten met behulp van wattenrolletjes waarop gekauwd moest worden. De deelnemers aan het onderzoek verschaften deze speekselmonsters op zes verschillende tijdstippen gedurende de meetdag: 's ochtends vroeg in het bijzijn van de onderzoeker, om elf uur 's ochtends, om drie uur 's middags en 's avonds om acht uur en om halfelf. Het laatste speekselmonster werd de volgende ochtend direct na het ontwaken, afgenomen.

In dit proefschrift werd geen relatie tussen het cortisol dagprofiel en depressie, zowel klinisch als niet-klinisch, gevonden. Slechts een klein aantal studies naar de relatie tussen cortisol en depressie had een steekproef van vergelijkbare grootte met de studie in dit proefschrift (n=338). De studies van Brandstadter et al. (1991; n=767), Strickland et al. (2002, n=343) en Mazur et al. (1992, n=4462) waren de enigen met een nog grotere steekproef en deze studies vonden ook geen relatie tussen cortisol en depressie.

Een van de sterke punten van de onderzoeksopzet die in Hoofdstuk 6 werd gebruikt was de beschikbaarheid van zowel gegevens over klinische depressie als gegevens van persoonlijkheidsvragenlijsten met betrekking tot angst en depressie, weergegeven in de factorscore voor angstige depressie. Daarnaast werd de relatie tussen cortisol en depressie niet alleen in de gehele populatie gemeten, maar werden ook families en broers en zussen met elkaar vergeleken. Op deze manier kon er gecorrigeerd worden voor factoren die variëren tussen families, maar niet binnen families. De meeste onderzoeken in tabel 1.1 (hoofdstuk 1 van dit proefschrift) rapporteren het cortisolniveau van klinisch depressieve patiënten, of van mensen met een hoge score op een persoonlijkheidsvragenlijst met betrekking tot depressie (vaak afgenomen op de meetdag zelf). Geen van de bestudeerde onderzoeken in Tabel 1.1 heeft depressie zo uitgebreid gemeten als de studie in dit proefschrift. Bovendien is er, gebaseerd op de studies die een sterk verband vinden tussen roken en cortisol en depressie en roken, speciale aandacht gegeven aan de rol van roken in de steekproef. Slechts een klein aantal studies die worden genoemd in Tabel 1.1, vermeldt roken als een belangrijke onderzoeksfactor. Het stimulerende

effect van roken op cortisol (Wilkins et al., 1982; Pomerleau & Pomerleau, 1990; Kirschbaum et al., 1992; Del Arbol et al., 2000) en bloeddruk (bv. Oncken et al., 2001; Kannel & Higgings, 1990; Sleight, 1993; Kochar & Bindra, 1996) werd bevestigd in dit proefschrift. Uit de gegevens met betrekking tot het cortisolniveau in Hoofdstuk 6 bleek dat rokers gedurende de dag een *hoger* cortisolniveau hadden dan niet-rokers, dit was significant op de tweede ochtendmeting en de middagmeting. Maar rokers hadden juist een *lager* cortisolniveau bij het ontwaken.

Het meten van cortisol in het speeksel is een relatief nieuwe methode in het cortisolonderzoek (zie voor een review: Bartels et al., in druk). Voor deze methode werd gekozen omdat het in tegenstelling tot bloedprikken, een niet-invasieve methode is en daarom nauwelijks stress veroorzaakt. Daarnaast is het eenvoudig door de deelnemers zelf uit te voeren waardoor er geen professionele medewerkers nodig zijn voor het afnemen van bloedmonsters. Ten slotte is gekozen voor deze methode omdat het onder bijna alle omstandigheden uitgevoerd kan worden, wat ideaal is voor ambulant onderzoek. De deelnemers aan de studie konden moeiteloos voldoen aan het protocol gedurende hun dagelijkse activiteiten. Dit geeft aan dat deze methode bruikbaar is voor het verzamelen van cortisol in grote steekproeven.

Er werd echter, ondanks de grootte van de steekproef, de controle voor factoren die mogelijk cortisol beïnvloeden en de zorgvuldigheid waarmee zowel cortisol als depressie werden gemeten, geen relatie gevonden tussen cortisol en depressie.

9.5 Bloeddruk

In Hoofdstuk 7 werd de relatie tussen ambulant gemeten bloeddruk en depressie onderzocht. Gedurende de ambulante meetdag werd elke dertig minuten (± 10 minuten) de bloeddruk gemeten met behulp van een ambulante bloeddrukmeter (SpaceLabs Medical, Redmond, USA). De deelnemers aan het onderzoek kregen een waarschuwingssignaal te horen wanneer de bloeddrukmeter ging meten en waren geïnstrueerd hun arm zo ontspannen mogelijk te houden, of wanneer dit niet mogelijk was, de meting enige minuten uit te stellen. De bloeddrukmeter zat om de niet-dominante arm.

Er bleek geen significante relatie te zijn tussen bloeddruk en de factorscore voor angstige depressie. Er was echter wel een significante relatie tussen bloeddruk en de life-time diagnose voor depressie. Zowel de diastole als de systole bloeddruk waren hoger bij personen die ooit in hun leven een klinische depressie hadden gehad in vergelijking tot personen die nog nooit in hun leven een klinische depressie hadden gehad. Wanneer er echter werd gecorrigeerd voor factoren die van invloed konden zijn op de bloeddruk, zoals roken, body mass index (BMI), leeftijd, sekse, medicijngebruik en tijd van de dag, dan was het effect alleen nog significant voor de systole bloeddruk bij post-menopausale vrouwen en niet bij pre-menopausale vrouwen of mannen.

Deze resultaten lijken de bevindingen van andere studies die een relatie vonden tussen bloeddruk en depressie te bevestigen. Dit onderzoek verschilde echter op een aantal belangrijke

punten van andere studies. Ten eerste werd in deze studie zowel klinische depressie als genetische kwetsbaarheid voor klinische depressie gemeten. Uit de resultaten bleek dat niet de genetische kwetsbaarheid, weergegeven door een hoge factorscore voor angstige depressie, maar het ooit ervaren van een klinische depressie was gerelateerd aan een hogere bloeddruk. Ten tweede werd in deze studie rekening gehouden met factoren waarin depressieve personen verschilden van niet-depressieve personen en die mogelijk invloed hadden op de bloeddruk. Van mensen die depressief zijn wordt gedacht dat ze ook minder actief zijn gedurende de dag, daarom is het bij ambulante bloeddrukmetingen van belang ook te corrigeren voor houding en activiteit. Om deze reden werden alleen de bloeddrukregistraties gedurende de zittende houdingen gebruikt in de uiteindelijke analyses in Hoofdstuk 7. Ten derde werd er gecorrigeerd voor factoren die van invloed zouden kunnen zijn op de bloeddruk. Omdat zowel depressie als bloeddruk verband hebben met rookstatus, BMI, het gebruik van medicatie, leeftijd en sekse, werd met al deze factoren rekening gehouden. Uit de gegevens van de ambulante bloeddrukregistraties bleek bijvoorbeeld dat rokers en ex-rokers een hogere bloeddruk hadden dan niet-rokers. Na correctie voor deze factoren was het effect van de diagnose voor life-time depressie op bloeddruk alleen nog maar aanwezig bij oudere vrouwen. Helaas betekende het includeren van al deze factoren in de analyse ook dat de vergelijking tussen families en kinderen binnen één gezin zoals uitgevoerd in Hoofdstuk 4 (rookstatus) en 6 (cortisol), niet mogelijk was: het doen van deze analyses maakt een grotere steekproef met meer complete gezinnen noodzakelijk.

Samengevat kan gesteld worden dat dit de grootste studie is naar de relatie tussen depressie en ambulant gemeten bloeddruk. Deze relatie blijkt echter niet zo eenduidig te zijn als eerdere onderzoeken suggereren. Een hoge factorscore voor angstig depressie, een indicatie voor kwetsbaarheid voor klinische depressie, was niet gerelateerd aan een hoge bloeddruk, maar het hebben van een life-time diagnose voor klinische depressie wel. Na controle voor roken, BMI, medicatiegebruik, leeftijd en sekse, was deze relatie alleen significant voor post-menopausale vrouwen.

9.6 Synthese

In dit proefschrift werd a priori besloten om de risicofactoren voor hart- en vaatziekten gerelateerd aan depressie, te meten in een normotensieve, niet-klinische populatie die was geselecteerd op basis van een extreem hoge of lage factorscore voor angstige depressie. Hoewel de diagnose voor depressie bekend was, werd geen onderscheid gemaakt tussen depressie in de acute fase of remitterende depressie. De gedachtegang hierachter was dat als er volgens de stress-diathese hypothese voor depressie inderdaad een relatie is tussen depressie en biologische veranderingen, deze ook meetbaar zouden zijn bij mensen die ooit depressief waren en in mindere mate bij mensen die kwetsbaar zijn voor klinische depressie. De populatie voor dit onderzoek was afkomstig uit het Nederlands Tweelingen Register. Omdat tweelinggeboorten in principe

voorkomen in alle lagen van de bevolking en in elk deel van het land, is een steekproef uit een tweelingenregister een representatieve weergave van de bevolking en zouden de resultaten gegeneraliseerd kunnen worden.

In dit proefschrift werd depressie geassocieerd met een aantal gezondheidsrisico's. Personen die een diagnose voor klinische depressie hadden in vergelijking met personen zonder diagnose hadden een hogere BMI en waren vaker rokers of ex-rokers. Personen met een diagnose voor depressie waren over het algemeen ouder dan personen die geen diagnose voor depressie hadden en ze gebruikten vaker medicatie, waarschijnlijk juist omdat ze ouder waren. Personen met een hoge score voor angstige depressie, die kwetsbaar zijn voor klinische depressie, waren vaker rokers of ex-rokers dan personen met een lage score voor angstige depressie. Uit de resultaten kan worden geconcludeerd dat een klinische depressie is geassocieerd met een ongunstig gezondheidsprofiel voor hart- en vaatziekten (bv. roken, hogere leeftijd, hoger BMI). Bovendien werd in Hoofdstuk 6 (cortisol) en Hoofdstuk 7 (ambulant gemeten bloeddruk) gevonden dat de relatie tussen roken en depressie een belangrijke rol kan spelen in de interpretatie van de resultaten omdat roken een stimulerend effect heeft op zowel het cortisolniveau (bv. Wilkins et al., 1982; Pomerleau & Pomerleau, 1990; Kirschbaum et al., 1992; Del Arbol et al., 2000) als de bloeddruk (bv. Oncken et al., 2001; Kannel & Higgings, 1990; Sleight, 1993; Kochar & Bindra, 1996) Het is daarom sterk aan te raden de rookstatus te beschouwen als een factor die van invloed kan zijn op de relatie tussen depressie en cortisol en bloeddruk.

De resultaten uit de analyses van depressie met cortisol en ambulant gemeten bloeddruk waren minder eenduidig. De hypothese was dat er sprake zou zijn van een toegenomen sympathische activatie en een afgenomen parasympathische activatie bij depressieve personen, wat op den duur zou resulteren in hoger cortisol- en bloeddrukniveau. Er werd echter geen bewijs gevonden voor hogere cortisolwaarden bij angstige depressie of klinische depressie. Dit betekent echter niet dat er geen verstoring is in de CNS-CRF banen, aangezien cortisol een eindproduct is van een serie van hormonale reacties die worden gereguleerd door verschillende feedback mechanismen. Mogelijk is een belangrijke reden waarom in de huidige studie geen relatie wordt gevonden tussen depressie en cortisol, dat cortisol niet alleen is gemeten bij mensen met een life-time diagnose voor klinisch depressie, maar ook bij mensen zonder deze diagnose, maar wel met een hoge factorscore voor angstige depressie. Andere studies die eveneens werden uitgevoerd in niet-klinisch depressieve populaties, maar wel met een hoge score voor depressie op persoonlijkheidsvragenlijsten, vonden de relatie tussen cortisol en depressie ook niet (bv. Brändtstädter et al., 1991; Mazur et al., 1996; Strickland et al., 2002). Het zou kunnen zijn dat hogere cortisolwaarden alleen worden gevonden in klinisch depressieve populaties. In Hoofdstuk 6 van dit proefschrift werden echter ook geen hogere cortisolwaarden gevonden bij mensen die naast een hoge depressiescore op een persoonlijkheidsvragenlijst ook nog een DSM-IV diagnose voor life-time depressie hadden. Een andere verklaring voor het ontbreken van het verwachte verband tussen depressie en cortisolwaarden is dat er van deze associatie alleen sprake is tijdens

een acute depressie en niet in de niet-acute fase van een depressie. Deze mogelijkheid wordt ondersteund door de resultaten die werden gevonden in een studie van Trestman et al. (1995). Een verklaring voor het feit dat alleen in de acute fase van een depressie hogere cortisolwaarden worden gevonden zou kunnen zijn dat het ervaren van een depressie een stresvolle gebeurtenis is voor de patiënt zelf waardoor het cortisolniveau stijgt (Maes et al., 1994).

De resultaten uit de analyses van de bloeddrukmetingen toonden alleen een verhoogde bloeddruk bij personen die ooit een klinische depressie hadden gehad en niet bij personen die wel een hoge factorscore hadden, maar nooit een klinische depressie hadden gehad. Na controle voor factoren die de resultaten zouden kunnen beïnvloeden bleef het effect alleen significant gehandhaafd voor de systole bloeddruk van post-menopausale vrouwen en niet bij jonge vrouwen of mannen.

Aangezien verhoogde bloeddruk veroorzaakt kan worden door deregulatie van het autonome zenuwstelsel, is mogelijk dat dit toch het onderliggende mechanisme is, hoewel in dit onderzoek daar geen bewijs voor werd gevonden. Depressie wordt in verband gebracht met sympathische overactivatie (Dawson, Schell & Catania, 1977; Iacono et al., 1983; Ward, Doerr & Storrie, 1983) en een afname van parasympathische activatie (Carney et al., 1995; Krittayaphong et al., 1997; Watkins & Grossman, 1999). Deregulatie van het autonome zenuwstelsel kan een verklaring zijn voor de hogere systole bloeddrukwaarden die werden gevonden bij oudere vrouwen met een DSM-IV diagnose voor depressie. Om dit verder te onderzoeken is het noodzakelijk om hartslag, ademhaling en het impedantie cardiogram te registreren. Van deze variabelen kunnen betrouwbare maten van zowel de activatie van het sympathische zenuwstelsel als de vagale tonus afgeleid worden, namelijk de pre-ejectie periode (PEP) en de respiratoire sinus arythmie (RSA) of de root mean square of the successive differences [wortel van het gemiddelde gekwadrateerde verschil tussen twee R-toppen (RMSSD)]. Deze registraties zijn ook gemaakt en de resultaten zullen in de nabije toekomst worden gepubliceerd.

9.7 Toekomstig onderzoek

Dit onderzoek was het eerste deel van een groot onderzoek naar de genen die van belang zijn voor angst en depressie. Het identificeren van endofenotypen die de kennis over de mechanismen die ten grondslag liggen aan het ontstaan van angst en depressie vergroten, is hierbij van groot belang. Het vervolgonderzoek van de ambulante metingen van hartslag, bloeddruk en cortisol is inmiddels meer dan een jaar geleden begonnen en zal het aantal families vergroten. Er zullen tevens meer monozygote en dizygote tweelingparen gemeten worden. Het voordeel van meer complete families en van meer complete monozygote en dizygote tweelingparen is dat er dan ook genetische analyses gedaan kunnen worden. Hierdoor zal beter onderzocht kunnen worden of er mogelijk gemeenschappelijke genetische factoren zijn die ten grondslag liggen aan zowel depressie als de kans op hart- en vaatziekten.

Tenslotte is het interessant om op te merken dat in de analyse van verschillen tussen aangedane en niet-aangedane familieleden vaak wordt aangenomen dat overeenkomsten tussen hen zijn toe te schrijven aan familiale factoren die niet gerelateerd zijn aan depressie. In Hoofdstuk 4 werd echter gevonden dat de niet-aangedane kinderen uit families met een positieve familiehistorie voor depressie vaker zelf ook een hoge factorscore hadden voor angstige depressie dan de niet-aangedane kinderen van families met een negatieve familiehistorie voor depressie. De niet-aangedane kinderen van families met een positieve historie voor angstige depressie leken dus meer op hun aangedane broer of zus dan de niet-aangedane kinderen uit families met een negatieve historie voor angstige depressie. Deze overeenkomst kan een genetische oorzaak hebben, maar er moet niet vergeten worden dat de niet-aangedane broer of zus van een aangedane persoon zelf ook een negatieve gebeurtenis in zijn of haar leven heeft meegemaakt, namelijk dat zijn of haar broer of zus depressief is (geweest). Het hebben van een depressief familielid kan een zeer stressvolle gebeurtenis zijn voor een heel gezin. Het is daarom aan te raden om bij het vergelijken van aangedane en niet-aangedane familieleden een niet-aangedaan familielid te nemen die met betrekking tot de omgevingsinvloeden minder nauw verbonden is met het aangedane familielid, zoals een neef of een nicht.

