Prenatal stress

and its effect on infant development

This study was supported by a grant from the Van der Gaag Stichting, the Praeventie fonds (Zorg Onderzoek Nederland), Hersenstichting Nederland and the J.E. Jurriaanse Stichting

Coverdesign by Annemieke and Mark van Westervoort
Realization by Studio van Westervoort, Koog aan de Zaan

ISBN: 90-393-2497-2

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(Met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de Rector Magnificus, Prof. Dr. H.O. Voorma,
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
op dinsdag 10 oktober 2000 des namiddags te 12:45
door

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geboren op 11 april 1969, te Wormerveer

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Voor mijn ouders
Aan Marjolein

Contents

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From postnatal to prenatal determinants of development: a shift of a paradigm

Anja C. Huizink & Jan K. Buitelaar

1.1 Abstract

Traditionally, the study of child development has taken birth as its starting point. However, current insight suggests that prenatal influences explain a significant part of variation in later behavior and development. Small variations in the fetal physiological environment induced by internal or external factors can be of critical and long lasting importance, given an increased sensitivity of the rapidly developing brain. This calls for the need of a shift in a paradigm in infant studies from postnatal to prenatal determinants of development.

1.2 Introduction

In 13-16 % of all young children psychosocial adaptation is hampered by mild or severe neurodevelopmental disorders, that range from hyperactivity, learning disabilities, language delays and motor abnormalities to autistic spectrum disorders and cerebral palsy (Kirby & Brewster, 1995; Robert et al., 1998; Thompson et al., 1996). Possible harmful effects on the brain during delivery explain only a proportion of these disorders in children's behavior and development (Goodman & Stevenson, 1989; Gillberg et al., 1983; Uljas et al., 1999; O'Callaghan et al., 1997; Taylor et al., 2000). Moreover, improved obstetric care during the last decades has not been able to reduce the rate of these neurodevelopmental disorders (Casaer, 1993; Hjalmorson et al., 1988; Visser & Narayan, 1996). Animal studies have shown that the prenatal period should not be neglected when one is interested in early determinants of development (Weinstock, 1997). As a result, there is now an increasing recognition of the role played by prenatal factors in the development of subsequent neuropsychiatric impairment, particularly in term born infants.

Interest in prenatal risk factors can be found in various fields of research. First, prenatal influences may affect the general development of the fetus. Food deprivation (Creasy, 1991), alcohol-intake (Faden et al., 1997), smoking (Cnattingius et al., 1999; Eyler et al., 1998) and drugs during pregnancy (Tuthill et al., 1999) may result in adverse birth outcome, such as preterm birth and low birth weight. Internal factors, like elevated prenatal stress, are likewise associated with premature delivery, and low birth weight. (Dunkel-Schetter, 1998; Copper et al., 1996; Wadhwa et al., 1993).

Second, effects of prenatal influences on physical development have been found, which may result in specific illnesses. For instance, food deprivation during pregnancy has been found to have an effect on health status at adult age (Barker, 1995; Ravelli et al, 1998). The Barker hypothesis states that children with low birth weight, possibly as a result of prenatal food deprivation of the pregnant woman and secondary of the fetus, have an increased risk for diabetes, obesity, high blood pressure and cardiovascular disease at adult age. After alcohol-intake in pregnancy disturbances of physical development occur, resulting in heart defects, distortions of the joints and minor physical abnormalities (Day & Richardson, 1991; Sokol & Clarren, 1989). Prenatal smoking is associated with increased susceptibility to emphysema (Maritz et al., 1993) and abnormal early pulmonary maturity (Lieberman et al., 1992). The use of pharmacological agents during pregnancy may result for instance in deformed limbs (thalidomide) or in an increased risk for abnormalities in the structure of the reproductive organs and for the development of cancers in the vagina or cervix (diethylstilbestrol, DES; in: Seifert & Hoffnung, 1987).

Third, variations in the fetal physiological environment, caused by the aforementioned external factors, appear to have effects on brain development, which may lead to neonatal brain disease or psychopathology later in life. In the field of child neurology, etiological factors for cerebral palsy are sought in the prenatal period, because perinatal complications seemed to explain only part of the occurrence of this brain dysfunction in at term born infants (Bottos et al., 1999; Casaer, 1993; Sugimoto et al. , 1995; Truwit et al., 1992).The harmful effects of prenatal exposure to tobacco, alcohol, medication or drugs on human brain development are well established (see for a review: Ferreiro & Dempsey, 1999). In short, gestational alcohol exposure may result in microcephaly and central nervous system

malformations (Sokol & Clarren, 1989; Day & Richardson, 1991), disturb neuronal migration (Day & Richardson, 1991) and reduce neuronal numbers (Kumari & Ticku, 1998; Pantazis et al., 1998). According to these studies, alcohol exerts its effects on a variety of genes and can modify the composition of the postsynaptic membrane (Ferreiro & Dempsey, 1999). Likewise, fetal nicotine exposure results in alterations of cholinergic, noradrenergic and dopaminergic projections in postnatal life and appears to elicit a premature switch from proliferation to differentiation. In addition, fetal cell damage and cell loss is found (Slotkin, 1998). Besides the direct effect of alcohol, nicotine and cocaine on fetal brain development, the prenatal exposure to these substances may further interfere with normal brain processes. This may result in later cognitive dysfunction (Fried et al., 1998; Richardson et al., 1995; Frydman, 1996; Naeye & Peters, 1984), behavioral deficits (Weissman et al., 1999; Eckhardt et al., 1998; Fergusson et al., 1998; Williams et al., 1998; Olson et al., 1997; Sampson et al., 1997; Wakschlag, et al. , 1997; Orlebeke et al., 1997; Milberger et al., 1996) and even mental retardation (Drews et al., 1996). Exposure to high levels of radiation in pregnancy has also been related to mental retardation (Otake & Schull, 1984). Other prenatal factors linked with later compromised development, in particular schizophrenia and major affective disorders, included malnutrition (Hoek et al., 1998; Susser et al., 1998; Geddes, 1999) and influenza (Machon et al., 1997).

The effects of these external prenatal factors generally result in explicit physical pathology, growth retardation or psychopathology. Internal prenatal factors such as maternal stress may lead to a more subtle disruption in normal development besides the obvious effects on general fetal development that resulted in preterm birth and lower birth weight. In humans, prenatal stress has been associated with a smaller head circumference of the neonates (Lou et al., 1994). Severe maternal stress in pregnancy has been linked with an increased risk for schizophrenia at adult age for the infants born of these mothers (Van Os & Selten, 1998). In non-human primates, it was found that prenatal stress had long-term effects on behavioral regulation in the offspring that persist into adolescence (Clarke & Schneider, 1997). Offspring of prenatally stressed rats had stronger and prolonged responses of the Hypothalamic-Pituitary-Adrenal (HPA) axis in stressful situations (Weinstock, 1997). The effects of prenatal stress on animal offspring will be discussed in detail elsewhere in this thesis.

The early effects of stress hormones on the developing brain may account for a part of variation in human brain development. So far, the effects of psychosocial factors, and the direct or mediating effects of stress hormones and gonadal hormones on fetal brain development have received little attention from the field of infant studies until now.

Therefore, this chapter aims to affirm that it is worthwhile to study the influence of prenatal determinants, and in particular of prenatal stress, on infant development. The concepts of early programming and prenatal maternal stress are briefly introduced. Fetal brain development and possible sensitive periods in fetal brain developement are described. The role of prenatal hormones as mediators for the effect on human development is elaborated. Moreover, the importance of longitudinal, prospectively designed studies is endorsed.

1.2.1 Early programming effects on the brain

The study of early 'programming' effects on the brain has the potential of gaining more insight in normal and abnormal neurodevelopmental processes. The term 'programming' has been used to describe the process whereby a stimulus or input during a sensitive period of development has permanent effects on the structure, physiology and metabolism of the body, including the brain (Barker, 1995). From various fields of research, the concept of early programming of the fetal brain and the influence on subsequent development emerges. Small variations in the fetal environment may result in developmental and behavioral problems later in life, as the aforementioned studies have shown.

1.2.2 The concept of prenatal maternal stress

Prenatal maternal stress may induce changes in the metabolic environment of the fetus and account for some of these early programming effects on brain development. A problematic issue of the concept of prenatal maternal stress in human pregnancy is the lack of consensus on the definition and operationalization of prenatal stress. Older studies frequently used single questionnaires to assess an aspect of prenatal stress, such as major life events or general anxiety, whereas more recent studies have focused on prenatal stress as a multidimensional concept. In line with these recent studies, we formulated and tested a multidimensional model of prenatal stress in this thesis. As a theoretical starting point, we used the model of Lazarus and Folkman (1984) which differentiates between stress-provoking, stress-mediating or -moderating, and stress-resulting factors.

It is difficult to compare animal findings with human findings with regard to prenatal stress effects on the offspring. Prenatal stressors in animal studies are well-defined and circumscribed and are externally inflicted upon the animal. Many forms of human stress are linked to the occurrence of life events of daily hassles and are partly attributable to the person and may be interwoven with personality and lifestyle factors. It is therefore of great importance to clearly define prenatal maternal stress in humans. In the present thesis the concept of prenatal maternal stress will be elaborated in detail in normal risk pregnant women.

1.2.3 Fetal brain development

From conception onwards the human brain develops rapidly. During early gestation, the neural tube is formed which will differentiate into diverse sections of the brain. Cells also differentiate into neurons and supportive cells, followed by a period of neuronal migration. If neuronal migration is disrupted, an abnormality in cell position results and neurons are then said to be heterotopic. Behavioral disorders that have been associated with such a disruption include some forms of schizophrenia (Korelman & Scheibel, 1983) and of dyslexia (Sherman et al., 1985). Middle gestation is known as a period during which there is neuroblast proliferation and adult numbers of neurons are virtually achieved by the time it is over (Dobbings & Sands, 1979). The later part of gestation corresponds to the brain growth spurt, a period during which brain weight and developmental processes proceed very quickly, which continues

postnatally, and to early synaptogenesis. The timing of brain development differs from one cell type to another and from region to region, however, resulting in an extremely complex developmental pattern.

1.2.4 Sensitive periods in fetal brain development

It has been suggested that sensitivity to harmful effects of various fetal environmental factors on fetal brain development is increased in specific periods of pregnancy. A sensitive period is defined as a specific time period during central nervous system development in which the effects of experience can alter neuronal connectivity (Bear, 1995). When the fetal brain is confronted during a sensitive period, small variations could therefore result in large and lasting effects on brain functioning. Exposure to a substance during a sensitive period may alter normal development and cause malformations or defects. It could be that a specific lack of a substance essential for fetal brain development or an overdose of a harmful essence during such a sensitive period, would cause a detrimental effect with lasting consequences.

To investigate if an increased vulnerability can be detected for the effects of internal prenatal factors such as elevated prenatal stress hormone levels, Schneider et al. (1999) conducted a study in nonhuman primates. The primate offspring proved to be more vulnerable to stress in early gestation as compared to stress exposure in mid-late gestation. However, no specific sensitive period for prenatal stress was found, because the effects of prenatal stress were leveling off from early to mid-late gestation, without a clear demarcation. Although these findings do suggest that early pregnancy could be a sensitive period for brain development, only limited empirical proof is available as yet. This could be attributed to methodological problems in many studies, and therefore further research on this topic is warranted. In the present thesis, we will explore the possible sensitive periods in human fetal brain development for prenatal maternal stress effects.

1.2.5 Importance of maternal hormones for fetal brain development

Even small variations in the fetal hormonal environment have been shown to exert programming effects on the developing brain. Especially the internal steroid hormones produced by the gonads, the thyroid, and the adrenal glands have been found to exert a great influence on the development of the fetal brain (Collaer & Hines, 1995; Sikich & Todd, 1988). Steroid hormone effects occur at the level of gene transcription, via the actions of intracellular hormone receptors. Other effects occur at the level of the membrane via receptors on the cell surface that produce rapid effects on bioelectrical activity and secondary messenger systems. These hormones are regarded as links between the gene and the environment (McEwen, 1992). Increased or decreased levels of maternal steroid hormones during pregnancy may be the mediating factors in explaining disruptions in development.

First, products of the gonads, such as androgens, estrogens and progesterone have shown to program the development of the brain and to affect social development of the infant. Higher levels of testosterone were associated with timidity in preschool boys (Marcus et al.,

1985) and increased visual-spatial performance in girls at the age of 6 years (Jacklin & Maccoby, 1988). More recent studies (Finegan et al., 1992; Grimshaw et al., 1995) found indications of an effect of gonadal hormones on the cerebral lateralization and of cognitive abilities at 4-year-old girls. Prenatal testosterone levels showed a curvilinear (inverted U-shape) relation to language comprehension and classification abilities, and a linear relation to counting, number facts and block building. Furthermore, girls with higher prenatal testosterone levels were more strongly right-handed and had stronger left-hemisphere speech representation, thus had a greater lateralization of function than girls with lower prenatal testosterone levels.

Second, thyroid hormone levels were found to be critical signals for brain development. The presence of sufficient levels of maternal thyroid hormones during the first 10-12 weeks of gestation is important because only from that period on does the fetus start to produce its own thyroid hormones. It has been shown that subtle and subclinical abnormalities of maternal thyroid status in this early period in pregnancy were associated with a compromised cognitive development at age five years (Pop et al., 1995).

Finally, the adrenal gland produces hormones, like cortisol, that are involved in stress responses. From animal studies we know that prenatally stressed rodents and monkeys showed an abnormal reactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis early and later in life. Also, prenatal exposure to prolonged increased levels of corticosteroids resulted in adverse social and exploratory behavior and motor developmental delays in offspring. Studies of Mary Schneider et al. (1992a,b,c; 1993; 1997; 1999) showed that prenatal stress (daily exposure to unpredictable noise stimuli) in nonhuman primates was associated with slower motor development, less exploration in a novel environment and more disturbed behavior of the offspring, even in the absence of clear effects on physical development.

In short, small variations in maternal hormone levels during gestation may result in developmental problems later in life. Therefore, these variations in maternal hormone levels may be mediating the effect on brain development. In this thesis the focus will be on the potential mediating effects of maternal stress hormones, in particular the HPA axis activity, on postnatal developmental delays and behavioral problems.

1.2.6 Methodological guidelines

Precise analysis of the relationship between prenatal events or elevated stress hormones and disruption of fetal brain development and later deviant development is very difficult, especially in humans. Many factors may confound assignments of causation to specific agents. Increased prenatal stress hormones is often accompanied by exposure to prenatal alcohol or nicotine. Furthermore, intrinsic genetic variation could influence the susceptibility of the mother or fetus to potential harmful hormones, which may further complicate efforts to identify factors that are harmful for the developing brain. In this field of research, retrospectively designed studies are commonly used in order to examine possible prenatal stress influences on later compromised development (Van Os & Selten, 1998; Huttunen et al., 1994; Clements, 1992; Meier, 1985; Stott, 1973; Davids, 1963). Since most of these retrospective studies depend on maternal reports, the effects cannot be attributed to specific forms and amounts of stress and confounding factors cannot be adequately controlled for. Recall bias is

a major disadvantage of such a design. Moreover, it is almost impossible to study the effects of a particular factor in a specific period during pregnancy. Prospective longitudinal studies are therefore needed to collect data throughout pregnancy at several gestational periods and from birth onwards. In the present thesis we therefore used a prospective longitudinal design.

It is important to appreciate that the design of the present studies does not allow to examine the relative contribution of genetic versus environmental versus gene by environmental interactional influences on infant development and temperament. Twin studies in humans have shown that the exposure to stressful life events and daily hassles is not independent of the genetic make-up of the individual (Kendler et al., 1993). Especially, for personal and network dependent stressful life events a significant aetiological role for genetic factors has been found (Kendler et al., 1999). Therefore, any association between stress of the pregnant mother and infant development cannot simply be interpreted as reflecting environmental influences on infant development. Rather, these assocations will be due to a complex interplay between genetic and environmental influences that can be unraveled only by using genetic sensitive designs like twin and (half-)sibling studies.

1.3 Conclusion

The concept of early programming of the development of behavior and cognition with a focus on prenatal instead of postnatal influences offers a new paradigm for researchers interested in infant and child development.

Even small variations in fetal physiological environment in specific periods of time may be mediating the effects on later development. It is still questionable if specific sensitive periods exist for the harmful effects of prenatal exposure to maternal stress hormones. Prospective longitudinal studies will offer the opportunity to investigate the effects on the developing fetal brain more precisely. Researchers in the field of infant studies, should take advantage of the knowledge already available in other fields of research and start studying children from their earliest days, instead of having birth as a starting point of their focus of attention. Since prenatal stress is common in today's and probably tomorrow's society, we strongly recommend research with a focus on the effects of psychosocial factors, such as stress, on development from the earliest stages of human life onwards.

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2

Outline and aims of this thesis

The main purpose of this thesis is to focus on the role of prenatal maternal stress in relation to infant development in a prospective longitudinal design. In this design, data was gathered on various aspects of maternal and fetal aspects. In Table 2.1 the design is presented. The aspects in italics in Table 2.1 reflect data on which this thesis reports. The gray parts on Figure 2.1 summarize the focus of this thesis. The relationships between prenatal maternal stress and fetal behavior and birth outcome (represented in Figure 2.1 by means of white arrows) will be discussed in an accompanying thesis entitled 'The effects of maternal stress on fetal development' by P.G. Robles de Medina.

Table 2.1 **Design of the large prospective study of which a part is discussed in this thesis.**

	Time period	Maternal measures	Fetal/Infant measures	General measures/ combination maternal-infant measures
Pregnancy	15 - 17 weeks	General demographic information Socio-economic status Smoking and drinking habits Stress questionnaires Cortisol in saliva during ultrasound Cortisol (saliva) day curve	Ultrasound recording of fetal behavior and fetal heart rate during 1 hour	
	24 weeks	ACTH & ß-endorphin in plasma (subsample)		
	27 - 28 weeks	Stress questionnaires Pregnancy complaints Smoking and drinking habits Cortisol in saliva during ultrasound Cortisol (saliva) day curve	Ultrasound recording of fetal behavior and fetal heart rate during 1 hour	
	32 weeks	ACTH & ß-endorphin in plasma (subsample)		
	37 - 38 weeks	Stress questionnaires Pregnancy complaints Smoking and drinking habits Cortisol sample in saliva during ultrasound Cortisol day curve	Ultrasound recording of fetal behavior and fetal heart rate during 2 hours	
Birth			Gestational age Birth weight Apgar score 1/5 minutes	General information on delivery (duration, mode of delivery etc.) Complications Medication use
Neonatal	10 ± 2 days postpartum	Stress questionnairesNeonatal perception questionnaireGeneral information on health status of the baby	Neurological status (Prechtl) of the baby Head circumference	Observation of Mother-Infant-Interaction during bathing session
Infancy	2 months		Cortisol day curve in subsample	
	3 months	Stress questionnaires Infant temperament questionnaire (ICQ) General information on health status of the infant	Bayley Scales of In-fant Development (BSID) Head circumference Vagal tone during BSID in subsample	
	4 months		Cortisol day curve in subsample	
	5 months		Cortisol day curve in subsample	
	8 months	Stress questionnaires Infant temperament questionnaire (ICQ) General information on health status of the infant	Bayley Scales of In-fant Development (BSID) Head circumference Vagal tone during BSID in subsample	Observation of Mother-Infant-Interaction during semi-structured play-session and BSID
	2 years; data collection on-going	Stress questionnaires Childrearing questionnaire Marital status questionnaire Child Behavior Checklist (CBCL) Infant temperament questionnaire General information on health status of the infant	Bayley Scales of In-fant Development (BSID) Head circumference Vagal tone during BSID in subsample	Observation of Mother-Infant-Interaction during semi-structured play-session and BSID

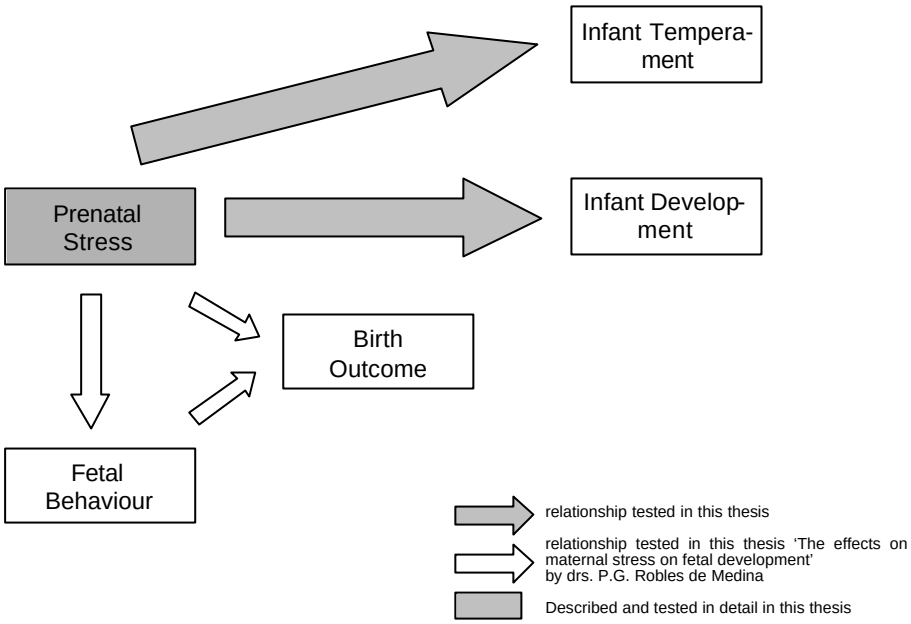

Figure 2.1. Schematical presentation of the tested relationships in this thesis presented in gray.

The present thesis addresses the following specific aims:

1. To provide a summary of the preclinical literature of the effects of prenatal stress;
2. To clarify the concept of prenatal stress;
3. To test the hypothesis that prenatal stress in humans has adverse effects on the mental and motor development and on the temperament of the infant.

The influence of prenatal maternal stress on animal offspring is systematically reviewed (**chapter 3**). First, the prenatal stress paradigm in animal experimental studies is described, after which the effects of prenatal stress on behavior of rodent offspring and nonhuman primate offspring is discussed separately. The mechanisms underlying alterations in offspring behavior and development due to prenatal stess are reviewed, with a main focus on the role of the maternal hypothalamic-pituitary-adrenal (HPA) axis. Transduction of stress from the mother to the fetus may involve transplacental transport of maternal stress hormones to the fetus, maternal stress-induced release of placental hormones that may enter the fetal circulation and maternal stress-induced effects on the blood flow to the placenta. These possibilities are elaborated. An important effect of prenatal stress is an altered HPA axis regulation in the offspring. Several mechanisms underlying this effect are discussed. In addition, the effect of prenatal stess on the brain opioid system and brain neurotransmitter systems (serotonergic, noradrenergic, dopaminergic and cholinergic system) are described. Modification by postnatal influences on the prenatal stress effects in animals are briefly summarized. Finally, the relevance of animal prenatal stress models for the human and implications for human psychopathology are discussed.

Although the evidence of harmful effects of prenatal maternal stress on the developing fetal brain and offspring development and behavior is abundant, animal findings cannot be extrapolated directly to the human situation. Besides the differences in brain development and potential transfer mechanisms of maternal stress hormones to the fetus, a major problem concerns the concept of prenatal stress used in animal studies which differs from stressors that may be encountered in human pregnancy. Therefore, it is important to conceptualize and operationalize clearly what is meant by prenatal stress in human pregnancy. Various concepts of prenatal stress have been used by reseachers who have studied the harmful effects of stress during pregnancy on birth outcome. A second aim of this thesis is therefore to analyze the concept of prenatal stress in detail in a sample of nulliparous pregnant women. For that purpose, various aspects of prenatal stress in human pregnancy were studied. First, **chapter 4** focuses on a unique element of human pregnancy: pregnancy-related anxieties. Confirmatory factor analysis was carried out on a questionnaire aimed to assess these anxieties to test the structure, internal consistency, stability and change of pregnancy-related anxieties. To test if prenatal anxiety is a syndrome that may be differentiated from general anxiety and other personality factors, multiple regression analysis was performed.

Second, coping with stress is another aspect of human pregnancy that has not received much attention thusfar. Especially, studying coping in a normal risk population of pregnant women may give more insight into coping processes that naturally occur during pregnancy. This topic will be addressed in **chapter 5**. The potential mediating role of coping on the distress response is elaborated in **chapter 6**.

Third, in **chapter 7**, multidimensional models of prenatal stress will be formulated and tested by means of structural equational modeling. These models incorporate various aspects of stress that may cause an emotional response, i.e. distress. According to the stress model of Lazarus and Folkman (1984) several factors can be differentiated: stress-provoking factors, stress-mediating or -moderating factors and a stress-resulting factor. Pregnancy-related anxieties may provoke a stress response and are therefore included as stress-provoking factors, besides more common potentially stress-provoking factors like daily hassles and life events. Stress-mediating or - moderating factors included in the present study are coping style (as described in chapter 5), social support and neuroticism. Finally, a latent construct of distress is formulated by means of several questionnaires that measure the amount of perceived stress or anxiety.

After describing the emotional aspects of pregnant women in chapter 4 through 7, resulting in a multidimensional model of distress in pregnancy, the possible harmful effects of prenatal maternal distress on the infant at the age of 3 and 8 months is analyzed in the remaining chapters to address the third aim of this thesis. From animal studies, described in chapter 3, we learned that prenatal stress may result in neuromotor and attentional problems in the offspring. Therefore, in **chapter 8** the focus is on the influence of prenatal stress on infant mental and motor development at these ages. In **chapter 9**, the temperament of the infant after exposure to prenatal maternal stress is the topic of study. In both chapters, exploratory analyses are carried out to test for the possible role of the HPA axis in mediating the effects of prenatal stress.

Finally, in **chapter 10**, a general discussion of our findings is presented and recommendations for future research are provided.

3

Prenatal stress and risk for psychopathology early or later in life: specific effects or induction of general susceptibility?

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Submitted

3.1 Abstract

This review focuses on prenatal stress as a risk factor for psychopathology early or later in life. Evidence from animal studies is summarized and the relevance of prenatal stress models in animals for human studies is discussed. In the offspring of prenatally stressed animals, overactivity and impaired negative feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis are consistent findings, and may reflect a pathophysiological mechanism involved in the development of psychopathology. Reduced activity of the opioid, GABA/benzodiazepine, 5-HT and dopamine systems and increased activity of the sympathico-adrenal system been found as well. These alterations have been linked to a diverse spectrum of psychopathology. Therefore, the evidence supports the view that exposure to prenatal stress may result in a general susceptibility to psychopathology, rather than exerting a direct effect on a specific form of psychopathology. Recommendations for future research are provided.

3.2 Introduction

In ancient times, it was already a common belief that the emotional state of a mother may affect the child she is carrying (Ferreira, 1965). Prospective studies have substantiated this belief by reporting that maternal stress or anxiety in pregnancy is associated with an adverse obstetric outcome. This is reflected in increased risk for premature delivery, or low birth weight for gestational age (Pagel et al., 1990; Hedegaard et al., 1993; Copper et al., 1996; Lou et al., 1994). Recently, it was further found that prenatal stressors of human life were associated with a significantly smaller head circumference, when corrected for birth weight (Lou et al., 1994). Prenatal stress also significantly worsened the scores on the neonatal neurological examination. This indicates that prenatal stress is able to directly affect fetal brain development in humans. Along similar lines, work in internal medicine and epidemiology has adduced growing evidence that variations in the prenatal environment can influence the physiological responses of the offspring for life. For example, undernutrition in utero changes the body's structure, physiology and metabolism, and predicts the susceptibility to hypertension, coronary heart disease and stroke in adult life (Barker, 1995). The principle that the endocrinologic and metabolic environment afforded by the mother has lasting or lifelong significance has been called fetal programming (Lucas, 1998).

The aim of this paper is to review the importance of prenatal stress as a determinant of the susceptibility to human psychopathology later in life. Such a relationship has been alluded to in papers that described maternal stress in pregnancy as a potential risk factor for schizophrenia (Huttunen et al., 1994; van Os & Selten, 1998; Selten et al., 1999). Although these studies suggest a direct effect of prenatal stress on a specific form of psychopathology, schizophrenia, the question is if there is any evidence for this hypothesis. The present paper explores if rather than having a specific effect, prenatal stress may result in a general susceptibility to psychopathology. Several issues concerning the relationship between prenatal stress and the subsequent fetal and infant development are highlighted, such as: "Which behavioral domains or physiologic systems are particularly affected by prenatal stress? Which effects of prenatal stress are short-lived, and which effects are long-lasting? Does individual sensitivity to prenatal stress play a major role? What are the physiologic mechanisms that mediate the long-term effects of prenatal stress?"

Animal models of prenatal stress are of great value in answering these questions because they allow for a much greater experimental control and afford the opportunity to standardize the exposure to stress and to isolate stress from other lifestyle factors that are interwoven with stress in the human situation. Therefore, results of animal experiments on prenatal stress will be reviewed extensively first. Then we will examine to what extent animal findings are relevant to the human situation. Finally, we will draw implications for our understanding of how the susceptibility to psychopathology in humans may evolve and focus on the issue if this susceptibility is specific or nonspecific.

3.2.1 The prenatal stress paradigm in animal experimental studies

The common element of prenatal stress studies in animals is that pregnant females are subjected to an experimentally controlled stressful situation that leads to changes in the maternal physiology and presumably to alterations in the fetal environment. Subsequently, the influence of the altered fetal environment is examined by pregnancy outcome measures, such as litter size, birth weight, presence of malformations, and by testing the behavioral and physiologic responses of the offspring under standardized conditions. However, studies vary widely according to the methodology employed and the rigor of control for confounding influences. This complicates the comparability of results across studies and may explain discrepant findings.

First, pregnant animals have been exposed to a variety of stressors, including conditioned avoidance training (Thompson, 1957), suspension (Alonso et al., 1991), crowding (Dahlof et al., 1978), rehousing with unfamiliar confederates (Schneider & Coe, 1993), social isolation, repeated electric tail shocks (Takahashi et al., 1991), noise (Clarke et al., 1994), saline injections (Peters, 1982; Cratty et al., 1995), immobilization (Ward & Weisz, 1984) or restraint (Deminière et al., 1992). These stressors are not readily comparable with each other, because some comprise the infliction of pain, some are non-painful physical stressors, and for still other stressors, like crowding, the social element is rather prominent. As a consequence, each of these stressors may give rise to partly different physiologic stress responses in the pregnant animal. Furthermore, the removal of the dam from the home cage in order to inflict stress could be the crucial variable to cause effects in itself, as there were no behavioral differences between the offspring from mothers who were only handled and from those given conditioned avoidance training (Hutchings & Gibbon, 1970). Few studies have controlled for handling and other nonspecific factors when reporting about prenatal stress (Sapolsky, 1997).

Second, the timing, frequency, and duration of the stress application are major variables (Weinstock et al.,1988). Exposure to stress at different times during pregnancy could produce different responses in the offspring (Archer & Blackman, 1971). For instance, the effects have been compared of noise and light stress applied either daily throughout pregnancy, randomly three times weekly throughout pregnancy, or daily only in late pregnancy (Fride & Weinstock, 1984). Following randomly applied stress, pups exhibited an overall delay in motor development, whereas following late daily stress a somewhat accelerated motor development was observed. Thus, the same type of prenatal stress proved to either increase or delay the rate of maturation of early motor behavior of the offspring according to the timing of its application. Daily stress applied throughout pregnancy did not differ in its effects on development from the control condition, whereas exposure to stress in only the last week of pregnancy resulted in altered development (Fride & Weinstock, 1984). Stress exposure on a regular base, for example the presentation of noise in a consecutive series of days at fixed times, may lead to rapid adaptations and may differ in its effect on the physiology of the dam from stress delivered on an unpredictable basis (Fride & Weinstock, 1984). A related issue is the influence of the intensity of the stressor. Due to the mentioned methodological variation, the findings of various studies with regard to intensity and dose-response relationship of the stressor are ambiguous. It seems self-evident that the intensity of the mother's

physiological response rather than the stimulus intensity is the decisive factor, as suggested by Archer and Blackman (1971).

Third, not all designs have included the measurement of the changes in the pregnant animal's physiology brought about by stress exposure. This would be both a methodological control on the effectiveness of stress application and a strategy to learn about the stress sensitivity and stress reactivity of pregnant animals and about dose-response relationships of various types of stressors. In any case, each of the stressors described earlier, including handling, has been shown to activate the HPA axis and to elevate the plasma levels of glucocorticoids of the pregnant animal, when the animal is exposed for the first time (Hennessy & Levine, 1978). Prolonged activation of the HPA axis of the mother, as reflected by persistently elevated levels of glucocorticoids following exposure to chronic stress, was established for the application of restraint stress (Ward & Weisz, 1984) and repeated saline injections (Peters,1986). However, exposure of rats to noise on a regular daily basis led to habituation of the endocrinologic stress response, in contrast to unpredictable noise stress (Fride & Weinstock, 1984). Noise stress or repeated social stress presented to pregnant nonhuman primates also led to an activation of the HPA axis (Mendoza et al., 1979; Schneider et al., 1993). In a similar vein, it is important to document physiological changes in the fetus during and immediately following the stress application to the mother. The two noise schedules described above (Fride & Weinstock, 1984) resulted in different patterns of fetal rat plasma corticosterone. Regular daily stress was associated with a corticosterone peak occurring one day earlier than that in controls. By contrast, unpredictable noise stress produced lower and later fetal peak levels of corticosterone (Barbazanges et al., 1996; Ward & Weisz, 1984; Douglas, 1975).

Fourth, the species that is studied is of importance. Most prenatal stress work has been done in rodents, but a couple of interesting studies have been performed in rhesus monkeys. One should appreciate that there are clear differences between rodents, nonhuman primates and humans in the timing of birth relative to the degree of maturation of the brain and the body at birth (Dobbing & Sands, 1979). At birth, the rat's brain is only about 12 percent its adult weight. However, the rat's postnatal development is extremely rapid and it attains its adult weight within 40 days. Brain development in the rat at postnatal days 12-14 is comparable to that in human babies near term. In contrast, the rhesus monkey's brain growth is nearly complete at birth. The growth of the human brain has a slower speed than that of rat and monkey and is rather prolonged until childhood for most parts and even until young adulthood for some structures of the brain. As a consequence, early programming effects may be expected to occur foremost during the perinatal and early postnatal period in rats, primarily prenatally in rhesus monkeys and over a much more prolonged period encompassing both gestation and the first years of life in humans (Sikich & Todd, 1988). Nonhuman primate models of prenatal stress are rather suitable for extrapolations to the human situation because of long gestations that make it more easy to contrast stress exposure during different periods of gestation, single births, and slower postnatal growth that allows more refined evaluation of neuromotor development than in rapidly developing rodents (Schneider et al., 1999).

Fifth, a number of other methodological issues matter. It is necessary to control for maternal weight loss during pregnancy following stress exposure, particularly when immobilization or restraint stress is used, since weight loss by itself has been shown to adversely

affect fetal development (Guo et al. 1993; Ward & Wainwright, 1988). Some of the stress procedures could also directly and physically affect the fetus, independent of eventually mediating changes of the fetal environment (Hultman et al.,1997; Giberson & Weinberg, 1995). Last but not least, the results of prenatal stress studies may also depend on the choice of the control conditions employed. In some studies comparison was made between a stressed and a nonstressed group, whereas in others it was between two groups that have been stressed under different regimes (Archer & Blackman, 1971). Since it is likely that a prenatally stressed mother will present alterations of postnatal maternal behavior compared to nonstressed mothers, another methodological requirement is to differentiate prenatal effects on the offspring from postnatal effects. This is usually accomplished by using a cross-fostering design, in which the offspring of prenatally stressed mothers are raised by either stressed or nonstressed foster dams (e.g., Maccari et al., 1995). In addition, to not confound treatment with litter effects, it is essential to use the whole litter as the unit of analysis (as opposed to the split litter method), and to retain both female and male pups in the litter. It is known that dams differentially attend to female and male pups with respect to anogenital licking and this differential attention contributes to adverse sexually dimorphic behaviors (Moore, 1984). However, these requirements have often been violated, when researchers used split or single sex litters.

3.2.2 Effects of prenatal stress on behavior of rodent offspring

Aspects of development that have been studied as a function of prenatal stress particularly include early physical and motor development, exploration in a novel environment, disturbance behavior under stressful conditions, learning abilities, and social and sexual behaviors. Many studies report lower birth weights of the pups following prenatal stress (Weinstock et al., 1988), though some studies found no effect on birth weight (e.g. Rojo et al., 1985) or even an increase in birth weight (Dahlof et al., 1978). Early motor development appears to be sensitive to maternal stress as well. The offspring of vehicle-injected damns showed decreased motor abilities, as reflected by the quality of the righting reflex and of climbing at the age of five days, when compared to the offspring of untreated damns (Grimm & Frieder, 1987). Similar findings of a delay in early motor development resulting from prenatal stress have been described by others (Fride & Weinstock, 1984; Barlow et al., 1978).

Most early studies found that prenatal stress affected the behavior of rodent offspring in a novel situation, with prenatally stressed offspring showing a decreased exploration and more defecation in an open field (Archer & Blackman, 1971). Later studies replicated the finding that in a novel environment, such as an open field or a plus-maze, prenatally stressed animals show less exploratory behavior and display signs of heightened emotionality or fear and anxiety, as is reflected by a decrease in locomotor activity and increased defecation (Weinstock et al.,1992; Wakshlak & Weinstock, 1990; Grimm & Frieder, 1987). Other studies, however, revealed a reverse trend, with shorter latencies to explore in the prenatally stressed rodents and more active behavior in a novel situation (Deminière et al., 1992), or found no influence of prenatal stress on these behavioral measures (Chapman & Stern, 1979; Moore & Power, 1986). Genetic factors might contribute to these differing results, since it was found

that prenatal stress caused different offspring activity levels depending on the characteristics of their breed (Thompson & Olian, 1961; Weir & DeFries, 1964). For instance, two inbred strains of mice, which showed either high or low activity levels, were used to study the influence of genetic factors on the offspring response to prenatal stress. The male offspring of a low-activity strain of prenatally stressed mothers were more active than control males, whereas prenatally stressed male offspring of a high activity strain were less active. Female offspring of both strains were less active (Stohr et al., 1998). Thus, both sex effects and genetic effects seem relevant to explain different results following the exposure to prenatal stress. Environmental variables also proved to be relevant, since exploratory activity in reaction to novelty was significantly less in a bright light but not in dim light conditions (Poltyrev et al., 1996). In fact, in the latter condition an increase in locomotor response to novelty was observed (Deminière et al., 1992).

Prenatal stress further affects the adaptation to postnatal stressful conditions. During the preweaning period, 14-day-old prenatally stressed rat pups emit fewer ultrasonic vocalizations than control pups when placed in social isolation (Takahashi et al., 1990). Reduced vocalization is an index of behavioral inhibition that generally occurs in response to threatening situations (Takahashi, 1994). Shock-induced freezing, when tested between postnatal days 70 and 90, was significantly longer in prenatally stressed rats than in control rats (Takahashi et al., 1992). This suggests that the early predisposition toward heightened behavioral defensiveness, which was experimentally induced in rats by prenatal stress, is present at young age and remains unchanged into early adulthood. In general, behavioral differences between prenatally stressed animals and controls are magnified in aversive conditions, such as forced swimming (Alonso et al., 1991), electric foot shocks, or air puffs (Fride et al., 1985, 1986).

Studies on learning abilities of the offspring of stressed damns have revealed impairments on a number of tasks. Early work has adduced evidence for impairments of discrimination learning (Archer & Blackman, 1971; Grimm & Frieder, 1987), reversal of a learning set on a T-maze and acquisition of an operant response (Smith, 1981) in the offspring of prenatally stressed rats. The results of two recent studies, however, are conflicting. Using crowding combined with one daily painful experiences as stressors in Wistar rats, learning acquisition in a water-maze at day 30 did not differ between the prenatal stress and control conditions (Hayashi et al., 1998). In the reversal task, however, prenatally stressed rats spent more time than control animals searching for the platform. After the application of restraint stress in Sprague Dawley rats in the last week of pregnancy, the cognitive performance of the adult offspring (age 120 days) was tested in the water-maze and using a two-trial memory test in a Y-maze with progressive inter trial intervals (Vallee et al., 1997). Though in this design the animals showed problems in coping with novelty, expressed as an increased escape behavior, spatial learning or memory performance proved not to be affected by prenatal stress. These findings suggest that learning impairments due to prenatal stress may be present at young rather than at older age in rats, although the difference in strain and also other design variance of the studies may explain the discrepancies.

The study of social behavior of the offspring of prenatally stressed dams has been a relatively disregarded topic. In an experiment by Takahashi et al. (1992), stress procedures began on day two of pregnancy and lasted throughout pregnancy (day 20) and consisted of an uncontrollable electric shock every other day for ten sessions. On postnatal days 25 and 26,

the social interactions of sibling pairs, consisting of an experimental and a control juvenile male rat, were observed. When the behavioral responses that are indicative of social play were measured by means of the latency to pounce on the opponent, prenatally stressed rats exhibited significantly longer latencies to initiate social play than control rats.

The effect of prenatal stress on altered sexual behavior in the offspring has received a lot of attention by researchers. Restraint stress in the third week of pregnancy was associated with a significant reduction in the testes weight and anogenital distance at birth (Dahlof et al., 1978). It has been shown that maternal stress in the third week of pregnancy, in both mice and rats, demasculinized and feminized the sexual behavior of male offspring (Holson et al., 1995). Prenatal stress was associated with disruptions in the normal course of sexual differentiation and subsequent alterations in reproductive behavior (Rhees & Fleming, 1981), such as impaired ejaculatory behavior and increased female lordotic behavior in male offspring of stressed pregnant rats (Ward, 1972; Ward & Reed, 1985). These behavioral alterations have collectively been named the 'prenatal stress syndrome' (Ward, 1984). The etiology of this syndrome could stem from the same hormonal mechanism underlying sexual behavior differentiation in both normal males and females (see below).

The effects of prenatal stress in rodent offspring behavior are summarized in Table 3.1.

Table 3.1
Prenatal stress effects in rodent offspring behavior

		References
Behavioral effects	Less activity, more defecation, more emotionality in novel situation	Thompson, 1957; Wakshlak & Weinstock, 1990; Fride & Weinstock, 1988; Fride et al., 1986; Grimm & Frieder, 1987; Takahashi et al., 1988, 1990, 1992; Weinstock et al., 1992
	More shock-induced defensive freezing	Takahashi et al., 1992
	Fewer ultrasonic vocalizations when placed in social isolation	Takahashi et al., 1990
	Changes in sexual dimorphic behaviors (play, maternal behaviors)	Ward, 1991
	Impaired sexual function	Holson et al., 1985; Rhees & Fleming, 1981; Ward & Reed, 1985; Ward, 1972
	Reduced propensity for social interaction	Takahashi et al., 1992
	Increased locomotor response to novelty	Deminière et al., 1992
Learning ability	Impairment of maze learning	Archer & Blackman, 1971; Grimm & Frieder, 1987
	Reversal of learning set	Smith et al., 1981; Hayashi et al., 1998
	No effect	Vallee et al., 1997
Motor development	Decreased quality of righting reflex and of climbing	Barlow et al., 1978; Fride & Weinstock, 1984; Grimm & Frieder, 1987

3.2.3 Prenatal stress and behavior of nonhuman primate offspring

Schneider and colleagues have looked at prenatal stress effects in nonhuman primate offspring. In an initial study, rhesus macaques were mildly stressed five times per week during pregnancy from day 90 to day 145 by loud noise. The neuromotor responses of the prenatally stressed infants were assessed at two weeks after birth, employing a modified procedure of the human neonatal assessment protocol used in the clinical setting, the Brazelton Neonatal Assessment Scale. The stressed infants had lower birth weights and compromised physical growth, and exhibited retarded motor development, shorter attention spans, and delays in the development of Piagetian object permanence, when compared to infants born to nonstressed mothers (Schneider, 1992b). When the prenatally stressed subjects were tested at six months of age in a novel environment, they showed significantly more disturbance behaviors and lower amounts of exploratory behaviors compared to controls (Schneider, 1992a). In addition, half of the prenatally stressed infants showed an abnormal response to novelty in the form of falling asleep, while none of the control infants displayed this behavior (Schneider, 1992a).

In a somewhat similar design, the effects of prenatal stress on the offspring of pigtail macaques were examined (Worlein & Sackett, 1995). The applied stressor was restraint in the period from 30 to 130 days of gestation. Infants of prenatally stressed mothers appeared to spend almost twice as much time as nonstressed infants exhibiting fearful behavior in a novel environment. Furthermore, during the first eight months postpartum the stressed infants appeared to be less socially adept, to initiate fewer social interactions and to withdraw from social interactions more often. The adverse influence on motor development as observed by Schneider et al. (1992a) in 6-months-old infants was not confirmed. An explanation for these discrepant findings may be that the prenatally stressed population of Schneider et al. 's study had lower birth weights compared to their nonstressed sample, whereas both the stressed and nonstressed groups of Worlein and Sackett's study (1995) had similar birth weights. Since birth weight in itself was positively correlated with motor maturation ($r=.58$, $p<0.01$; Schneider et al., 1992b), the influence on motor development may be due to lower birth weight rather than to a direct effect of prenatal stress. It is a common finding also in human infants that low-birth weight is associated with a higher incidence of developmental deficits (e.g. Dewey et al., 1999; Pharoah et al., 1994).

Another interpretation problem of both studies includes stress-related changes in the pregnant animal's food and water intake and weight gain. Restraint stress has been found to result in reductions in maternal food and water intake, and the concomitant decrease in maternal nutritional status might account for some effects of prenatal stress on offspring behavior. It has been demonstrated that the offspring of prenatally stressed mice and of pair-fed mice that were fed according to the intake of the stressed mice but did not receive stress themselves, did not differ from each other on a number of brain and behavior developmental factors, whereas both differed from the offspring of controls (Ward & Wainwright, 1988). In a similar vein, Schneider et al. (1992b) noted that the stressed females gained less weight during their pregnancy than nonstressed females. This weight difference could confound possible effects of maternal stress on the infant outcome.

In another study, Schneider and Coe (1993) tried to replicate and extend the earlier findings in three ways: squirrel monkeys were used as another primate species, repeated social stress was applied instead of noise stress, and chronic stress was contrasted with only mid gestational stress and a control condition. The social stress procedure involved changes in housing conditions after which the pregnant animals were exposed to unfamiliar confederates. There were no differences in birth weight between the infants from the three experimental conditions. Assessment of neuromotor functions at two weeks postpartum revealed that infants born following chronic stress were significantly behind in motor maturity and activity than the controls, with the scores of the mid gestation stressed infants falling in-between. Furthermore, infants from pregnancies under chronic stress maintained shorter attention spans, and had shorter durations of orienting episodes, a shorter post rotary nystagmus and less well developed balance control compared to controls. Thus, the adverse influence of prenatal stress on neuromotor development could be replicated, even in the absence of an overt effect on physical growth. When the infant monkeys were examined at the average age of 18.5 months using a separation stress design, prenatally stressed infant monkeys did not differ from controls on environmental exploration or on nonsocial behavior, such as locomotion. They turned out to be different, however, in their social repertoire in that they showed more mutual clinging both in baseline and stress conditions (Clarke & Schneider, 1993). Mutual clinging can be considered as abnormal behavior, since it is rarely observed in normally reared animals.

The aim of a next study was to clarify the period of greatest vulnerability to prenatal stress in rhesus monkeys by contrasting the effects of unpredictable noise stress in early gestation to that in mid-late gestation and to a nonstress condition (Schneider et al., 1999). Early gestation stress was associated with significantly lower birth weights than the mid-late gestation stress and the control condition. Further, infants born following either one of the stress conditions were clearly behind compared to the controls on measures of motor development and attention, but early gestation stress was associated with more pronounced and pervasive motor impairments than mid-late gestation stress (Schneider et al., 1999). Early rather than mid-late gestation stress was also associated with a significant decrease of activity levels. The conclusion is that susceptibility to prenatal stress in nonhuman primates peaks during early gestation and tapers off during mid-late gestation.

The effects of prenatal stress in nonhuman primate offspring are summarized in Table 3.2.

Table 3.2
Prenatal stress effects in nonhuman primate offspring behavior

		References
Behavioral effects	Shorter attention span	Schneider, 1992bSchneider & Coe, 1993
	In novelty: More disturbance behavior	Schneider, 1992a; Worlein & Sackett, 1995
	Less exploration	Schneider, 1992a
	Abnormal response: falling asleep	Schneider, 1992a
	Social behavior: Fewer social interactions and withdrawal from social interactions	Worlein & Sackett, 1995
	More mutual clinging in baseline and stress conditions	Clarke & Schneider, 1993
Development	Retarded motor development	Schneider, 1992b; Schneider et al., 1999; Schneider & Coe, 1993
	Delay in development of Piagetian object permance	Schneider, 1992b

3.2.4 Prenatal stress and offspring behavior: Summary of animal experimental results

Before proceeding, it is useful to summarize the effects of prenatal stress on behavior and learning abilities of offspring. Prenatal stress causes a delay in motor development in both rodents and nonhuman primates. In rodents, prenatal stress is further associated with decreased exploratory behavior, increased emotionality, and impaired adaptation to conditions of conflict or aversion. Exploratory behavior in response to stress or novelty does not seem to be affected in nonhuman primates, although the subjects show more fearful behavior in these contexts. Furthermore, sexual behavior in rats and social behavior in both rodents and nonhuman primates are altered after exposure to prenatal stress. Finally, learning deficits can be found in prenatally stressed rat offspring.

We confined ourselves to reviewing the reported effects of prenatal maternal stress on offspring behavior in rodents and nonhuman primates, two groups of placental mammals that have been studied most extensively. Therefore, the above mentioned summary statements seem acceptable. However, as no attempt was made to critically evaluate the relevant literature, the possibility exists that observed effects in some studies were the result of some variable other than gestational stress alone, due to methodological and procedural shortcomings.

It appeared from the presented evidence that the occurrence of some sort of behavioral effect is not limited to a particular type of maternal stressor and that the same stressor may produce different effects in different species and even in different strains of the same species. These generalized conclusions have important implications for our understanding of the origin of human psychopathology.

3.2.5 Mechanisms underlying alterations in offspring behavior and development

Numerous studies have attempted to uncover the mechanism by which prenatal maternal stress affects offspring. Early attempts involved the possible role of the sympathico-adrenergic system. Maternal administration of catecholamines (e.g., adrenalin), which to some extent mimic the effects of stress, has indeed been found to induce behavioral effects in the offspring, particularly on open-field behavior, comparable to those seen after some forms of prenatal stress. However, this kind of experiment has as yet not provided conclusive proof that it is the maternal catecholamine secretion resulting from stress that is responsible for the alterations in offspring behavior (Thompson & Quinty, 1964; Lederman et al., 1981).
In contrast, during the past two decades a large body of evidence has emerged relating stress-induced disturbances in the maternal HPA axis activity to impaired offspring development. Although the regulation of the stress system, including the HPA axis, under normal and stress conditions is well understood in adult animals and man (see for reviews Chrousos, 1998 and Vázquez, 1998), little is known about the route and mechanism(s) by which maternal stress affects intrauterine development. It will become clear in the next section that no single factor or hormone is to be implicated conclusively as the sole causative agent. After description of the general stress response of the HPA axis in adults, presumed mechanisms of transduction of maternal 'stress' to the fetus will be discussed. Then we will provide evidence that excess of hormones involved in HPA regulation may exert deleterious effects on the offspring's behavior and development. Finally, we will review the effects of prenatal stress on the offspring's brain, including HPA axis regulation and the possible roles of glucocorticoid receptors, opioid receptors, and brain neurotransmitters.

