PSYCHOLOGICAL FUNCTIONING AND THE AUTONOMIC NERVOUS SYSTEM DURING PREGNANCY

IMPACT ON MOTHER AND CHILD

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Introduction

1.1 Introduction

Since the middle of the 20th century, when the concepts underpinning psychological stress were first defined, there has been an explosion of interest in how stress influences health and disease. Stress may affect health directly, by activating specific physiological responses, or indirectly through its influence on health-related behaviors, e.g. alcohol use or smoking (Segerstrom & O'Connor, 2012). Stress can be defined as an individual's response to an environmental condition or a stimulus. Although, stress responses can be adaptive by preserving homeostasis or maladaptive if responses become chronic, the term stress is often only used as a synonym for distress and not for eustress (Koolhaas et al., 2011; Selye, 1976). Stimuli can trigger both physiological and psychological or behavioral responses. From a psychological point of view, a stimulus becomes a stressor when it is appraised or perceived as a threat (Lazarus & Folkman, 1984). Such an appraisal process can elicit psychological stress responses such as state anxiety or anger (Spielberger & Sarason, 2013). Typical physiological stress responses are an elevated heart rate (HR) and reduced heart rate variability (HRV), which express alterations in autonomic nervous system (ANS) activity (Berntson & Cacioppo, 2004).

In non-pregnant populations it is extensively studied that the ANS plays a key role in psychological and physical wellbeing. It is believed that the ANS provides a common pathway linking negative affective states and conditions to ill health (Kemp & Quintana, 2013; Thayer, Yamamoto, & Brosschot, 2010). For instance, anxiety and depression are known to be strongly associated with reduced HRV (Friedman & Thayer, 1998a; Kemp et al., 2010). In contrast, there is much less known about ANS activity during pregnancy and its relationship with the psychological functioning of the pregnant woman. Nevertheless, it is suggested that altered activity in stress response systems such as the ANS and the hypothalamic-pituitary-adrenal (HPA) axis might mediate and thus provide insight into the relationship between psychological functioning (e.g. distress or anxiety) during pregnancy and fetal (brain) development and later cognitive, behavioral and social-emotional development of the child (Glover, 2011; Talge, Neal, Glover, Stress, & Translational Research and Prevention Science Network: Fetal and Neonatal Experience on Child and Adolescent Mental Health, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005; Weinstock, 2008). Research about the relation between the emotional state of the pregnant mother and consequences for her (unborn) child regained attention when the developmental origins of health and disease (DOHaD) hypothesis was formed. According to this hypothesis, which is fundamentally based on Barker's idea on fetal programming (Barker, Winter, Osmond, Margetts, & Simmonds, 1989), health and disease in adulthood have their origin in early life, namely during fetal and early childhood development (Barker, 2007). Maternal psychological functioning and associated physiological responses may affect fetal development as it may cause fetal cortisol exposure and altered blood flow to the uterus (Dipietro, 2012; Mulder et al., 2002; Räikkönen, Seckl, Pesonen, Simons, & Van den Bergh, 2011; Van den Bergh et al., 2005).

The main goal of this dissertation is to study the association between ANS activity and the psychological functioning of a pregnant woman. Since women undergo marked physiological changes during pregnancy (Silversides & Colman, 2007), ANS functioning is studied in all three trimesters of pregnancy. ANS functioning is examined from both the perspective of physiological responses to (acute) stressors and basal ANS activity.

In the next section of this introductory chapter the ANS and important ANS-related cardiovascular measures such as HRV are described. Subsequently, Sections 1.3.1 and 1.3.2 present earlier findings in the literature on respectively the basal ANS function and ANS-related responses to stress during pregnancy. Section 1.4 then presents existing knowledge on the relationship between psychological functioning and ANS. Section 1.5 describes how psychological functioning can affect child outcomes. This chapter ends with Section 1.6, which provides an overview of the aims of this dissertation, the main research questions addressed and an outline of the following chapters.

1.2 The autonomic nervous system

The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) are the key physiological mechanisms by which an organism reacts to stress. HPA axis activation can be measured in a non-invasive and reliable manner, e.g. by the level of salivary cortisol, which is the HPA axis' end product. However, HPA axis responses are beyond the scope of this dissertation, which focuses solely on ANS activity.

The activities of the ANS are considered automatic or self-regulating; it controls responses without intervention of the conscious mind (Carlson, 2007). The main function of the ANS is to keep the body in a balanced internal state (Andreassi, 2007; Silbernagl & Despopoulos, 2009), by regulating, e.g., the respiration, digestion, body temperature, and metabolism. The ANS has thus a clear homeostatic function and is therefore of vital importance for the wellbeing of the organism (Berntson & Cacioppo, 2004).

The ANS is traditionally divided into three distinct branches, based on their anatomical and functional differences. These are the sympathetic nervous system (SNS), the parasympathetic nervous system (PNS) and the enteric nervous system. Since the latter branch is not involved in the regulation of the cardiovascular system, the rest of the dissertation will solely focus on activity in the SNS and PNS. Both the parasympathetic and sympathetic nerves synapse on the sinoatrial node in order to influence heart rate (Levy & Pappano, 2007). Traditionally, the sympathetic and parasympathetic branches have been regarded as acting in an opposite (antagonistic or reciprocal) manner. Nowadays, it has become clear that this is an oversimplification and that both systems may be concurrently active or operate separate of each other (Andreassi, 2007; Berntson & Cacioppo, 2004).

1.2.1 Sympathetic Nervous System (SNS)

The sympathetic nervous system helps to mediate vigilance, arousal, and activation, and prompts bodily resources to cope with increased metabolic needs during challenging situations (Sapolsky, 2004). It prepares the body for high levels of somatic activity that may be required from an interaction with a stimulus in the environment (Andreassi, 2007). The SNS tends to be continuously active, but the degree of activity varies from moment to moment. However, during emergencies or threat, activity of the SNS comes to a maximum. The SNS is sometimes referred to as the fight-or-flight branch of the ANS, because it is activated to quickly respond to a physically or emotionally stressful situation (e.g. to fight against or flight from a threatening animal). Figure 1.1 shows the major organs that are innervated by the SNS[1]. As a result of the SNS activation many physiological stress responses are elicited, e.g. an increase in heart rate (HR), breathing rate, blood flow to skeletal muscles, sweating and dilation of eye pupils (Andreassi, 2007; Carlson, 2007). The responses of the SNS to threat are considered to be adaptive, because it enhances survival during stressful situations.

1.2.2 Parasympathetic Nervous System (PNS)

The parasympathetic nervous system is primarily concerned with the conservation of energy and maintenance of organ function during periods of minimal activity (Sapolsky, 2004). The PNS is sometimes called the rest-and-digest branch, which is mainly activated during rest and facilitates digestion and absorption of nutrients and excretion of waste products. Figure 1.2 shows the major organs that are innervated

[1]Figures 1.1 and 1.2 are reproduced from the PhD thesis entitled *Prenatal development and later neuroendocrine control of cardiovascular function: testing the stress hypothesis* (Jones, 2006) with permission from Dr. Alexander Jones.

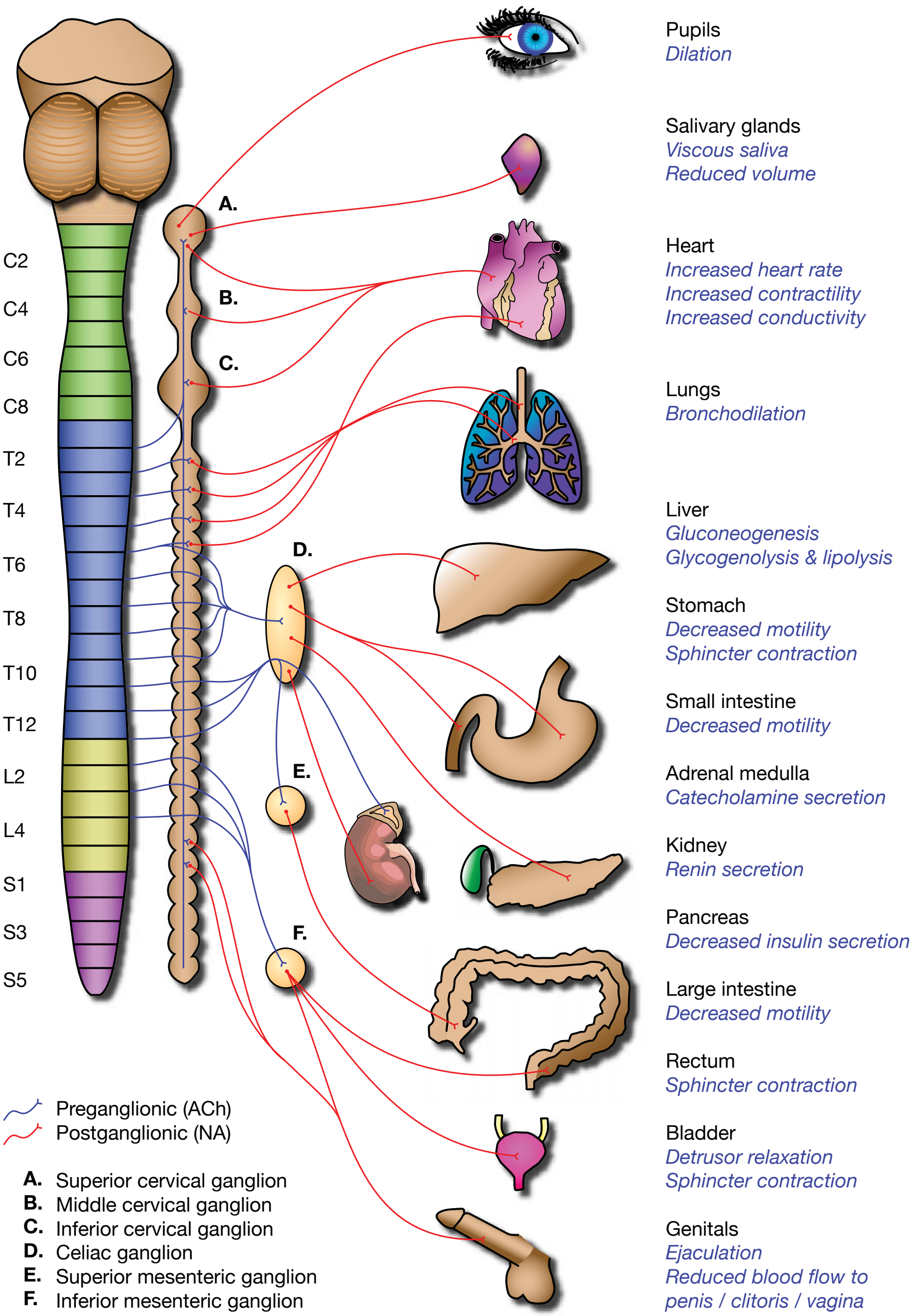

Figure 1.1. The sympathetic nervous system (innervation of major organs). Sympathetic innervation not shown includes that to blood vessels, spleen, piloerector muscles, sweat glands (cholinergic neurotransmission), adipose tissue, thyroid gland, ovaries, testes and uterus. Typical effects on target organs are listed. Ach, Acetylcholine; NA, Noradrenaline.

by the PNS. Activity in the PNS elicits among others a decrease in HR and blood pressure, constriction of pupils and many other responses that are not crucial during stressful situations (Andreassi, 2007). The level of activity in the PNS is also referred to as the vagal control or tone, because it resembles the inhibitory control of the vagus nerve over HR and atrioventricular conduction.

1.2.3 ANS-related cardiovascular measures

Heart Rate (HR) is regulated through both branches of the ANS. Heart rate variability (HRV) measures oscillations in the interval between consecutive heart beats (or cardiac cycle length variability), specifically, variability in the intervals between R waves (i.e., the RR interval). HRV is a non-invasive, accurate means of studying the beat-by-beat autonomic control of the cardiovascular system. It has become an important measure in health research. A decreased HRV has been linked to an increased risk of death and has a predictive value for life expectancy and health (Bigger, Fleiss, Rolnitzky, & Steinman, 1993; Tsuji et al., 1996).

Typically, standardized HRV measures are calculated from an electrocardiogram (ECG), which is a recording of the electrical activity of the heart over time produced by an electrocardiograph, usually in a noninvasive recording via skin electrodes. There are two main methods of HRV analysis: time-domain analysis and frequency-domain analysis. Commonly used HRV measures in the time domain are the standard deviation of interbeat intervals (SDNN), square root of the mean squared differences of successive interbeat intervals (RMSSD) and the portion of interval differences of successive interbeat intervals greater than 50 ms (pNN50). Frequency-domain HRV measures describe cardiovascular oscillations at certain frequency ranges and are calculated using a standard spectral analysis. As a result, one can distinguish, for example, between high frequency HRV (HF HRV) and low frequency HRV (LF HRV). RMSSD, pNN50 and HF HRV are accepted measures of parasympathetic activity, whereas LF is a marker for both sympathetic and parasympathetic modulation (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

The pre-ejection period (PEP) is a promising pure measure of activity in the SNS and relies on thoracic impedance cardiography (Newlin & Levenson, 1979), which aims to record the change in impedance over the thorax. These impedance changes are correlated to the aortic blood flow. PEP is derived from the impedance cardiogram (ICG) as the interval (measured in milliseconds) between Q-wave onset, which marks the depolarization of the ventricles, and the B-point, which represents the opening of the aortic valve and subsequent ventricular ejection.

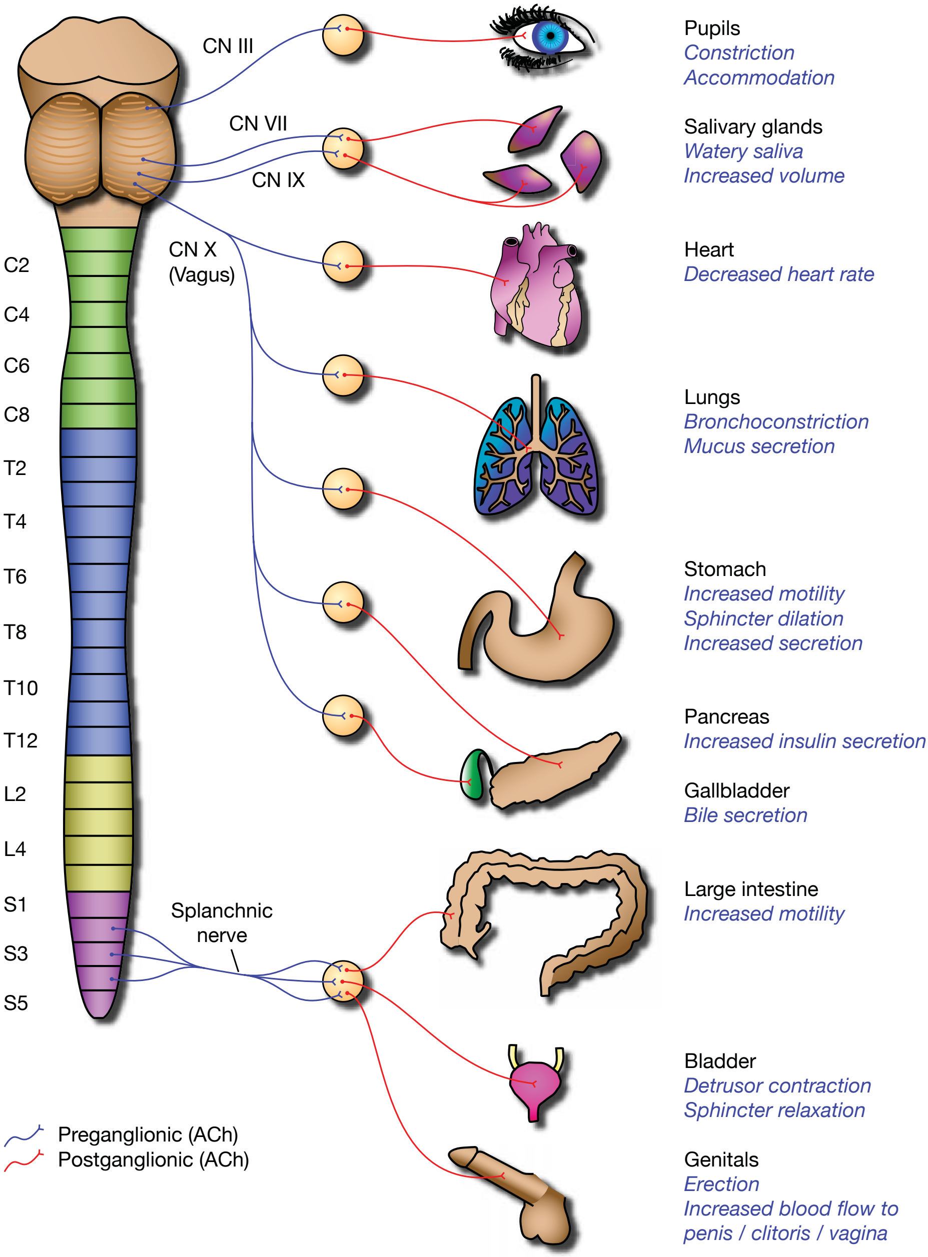

Figure 1.2. The parasympathetic nervous system (innervation of major organs). Parasympathetic ganglia are represented as discrete but are usually very close or embedded within target organs.

Chapter 2 contains more details on how HRV measures and PEP are calculated from ECG and ICG recordings.

1.3 The autonomic nervous system during pregnancy

1.3.1 Basal ANS functioning during pregnancy

From a cardiovascular point of view, pregnancy is associated with marked adaptations such as increased stroke volume (SV) and heart rate (HR) (Abbas, Lester, & Connolly, 2005; Moertl et al., 2009; Silversides & Colman, 2007). The autonomic nervous system plays a central role in these changes. It is known that ANS activity is shifted towards higher sympathetic and lower vagal modulation (e.g. reduced heart rate variability (HRV)) over the course of pregnancy (Kuo, Chen, Yang, Lo, & Tsai, 2000). The increased SV and HR generate higher cardiac output to offset the drop in systemic vascular resistance that occurs early in pregnancy (Abbas et al., 2005; Thornburg, Jacobson, Giraud, & Morton, 2000). The net effect is a slight decrease in mean blood pressure with the decline in DBP being larger than the SBP fall (Abbas et al., 2005). Since cardiac output keeps rising until the end of pregnancy and systemic vascular resistance regains again (Clark et al., 1989; Easterling, Benedetti, Schmucker, & Millard, 1990; Thornburg et al., 2000), blood pressure parameters increase again to pre-pregnancy levels by term (Christian, 2012; Hermida et al., 2000).

Most studies on physiological changes during pregnancy are based on relatively short-term recordings. While short-term HRV recordings can provide relevant data on autonomic function under specific controlled conditions, 24-hour ambulatory ECG recordings might provide a better view on individuals' basal autonomic nervous system activity in normal day-to-day activities. Moreover, it allows the analysis and comparison of ultra low frequency (ULF) HRV and sleep and circadian rhythm data. Nevertheless, so far there only exist two studies on 24-hour recordings of HRV during pregnancy. One study was based on 24-hour ECG recordings in 16 healthy women between 11 and 27 weeks of pregnancy and in 12 women before pregnancy, and found that pregnancy was associated with lower SDNN, VLF HRV and HF HRV (Ekholm, Hartiala, & Huikuri, 1997). Remarkably, HF HRV in pregnant women was only blunted during sleeping hours, suggesting that pregnancy reduces the capability of the vagus to activate normally during sleep (Ekholm et al., 1997). Similar results were found in a study with 24-hour ECG recordings for eight healthy pregnant volunteers (Stein et al., 1999). With the exception of a decrease in LF HRV, the latter study found no significant changes in daytime or nighttime HRV as pregnancy advances (Stein et al., 1999). These findings, clearly, need to be carefully interpreted, as these are based on rather small samples. Moreover, no single study included PEP as a measure of SNS activity, limiting the knowledge about circadian ANS function during pregnancy.

1.3.2 ANS reactivity to acute stressors during pregnancy

Given the marked basal physiological changes during pregnancy, one could also wonder how pregnant women physiologically react to stressful situations in everyday life. Stress reactivity is typically studied in the laboratory by means of standardized stress tests or protocols, in which physiological responses to acute stressors are measured. Stress tests mostly involve pain and discomfort (cold, heat and noise) or psychological stress (cognitive tests: color-word, mental arithmetic, mirror image tracing) (de Weerth & Buitelaar, 2005).

Research on physiological stress reactivity during pregnancy is mostly focused on HR and blood pressure and only rarely includes HRV or PEP as a physiological measure for stress responses. A review study concluded that physiological stress reactivity to laboratory challenges in human pregnancy is clearly present (de Weerth & Buitelaar, 2005). More specifically, HR and blood pressure of pregnant women tend to increase during mental arithmetic stress tests (Matthews & Rodin, 1992; McCubbin et al., 1996; Monk et al., 2000). Other frequently used stress tests during pregnancy are the Stroop color-word test and cold pressor test, which also show significant increases in BP and HR (de Weerth & Buitelaar, 2005). Notably, the design of the study (e.g., test protocol and kind of stressor used in experimental settings) can influence the kind of association found, as, for instance, in a sample of third-trimester pregnant women, reactivity to the Stroop color-word test was found to be greater than the reactivity to a mental arithmetic task (Monk et al., 2001).

To the best of our knowledge, only one study examined HRV reactivity to induced stress during pregnancy and found that exposure to the Trier Social Stress Test was linked to a significant decrease in HF HRV and a significant increase in LF/HF ratio (Klinkenberg et al., 2009). No studies exist examining PEP reactivity to laboratory stressors during pregnancy.

Besides marked changes in basal ANS activity (see Section 1.3.1), over the course of pregnancy women also have reduced cardiovascular responses to stress, as HR and blood pressure reactivity is typically attenuated at later compared to earlier pregnancy (DiPietro, Costigan, & Gurewitsch, 2003; Entringer et al., 2010). Likewise, although examined in only one study, there is a trend-significant decrease in autonomic response as expressed in HRV as pregnancy advances (Klinkenberg et al., 2009). To obtain a better understanding of autonomic reactivity in pregnancy and how it evolves, more studies focusing on different stages in the pregnancy are needed (de Weerth & Buitelaar, 2005).

1.4 Psychological correlates of autonomic activity

It is generally known from non-pregnant populations that many factors might have an influence on the ANS. Some variables thought to influence HRV are widely investigated, e.g. gender (less relevant for this dissertation), age and BMI. Both LF HRV and HF HRV decrease with increasing age (Umetani, Singer, McCraty, & Atkinson, 1998; Zhang, 2007). BMI is inversely related to HF HRV, indicating that the parasympathetic activity is greater in individuals with a lower BMI (Molfino et al., 2009; Vallejo, Márquez, Borja-Aburto, Cárdenas, & Hermosillo, 2005). Besides subject characteristics such as age and BMI, there also exist psychological correlates of autonomic activity, which play a key role in this dissertation. In particular, this section presents important earlier findings on the relationship between anxiety (Section 1.4.1), depression (Section 1.4.2) and ANS functioning. However, by solely focusing on the stressful nature of pregnancy, potential positive associations with ANS functioning might be overlooked. In this dissertation, it is studied how ANS functioning is associated with mindfulness (Section 1.4.3) and physical exercise during pregnancy (Section 1.4.4).

Although anxiety and depression are clearly related to each other and share a substantial component of general affective distress, empirical studies have shown that they are two separate, but often comorbid, constructs (Watson, 2005). Anxiety and depression can be differentiated on the basis of positive and negative affectivity. Negative affectivity represents a nonspecific factor common to both anxiety and depression, whereas low positive affectivity is a specific factor that is mainly related to depression (Watson, 2005).

It must be noted that mindfulness is related to anxiety and depression. Mindfulness predicts self-regulated behavior, positive emotional states and lower mood disturbance and less distress (Baer, Smith, Hopkins, Krietemeyer, & Toney, 2006; Baer et al., 2008; Brown & Ryan, 2003). Moreover, mindfulness-based therapy can reduce feelings of both anxiety and depression (Hofmann, Sawyer, Witt, & Oh, 2010). Techniques enhancing mindfulness during pregnancy also reduce anxiety and perceived stress for up to two months after the intervention (Bastani, Hidarnia, Kazemnejad, Vafaei, & Kashanian, 2005; Beddoe, Paul Yang, Kennedy, Weiss, & Lee, 2009; Vieten & Astin, 2008). Likewise, it is also worth mentioning that physical exercise is linked to less anxiety and depression (Mello et al., 2013; Salmon, 2001; Ströhle, 2009). Regular physical exercise during pregnancy has also been associated with improved psychological functioning (Poudevigne & O'Connor, 2006).