References

Aardal E, Holm AC. (1995). Cortisol in saliva — reference ranges and relation to cortisol in

Abecasis GR, Cardon L, Cookson WOC. (2000) A general test of association for quantitative traits in nuclear families, *Am J Hum Genet*, 66, 279-292

Achenbach TM. (1990). *The Young Adult Self Report*. University of Vermont, Dept of Psychiatry: Burlington, VT.

Acton GS, Prochaska JJ, Kaplan AS, Small T, Hall SM. (2001). Depression and stages of change for smoking in psychiatric outpatients. *Addictive Behaviors,* 26:621-631.

Akiskal HS Mood disorders: Introduction and overview. (1995) In: Kaplan HI, Sadock BJ (Eds.). *Comprehensive textbook of psychiatry.* (pp. 1067-1079). 6th ed baltimore, Md: Lippincott, Williams & Wllkins.

Allen MG. (1976). Twin Studies of Affective Illness. *Arch Gen Psychiatry,* 33:1476-1480.

Anda RF, Williamson D, Jones D, Macera C, Eaker E, Glassman A, Marks J. (1993). Depressed affect, hopelessness, and the risk of ischemic heart disease in a cohort of U.S. adults. *Epidemiology* 4:285-294.

Anda RF, Williamson DF, Escobedo LG, Mast EE, Giovino GA, Remington PL. (1990). Depression and the dynamics of smoking. A national perspective. *JAMA,* 264:1541-1550.

Andrews G, Peters L. (1998). The psychometric properties of the Composite International Diagnostic Interview. *Soc Psychiatry Psychiatr Epidemiol* , 33:80-88.

Angst J, Clayton P. (1986). Premorbid personality of depressive, bipolar, and schizophrenic patients with special reference to suicidal issues. *Compr Psychiatry,* 27:511-532.

Anthony JC, Petronis KR. (1991). Suspected risk factors for depression among adults 18-44 years old. *Epidemiology,* 2:123-132.

Arana GW, Baldessarini RJ, Ornsteen M. (1985). The dexamethasone suppression test for diagnosis and prognosis in psychiatry. Commentary and review. *Arch Gen Psychiatry,* 42:1193-1204.

Ariyo AA, Haan M, Tangen CM, Rutledge JC, Cushman M, Dobs A, Furberg CD. (2000). Depressive symptoms and risk of coronary heart disease and mortality in elderly Americans.. *Circulation,* 102:1773-1779.

Arnau RC, Meagher MW, Norris MP, Bramson R. (2001). Psychometric evaluation of the Beck Depression Inventory-II with primary care medical patients. *Health Psychol,* 20:112-119.

Åström M. (1996). Generalized anxiety disorder in stroke patients. A 3-year longitudinal study. *Stroke,* 27:270-275.

August P, Oparil S. (1999). Hypertension in women. *J Clin Endocrinol Metab,* 84:1862-1866.

Barden N, Reul JMHM, Holsboer F. (1995). Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system. *Trends Neurosci,*18:6-11.

Barrett-Connor E, Bush TL. (1991). Estrogen and coronary heart disease in women. *JAMA,* 265:1861-1867.

Bartels M, Van den Berg M, Sluyter F, Boomsma DI, De Geus EJC. (2001). Heritability of cortisol levels; review and simultaneous analysis of twin studies. *Psychoneuroendocrinology* (in press).

Baum A, Garofalo JP, Yali AM. (1999). Socioeconomic status and chronic stress. Does stress account for SES effects on health?. *Ann N Y Acad Sci,* 896:131-144.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. (1961). An inventory measuring depression. *Arch Gen Psychiatry,* 4:53–63.

Beerda B, Schilder MB, Janssen NS, Mol JA. (1996). The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. *Horm Behav,* 30:272-279.

Beique JC, de Montigny C, Blier P, Debonnel G. (1998). Blockade of 5-hydroxytryptamine and noradrenaline uptake by venlafaxine: a comparative study with paroxetine and desipramine. *Br J Pharmacol,* 125:526-532.

Berlin I, Said S, Spreux-Varoquaux O, Olivares R, Launay JM, Puech AJ. (1995). Monoamine oxidase A and B activities in heavy smokers. *Biol Psychiatry,*. 38:756-761.

Bernardi L, Chau NP, Chanudet X, Vilar J, Larroque P. (1992). Ambulatory blood pressure monitoring: a critical review of the current methods to handle outliers. *J Hypertension 10:1243-1248.*

Bjorntorp P, Rosmond R. (1999). Visceral obesity and diabetes. *Drugs* ,58 (1):13-18; discussion 75-82.

Blackwelder WC, Elston RC, Power and robustness of sib-pair linkage tests and extension to larger sibships, *Comm Stat Theory Meth*, 449-484, 1982

Blazer DG, Kessler RC, McGonagle KA, Swartz MS. (1994). The prevalence and distribution of major depression in a national community sample: the national comorbidity survey. *Am J Psychiatry,* 151:979-986.

Boomsma DI, Beem AL, Van den Berg M, Dolan CV, Koopmans JR, Vink JM et al. (2000). Netherlands twin study of anxious depression (NETSAD). *Twin Research* 3:323-334.

Boomsma DI, Dolan CV. (2000) Multivariate QTL analysis using structural equation modeling: A look at power under simple conditions, in: *Advances in Twin & Sib-Pair Analysis*, ed. by T Spector, H Snieder, A MacGregor, 203-208.

Boomsma DI, Dolan CV. (1998). A comparison of power to detect a QTL in sibpair data using multivariate phenotypes, mean phenotypes, and factor-scores. *Behav Genet* 28:329–340.

Boomsma DI, Geus EJC de, Baal GCM van, Koopmans JR. (1999) Religious upbringing reduces the influence of genetic factors on disinhibition: Evidence for interaction between genotype and environment, *Twin Research*, 2, 115-125,

Boomsma DI, Koopmans JR, Doornen LJP van, Orlebeke JF.(1994). Genetic and social influences on starting to smoke: a study of Dutch adolescent twins and their parents. *Addiction,* 89:219–226.

Boomsma DI, Molenaar PCM, Orlebeke JF. (1990). Estimation of individual genetic and environmental factor scores. *Genetic Epidemiology*, 7:83-91.

Boomsma DI. (1996) Using multivariate genetic modeling to detect pleiotropic quantitative trait loci, *Behav Genet*, 26, 161-166,

Brandtstadter J, Baltes-Gotz B, Kirschbaum C, Hellhammer D. (1991). Developmental and personality correlates of adrenocortisal activity as indexed by salivary cortisol: observations in the age range of 35 to 65 years. *J Psychosom Res* 35:173-185.

Breslau N, Peterson EL, Schultz LR, Chilcoat HD, Andreski P. (1998). Major depression and stages of smoking. A longitudinal investigation. *Arch Gen Psychiatry* 55:161-166.

Brindley DN, Rolland Y. (1989). Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin Sci (Lond)* 77:453-461.

Brioni JD, O'Neill AB, Kim DJ, Decker MW. (1993). Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus-maze test. *Eur J Pharmacol,* 238:1-8.

Britz B, Siegfried W, Ziegler A, Lamertz C, Herpertz-Dahlmann BM, Remschmidt H, Wittchen HU, Hebebrand J. (2000). Rates of psychiatric disorders in a clinical study group of adolescents with extreme obesity and in obese adolescents ascertained via a population based study. *Int J Obes Relat Metab Disord,* 24:1707-1714.

Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. (1982) The cognitive failures questionnaire (CFQ) and its correlates, *Br J Clinical Psychol*, 21, 1-16

Brochu M, Poehlman ET, Ades PA. (2000). Obesity, body fat distribution, and coronary artery disease. *J Cardiopulm Rehabil,* 20:96-108.

Brown AS, Gershon S. (1993). Dopamine and depression. *J Neural Transm Gen Sect* 91:75-109.

Brown C, Madden PA, Palenchar DR, Cooper-Patrick L. (2000). The association between depressive symptoms and cigarette smoking in an urban primary care sample. *Int J Psychiatry Med,* 30:15-26.

Brownley, Hurwitz & Schneiderman. (2000). Cardiovascular psychophysiology. In: Cacioppo JT, Tassinary LG & Berntson GG. (Eds.). 2nd edition handbook of psychophysiology.

Bushong DM, Friend TH, Knabe DA. (2000).Salivary and plasma cortisol response to adrenocorticotropin administration in pigs. *Lab Anim,* 34:171-181.

Caggiula AR, Donny EC, Epstein LH, Sved AF, Knopf S, Rose C, McAllister CG, Antelman SM, Perkins KA. (1998). The role of corticosteroids in nicotine's physiological and behavioral effects. *Psychoneuroendocrinology*, 23:143-159.

Caldarone B, Saavedra C, Tartaglia K, Wehner JM, Dudek BC, Flaherty L. (1997) Quantitative trait loci analysis affecting contextual conditioning in mice, *Nat Genet*, 17, 335-337,

Cam GR, Bassett JR, Cai rncross KD. (1979). The action of nicotine on the pituitary-adrenal cortical axis. *Arch Int Pharmacodyn Ther,* 237:49-66.

Camacho TC, Roberts RE , Lazarus NB, Kaplan GA, Cohen RD. (1991). Physical activity and depression: evidence from the Alameda County Study. *Am J Epidemiol,* 134:220-231.

Cannon WB. (1914). The emergency function of the adrenal medulla in pain and in the major emotions. *Am J Physiol,* 33:356-372.

Carels RA, Blumenthal JA , Sherwood A. (2000). Emotional responsivity during daily life: relationship to psychosocial functioning and ambulatory blood pressure. *Int J Psychophysiology,* 36 :25-33.

Carney RM, Freedland KE , Stein PK, Skala JA, Hoffman P, Jaffe AS. (2000). Change in heart rate and heart rate variability during treatment for depression in patients with coronary heart disease. *Psychosom Med,* 62:639-647.

Carney RM, Rich MW, teV elde A, Saini J, Clark K, Freedland KE. (1988). The relationship between heart rate, heart rate variability and depression in patients with coronary artery disease. *J Psychosom Res,* 32:159-164.

Carney RM, Rich MW, Te velde A, Saini J, Clark K, Jaffe AS. (1987). Major depressive disorder in coronary artery disease. *Am J Cardiol,* 60:1273-1275.

Carney RM, Saunders RD , Freedland KE, Stein P, Rich MW, Jaffe AS. (1995). Association of depression with reduced heart rate variability in coronary artery disease. *Am J Cardiol*, 76:562-564.

Carpenter KM, Hasin DS, Allison DB, Faith MS. (2000). Relationships between obesity and DSM-IV major depressive disorder, suicide ideation, and suicide attempts: results from a general population study. *Am J Public Health,* 90:251-257.

Carvalhaes-Neto N, Ram os LR, Vieira JG, Kater CE. (2002). Urinary free cortisol is similar in older and younger women. *Aging Res,* 28:163-168.

Catalán R, Gallart JM, Ca stellanos JM, Galard R. (1998). Plasma Corticotropin-Releasing Factor in Depressive Disorders. *Biol Psychiatry,* 44:15-20.

Charlton BG, Ferrier IN. (1 989). Hypothalamic-pituitary-adrenal axis abnormalities in depression: a review and a model. *Psychol Med,* 19:331-336.

Charlton BG, Leake A, W right C, Griffiths HW, Ferrier IN. (1987). A combined study of cortisol, ACTH and dexamethasone concentrations in major depression. Multiple time-point sampling. *Br J Psychiatry,* 150:791-796.

Cheeta S, Irvine EE, Tucc i S, Sandhu J, File SE. (2001). In adolescence, female rats are more sensitive to the anxiolytic effect of nicotine than are male rats. *Neuropsychopharmacology,* 25:601-607.

Chemerinski E, Robinson RG. (2000). The neuropsychiatry of stroke. *Psychosomatics,* 41:5-14.

Christensen L, Somers S. (1996). Comparison of nutrient intake among depressed and nondepressed individuals. *Int J Eat Disord,* 20:105-109.

Clement DL, De Buyzere M, Duperez D. (1994). Prognostic value of ambulatory blood pressure monitoring. *J Hypertension,* 12:857-864.

Cooke RR, McIntosh JE, Murray-McIntosh RP. (1996). Effect of cortisol on percentage of non-sex-hormone-bound steroid: implications for distribution of steroids on binding proteins in serum. *Clin Chem.* 42:249-454.

Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. (1996). Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci USA,* 93:1619-1623.

Costall B, Kelly ME, Naylo r RJ, Onaivi ES. (1989). The actions of nicotine and cocaine in a mouse model of anxiety. *Pharmacol Biochem Behav,* 33:197-203.

Cryer PE, Haymond MW, Santiago JV, Shah SD. (1976). Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N Engl J Med,* 295:573-577.

Dahl RE, Ryan ND, Puig-A ntich J, Nguyen NA, al-Shabbout M, Meyer VA, Perel J. (1991). 24-hour cortisol measures in adolescents with major depression: a controlled study. *Biol Psychiatry,* 30:25-36.

Dawson ME, Schell AM, C atania JJ. (1977). Autonomic correlates of depression and clinical improvement following electroconvulsive shock therapy. *Psychophysiology,* 14:569-578.

De Bellis MD, Dahl RE, P erel JM, Birmaher B, al-Shabbout M, Williamson DE, Nelson B, Ryan ND. (1996). Nocturnal ACTH, cortisol, growth hormone, and prolactin secretion in prepubertal depression. *J Am Acad Child Adolesc Psychiatry.* 35:1130-1138.

De Geus EJC, Van Doorn en LJP. (1996). Ambulatory assessment of parasympathetic/sympathetic balance by impedance cardiography. In: J. Fahrenberg & M. Myrtek (Eds.) *Ambulatory assessment. Computer-assisted psychological and .psychophysiological methods in ambulatory monitoring and field studies*. Seattle, Hogrefe & Huber Publishers, 141-164.

De Geus EJC, Willemsen G, Klaver CHAM, Doornen LJP van. (1995). Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. *Biol Psychol,* 41: 205–227.

Del Arbol JL, Munoz JR, O jeda L, Cascales AL, Irles JR, Miranda MT, Ruiz Requena ME, Aguirre JC. (2000). Plasma concentrations of beta-endorphin in smokers who consume different numbers of cigarettes per day. *Pharmacol Biochem Behav,* 67:25-28.

Der G, Bebbington P. (19 87). Depression in inner London. A register study. *Soc Psychiatry,* 22:73-84.

Deuschle M, Schweiger U , Gotthardt U, Weber B, Korner A, Schmider J, Standhardt H, Lammers CH, Krumm B, Heuser I. (1998). The combined dexamethasone/corticotropin-releasing hormone stimulation test is more closely associated with features of diurnal activity of the hypothalamo-pituitary-adrenocortical system than the dexamethasone suppression test. *Biol Psychiatry,* 43:762-766.

Deuschle M, Schweiger U , Standhardt H, Weber B, Heuser I. (1996). Corticosteroid-binding globulin is not decreased in depressed patients. *Psycho-neuroendocrinology*, 21:645-649.

Deuschle M, Schweiger U , Weber B, Gotthardt U, Korner A, Schmider J, Standhardt H, Lammers CH, Heuser I. (1997): Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. *J Clin Endocrinol Metab,* 82:234-238.

Diehl DJ, Gershon S. (199 2). The role of dopamine in mood disorders. *Compr Psychiatry,* 33:115-120.

Dolan CV, Boomsma DI, Neale MC. (1999) A simulation study of the effects of assigning prior IBD probabilities to unselected sib-pairs in covariance structure modeling of a QTL test, *Am Human Genet*, 268-280,

Dolan CV, Boomsma DI, Neale MC. (1999). A note on the power provided by sibships of size 3 and 4 in genetic covariance modeling of a codominant QTL. *Behav Genet*; 29:163–170.

Dolan CV, Boomsma DI. (1998). Optimal selection of sibpairs from random samples for linkage analysis of a QTL using the EDAC test. *Behav Genet*; 28:197–206.

Dotsch J, Dorr HG, Stalla GK, Sippell WG. (2001). Effect of glucocorticoid excess on the cortisol/cortisone ratio. *Steroids,* 66:817-820.

Dressendorfer RA, Kirsch baum C, Rohde W, Stahl F, Strasburger CJ. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *J Steroid Biochem Mol Biol,* 43:683-692.