3.2.6 Regulation of the stress system under normal and stress conditions

The HPA axis is considered a peripheral limb of the stress system and consists of three components: the hypothalamus, specifically the paraventricular nucleus (PVN), the anterior pituitary and the adrenal cortex. Within these parts, corticotropin releasing hormone (CRH),

vasopressin (AVP), adrenocorticotropin hormone (ACTH) and ß-endorphin (ß-E) and glucocorticoid hormones (cortisol in humans and nonhuman primates; corticosterone in rodents) are secreted. CRH and AVP are produced in the PVN in response to different stressors. Their role consists of stimulation of receptor systems in the pituitary for ACTH production and secretion. ACTH is mainly produced in the anterior pituitary and stimulates glucocorticoid synthesis and secretion in the adrenal cortex. CRH further influences cells in the arcuate nucleus of the hypothalamus that contain pro-opiomelanocortin (POMC), a large precursor molecule from which ACTH and ß-E are processed. Opioid peptides, such as ACTH and ß-E, contribute to the feedback regulation of CRH release in response to stress, among others by a direct action on CRH terminals in the PVN. Another level of feedback regulation of the HPA axis consists of glucocorticoid receptors. Two types of glucocorticoid receptors have been described, type I and type II receptors. Type I receptors, which are mainly confined to the septohippocampal system, are essentially fully occupied at normal physiological concentrations of cortisol (about 10 ng/ml). Type II receptors are more diffusely distributed throughout the brain and are partially occupied at low circulating cortisol concentrations, but are essentially fully occupied at circulating cortisol concentrations typical of stressed individuals (50-60 ng/ml) (Uno et al.,1994; Weinstock, 1997). Glucocorticoids exert a negative feedback effect on both CRH and ACTH production and secretion at the levels of the hypothalamus and the pituitary, respectively. Glucocorticoids, which are the final effectors of the HPA axis, take part of a complex signaling system between the external environment, the brain and the periphery (McEwen et al., 1997).

The central components of the stress system include the PVN-CRH and the locus coeruleus/noradrenergic-sympathetic (LC/NE) systems. The stress system interacts with other brain systems, a.o. the mesocorticolimbic dopaminergic system, the amygdala, the hippocampus, and the arcuate nucleus POMC neuronal systems. All are activated during stress and, in turn, influence the activity of the stress system. The hippocampus exerts a mostly inhibitory influence on the PVN-CRH and LC/NE systems and the amygdala. The latter can directly stimulate the central components, as well as influence the activity of the dopaminergic system, probably in a lateralized fashion (Chrousos,1998).

Prenatal stressors, including handling, have been shown to activate the maternal HPA axis and to elevate the plasma levels of glucocorticoids in the pregnant animal. The final result is dysregulation of the HPA axis with chronically elevated levels of circulating glucocorticoids and altered feedback regulation. There are several possibilities to explain this: (1) down regulation of receptors in the hippocampus, the hypothalamus, the pituitary or adrenal glands; (2) decreased sensitivity of the receptors at any of these levels; (3) alteration in CRH levels, or altered levels or affinity of plasma binding proteins (Clarke, Wittwer, Abbott, & Schneider, 1994).

3.2.7 Mechanisms of transduction of stress from mother to fetus

The mechanisms presumably involved in transducing stress from the pregnant mother to the fetus are only partly understood. We consider three possibilities which may act in concert: (1) transplacental transport of maternal stress hormones to the fetus; (2) maternal stress-

induced release of placental hormones that in turn enter the fetal circulation; and (3) maternal stress-induced effects on the blood flow to the placenta.

3.2.7.1 Transplacental transport of maternal stress hormones

Abnormal offspring development may be due to in utero exposure to high levels of maternal glucocorticoids. The main argument is that maternal stress is associated with increased secretion of glucocorticoids and that corticosterone, the main glucocorticoid in rodents, easily crosses the placental and blood-brain barriers (Arishima et al., 1977; Zarrow, 1970). Direct evidence for this mechanism is provided in a study in which offspring of prenatally stressed mothers were compared to offspring of prenatally stressed mothers with blocked corticosterone secretion, the latter achieved by removing the adrenal gland and corticosterone substitution (Barbazanges et al., 1996). The offspring of the intact animals showed prolonged stress-induced secretion of corticosterone and a decrease in hippocampal type I corticosteroid receptors, whereas these responses were abolished in the offspring of the mothers with blocked corticosterone secretion. When the adrenalectomized mothers with corticosterone substitution were in addition given an injection of corticosterone to mimic stress levels of glucocorticoids, the usual effects of prenatal stress could be reinstated (Barbazanges et al., 1996). However, adrenalectomy not only affects circulating levels of glucocorticoids, but also influences the levels of other hormones, including ACTH, ß-E, and catecholamines. Alterations of these hormones may play a role as well in producing long-term changes in behavior and neuroregulation in the offspring, in addition to an excess of glucocorticoids.

In contrast to the situation in rodents, where corticosterone easily crosses the placenta, human and nonhuman primate fetuses are relatively protected from the 2-10 times higher maternal levels of cortisol by the placental enzyme 11ß-hydroxysteroid dehydrogenase (11ß-HSD). This enzyme, which exists in at least two isoforms, converts cortisol into the bio-inactive cortisone. Synthetic glucocorticoids (betamethasone, dexamethasone) escape from placental inactivation and readily enter the fetal circulation. In a study in dually perfused freshly isolated intact human placentas a considerable variation between individual placentas was observed in 11ß-HSD activity with a minimum of around 50% and up to 80-90% conversion of cortisol to cortisone (Benediktsson, 1997). Placental 11ß-HSD activity was further positively correlated with birth weight (Stewart, 1995). However, in a study that measured plasma cortisol levels in paired maternal and fetal venous samples at 13-35 weeks' gestation, fetal concentrations of cortisol were found to be linearly related ($r = .63$) to maternal cortisol levels (Gitau et al., 1998). Thus, in spite of the importance of variation in the placental 11ß-HSD barrier to maternal cortisol, maternal cortisol did account for about 40% of the variance in fetal concentrations. Further, a contribution of 10-20% from the mother could still double fetal concentrations, given that fetal concentrations are much lower.

3.2.7.2 Production of stress hormones by the placenta

Maternal 'stress' may also be transduced to the fetus by increased production of CRH and related stress hormones by placental cells under the influence of maternal stress. This mechanism has been found only in primates. During pregnancy the placenta becomes an important transient endocrine unit that is the source of ACTH, CRH and many other hormones (Petraglia et al., 1996). Placental CRH is identical to that present in the hypothalamus and shows the same immunoactivity and bioactivity, but is not subjected to the negative feedback regulation within the HPA axis (Majzoub & Karalis, 1999; Challis et al., 1995). Placental CRH is secreted into both the maternal and fetal circulations and participates, on either side of the placenta, in positive feedback loops. Paradoxically, glucocorticoids stimulate placental CRH, which, in turn, activates the maternal HPA axis, as evidenced by steeply increasing levels of cortisol and ACTH in maternal blood near the end of pregnancy (maternal positive feedback loop). Similarly, placental CRH, entering the fetal circulation via the umbilical vein, stimulates the fetal HPA axis, resulting in increased levels of fetal ACTH and cortisol. The latter enters the placental circulation through the umbilical artery and stimulates further placental CRH secretion, thereby completing the fetal positive feedback loop (Majzoub & Karalis, 1999). A possible physiologic implication of placentally derived CRH is to contribute to fetal maturation, including the early maturation and regulation of the fetal HPA axis. CRH has also been implicated in the control of fetal-placental blood flow and the timing of delivery (McLean & Smith, 1999).

Noradrenaline, acetylcholine, oxytocin and AVP are also known to increase the release of placental CRH (Petraglia et al., 1996). Enhanced activity of maternal stress systems, i.e., an increase in circulating glucocorticoids and noradrenaline, could easily lead to further stimulation of placental CRH and the positive feedback loops. Abnormalities of the placental CRH system, therefore, might be involved in the pathogenesis of preterm delivery and fetal growth retardation (McLean & Smith, 1999), and in the altered regulation of the HPA axis in offspring of prenatally stressed animals.

3.2.7.3 Changes in uteroplacental bloodflow

Maternal stress may reduce uteroplacental blood flow, since cortisol and catecholamines in particular are known to affect vessel tone. Stress-induced activation of the sympathetic nervous system in the pregnant mother could thus reduce the blood flow through the placenta, the more so as placental tissue contains a high density of adrenergic receptors. Indeed, it has been shown that high anxious women have a significant reduction of uterine blood flow in the third trimester of pregnancy as determined by means of Doppler ultrasound, when compared to low anxious women (Teixeira et al., 1999). Moreover, maternal stress is associated with increased uterine activity. The regularly occurring uterine contractions, especially when they are long-lasting, may repeatedly hamper the transplacental transport of oxygen and nutrients to the fetus and thus impair fetal development (Mulder & Visser, 1987). In turn, reduced supply of oxygen and nutrients to the fetus constitutes a significant source of stress for the fetus and may lead to increased release of placental CRH, thereby contributing to the above mentioned positive feedback loops (Challis, 1989).

3.2.8 Effects of excess maternal HPA axis hormones on offspring development

3.2.8.1 Behavioral effects

Prolonged elevated levels of glucocorticoids due to repeated stressors or maternal administration of natural or synthetic glucocorticoids have been shown to affect behavioral development of offspring. Indirect evidence for the excess of glucocorticoid hypothesis may be found by comparing the effects in the offspring of administering HPA axis hormones (CRH, ACTH and glucocorticoids) to pregnant dams with those following prenatal stress. An interpretation problem involved in using this approach is that maternal injection of vehicle alone is quite stressful in rats and able to produce a rise of circulating glucocorticoids and behavioral changes in adult offspring (Grimm & Frieder, 1987).

Activation of the entire HPA axis by administering CRH to pregnant rat females led to increased vocalizations in a novel environment and a shorter anogenital distance in male offspring (Williams et al., 1995). These findings closely resemble abnormalities observed following a combination of heat, light and restraint stressors in the third week of pregnancy (Williams et al., 1998).

The administration of ACTH during pregnancy in rodents has been found to exert inconsistent effects on offspring behavior, with some studies reporting alterations of sexual behavior (Harvey & Chevins, 1984; Rhees & Fleming, 1981) and others failing to observe any changes (Holson et al., 1995; de Cantanzaro et al., 1986). Endocrine activation of pregnant rhesus monkeys by means of a 2-week period of ACTH administration resulted in similar early impairments in motor coordination, attention and temperament as found after prenatal exposure to intermittent noise stress (Schneider et al., 1992). Since ACTH has not been found to cross itself the placental barrier (Dupouy, 1980; Milkovic, 1961), other hormones of the HPA axis (e.g., cortisol and ß-E) likely mediate the ACTH effect.

To determine the possible influence of maternal corticosterone, pregnant rats were subjected to a low dose in the last week of pregnancy (Diaz et al., 1995). The locomotor activity in the prepubertal offspring was increased in the prenatally treated animals as compared to controls. In another study by Diaz et al. (1997), corticosterone administration to pregnant rats resulted in sex-specific alterations in behavior in the offspring at adult age. Males exhibited more exploratory activity than females, and females showed more spontaneous locomotion with no change in exploratory behavior. Corticosterone administration to mice also resulted in abnormal motor behavior, including hyperactivity, impaired avoidance reaction, and decreased motor coordination (Benesova & Pavlik, 1989). Feminized sexual behavior has been observed in male offspring of rats treated with daily dexamethasone injections during the last week of pregnancy (Holson et al., 1995). Mice offspring exposed to a single dose of betamethasone or dexamethasone on gestational day 14 showed specific alterations in anxiety, memory, and socialization when compared to control animals, but no changes in sensory, motor, motivation, and learning performance (Rayburn et al., 1997). Rat pups exposed to either betamethasone or dexamethasone when in utero showed impaired walking in the first week of life, characterized by an abnormal postural tremor and deviant postural control. Complex motor reactions to vestibular stimulation (negative geotaxis and free-fall righting) were markedly retarded. These results, which were previously found in postnatally treated

pups, indicate that synthetic corticosteroids interfere with the development of cerebellar functions involved in complex motor patterns (Gramsbergen & Mulder, 1998). Rhesus monkeys prenatally treated with dexamethasone exhibited no motor or behavioral deficits at young age (Uno et al., 1994).

Activation of the maternal HPA axis under the influence of prenatal stress leads also to increased levels of ß-E that is able to cross the placenta and enters the fetal circulation. There is evidence from rodents that the impairment of early motor development, the feminization of male sexual behavior, and the signs of increased emotionality in the open field following prenatal stress are mediated, in part, by an excess of ß-endorphin. First, similar changes in the offspring can be induced by opioid administration to pregnant animals (Zagon et al., 1970; Ward & Weisz, 1984; Vathy et al., 1985; Ward et al., 1986). Second, treatment of stressed pregnant rats with naltrexone, a long-acting blocker of the mu-opioid receptors, prevents the delay in early motor development, the reduction in anogenital distance in males, and the emergence of increased anxiety in the plus-maze (Keshet & Weinstock, 1995).

3.2.8.2 Morphological effects

It has repeatedly been reported that corticosteroid treatment during neuro-ontogeny leads to abnormalities in brain development, characterized by a.o. decreased proliferation of neural and glial elements, retarded myelination, and increased cell death (see for review Matthews, 2000). The hippocampus is a brain structure that appears to be particularly vulnerable to insult during early development (Levitt et al., 1996; Meaney et al., 1989; Henry et al., 1994; O'Donnell et al., 1994). The hippocampal pyramidal neurons contain a high concentration of glucocorticoid receptors that are highly sensitive to either hypercortisolemia caused by severe stress or to exposure to exogenous glucocorticoids (Uno et al., 1994). Treatment of pregnant rhesus monkeys with dexamethasone, that binds preferentially to type II receptors, led already in low physiologic dosages and in a dose-dependent way to neurotoxic effects on hippocampal neurons (Uno et al., 1990, 1994). Severity and extent of the neurotoxicity of dexamethasone further appeared to depend on the age of the individual. Administration of dexamethasone in the early fetal stage was found to induce severe cerebral deformities, whereas administration in the late fetal stage affects the hippocampal pyramidal neurons that provide the glucocorticoid receptors (Uno et al., 1994).

The effects of excessive amounts of HPA axis hormones during pregnancy have not only been examined with regard to the behavioral and morphological development of offspring, but also in terms of their influence on the HPA axis and neurochemistry of offspring.

3.2.9 Altered activity of the HPA axis in prenatally stressed offspring

There is abundant evidence of an overactive and dysregulated HPA axis in the offspring of prenatally stressed animals. First, a number of studies have measured higher levels of circulating glucocorticoids under baseline conditions in adult rodents that were exposed to stress when in utero (Fride et al., 1986; Weinstock et al., 1992; Ader & Plaut, 1968; McCormick,

1995; Weinstock, 1998). Second, prenatally stressed rats exhibit faster (Fride et al., 1986), stronger (Fride et al., 1986; Takahashi et al., 1988; Takahashi, 1992; Peters, 1982; McCormick, 1995; Weinstock, 1995), and/or more prolonged (Henry et al., 1994; Vallee et al., 1996; Weinstock, 1995) endocrine responses than control animals in reaction to novelty stress, tail shocks or open field observation. For example, Fride et al. (1986) reported that unpredictable noise and light stress, administered weekly in rat pregnancy, changed the corticosterone release of the offspring in adulthood in response to repeated exposure to a novel environment. The release of corticosterone was significantly higher in prenatally stressed rats in response to stress than in controls. More specifically, there was a faster onset of the corticosterone response to a novel environment and delayed habituation upon repeated exposure in prenatally stressed offspring.

Female rodent offspring appear to be more sensitive to the effects of prenatal stress on the HPA axis than male offspring (McCormick, 1995; Weinstock et al., 1992). This is in line with the observation that normal baseline levels of glucocorticoids have been measured exclusively in prenatally stressed males (Henry et al., 1994; Maccari et al., 1995; Takahashi, 1992). In contrast, following maternal ACTH administration in the last week of pregnancy, both male and female adult offspring had elevated basal levels of corticosterone, but lower corticosterone responses than control rats after exposure to stress (Fameli et al., 1994). This suggests that the basal level of adrenal function is programmed at a higher set-point than normal. The continuous hyperactivity of the HPA axis could have led to its exhaustion, since the applied stress challenge did not induce an appropriate endocrine response.

The age at which the activity of the HPA axis is examined in the offspring should also be considered. Basal levels and the stress response of corticosterone were determined in 3-, 21-, and 90-day-old male rats after restraint stress in the third week of pregnancy (Henry et al., 1994). Basal levels of glucocorticoids did not differ between the control and the prenatally stressed groups at all ages. In 3- and 21-days-old prenatally stressed animals, a higher corticosterone secretion in response to novelty was found as compared to controls. At 90 days of age, the experimental animals showed a prolonged response and diminished recovery of circulating glucocorticoids after stress as compared to controls (Henry et al., 1994). These animals also had higher glucose levels, which is consistent with overactivity of the HPA axis (Vallee et al., 1996). Another study showed the presence of an overactive HPA axis in young prenatally stressed rats, while the secretion of HPA hormones did not differ between prenatally stressed and nonstressed groups at adult age (Takahashi, 1992).

In rhesus monkeys the picture is less clear. In prenatally stressed subjects, the ACTH response rather than the cortisol response to a stressor proved to be increased, and the disparity between the ACTH and cortisol levels was greatest in the most stressful condition (Clarke et al., 1994; Clarke & Schneider, 1993; Schneider, 1998). Baseline levels of cortisol and ACTH were normal in 8-months-old monkeys following prenatal noise stress, but were elevated in 18-months-old animals following social stress during pregnancy (Clarke et al., 1994; Schneider, 1998). These data demonstrate that previously stressed and nonstressed monkeys differ in some aspect of feedback regulation within the HPA axis, but both the response type and age effect appear to be different from those seen in rodents.

In several of the above cited studies, an association was found between overactivity and altered feedback regulation of the HPA axis and the earlier described behavioral changes in prenatally stressed animals (Fride et al., 1986; Takahashi, 1992). The altered physiology and

morphology of the HPA axis in prenatally stressed offspring is summarized in Table 3.3.

Table 3.3

Altered physiology/morphology of the HPA axis in prenatally stressed offspring

Rodents	**References**
Higher levels of glucocorticoids (CORT) in baseline	Fride et al., 1986; Weinstock et al., 1992; Weinstock, et al., 1998; Ader & Plaut, 1968; McCormick, 1995
Faster & stronger CORT response to novelty, shock, open field	Fride et al., 1986; Takahashi et al., 1988; Takahashi, 1992; Peters, 1982; McCormick, 1995; Weinstock, 1995
Prolonged CORT response to stress	Henry et al., 1994; Vallee et al., 1996; Weinstock, 1995
Reduction in hippocampal corticosteroid receptors	Barbazanges et al., 1996; Maccari et al., 1995; Henry et al., 1994; Weinstock et al., 1992
Decreased synaptic density in hippocampus	Hayashi et al., 1998
Higher levels of CRH in the amygdala	Cratty et al., 1995; Makino et al, 1994
Nonhuman primates	**References**
Baseline levels of cortisol and ACTHElevated (18 months postpartum)	Clarke et al., 1994
Baseline levels of cortisol and ACTH normal (8 months postpartum)	Schneider, 1998
Increased ACTH response to stressor	Clarke et al., 1994; Clarke & Schneider, 1993; Schneider, 1998

3.2.10 Mechanisms underlying altered HPA axis regulation in prenatally stressed offspring

The generally held hypothesis is that (1) prenatal stress leads to enhanced release of maternal stress hormones, (2) maternal and/or placental stress hormones enter the fetal circulation and, in turn, (3) affect fetal hippocampal ontogeny by down-regulating glucocorticoid receptors, altering receptor sensitivity, and/or exerting neurotoxic effects on hippocampal cells, finally resulting in altered HPA axis regulation (McEwen, 1991; Sapolsky, 1987; Sapolsky et al., 1990). Though not all of these mechanisms which may underlie alterations in feedback regulation of the HPA axis after prenatal stress have been thoroughly investigated as yet, there are at least two clues: a decrease in hippocampal corticosteroid receptors, and higher levels of CRH in the amygdala in offspring exposed to in utero stress.

Clear-cut changes in the hippocampal corticoid receptors have been observed in the offspring of prenatally stressed rodents, with a 70% reduction of type I receptors and a 30% reduction of type II receptors (Barbazanges et al., 1996; Maccari et al., 1995; Henry et al., 1994; Weinstock et al., 1992). Since very young (3-days-old) pups have higher levels of corticosterone but still an almost equal density of hippocampal glucocorticoid receptors, it is likely that the lower amount of receptors at older age is due to exposure to higher levels of steroid hormones, and not vice versa (Henry et al., 1994).

The offspring of rhesus monkeys that had been treated with dexamethasone during pregnancy had at nine months of age higher baseline and post-stress levels of cortisol than vehicle-treated controls. Furthermore, MRI scans of the brain at 20 months of age showed an approximately 30% reduction in size and segmental volumes of the hippocampus following prenatal exposure to dexamethasone. These results therefore indicate that the hippocampus mediates the negative feedback of cortisol release and is especially vulnerable to elevations of glucocorticoids during early development.

Another neural structure that may contribute to increased responsiveness of the HPA axis following prenatal stress is the amygdala. The content of CRH appeared to be substantially increased in the amygdala of prenatally stressed rats compared to levels in control animals (Cratty et al., 1995). Because glucocorticoids have been shown to influence the regulation of the expression of neuropeptide genes (Harlan, 1988), elevated levels of stress hormones induced by prenatal stress may result in increasing CRH mRNA expression in the amygdala (Makino et al., 1994). In turn, increased levels of CRH in the amygdala have been found to be associated with increased emotionality and anxiety-like behaviors and seem to facilitate stress-induced behavior and autonomic activation (Brown & Gray, 1988; Takahashi, 1998).

Thus, prenatal stress significantly augments the responsivity and decreases the feedback regulation of the HPA axis of offspring. During fetal brain development both glucocorticoid receptor immunoreactivity and mRNA levels are present in multiple areas of the brain, including regions containing the monoaminergic neurotransmitter systems (Cintra et al., 1993). The appearance of glucocorticoid receptors in these areas of the fetal brain suggests a possible role of glucocorticoids in normal brain development. Therefore, prenatal stress may result in alterations of other biochemical systems in the brain, such as the opioid and neurotransmitters systems. These alterations could underlie some of the behavioral and physiological effects observed after prenatal stress and may further affect later stress responses. This will be discussed in the following sections.

3.2.11 Prenatal stress and the brain opioid system

It has been noted for some time that endogenous opiates released during stress early in fetal development may mediate stress effects attributed to gonadal steroids. For example, the feminizing effects of prenatal stress on sexual behavior in male offspring can be blocked if naltrexone, an opiate antagonist, is administered to the mother prior to the stressor (Ward et al., 1986). Also, exogenous opiates administered to a pregnant female have been shown to suppress plasma levels of testosterone in the fetus (Singh et al., 1980) and to result in long-term changes in sexual behavior (Ward et al., 1983). In addition to effects on gonadal steroids, opiates have trophic roles during neural development by altering the elaboration of processes, the formation of synapses and the normal rate of cell attrition (Insel et al., 1990).

Prenatal stress has been found to be associated with a diminished activity of the opioid system and with fewer brain opioid receptors in the offspring compared to controls (Insel et al., 1990). In the latter study, female rats were exposed to heat and restraint stress from gestational day 15 through day 22, with a second group of pregnant females left undisturbed. The offspring from stressed females showed a decreased binding of a selective mu opiate receptor ligand in homogenates of the striatum on postnatal day 42. The decrease was largely due to a reduced number of receptors and not to changes in affinity. In vitro analysis revealed decreases in ligand binding in various areas of the brain, such as the caudate-putamen, nucleus accumbens, lateral amygdala and the endopiriform nucleus. On the behavioral level, this was reflected by a reduction of opioid-mediated behaviors, such as morphine and stress-induced analgesia or forced swimming (Kinsley et al., 1986; Alonso et al., 1991). Higher circulating levels of glucocorticoids following prenatal stress seem to be responsible for the reduction of opioid receptors, as well as for the reduction of GABA/benzodiazepine (BZD) receptors in the hippocampus (Fride et al., 1985). In turn, both opioid and GABA/BZD receptor activities contribute to the inhibitory control of CRH release, as administration of naloxone, an opioid blocker, leads to an increased release of glucocorticoids in both normal and prenatally stressed rats, though the increase is much larger in the stressed animals (Poltyrev & Weinstock, 1997). Further, the increased activation of the HPA axis following opioid blockade can be prevented by stimulation of the GABA/BZD receptors (Torpy et al., 1993). As a consequence, the reduced activity of both opioid and GABA/BZD systems following prenatal stress augments the increased activity of the HPA axis.

3.2.12 Prenatal stress and brain neurotransmitter systems

3.2.12.1 The serotonergic system

There is a close relationship between the regulation of the HPA axis and the serotonin (5-HT) system (Mitchell et al., 1990). Further, 5-HT is believed to play a role in early brain development through facilitating synapse formation and maintenance (Hayashi, 1998). Therefore, the 5-HT system could hold the key to the problem of identifying where stress effects on the fetal brain take place. Earlier work already showed that prenatal stress is able to change 5-HT turnover in the fetal brain (Peters, 1990). After daily saline injections combined

with crowding during the third week of pregnancy in rats as a stress procedure, the levels of free tryptophan in plasma and therefore the amount of tryptophan available to the fetal brain were significantly elevated compared to control animals. Since tryptophan is a precursor to 5-HT, the amounts of 5-HT and 5-HIAA, a 5-HT metabolite, were increased in the fetal brain. These changes were maintained after birth until postnatal day 10, and were found to be associated with a reduced number of 5-HT receptors in the brain of adult rats (Peters, 1986; 1988). A reduction of 5-HT binding sites in the hippocampus in particular is consistent with suppression of the negative feedback by corticosteroids on type I and II glucocorticoid receptors in this area. Decreased levels of 5-HT and increased levels of 5-HIAA were measured on day 35 in the brains of offspring of prenatally stressed rats (Hayashi, 1998) and in experimental males following ACTH injections during pregnancy (Fameli et al., 1994). This reflects a substantially increased metabolic rate of 5-HT. Moreover, the synaptic density in the hippocampus of stressed offspring was decreased by about 30%. Thus, a stress-induced increase in fetal brain 5-HT synthesis and diminished synaptic density in the hippocampus seem to be mechanisms through which prenatal maternal stress may lead to increased activation of the HPA axis and affects postnatal development and behavior (Peters, 1990).

3.2.12.2 The noradrenergic system

The majority of noradrenergic cell bodies in the brain originate in the locus coeruleus, located in the dorsolateral pons, and project widely to various cortical areas. The noradrenaline-coeruleus system is the central component of the sympathico-adrenal stress system and is involved in attentional processes and in stress responses. Prenatal stress has been reported to elevate the basal concentration of noradrenaline (NE) in the hypothalamus (Peters, 1982) and to diminish it in the medial preoptic area and the median eminence (Moyer, 1978), whereas no alterations were measured in NE levels in cortical, brain stem, and cerebellar regions under basal conditions (Peters, 1982). More important, the concentration of noradrenaline in the cerebral cortex and the locus coeruleus was significantly reduced in prenatally stressed adult rats when measured immediately following a shock stress rather than under basal conditions (Takahashi et al., 1992). Combined with the finding of elevated concentrations of NE metabolites in these brain areas, this suggests that prenatal stress produces an overactive noradrenergic system with increased central NE turnover. Plasma levels of NE and its metabolites did not differ between prenatally stressed and control animals under baseline conditions (Weinstock et al., 1998). Foot shock stress, however, produced a significantly greater activation of the sympathetic nervous system in the prenatally stressed than in the control animals. Furthermore, the activities of the HPA axis and the sympathico-adrenal system were correlated in the control group under both basal and stress conditions, but there was no correlation between plasma corticosterone and indices of sympathetic activity in the prenatally stressed group, indicating a differential activation of the two systems in these animals (Weinstock et al., 1998).

3.2.12.3 The dopaminergic system

Several effects on the offspring of prenatal stress, such as delayed early motor development (Barlow et al., 1978), a stronger locomotor response to novelty (Deminière et al., 1992), and increased fearfulness to stressful situations (Fride & Weinstock, 1988), have been attributed to changes in the dopaminergic system. After prenatal stress in rats, elevated rates of dopamine turnover were observed in the right prefrontal cortex and reduced dopamine activity in the right nucleus accumbens and left corpus striatum (the mesolimbic and nigrostriatal dopamine pathways) (Fride & Weinstock, 1988). Also, a reduction in the degree of hemispheric asymmetry for dopamine and 5-HT turnover rates was seen in the experimental animals (Alonso et al., 1994). In the locus coeruleus reduced levels of dopamine were found following prenatal stress, concomitantly with increased levels of DOPAC (Takahashi et al., 1992). All these changes in the dopamine turnover are similar to those measured following conditioned fear in adult animals and are commonly associated with increased suppression of behavior in the forced swimming test (Alonso et al., 1994). These data suggest that maternal stress during gestation alters the cerebral lateralization of dopaminergic activity and increases the risk for depression and anxiety in the offspring.

Alterations of dopamine receptor systems of offspring following prenatal stress have been documented as well (Henry et al., 1995). The density of the D2 receptor increased markedly in the nucleus accumbens, while that of the D3 receptor decreased considerably in the core and the shell of the nucleus accumbens. In addition, the offspring were also more rapidly sensitized to amphetamine (Henry et al., 1995).

It is surmised that these changes in dopamine receptor densities develop when the animals are adults, with a potential role for impaired control of corticosterone secretion in the offspring (Henry et al., 1995). To determine the possible influence of maternal corticosterone on the nigrostriatal and mesolimbic dopamine pathways, pregnant rats were subjected to a low dose of corticosterone in the last week of pregnancy (Diaz et al., 1995). The locomotor activity in the prepubertal offspring was increased in the prenatally treated animals as compared to controls, and this was found to be associated with an increased dopamine metabolism in the ventral striatum. Furthermore, maternal corticosterone treatment resulted in a disappearance of the asymmetry of dorsal striatal dopamine metabolism in males only. These effects may be mediated by direct activation of corticosteroid receptors in the brain.

3.2.12.4 The cholinergic system

Interest in possible changes of the cholinergic systems after prenatal stress has been stimulated by the modulatory influence of cholinergic neurotransmission on the activity of the HPA axis (Sitichocke & Marotta, 1978) and the regulation of hippocampal glucocorticoid receptors (Yau et al., 1992; Alema et al., 1995). Furthermore, stress increases the release of acetylcholine in the hippocampus (Mark, 1996) and cholinergic tone may be involved in emotional affect (Janowsky, 1994). The female and male adult offspring of pregnant rats that were exposed to restraint stress and bright light exhibited no differences in basal release of acetylcholine, when compared to control animals (Day et al., 1998). Mild stress, however, was found to increase hippocampal acetylcholine release to a greater extent in prenatally

stressed rats than in controls. Furthermore, administration of CRH produced greater release of acetylcholine in prenatally stressed rats than in controls (Day et al., 1998). These findings underscore that prenatal stress has long-term effects on the development of forebrain cholinergic systems and that these changes in cholinergic systems could mediate the increased responsivity of the HPA axis.

The effects of prenatal stress on the aforementioned neurotransmitter systems are summarized in Table 3.4.

Table 3.4
Effects of prenatal stress on neurotransmitter systems

Rodents		References
Serotonergic system	Increased 5-HT in fetal brain	Peters, 1986; 1988
	Increased metabolic rate of 5-HT	Hayashi et al., 1998; Fameli et al., 1994; Peters, 1990
Noradrenergic system	Elevated basal concentrations of noradrenaline (NE) in hypothalamus	Peters, 1982
	Diminished NE levels in medial preoptic area & median eminence	Moyer, 1978
	Reduced NE in cerebral cortex & locus coeruleus after stress	Takahashi et al., 1992
Dopaminergic system	Elevated rates of dopamine turnover in right prefrontal cortex	Fride & Weinstock, 1988
	Reduced dopamine acitivity in right nucleus accumbens & left corpus striatum	Fride & Weinstock, 1988
	Reduced levels of dopamine & increased levels of DOPAC	Takahashi et al., 1992
	Increased density of D2 and decreased density of D3 receptors in nucleus accumbens	Henry et al., 1995
Cholinergic system	Increased hippocampal acetylcholine release	Day et al., 1998

In summary, the mechanisms involved in prenatal stress effects on the fetus are shown in Figure 3.1.

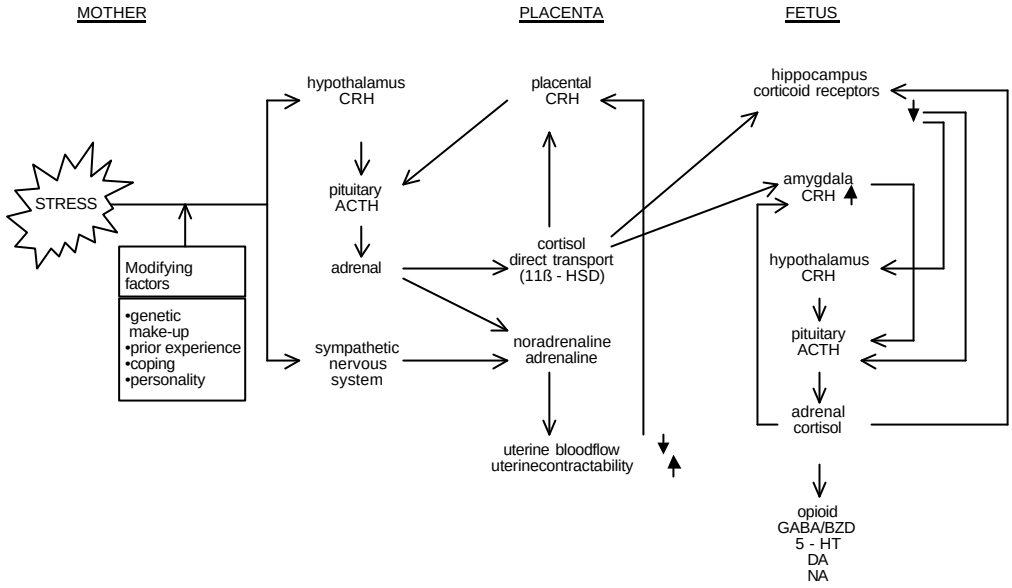

Figure 3.1. Mechanisms involved in the prenatal stress effects on the fetus including several positive feedback loops.

3.2.13 Effects of prenatal stress: modification by postnatal influences

A specific experiential variable can have opposing effects depending on an individual's maturational stage at exposure. For instance, prenatal handling inhibits postnatal exploration, whereas postnatal handling facilitates later exploration (Denenberg & Zarrow, 1971). In fact, a body of evidence exists to show that postnatal handling attenuates fearfulness in novel environments (Levine et al., 1967) and is able to reverse the increase in emotional reactivity induced by prenatal stress (Wakshlak & Weinstock, 1990). Memory performance in a water maze and a two-trial memory test, however, was not affected by prenatal stress nor postnatal handling (Vallee et al., 1997).

On a neuroendocrinologic level, early in postnatal life handled young adult rats were found not to differ from nonhandled rats in basal corticosterone levels at any time point over the diurnal cycle (Meaney et al., 1992). However, the responsivity of the HPA axis of handled rats was significantly attenuated compared to that of nonhandled animals, as was reflected by a decreased secretion of corticosterone and ACTH to a variety of stressors compared to nonhandled rats (Meaney et al., 1993; Vallee et al., 1997). Corticosterone levels of handled

rats also showed a faster return to basal levels following the termination of stress than do corticosterone levels of nonhandled animals (Meaney et al., 1989). In accordance with the altered feedback regulation of the HPA axis and the decreased number of hippocampal corticoid receptors following prenatal stress (see above), postnatal handling was associated with improved feedback regulation and increased density of glucocorticoid receptors in the hippocampus and frontal cortex (Meaney et al., 1989). A recent study showed that these effects of postnatal handling on the development of the HPA axis were mediated by effects on the mother-pup interaction (Sapolsky, 1997). Handling almost doubled the frequency of licking and grooming, and these maternal behaviors were associated with reduced plasma ACTH and corticosterone responses to restraint stress in adult offspring. Apparently, the effects of prenatal stress on the development of HPA responses to stress can be compensated by early postnatal environmental factors. In addition, on a behavioral level, well-groomed pups showed more open-field exploration. Thus, behavior itself can be influenced by the mother-pup interaction shortly after birth (Sapolsky, 1997).

Another method to examine the reverse effects of the postnatal environment on deleterious effects of prenatal stress is by using a cross-fostering design. In such a design, a group of prenatally stressed pups was placed with an adoption mother shortly after birth and compared to prenatally stressed pups raised by their biological mother (Maccari et al., 1995). Adoption increased maternal behavior, since foster mothers spent more time licking and picking up pups than did the biological mothers. The adopted pups showed an attenuated responsiveness of the HPA axis to stress compared to pups who were raised by their biological mother. Adoption led also to an increase in type I glucocorticoid receptors in the hippocampus (Maccari et al., 1995). This long-term effect of adoption in the early postnatal period needs to be examined more thoroughly in order to elucidate the exact mechanisms behind it.

Postnatal factors not only are able to compensate for prenatal negative effects, but can also be harmful. Maternal separation during the neonatal period results in higher basal corticosterone levels and greater corticosterone responses to stress (Thomas et al., 1968), which last until adulthood (Plotsky & Meaney, 1993). This effect could be explained by a significant reduction in glucocorticoid receptor density in the hypothalamus, hippocampus, and frontal cortex, resulting in a decreased negative feedback sensitivity of the HPA axis. In addition, hypothalamic hypersecretion of CRF was observed after maternal deprivation which in turn could predispose to the development of depression in adulthood (Gold et al., 1988; Coplan et al., 1997).

3.2.14 Relevance of animal prenatal stress models for the human

Although corticosteroids are essential for normal brain development, exposure to excessive amounts of these hormones can have long-lasting effects on neuroendocrine function and behavior. Several animal studies have shown evidence of permanent programming of the brain, including the hippocampus and HPA axis, and other organ systems, such as the endocrine pancreas (insulin) and the cardiovascular system (Matthews 2000). These studies have deepened our insight into the possible mechanisms underlying at least some physical problems in later life (high blood pressure, diabetes). So, with no hesitation, one may say

that animal models of prenatal stress are of significant importance for human development. However, when generalizing the results of prenatal stress models in animals to humans there are some caveats, especially as regards to the pathogenesis of human mental health problems.

We already discussed the differences in the timing of brain maturation between rodents, nonhuman primates, and humans. It is important to scale developmental processes in animals to those in man, and particularly to take into account differences in the stage of brain development at the time of birth.

Species differences also apply to transducing maternal 'stress' to the fetus. In contrast to most other mammals, the human and non-human primate fetus are relatively protected from increased maternal cortisol levels due to placental 11ß-HSD activity, although cortisol may cross the placenta to some extent in these species, especially in stressful conditions. On the other hand, only in primates, two positive feedback loops involving placental CRH are present in the maternal-placental-fetal unit, by which mechanism maternal stress signals reach the developing fetus.

The third comment relates to the experimental character of stress in animal models. Common across these studies is a circumscribed and well-defined form of stress that is externally inflicted upon the animal. Similarly, stress encountered during human pregnancy may be entirely due to external circumstances and be independent of the actions of the individual, like in case of earth quakes, sudden floods, war, or sudden death of significant others. By contrast, many other forms of human stress that are linked to the occurrence of life events or daily hassles, are, at least partially, attributable to the person and may be interwoven with personality and lifestyle factors. This implies the importance to differentiate between personality factors and exogenous life stress in human studies on prenatal stress. The human situation is more complicated with regard to stress responses, since social support and coping style may have mediating or moderating effects on the stress response and may reflect aspects of cognitive control over a stressful situation (Huizink et al., 2000). Furthermore, pregnancy-related anxieties have appeared to be unique elements of stress in human pregnancy and may give rise to altered behavior and development in infants as well (Huizink et al., 2000).

Experimental studies in pregnant animals have, in general, not focused on stress research in a naturalistic social setting. The important roles of the social environment as a main source of stress and of individual differences in stress responsivity and adaptation have been largely neglected. Most of the applied stressors in the earlier mentioned experiments (noise, restraint, electric foot-shock etc.) have little heuristic value as they bear little or no relation to the environmental challenges an animal may meet in its everyday life, although crowding might be an exception. To better mimic the etiology of human stress pathology, one may think of study designs that take into account housing condition (animals living in colonies, in small groups, in pairs, or individually), the social structure (hierarchy, pair bonding) and stability of a group of animals, and the way and ease of food acquisition (food ad libitum vs active foraging under unpredictable circumstances). Thus far, only a few studies of ethological and ecological relevance have been performed in relation to perinatal stress and offspring development. Of particular interest are the studies by Sachser in guinea pigs (Sachser & Kaiser, 1997) and by Nemeroff and colleagues in monkeys (see Coplan et al., 1998), which emphasized the impact of social support and daily hassles, respectively.

Finally, two different coping strategies have been found in adult rats (Koolhaas et al., 1998, 1999) and pigs (Schouten & Wiegant, 1997). Individuals are predisposed to either an active or passive coping style determined by their genetic constitution and early life experiences. Actively coping animals appear to differ from those using a passive coping style as regards to behavior, physiology, endocrinology, and immunology. However, we are unaware of any study of gestational stress that has incorporated maternal coping style and individual differences in response to stress.

3.2.15 Human studies

Much interest has been paid to the possible effects of gestational stress in humans on the duration of pregnancy, birth weight, and related measures of obstetric outcome (Pagel et al., 1990; Hedegaard et al., 1993; Copper et al., 1996; Lou et al., 1994). These studies did not only include the effect of psychological stress during pregnancy, but also the effect of physical stress such as chronic exposure to loud noise in the vicinity of an international airport (Schell, 1981) or fatigue associated with occupational working conditions during pregnancy (Landbergis & Hatch, 1996; Mamelle & Munoz, 1987). Relatively few studies, however, have looked at the influence of prenatal stress on postnatal development in humans, and most of these are limited by the use of retrospective designs, small sample sizes, and/or non-standardized measurements. For example, infants of emotionally disturbed or high anxious pregnant women have been described as restless, irritable, overactive, poor sleepers, and less alert and responsive compared to infants of undisturbed or low anxious women (Ferreira, 1960; Turner, 1956; Ottinger & Simmons, 1964; Farber et al., 1981). Infants of high anxious women further had lower scores on the mental scale of the Bayley Scales of Infant Development than infants of low anxious women (Davids, 1963). In a birth-cohort study of about 1300 children, marital discord and interpersonal tensions during pregnancy produced elevated rates of both physical disease and behavioral problems at later age (Stott & Latchford, 1976). Furthermore, the prenatal history of severely emotionally disturbed children that were seen in partial hospitalization or in-patient programs revealed exposure above chance level to stressors like unplanned and/or rejected pregnancies, marital discord, and affective problems in the mother (Ward, 1991). Of course, all these reports focused on the influence of maternal personality variables on the postnatal development of the child rather than on the influence of prenatal stress per se. Other retrospective studies reported that specific forms of stress during pregnancy, such as the threat of and exposure to the six-day Arab-Israeli war (Meijer, 1985) and severe familial and marital discord (Stott, 1973), were associated with delays in early motor development and increased amounts of behavioral problems as excessive clinging, crying, hyperactivity, low frustration threshold and antisocial behavior at age 2-10 years.

Some studies have focused on the relation between prenatal stress and the risk for later severe psychopathology. Severe psychological stress in the pre- and perinatal period was retrospectively linked to a relatively high incidence of attention-deficit hyperactivity disorder (Clements, 1992). In a follow-up study to age 15 of the children born to mothers who faced the death of their spouse during pregnancy, a relatively high incidence of psychiatric disorders was found, when compared to infants that lost their father in the first year of life (Hut-

tunen, 1994). The disorders included schizophrenic episodes, depressive and neurotic symptoms, alcoholism and antisocial behavior. Children born from unwanted pregnancies have also been reported to have increased risk to develop schizophrenia (Myhrman, 1996). In a case-control design and using data from psychiatric case registers, prenatal stress exposure in the first trimester of pregnancy caused by the German invasion during the second World War in the Netherlands was associated with a small but significantly elevated risk for schizophrenia (van Os & Selten, 1998). In a similar design, a slight nonsignificant increase was found in the incidence of non-affective psychosis following prenatal exposure to stress induced by the Dutch flood disaster of 1953 (Selten et al., 1999).

Few studies have employed a prospective set-up. In a sample of 337 pregnant women, maternal anxiety, assessed prospectively during pregnancy by means of a self-report anxiety scale, proved to be significantly related to a difficult temperament of the baby at four months after birth (Vaughn et al., 1987). Since temperamental ratings were also obtained from the mothers, the association between pregnancy anxiety and later temperament may well be due to report bias, i.e., personality factors of the mother. Plasma levels of maternal cortisol, ACTH and ß-endorphin obtained during the third trimester of pregnancy, during the early stages of labor, at day one after birth, and from umbilical blood failed to show consistent relations with either maternal anxiety or temperamental variables of the infant (Vaughn et al., 1987). In another study among 70 nulliparous women, self-report measures of state and trait anxiety obtained during pregnancy showed significant positive correlations with fetal motor activity and fetal sleep state organization, assessed by means of ultrasound recordings in the third trimester or pregnancy (Van den Bergh, 1990). In addition, prenatally assessed maternal anxiety was correlated with temperamental problems in the infants at seven months of age. Path analysis suggested that maternal anxiety had indirect (by modifying fetal behavior) rather than direct effects on infant behavior. However, no differences were found between infants from high and low anxious mothers during neurological examination and standardized observations of feeding behavior, nor on the Bayley Scales of Infant Development between 1 and 28 weeks after birth. All in all, these studies also suggested the influence of maternal personality rather than that of prenatal stress on fetal and infant behavior.

3.2.16 Prenatally induced physiological changes and implications for human psychopathology

As reviewed above animal experimental work on prenatal stress has shown that the HPA axis in the offspring is overactive and has an impaired feedback regulation. Abnormalities of brain transmitter systems have been documented as well. In this section we will consider the potential implications of these physiologic changes for our understanding of the risk for human psychopathology later in life.

The neuroendocrinologic profile of the offspring of prenatally stressed animals is very similar to that found in humans with major depressive disorders. In about 50% of depressed patients, there is an increased secretion of cortisol throughout the 24 hours and loss of circadian rhythm. In addition, like in chronically stressed animals, there is an impaired negative feedback control of the HPA axis and hypertrophy of the adrenal gland (Checkley, 1996). Evi-

dence for an increased central drive to the HPA axis in depressed patients is that the brains of depressed suicides were shown to have a substantial increase in the number of CRH expressing cells in the paraventricular nucleus of the hypothalamus and an increase in the co-expression of CRH and arginine-vasopressin in the same region (Scott & Dinan, 1998). Thus, prenatal exposure to stress in humans may lead to an altered set-point of the HPA axis and increase susceptibility to later depressive disorders.

Though far less extensively documented than in depression, children with an inhibited temperament are characterized by a higher baseline tone of the HPA axis and the sympathico-adrenergic system (Kagan et al., 1988). These children show high levels of anxiety and behavioral inhibition in unfamiliar environments and when exposed to novelty and uncertainty (Kagan et al., 1987), and they are at increased risk to develop later anxiety disorders (Kagan et al., 1988). Another consequence of prenatally induced hyperactivation of the HPA axis may be later deficits in memory and cognition through neurotoxic effects on hippocampal neurons (O'Brien, 1997). Studies in rats and primates have revealed that hippocampal neurons and glucocorticoid receptors are lost during ageing (Djordjevic-Markovic et al., 1999; Ball, 1977). In parallel, during ageing the negative feedback regulation of the HPA axis is weakened and dexamethasone resistance increased. Cell loss in the hippocampus by early exposure to an excess of glucocorticoids thus could lead to further activation of the HPA axis and greater susceptibility to develop impairments in memory and learning at old age (Sapolsky, 1997).

A second neurochemical change following prenatal stress that may be of relevance for later psychopathology is a low central activity of the 5-HT system. The 5-HT system plays an important role in regulating early developmental processes, including differentiation, cell migration, and synaptogenesis. Hence, early disruption of the 5-HT system may have widespread effects on various brain functions. An abnormally low activity of central 5-HT has been implicated in an array of psychopathological conditions, varying from autism, impulsive aggression, depression, eating disorders, and chronic pain syndromes (Halperin et al., 1997; O'Dwyer et al., 1996; Petty et al., 1996; Lopez-Ibor, 1992; Haze, 1991; Hendler, 1982).

Thus, rather than exhibiting a direct effect on a specific psychopathology, prenatal stress hampers several physiological systems that are involved in a wide spectrum of psychopathology.

3.3 Conclusion

The main arguments of this review have been summarized in Figure 3.1. A voluminous literature in rats and nonhuman primates contains consistent evidence that exposure to a variety of stressors during pregnancy is associated with delays in neuromotor development, increased emotionality, decreased exploratory behavior, and impaired adaptation to conditions of conflict. In addition, altered sexual behavior and learning deficits have been described. Physiological changes include overactivity and impaired negative feedback regulation of the HPA axis. Furthermore, reduced activity of the opioid, GABA/benzodiazepine, 5-HT, and dopamine systems and increased activity of the sympathico-adrenal system have been found. Likely mechanisms to explain the transfer of stress from the mother to the fetus are the direct transport of glucocorticoids and ß-endorphin across the placenta, increased

placental production of ACTH and CRH under the influence of maternal stress, and changes in the uteroplacental blood flow.

In contrast, our knowledge about the short-term and long-term effects of maternal stress during pregnancy on the developing fetus and the child in man is rather scanty. Though the human fetus seems to be relatively protected from a massive direct transport of cortisol across the placenta through the 11ß-HSD enzyme, elevations of maternal cortisol levels seem to be able to affect a significant amount of variation in fetal cortisol levels. Genetic polymorphisms of 11ß-HSD and the quality of the placenta may further add to explain individual variation in the permeability of the placenta to maternal stress hormones.

Animal studies indicate that it is quite plausible that prenatal stress leads to increased predispositions for later psychopathology, including depression, anxiety disorders, and memory and cognitive deficits. Evidence for a specific effect of prenatal stress on schizophrenia and other mental disorders is rather weak and has been generally overstated. All studies have used a retrospective design which makes it very hard to control for many other factors involved in the development of psychopathology. Moreover, pathophysiological mechanisms were not studied. The findings summarized in this review suggest that prenatal stress may exert its effects on various physiological systems, which are more generally involved in the development of psychopathology. Thus, the effects of prenatal stress appear to be nonspecific by early programming of, for instance, the HPA axis reactivity. This calls not only for further prospective human studies, but also for reconsidering health system routines around prenatal care and thinking about new strategies to reduce stress in pregnancy.