1.4.1 Anxiety

Symptoms

There is not much known about the association between maternal anxiety symptoms and basal ANS function during pregnancy. One study divided third-trimester pregnant participants based on the trait anxiety scale of the STAI pregnant participants into a low, middle and high anxiety group and could not find significant differences in basal HR, SBP and DBP between groups (Monk et al., 2004). HRV was not measured in this study. Studies with non-pregnant populations have similar results on basal levels of HR and BP, and additionally report no significant association between anxiety and various HRV measures (Choi, Kim, Kim, Kim, & Choi, 2011; Shinba et al., 2008; Stewart, Buffett-Jerrott, & Kokaram, 2001).

Research on the relationship between anxiety symptoms and stress responsiveness during pregnancy is also limited and consists of inconsistent results (Christian, 2012; de Weerth & Buitelaar, 2005). For example, some studies suggest that more anxious pregnant women have lower HR and blood pressure responses (Monk et al., 2000; Saisto, Kaaja, Helske, Ylikorkala, & Halmesmäki, 2004). In another study no association between prenatal anxiety and blood pressure or HR reactivity to a psychological stress test was found (Monk, Myers, Sloan, Ellman, & Fifer, 2003). These diverse findings might be explained by the use of different stress protocols (Monk et al., 2003). Previous studies typically do not take into account physiological changes throughout pregnancy and studies investigating a potential link between anxiety and HRV reactivity to stress during pregnancy are lacking. Studies with non-pregnant humans generally suggest that high anxiety is associated with exaggerated cardiovascular responses (i.e. HR and BP) (Gramer & Saria, 2007; Pointer et al., 2012).

Disorders

To the best of our knowledge, there are no studies about the autonomic functioning of *pregnant* woman with an anxiety disorder so far. Studies with men and non-pregnant women have linked various types of anxiety disorders (i.e. panic disorder, social phobia, generalized anxiety disorder) to significantly reduced basal parasympathetic activity, as indicated by various HRV measures (Blom et al., 2010; Cohen et al., 2000; Friedman & Thayer, 1998b; Licht, de Geus, van Dyck, & Penninx, 2009; Thayer, Friedman, & Borkovec, 1996; Yeragani et al., 1991).

Besides significant basal cardiovascular differences between humans with a current anxiety disorder and healthy persons, anxiety disorders are also associated with altered cardiovascular stress responsiveness. For example, patients with a panic or a post-traumatic stress disorder are shown to have a smaller increase in HR and less reduction in HF HRV during stress compared to controls (Cuthbert et al., 2003; Keary, Hughes, & Palmieri, 2009). A similar trend (i.e. reduced SBP, DBP, HR and cortisol reactivity) was found in men and women that were ever diagnosed with an anxiety disorder (de Rooij, Schene, Phillips, & Roseboom, 2010).

Only a few studies have investigated the impact of a prior history of anxiety disorder on HRV. Patients with a remitted anxiety disorder also tend to have blunted HRV (Licht et al., 2009). In a study with post-myocardial infarction patients 24-hour ambulatory ECG data lifetime anxiety disorder predicted reduced basal RMSSD HRV and HF HRV, even after additional adjustment of anxiety symptoms (Martens, Nyklíček, Szabó, & Kupper, 2008).

1.4.2 Depression

Research on the autonomic function and depressiveness is mostly focused on depressive disorders. To the best of our knowledge, there is only one study on maternal depression during pregnancy and HRV, reporting a reduced basal HRV (SDNN) (Shea et al., 2008). This is in line with findings in non-pregnant populations, as a review and meta-analysis showed that a depressive disorder is associated with reduced basal HRV, which decreases with increasing depression severity (Kemp et al., 2010). Critically, a variety of antidepressant treatments did not resolve these decreases despite resolution of symptoms (Kemp et al., 2010). Most studies show that patients with a depression have significantly lower HF HRV at rest (Carney et al., 2001; Hofmann, Schulz, Heering, Muench, & Bufka, 2010; Yeragani et al., 2002). In summary, depression is associated with alteration of cardiac autonomic tone towards decreased parasympathetic activity and an increased sympathetic activity (Udupa et al., 2007).

1.4.3 Mindfulness

Mindfulness is an adaptive mental state, often described as the attention to moment-to-moment experience with an accepting and nonjudgmental attitude (Baer et al., 2006; Williams, 2008) or as a receptive attentiveness to present experience (Brown & Ryan, 2003; Holt, 2012). Mindfulness can improve both mental and physical/physiological health, but studies concerning the physiological effects of mindfulness during pregnancy are lacking.

To the best of our knowledge, there are no studies published on the association between mindfulness as a mental resource and ANS activity during pregnancy. Therefore, this section presents the main findings on the relationship between ANS functioning during pregnancy and mindfulness-based stress reduction interventions and their the key components.

Meditation and yoga, which are both key components of mindfulness-based stress reduction interventions (Kabat-Zinn, 2003), breathing exercises and relaxation therapy are all possible options to reduce stress, anxiety and depressed feelings. Mindful yoga and meditation during the second pregnancy trimester reported reduced physical pain (Beddoe et al., 2009), better quality and quantity of sleep (Beddoe, Lee, Weiss, Kennedy, & Yang, 2010), and reduced anxiety and perceived stress (Bastani et al., 2005). Research also indicates reductions of anxiety, stress (Beddoe et al., 2009; Vieten & Astin, 2008) and negative affect (Vieten & Astin, 2008) at the end of pregnancy. There even exists evidence for improved levels of perinatal stress and mood (Newman, 2005; Vieten & Astin, 2008).

Only few studies have evaluated the effect of meditation kind exercises on physiological parameters. Most consistently, a reduction in heart rate has been reported (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008). In general, research in healthy populations shows that mediation and yoga tend to decrease LF HRV, while increasing HF HRV (Leonaite & Vainoras, 2010; Paul-Labrador et al., 2006; Sarang & Telles, 2006; Takahashi et al., 2005; Tang et al., 2009; Wu & Lo, 2008). Accordingly, these changes go together with significant decreases in the LF/HF ratio, reflecting a better sympathovagal balance (Sarang & Telles, 2006; Takahashi et al., 2005; Wu & Lo, 2008).

Studies about mindfulness-related interventions and its effect on physiological parameters during pregnancy are lacking, except one study that showed an increase in HF HRV during a yoga relaxation intervention - this effect was enhanced during later stages of the pregnancy (Satyapriya, Nagendra, Nagarathna, & Padmalatha, 2009).

1.4.4 Physical exercise

Many associations of obstetricians and gynecologists around the world recommend 30 minutes of daily moderate-intensity physical exercise for pregnant women (see review of country-specific guidelines on physical exercise during pregnancy by Evenson et al. (2013)).

Regular physical exercise during pregnancy has been associated with improved psychological functioning (Poudevigne & O'Connor, 2006), as well as with positive physical outcomes such as shorter labor and delivery, faster recovery after delivery, fewer pregnancy complications (e.g. gestational hypertension and diabetes) and less weight gain (Mudd, Owe, Mottola, & Pivarnik, 2013; Streuling et al., 2011).

There is also evidence of improved ANS functioning (i.e. enhanced vagal tone) during rest in pregnant women who exercise more often (Melzer, Schutz, Boulvain, & Kayser, 2010). In non-pregnant women regular physical exercise is also linked to enlarged circadian variation in HRV (Adamopoulos et al., 1995). However, no studies have been conducted on the association between regular physical exercise during pregnancy and circadian rhythms in HRV.

1.5 Child outcomes

Psychological functioning during pregnancy may not only affect mother's health (see Section 1.4), but is also associated with altered psychophysiological and fetal brain development and in this way it might influence the later cognitive, behavioural and social-emotional development of the child (Glover, 2011; Talge et al., 2007; Van den Bergh et al., 2005; Weinstock, 2008). In this section we briefly review known associations with birth outcomes, offspring ANS functioning, and offspring social-emotional development. First, potential mechanisms underlying these associations are discussed.

1.5.1 Fetal programming and modulation of fetal programming

Maternal anxiety during pregnancy may influence later development, possibly by modulating the programming of offspring (neuro)physiology and central nervous system structures or structure-function relationships (Van den Bergh, 2011a, 2011b). The developmental programming of health and disease (DOHaD) hypothesis indeed supposes that the physiological and metabolic adaptation that enable the fetus to adapt to alteration in its life environment may result in a permanent (re)programming of the developmental pattern within key tissues and organ systems (Gluckman, Hanson, Cooper, & Thornburg, 2008). The exact mechanism by which prenatal anxiety and stress in humans can modulate developmental programming is still unclear, but most probably includes the activation of the maternal stress system, i.e. the hypothalomo pituitary adrenal (HPA)-axis and the autonomic nervous system (ANS) (de Weerth & Buitelaar, 2005; Owen, Andrews, & Matthews, 2005). In Chapter 7 (general discussion) these two mechanisms are discussed in more detail and placed in context of the dissertation's results. Below the role of the HPA axis and ANS in (the modulation of) fetal programming is only briefly discussed. (1) So, one possibility is that cortisol levels, which are increased when the pregnant woman is anxious or experiences stress, can pass through the placental barrier (Mulder et al., 2002). Although cortisol measures have proved to be valuable measures when investigating anxiety and stress during pregnancy (Evans, Myers, & Monk, 2008; Kammerer, Adams, von Castelberg, & Glover, 2002; Obel et al., 2005; Pluess, Bolten, Pirke, & Hellhammer, 2010), use of this HPA-axis measure nevertheless leads to inconsistent results, with some studies reporting increased activation of the HPA-axis, and others reporting the opposite (Glover, O'Connor, & O'Donnell, 2010; O'Donnell, O'Connor, & Glover, 2009). This inconsistency is also revealed in studies conducted in non-pregnant populations, e.g. (Miller, Chen, & Zhou, 2007) and may be related to the type and timing of the stressor but also to the fact that the cortisol measures used are too imprecise. (2) Another possibility, involving ANS-activity, is that uteroplacental blood flow is reduced due to increased catecholamines, which are released during periods of anxiety and distress. For example, highly anxious women show a significant reduction of uterine blood flow as compared to low anxious women (Teixeira, Fisk, & Glover, 1999). Moreover, women with abnormal uterine perfusion were found to have reduced HRV compared to healthy women and gave birth to infants of smaller birth weight (Walther et al., 2006).

1.5.2 Birth outcomes

No association was found between anxiety symptoms during pregnancy and adverse birth outcomes in a meta-analysis with 50 studies (Littleton, Breitkopf, & Berenson, 2007; Littleton, Bye, Buck, & Amacker, 2010). In contrast, a recent review concluded that there exists strong evidence that stress and anxiety during pregnancy are important risk factors for preterm delivery and low birth weight (Dunkel Schetter & Tanner, 2012). Cardiovascular reactivity to induced stress (e.g. mental arithmetic task) during pregnancy is also associated with gestational age and birth weight. In particular, greater diastolic blood pressure is linked to a shorter gestation and lower birth weight (Gómez Ponce de León, Gómez Ponce de León, Coviello, & De Vito, 2001; Hatch et al., 2006; McCubbin et al., 1996), and a lower systolic blood pressure predicted small for gestational age (Harville, Gunderson, Matthews, Lewis, & Carnethon, 2010). So far, there is no association found between HR or HRV reactivity and any birth outcome.

Failure of cardiovascular adaptation during pregnancy is also a risk factor for adverse birth outcomes. Both low and high diastolic blood pressures during pregnancy are associated with small babies and high perinatal mortality (Steer, Little, Kold-Jensen, Chapple, & Elliott, 2004). A significant inverse association was found between daytime ambulatory DBP measurement and birth weight (Waugh et al., 2000).

1.5.3 ANS functioning

The functioning of the stress systems is often seen as a biological marker of psychopathology. In studies focusing on offspring biological systems significant empirical evidence has been found for an association between altered basal or stress-related cortisol secretion and prenatal exposure to maternal anxiety or stress (O'Connor et al., 2005; Van den Bergh et al., 2005; Yehuda et al., 2005). Theoretically, the fetus makes adaptations in response to changes in its environment, which prepare the fetus for a postnatal life in a prospected similar environment, i.e. fetal programming. This adaptation may alter set points of physiological systems (Gluckman & Hanson, 2004). Studies in this regard found that anxiety and depression during pregnancy were a significant predictor for a decreased cardiac vagal tone during rest and "interaction" in the offspring (Ponirakis, Susman, & Stifter, 1998). HRV of the developing fetus tends to be altered when mothers have a number of psychiatric conditions, including anxiety disorders, and these differences in ANS functioning persist postnatally (Dierckx et al., 2009; DiPietro, Costigan, Pressman, & Doussard-Roosevelt, 2000; Monk et al., 2004). However, in more recent studies no similar association was found between prenatal maternal psychosocial stress or disorders and the cardiac ANS balance in rest in the offspring of 4 months or five-six years (van Dijk, van Eijsden, Stronks, Gemke, & Vrijkotte, 2012). With regard to lifetime disorders, HRV of the offspring was significantly lower in those born to mothers reporting past mood disorder (Jacob, Byrne, & Keenan, 2009).

1.5.4 Social-emotional development

Maternal emotional distress during pregnancy is not only a significant risk factor for preterm delivery (Copper et al., 1996; Lobel et al., 2008), but it plays also a role in determining individual differences in cognitive, social and emotional functioning, and mental health problems in the offspring (Räikkönen et al., 2011; Van den Bergh et al., 2005).

In this dissertation two aspects of social-emotional development were taken into account, more specifically, adaptive functioning and anxiety. Maternal anxiety is associated with the anxiety level of the child as reflected in temperament, anxiety or internalizing problems (Huizink, de Medina, Mulder, Visser, & Buitelaar, 2002; Mulder et al., 2002; O'Connor, Heron, Golding, Beveridge, & Glover, 2002; Van den Bergh & Marcoen, 2004). Related to adaptive functioning, pregnancy-related factors such as increased maternal stress, anxiety or depression have been reported as precursors of regulatory problems (Dahl, Eklund, & Sundelin, 1986; Papousek & Von Hofacker, 1998; Wurmser et al., 2006), such as eating and sleeping problems (Schmid, Schreier, Meyer, & Wolke, 2011).

1.6 Aims, research questions and outline

The main aim of this dissertation is to provide insight into how ANS activity changes during pregnancy and how psychological functioning is associated with the ANS activity. Furthermore, it is also examined how maternal psychophysiological functioning is related to offspring birth weight and psychophysiological development. The following research questions are addressed in the chapters to come:

1. What is the typical autonomic reactivity and recovery during pregnancy and how is it related to the pregnant woman's level of trait anxiety? (Chapter 3)
2. Is there an association between past anxiety disorder, women's heart rate variability during pregnancy, offspring's heart rate variability and fearfulness? (Chapter 4)
3. Is mindfulness associated with better cardiovascular adaptation during pregnancy, better maternal mental health and better social-emotional development of the infant at 4 months? (Chapter 5)
4. Is there an association between physical exercise during pregnancy, circadian ANS functioning and offspring birth weight? (Chapter 6)

This dissertation is divided into seven chapters. The present introductory chapter (Chapter 1) is followed by Chapter 2, which describes the general research design for the studies included in this dissertation. In Chapters 3 to 6, the research questions as presented above will be examined. Finally, a general discussion and a summary of the main findings are presented in Chapter 7.

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2

General Research Design

2.1 Prenatal Early Life Stress (PELS) study

The subjects of the studies presented in this dissertation are mothers and infants who have been recruited within the Dutch branch of the prenatal early life stress (PELS) study. The PELS study is a collaborative study running in three countries participating in the Eurocores' "Stress and Mental Health" (EuroSTRESS) programme from the European Science Foundation (ESF). The PELS study was designed by B.R.H. Van den Bergh, project leader and principal investigator from Tilburg University (the Netherlands). Other partners were V. Glover, principal investigator from the Imperial College London (United Kingdom), S. Claes, principal investigator from KU Leuven (Belgium) and A. Rodriguez, associated partner from Uppsala University (Sweden). Research funding was generated from three national funding agencies through ESF, i.e. the Brain and Cognition Programme of the Dutch Organisation for Scientific Research (NWO) in the Netherlands, the Medical Research Council (MRC) in the United Kingdom and the Flemish Research Foundation (FWO) in Belgium. In the Netherlands, PELS focuses on associations between prenatal stress risk factors, birth outcomes and altered child psychophysiology and neurodevelopment. The study started in May 2009 and participants were enrolled until June 2010. Until now five waves of data collections are conducted, three during pregnancy (three pregnancy trimesters) and two after birth (2-4 and 9-10 months old offspring). The data collection took place between May 2009 and January 2012. Table 2.1 shows an overview of the data collection during the first five waves of the PELS study. The sixth wave (children of 4 years old) is running.

2.2 Procedure

The St. Elisabeth hospital and midwiferies in and around Tilburg were contacted and we explained the purpose of our study and asked them to collaborate. Most of them agreed to contribute to our study and engaged to introduce the study to their clients when they came for their first check-up at the beginning of pregnancy. If their clients were interested they gave permission to pass on their contact information to the researchers. The researchers made an appointment with the pregnant woman to explain the study in more detail. This is in accordance with the declaration of Helsinki, letting these pregnant women decide whether or not to participate. If the pregnant woman and her partner agreed to participate, they both signed the informed consent and an appointment for the first wave was made.

In most cases we visited the women at home during pregnancy at a time chosen by them. After explaining the procedures, the cardiovascular monitoring device was attached to record ECG (electrocardiogram) and ICG (impedance cardiogram)

Table 2.1
Overview on the data collection during first five waves of the PELS study

	1st pregnancy trimester	2nd pregnancy trimester	3rd pregnancy trimester	2-4 months after birth	9-10 months after birth
Mothers					
Questionnaires (e.g. psychological functioning)	•	•	•	•	•
ECG & ICG (mental arithmetic task)	•		•		
ECG & ICG (24 hours)	•	•	•		
Salivary Cortisol	•	•	•		•
Cortisol from hair	•		•	•	•
Fathers					
Questionnaires (e.g. Psychological functioning)	•	•	•	•	•
Infants					
Questionnaires (e.g. Psychological functioning)				•	•
Bayley Scales of Infant Development					•
Laboratory Temperament Assessment					•
Auditory ERP task				•	•
Audio-visual ERP task					•
ECG during ERP task				•	•
Salivary cortisol				•	•
Cortisol from hair					•

for 24 hours. Instruction cards for potentially reattaching the equipment after a shower were available, as well as telephone and e-mail support during waking hours. The total visit took about one hour. The following day subjects were visited again to collect the equipment. This procedure was repeated three times during pregnancy, namely before 15 weeks (first visit and pregnancy trimester), between 15 and 22 weeks (second trimester), and between 31 and 37 weeks (third trimester) of gestation. In addition to the 24h ambulatory recordings, the participants also filled out questionnaires about their feelings, their work and how they experience their pregnancy in all three trimesters.

Before 15 weeks of gestation and between 31 and 37 weeks of gestation the mothers completed also a 25-minutes task that consisted of five distinct phases, each lasting 5 minutes. In the second and fourth phases stress was induced, i.e., pregnant women needed to solve a mental arithmetic task. Continuous series of sums of five operations with a two- or three-digit number had to be performed without any verbalization. Using the mouse cursor, they indicated the correct answer by choosing between three alternatives. Subsequently, the next sum appeared. The first, third and fifth phase were resting phases: peaceful pictures were presented to the participants, while they were listening to relaxing music. The participants were encouraged to solve as many sums as possible with the promise that the 10 women with the highest scores received a little present. After the whole task was accomplished feedback was provided (Taelman, Vandeput, Vlemincx, Spaepen, & Van Huffel, 2011; Vlemincx, Taelman, De Peuter, Van Diest, & Van den Bergh, 2011). Most participants completed this task in a quiet room at home, so that they were on ease and felt quite comfortable before starting the stress task. One of the two researchers was always present, but they were out of sight. At the second wave (between 15 and 22 weeks of gestation) the participants were not asked to perform the computer task. Instead, we assessed their current and past psychiatric status using the Mini International Neuropsychiatric interview (MINI).

After birth, the participants came to our lab at Tilburg University to study the development of the child. We recorded the infants' EEG and ECG during an auditory information processing experiment performed at the ages of 2 to 4 months and 9 to 10 months. Event-related potentials (ERPs) were studied during an auditory oddball task to retrieve indices of the infants' neurocognitive development (Otte et al., 2013). During these 5 blocks, each lasting for 2.5 minutes, the ECG was also recorded. At the age of 9 to 10 months, the infants' temperament was assessed using the Laboratory Temperament Assessment Battery (Lab-TAB) (Goldsmith & Rothbart, 1999).

2.3 Participants

One hundred ninety different pregnant women were recruited and started in our study. The mothers had a mean age of 31.56 years (SD=4.42). In Table 2.2 more demographical information about the mothers can be found. One hundred seventy-eight of them were enrolled before 15 weeks of pregnancy (gestational age: M=11.96 weeks; SD=1.73), while 12 were enrolled between 15 en 22 weeks of pregnancy (gestational age: M=17.33; SD=2.10). All infants were born between September 23rd 2009 and March 24th 2011. Table 2.3 presents more characteristics of the infants.

Figure 2.1 shows how many participants were included in each study that is presented in one of the subsequent chapters of this dissertation.

Table 2.2
Demographical information for mothers (at recruitment) (N=190)

		n	%
Nationality			
	Dutch	185[⋆]	97.4
	German	2	1.1
	French	1	0.5
	Russian	1	0.5
	Thai	1	0.5
Marital Status			
	Married	96	50.5
	Cohabiting	89	46.8
	Single	5	2.6
Educational level			
	Primary or Secondary school	19	10
	General vocational training	4	25.8
	Higher vocational training	73	38.4
	University degree or higher	49	25.8
Currently has a job?			
	Yes	176	92.6
	No	14	7.4
Family net income (monthly)			
	< € 2100	9	4.7
	€ 2200-3600	38	20.0
	> € 3600	131	68.9
	No information	12	6.4
Smoker[•]			
	No	161	84.7
	Yes	9	4.7
	No information	20	10.5
Diabetes gravidarum[*]			
	No	169	88.9
	Yes	4[+]	2.1
	No information	17	8.9
Gestational hypertension[*]			
	No	166	87.4
	Yes	7[+]	3.7
	No information	17	8.9
Assisted Pregnancy			
	None	153	80.6
	In vitro fertilisation	15	7.9
	Intracytoplastmatic Sperm Injection	8	4.2
	Hormonal therapy	7	3.7
	Intra-uterine insemination	4	2.1
	Other	3	1.6
Previous pregnancies			
	Primigravida	74	38.9
	Miscarriage[†]	47	24.7

[⋆] Six were besides Dutch, also Bolivian, Moroccan, Romanian, Russian or Turkish
[•] In second pregnancy trimester [*] In third pregnancy trimester
[+] No ECG/ICG recording (used) [†] Miscarriage before 12 weeks of gestation

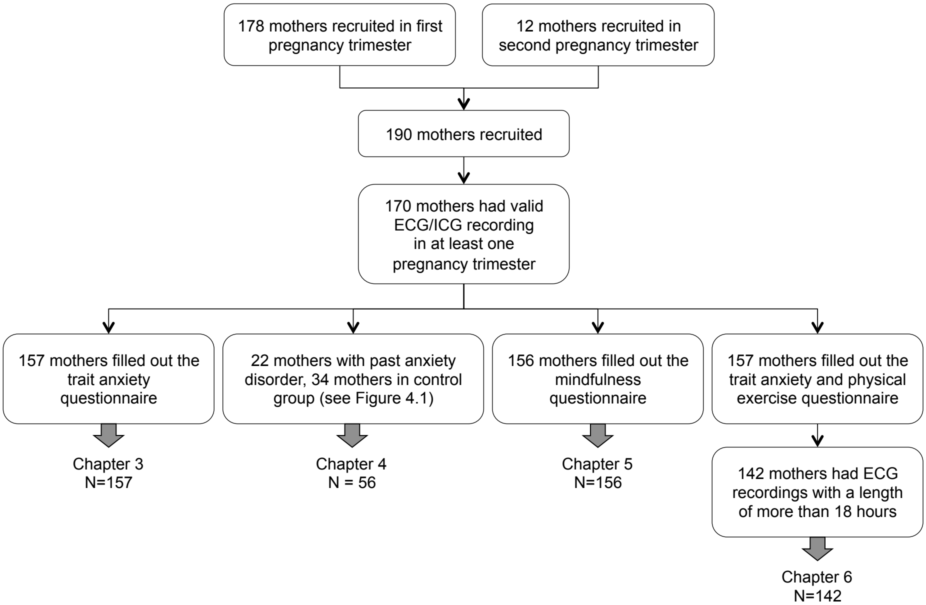

Figure 2.1. Overview on the sample sizes of each study in this dissertation

Table 2.3
Infant characteristics (N=192)

		n	%	M (SD)
Sex				
	Boy	92	48.2	
	Girl	96	49.7	
	Missing	4	2.1	
Birth weight (*g*)				3444 (519)
Gestational age at birth (*weeks*)				39.69 (1.6)
Preterm birth (before 37 *weeks*)		9⋆	4.7	
Apgar score above 8*				
At 5 minutes		160/165	97.0	
At 10 minutes		165/165	100.0	

⋆ 8 infants born between 32 and 37 weeks.