Duggan C, Sham P, Lee A , Minne C, Murray R. (1995). Neuroticism: a vulnerability marker for depression evidence from a family study. *J Affective Disorders,* 35:139-143.

Dunn AJ, Berridge CW. (1 990). Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Brain Res Rev,* 15:71-100.

Eaves LJ, Eysenck HJ. (1 980). The relationship between smoking and personality. In: Eysenck HJ, Eaves LJ, Kasriel J. (Eds.) *The causes and effects of smoking.* (pp. 283-310). Billings and Sons London, Guilford, Worcester and Oxford.

Eaves LJ, Neale M, Maes H, Multivariate multipoint linkage analysis of quantitative trait loci, *Behav Genet*, 26, 519-525, 1996

Ernst ND, Obarzanek E, C lark MB, Briefel RR, Brown CD, Donato K. (1997). Cardiovascular health risks related to overweight. *J Am Diet Assoc* ,97:S47-51.

Evans DL, Burnett GB, Ne meroff CB. (1983). The dexamethasone suppression test in the clinical setting. *Am J Psychiatry,* 140:586-589.

Everson SA, Kaplan GA, Goldberg DE, Salonen JT. (2000). Hypertension incidence is predicted by high incidence of

hopelessness in Finnish men. *Hypertension* 35, 561-567.
Everson SA, Roberts RE, Goldberg DE, Kaplan GA. (1998). Depressive symptoms and increased risk of stroke mortality over a 29-year period. *Arch Intern Med,* 158:1133-1138.
Ewart CK, Kolodner KB. (1994). Negative affect, gender and expressive style predict elevated ambulatory blood pressure in adolescents. *J Pers Soc Psychology,* 66:596-605.
Fa M, Cargangiu G, Passino N, Ghiglieri V, Gessa GL, Mereu G. (2000). Cigarette smoke inhalation stimulates dopaminergic neurons in rats. *Neuroreport*, 11:3637-3639.
Farrell M, Howes S, Bebbington P, Brugha T, Jenkins R, Lewis G, Marsden J, Taylor C, Meltzer H. (2001). Nicotine, alcohol and drug dependence and psychiatric comorbidity. *Br J Psychiatry* ,179:432-437.
Farrell M, Howes S, Taylor C, Lewis G, Jenkins R, Bebbington P, Jarvis M, Brugha T Gill B, Meltzer H. (1998). Substance misuse and psychiatric comorbidity: an overview of the OPCS national psychiatric comorbidity survey. *Addictive behaviors,* 23:909-918.
Feher MD, Rampling MW , Brown J, Robinson R, Richmond W, Cholerton S, Bain BJ, Sever PS. (1990). Acute changes in atherogenic and thrombogenic factors with cessation of smoking. *J R Soc Med,* 83:146-148.
Ferketich AK, Schwartzbaum JA, Frid DJ, Moeschberger ML. (2000). Depression as an antecedent to heart disease among women and men in the NHANES I study. National Health and Nutrition Examination Survey. *Arch Intern Med,* 160:1261-1268.
File SE, Fluck E, Leahy A. (2001). Nicotine has calming effects on stress-induced mood changes in females, but enhances aggressive mood in males. *Int J of Neuropharmacol* 4:371-376.
Fitzgerald SJ, Kriska AM, Pereira MA, et al. (1997). Associations among physical activity, television watching, and obesity in adult Pima Indians. *Med Sci Sports Exerc* 27:910–915.
Fleming CB, Kim H, Harachi TW, Catalano RF. (2002). Family processes for children in early elementary school as predictors of smoking initiation. *J Adolesc Health*, 30:184-189.
Flint J, Corley R, DeFries JC, Fulker DW, Gray JA, Miller S, Collins AC. (1995) A simple genetic basis for complex psychological trait in laboratory mice, *Science*, 269, 1432-1435,
Ford DE, Mead LA, Chang PP, Cooper-Patrick L, Wang N-Y, Klag MJ. (1998). Depression is a risk factor for coronary artery disease in men. *Arch Intern Med,* 158:1422-1426.
Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, MacGregor R, Alexoff D, Shea C, Schlyer D, Wolf AP, Warner D, Zezulkova I, Cilento R. (1996a). Inhibition of monoamine oxidase B in the brains of smokers. *Nature,* 379:733-736.
Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, Shea C, Alexoff D, MacGregor RR, Schlyer DJ, Zezulkova I, Wolf AP. (1996b). Brain monoamine oxidase A inhibition in cigarette smokers. *Proc Natl Acad Sci USA,* 93:14065-14069.
Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JM. (1999). Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension,* 33:1364-1368.
Frasure-Smith N, Lespérance F, Juneau M, Talajic M, Bourassa MG. (1999). Gender, depression, and one-year prognosis after myocardial infarction. *Psychosomatic medicine,* 61:26-37.
Frasure-Smith N, Lesperance F, Talajic M. (1993). Depression following myocardial infarction. Impact on 6-month survival. *JAMA*, 270:1819-1825.
Frasure-Smith N, Lesperance F, Talajic M. (1995). Depression and 18-month prognosis after myocardial infarction. *Circulation,* 91:999-1005.
Frederick SL, Reus VI, Ginsberg D, Hall SM, Munoz RF, Ellman G. (1998). Cortisol and response to dexamethasone as predictors of withdrawal distress and abstinence success in smokers. *Biol Psychiatry,* 43:525-530.
Friedman R, Schwartz JE, Schnall PL, Landsbergis PA, Pieper C, Gerin W, Pickering TG. (2001). Psychological variables in hypertension: relationship to causal or ambulatory blood pressure in men. *Psychosomatic medicine,* 63:19-31.
Fulker DW, Cherny SS. (1996) An improved multi-point sib-pair analysis of quantitative traits, *Behav Genet*, 26, 527-532,
Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, Cerutti S, Lombardi F, Pagani M, Malliani A. (1990). Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation,* 81:537-547.
Garrison RJ, Higgins MW, Kannel WB. (1996). Obesity and coronary heart disease. *Curr Opin Lipidol,* 7:199-202.
Gass P, Reichardt HM, Strekalova T, Henn F, Tronche F.(2001). Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: Models for depression and anxiety? *Physiol Behav,* 73:811-825.
Gauvin L, Rejeski WJ, Norris JL. (1996). A naturalistic study of the impact of acute physical activity on feeling states and affect in women. *Health Psychology,* 15:391-397.
Gerin W, Pickering TG, Glynn L, Christenfeld N, Schwartz A, Carroll D, Davidson K. (2000). An historical context for behavioral models of hypertension. *J Psychosom Res,* 48:369-377.
Gershenfeld HK, Neumann PE, Mathis C, Crawley JN, Li X, Paul SM. (1997) Mapping quantitative trait loci for open-field behavior in mice, *Behav Genet*, 27, 201-210,
Gershenfeld HK, Paul SM, Mapping quantitative trait loci for fear-like behaviors in mice, *Genomics*, 46, 1-8, 1997 10 Wehner JM, Radcliffe RA, Rosmann ST, Christensen SC,
Gershenfeld HK, Paul SM. (1998) Towards a genetics of anxious temperament: mice to men, *Acta Psychiatr Scand*, 98 (Suppl 393), 56-65,
Gilbert DG, McClernon FJ, Rabinovich NE, Dibb WD, Plath LC, Hiyane S, Jensen RA, Meliska CJ, Estes SL, Gehlbach BA. (1999). EEG, physiology, and task-related mood fail to resolve across 31 days of smoking abstinence: relations to depressive traits, nicotine exposure, and dependence. *Exp Clin Psychopharmacol,* 7:427-443.
Glassman AH, Covey LS, Dalack GW, Stetner F, Rivelli SK, Fleiss J, Cooper TB. (1993). Smoking cessation, clonidine, and vulnerability to nicotine among dependent smokers. *Clin Pharmacol Ther,* 54: 670-679.
Glassman AH, Helzer JE, Covey LS, Cottler LB, Stetner F, Tipp JE, Johnson J. (1990). Smoking, smoking cessation, and major depression. *JAMA*, 264:1546-1549.
Glassman AH, Shapiro PA. (1998). Depression and the course of coronary artery disease. *Am J Psychiatry,* 155:4-11.
Glassman AH, Stetner F, Walsh BT, Raizman PS, Fleiss JL, Cooper TB, Covey LS. (1988). Heavy smokers, smoking cessation, and clonidine. Results of a double-blind, randomized trial. *JAMA*, 259:2863-2866.
Gold PW, Chrousos GP. (1985). Clinical studies with corticotropin releasing factor: implications for the diagnosis and pathophysiology of depression, Cushing's disease, and adrenal insufficiency. *Psychoneuroendocrinology*; 10:401-419.
Gold PW, Goodwin FK, Chrousos GP, Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress. *New Engl J Med*, 1988, 319, (part1) 348-353, (part2) 413-420
Goldman J, Klinger M. (2001). Effectof smoking on the course of essential hypertension: a follow-up study of a group composed predominantly of women. *Med Sci Monit,* 7:1280-1284.
Goodyer IM, Herbert J, Tamplin A, Altham PME. (2000). First-episode major depression in adolescents. Affective, cognitive and endocrine characteristics of risk status and predictors of onset. *Br J Psychiatry,* 176:142-149.
Goodyer IM, Park RJ, Herbert. (2001). Psychosocial and Endocrine Features of Chronic First-Episode Major Depression in 8-16 Year Olds. *Biol Psychiatry*; 50:351-357.
Goodyer, IM, Herbert J, Tamplin A, Altham PME. (2000). Recent life events, cortisol, dehydropiandrosterone and the onset of major deppression in high-risk adolescents. *Br J Psychiatry*, 177:499-504.
Gotthardt U, Schweiger U, Fahrenberg J, Lauer CJ, Holsboer F, Heuser I. (1995). Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. *Am J Physiol,* 268:R865-873.
Gritz ER, Thompson B, Emmons K, Ockene JK, McLerran DF, Nielsen IR. (1998). Gender differences among smokers and quitters in the Working Well Trail. *Prev Med,* 27:553-561.
Grunberg NE, Singer JE. (1990) Biochemical measurement. In: Cacioppo JT, Tassinary LG. (Eds.) *Principles of psychophysiology.* (pp.149-176) Cambridge University Press,.
Haapanen-Niemi N, Miilunpalo S, Pasanen M, Vuori I, Oja P, Malmberg J. (2000). Body mass index, physical inactivity and low level of physical fitness as determinants of all-cause and cardiovascular disease mortality--16 y follow-up of middle-aged and

elderly men and women. *Int J Obes Relat Metab Disord,* 24:1465-1474.
Hämäläinen J, Kaprio J, Isometsä E, Heikkinen M, Poikolainen K, Lindeman S, Aro H. (2001). Cigarette smoking, alcohol intoxication and major depressive episode in a representative population sample. *J Epidemiol Community Health,* 55:573-576.
Haseman JK, Elston RC. (1972) The investigation of linkage between a quantitative trait and a marker locus, *Behav Genet*, 2, 3-19,
Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fujinami T, Yokoyama K, Watanabe Y, Takata K. (1991). Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *Am J Cardiol*, 67:199-204.
Heim C, Ehlert U, Hellhamer DH. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bod-ily disorders. *Psychoneuroendocrinol,* 25:1–35.
Heim C, Nemeroff CB. (1999). The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biol Psychiatry*, 46:1509-1522.
Heim C, Nemeroff CB. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry,* 49:1023-1039.
Heim C, Owens MJ, Plotsky PM, Nemeroff CB. (1997). Persistent changes in corticotropin-releasing factor systems due to early life stress: relationship to the pathophysiology of major depression and post-traumatic stress disorder. *Psychopharmacol Bull,* 33:185-192.
Heim C, Owens MJ, Plotsky PM, Nemeroff CB. (1997). The role of early adverse life events in the etiology of depression and posttraumatic stress disorder. Focus on corticotropin-releasing factor. *Ann N Y Acad Sci,* 821:194-207.
Hillhouse EW, Milton NG. (1989). Effect of noradrenaline and gamma-aminobutyric acid on the secretion of corticotrophin-releasing factor-41 and arginine vasopressin from the rat hypothalamus in vitro. *J Endocrinol,* 122:719-723.
Hippisley-Cox J, Fielding K, Pringle M. (1998). Depression as a risk factor for ischaemic heart disease in men: population based case-control study. *BMJ,* 316:1714-1719.
Hitsman B, Pingitore R, Spring B, Mahableshwarkar A, Mizes JS, Segraves KA, Kristeller JL, Xu W. (1999). Antidepressant pharmacotherapy helps some cigarette smokers more than others. *J Consult Clin Psychol,* 67:547-554.
Holsboer F, Barden N. (1996). Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocrine reviews,* 17:187-205.
Holsboer F, Dorr HG, Sippell WG. (1982). Blunted aldosterone response to dexamethasone in female patients with endogenous depression. *Psychoneuroendocrinology,* 7:155-162.
Holsboer F, Lauer CJ, Schreiber W, Krieg JC. (1995). Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. *Neuroendocrinology,* 62:340-347.
Holsboer F, Müller OA, Doerr HG, Sippell WG, Stalla GK, Gerken A, et al. (1984). ACTH and multisteroid responses to corticotropin-releasing factor in depressive illness: relationship to multisteroid responses after ACTH stimulation and dexamethasone suppression. *Psychoneuroendocrinology,* 9:147-160.
Holsboer F, Winter K, Dorr HG, Sippell WG. (1982). Dexamethasone suppression test in female patients with endogenous depression: determinations of plasma corticosterone, 11-deoxycorticosterone, 11-deoxycortisol, cortisol and cortisone. *Psychoneuroendocrinology,* 7:329-338.
Holsboer F. (1999). The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiatr Res,* 33:181-214.
Holsboer F. (2000). the corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology,* 23:477-501.
Holsboer F. (2001). Prospects for antidepressant drug discovery. *Biol Psychol,* 57:47-65.
Hughes JR, Hatsukami DK, Mitchell JE, Dahlgren LA. (1986). Prevalence of smoking among psychiatric outpatients. *Am J Psychiatry,* 143:993-997.
Hughes JR, Stead LF, Lancaster T. (2000). Antidepressants for smoking cessation. *Cochrane Database Syst Rev,* (4):CD000031.
Hughes JR. (1988). Clonidine, depression, and smoking cessation. *JAMA,* 259:2901-2902.