Recommendations for such future studies include research on the stress reactivity and endocrinology of pregnant women in order to elucidate potential pathophysiological mechanisms that may mediate the effect of prenatal stress on the developing fetus. Genetic studies may shed more light on the genetic contribution on individual sensitivity of infants for exposure to prenatal stress. Future animal studies could try to link preclinical topics with clinical issues by designing prenatal stress studies in line with the stressors that may be encountered in human pregnancy. Thus, a lifestyle stressor approach, with daily hassles or social stress as predictors of offspring development and behavior, may provide more relevant and comparable results for human studies. In addition, coping behavior of pregnant animals confronted with stress also offers an interesting approach for future studies, since in human studies coping is regarded as a potential mediator of the stress response.

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4

Is pregnancy anxiety a relatively distinctive syndrome?

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Under reference process

4.1 Abstract

Background: Assessment of general anxiety during pregnancy may underestimate anxiety specifically related to pregnancy. Therefore, a questionnaire on pregnancy anxieties was used to test their structure, internal consistency, stability and change in the course of pregnancy. In addition, associations with general anxiety and depression measures were examined, and possible clinical correlates of pregnancy anxieties were investigated.

Methods: Nulliparous pregnant women (N=230) filled out a 34-item questionnaire on pregnancy-related anxiety and several other questionnaires covering general personality factors, such as general anxiety (STAI), locus of control (IPC), appraisal of pregnancy and neuroticism (ABV-N). These questionnaires were filled out at 15-17 weeks, 27-28 weeks and 37-38 weeks of gestation.

Results: A three-factor model of pregnancy anxiety was found by means of confirmatory factor analysis, reflecting 'fear of giving birth', 'fear of bearing a physically or mentally handicapped child' and 'concern about one's appearance'. The factor structure is stable throughout pregnancy. Mean scores change over time, with highest levels of fear during early pregnancy, slightly lower levels in late pregnancy and lowest scores during mid pregnancy. Personality factors can explain only a small part of the variance of these fears. High risk groups, such as women suffering from mental problems or women with a previous miscarriage, have more fear of bearing a physically or mentally handicapped child and are less concerned about their personal appearance than are low risk pregnant women.

Conclusion: Pregnancy anxiety should be regarded as a relatively distinctive syndrome. Its measurement can make a unique contribution to the evaluation of anxiety during pregnancy.

4.2 Introduction

Pregnancy is an event that changes many perspectives of a woman's life. It has been regarded as a time of psychological and biological crisis and of emotional upheaval, and as a life event for first-time mothers which initiates a new social role (Thorpe et al., 1992). A more optimistic standpoint views pregnancy as a period that brings marvellous feelings of well-being and psychological strength, while others view it simply as a relatively normal and largely positive developmental experience (Brown, 1979). Although the individual experience may vary between these extremes, pregnancy has potentially important short- and long-term implications for women's health, well-being and social roles (Striegel?Moore et al., 1996). Since a pregnant woman is the environment for the developing fetus, psychological alterations or even mental disorders may affect the fetus. Given the fact that about 90% of all women become pregnant at least once in their life, it seems highly relevant to investigate psychological changes during pregnancy.

However, over the years research interest has focused on the marked changes in women' s emotional reactions following delivery rather than on their psychological state during pregnancy. This is understandable in so far as epidemiological studies suggest that puerperium is a period of increased vulnerability to severe psychiatric disorders like psychoses, whereas the prevalence of such disorders during pregnancy may even be slightly less than among age-matched females in the general population (Pugh et al., 1963; Kendell et al., 1976, 1987). When less severe manifestations of psychopathology are considered, however, a different picture emerges. The prevalence rates of depression in pregnancy, measured by means of interviews and recognized diagnostic criteria, are similar to those found after delivery and range from 3.5 % to 16 % (Green and Murray, 1994). Studies using self-report symptom scales show even higher depression scores in pregnancy than postnatally (Green and Murray, 1994). In a more recent study among a population sample of pregnant women, levels of dysphoria assessed by means of a composite score of the anxiety and depression subscales of the Symptoms Checklist (SCL-90R) were rather stable throughout pregnancy and were comparable to baseline data on the emotional state of the same women before pregnancy (Striegel?Moore et al., 1996). Moodiness, on the other hand, was experienced more often in pregnant women than in non-pregnant controls, but only in the first trimester of pregnancy.

All these studies have centred around the issue of whether the presence and course of common symptoms of depression and anxiety are influenced by pregnancy or childbirth. The interpretation of the results, however, is limited by the use of general scales of depression and of anxiety, such as the General Health Questionnaire-30 (GHQ-30; Goldberg, 1972), the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970) and the Manifest Anxiety Scale (MAS; Taylor, 1953). These scales have not been designed to assess anxieties and worries related specifically to pregnancy. Various descriptive and exploratory studies suggest that pregnant women may experience specific and intense fears, such as fear of incompetence and concerns about pain and loss of control during delivery, fear for their own life and the life of their baby, and worries about changes in their personal life due to pregnancy and childbirth (Dunkel?Schetter, 1998; Sjögren, 1997).

Few studies have systematically assessed the specific fears and worries related to pregnancy and examined the structure of pregnancy anxiety. In the early seventies, the Pregnancy Anxiety Scale (PAS) was created by Burstein et al. (1974). A later confirmatory factor

analysis performed on the original items of the PAS collected retrospectively after childbirth in a sample of 266 women suggested a three-dimensional model of pregnancy anxiety: 'anxiety about being pregnant', 'anxiety about childbirth', and 'anxiety about hospitalization' (Levin, 1991). Standley et al. (1979) obtained data concerning the presence of one general anxiety and five specific pregnancy anxieties (physical anxiety, anxiety about the integrity of the fetus, childbirth anxiety, child care anxiety and infant feeding anxiety) during the last month of pregnancy in 73 near-term nulliparous pregnant women. Exploratory factor analysis showed that the specific pregnancy anxieties could be clustered in two dimensions: 'anxiety about pregnancy and childbirth' and 'anxiety about future parenting'.

A Dutch questionnaire about pregnancy anxiety was developed by Van den Bergh (1990). This Pregnancy Related Anxiety Questionnaire (PRAQ) consisted of 58 items, with 33 items from the PAS and the remaining items based on other questionnaires (Blau et al., 1984; Kumar & Robson, 1984; Pleshette et al., 1956; Schaefer & Manheimer, 1960) and based on their clinical relevance. Exploratory factor analysis performed on data from the PRAQ completed by 231 women in the third trimester of pregnancy revealed five factors: fear of childbirth, fear of bearing a physically or mentally handicapped child, fear of changes in the relationship with the partner, fear of changes in the mother's personal life, and fear of changes in the mother's mood and problems in the mother-child relationship. A revised version (PRAQ-R) of this questionnaire was used in the present study.

Until now, only general anxiety indices have been used to predict birth outcome and the postnatal development of children (Allen et al. 1998; Pagel et al. 1990; Dorn et al. 1993; Beck et al. 1980; McCool et al. 1994; McCool & Susman, 1994; Istvan, 1986) and the aspects of anxiety specifically related to pregnancy have been ignored. Research into the effects of prenatal maternal psychological influences on birth outcome and on the postnatal development of children requires as a first step a full assessment of both general anxiety, depression and specific fears and worries during pregnancy. The present prospective study was therefore designed to achieve the following aims:

1. To investigate the structure of specific fears and worries related to pregnancy ('pregnancy anxieties') in the course of pregnancy.
2. To examine changes in the level of pregnancy anxiety throughout pregnancy.
3. To differentiate pregnancy anxiety from symptoms of general anxiety and depression and to study the personality predictors of pregnancy anxiety.
4. To examine clinical correlates of pregnancy anxiety.

4.3 Methods

4.3.1 Participants

All the participants in this study were deliberately included in a larger prospective longitudinal project which also investigated the influence of prenatal psychosocial factors on fetal behaviour and on the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Centre Utrecht (UMCU), which is a first-line referral center for low-risk

pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of approximately 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the study. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants did not differ from those of non-participants, except in the case of women with full-time jobs, who were less likely to participate. The descriptives of the participants are summarized in Table 4.1. As shown, the sample of participants consisted largely of middle class women, although both lower social and higher social classes were represented. The majority of women (92.4%) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job, 54.2 % working less than 38 hours a week and 45.8 % working full-time.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion. The main reason for the drop in the number of participants towards late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

Table 4.1 **Demographic characteristics of participants**

Variable	early pregnancy (N=230)
age (yr), mean (sd)	30.9 (5.1)
range	17 - 45
having a paid job (%)	93.5 %
part-time (less than 38 hr)	54.2 %
full-time	45.8 %
smokers (%)	22.9 %
use of alcohol (%)	19.9 %
socio-economic status	
low	23.4 %
middle	57.6 %
high	19.0 %
married or cohabiting (%)	92.4 %

smokers are women who smoke at least 1 cigarette a day during the relevant part of pregnancy;
use of alcohol is defined as using at least one glass of an alcohol-containing beverage during the relevant part of pregnancy;
socio-economic status is described by a combination of educational and professional levels.

4.3.2 Questionnaire measures

Among the package of questionnaires that were compiled to measure various aspects of the mental status of the pregnant women, some measured pregnancy-related fears and worries, while others assessed common symptoms of anxiety and depression and aspects of personality such as neuroticisms and locus of control, which may be important predictors of pregnancy-related fears.

Pregnancy Related Anxieties Questionnaire-Revised. Specific fears and worries related to pregnancy were measured on each occasion by means of an abbreviated version of the PRAQ developed by Van den Bergh (1989). This shortened 34-item version, the PRAQ-R, was derived from the original version by retaining the items with the highest factor loadings on each of the five subscales: 'fear of giving birth' (8 items), 'fear of bearing a physically or mentally handicapped child' (5 items), 'fear of changes and disillusion in partner relationship' (6 items), 'fear of changes' (8 items) and 'concern about one's mental well-being and the mother-child relationship' (4 items). The PRAQ-R can be obtained from the corresponding author on request.

State-Trait Anxiety Inventory (STAI). The STAI (Spielberger et al., 1970) comprises two self-report scales for measuring two distinct anxiety concepts, state-anxiety and trait-anxiety. Both scales contain 20 statements that ask the respondent to describe how she feels at a particular moment in time (state-anxiety) or how she generally feels (trait-anxiety). State anxiety is conceptualized as a transitory emotional state, whereas trait-anxiety refers to relatively stable individual differences in proneness to anxiety. Cronbach's alpha in this study was .88 for state anxiety and .83 for trait anxiety. The STAI was filled out on each occasion.

Edinburgh Postnatal Depression Scale (EPDS). The EPDS (Cox et al., 1987) is a 10-item questionnaire that can be used to measure prenatal and postnatal depression and has been validated for use in pregnancy (Green and Murray, 1994). The EPDS was completed twice during pregnancy (mid and late pregnancy) and Cronbach's alphas in this study were .86 and .87, respectively.

Neuroticism was determined with a subscale of the Amsterdam Biographical Questionnaire (Wilde, 1963). This questionnaire was filled out only during early pregnancy because it is believed to reflect a stable personality trait. Cronbach's alpha of the items on the subscale Neuroticism was .83.

Locus of control was measured by means of the items with the highest factor loadings on the subscales of the Internal locus of control, Powerful Others and Chance-Scale (IPC; Brosschot et al. 1994), with two items reflecting the Powerful Others scale, two items representing the Internal locus of control scale and two items for the Chance scale. Both the Powerful Others scale and the Chance scale reflect external locus of control. Powerful Others means that an individual believes that other powerful persons are in control of her life, whereas the Chance scale reflects the idea that the world is unordered and unpredictable, and is thus controlled by chance. Internal locus of control is found in individuals who believe they have their own life under control. Since locus of control is believed to reflect a stable personality trait, this questionnaire was completed only in early pregnancy.

Appraisal of pregnancy was measured by two single-item instruments. The perceived threat of the situation, or primary appraisal, was measured by the question 'Can you indicate on a ten-point scale the degree to which your pregnancy relates to the most upsetting (=1)

and most pleasant event (=10) in your life?'. Secondary appraisal, or the perceived options to control the situation, was assessed with the question 'To what extent do you think you are able to influence the course of your pregnancy?'. Participants could answer on a 5-point scale ranging from 'considerably' to 'not at all'. These two items were answered on each occasion.

4.3.3 High-risk groups

To examine the clinical correlates of pregnancy anxiety, we formed two mutually exclusive high-risk groups. First of all, women without a history of mental health problems (N=163, numbers refer to early pregnancy) were differentiated from women with previous mental problems that required treatment in the past and from women who were suffering from mental problems for which they received therapy during their pregnancy (total N=66). Secondly, we explored whether women with a previous miscarriage (N= 45) could be differentiated from women without such an obstetric history with regard to their pregnancy anxiety.

4.3.4 Statistical analysis

The factor structure of the PRAQ-R was examined by means of exploratory and confirmatory factor analysis (CFA), using SPSS version 6.1 for Windows and LISREL 8.30, respectively. CFA was performed by means of structural equation modelling and postulates relations between the observed measures and the underlying factors a priori. The goodness of fit between the hypothesized structure and the sample data was subsequently tested. This provided information about the reliability and validity of the model while taking measurement errors into account. Goodness of fit measures used were Chi Square (χ^2) and Chi-square divided by degrees of freedom. The latter is sensitive to sample size, and is therefore regarded as a measure of fit instead of a test statistic. When chi-square is divided by its degrees of freedom the result should be less than 3 if it is to indicate a reasonable fit to the data. Other fit criteria include: Comparative Fit Index (CFI ; >.9 indicates a good fit), Non-Normed Fit Index (NNFI; >.9 indicates a good fit), Root Mean Square Error of Approximation (RMSEA), which should be at least less than .08 and Root Mean Square Residual (RMR), which should be less than .05.

The next step was to examine the stability of pregnancy anxiety in the course of pregnancy by means of LISREL tests for stability of factor loadings and with Pearson intercorrelation coefficients. The change in the level of pregnancy anxiety over the trimesters was examined with MANOVA with repeated measures.

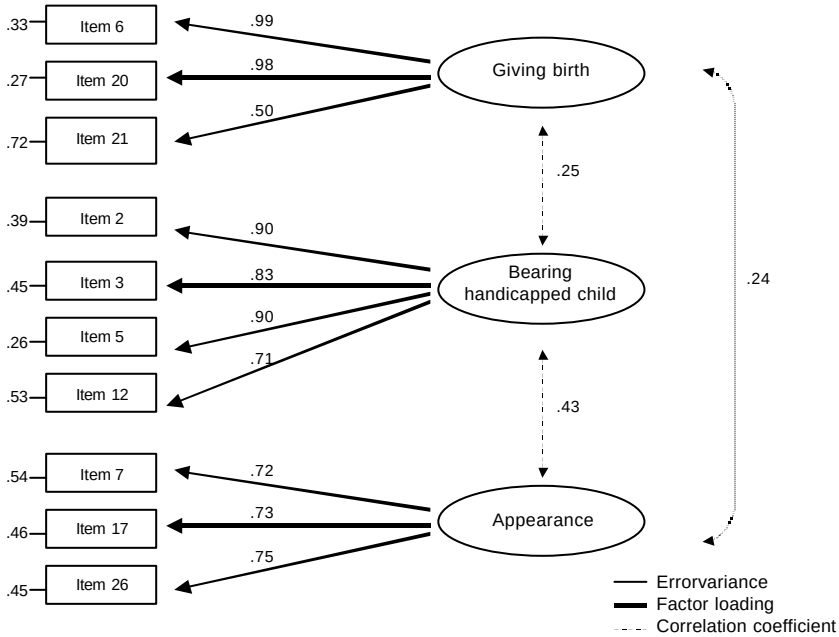
The associations of pregnancy anxiety with common symptoms of anxiety and depression and with personality factors were examined with linear regression models. MANOVA was used to compare the mean level of pregnancy anxiety of the high risk groups and the level of fear of the low risk group.

4.4 Results

4.4.1 The factor structure of pregnancy anxiety

Exploratory factor analysis of the PRAQ-R data collected three times during pregnancy revealed five factors in each part of pregnancy, similar to those found by Van den Bergh (1990), with eigenvalues larger than 1. Since two of these factors (fear of changes in the relationship with the partner and fear of changes in one's own mood and problems in the mother-child relationship) accounted only for 5.6 % and 4.9 % of the total explained variance, respectively, a CFA was performed with only three factors despite the fact that the theoretical concept of the questionnaire included five factors. As a check, CFA was first run testing a model with five factors. The results showed a bad fit, with very high error variances (>.85) of the items of the last two factors, even if they were allowed to load on the three other factors. Then, the three-factor model was fitted to the data of early pregnancy. Several items were removed due to high error variances. The remaining model included three items for the factor *Fear of giving birth*, four items for the factor *Fear of bearing a physically or mentally handicapped child*, and three items representing the factor *Concern about one's appearance*. These items are shown in appendix I. The model showed a good fit to the data (χ^2= 32.48, df = 29, p = .30, RMSEA = .03, RMR= .05, CFI= .99, NNFI=.99) and is shown in Figure 4.1.

Figure 4.1 Best fitting model of the factor structure of the pregnancy-related anxiety questionnaire in early pregnancy.



Likewise, the PRAQ-R data obtained during mid and late pregnancy were tested with CFA, as an attempt to build a three-factor model. With data obtained during mid- pregnancy, a model was fitted similar to the one fitted with data from early pregnancy (χ^2= 44.63, df = 32,

p = .07, RMSEA = .05, RMR= .05, CFI= .98, NNFI=.98). With data derived during late pregnancy, again a three factor model of pregnancy anxiety was fitted (χ^2= 40.41, df = 32, p = .15, RMSEA = .04, RMR= .04, CFI= .99, NNFI=.98).

The internal consistency of the three-factor scores during each trimester of pregnancy, as reflected in Cronbach's alpha, proved to be quite sufficient. The consistency of *Fear of giving birth* varied between .79 and .83, that of F*ear of bearing a physically or mentally handicapped child* between .87 and .88, and that of *Concern about one's appearance* between .76 and .83.

4.4.2 Stability of the factor structure

To test for the stability of the factor structure of pregnancy anxieties in the course of gestation, we set the factor loadings of items equal in early, mid, and late pregnancy. In addition, measurement error of an item measured early in pregnancy was allowed to be correlated to the measurement error of the same item measured during mid and late pregnancy and so forth. Fit indices showed a good fit for the factors *Fear of giving birth* (χ^2 = 24.78, df=20, p=.21, RMSEA=.04, RMR = .05, CFI= .99, NNFI=.99) and for *Concern about one's appearance* (χ^2=23.12, df=20, p=.28, RMSEA=.03, RMR=.04, CFI=.99, NNFI=.99) and an adequate fit for Fear of bearing a physically or mentally handicapped child (χ^2= 66.88, df=44, p=.02, RMSEA= .06, RMR=.06, CFI= .97, NNFI=.96).

Another procedure to determine the stability of the factor structure is to calculate intercorrelation coefficients of the three factors from early, to mid, to late pregnancy. Pearson correlation coefficients between factor scores of *Fear of giving birth* derived at early, mid and late pregnancy were all significant and ranged between .69 and .78 (p< .0005). Likewise, the scores on the factor *Fear of bearing a physically or mentally handicapped child* were significantly intercorrelated between early, mid and late pregnancy (r= .62 - .73, p < .0005) as well as the scores on the factor *Concern about one's appearance* (r = .55- .75, p < .0005).

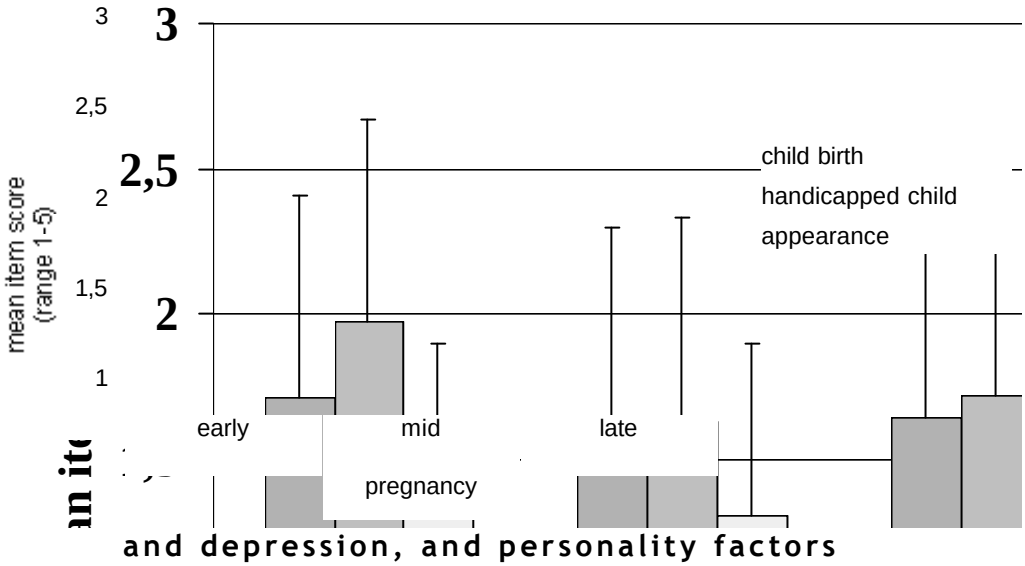
4.4.3 Change in the level of pregnancy anxiety in the course of pregnancy

MANOVA with repeated measures and polynomial contrasts was performed with two within- subjects factors: time and anxiety. These results are presented graphically in Figure 4.2. Significant main effects were found for the factor time (F= 4.71, df=2, p<.01), reflecting the changes in anxiety scores from early to late pregnancy, and for the factor anxiety (F=24.49, df=2, p< .0005), reflecting that within a certain time period mean item scores on the three different anxieties differed significantly. In addition, a significant interaction effect was found (F=2.65, df=4, p<.05). Thus, depending on the time period in pregnancy, differences could be found in the ranking of the anxiety scores.

Follow-up tests showed that *Fear of giving birth* and *Fear of bearing a physically or mentally handicapped child* had highest scores in early pregnancy, decreased in mid pregnancy and were elevated again in late pregnancy. In contrast, *Concern about one's appearance* remained rather stable throughout the course of pregnancy. In early pregnancy, *Fear of bear-*

ing a physically or mentally handicapped child was highest, followed by *Fear of giving birth*. *The Concern about one's appearance* scores were rather low, compared to the other two fears. In mid-pregnancy, both *Fear of bearing a physically or mentally handicapped child* and *Fear of giving birth* no longer differed significantly from each other on the mean item score. Both had significantly higher mean item scores than *Concern about one's appearance*. In late pregnancy the pattern was identical to the one found in mid-pregnancy.
Figure 4.2 Changes in level of pregnancy-related anxiety in the course of gestation.

4.4.4 Pregnancy anxiety, common symptoms of anxiety



and depression, and personality factors

To examine whether symptoms of general anxiety and depression and personality factors were associated with pregnancy anxiety, we performed a series of multiple stepwise regression analyses with the three factors of pregnancy anxiety as dependent variables. Predictors were trait and state anxiety, prenatal depression, neuroticism, locus of control indices, and primary and secondary appraisal of the pregnancy. Possible covariates such as maternal age and socio-economic status (SES) were first tested for their linear relationship with the dependent variables, by means of correlation and regression analyses. When found to be a significant covariate, this variable was entered in the regression analysis as a first block. The second block consisted of the predictor variables. Although SES was found to be correlated significantly with the dependent variables, in none of the regression analyses its contribution was significant. The results of the regression analyses are shown in Table 4.2.

Concerning early pregnancy, trait anxiety explained 17.9 % of *Fear of giving birth*. Neuroticism explained 20.4 % of *Fear of bearing a physically or mentally handicapped child*. In addition, secondary appraisal of the pregnancy explained another 7.5 % of this fear. External

locus of control explained 18.7% of the third factor, *Concern about one's appearance*, and primary appraisal of the pregnancy and state anxiety explained 6.8% and 6.1 % of the variance of this factor.

In mid-pregnancy, neuroticism explained 20 % of the variance of the *Fear of giving birth* and external locus of control (powerful others) an additional 4.8 %. Trait anxiety explained a significant part (37.2 %) of the variance in *Fear of bearing a physically or mentally handicapped child*. External locus of control contributed to another 6 % of the total variance and state anxiety contributed 6.9 %. *Concern about one's appearance* was explained partly by prenatal depression (18 %), with an additional contribution of 6.2 % by an external locus of control (powerful others).

In late pregnancy, prenatal depression explained a significant part of the F*ear of giving birth* (13.6 %) rather than trait anxiety or neuroticism. *Fear of bearing a physically or mentally handicapped child* was explained by trait anxiety (21.7 %). Trait anxiety explained 18.2% of the variance of *Concern about one's appearance*.

Table 4.2
Results of multiple regression analyses

Dependent variable	Predictors	R^2	F	Beta	Significance
Fear of child birth	*Early pregnancy*				
	X_1 = Trait anxiety	.18	8.36	.42	.0001
	Mid pregnancy				
	X_1 = Neuroticism	.20	19.71	.39	< .0001
	X_2 = Powerful others	.25	12.83	-.23	.0288
	Late pregnancy				
	X_1 = EPDS	.14	11.65	.37	.001
Fear of handicapped child	*Early pregnancy*				
	X_1 = Neuroticism	.20	9.85	.43	<.0001
	X_2 = Secundary appraisal	.28	9.79	.28	.0063
	Mid pregnancy				
	X_1 = Trait anxiety	.37	46.81	.24	<.0001
	X_2 = Powerful others	.43	29.63	-.33	.0004
	X_3 = State anxiety	.50	25.72	.37	.0017
	Late pregnancy				
	X_2 = Trait anxiety	.22	20.73	.47	<.0001
Concern about own appearance	*Early pregnancy*				
	X_1 = Powerful others	.19	17.88	-.41	<.0001
	X_2 = Primary appraisal	.25	13.12	-.25	.0104
	X_3 = State anxiety	.32	11.67	.25	.0110
	Mid pregnancy				
	X_1 = EPDS	.18	17.38	.32	.0044
	X_2 = Powerful others	.24	12.44	-.27	.0137
	Late pregnancy				
	X_1 = Trait anxiety	.18	16.66	.43	.0001

4.4.5 High-risk groups and pregnancy anxiety

To explore the clinical correlates of pregnancy anxiety, we performed MANOVA to compare pregnancy anxiety scores of high-risk and low risk samples as defined earlier.

Early pregnancy. The results showed that women with mental problems tended to have increased fear of bearing a physically or mentally handicapped child when compared to low risk women (10.1 versus 8.9; F=3.23, df=1, p=.074). Women with a previous miscarriage worried less about their appearance during early pregnancy than did women without a previous miscarriage (3.2 versus 4.0; F= 4.69, df=1, p=.032).

Mid-pregnancy. Analyses of the two risk groups showed that women with mental problems have increased fear of bearing a physically or mentally handicapped child when compared to the low risk group (7.4 versus 5.9; F=7.3, df=1, p=.008). Likewise, women with a previous miscarriage had an elevated level of anxiety of bearing a physically or mentally handicapped child when compared to low risk women (5.9 versus 7.4; F= 5.29, df=1, p =.025).

Late pregnancy. The results showed that women with mental problems had increased fear of bearing a physically or mentally handicapped child when compared to women without mental problems (7.4 versus 6.4; F=3.80, df=1, p=.050). With regard to pregnancy-related fears, women with a previous miscarriage no longer differed from women without such an obstetric history.

4.5 Discussion

The first aim of the study was to examine the structure of pregnancy anxiety. Using structural equation modelling, we were able to specify a measurement model of pregnancy anxiety. Starting with the PRAQ-R questionnaire which consisted of 34 items, only ten items were found to offer a good indication of pregnancy-related fears and worries. A three-factor model emerged in early, mid and late pregnancy. The stability of the three-factor model was tested and found to be good from early to late pregnancy. The low to moderate size correlations (.24 - .46) between factors indicated that they are not derived from a single underlying latent variable. In fact, three aspects of pregnancy related anxiety could be distinguished; 'Fear of giving birth', 'Fear of bearing a physically or mentally handicapped child', and 'Concern about one's appearance'. These findings need to be replicated in another and perhaps larger sample. They could form the basis of the development of a rather short questionnaire that would provide sufficient information about a pregnant woman's amount of pregnancy-related fears and could be used in obstetric practice or in research.

In contrast to prior studies which described the structure of pregnancy anxiety, this study used a prospective design and thus information was gathered during pregnancy, which avoided recall bias. This could explain the differences between our results and those of Levin (1991). In addition, the population in the study of Levin (1991) was rather different from ours; Anglo and Black and Hispanic women were possibly from a lower socio-economic status than our population. Moreover, Levin (1991) did not report on the parity of the women.

The second aim was to examine changes that occurred in the level of pregnancy anxiety in the course of gestation. We found that the amount of fear of giving birth decreased from early to mid pregnancy but thereafter remained stable. Fear of bearing a physically or men-

tally handicapped child was highest during early pregnancy, lowest during mid-pregnancy and increased from mid to late pregnancy. Concern about one's appearance was rather stable throughout pregnancy. Thus, the highest levels of pregnancy related anxieties were found in early and late pregnancy. During these periods there is also the highest risk for adverse effects on birth outcome and the development and behaviour of the child (Barker, 1995; Korelman & Schneibel, 1983; Otake & Schull (1984); Ravelli et al. 1998; Schneider et al. (1999); Sherman et al., 1985).

With regard to the third aim of this study, it was found that pregnancy anxiety can be differentiated from general anxiety and other personality characteristics. However, various personality factors were found to have effects on the amount of pregnancy anxieties reported. First of all, trait anxiety was significantly positively related to all pregnancy-related anxieties. The feeling of being in control of the course of pregnancy appeared to have an influence on the amount of pregnancy anxiety. Women who experienced the course of pregnancy as uncontrollable had increased fear of bearing a physically or mentally handicapped child, but they did not have increased fear of giving birth, nor did they have more worries about their appearance. Related to the appraisal of the pregnancy are factors that represent the locus of control a woman experiences. When she put her locus of control in powerful others (external locus of control), she showed less concern about her appearance during early pregnancy and displayed a surprisingly lower level of all pregnancy-related fears in mid-pregnancy. Perhaps, putting her trust in the medical staff or in other significant persons in her surroundings reduced her worries. This suggestion was also proposed by Sjögren (1997), who documented lack of trust in the obstetric staff as an important reason for anxiety about childbirth in a sample of extremely anxious pregnant women. Likewise, in the present study, primary appraisal of pregnancy was negatively related to the amount of concern about one's appearance during pregnancy. Furthermore, personality factors such as neuroticism and prenatal depression were found to be associated with increased fears at some time periods during pregnancy. All these factors together, however, only explained about 20-25 % of pregnancy anxieties during early and late pregnancy. Thus, the largest part of pregnancy anxiety was not explained by these factors and it is therefore concluded that pregnancy anxiety and general anxiety are different entities.

Mid-pregnancy appeared to differ in some respects from early and late pregnancy. During mid-pregnancy, 50 % of the fear of bearing a physically or mentally handicapped child could be explained by various personality factors. Neuroticism explained about 20 % of the fear of giving birth, whereas trait anxiety did not predict this fear at all. In other words, during mid-pregnancy pregnancy-related anxieties and personality factors were more related. The decreased levels of pregnancy anxieties during mid pregnancy could reflect the impression that during this period, women tend to worry less about their pregnancy and are more or less used to the changed situation in their lives. Therefore, it could well be that pregnancy-related anxieties can be less well differentiated from other personality factors during this period, due to the relative absence of the former. In this respect, it is interesting to notice that at the end of pregnancy, when the woman is more aware of the approaching delivery and of the period of life thereafter, only trait anxiety and prenatal depression explained a small part of the variance of pregnancy related anxieties (13.6 % - 21.7 %). Thus, when levels of pregnancy-related anxieties are increased, it is clear that they should be differentiated from more general anxieties and other personality factors.

The fourth aim of this study was to explore the clinical correlates of pregnancy anxiety. In our sample of normal nulliparous pregnant women, some women suffered from mental health problems for which they either had received psychological or psychiatric treatment in the past or were currently having therapy. These women had increased levels of fear of bearing a physically or mentally handicapped child from early pregnancy onwards. Further research is needed to clarify the relation between pregnancy-related anxieties and general anxiety in this group. Also, another risk group, women with a previous miscarriage, were found to worry less about bodily changes during early pregnancy than low risk women. Perhaps, the event of a miscarriage has led them to focus solely on the well-being of the child, instead of on their own physical well-being. During mid pregnancy, women with a previous miscarriage had increased levels of fear of bearing a physically or mentally handicapped child, when compared to low risk women, whereas the level of this fear was comparable to that of low risk women in late pregnancy.

In conclusion, our results show that pregnancy anxiety is a relatively distinctive syndrome that is different from general indices of anxiety and depression, at least during early and late pregnancy. Furthermore, pregnancy anxiety is not a single construct, but can be differentiated in several aspects. In a normal population of pregnant women levels of pregnancy-related anxieties change from early to late pregnancy. Authors of studies using anxiety during pregnancy as predictors of birth outcome or postnatal development should be aware of the specific fears related to pregnancy. Clinical significance and possibly harmful effects of pregnancy-related anxieties on birth outcome and postnatal development should be carefully examined in prospectively designed studies. In addition, when studying the development of psychopathology in women during or after pregnancy, one needs to take these normative changes in pregnancy related anxieties into account. Our results suggest that pregnancy itself should not be regarded simply as a common life event; it includes specific fears that might be increased in women with mental problems. An elevated amount of pregnancy-related anxieties could be a precursor of general anxiety syndromes or other psychopathology. More research is therefore warranted with regard to pregnancy-related anxieties in women who risk developing psychopathology during or after pregnancy.

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APPENDIX 1:

List of items included in the factors as derived with CFA:

Fear of giving birth:

Item 6: I am worried about the pain of contractions and the pain during delivery.
Item 20: I am anxious about the delivery because I have never experienced one before.
Item 21: I am worried about not being able to control myself during labour and fear that I will scream.

Fear of bearing a physically or mentally handicapped child:

Item 2: I am afraid the baby will be mentally handicapped or will suffer from brain damage.
Item 3: I am afraid our baby will be stillborn, or will die during or immediately after delivery.
Item 5: I am afraid that our baby will suffer from a physical defect or worry that something will be physically wrong with the baby.
Item 12: I sometimes think that our child will be in poor health or will be prone to illnesses.

Concern about one's appearance:

Item 7: I am worried about the fact that I shall not regain my figure after delivery.
Item 17: I am concerned about my unattractive appearance.
Item 26: I am worried about my enormous weight gain.

5

Coping in normal pregnancy

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Accepted pending revision

5.1 Abstract

Background: In high-risk populations (e.g. adolescents, substance abusers) coping strategies in pregnancy have been studied. Avoidance of the stressful situation and aggressive coping are frequently used and are often related to postnatal depression and other negative outcomes. Little is known about coping strategies used to deal with stress and the necesarry adjustments involved with normal risk pregnancy.

Objective: To examine the factor structure of the Utrecht Coping List-19 (UCL-19) in a sample of nulliparous normal risk pregnant women and to test the stability, change and predictors of coping strategies throughout pregnancy. The effectiveness of a particular coping strategy is examined by predicting reported pregnancy complaints.

Methods: The UCL-19 was filled out and self-report data about various aspects of personality (neuroticism, locus of control, depression, perceived stress), general characteristics (socioeconomic status, maternal age) and pregnancy complaints were collected in nulliparous women in early, mid- and late pregnancy.

Results: Two coping strategies were found with confirmatory factor analysis on the UCL-19: emotion-focused coping and problem-focused coping. The factor structure of the UCL-19 had a good stability throughout pregnancy. Some changes in emotion-focused coping and problem-focused coping scores were found, although the absolute differences were rather small. Most women (48.4%) favor emotion-focused coping during early pregnancy, whereas 33.2 % used more problem-focused coping and 18.4 % used both coping styles in equal amounts in this period of pregnancy. High educational level and low internal locus of control predicted high score on emotion-focused coping in this period of pregnancy ($F(2, 228) = 11.49$, $p < .005$, $R^2 = .22$). Emotion-focused coping was negatively and problem-focused coping was positively related to pregnancy complaints ($r = -.23$, $p < .05$ and $r = .25$, $p < .005$, respectively).

Conclusion: Coping in normal risk pregnancy is a process with small temporal variations. Emotion-focused coping is favored by most nulliparous pregnant women in early pregnancy and is most effective in reducing the number of reported pregnancy complaints.

5.2 Introduction

Coping strategies associated with stress provoking factors in pregnancy have been studied in high risk populations, such as homeless pregnant women (Killison, 1995), adolescent expectant mothers (Dukewich et al., 1996), pregnant substance abusers (Blechman et al., 1999), women fertilized by means of in vitro fertilization (Eugster & Vingerhoets, 1999; Lukse & Vacc, 1999) and women with antenatal fetal death (Nikcevic et al., 1998). Also, coping styles during pregnancy as predictors of postnatal depression have been thoroughly studied (Bifulco & Brown, 1996; Demyttenaere et al., 1995; Righetti-Veltema et al., 1998; Terry et al., 1996). Overall, these studies show that avoidance of the stressful situation (or disengagement coping) is often related to increased negative outcomes, such as postnatal depression and a lower pregnancy rate following IVF-procedures. In contrast, high self-esteem has been found to prevent women from developing postnatal depression. Substance abusers and adolescents use more aggressive coping, which does not reduce levels of distress.

On the population level, however, the majority of pregnant women fall within the normal risk category because they are exposed to about average levels of psychosocial stress. They have to make the necessary adjustments to changing life conditions due to pregnancy and expectant parenthood, while other potential stress provoking factors (e.g. life events, daily hassles, work stress) could cause distress and anxiety superimposed on the stress and concerns resulting from the event of pregnancy itself. The study of coping strategies in a normal risk pregnant population could offer more insight into the naturally occurring processes of coping during pregnancy. Moreover, it may be informative if coping strategies applied in normal risk pregnancy can be compared to the strategies used in high-risk pregnancy.

A basic distinction is usually made between emotion-focused and problem-focused coping. Emotion-focused coping is directed toward regulating affect surrounding a stressful encounter and typically includes expression of feelings to others, positive reappraisal of the situation and so on. In contrast, problem-focused coping is directed toward alleviating the circumstances which produce stress, and includes planning, information seeking and finding solutions for the problems. The distinction in emotion- and problem-focused coping should not be regarded as a dichotomy. That is, rather than being totally independent coping strategies, emotion- and problem-focused coping can both facilitate and impede each other in the coping process (Lazarus & Folkman, 1984). Another coping dimension, avoidance, is sometimes identified, and may include either person-oriented or task-oriented strategies. Avoidance is not a successful way of coping in a long-term perspective but may reduce stress levels at short term, by escaping the situation that causes stress.

The concept of coping was originally derived from the psychoanalytic ego psychological theory. In this model, coping was defined as: 'realistic and flexible thoughts and acts that solve problems and thereby reduce stress' (Lazarus & Folkman, 1984). Since this model of coping has dominated for decades, the emphasis on problem-focused coping as the most effective and, perhaps, most mature ego process is seen in many studies. In addition, research on the effectiveness of coping strategies has often been based on the presumed superiority of male-gender role behavior, thus promoting problem-focused coping styles that men use more frequently than women. Women, however, are generally believed to make greater use of social support as a coping strategy and therefore it could be that this kind of coping is more effective in women (Cameron et al., 1996). Many studies have failed to con-

sider more female styles of coping as potentially effective. Even in studies regarding maternal coping in pregnancy, thus per definition including female subjects, most authors tend to focus on problem-focused coping strategies. An exception is a recent study on the efficacy of emotion-focused and problem-focused group therapies for women with fertility problems (McQueeney et al., 1997). It appeared that emotion-focused coping resulted in greater improvements with regard to well-being and distress than problem-focused coping. This specific group of subjects had to deal with partly uncontrollable circumstances, which could explain why problem-focused coping is not successful in solving the problem and therefore has no clear effect on well-being and distress. These findings suggest that the context of an individual's life and the sort of event taking place are of importance in predicting the most effective coping strategy to be used to decrease levels of distress. This was also demonstrated by a study of Yali & Lobel (Yali & Lobel, 1999), in which the association between coping and pregnancy-specific distress in mid-pregnancy was examined in high medical risk women. Surprisingly, preparation for motherhood, a coping style that can be regarded as problem-focused coping, was also associated with increased pregnancy-specific distress. Since the subjects in this study were at high medical risk, they were under threat, and preparing for motherhood may have put their focus of attention on this threat and could therefore have increased their levels of distress. In general, when conditions of stress are appraised as changeable, problem-focused coping predominates. However, when the conditions are appraised as unchangeable, emotion-focused coping predominates (Lazarus, 1999). For these reasons, we used a questionnaire in the present study that could assess problem-focused coping, emotion-focused coping and avoidance. These three styles of coping were found after exploratory factor analysis in a general population, including both male and female subjects. Since our sample consisted of pregnant women, the first purpose of this study is to examine the factor structure of coping in a sample of normal risk pregnant women.

Another consequence of the psychoanalytical ego psychology approach of coping was that coping was viewed from a trait/style perspective for many years. That is, coping was presumed to remain stable over time or across conditions. An alternative view is offered by Lazarus (Lazarus & Folkman, 1984; Lazarus, 1999), which defines coping as a process. A key principle of this process-model of coping is that the choice of coping strategy will usually vary with the adaptational significance and requirements of each threat and its context, which will change over time. As pregnancy is a process itself with changing demands in the course of gestation, it is expected that the choice of coping strategies will change throughout pregnancy. In addition, certain personality dispositions or traits, such as age, socio-economic status, neuroticism, internal or external locus of control or depression can influence coping styles. These variables were examined in the present study as potential predictors of coping strategies. The health-related significance of using an effective coping strategy in pregnancy may be a reduction in reported pregnancy complaints, such as nausea, changes in appetite, backache, reduced concentration, emotional lability and so on. Therefore, we tested if a particular coping style was negatively related to reported pregnancy complaints.

In summary, the present study aims to examine:

1. the factor structure of a coping questionnaire in a sample of normal risk pregnant women;
2. the stability and change of coping strategies in the course of gestation;
3. predictors of coping strategies used throughout pregnancy;
4. the effect of coping strategies on reported pregnancy complaints.

5.3 Methods

5.3.1 Participants

All the participants in this study were included in a larger prospective longitudinal project which also investigated the influence of prenatal psychosocial factors on fetal behaviour and on the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Centre Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of approximately 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the study. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants did not differ from those of non-participants, except in the case of women with full-time jobs, who were less likely to participate. The descriptives of the participants are summarized in Table 4.1. As shown, the sample of participants consisted largely of middle class women, although both lower social and higher social classes were represented. The majority of women (92.4%) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job, 54.2 % working less than 38 hours a week and 45.8 % working full-time.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion. The main reason for the drop in the number of participants towards late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, twin pregnancy, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

5.3.2 Questionnaire measures

The package of questionnaires was composed to measure, among others, coping behavior and aspects of personality such as neuroticism, locus of control, prenatal depression and primary and secondary appraisal of pregnancy.

Utrecht Coping List. To assess the coping style of our subjects, we used the 'Utrecht Coping List-19' (Schreurs et al., 1988), which is an abbreviated form of the 'Utrecht Coping List-30'. It contains 19 items to be answered at a 5-point scale, which have been found to be dispersed over 3 factors: 'problem-focused coping', 'emotion-focused coping' and 'avoidance'. The first two factors consisted of 5 items, whereas the third factor contained 4 items. Thus,

5 items of the questionnaire were not used in calculating these factor scores. This questionnaire was filled out in early, mid- and late pregnancy and women were asked to describe their coping style during that particular time period of pregnancy. The factor structure of the questionnaire was reanalyzed in this study on pregnant women.

State-Trait Anxiety Inventory (STAI). The STAI (Spielberger et al., 1970) is comprised of two self-report scales for measuring two distinct anxiety concepts, state-anxiety and trait-anxiety. Both scales contain 20 statements that ask the respondent to describe how they feel at a particular moment in time (state-anxiety) or how they generally feel (trait-anxiety). State-anxiety is conceptualized as a transitory emotional state, whereas trait anxiety refers to relatively stable individual differences in anxiety proneness. In the present study only the trait-anxiety score was examined as predictor of coping style. Cronbach's alpha in this study was .83 for trait anxiety. The STAI was filled out on each occasion.

Edinburgh Postnatal Depression Scale (EPDS). The EPDS (Cox et al., 1987) is a 10-item questionnaire that can be used to measure prenatal and postnatal depression and has been validated for use in pregnancy (Green & Murray, 1994). The EPDS was filled out twice during pregnancy (mid and late pregnancy) and Cronbach's alphas in this study were .86 and .87, respectively.

Neuroticism was determined with a subscale of the Amsterdam Biographical Questionnaire (Wilde, 1963). This questionnaire was filled out only during early pregnancy because it is believed to reflect a stable personality trait. Cronbach's alpha of the items of the subscale Neuroticism was .83.

Locus of control was measured by means of the items with the highest factor loadings on the subscales of the Internal locus of control, Powerful Others and Chance-Scale (IPC; Brosschot et al., 1994), with two items reflecting the Powerful Others scale, two items representing the Internal locus of control scale and two items for the Chance scale. Both Powerful Others and Chance reflect external locus of control. Powerful Others means that an individual believes that powerful others are in control of one's life, whereas the Chance scale reflects the idea that the world is unordered and unpredictable, and is thus controlled by chance. Internal locus of control is found in individuals who believe they have their own life under control. Since locus of control is believed to reflect a stable characteristic the questionnaire was filled out only once during early pregnancy.

Appraisal of pregnancy was measured by two single-item instruments. The perceived threat of the situation, or primary appraisal, was measured by the question 'Can you indicate on a ten-point scale the degree to which your pregnancy relates to the most upsetting (=1) and most pleasant event (=10) of your life?' Secondary appraisal, or the perceived options to control the situation, was assessed with the question 'To what extent do you think you are able to influence the course of your pregnancy?' Participants could answer on a 5-point scale ranging from 'much' to 'not at all'. These two items were answered on each occasion.

Socioeconomic status. Two aspects of social economical status were assessed; educational level and professional level (Westerlaak et al., 1976). A stratification was provided for educational level, ranging from primary school ('1') to university level ('7') and for professional level, ranging from unschooled jobs ('1') to jobs on academic level or jobs with very high responsibilities, such as senior managers ('7'). In table 1 an aggregated descriptive frequency score of the total sample is provided.

Perceived stress. Perceived stress was assessed by means of a Dutch translation of the

Perceived Stress Scale of Cohen & Williamson (1987). It contains 14 items on perceived stress of an individual during the preceeding four weeks to be answered on a 4-point scale, ranging from 'never' to 'always'. Items assess the degree to which respondents perceive their lives as unpredictable, uncontrollable, and burdensome. This questionnaire was filled out on each occasion.

Pregnancy complaints. Pregnancy complaints were assessed with a self-developed questionnaire, containing 33 items that include various physical and psychological complaints that may be associated with pregnancy. The items are given in Appendix B. Items were answered on a 4-point scale, ranging from 'never' to 'very often' and a total score was calculated. This questionnaire was filled out twice during pregnancy (mid and late pregnancy).

5.3.3 Statistical analysis

The factor structure of the UCL-19 was examined by means of exploratory and confirmatory factor analysis (CFA), using SPSS version 6.1 for Windows and LISREL 8.30, respectively. CFA can be performed by means of structural equation modelling and postulates relations between the observed measures and the underlying factors a priori. The goodness of fit between the hypothesized structure and the sample data is subsequently tested. This provides information on the reliability and validity of the model while taking measurement errors into account. Goodness of fit measures used are for instance Chi Square (χ^2) and Chi-square divided by degrees of freedom. Chi-square with degrees of freedom is sensitive for sample size, and is therefore regarded as a measure of fit instead of a test statistic. When chi-square is divided by its degrees of freedom it should be smaller than 3 to indicate a reasonable fit to the data. Other fit criteria include: Comparative Fit Index (CFI ; >.9 indicates a good fit), Non-Normed Fit Index (NNFI; ; >.9 indicates a good fit), Root Mean Square Error of Approximation (RMSEA) which should be at least smaller than .08 and Root Mean Square Residual (RMR) which should be smaller than .05.

In a next step, the stability of the factors representing coping styles throughout pregnancy was examined by means of LISREL tests for stability of factor loadings and with Pearson intercorrelation coefficients. The change in level of coping behavior throughout pregnancy was examined with ANOVA with repeated measures.

The associations of coping behavior with common symptoms of anxiety and depression and with personality factors were examined with stepwise linear regression models. Correlations were calculated between coping scores and the total pregnancy complaints scores. Finally, exploratory analyses with t-tests were performed to test for differences in mean scores on anxiety and neuroticism in women with high levels of emotion-focused coping as compared to women with low levels of this coping strategy.

5.4 Results

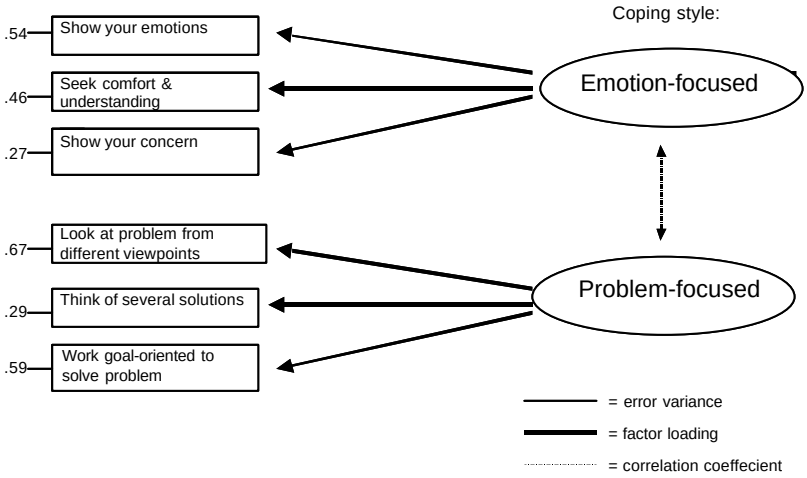
5.4.1 The factor structure of coping

Exploratory factor analysis with varimax rotation of the coping questionnaire filled in in early pregnancy resulted in 5 factors with eigenvalues greater than 1 and explained 58.3 % of the total variance. However, two of these factors consisted of only two items each, and showed only a small contribution to the total explained variance (7.3 % and 6.3 %, respectively, with eigenvalues of 1.39 and 1.19); these two factors were considered as not meaningful. Therefore, we used a three-factor solution in our conceptual model as suggested by Schreurs (1996). 'Emotion-focused coping' contained 5 items, had an eigenvalue of 3.83 and contributed 20.1 % to the total explained variance. 'Problem-focused coping' consisted of 4 items, had an eigenvalue of 2.73 and contributed 14.3 % to the total explained variance. 'Avoidance' consisted of 4 items, had an eigenvalue of 1.96 and contributed 10.3 % to the total explained variance. This solution showed minor deviations from the proposed factor solution by Schreurs.