* No Apgar information on 27 infants.

2.4 Materials

2.4.1 Data related to the mothers

In a general questionnaire at the beginning of the study, participants reported about their own birth date, work, education level, height and weight before pregnancy, parity, gravidity, experiences getting pregnant, first day of the last period, medical diseases and expected delivery date. Age and BMI were calculated from these data. Data about mothers' weight gain and blood pressure were obtained from medical records of the hospital or midwiferies.

Autonomic nervous system measurements

The VU University Ambulatory Monitoring System (VU-AMS)[1] was developed by the department of Biological Psychology and the Technical Department (ITM) of the Faculty of Psychology and Education to allow recording of autonomic and cardiovascular activity in a variety of research settings, including ambulatory monitoring in naturalistic settings (de Geus & van Doornen, 1996). It is a worldwide used monitoring device for ambulatory measurements with a sampling rate of 1024Hz. In this dissertation, the VU-AMS was used to monitor the ECG and ICG of the mothers during pregnancy. The ECG and ICG signals were recorded using seven disposable, pregelled Conmed Ag/AgCl electrodes. The first ECG electrode is placed on the sternum over the first rib between the two collarbones (No. 1 in Figure 2.2). The second ECG electrode is a ground electrode and is placed over the right abdomen (No. 2 in Figure 2.2). The third ECG electrode is placed on the apex of the heart over the ninth rib at the left lateral margin of the chest approximately 3 cm under the left nipple (No. 3 in Figure 2.2). The first ICG electrode is placed next to the first ECG electrode (No. 6 in Figure 2.2). A second ICG measuring electrode is placed over the tip of the xiphoid complex of the sternum (No. 7 in Figure 2.2). The ICG current electrodes are placed on the back over cervical vertebra C4 and between thorax vertebras T8-T9 (No. 4 and No. 5 in Figure 2.2). Electrode resistance is kept below 10 KOhm by cleaning the skin with alcohol and rubbing before placing the electrodes. After placing the electrodes, the quality of the recording signals was checked on a computer, the VU-AMS device was put in a carrier bag and gird on with a belt with the lead wire connector facing up. This was important because the device also contained an accelerometer, which is sensitive to changes in vertical acceleration.

[1] Instruction manual available at http://www.vu-ams.nl/support/instruction-manual/

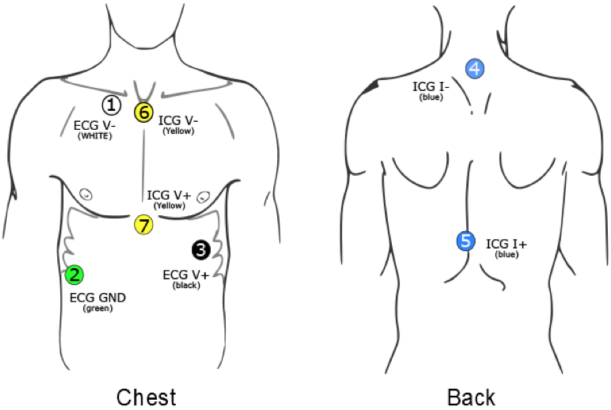

Figure 2.2. Attachment of the electrodes on the chest and back as suggested in the instruction manual from the VU University Ambulatory Monitoring System.

State Trait Anxiety Inventory (STAI)

The State Trait Anxiety Inventory (STAI) is a 40-item measure (Spielberger, Gorsuch, & Lushene, 1970). We used the Dutch, psychometrically validated version of the STAI (Van der Ploeg, Defares, & Spielberger, 1980). In this study the participants filled out the questions constituting the state or trait anxiety subscale in each pregnancy trimester. State anxiety is conceptualized as a transient emotional condition, while trait anxiety refers to differences in anxiety proneness and is seen as a personality trait. Recently, the STAI is proclaimed as the best instrument to assess prenatal anxiety, in particular the trait anxiety subscale (Nast, Bolten, Meinlschmidt, & Hellhammer, 2013). The subscales contain each 20 items scored from 1 to 4 and with a reliability coefficient, Cronbach's alphas of 0.92 (first and third trimester) in our sample for state anxiety and a Cronbach's alpha of 0.75 for trait anxiety (second trimester).

Edinburgh Depression Scale (EDS)

The Edinburgh Depression Scale (EDS) (Cox, Holden, & Sagovsky, 1987) is a 10 item self-report measure designed to screen women for symptoms of depression and emotional distress during pregnancy and the postnatal period. It is widely used for postpartum depression screening. Cut-offs used are between 11 and 13 (Harris, Huckle, Thomas, Johns, & Fung, 1989; Murray & Carothers, 1990), however during pregnancy a cut-off of 11 during the first pregnancy trimester and a cut-off of 10 during the second and third trimester of pregnancy are sometimes advised (Bergink et al., 2011). The best currently available instrument to measure prenatal depression is the EDS (Nast et al., 2013). Women indicate at the beginning (<15 weeks of gestation) and the end (31-37 weeks of gestation) of pregnancy how they felt during the last seven days. The 10 items like “I have felt sad or miserable” are scored on a 4-point Likert scale. The reliability and validity of the original and Dutch versions are adequate (Nyklíček, Scherders, & Pop, 2004). In our sample the internal consistency is adequate during the first ($\alpha = .82$) and third ($\alpha = .85$) trimester of pregnancy.

Mini-International Neuropsychiatric Interview (MINI)

Current and lifetime maternal psychopathology was assessed using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan & Lecrubier, 2010). The MINI is a structured interview based on Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria. The pregnant women were interviewed between week 15 and 22 of gestation. Lifetime anxiety disorders included panic disorder, agoraphobia, generalized anxiety disorder, social phobia, obsessive-compulsive disorder and posttraumatic stress disorder. Seventeen women had only one of these anxiety diagnoses, while five women had two of them. Nine women had remitted comorbid depression and five women had a remitted comorbid eating disorder.

Freiburg Mindfulness Inventory

Between 15 and 22 weeks of gestation women filled out the Freiburg Mindfulness Inventory - short form (FMI-s) to assess self-reported mindfulness (Walach, Buchheld, Buttenmüller, Kleinknecht, & Schmidt, 2006). It consists of 14 items covering the central aspects of mindfulness and the scale is sensitive to change, so it can be used in subjects without previous meditation experience. The items are scored on 4-point scales ranging from “Rarely” to “Almost Always”. Two examples of items are “I feel connected to my experience in the here-and-now” and “I accept unpleasant experiences”. The short version has an adequate internal consistency, in the original ($\alpha = .86$) and Dutch ($\alpha = .79$) versions (Klaassen, Nyklíček, Traa, & De Nijs, 2012) as well as in this study ($\alpha = .85$).

2.4.2 Data related to the offspring

Cardiovascular measurements

The infants their ECG and EEG were simultaneously recorded during an auditory task in our lab at Tilburg University with a sampling rate of 512 Hz. BioSemi FLAT Active electrodes were used and placed on the thorax of the offspring. The flat shape makes them ideal for body surface applications and they have a sintered Ag-AgCl electrode pallet providing very low noise, low offset voltages and very stable DC performance. More details about the device can be found on the manufacturer's website (www.biosemi.nl).

Ages and Stages Questionnaires - Social-Emotional (ASQ-SE)

Mothers filled out the Ages and Stages Questionnaires - Social-Emotional (ASQ-SE) (Squires, Bricker, & Twombly, 2002). It measures social-emotional problems, behavioral problems, and social competencies on a three-point Likert scale. It is a parent reported screening instrument for infants, which has an adequate internal consistency ($\alpha = .69$) and consists of 5 subscales. The subscales of self-regulation (5 items), communication (2 items), affect (2 items), interaction with people (3 items) and adaptive functioning (6 items) represent the child's ability or willingness to calm or settle down or adjust to physiological or environmental conditions or stimulation; to respond to or initiate verbal or nonverbal signals to indicate feelings, affective, or internal states; to demonstrate his or her own feelings and empathy for others; to respond to or initiate social responses to parents, other adults, and peers; and to cope with physiological needs (e.g., sleeping, eating, elimination, safety), respectively. A total score above 45 indicates in clinical use of this questionnaire that further social or psychological evaluation is needed.

Laboratory Temperament Assessment Battery (Lab-TAB)

The Laboratory Temperament Assessment Battery (Lab-TAB) (Locomotor Version) (Goldsmith & Rothbart, 1999) is a leading observational measure of childhood temperament, with considerable support for its validity and clinical value (e.g., Aksan and Kochanska (2004)). We used one part of this battery, namely the unpredictable mechanical toy paradigm from the fear subscale. A robotic dog was presented to the child, the dog was barking and walking toward the child as its eyes, mouth, and head moved. Indicators of fear were assessed for the first 20 seconds from facial expression, body posture, vocalizations, and escape behavior. A researcher blind to maternal data rated the recorded videotapes by using the standard scoring procedures. A composite fear score was calculated based on the fear indicators (Bergman, Sarkar, O'Conner, Modi, & Glover, 2007).

Birth outcomes

Data about the offspring's sex, birth weight and exact date of birth were retrieved from medical records, allowing us to calculate the gestation length and the children's age. Based on these calculations it was possible to determine the appropriate timing to invite mothers and their infant to our babylab (at the age of 2-4 and 9-10 months old). The gestation length was calculated as the number of weeks between the first day of the last period and the day of birth.

2.4.3 Autonomic nervous system functioning

ECG Preprocessing

The primary step in processing ECG recordings is to reliably identify QRS complexes in the signal (see Figure 2.3)[2]. A common algorithm to detect QRS waveforms is the Pan-Tompkins algorithm (Pan & Tompkins, 1985). This technique identifies complexes on the basis of slope, amplitude and width. Another recognized method is based on the Hilbert transform. The data that underlies this dissertation is processed using the Hilbert transform-based algorithm (Benitez, Gaydecki, Zaidi, & Fitzpatrick, 2001). The latter algorithm has the advantage that it performs slightly better in the presence of severe noise contamination (Jones, 2006). Potentially erroneous beat detections were identified on the basis of sudden changes in inter-beat intervals (i.e. RR intervals), which most often occur in case of ectopic beats or errors made by the beat identification algorithm (Berntson, Quigley, Jang, & Boysen, 1990). All RR intervals constitute a new signal, commonly referred to as the RR interval time series, and is often graphically represented in a RR tachogram. The Y-axis of a RR tachogram denotes the length of the RR intervals; the X-axis represents the time at which the heart beats. All HRV measures are based on the RR interval time series. As RR intervals are sometimes also called normal-to-normal (NN) intervals, both terms are used in this dissertation.

HRV measures

Measures for HRV were standardized by the Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology (1996). There are two main groups of HRV measures: time domain and frequency domain HRV measures.

Time domain measures Simple time-domain variables that can be calculated include the mean NN interval, the mean HR and the difference between the longest and shortest NN interval. More complex time-domain measures are based on statistical properties of the RR tachogram. Fundamentally, these measures may be divided into two classes, (1) those derived from direct measurements of the NN

[2] Figure 2.3 is reproduced from the PhD thesis entitled *Prenatal development and later neuroendocrine control of cardiovascular function: testing the stress hypothesis* (Jones, 2006) with permission from Dr. Alexander Jones.

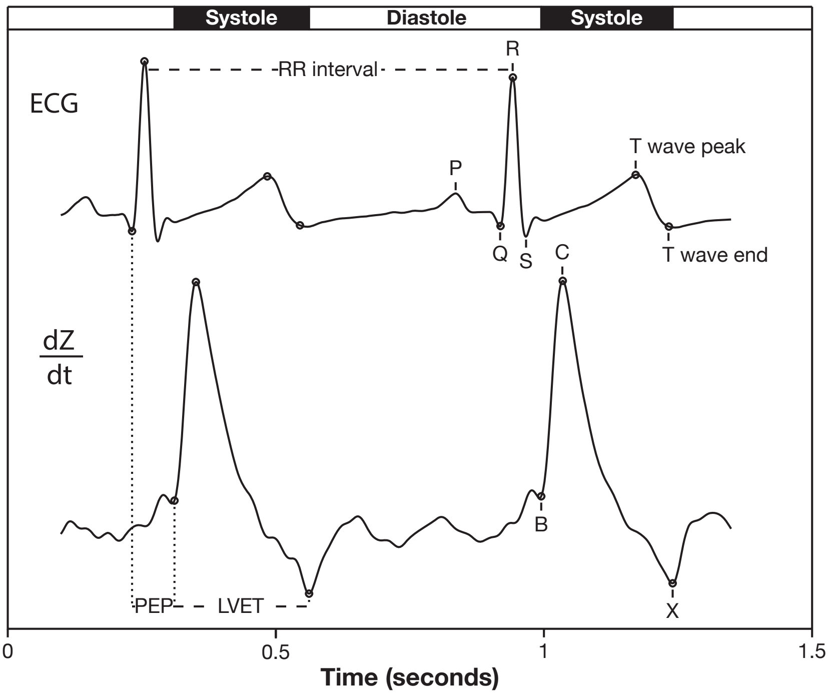

Figure 2.3. A representative recording of the electrocardiogram (ECG) and first time-derivative of the impedance cardiogram (dZ/dt) for two heart beats. Following convention, impedance is shown such that a decrease in impedance results in a greater y-axis magnitude. The cardiac cycle and the derivation of cardiac time intervals - RR interval, pre-ejection period (PEP) and left ventricular ejection time (LVET) - are indicated. Hollow circles indicate fiducial points on both signals identified automatically by computer. In addition to the well-known PQRST sequence of the ECG, major time points on dZ/dt are marked. These include the B-point, which coincides with aortic valve opening, the X-point which coincides with closure of the aortic valve, and the C-point which marks the maximum rate of decline in thoracic impedance which coincides with peak systolic ejection rate

intervals or instantaneous heart rate, and (2) those derived from the differences between RR intervals. These measures may be derived from analysis of the total ECG recording or may be calculated using smaller segments of the complete recording period. The latter method allows comparison of HRV to be made during varying activities, e.g. rest, sleep, etc., which formed the basis for the study on the 24h recordings (see Chapter 6).

The most common time domain measures are:

- Mean NN is the average duration or length of the NN intervals (in milliseconds) over the defined period (e.g. total ECG recording or a specific segment). Notably, this measure does not really describe the variations in heart rate. The mean HR (in beats per minute) can be derived by dividing 60 x 1000 by the mean NN.

- diffNN is the difference between the longest and shortest NN interval.
- SDNN is the standard deviation of the NN interval (SDNN), i.e. the square root of variance. Since variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording. As the total variance of HRV increases with the length of recording (Saul, Albrecht, Berger, & Cohen, 1988), SDNN depends on the length of recording period. Thus, in practice, it is inappropriate to compare SDNN measures obtained from recordings of different durations. Therefore, it is recommended to calculate SDNN for short-term 5 minutes recordings or 24h long-term recordings.
- pNN50 is the percentage of successive NN intervals that differ more than 50 ms from the previous interval.
- RMSSD is the square root of the mean squared differences of successive NN intervals. This measure can be considered giving an estimate of short-term components of HRV.
- SDSD is the standard deviation of differences between adjacent NN intervals.

pNN50 and RMSSD are highly correlated measurement of short-term variation in heart rate, which are considered markers of parasympathetic activity. The RMSSD method is preferred to pNN50 because it has better statistical properties (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). High Frequency (HF) HRV is considered an approximate frequency domain correlate.

Frequency domain measures Already in the early 18th century, physiologists have documented the existence of regular oscillations of HR. Initial findings were mainly focused on HR oscillations occurring at the respiratory rate. However, since the widespread use of power spectrum analysis (frequency analysis) using computers there is strong evidence that additional oscillations at frequencies slower than the respiratory rate provide meaningful information about cardiovascular control (Jones, 2006). Power spectral density (PSD) analysis provides the basic information on how power (variance) distributes as a function of frequency (Task Force of the European

Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). Although there are various methods to compute the PSD from a tachogram (e.g. fast Fourier transform (FFT) or autoregressive (AR) modeling), in practice, all of these methods produce comparable results with most physiological data (Jones, 2006).

A number of frequency domain HRV measures can be considered by distinguishing specific spectral bands:

- High frequency (HF), from 0.15 Hz to 0.4 Hz (0.15 Hz to 1.04 Hz for infants)
- Low frequency (LF), from 0.04 Hz to 0.15 Hz
- Very low frequency (VLF), from 0.003 Hz to 0.04 Hz
- Ultra low frequency (ULF), below 0.003 Hz

HF, LF and VLF are appropriate HRV measures for both short-term and long-term (e.g. 24h) recordings, whereas ULF requires a long-term recording.

Parasympathetic activity is the main contributor to the HF component. LF HRV reflects a combination of both sympathetic and parasympathetic activity. Consequently, the LF/HF ratio is considered by some scientists to mirror sympathovagal balance or to reflect the sympathetic modulations (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). However, a detailed investigation of the association between LF/HF and PEP, which is a better measure of sympathetic activity, showed no significant correlations between the two measures. Moreover, only PEP revealed the expected reciprocal relation to HF power, a proxy measure of cardiac vagal control, and it co-varies more systematically with the expected changes in cardiac sympathetic control in response to mental and physical stressors (Goedhart, Willemsen, Houtveen, Boomsma, & De Geus, 2008).

Impedance cardiography and pre-ejection period (PEP)

The PEP is a measure of sympathetic activity and relies on thoracic impedance cardiography, which aims to record the change in impedance over the thorax. These impedance changes are correlated to the aortic blood flow. Other ICG parameters such as cardiac output, stroke volume and systolic time ratio were considered, but the measurements were insufficiently accurate due to the electrode configuration used in the longterm ICG recording. These measurements might have been more accurate with a band electrode configuration, but this was not tolerable for a 24-hour recording.

For the calculation of PEP the ICG dZ/dt signal (first derivative) was ensemble averaged from consecutive cardiac cycles and synchronized with the ECG signal. Once R peaks in the ECG were detected (see Section 2.4.3), the ECG R onset (Q point), which marks the depolarization of the ventricles, was automatically detected by searching it in a window between 60ms before the R peak and the R peak itself. The B point in the impedance cardiogram (ICG), which represents the opening of the aortic valve, was determined as the 15% response point of the negative peak of the dZ/dt waveform (dZ/dt_{min}) from the baseline (Ono et al., 2004). PEP was then calculated as the interval between R onset (Q point) in the ECG and the B-point in the ICG dZ/dt signal (see Figure 2.3).

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3

Anxious women do not show the expected decrease in cardiovascular stress responsiveness as pregnancy advances

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Abstract

Altered stress responsiveness is a risk factor for mental and physical illness. Characterization of these risks for mothers and offspring requires greater understanding of how stress reactivity and recovery are influenced by pregnancy. Pregnant women were presented repeatedly with mental arithmetic stress tasks in the first and third pregnancy trimester and reported their trait anxiety using the State Trait Anxiety Inventory. Cardiovascular stress reactivity in late pregnancy was lower than reactivity in the first pregnancy trimester (Heart Rate (HR): $t(197) = 4.98, p < .001$; Root Mean Square of Successive Differences (RMSSD): $t(201) = -2.03, p = .04$; High Frequency Heart Rate Variability (HF HRV): $t(196) = -2.09, p = .04$). Less attenuation of stress reactivity occurred in more anxious women (HR: $b = 0.15, SE = 0.06, p = .008$; RMSSD: $b = -0.35, SE = 0.12, p = .004$; HF: $b = -10.97, SE = 4.79, p = .02$). Higher (i.e., less dampened) stress reactivity through pregnancy may pose long-term risks for anxious women and their offspring. Follow-up studies are required to determine these risks.

Highlights:

- Stress responsiveness usually decreases over the course of pregnancy
- Anxious women do not show expected decrease of stress responses through pregnancy
- Reduced dampening of stress responses pose risks for pregnant women and offspring

3.1 Introduction

Pregnant women undergo marked changes in maternal cardiovascular function during pregnancy, such as increased basal stroke volume (SV) and heart rate (HR) (Abbas et al., 2005; Silversides & Colman, 2007). The autonomic nervous system (ANS) plays a central role in these changes. Basal ANS activity is shifted towards higher sympathetic (e.g. shorter Pre-Ejection Period (PEP) and lower vagal modulation (e.g. reduced Heart Rate Variability, HRV) over the course of pregnancy (Ekholm & Erkkola, 1996; Kuo et al., 2000). These changes go along with attenuated cardiovascular responses to stress, as HR and blood pressure reactivity are typically attenuated as pregnancy progresses (DiPietro et al., 2003; Entringer et al., 2010). Studies of ANS stress responsiveness during pregnancy are lacking but one study found a decrease in HRV responsiveness with advancing pregnancy, although this finding had marginal statistical significance (Klinkenberg et al., 2009).

Considering these marked physiological adaptations, a number of scientists have conceptualized pregnancy as a stressor (Christian, 2012; Williams, 2003), which potentially amplifies the effect of underlying mental disorders or cardiovascular risk factors (Magnussen, Vatten, Smith, & Romundstad, 2009). For example, women who have peripartum depression or anxiety are more at risk for these conditions outside of pregnancy (Heron et al., 2004; Kammerer et al., 2009). Moreover, women with gestational hypertension have greater risks of chronic hypertension (Cuevas & Germain, 2011; Magnussen et al., 2009; Young, Levine, & Karumanchi, 2010), for which anxiety and depression are also strong independent risk factors (Everson-Rose & Lewis, 2005; Yan et al., 2003).

Research on predictors and consequences of stress responsiveness during pregnancy is limited (Christian, 2012; de Weerth & Buitelaar, 2005), although mental and emotional states such as depression and anxiety have been linked to stress responsiveness in pregnant women. Findings in this area have been inconsistent. Some studies suggest that more anxious pregnant women have lower HR and blood pressure reactivity (Monk et al., 2000; Saisto et al., 2004). In another study, no association between maternal anxiety during pregnancy and blood pressure or HR reactivity to a psychological stress test was found (Monk et al., 2003). These diverse findings might be explained by the use of different stress protocols (Monk et al., 2003).

The aims of this study were (a) to determine the typical autonomic responsiveness (i.e. reactivity and recovery) through different pregnancy trimesters because these are not currently known, and (b) to test the hypothesis that women with higher anxiety have altered autonomic stress responsiveness that could confer risk of somatic or mental disease and/or adverse fetus development.

3.2 Methods

3.2.1 Participants

The prenatal early life stress (PELS) study is a longitudinal study and focuses on associations between prenatal stress risk factors, birth outcomes, altered mother's physiology and child's psychophysiology and neurodevelopment. The study protocol was approved by the ethics committee of the St. Elisabeth hospital, Tilburg, the Netherlands. Pregnant women were recruited from a hospital and midwiferies around Tilburg. They filled out questionnaires about their emotions and their ECG was recorded during each pregnancy trimester. After birth psychophysiological measurements of the children and mothers took place. All participants and their partners provided written informed consent. None of the participants were under treatment for a current mental disorder, or used cardiovascular medications or antidepressants.

One hundred and seventy women completed stress tasks during the first (8th-14th week of gestation, N = 133) and/or third pregnancy trimester (31st-37th pregnancy week, N = 138) and 157 of these 170 participants filled out a standardized anxiety self-report questionnaires at 15-22 weeks of pregnancy.

3.2.2 Materials

Relaxation and Stress Tasks

In the first and third pregnancy trimesters, each mother undertook a 25-minute task consisting of five testing phases, lasting for 5 minutes each. Stress was induced in the second and fourth phase, with the remainder being relaxation phases. Participants viewed peaceful pictures and listened to restful music during the relaxation phases (Taelman et al., 2011). During the stress phases, participants were asked to solve complex mental arithmetic problems, involving five mathematical operations on 2-3 digit numbers without verbalization (e.g., $361 + 17/242 + 13$). They were asked to choose the correct answer from three possibilities presented by a computer. Feedback on the task was given after completion of the last phase (Vlemincx et al., 2011).

ECG and ICG Recording

Maternal Electrocardiography (ECG) and Impedance cardiography (ICG) was recorded with the Vrije Universiteit Ambulatory Monitoring system (VU-AMS) during the stress task using seven Ag/AgCl electrodes placed according to the VU-AMS configuration guidelines (Goedhart, van der Sluis, Houtveen, Willemsen, & de Geus, 2007). The skin was cleaned with alcohol to keep electrode resistance low. Cardiovascular measurements were determined for the three relaxation phases and two stress phases, each lasting 5 minutes. The first relaxation phase was used as baseline measure. Cardiovascular reactivity to the stress phase was calculated by subtracting the cardiovascular measurements of each of the stress phases with the level of the previous relaxation phase. Cardiovascular recovery from a stressor presentation was derived from the subtraction between cardiovascular measurements in the relaxation phase that follows the stressor presentation and baseline cardiovascular measurements (i.e. in the first relaxation phase) (Stewart & France, 2001).