Hughes JW, Stoney CM. (2000). Depressed mood is related to high-frequency heart rate variability during stressors. *Psychosom Med,* 62:796-803.
Huikuri HV, Jokinen V, Syvanne M, Nieminen MS, Airaksinen KE, Ikaheimo MJ, Koistinen JM, Kauma H, Kesaniemi AY, Majahalme S, Niemela KO, Frick MH. (1999). Heart rate variability and progression of coronary atherosclerosis. *Arterioscler Thromb Vasc Biol,* 19:1979-1985.
Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE, de Jong FH, Lamberts SW. (1998). Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. *J Clin Endocrinol Metab,* 83:47-54.
Hurt RD, Dale LC, Croghan GA, Croghan IT, Gomez-Dahl LC, Offord KP. (1998). Nicotine nasal spray for smoking cessation: pattern of use, side effects, relief of withdrawal symptoms, and cotinine levels. *Mayo Clin Proc,* 73:118-125.
Iacono W, Lykken D, Peloquin L, Lumry A, Valentine R, Tuason V. (1983). Electrodermal activity in euthymic unipolar and bipolar affective disorders. *Arch Gen Psych,* 40:557-568.
Inglis GC, Ingram MC, Holloway CD, Swan L, Birnie D, Hillis WS, et al. (1999). Familial pattern of corticosteroids and their metabolism in adult human subjects –the Scottish adult twin study-.*J clin end met,* 84:4132-4137.
Ishibashi F. (1993). Higher serum insulin level due to greater total body fat mass in offspring of patients with essential hypertension. *Diabetes Res Clin Pract,* 20:63-68
Jaarverslag, DEFACTO, Den Haag 2000.
Jacobson L, Sapolsky R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev,* 12:118-134.
Jardine R, Martin NG, Henderson AS. (1984) Genetic covariation between neuroticism and the symptoms of anxiety and depression, *Genetic epidemiology*, 1, 89-107,
Jenkins CD, Rosenman RH, Zyzanksi S. (1974) Prediction of clinical coronary heart disease by a test for the coronary prone behavior pattern, *New Engl J Medicine*, 290, 1271-1275,
Jonas BS, Franks P, Ingram DD. (1997). Are symptoms of anxiety and depression risk factors for hypertension? Longitudinal evidence from the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch Fam Med,* 6:43-49.
Jonas BS, Lando JF. (2000). Negative affect as a prospective risk factor for hypertension. *Psychosomatic medicine,* 62:188-196.
Jonas BS, Mussolino ME. (2000). Symptoms of depression as a prospective risk factor for stroke. *Psychosomatic medicine,* 62:463-471.
Joyce PR, Mulder RT, Cloninger C.R. (1994). Temperament and hypercortisolemia in depression. *Am J Psychiatry,* 151:195-198.
Judd LL, Akiskal HS, Zeller PJ, Paulus M, Leon AC, Maser JD, Endicott J, Coryell W, Kunovac JL, Mueller TI, Rice JP, Keller MB. (2000). Psychosocial disability during the long-term course of unipolar major depressive disorder. *Arch Gen Psychiatry,* 57:375-380.
Julius S, Jamerson K. (1994). Sympathetics, insulin resistance and coronary risk in hypertension: the 'chicken-and-egg' question. *Journal of Hypertension,* 12:495-502.
Julius S, Pascual AV, London R. (1971) Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension. *Circulation,* 44:413-418.
Julius S. (1993). Sympathetic hyperactivity and coronary risk in hypertension. *Hypertension,* 21:886-893.
Julius S. (1996). The evidence for a pathophysiologic significance of the sympathetic overactivity in hypertension. *Clin exp hypertens,* 18:305-321.
Kalin NH. (1990). Behavioral and endocrine studies of corticotrophin releasing hormone in primates. In: De Souza EB, Nemeroff CB. (Eds). *Corticotrophin-releasing factor: Band and clinical studies of a neuropeptide.* Boca Raton. Fla CRC press.
Kannel WB, D'Agostino RB, Cobb JL. (1996). Effect of weight on cardiovascular disease. *Am J Clin Nutr,* 63:419S-422S.
Kannel WB, Higgins M. (1990). Smoking and hypertension as predictors of cardiovascular risk in population studies. *J Hypertens Suppl,* 8:S3-8.
Kaplan JR, Pettersson K, Manuck SB, Olsson G. (1991). Role of the sympathoadrenal medullary activation in the initiation and

progression of artherosclerosis. *Circulation,* 84(suppl VI):23-32.
Kaplan, N. (1990). Changing hypertension treatment to reduce the overall cardiovascular risk. *J Hypertension,* 8 (suppl 7):S175-S179.
Kario K, Schwartz JE, Davidson KW, Pickering TG. (2001). Gender differences in associations of diurnal blood pressure variation, awake physical activity, and sleep quality with negative affect. The work site blood pressure study. *Hypertension,* 38:997-1002.
Kasckow JW, Regmi A, Sheriff S, Mulchahey J, Geracioti TD Jr. (1999). Regulation of corticotropin-releasing factor messenger RNA by nicotine in an immortalized amygdalar cell line. *Life Sci,* 65:2709-2714.
Kathol RG, Jaeckle RS, Lopez JR, Meller WH. (1989). Consistent reduction of ACTH responses to stimulation with CRH, vasopressin and hypoglycaemia in patients with major depression. *Br J Psychiatry,* 155:468-478.
Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biol Psychiatry,* 48:778-790.
Kelly JJ, Mangos G, Williamson PM, Whitworth JA. (1998). Cortisol and hypertension. *Clin Exp Pharmacol Physiol,* Suppl 25:S51-6.
Kendler KS, Gardner CO, Prescott CA. (1999). Clinical characteristics of major depression that predict risk of depression in relatives. *Arch Gen Psychiatry,* 56:322-327.
Kendler KS, Heath A, Martin NG, Eaves LJ. (1986). Symptoms of anxiety and depression in a volunteer twin population. The etiologic role of genetic and environmental factors. *Arch Gen Psychiatry,* 43:213-221.
Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, et al. (1995). Stressful life events, genetic liability and onset of of an episode of major depression. *Am J Psychiatry,* 152:833-842.
Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. (1992) Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch Gen Psychiatry,* 49:716-722.
Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. (1992). A population-based twin study of major depression in women. The impac of varying definitions of illness. *Arch gen Psychiatry,* 49:257-266.
Kendler KS, Neale MC, kessler RC, heath AC, Eaves LJ. (1993). A Longitudinal twin study of 1-year prevalence of major depression in women. *Arch Gen Psychiatry,* 50:843-852.
Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. (1993b): A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry,* 50:853-862.
Kendler KS, Neale MC, MacLean CJ, heath AC, Eaves LJ, Kessler RC. (1993). Smoking and major depression. *Arch Gen Psychiatry,* 50:36-43.
Kendler KS, Prescott CA. (1999). A population-based twin study of lifetime major depression in men and women. *Arch Gen Psychiatry,* 56:39-44.
Kendler KS. (1993). Twin studies of psychiatric illness: current status and future directions. *Arch Gen Psychiatry,* 50:905-915.
Kendler KS. (1996). Major depression and generalised anxiety disorder. Same genes, (partly)different environments--revisited. *Br J Psychiatry,* 30:68-75.
Kendler KS. (2001). Twin studies of psychiatric illness. An update. *Arch Gen Psychiatry,* 58:1005-1014.
Kenyon & Fraser. (1992) Biochemestry of steroid hypertension. In: James VHT, (Ed.) *The adrenal gland.* New york, Raven Press.
Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS. (1994). Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry,* 51:8-19.
Kessler RC, Nelson CB, McGonagle KA, Liu J, Swartz M, Blazer DG. (1996). Comorbidity of DSM-III-R major depressive disorder in the general population: results from the US National Comorbidity Survey. *Br J Psychiatry Suppl,* 30:17-30.
Khattar RS, Swales JD, Banfield A, Dore C, Senior R, Lahiri A. (1999). Prediction of coronary and cerebrovascular morbidity and mortality by direct continuous ambulatory blood pressure monitoring in essential hypertension. *Circulation,* 100:1071-1076.
Kirschbaum C, Hellhammer DH. (1989). Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology,* 22:150-169.

Kirschbaum C, Hellhammer DH. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* , 19:313-333.
Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med,* 61:154-162.
Kirschbaum C, Strasburger CJ, Jammers W, Hellhammer DH. (1989). Cortisol and behaviour: 1. Adaptation of a radioimmunoassay kit for reliable and inexpensive salivary cortisol determination. *Pharmacol Biochem Behav,* 34:747-751.
Kirschbaum C, Wüst S, Faig H-G, Hellhammer DH. (1992). Heritability of cortisol response to human corticotropin-releasing hormone, ergometry and psychological stress in humans. *J Clin Endocrinol Metab,* 75:1526-1530.
Kirschbaum C, Wust S, Hellhammer D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med,* 54:648-657.
Kochar MS, Bindra RS. (1996)The additive effects of smoking and hypertension. More reasons to help your patients kick the habit. *Postgrad Med,* 100:147-148, 151-154, 159-160.
Kok FW, Heijnen CJ, Bruijn JA, Westenberg HG, van Ree JM. (1995). Immunoglobulin production in vitro in major depression: a pilot study on the modulating action of endogenous cortisol. *Biol Psychiatry,* 38:217-226.
Koopmans JR, Boomsma DI. (1996). Familial resemblances in alcohol use: genetic or cultural transmission? *J Stud Alcohol,* 57:19-28.
Koopmans JR, Doornen LJP van, Boomsma DI. (1997). Association between alcohol use and smoking in adolescent and young adult twins: a bivariate genetic analysis. *Alcoholism, Clin Exper Res,* 21:537–546.
Koopmans JR, Slutske WS, Heath AC, Neale MC, Boomsma DI. (1999). The genetics of smoking initiation and quantity smoked in Dutch adolescent and young adult twins. *Behav Genet,* 29:383-393.
Koopmans JR. (1997). The genetics of health-related behaviors. A study of adolescent twins and their parents. Thesis, Vrije Universiteit Amsterdam, The Netherlands
Kovacs GL Fekete M, Szabo G, Telegdy G. (1987). Action of the ACTH-corticosteroid axis on the central nervous systems. *Front Horm Res,* 15:79-127.
Krantz DS, Manuck SB. (1984). Acute psychophysiologic reactivity and risk of cardiovascular disease: a review and methodologic critique. *Psychol Bull,* 96:435-464.
Krieg J-C, Lauer CJ, Schreiber W, Modell S, Holsboer F. (2001). Neuroendocrine, Polysomnographic and Psychometric observations in Healthy subjects at High Familial Risk for affective Disorders: the Current State of The 'Munich Vulnerability Study'. *J Affective Disorders*; 62:33-37.
Krittayaphong R, Cascio WE, Light KC, Sheffield D, Golden RN, Finkel JB, Glekas G, Koch GG, Sheps DS. (1997). Heart rate variability in patients with coronary artery disease: differences in patients with higher and lower depression scores. *Psychosom Med,* 59:231-235.
Kruglyak L, Lander E. (1995) Complete multipoint sib pair analysis of qualitative and quantitative traits, *Am J Hum Genet*, 57, 439-454,
Kubzansky LD, Kawachi I, Weiss ST, Sparrow D. (1998). Anxiety and coronary heart disease: a synthesis of epidemiological, psychological, and experimental evidence. *Ann Behav Med,* 20:47-58.
Kutcher S, Malkin D, Silverberg J, Marton P, Williamson P, Malkin A, Szalai J, Katic M. (1991). Nocturnal cortisol, thyroid stimulating hormone, and growth hormone secretory profiles in depressed adolescents. *J Am Acad Child Adolesc Psychiatry,* 30:407-414.
Lassila R, Laustiola KE. (1992). Cigarette smoking and platelet-vessel wall interactions. *Prostaglandins Leukot Essent Fatty Acids,* 46:81-86.
Lauer CJ, Bronnisch T, Kainz M, Schreiber W, Holsboer F, Krieg J-C. (1997). Pre-Morbid Psychometric profile of Subjects at High Familial Risk for Affective Disorder. *Psychological medicine*, 27:355-362.
Lawley DN, Maxwell AE. (1971) Factor Analysis as a Statistical Method, Butterworths, London,
Lehofer M, Moser M, Hoehn-Saric R, McLeod D, Liebmann P, Drnovsek B, Egner S, Hildebrandt G, Zapotoczky HG. (1997). Major depression and cardiac autonomic control. *Biol Psychiatry,* 42:914-919.

Leonard S, Adler LE, Benhammou K, Berger R, Breese CR, Drebing C, Gault J, Lee MJ, Logel J, Olincy A, Ross RG, Stevens K, Sullivan B, Vianzon R, Virnich DE, Waldo M, Walton K, Freedman R. (2001). Smoking and mental illness. *Pharmacol Biochem Behav,* 70:561-570.

Lespérance F, Frasure-Smith N, Juneau M, Théroux P. (2000). Depression and 1-year prognosis in unstable angina. *Arch Int Med,* 160 :1354-1360.

Lesperance F, Frasure-Smith N, Talajic M. (1996). Major depression before and after myocardial infarction: its nature and consequences. *Psychosom Med,* 58:99-110.

Lespérance F, Frasure-Smith N. (2000). Depression in patients with cardiac disease : a practical review. *J Psychosom Res,* 48:379-391.

Leutwyler K. (1995). Depression's double standard. Clues emerge as to why women have higher rates of depression. *Sci Am,* 272:23, 26.

Lewinsohn PM, Zinbarg R, Seeley JR, Lewinsohn M, Sack WH. (1997) Lifetime comorbidity among anxiety disorders and between anxiety disorders and other mental disorders in adolescents. *J Anxiety Disord,* 11:377-394.

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 Clin Endocrinol Metab,* 61:429-438.

Linkowski P, Van Onderbergen A, Kerkhofs M, Bosson D, Mendelwicz 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 (*Endocrinol Metab.* 27), E173-E181.

Linthorst AC. (2000). Glucocorticoid receptor impairment alters CNS responses to a psychological stressor: an in vivo microdialysis study in transgenic mice. *Eur J Neurosci,* 12:283-291.

Litchfield WR, Hunt SC, Jeunemaitre X, Fisher ND, Hopkins PN, Williams RR, Corvol P, Williams GH. (1998). Increased urinary free cortisol: a potential intermediate phenotype of essential hypertension. *Hypertension,* 31:569-574.

Lovallo WR, Thomas TL. (2000). Stress hormones in psychophysiological research: emotional behavioral and cognitive implications. In: JT Cacioppo, LG Tassinary & GG Berntson (Eds.) *Handbook of psychophysiology.* (pp 342-367). New York Cambridge University press.

Lustman PJ, Clouse RE, Griffith LS, Carney RM, Freedland KE. (1997). Screening for depression in diabetes using the Beck Depression Inventory. *Psychosom Med,* 59:24-31.

Maes M, Calabrese J, Meltzer HY. (1994). The Relevance of the In- Versus Outpatient Status on HPA-axis in depression: Spontaneous Hypercortisolism is a Feature of Major Depressed Inpatients and not of Major Depression Per Se. *Prog Neuropsychopharmacol Biol Psychiatry*; 18: 503-517.

Maes M, Claes M, Vandewoude M, Schotte C, Martin M, Blockx P, Cosyns P. (1992). Adrenocorticotropin hormone, beta-endorphin and cortisol responses to oCRF in melancholic patients. *Psychol Med,* 22:317-329.

Malik M, Camm AJ. (1993). Components of heart rate variability--what they really mean and what we really measure. *Am J Cardiol,* 72:821-822.

Marin P, Darin N, Amemiya T, Andersson B, Jern S, Bjorntorp P. (1992). Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism,* 41:882-886.

Marmot M, Shipley E, Brunner E, Hemingway H. (2001). Relative contribution of early life and adult socioeconomic factors to adult morbidity in the whitehall II study. *J Epidemiol Community Health,* 55:301-307.

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

Martin NG. (2000) Gene-environment interaction and twin studies, in: *Advances in Twin & Sib-Pair Analysis*, ed. by TD Spector, H Snieder, AJ MacGregor, 143-150, Greenwich Medical Media Ltd, London,

Martin NG, Jardine R, Andrews G, Heath AC, Anxiety disorders and neuroticism: Are there genetic factors specific to panic?, *Acta Psychiatrica Scandinavia*, 77, 698-706, 1988

Mathers CD, Vos T, Stevenson CE, Begg SJ. (2001). The burden of disease and injury in Australia. *Bulletin of the world health organization,* 79:1076-1084.

Mathis C, Neumann PE, Gershenfeld H, Paul SM, Crawley JN, Genetic analysis of anxiety-related behaviors and responses to benzodiazepine-related drugs in AXB and BXA recombinant inbred mouse strains, *Behav Genet*, 25, 557-568, 1995

Matta SG, Beyer HS, McAllen KM, Sharp BM. (1987). Nicotine elevates rat plasma ACTH by a central mechanism. *J Pharmacol Exp Ther,* 243:217-226.

Matta SG, Fu Y, Valentine JD, Sharp BM. (1998). Response of the Hypothalamic-Pituitary-Adrenal Axis to Nicotine. *Psychoneuroendocrinology*; 23:103-113.

Matta SG, Singh J, Sharp BM. (1990). Catecholamines mediate nicotine-induced adrenocorticotropin secretion via alpha-adrenergic receptors. *Endocrinology,* 127:1646-1655.

Mazur A. (1994). Do cortisol and thyroxin correlate with nervousness and depression among male army veterans. *Biol Psychology*, 37:259-263.

McGuffin P, Katz R, Rutherford J. (1991). Nature, nurture and depression: a twin study. *Psychol Med,* 21:329-335.

McKinlay SM, Brambilla DJ, Posner JG. (1992). The normal menopause transition. *Maturitas,* 14:103-115.

Meade TW, Imeson J, Stirling Y. (1987). Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. *Lancet,* 2:986-988.

Meikle AW, Stringham JD, Woodward MG, Bishop DT. (1988). Heritability of variation of plasma cortisol levels. *Metabolism,* 37:514-517.

Melamed S, Kushner T, Strauss E, Vigiser D. (1997). Negative association between reported life events and cardiovascular disease risk factors in employed men: the cordis study. *J Psychosomatic Res,*. 43 247-258.

Melse JM, Essink-Bot M-L, Kramers PGN, Hoeymans N. (2000). A National Burden of Disease Calculation: Dutch Disability-Adjusted Life-Years. *Am J Pub Health,* 90:1241-1247.

Meltzer H Gill B Petticrew M Hinds K. (1995). The prevalence of psychiatric morbidity among adults living in private households. OPCS survey of psychiatric morbidity in great britain. Report I. HMSO, London.

Mercuro G, Podda A, Pitzalis L, Zoncu S, Mascia M, Melis GB, Rosano GM. (2000). Evidence of a role of endogenous estrogen in the modulation of autonomic nervous system. *Am J Cardiol,* 85:787-789, A9.

Merikangas KR, Angst J, Eaton W, Canino G, Rubio-Stipec M, Wacker H, Wittchen HU, Andrade L, Essau C, Whitaker A, Kraemer H, Robins LN, Kupfer DJ. (1996). Comorbidity and boundaries of affective disorders with anxiety disorders and substance misuse: results of an international task force. *Br J Psychiatry Suppl,* 58-67.