The suggested factor scores in our first conceptual model were tested with confirmatory factor analysis in LISREL.This model did not fit the data well (χ^2 = 257.57, df = 101, p < .0001, RMSEA= .080, RMR = .077, CFI= .83, NNFI=.80). Several items of the three proposed factors showed high error variances (.80 - .88) and were removed in the next model, including all items of the factor 'avoidance'. Therefore, the remaining model only included two factors: 'emotion-focused' and 'problem-focused' coping. This model fitted the data best (χ^2= 12.24, df = 8, p = .14, RMSEA= .05, RMR = .04, CFI= .99, NNFI=.98) and can be theoretically explained. The model is presented in Figure 5.1.

Figure 5.1. Best fitting model of the factor structure of the UCL-19 in early pregnancy.

The same procedure was followed with data of the UCL-19 questionnaire collected in mid



and late pregnancy. The results showed factor structures, similar to the one found in early pregnancy in mid (χ^2= 9.06, df = 8, p = .25, RMSEA= .04, RMR = .04, CFI= 1.00, NNFI=.99) and late pregnancy (χ^2 = 14.08, df = 8, p = .08, RMSEA= .07, RMR = .05, CFI= .99, NNFI=.97).

The rather low correlation coefficients (.20, .18 , and .26 at the three occassions, respec-

tively) between the total scores of emotion-focused coping and problem-focused coping reflect that these two coping styles are rather independent.

Cronbach's alphas for the factor emotion-focused coping fell within the range of .78 - .80 whereas the Cronbach's alphas for problem-focused coping were .71, .84, and .87 for early, mid and late pregnancy, respectively.

5.4.2 Stability of factor structure

Stability of the CFA model and its factor loadings across time was tested with LISREL. Factor loadings of items were set equal in early, mid and late pregnancy. In addition, the measurement error of an item assessed during early pregnancy was allowed to correlate with the measurement error of the same item determined during mid and late pregnancy. Fit indices showed an excellent fit for the stability of the factor loadings of the items of 'emotion-focused coping' (χ^2=21.01, df=20, p= .40, RMSEA= .017, RMR = .045, CFI= 1.00, NNFI=.99) and for the items of 'problem-focused coping' (χ^2=17.50, df=20, p= .62, RMSEA< .0001, RMR= .040, CFI= 1.00, NNFI=1.00).

Another procedure to determine the stability of the factor structure is to calculate intercorrelation coefficients of the two factors from early to late pregnancy. Intercorrelations for emotion-focused coping during the course of pregnancy were significant, varying from .52 to .62 ($p < .0005$). Scores derived at early, mid and late pregnancy on problem-focused coping were also significantly intercorrelated (. 53- .65; $p < .0005$).

5.4.3 Changes in coping behavior during pregnancy

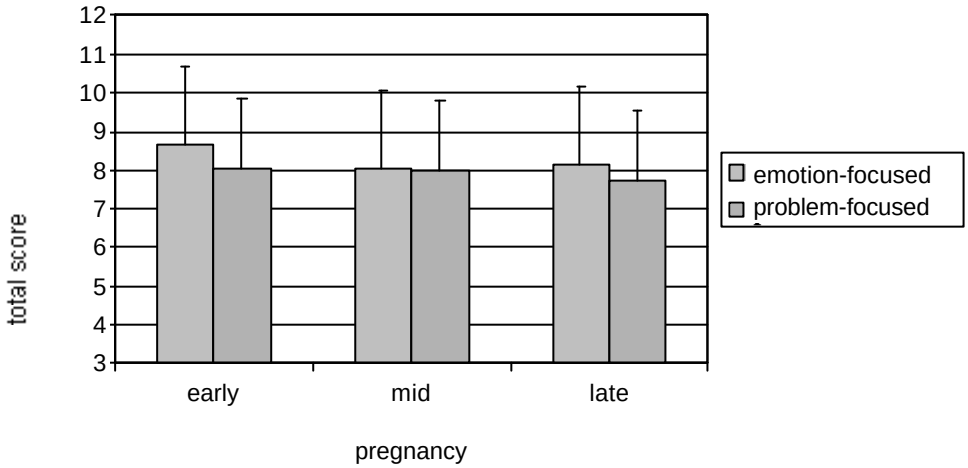
ANOVA with repeated measures, two within-subject factors (time and coping style) and polynomial contrasts was performed to examine the changes in mean scores of both emotion-focused coping and problem-focused coping from early to late pregnancy. These results are graphically presented in Figure 5.2. A significant main effect was found for the factor time ($F(2,172)=10.51$, df=2, $p<.0005$). Univariate analyses showed that emotion-focused coping changed over time ($F(2,172)=11.75$, df=2, $p<.005$) with highest scores in early pregnancy as compared to mid and late pregnancy. The scores derived in mid and late pregnancy did not differ significantly. Problem-focused coping also changed over time ($F(2,172)=4.76$, df=2, $p=.010$) with highest scores during early and mid pregnancy as compared to late pregnancy. Scores in early and mid pregnancy were comparable. Thus, scores on both emotion- and problem-focused coping styles were decreased in the last part of pregnancy, although the decrease in scores was rather small. A significant main effect of the factor 'coping style' was not found. However, a significant interaction effect of time by coping style was found ($F(2,172)=5.43$, df=2, $p<.005$). Post hoc analyses (t-tests) showed that in early pregnancy women used emotion-focused coping more frequently than problem-focused coping (t= 4.22, df= 230, $p<.0005$). In mid pregnancy both coping strategies were used equally frequently. In late pregnancy, emotion-focused coping was slightly favored, although this difference was not significant (t= 1.81, df= 170, p=.07).

A difference score was calculated in each period of pregnancy by subtracting the score on

problem-focused coping from the score of emotion-focused coping. A negative difference score of a subject reflected that problem-focused coping was favored, whereas a positive difference score reflected that emotion-focused coping was preferred. The frequencies of these differences scores were then calculated. In early pregnancy, 33.2 % of women preferred problem-focused coping, 48.4 % preferred emotion-focused coping and 18.4 % used both coping styles in equal amounts. In mid pregnancy, 41.7 % of women preferred problem-focused coping, 38.9 % preferred emotion-focused coping and 19.4 % used both coping styles in equal amounts.

In late pregnancy, 33.3 % of women preferred problem-focused coping, 46.4 % preferred emotion-focused coping and 20.1 % used both coping styles in equal amounts.

Figure 5.2. Changes in coping scores in the course of gestation. The error bars reflect the standard deviations.



5.4.4 Correlates of coping style

Significant positive correlation coefficients were found between emotion-focused coping style and educational level (r=.29, p < .001), age of the mother (r=.20, p=.003), primary appraisal of her pregnancy (r=.16, p=.036), and external locus of control (r=.19, p=.05). Significant negative correlation coefficients were found between emotion-focused coping and neuroticism (r= -.22, p=.021), internal locus of control (r= -.31, p=.001), and depression (only in mid pregnancy; r= -.17, p=.012). For problem-focused coping, positive correlates were also educational level (r=.21, p=.002), primary appraisal of the pregnancy (only in early pregnancy; r=.15, p=.041), and depression (only in mid pregnancy; r = .24, p=.001). No negative correlates of problem-focused coping were found.

To examine if any of these personality factors could predict the score on emotion- or

problem-focused coping in a multivariate model, a set of stepwise multiple regression analyses was performed. These results are presented in table 5.1. The results showed that for emotion-focused coping in *early pregnancy*, internal locus of control (13 %; Beta = -.33) and educational level (9 %; Beta= .29) explained significant parts of the variance. In *mid pregnancy*, educational level (17%; Beta= .39), maternal age (5%; Beta= .20), and depression (4%; Beta = -.28) explained part of the variance, whereas in *late pregnancy*, educational level (18%; Beta= .38), and age (5%; Beta= .24) accounted for some variance of emotion-focused coping. For problem-focused coping, only educational level was found to explain part of the variance in *early and mid pregnancy* (6 % and 8 %; Beta's .23 and .28, respectively). In addition to the variance explained by educational level (16%; Beta= .36), secondary appraisal, that is, the appraised uncontrollableness of the course of pregnancy, contributed for 7 % (Beta= -.26) of the explained variance of problem-focused coping in *late pregnancy*. This latter effect was negative, i.e., women who regarded their pregnancy as uncontrollable, have lower scores on problem-focused coping. Likewise, in early pregnancy a negative effect of internal locus of control on emotion-focused coping was found, whereas in mid pregnancy prenatal depression resulted in decreased levels of emotion-focused coping.

Dependent variable	Predictors	R^2	DR^2	F	Beta	Significance
Emotion-focused coping	*Early pregnancy*					
	X_1 = Internal locus of control	.13	.13	12.87	-.33	.0006
	X_2 = Educational level	.22	.09	11.49	.29	.004
	Mid pregnancy					
	X_1 = Educational level	.17	.17	17.88	.39	.0001
	X_2 = Maternal age	.22	.05	12.29	.20	.03
	X_3 = Depression	.26	.04	9.98	-.28	.04
	Late pregnancy					
	X_1 = Educational level	.18	.18	14.43	.38	.001
	X_2 = Maternal age	.23	.05	9.89	.24	.04
Problem-focused coping	*Early pregnancy*					
	X_1 = Educational level	.06	.06	4.80	.23	.03
	Mid pregnancy					
	X_1 = Educational level	.08	.08	6.68	.28	.01
	Late pregnancy					
	X_1 = Educational level	.16	.16	12.73	.36	.002
	X_2 = Secundary appraisal	.23	.07	9.58	-.26	.04

Table 5.1 **Results of multiple regression analyses**

5.4.5 Profile of women using emotion-focused coping in early pregnancy

The results suggested that almost 50 % of the women prefer to use emotion-focused coping in early pregnancy. Moreover, the personality factors predicting a higher score on this coping strategy reveal that older women who were better educated and had less depressive symptoms use emotion-focused coping more frequently than younger, less educated women suffering from more depressive symptoms. Therefore, we performed exploratory analyses by means of t-tests to get an indication of the general psychological profile of women using emotion-focused coping. Two groups were formed based on a median split method.

Results of these t-tests showed that women scoring above the median on emotion-focused coping differed from women scoring below the median on several aspects; neuroticism (36.1 versus 48.4, p=.006), state-anxiety (30.6 versus 33.8, p=.005) and trait-anxiety (33.6 versus 36.5, p=.033). Thus, women who used emotion-focused coping more frequently were less anxious and neurotic as compared to women who used this coping style less often. In addition, exploratory analysis showed that the amount of perceived stress was lower in women with a rather high emotion-focused coping style when compared to women with a low amount of emotion-focused coping (26.6 versus 29.4, p=.001).

5.4.6 Effect of coping style on pregnancy complaints

Correlation coefficients between emotion-, and problem-focused coping scores and a total score on pregnancy complaints in mid- and late pregnancy were calculated. Emotion-focused coping in early pregnancy was negatively related to pregnancy complaints in late pregnancy ($r = -.23$, $p < .05$). In mid-pregnancy emotion-focused coping was negatively related to pregnancy complaints in mid and late pregnancy ($r = -.17$, $p<.05$ and $r= -.23$, $p<.05$, respectively), whereas problem-focused coping in mid pregnancy was positively related to pregnancy complaints in mid pregnancy ($r= .25$, $p <.005$).

5.5 Discussion

With regard to the first aim of the study, two important factors could be found when using the UCL-19 questionnaire in a sample of nulliparous pregnant women. These factors represent emotion-focused coping and problem-focused coping. Each aspect of coping was found to be represented by only three items, which is a major advantage for future practical use. The items of the factor 'avoidance coping' of the UCL-19 had very high error variances in the present sample of pregnant women, and this factor was therefore excluded from the model. When we compare the contents of the emotion- and problem-focused coping factor scores with the various coping strategies represented in the Ways of Coping Questionnaire-Interview of Lazarus & Folkman (1984) it is obvious that only limited aspects of coping were assessed with the UCL-19. In the questionnaire developed by Lazarus and Folkman, more detailed aspects of the concept of emotion-focused coping can be obtained (e.g. 'seeking social support', 'positive reappraisal' etc.). Also, problem-focused aspects of coping were differentiated

in, for instance, 'confrontive coping' and 'planful problem solving'. The items of the factor solution of the UCL-19 resemble 'seeking social support' (emotion-focused coping) and 'planful problem solving' (problem-focused coping). We therefore suggest to examine the validity of the Ways of Coping Questionnaire-Interview in pregnant women in future studies, and to perform confirmatory factor analysis. Moreover, a replication of the factor structure of the UCL-19 in an independent sample of normal risk pregnant women is warranted. Nevertheless, we felt confident in using both factor scores of the UCL-19 in further analyses, since the internal consistency was found to be sufficient and the CFA results showed an excellent fit to the data.

The second purpose of the present study was to examine stability and change in coping strategies used throughout pregnancy. The stability of the factor structure of the UCL-19 turned out to be good. Some changes in total scores on emotion-focused coping style were found in the course of gestation, although the differences in scores from early to late pregnancy are rather small. Early pregnancy appeared to be the period during which most women depend on emotion-focused coping. The event of pregnancy and all its associated expectations and changes for the future represented a situation that pregnant women wanted to discuss with their partner or close friends. Especially in nulliparous women, pregnancy involves many necessary adaptations and involve a novel siuation that could give rise to the need to ventilate emotions, since these women have no prior experience with pregnancy. In mid pregnancy, women could be more or less adapted to the changed life situation, thus the need for emotion-focused coping could have been decreased. This is exactly what we found in the present study. Problem-focused coping strategies were also mostly used in early pregnancy with declining scores in the course of gestation. These findings fit well into the concept of coping as a process as described by Lazarus (1999). Overall, however, women favor emotion-focused coping instead of problem-focused coping in this period of pregnancy, although individual differences exist. It is concluded that the coping process in pregnancy showed small temporal variations and since the situational demands during pregnancy are changing, and as a result coping appeared to change as well.

The third aim of the present study was to examine the predictors of coping strategies used throughout pregnancy. In early pregnancy, educational level and internal locus of control were found to predict the score on emotion-focused coping. Women with higher educational level used this coping style more frequently than pregnant women who were less well educated. Women with internal locus of control were found to use less emotion-focused coping. Since these women tend to believe that they are or should be in control of their lives, they probably feel less need to ventilate their emotions with family or close friends. In mid pregnancy, higher educational level, less depressive symptoms and older maternal age predicted higher scores on emotion-focused coping. In late pregnancy, again older age and higher educational level resulted in higher levels of emotion-focused coping. These results suggest that in normal risk pregnancy, highly educated women who have more life-experience because of an older age and who do not suffer from depressive symptomatology employ an emotion-focused coping style. The psychological profile of women using this coping style more frequently throughout pregnancy suggests that these women were less anxious and neurotic and could therefore have a more stable personality. With regard to problem-focused coping, women with higher educational level have higher scores on this coping strategy as compared to women with less education. Other personality factors (such as internal locus of control,

maternal age and primary appraisal) accounted for some variation in emotion-focused coping and only secondary appraisal contributed significantly to a part of the explained total variance of problem-focused coping. In other studies (Lazarus & Folkman, 1984; Lazarus, 1999) it has been found that in situations that are perceived as unchangeable, emotion-focused coping predominated. The findings of the present study show that problem-focused coping declined in these situations, although emotion-focused coping did not increase. Overall, women with higher educational level had higher scores on either coping style. It might be that women who were less well educated used other coping styles, such as avoidance, instead. Since the UCL-19 did not provide a good and reliable factor score of this specific coping style in pregnant women, we were not able to test for this effect. Further research is therefore warranted with regard to other aspects of coping in a normal risk population of pregnant women.

The last aim of this study was to test the effectiveness of emotion- and problem-focused coping on reducing pregnancy related health and psychological complaints. Our results suggest that emotion-focused coping was able to reduce these pregnancy complaints, whereas problem-focused coping in mid pregnancy was associated with more pregnancy complaints. Thus, emotion-focused coping is the most effective coping style for normal risk pregnant women with regard to preventing the occurence of pregnancy complaints.

The findings of the present study show that coping in pregnancy is a process that changes across time, perhaps due to changing demands. From exploratory analysis it appeared that women high on emotion-focused coping had less perceived stress than women low on this coping score. Since stress in pregnancy has been associated with adverse birth outcome, the role of coping with stressors in pregnancy offers an interesting topic of research. This study shows that an effect of coping on health complaints could be found. Perhaps, coping has an effect on the emotional response to stress provoking factors as well. We will therefore investigate the effectiveness of the coping strategy used by pregnant women and the role of coping as mediator in more detail using longitudinal data of the present project that will include measures of stress provoking factors and distress.

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6

Does coping mediate the effects of stress in pregnancy?

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Submitted

6.1 Abstract

Background: The process view of coping suggests that coping may mediate the effect of a stressor on distress. In pregnancy, several specific anxieties and the primary and secondary appraisal may be regarded as stressors besides more common stress-provoking factors such as life events and daily hassles.

Objective: To investigate the role of coping as a mediator in a multidimensional model of distress in a population of normal risk nulliparous pregnant women.

Methods: Self-report data about various aspects of stress-provoking (life events, daily hassles, appraisal of pregnancy and pregnancy-specific anxieties), coping style (Utrecht Coping List-19; emotion-focused coping and problem-focused coping), and stress-resulting (perceived stress, state-anxiety and general psychological well-being) factors were collected in nulliparous pregnant women in early (n=230), mid (n=217) and late (n=172) pregnancy. Path analysis was performed by means of LISREL 8.30.

Results: Coping had mostly direct effects on the distress level, rather than having a mediating role between stressors and the distress response. An exception was found in early pregnancy, when the stress response to primary appraisal of pregnancy was mediated by emotion-focused coping. The best model fit in early pregnancy showed that emotion-focused coping was most effective in reducing levels of distress (χ^2 = 12.45, df=14, p=.57, RMSEA < .05, RMR= .04, CFI=1.00, NNFI=1.02), whereas later in pregnancy problem-focused coping was related to a reduction in distress levels (χ^2 = 13.51, df=10, p=.20, RMSEA = .05, RMR= .03, CFI=.99, NNFI=.98).

Conclusion: Coping is a process that responds to the specific demands of a particular period in normal risk pregnancy. Coping may be regarded as a part of a multidimensional model of distress in pregnancy.

6.2 Introduction

Pregnancy is an important period during which the mother and the fetus are potentially exposed to the negative impact of stress (Cameron et al., 1996). It furthermore entails many life adjustments which are to be dealt with. Many pregnant women report distress during pregnancy, including worries over physical changes, medical problems, and parenting competence (Lederman, 1984; Lobel, 1998; Arizmendi & Affonso, 1987). Moreover, distress during pregnancy has been found to be associated with an adverse birth outcome. Studies on this issue, however, differ in the conceptualization and measurement of prenatal stress, which could account for the inconsistency in findings across studies (for reviews see e.g. Levin & DeFrank, 1988; Lederman, 1995; Paarlberg et al. 1995; Omer & Everly, 1988; Hoffman & Hatch, 1996). In short, some studies have looked at the effect of major life events on birth outcome (e.g. Berkowitz & Kasl, 1983; Newton et al., 1979), while others have used a score on an anxiety questionnaire as conceptualization of stress (Beck et al., 1980). More recently, the effect of daily hassles on birth outcomes has been a topic of research (e.g., Paarlberg et al., 1999).

Stress is presumably most accurately described through a multidimensional concept, with the model of Lazarus & Folkman (1984) as a useful theoretical starting point. In this model a differentiation is made between stress-provoking factors (e.g., life events, daily hassles), stress-mediating or -moderating factors (e.g., coping, social support) and stress-resulting factors (e.g., perceived distress). Although some studies (Newton & Hunt, 1984; Magni et al., 1986) have used anxiety measures, personality factors and life events scores of the same subjects as predictors of birth outcome, these factors were regarded as independent predictors and were not combined in a multidimensional model of prenatal stress. A first attempt to describe prenatal stress as a multidimensional concept was undertaken by Lobel and Dunkel-Schetter (1990) in a study among socioeconomically disadvantaged pregnant women. Structural equation modeling techniques were used to test a single construct of prenatal stress underlying the environmental (life events), perceptual (event distress and perceived distress), and response-based (state anxiety) indicators of stress. The results indicated that stress could be predicted by event distress, state anxiety and perceived stress suggesting that appraisal and emotion may be the central issue to one component of distress (Lobel and Dunkel-Schetter, 1990). These authors did not include environmental conditions (stress-provoking factors) or the mediators or moderators such as coping, social support and personality factors in their model. In fact, the model found consisted only of the stress-resulting part of stress and is therefore unidimensional rather than multidimensional in our view. More recently, Sheehan (1996; 1998) used structural equation techniques to develop a psychosocial measurement model for stressful life events during pregnancy. Various life events and other experiences that were regarded as potentially stressful could be clustered in three factors: economic stressors, social support and family stressors (Sheehan, 1996). These three factors appeared to exert an indirect effect on birth weight rather than a direct effect (Sheehan, 1998). Although these studies provided insight into the relationships between stress provoking factors and a potential mediating factor (social support) on birth outcome, the influence of important stress provoking factors such as daily hassles or other stress mediating factors like coping were not taken into consideration.

Coping style in particular should be taken into account as a possible mediator when study-

ing the relationship between stressors and distress. This is in accordance with a process view of coping, as has been strongly endorsed by Lazarus (1999). Lazarus considered coping to be a mediator rather than a moderator because the coping process arises *de novo* from the transaction between the person and the environment. As has been outlined by Baron and Kenny (1986), a mediator represents the generative mechanism through which the independent variable is able to influence the dependent variable of interest and that, in statistical terms, accounts for the relation between the independent and dependent variables. In contrast, a moderator modifies the strength of the relation between the independent and dependent variables. To test if coping behavior functions as a mediator, it should explain how daily hassles or life events lead to perceived stress, in a similar way as has been found that coping explained a significant amount of the variance in several emotions in a sample of middle-aged women (Folkman and Lazarus, 1988).

An important factor in the stress-coping process is the appraisal of an event, which can be differentiated in two aspects. Firstly, primary appraisal reflects the perceived stressfulness of an event. Secondly, secondary appraisal is the perceived coping options for the event. Both forms of appraisal are supposed to interact (Komproe et al., 1997). Secondary appraisal is in fact an evaluation of coping options and most often the cognitive underpinning of coping. Appraising pregnancy as a pleasant event should provoke fewer stress reactions than viewing pregnancy as an unpleasant and stressful event. Likewise, regarding pregnancy as uncontrollable would result in more distress. Coping may in fact mediate the stress response to the primary and secondary appraisal of pregnancy.

Pregnancy not only is a specific event that may be appraised on an individual basis, it also may result in specific stressors and fears associated with pregnancy itself (DiPietro et al., 2000; Huizink et al., 2000a). Results showed that pregnancy anxiety can be regarded as a relatively distinctive syndrome that is different from general indices of anxiety and depression. Therefore, these pregnancy-related anxieties may provoke a more general stress response.

In an earlier study of the same sample of subjects, we performed confirmatory factor analyses on a coping questionnaire and found two different coping concepts: emotion-focused coping and problem-focused coping (Huizink et al., 2000b). These concepts resembled the 'seeking social support' and 'planful problem solving' scales of the Ways of Coping Questionnaire of Lazarus and Folkman (1984). In the present study, we have used these two concepts as potential mediators in the stress process.

Although research has been carried out on the role of coping in high risk pregnancy (e.g., Killison, 1995; Dukewich, Borkowski & Whitman, 1996; Blechman, Lowell & Garrett, 1999; Eugster & Vingerhoets, 1999; Lukse & Vacc, 1999), the present study is, to our knowledge, the first study to examine the role of coping in a multidimensional model of distress in normal pregnancy. The main questions to be examined were whether (1) coping mediates the distress response when exposed to daily hassles and life events during pregnancy, and (2) coping mediates the distress response to the appraisal of pregnancy and to pregnancy-related anxiety. In spite of the fact that several studies found correlations between coping and distress, this does not necessarily mean that coping influences the degree of distress. The relation may also be the other way around, with distress having an influence on the way people cope. Therefore, as a third question the alternative possibility will be examined: (3) Is coping a response to distress?

6.3 Methods

6.3.1 Participants

All the participants in this study were deliberately included in a larger prospective longitudinal project which also investigated the influence of prenatal psychosocial factors on fetal behavior and on the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of approximately 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the study. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants did not differ from those of nonparticipants, except in the case of women with full-time jobs, who were less likely to participate. The descriptives of the participants are summarized in Table 4.1. As shown the sample of participants consisted largely of middle class women, although both lower social and higher social classes were represented The majority of women (92.4%) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job, 54.2 % working less than 38 hours a week and 45.8 % working full-time.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion. The main reason for the drop in the number of participants toward late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, twin pregnancy, stillbirth, pregnancy complications that required intensively follow up, or relocation to another city.

6.3.2 Questionnaire measures

The package of questionnaires was composed principally to measure stress related factors, including stress provoking factors such as life events and daily hassles, stress resulting factors such as perceived stress or distress, coping behavior and common symptoms of anxiety and depression, and aspects of personality such as neuroticism and locus of control which may be important as predictors of coping behavior.

6.3.2.1 Stress-provoking factors

Life events Questionnaire (Vragenlijst meegemaakte gebeurtenissen, van de Willege et al., 1985). The life events impact score of this questionnaire was used, which was based on the Social Readjustment Rating Questionnaire (Holmes & Rahe, 1967).

Daily Hassles (Alledaagse Problemen Lijst, Vingerhoets et al., 1989). The daily hassles questionnaire used in this study is a Dutch translation of a selection of items of questionnaires, including the Daily Hassles Scale (Kanner et al., 1981), the Everyday Problem Scale (Burks & Martin, 1985) and the Daily Life Experience Questionnaire (Stone & Neale, 1982). It measures the frequency of occurrences of daily hassles in the past month and gives an intensity score which is the subjective experience of the subject of the unpleasantness of the hassles. In this study, only the frequency score was used in order to stay free from confounding stress provoking and stress resulting factors in the intensity score.

Pregnancy anxieties were assessed by means of the Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R). Specific fears and worries related to pregnancy were measured on each occasion by means of an abbreviated version of the PRAQ developed by Van den Bergh (1989). An earlier study (Huizink et al., 2000a) showed that three different fears specifically related to pregnancy could be measured by using three items per subscale: fear of giving birth, fear of bearing a physically or mentally handicapped child and concern about one's appearance. This questionnaire was filled out in early, mid and late pregnancy. Cronbach's alpha's of the subscales were all > . 76 throughout pregnancy.

The appraisal of pregnancy was measured by two single-item instruments. The perceived threat of the situation, or primary appraisal, was measured by the question 'Can you indicate on a ten-point scale the degree to which your pregnancy relates to the most upsetting (=1) and most pleasant event (=10) in your life?'. Secondary appraisal, or the perceived options to control the situation, was assessed with the question 'To what extent do you think you are able to influence the course of your pregnancy?'. Participants could answer on a 5-point scale ranging from 'considerably' to 'not at all'. These two items were answered on each occasion.

6.3.2.2 Stress-mediating factors

Utrecht Coping List (Schreurs et al., 1988). To assess the coping style of our subjects, we used the 'Utrecht Coping List-19' which is an abbreviated form of the 'Utrecht Coping List-30'. An earlier study (Huizink et al., 2000b) using confirmatory factor analyes showed that two factors of coping could be found when using this questionnaire in a sample of pregnant women: emotion-focused coping and problem-focused coping. Each factor contained three items. This questionnaire was filled out three times during pregnancy. Cronbach's alpha's of the factor scores were all > .71 throughout pregnancy.

6.3.2.3 Stress-resulting factors

*State-Trait Anxiety Inventory (STAI).*The STAI (Spielberger et al., 1970) comprises two self-report scales for measuring two distinct anxiety concepts, state-anxiety and trait-anxi-

ety. Both scales contain 20 statements that ask the respondent to describe how she feels at a particular moment in time (state-anxiety) or how she generally feels (trait-anxiety). State anxiety is conceptualized as a transitory emotional state, whereas trait-anxiety refers to relatively stable individual differences in proneness to anxiety. Cronbach's alpha in this study was .88 for state anxiety and .83 for trait anxiety. The STAI was filled out on each occasion.

General Health Questionnaire (GHQ-30; Goldberg, 1972). A Dutch translation of the GHQ-30 (Koeter & Ormel, 1991) was used in this study to measure the psychological well-being of our subjects. The questionnaire contains 30 questions to be answered on a four-point scale. This questionnaire was filled out on each occasion.

Perceived Stress Scale (Vragenlijst Ervaren Stress). Perceived stress was assessed by means of a Dutch translation by Vingerhoets (1989) of the Perceived Stress Scale of Cohen & Williamson (1988). It contains 14 items on perceived stress of an individual during the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This questionnaire was filled out on each occasion.

6.3.3 Statistical analysis

The mediating role of both emotion- and problem-focused coping was examined by means of path analyses with LISREL 8.30, a structural equation modeling technique. In our path model, the latent construct distress was composed of three indicators (GHQ-30, PSS, State-anxiety) and predicted by various independent latent constructs of stress provoking factors (daily hassles, life events, primary appraisal of pregnancy, secondary appraisal of pregnancy, fear of giving birth, fear of bearing a physically or mentally handicapped child, concern about one's appearance) and two dependent latent constructs of coping (emotion-focused coping and problem-focused coping). Thus, distress is the outcome variable in the recursive path model. The three indicators showed high intercorrelations (> .60). Moreover, Cronbach's alpha of the items of all three questionnaires was >.90. The goodness of fit between the hypothesized path model and the sample data was subsequently tested. This provided information about the reliability and validity of the model while taking measurement errors into account. Goodness of fit measures used were Chi Square (χ^2) and Chi-square divided by degrees of freedom. The latter is sensitive to sample size, and is therefore regarded as a measure of fit instead of a test statistic. When chi-square is divided by its degrees of freedom the result should be less than six if it is to indicate a reasonable fit to the data. P values of the Chi Square statistic should be > .05, thus indicating that the model is not significantly different from the data. Other fit criteria include: Comparative Fit Index (CFI ; >.9 indicates a good fit), Non-Normed Fit Index (NNFI; ; >.9 indicates a good fit), Root Mean Square Error of Approximation (RMSEA), which should be at least less than .08 and Root Mean Square Residual (RMR), which should be less than .05.

When several a priori nested models are tested, the model Akaike's Information Criterion (AIC) can be compared among the models, with the lowest values indicating the best model. In addition, the difference between chi-square can be tested for significance.

6.4 Results

6.4.1 Coping as a mediator of the distress response to daily hassles and life events

Early pregnancy. Firstly, a model was formulated which included both direct effects of daily hassles and life events on distress and mediating effects of coping. Two coping styles were included, problem-focused coping and emotion-focused coping. This model showed a good fit to the data (χ^2 = 9.45, df = 9, p = .40, RMSEA= .015, RMR= .033, CFI=.99, NNFI=.98). However, the path from problem- focused coping to distress was not significant (t=.38) and was therefore left out in the next model. This model showed an excellent fit, although the paths from daily hassles, life events to the mediator emotion-focused coping were not significant. Therefore, a model was constructed with only direct effects on distress of daily hassles, life events and emotion-focused coping. This model had an excellent fit to the data (χ^2 = 5.24, df = 6, p = .51, RMSEA< .01, RMR= .023, CFI=1.00, NNFI=1.01) and shows that emotion-focused coping indeed has a direct rather than an indirect effect on distress. The model is presented in Figure 6.1. More daily hassles and more life events lead to more distress, whereas more emotion- focused coping leads to less distress.

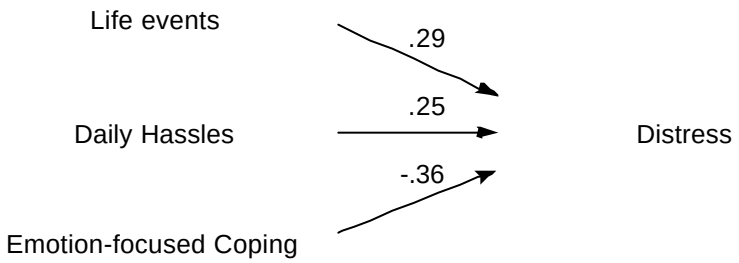
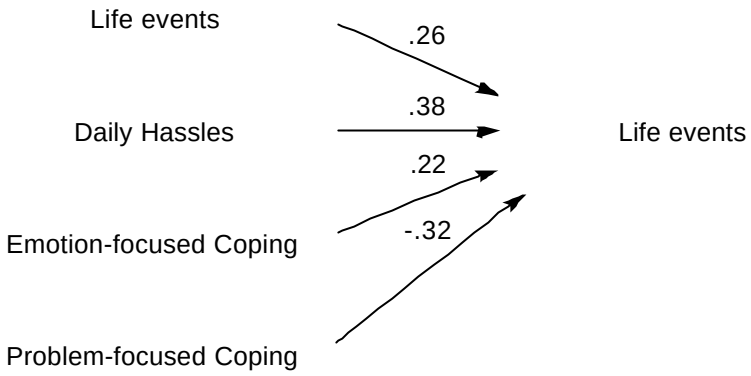


Figure 6.1. Best fitting model in early pregnancy for direct effects of life events, daily hassles and emotion-focused coping on distress.

Mid pregnancy. The first model tested included both direct effects of daily hassles and life events on distress and mediating effects of emotion- and problem-focused coping on distress. Fit criteria showed that the model fitted reasonably well (χ^2= 20.21, df = 9, p =.02, RMSEA= .08, RMR= .05, CFI=.97, NNFI=.96). However, the paths from life events to problem-focused coping and from daily hassles to problem-focused coping (thus, the mediating paths of problem-focused coping) were not significant (t= -.38 and t= 1.47, resp.). Therefore, these three paths were left out in the next model. The second model therefore included direct effects on distress of life events, daily hassles, problem- and emotion-focused coping. This model shows a good fit to the data (χ^2 = 12.97, df = 8, p =.11, RMSEA= .06, RMR= .03, CFI=.98, NNFI=.96)

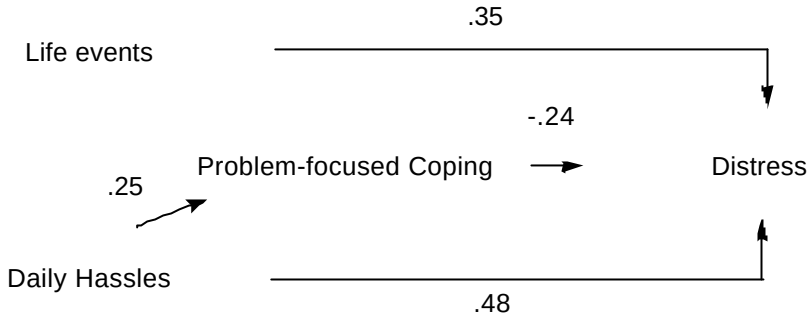
and is presented in Figure 6.2. Thus, in mid pregnancy, emotion- focused coping and problem-focused coping had a direct effect on distress. However, emotion-focused coping appeared to be associated with more distress, which is in contrast to our hypothesis.

Figure 6.2. Best fitting model for direct effects of life events, daily hassles , emotion- and problem-focused coping on distress.



Late pregnancy. In late pregnancy, again a model was first tested including both direct effects of life events and daily hassles on distress and mediating effects of emotion-focused coping and problem-focused coping on distress. This model did not show a good fit to the data (χ^2 = 20.35, df = 9, p =.02, RMSEA= .09, RMR= .06, CFI=.96, NNFI=.94). The paths from daily hassles to emotion-focused coping and from life events to emotion-focused coping were not significant (t = .47 and t = .94 resp.) Also, the path from life events to problem-focused coping was not significant (t=1.66). Moreover, the path from emotion-focused coping to distress was not significant (t= -1.36). Therefore, the second model to be tested included direct effects of daily hassles and life events on distress as well as a mediating effect of problem-focused coping for the effects of daily hassles on distress. This mediating effect was as expected. Thus, more daily hassles were associated with more problem-focused coping and in turn this coping style was associated with less distress. The model showed an excellent fit to the data (χ^2 = 8.50, df = 7, p =.29, RMSEA= .04, RMR= .04, CFI=.99, NNFI=.98) and is presented in Figure 6.3a.

Figure 6.3a Model of distress in late pregnancy including a mediating function for problem-



focused coping.

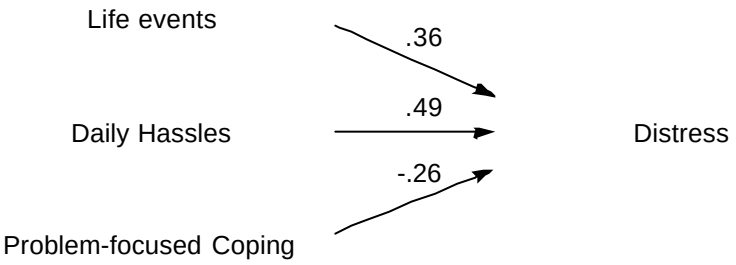


Figure 6.3b Model of distress in late pregnancy including only direct effects. This model showed the best fit to the data.

However, problem-focused coping functions only as a mediator when it meets the following conditions (Baron and Kenny, 1986): 1] Variations in the levels of daily hassles account significantly for variation in problem-focused coping; 2] Variations in problem-focused coping account significantly for variation in distress; and 3] When both paths of condition 1 and 2 are controlled for, a previously significant relation between daily hassles and distress is no longer significant. Although condition 1 and 2 were met, condition three needed to be tested. So, a model was tested including only direct effects of life events, daily hassles, and problem-focused coping on distress. The results showed an excellent fit to the data (χ^2 = 5.72, df = 6, p =.46, RMSEA< .0005, RMR= .03, CFI=1.00, NNFI=1.01) and are presented in Figure 6.3b. When we compared the path from daily hassles to distress in Figure 6.3b with the same path in Figure 6.3a, the difference was very small (.49-.48=.01) and not significant.

Moreover, when we compared both models based on the χ^2 method, an almost significantly better fit is found for the model in Figure 6.3b (χ^2 = 2.78, df=1, p=.10). The latter model had a slightly lower model AIC (35.72 versus 36.50).

In short, a direct rather than an indirect effect of coping style on distress is found throughout pregnancy.

6.4.2 Coping as a mediator of the distress response to the primary and secondary appraisal of the pregnancy

Pregnancy itself can be regarded as a stress-provoking factor. In the present study, primary and secondary appraisal of the pregnancy were therefore included in a more complex model of prenatal stress. This model included direct effects of daily hassles and life events on distress and a mediating effect for coping between the appraisal of pregnancy and distress. The results are summarized in the next section.

Early pregnancy. The model that fitted best to the data (χ^2= 12.45, df = 14, p =.57, RMSEA< .05, RMR= .04, CFI=1.00, NNFI=1.02) included a mediating effect of emotion-focused coping for the primary appraisal of pregnancy, and direct effects of daily hassles, life events and secondary appraisal of the pregnancy. When women regarded their pregnancy as a pleasant event, more emotion-focused coping was found, which resulted in decreased levels of distress. More daily hassles and life events were related to increased levels of distress and when pregnancy was regarded as a situation that can be controlled only to a small extent, also increased levels of distress were found. No significant correlation coefficients were found between the appraisal of pregnancy and daily hassles or life events, suggesting that these factors were independent predictors of distress.

Mid-pregnancy. No mediating effects for coping were found with regard to the relation between the appraisal of pregnancy and distress. The model that fitted the data best (χ^2 = 10.69, df = 10, p =.38, RMSEA< .05, RMR= .03, CFI=1.00, NNFI=.99) showed a direct effect of secondary appraisal of the pregnancy on distress, in combination with direct effects on distress of daily hassles, life events and problem-focused coping. No effect of primary appraisal on distress was found in mid-pregnancy. Again, when pregnancy was regarded as a relatively uncontrollable situation, more distress was found. No significant correlation coefficients were found between secondary appraisal of the pregnancy and daily hassles or life events.

Late pregnancy. Coping did not mediate between appraisal of pregnancy and distress in late pregnancy. Direct effects were found for daily hassles, life events, problem-focused coping, and primary appraisal of the pregnancy on distress levels in the model that fitted best to the data (χ^2 = 8.53, df = 8, p =.38, RMSEA<.05, RMR= .03, CFI=1.00, NNFI=.99). Increased amounts of daily hassles and life events resulted in increased levels of distress, whereas more problem-focused coping and regarding pregnancy as a pleasant event reduced the level of distress. Daily hassles, life events and primary appraisal of the pregnancy were not significantly intercorrelated and can be regarded as independent predictors of distress.

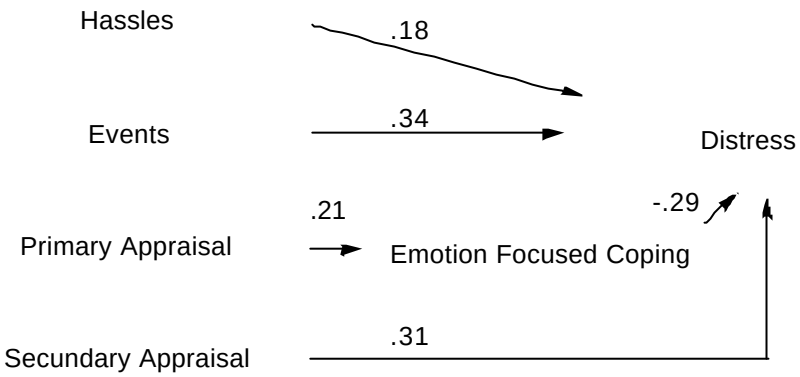
6.4.3 Coping as a mediator of the distress response

to pregnancy-specific anxieties

It is possible that stressors specifically related to pregnancy may provoke a stress response which may be mediated by coping. Therefore, we included three pregnancy-related anxiety scores (fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one's appearance) as potentially stress provoking factors into our model of prenatal distress.

In *early pregnancy* it was found that fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one's appearance did not account statistically for the level of distress. In addition, neither of these fears resulted in changed levels of emotion-focused coping. Therefore, the model of distress as described in the previous section was found to predict the level of distress in early pregnancy best (χ^2 =12.45, df = 14, p =.57, RMSEA<.05, RMR= .04, CFI=1.00, NNFI=.99), and accounted for 35% of the total variance of distress. The model is shown in figure 6.4.

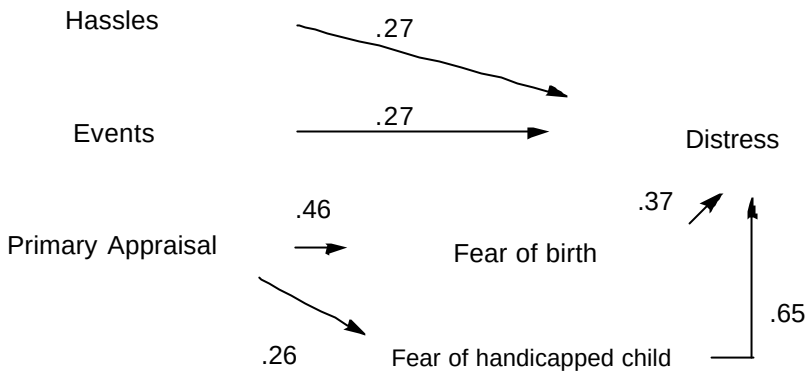
Figure 6.4 Emotion-focused coping as mediator for the effect of primary appraisal of pregnancy on distress in early pregnancy.



Data derived from *mid-pregnancy* showed that mediating effects of problem-focused or emotion-focused coping were absent for the stress response of pregnancy-related fears on general distress levels. However, when pregnancy was appraised as more uncontrollable, increased levels of fear of giving birth and fear of bearing a physically or mentally handicapped child were found, which, in turn, were associated with increased levels of distress. When these fears were included in the path model of distress, problem-focused coping no longer explained significantly a part of the variance of distress. The model with the best fit to the data (χ^2=25.47, df = 15, p =.05, RMSEA=.07, RMR= .04, CFI=.98, NNFI=.97) included daily hassles, life events, secondary appraisal of the pregnancy, fear of giving birth and fear of bearing a physically or mentally handicapped child as predictors of the dependent variable

distress. In total, this model explained 66 % of the variance of distress. The model is shown in Figure 6.5.

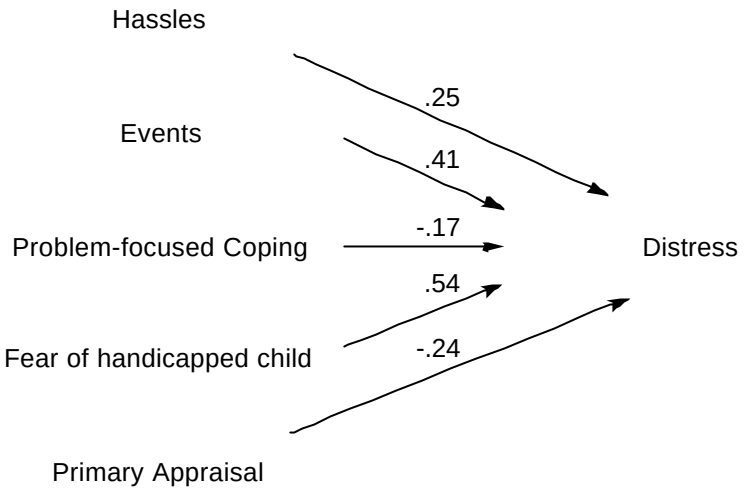
Figure 6.5 Best fitting model of distress in mid-pregnancy when pregnancy-related anxieties



are added to the model.

Late pregnancy. In late pregnancy, problem-focused coping nor emotion-focused coping did mediate the effects of pregnancy related fears on distress. Rather, a direct effect on distress of fear of bearing a physically or mentally handicapped child was found in addition to the direct effects of daily hassles, life events, problem-focused coping and primary appraisal of the pregnancy. The model that fitted best to the data (χ^2= 13.51, df = 10, p =.20, RMSEA=.05, RMR= .03, CFI=.99, NNFI=.98) is shown in Figure 6.6. In total, this model explained 74 % of the variance in the level of distress.

Figure 6.6. Best fitting model of distress in late pregnancy.



6.4.4 Causal relationship between coping and distress

To examine whether coping scores and the amount of perceived distress were confounded, several models were tested in which distress predicted scores on emotion- or problem-focused coping. None of these models did fit to the data ($p<.0005$) in any part of pregnancy. Therefore, the results suggested a causal relation between coping and distress instead of the other way around.

In addition, it could be that coping would have a prolonged effect on distress. That is, coping in early pregnancy may predict distress levels in mid-pregnancy and coping in mid-pregnancy may predict distress in late pregnancy. The findings of these longitudinal models showed that neither emotion-focused coping nor problem-focused coping could account for distress in the subsequent part of pregnancy.

6.5 Discussion

The purpose of the present study was to examine the role of coping in a multidimensional model of prenatal stress in a population of normal risk first-time expectant mothers. With regard to the first aim of this study, coping appeared to have direct effects on the emotional status of pregnant women rather than having a mediating role between stress provoking factors and distress.

In early pregnancy, increased emotion-focused coping caused decreased levels of distress. In mid and late pregnancy, increased problem-focused coping was related to decreased levels of distress. Also, direct effects of the amount of life events and daily hassles accounted for a significant part of the variation in distress. As pregnancy proceeded, daily hassles in particular had strong effects on distress. These findings partly contradict the process model of coping (Lazarus, 1999), which states that coping arises from the transaction between the person

and the environment and mediates the effect on emotional outcome. Clearly, daily hassles and life events did not provoke a specific coping response in our subjects. In fact, our results suggested that emotion- and problem-focused coping tend to reduce distress, independent of daily hassles and life events. This could be explained by viewing coping as a personality trait, as many have done before. However, several findings argue against this interpretation. First of all, if coping would be a trait aspect of the personality rather than a changing process, the effect of coping on distress would be stable over time. However, our longitudinal models showed that coping scores in early pregnancy did not predict levels of distress in mid-pregnancy, nor did coping scores obtained in mid-pregnancy account for variation in the distress level in late pregnancy. Secondly, a previous study in the same population showed that coping changes throughout pregnancy. In early pregnancy, emotion-focused coping appeared to prevail, whereas emotion-focused coping scores declined with increasing gestation (Huizink et al., 2000b).

These latter findings are even more interesting, when we combine them with the pattern found in the path models of prenatal stress, as described in the present study. In early pregnancy, emotion-focused coping has a negative influence on distress, whereas in mid- and late pregnancy problem-focused coping predicted distress levels negatively. Perhaps, in early pregnancy the emotional adaptations involved with the discovery of being pregnant call for a need of emotion-focused coping. Mid and late pregnancy may involve more practical adaptations, such as preparation of the baby's room and arranging public nursery. Problem-focused coping, therefore, would be a better way of dealing with these practical issues. In mid pregnancy it was found that emotion-focused coping increased distress, whereas problem-focused coping tended to reduce distress levels. This finding suggest that problem-focused coping may be more effective in this particular period of pregnancy. In that respect, coping is a process that responds to the necessary demands and adaptations needed in a certain condition or situation.

It is obvious, however, that in our sample of normal risk pregnant women, daily hassles and life events both had an independent effect on distress and did not give rise to increased levels of either emotion- or problem-focused coping. Presumably, the adaptations to pregnancy are independent of more common stress-provoking factors such as daily hassles and life events.

In that respect it is interesting to notice that the second question addressed in the present study showed that the appraisal of pregnancy contributed significantly to the level of distress. In early pregnancy, regarding pregnancy as a pleasant event gives rise to increased levels of emotion-focused coping, which in turn is related to reduced distress levels. Thus, emotion-focused coping mediates the stress response to the uplift of pregnancy itself. Secondary appraisal of the pregnancy, or the uncontrollableness of the situation, is positively related to levels of distress, independent of the occurrence of daily hassles or life events. Thus, the appraisal of pregnancy indeed results in some degree of distress. What exactly contributes to this distress response is unknown, and it should therefore be studied in more detail, by using a questionnaire that includes pregnancy-related stressors.

Although we did not include the stressors specifically related to pregnancy as described by DiPietro et al. (2000.), some aspects of the pregnancy itself were included in our more complex models. In order to test the aforementioned hypothesis, we examined the role of coping in the stress response to pregnancy-related anxieties. These anxieties have been shown to be

specifically related to pregnancy and could be differentiated from more general anxiety and depression symptoms (Huizink et al., 2000a). The results showed that coping did not mediate the stress provoking effects of these fears. Rather, a direct effect of these fears on distress was found. In mid-pregnancy, the secondary appraisal of pregnancy was associated with increased fears, which in turn raised the amount of distress.

In the present study, coping and distress measures were not confounded. It was found that coping predicted the level of distress, instead of vice versa. Therefore, the findings of the present study demonstrate that coping has an effect on distress independent of daily hassles and life events and should therefore be included in a multidimensional model of prenatal stress.

Thus, in the present study, emotion-focused coping only mediated the effect of primary appraisal of the pregnancy on distress. It could be that the coping scores we have used in this study were not used by pregnant women to mediate their emotional reaction. Therefore, further research is needed to clarify the role of other coping strategies in mediating stress responses. Clearly, emotion-focused coping or problem-focused coping did not mediate the stress response in our sample of relatively normal risk pregnancies. Perhaps, more intense stress provoking factors in high-risk subjects would provoke more coping processes than in our sample, which in turn would mediate the stress response

The models of distress that we found predicted a large amount of the variance of distress in mid (66%) and late pregnancy (74%), and a moderate amount of variance in early pregnancy (35%). A potential moderator of the effect on distress of daily hassles, life events, primary or secondary appraisal of the pregnancy, and pregnancy-related fears is the available or received social support of the pregnant women. Also, personality aspects such as neuroticism and trait anxiety may modify the distress response to these stress provoking factors. Therefore, in a next study, we will examine if and to what extent these factors will account for the remaining variance of distress, especially in early pregnancy.