The State Trait Anxiety Inventory (STAI)

In the second pregnancy trimester the participants filled out the questions constituting the trait anxiety subscale of the Dutch, psychometrically validated version of the State Trait Anxiety Inventory (STAI) (Van der Ploeg et al., 1980). The trait subscale has been identified recently as the best instrument to assess general maternal anxiety during pregnancy (Nast et al., 2013). Trait anxiety refers to differences in anxiety proneness and is seen as a personality trait. This subscale contains 20 items scored from 1 to 4 and has a reliability coefficient (Cronbach's alpha) of 0.75 in our sample.

3.2.3 Data Processing and Analyses

ECG data were processed using custom software written in Matlab R2012b (Mathworks, Natick, USA) to obtain indices of parasympathetic and sympathetic ANS activity, according to published standards (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). These measures were HR, the parasympathetic measures root mean square of successive differences (RMSSD) HRV and high frequency heart rate variability (HF HRV), and the sympathetic measure pre-ejection period (PEP). ECG beat detection was carried out with the Hilbert transform algorithm (Benitez et al., 2001). Potentially erroneous beat detections were identified and screened using a standard approach (Berntson et al., 1990). Variables that were right-skewed were transformed to normality. Data of four mothers were excluded due to extreme HRV values; visual inspection of the ECGs suggested that these outliers were due to cardiac arrhythmias. For the calculation of PEP the ICG dZ/dt signal (first derivative) was ensemble averaged from consecutive cardiac cycles and synchronized with the ECG signal. PEP, which is an indicator of sympathetic activity, was calculated from the ICG as the interval between ECG's R-wave onset and the ICG's B-point. The B-point, which represents the opening of the aortic valve, was determined as the 15% response point of the negative peak of the dZ/dt waveform (dZ/dt_{min}) from the baseline (Ono et al., 2004).

3.2.4 Statistical analysis

All analyses were conducted using Stata 12.1. Repeated measures ANOVA was conducted to validate that the mental arithmetic task evoked significant responses in HR, HRV and PEP. ANOVA was also used to determine HR, HRV and PEP differences between pregnancy trimesters.

Multilevel (i.e. pregnancy trimester and first/second stressor presentation) regression analyses were conducted to examine the potential association between trait anxiety and cardiovascular responsiveness (reactivity and recovery) during pregnancy. Two-way and three-way interaction effects were assessed to test whether reactivity and recovery in HR, HRV and PEP differ across levels of anxiety, pregnancy trimesters and first and second presentation of the stressor.

All regression models were adjusted for levels of HR, HRV or PEP in the first phase of the mental task (baseline), as well as for common confounders such as age and pre-pregnancy BMI (Vallejo et al., 2005).

3.3 Results

Characteristics of mothers are shown in Table 3.1.

3.3.1 HR, HRV and PEP differences across tasks phases and pregnancy trimesters

Repeated measures ANOVA (pregnancy trimester × task phase) were executed to validate that the mental arithmetic task evoked significant responses and indicated significant differences between the five phases of the mental arithmetic task for mothers' HR and HRV (HR: $F(4,161) = 49.02, p < .001$; RMSSD: $F(4,161) = 20.82, p < .001$; HF: $F(4,161) = 11.39, p < .001$) but not for PEP ($p > .1$). Therefore, no PEP reactivity and recovery measures were calculated. HR, HRV and PEP were significantly different between pregnancy trimesters (HR: $F(1,161) = 581.87, p < .001$; RMSSD: $F(1,161) = 417.02, p < .001$; HF: $F(1,161) = 362.44, p < .001$; PEP: $F(1,157) = 219.53, p < .001$).

Table 3.1
Descriptive statistics for variables related to mothers. Observed differences between the first and third pregnancy trimester were tested using paired samples t tests.

	M	SD
Trait anxiety	36.67	5.29
BMI before pregnancy (kg/m^2)	24.19	4.09

	1st trimester			3rd trimester			
	n	M	(SD)	n	M	(SD)	p
Age ($years$)	125	32.58	(4.26)	129	32.77	(4.33)	
Baseline HR (bpm)	125	80.25	(9.45)	129	89.29	(10.01)	$< .001$
Baseline RMSSD ($ln\,ms$)	125	3.45	(0.57)	129	3.03	(0.59)	$< .001$
Baseline HF ($ln\,ms^2$)	124	6.07	(1.08)	128	5.30	(1.21)	$< .001$
Baseline PEP (ms)	122	74.36	(20.51)	124	60.95	(20.64)	$< .001$
HR reactivity (bpm)	125	3.90	(4.00)	129	2.27	(3.88)	$< .001$
RMSSD reactivity (ms)	125	−3.84	(9.16)	129	−2.09	(8.70)	0.04
HF reactivity (ms^2)	124	−105.00	(454.53)	128	−33.05	(197.26)	0.04
HR recovery (bpm)	125	0.37	(4.03)	129	−0.65	(4.07)	0.01
RMSSD recovery (ms)	125	−1.23	(7.99)	129	−0.56	(7.06)	0.38
HF recovery (ms^2)	124	−63.82	(421.54)	128	−15.78	(210.71)	0.14

3.3.2 Cardiovascular reactivity and recovery

Differences in reactivity and recovery between the first and third pregnancy trimester were tested using paired samples t tests (see Table 3.1). Cardiovascular stress reactivity in late pregnancy was lower than reactivity in the first pregnancy trimester (HR: $t(197) = 4.98, p < .001$; RMSSD: $t(201) = -2.03, p = .04$; HF: $t(196) = -2.09, p = .04$).

Furthermore, HR recovery, but not HRV recovery, was greater in the third pregnancy trimester compared to the first trimester ($t(201) = 2.74, p = 0.01$), indicating that the difference between HR after a stressor presentation and baseline HR was smaller in the third pregnancy trimester compared to the first trimester.

Stress presentation was significantly related to HR reactivity, indicating that HR reactivity was significantly lower during the second stressor presentation ($b = -1.67, SE = 0.28, p < .001$). Analogously, both RMSSD and HF decrease less during the second stressor presentation (RMSSD: $b = 2.51, SE = 0.59, p < .001.$; HF: $b = 94.27, SE = 23.46, p < .001$). Recovery from the second stressor presentation was significantly larger than recovery from the first stressor presentation (HR: $b = -1.27, SE = 0.26, p < .001$; RMSSD: $b = 1.27, SE = 0.54, p = .02$; HF: $b = 43.61, SE = 18.11, p = .02$), indicating that the difference between HR or HRV after the stressor presentation and baseline HR or HRV was smaller after the second presentation compared to after the first presentation.

Multilevel regression analyses showed significant two-way interactions between trait anxiety and pregnancy trimester for HR, RMSSD and HF reactivity, adjusted for baseline levels of HR, RMSSD and HF (HR: $b = 0.15, SE = 0.06, p = .008$; RMSSD: $b = -0.35, SE = 0.12, p = .004$; HF: $b = -10.97, SE = 4.79, p = .02$), indicating that in the third pregnancy trimester, compared to less anxious women, more anxious women have higher HR, RMSSD and HF responses to stress, independently of the baseline level of HR, RMSSD or HF (Table 3.2 and Figures 3.1a, 3.1b and 3.1c). Given the significant decrease in HR and HRV reactivity between the first and third pregnancy trimester, this implies that compared to less anxious women, more anxious women have less dampened HR and HRV reactivity in the third trimester compared to the first trimester[1]. There was no main effect of trait anxiety on HR or HRV reactivity ($p > .05$), indicating that in the first trimester there were no significant differences in cardiovascular reactivity between high and low anxious women. There was no main effect of trait anxiety on HR or HRV recovery ($p > .05$), nor were there significant trait anxiety × pregnancy trimester interactions for HR or HRV recovery ($p > .05$) (see Table 3.2 and Figures 3.1d, 3.1e and 3.1f).

The multilevel regression analyses showed that stressor presentation interactions with pregnancy trimester and/or trait anxiety were not significant for any reactivity or recovery measure. Nevertheless, separate single-level regression analyses showed that in the third pregnancy trimester reactivity to the second stressor presentation is higher in more anxious women (HR: $b = 0.14, SE = 0.07, p = .05$; RMSSD: $b = -0.32, SE = 0.14, p = .02$; HF: $b = 11.79, SE = 5.11, p = .02$) compared to less anxious women.

3.4 Discussion

To the best of our knowledge, this is the most detailed longitudinal study that gives insight into cardiovascular and autonomic reactivity in pregnancy. Previous studies either focused on only one pregnancy trimester, had significantly less participants, or did not include HR as well as measures of parasympathetic and sympathetic activity. HR and HRV measures, but not PEP, differed over the various phases of the mental

[1] Additional adjustment for maternal emotional distress (as measured by the Edinburgh Depression Scale, see Section 2.4.1) or maternal mindfulness (as measured by Freiburg Mindfulness Inventory, see Section 2.4.1) had no effect on the significance of these results. The two-way interaction between maternal emotional distress and pregnancy trimester for HR, RMSSD and HF reactivity was also significant (HR: $b = 0.03, SE = 0.01, p = 0.02$; RMSSD: $b = -0.08, SE = 0.03, p = 0.002$; HF: $b = -2.87, SE = .99, p = 0.004$), indicating that in the third pregnancy trimester, compared to less emotionally distressed women, more emotionally distressed women have higher HR, RMSSD and HF responses to stress. The two-way interaction between maternal mindfulness and pregnancy trimester was not significant for any cardiovascular reactivity or response measure, implying that mindfulness was not significantly associated with the change in cardiovascular responsiveness to stress across pregnancy trimesters.

Table 3.2

Multilevel regression analyses between Trait anxiety and reactivity/recovery of HR, RMSSD HRV and HF HRV (n=157)

	HR reactivity			RMSSD HRV reactivity			HF HRV reactivity		
	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*
Baseline level	−0.05	0.02	.04	−0.14	0.02	< .001	−0.28	0.03	< .001
Trait Anxiety	−0.04	0.06	.51	0.13	0.11	.25	3.04	4.14	.46
Trimester (3rd vs. 1st)	−6.85	2.16	.008	13.44	4.54	.003	397.78	178.75	.03
Trait Anxiety × Trimester	0.15	0.06	.01	−0.35	0.12	.004	−10.97	4.79	.02
Presentation of stressor (2nd vs. 1st)	−1.67	0.28	< .001	2.51	0.59	< .001	94.27	23.46	< .001
Age	−0.03	0.06	.63	0.07	0.12	.59	−0.40	4.27	.92
BMI	−0.01	0.06	.84	0.20	0.13	.12	0.61	4.36	.89
Constant	11.32	3.94	.004	−13.04	7.24	.07	−109.41	255.29	.67
	HR recovery			RMSSD HRV recovery			HF HRV recovery		
	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*
Baseline level	−0.17	0.02	< .001	−0.19	0.03	< .001	−0.22	0.02	< .001
Trait Anxiety	−0.03	0.05	.51	0.08	0.11	.49	−0.51	3.60	.89
Trimester (3rd vs. 1st)	−2.47	2.03	.22	2.24	4.14	.59	69.70	139.36	.62
Trait Anxiety × Trimester	0.08	0.05	.13	−0.09	0.11	.40	−2.90	3.74	.44
Presentation of stressor (2nd vs. 1st)	−1.27	0.26	< .001	1.26	0.54	.02	43.61	18.11	.02
Age	−0.14	0.05	.01	−0.03	0.12	.81	−1.18	3.88	.76
BMI	−0.06	0.06	.28	0.01	0.12	.91	2.00	3.94	.61
Constant	21.42	3.55	< .001	2.73	7.04	.70	73.08	225.08	.75

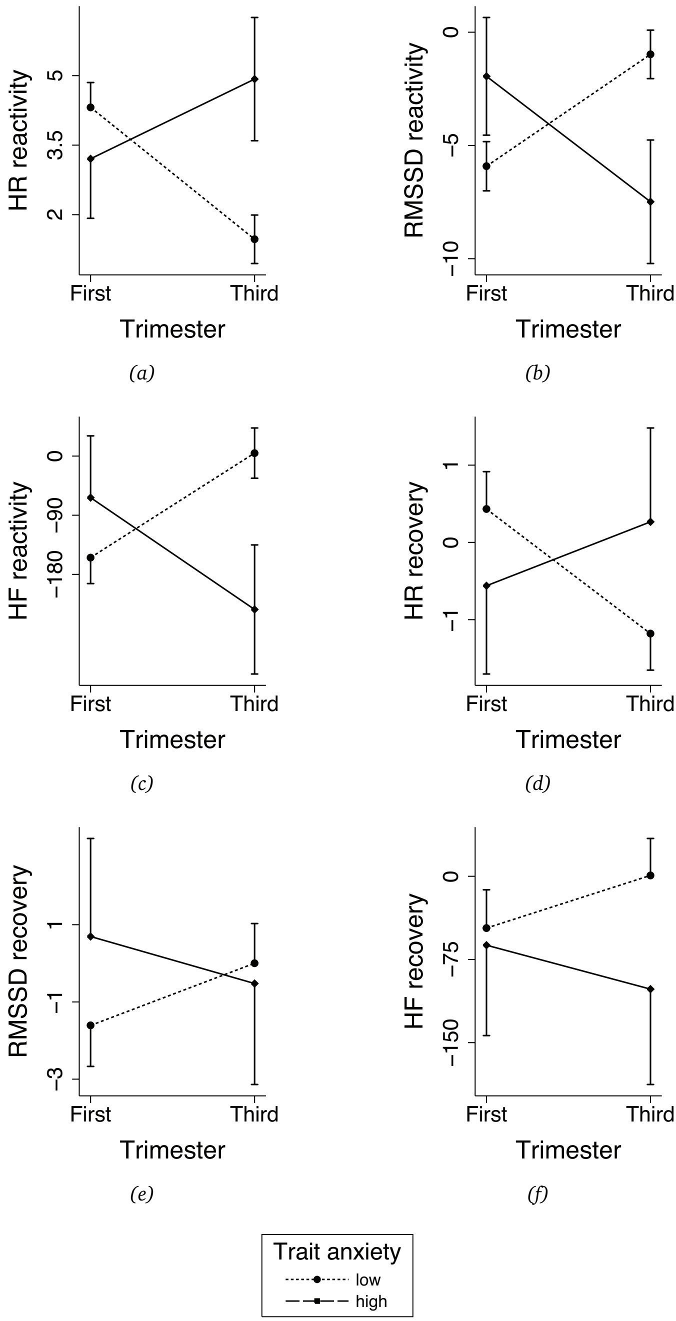

Figure 3.1. The association between trait anxiety and cardiovascular reactivity (significant interaction) and recovery. Plots are based on estimated marginal means from multilevel regression analyses showing interactions between trait anxiety and pregnancy trimester. Minimum and maximum scores for trait anxiety in our sample were respectively 29 and 59.

arithmetic task, in the first as well as in the third trimester. Stress reactivity was lower in the third pregnancy trimester but the decrease in reactivity between early and late pregnancy was smaller in more anxious women compared to less anxious women.

Induced stress was associated with increased HR and decreased HRV in the mental arithmetic task. This is in line with previous research focusing only on the third pregnancy trimester (Klinkenberg et al., 2009). The stress task that we used did not lead to significant changes in PEP. This suggests that stress responses elicited with the mental arithmetic task are mainly reflected in a decreased parasympathetic nervous system activity but not in a significant change in sympathetic nervous system. Autonomic responses to stressful tasks vary according to the type and severity of the stressors and according to populations. Further study will be required to determine whether the lack of sympathetic responsiveness to this particular task was due to a particular robustness due to pregnancy or an insufficiently stimulating stressor.

In women without a tendency for anxiety, HR and HRV reactivity to the stress phases were significantly reduced in the third pregnancy trimester, compared to the first trimester. Previous studies presented similar results for attenuated HR and blood pressure reactivity in late pregnancy (DiPietro et al., 2003; Entringer et al., 2010). Importantly, the present study also found significantly reduced autonomic stress reactivity in the third pregnancy trimester, for which so far only marginal evidence existed (Klinkenberg et al., 2009). It must be noted that from the present study design it cannot ruled out that the attenuated stress responses in the third pregnancy trimester are (partially) the result of a habituation process, as the same stress task was already presented in the first trimester. Therefore, future studies need to include a control group consisting of non-pregnant women to assure that the current findings are not driven by habituation to the stress task. Additionally, it is also recommended to compare two independent samples of women in their first pregnancy trimester and women in their third pregnancy trimester.

The results in the present study indicated significant interactions between pregnancy trimester and mother's trait anxiety on HR and HRV reactivity. These interactions can be interpreted in two ways. Firstly, it suggests that more anxious mothers have less dampened stress reactivity in late pregnancy. From previous studies it is known that cardiovascular stress reactivity is generally attenuated in late pregnancy (DiPietro et al., 2003; Entringer et al., 2010). Besides the attenuation in cardiovascular responses, women in late pregnancy also have reduced hormonal (Kammerer et al., 2002) and psychological reactivity (Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001). It is thought that the stress sensitivity is dampened as pregnancy advances to protect the growing fetus (Christian, 2012). Attenuated stress reactivity prevents mother and fetus from excessive exposure to stress hormones and marked alterations in cardiovascular parameters such as uterine blood flow. Elevated cardiovascular reactivity to stress may contribute to vasoconstriction, which is thought to alter uteroplacental blood flow, causing subsequent oxygen and nutrition reduction to the fetus and thereby affecting fetal growth (Copper et al., 1996; McCubbin et al., 1996) and nervous system development (Sjöström, Valentin, Thelin, & Marsál, 1997). Additionally, there is evidence that abnormal stress responsiveness in pregnant women predicts risk of adverse cardiovascular outcomes in pregnancy (Christian, 2012) or later in life (Chida & Steptoe, 2010; Schwartz et al., 2003; Steptoe & Kivimäki, 2013). Increased reactivity during pregnancy has also been linked to greater risks of chronic hypertension, maternal cardiovascular morbidity and neonatal morbidity/mortality (Cuevas & Germain, 2011; Magnussen et al., 2009; Young et al., 2010).

Secondly, the interaction between pregnancy trimester and mother's trait anxiety can also be interpreted as an indication that the relationship between trait anxiety and acute stress reactivity only exists in the third pregnancy trimester. More specifically, the results indicate that in the third pregnancy trimester, more anxious women tend to have greater HR and HRV reactivity to stress than less anxious women have in the third pregnancy trimester. This is in contrast to previous studies with pregnant women, which either found no association between anxiety and HR or blood pressure response (Monk et al., 2003), or that anxiety is negatively associated with HR and blood pressure responses to stress (Monk et al., 2000; Saisto et al., 2004). These inconsistent findings might be the consequence of different stress tasks being used in those studies. Furthermore, those studies should be interpreted cautiously given their small sample sizes (i.e. 32, 17 and 40 mothers). Moreover, studies with non-pregnant participants generally suggest that high anxiety is associated with exaggerated cardiovascular responses (i.e. HR and blood pressure) (Gramer & Saria, 2007; Pointer et al., 2012). A possible reason why anxiety is only related to stress responsiveness in the third pregnancy might be that the physiological demands of pregnancy are large enough only in late pregnancy to reveal significant differences in reactivity between more anxious and less anxious women. However, this possibility would be inconsistent with the general finding of such an association in non-pregnant individuals.

We also found that in the third pregnancy trimester, more anxious women react more strongly to the second presentation of the stressor than less anxious women. Similar patterns of individual differences in cardiovascular response adaptation have been found in non-pregnant people with neuroticism (Hughes, Howard, James, &

Higgins, 2011), which is fundamentally related to trait anxiety (Reiss, 1997). It is important to mention that our findings with respect to the second presentation of the stressor were based on single-level regression analyses. Future research needs to further address the role of anxiety during pregnancy in responses to repeated stressors.

Both HR and HRV reactivity differed significantly between pregnancy trimesters but only HR recovery and not HRV recovery was significantly different between trimesters. Furthermore, no significant interactions between trait anxiety and pregnancy trimester with HR or HRV recovery were found. The stress task used in the present study has five-minute relaxation phases for each participant. As individuals can vary greatly in the speed of physiological recovery from a stressor, five minutes might be not enough to truly measure physiological recovery (de Weerth & Buitelaar, 2005). A better approach may be to observe how long it takes to return to baseline HR and HRV levels after termination of a stressor. Such a recovery measure might reveal more inter-individual differences, which might have stronger relationships to anxiety. Further research is required to investigate if the present findings can be confirmed or opposed when recovery is measured in a different way.

Our study has a number of strengths: compared to previous studies, we studied a relatively large population of pregnant women, included data from the first pregnancy trimester, presented stressors more than once and used autonomic measures such as HRV and PEP. Finally, the present study measured anxiety using the STAI, which is generally acknowledged as the best instrument to assess maternal anxiety during pregnancy, and in particular the trait anxiety subscale (Nast et al., 2013).

In summary, this study provides insight into how autonomic control evolves throughout pregnancy. Moreover, it offers good evidence that trait anxiety is associated with higher cardiovascular reactivity in late pregnancy (i.e. less dampening than typically occurs). Existing evidence suggests that high reactivity in late pregnancy is linked to worse outcomes for mothers and their offspring. Therefore, further research should test whether anxiety-reducing interventions in anxiety-prone pregnant women reduce these risks.

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4

Pregnant mothers with resolved anxiety disorders and their offspring have reduced heart rate variability: Implications for the health of children

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Abstract

Objective: Active anxiety disorders have lasting detrimental effects on pregnant mothers and their offspring but it is unknown if historical, non-active, maternal anxiety disorders have similar effects. Anxiety-related conditions, such as reduced autonomic cardiac control, indicated by reduced heart rate variability (HRV) could persist despite disorder resolution, with long-term health implications for mothers and children. The objective in this study is to test the hypotheses that pregnant mothers with a history of, but not current anxiety and their children have low HRV, predicting anxiety-like offspring temperaments.

Methods: The participants in this case-control study consist of 56 women during their first trimester and their offspring (15 male, 29 female). Women had a history of an anxiety disorder (n=22) or no psychopathology (n=34) determined using the Mini-International Neuropsychiatric Interview. The main outcome measures were indices of autonomic cardiac control including root mean square of successive differences (RMSSD) and high frequency (HF) variability. Children's fearfulness was also assessed using the Laboratory Temperament Assessment Battery (Lab-TAB)-Locomotor Version.

Results: HRV was lower in women and children in the past anxiety group compared to controls. HRV measures for mothers and children were positively correlated in the anxiety group only. In all children, low HRV measures at 2-4 months were associated with a higher chance of fearful behavior at 9-10 months.

Conclusions: Pregnant women with previous but not current anxiety and their children have low HRV. Children with low HRV tend to show more fearfulness. These findings have implications for identifying children at risk of anxiety disorders and point to possible underlying mechanisms of child psychopathology.

Keywords: anxiety disorder; heart rate variability; fearfulness; pregnancy; offspring psychopathology.

4.1 Introduction

More than one third of adults have a history of anxiety disorders and women are twice as likely to experience such disorders (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2006). Though the presence of anxiety disorders during pregnancy has an impact on both mothers and their offspring (Alder, Fink, Bitzer, Hösli, & Holzgreve, 2007; Ross & McLean, 2006), it is not known if a resolved anxiety disorder is similarly important. Mood and anxiety disorders are associated with abnormal autonomic nervous system (ANS) function, indexed by reduced heart rate variability (HRV). Reduced HRV is a marker of physical and mental ill health and has been identified as a risk factor for various diseases, including cardiovascular disease (CVD) and mortality (Kemp & Quintana, 2013; Kemp, Quintana, Felmingham, Matthews, & Jelinek, 2012; Kemp et al., 2010; Thayer & Lane, 2007; Thayer et al., 2010). If autonomic abnormalities (e.g., low HRV) also persist in pregnant women who have had an anxiety disorder, these could have important, under-appreciated health risks in both, mothers and their children. However, it is not known whether past mental disorders could affect pregnant women and their children via reduced HRV, or other uncharacterized mechanisms. HRV of the developing fetus is altered in the offspring of mothers with a number of psychiatric conditions, including anxiety disorders, and these differences persist postnatally (Dierckx et al., 2009; DiPietro et al., 2000; Monk et al., 2004). This suggests that the development of the ANS may be susceptible to the influence of maternal characteristics, with potential long-term consequences for the health of the offspring. Therefore, improved understanding of the relationship between maternal psychiatric illness, HRV and infant physical and mental wellbeing is an important goal.