Meulenbelt I, Droog S, Trommelen GJM, Boomsma DI, Slagboom PE. (1995) High yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations, *Am J Hum Genet*, 57, 1252-1254,

Michael A, Jenaway A, Paykel ES, Herbert J. (2000). Altered salivary dehydroepiandrosterone levels in major depression in adults. *Biol Psychiatry*, 48:989-995.

Milani RV, Lavie CJ, Cassidy MM. (1996). Effects of cardiac rehabilitation and exercise training programs on depression in patients after major coronary events. *Am Heart J,* 132:726-732.

Miller MB, Monozygotic twins increase power of genetic association studies of complex phenotypes, *Behav Genet,* 28, 476, 1998

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. (2001). Chronic stress differentially regulates glucocorticoid negative feedback response in rats. *Psychoneuroendocrinology,* 26:443-459.

Modell S, Lauer CJ, Schreiber W, Huber J, Krieg JC, Holsboer F. (1998). Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. *Neuropsychopharmacology*, 18:253-262.

Muris P, Merckelbach H, Collaris R. (1997). Common childhood fears and their origins. *Behav Res Ther,* 35:929-937.

Muris P, Schmidt H, Merckelbach H. (1999). The structure of specific phobia symptoms among children and adolescents. *Behav Res Ther,* 37:863-868.

Murray CJL, Lopez AD. (1997). Global mortality, disability, and the contribution of risk factors: global burden of disease. *Lancet,* 349:1436-1442.

Murrell SA, Himmelfarb S, Wright K. (1983). Prevalence of depression and its correlates in older adults. *J epidemiol,* 117:173-185.

Musselman DL, Evans DL, Nemeroff CB. (1998). The relationship of depression to cardiovascular disease. Epidemiology,

biology and treatment. *Arch gen psychiatry,* 55: 580-592.
Neale MC, Cardon LR. (1992). Methodology for Genetic Studies of Twins and Families (NATO ASI Series D: Behavioural and Social Sciences, vol 67). Kluwer Academic Publishers: Dordrecht, The Netherlands.
Neale MC (1997) Mx: Statistical Modeling (Medical College of Virginia, USA),
Nemeroff CB. (1996). The corticotropin releasing factor (CRF) hypothesis of depression: new findings and new directions. *Mol Psychiatry*; 1:336–342.
Nemeroff CB. (1999). The preeminent role of early untoward experience on vulnerability to major psychiatric disorders: the nature-nurture controversy revisited and soon to be resolved. *Mol Psychiatry,* 4:106-108.
Newmann JP. (1989). Aging and depression. *Psychology & Aging,* 4:150-165.
Nisell M, Nomikos GG, Svenson TH. (1994). Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse,* 16:36-44.
Nurnberger JL, Gershon ES. (1981). Genetics of affective disorders. In: E. Friedman, (Ed.). *Depresion and antidepressants: Implications for courses and treatment.* (pp. 23-39). New York:, Raven Press.
Nutt D. (2000). Treatment of depression and concomitant anxiety. *Eur Neuropsychopharmacol,* 4:S433-437.
Okugawa G, Omori K, Suzukawa J, Fujiseki Y, Kinoshita T, Inagaki C. (1999). Long-term treatment with antidepressants increases glucocorticoid receptor binding and gene expression in cultured rat hippocampal neurones. *J Neuroendocrinol,* 11:887-895.
Omvik P. (1996). How smoking affects blood pressure. *Blood Press,* 5:71-77.
Oncken CA, White WB, Cooney JL, Van Kirk JR, Ahluwalia JS, Giacco S. (2001). Impact of smoking cessation on ambulatory blood pressure and heart rate in postmenopausal women. *Am J Hypertens,* 14:942-949.
Oparil S, Chen YF, Naftilan et al. Pathogenesis of hypertesnion. (1992). In: HA Fozzard, RB Jennings RB, Katz AM et al (Eds). *The heart and cardiovascular system.* (pp295-333). New York, Raven Press.
Osran H, Reist C, Chen CC, Lifrak ET, Chicz-DeMet A, Parker LN. (1993). Adrenal androgens and cortisol in major depression. *Am J Psychiatry,* 150:806-809.
Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, et al. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res,* 59:178-193.
Palsson SP, Ostling S, Skoog I. (2001). The incidence of first-onset depression in a population followed from the age of 70 to 85. *Psychol Med,* 31:1159-1168.
Patel YC, Eggen DA, Strong JP. (1980). Obesity, smoking and atherosclerosis. A study of interassociations. *Atherosclerosis,* 36:481-490.
Paterniti S, Alperovitch A, Ducimetiere P, Dealberto MJ, Lepine JP, Bisserbe JC. (1999). Anxiety but not depression is associated with elevated blood pressure in a community group of French elderly. *Psychosom Med,* 61:77-83.
Patton GC, Hibbert M, Rosier MJ, Carlin JB, Caust J, Bowes G. (1996). Is smoking associated with depression and anxiety in teenagers? *Am J Public Health,* 86:225-230.
Pauly JR, Ullman EA, Collins AC. (1988). Adrenocortical hormone regulation of nicotine sensitivity in mice. *Physiol Behav,* 44:109-116.
Penninx BW, Beekman AT, Honig A, Deeg DJ, Schoevers RA, van Eijk JT, van Tilburg W. (2001). Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry,* 58:221-227.
Pepin MC, Govindan MV, Barden N. (1992). Increased glucocorticoid receptor gene promoter activity after antidepressant treatment. *Mol Pharmacol,* 41:1016-1022.
Perkins KA. (1999). Nicotine discrimination in men and women. *Pharmacol Biochem Behav,* 64: 295-299.
Perkins KA. (2001). Smoking cessation in women. Special considerations. *CSN Drugs,* 15: 391-411.
Peters L, Andrews G. (1995). Procedural validity of the computerized version of the Composite International Diagnostic Interview (CIDI-Auto) in the anxiety disorders. *Psychol Med,* 25:1269–1280.
Peters L, Clark D, Carroll F. (1998). Are computerized interviews equivalent to human interviewers? CIDI-Auto versus CIDI in anxiety and depressive disorders. *Psychol Med,* 28:893-901.
Pike JL, Smith TL, Hauger RL, Nicassio PM, Patterson TL, McClintick J, Costlow C, Irwin MR. (1997). Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. *Psychosom Med,* 59:447-457.
Plotsky PM, Owens MJ, Nemeroff CB. (1998). Psychoneuroendocrinology of depression. *Psychiatric clin n am,* 21:293-307.
Pocock SJ, McCormack V, Gueyffier F, Boutitie F, Fagard RH, Boissel J-P. (2001). A score for predicting risk of death from cardiovascular disease in adults with raised blood pressure, based on individual patient data for randomised controlled trials. *BMJ,* 323:75-81.
Pomerleau OF, Pomerleau CS. (1984) Neuroregulators and the reinforcement of smoking: towards a biobehavioral explanation. *Neurosci Biobehav Rev,* 8:503-513.
Pomerleau OF, Pomerleau CS. (1990). Cortisol response to a psychological stressor and/or nicotine. *Pharmacol Biochem Behav,* 36:211-213.
Porges SW. (1992). Vagal tone: a physiologic marker of stress vulnerability. *Pediatrics,* 90:498-504.
Porges SW. (1995). Cardiac vagal tone: a physiological index of stress. *Neurosci Biobehav Rev,* 19:225-233.
Posener JA, DeBattista C, Williams GH, Chmura Kraemer H, Kalehzan BM, Schatzberg AF. (2000). 24-hour monitoring of cortisol and cortisotropin secretion in psychotic and nonpsychotic major depression. *Arch Gen Psychiatry,* 57:755-760.
Posthuma D, de Geus EJC, Boomsma DI, and Neale MC. (2002). Combined linkage and association tests in Mx. *Behavior Genetics*, 32, submitted.
Price JF Fowkes FG. (1997). Risk factors and the sex differential in coronary artery disease. Epidemiology, 8:584-591.
Province MA, Tishler P, Rao DC. (1989). Repeated-measures model for the investigation of temporal trends using longitudinal family studies: application to systolic blood pressure. *Genet Epidemiol,* 6:333-347.
Pruessner JC, Hellhammer DH, Kirschbaum C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosom Med,* 61:197-204.
Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, Von Auer K, Jobst S, Kapers F, Kirschbaum C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences,* 61:2539-2549.
Räikkönen K, Matthews KA, Flory JD, Owens JF, Gump BB. (1999). Effects of optimism, pessimism and trait anxiety on ambulatory blood pressure and mood during everyday life. *J Pers Soc Psychol,* 76:104-113.
Rasmussen DL, Fulker DW, Wiles M. (1997) Quantitative trait locus analysis of contextual fear conditioning in mice, *Nat Genet*, 17, 331-334,
Rechlin T, Weis M, Spitzer A, Kaschka WP. (1994). Are affective disorders associated with alterations of heart rate variability? *J Affect Disord,* 32:271-275.
Reul JM, Holsboer F. (2002). Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr Opin Pharmacol,* 2:23-33.
Riad-Fahmy D, Read GF, Walker RF, Griffiths K. (1982). Steroids in saliva for assessing endocrine function. *Endocr Rev,* 3:367-395.
Rickerby J. (2002). The role of home blood pressure measurement in managing hypertension: an evidence-based review. *J Hum Hypertens,* 16:469-472.
Riese H. (2000). *Job strain and risk for cardiovascular disease in female nurses.* Thesis: Vrije Universiteit, Amsterdam, The Netherlands.
Risch N, Zhang H. (1995). Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science,* 268:1584-1589.
Robinson GK. (1991) That BLUP is a good thing - the estimation of random effects. Statistical *Science*, 6, 15-51,
Rosmond R, Bjorntorp P. (1998). Blood pressure in relation to obesity, insulin and the hypothalamic-pituitary-adrenal axis in Swedish men. *J Hypertens,* 16:1721-1726.
Roy K, Parker G, Mitchell P, Wilhelm K. (2000). Depression and smoking: examining correlates in a subset of depressed patients. *Australian and New Zealand J Psychiatry,* 35: 329-335.
Roy MA, Neale MC, Pedersen NL, Mathe AA, Kendler KS. (1995). A twin study of generalized anxiety disorder and major depression. *Psychol Med,* 25:1037-1049.
Rozanski A, Blumenthal JA, Kaplan J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and

implications for therapy. *Circulation,* 99:2192-2217.
Rubin RT, Phillips JJ, McCracken JT, Sadow TF. (1996). Adrenal gland volume in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biol Psychiatry*, 40:89-97.
Sanchez C, Hyttel J. (1999). Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol*, 19:467-489.
Sapolsky RM, Romero LM, Munck AU. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev,* 21:55-89.
Saris WE, Pijper M de, Mulder J, Optimal procedures for estimation of factor scores, *Sociological Methods & Research*, 7, 85-106, 1978
Schmidt-Reinwald A, Pruessner JC, Hellhammer DH, Federenko I, Rohleder N, Schurmeyer TH, Kirschbaum C. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sci,* 64:1653-1660.
Schnall PL, Schwartz JE, Landsbergis PA, Warren K, Pickering . TG. (1998). A longitudinal study of job strain and ambulatory blood pressure. Results from a three year follow-up. *Psychosom Med,* 60:697-706.
Scott LV, Dinan TG. (1998). Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers. *J Affect Disord,* 47:49-54.
Selye H. Thymus and adrenals in the response of the organism to injuries and intoxications. *Br J Exp Pathol*, 17:234-248.
serum. *Eur J Clin Chem Clin Biochem,* 33:927-32.
Sesso HD, Kawachi I, Vokonas PS, Sparrow D. (1998). Depression and the risk of coronary heart disease in the Normative Aging Study. *Am J Cardiol,* 82:851-856.
Shapiro D, Jamner LD, Goldstein IB, Delfino RJ. (2001). Stiking a chord : moods, blood pressure, and heart rate in everyday life. *Psychophysiology,* 38:197-204.
Shiffman S. (1982). Relapse following smoking cessation: a situational analysis. *J Consult Clin Psychol,* 50:71-86.
Shinn EH, Poston WS, Kimball KT, St Jeor ST, Foreyt JP. (2001). Blood pressure and symptoms of depression and anxiety: a prospective study. *Am J Hypertens,* 14:660-664.
Silverstein B. (2002). Gender difference in the prevalence of somatic versus pure depression: a replication. *Am J Psychiatry,* 159:1051-1052
Sinaiko AR, Gomez-Marin O, Prineas RJ. (1997). Relation of fasting insulin to blood pressure and lipids in adolescents and parents. *Hypertension,* 30:1554-1559.
Sleight P. (1993). Smoking and hypertension. *Clin Exp Hypertens,* 15:1181-1192.
Solomon A, Haaga DA, Arnow BA. (2001). Is clinical depression distinct from subthreshold depressive symptoms? A review of the continuity issue in depression research. *J Nerv Ment Dis,* 189:498-506.
Specker S, de Zwaan M, Raymond N, Mitchell J. (1994). Psychopathology in subgroups of obese women with and without binge eating disorder. *Compr Psychiatry,* 35:185-90.
Spielberger CD, Gorsuch RL, Lushene RE. (1970). STAI Manual for the State-Trait Anxiety Inventory. *Consulting Psychologists Press*, Palo Alto, CA.
Spielberger CD, Jacobs GA, Russell SF, Crane RS. (1983) Assessment of anger: The State-Trait Anger Scale, in: *Advances in personality assessment*, JN Butcher and CD Spielberger (Eds), 2, 159-187, Lawrence Erlbaum Ass, Hillsdale,
Stamler J, Stamler R, Riedlinger WF, Algera G, Roberts RH. (1976). Hypertension screening of 1 million Americans. Community Hypertension Evaluation Clinic (CHEC) program, 1973 through 1975. *JAMA,* 235:2299-2306.
Steer RA, Rissmiller DJ, Beck AT. (2000). Use of the Beck Depression Inventory-II with depressed geriatric inpatients. *Behav Res Ther,* 38:311-318.
Steiger A, Holsboer F. (1997). Nocturnal secretion of prolactin and cortisol and the sleep EEG in patients with major endogenous depression during an acute episode and after full remission. *Psychiatry res,* 72:81-88.
Steptoe A, Butler N. (1996). Sports participation and well-being in adolescents. *Lancet*, 347: 1789-1792.
Steptoe A, Kimbell J, Basford P. (1998). Exercise and the experience and appraisal of daily stressors. A Naturalistic study. *J Behavioral Medicine*, 21: 363-374.
Steptoe A, Roy MP, Evans O, Snashall D. (1995). Cardiovascular stress reactivity and job strain as determinants of ambulatory blood pressure at work. *J Hypertens,* 13:201-210.
Stone AA, Schwartz JE, Smyth J, Kirschbaum C, Cohen S, Hellhammer D, Grossman S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. *Psychoneuroendocrinology,* 26:295-306.
Strachan T, Read AP, Human Molecular Genetics, 2nd edition, BIOS Scientific Publishers, Oxford, 1999
Strickland P, Morriss R, Wearden A, Deakin B. (1998) A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls. *J Affect Disord,* 47:191-194.
Strickland PL, Deakin JFW, Percival C, Dixon J, Gater RA, Goldberg DP. (2002). Bio-social origins of depression in the community. Interactions between social adversity, cortisol and serotonin neurotransmission. *Brittish J Psychiatry,* 180:168-173.
Sullivan PF, Neale MC, Kendler KS. (2000). Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry,* 157:1552-1562.
Talbot CJ, Nicod A, Cherny SS, Fulker DW, Collins AC, Flint J. (1999) High-resolution mapping of quantitative trait loci in outbred mice, *Nat Genet*, 21, 305-308,
Tarantino LM, Bucan M. (2000) Dissection of behavior and psychiatric disorders using the mouse as a model, *Human Molecular Genetics*, 9, 953-965,
Taylor RG. (1987). Smoking and the leucocyte count. *Eur J Respir Dis,* 71:65-68.
Tersman Z, Collins A, Eneroth P. (1991). Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. *Psychosom Med,* 53:185-197.
Thakor NV, Webster JG, Tompkins WJ. (1983). Optimal QRS detector. *Med Biol Eng Comput,* 21:343-350.
Thompson GH. (1951) The Factorial Analysis of Human Ability, University Press, London,
Thompson LM, Rubin RT, McCracken JT. (1992). Neuroendocrine aspects of primary endogenous depression: XII. Receiver operating characteristic and kappa analyses of serum and urine cortisol measures in patients and matched controls. *Psychoneuroendocrinology*,17:507-515.
Thurstone LL. (1935) The Vectors of the Mind, University of Chicago Press, Chicago,
Trainer PJ, McHardy KC, Harvey RD, Reid IW. (1993). Urinary free cortisol in the assessment of hydrocortisone replacement therapy. *Horm Metab Res,* 25:117-120.
Trainer PJ, Woods RJ, Korbonits M, Popovic V, Stewart PM, Lowry PJ, Grossman AB. (1998). The pathophysiology of circulating corticotropin-releasing hormone-binding protein levels in the human. *J Clin Endocrinol Metab,* 83:1611-1614.
Trestman RL, Yehuda R, Coccaro E, Horvath T, Knott P, gabriel S, Siever LJ. (1995). Diurnal endocrine and autonomic function in acute and remitted depressed male patients. *Biol Pssychiatry*, 37:448-456.
Turri MG, Talbot CJ, Radcliffe RA, Wehner JM, Flint J. (1999) High-resolution mapping of quantitative trait loci for emotionality in selected strains of mice, *Mamm Genome*, 10, 1098-1101,
Ursin R. (1980). Does para-chlorophenylalanine produce disturbed waking, disturbed sleep or activation by ponto-geniculo-occipital waves in cats? *Waking Sleeping,* 4:211-221.
Van Cauter E, Leproult R, Kupfer DJ. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab,* 81:2468-2473.
Van Cauter E, Turek FW. (1994). Endocrine and other biological rhythms. In: DeGroot LJ. (Ed.). *Endocrinology*. (pp. 2487-2548). Saunders, Philadephia.
Van Londen L, Goedkoop JG, Zwinderman AH, Lanser JBK, Wiegant VM, De Wied D. (1998). Neuropsychological Performance and Plasma Cortisol, Arginine Vasopressin and Oxytocin in Patients with Major Depression. *Psychol Med,* 28:275-284.
Vander A, Sherman J, Luciano D. (1998). Human physiology, the mechanisms of body function. Boston, WCB-McGraw-Hill.
Vink JM, Groot AS, Kerkhof GA, Boomsma DI. (2001). Genetic analysis of morningness and eveningness. *Chronobiol Internat,* 18:809-822.
Von Bardeleben U, Holsboer F, Stalla GK, Muller OA. (1985). Combined administration of human corticotropin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. *Life Sci,* 37:1613-1618.
Vrijkotte T. (2001). *Work stress and cardiovascular disease risk.* Thesis: Vrije Universiteit, Amsterdam, The Netherlands.