In conclusion, coping has a main effect on distress in pregnant women rather than a mediating effect. Furthermore, the most effective coping style to reduce distress varies with increasing gestation. To reduce levels of distress in pregnancy by intervention studies, it is therefore important to take account of this time-specificity of effective coping and to focus on the emotional and practical needs in that particular period of pregnancy.

In future studies, pregnancy-specific stressors should be included as stress-provoking factors, and social support and personality aspects should be studied with regard to their moderating role. In addition, these models of coping strategies in normal risk pregnancy have to be confirmed in a future and independent study group.

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7

Multidimensional models of prenatal distress in normal risk pregnancy

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Submitted

7.1 Abstract

Background: Prenatal stress has been operationalized in numerous ways across studies on the effects of stress during pregnancy on birth outcome and later development. A multidimensional concept of stress in the most likely one to describe what prenatal stress actually is.

Objective: To formulate and test multidimensional models of prenatal stress in a population of normal risk nulliparous pregnant women.

Methods: Self-report data about various aspects of stress-provoking (life events, daily hassles, appraisal of pregnancy and pregnancy-specific anxieties), stress-mediating or -moderating (coping style, social support, neuroticism), and stress-resulting (perceived stress, state-anxiety and general psychological well-being) factors were collected in nulliparous pregnant women in early (n=230), mid (n=217) and late (n=172) pregnancy. Path analysis was performed by means of LISREL 8.30.

Results: In each period of pregnancy a significant multidimensional model of prenatal distress was found which explained most of the variance in the dependent construct of distress (86%, 88% and 94% for early, mid and late pregnancy, respectively). In early pregnancy the best fitting model included direct effects on distress of life events, neuroticism, secondary appraisal of the pregnancy and emotion-focused coping and indirect effect of primary appraisal of pregnancy and a moderating variable daily hassles by available support (χ^2 =26.74, df=17, p=.06, RMSEA=.06, RMR=.05, CFI=.96, NNFI=.92). In mid-pregnancy the best fitting model included direct effects on distress of life events, neuroticism, daily hassles and pregnancy anxiety, and indirect effects of secondary appraisal of the pregnancy and instrumental support (χ^2 =21.95, df=22, p=.46, RMSEA<.01, RMR=.04, CFI=1.00, NNFI=.99). In late pregnancy the best fitting model included direct effects on distress of life events, neuroticism, pregnancy anxiety and problem-focused coping and indirect effects of instrumental support and the moderating variable daily hassles by available support (χ^2 =31.58, df=17, p=.02, RMSEA=.07, RMR=.05, CFI=.97, NNFI=.94).

Conclusion: Each period of pregnancy had some unique aspects that accounted for increased or decreased levels of distress, but life events and especially neuroticism were found to predict distress throughout pregnancy. The multidimensional models of prenatal stress offer more insight into the processes that lead to increased distress levels in pregnant women and may be used to predict birth outcome and postnatal development.

7.2 Introduction

Stress in pregnancy has been associated with premature delivery, reduced birth weight and smaller head circumference in humans (Dunkel-Schetter, 1998; Copper et al., 1996; Lou et al., 1994; Wadwha et al., 1993). Furthermore, animal studies performed under well-controlled conditions have shown deleterious effects of prenatal stress on behaviour and development of the offspring (Schneider, 1992; Schneider & Coe, 1993). In human studies, however, research on severe prenatal stressors has been limited to naturally occurring disasters or stressors during pregnancy, such as war (Rajab et al., 2000), flood (Selten et al., 1999) and major life events (e.g. Newton & Hunt, 1984; Nuckolls et al., 1972). Across these human studies stress has been operationalized in numerous ways, which makes comparison difficult. Results of animal studies are also not readily comparable to results of these varying human studies, since the stressors used in animal studies are of experimentally nature and not comparable to stressors encountered in human pregnancy. Therefore, there is a need for a well described concept of human prenatal stress in order to gain more insight into the complex aspects of human prenatal stress as compared to animal prenatal stress. The purpose of the present study is to develop a multidimensional measurement model of prenatal stress, which could later be incorporated into a biopsychosocial model of birth outcome and postnatal development. To do so, one needs to carefully consider the methodological issues regarding general stress research.

7.2.1 General considerations with regard to stress

For years, the impact of stress on various aspects of health has been assessed with major life changes as stressors. However, the relationship between life event scores and health outcome has been found to be rather modest (e.g. McEwen & Seeman, 1999; Rabkin & Struening, 1976). As an alternative, the focus shifted toward daily hassles, or the ongoing stresses and strains of daily living (DeLongis, et al., 1982). DeLongis et al. (1982) proposed a model in which daily hassles are seen as proximal measures of stress while life events are considered distal. Therefore, hassles should have a more direct impact on health than life events.

Daily hassles, sometimes called minor stressors, may occur simultaneously with major life events. Some researchers have even suggested that hassles could act as an important route of transmission in explaining the effects of major events (Pillow et al., 1996). In a study to identify mediational links between major life events and daily hassles in the stress process, Pillow et al. (1996) tested two alternative models, which can be distinguished in the literature. In the *event vulnerability model*, major life events may sensitize the individual to the occurrence of daily hassles, causing a better remembrance of these minor stressors, which, in addition, are experienced in a surplus negative fashion. On the other hand, the *inoculation model* suggests that exposure to a major stressful life event may reduce the adverse impact of a subsequent negative minor event by means of reduced sensitization to other negative occurrences. Neither of these models could be supported by the results of Pillow et al. (1996). It therefore seems plausible that the effects on health outcome of daily hassles and of life events should be considered independently. Another argument for this view is found in the results of DeLongis et al. (1982). They showed that the effects of life events on health

were only noticeable after a considerable amount of time, whereas the effects of daily hassles on health occurred more rapidly. Therefore, the underlying mechanism by which stress affects health may essentially differ between daily hassles and major life events. Moreover, it has been demonstrated that chronic and daily stressors may be better predictors of subjective and objective health complaints than the more intense but rarely occurring life events (DeLongis et al., 1982; Kanner et al., 1981; Herbert & Cohen, 1993). The many small events of daily life may elevate activities of physiological systems to cause health problems (McEwen & Seeman, 1999). If we expect to find stronger and more direct effects on health of daily hassles, we are confronted with a methodological problem. Changes in levels of hassles may affect health and vice versa, which renders causal interpretation complex. A longitudinal study design in which both aspects are measured repeatedly offers the best possible solution to this dilemma. A study by Dohrenwend et al. (1984) showed that instruments measuring daily hassles may confound stressful circumstances with symptom outcomes. This has also been described as one of the major problematic issues with life events research. This fact should be carefully considered when studying the relationship between daily hassles and health outcome.

7.2.1.1 Social support

With regard to some factors that have been frequently regarded as mediators or moderators in the relationship between stressors and (perceived) distress or health outcome, the role of social support and coping is of special interest. The study of social support has been difficult because various definitions have been used and consensus on the definition of the construct has not been achieved (Heitzmann & Kaplan, 1988). The studies by Caplan (1974) and Cobb (1976) have been frequently cited. Their results emphasize the positive effects of the resources offered by a person's social network on his/her well-being. However, these definitions are not useful because their description of social support is based in fact on the perceived effects of social support (Komproe et al,, 1997). Therefore, Komproe et al. (1997) propose the use of the definition of Shumaker and Brownell (1984). This definition describes social support *as an exchange of resources between at least two individuals generally perceived to be intended to enhance the well-being of the recipient (which may actually have positive, neutral and/or negative effects on the recipient's well-being).* In this view the intention is more important than the actual act or result of social support. A complicating aspect of this definition, however, is that it assumes knowledge of the perceptions of those involved in the interaction. In other words, the intentions of the support provider are assumed to be known. In most studies, social support is judged only from the point of view of the recipient and the intentions of the support provider are ignored. More specifically, most studies only focus on social support when it is perceived by both the recipient and the provider as such, i.e., it is assumed to be helpful by both actors in the interaction and is therefore congruent. However, the perceptions of the provider and recipient with regard to the exchange may also be incongruent when, for example, the provider views his/her support to the recipient as helpful, whereas the recipient perceives the same support as neutral or harmful. Other studies have neglected these possibilities of social support and have only focused on the congruent exchanges. Thus, they view social support only if it is meant and

perceived as helpful. Shumaker and Brownell (1984), on the other hand, emphasize this interactional aspect of social support. In the present study, we do focus on the recipient of social support, for mostly pragmatic reasons. Therefore, by applying the concept of social support as defined by Shumaker and Brownell (1984), we will have to estimate what is generally perceived as the intention of a specific behaviour or interaction.

An issue that has been frequently discussed in the literature is the 'main or buffer effect' of social support. Some researchers state that social support has a main positive effect on health, even in normal situations without stress. Others suggest that only in case of distress, adequate social support would buffer the negative effects on health of this distress. With regard to this controversy, Thoits (1982) describes several methodological issues that should be considered when studying the buffering hypothesis. Most studies suffer from inadequate conceptualization and operationalization of social support. Although some investigators have offered more elaborate conceptual statements (e.g. Cobb, 1976; Kaplan, 1977; House, 1981), efforts to assess the validity and reliability of social support indicators are still noticeably lacking in the literature. Furthermore, most studies have either theoretically or operationally confounded the direct effect of life events upon social support with the interactive (or buffering) effects of events upon support. That is, when examining life-event scales, many important events (like divorce, death of a spouse, marriage) are interpretable as losses or gains of supportive relationships. Moreover, life events may produce additional alterations in the social support system. For instance, divorce may cause other relatives and friends to either increase or reduce their support. Thus, life events can be direct indicators of changes in social support, may cause additional changes in social support, or may exert both effects. Therefore, adequate testing of the buffering hypothesis implies measuring social support repeatedly in a longitudinal design. Again, daily hassles could prove to be less influenced by confounding matters with respect to social support, since most daily hassles do not include changes in social support. Finally, most studies have failed to examine the main effects of social support upon distress but solely investigated the buffer effect. Nevertheless, there are several good reasons to expect a main effect of social support on distress levels. For instance, perception of oneself and one's identity may originate in social interaction and could therefore reflect important aspects of psychological well-being. Furthermore, social support helps to strengthen or maintain self-esteem and could therefore have a direct effect on the psychological state.

7.2.1.2 Coping

Like social support, coping style is another factor to take into account as a possible mediator when studying the relationship between stressors and distress. As we have seen with the concept of social support, it is important to conceptualize clearly what is meant by coping. The word in itself can be confusing in that coping means literally "successfully dealing with the circumstances." However, one could argue that attempts to cope are a way of coping as well. Therefore, we apply the definition of Lazarus & Folkman (1984) which states that coping includes *the efforts, both action oriented and intra-psychic, to manage environmental and internal demands and conflicts among them, which exceed a person's resources.* Coping has been differentiated in, among others, the dimensions of problem-focused coping and

emotion-focused coping, both of which have been found to be related to psychological well-being (Bruder?Mattson & Hovanitz,1990). Strategies of problem-focused coping refer to a task-orientation, like solving a problem, reconceptualizing it, or minimizing its effects. Emotion-focused coping, on the other hand, refers to a person-orientation that may include emotional responses, self-preoccupation, and fantasizing reactions (Endler & Parker, 1993). Another coping dimension, avoidance, is sometimes identified, and may include either person-oriented or task-oriented strategies. Avoidance is not a successful way of coping in a long-term perspective but can reduce stress levels at short term, by avoiding the situation that causes stress. Coping has also been considered an important determinant of social support (Billings & Moos, 1981). For instance, people who use avoidance coping responses have fewer social resources. From a coping point of view, social support is conceptualized as a resource of coping strategies. For example, one can use other persons as a source of emotional regulation in the service of emotion-oriented coping. Thus, coping could have moderating effects on the relationship between social support and physical well-being. In addition, coping strategies would appear to mediate between stressful events and psychological distress.

Besides the effect of social support and coping on distress, personality traits such as neuroticism have also been found to be strong predictors of levels of distress. Neuroticism, or negative emotionality, has been associated with a stronger susceptibility to psychological distress and with increased vulnerability to stress-provoking factors. Individuals with higher negative emotionality also have a tendency to have unrealistic ideas and inefficient ways of coping with stress. It has also been suggested that people who score high on negative affectivity seem to be particularly sensitive to minor failures, frustrations, and irritations of daily life (Watson & Clark, 1984). Thus, personal characteristics could have mediating or moderating effects on the distress response to life events or daily hassles or could perhaps even have a direct influence on distress. Moreover, neuroticism could bias the amount of recollected minor or major stressful events.

All abovementioned methodological issues should be considered carefully when studying the effects of stress on health outcome.

7.2.2 Prenatal stress

With this information in mind and aware of the methodological pitfalls involved in stress research, we tried to apply this knowledge to the literature on prenatal stress. Since our future aim is to analyze the effects of prenatal stress on the fetus, birth outcome, and later child development and behavior, it is important to conceptualize precisely what is meant by prenatal stress.

A multidimensional concept of stress is the most likely one to describe what prenatal stress actually is. Such a concept should involve various aspects of stress. As a theoretical starting point, it is useful to incorporate the model of Lazarus and Folkman (1984), in which a differentiation is made between stress-provoking factors, stress-mediating or -moderating factors, and stress-resulting factors. With regard to stress-provoking factors, major life events and daily hassles are assumed to potentially provoke a stress reaction. As we have learned from the general stress literature, daily hassles have some advantages over life

events, because they hold more potential for modification, intervention, or prevention than do life events, which are sometimes unavoidable.

Mediating factors are factors that may interact with the effect of stress-provoking factors on stress-resulting factors. As outlined by Baron and Kenny (1986), a mediator represents the generative mechanism through which the independent variable is able to influence the dependent variable of interest and that, in statistical terms, accounts for the relation between the independent and dependent variable. In contrast, a moderator modifies the strength of the relation between the independent and dependent variables. Social support, coping style, and personality factors are such factors that may either increase or decrease the effect of a life event or daily hassles on the amount of stress that an individual perceives. For instance, in their review of controlled trials of enhanced social and psychological support in pregnancy, Elbourne et al. (1996) concluded that social support has a number of beneficial psychological and behavioural effects on the pregnant woman. Pregnant women receiving social support were less likely than controls to feel unhappy, nervous, and worried during pregnancy. Thus, social support might have an indirect effect on birth outcome by reducing stress or anxiety levels. When these factors are also taken into account, a more informative concept of stress is formed.

Stress-resulting factors are generally reflected as the amount of stress an individual actually perceives or reports. Thus, subjective feelings of stress are part of the stress-resulting factors of the model of Lazarus and Folkman. This is also known as distress.

Recent studies (Sheehan, 1998; Sheehan, 1996; Lobel & Dunkel?Schetter, 1990) have integrated the various stress definitions into a comprehensive and robust multidimensional definition of prenatal stress. The results of Lobel and Dunkel-Schetter (1990) showed that the construct of prenatal distress was best predicted by combining life events distress, perceived chronic distress, and state anxiety. In other words, appraisal and emotion may be central components of distress. Sheehan (1996; 1998) created a measurement model that included socio-economic stressors, family stressors and support as part of the prenatal stress concept. These stressors are environmental factors, unlike the stressors in the study of Lobel and Dunkel-Schetter (1990) which appeared to be personality factors. Although either model is multidimensional, an overall model combining both environmental and personality factors may prove to be a more complete model of prenatal stress. Personality aspects, like neuroticism, coping style, and available or perceived social support may moderate or mediate the effects of stress provoking factors on the amount of perceived distress. Although both Sheehan (1996; 1998) and Lobel and Dunkel-Schetter (1990) do mention these potentially mediating or moderating factors, these factors were not included in their models of prenatal stress.

We previously showed that coping changes during pregnancy (Huizink et al., 2000b). Overall, pregnant women tended to prefer emotion-focused coping styles. However, the effectiveness of coping in reducing distress also changed throughout pregnancy. A negative association between emotion-focused coping and distress was found only in early pregnancy, whereas problem-focused coping was related to reduced levels of distress in mid and late pregnancy. The same temporal specificity may apply to the possibly moderating function of social support. Thus, it is important to test the multidimensional construct of prenatal stress at various moments of pregnancy. Therefore, data on stress-related factors were collected three times during pregnancy in the present study.

7.2.3 The model of prenatal stress to be tested in

the present study

In this study, we formulated a multidimensional model of distress in pregnancy, which is shown in Figure 7.1. Distress is determined by a combination of questionnaires which assess the perceived feelings of stress. General stress-provoking factors included the impact scores of life events and the frequency of daily hassles. The impact score of life events was assumed to have an effect independent of daily hassles. In addition, the appraisal of pregnancy will be included as a stress-provoking factor. Primary appraisal of pregnancy indicates whether or not the event of pregnancy itself is regarded as a threat. Secondary appraisal describes the amount of control one perceives to have over the course of pregnancy. Both aspects of appraisal of pregnancy may potentially provoke a stress response. For instance, when pregnancy is regarded as an unpleasant event, perhaps due to physical symptoms involved with pregnancy or to relational problems with the father of the child, pregnancy itself is a stressor. In addition, the uncontrollableness of all changes involved with pregnancy may give rise to increased distress. This was indeed found in a previous study (Huizink et al., 2000a), which also showed effects of primary appraisal of pregnancy on distress.

Likewise, anxieties specifically related to pregnancy have been shown to increase levels of distress (Huizink et al., 2000a). In particular, increased levels of fear of giving birth and fear of bearing a physically or mentally handicapped child resulted in increased levels of general distress. Therefore, we included these pregnancy-specific anxieties as potentially stress-provoking factors in our model.

With regard to potentially mediating or moderating factors, we included both direct and indirect effects of social support on distress in our multidimensional model of prenatal stress. We hypothesized that various aspects of social support may be effective in pregnant women. First of all, the perceived available social support may have a direct or buffering effect on distress. Secondly, the received emotional and instrumental support may be of particular relevance in this study. Previous studies on coping in pregnancy (Huizink et al., 2000b) showed that pregnant women preferably use emotional coping strategies. Emotional social support may be a source for this coping strategy. Moreover, since pregnancy also involves physical changes that may result in less mobility, instrumental support may be especially appreciated in this period of life.

Another potentially moderating effect is expected of neuroticism, for which a main effect is formulated in our model.

In order to gain more insight into the concept of prenatal stress, we formulated and tested a multidimensional model of prenatal stress in early, mid-, and late pregnancy in a sample of normal risk pregnant women, including the aforementioned factors. The distress model based on the aforementioned general and pregnancy-specific considerations with regard to the various factors involved with stress is presented in Figure 7.1.

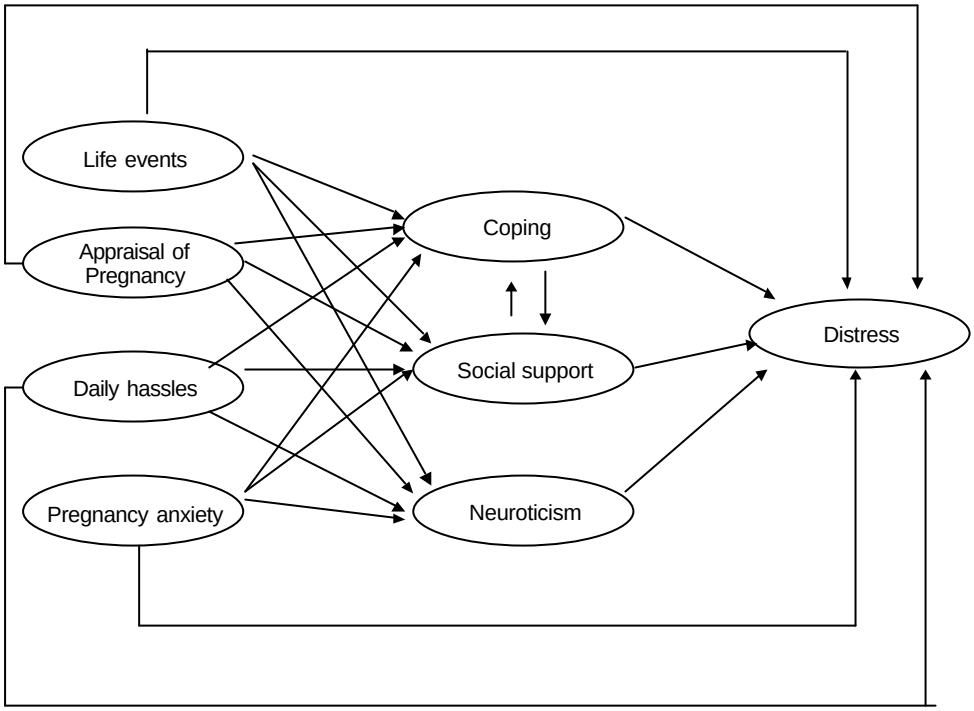

Figure 7.1. Theoretical path model to be tested with LISREL.

From this model, we could specify more specific hypotheses to be tested, which were based on findings of a previous study on the mediating role of coping in a multidimensional model of distress (Huizink et al., 2000c). The general aims to be examined in the present study were:

1. To formulate and test a multidimensional model of prenatal stress cross-sectionally in early, mid- and late pregnancy, including environmental and personality factors;
2. To analyze the different effects of the predictors of prenatal stress in early, mid-, and late pregnancy;
3. To test for longitudinal effects on prenatal stress of stress-provoking factors.

7.3 Methods

7.3.1 Participants

All participants in this study were included in a larger prospective longitudinal project which also investigated the influence of prenatal psychosocial factors on fetal behavior and the postnatal development of the children. Subjects were recruited from a consecutive series

of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of approximately 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the study. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants did not differ from those of non-participants, except in the case of women with full-time jobs, who were less likely to participate. The descriptives of the participants are summarized in Table 4.1. As shown, the sample of participants consisted largely of middle class women, although both lower and higher social classes were represented. The majority of women (92.4%) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job, 54.2 % working less than 38 hours a week and 45.8 % working full-time.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 did so on the third occasion. The main reason for the drop in the number of participants towards late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

7.3.2 Questionnaires

The package of questionnaires was composed to measure stress-provoking aspects, potential stress-mediating or -moderating factors and stress-resulting aspects.

Stress-provoking factors were assessed by means of a life events questionnaire and a daily hassles list. Also, pregnancy-related anxieties and primary and secondary appraisal of pregnancy were determined.

Life events were assessed with the Life Events Questionnaire (Vragenlijst meegemaakte gebeurtenissen; Willige van de et al., 1985). The life events impact score of this questionnaire was used, which was based on the Social Readjustment Rating Questionnaire (Holmes & Rahe, 1967).

Daily Hassles were assessed by means of the Everyday Problem Checklist (Alledaagse Problemen Lijst) (Vingerhoets et al., 1989). The daily hassles questionnaire used in this study is a Dutch translation of a selection of items of questionnaires, including the Daily Hassles Scale (Kanner et al., 1981), the Everyday Problem Scale (Burks & Martin, 1985) and the Daily Life Experience Questionnaire (Stone & Neale, 1982). It measures the frequency of daily hassles in the past month and gives an intensity score which is the subjective experience of the participant of the unpleasantness of the hassles. In this study, only the frequency score was used in

order to stay free from confounding stress-provoking with stress-resulting factors in the intensity score.

Pregnancy anxieties were assessed by means of the Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R). Specific fears and worries related to pregnancy were measured on each occasion by means of an abbreviated version of the PRAQ developed by Van den Bergh (1990). A previous study showed that three different fears specifically related to pregnancy could be measured by using three items per subscale: fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one's appearance (Huizink et al., 2000a). This questionnaire was filled out in early, mid and late pregnancy. Cronbach's alpha's of the subscales were all > .76 throughout pregnancy.

The appraisal of pregnancy was measured by two single-item instruments. The perceived threat of the situation, or primary appraisal, was measured by the question 'Can you indicate on a ten-point scale the degree to which your pregnancy relates to the most upsetting (=1) and most pleasant event (=10) in your life'? Secondary appraisal, or the perceived options to control the situation, was assessed with the question 'To what extent do you think you are able to influence the course of your pregnancy'? Participants could answer on a 5-point scale ranging from 'considerably' to 'not at all'. These two items were answered on each occasion.

Information on potential **stress-mediating or -moderating factors** was gathered on each occasion by means of questionnaires on social support, coping behavior, and a general personality factor. *Social support* questionnaires included two subscales of the Social Provisions Scale (SPS; Russell and Cutrona, 1984; Cutrona and Russell, 1987) which have been translated into Dutch (Komproe et al., 1991), and two subscales of the Social Support List-Interactions: emotional and instrumental support (SSL-I; Sonderen, 1991, 1993). The two subscales of the SPS measured the perceived available emotional and instrumental support. Each subscale contains four items, with two of them worded in a positive direction and two worded in a negative direction. Respondents indicate on a four-point scale the level of (dis-) agreement with the statements. Cronbach's alpha's of the subscale emotional support were moderate (.66 - .68). However, Cronbach's alpha's of the subscale instrumental support in this study were < .60 and therefore not appropriate for further analysis (Cronbach, 1951). Since the SPS questionnaire has never before been used in a sample of pregnant women, we performed a confirmatory factor analysis (CFA) by means of LISREL 8.30* to test if a single factor could be found in our sample, instead of two separate factors. The results showed that four items indicated a latent factor 'available support' best in early and mid pregnancy (Appendix 1). Four other items were removed due to high error variances (>.75). The Cronbach's alpha of this factor was .78 in early pregnancy and .63 in mid pregnancy. In late pregnancy, a slightly different factor solution was found, including three items that fitted the data best (Appendix 1). The Cronbach's alpha of this factor was .89. These factor scores were included in further analyses. The two subscales of the SSL-I assessed received emotional and instrumental support, and both measure the frequency of supportive interactions. The subscale emotional support consists of eight items and that of instrumental support consists of seven items. A four-category response format was used, ranging from 'hardly ever or never' to 'very often'. Cronbach's alpha's of the subscales in this study were .91 for emotional support throughout

* The CFA showed the following results. In early pregnancy: χ^2 = 1.36, df=2, p= .50, RMSEA<.005, RMR=.02, CFI=1.00, NNFI=1.00. In mid pregnancy: χ^2 = .13, df=2, p= .94, RMSEA<.005, RMR=.007, CFI=1.00, NNFI= 1.00. In late pregnancy: χ^2 = .12, df=2, p= .95, RMSEA<.005, RMR=.006, CFI=1.00, NNFI=1.00. These results indicate good fits of the models.

pregnancy, and .83, .77 and .79 for instrumental support in early, mid, and late pregnancy, respectively.

Coping style was assessed by means of the 'Utrecht Coping List-19' (Schreurs, 1988), which is an abbreviated form of the 'Utrecht Coping List-30'. It contains 19 items to be answered at a 5- point scale. A previous confirmatory factor analysis of this questionnaire in the same sample of pregnant women, showed the presence of two coping factors: emotion-focused coping and problem-focused coping. Only three items per factor were needed to predict these coping styles accurately (Huizink et al., 2000b) and were used in the present study. They were determined on each occasion during pregnancy.

Trait anxiety was assessed by means of the State-Trait Anxiety Inventory (STAI). The STAI (Spielberger et al., 1970) comprises two self-report scales for measuring two distinct anxiety concepts, state-anxiety and trait-anxiety. Both scales contain 20 statements that ask the respondent to describe how she feels at a particular moment in time (state-anxiety) or how she generally feels (trait-anxiety). State anxiety is conceptualized as a transitory emotional state, whereas trait-anxiety refers to relatively stable individual differences in proneness to anxiety. Cronbach's alpha in this study was .88 for state anxiety and .83 for trait anxiety. The STAI was filled out on each occasion. A subgroup of 159 pregnant women also filled out a neuroticism questionnaire (ABV-N; Wilde, 1963). Trait anxiety and neuroticism appeared to be highly significantly correlated ($r > .80$, $p < .005$). Therefore, we preferred to label 'trait anxiety' as neuroticism throughout the present paper.

Stress-resulting factors involved a perceived stress scale, a general index on mental well-being, and state-anxiety.

State-Trait Anxiety Inventory (STAI). The STAI (Spielberger et al., 1970) as described above was used to assess state anxiety three times during pregnancy.

General Health Questionnaire (GHQ-30; Goldberg, 1972). A Dutch translation of the GHQ-30 (Koeter & Ormel, 1991) was used to measure psychological well-being of our subjects. The questionnaire contains 30 questions to be answered on a four-point scale. This questionnaire was filled out on each occasion.

Perceived Stress Scale (Vragenlijst Ervaren Stress). Perceived stress was assessed by means of a Dutch translation of the Perceived Stress Scale of Cohen & Williamson (1987). It contains 14 items on an individual's perceived stress over the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This questionnaire was filled out on each occasion.

The dependent variable in this study is a latent construct distress, composed of the three abovementioned questionnaires. Thus, distress is the outcome variable in the recursive path model. The three indicators showed high intercorrelations ($r > .60$). Moreover, Cronbach's alpha of the items of all three questionnaires was >.90.

7.3.3 Statistical approach

The predictors of distress in the multidimensional model were examined by means of path analyses with LISREL 8.30, a structural equation modeling technique (Jöreskog & Sörbom, 1993; Byrne, 1998). In our path model, the latent construct distress was composed of three indicators (GHQ-30, PSS, State-anxiety) and predicted by various aspects of stress provoking factors (daily hassles, life events, primary and secondary appraisal of pregnancy, fear of giv-

ing birth, fear of bearing a physically or mentally handicapped child, and concern about one's appearance), two aspects of coping (emotion-focused coping and problem-focused coping), three aspects of social support (available support, received emotional support, and received instrumental support) and one personality characteristic (trait anxiety).

Various steps were undertaken in the statistical analyses. First, descriptive analyses were performed, yielding mean and standard deviations and Pearson correlation coefficients between the dependent variable and the predictors. The results of these analyses gave some insight into the correlational structure, which was further elucidated with cross-sectional path analyses of distress. Finally, longitudinal analyses were performed.

In more detail, cross-sectional analyses included several steps. First, a basic path model was postulated in each period of pregnancy according to previous findings (Huizink et al., 2000b) and tested with LISREL. Included in this model were direct (main) effects on distress of various aspects of social support and personality. Second, moderating effects of social support were tested in a next model. Model fits of the first and second models were determined. Finally, a more complex model was formulated, according to the findings and modification indices offered by LISREL of the first two models. The best fitting model will then be presented.

Longitudinal analyses also involved several steps. First, it was tested if predictors of distress in early pregnancy could also predict distress in mid-pregnancy directly or indirectly by means of the distress level in early pregnancy. The same step was made for the predictors of distress in mid-pregnancy and the distress level in late pregnancy. A next step tested if the cross-sectional model of distress could be improved by adding the distress level found in the preceding period of pregnancy.

The goodness of fit between the hypothesized path model and the sample data was subsequently tested for each model. This provided information about the reliability and validity of the model while taking measurement errors into account. Goodness of fit measures used were chi square (χ^2) and chi-square divided by degrees of freedom. The latter is sensitive to sample size and is therefore regarded as a measure of fit rather than a test statistic. When chi-square is divided by its degrees of freedom the result should be less than 3 if it is to indicate a reasonable fit to the data. P-values of the Chi-square statistic should be > .05, thus indicating that the model is not significantly different from the data. Other fit criteria include: Comparative Fit Index (CFI ; >.9 indicates a good fit), Non-Normed Fit Index (NNFI; >.9 indicates a good fit), Root Mean Square Error of Approximation (RMSEA), which should be at least less than .08 and Root Mean Square Residual (RMR), which should be less than .05 (Jöreskog & Sörbom, 1993; Byrne, 1998).

When several a priori nested models are tested, the models' Akaike's Information Criterion (AIC) can be compared among the models; the lowest value indicates the best model. In addition, the difference between chi-square can be tested for significance.

7.4 Results

7.4.1 Descriptive analyses

Means and standard deviations were calculated for all predictors and depending variables and their correlation coefficients are presented in Tables 7.1 and 7.2.

Table 7.1 Means (standard deviations) of the stress-provoking, stress-mediating, and stress-resulting factors on three occasions in pregnancy

Predictors	Early pregnancy	Mid-pregnancy	Late pregnancy
Daily hassles	10.2 (6.4)	7.8 (5.5)	6.4 (4.4)
Impactscore Life Events	301.9 (138)	164.4 (114)	150.4 (106)
Emotion-focused coping	8.7 (1.8)	8.0 (1.9)	8.1 (2.2)
Problem-focused coping	8.0 (1.9)	8.0 (1.9)	7.7 (2.0)
Available support	14.8 (1.9)	14.7 (1.6)	10.9 (2.5)
Emotional support	18.5 (4.4)	19.0 (4.4)	18.2 (4.5)
Instrumental support	14.0 (3.9)	13.8 (3.4)	13.4 (3.5)
Trait anxiety	35.7 (9.1)	34.4 (9.3)	33.7 (8.9)
GHQ-30	4.6 (5.0)	4.9 (5.0)	5.4 (4.8)
State anxiety	32.9 (7.8)	31.4 (7.3)	31.1 (8.4)
Perceived Stress Scale	28.1 (5.5)	28.1 (5.9)	27.0 (5.5)

Table 7.2 Pearson correlations between distress and stress-provoking and -mediating factors

	Distress T1			Distress T2			Distress T2		
Predictor	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
Daily hassles	.30**	.28**	.32**	.28**	.37**	.36**	.19*	.33**	.41**
Life events	.32**	.18*	.19*	.26**	.28**	.25*	.32**	.32**	.34**
Emotion-focused coping	-.27*	.15*	-.21*	-.17*	.18*	n.s.	-.18*	n.s.	n.s.
Problem-focused coping	n.s.	-.22*	n.s.	n.s.	-.18*	n.s.	n.s.	n.s.	n.s.
Available support	-.25**	-.29**	n.s.	-.19*	-.30**	n.s.	n.s.	-.36**	n.s.
Emotional support	.21**	n.s.	n.s.	.16*	.26**	.22*	n.s.	n.s.	.20**
Instrumental support	.16*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Trait anxiety	.72**	.60**	.55*	.62**	.82**	.72**	.54**	.60**	.79**
Primary appraisal	-.25**	-.16*	-.24*	n.s.	-.20*	-.25*	-.19*	n.s.	-.28**
Seconcary appraisal	.20**	.25**	.22*	n.s.	.28**	.22*	n.s.	n.s.	n.s.
Fear of giving birth	.21**	.26**	.21*	.21*	.28**	.26**	.21*	.30**	.34**
Fear of handicapped child	.25**	.38**	.37**	.35**	.49**	.49**	.25**	.38**	.48**
Concern for appearance	.24**	.27**	.33**	.22*	n.s.	.32**	.35**	.28**	.27**

T1 = early pregnancy; T2 = mid-pregnancy; T3 = late pregnancy * : p <.05; **: p<.005; n.s. = not significant

The impactscore of life events and the frequency of daily hassles (stress-provoking factors) decreased by almost 50% in the course of gestation (Table 7.1). In early pregnancy, many women encountered life events associated with being pregnant, such as marriage, changing houses, changes in the financial situation, and changes in the relationship with the partner. Later in pregnancy, pregnant women had less quarrels with their partner or with other family members. In contrast, the GHQ-30 score slightly increased, whereas state-anxiety and perceived stress hardly changed. The latter three are all indicators of the dependent variable. Thus, despite the decrease in some stress provoking factors, the amount of distress remained rather stable.

7.4.2 Cross-sectional analyses

7.4.2.1 Early pregnancy

Previously we showed that direct effects on distress exist of daily hassles, life events, and secondary appraisal of the pregnancy in early pregnancy (Huizink et al., 2000b). In addition, a mediating effect was found of emotion-focused coping for the distress response to the uplift of pregnancy (primary appraisal). These paths were included in the present basic path model. Direct effects on distress of available support, received emotional and instrumental support and anxious personality were initially added to this model. This model did not fit the data well and no direct effects of available or received support were found. In contrast, there was a strong direct effect of neuroticism on the level of distress. An alternative model was postulated including moderating effects of available support and received emotional and instrumental support for daily hassles and life events. None of these moderating variables contributed significantly to the level of distress. Therefore, a more complex model was formulated in which the effects on distress of the moderating variables daily hassles by available support and life events by available support were mediated by emotion focused coping. The path of the moderating variable life events by available support to emotion focused coping was not significant. The remaining best fitting model (χ^2 = 26.74, df=17, p= .06, RMSEA=.06, RMR = .05, CFI= .96, NNFI=.92) is presented in Figure 7.2 and explained 86% of the variance of the latent construct distress. The model included both direct and indirect effects. Increased levels of the impact score on life events, a more anxious personality ('neuroticism'), and regarding pregnancy as a largely uncontrollable event resulted in increased levels of distress. The uplift of pregnancy resulted in increased emotion focused coping. When daily hassles were accompanied by available support in one's surroundings, increased emotion focused coping strategies were also found. In turn, the latter resulted in decreased levels of distress. The direct effect of daily hassles on distress was no longer significant when the moderator daily hassles by available support was included in the model. Specific pregnancy-related fears did not play a significant role in early pregnancy.

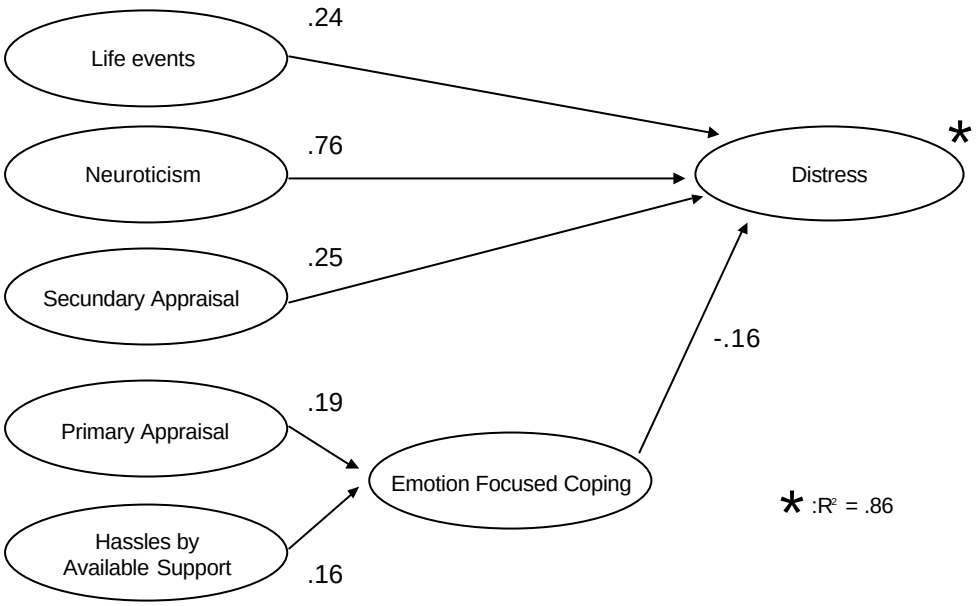

Figure 7.2 Best fitting model of distress in early pregnancy. The numbers above the arrows reflect the standardized Beta coefficients.

7.4.2.2 Mid pregnancy

The basic path model of distress in mid-pregnancy included direct effects of daily hassles and life events on distress. Secondary appraisal was related to increased levels of fear of giving birth and fear of bearing a physically or mentally handicapped child, which in turn were associated with increased levels of distress (Huizink et al., 2000b). First, direct paths of available, received instrumental and emotional support, and neuroticism to distress were added to this model. No significant direct effects of social support on the level of distress were found. Neuroticism was positively related to the level of distress. Second, the proposed direct effects of social support were replaced by moderating effects of the various aspects of social support for daily hassles and life events. None of these moderating variables contributed significantly to the prediction of distress. Finally, we hypothesized in a more complex model that received instrumental and emotional support would reduce the levels of pregnancy-specific anxiety, which in turn would decrease the level of distress. Instrumental support was found to significantly reduce the fear of giving birth and the fear of bearing a physically or mentally handicapped child, thereby indirectly reducing the level of distress. No such effect was found for received emotional support in mid-pregnancy. This model fitted the data well (χ^2 = 21.95, df=22, p= .46, RMSEA<.01, RMR = .04, CFI= 1.00, NNFI=.99) and accounted for 88% of the total variance of distress. The model is shown in Figure 7.3.

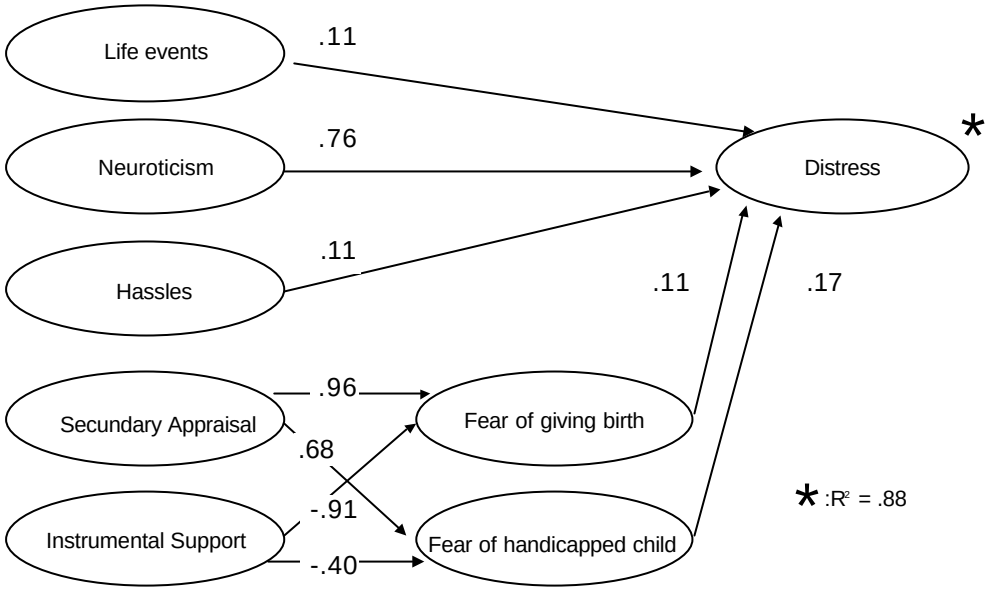

Figure 7.3. Best fitting model of distress in mid-pregnancy.

7.4.2.3 Late pregnancy

The basic path model of distress in late pregnancy included direct effects on distress of daily hassles, life events, problem-focused coping and fear of bearing a physically or mentally handicapped child (Huizink et al., 2000b). As a first step, direct effects of social support and neuroticism on distress were added to this model. Neuroticism showed a significant effect on distress, whereas no significant direct effects were found for available and received social support. The direct effects of social support were therefore replaced by moderating effects of social support by daily hassles or by life events in the next model. None of these moderating variables was found to be a significant predictor of distress. A complex final model was postulated, according to our hypotheses that received instrumental support would increase problem-focused coping and that daily hassles by available support would also increase the level of problem-focused coping, which in turn would lead to reduced levels of distress. This model showed a good fit to the data (χ^2 = 31.58, df=17, p=.02, RMSEA=.07, RMR = .05, CFI= .97, NNFI=.94) and accounted for 94 % of the total variance of distress in late pregnancy. The model is presented in Figure 7.4.

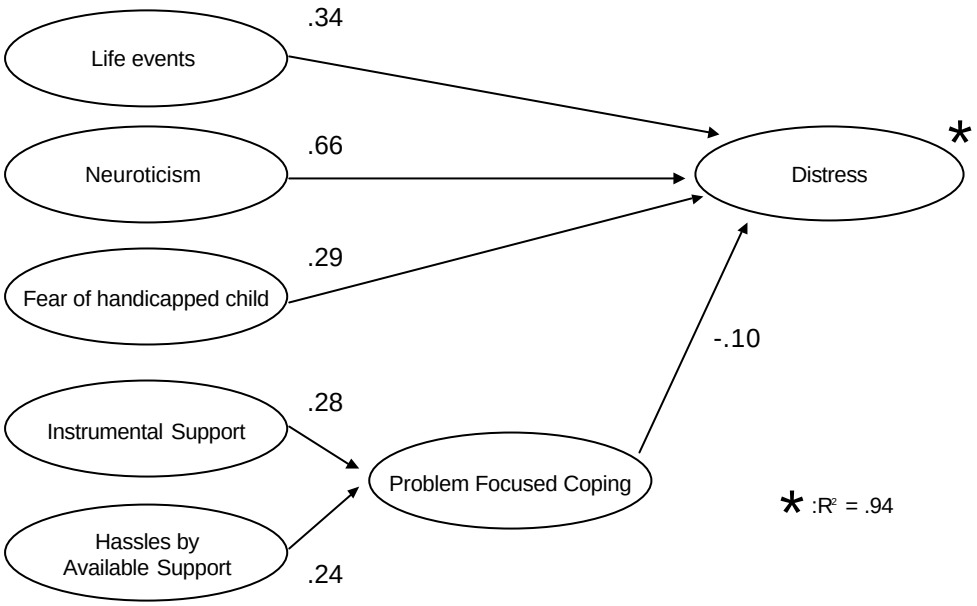

Figure 7.4. Best fitting model of distress in late pregnancy.

7.4.3 Longitudinal analyses

To test for longitudinal effects of potentially stress provoking factors on distress, several steps were undertaken. First, correlation coefficients between the distress scores in early, mid- and late pregnancy were found to be moderately high ($r > .57$) and statistically significant ($p < .0005$). Second, predictors of distress in early pregnancy (see Figure 7.2) were postulated as predictors of distress in mid-pregnancy in a path model and tested with LISREL. The results showed that neuroticism, secondary appraisal of pregnancy, and life event impact scores determined in early pregnancy accounted for 63 % of the distress in mid-pregnancy. All these paths were significant ($t > 2$) and the model fit was found to be adequate ($\chi^2 = 18.17$, $df=17$, $p= .38$, RMSEA=.03, RMR = .06, CFI= .99, NNFI=.98). Third, secondary appraisal of pregnancy and the life event impact scores determined in early pregnancy were added to the cross-sectional predictors of distress in mid-pregnancy (see Figure 7.3). Although neuroticism determined in early pregnancy contributed significantly to distress in mid-pregnancy, this variable was not added to the cross-sectional model, because neuroticism in early and mid-pregnancy were strongly correlated ($r=.76$, $p< .0005$). They cannot be regarded as independent predictors and, therefore, reflect a relatively stable characteristic of an individual. When the cross-sectional predictors were entered in the model, the longitudinal effects of secondary appraisal of pregnancy and the life event impact scores were no longer significant. Fourth, cross-sectionally determined predictors (see Figure 7.3) and distress scores of early pregnancy were postulated as predictors of distress in mid-pregnancy. Distress in early pregnancy did not significantly contribute to the variance of distress in mid-pregnancy when the

cross-sectional predictors of distress in mid-pregnancy were taken into account. Finally, according to the modification indices offered by LISREL, two paths were added to the latter model. Distress in early pregnancy was found to be related to increased fear of giving birth and fear of bearing a physically or mentally handicapped child (see Table 3). This model fitted adequately to the data (χ^2 = 48.07, df=44, p=.31, RMSEA=.03, RMR = .05, CFI= .98, NNFI=.96), although the fit is not optimal. When we compared this longitudinal model with the cross-sectional model in Figure 7.3, the latter is preferred according to the AIC criteria (142.07 versus 87.95) and according to the CFI (.98 versus 1.00) and NNFI (.96 versus .99).

Similar steps were followed to test the longitudinal effects on distress in late pregnancy of stress provoking factors determined in mid-pregnancy. The first step involved predictors of distress in mid-pregnancy (see Figure 7.3) as potentially stress-provoking factors for distress in late pregnancy. This model showed no satisfactory fit to the data. However, the paths from the life event impact scores and neuroticism determined in mid-pregnancy to distress in late pregnancy were found to be significant (t >2). However, when the cross-sectional predictors of distress in late pregnancy (see Figure 7.4) were taken into account, these factors from mid-pregnancy were no longer significant predictors of distress in late pregnancy. The second step included stress-provoking factors from late pregnancy and distress scores of mid-pregnancy as predictors of distress in late pregnancy. Distress in mid-pregnancy did not significantly contribute to the variance of distress in late pregnancy.

7.5 Discussion

Pregnancy is a period during which many aspects of a woman's life change. With regard to stress, the descriptives of the present study showed that early pregnancy is the period in which most women reported most daily hassles and life events. In the course of pregnancy, these stress-provoking factors were found to decrease with almost 50%, whereas the psychological distress did not change with time. Therefore, it was of interest to test multidimensional models of distress three times in pregnancy to gain more insight into the similar and different predictors of distress throughout pregnancy.

The first aim of the study was to formulate and test a multidimensional model of prenatal stress cross-sectionally in early, mid-, and late pregnancy. In each period of pregnancy we found a significant multidimensional model of distress which explained most of the variance in the dependent construct (86%, 88% and 94%, respectively).

Two common predictors of distress were found throughout pregnancy. The general stress-provoking factor life events accounted for a significant part of the variance of distress in early, mid- and late pregnancy. In addition, neuroticism had a strong main effect on distress in each period of pregnancy. The main effect of life events on distress was somewhat unexpected, since other studies showed that life events had only a modest effect on health outcome (e.g. McEwen & Seeman, 1999; Rabkin & Struening, 1976). Instead, we had expected more effects of daily hassles on distress, according to other studies (DeLongis et al., 1982; Paarlberg et al., 1999). Neuroticism had a main effect rather than a moderating effect on the distress level, a finding in line with other studies (Ormel & Wohlfart, 1991; Cimbolic Gunthert et al., 1999). These authors argued that neuroticism is frequently associated with a chronic negative affectivity, which could explain the high scores on distress levels throughout preg-

nancy.

Daily hassles no longer accounted for increased distress in early and late pregnancy when personality factors and social support were included in our model. Only a small effect was found during mid-pregnancy. In contrast, we previously found that daily hassles have strong effects on distress, when personality factors and social support are not included (Huizink et al., 2000b). It has been suggested that negative emotionality, like anxiousness, is associated with an increased vulnerability to everyday problems and small irritations (Watson & Clark, 1984). Therefore, including neuroticism in the present models may have overruled the effect of daily hassles on distress. Another possibility is that distress and daily hassles are separable concepts, with different mechanisms underlying the possible effect on birth outcome and later development. After all, effects of both daily hassles (Paarlberg et al., 1999) and distress (Dunkel-Schetter, 1998) on birth outcome have been found This possibility needs to be examined in future studies.

Other stress-provoking factors specifically related to pregnancy were found to result in increased levels of distress. In early pregnancy, the perceived uncontrollableness of the course of pregnancy resulted in more distress, whereas in mid-pregnancy the same resulted in increased pregnancy-related fears. The specific pregnancy-related anxieties also explained a significant part of the variance of distress in mid- and late pregnancy.

Emotion-focused coping appeared to buffer the stress response to the uplift of pregnancy in early pregnancy. Problem-focused coping had a direct inhibiting effect on distress in late pregnancy. If instrumental support was provided in the last part of pregnancy, more problem-focused coping was found. This finding corresponds well with our hypothesis that instrumental support would be welcomed during pregnancy. Especially in late pregnancy, when women are less mobile and preparations for the nursery are commonly carried out, instrumental support may be needed. Our findings suggest at least that when problems are encountered, instrumental support facilitates problem-focused coping. When social support is available in early and late pregnancy, the occurrence of daily hassles also increased coping. Thus, our findings support the view that social support must be considered a resource of coping strategies, rather than considering coping as an important determinant of social support. In early pregnancy, emotion-focused coping is activated, whereas in late pregnancy problem-focused coping is increased. Apparently, available social support buffered the effect of daily hassles on distress indirectly.