In this study, we aimed to assess the physiological correlates of past maternal anxiety disorders in both pregnant mothers and their offspring, focusing on HRV as a potential shared risk factor for ill health and abnormal autonomic function. To optimize our study, HRV was measured at rest and during mental stress. Although associations with HRV are apparent at rest (Blom et al., 2010; Pittig, Arch, Lam, & Craske, 2013), they are often more evident when participants are exposed to stress (Hanson, Outhred, Brunoni, Malhi, & Kemp, 2013), and anxious individuals may be particularly sensitive to such stress exposure (Friedman & Thayer, 1998; Thayer, Friedman, Borkovec, Johnsen, & Molina, 2003). We assessed HRV in mothers with a remitted anxiety disorder and healthy controls in their first trimester of pregnancy, a critical period of fetal development. We then assessed the HRV of their offspring at 2-4 months of age and the child's temperament at 9-10 months.

We hypothesized the following: 1) mothers with a past, but no current, anxiety disorder will demonstrate abnormal autonomic physiology (particularly low HRV); 2) these differences will be more apparent under stress than under rest; 3) maternal HRV will be positively correlated with offspring HRV; 4) offspring of mothers with a history of an anxiety disorder will display abnormal autonomic physiology (low HRV) and; 5) this will be associated with temperamental disturbances in the offspring that might be considered predictive of later psychopathology.

4.2 Materials and Methods

4.2.1 Participants

Participants in the present study were recruited as part of the Prenatal Early Life Stress (PELS) study by midwives and hospitals, between May 2009 and July 2010. The overarching goal of PELS is to study the associations between prenatal risk factors, birth outcome and altered child psychophysiology and neurodevelopment. For the purposes of the present study, a group of women with a past, but no current, anxiety disorder (PAD group) and a healthy control group without any history of psychopathology were defined. No participants with a current diagnosis of an anxiety disorder were available from the PELS study. PAD included panic disorder, agoraphobia, generalized anxiety disorder, social phobia, obsessive-compulsive disorder and posttraumatic stress disorder. In the PAD group, 17 women had only one of these anxiety diagnoses, while five women had two of them. Nine women also had a history of comorbid depression and five had a history of a comorbid eating disorder. None of the participants were under treatment for a current mental disorder, used cardiovascular medications or antidepressants. Loss to follow-up or attrition due to data loss in the groups is shown in Figure 4.1.

4.2.2 Ethics Statement

The Medical Ethics Committee of the St. Elisabeth hospital in Tilburg (the Netherlands) approved the study. All participants provided informed, written consent for themselves and their children.

4.2.3 Procedures

Psychological and Psychiatric Assessment of Mothers

The Mini-International Neuropsychiatric Interview 6.0 (Sheehan & Lecrubier, 2010) was administered within the second pregnancy trimester to assess past maternal psychopathology. This is a structured interview based on Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria. In the first pregnancy

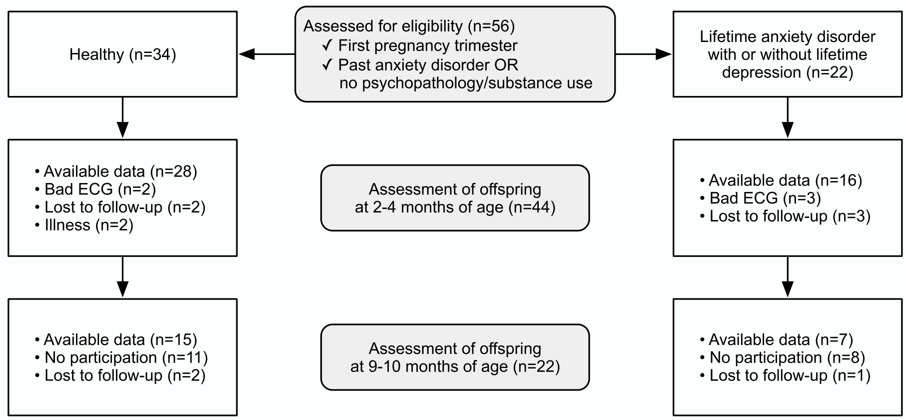

Figure 4.1. Consort diagram

trimester women completed the state anxiety subscale of the State Trait Anxiety Inventory (Van der Ploeg et al., 1980). The women rated themselves on twenty items on a four-point Likert scale ranging from "(1) – not at all" to "(4) – very much", resulting in an anxiety total score ranging between 20 and 80.

Relaxation and Stress Tasks

In the first pregnancy trimester each mother undertook a 25-minute task that consisted of five testing phases, lasting 5 minutes each. Stress was induced in the second and fourth phase, with the remainder being relaxation phases. During the stress phases, participants were asked to solve complex mental arithmetic problems, involving five mathematical operations on 2-3 digit numbers without verbalization (e.g., $(361 + 11)/(34) + 137$). They were asked to choose the correct answer from three possibilities presented by a computer. Feedback on the task was given after completion of the last phase (Vlemincx et al., 2011). Participants viewed peaceful pictures and listened to restful music during the relaxation phase (Taelman et al., 2009).

ECG Recording

During the relaxation and stress tasks maternal ECG was recorded using the Vrije Universiteit Ambulatory Monitoring system (VU-AMS) (Goedhart et al., 2007) in a three Ag/AgCl electrode placement configuration: (1) on the sternum over the first rib between the two collarbones, (2) at the apex of the heart over the ninth rib on the left lateral margin of the chest, and (3) at the lower right abdomen (ground electrode). The skin was cleaned with alcohol to ensure impedance was low. Five-minute HRV measurements were determined for the relaxation and stress phases separately.

Children's ECGs were collected when they were 2-4 months of age. The active electrode was placed on the child's thorax, while the reference and ground electrode were on an EEG cap (Otte et al., 2013). These recordings were made using a Biosemi ActiveTwo biopotential measurement system. Although some data were discarded due to motion artifacts, HRV measurements were determined from the average of 3 to 5 blocks of ECG, lasting 2.5 minutes each.

Behavioral Assessment of the Children

An additional assessment of child behavior at 9-10 months of age was conducted using the Laboratory Temperament Assessment Battery (Lab-TAB)-Locomotor Version (Goldsmith & Rothbart, 1999), a more ecologically valid and less biased assessment of child behavior and temperament than parental-report measures with demonstrable clinical value (Aksan & Kochanska, 2004). The child was presented with a robotic dog (the unpredictable mechanical toy paradigm from the fear subscale) that was barking and walking towards them. Based on the children's facial expression, body posture, vocalizations, and escape behavior, indicators of fear were assessed for the first 20 seconds (Bergman et al., 2007). A researcher, blind to maternal data, rated the recorded videotapes using the standard scoring procedures. Observed fearfulness was calculated from the fear indicators. As the fearfulness measure could not be normalized, children with a score of 0 or 1 were categorized as not fearful, while children with a score above 1 were considered fearful.

4.2.4 Data Processing and Analyses

ECG data were processed using custom software written in Matlab (Mathworks, Natick, USA) to obtain indices of parasympathetic ANS activity, according to published standards (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). These measures were root mean square of successive differences (RMSSD) and high frequency (HF) variability. ECG beat detection was carried out with the Hilbert transform algorithm (Benitez et al., 2001). Potentially erroneous beat detections were identified using a standard approach and screened for validity (Berntson et al., 1990). As HRV measures were right-skewed, they were natural log transformed to normality. Data for three mothers were excluded due to extreme values; visual inspection of the ECGs suggested that these outliers were due to cardiac arrhythmias.

Statistical analyses were performed using SPSS 19 (IBM Corp., 2010). A repeated measures ANOVA was used to examine the differences between mothers' HRV during the various phases of the mental arithmetic task across the two groups (healthy group versus PAD group). Mother's age and pre-pregnancy BMI were included as covariates. To account for mothers' state anxiety, we conducted a propensity score matching to balance state anxiety, age and BMI in both groups. This procedure was executed using a custom designed plugin (Thoemmes, 2012) for IBM SPSS Statistics. Logistic regression is used to determine a predicted score that relates to the propensity for participants to belong to a particular group based on a given set covariates. The matching algorithm was fine-tuned by discarding units outside the area of common support to improve balance on covariates, and using a 'caliper' of 0.5 - this is the standard deviation of the logit of the propensity score - to prevent 'bad' matches. After the propensity score is estimated, cases with the closest score are matched using a simple 1:1 nearest neighbour matching routine based on a 'greedy' matching algorithm. A series of model adequacy checks were then performed including inspection of numerical balance measures, diagnostic plots and re-examination of group differences across the covariates entered into the PS analysis. Next, mothers' state anxiety was added as covariate in the repeated measures ANOVA. Independent t-tests were conducted to investigate group differences in children's HRV. Linear regression analyses were conducted to determine whether maternal HRV was associated with that of their children, and logistic regression analyses to determine whether children's HRV at 2-4 months of age was associated with fearfulness at 9-10 months of age. Regression analyses were controlled for sex, age, birth weight, and gestation length of the child and mother's state anxiety. The effect of offspring HRV on the continuous variable fearfulness was also tested for significance by generating bootstrapped 95% confidence intervals as the fearfulness variable was not normal.

4.3 Results

4.3.1 Participant Characteristics

Characteristics of mothers and children in the healthy and PAD groups are shown in Table 4.1. Importantly, no differences between groups were observed for variables that might have a confounding influence on HRV, except for mothers' state anxiety. Age and BMI of mothers, and age, sex distribution, birth weight, fearfulness and gestation length of the children did not differ significantly between the groups. Propensity score matching identified and matched 18 mothers in each group, providing a matched sample across all of the critical confounding factors, including mothers' state anxiety.

Table 4.1
Comparison of mean (SD) characteristics of mothers and children in the healthy group versus the past anxiety disorder group.

	Healthy	Past anxiety	p value
Mothers			
N	34	22	
AGE (yr)	34.31 (3.14)	33.47 (4.95)	.44
BMI (kg/m^2)	24.51 (4.98)	24.13 (3.13)	.75
STAI (state)[a]	33.29 (5.79)	39.05 (6.98)	.001
RMSSD Phase 1 (rest) ($ln\,ms$)	3.46 (0.53)	3.2 (0.44)	.06
RMSSD Phase 2 (stress) ($ln\,ms$)	3.31 (0.48)	2.92 (0.48)	.005
RMSSD Phase 3 (rest) ($ln\,ms$)	3.38 (0.45)	3.15 (0.51)	.08
RMSSD Phase 4 (stress) ($ln\,ms$)	3.28 (0.43)	3.08 (0.51)	.13
RMSSD Phase 5 (rest) ($ln\,ms$)	3.47 (0.46)	3.17 (0.45)	.02
HF Phase 1 (rest) ($ln\,ms^2$)	6.16 (1.02)	5.69 (0.77)	.07
HF Phase 2 (stress) ($ln\,ms^2$)	5.92 (0.92)	5.25 (0.86)	.009
HF Phase 3 (rest) ($ln\,ms^2$)	5.99 (0.91)	5.49 (0.93)	.05
HF Phase 4 (stress) ($ln\,ms^2$)	5.97 (0.72)	5.39 (0.8)	.006
HF Phase 5 (rest) ($ln\,ms^2$)	6.25 (0.84)	5.54 (0.85)	.003
Children at 2-4 months			
N	28	16	
Age (wk)	15.2 (5.1)	16.6 (6.2)	.41
Sex	8 boys, 20 girls	7 boys, 9 girls	.31
Gestation (wk)	39.4 (1.1)	39.1 (2.1)	.54
Birth weight (g)	3330 (348)	3269 (562)	.87
RMSSD Rest ($ln\,ms$)	2.47 (0.51)	2.16 (0.44)	.048
HF Rest ($ln\,ms^2$)	4.43 (1.04)	3.78 (1.00)	.048
Children at 9-10 months			
N	15	7	
Age (wk)	35.7 (3.1)	35.7 (3.6)	$> .99$
Sex	4 boys, 11 girls	4 boys, 3 girls	.17[b]

[a] Analysis focused on the state anxiety subscale of the STAI (20 items measuring the intensity of anxiety-related symptoms).
[b] χ^2 test.

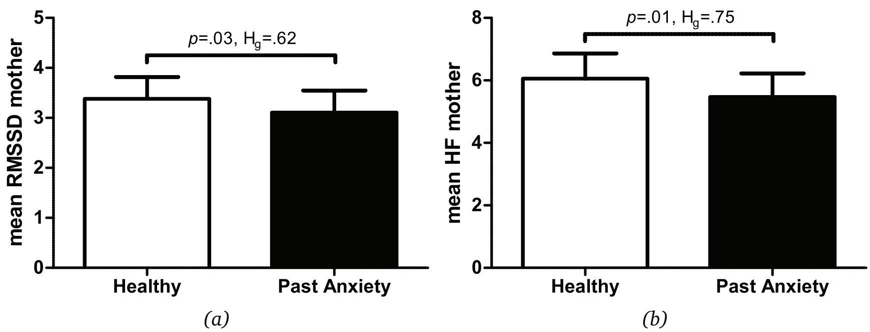

Figure 4.2. Comparison of mother's HRV between healthy group and the past anxiety disorder group.

4.3.2 Impact of Past Anxiety Disorder in Women

Repeated measures ANOVA analysis, adjusted for mother's age and pre-pregnancy BMI, revealed a significant between-subjects effect of group (i.e. PAD or healthy) on both RMSSD HRV (RMSSD: $F(1,52) = 4.37, p = .04$, partial $\eta^2 = 0.08$) and HF HRV ($HF : F(1,52) = 6.68, p = .01$, partial $\eta^2 = 0.12$), indicating reduced HRV in women with PAD (Figures 4.2a and 4.2b). In addition, there was a significant within-subjects effect of phase, indicating that mothers' HRV was significantly different across phases (RMSSD: $F(1,52) = 11.16, p < .001$, partial $\eta^2 = 0.18$; HF: $F(1,52) = 5.73, p < .001$, partial $\eta^2 = 0.10$) (see Table 4.1). There was no interaction effect of group by phase, therefore, subsequent analyses are based on the mean HRV over all five phases, since the aim of our study was to study differences between women with a PAD and the healthy group. Between-subjects effects of group remained significant for HF HRV ($F(1,36) = 4.23, p = .048$, partial $\eta^2 = 0.12$), but not for RMSSD ($F(1,36) = 1.60, p = .22$, partial $\eta^2 = 0.05$) when tests were run on the propensity score matched sample.

4.3.3 Associations Between Maternal and Offspring HRV

Regression analysis, adjusted for mother's state anxiety, offspring's sex and age, revealed a significant group $\times$ mother's HRV interaction effect on offspring's HRV RMSSD ($\beta = 0.98, p = .01$) and HRV HF ($\beta = 0.95, p = .046$), indicating that there is a positive significant association between maternal and offspring HRV in the PAD group, but not in the healthy group. Adding birth weight and gestation length as additional covariates showed that the relationship between maternal and infant HRV was not influenced by changes in birth weight and gestational age.

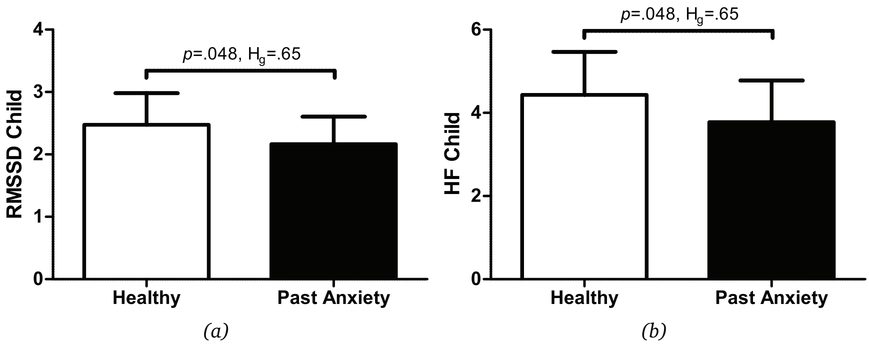

Figure 4.3. Comparison of child's HRV between healthy group and the past anxiety disorder group.

4.3.4 Impact of Maternal Past Anxiety Disorder on Offspring HRV

An independent t-test showed that children of mothers with a PAD have lower HRV than those born to mothers without a PAD (RMSSD: $t(42) = 2.04, p = .048, H_g = .65$; HF: $t(42) = 2.05, p = .048, H_g = .65$; Table 4.1 and Figures 4.3a and 4.3b). Maternal state anxiety was inversely correlated with offspring RMSSD HRV but this had marginal significance ($r = -0.27, p = .08$), while the correlation for HF HRV was significant ($r = -0.33, p = .03$). These were correlations in the total group (consisting of the healthy group and PAD group); no correlations were found between maternal state anxiety and offspring HRV when focusing on groups separately ($p > .05$).

4.3.5 Relationship between Offspring Temperament and Offspring HRV

Logistic regression analyses controlling for age, sex and gestation length indicated that the children's HRV at 2-4 months of age was inversely associated with measures of their fearfulness carried out at 9-10 months of age (RMSSD: odds ratio $= 0.01, p = .047$; HF: odds ratio $= 0.20, p = .07$). Bootstrapped regression analyses with fearfulness as a continuous variable also indicated a significant effect of offspring HRV on fearfulness (RMSSD: 95% CI $= [-1.63, -1.19]$; HF: 95% CI $= [-0.09, -0.01]$).

4.4 Discussion

To our knowledge, this is the first study to show that pregnant women with a PAD have autonomic abnormalities early in pregnancy and that these abnormalities may impact on the future physiological (reduced parasympathetic function) and mental (fearfulness) wellbeing of their offspring. Pregnant women with PAD displayed reduced parasympathetic nervous system activity (indexed by HRV), compared to those without PAD. Furthermore, the offspring of mothers with a PAD also had lower HRV than those whose mothers had been healthy. In the group of mothers with PAD, an association between maternal HRV and offspring HRV was observed. In the offspring, lower HRV at 2-4 months was associated with a fearful temperament assessed approximately 7 months later. These findings were independent of variations in state-anxiety, age, sex, BMI and mother-child associations were not explained by the children's birth weight or gestational age.

Consistent with prior research, this study shows that people with past psychiatric disorders have lower HRV (Kemp & Quintana, 2013; Kemp et al., 2012, 2010; Licht et al., 2008). The novel contribution we make here is the result that pregnant women with a past anxiety disorder also have reduced HRV and these psychophysiological alterations may impact on the future physiological and mental wellbeing of their offspring. It is important to note that the HF HRV differences between women with and without PAD - findings associated with a moderate to large effect size - remained after controlling for confounding factors including state anxiety, age and BMI by propensity score matching. Future studies are needed to confirm this finding in a larger sample in order to generalize to both pregnant and non-pregnant women populations. We note here that women with PAD have a higher level of a subclinical trait (e.g., anxiety), although it no longer reaches criteria for clinical diagnosis. Although we controlled for state anxiety as a confounding factor, it is possible that state anxiety also impacts on maternal HRV as well as the physiological and mental state of the offspring.

There are numerous possible means by which maternal PAD and/or HRV might be associated with parasympathetic nervous system function in the offspring. Clearly, mothers and their children share genes and environmental exposures and the health of mothers in pregnancy is an important determinant of child development and health. Maternal behavior may also be an important factor in this association (Rutter, 2002; Stern, 2009; Weaver et al., 2004). In our study, the mothers with a PAD not only had significantly lower HRV compared to the healthy group, but also had altered state anxiety, albeit to a lesser extent than in the past. In comparison to the healthy group, the offspring of mothers with a PAD also had lower HRV. Lower HRV in all children was shown to be associated with a fearful temperament. Therefore, it is possible that mothers with a PAD and their offspring share an underlying psychological disorder, which might explain links between maternal and offspring HRV. Our study highlights the need for more research into how mothers might transmit their pro-anxiety phenotype to their offspring.

A variety of genes influence vulnerability to anxiety disorders. These include serotonin-transporter linked polymorphic region (5-HTTLPR), Catechol-O-methyl-transferase (COMT), and brain-derived neurotrophic factor (BDNF) gene variants (Lee & Park, 2011). Previously, it was reported that the combination of a BDNF V/V genotype and early life stress predicts changes in brain structure that are associated with lower HRV and higher anxiety (Gatt et al., 2009). These findings provide a potential explanation for our observed relationship between HRV of mothers with a PAD and their children, but not in healthy controls.

Another possibility is that altered autonomic function in pregnant mothers may impact on the development of their offspring, as a form of developmental programming. Reduced HRV is associated with dysregulation of various allostatic systems, including glucose regulation, hypothalamic-pituitary-adrenal (HPA) axis function and inflammatory processes (Thayer & Sternberg, 2006), all of which may program fetal development (Lupien, McEwen, Gunnar, & Heim, 2009; Matthews & Phillips, 2010; Meyer et al., 2006; Räikkönen et al., 2011; Van den Bergh, 2011; Van den Bergh et al., 2005; Young, 2002). However, it is not clear whether the abnormal ANS function, reflected by lower HRV, is an important causative factor in these disorders, or simply the result of shared underlying processes.

Observed fearfulness in the children at 9 months was associated with their HRV at 2-4 months. It is possible that 9 month olds with higher fearfulness already had characteristics of a fearful temperament at 2-4 months, which could explain the association between reduced HRV and future fearful temperament as a persistence of these related factors. However, it is very challenging, if not impossible, to detect these psychological features in young infants (Kagan, 1982; Rapee, 2002). Therefore, we were unable to determine whether reduced HRV came before the development of fearfulness or not, making it difficult to infer causation in this association. Nevertheless, reduced HRV in infancy may be an early risk marker for the development of psychological abnormalities, such as fearfulness, in later life. This observation makes an important contribution to the literature, which indicates strong associations between low HRV and fearful temperament (reflected in inhibited behavior, shyness, harm avoidance, anxiety and distress) (Calkins & Swingler, 2012; Kagan, 1982; Kagan, Reznick, & Snidman, 1987). Such behavioral characteristics have been linked to internalizing problems and psychiatric diagnoses (Degnan, Almas, & Fox, 2010; Rettew & McKee, 2005). Low HRV may indicate a vulnerability to the development of psychopathology, as suggested by our observation that infant's HRV is related to later fearfulness.

Our study has a number of advantages, including a prospective design and longitudinal assessment; assessment of maternal HRV during the first 14 weeks of pregnancy, which is the period of greatest fetal developmental vulnerability to external influences; exclusion of women on antidepressants, which is an important consideration in studies of HRV; and use of an infant temperament (fearfulness) measure, which avoids bias in parental perceptions.

In summary, our study showed that pregnant women with a PAD had altered ANS function that was also found in their infants. Alterations in the offspring were associated with fearfulness in later infancy, suggesting transmission of a pro-anxiety phenotype from mothers to their children. Determination of the mechanisms of this transmission should be an important goal of future research. Our findings may have implications for identifying early risk factors in childhood for the development of psychological disorders in later life. Thus, future research might build on our findings to establish novel risk factors for psychopathology in childhood and use those factors to study the etiology of psychiatric disease in children.

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5

Mindfulness during pregnancy may benefit maternal autonomic nervous system and infant development

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Submitted.

Abstract

Objective: Mindfulness, or present-moment awareness, is known to decrease psychological distress and may be useful during pregnancy. Therefore, the aim was to examine the prospective association of mindfulness with autonomic nervous system functioning during pregnancy and with later social-emotional development of the infant.

Methods: 156 pregnant women completed self-report mindfulness and emotional distress, and had their autonomic function assessed in their first and third trimesters, including heart rate (HR), indices of heart rate variability (HRV), pre-ejection period (PEP), systolic (SBP) and diastolic blood pressure (DBP). Their infants' social-emotional development was assessed at 4 months of age.

Results: More mindful pregnant women had less prenatal and postnatal emotional distress ($b = -0.06, SE = 0.01, p < .001$) and higher cardiac parasympathetic activity: root mean square of successive differences (RMSSD: $b = 0.01, SE = 0.01, p = 0.03$) and high-frequency (HF) HRV ($b = 0.03, SE = 0.01, p = 0.02$). Between the first and third trimesters, HR increased ($b = 7.42, SE = 0.34, p < 0.001$) and RMSSD ($b = -0.07, SE = 0.02, p < 0.001$), HF HRV ($b = -0.16, SE = 0.04, p < 0.001$), low-frequency (LF) HRV ($b = -0.23, SE = 0.04, p < 0.001$) and PEP decreased ($b = -17.71, SE = 1.04, p < 0.001$). In more mindful mothers, however, parasympathetic activity decreased less (RMSSD: $p = 0.01$; HF HRV: $p = 0.03$) and sympathetic activity (inversely related to PEP) increased less (PEP: $p = 0.02$) between trimesters. Their offspring had fewer social-emotional problems (observed coefficient $= -0.19$; 95% CI$= [-0.37, -0.02], p = 0.03$) compared to offspring of less mindful mothers.