Vrijkotte TG, de Geus EJ. (2001). Ambulatory heart rate is underestimated when measured by an ambulatory blood pressure device. *J Hypertens,* 19:1301-1307.
Vrijkotte TG, van Doornen LJ, de Geus EJ. (2000). Effects of work stress on ambulatory blood pressure, heart rate, and heart rate variability. *Hypertension,* 35:880-886.
Walker BR, Phillips DI, Noon JP, Panarelli M, Andrew R, Edwards HV, Holton DW, Seckl JR, Webb DJ, Watt GC. (1998). Increased glucocorticoid activity in men with cardiovascular risk factors. *Hypertension,* 31:891-895.
Ward KD, Klesges RC, Zbikowski SM, Bliss RE, Garvey AJ. (1997). Gender differences in the outcome of an unaided smoking cessation attempt. *Addict Behav,* 22:521-533.
Ward NG, Doerr HO, Storrie MC. (1983). Skin conductance: a potentially sensitive test for depression. Psychiatry Res, 10:295-302.
Wassertheil-Smoller S, Applegate WB, Berge K, Chang CJ, Davis BR, Grimm R Jr, Kostis J, Pressel S, Schron E. (1996). Change in depression as a precursor of cardiovascular events. SHEP Cooperative Research Group (Systoloc Hypertension in the elderly). *Arch Intern Med,* 156: 553-561.
Watkins LL, Grossman P, Krishnan R, Blumenthal JA. (1999). Anxiety reduces baroreflex cardiac control in older adults with major depression. *Psychosom Med,* 61:334-340.
Watkins LL, Grossman P. (1999). Association of depressive symptoms with reduced baroreflex cardiac control in coronary artery disease. *Am Heart J,* 137:453-457.
Watson D, Tellegen A. (1985). Toward a consensual structure of mood. *Psychol Bull,* 98:219-235.
Watt GC, Harrap SB, Foy CJ, Holton DW, Edwards HV, Davidson HR, Connor JM, Lever AF, Fraser R. (1992). Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four-corners approach to the identification of genetic determinants of blood pressure. *J Hypertens,* 10:473-482.
Weber B, Lewicka S, Deuschle M, Colla M, Vecsei P, Heuser I. (2000). Increased diurnal plasma concentrations of cortisone in depressed patients. *J Clin End Met,* 85:1133-1136.
Wedekind D, Bandelow B, Broocks A, Hajak G, Ruther E. (2000). Salivary, total plasma and plasma free cortisol in panic disorder. *J Neural Transm,* 107:831-837.
Wilde GJS. (1970). [Neurotische labiliteit gemeten volgens de vragenlijstmethode (The questionnaire method as a means of measuring neurotic instability)]. Van Rossen, Amsterdam.
Wilkins JN, Carlson HE, Van Vunakis H, Hill MA, Gritz E, Jarvik ME. (1982). Nicotine from cigarette smoking increases circulating levels of cortisol, growth hormone, and prolactin in male chronic smokers. *Psychopharmacology,* 78:305-308.
Willemsen GHM, Geus EJC de, Klaver CHAM, Doornen LJP van, Carroll D. (1996). Ambulatory monitoring of the impedance cardiogram. *Psychophysiol,* 33:184–193.
Willett WC, Green A, Stampfer MJ, Speizer FE, Colditz GA, Rosner B, Monson RR, Stason W, Hennekens CH. (1987). Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. *N Engl J Med,* 317:1303-1309.
Wilson & Foster. (1992). *Williams textbook of endocrinilogy.* Philadelphia, Saunders.
Wittchen HU, Carter RM, Pfister H, Montgomery SA, Kessler RC. (2000). Disabilities and quality of life in pure and comorbid generalized anxiety disorder and major depression in a national survey. *Int Clin Psychopharmacol,* 15:319-328.
Wittchen H-U, Robins LN, Cottler LB, Sartorius N, Burke JD, Regier D. (1991). Cross-cultural feasibility, reliability and sources of variance of the Composite International Diagnostic Interview (CIDI). *Br J Psychiatry,* 159:645-653.
Wittchen HU. (1994). Reliability and validity studies of the WHO-Composite International Diagnostic Interview (CIDI): a critical review. *J Psychiatr Res,* 28:57–84.
Witte JS, Gauderman WJ, Thomas DC. (1999). Asymptotic bias and efficiency in case control studies of candidate genes and gene-environment interactions:basic family designs. *Am J Epidemiol,* 149:693-705.
Wong M-L, Kling MA, Munson PJ, Listwak S, Prolo P, Karp B, et al. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancolic features: relation to hypercortisolism and corticotropin-releasing hormone. *Proc Nat Acc Sci,* 97:325-330.
Wüst S, Federenko I, hellhammer DH, Kirschbaum C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology,* 25:707-720.
Yehuda R, Kahana B, Binder-Brynes K, Southwick SM, Mason JW, Giller EL. (1995). Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. *Am J Psychiatry,* 152:982-986.
Yehuda R, Teicher MH, Trestman RL, Levengood RA, Siever LJ. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biol Psychiatry,* 40:79-88.
Yeung RR. (1996). The acute effects of exercise on mood state. *J Psychosom Res,* 40:123-141.
Yong LC, Kuller LH, Rutan G, Bunker C. (1993). Longitudinal study of blood pressure: changes and determinants from adolescence to middle age. The Dormont High School follow-up study, 1957-1963 to 1989-1990. *Am J Epidemiol,* 138:973-983.
Young, E.A., Aggen, S.H., Prescott, C.A., and Kendler, K.S. (2000). Simmilarity in saliva cortisol measures in monozygotic twins and the influence of past major depression. *Biolog Psychiatry,* 48:70-74.
Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J., Akil, H. (2000). Hormonal evidence for altered responsiveness to social stress in major depression. *Neuropsychopharmacology,* 23:411-418.
Zieske AW, Takei H, Fallon KB, Strong JP. (1999). Smoking and atherosclerosis in youth. *Atherosclerosis,* 144:403-408.
Zisook S, Downs N. (1998). Diagnosis and treatment of depression in late life. *J Clin Psychiatry,* 59:80-91.
Zuckerman M. (1970) Dimensions of sensation seeking, *J Consult Clin Psychol,* 36:45-52

Appendices

Appendix I

Letter of invitation and brochure for the fourth wave of the survey

020-4448832

020-4448787

Geachte lezer,

U heeft zojuist vragenlijst vier van het onderzoek "gezondheid en leefgewoonten" ontvangen. **Deze vragenlijst is bestemd voor de tweeling en/of broers en zussen van de tweeling die nog thuis wonen.** Tot nu toe zijn voornamelijk de tweeling en de ouders/verzorgers benaderd. Door deze keer het onderzoek op zowel de tweelingen als op hun broers en zussen te richten kan nòg nauwkeuriger worden nagegaan welke combinatie van erfelijke eigenschappen en omgevingsinvloeden verantwoordelijk is voor een groot aantal aspecten van de gezondheid. Meer informatie hierover staat in de folder.

Wij zouden de thuiswonende tweeling en/of broers en zussen van de tweeling willen verzoeken de vragen in dit boekje in te vullen en het boekje zo spoedig mogelijk te retourneren in de antwoordenvelop. Het boekje bevat een aantal algemene vragen en een aantal persoonlijkheidsvragenlijsten.

Medewerking aan dit onderzoek is vrijwillig en verplicht niet tot verdere medewerking in de toekomst. De antwoorden die gegeven worden, worden vertrouwelijk behandeld. Dit betekent dat persoonlijke gegevens zoals naam en adres zullen worden losgekoppeld van de antwoorden. Naar aanleiding van de resultaten uit dit onderzoek wordt mogelijk een klein groepje tweelingen en hun broers en zussen verzocht mee te werken aan een uitgebreider onderzoek.

Mochten er verder nog vragen zijn, dan kunt u contact opnemen met mevr. J.R Koopmans of met mevr. M. Van den Berg, tel: 020-4448809.

Wij willen u alvast hartelijk bedanken voor de moeite. Indien de lijst niet na vier weken is geretourneerd, dan bestaat de mogelijkheid dat wij telefonisch contact opnemen. Hiermee proberen wij een zo groot mogelijke respons te krijgen.

Vriendelijke groeten,

Drs. J.R. Koopmans Drs. M. van den Berg

blijven van tweelingonderzoek. Dit onderzoek blijft **de manier** om een eerste indruk te krijgen hoe belangrijk erfelijke factoren zijn. De volgende stap is op zoek te gaan naar de specifieke genen die van belang zijn. Voor veel ziekten die door een enkel gen worden bepaald, is dat inmiddels bekend. Maar bij complexere eigenschappen zullen meerdere genen een rol spelen. Door nieuwe technieken wordt het nu mogelijk op zoek te gaan naar deze genen. **Voor dit soort onderzoek is ook de medewerking van de broers en zussen van de tweelingen van groot belang.** Door de broers en zussen bij het onderzoek te betrekken, kan op nog nauwkeurigere wijze worden nagegaan welk gen verantwoordelijk is voor bepaalde complexe eigenschappen. Hiermee kan een eerste stap worden gezet op de weg naar lokalisatieonderzoek, waarmee de precieze plaats van het gen kan worden ontdekt. Kennis over de genen die verantwoordelijk zijn voor een ziekte of psychische aandoening zal uiteindelijk leiden tot verbeterde diagnoses, de ont-wikkeling van medicijnen en verbetering van de behandeling.

Meedoen aan onderzoek.

Met deze folder hopen wij het belang van het tweeling- en familieonderzoek toegelicht te hebben. **De medewerking van zoveel mogelijk tweelingen en hun familieleden is van essentieel belang** voor de voortgang van het onderzoek naar risicofactoren van hart- en vaatziekten, CARA, verschillende psychische aandoeningen en probleemgedrag.

Literatuur

Hieronder worden een aantal titels genoemd waarin de uitkomsten worden beschreven van lopende onder-zoeken. Zonder de inzet van honderden tweelingen paren verspreid over heel Nederland was dit onder-zoek niet mogelijk geweest.

Boomsma, DI. et al. ***Een onafscheidelijke tweeling.*** **Natuur en techniek, 56, 662-673, 1988.**

Boomsma, DI. et al. ***Genetic and social influences on starting to smoke: a study of Dutch adolescent twins and their parents.*** **Addiction, 89: 219-226, 1994**

Boomsma, DI. & Verhulst, FC . ***Gentisch onderzoek naar psychopathologie bij jonge tweelingen*** **in: Jaarboek psychiatrie en psychotherapie 5, 90-102, 1995**

Boomsma, DI. *Gedragsgenetisch onderzoek naar angst: enkele ontwikkelingen sinds Heymans en Wiersma (1905).* De Psycholoog, 438-441, 1996

Koopmans JR et al., *Smoking and sports participation* **in: Genetic factors in Coronary Heart Disease**, Dordrecht: Academic Publishers, 217-235, 1994

Koopmans JR et al., *A multivariate genetic analysis of sensation seeking.* Behavioral genetics, 25: 349-356, 1995

Koopmans JR & Boomsma, DI. *Familial resemblances in alcohol use: genetic or cultural transmission?* Journal of studies on alcohol, 57: 19-28, 1996

Koopmans JR & Boomsma, D I. ***Individuele verschillen in alcoholgebruik. De rol van erfelijke aanleg en de omgeving.*** **De Psycholoog, maart 1996.**

Het Nederlands Tweelingen Register
Postadres:
De Boelelaan 1111
1081 HV Amsterdam tel: 020-444 8787

Nederlands Tweelingen Register

Wat is het NTR?

Het Nederlands Tweelingen Register (NTR) werd op 1 februari 1987 opgericht aan de Vrije Universiteit te Amsterdam. Tweelingen van alle leeftijden staan bij het register ingeschreven. De tweelingen, en soms ook hun familie, kunnen het verzoek krijgen om mee te werken aan wetenschappelijk onderzoek. Dit komt in de meeste gevallen neer op het invullen en retour-neren van vragenlijsten. Soms kan een kleine groep tweelingen uitgenodigd worden voor een bezoek aan de Vrije Universiteit voor een specifiek onderzoek, bijvoorbeeld naar astma. **Een registratie in het NTR verplicht tot niets.** Deelname aan het onderzoek is altijd vrijwillig. Iedereen die bij het NTR staat inge-schreven, ontvangt het jaarlijkse informatie magazine, de TWINFO. Daarin staan wetenswaardigheden over tweelingen en wordt een beschrijving gegeven van de resultaten van onderzoek waaraan de tweelingen hebben meegewerkt.

Lopend onderzoek bij het NTR

Op dit moment doet het NTR onder meer onderzoek bij jonge kinderen naar het ontstaan van gedrags-problemen. Bij tweelingen tussen de 12 en 25 jaar en hun familieleden wordt onderzoek gedaan naar persoonlijkheid en leefgewoonten die een risico kun-nen vormen voor het ontstaan van hart- en vaat-ziekten. Bij groepen tweelingen van verschillende leeftijden is er een onderzoek gaande naar de ontwik-keling van de hersenen. Daarnaast is er in samen-werking met het Academische Ziekenhuis van de Vrije Universiteit een onderzoek naar de erfelijke aanleg voor CARA.

Vertrouwelijk

Alle informatie die wij door onderzoek verkrijgen, wordt door ons **vertrouwelijk** behandeld. **Persoon-lijke gegevens, zoals naam en adres, worden los-gekoppeld van beantwoorde vragen**. De antwoor-den worden gecodeerd in cijfers en opgeslagen in de computer. De direct bij het onderzoek betrokken medewerkers gebruiken alleen de gecodeerde infor-matie. Alleen de projectleider kan over de gecodeer-de informatie en de adresgegevens beschikken. De gegevens worden alleen voor wetenschappelijke doel-einden gebruikt.

Tweelingen: één- of tweeëiig?

Bij het doen van tweelingen onderzoek moet er een onderscheidt gemaakt worden tussen één- en twee-ëiige tweelingen. Tweeëiige tweelingen ontstaan wanneer er in plaats van één eicel, twee eicellen worden bevrucht en beide zich ontwikkelen tot een embryo. Deze tweelingen verschillen evenveel van elkaar als 'gewone' broers en zussen. Dat wil zeggen dat 50% van hun erfelijke materiaal overeenkomt. In uiterlijk hoeven de tweeëiige tweelingen ook niet op elkaar te lijken, dit is natuurlijk niet uitgesloten. Eéneiige tweelingen onstaan wanneer een bevruchte eicel zich splitst. Beide helften bevatten dan hetzelfde erfelijke materiaal. Eéneiige tweelingen hebben altijd hetzelfde geslacht en lijken meer op elkaar dan 'gewone' broers en zussen.