Another potentially moderating factor for the response to stress provoking factors was neuroticism (Fig. 7.1). In the present study, a strong direct effect was found for this personality aspect, rather than a moderating effect. Our sample consisted of normal risk pregnant women without very disturbing life conditions or great stressors. It may be that in situations of extreme stress provoking factors neuroticism would modify the stress response. Therefore, the models obtained in this study should be tested in high-risk pregnant women as well.

The second aim of the study was to analyze in a multidimensional model the differences in prenatal stress in early, mid- and late pregnancy. The results clearly showed that each period of pregnancy has some unique aspects that accounted for increased or decreased levels of distress. As previously reported, the most effective coping style changes from emotion-focused coping in early pregnancy to problem-focused coping in late pregnancy (Huizink et al., 2000b). In mid and late pregnancy, pregnancy-specific anxieties were found to increase distress, whereas in early pregnancy no such effect was found. In a previous study, we

showed that the highest levels of pregnancy-related anxieties were found in early pregnancy. Since these fears resulted in a measurable increase in distress only after several months of pregnancy had elapsed, perhaps the chronic effects of these fears may have resulted in more distress in the later stages of pregnancy.

The third aim of the study was to test for longitudinal effects of stress-provoking factors on distress. As described in the introduction, some stress provoking factors may have long-term effects on distress. Moreover, certain aspects of potential stressors could be confounded by distress. Therefore, we performed some longitudinal analyses. Simple correlation coefficients between distress scores in early, mid- and late pregnancy were found to be moderately high and statistically significant, suggesting that distress is a rather stable emotion throughout pregnancy. However, more detailed analyses showed that the factors contributing to the total amount of explained variance of distress differed in each period of pregnancy. This becomes clear from visual inspection of Figures 7.2 through 7.4. Statistically, it was found that the cross-sectional predictors of distress resulted in the best models of distress. Nevertheless, some similarities were found among the models of distress in early, mid- and late pregnancy. Neuroticism was a strong predictor of distress in each period of pregnancy. Since neuroticism reflects a relatively stable personality characteristic, this may explain the strong relationship between the levels of distress over time. On top of neuroticism, different factors accounted for the remaining variance of distress in early, mid-, and late pregnancy. These factors appeared to be rather specific for a particular period of pregnancy. This finding is supported by results of exploratory analyses in which we tried to fit the model of distress in early pregnancy with the data from mid- and late pregnancy. Similarly, the models of distress in mid- and late pregnancy were fitted with data from the other pregnancy periods. All resulting models had poor fits (results not shown), suggesting that they contain specific elements which contribute to distress. Life events had the strongest effect at the moment they were encountered in pregnancy, although a long term effect, three months later, was also found. However, current life events overruled the long-term effect of life events occuring in an earlier period of pregnancy. It is likely that the repeated occurrence of life events throughout pregnancy results in accumulating effects on distress. This calls for another type of analysis of these data in the future.

Pregnancy is a unique event in a woman's life, involving many adaptational changes but also naturally occurring biological changes in the body. In the present study, we have tested models of stress in this changing biological system and have found temporal changes in distress. However, our analyses have focused on the psychological level of distress. The biological system of pregnant women is unstable due to changes in hormone levels associated with pregnancy and offers a unique situation to test the effect of stress during a relatively short period of time. Perhaps the biological changes could have attributed to the differences found in the models of distress throughout pregnancy. In a next study we will examine the relationship between biological changes as reflected by levels of cortisol, ß-endorphine, and ACTH, and the models of stress.

Our findings clearly show that a single measurement of distress in pregnancy is not sufficient to accurately describe prenatal stress. The multidimensional models of prenatal stress offer more insight into the processes that lead to increased distress levels in pregnant women. Prevention and intervention programs should take notice of the temporal specificity of certain aspects of the models. Although neuroticism is a stable and strong predictor of dis-

tress, other aspects related to emotions and cognition regarding pregnancy hold potential for modification. Since there is growing evidence that women with high prenatal distress are at increased risk for poor pregnancy outcome, prevention of high levels of distress is needed. The general models of prenatal distress described in the present study should be tested in specific high risk groups in the future, to identify the major distress causing factors. Then, specific prevention or intervention programs can be developed, which may have more potential to reduce the levels of distress and subsequently poor birth outcome. The temporal specificity of the models suggests that prevention and intervention programs of distress must take into account the period of pregnancy. It is recommended to take at least the following predictors of distress into account when testing the effect of prenatal stress on birth outcome or postnatal development: life events, neuroticism, secondary appraisal of pregnancy (early and mid-pregnancy), fear of bearing a handicapped child (mid- and late pregnancy), emotion-focused coping in early pregnancy and problem-focused coping in late pregnancy (Fig. 7.2-7.4).

In future studies we will examine if the concept of prenatal stress as described in the present study predicts birth outcome and later postnatal development. Our findings of this study suggest to take the amount of daily hassles into account as an independent predictor of birth outcome and development as well.

7.6 References

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APPENDIX 1

Items of the factor 'available support' from the SPS:

Early pregnancy and mid-pregnancy:

1. People are available for support when I really need them.
2. I have close relationships which give me a sense of security and well-being.
3. If anything should go wrong, no one would come to help me.
4. I miss a sense of intimacy with someone else.

Late pregnancy:

1. I feel that I do not have close relationships with other people.
2. If anything should go wrong, no one would come to help me.
3. I miss a sense of intimacy with someone else.

8

Psychosocial and endocrinologic measures of prenatal stress as predictors of mental and motor development in infancy

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Submitted

8.1 Abstract

Background: Animal studies have found that maternal stress during pregnancy can influence the developing fetus, resulting in delay of motor and cognitive development. These effects may be mediated by the hypothalamic-pituitary-adrenal (HPA) axis.

Objective: To investigate the effect of prenatal maternal stress on motor and cognitive (mental) development in a prospective design.

Methods: Self-report data about various aspects of prenatal stress (pregnancy specific anxiety, daily hassles and an overall construct of distress) were collected in nulliparous women in early, mid- and late pregnancy. In addition, cortisol in saliva and ACTH plasma levels (mid and late pregnancy) were determined. The development of the infant was measured at 3 and 8 months by means of the Bayley Scales of Infant Development.

Results: Complete data were available of 170 term-born infants. A high level of pregnancy specific anxiety (mid and late pregnancy) predicted a lower mental and motor development index score at the age of 8 months ($p < .005$) High amounts of daily hassles in early pregnancy were associated with a significant decrease of mental development at 8 months ($p<.01$). Early morning values of cortisol in late pregnancy were negatively related to both mental and motor development at the age of 3 months (mental development and motor development; $p <.05$ and $p < .005$, respectively) and 8 months (motor development; $p<.01$). All results were adjusted for a large number of covariates. Psychological and endocrinologic stress measures were unrelated in early and mid pregnancy. In late pregnancy rather moderate assocations were found between cortisol levels determined at 8 AM and daily hassles ($r=.27$, $p<.05$) and distress ($r=.25$, $p<.05$).

Conclusion: Prenatal stress, in particular pregnancy specific anxiety, appears to be one of the determinants of delay in motor and mental development in infants of 8 months of age and may be a risk factor for later developmental problems. Further systematic follow-up of the present sample is planned.

8.2 Introduction

In a series of studies, Schneider and co-workers have shown that prenatal stressors adversely affect the motor and mental development of rhesus monkeys (Schneider 1992a; Schneider 1992b; Schneider et al., 1992; 1999). Exposure to mild stress during mid pregnancy, operationalized as three noise bursts over a 10 minute period five times a week, resulted in decreased motor maturity, as evidenced by a delay in learning to self-feed, low muscle tone, inferior balance reactions, a slowed response speed, poorer coordination and a declined attention in the first months of life in comparison to control infants (Schneider, 1992a). These effects of a mild stressor could be mimicked by prenatal exposure to adrenocorticotropic hormone (ACTH) during a 2-week period (Schneider, 1992b). Recently, Schneider et al. (1999) showed that these effects were most profound after exposure to stress in early gestation, but could still be found after mid to late gestational stress. The same mild prenatal stressor appeared to have a negative effect on cognition as well. A delay in object permanence was found on a sequence of Piagetian tasks after prenatal stress (Schneider et al., 1992).

Most human studies on prenatal stress have focused on pregnancy outcome. Stress in pregnancy has been associated with premature delivery and lower birth weight adjusted for gestational age (Dunkel-Schetter, 1998; Copper et al., 1996; Lou et al., 1994; Wadwha et al., 1993). In a prospective study, it was even found that prenatal maternal stress was associated with a reduced head circumference (Lou et al., 1994). The effect on fetal head growth may reflect suboptimal brain development and may be a predictor of impaired cognitive development (Hack et al., 1991; Stanley et al., 1989; Greisen & Petersen, 1989; Ounsted et al., 1988; Chase et al., 1972). Retrospective reports indicated that infants of high-anxious pregnant women have lower scores on the mental scale of the Bayley Scales of Infant Development than infants of low anxious women (Davids et al., 1963) and that stress during pregnancy was associated with delays in early motor development and increased amounts of behavioral problems (Meijer, 1985; Stott, 1973).

We were able to identify one prospective study on the effect of prenatal stress on postnatal development in humans (Van den Bergh, 1990). In a sample of 70 healthy nulliparous women state and trait anxiety scores measured in the third trimester of pregnancy were positively correlated with a difficult temperament of the infant at 10 weeks and 7 months after birth. However, no association was found between prenatal general anxiety measures and mental or motor developmental status of the infant at this early age.

The present prospective longitudinal study was designed to examine the effects of stress in human pregnancy on both motor and mental development early in life. We took account of several potential confounders and used multidimensional models of stress in early, mid, and late pregnancy, according to previous findings (Huizink et al., 2000b). These models are specific for the early, mid and late periods of pregnancy and include various stress-provoking (e.g. life events, secondary appraisal of the pregnancy, pregnancy-related anxiety, neuroticism) and stress-mediating factors (coping styles) that contribute to the amount of distress of pregnant women. Previous results also suggested that distress and daily hassles may be relatively unrelated concepts, with different mechanisms underlying the possible effect on birth outcome and later development (Huizink et al., 2000b). In another study, we showed the existence of pregnancy anxieties which were only partly related to personality characteris-

tics; these were regarded as pregnancy-specific stress provoking factors (Huizink et al., 2000a).

The hypothalamic-pituitary-adrenal (HPA) axis has found to be one of the mediators of the effects of prenatal maternal stress on the developing fetus in animal studies (e.g. Weinstock, 1997; Weinstock et al., 1992; Fride et al., 1986; McCormick, et al., 1995). Therefore, physiological parameters reflecting the activity of the maternal HPA axis during pregnancy were also included as predictors of postnatal infant development. For that purpose, cortisol day profiles were assessed in early, mid, and late pregnancy. Adrenocorticotropic hormone (ACTH) was assessed in mid and late pregnancy in a subsample.

The following questions were addressed in this study:

1. Does prenatal maternal stress or anxiety have a negative effect on mental and/or motor development of the infant at the age of 3 and 8 months?
2. Which aspects of prenatal stress or anxiety show negative effects on the infants' developmental status at 3 and 8 months?
3. Can specific periods in pregnancy be identified which are most vulnerable for prenatal stress effects on later infant development?
4. Are indices of maternal HPA axis activity (cortisol and ACTH) related to the infants'developmental status at 3 and 8 months?

8.3 Methods

8.3.1 Participants

All participants in this study were included in a large prospective longitudinal project which investigated the influence of prenatal psychosocial factors on fetal behavior and the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the prenatal part of the study, which included ultrasound recordings of the fetus. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants, such as maternal age, socio-economic status, and biomedical risks did not differ from those of non-participants. However, women with full-time jobs were less likely to participate.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion. The main reason for the drop in the number of participants towards late pregnancy was delivery

before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

Only healthy infants born near term (> 37 completed weeks of gestation) were included in the follow-up study after birth, to remain free from confounding factors involved with prematurity or health problems of the infant. The total number of participants, both the mothers and their infants, who completed the postnatal part of the study, which included an examination of the infants' development at 3 and 8 months of age, was 170. The sample of participants consisted largely of caucasian middle class women, although both lower and higher social classes were represented (Table 8.1). On average, the women were 31 years old. The majority of women (93.7%) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job (87.4 %), 55.3 % working less than 38 hours a week and 44.7 % working full-time. Of the infants, 84 were boys and 86 were girls.

8.3.2 Psychosocial predictors during pregnancy

To predict infant development we used three aspects of prenatal maternal stress in early, mid, and late pregnancy, which were only moderately intercorrelated (r ranging from -.03 to .26). First, a higher-order distress score was calculated for each period of pregnancy. This score was derived by means of LISREL, a structural equation technique, according to a multidimensional model of prenatal distress that has been described in detail elsewhere (Huizink et al., 2000b). In short, the distress concept in early pregnancy involved a life event impact score, neuroticism, perceived lack of control over the course of pregnancy, and emotion-focused coping. In mid pregnancy, the life event impact score, neuroticism, daily hassles, and pregnancy-related fears (fear of giving birth and fear of bearing a handicapped child) explained significant parts of the variance in distress. In late pregnancy, the distress concept included a life event impact score, neuroticism, fear of bearing a handicapped child and problem-focused coping. Throughout pregnancy, neuroticism was the strongest predictor of distress.

Second, since we found that the frequency of daily hassles was hardly predictive of the amount of distress, it was regarded as an independent predictor of infant development. Daily hassles were measured by means of the Everyday Problem List (Alledaagse Problemen Lijst, Vingerhoets et al., 1989). This Dutch questionnaire is based on a selection of items of other questionnaires, including the Daily Hassles Scale (Kanner et al., 1981), the Everyday Problem Scale (Burks & Martin, 1985) and the Daily Life Experience Questionnaire (Stone & Neale, 1982). It measures the frequency of occurrence of daily hassles in the past month and gives an intensity score which is the subjective experience of the subject of the unpleasantness of the hassles. Examples of items are: 'You could not find important belongings', 'You were trapped in a traffic jam'. In this study, only the frequency score was used in order to stay free from confounding stress provoking and stress resulting factors in the intensity score.

Third, pregnancy-related anxiety was assessed by means of the Pregnancy Related Anxi-

eties Questionnaire-Revised (PRAQ-R), an abbreviated version of the PRAQ developed by Van den Bergh (1990). We used two subscales in the present study: fear of giving birth (3 items) and fear of bearing a physically or mentally handicapped child (4 items). This questionnaire was developed from the PRAQ of Van den Bergh (1990) and consisted of nine items that fitted to a three factor model (3 items per factor): fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one's own appearance (Huizink et al., 2000a). Examples of items are: 'I am worried about the pain of contractions and the pain during delivery' (fear of giving birth) and 'I am afraid the baby will be mentally handicapped or will suffer from brain damage' (fear of bearing a physically or mentally handicapped child). The items were answered on a 5-point scale, ranging from 'never' to 'very often'. The Cronbach's alpha's of the subscales were all > .76 throughout pregnancy.

8.3.3 Endocrinological predictors during pregnancy

Two representatives of the HPA axis were determined. Cortisol was measured by determining the concentration of salivary cortisol which has been proven to be a valid and reliable reflection of the unbound hormone in blood (Kirschbaum & Hellhammer, 1989; Meulenberg & Hofman, 1990). Seven saliva samples were collected every two hours between 8:00 AM and 8:00 PM, to obtain cortisol day time curves in each of the three periods of pregnancy. All samples were stored at -70 0C until assayed. Cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2]-^{3}H(N)-Hydrocortisone (NET 185, NEN-DUPONT, Dreiech, Germany) was used as a tracer following chromatic verification of its purity. The lower limit of detection was 0.5 nmol/L and interassay variation was 11.0%, 8.2%, and 7.6% at 4.7, 9.7 and 14.0 nmol/L, respectively (n = 20). Reference values for adults are 4-28 nmol/L at 8:00 AM. For each cortisol day profile, the mean, and early morning (8 AM) values were chosen to reflect a part of the maternal HPA axis activity.

At 24 and 32 weeks of gestation, 30 ml of venous blood was collected for the assessment of ACTH in a subsample of subjects (at 24 weeks: n=43, and at 32 weeks n= 37). ACTH was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 1.0 ng/L and inter-assay variation was 11.4, 10.7 and 6.8% at 11, 68 and 310 ng/L respectively (n = 32).

The correlation coefficients between the psychosocial and endocrinological predictors were calculated. The only significant associations found between the psychosocial and endocrinological predictors, were those between the 8 AM cortisol value in late pregnancy and daily hassles ($r = .27$, $p < .05$) or distress ($r = .25$, $p < .05$) in this period of pregnancy.

8.3.4 Dependent measures of infant development

The main dependent measures were the developmental indices of the infant at the age of 3 and 8 months after birth as assessed by means of the Bayley Scales of Infant Development (BSID; Bayley, 1969) in a stardard test situation. The BSID has been translated and validated

for the Dutch population of infants (van der Meulen & Smrkovsky 1983; 1984) and therefore offers a good tool to investigate the infants' development. The mental scale results in a standard score, the *Mental Developmental Index (MDI)*, and is designed to assess sensory-perceptual acuities, discriminations, and the ability to respond to these; the early acquisition of 'object constancy' and memory, learning, and problem-solving ability; vocalizations and the beginnings of verbal communication; and early evidence of the ability to form generalizations and classifications. The second part of the BSID consists of the motor scale, which results in the *Psychomotor Developmental Index (PDI)*. The motor scale is designed to provide a measure of the degree of control of the body, coordination of the large muscles, and finer manipulatory skills of the hands and fingers.

8.3.5 Potential covariates

Data was gathered on various other aspects besides prenatal stress that may influence infant development. Descriptives of these potential covariates are shown in Table 8.1.

Prenatal factors included maternal age (in years), socio-economic status (SES), smoking and alcohol-intake during pregnancy and biomedical risks. *SES* was defined by educational level and professional level of the pregnant woman and her partner (Westerlaak et al., 1976). *Smoking behavior* was assessed by self-report, expressed as the number of cigarettes per day (cig/day) and categorized in three groups: 1) non-smokers; 2) smoking 1-10 cig/day; 3) smoking > 10 cig/day. The latter group consisted of only 7 subjects, and therefore a dichotomous variable was created: 1) non-smokers (n= 141); 2) smokers (n= 31); > = 1 cig/day. *Alcohol-intake* during pregnancy was likewise determined by self-report, and was expressed as the number of alcohol-containing beverages per week. Only 11 subjects consumed more than 2 alcohol-containing beverages per week and therefore a dichotomous variable was created: 1) non-drinkers (n= 144); 2) drinkers (n = 28); > = 1 drink per week. Our sample thus contained very few heavy smokers or drinkers, and only a relatively small number of modest smokers and drinkers. *Biomedical risk factors* included the use of medication during pregnancy, pre-existent health problems, fertility problems, gynecological risk factors (DES daughters etc.), high bloodpressure, excessive vomitting and diabetes mellitus caused by pregnancy. The risk factors were added up in a categorical variable; scores ranged from 0 - 5.

Perinatal covariates that may confound the effect of prenatal stress on infant development included birth weight (in grams) and gestational age at birth (in weeks). Also, complications during delivery, the use of medication during delivery, fetal distress, and mode of delivery (elective caesarean section or artifical delivery) were taken into account, by calculating a cumulative score of these perinatal complications (range 0-5).

Postnatal potentially covariates included in the present study are postnatal stress levels of the mother which were determined at 3 and 8 months following child birth. *Psychological well-being* was determined by means of the Dutch translation (Koeter & Ormel, 1991) of the General Health Questionnaire (GHQ-30; Goldberg, 1972). This questionnaire contains 30 questions to be answered on a four-point scale. *Perceived stress* was assessed with the Perceived Stress Scale of Cohen & Williamson (1987), using a Dutch translation. It contains 14 items on an individual's perceived stress over the last month to be answered on a 4-point scale, ranging from 'never' to 'always'.

Table 8.1
Descriptives of potential prenatal, perinatal and postnatal confounders

Confounders	
Prenatal	
Maternal age (years) ± SD	31.3 (4.9)
SES	**Educational level mother** * Low 13.6 % Middle 67.5 % High 18.9 % **Educational level partner** Low 23.4 % Middle 59.8 % High 16.8 % **Professional level mother** Low 8.0 % Middle 54.6 % High 37.4 % **Professional level partner** Low 18.0 % Middle 29.2 % High 52.8 %
Smoking	Smokers: n = 29; > = 1 cigarette per day Non-smokers: n = 141
Alcohol-intake	Drinkers: n = 26; > = 1 drink per week Non-drinkers: n = 144
Biomedical risks	No risk: n = 102 Pregnancy complications: n = 30 Medication during pregnancy: n = 25 Risk for fetus of medication: n = 4 Fertility problems: n = 48 IVF: n = 13 High bloodpressure: n = 15 Diabetus mellitus due to pregnancy: n = 3 Gynecological risk: n = 12 Pre-existent disease: n = 12 Mean score (± SD): 1 (1.2)
Perinatal	
Birth weight (grams) ± SD	3385.5 (487.3)
Gestational age at birth (weeks) ± SD	39.6 (1.9)
Perinatal complications	Partus complications: n= 25 Medication during delivery: n=88 Elective caeserean section: n= 24 Artifical delivery due to fetal distress: n=20 Mean score (± SD): 1 (1.3)
Postnatal	
Psychological well-being (GHQ-30) ± SD	3 months postpartum: 4.7 (5.1) 8 months postpartum: 3.6 (5)
Perceived stress ± SD	3 months postpartum: 25.9 (5.8) 8 months postpartum: 25.5 (5.7)

* low level: primary school, high-school education; middle level: secondary school education; high level: college or academic education

8.3.6 Statistical analysis

First, descriptive analyses were performed on all independent and dependent variables. Second, possible categorical or interval scaled covariates (SES, maternal age, gestational age, birth weight, postnatal stress of the mother) were tested for their relationships with the dependent variables by means of correlations (Pearson or Spearman when appropriate) and regression analysis. Only covariates which were significantly related to the dependent variables were included in further analyses on main effects of prenatal stress. Non-linear effects were tested within a MANCOVA with MDI and PDI scores of 3 and 8 months as dependent variables with a high/low contrast on the between-subjects factor that represented the upper and lower quartile scores in the predictors. For ACTH a median split method was used to form two groups, due to the small sample size. Dichotomous covariates (smoking and alcohol-use during pregnancy, infants' sex) were entered as a between-subjects factor in the MANCOVA. In case of a significant multivariate Hotelling's T2 test, univariate analyses were performed subsequently to locate the source of the difference. Linear effects of significant stress predictors were then examined with multiple regression analysis. The clinical relevance of prenatal predictors was explored in logistic regression analyses that attempted to differentiate mental and motor scores in the lowest quartile from scores in the higher quartile. The associations between continuous predictor variables and the dichotomized dependent variables in the logistic regression models are reported as standardized odds ratios (SOR) and 95% confidence intervals (CI). The SOR represents the change in risk due to one standard deviation change in the independent variable. To control for the possibility of chance findings due to the relatively high number of independent and dependent variables, we used multivariate techniques of analysis. With all tests, statistical significance was assumed at the level of $p < .05$.

8.4 Results

8.4.1 Descriptive analysis

In Table 8.2, means, standard deviations and range in scores of the predictors are presented. With regard to the dependent variables, the mean MDI scores were 114.9 (SD 15.0; range 71-150) and 117.7 (SD 15.5; range 76-150) at 3 and 8 months of age, respectively. The mean PDI scores were 101.3 (SD 13.7; range 61-150) and 109.4 (SD 13.5; range 77-150) at 3 and 8 months of age, respectively. The MDI scores at 3 and 8 months of age were significantly correlated ($r = .26$, $p < .0005$), and so were the PDI scores at 3 and 8 months ($r = .23$, $p < .0005$). Stronger associations were found between the MDI and PDI scores at 3 months ($r = .52$, $p < .0005$) and those at 8 months ($r = .38$, $p < .0005$).

Table 8.2
Mean, SD and range in scores of the psychosocial and endocrinologic predictors

Predictors	*Mean*	*SD*	*Range*	*N*
Psychosocial				
Daily hassles T1	9.96	6.3	0-45	170
Daily hassles T2	7.83	5.5	0-26	170
Daily hassles T3	6.41	4.3	0-23	170
Distress T1	-.02	1.0	-2.1-2.6	170
Distress T2	-.02	1.0	-1.2-7.6	170
Distress T3	.00	1.0	-1.2-8.2	170
Fear of giving birth T1	6.17	2.9	3-15	170
Fear of giving birth T2	5.92	2.7	3-15	170
Fear of giving birth T3	5.99	2.7	3-15	170
Fear of handicapped child T1	9.25	3.5	4-20	170
Fear of handicapped child T2	8.56	3.1	4-19	170
Fear of handicapped child T3	8.49	3.2	4-20	170
Endocrinologic				
Mean cortisol T1	10.57	2.3	5.2-19.8	142
Mean cortisol T2	14.38	3.1	6.3-22.3	130
Mean cortisol T3	17.35	3.8	2.8-30.8	85
Cortisol 8 AM T1	19.78	7.4	6-44	142
Cortisol 8 AM T2	23.29	6.8	9.3-41	130
Cortisol 8 AM T3	23.64	6.3	2.5-43	85
ACTH 24 weeks	16.95	9.3	5-44	43
ACTH 32 weeks	25.97	15.7	11-89	37

T1= early pregnancy; T2= mid-pregnancy; T3= late pregnancy.

8.4.2 Tests for the effects of potential covariates

Correlation coefficients were calculated between the infant developmental scores at 3 and 8 months and prenatal (SES, maternal age, biomedical risks), perinatal (gestational age at birth, birth weight and perinatal complications), and postnatal (mothers' stress levels) factors.

Infant mental development at 8 months of age was positively correlated with gestational

age at birth (r = .17, p < .05), birth weight (r = .21, p < .01), and the educational level of the mother (r = .15, p < .05), but negatively correlated with her amount of perceived stress at 3 months after delivery (r = -.28, p < .001). Infant motor development was positively related to gestational age at birth, both at 3 months (r = .17, p < .05) and at 8 months (r = .21, p < .01). No other linear relationships between potential covariates and the dependent variables were found. Multiple regression analysis with the significant correlates of infant development showed independent effects on infant mental development at 8 months of birth weight (F(1,172) = 7.56, p < .01), gestational age at birth (F(1,172) = 7.53, p < .01), and the amount of perceived maternal stress at 3 months (F(1,172) = 6.12, p < .05). These variables explained 4.2%, 4.3%, and 5.6% of the total variance in the MDI scores at 8 months, respectively. These covariates were taken into account in further analyses.

No main effects or interaction effects of the dichotomous variables reflecting smoking, alcohol-intake during pregnancy, or sex of the infant were found on MDI or PDI scores.

8.4.3 Infant mental and motor development in relation to measures of maternal prenatal stress

In this section, the MDI and PDI scores at 3 and 8 months were compared between infants of mothers who had low (<= P25) and high (> = P75) stress levels during pregnancy. This analysis was carried out using MANCOVA (including MDI and PDI scores of the infants at 3 and 8 months of age) and univariate analyses for early, mid, and late pregnancy separately to test for non-linear effects, followed by multiple regression analysis to test for linear effects. Finally, logistic regression was performed. The results are summarized in Table 8.3 and Figures 8.1-8.4.

MANCOVA showed that an overall decrease in MDI and PDI scores of infants whose mothers had reported a high amount of daily hassles in early pregnancy (F(12,188) = 5.43, p <.05), and a high level of fear of giving birth in mid- and late pregnancy (F (12, 188) = 4.37, p <.005 and F (12,188)= 4.02, p <.05, respectively), after adjusting for gestational age at birth, birth weight and the postnatal stress level of the mother. Subsequent univariate analyses showed that the decline in MDI scores at the age of 8 months is significant for daily hassles in early pregnancy, and for fear of giving birth in mid- and late pregnancy (see Figs. 8.1-8.3). No linear effect of psychosocial stress on MDI scores could be found. Univariate analyses furthermore showed that the difference in PDI scores, when comparing women high on fear of giving birth in mid-pregnancy with women low on this fear, was significant in infants at the age of 8 months (see Table 8.3 and Figure 8.4). Multiple regression analysis showed a linear negative effect of fear of giving birth in mid-pregnancy on PDI scores, explaining 5 % of the total variance.

Logistic regression showed that daily hassles and distress in early pregnancy were independent risk factors for low (i.e. <= P25) MDI scores of infants at 8 months of age (SOR = 1.1, 95% CI 1.02 - 1.18 and SOR = 1.7, 95% CI 1.04 - 2.7, respectively). Logistic regression furthermore showed that high levels of fear of giving birth in mid-pregnancy increased the risk of having an infant with a low (i.e. <= P25) PDI score at 8 months of age (SOR=1.3, CI 1.12 - 1.56).

		MANCOVA high/low contrast			Univariate post-hoc analyses			Multiple regression analyses				
Predictors	*Da*	*F*	*df* (m,n)	*P*		*F*	*P*	*β*	R^2 total	*F*	*df*	*P*
Psychosocial												
Early pregnancy					3 months	--	n.s.	--	--	--	--	--
Daily Hassles	MDI	5.43	12,188	<.05	8 months	7.0	<.01	--	--	--	--	n.s.
	PDI				3 months	--	n.s.	--	--	--	--	--
					8 months	--	n.s.	--	--	--	--	--
Mid pregnancy					3 months	--	n.s.	--	--	--	--	--
Fear of giving birth	MDI	4.37	12,188	<.005	8 months	8.35	<.005	--	--	--	--	n.s.
	PDI				3 months	--	n.s.	--	--	--	--	--
					8 months	13.73	<.005	-.21	.05	5.30	16	<.05
Late pregnancy					3 months	--	n.s.	--	--	--	--	--
Fear of giving birth	MDI	4.02	12,188	<.05	8 months	5.34	<.05	--	--	--	--	n.s.
	PDI				3 months	--	n.s.	--	--	--	--	--
					8 months	--	n.s.	--	--	--	--	--
Endocrinologic												
Early pregnancy					3 months	6.38	<.05	-.31	.10	7.19	85	<.01
Daily Hassles	MDI	4.20	12,83	<.01	8 months	--	n.s.	--	--	--	--	--
	PDI				3 months	9.15	<.005	-.28	.14	5.16	85	<.01
					8 months	8.50	<.01	-.28	.08	7.08	85	<.01

n.s. = not significant; -- = not relevant, Da = Developmental aspects

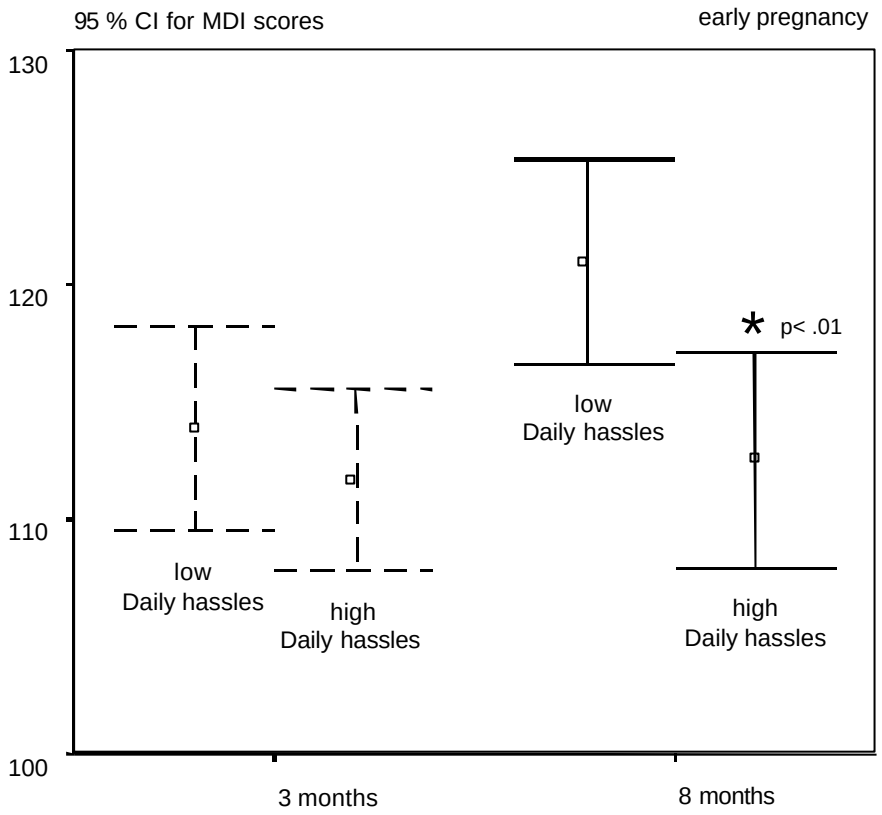

Figure 8.1
The effect of daily hassles in early pregnancy on MDI scores of infants at 3 and 8 months of age.

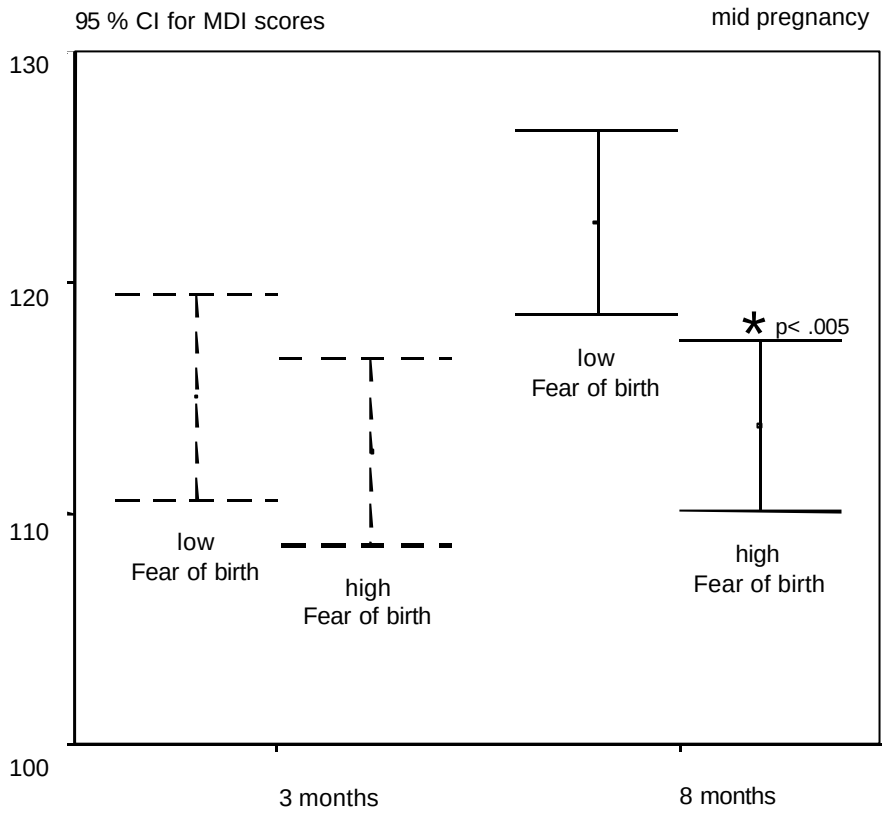

Figure 8.2
The effect of fear of birth in mid-pregnancy on MDI scores of infants at 3 and 8 months of age.

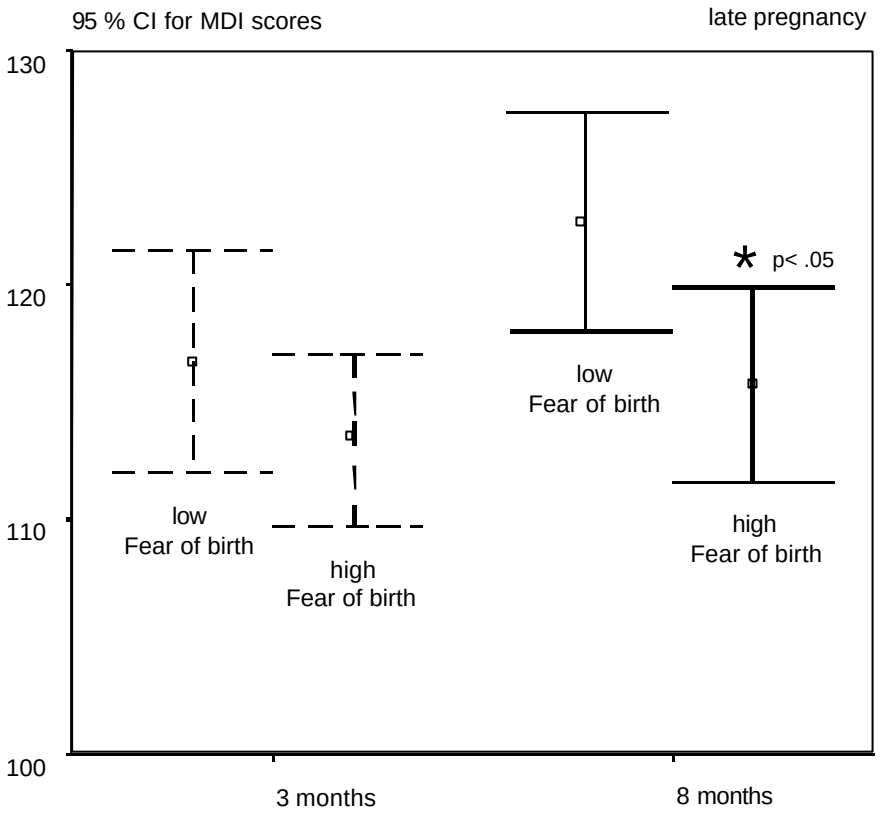

Figure 8.3
The effect of fear of birth in late pregnancy on MDI score of infants at 3 and 8 months of age.

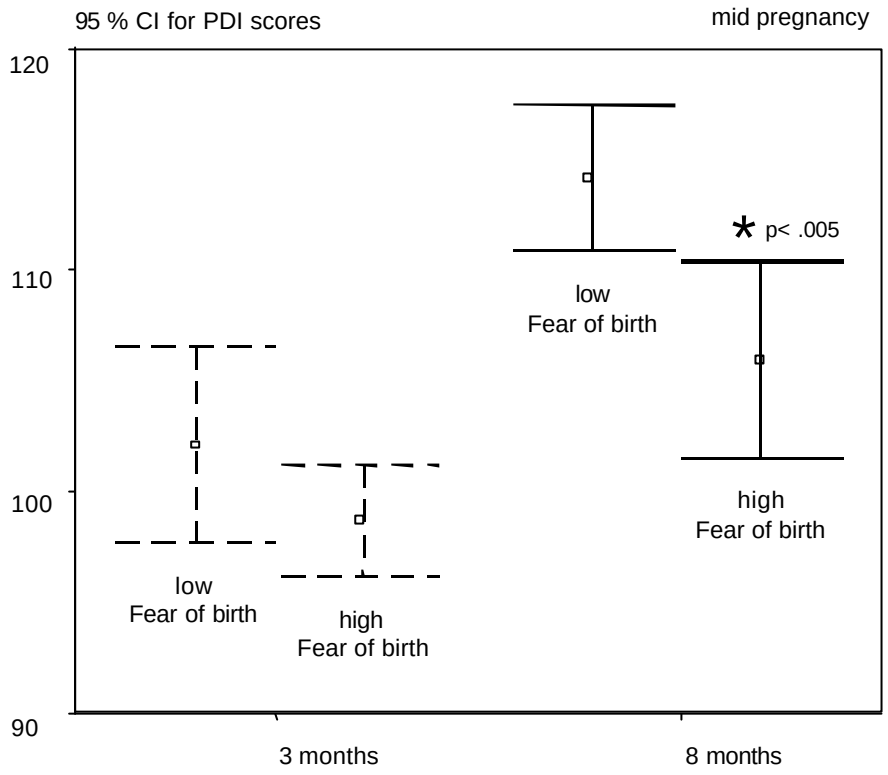

Figure 8.4
The effect of fear of birth in mid-pregnancy on PDI scores of infants at 3 and 8 months of age.

8.4.4 Infant mental and motor development in relation to measures of maternal HPA axis activity during pregnancy

In this section, analyses are presented analogous to those performed above. The results are presented in Table 8.3 and Figures 8.5 and 8.6.

MANCOVA showed that high levels of cortisol at 8 AM in late pregnancy as compared to low levels of cortisol at 8 AM in this period of pregnancy were associated with an overall decrease in MDI and PDI scores ($F(12,83) = 4.20$, $p<.01$), after adjusting for confounders. Subsequently performed univariate analyses showed that the decline in MDI scores was significant in infants at 3 months of age ($F(1,32)= 6.38$, $p< .05$), whereas the effect of high cortisol on PDI was significant for infants at both 3 ($F(1, 32) = 9.15$, $p<.005$) and 8 months of age ($F(1,32) = 8.50$, $p<.01$; see Figs. 8.5-8.6). Multiple regression analyses showed a linear negative effect of cortisol determined at 8 AM in late pregnancy on the MDI scores of 3-months-old infants and on the PDI scores of infants both at 3 and 8 months of age.

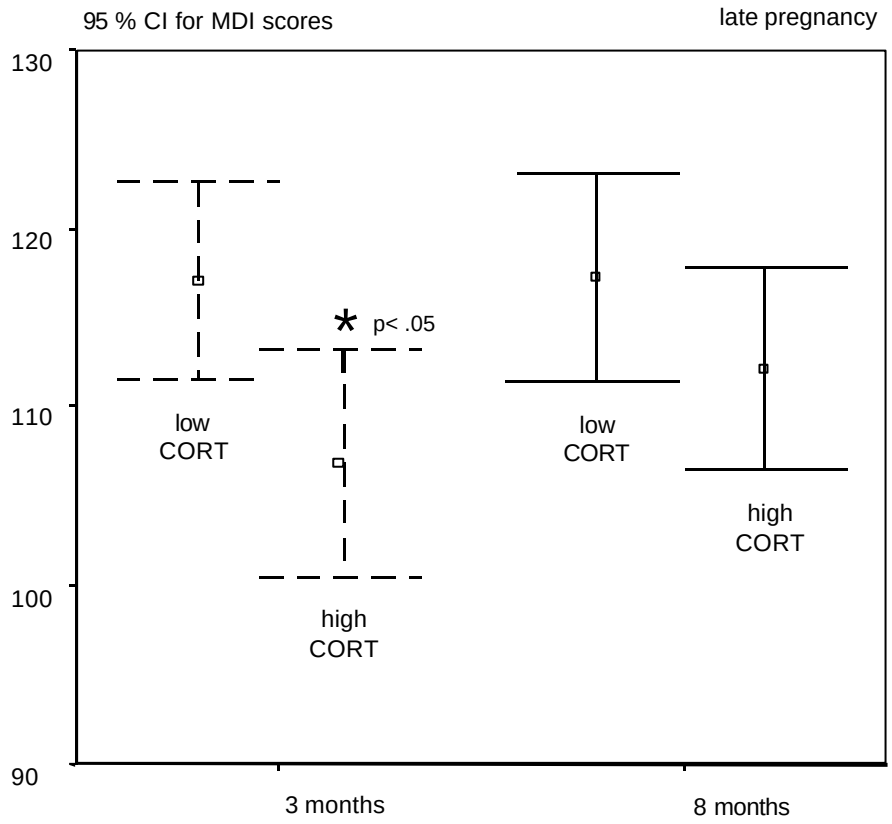

Figure 8.5 The effect of cortisol (CORT) level in saliva at 8 AM in late pregnancy on MDI scores of infants at 3 and 8 months of age.

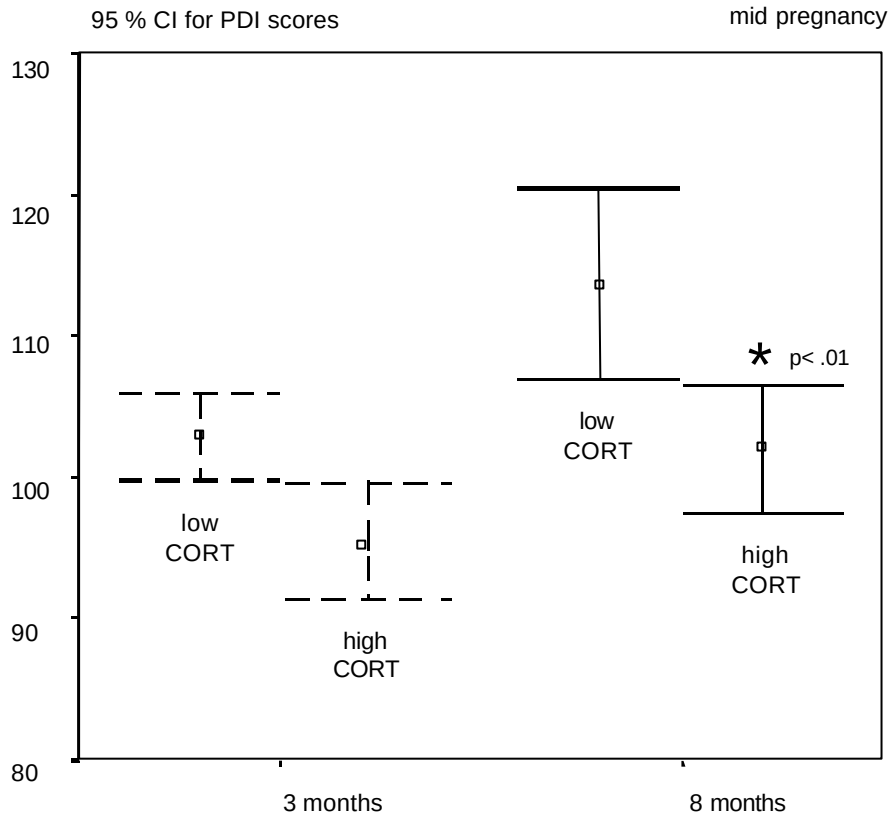

Figure 8.6
The effect of cortisol level in saliva at 8 AM in late pregnancy on PDI scores of infants at 3 and 8 months of age.

8.5 Discussion

The present study examined the effect of prenatal stress on infant development in the first 8 months of life in infants born near term. The results of this investigation indicated that prenatally stressed infants have declined MDI and PDI scores when compared to non-stressed infants. These effects of prenatal psychosocial stress were evident at the age of 8 months postpartum and remained significant after adjusting for possible confounders, such as SES, maternal age, birth weight, gestational age, biomedical risks, perinatal complications, and the mothers' postnatal stress levels (see Table 8.1). On average an 8-point decline in MDI and PDI scores was found after exposure to prenatal maternal stress, as determined by psychosocial measures. The effect on MDI scores was only found when high contrast groups were formed, whereas the negative effect of prenatal maternal stress on PDI scores was also linear.

Although the effects of prenatal stress may seem rather mild, they were adjusted for various pre-, peri- and postnatal covariates and they were found in a sample of fullterm infants. Since prenatal stress has been found to be associated with preterm delivery and low birth weight (Dunkel-Schetter, 1998; Copper et al., 1996; Wadwha et al., 1993), the present study may underestimate the influence of prenatal stress on infant development, possibly mediated by adverse pregnancy outcomes. Moreover, an increased risk of obtaining a MDI or PDI score in the lowest 25th percentile was found for specific stressors in early (daily hassles and distress) and mid-pregnancy (fear of giving birth). Thus, the present findings have clinical relevance. It is important to note that there are wide individual differences in responsivity to stressors among adults (Steptoe et al., 1996; Stansbury & Gunnar, 1994). It is therefore highly unlikely that psychological disturbances such as prenatal stress or anxiety would affect all mothers and their infants in an identical manner. The present study analyzed the effect on a group level, and therefore it could be that we underestimated the effects of stress for especially reactive women and their infants. In addition, the stressors used in this study are naturally occurring stressors and no experimental stressors such as used in animal studies, nor a circumscribed stressor by accident like a period of war of earthquake. Therefore, it is most likely that the prenatal stress levels in our sample are rather mild as compared to stressors used in animal studies. However, our overall results concur with other evidence indicating that prenatal stress administered to pregnancy monkeys induced neuromotor deficits in their offspring (Schneider, 1992; 1992a; 1992b; Schneider et al., 1999). We should note that although the MDI is classified as a 'mental developmental index', between the age of 3 and 8 months over half of the items contributing to the MDI are motor or sensorimotor tasks.

With regard to some potential confounders or modifying factors, we did not find a main effect or modifying effect of the infants' sex on developmental outcome after prenatal stress. Some animal studies have found that the male and female offspring of a low-activity strain of prenatally stressed mothers differed in their postnatal behavior (Stohr et al., 1998), suggesting that sex effects seem relevant to explain different results following the exposure to prenatal stress. In our sample, only very few women were heavy smokers or drinkers and a relatively small number of pregnant women were moderate smokers or drinkers during pregnancy. Therefore, it is not surprising that we did not find a main or interaction effect of smoking or alcohol-intake during pregnancy on infant development.

We furthermore tested which aspects of prenatal stress and anxiety had the most profound adverse effects on MDI and PDI in early infancy. Pregnancy-specific fears had a negative effect on infant development. In particular, an increased level of fear of giving birth during mid-pregnancy was found to be associated with a linear decrease in PDI scores at 8-months-old infants. Daily hassles and the multidimensional concept of distress in pregnancy also had an adverse effect on MDI scores at 8 months. These aspects of stress are only moderately intercorrelated and possibly reflect various aspects of the emotional state of pregnant women. Thus, it appears that various prenatal stress aspects and fear of giving birth were associated with reduced MDI scores at 8 months of age, but the strongest effects were found for pregnancy-related anxiety. Since the stress measures used in the present study are not comparable with the stressors used in non-human primate studies, the present findings should be replicated in another human study. Pregnancy-related anxiety has been found to predict adverse pregnancy outcome as well (Killingsworth Rini et al., 1999), and reflects a unique element of human pregnancy. Rather than studying the effects of life events and daily

hassles on birth outcome and postnatal development, the present findings suggest to focus on anxieties and stressors specifically related to pregnancy in humans.

Our results show evidence for the notion that prenatal stress factors do affect mental and motor development. However, it remains difficult to be clear about which period of pregnancy in particular is involved. First, our prenatal stress measures throughout pregnancy are not independent, that is, they are correlated over time. Second, a direct effect of stress during a particular period in pregnancy on the developing fetus would offer stronger evidence for the assumption that a sensitive period for prenatal stress would exist. These effects are tested as well in our large prospective study and will be described in detail elsewhere (Robles de Medina, 2000). Although we employ cautiousness in drawing conclusions about sensitive periods for prenatal stress, our findings are partly in line with findings of Schneider et al. (1999), who showed that sensitivity to prenatal stress of rhesus macaques peaks during early gestation and tapers off during later gestation. In our study, the strongest effects of prenatal stress on mental development were found in early and mid-pregnancy. The effects tapered off until late pregnancy, but could still be found. For motor development, mid-pregnancy stress exhibited the strongest effects. Early gestation reflects a period of generation of neurons and neuronal migration. Middle gestation is known as a period during which there is neuroblast proliferation. Late gestation and the subsequently postnatal first 18 months of life correspond to the brain growth spurt, a period during which brain weight and developmental processes proceed very quickly (Dobbings & Sands, 1979). Studies have indicated that cell neuronal migration is highly sensitive to various perturbations, such as toxins, viruses, and genetic mutations (Barth, 1987; Caviness et al., 1989; Rakic, 1988). Proper neuronal migration results in an appropriate acquisition of neuron position, which enables communication between early and late forming neurons at the critical developmental stages, before they make their synaptic connections (Rakic, 1985). Thus, theoretically, one would expect a stronger effect of prenatal stress in the early period of pregnancy. However, the negative effect of cortisol on MDI and PDI scores was only found in late pregnancy. These results should be interpreted with caution, since an effect was found for the early morning level of cortisol only, and this value is vulnerable to variation due to the circadian rhythm of cortisol secretion, where early morning values decline steeply around that time. Interestingly, only in late pregnancy, the early morning value of cortisol was moderately correlated with psychosocial measures of stress. Perhaps, this indicates that the HPA axis is reactive to stress only in late pregnancy in humans and may mediate the effects on the developing fetus in this period. Further research is warranted to elucidate this potential pathophysiologic mechanism in more detail.