Conclusions: Mindfulness in pregnancy was associated with ANS changes likely to be adaptive and with better social-emotional offspring development. Interventions to increase mindfulness during pregnancy might improve maternal and offspring health but randomized trials are needed to demonstrate this.

Keywords: mindfulness, autonomic nervous system, emotional distress, pregnancy, offspring's social emotional development

5.1 Introduction

Pregnancy is associated with marked cardiovascular adaptations such as increased stroke volume (SV) and heart rate (HR) (Abbas et al., 2005; Silversides & Colman, 2007). The autonomic nervous system (ANS) plays a central role in these changes. It is known that ANS activity is shifted towards higher sympathetic and lower vagal modulation (e.g. reduced HRV) over the course of pregnancy (Kuo et al., 2000). The increased SV and HR generate higher cardiac output to offset the drop in total peripheral resistance that occurs early in pregnancy (Abbas et al., 2005). The net effect is a slight decrease in mean blood pressure with the decline in DBP being larger than the SBP fall (Abbas et al., 2005). Since cardiac output keeps rising until the end of pregnancy and systemic vascular resistance steadily recovers (Clark et al., 1989; Easterling et al., 1990; Thornburg et al., 2000), blood pressure measures return to pre-pregnancy levels by term (Christian, 2012; Hermida et al., 2000).

Failure of cardiovascular adaptation during pregnancy may lead to complications such as hypertension, pre-eclampsia or other cardiovascular diseases (CVD) (Faber et al., 2004; Thayer et al., 2010; Voss et al., 2000; Walther et al., 2005, 2006). These disorders are associated with greater risks of preterm birth, chronic hypertension, maternal cardiovascular morbidity and neonatal morbidity/mortality (Cuevas & Germain, 2011; Magnussen et al., 2009; Young et al., 2010; Zhang et al., 2007).

A number of mental disorders have also been linked to detrimental cardiovascular changes in pregnancy. For example, maternal emotional distress or depressive symptoms are associated with abnormal cardiovascular function, e.g. more reduction in parasympathetic activity (Shea et al., 2008) and larger increase in blood pressure (hypertension and pre-eclampsia) (Kurki, Hiilesmaa, Raitasalo, Mattila, & Ylikorkala, 2000; Qiu, Sanchez, Lam, Garcia, & Williams, 2007; Qiu, Williams, Calderon-Margalit, Cripe, & Sorensen, 2009). Furthermore, both hypertension disorders (Robinson et al., 2009, 2013; Whitehouse, Robinson, Newnham, & Pennell, 2012) and maternal emotional distress (Räikkönen et al., 2011; Van den Bergh et al., 2005) during pregnancy are linked to adverse behavior and developmental outcomes in infancy, childhood and adulthood.

Psychopharmaceuticals reduce maternal prenatal stress/depression and elevated levels of blood pressure effectively, but their use during pregnancy is often inappropriate because they bear a risk for the fetus (Duley, Henderson-Smart, & Meher, 2006; Ericson, Källén, & Wiholm, 1999; Kulin et al., 1998; Mulder, Ververs, de Heus, & Visser, 2011).

A better way to cope effectively with stress, anxiety and depression during pregnancy may be to make enhanced use of mental resources such as mindfulness. Mindfulness is an adaptive mental state, often described as the attention to moment-to-moment experience with an accepting and nonjudgmental attitude (Baer et al., 2006; Williams, 2008), or as a receptive attentiveness to present experience (Brown & Ryan, 2003; Holt, 2012). Mindfulness therapy can improve both mental and physical/physiological health (Hofmann et al., 2010; Nyklíček, Mommersteeg, Van Beugen, Ramakers, & Van Boxtel, 2013), but studies concerning the physiological effects of mindfulness during pregnancy are lacking.

Therefore, the present study was designed to test the hypotheses that mindfulness would be associated with (1) better cardiovascular adaptation during pregnancy, (2) lower maternal emotional distress and (3) better social-emotional development of the infant at 4 months.

5.2 Methods

5.2.1 Participants

This sample consisted of 156 pregnant women who were recruited at midwiferies and a hospital in Tilburg and surroundings. They had low obstetric risk as judged by the midwiferies and hospitals and made no use of medication or drugs. The longitudinal nature of the study and the fact that women participated on a voluntary basis, may explain the biased sample with a higher education in comparison with the general population of pregnant women.

We had access to medical records of 145 women of whom blood pressure data were obtained and 110 mothers completed the questionnaire about the social-emotional development of the infant after birth. The Medical Ethics Committee of the St. Elisabeth hospital, Tilburg approved the study. All participants provided informed, written consent.

5.2.2 Procedure

The design of the study is prospective with the following assessment times:

1. First trimester (between 8 and 14 weeks of gestation): for the first assessment of mothers' cardiovascular functioning and emotional distress.

2. Second trimester (between 15 and 22 weeks of gestation): for the assessment of mothers' mindfulness. This was only performed once, because it is often

considered as a stable disposition, which has strong relationships with Big Five personality traits (Giluk, 2009). It was intended to measure mindfulness as trait-like component by not suggesting a specific time frame of reference when participants filled out the questionnaire to measure this construct (Walach et al., 2006).

3. Third trimester (between 31 and 37 weeks of gestation): for the same assessments as in the first trimester.

4. Postnatal (mothers: 2-4 months after delivery): for the third assessment of mothers' emotional distress.

5. Postnatal (infant: at 4 months of age): for the assessment of social-emotional development by the mother of the infant (see below).

5.2.3 Instruments

Mindfulness

The Freiburg Mindfulness Inventory - short form (FMI-s) was used to assess mindfulness (Walach et al., 2006). It consists of 14 items covering central aspects of mindfulness, including attention to external and internal phenomena in the present moment (e.g. "I am open to the experience of the present moment"), and acceptance (e.g., "I accept unpleasant experiences"). Items are scored on a scale from 1 to 4. The short form has an adequate internal consistency, in the published original ($\alpha = .86$) and Dutch ($\alpha = .79$) version (Klaassen et al., 2012), as well as in this study ($\alpha = .85$).

Emotional distress

The Edinburgh Depression Scale (EDS) is a 10 item self-report measure designed to screen women for symptoms of depression and emotional distress during pregnancy and the postnatal period (Cox, Holden, Sagovsky, 1987). Women indicate before 15 weeks of gestation, at 31-37 weeks of gestation and 2-4 months after birth how they felt during the last seven days. The reliability and validity of the original and Dutch versions are adequate (Nyklíček et al., 2004). In our sample the internal consistency was adequate during the first ($\alpha = .82$) and third ($\alpha = .85$) trimester of pregnancy. Both questionnaires were completed at home.

ECG and ICG recording

A 25-minute experimental session assessing the cardiovascular functioning was conducted at the participants' home in a quiet room. The session consisted of five testing phases, lasting 5 minutes each. Stress was induced in the second and fourth phase with complex mental arithmetic problems, while the other three phases consisted of relaxation with peaceful pictures and restful music as applied before (Taelman et al., 2009; Vlemincx et al., 2011). Mothers' ECG and ICG was recorded using seven Ag/AgCl electrodes and the Vrije Universiteit Ambulatory Monitoring system (VU-AMS) (Goedhart et al., 2007). The electrodes were arranged according to the VU-AMS configuration guidelines. This system provides continuous measurements of heart rate (HR), indices of heart rate variability (HRV), and pre-ejection period (PEP, an index of cardiac sympathetic drive, see below).

Blood pressure

Maternal systolic (SBP) and diastolic blood pressure (DBP) data were retrieved from medical records. Similar to the ECG/ICG recording, blood pressure was measured in the first and third pregnancy trimester, each time within less than 3 weeks from the day the ECG/ICG were recorded. For both pregnancy trimesters, there was only one blood pressure measurement. Three pregnant women with extremely elevated blood pressure (higher than 140/90 mmHg) were excluded as outliers.

Birth outcomes

All pregnancies were dated using the first day of the last menstrual period, which was provided by the participants at recruitment. The infant's day of birth was retrieved from medical records. Based on these data the gestation length was calculated. The offspring birth weight was retrieved from medical records, as well as information on the infant's health immediately after birth. The infant's health was assessed using the Apgar score, which evaluates a newborn baby on five simple criteria (i.e. Appearance, Pulse, Grimace, Activity, Respiration) (Apgar, 1953).

Infant social-emotional development

When the children were between 4 and 5 months old, mothers filled out the Ages and Stages Questionnaire - Social-Emotional (ASQ-SE) (Squires et al., 2002) at home. It measures social-emotional problems, behavioral problems, and social competencies on a three-point Likert scale. It is a parent reported screening instrument for infants, which consists of 5 subscales, namely self-regulation, communication, affect, interaction with people and adaptive functioning. All infants had a total score below 45. In a clinical setting, scores higher than 45 would be required to meet criteria for further psychological evaluation. One outlier was removed (2.5 standard deviations from the mean).

5.2.4 Data Processing and Statistical Analyses

ECG and ICG data were processed using custom software written in Matlab R2012b (Mathworks, Natick, USA) to obtain HR and indices of sympathetic (PEP) and parasympathetic (RMSSD and HF HRV) or both (LF HRV) ANS activity, according to published standards (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). ECG beat detection was carried out with the Hilbert transform algorithm (Benitez et al., 2001). Potentially erroneous beat detections were identified using a standard approach and screened by the researchers for validity (Berntson et al., 1990). As HRV measures are right-skewed, they were log transformed to normality. PEP was calculated from the ICG as the interval between R-wave onset and the B-point. This B-point was identified using an extrapolation technique (Jones, 2006).

All analyses were conducted using Stata 12.1. Multilevel (i.e. pregnancy trimester and mental arithmetic task phase) regression analyses were conducted to examine the potential association between mindfulness and cardiovascular adaptation during pregnancy. Interaction effects between mindfulness and task phase were computed to test whether the association between mindfulness and HR, HRV or PEP differed between stress versus rest. Interaction effects between mindfulness and the pregnancy trimester were also computed to test whether potential changes in HR, HRV, PEP or BP over the course of pregnancy are related to the mother's level of mindfulness. The multilevel models were adjusted for emotional distress, level of education, age and BMI (Vallejo et al., 2005). The models predicting HRV and PEP were also adjusted for HR (Ramaekers, Ector, Aubert, Rubens, & Van de Werf, 1998).

In addition, a multilevel (i.e. time of measurement: first and third pregnancy trimester, and 2-4 months postpartum) regression analysis, adjusted for the common potential confounders level of education, age and BMI, was used to investigate how mindfulness is related to maternal emotional distress. Finally, the associations between maternal mindfulness and the continuous variables of social-emotional development were tested for significance by generating bootstrapped regression analyses with 95% confidence intervals (5000 iterations) as no normal distribution of the residuals could be achieved by transforming the data. In these analyses, maternal age, education and emotional distress, and infants' sex, birth weight, and gestation length were controlled.

5.3 Results

Characteristics of mothers and children are shown in Table 5.1. Mothers who provided data on the social-emotional development of the offspring (N = 109) did not differ from mothers who did not provide these data (N = 47) regarding age, education level, emotional distress or mindfulness ($p > .10$).

Table 5.1
Descriptive statistics for variables related to infants and mothers

	n	M	(SD)	Percent
Infants				
Gestation length (*weeks*)	106	39.57	(1.49)	
Birth weight (*g*)	109	3357.38	(679.63)	
Apgar score above 8				
At 5 minutes	108/109			99.08%
At 10 minutes	109			100.00%
Males	51/109			46.79%
Age (*weeks*)	109	13.48	(5.30)	
Social-emotional development (total)	109	13.94	(10.67)	
Self-regulation	109	5.55	(6.64)	
Communication	109	0.78	(2.06)	
Affect problems	109	2.94	(3.98)	
Interaction with people	109	0.78	(2.47)	
Adaptive functioning	109	3.76	(5.58)	
Mothers				
Mindfulness	156	39.91	(6.19)	
Emotional distress (postpartum)	113	4.76	(4.30)	
BMI before pregnancy (kg/m^2)	156	24.19	(4.11)	
Education (Higher Education)	156			67.74%

	1st trimester			3rd trimester			
	n	M	(SD)	n	M	(SD)	*p* value
HR, HRV and PEP [a]							
HR (*bpm*)	124	82.43	(8.89)	128	90.03	(9.44)	< .001
RMSSD ($ln\,ms$)	124	3.36	(0.51)	128	2.96	(0.54)	< .001
HF ($ln\,ms^2$)	124	5.96	(0.98)	128	5.20	(1.06)	< .001
LF ($ln\,ms^2$)	124	6.44	(0.70)	128	5.84	(0.74)	< .001
PEP (*ms*)	119	73.04	(20.38)	118	60.02	(21.27)	< .001
Systolic BP (*mmHg*)	101	113.05	(10.46)	84	113.77	(10.29)	.75
Diastolic BP (*mmHg*)	100	67.06	(7.42)	84	68.77	(9.54)	.12
Emotional distress (prenatal)	124	5.69	(4.20)	128	5.16	(4.15)	.84

[a] averaged across mental arithmetic task phases
BMI = Body Mass Index, HR = Heart Rate, HRV = HR variability, PEP = pre-ejection period, RMSSD = root mean squared of successive differences, HF = high-frequency, LF = low-frequency, BP = blood pressure.

5.3.1 Mindfulness and Cardiovascular Adaptation During Pregnancy

Adjusted for age, education level, emotional distress and BMI, mindfulness was significantly positively associated with RMSSD HRV ($b = 0.01, SE = 0.01, p = .03$) and HF HRV ($b = 0.03, SE = 0.01, p = .02$)[1]. There were also marginally significant relationships between mindfulness and LF HRV ($b = 0.02, SE = 0.01, p = .07$), PEP ($b = 0.46, SE = 0.25, p = .06$) and HR ($b = -0.20, SE = 0.11, p = .07$). These associations between mindfulness and HRV and PEP were not significant after adjustment for HR ($p > .05$). No association was found between mindfulness and baseline SBP or DBP ($p > .05$).

No evidence was found for an interaction between mindfulness and the different phases of the experimental session for any ANS measure ($p > .05$). HR increased across trimesters ($b = 7.42, SE = 0.34, p < .001$), while RMSSD ($b = -0.07, SE = 0.02, p < .001$), HF HRV ($b = -0.16, SE = 0.04, p < .001$), LF HRV ($b = -0.23, SE = 0.04, p < .001$) and PEP ($b = -11.71, SE = 1.04, p < .001$) all decreased. DBP increased marginally across trimesters ($b = 1.88, SE = 1.02, p = .06$).

The results depicted in Table 5.2 show a significant interaction between mindfulness and the trimester of pregnancy on RMSSD HRV ($p = .01$), HF HRV ($p = .03$), and PEP ($p = .02$), after adjustment for HR, emotional distress, age, BMI and level of education[2]. This indicates that the more mindful pregnant women were, the less RMSSD, HF HRV, and PEP declined during pregnancy (Figures 5.1b, 5.1c and 5.1e). The decrease in LF HRV and increase of HR, DBP and SBP over the course of pregnancy were not significantly different for different levels of mindfulness ($p > .05$) (see Table 5.2 and Figures 5.1d, 5.1a, 5.1f and 5.1g).

5.3.2 Mindfulness and Emotional Distress

Mindfulness was negatively associated with emotional distress, after adjustment for education level, age and BMI ($b = -0.06, SE = 0.01, p < .001$). There was no significant effect of time of measurement ($p > .05$) or an interaction between mindfulness and time of measurement ($p > .05$). This indicates that women who are more mindful tend to suffer less from emotional distress, independently when emotional stress was measured (i.e. in first trimester, in third trimester or 2-4 months postpartum).

[1] Additional adjustment for maternal anxiety (as measured with the State Trait Anxiety Inventory, see Section 2.4.1) had no effect on the significance of these results.

[2] see footnote 1

Table 5.2

Multilevel regression analyses relating mindfulness to HR, RMSSD HRV, HF HRV, LF HRV, PEP, SBP and DBP

	HR[a]			Ln RMSSD HRV[a]			Ln HF HRV[a]			Ln LF HRV[a]		
	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*
Mindfulness	−0.16	0.11	.16	0.004	0.005	.4	0.01	0.01	.31	0.01	0.01	.45
Trimester (3rd vs. 1st)	7.84	2.13	< .001	−0.29	0.09	.001	−0.57	0.19	.003	−0.39	0.24	.09
Mindfulness × Trimester	−0.01	0.05	.84	0.006	0.002	< .01	0.01	0.005	.03	0.004	0.01	.49
Emotional distress	0.01	0.08	.88	< 0.001	.003	.90	0.01	0.01	.45	0.01	0.01	.13
Higher education	0.04	1.44	.98	0.006	0.06	.92	0.04	0.13	.74	0.03	0.10	.77
HR				−0.04	0.001	< .001	−0.07	0.003	< .001	−0.04	0.003	< .001
Age	-0.29	0.16	.06	−0.03	0.01	< .001	−0.06	0.01	< .001	−0.04	0.01	< .001
BMI	0.25	0.16	.13	0.001	0.01	.86	−0.01	0.01	.62	−0.02	0.01	.09
Constant	91.95	7.45	< .001	7.31	0.32	< .001	13.61	0.71	< .001	11.24	0.60	< .001

	PEP[b]			SBP[c]			DBP[c]		
	Coef.	SE	*p*	Coef.	SE	*p*	Coef.	SE	*p*
Mindfulness	0.23	0.26	.37	0.15	0.18	.41	0.03	0.14	.83
Trimester (3rd vs. 1st)	−24.66	5.71	< .001	6.21	7.40	.40	5.66	6.45	.38
Mindfulness × Trimester	0.33	0.14	.02	−0.14	0.18	.44	−0.09	0.16	.55
Emotional distress	−0.11	0.20	.59	0.05	0.20	.79	0.27	0.16	.09
Higher education	1.42	3.18	.66	0.82	1.99	.68	0.56	1.54	.72
HR	−0.23	0.07	.001						
Age	−0.17	0.35	.62	−0.05	0.22	.81	0.15	0.17	.38
BMI	−1.13	0.36	.002	0.75	0.26	.004	0.43	0.21	.04
Constant	116.11	17.82	< .001	91.01	11.79	< .001	48.79	9.26	< .001

[a] n=156; [b] n=150; [c] n=124

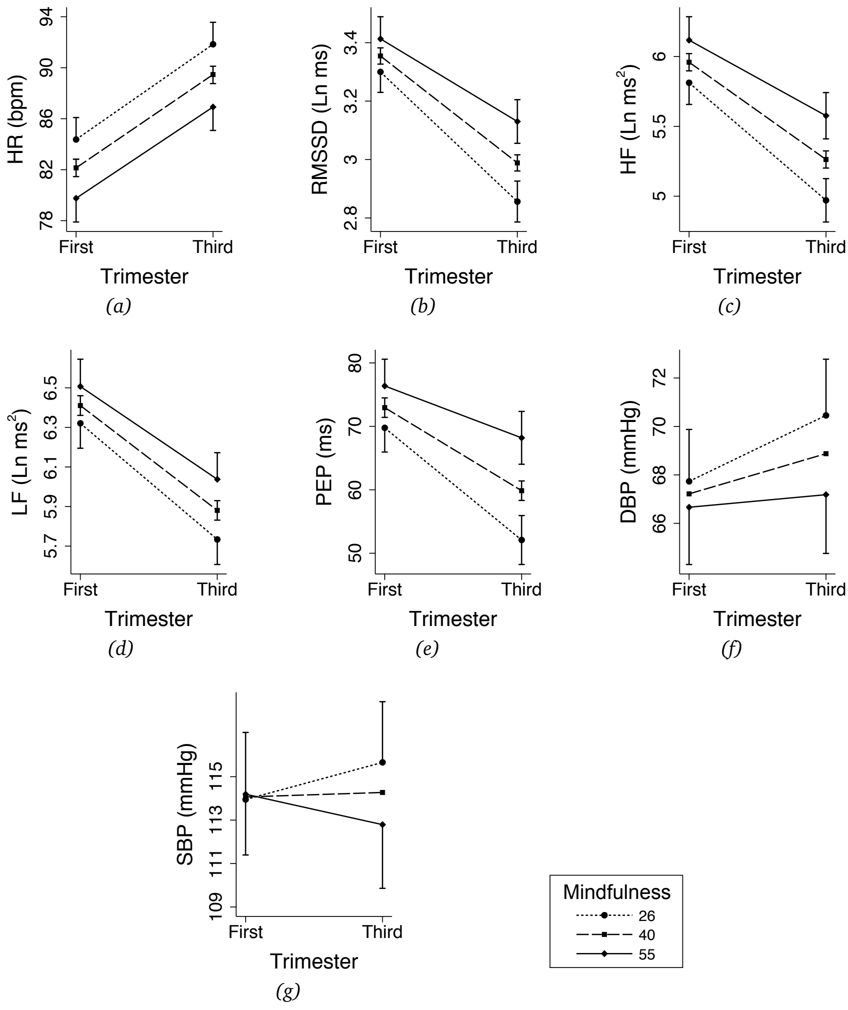

Figure 5.1. The association between mindfulness and cardiovascular measures over the course of pregnancy, namely HR, RMSSD HRV, HF HRV, LF HRV, PEP, DBP and SBP. Plots are based on estimated marginal means from multilevel regression analyses showing interactions between mindfulness and pregnancy trimester. Minimum, average and maximum scores for mindfulness in our sample are respectively 26, 40 and 55.

5.3.3 Mindfulness and Infant Social-Emotional Development

While the subscales 'self-regulation', 'affect', 'communication' and 'interaction with people' of social-emotional development as scored by the mother 4 months after birth were not significantly related with mindfulness, bootstrapped regression analyses showed evidence for an association between the subscale 'adaptive functioning' and mindfulness (observed coefficient (OC) $= -0.18$; 95% CI$= [-0.29, -0.06], p = 0.003$), indicating that infants of mothers who are more mindful tend to have less problems in adaptive functioning. The association remained significant after controlling for sex, birth weight, gestation length, infant's age, mother's education level and postnatal emotional distress (OC $= -0.19$; 95% CI$= [-0.37, -0.02], p = 0.03$). None of the covariables showed significant associations with adaptive functioning (95% CI includes zero, $p > .05$).

5.4 Discussion

5.4.1 Conclusions

To the best of our knowledge, this is the first study to investigate whether mindfulness is associated with ANS function during pregnancy. It also assessed the association of mindfulness with future maternal mental health and offspring social-emotional development. More mindful pregnant women had less emotional distress, both during and after pregnancy and higher cardiac parasympathetic activity (reflected by RMSSD and HF HRV).

Over the course of pregnancy, all mothers experienced significant increases of HR and marginally significant increases of DBP. PEP, RMSSD, HF and LF HRV fell between the first and third pregnancy trimester. These findings are in line with previous research (Abbas et al., 2005; Christian, 2012; Hermida et al., 2000; Kuo et al., 2000; Silversides & Colman, 2007). Cardiac parasympathetic activity (RMSSD and HF) did not decrease as much and sympathetic activity (inversely related to PEP) did not increase as much in mothers who were more mindful. Decreased parasympathetic activity and increased sympathetic activity are characteristic of a hyperactivated cardiovascular system. Therefore, it can be argued that these mothers maintained a more relaxed physiological state through pregnancy. The benefits of doing so for the offspring are unknown but we found that offspring of more mindful mothers had fewer adaptive functioning problems in infancy, suggesting a possible benefit. If replicated, further studies will be required to determine whether this benefit arises from improved fetomaternal health in pregnancy, enhancing fetal neurodevelopment or from postnatal benefits of being raised by more mindful mothers.

Despite published calls for studies to examine physiological concomitants of stress when examining the effectiveness of body-mind interventions during pregnancy (Beddoe & Lee, 2008), we could only find two published studies that have done this (Little et al., 1984; Satyapriya et al., 2009). These studies evaluated the effects of a relaxation intervention on HRV and BP during pregnancy. During relaxation exercises, HF HRV increased and was even enhanced during later stages of pregnancy (Satyapriya et al., 2009). Both SBP and DBP were lower than in controls (Little et al., 1984). In the present study mindfulness was related to RMSSD and HF HRV, but significant associations were not found with HR, LF HRV, PEP or blood pressure (SBP and DBP). This suggests that mindfulness is predominantly related to parasympathetic activity. This is in line with studies with non-pregnant women, which found that components of mindfulness-based stress reduction interventions tend to increase the parasympathetic ANS mediated component (HF) of HRV (Ditto, Eclache, & Goldman, 2006; Leonaite & Vainoras, 2010; Paul-Labrador et al., 2006; Sarang & Telles, 2006; Takahashi et al., 2005; Tang et al., 2009; Wu & Lo, 2008), although this is not found in all studies (Nyklíček et al., 2013). In contrast, we could not find a relationship between mindfulness and blood pressure during pregnancy, despite findings that meditation can reduce blood pressure (SBP and DBP) in non-pregnant participants (Anderson, Liu, & Kryscio, 2008; Chiesa & Serretti, 2010; Goldstein, Josephson, Xie, & Hughes, 2012; Nyklíček et al., 2013). Almost three decades ago, a study with 60 pregnant women showed that systematic relaxation training lowered SBP and DBP in comparison to a control group (Little et al., 1984). This finding has not been replicated.