Waarom tweelingonderzoek?

Voor een groot aantal eigenschappen die een mens heeft, is een bepaald gen of een bepaalde combinatie van genen en omgeving verantwoordelijk. Een gen is een klein deeltje erfelijk materiaal dat in elke lichaamscel voorkomt. Voor sommige ziekten, zoals bijvoorbeeld PKU of de ziekte van Huntington, is bekend welk gen de ziekte veroorzaakt. Voor comple-xere ziekten, zoals hart- en vaatziekten, en voor com-plexe eigenschappen, zoals iemands persoonlijkheid, is het veel moeilijker na te gaan wat de invloed is van de erfelijke en de niet-erfelijke factoren. In dit geval vormt het vergelijken van één- en tweeëiige twee-lingen vaak de eerste aanwijzing in welke mate deze eigenschappen erfelijk bepaald zijn.

Ieder mens heeft zijn eigen unieke genetische opmaak. Met uitzondering van ééneiige tweelingen, die hebben dezelfde genetische opmaak. Door deze bijzondere speling van de natuur bieden tweelingen de mogelijkheid om na te gaan wat de invloed is van erfelijke aanleg en de omgeving op een eigenschap. In het kort komt het er op neer dat de overeenkomst tussen eeneiige tweelingen wordt vergeleken met de overeenkomst tussen tweeëiige tweelingen. Wanneer ééneiige tweelingen voor een bepaalde eigenschap meer op elkaar lijken dan tweeëiige tweelingen, mag gezegd worden dat die eigenschap gedeeltelijk gene-tisch bepaald is.

Familie-onderzoek

Naast de tweeling kunnen ook andere familieleden, zoals de ouders of de broers en zussen, belangrijke informatie verschaffen over de invloed van erfelijke aanleg en de omgeving. Door ouders en kinderen te vergelijken kunnen we meer te weten komen over het belang van de gemeenschappelijke gezinsomgeving. Door broers en zussen bij het onderzoek te betrekken kunnen we nagaan in hoeverre broers en zussen el-kaars gedrag beïnvloeden. Ook kunnen we dan na-gaan of 'gewone' broers en zussen net zoveel op elkaar lijken als tweeëiige tweelingen.

Uitkomsten van tweelingonderzoek

Tweeling- en familieonderzoek geeft een eerste aanwijzing dat erfelijke aanleg een rol speelt bij een bepaalde eigenschap. Als we vinden dat een eigen-schap wordt beïnvloed door erfelijke factoren wil dat niet zeggen dat die eigenschap onveranderlijk is. Naast erfelijke aanleg is ook de invloed van de om-geving belangrijk. Deze omgevingsfactoren kunnen veranderd worden. Bijvoorbeeld, mensen die door erfelijke aanleg een risico lopen een te hoge bloed-druk te ontwikkelen, kunnen door middel van veran-deringen in het dieet het risico hierop verkleinen. Tweeling -en familieonderzoek biedt ook de mogelijk-heid om na te gaan welke omgevingsfactoren een rol spelen. Op die manier kunnen we aangeven welke omgevingsfactoren het meest aangewezen zijn om veranderingen te bewerkstelligen.

Toekomst van tweelingonderzoek

Er is de laatste jaren een enorme ontwikkeling gaande op het gebied van erfelijkheidsonderzoek. Naar alle waarschijnlijkheid zal eind 1997 de helft van de circa 100.000 menselijke genen in kaart gebracht zijn. Is er met de opkomst van het moderne onderzoek op het niveau van chromosomen en genen nog een rol weggelegd voor tweelingonderzoek? Voor veel eigen-schappen die geen eenvoudig patroon van overerving vertonen, zal de wetenschap voorlopig afhankelijk

Appendix II

Letter and folder first introducing the NETSAD project

datum postmerk 020-4448832

MvB/ns 020-4448787

Geachte mevrouw, mijnheer,

In de afgelopen jaren hebben u en een aantal van uw familieleden meegedaan aan vragenlijstonderzoek van het Nederlands Tweelingen Register (NTR). Uw medewerking hieraan was van groot belang en werd zeer gewaardeerd. Uit het gedane onderzoek is gebleken dat de erfelijke aanleg een belangrijke rol speelt, zowel bij lichamelijke als bij geestelijke gezondheid. Daarnaast zijn uiteraard ook niet-erfelijke invloeden van belang. Wij willen u en een aantal van uw familieleden uitnodigen voor een vervolgonderzoek.

Dit vervolgonderzoek gaat over de invloed van erfelijkheid op angstige en depressieve gevoelens. Deze gevoelens hebben, meer dan tot voor kort werd aangenomen, een negatieve invloed op de gezondheid. De resultaten van dit onderzoek moeten onder meer leiden tot verbetering van diagnose en behandeling van angst en depressie. Ook wordt het effect van depressieve en angstige gevoelens op de bloeddruk onderzocht. Wij verzoeken u mee te doen ook al heeft u zelden of nooit angstige of depressieve gevoelens. Uw deelname geeft namelijk inzicht in de vraag waarom de ene mens gezond is en de andere lijdt aan angst of depressie. Over de details van dit onderzoek leest u meer in de bijgevoegde folder.

Het onderzoek zal in drie stappen worden uitgevoerd. Voor de eerste stap worden tweelingen en hun broers/zusters benaderd die in de afgelopen jaren een vragenlijst van het NTR hebben ingevuld, en indien mogelijk, hun ouders. We vragen hen een monduitstrijkje te maken. Hieruit kan erfelijk materiaal worden verkregen. Het maken van een monduitstrijkje kost u bij elkaar ongeveer 10 minuten tijd. Voor stap twee en drie worden twee of drie gezinsleden verzocht mee te doen aan een telefonisch interview en een onderzoek naar bloeddruk. Alle onderdelen van dit onderzoek kunnen thuis gebeuren. Al uw gegevens worden vertrouwelijk behandeld.

Medewerking aan één deel van het onderzoek verplicht u niet tot verdere deelname; ook uw medewerking aan alleen het eerste deel is zeer waardevol. Mocht u nog vragen hebben, dan kunt u contact opnemen met mw. prof. dr. D.I. Boomsma of mw. drs. M. van den Berg, tel: 020-444.8787.

De eerste stap van het onderzoek gaat binnenkort van start. Hiervoor worden de leden van 500 families benaderd. Zij ontvangen in de komende twee weken thuis het afnamemateriaal voor het maken van een monduitstrijkje. Uw medewerking wordt zeer gewaardeerd.

Met vriendelijke groet,

prof. dr. D.I. Boomsma

De Vrije Universiteit doet met behulp van het Nederlands Tweelingen Register (NTR) onderzoek naar de invloed van erfelijke aanleg op gedrag en gezondheid. Dit gebeurt met vragenlijsten en door middel van onderzoek op de universiteit en in het VU ziekenhuis.

Sinds 1991 loopt bij tweelingen, hun ouders en hun broers/zusters een groot onderzoek naar leefgewoonten en gezondheid. In 1998 gaat de volgende fase van dit onderzoek van start. In deze fase wordt speciale aandacht besteed aan de invloed van erfelijkheid op de geestelijke gezondheid en de invloed van psychische klachten op de lichamelijke gezondheid. Een groot aantal Nederlanders heeft wel eens last van angst of depressie. Uit ons tweelingonderzoek blijkt dat de reden dat de ene mens wel en de andere mens geen last heeft van angstige en neerslachtige gevoelens voor een deel bepaald wordt door verschillen in erfelijke aanleg. Het is nog onbekend welke genen (dragers van erfelijk materiaal) bijdragen aan deze verschillen tussen mensen. Ook is nog maar heel beperkt bekend hoe de omgeving van invloed is op deze klachten. Het is bijvoorbeeld mogelijk dat de vatbaarheid voor angstige en depressieve gevoelens met name tot uiting komt onder invloed van bepaalde omgevingsfactoren. Met uw medewerking willen we proberen een antwoord te krijgen op deze vragen.

Voor de ontwikkeling van een effectieve diagnose en behandeling is het van groot belang dat er meer inzicht komt in de manier waarop erfelijkheid en de omgeving leiden tot depressieve klachten. Dit willen wij doen door middel van een familieonderzoek. Hiervoor worden meerdere leden van een gezin met elkaar vergeleken. Met name als daar één- en twee-eiige tweelingen bij zijn, kan worden nagegaan of erfelijke aanleg een rol speelt in het ontwikkelen van angstige en neerslachtige gevoelens. Als dit zo is, kan nader onderzoek worden gedaan naar deze erfelijke factoren: hoeveel genen spelen een rol en wat is hun plaats in het totale erfelijke materiaal?

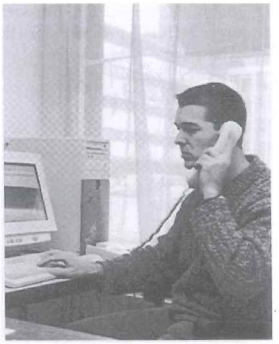
Waarschijnlijk is een groot aantal genen betrokken bij een aangeboren gevoeligheid voor angst en depressie. Om inzicht te krijgen in deze problematiek, zijn zowel gegevens over gedrag als gegevens over erfelijk materiaal nodig. Daarom vraagt het NTR de medewerking van een groot aantal gezinnen.

Het onderzoek wordt uitgevoerd in drie stappen:

1. Voor de eerste stap moet een monduitstrijkje worden gemaakt. Dit gebeurt bij tweelingen, hun ouders en hun broers/zusters, die eerder hebben meegedaan aan vragenlijstonderzoek. Met dit monduitstrijkje kan, op een eenvoudige manier, erfelijk materiaal worden verzameld. Het is bovendien een volstrekt pijnloze methode. Het monduitstrijkje wordt verkregen door met een wattenstaafje zachtjes langs de binnenkant van de mond te wrijven. Op deze manier wordt er wangslijmvlies verzameld. Dit is geschikt voor bepalingen in het erfelijk materiaal omdat de cellen van het wangslijmvlies zeer vaak vernieuwd worden. Ook kan dit materiaal worden gebruikt om te bepalen of een tweeling één- of twee-eiig is. De tweelingen die aan dit onderzoek meedoen krijgen de uitslag hiervan kosteloos toegezonden.

Wattenstaafje zachtjes langs de binnenkant van de mond strijken. De staafjes worden in een buisje gedaan en per post teruggestuurd aan de onderzoekers.

2. De tweede stap bestaat uit een kort telefonisch interview waarvoor twee of drie familieleden worden benaderd. Met dit interview wordt nagegaan welke persoonlijkheidskenmerken van belang zijn voor het hebben van angstige of neerslachtige gevoelens. Voor dit interview wordt u thuis gebeld door een medewerker van het NTR die hier speciaal voor getraind is. Het is niet onze bedoeling diagnoses te stellen met behulp van het interview. Er worden om deze reden ook geen individuele uitslagen verstrekt. U kunt zelf bepalen op welk tijdstip het interview wordt afgenomen. Wij zullen u hierover ruim van te voren informatie thuis sturen.



U ontvangt van tevoren thuis een boekje dat u tijdens het interview nodig heeft. Het interview vindt plaats op een voor u geschikt tijdstip.

3. De derde en laatste stap, tenslotte, bestaat uit een hartslag- en bloeddruk-onderzoek. De gezinsleden die aan het tweede deel van het onderzoek hebben meegedaan, zullen worden uitgenodigd ook aan dit laatste deel mee te doen. Dit houdt in dat zij worden verzocht gedurende een etmaal kleine meetapparatuur te dragen (ter grootte van een walkman). Hiermee kunnen de hartslag en de bloeddruk gemeten worden. Deze methode van meten is ontwikkeld aan de Vrije Universiteit in Amsterdam. Het is een unieke methode die het mogelijk maakt om hartslag en bloeddruk te meten in de normale dagelijkse situatie, in plaats van bij een arts of in een laboratorium. Dit deel van het onderzoek zal in de loop van de komende twee jaar plaatsvinden. Door het formaat van de meetapparatuur kan deze gewoon onder de kleding worden gedragen zonder dat u hier hinder van ondervindt bij uw dagelijkse bezigheden. Een medewerker van het NTR zal op afspraak, bij u thuis, het apparaatje 's ochtends bij u om doen en het de volgende ochtend weer bij u ophalen. U hoeft er dus niet voor naar Amsterdam te komen.

De meetapparatuur wordt bij u thuis door één van de onderzoekers bevestigd.

Voor dit onderzoek worden 500 families in Nederland benaderd. Het onderzoek beperkt zich niet tot mensen die reeds klachten van angstige of neerslachtige aard hebben. Ook de deelname van mensen die nooit last hebben van deze klachten is van groot belang. Juist het verschil tussen mensen verschaft waardevolle informatie over de oorzaken van angstige gevoelens, nervositeit of neerslachtigheid. We hopen van harte dat u bereid zult zijn aan dit onderzoek deel te nemen. Al uw gegevens worden vertrouwelijk behandeld. Ze worden op gecodeerde wijze bewaard en zijn alleen toegankelijk voor de directe medewerkers aan dit project. Het is de medewerkers niet toegestaan om de gegevens voor andere dan wetenschappelijke doeleinden te gebruiken. Door uw medewerking kunnen we meer te weten komen over de oorzaken van angst en depressie en hun invloed op lichamelijke gezondheid. Dit kan een bijdrage leveren aan de kwaliteit van de diagnose en de behandeling voor angst en depressie.

Namens het onderzoeksteam:

drs. M. van den Berg
dr. J.R. Koopmans
dr. C.V. Dolan
dr. J.C.N. de Geus
prof. dr. D.I. Boomsma
prof. dr. J. F. Orlebeke

Appendix III
Letter and instruction for buccal swap

datum postmerk 020-4448832

MvB/ns 020-4448787

Geachte heer/mevrouw,

Kort geleden verzochten wij u deel te nemen aan familieonderzoek naar leefgewoonten en geestelijk en lichamelijke gezondheid. Wij stuurden u daarbij een folder met alle informatie over het onderzoek.

Bij deze brief ontvangt u het materiaal voor het maken van een monduitstrijkje:
_ een instructie (geel vel)
_ een verklaring (blauw vel)
_ een grote buis met 12 wattenstaafjes
_ drie dunne buisjes met vloeistof
_ een antwoordenveloppe (NB geen postzegel nodig)

Het is de bedoeling dat u eerst de instructie goed doorleest. Daarin wordt uitgelegd hoe u het monduitstrijkje maakt met de wattenstaafjes. Na afname stuurt u de drie dunne buisjes samen met de ingevulde verklaring terug. Uw medewerking aan dit onderzoek is vrijwillig, u kunt dus ook verkiezen niet mee te werken. Dit kunt u zonder opgaaf van reden, op elk moment tijdens het onderzoek doen.

De informatie die met de monduitstrijkjes wordt verkregen wordt vertrouwelijk behandeld en gecodeerd opgeslagen. Voor de codering treft u op de buizen een nummer aan. Voor u gemak hebben we een voornaam toegevoegd of de aanduiding 'vader' of 'moeder'. Met vader en moeder worden hier de ouders van de tweeling bedoeld. Over resultaten van dit deel van het onderzoek worden geen individuele uitslagen verstrekt; wel krijgt u een verslag van de uitkomsten van het totale onderzoek. Ook laten we de tweelingen die meedoen weten of ze een_ of twee_eiig zijn.
Mocht u nog vragen hebben met betrekking tot de aard en de procedures van het onderzoek dan kunt u contact opnemen met mevr drs van den Berg of professor dr Boomsma (tel. 020_444 8787).

Wij hopen op uw medewerking,

Met vriendelijke groet,

prof dr. D.I. Boomsma

Vrije Universiteit Faculteit
Nederlands Tweelingenregister
Van der Boechorststraat 1
1081 BV AMSTERDAM
020-4448787

INSTRUCTIE VOOR HET MAKEN VAN MOND-UITSTRIJKJES

U ontvangt hierbij het materiaal voor afname van een mond-uitstrijkje.
Wilt u bij de afname a.u.b. letten op de volgende punten.

1. U heeft per persoon 4 buizen ontvangen: een dikke buis met 12 wattenstaafjes en 3 dunne buisjes met alleen een beetje vloeistof. U gebruikt per keer 4 staafjes voor het afnemen van de mond-uitstrijkjes. Het is de bedoeling dat na afname van de mond-uitstrijkjes de wattenstaafjes in de dunne buisjes worden gedaan, en in de bijgesloten antwoordenveloppe op de brievenbus worden gedaan. De dikke buis heeft u niet meer nodig.