Although we controlled for postnatal stress levels and psychological well-being in the present study, it is almost impossible to control for all life-style variables of the postnatal environment of the infant. Most likely, a risk profile for postnatal development would predict the development of the infant most accurately. Women high on prenatal stress may have more adverse life-styles, which would contribute to an accumulating negative effect on the infants' development. Postnatal environmental factors may contribute in an additional way to the early programmed vulnerabilty of prenatally stressed infants. In that light, it is interesting to see that the effect of prenatal psychosocial stress and anxiety were only significant when the infant had reached the age of 8 months, although a similar pattern was already found at 3 months. In contrast, the effects of cortisol were already significant for 3 months-old-infants.

Thusfar, the results of existing intervention programs designed to reduce distress during

pregnancy are inconclusive (Villar et al., 1992; Elbourne et al., 1996; Norbeck, 1994). Our results suggest that a focus on pregnancy related anxiety may increase the effectiveness of intervention studies. Moreover, the findings of the present study offer a guideline for future studies on prenatal stress effects in high-risk populations. Although the effects of stress in pregnancy are only mild in this early part of life, animal studies have shown that they could persist until later in life and retrospective human studies suggest that the effects may even increase at a later age. Early neuromotor dysfunction has been found to be associated with academic, cognitive, and behavioral problems at later ages (Gillberg & Gillberg, 1989; Marlow et al., 1993; Brumback, 1993). Already decades ago Bayley (1969) stated: `Motor abilities play important roles in the development of the child's orientation toward its environment, and they influence the quality of its interaction with the environment. Locomotion and control of the body serve to enlarge the potential sphere for new and varied experiences and for individual choices in seeking or avoiding different kinds of experience'. Thus, the effects of prenatal stress on motor development may hamper infant development in various ways. Therefore, a longer follow-up of prenatally stress-exposed children is warranted.

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9

Prenatal psychosocial and endocrinologic predictors of infant temperament

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9.1 Abstract

Background: Preclinical data in nonhuman primates and rodents indicate that prenatal stress adversely affects the ability of offspring to cope in challenging situations. These effects may be mediated by the hypothalamo-pituitary-adrenal (HPA) axis.

Objective: To examine the effects of psychological stress of pregnant women on temperament of infants in a prospective design.

Methods: Self-report data about various aspects of prenatal stress (pregnancy specific anxiety, daily hassles, perceived stress, and an overall construct of distress) were collected of nulliparous women in early pregnancy. In addition, cortisol saliva and ACTH plasma levels (mid pregnancy) were determined. The temperament of the infant was measured at 3 and 8 months by observation at the administration of the Bayley Scales and by parent reports on the Infant Characteristics Questionnaire (ICQ).

Results: Complete data were available of 170 term-born infants. Pregnancy specific anxieties explained 7% of the variance (p's < .05) of test-affectivity and goal-directedness of the infant at the test situation at 8 months. Perceived stress explained 12% of the variance ($p < .01$) of unadaptability of the ICQ at 3 months but not at 8 months. Levels of ACTH explained 29% of the variance ($p < .01$) of unadaptability at 8 months. All results were adjusted for a large number of covariates. Psychological and endocrinologic stress measures were unrelated.

Conclusion: Increased stress in human pregnancy seems to be one of the determinants of temperamental variation of infants at 8 months and may be a risk factor for developing psychopathology later in life. Further systematic follow-up of the present sample is planned.

9.2 Introduction

Harmful events during delivery explain only a small proportion of disorders in child behavior and development (Goodman & Stevenson, 1989; Gillberg et al., 1983; Uljas et al., 99; O'Callaghan et al., 1997; Taylor et al., 2000), and improved obstetric and neonatal care during the last decades has not been able to reduce the rate of these neurodevelopmental disorders (Casaer, 1993; Hjalmorson et al., 1988; Visser & Narayan, 1996).Therefore, prenatal factors and their presumed programming effects on the fetal brain have been incriminated to play a role in developmental psychopathology (Huttunen et al., 1994; van Os & Selten, 1998; Selten et al., 1999). However, prospective studies on the possible involvement of prenatal stress and anxiety are almost lacking in the fields of child psychiatry and developmental psychology.

9.2.1 Long-term effects of prenatal stressors: evidence from animal studies

There is a large body of animal studies to show that prenatal maternal stress may influence offspring behavior. For instance, prenatally stressed rats showed decreased exploration and more defecation (a measure of distress) in an open field test condition (Archer & Blackman, 1971). Long-term effects on behavior have also been demonstrated in prenatally stressed non-human primates. When studied in a novel environment, these infants exhibited more disturbance and fearful behaviors and reduced exploration (Schneider et al., 1992), showed more mutual clinging (Clarke & Schneider, 1993), had less social interactions, and withdrew more often from social interactions (Worlein & Sackett, 1995). Some of the alterations in infant behavior seen after prenatal exposure to noise stress (impairments in attention, and temperament) could also be induced by endocrine activation of the pregnant monkey by means of a 2-week period of adrenocorticotrophic hormone (ACTH) administration (Schneider, 1992a). It has, therefore, been suggested that the maternal hypothalamic-pituitary-adrenal (HPA) axis is one of the mediators of the effects on the developing brain of prenatal stress (Barbazanges et al., 1996).

9.2.2 Long-term effects of prenatal human stressors

Although the belief that the emotional state of the mother may affect the child she is carrying has a very long history (Ferreira, 1965), only recent studies provided some evidence that maternal stress in pregnancy is associated with increased risk for preterm delivery, low birth weight, and a smaller head circumference as a measure of brain development (Dunkel-Schetter, 1998; Wadhwa et al., 1993; Pagel et al., 1990; Hedegaard et al., 1993; Copper et al., 1996; Lou et al., 1994). A few prospective studies have examined the effect of prenatal maternal stress or anxiety on infant behavior. Van den Bergh (1990), in a study on 70 healthy pregnant women, found a significant relationship between high maternal state and trait anxiety scores in late pregnancy and a difficult temperament in 10-week-old and 7-months-old infants. DiPietro et al. (1996) showed that a particular index of fetal maturation predicted

22-60% of the variance in infant temperament scores, such as difficulty, unadaptability, and activity, at the age of 3 and 6 months. Although the latter study did not include maternal stress measures, these findings suggest the existence of some prenatal antecedents of human postnatal behavior.

A difficult aspect of studying stress in human pregnancy is the operationalization of the concept of prenatal stress. Therefore, in the present study we used a multidimensional model of distress in pregnancy. This model includes various stress-provoking factors (life events, secondary appraisal of pregnancy, pregnancy-related anxiety, neuroticism) and a stress-mediating factor (coping) that contribute to the amount of distress a pregnant woman perceives (Huizink et al., 2000b). Neuroticism was found to be the strongest predictor of distress. This model reflects a more detailed picture of prenatal stress than was given in the study of Van den Bergh (1990). Previous studies suggested that distress and daily hassles may be separable concepts, and that pregnancy-related anxieties may be regarded as stress-provoking factors specifically related to pregnancy (Huizink et al., 2000a; Huizink et al., 2000b). Thus, besides a multidimensional concept of distress, daily hassles and pregnancy-related anxieties were regarded as prenatal predictors of infant temperament. In addition, we focused on the effects of perceived stress, which may be regarded as a very subjective measure of prenatal stress, on infant temperament.

9.2.3 The role of possible confounders in the study of prenatal stressors

Various factors, both prenatal and postnatal, complicate the study of possible effects of prenatal stressors on infant behavior. Prenatal confounders include, for instance, smoking and alcohol intake during pregnancy, SES and maternal age (Creasy, 1991; Landry et al., 1997; Pollock, 1996; Hemminki & Gissler, 1996; Fergusson & Lynskey, 1993; Trasti et al., 1999; Makin et al., 1991; Frydman, 1996; Naeye & Peters, 1984; Kaplan-Estrin et al., 1999; Gusella & Fried, 1984; Jacobson et al., 1993). Unlike animal studies, it is impossible to control for the postnatal environment in which the child is raised. Several lifestyles may contribute to the infants' behavior and the mother-infant interaction is likely to influence at least some aspects of the infant's behavior (Cohn & Tronick, 1989; Field, 1992; Lyons-Ruth et al., 1990). If the mother is highly stressed in the postnatal period, her behavior may influence the reactions of her infant and her perceptions of the infant may differ from those of low postnatally stressed mothers. Other environmental factors that may influence infant behavior are family and social risk factors, such as one parent families and low SES.

9.2.4 Infant temperament

Although researchers have focused for many years on environmental variables that are responsible for a child's development and behavior, lately the focus has been shifted to interest in how children's own tendencies affect transactions with caregivers and consequently their own personality development. These behavioral tendencies are often described in terms of temperament (Bates, 1989). The most general definition of temperament is that it consists

of biologically rooted individual differences in behavior tendencies that are present in early life and are relatively stable across various kinds of situations and over the course of time (Bates, 1986; Goldsmith et al., 1987). Furthermore, there is general agreement that temperament is manifest largely in the context of social interaction. The concept of temperament is not just a general concept, but also describes specific aspects of individual differences in behavior. Several constructs are frequently mentioned in the literature on temperament, including difficultness, adaptability, and attention regulation. Difficultness refers to a negative mood, withdrawal, high intensity and low regularity of biological rhythms. Adaptability reflects adaptation to novelty, thus individual differences in responding to new people and other new stimuli. Finally, attention regulation reflects the attention span and task persistence (Bates, 1989).

Evidence suggests that temperamentally difficult infants may be at risk for later adjustment problems (Bates et al., 1985; Wolkind & De Salis, 1982; Rutter et al., 1964). In a longitudinal-epidemiological study Caspi et al. (1996) showed that behavioral differences among children in the first 3 years of life are linked to specific adult psychiatric disorders, such as anxiety and mood disorders, antisocial personality disorder, recidivistic and violent crime, alcholism, and suicidal behavior. Thus variations in early temperamental characteristics may be an early sign of or a risk factor for the development of psychopathology in children.

9.2.5 Aims of the study

The present study analyzed the effects of prenatal maternal stress and anxiety on both infant temperament reported by the mother and objectively observed behavior in a standard novel situation. In line with the findings of animal studies, we hypothesize that prenatal stress and anxiety is related to more difficult behavior of the child and to more problematic adaptation to a novel situation. Moreover, we expect less exploratory behavior in prenatally stressed infants in a standard novel test condition, less attention during the test, and more problems in the social interaction with the testleader. Schneider et al. (1999) showed in a study on the ontogenetic vulnerability for prenatal stress in nonhuman primates, that sensitivity to prenatal stress may peak during early gestation and tapers off during mid-late gestation. Therefore, we used planned comparisons for prenatal stress effects on infant temperament derived during early and explored the effect of mid- and late pregnancy stress. The possibly mediating role of the maternal HPA axis was also explored. The following questions were addressed:

1. Does maternal stress or anxiety in early pregnancy have a negative effect on behavioral aspects of the infant at the age of 3 and 8 months?
2. Which aspects of prenatal stress or anxiety have negative effects on behavioral aspects of 3- and 8-months-old infants?
3. Are indices of maternal HPA axis activity (cortisol and ACTH) related to behavioral aspects of 3- and 8-months old infants?

9.3 Methods

9.3.1 Participants

All participants in this study were included in a large prospective longitudinal project which investigated the influence of prenatal psychosocial factors on fetal behavior and the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the prenatal part of the study, which included ultrasound recordings of the fetus. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants, such as maternal age, socio-economic status, and biomedical risks did not differ from those of non-participants. However, women with full-time jobs were less likely to participate.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion.The main reason for the drop in the number of participants towards late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

Only healthy infants born near term (> 37 completed weeks of gestation) were included in the follow-up study after birth to remain free from confounding factors involved with prematurity or health problems of the infant. The total number of participants, both the mothers and their infants, who completed the postnatal part of the study, which included an examination of the infants' development at 3 and 8 months of age, was 170. The sample of participants consisted largely of caucasian middle class women, although both lower and higher social classes were represented (Table 9.3). On average, the women were 31 years old. The majority of women (93.7 %) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job (87.4 %), 55.3 % working less than 38 hours a week and 44.7 % working full-time. Of the infants, 84 were boys and 86 were girls.

Postnatally, at the age of 3 months and the age of 8 months, the Bayley Developmental Scales was performed on the infants, while the mother was asked to fill out questionnaires on the infant's behavior and her own stress level.

9.3.2 Measures

9.3.2.1 Psychosocial predictors

To predict infant development we used four aspects of prenatal maternal stress, namely daily hassles, pregnancy anxiety, perceived stress, and distress, which were only moderately intercorrelated (r ranging from -.03 to .26), except for the correlation between perceived stress and distress: r = .41, p <. 005). First, the frequency of daily hassles was assessed with a daily hassles questionnaire, which is a Dutch translation of a selection of items of questionnaires (Alledaagse Problemen Lijst, Vingerhoets et al., 1989), including the Daily Hassles Scale (Kanner et al., 1981), the Everyday Problem Scale (Burks & Martin, 1985) and the Daily Life Experience Questionnaire (Stone & Neale, 1982). It measures the frequency of occurrence of daily hassles in the past month and gives an intensity score which is the subjective experience of the subject of the unpleasantness of the hassles. Examples of items are: 'You could not find important belongings', 'You were trapped in a traffic jam'. In this study, only the frequency score was used in order to stay free from confounding stress provoking and stress resulting factors in the intensity score. In early, mid, and late pregnancy, the frequency of daily hassles was calculated and used as a predictor variable.

Second, pregnancy-related anxiety was shown to reflect another aspect of the emotional status of pregnant women (Huizink et al., 2000a) and was therefore included as predictor of infant development as well. Pregnancy-related anxieties were assessed by means of the Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R), an abbreviated version of the PRAQ developed by Van den Bergh (1990). We used two subscales in the present study: fear of giving birth (3 items) and fear of bearing a physically or mentally handicapped child (4 items). Examples of items are: 'I am worried about the pain of contractions and the pain during delivery' (fear of giving birth) and 'I am afraid the baby will be mentally handicapped or will suffer from brain damage' (fear of bearing a physically or mentally handicapped child). The items were answered on a 5-point scale, ranging from 'never' to 'very often'. This questionnaire was filled out in early, mid, and late pregnancy. The Cronbach's alpha's of the subscales were all > .76 throughout pregnancy.

Third, perceived stress was assessed by means of a Dutch translation of the Perceived Stress Scale of Cohen & Williamson (1987). It contains 14 items on perceived stress of an individual during the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This scale reflects a very subjective measure of stress and is a part of the higher-order construct of distress. This questionnaire was filled out on each occasion. For the present study, we used only the score derived in early pregnancy.

Fourth, a higher-order distress score was calculated for each period of pregnancy based on multidimensional models of prenatal distress (Huizink et al., 2000b). In short, the distress concept in early pregnancy involved a life event impact score, neuroticism, perceived lack of control over the course of pregnancy, and emotion-focused coping. In mid pregnancy, the life event impact score, neuroticism, daily hassles, and pregnancy-related fears (fear of giving birth and fear of bearing a handicapped child) explained significant parts of the variance in distress. In late pregnancy, the life event impact score, neuroticism, fear of bearing a handicapped child and problem-focused coping were part of the distress concept. Throughout pregnancy, neuroticism was the strongest predictor of distress.The factor score of distress was calculated with LISREL and used as a predictor variable.

9.3.2.2 Endocrinological predictors

In addition to the psychosocial predictors of infant development, we assessed two endocrinologic parameters to determine the relationship between the maternal HPA axis activity during pregnancy and the postnatal development of the infants. Cortisol was measured by determining the concentration of salivary cortisol which has been proven to be a valid and reliable reflection of the unbound hormone in blood (Kirschbaum & Hellhammer, 1989; Meulenberg & Hofman, 1990). Seven saliva samples were collected every two hours between 8:00AM and 8:00 PM, to obtain cortisol day time curves in each of the three periods of pregnancy. All samples were stored at -70^{0}C until assayed. Cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2]-^{3}H(N)-Hydrocortisone (NET 185, NEN-DUPONT, Dreiech, Germany) was used as a tracer following chromatic verification of its purity. The lower limit of detection was 0.5 nmol/L and interassay variation was 11.0%, 8.2%, and 7.6% at 4.7, 9.7 and 14.0 nmol/L, respectively (n = 20). Reference values for adults are 4-28 nmol/L at 8:00 AM. For each cortisol day profile, the mean, and early morning (8 AM) values were chosen to reflect a part of the maternal HPA axis activity.

ACTH. At 24 and 32 weeks of gestation, 30 ml of venous antecubital blood was collected for assessment of ACTH in a subsample of subjects. For the present study, we only used the ACTH collected at 24 weeks of gestational age as predictors of infant temperament (n= 41). ACTH was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 1.0 ng/L and inter-assay variation was 11.4 %, 10.7 % and 6.8% at 11, 68 and 310 ng/L respectively (n = 32).

The correlation coefficients between the psychosocial predictors and cortisol measures assessed in early pregnancy were calculated. No significant associations were found.

9.3.2.3 Infant measures of temperament

The infants were tested in a standard novel situation at 3 and 8 months of age. The Bayley Scales of Infant Development were performed by the first author, or by a trained junior researcher in a standard testing room and provided a mild challenging situation for the infant, since the testleader, the test session and the environment were novel situations to cope with. The testleaders were blinded for the prenatal stress data of the mothers. A temperament questionnaire and two stress questionnaires were filled out by the mother either during the test, or within a week at home. The data gathered during these sessions and analyzed in the present study included.

9.3.2.4 Infant Temperament

Infant temperament at 3 and 8 months was assessed by a Dutch translation of the Infant Characteristics Questionnaire (ICQ; Bates et al., 1979), which was filled out by the mother. The factor structure of the Dutch ICQ has been studied over different ages and reference

data have been collected. At the age range 5-6 and 12-14 months, factors were derived that were closely similar to the factors of the original ICQ of Bates (1979). Difficult behavior was assessed by 10 items, unadaptability ws assessed by 5 items. Data was quantified in two factors of Difficult and Unadaptable behavior based on scoring procedures developed through factor analyses by Kohnstamm (personal communication). Cronbach's alpha's of the factors were all > .76 in the present study.

9.3.2.5 Observed behavior of the infant

The behavior of the infants during developmental testing (BSID) was assessed by means of ratings by the testleader on the Infant Behavior Record (IBR), the third component of the Bayley Scales (Bayley, 1969). The ratings were mainly performed by the first author, with contributions from a junior researcher, who was trained by the first author to achieve an interrater reliability of > .85. This test session took place in a standard novel situation. The IBR consists of a number of descriptive rating scales for behavior characteristics of infants up to 30 months of age. The scales included in the present study reflected: exploration, goal-directedness, and test-affectivity. These scales were derived by a factor analytic approach which identifies the IBR scales that cluster together (van der Meulen & Smrkovsky, 1983, 1984).

9.3.2.6 Potential covariates

Data was gathered on various other aspects besides prenatal stress that may influence infant development. Descriptives of these potential covariates are shown in Table 9.1.

Prenatal factors included maternal age (in years), socio-economic status (SES), smoking and alcohol-intake during pregnancy, and biomedical risks. *SES* was defined by educational level and professional level of the pregnant woman and her partner (Westerlaak et al., 1976). *Smoking behavior* was assessed by self-report, expressed as the number of cigarettes per day (cig/day) and categorized in three groups: 1) non-smokers; 2) smoking 1-10 cig/day; 3) smoking > 10 cig/day. The latter group consisted of only 7 subject, and therefore a dichotomous variable was created: 1) non-smokers (n= 141); 2) smokers (n= 29); >= 1 cig/day. *Alcohol-intake* during pregnancy was likewise determined by self-report, and was expressed as the number of alcohol-containing beverages per week. Only 11 subjects consumed more than 2 alcohol-containing beverages per week and therefore a dichotomous variable was created: 1) non-drinkers (n= 144); 2) drinkers (n = 26); > = 1 drink per week. Our sample thus contained very few heavy smokers or drinkers, and only a relatively small number of modest smokers and drinkers. *Biomedical risk factors* included the use of medication during pregnancy, pre-existent health problems, high bloodpressure, fertility problems, gynecological risk factors (DES daughters etc), excessive vomitting and diabetes mellitus caused by pregnancy.The risk factors were added up in a categorical variable; scores ranged from 0 - 5.

Perinatal covariates that may confound the effect of prenatal stress on infant development include birth weight (in grams) and gestational age at birth (in weeks). Also, complications during delivery, the use of medication during delivery, fetal distress, and mode of deliv-

ery (elective caesarean section or assisted delivery) were taken into account, by calculating a cumulative score of these perinatal complications (range 0-5).

Potentially postnatal covariates included in the present study are the postnatal stress levels of the mother. *Psychological well-being* was determined by means of the Dutch translation (Koeter & Ormel, 1991) of the General Health Questionnaire (GHQ-30; Goldberg, 1972). This questionnaire contains 30 questions to be answered on a four-point scale and was filled out on both postnatal occasions. *Perceived stress* was assessed with the Perceived Stress Scale of Cohen & Williamson (1988), using a Dutch translation. It contains 14 items on an individual's perceived stress over the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This questionnaire is the same as used during pregnancy and was filled out on both postnatal occasions.

Table 9.1
Descriptives of potential prenatal, perinatal and postnatal confounders

Confounders	
Prenatal	
Maternal age (years) ± SD	31.3 (4.9)
SES	**Educational level mother ***
	Low 13.6 %
	Middle 67.5 %
	High 18.9 %
	Educational level partner
	Low 23.4 %
	Middle 59.8 %
	High 16.8 %
	Professional level mother
	Low 8.0 %
	Middle 54.6 %
	High 37.4 %
	Professional level partner
	Low 18.0 %
	Middle 29.2 %
	High 52.8 %
Smoking	Smokers: n = 29; > = 1 cigarette per day
	Non-smokers: n = 141
Alcohol-intake	Drinkers: n = 26; > = 1 drink per week
	Non-drinkers: n = 144
Biomedical risks	No risk: n = 102
	Pregnancy complications: n = 30
	Medication during pregnancy: n = 25
	Risk for fetus of medication: n = 4
	Fertility problems: n = 48
	IVF: n = 13
	High bloodpressure: n = 15
	Diabetus mellitus due to pregnancy: n = 3
	Gynecological risk: n = 12
	Pre-existent disease: n = 12
	Mean score (± SD): 1 (1.2)
Perinatal	
Birth weight (grams) ± SD	3385 (487)
Gestational age at birth (weeks) ± SD	39.6 (1.9)
Perinatal complications	Partus complications: n= 25
	Medication during delivery: n=88
	Elective caeserean section: n= 24
	Artifical delivery due to fetal distress: n=20
	Mean score (± SD): 1 (1.3)
Postnatal	
Psychological well-being (GHQ-30) ± SD	3 months postpartum: 4.7 (5.1)
	8 months postpartum: 3.6 (5)
Perceived stress ± SD	3 months postpartum: 25.9 (5.8)
	8 months postpartum: 25.5 (5.7)

* low level: primary school, high-school education; middle level: secondary school education; high level: college or academic education

9.3.3 Statistical Analysis

First, descriptive analyses were performed on all dependent variables: difficulty, unadaptability, test-affectivity, goal-directedness and exploration scores assessed in 3- and 8-months-old infants. Second, possible categorical or interval scaled covariates (SES, maternal age, gestational age at birth, birth weight, postnatal stress of the mother) were tested for their linear relationship with the dependent variables by means of correlations (Pearson or Spearman when appropriate) and regression analysis. If covariates were significantly related to the dependent variables, they were included in the next steps of analysis. Since we expected to find a small and possibly nonlinear effect of prenatal maternal stress in our sample of healthy fullterm infants, we formed two groups: one group of women who scored in the lower 25 % of a particular questionnaire and one group of women who scored in the upper 25% of that questionnaire. Thereby, high/low (P75/P25; P= percentile) contrasts were set on the predictors and behavioral scores were compared by means of MANCOVA. For ACTH a median split method was used to form two groups, due to the small sample size. Two MANCOVAs were performed for each predictor; one including the observed behaviors at 3 and 8 months (test-affectivity, goal-directedness, exploration) and one including the maternal reports of difficulty and unadaptability at 3 and 8 months. Dichotomous covariates (smoking and alcohol-use during pregnancy, infants' sex) were entered as a factor in the MANCOVAs (see Figure 9.1 step A). Also, perinatal covariates were included in step A. When the MANCOVA showed an overall significant main effect by means of a significant Hotelling's T^2 test for prenatal stress, post-hoc univariate analyses were performed. Next, postnatal stress levels of the mother were added as covariates in subsequently performed ANCOVAs (see Figure 9.1 step B). If the latter were significant, a more detailed analysis of the effect of prenatal stress was performed by means of multiple linear regression analysis to test for a linear association between prenatal stress and temperament of the infant. With all tests, statistical significance was assumed at the level of $p < .05$.

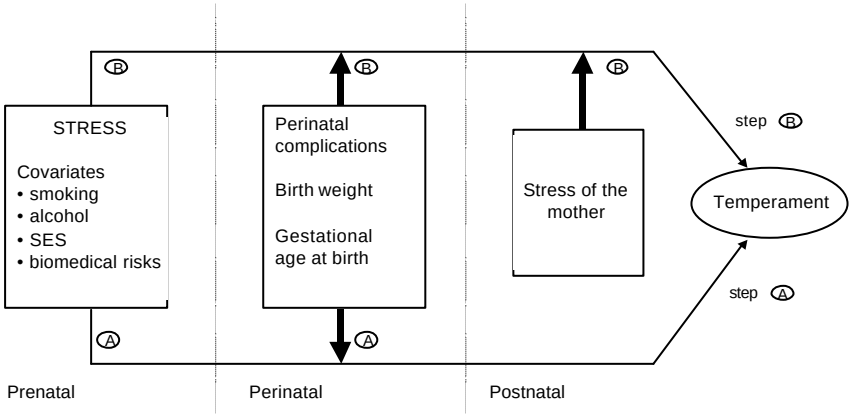

Figure 9.1. Steps in statistical analysis. Step A represents MANCOVA in which prenatal and perinatal confounders are taken into account. Step B is the next step of analysis in which postnatal stress of the mother is added as confounder in the ANCOVA.

9.4 Results

9.4.1 Descriptive analyses

Means, standard deviations and ranges in scores were calculated for all predictors and depending variables (Tables 9.2 & 9.3).

Table 9.2

Means, standard deviations (SD) and ranges in scores of the predictors in early pregnancy

Predictors	*Mean*	*SD*	*Range*	*N*
Psychosocial				
Daily hassles	9.96	6.3	0-45	170
Distress	-.02	1.0	-2.1-2.6	170
Perceived stress	28.07	5.49	18-48	170
Fear of giving birth	6.17	2.9	3-15	170
Fear of handicapped child	9.25	3.5	4-20	170
Endocrinologic				
Mean cortisol	10.57	2.3	5.2-19.8	142
Cortisol 8 AM	19.78	7.4	6-44	142
ACTH 24 weeks	16.95	9.3	5-44	43

Table 9.3

Descriptives of the dependent variables: Difficult behavior, Unadaptability and Reactivity (ICQ) and observed behaviors (IBR) at 3 and 8 months

	Mean (N=170)	*S.D.*	*Range in scores*
ICQ:			
Difficulty 3 months	27.1	6.9	11-46
Difficulty 8 months	29.7	7.1	13-56
Unadaptability 3 months	12.0	4.1	5-26
Unadaptability 8 months	12.3	4.3	5-39
Reactivity 3 months	12.1	2.1	4-18
Reactivity 8 months	12.2	1.9	6-18
Observed behaviors IBR:			
Activity 3 months	16.1	1.9	10-21
Activity 8 months	17.0	2.4	8-23
Exploration 3 months	9.6	3.6	3-18
Exploration 8 months	20.6	1.8	12-24
Test-affectivity 3 months	27.5	4.6	5-38
Test-affectivity 8 months	30.5	4.0	17-37
Goal-directedness 3 months	19.7	3.1	8-27
Goal-directedness 8 months	25.0	2.3	16-31

The two factor scores of infant temperament reported by the mother were significantly correlated in time. That is, difficult behavior at 3 month was positively associated with difficult behavior at 8 months ($r = .52$, $p < .0005$), and unadaptability at 3 months was related to unadaptability at 8 months ($r = .41$, $p < .0005$). Of the observed behaviors during the BSID, goal-directedness at 3 months was associated with goal-directedness at 8 months ($r = .27$, $p<.001$). Likewise, test-affectivity scores determined at 3 months were positively correlated with the test-affectivity scores at 8 months ($r = .28$, $p <. 001$).

Temperamental ratings of the infant by the mother were correlated with some observed behaviors. Difficulty of the infant at 3 months of age was negatively correlated with exploration of the infant at 3 months of age ($r= -.16$, $p<.05$). Unadaptability of the infant, reported by the mother, was negatively associated with exploration, test-affectivity and goal-directedness at both 3- and 8-months-old infants (r ranging from -.15 to -. 21; p's <.05).

Correlations between prenatal and postnatal stress levels were calculated and ranged from .23 to .61 (p's < .005), with the highest correlation coefficients between late pregnancy stress and stress determined at 3 months postpartum.

9.4.2 Preliminary analyses for potential covariates

Correlation coefficients were calculated between the infant temperament scores and observed behavior at 3 and 8 months and prenatal (SES, maternal age, biomedical risks), perinatal (gestational age at birth, birth weight and perinatal complications), and postnatal (mothers' stress levels) factors. Difficult behavior of the infant at 3 months of age was negatively correlated with birth weight ($r = -.15$, $p < .05$) and the educational level of the mother ($r = -.15$, $p < .05$). Difficult behavior of the infant at 8 months of age was negatively associated with the educational levels of the mother ($r = -20$, $p<.01$). Unadaptability of the infant at 3 and 8 months of age was negatively correlated with the professional level of the mother ($r = -.21$, $p<.01$ and $r = -.39$, $p< .0005$, respectively) and her educational level ($r = -.18$, $p<.05$ and $r = -.28$, $p < .005$, respectively). Unadaptibility of the infant at 8 months of age was furthermore negatively associated with maternal age ($r = -.17$, $p<.05$). Test-affectivity of the infant at 8 months of age was positively associated with the professional and educational levels of the mother ($r = .18$, $p<.05$ and $r = .16$, $p<.05$, respectively). No other linear relationships between potential covariates and the dependent variables were found. Multiple regression analysis showed independent effects on unadaptability at 3 and 8 months of the professional level of the mother ($F= 10.16$, $p < .01$ and $F= 21.64$, $p < .001$, respectively). This variable explained 6.3% and 12.5% of the total variance in the unadaptability scores at 3 and 8 months, respectively. Maternal educational level furthermore contributed to the model of unadaptability at 8 months of age ($F = 13.10$, $p < .005$, $R^2 = 0.02$). Other potential covariates did not significantly contribute to these models, and were excluded from further analysis.

There were no significant main effects or interaction effects with prenatal stress of the infant's sex on the infant's temperamental characteristics. Likewise, no main or interaction effects with prenatal stress and alcohol-intake on infant temperament were found. However, when several high/low contrasts were formed for the psychosocial predictors and the variable 'smoking' was added, a main effect for smoking was found in addition to a main effect of fear of giving birth on test-affectivity of infants at the age of 8 months ($F (1,93) = 4.12$, $p <.05$; non-smoking: 31.40 versus smoking: 28.46).

9.4.3 Infants' observed behavior in relation to measures of prenatal maternal stress

In this section, the test-affectivity, goal-directedness and exploration scores at 3 and 8 months were compared between infants of mothers who had low (<= P25) and high (>= P75) stress levels during pregnancy. This analysis was carried out using MANCOVA (including the observed behavior scores of the infants at 3 and 8 months of age) and univariate analyses for early pregnancy, to test for non-linear effects. For significant univariate tests, the postnatal stress levels of the mother were entered in a subsequently performed ANCOVA (see Figure 9.1 step B), followed by multiple regression analysis, to test for linear effects. The results are summarized in Table 9.4 and Figures 9.2 - 9.4.

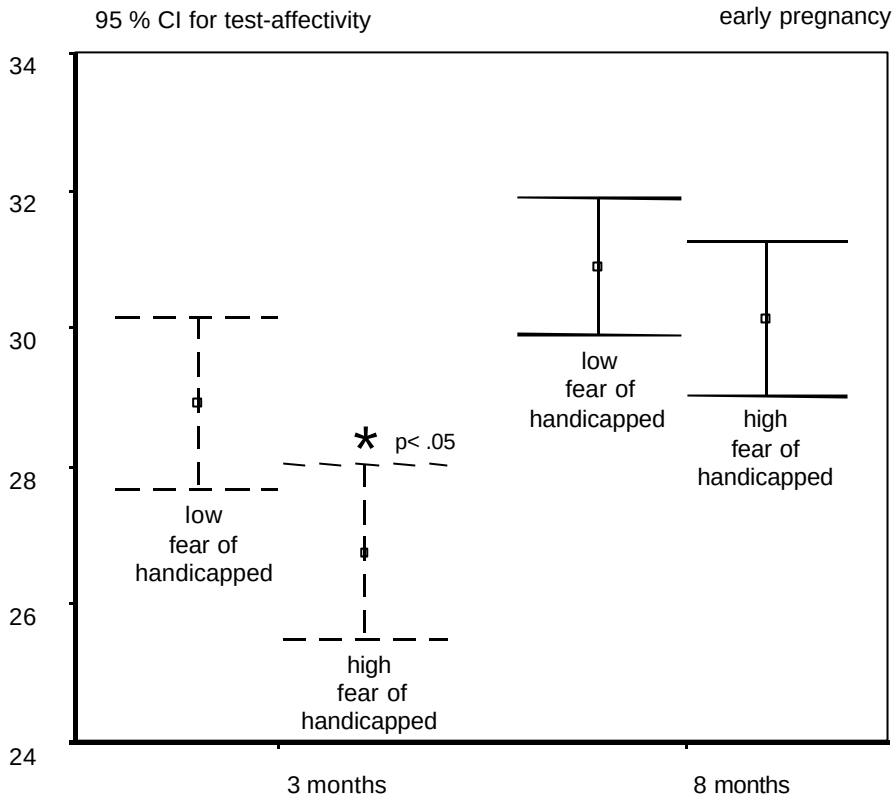

Figure 9.2. The effect of fear of bearing a handicapped child in early pregnancy on test-affectivity of infants at 3 and 8 months of age.

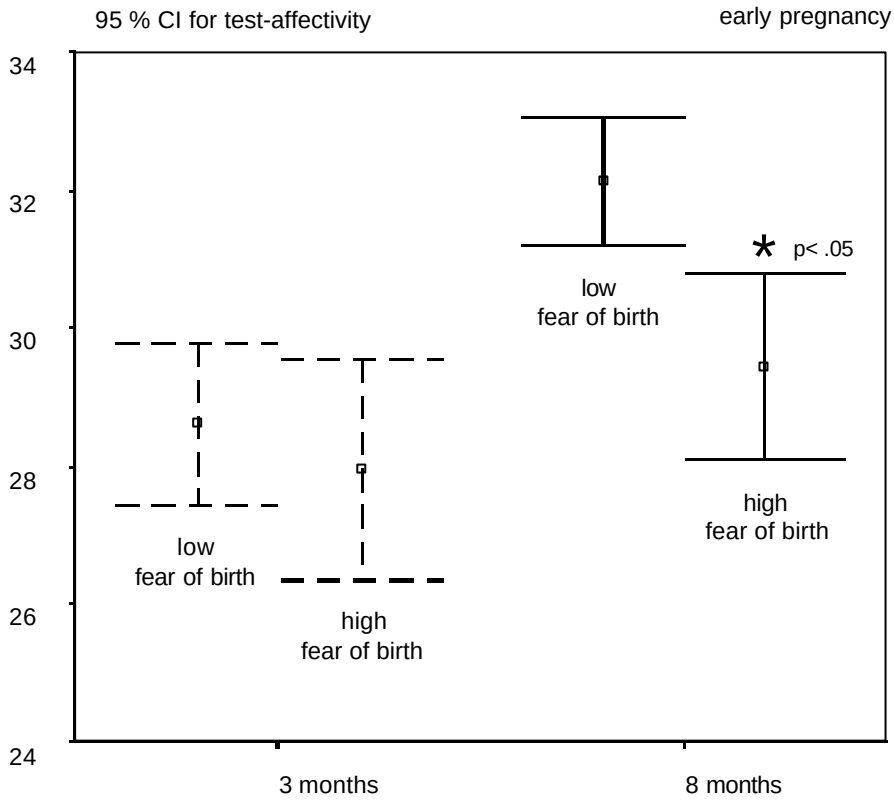

Figure 9.3 The effect of fear of birth in early pregnancy on test-affectivity of infants at 3 and 8 months of age.

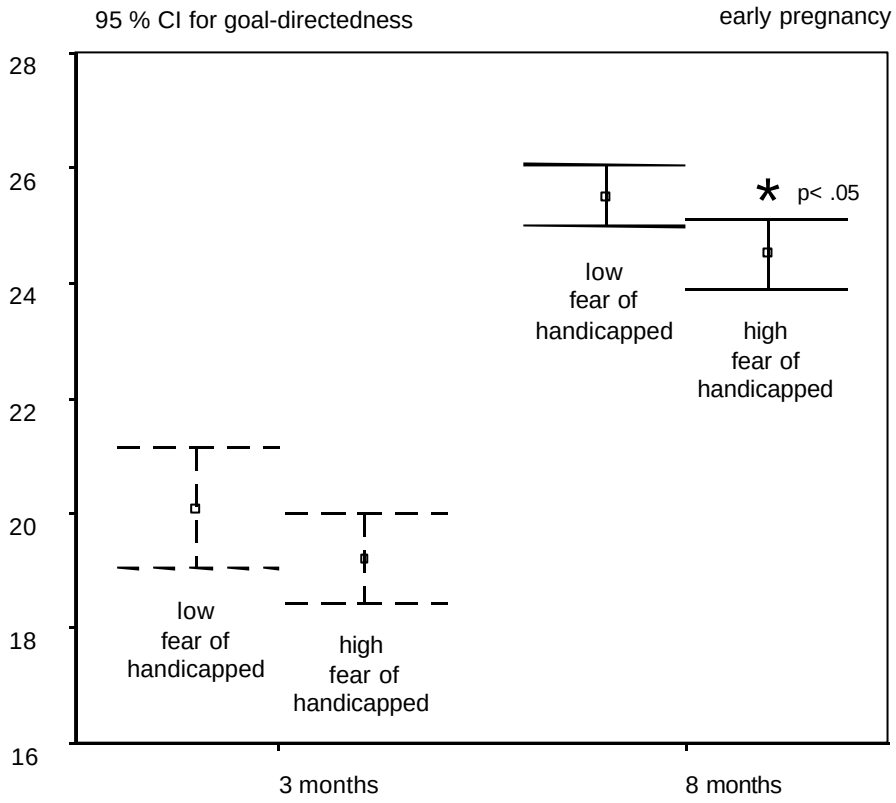

Figure 9.4 The effect of fear of bearing a handicapped child in early pregnancy on goal-directedness of infants at 3 and 8 months of age.

Table 9.4

Results of MANCOVAs, univariate analyses, ANCOVA corrected for postanatal stress and multiple regression analayses

	MANCOVA high/low contrast			*Univariate post-hoc analyses*			*Univariate post-hoc analyses*			*Multiple regression analyses*			
Predictors	*Behavior*	*F*	*P*		*F*	*P*		*F*	*P*	*β*	R^2 *total*	*F*	*P*
Fear of giving birth	Exploration Testaffectivity Goal-directedness	2.74	<.05	3 months --	--	n.s.	–	--	--	--	--	--	--
				8 months testaffectivity	11.38	<.001	8 months testaffectivity	6.39	<.05	-.21	.07	2.73	<.05
Fear of handicapped child	Exploration Testaffectivity Goaldirectedness	2.87	<.05	3 months testaffectivity	5.93	<.05	3 months testaffectivity	5.85	<.05	--	--	--	n.s.
				8 months goal-directedness	6.27	<.05	8 months goal-directedness	5.49	<.05	-.23	.07	2.65	<.05
Perceived stress	Exploration Testaffectivity Goaldirectedness	2.45	<.05	3 months --	--	n.s.	–	--	--	--	--	--	--
				8 months exploration	7.80	<.01		--	n.s.	--	--	--	--
Perceived stress	Difficulty Unadaptability	4.44	<.005	3 months difficulty	12.32	<.001	3 months difficulty	9.02	<.005	--	--	--	n.s.
				unadaptability	7.80	<.01	unadaptability	7.1	<.05	.28	.12	5.46	<.01
				8 months difficulty unadaptability	8.11 4.55	<.01 <.05	8 months	--	n.s.	--	--	--	--
ACTH 24 week	Difficulty Unadaptability	3.19	<.05	3 months difficulty unadaptability	--	n.s. n.s.	3 months –	--	--	--	--	--	--
				8 months difficulty unadaptability	-- 5.73	n.s. <.05	8 months – unadaptability	-- 6.8	n.s. <.05	-- .37	-- .29	-- 6.12	-- <.01

MANCOVA showed an overall significant effect of fear of giving birth ($F(6,89) = 2.74$, $p < .05$), fear of bearing a handicapped child ($F(6,89) = 2.87$, $p < .05$) and perceived stress ($F(6,88) = 2.45$, $p < .05$) on the observed behaviors exploration, test-affectivity, and goal-directedness. Subsequently performed univariate analyses showed that test-affectivity was declined in 3-months-old infants after a high level of fear of bearing a handicapped child ($F(1,84) = 5.93$, $p < .05$), whereas in infants at 8 months of age, a negative effect on test-

affectivity was found for fear of giving birth ($F(1,84)=11.38$, $p < .001$). Goal-directedness of 8-months-old infants was reduced in infants of mothers with high levels of fear of bearing a handicapped child ($F(1,84)= 6.27$, $p<.05$). Exploration of 8-months-old infants was decreased after exposure to high levels of maternal perceived stress.

After adding postnatal maternal stress levels (see Figure 9.1, step B) only to the significant univariate tests, the negative effects of prenatal stress on test-affectivity and goal-directedness were slightly adjusted and the significant effects of prenatal stress could still be found (see Table 9.4, fourth column), except for the effect of prenatally perceived stress on exploration.

Multiple regression showed a linear negative effect of fear of giving birth on test-affectivity and of fear of bearing a handicapped child on goal-directedness of 8-months-old infants, both explaining 7 % of the total variance (see Table 9.4, fifth column).

No effect on observed behavior was found for daily hassles or distress in early pregnancy. Exploratory analysis with stress measures from mid- and late pregnancy were only carried out for the significant effects found of stress in early pregnancy, after controlling for postnatal stress. Only a trend toward a significant differences in test-affectivity and goal-directedness of infants at 8 months of age was found for fear of giving birth and fear of bearing a handicapped child in mid-pregnancy, respectively. No effects were found for pregnancy related anxiety determined in late pregnancy.

9.4.4 Infants' temperament rated by maternal report in relation to measures of prenatal maternal stress

In this section, analyses are presented analogous to those performed above. The results are presented in Table 9.4 and Figures 9.5 and 9.6.

An overall effect of perceived stress was found with MANCOVA on difficulty and unadaptability at the age of 3 and 8 months ($F(4,81)=4.44$, $p<.005$). Univariate analyses showed that more difficult and unadaptable behavior was found in infant at both 3 and 8 months of age (Table 9.4). After correction for postnatal stress levels of the mother, the effects of prenatal stress on difficulty and unadaptability of 8-months-old infants was no longer found, whereas the effect on difficulty and unadaptability of 3-months-old infants remained significant (Table 9.4, fourth column).

Multiple regression showed a linear negative effect of perceived stress on unadaptability at 3-months, explaining 12% of the total variance.

No effect was found for daily hassles, distress, or pregnancy anxiety on temperamental ratings of the infant by the mother. Exploratory analysis carried out with perceived stress in mid- and late pregnancy showed no significant effect on the temperament of the infant.

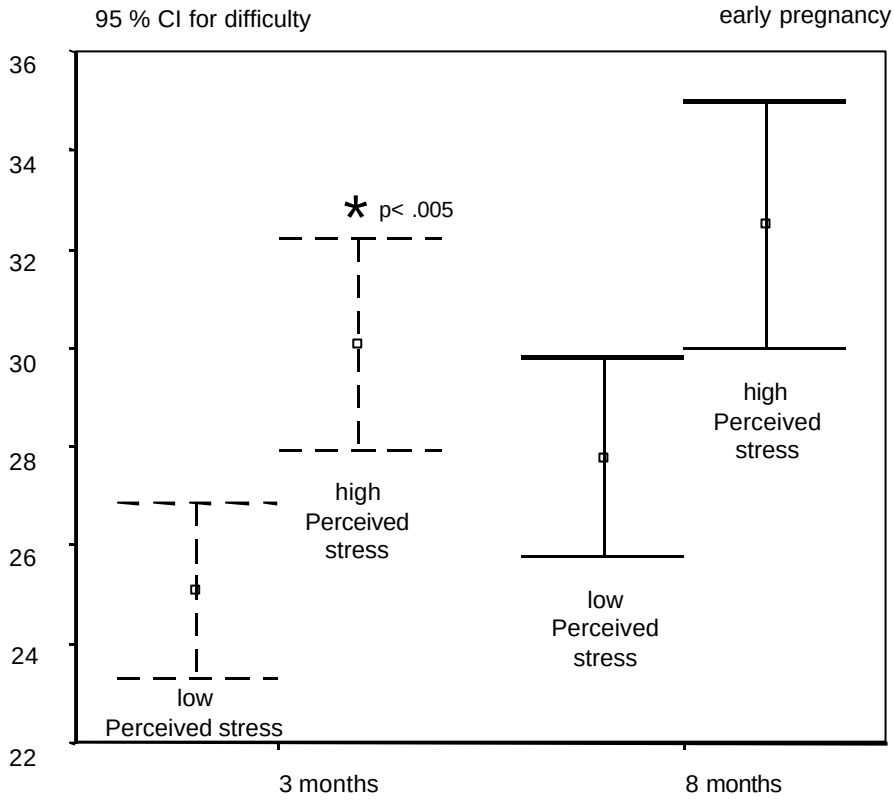

Figure 9.5 The effect of perceived stress in early pregnancy on difficulty based on maternal report of infants at 3 and 8 months of age.

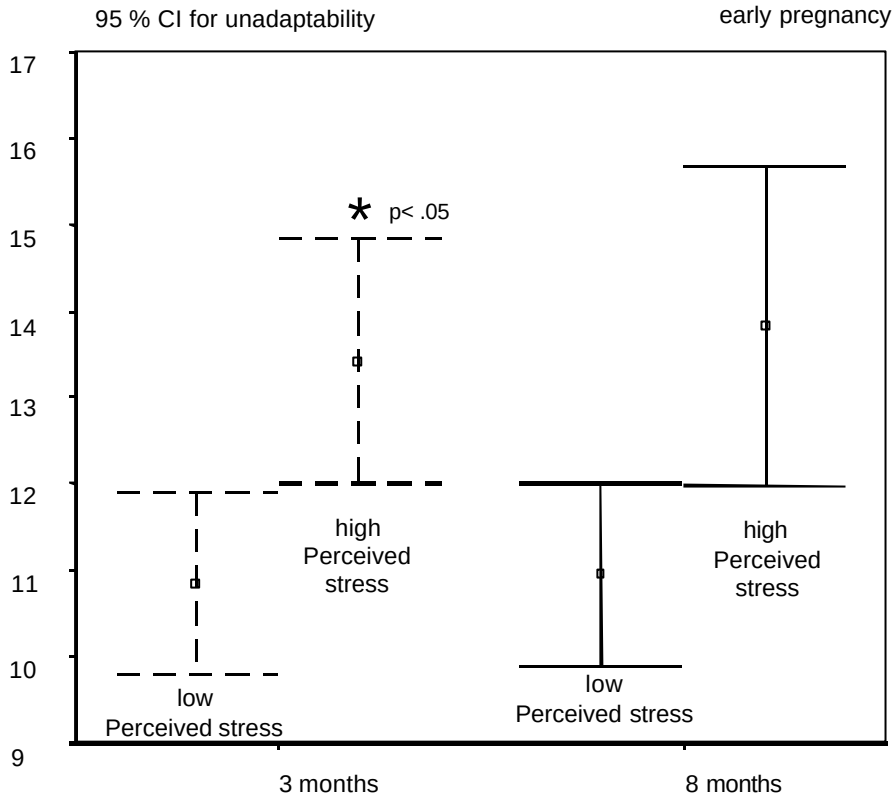

Figure 9.6 The effect of perceived stress in early pregnancy on unadaptability of the infants at the age of 3 and 8 months based on maternal report.

9.4.5 Infants' observed behavior and temperament in relation to measures of maternal HPA axis activity during pregnancy

The results are presented in Table 9.4 and Figure 9.7.

MANCOVA showed an overall effect of maternal ACTH content at 24 weeks of gestation on difficulty and unadaptability of infants of 3 and 8 months of age ($F(1, 41)= 3.19$, $p <.05$). Subsequently performed univariate analyses showed that the effect on unadaptability of 8-months-old infants was significant ($F(1,41)= 5.73$, $p<.05$). After correction for postnatal stress, the effect of ACTH remained significant ($F(1,41)=6.80$, $p<.05$).

It was further found that after correction for the educational level of the mother and the postnatal level of stress, 14.6 % of the total variance in unadaptability at the age of 8 months could be attributed to the level of ACTH at 24 weeks of gestation ($F (1,41)= 6.12$, Beta = .37,

p < .01). In total, 29.0 % of the variance was explained by ACTH and the mothers' educational level. Postnatal stress of the mother did not significantly contribute to this model. No other significant contributions of ACTH and no contributions of cortisol to infant temperament or observed behavior were found.