The association between mindfulness and the parasympathetic activity did not differ between resting versus stress conditions, suggesting that mindfulness may be related to increased parasympathetic activity in general rather than to stress reactivity. However, we used a mild stressor in consideration of the pregnant status of our participants. Therefore, we cannot exclude the possibility that a stronger stressor such as the Trier Social Stress Test might have provoked different stress responses in mindful women compared to controls (Holt, 2012; Kemeny et al., 2012; Nyklíček et al., 2013).

Previous research has shown that HRV tends to decrease as HR and BP rise (Christian, 2012; Hermida et al., 2000) over the course of pregnancy (Kuo et al., 2000). Our findings were compatible with this. We also found significant decreases of PEP and the HRV variables. Furthermore, the changes in RMSSD, HF and PEP were still related to mindfulness after adjustment for HR, emotional distress, age, BMI and level of education. Thus, the autonomic changes that we observed, which were related to mindfulness, were not dependent on HR, emotional distress or the other potential confounders. Although we found a relationship between mindfulness and PEP, a marker of cardiac sympathetic nervous system stimulation, we found no similar relationship with LF HRV. LF HRV has been considered a marker of sympathetic ANS activity but it is now clear that both the sympathetic and parasympathetic ANS influence LF HRV, making interpretation difficult. Therefore, PEP is considered a better indicator of sympathetic activity (Houle & Billman, 1999).

We did not find strong relationships between mindfulness and BP. More mindful mothers did demonstrate less increase in DBP over the course of their pregnancies but this finding was only marginally significant. In our study, BP was not measured at the same time as our other measures, relying upon clinical measurements recorded in the obstetric record. A limitation of this study, therefore, is that our BP measures were carried out according to standard clinical practice but not recommended research guidelines. This may have resulted in an increased measurement error in this variable and may have weakened associations with mindfulness.

Previous studies have suggested that reduced HRV during pregnancy is an indicator of increased risk of developing gestational hypertension or preeclampsia (Macdonald-Wallis et al., 2012), which may be detrimental to the offspring with effects such as low birth weight (Ananth, Peedicayil, & Savitz, 1995). Therefore, the potential benefit of mindfulness in protecting against declining HRV during pregnancy should be examined to potentially reduce the risk associated with such pregnancies in the future. However, this cannot be determined without randomized controlled trials of interventions designed to increase mindfulness during pregnancy.

Previously, several authors have shown that mindfulness in non-pregnant women predicts self-regulated behavior and positive emotional states and that it is related to lower mood disturbance and less distress (Baer et al., 2006, 2008; Brown & Ryan, 2003). To our knowledge, our study is the first to demonstrate a similar relationship in pregnancy, finding negative associations between mindfulness and emotional distress. This is in line with earlier findings that techniques enhancing mindfulness during pregnancy reduce anxiety and perceived stress for up to two months after the intervention (Bastani et al., 2005; Beddoe et al., 2009; Vieten & Astin, 2008). Remarkably, the relationship between mindfulness and emotional distress was significant both during pregnancy and postpartum. Previous research demonstrated that mindfulness-based cognitive therapy during pregnancy might cause a decline in measures of depression and that this improvement can continue into the postnatal period (Dunn, Hanieh, Roberts, & Powrie, 2012). Our findings offer further support for this notion, suggesting that mindfulness might indeed reduce the risk of postpartum emotional distress, independently of prenatal emotional distress. We would recommend that future studies include a postnatal mindfulness measurement to better understand the relationship between (prenatal) mindfulness and postnatal emotional distress.

Mindfulness was associated with the social-emotional development of the offspring. Specifically, more mindful mothers had offspring with fewer problems in adaptive functioning. The subscale measuring this construct assesses the infant's success or ability to cope with physiological needs (e.g., sleeping, eating, elimination, safety) (Squires et al., 2002). This kind of regulatory problems are known to predict adverse social and adaptive behavior and lower cognition in pre-school age children (Schmid, Schreier, Meyer, & Wolke, 2010; Wolke, Schmid, Schreier, & Meyer, 2009). Other social-emotional development measures in this study were not associated with mindfulness. We are not certain whether this fact (a) truly reflects a very specific altered social-emotional development in adaptive functioning, (b) whether other subscales of the questionnaire were not sufficiently sensitive in our young age group, or (c) reflects a chance finding. Although the questionnaire was designed for 3 to 8 months old infants, many of the measures are likely to be more suitable to children at the upper end of this age range. Our participants were 4 months old and their data on communication and interaction with others, for example, may not have been clearly observable to mothers and may have had limited power to reveal the influence of maternal mindfulness. The possibility of a chance finding, although it cannot be ruled out, seems unlikely, in light of the fact that this specific effect was highly significant, and would remain significant if a correction for multiple testing was applied.

5.4.2 Study Limitations

The fact that infant's development was rated by the mother, who was the same person as the one rating her own mindfulness may be regarded as a limitation of the present study. However, the potential reporting bias introduced here was minimized by including mother's mood (emotional distress) as a variable that was controlled in the analyses. Besides the suboptimal blood pressure assessment mentioned above, another limitation is the reduced number of observations regarding the infant's development and mother's emotional distress. This is the result of mothers not returning the filled-out questionnaire, which possibly is related to the fact that mothers might be quite busy taking care of their newborn child. This has decreased power regarding associations with infant's development variables, but has probably not limited generalizability of findings, because women who complied with all aspects of the study did not differ from those not completing these questionnaires regarding age, education level, emotional distress or mindfulness.

5.4.3 Strengths and Conclusion

Our study has a number of strengths. Compared to previous studies, we examined a relatively large population of pregnant women and included data from the first pregnancy trimester. Most prior studies have focused on the second or third trimester only. There are numerous studies that have focused on the effects of negative emotions (anxiety and depression) during pregnancy. However, very few studies have sought to establish resources able to counteract the effects of these negative emotional states. Our study provides support for the possibility that enhancing psychological characteristics such as mindfulness could be beneficial. This should support the conduct of intervention trials examining the effect of psychological interventions on positive factors such as mindfulness and their effects in pregnancy. Perhaps, there is the potential for significant mental and physiological benefits for both mothers and their children.

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6

Physical exercise during pregnancy and circadian variation in parasympathetic activity are negatively associated with offspring birth weight

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Submitted.

Abstract

Regular physical exercise has a beneficial effect on the autonomic regulation during rest, as indexed by increased heart rate variability (HRV) and enlarged circadian variation in HRV. During pregnancy regular physical exercise has also been linked to an enhanced vagal tone. However, no studies have been conducted on the association between regular physical exercise during pregnancy and circadian rhythms in HRV.

Between 15 and 22 weeks of gestation, 157 pregnant women reported about their physical exercise and wore a holter monitor for 24 hours to collect data on their heart rate (HR), HRV and pre-ejection period (PEP). Offspring birth weight was retrieved from medical records.

Regular maternal moderate-intensity physical exercise was negatively associated with offspring birth weight ($\beta = 0.22, p < .005$). The circadian variation in HRV was significantly related to offspring birth weight (RMSSD HRV: $\beta = -0.19, p = .007$; HF HRV: $\beta = -0.18, p = .01$), adjusted for regular maternal physical exercise. Bootstrapping mediation analysis showed that the association between regular physical exercise and birth weight is partially mediated through circadian variation in RMSSD HRV (Percentile CI $= [-216.04, -13.17]$; Bias-corrected CI $= [-236.27, -22.23]$).

Regular moderate-intensity physical exercise during pregnancy might enhance circadian variation in parasympathetic activity and partially mediate the negative association between regular moderate-intensity physical exercise and offspring birth weight.

6.1 Introduction

Marked increases in heart rate (HR) occur during acute physical exercise. These changes are primarily driven by alterations in autonomic nervous system (ANS) activity (Hautala, Kiviniemi, & Tulppo, 2009). Increased HR is linked to reduced parasympathetic activity and increased sympathetic activity (Hautala et al., 2009). Previous studies have shown that regular physical exercise improves autonomic regulation of HR during rest, in the absence of physical exercise, as indexed by increased HR variability (HRV) (Davy, DeSouza, Jones, & Seals, 1998; De Meersman, 1993; Stein, Ehsani, Domitrovich, Kleiger, & Rottman, 1999) and enlarged circadian variation in HRV (Adamopoulos et al., 1995).

Regular physical exercise during pregnancy has been associated with multiple beneficial psychological effects such as decreased pain/discomfort and less depression, as well as with positive physical outcomes such as shorter labor and delivery, faster recovery after delivery, fewer pregnancy complications (e.g. gestational hypertension and diabetes) and less weight gain (Mudd et al., 2013; Streuling et al., 2011). Pregnant women who exercise more often tend to have a lower HR during rest, as well as reduced blood pressure and enhanced vagal tone (Melzer et al., 2010). Although it is known that an ANS circadian rhythm persists during pregnancy (Ekholm et al., 1997; Stein, Hagley, et al., 1999), no studies have been conducted on the association between regular physical exercise during pregnancy and circadian rhythms in HRV.

Considering the potential maternal benefits, pregnant women are often encouraged to exercise before, during and after pregnancy (Artal & O'Toole, 2003; Hassall, 2011). However, the possibility that regular physical exercise may adversely affect birth weight has been raised but evidence for this remains controversial. Theoretically, the redistribution of blood flow to working muscles during physical exercise might reduce placental circulation, thereby compromising fetal growth and impacting birth weight (Clapp, 2003). Evidence exists for both positive (Clapp, Kim, Burciu, & Lopez, 2000) and negative (Hopkins, Baldi, Cutfield, McCowan, & Hofman, 2010) associations between physical exercise during pregnancy and birth weight. Researchers in this area have concluded that there are still insufficient data to infer important risks or benefits of maternal physical exercise on birth weight (Kramer, Vivrette, Satter, Jouldjian, & McDonald, 2009). This is important because birth weight is known to be a major factor determining many long-term health risks. Low birth weight has been associated with infant mortality and morbidity, childhood development, and health later in life (Frankel, Elwood, Sweetnam, Yarnell, & Smith, 1996; Osler, Lund, Kriegbaum, & Andersen, 2009; Strauss, 2000). Macrosomia has been suggested as a risk factor for diabetes, obesity and metabolic syndrome (Bogaerts et al., 2013; Egeland, Skjaerven, & Irgens, 2000; Kajantie et al., 2005).

The aim of the present study was to address the hypothesis that regular physical exercise during pregnancy is associated with reduced birth weight in the offspring. A second hypothesis was that regular physical exercise during pregnancy is associated with altered ANS activity. Finally, our study aimed to determine whether effects of physical exercise during pregnancy on the offspring birth weight and on the maternal ANS are likely to be independent or might be on a common causal pathway.

6.2 Methods

6.2.1 Participants

Participants in the present study were recruited as part of the Prenatal Early Life Stress (PELS) study, a prospective follow-up study. The overarching goal of PELS is to collect comprehensive data on parents and their children from early pregnancy onwards and followed-up throughout childhood. It focuses on associations between prenatal risk factors, birth outcome and altered child psychophysiology and neurodevelopment.

As part of the PELS study, between 15 and 22 weeks of gestation, 157 pregnant women filled out questionnaires and wore a holter monitor for 24 hours to record their electrocardiogram (ECG) and impedance cardiogram (ICG). This sample of pregnant women had low obstetric risk and made no use of medication or drugs. The Medical Ethics Committee approved the study and all participants provided informed, written consent before participation.

6.2.2 Procedures

Questionnaires filled out by pregnant women

Women were asked at recruitment to provide their height, weight before pregnancy, date of birth and first day of the last menstrual period.

Participants provided more information on the type of physical exercise they recently did. Possible answer options were none, walking, cycling, running and other, in which they needed to specify the type of physical exercise. Popular alternative types were swimming and yoga. Regular physical exercise was determined as follows: no physical exercise, low-intensity physical exercise (e.g. walking or yoga) and moderate-intensity physical exercise (e.g. running, cycling or swimming).

Participants filled out questions constituting the trait anxiety subscale of the Dutch, psychometrically validated version of the State Trait Anxiety Inventory (STAI) (Van der Ploeg et al., 1980). The trait subscale has been identified recently as the best instrument to assess general maternal anxiety during pregnancy (Nast et al., 2013). Trait anxiety refers to differences in anxiety proneness and is seen as a personality trait. This subscale contains 20 items scored from 1 to 4 and has a reliability coefficient (Cronbach's alpha) of 0.75 in our sample.

Maternal ANS functioning

The Vrije Universiteit Ambulatory Monitoring system (VU-AMS) (Goedhart et al., 2007) was used to record mothers' ECG and ICG for 24 hours. Seven Ag/AgCl electrodes were arranged according to the VU-AMS configuration guidelines. The device contained a single axis accelerometer and recorded vertical acceleration as a proxy for gross body movements. The skin was cleaned with alcohol to keep electrode resistance low.

Infant data

All pregnancies were dated using the first day of the last menstrual period and obstetric data including birth weight, gestation length and sex of the baby, were obtained from medical chart review.

6.2.3 Data processing and statistical analyses

ECG and ICG data were processed using custom software written in Matlab (Mathworks, Natick, USA) to obtain HR and indices of sympathetic (pre-ejection period (PEP)) and parasympathetic (RMSSD and HF HRV) ANS activity, according to published standards (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). ECG beat detection was carried out with the Hilbert transform algorithm (Benitez et al., 2001). Potentially erroneous beat detections were identified using a standard approach and screened by the researchers for validity (Berntson et al., 1990). As HRV measures are right-skewed, they were log transformed to normality. PEP, an indicator of sympathetic activity, was calculated from the ICG as the interval between R-wave onset and the B-point. This B-point was identified using an extrapolation technique (Jones, 2006).

We excluded ECG and ICG recordings with a duration of less than 18 hours. For all remaining recordings, average HR, HRV and PEP were calculated for sequential 30-minute periods. To reliably model diurnal variation in parameters, a sinusoidal curve was fitted with robust least square regression (the fundamental frequency of

Fourier analysis) to each of the 24-hour HR, HRV and PEP recordings. The peak and trough of this curve was detected and the difference between these points was calculated (peak-trough difference) to measure the magnitude of the circadian variation in HR, HRV and PEP. Additionally, mean daytime HR, HRV and PEP (11 am till 5 pm) and mean nighttime HR, HRV and PEP (0 am till 6 am) were calculated.

All analyses were conducted using Stata 12.1. Three groups of regression analyses were conducted to examine the association between maternal regular physical exercise during pregnancy and birth weight, and the potential mediating role of maternal ANS functioning (i.e. circadian variations in ANS activity, and daytime and nighttime ANS activity) in this relationship: (a) regression analysis was used to study the potential association between maternal regular physical exercise during pregnancy and birth weight; (b) regression analyses were used to study how maternal regular physical exercise is related to circadian variations (peak-trough difference) in ANS activity, daytime ANS activity and nighttime ANS activity; (c) regression analyses were conducted to examine how circadian variations (peak-through difference) in ANS activity, daytime ANS activity or nighttime ANS activity are related to birth weight, after adjustment for maternal regular physical exercise. If maternal regular physical exercise was a significant predictor in models (a) and (b), and if ANS functioning was a significant predictor for birth weight in model (c), then the mediation of maternal regular physical exercise on birth weight through ANS functioning was evaluated using a bootstrapping method (Preacher & Hayes, 2008). This method provides point estimates and 95% confidence intervals (i.e., percentile confidence interval and bias-corrected confidence interval) for indirect effects. We considered point estimates of indirect effects significant if zero was not contained in the 95% confidence interval.

In regression models (a) and (c) adjustments were made for potential confounders, including maternal trait anxiety, maternal BMI before pregnancy, gestation length and offspring sex[1]. Since diurnal changes in cardiovascular parameters (e.g. HRV) are the result of the influence of both external stimuli (e.g. physical movements) and endogenous homeostatic control mechanisms, in a second step, the regression models (c) were also controlled for physical movements as measured by the accelerometer. The regression analyses (b) were adjusted for maternal trait anxiety and maternal BMI before pregnancy.

[1]Additional adjustment for maternal emotional distress (as measured by the Edinburgh Depression Scale, see Section 2.4.1) or maternal mindfulness (as measured by Freiburg Mindfulness Inventory, see Section 2.4.1) had no effect on the significance of the results.

Table 6.1
Descriptive statistics for variables related to infants and mothers

	M	(SD)
BMI before pregnancy	24.19	(4.29)
Trait anxiety	36.81	(5.54)
Physical movements* (sum of 30 minutes)		
Full day (g)	24.16	(8.21)
Between 11 am and 5 pm (g)	40.76	(16.07)
Between 0 am and 6 am (g)	3.43	(4.32)
Circadian variation (peak-trough difference)		
HR ($ln\,bpm$)	25.38	(10.05)
RMSSD HRV ($ln\,ms$)	2.91	(0.84)
HF HRV ($ln\,ms^2$)	6.04	(1.25)
PEP ($ln\,ms$)	2.96	(0.78)
Nighttime (Between 0 am and 6 am)		
HR (bpm)	76.11	(17.01)
RMSSD HRV ($ln\,ms$)	3.67	(0.55)
HF HRV ($ln\,ms^2$)	6.57	(1.10)
PEP ($ln\,ms$)	4.23	(0.57)
Daytime (Between 11 am and 5 pm)		
HR (bpm)	93.31	(10.65)
RMSSD HRV ($ln\,ms$)	3.23	(0.45)
HF HRV ($ln\,ms^2$)	5.82	(0.91)
PEP ($ln\,ms$)	4.16	(0.35)
Gestational length ($weeks$)	39.56	(1.50)
Offspring birth weight (g)	3436.46	(493.80)

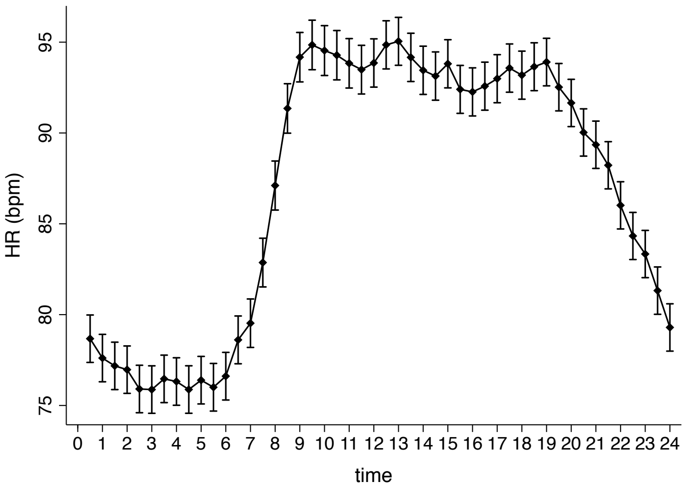
* Physical movements are expressed in units of gravity (g).

6.3 Results

Out of 157 pregnant women who wore a holter monitor and filled out the questionnaires, 15 women had ECG recordings with a length of less than 18 hours. Characteristics of mothers and offspring are presented in Table 6.1. There was a circadian rhythm in each cardiovascular parameter. Figures 6.1a, 6.1b, 6.2a and 6.2b show estimated marginal means of HR, RMSSD HRV, HF HRV and PEP by time.

Regression model (a) There was a significant negative association between regular maternal moderate-intensity physical exercise and offspring birth weight ($\beta = -0.22, b = -210.936, SE = 73.86, p < .005$), indicating that the offspring of women who exercise with moderate intensity weighs 211g less than the offspring of sedentary women. Maternal physical exercise explained an additional 4% of the variance in offspring birth weight, beyond the 34% explained by the control variables.

Regression model (b) There was a trend towards a significant relation between maternal low-intensity physical exercise and the circadian variation in RMSSD HRV ($\beta = 0.19, p = .07$). Moreover, there were significant and marginally significant as-



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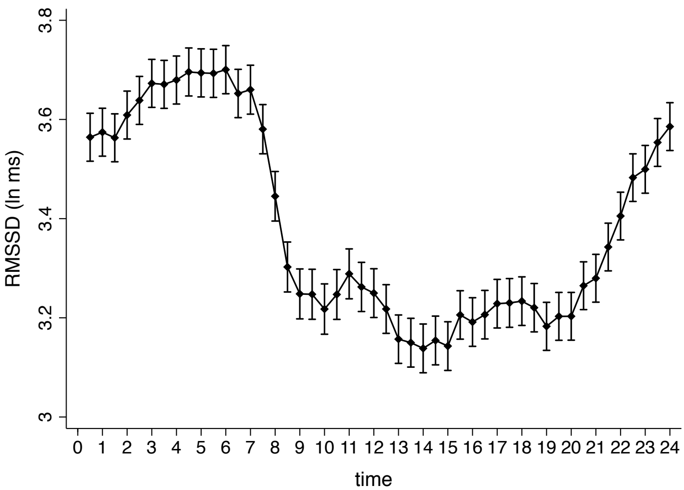

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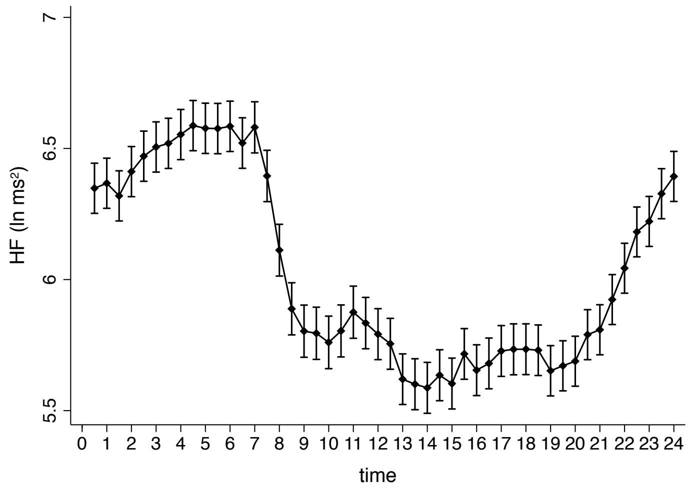
Figure 6.1. Estimated marginal means of HR and RMSSD HRV by time



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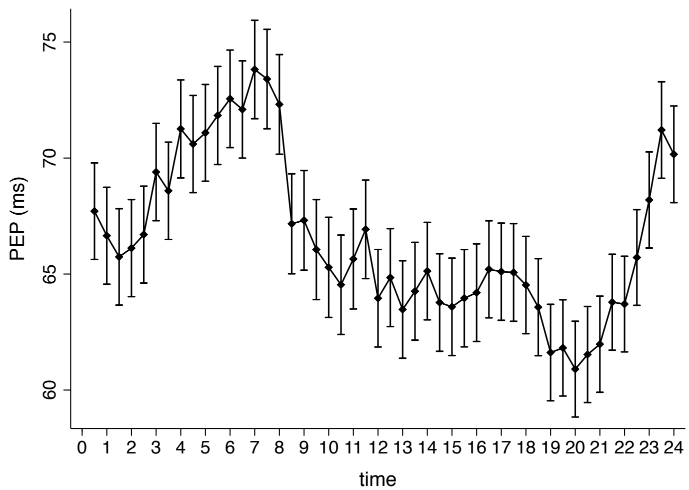


(b)

Figure 6.2. Estimated marginal means of HF HRV and PEP by time

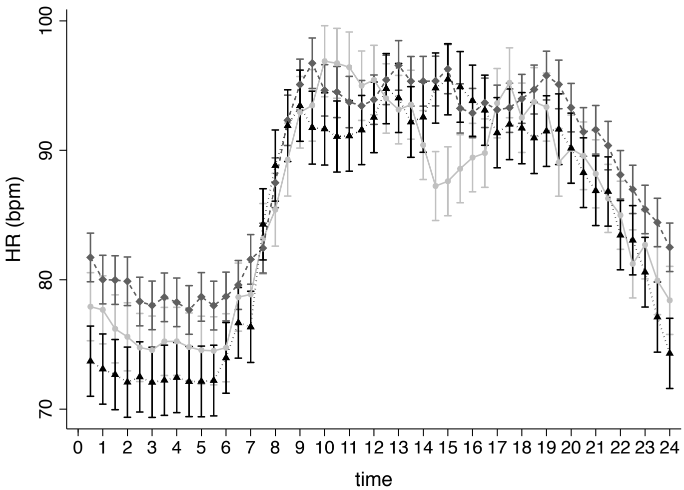
sociations between moderate-intensity physical exercise and the circadian variation in RMSSD HRV ($\beta = 0.23, p = .03$) and HF HRV ($\beta = 0.18, p = .08$) respectively. Maternal physical exercise explained 2% of the variance in the circadian variation. Control variables were not significant. Maternal physical exercise was not significantly associated with circadian variation in HR and PEP ($p > .05$). No significant associations were found between maternal regular physical exercise and daytime and nighttime ANS activity ($p > .05$).