2. Wilt u de uitstrijkjes direct na afname met de gewone post opsturen. Let op: als het buiten vriest, de enveloppe niet in een buitenbrievenbus gooien.

3. De mond-uitstrijkjes moeten 3 keer worden afgenomen, verspreid over één of twee dagen (bijv. voor alle maaltijden). Niet van te voren de mond spoelen, tandenpoetsen of eten!

4. Per keer gebruikt u 4 wattenstaafjes:
   - 1 staafje voor de binnenkant van de bovenlip en het tandvlees van de bovenkaak.
   - 1 staafje voor de binnenkant van de onderlip en het tandvlees van de onderkaak.
   - 1 staafje voor de binnenkant van de linkerwang.
   - 1 staafje voor de binnenkant van de rechterwang.

   Doe de 4 staafjes in een van de dunne buisjes. De andere dunne buisjes zijn voor de volgende monduitstrijkjes.

5. U wrijft per wattenstaafje ongeveer 10-20 seconden, zorgvuldig en met enige druk langs de wangen, tandvlees en binnenkant van de lip (het hoeft niet hard; het mag geen pijn doen).

6. Na het wrijven deponeert u het staafje, met het watje naar beneden, in de vloeistof in een dun buisje. Wilt u het deksel van het buisje goed dichtdraaien? (Niet te hard om barsten van de buis te voorkomen).

Nadat het erfelijk materiaal is geanalyseerd, zullen eventuele restanten voorlopig opgeslagen worden voor eventuele aanvullende bepalingen in de toekomst. Wanneer u dit niet wilt, stelt u ons dan daarvan s.v.p. op de hoogte. In dat geval zullen wij het overgebleven materiaal vernietigen.

Als u nog vragen heeft, kunt u bellen naar het secretariaat, en vragen naar mevr. drs. Van den Berg of mevr. prof.dr. Boomsma (tel. 020-4448787).

Appendix IV
Letter of invitation for CIDI interview

datum postmerk 020-4448832

MvB/ns 020-4448787

Geachte lezer,

Wij vragen u om uw verdere medewerking in het grote familieonderzoek naar lichamelijke en geestelijke problemen bij angst en depressie.

Deze medewerking bestaat uit deelname aan een telefonisch interview. Hiermee wordt nagegaan welke karakter-eigenschappen een rol spelen bij het ontstaan van angstige en depressieve klachten. Het interview wordt afgenomen op een voor u geschikt tijdstipt (overdag of 's avonds) en duurt tussen de 10 en 40 minuten. Het interview bestaat uit een aantal vragen die u worden voorgelezen vanaf een computerscherm. Uw antwoorden worden strikt vertrouwelijk behandeld. In de rapportage over het onderzoek zijn uitslagen nooit herleidbaar tot individuele deelnemers.

De bijgesloten gele kaart wordt voor dit interview gebruikt. **Bewaart u deze kaart alstublieft goed!**

Dit interview is onderdeel van een groot, werelwijd, onderzoek. Ook in de Verenigde Staten en Australië worden tweelingen en hun broers en zussen op dezelfde wijze geïnterviewd.

Wij verzoeken u mee te doen ook als u zelf geen klachten heeft. Uw antwoorden kunnen inzicht geven in de vraag waarom de ene mens gezond is en de andere mens lijdt aan depressie of angststoornissen. In Nederland zijn een groot aantal mensen onder behandeling vanwege deze klachten. Dankzij onderzoek bij tweelingfamilies staat inmiddels vast dat erfelijke aanleg een rol speelt bij angst en depressie. Voor de hulpverlening is het van groot belang dat er meer inzicht komt in de manier waarop erfelijke aanleg en omgeving kunnen leiden tot deze problemen.

De komende weken wordt er contact met u opgenomen om een afspraak te maken voor het telefonische interview. Deze afspraak wordt gemaakt door de mensen die de interviews afnemen. Zij zijn zeer beperkt op de hoogte van de andere delen van het familieonderzoek. Informatie over alle delen van het familieonderzoek vindt u in de folder die wij u toestuurden. Mocht u vragen hebben over het telefonische interview of over andere onderdelen van het onderzoek, aarzel dan alstublieft niet om contact met ons op te nemen.

Met vriendelijke groet, prof.dr. D.I. Boomsma
Drs. M. van den berg

Appendix V

Example diary

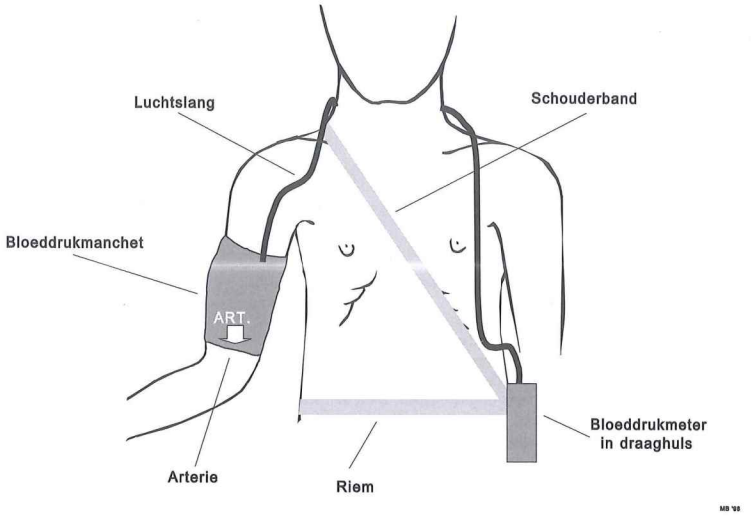
DATUM: 27-07 19 99/ 20 **VOORBEELD van een ingevuld dagboek.**

TIJD: 7.15- 7.45	Kastjes werden omgehangen Vu-onderzoeker om 7.30 weg Alleen ontbeten
TIJD: 7.45 - 8.15	Alleen in huis Beetje opgeruimd, katten eten gegeven Om 8 uur de deur uit en vuilnis buiten gezet plus minus 20 minuten fietsen
TIJD: 8.15-8.45	Aangekomen op werk. Computer aan, koffie halen. Kletsen met collega's
TIJD: 8.45-9.15	Kletsen met collega's, om 09.00u aan het werk PC werk, alleen
TIJD: 9.15- 9.45	Zitten achter de PC (alleen)
TIJD: 9.45-10.15	Zitten achter de PC (alleen) 2x opgestaan en naar de printer gelopen

Appendix VI

Short manual Spacelabs device

HANDLEIDING SPACELABS BLOEDDRUKMETER



Bevestigen

- Schuif de draaghuls waarin de bloeddrukmeter komt te hangen aan de riem. Bevestig de riem en schuif de draaghuls op uw rechterzij * (zie figuur 1). Als u zelf een riem draagt, kunt u de draaghuls ook om deze riem schuiven.
- Bevestig de manchet om uw **linkerarm** * zodanig dat het woord 'ARTERY' aan de binnenkant van uw arm zit, het pijltje eronder naar de knik in uw arm wijst en de onderkant van de manchet 2,5 cm boven de knik in uw arm zit. De manchet mag niet over uw arm kunnen schuiven en moet dus niet te los zitten.
- Daarna laat u de slang over uw schouder naar de bloeddrukmeter lopen. U maakt vervolgens de slang vast aan de luchtslangaansluiting aan de voorkant van de bloeddrukmeter door de slang op het aansluitpunt te draaien.

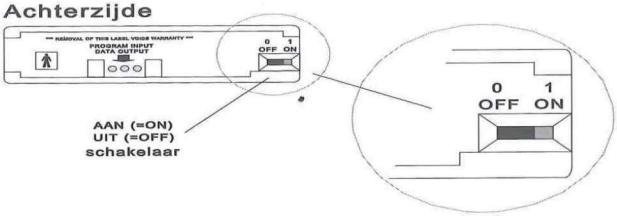
Aanzeten van de bloeddrukmeter

Om de bloeddrukmetingen te starten moet u de bloeddrukmeter op "ON" zetten ("OFF / ON" knop zit aan de onderkant van de bloeddrukmeter). Het digitale-venster op de bovenkant geeft nu de tijd aan. Zolang de bloeddrukmeter op "ON" blijft staan komt er om het halfuur automatisch een bloeddrukmeting.

Voorzijde

Aansluitpunt START/STOP knop Display

Achterzijde



* Als u links bent, neem dan de rechterarm en linkerheup.

Vermijd scherpe voorwerpen bij de manchet en de luchtslang.

Uitzetten van de bloeddrukmeter.

korte onderbreking: om een bloeddrukmeting even uit te stellen, bijvoorbeeld als u de arm even niet stil kunt houden, kunt u tijdens de meting op de 'START/STOP' knop drukken. De meting wordt gestopt, maar na 3 minuten wordt er automatisch een nieuwe meting gedaan.

lange onderbreking: Om de bloeddrukmetingen voor langere tijd te onderbreken, bijvoorbeeld wanneer u gaat douchen of gaat slapen, zet u de meter op 'OFF', maakt de manchet los en legt u de meter af. Als u weer verder wilt gaan met de meting, doet u de meter weer om en zet u de knop weer op 'ON'.

□s nachts □s nachts legt u de bloeddruk af zoals hierboven staat beschreven.

Er gaat iets mis...

□ De metingen worden om de drie minuten herhaald.

diagnose: De bloeddrukmetingen mislukken door teveel beweging of door een te los manchet. Dit schakelt de automatische herhaalmetingen in.

oplossing: a. U dient uw arm tijdens de meting goed stil te houden
b. Controleer of de manchet strak genoeg zit.

□ Er komen geen nieuwe metingen hoewel u de bloeddrukmeter niet bewust uit heeft gezet.

diagnose: Oorzaak hiervan kan zijn dat de luchtslang los is geraakt van de luchtslang-bevestiging, of dat u per ongeluk de 'ON/OFF' knop hebt verschoven.

oplossing: controleer of de luchtslang op de aansluiting zit en of de 'ON/OFF' knop op 'ON' staat.

□ Er staat iets anders dan de tijd in de digitale venster, bijv. een code als 'EC00' of 'LLL'.

diagnose: de bloeddrukmeter is stuk of in de war.

oplossing: kunt dan het beste de meter op 'OFF' zetten zodat de voorafgaande metingen bewaard blijven. U kunt de meter dan op een veilige plaats opbergen.

□ Anders, bellen naar het telefoonnummer in het dagboekje

Legt u de manchet af, vergeet dan niet de bloeddrukmeter op □OFF□ te zetten.

Appendix VII

Protocol cortisol sampling

Gebruiksaanwijzing speekselverzameling met de 'Salivette' wattenrolletjes.

Het is van belang dat u niet vlak voor de speekselverzameling uw tanden poetst of zure voedingswaren gebruikt, zoals fruit of koolzuurhoudende frisdrank. Het beste kunt u uw mond even kort spoelen met water.

De speekselverzameling vindt plaats op de volgende tijden:

1. direct na aankomst van de onderzoeker
2. 11.00 uur 's morgens
3. 15.00 uur 's middags
4. 20.00 uur 's avonds
5. 22.30 uur 's avonds
6. 's morgens direct na het opstaan

Deze tijdstippen staan op de buisjes aangegeven.

Hieronder volgen de **instructies voor het verzamelen van speeksel**:

1) Op de aangegeven tijdstippen neemt u een genummerde buis uit de Salivette-houder.
2) U draait de dop van de buis en haalt het wattenrolletje eruit.
3) Legt u het wattenrolletje in uw mond gedurende tenminste 45 seconden. Door de gaatjes in het plastic hulsjes wordt speeksel in het wattenrolletje opgenomen. Het is belangrijk (!) dat het wattenrolletje goed doordrenkt wordt. U kunt dit bevorderen door niet te slikken en licht op het wattenrolletje te kauwen.
4) Stop het wattenrolletje in de genummerde buis, draai de dop erop.
5) Bewaar de gebruikte genummerde buizen op een donkere koele plaats.

Hartelijk dank!

SCHEMA VOOR SALIVETTE

1e salivette: _____ tijdstip _____ : _____
2e salivette: _____ tijdstip _____ : _____
3e salivette: _____ tijdstip _____ : _____
4e salivette: _____ tijdstip _____ : _____
5e salivette: _____ tijdstip _____ : _____
6e salivette: _____ tijdstip _____ : _____

Dankwoord

Het dankwoord van een proefschrift wordt vaak als eerste gelezen en helaas is het soms het enige wat wordt gelezen. Toch zou ik u willen zeggen: begin bij het begin, het is de moeite waard wat hier voor u ligt. Bijna zes jaar heb ik aan dit boekje gewerkt, waarvan vijf jaar als AiO. In die tijd is er veel gebeurd. Dit boekje sluit voor mij een heel tijdperk af, misschien dat het de inhoud voor u waardevoller maakt

Als eerste wil ik mijn promotor Dorret Boomsma bedanken. Toen ik zes jaar geleden bij haar solliciteerde kreeg ik de baan niet, maar 's avonds belde ze me toch op om te vragen of ik niet op een ander project bij haar wilde komen werken. Dorret, ik wil je vanaf deze plek heel erg bedanken voor het vertrouwen dat je altijd in mij hebt gesteld. Bovendien ben ik er erg trots op dat ik ben gepromoveerd met jou als promotor.

Zo ging ik dus longitudinaal vragenlijstonderzoek doen bij het Nederlands Tweelingen Register, maar ik wilde meer. Ik wilde ook fysiologisch onderzoek doen. Eco de Geus bood me de mogelijkheid om cardiovasculaire metingen te gaan doen en hij werd mijn co-promotor in het ambulante onderzoek naar de relatie tussen depressie en het risico op hart- en vaatziekten. Bedankt Eco voor je input. Ook bedank ik Paul Groot en de technische dienst voor hun onmisbare hulp. Paul heeft een groot aantal keer de software voor mij aangepast. De data hadden niet verzameld kunnen worden zonder de inzet van Hans Elich. Hem wil ik heel hartelijk bedanken voor de inzet en de gezelligheid.

Beste Harriëtte en Gonneke, met jullie doortastendheid en kordaatheid sleurden jullie me door de laatste 9 maanden heen. Alle data waren verzameld, maar er stond nog niets op papier. Zonder jullie had ik het niet gered! Ik heb in 9 maanden dit proefschrift geschreven, maar jullie bijdrage daarbij is voor mij volstrekt onmisbaar geweest.

Het onderzoek werd deel van een groot internationaal project. Er moest DNA-materiaal en cortisol verzameld worden en psychiatrische interviews worden afgenomen. Dit heb ik natuurlijk niet allemaal alleen gedaan. Zonder de hulp van Ilja, Marlies, Susanne, Christine, Anna, Saskia en Lex, had ik nooit al het werk kunnen doen. Ook de andere stagaires hebben zich altijd enorm ingezet voor dit bijzondere project: Suzanne, Sandra, Judith, Silvia, Thessa, Berniek en Juanita van harte bedankt en veel goeds toegewenst

De verwerking van al het DNA-materiaal is gebeurd bij de afdeling van prof. Eline Slagboom in Leiden. De cortisolbepalingen werden gedaan in het laboratorium van prof. Kirschbaum. Voor het aanschrijven van de deelnemers, het verwerken van alle post en de hulp bij het persklaar maken van dit proefschrift wil ik het secretariaat van de afdeling Biologische Psychologie zeer hartelijk bedanken.

Mijn bijzondere dank gaat uit naar alle families die hebben meegewerkt aan dit onderzoek. Ik kwam op de gekste tijden (om zes uur 's ochtends was geen uitzondering). Er was altijd koffie, iedereen was altijd goedgehumeurd, geïnteresseerd en bijna zonder uitzondering deed iedereen graag mee. Uw deelname is van het grootste belang geweest en daar heb ik bijzonder veel respect voor.

Lieve Fred, ik kan me voorstellen dat het voor jou niet altijd makkelijk is geweest. We kregen in de afgelopen jaren soms zoveel voor onze kiezen dat mijn proefschrift maar een bijzaak leek. Ik moest soms door met mijn proefschrift op momenten waarop ik liever had willen stoppen. Dat is niet altijd even eerlijk tegenover jou geweest. Ik hoop dat wij samen later hele ouwe gerimpelde mensjes worden die met veel voldoening op deze periode terugkijken.

Tenslotte wil ik natuurlijk iedereen bedanken: mijn kamergenoten Elisa en Jacqueline, die ook mijn paranimf is, voor de gezelligheid, mijn collega's op de VU, de AMS-leesgroep, Ewald en Monica, paranimf Zamarra, Val en Koos (thank you so much for everything en merci beaucoup), mijn nichtje Samantha en mijn zus Nathalie, in het bijzonder mijn vader voor het feit dat hij altijd op mij vertrouwt, en verder iedereen die ik vergeten ben.