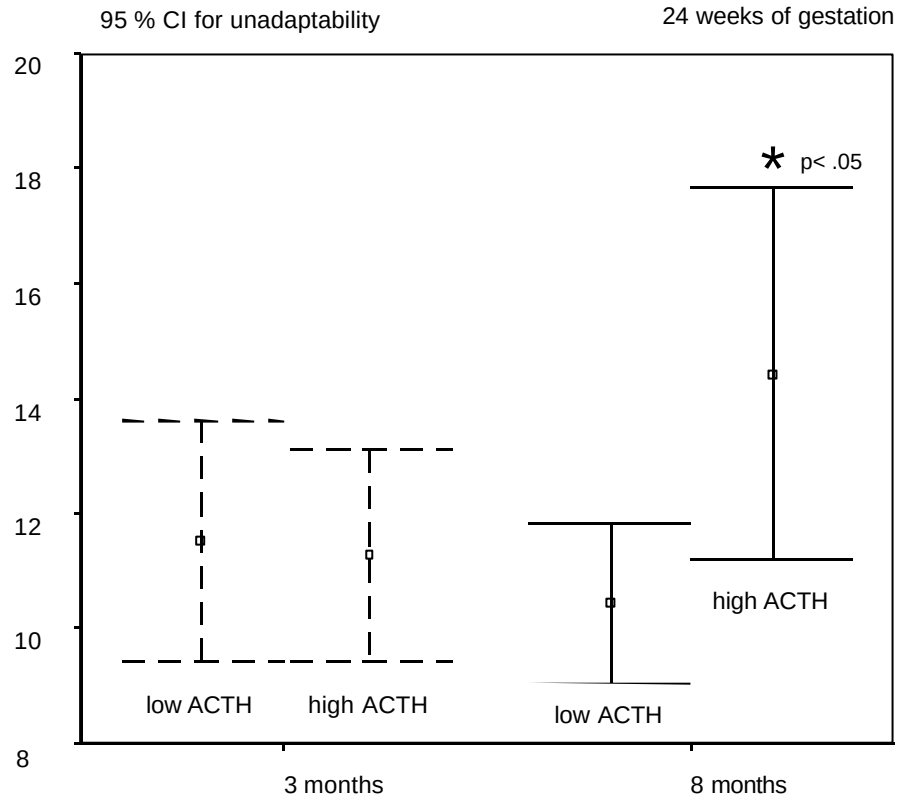

Figure 9.7 The effects of ACTH in plasma at 24 weeks of gestation on unadaptability of the infant at 3 and 8 months of age based on maternal report..

9.5 Discussion

The present study showed that various aspects of prenatal maternal stress or anxiety had a negative effect on the behavior infants at age 3 and 8 months. Pregnancy-related fears were related to a decrease in attention regulation during a standard test situation, as was evident from changes in test-affectivity and goal-directedness. Levels of perceived stress and ACTH were associated with more problems in adaptation to a new situation or to the presence of unfamiliar persons. These findings are in line with animal studies which showed problems in coping with novel situations in offspring of prenatally stressed rats (Archer & Blackman, 1971; Deminière et al., 1992) and nonhuman primates (Schneider, 1992a) and more attentional problems after prenatal stress (Schneider, 1992b). Contrary to our expectations we did not find an effect on observed exploration of the infant during a standard test situation, after we corrected for the postnatal stress levels of the mothers. Although the temperamental characteristic unadaptability was determined by maternal report and thus may be confounded by rater bias, the finding that ACTH was also related to more adaptational problems of the infant suggests that prenatal stress may indeed have an effect on temperament of the infant.

Effects of early pregnancy stress on observed behavior of infants were found at age 8 months rather than at 3 months. This is an interesting finding, when we realize that around the age of 8 months infants start to develop a greated awareness of and interest in the outside world. As a result of this and of concomitant cognitive development as well, infants around 8 months generally are more sensitive to unfamiliarity than at earlier ages. This may be apparent, among others, from increased stranger anxiety. Thus, the test situation at 8 months likely has been much more challenging for the infant than that at 3 months, and therefore may have resulted in a more pronounced effect of prenatal maternal stress on the test-affectivity and goal-directedness of the infant. In contrast, the effects of perceived stress in pregnancy were only significant for 3-months-old infants and were less clear when the infant had reached the age of 8 months. This finding argues against the idea that perceived stress in pregnancy merely reflects a general stress level of women which may influence her perception of the infant. In that case, her infant would also have been significantly more difficult and unadaptable at 8 months.

It is noteworthy to mention that in early pregnancy daily hassles nor the multidimensional construct distress had an effect on infant temperament. Others have suggested that daily hassles could be potentially more harmfull than other stressfactors (DeLongis, 1982; Paarlberg et al., 1999). Our sample consisted of a population of relatively normal risk pregnant women, mostly from a middle class socioeconomic status. Perhaps, the amount of daily hassles in our sample was therefore relatively low. The fact that the prenatal distress level was not associated with temperamental scores of the infant argues against the interpretation that maternal characteristics are mainly responsible for the temperamental scores, since a main predictor of the multidimensional concept of prenatal distress was neuroticism (Huizink et al., 2000b). Pregnancy-specific fears were the most powerful predictors of postnatal behavior of the infant in the present study, and were also found to have an effect on mental and motor development of infants in another study (Huizink et al., 2000a). Moreover, these fears have been found to predict adverse pregnancy outcome (Killingsworth Rini et al., 1999). Clearly, these anxieties of pregnant women deserve more attention in future studies.

It may be that periods of vulnerability exist for behavioral impairments associated with prenatal stress or anxiety. A study of Schneider et al. (1999) showed that in nonhuman primates early gestation stress was associated with more pervasive motor impairments than mid-late gestation. Huttunen (1988) reported a significant relation between maternal stress during the first trimester of pregnancy and temperamental features of the infant (slow adaptability, negative mood and easy distractibility), whereas second and third trimester stress were unrelated to infant temperament. We therefore tested for behavioral problems in infants following early prenatal stress. Our results suggest that for the observed behaviors such as test-affectivitiy, goal-directedness, and the temperament aspects difficulty and unadaptability stress in early pregnancy is harmful, whereas mid- and late prenatal stress did not result in significant effects. ACTH exhibited an effect on postnatal behavior when the concentrations of 24 weeks gestation were analyzed, thus suggesting that mid-pregnancy may be a period with increased vulnerability for increased HPA-axis activity. However, several limitations of the present study warn against strong conclusions. First, we gathered no endocrinologic data on the very early period of pregnancy, which may be especially sensitive to small variations in the fetal environment, since this is the period of neural migration and for instance teratogen exposure during this period can induce gross irreversible malformations. Second, to study the possible vulnerable periods more precisely in pregnancy for prenatal stress effects on the infants, a different design should be used. For instance, exposure to a stressor by nature like the explosion of a fireworks storage located in a residential area in a town of the Netherlands (Enschede) in only early, mid or late pregnancy may result in different outcomes of infant behavior when these three groups divided by the timing of exposure are compared. Third, our stress measures throughout pregnancy were not independent but were correlated over time, which could have biased our results.

With regard to the possibly mediating role of the HPA- axis activity of pregnant women on postnatal behavior it was shown that increased levels of ACTH at 24 weeks of gestation were linearly related to unadaptability at 3 months and explained almost 15 % of this behavior. Thus, the HPA-axis may indeed play a part in explaining the effects of prenatal stress on postnatal behavior in human infants, as has been shown before in animal studies (Weinstock et al., 1997). Evidence is found for an altered HPA axis reactivity in prenatally stressed offspring (Weinstock et al., 1992; McCormick, 1995; Fride et al., 1986), which may result in a general vulnerability for psychopathology later in life (Huizink et al., 2000c). Reactivity of the prenatally stressed infants' HPA axis to novelty or more challenging situations may shed more light on the difficult issue of pathophysiological mechanisms causing the effect of prenatal stress on later altered behavior. However, the exact mechanism by which prenatal distress, prenatal perceived stress or prenatal pregnancy-related anxieties could result in altered postnatal behavior remains rather unclear, since the psychosocial and endocrinologic measures of prenatal stress were unrelated in early pregnancy.

Certain personality factors of the mothers may influence the mother-infant-interaction after birth which could in turn affect the infants' behavioral development. For instance, postnatally depressed women are less responsive and sensitive to their children (Cohn & Tronick, 1989; Field, 1992; Lyons-Ruth et al., 1990) which has an effect on the behavior of the child as well. Moreover, women high on prenatal distress have a tendency to have high overall levels of distress also after birth, which could alter their perception of their infant in a negative way, resulting in higher scores on difficult behavior and unadaptability. Therefore, we per-

formed an analysis in which we controlled for postnatal stress levels and psychological well-being of the mothers. Our results showed that the effect of prenatal stress were declined when postnatal stress was taken into account, but in general the effects of prenatal stress were still noticeable. In human studies, these postnatal influences are hard to control for in an experimental design and it is hardly impossible to conclude from our results that prenatal factors are mainly responsible for a more difficult pattern of behavior in these young infants.

It is important to appreciate that the design of the present study does not allow to examine the relative contribution of genetic versus environmental versus gene by environment interactional influences on infant behavior. Infants could have a high genetic loading on temperamental difficulties, may encounter high environmental demands or may be influenced on a behavioral level by an interaction of these two aspects. Prenatal stress effects may increase the infants' vulnerability for genetic and environmental influences on behavioral problems. This line of research offers a challenge for future research.

The results of the present study show that variation in the exposure to relatively mild prenatal stress is associated with temperamental variation of the infant at 8 months of age. These changes may enhance the risk of developing later behavioral problems, such as anxiety and mood disorders, antisocial personality disorder, recidivistic and violent crime, alcholism, and suicidal behavior (Caspi et al., 1996). Therefore a follow-up study of the infants is warranted. In future studies, we will focus on detailed observation of the mother-infant interaction during a semi-structured play session and the Bayley Scales of Infant Development examination to remain free from the rater bias caused by the mothers'subjective perception of the infant when using parent reports and to perform fine-grained analysis on the (behavioral) mechanisms involved in the effects of prenatal stress on infant temperament.

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10

Summary of results and general discussion

10.1 Summary of results

In this thesis on the effects of prenatal stress on infant development, three parts can be differentiated.

The first part introduces the concept of prenatal influences and in particular prenatal stress. The importance of studies on prenatal determinants of later development is elucidated in chapter 1 and evidence of harmful effects of prenatal stress on postnatal development is provided by a review on animal studies on this topic in chapter 3. The aims of this thesis are formulated in chapter 2.

The second part describes and empirically tests the various aspects involved in the psychological stress of pregnant women who are expecting their first child. In this way, the concept of prenatal stress is clarified. The aspects studied include pregnancy-specific anxieties (chapter 4) and coping styles that can be found in normal-risk pregnancy (chapters 5 and 6). As a next step, multidimensional modelsare described in chapter 7 that specify which elements of stress contribute to perceived distress in early, mid- and late pregnancy.

Finally, the third part attempts to answer the question whether prenatal maternal stress affects infant development (chapter 8) and infant temperament (chapter 9) at the age of 3 and 8 months postpartum.

With regard to the second part of this thesis which aims to clarify the concept of prental stress, several topics are addressed:

1. Are pregnancy-related anxieties specific or do they reflect a general predisposition for anxiety?
2. Which aspects of coping can be differentiated in normal-risk pregnancy using the Utrecht Coping List-19?
3. Which coping style is most effective in reducing the level of distress?
4. Which aspects contribute to a multidimensional concept of distress and are these aspects specific for the different periods in pregnancy?

The outcome of these empirically tested questions is now summarized.

10.2 Are pregnancy-related anxieties specific or do they reflect a general predisposition for anxiety?

A sample of 230 nulliparous pregnant women filled out a questionnaire on pregnancy-related anxieties and several other questionnaires covering general personality factors, such as general anxiety, locus of control, appraisal of pregnancy and neuroticism. Three anxieties specifically related to pregnancy were found by means of confirmatory factor analysis; 'fear of giving birth', 'fear of bearing a physically or mentally handicapped child' and 'concern about one's appearance'. Personality factors such as trait anxiety, neuroticism, external locus of control and appraisal of pregnancy explained only a small part of the variance of these fears. Therefore, it is concluded that pregnancy-specific anxiety can be differentiated for a major part from general anxiety and other personality characteristics. Assessment of merely

general anxiety during pregnancy may therefore underestimate the level of anxiety caused by fears specifically related to pregnancy.

10.3 Which aspects of coping can be differentiated in normal-risk pregnancy using the Utrecht Coping List-19?

To answer this question, the Utrecht Coping-List 19 was filled out three times during pregnancy in our sample of pregnant women and the factor structure and its stability throughout pregnancy was determined by means of confirmatory factor analysis. Two important coping factors were found: emotion-focused coping and problem-focused coping. Emotion-focused coping includes for instance expression of feelings to others or reappraisal of the situation, and is typically directed towared regulating affect surrounding a stressful encounter. Problem-focused coping is directed toward alleviating the circumstances which produce stress and includes for instance planning and finding solutions for the problem. Especially in early pregnancy most women depended on emotion-focused coping, whereas emotion-coping scores declined with increasing gestation. Perhaps the event of a first-time pregnancy and all its associated expectations and changes for the future represented a situation that pregnant women wanted to discuss with their partner or close friends in the early period of pregnancy. Furthermore, it is suggested that coping in normal-risk pregnancy is a process rather than a personality trait, and that this process changes across time due to changing demands of the situation.

10.4 Which coping style is most effective in reducing the level of distress?

In a multidimensional model of distress in normal-risk pregnancy, the role of coping was examined. Coping appeared to have direct effects on the level of distress of pregnant women, rather than having a mediating role between general stress-provoking factors and the distress response. A temporal specificity was found for the most effective coping style used to decrease the level of distress. In early pregnancy, emotion-focused coping was most effective in reducing the level of distress, whereas in late pregnancy problem-focused coping was most useful for this purpose.

10.5 Which aspects contribute to a multidimensional concept of distress and are these aspects specific for the different periods in pregnancy?

Results from animal studies showed that prenatal stress has a negative influence on postnatal development and behavior. However, these findings are not readily comparable to results of human studies, since the stressors used in animal studies are not very relevant and comparable to stressors encountered in human pregnancy. Prenatal stress has been opera-

tionalized in numerous ways across human studies on the effects of stress during pregnancy on birth outcome and later development, making it difficult to compare the results of these studies. A multidimensional concept is most likely to describe stress accurately. Therefore, we formulated multidimensional models of prenatal distress for early, mid- and late pregnancy, which were tested by means of structural equation modeling. Stress-provoking (life events, daily hassles, pregnancy-related anxieties and appraisal of pregnancy), stress-mediating or - moderating factors (social support, coping, personality characteristics) and stress-resulting factors (perceived stress, psychological well-being, state anxiety) were included in the theoretical models of prenatal distress. The models found were specific for the early, mid and late periods of pregnancy, but neuroticims and life events were found to predict distress throughout pregnancy. In addition, in early pregnancy the lack of control a woman perceives to have over the course of pregnancy (secondary appraisal) contributed to increased levels of distress, whereas emotion-focused coping reduced distress. In mid pregnancy, daily hassles and pregnancy-related fears increased the level of distress. In late pregnancy, a pregnancy-related fear (fear of bearing a physically or mentallly handicapped child) was positively related to the level of distress and problem-focused coping was negatively associated with the level of distress. The multidimensional models offer more insight into the processes that lead to increased distress levels in pregnant women and were used in this thesis to predict postnatal development.

After testing and describing the factors involved in prenatal maternal stress in humans, the following questions were addressed in the third part of this thesis to address the third aim of this thesis; to test the hypothesis of adverse effect of prenatal stress on mental/motor development and on temperament.

5. Are psychosocial and endocrinologic measures of prenatal maternal stress related to the mental and motor development of infant at 3 and 8 months of age?
6. Are psychosocial and endrocrinologic measures of prenatal maternal stress related to temperamental charachteristics of infants at 3 and 8 months of age?

The results of the studies on these topics, described in chapter 8 and 9, are now summarized.

10.6 Are psychosocial and endocrinologic measures of prenatal maternal stress related to the mental and motor development of infant at 3 and 8 months of age?

In a prospective study of 170 nulliparous pregnant women and their infants, we examined the effect of early, mid and late pregnancy prenatal stress on the mental and motor development of infants at 3 and 8 months of age, assessed with the Bayley Scales of Infant Development. Prenatally stressed infants had lower scores on both mental and motor developmental indices (MDI and PDI, respectively) at the age of 8 months, after adjusting for several pre-, peri-, and postnatal confounders. On average an 8-point decline in MDI and PDI scores was found after exposure to high levels of psychosocial measures of prenatal stress. Especially

pregnancy-related fears were found to be related to adverse developmental outcome in these infants. High levels of early morning cortisol in late pregnancy were likewise associated with decreased MDI scores in 3-months-old infants, and significantly reduced PDI scores in infants at 3 and 8 months of age.

10.7 Are psychosocial and endocrinologic measures of prenatal maternal stress related to temperamental characteristics of infant at 3 and 8 months of age?

We examined the effects of prenatal psychosocial (distress, daily hassles, perceived stress and pregnancy-specific anxiety) and endocrinologic (cortisol, ACTH) measures, determined in early pregnancy, on infant temperamental characteristics in the first 8 months of life of 170 healthy fullterm infants. The results show that pregnancy-specific fears were related to a decrease in attention regulation during a standard test situation. Perceived stress of pregnant women in early pregnancy and high levels of ACTH in mid-pregnancy showed to be related to increased unadaptability of the infant, even when postnatal stress levels of the mothers were taken into account. These findings are in line with studies with nonhuman primates and suggest that prenatal stress may have a deleterious effect on the behavior of infants, which may be a risk factor for developing psychopathology later in life. The exact psychopathological mechanism of prenatal stress remains unknown, but the results of this study suggest that the HPA axis may play a role.

10.8 General discussion

10.8.1 Importance of pregnancy-specific anxieties

The studies as presented in the third part of this thesis indicate that pregnancy-related anxiety is the most powerful predictor of adverse developmental and behavioral outcome of infants at 8 months of age. In contrast, no strong evidence was found for a negative effect of the multidimensional construct of distress on infant development and temperament in the first 8 months of life. Since pregnancy-related anxiety is an albeit minor part of the higher-order construct of prenatal distress in mid- and late pregnancy, as was described in chapter 7, the separate measurement of these anxieties resulted in stronger effects on development and temperament of the infant. In the second part of this thesis, we showed that pregnancy-related anxiety can be regarded as a relatively distinctive syndrome which can be differentiated from general anxiety for a major part. Thus, pregnancy may be regarded as a special period in life accompanied by unique anxieties related to the event of pregnancy, which should not be overlooked when testing the effect of prenatal maternal stress on birth outcome and postnatal development. It is interesting to find that these pregnancy-related anxieties are predictive of infant outcome, since they may be regarded as a unique element of human pregnancy. Nonhuman primate studies have found similar results of prenatal maternal stress on offspring development and behavior, but have used very different stressors. It

seems highly unlikely that a nonhuman primate or other animal model could be able to test pregnancy-related anxiety effects on the offspring. Therefore, more human research on these anxieties is warranted. Daily hassles showed no linear effect on infant development or temperament, whereas pregnancy anxiety did, suggesting a dose-response relation for pregnancy anxiety and perhaps a threshold effect for daily hassles. This thesis has focused only on negative aspects of stress. In future research, we will test the effect of uplifts that may be regarded as stressors as well.

The multidimensional concept of distress in early pregnancy was only able to predict very low (<P25) scores of mental development. No other associations were found between this concept of distress and motor development or temperament. It was found that distress throughout pregnancy was predicted for approximately 70% by neuroticism throughout pregnancy. Ano interpretation of these findings could be that in our sample of normal risk middle class women, the overall neuroticism and therefore distress level was rather low. In high-risk populations (e.g. low social class) neuroticism may be more frequently found. Several trends were found for a negative effect of distress on development and temperament, but no significant differences were found, suggesting that the power of the construct may have been too low. The models of distress found in our sample should be replicated in another study, before we can draw conclusions on its significance for prenatal stress research.

10.8.2 Effects of prenatal stress at 8 rather than at 3 months

It is of interest that we find most effects of prenatal stress on development and temperament when the infant has reached the age of 8 months. At this age, a major developmental transition occurs, termed by Emde (1984) the onset of focused attachment and by Stern (1985) the discovery of intersubjectivity. Infants at this age develop stranger anxiety and separation protests appears. These changes thus involve qualitatively different social experiences for the infant (Zeanah et al., 1997) and the demands on the infants may therefore have been increased as well. Our test situation may have been more challenging for the infants at the age of 8 months, because of their increased social awareness and stranger anxiety, resulting in a more pronounced difference between infants exposed to high versus low prenatal maternal stress. Daily life may also put more strain on the prenatally stressed infant at this age, and the maternal report of elevated unadaptability suggests that prenatally stressed infants may be at risk for increased sensitivity to novel situations and stranger anxiety.

10.8.3 Interrelationships between prenatal stress effects on mental/motor development and on temperament

In the present thesis we tested the effects of prenatal stress on infant development separately from the effects on temperament. However, mental development scores (MDI) at 8-months-old infants were negatively correlated with unadaptability ($r = -.19$, $p < .05$) and posi-

tively correlated with test-affectivity and goal-directedness (r= .37, p<.005 and r=.34, p<.005, respectively). Motor development scores (PDI) of 8-months-old infants were not related to maternal reports of infant temperament, but were positively correlated with test-affectivity (r= .30, p<.005).

Since test-affectivity and goal-directedness reflect observed behavior during the Bayley Scales of Infant Development, it is not surprising to find the positive associations with MDI and PDI scores. Unadaptability was determined by maternal report and the moderate negative correlation with MDI scores is therefore of more interest. Difficulty in adapting to novel environments, such as the test situation in this study, and to new persons (the testleader), may have underestimated the MDI scores of infants with high scores on unadaptability. Another interpretation may be that the general unadaptability of an infant results in an increased risk for developmental delay. Unadaptability is negatively correlated with exploration (data not shown) which may reduce the infant's exposure to new stimuli, thereby hampering learning by experience. Therefore, we performed exploratory analysis that included unadaptability as a covariate in an ANCOVA that tested the effect of prenatal stress on MDI scores. After adjusting for the significant covariate undaptability, the effects of daily hassles and pregnancy anxiety on MDI remained significant, although the significance level of the effect was reduced (from p <.005 to p <.01). We performed similar exploratory analyis on the effects of prenatal stress on temperament, while adjusting for developmental scores. MDI and PDI were not found to be significant covariates for the effect of stress on difficulty and unadaptability rated by maternal report and thus the effect of prenatal stress on temperament remained significant. To test the potentially mediating role of temperamental characteristics on the prenatal stress effects on development, and conversely, the mediating role of developmental scores in the relationship between prenatal stress and infant temperament in more detail, we aim to test path analytic models by means of structural equation modeling in the future. This fascinating interaction between developmental delays and temperamental difficulties after prenatal stress offer many possibilities future research on prevention and interventions programs as well.

10.8.4 Sensitive periods?

It is still questionable if a sensitive period for the effect of prenatal maternal stress on the developing fetal brain exist. Our results suggest that early and mid-pregnancy might be periods of increased vulnerability for small variations in the fetal physiological environment, caused by maternal stress, although no strong evidence for this hypothesis was found. First, the potential mediating role of the HPA axis reactivity of pregnant women was only explored in the present study and it was found that some effects of high cortisol in late pregnancy and high ACTH in mid-pregnancy were found on infant development and infant temperament, respectively. However, no relation was found between these endocrinologic measures of stress and the psychosocial measures of stress. These findings are in line with the general finding that only moderate or no correlations exist between psychological and endocrinologic stress measures. We do not know if pregnancy-related anxiety is able to cause small variations in the fetal physiological environment. At least, no evidence was found for an elevated stress hormone level in relation to pregnancy-related anxiety. In future studies we shall

analyse the relationship between cortisol day curve data and prenatal stress measures with refined statistical techniques such as multilevel modeling. Second, several limitations of the studies in this thesis should be taken into account when drawing conclusions on possible sensitive periods. A limitation of our studies is that we did not gather endocrinologic data in a very early period of pregnancy but started collecting data only from 15 weeks gestation onwards. Although for pragmatic reasons it might be hard to accomplish, it would certainly be preferred to focus on the early period of the first trimester in particular, since this reflects a period of generation of neurons and neuronal migration. Studies have indicated that cell neuronal migration is highly sensitive to various perturbations, such as toxins, viruses and genetic mutations. Moreover, to be able to test more precisely for a specific sensitive period for the harmful effects of stress another design is needed in which stress is inflicted only in a particular period of pregnancy and compared to the effects on infant development and behavior of stress exposure in another period of pregnancy. For such a design, a naturally occuring disaster such as the explosion of a fireworks storage in a residential quarter of Enschede, a town in the Netherlands, offers a well-circumscribed stressor. Infants of women living in the hitten residential quarter who were in their first trimester of pregnancy when exposed to this stressor may be compared to infants of women who were in their second or third trimester of pregnancy at the time of this disaster.

In sum, although our sample consisted of normal-risk pregnant women and healthy full-term infants, we still were able to find a small effect of prenatal stress on the development of infants in the first 8 months of life. We expect that in high-risk populations, these adverse effects of prenatal stress may be much more profound, especially if accumulating prenatal and postnatal risk factors, such as maternal smoking or alcohol-intake and adverse lifestyles, exist. The results of this thesis may then be regarded as a frame of reference.

10.9 Implications for clinical practice

Several implications for clinicians working with normal risk pregnant women may be deduced from the present thesis. First, since pregnancy-related anxieties, such as fear of giving birth and fear of bearing a physically or mentally handicapped child, are related to adverse infant development and temperament, they deserve more attention. The short questionnaire on these anxieties, as described in chapter 4, offers a tool to investigate the amount of pregnancy-related anxiety in patients. In early pregnancy a cut-off score of 8 for both fear for giving birth and fear of bearing a handicapped child may be regarded as a risk for the fetus. Providing information and reassurance may lower these fears, especially in nulliparous women. Second, although it might be impossible to avoid stress during pregnancy, it is important that women become more aware of the possible adverse effects stress may have on their fetus. The findings of the present human study first need to be replicated, but the results of animal studies strongly point to the negative effects of prenatal stress. Therefore, without alarming pregnant women, one should consider that prevention of stress during pregnancy is adviseable.

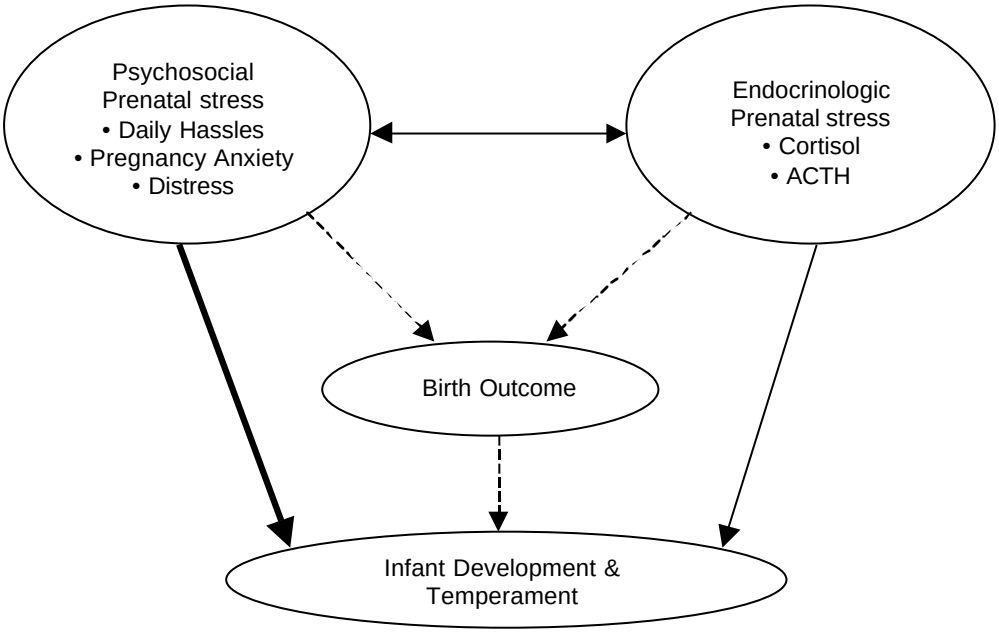
Several intervention studies have aimed to reduce distress during pregnancy, focusing on general stress and social support aspects, and thereby prevent adverse birth outcome. The results of these existing intervention programs are inconclusive. Our results suggest that per-

haps the focus of attention should be shifted towards alleviating pregnancy-specific anxiety in order to prevent adverse birth and developmental outcome.

10.10 Recommendations for future research

Several recommendations for future research may be deduced from Figure 10.1.

Figure 10.1 In this thesis we have tested the thick line representing the relationship



between psychosocial prenatal stress and infant development and temperament. The thinner line on the right hand side reflects the explored relationship between endocrinologic prenatal stress and infant development and temperament. We do not kwow as yet how psychsocial prenatal stress and endocrinologic prenatal stress are related. The interrupted lines reflect several paths that may be tested in the future.

First, prenatal stress may result in premature birth or low birth weight, thereby increasing the risk of developmental and behavioral problems later in life. Including both premature and at term born infants in a study will help to test for this effect. Second, more attention should be given to the role of the HPA axis reactivity in pregnant women, and the assocation between endocrinologic and psychosocial measures of stress in pregnancy. Since animal studies have found that the HPA axis reactivity of prenatally stressed offspring was altered, this offers an opportunity for research in human infants' HPA axis reactivity as well. Other recom-

mendations include research on gene/environment interaction with regard to individual sensitivity to prenatal stress effects.

Moreover, to be able to test the effect of stronger stressors on fetal and infant development, it is adviseable to prospectively study the effects of disasters. Retrospective studies of the effect on the developing fetus of such severe stressors, like the Flood in 1953 and the German Invasion during WO II in the Netherlands, suggested an effect on later psychopathology. Prospectively designed studies are preferrable for many reasons. Several years ago an El-Al Boeing 747-F crashed into two apartment buildings in the Bijlmermeer, a suburb of Amsterdam. Thirtynine people were killed on the ground, more than 260 people lost their apartments and 800 people experienced the disaster at close range. More recently, a fireworks storage, located in a residential quarter, exploded in Enschede, a town in the Netherlands, leaving many people homeless and wounded, while 18 were killed. It would be adviseable if pregnant women of these populations of victims would be asked shortly after the occurence of such a disaster to participate in a prospectively designed study on the effect of such severe stressors on their fetus. Ethical principles are of course involved and should be carefully considered. From a scientific point of view, however, much can be learned for future prevention of developmental and behavioral problem in infants, if strong stress effects on the developing fetus can be examined prospectively.

Nederlandse samenvatting

Bij een aanzienlijk deel van jonge kinderen (13-16 %) is er sprake van een ontwikkelingsachterstand, hetgeen zich kan uiten in diverse problemen, zoals hyperactiviteit, leerstoornissen, problemen met het gaan spreken en begrijpen van taal, en vertragingen in het leren zitten, staan en lopen, evenals gedragsproblemen. Een gedeelte van deze stoornissen zou verklaard kunnen worden doordat bij de geboorte complicaties op zijn getreden, met mogelijke hersenbeschadigingen tot gevolg. Echter, ondanks het feit dat er in de laatste jaren een enorme vooruitgang is geboekt op het gebied van de verloskunde, zodat de risico's op hersenletsel tijdens de geboorte aanzienlijk zijn verminderd, is dit percentage van kinderen met problemen nog niet afgenomen. Het is vooral opvallend dat vele van deze kinderen een normale zwangerschapsduur kenden en een op het oog normale zwangerschap en geboorte meemaakten.

Zodoende is de aandacht met betrekking tot de risicofactoren voor een latere ontwikkelingsachterstand of later probleemgedrag de laatste jaren verschoven van de geboorte naar de zwangerschapsperiode. Aanwijzingen voor deze zogenaamde 'prenatale' risicofactoren zijn te vinden in vele gebieden van onderzoek, welke in hoofdstuk 1 kort worden toegelicht. Het centrale idee hierachter is, dat al tijdens de vroege ontwikkeling van de foetus bepaalde factoren van invloed kunnen zijn op het zich snel ontwikkelende foetale brein. Dit noemt men 'vroege programmering', hetgeen zich later kan uiten in bijvoorbeeld ontwikkelingsachterstanden en gedragsproblemen.

In hoofdstuk 2 worden vervolgens de opbouw en de doelstellingen van het proefschrift weergegeven.

Het is opmerkelijk dat twee aspecten van de prenatale periode, namelijk stress en angst tijdens de zwangerschap, nog weinig aandacht hebben gekregen vanuit de onderzoekswereld, tenminste wanneer we spreken over de menselijke zwangerschap. Uit dieronderzoek is gebleken dat stress bij het zwangere vrouwtje wel degelijk een effect kan hebben op de ontwikkeling en het gedrag van het jonge dier na de geboorte. In hoofdstuk 3 worden deze dierexperimentele onderzoeken uitgebreid en systematisch besproken. De belangrijkste conclusie die hieruit te trekken valt, is dat prenatale stress bij dieren vooral negatieve gevolgen heeft voor de motorische ontwikkeling en de aanpassing aan een nieuwe omgeving. Diverse mogelijke mechanismen die deze effecten kunnen verklaren worden beschreven. Een mogelijke verklaring is dat bepaalde stresshormonen, waaronder cortisol, door de placenta heen kunnen dringen en zo het foetale brein kunnen bereiken. Daar kan cortisol vervolgens de ontwikkeling van de hypofyse-bijnieras verstoren, hetgeen een ontregeld stress mechanisme bij het jonge dier tot gevolg heeft. Hierdoor reageert het jonge dier sterker op een stressvolle situaties, zoals een nieuwe omgeving dat kan zijn.

Het is echter niet mogelijk om deze resultaten van dieronderzoek direct te betrekken op de menselijke situatie. Men stuit dan direct op het probleem wat 'prenatale stress' precies is. Bij dieronderzoeken is het immers eenvoudiger een duidelijk omschreven stressor toe te dienen (een hard geluid, of een elektrische shock bijvoorbeeld) dan bij mensen. Daarom is een belangrijk doel van dit proefschrift ook geweest om het begrip 'prenatale stress' duidelijker te omschrijven alvorens voorspellingen ten aanzien van de ontwikkeling bij mensen te kunnen doen.

Een uniek element van de menselijke zwangerschap is dat de zwangere vrouw zich zorgen kan maken om de gezondheid van haar nog ongeboren kind en over de pijnen die zij zal moeten doorstaan tijdens de bevalling. Deze gevoelens laten zich omschrijven als zwangerschap-specifieke angsten en worden in hoofdstuk 4 besproken.Tevens wordt aangetoond dat deze angsten maar voor een beperkt deel te verklaren zijn uit een algemeen angstig of neurotisch karakter. Eerder onderzoek naar de effecten van angst in de zwangerschap op de zwangerschapsduur en het geboortegewicht, richtte zich met name op deze meer algemene angsten. Ons onderzoek toont aan, dat het zeer zinvol is om ook te kijken naar de angsten die specifiek samenhangen met de zwangerschap.

Een ander element van stress in de menselijke zwangerschap is de mogelijkheid om op verschillende manieren met stress om te gaan. Voor het meten van deze zogenaamde 'coping' zijn er verschillende vragenlijsten beschikbaar. Omdat de meeste copingvragenlijsten nog nooit gebruikt zijn voor zwangere vrouwen, moest eerst onderzocht worden of zij geschikt waren voor deze specifieke groep. De resultaten daarvan staan in hoofdstuk 5 vermeld. Dit hoofdstuk laat zien dat er twee manieren van omgaan met stress zijn bij onze groep zwangere vrouwen. Ten eerste is dat de 'emotioneel gerichte coping', hetgeen inhoudt dat men zoekt naar een manier om met de emoties die een bepaalde situatie oproepen om te gaan. Daaronder valt bijvoorbeeld het praten over deze gevoelens met naasten. Ten tweede is er een 'probleemgerichte coping'mogelijk, waarbij meer naar praktische oplossingen voor een situatie wordt gezocht. Deze laatste vorm is meer rationeel van aard. In onderzoek naar de meest effectieve manier van omgaan met stress wordt deze probleemgerichte copingstijl vaak aangewezen als beste. Echter, dit is ook traditioneel de meest mannelijke manier van omgaan met problemen en het is goed mogelijk dat bij vrouwen juist de emotioneel gerichte coping strategie effectiever is in het verminderen van stress gevoelens. In hoofdstuk 6 worden de resultaten beschreven van onderzoek naar welke manier van coping in welke fase van de zwangerschap nu het beste bruikbaar lijkt. Vroeg in de zwangerschap bleek de emotioneel gerichte coping het meest geschikt te zijn, terwijl later in de zwangerschap de probleemgerichte coping juist effectiever was.

In hoofdstuk 7 worden de voorgaande elementen die betrokken kunnen zijn bij vroege programmering van het foetale brein samengenomen in een complexer model dat prenatale stress bij een zwangere vrouw omschrijft. Dit model toont aan dat bepaalde persoonlijke kenmerken, zoals een angstig of neurotisch karakter, maken dat men sneller stress ervaart. Ook het meemaken van ingrijpende gebeurtenissen en zwangerschap-specifieke angsten voorspellen een hogere mate van ervaren stress. Met behulp van dit model werd tevens getracht de ontwikkeling en het gedrag van jonge kinderen op de leeftijd van 3 en 8 maanden te voorspellen.

Met deze dissertatie is er voor het eerst onderzoek gedaan naar de relatie tussen stress van aanstaande moeders ten tijde van haar zwangerschap en de latere ontwikkeling van het kind tot 8 maanden na de geboorte. De uiteindelijke resultaten staan in hoofdstuk 8 en 9 vermeld. Eerdere onderzoeken vroegen òf achteraf (na de bevalling) naar de mate van stress die vrouwen meenden te hebben ondervonden tijdens hun zwangerschap, òf keken uitsluitend naar het effect van deze stress op de geboorteuitkomst, zoals de zwangerschapsduur of het geboortegewicht. Dit proefschrift bevestigt bevindingen vanuit dierexperimenteel onderzoek. Moeders die veel stress en angsten hebben ervaren tijdens hun zwangerschap lopen een verhoogd risico op het krijgen van een kind dat zich minder goed kan aanpassen aan een nieuwe

omgeving, moeilijker gedrag vertoont en bovendien een achterstand laat zien op de leeftijd van 8 maanden op een standaard test van de psychische en lichamelijke ontwikkeling van baby's. Vooral angsten die specifiek gerelateerd zijn aan de zwangerschap, zoals angst voor de gezondheid van het ongeboren kind en angst voor de bevalling, lijken deze latere problemen te voorspellen. Genoemde effecten van stress en angst tijdens de zwangerschap worden gevonden zelfs nadat er rekening werd gehouden met andere factoren die zouden kunnen samenhangen met de ontwikkeling en het gedrag van jonge kinderen, zoals een lager inkomen, roken en drinken in de zwangerschap en eventuele complicaties bij de bevalling. Het lijkt daarom veilig te concluderen dat vroege effecten van moederlijke stress mogelijk invloed hebben op het zich ontwikkelende brein van het ongeboren kind.

De implicaties van onze bevindingen zijn divers. Ten eerste zou het goed zijn wanneer de klinische praktijk rekenschap zou nemen van het feit dat stress en angst tijdens de zwangerschap eventueel schadelijk effect kunnen zijn voor het (ongeboren) kind. Door het invullen van een korte vragenlijst over zwangerschapsgerelateerde angsten kan een snelle indruk verkregen worden over de intensiteit van deze angsten. Goede voorlichting en gesprekken over de angsten zouden preventief kunnen werken ter voorkoming van een gestoorde ontwikkeling van het kind. Daarnaast zou soortgelijk onderzoek naar schadelijke effecten van stress in de zwangerschap op het ongeboren kind bij kunnen dragen aan het verder ontginnen van dit nog relatief onbekende onderzoeksgebied. Zodra er meerdere risicofactoren voor een vertraagde of afwijkende ontwikkeling, zoals een premature geboorte, bekend zijn, kan onderzocht worden in hoeverre stress de negatieve effecten van deze risicofactoren verder versterkt.

Al met al is met dit proefschrift een eerste stap gezet op een relatief on bekend onderzoeksterrein, dat veel meer aandacht verdient. Zeker gezien de toenemende stress beleving van vrouwen in de maatschappij, is het belangrijk manieren te vinden om stress tijdens de zwangerschap te voorkomen of te verminderen. Immers, de negatieve effecten van stress op het zeer jonge kind zijn een bron van zorg. Om te achterhalen of de gevonden effecten ook langdurig en blijvend zijn, zal de groep kinderen uit dit onderzoek op latere leeftijd nogmaals worden onderzocht.

Dankwoord

In een complex multidisciplinair onderzoek zijn per definitie meerdere mensen betrokken, ook al prijkt er maar één naam op de kaft van dit proefschrift. Ik wil een ieder bedanken die heeft meegewerkt aan ons project.

Vanzelfsprekend was dit onderzoek niet mogelijk geweest zonder alle enthousiaste deelneemsters aan ons project, die wij hebben overdonderd met de vele metingen en vragenlijsten. Alle huisbezoeken die ik heb afgelegd vlak na de geboorte waren voor mij een zeer aangenaam onderdeel van dit project en ik dank alle vrouwen dat zij ook zo vlak na de bevalling bereid waren mee te werken en mij zo gastvrij te ontvangen.

Prof. J.K. Buitelaar was als eerste promotor een zeer inspirerende kracht achter dit project. Jan, je bent in vele opzichten een groot voorbeeld geweest en je vele ideeën hebben mij gestimuleerd tot dit breed opgezette proefschrift. Je hebt me veel vrijheid gegeven in het vormgeven van de artikelen en daaruit bleek je vertrouwen in een goed eindprodukt.

Prof. G.H.A. Visser, als tweede promotor voor mij meer op de achtergrond aanwezig, maar altijd zeer enthousiast en daarmee zeer stimulerend. Gerard, ik heb je het laatste half jaar overdonderd met steeds maar nieuwe en vaak ook erg psychologisch getinte artikelen, maar je bleef enthousiast en hebt me daarmee zeker gemotiveerd.

Dr. E.J.H. Mulder, mijn co-promotor, heeft zeer intensief mijn teksten doorgelezen en geredigeerd, met zijn bijzondere oog voor detail en zijn grote kennis van de Engelse taal en daarmee heeft hij een grote bijdrage geleverd aan vele hoofdstukken in dit proefschrift. Edu, bedankt dat ook jij je door die enorme brei van woorden hebt heen geworsteld en er zinnige dingen over kon zeggen, terwijl dit toch echt jouw vakgebied niet was.

Mijn collega-AIO op dit project, Pascale Robles de Medina heeft er zorg voor gedragen dat alle prenatale data vakkundig verzameld werden, zonder welke dit proefschrift niet mogelijk was geweest. Bedankt voor de prikkelende samenwerking. Ik heb veel bewondering voor hoe jij je door alle >life events= hebt heengeslagen en wens je veel succes met het afmaken van je eigen proefschrift.

Prof. Dr. H. van Engeland heeft als hoofd van de afdeling Kinder- en Jeugdpsychiatrie gezorgd voor een klimaat waar vele AIO's in tuinen kunnen opbloeien tot zelfstandige onderzoekers. Dat ik niet in zo'n tuin terecht kwam, heeft met name te maken met de wortels die ik reeds had ontwikkeld op de afdeling Kinderpsychiatrie en die moeilijk los lieten. Bedankt Herman, voor je belangstelling in mijn onderzoek in met name de laatste fase van mijn onderzoeksproject.

Dr. S.H.N. Willemsen-Swinkels wil ik bedanken voor haar hulp bij het opzetten van een ethologisch deel van dit onderzoek. Inge Maitimu dank ik voor de laboratoriumanalyses van cortisol en ACTH; Gerard Maassen voor zijn nuttige adviezen met betrekking tot de LISREL analyses.

Rob Nelissen, Sabine Oomen en Sharin Mercera hebben respectievelijk als student-assistent,

onbezoldigd onderzoeksassistent en bezoldigd onderzoeksassistent bijgedragen aan de dataverzameling of - verwerking van dit project. Veel dank voor jullie enthousiasme en gezelligheid.

Vele studenten hebben ieder hun eigen aandeel geleverd in dit promotie-onderzoek. Ik heb veel van jullie geleerd en ik hoop jullie ook iets te hebben meegegeven. In willekeurige volgorde noem ik: Sabine, Arianne, Roelke, Bregje, Germine, Marlies Koolen, Marlies van de Berg, Robbert, Rob, Veronique, Jet, Franca, Simone, Lisette, Marco, Ronald, Els, Annejet, Inge, Goedele, Mirella, Leonoor, Bernadette.

Aan collegiale belangstelling geen gebrek. In het bijzonder wil ik Emma van Daalen noemen, die mij zeer heeft gemotiveerd en gesteund, ook in tijden dat ik zelf geen einde zag komen aan dit project. Vooral in een latere fase was jouw enthousiasme over mijn artikelen en je bereidheid die kritisch te

willen lezen heel belangrijk voor mij. Ook heb je mij vele levenslessen geleerd. We hebben laten zien dat kliniek en research goed samen kunnen gaan en tot synergie kunnen leiden.

Ditte Slabbekoorn heeft zeker bijgedragen aan een prettige tijd op het AZU. Samen zijn begonnen aan het promotie-avontuur en zeker in het begin was het erg plezierig om met jou te kunnen praten over allerlei facetten van onderzoek doen. Je hebt me bovendien voorgedaan hoe je moet promoveren. Ik was jouw paranimf, en nu ben jij de mijne. Je bent een enorme steun en bron van advies geweest, in praktisch en emotioneel opzicht, in de allerlaatste fase.

Met Nicolle van de Wiel heb ik menige rookpauze doorgebracht, als niet-roker. Bedankt voor de gezellige breaks. Stephanie van Goozen was altijd enthousiast over mijn onderzoek. Met Carolina de Weerth heb ik ook inhoudelijk kunnen praten over 'ons' onderzoeksgebied, hetgeen een zeer welkome aanvulling was. Alle AIO's dank ik voor jullie enthousiasme en gezelligheid, hoewel ik daar zelf de laatste maanden niet veel van heb kunnen meemaken door de drukke werkzaamheden.

Ik verlang soms terug naar de tijd dat het geschater van Anita en Hannemieke over de gang klonk. Dank jullie wel voor die gezelligheid en het eeuwige meeleven, vooral met mijn verre reisbestemmingen, waarin ook Joke en Irene telkens weer geïnteresseerd waren. Daarnaast waren jullie natuurlijk altijd beschikbaar voor veelal praktische vragen en ook toen ik al min of meer vertrokken was bij het AZU waren jullie zo attent om mij te helpen waar jullie konden.

Annemieke van Westervoort en Mark van Westervoort bedank ik voor het uitwerken van mijn vage concept tot de omslag van dit proefschrift. Het resultaat mag er zijn! Mark van Westervoort bedank ik tevens voor het verzorgen van de lay-out van dit proefschrift.

Het gewone leven, het alledaagse, maar zeker zo bijzondere, heeft veel te lijden gehad onder mijn promotie-onderzoek. Gelukkig is het wel doorgegegaan en heeft het mij bij tijd en wijle weer met beide benen op de grond weten te zetten. Dit heb ik zeker niet aan mezelf te danken, maar aan al die lieve mensen om mij heen.

In de eerste plaats denk ik daarbij natuurlijk aan Marjolein, die mij veel heeft moeten missen, ook als ik fysiek wel aanwezig was, maar met mijn hoofd nog op het AZU of in mijn onderzoek ronddwaalde. Zij liet mij mijn gang gaan, maar trok mij terug in de realiteit als dat nodig was. Ze houdt mij dagelijks een spiegel voor hoe je ook kunt genieten van de kleine dingen in plaats van alleen de grote dingen, zoals een promotie, na te jagen. We waren een echt en hecht team in de laatste zeer hectische en bewogen periode. Jouw onuitputtelijke vertrouwen in mijn kunnen, heeft me zeker in de laatste periode zeer gesteund.

In de tweede plaats denk ik aan mijn ouders, die hun dochter aan de wetenschap beschikbaar hebben gesteld, terwijl zij nog in leven was! Hier is moed voor nodig, ik onderschat dat niet, en ik besef dat het een ware opoffering is geweest.

Verschillende vriendinnen hebben mij in tijden van pre-promotie stress veel ontspanning geboden met al hun >clubjes=. Hoewel ik niet het meest trouwe lid ben geweest de afgelopen jaren, hoop ik als Dr. Vouwgleuf weer in jullie midden te mogen treden. Edith, Erica, Linda, Miranda, bedankt voor alle gezelligheid en het meeleven met alle promotieperikelen.

Speciale woorden zou ik willen richten aan Peter, Anuschka en Aletta, mijn zeer goede vrienden die mij altijd hebben ondersteund in alle fases die ik heb doorlopen tijdens de laatste jaren. Bedankt voor jullie rotsvaste vertrouwen in een goede afloop en de onvoorwaardelijke steun die ik heb mogen ontvangen. Zeker in de laatste fase zijn jullie onmisbaar gebleken. Aletta, ik ben er trots op dat je mijn paranimf wilde zijn en dat je op dit voor mij zo belangrijke moment ook weer achter me zult staan.

Allen zou ik willen toezeggen dat ik meer tijd en aandacht aan jullie wil besteden, nu deze proeve van bekwaamheid is afgelegd. Het proefschrift is af, leve het leven.

Curriculum vitae

Anja Huizink werd geboren op 11 april 1969 te Wormerveer. In 1987 behaalde zij haar VWO-diploma met een gemengd a/b pakket aan scholengemeenschap 'Bertrand Russell' te Krommenie. In dat zelfde jaar begon zij aan de HBO opleiding fysiotherapie. Daar volgde ze colleges Neuropsychologie die haar bijzonder boeide.

In 1991 volgde mede daardoor een overstap naar de wetenschappelijke opleiding Psychologie aan de Vrije Universiteit met als beoogde afstudeervariant Neuropsychologie. Tijdens deze studie ontdekte zij tevens de Fysiologische Psychologie. Zodoende doorliep zij twee afstudeerstages, waarbij zij onderzoek deed naar EEG metingen bij jonge kinderen voor de Neuropsychologische afstudeervariant en het effect van mentale stress op de bloedstolling en fibrinolyse onderzocht voor de Psychofysiologische afstudeervariant. In 1994 werd na 3.5 jaar het doctoraal examen 'met genoegen' in deze twee afstudeerrichtingen afgesloten.

Na het afstuderen volgde een periode waarin zij werkzaam was als testzaalassistente bij een psychologisch organisatie- en adviesbureau, als postbode en als thuiszorghulp, terwijl zij solliciteerde naar een geschikte assistent in opleiding (AIO) functie. In januari 1996 begon zij als AIO aan het toenmalige Academisch Ziekenhuis Utrecht (AZU) bij de faculteit Geneeskunde, afdeling Kinder- en Jeugdpsychiatrie, inmiddels verworden tot Universitair Medisch Centrum Utrecht. Als AIO was zij tevens aangesloten bij het Rudolf Magnus Instituut voor Neurowetenschappen.

Sinds 1 juli 2000 is Anja werkzaam als postdoc bij het Extramuraal Geneeskundig Onderzoeks (EMGO) Instituut aan de faculteit der Geneeskunde van de Vrije Universiteit Amsterdam. Ook daar bestudeert zij weer de relatie tussen stress en gezondheid, de rode draad die door haar academische leven loopt, in het kader van een grootschalig epidemiologisch onderzoek naar de effecten van de Bijlmerramp op de geestelijke en fysieke gezondheid van bewoners, hulpverleners en KLM medewerkers die betrokken zijn geweest bij de ramp of blootgesteld aan de brokstukken van het neergestorte El Al vliegtuig.

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