Regression model (c) The circadian variation in RMSSD HRV and HF HRV was related significantly to offspring birth weight (RMSSD HRV: $\beta = -0.19, p = .007$; HF HRV: $\beta = -0.18, p = .01$), adjusted for regular maternal physical exercise. Circadian variation in RMSSD HRV and HF HRV explained an additional 4% of the variance in birth weight beyond the 38% by maternal exercise and covariates. These associations between offspring birth weight and circadian variation in HRV remained significant when additionally controlled for maternal physical movements. The circadian variation in HR and PEP were not associated with offspring birth weight ($p > .05$). Nighttime RMSSD HRV and HF HRV were significantly associated with offspring birth weight (RMSSD HRV: $\beta = -0.15, p = .02$; HF HRV: $\beta = -0.15, p = .02$), explaining an additional 3% of the variance in birth weight beyond 38% explained by maternal physical exercise and control variables. These associations remained significant when adjusted for maternal physical movements (RMSSD HRV: $\beta = -0.16, p = .03$; HF HRV: $\beta = -0.17, p = .03$). Nighttime HR and PEP were not significantly related to offspring birth weight, nor were any daytime ANS measures. Figures 6.3a, 6.3b, 6.4a and 6.4b show estimated marginal means of respectively HR, RMSSD HRV, HF HRV and PEP by time and upper and lower quartiles of birth weight, compared to the central half of the birth weight distribution.

Given the significant association between maternal regular physical exercise and circadian variation in RMSSD HRV, a mediation of maternal regular physical exercise on offspring birth weight through circadian variation in RMSSD was evaluated. The point estimate of the indirect link between regular moderate-intensity physical exercise and offspring birth weight was significant (Percentile CI = $[-216.04, -13.17]$; Bias-corrected CI = $[-236.27, -22.23]$).

6.4 Discussion

The present study suggests that regular moderate-intensity physical exercise during pregnancy is negatively associated with offspring birth weight. Furthermore, evidence was provided that the relationship between regular physical exercise and



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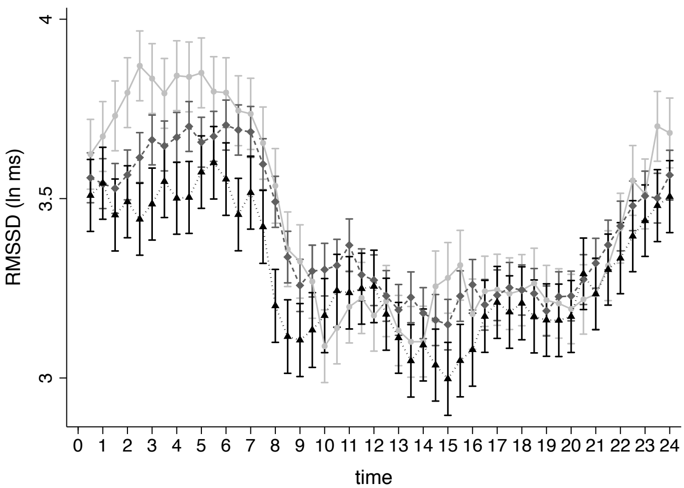

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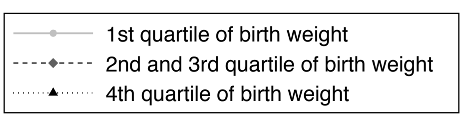


Figure 6.3. Estimated marginal means of HR and RMSSD HRV by time and upper and lower quartiles of birth weight, compared to the central half of the birth weight distribution.

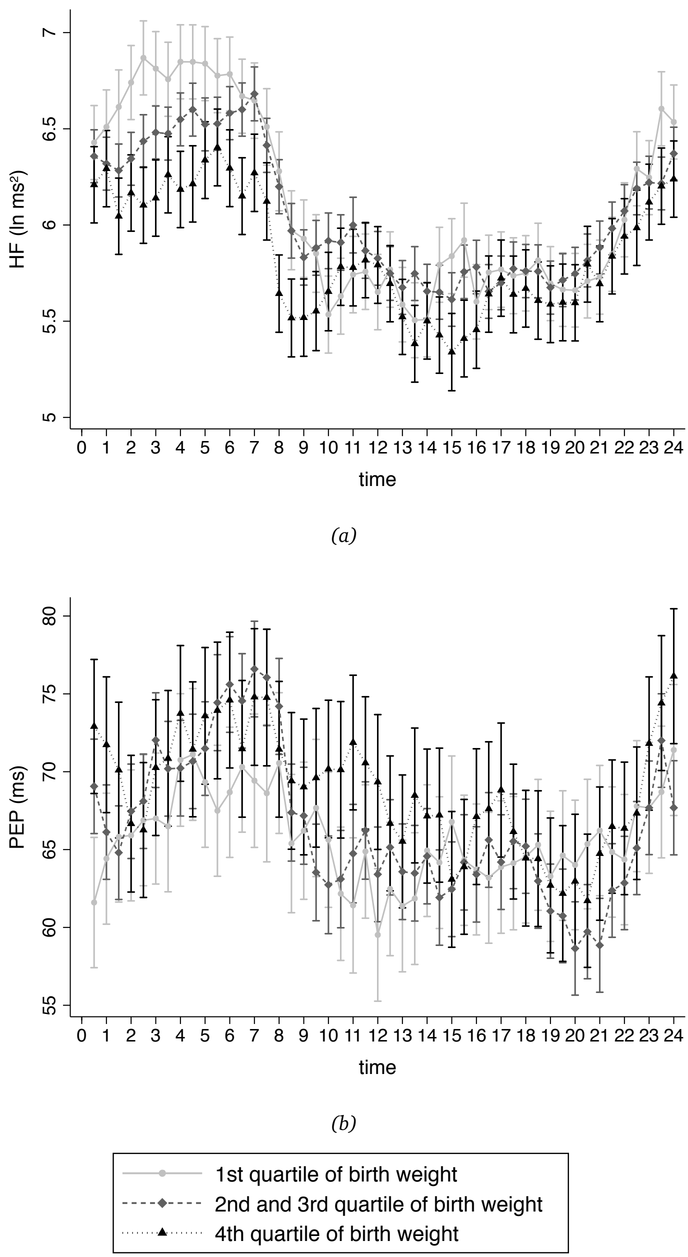


Figure 6.4. Estimated marginal means of HF HRV and PEP by time and upper and lower quartiles of birth weight, compared to the central half of the birth weight distribution.

offspring birth weight is partially mediated through circadian variation in parasympathetic activity.

To the best of our knowledge, this is the most detailed longitudinal study that gives insight into circadian variations in autonomic functioning in pregnancy. There only exist two previous studies on 24-hour recordings of HRV during pregnancy (Ekholm et al., 1997; Stein, Hagley, et al., 1999). However, these studies were based on much smaller samples and did not include PEP as a measure of sympathetic activity, limiting the knowledge about circadian ANS function during pregnancy. Moreover, previous studies did not examine how physical exercise during pregnancy potentially affects circadian variations in autonomic functioning, nor was it investigated how circadian variations in autonomic functioning are associated with offspring birth weight.

Whereas low-intensity physical exercise (e.g. walking or yoga) did not significantly affect offspring birth weight, there was found a significant negative association between moderate-intensity physical exercise (e.g. running, swimming and cycling) and offspring birth weight. Previous studies found both positive (Clapp et al., 2000) and negative (Hopkins et al., 2010) associations between physical exercise during pregnancy and birth weight. There seems to be a growing consensus that low-intensity or low-to-moderate intensity physical exercise is not a risk factor for lower birth weight and can even play a protective role during pregnancy in reducing risks of developing preeclampsia, hypertension and gestational diabetes mellitus (Melzer et al., 2010; Schlüssel, Souza, Reichenheim, & Kac, 2008). However, comparison of various studies requires careful attention, as measures of physical exercise can greatly differ.

A common shortcoming is that the type, intensity, frequency, and duration of the physical exercise are not measured (Melzer et al., 2010); this is also the case in the present study. Furthermore, as physical exercise often changes in terms of type and intensity as pregnancy advances (Haakstad, Voldner, Henriksen, & Bø, 2009), future research ideally determines physical exercise in multiple stages of pregnancy, as well as before pregnancy (Henriksen, 2008). It must be noted that advice regarding the optimal level of physical exercise during pregnancy probably always needs to be personalized on the basis of personal health status (e.g. obesity) and risk factors of both low birth weight and fetal macrosomia. The results in the present study suggest that ANS monitoring has the potential to play a guiding role for health professionals in determining the optimal exercise level.

An important contribution to the literature is the result that the relationship between maternal regular physical exercise and offspring birth weight might be partially mediated through the circadian variation in parasympathetic activity. The enhanced circadian variation in ANS activity is probably the result and/or a marker of chronic physical exercise, which is linked to fetal growth and birth weight in various ways. For example, during physical exercise circulatory redistribution can shift the blood concentration from the uterus and placenta to the extremities (Clapp, 2003; Schlüssel et al., 2008). Interestingly, the net adverse outcome of the altered uterine blood flow might be still minimal as fluctuations in oxygen and nutrient delivery to the placenta caused by physical activities have been associated with greater placental villous vascular volume, a higher proliferation index, and a stimulated placental growth (Bergmann, Zygmunt, & Clapp, 2004; Clapp, 2006). However, regular physical exercise might also decrease the normal insulin resistance in pregnancy, leaving the fetus and mother to compete with each other for glucose (Hopkins et al., 2010; Jukic et al., 2012).

Our study has a number of strengths. Compared to previous studies, we examined a relatively large population of pregnant women and assessed ANS functioning based on 24-hour ambulatory ECG and ICG recordings. Short-term ECG and ICG recordings can provide relevant data on ANS activity under specific controlled conditions. However, 24-hour ambulatory ECG and ICG recordings provides a better view on individuals' ANS activity in normal day-to-day activities and allows the analysis and comparison of sleep and circadian rhythm data.

All regression models were controlled for potential confounders of ANS activity and birth weight, including maternal trait anxiety, BMI, gestational length and offspring's sex. Additionally, adjustments were made for current physical movements to obtain a more pure measure of circadian rhythm. The present study was part of a larger study (cfr. PELS) in which pregnant women needed to fill out various questionnaires. Therefore, it was decided to use a relatively short questionnaire for the physical exercise assessment. Future research should attempt to collect more detailed and objective exercise data and use exercise diaries to improve the physical exercise measure with information on the intensity, frequency and duration of physical activities. Nevertheless, short questionnaires to assess physical exercise have been reported to be fairly reliable and reasonably valid (Wendel-Vos, Schuit, Saris, & Kromhout, 2003).

Besides extending the measure of physical exercise, it might also be important for future studies to include posture information as well, as standing and supine positions influence maternal ANS activity and the uterine blood flow (Jeffreys, Stepanchak, Lopez, Hardis, & Clapp, 2006; Sohn, Kesternich, & Fendel, 1989). Likewise, it has been reported that the parasympathetic activity in pregnant women during sleep is greater in the left lateral position compared to the supine and right lateral positions (Kuo, Chen, Yang, & Tsai, 1997).

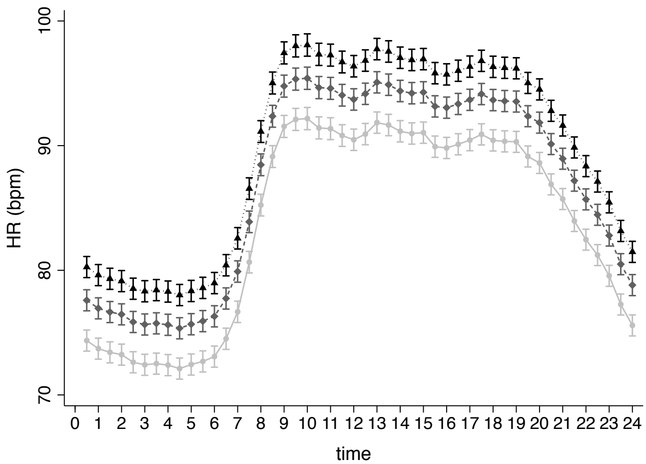
In conclusion, the present study provided comprehensive insight into circadian variations in autonomic functioning in pregnancy. The results suggest that regular physical exercise during pregnancy might be beneficial for the pregnant women, as it has the potential to enhance circadian variation in ANS activity, and at the same time it might have an adverse birth outcome, as regular moderate-intensity maternal physical exercise during pregnancy and circadian variations in ANS activity were negatively associated with offspring birth weight.

Supplement:
Circadian rhythms in all three pregnancy trimesters

In this chapter only the 24-hour ECG and ICG data collected in the second pregnancy trimester was used, because the PELS study only collected physical exercise data in the second trimester. There only exist two previous studies on 24-hour recordings of HRV during pregnancy (Ekholm et al., 1997; Stein, Hagley, et al., 1999). However, these studies were based on much smaller samples (i.e. respectively 16 and 20 pregnant women) and/or only focused on one pregnancy trimester and did not include PEP as a measure of sympathetic activity, limiting the knowledge about circadian ANS functioning during pregnancy. Therefore, in this supplementary section the 24-hour data from all pregnancy trimesters are presented (see Table 6.2), showing that circadian rhythms of HR, HRV and PEP persist in all three trimesters of pregnancy. Repeated measures ANOVA showed that HR, HRV and PEP significantly differed across pregnancy trimesters ($p < .001$). Figures 6.5a, 6.5b, 6.6a and 6.6b show estimated marginal means of HR, RMSSD HRV, HF HRV and PEP by time in each pregnancy trimester.

Table 6.2
Cardiovascular characteristics of mothers throughout pregnancy (24-hour recordings)

	1st pregnancy trimester (n=147)		2nd pregnancy trimester (n=142)		3rd pregnancy trimester (n=126)	
	M	(SD)	M	(SD)	M	(SD)
Circadian variation (peak-trough difference)						
HR ($ln\, bpm$)	25.33	(9.77)	25.38	(10.05)	25.74	(10.69)
RMSSD HRV ($ln\, ms$)	2.88	(0.91)	2.91	(0.84)	2.57	(1.06)
HF HRV ($ln\, ms^2$)	6.08	(1.25)	6.04	(1.25)	5.73	(1.38)
PEP ($ln\, ms$)	2.99	(0.766)	2.96	(0.78)	2.84	(0.91)
Nighttime						
HR (bpm)	72.32	(10.76)	76.11	(17.01)	79.12	(15.27)
RMSSD HRV ($ln\, ms$)	3.73	(0.53)	3.67	(0.55)	3.52	(0.59)
HF HRV ($ln\, ms^2$)	6.63	(1.05)	6.57	(1.10)	6.36	(1.15)
PEP ($ln\, ms$)	4.31	(0.41)	4.23	(0.57)	4.07	(0.66)
Daytime						
HR (bpm)	90.84	(9.75)	93.31	(10.65)	97.73	(11.84)
RMSSD HRV ($ln\, ms$)	3.30	(0.45)	3.23	(0.45)	3.08	(0.50)
HF HRV ($ln\, ms^2$)	5.94	(0.88)	5.82	(0.91)	5.67	(1.17)
PEP ($ln\, ms$)	4.24	(0.27)	4.16	(0.35)	3.97	(0.40)



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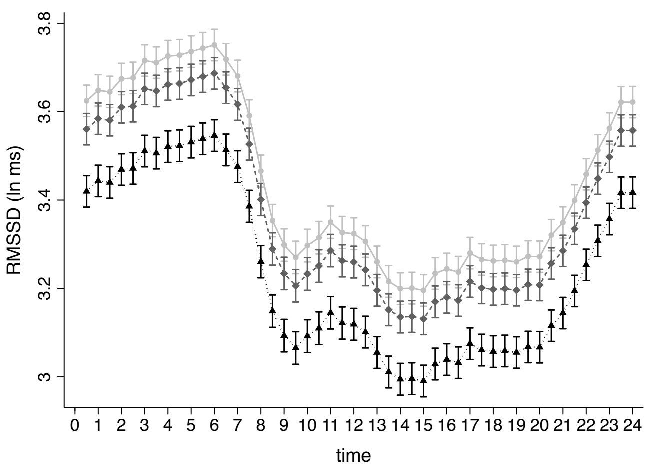

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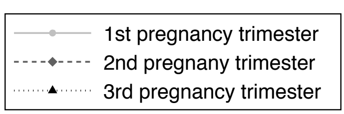
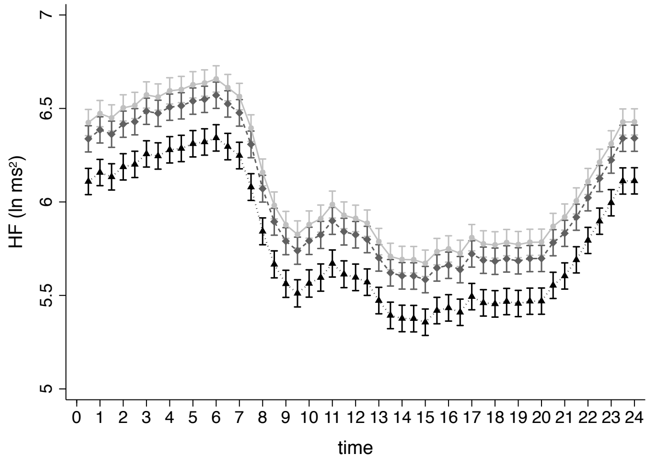


Figure 6.5. Estimated marginal means of HR and RMSSD HRV by time and in each pregnancy trimester.



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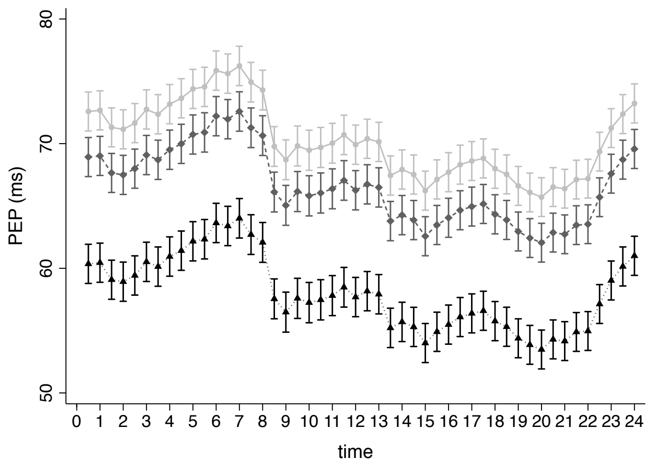

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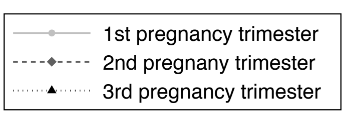

Figure 6.6. Estimated marginal means of HF HRV and PEP by time and in each pregnancy trimester.

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7

General discussion

7.1 Introduction

The main aim of this dissertation was to provide insight in how ANS activity changes during pregnancy and how psychological functioning is associated with ANS activity. Furthermore, it was also examined how maternal psychophysiological functioning is related to offspring birth weight and psychophysiological development.

First, the main findings of the four studies included in this dissertation are summarized (Section 7.2). Next, based on the main results and supported by discussion sections from previous chapters, a detailed and critical discussion on the research methodology is presented, highlighting the dissertation's strengths and limitations, complemented with concrete suggestions for improvement (Section 7.3). Subsequently, underlying mechanisms and explanations for the main results are described (Section 7.4). Finally, directions for future research (Section 7.5) and clinical implications are discussed (Section 7.6). This dissertation ends with a general conclusion (Section 7.7).

The research questions that were addressed in this dissertation are:

1. What is the typical autonomic reactivity and recovery during pregnancy and how is it related to the pregnant woman's level of trait anxiety? (Chapter 3)

2. Is there an association between past anxiety disorder, women's heart rate variability during pregnancy, offspring's heart rate variability and fearfulness? (Chapter 4)

3. Is mindfulness associated with better cardiovascular adaptation during pregnancy, better maternal mental health and better social-emotional development of the infant at 4 months? (Chapter 5)

4. Is there an association between physical exercise during pregnancy, circadian ANS functioning and offspring birth weight? (Chapter 6)

7.2 Main findings

The main results presented in Chapter 3, showed that HR and HRV reactivity to the stress phases were significantly reduced in the third pregnancy trimester, compared to the first trimester. Previous studies presented similar results for attenuated HR and blood pressure reactivity in late pregnancy (DiPietro et al., 2003; Entringer et al., 2010). Importantly, the present study also found significant reduced autonomic stress reactivity (as reflected in HRV reactivity) in the third pregnancy trimester, for which so far only marginal evidence existed (Klinkenberg et al., 2009). Additionally, the results in Chapter 3 also suggest that mothers with a high level of trait anxiety have less dampened stress reactivity in late pregnancy. From previous studies it is known that cardiovascular stress reactivity is attenuated in late pregnancy (DiPietro et al., 2003; Entringer et al., 2010), as well as reduced hormonal (Kammerer et al., 2002) and psychological reactivity in late pregnancy (Glynn et al., 2001). Whereas HR and HRV reactivity significantly differed between pregnancy trimesters, only HR recovery, and not HRV recovery, was significantly different between trimesters. Furthermore, no significant interaction between trait anxiety and pregnancy trimester on HR or HRV recovery was found.

Chapter 4 indicated that pregnant women with a past, but no current, anxiety disorder have autonomic abnormalities (i.e. reduced parasympathetic activity) in the first pregnancy trimester and that these abnormalities may impact on the future physiological (i.e. reduced parasympathetic activity) and mental (fearfulness) wellbeing of their offspring. Pregnant women with a past anxiety disorder displayed reduced parasympathetic nervous system activity (indexed by HRV), compared to those without a past anxiety disorder. This result is consistent with prior research that shows that people with past psychiatric disorders have lower HRV (Kemp & Quintana, 2013; Kemp et al., 2012, 2010; Licht et al., 2008). The novel contribution made is the result that pregnant women with a past anxiety disorder also have reduced HRV and these psychophysiological alterations may impact on the future physiological and mental wellbeing of their offspring. In the study described in Chapter 4 the offspring of mothers with a past anxiety disorder also had lower parasympathetic activity (i.e. lower HRV) than those whose mothers had been healthy. Furthermore, in the group of mothers with past anxiety disorder there was also a positive association between maternal HRV and offspring HRV. Finally, reduced parasympathetic activity in the offspring was also linked to psychological consequences. HRV of children at 2-4 months was associated with observed fearfulness at the age of 9 months, which is consistent with previous research that indicates strong associations between low HRV and fearful temperament (reflected in inhibited behavior, shyness, harm avoidance, anxiety and distress) (Calkins & Swingler, 2012; Kagan, 1982; Kagan et al., 1987).

The results in Chapter 5 showed that mothers experienced significant increases of HR and marginally significant increases of DBP over the course of pregnancy. PEP and HRV significantly fell between the first and third pregnancy trimester. This is in line with previous studies, which found that HRV tends to decrease as HR and BP rise (Christian, 2012; Hermida et al., 2000) over the course of pregnancy (Kuo et al., 2000). Importantly, the more mindful a mother was the less there was a fall in parasympathetic activity (as expressed in RMSSD HRV and HF HRV) and an increase in sympathetic activity (inversely related to PEP) between trimesters. Mindfulness trait was also related to baseline RMSSD and HF HRV, but not to baseline HR, LF HRV, PEP or blood pressure (SBP and DBP). This suggests that mindfulness trait is predominantly related to parasympathetic activity. This is in line with studies with non-pregnant women, which found that components of mindfulness-based stress reduction interventions tend to increase the parasympathetic ANS mediated component (HF) of HRV, (Ditto et al., 2006; Leonaite & Vainoras, 2010; Sarang & Telles, 2006; Takahashi et al., 2005; Tang et al., 2009; Wu & Lo, 2008) although this is not found in all studies (Nyklíček et al., 2013; Paul-Labrador et al., 2006). No association was found between mindfulness trait and blood pressure during pregnancy, despite findings that meditation can reduce blood pressure (SBP and DBP) in non-pregnant participants (Anderson et al., 2008; Chiesa & Serretti, 2010; Goldstein et al., 2012; Nyklíček et al., 2013). Almost three decades ago a study with 60 pregnant women showed that systematic relaxation training lowered SBP and DBP in comparison to a control group (Little et al., 1984), but this finding has not been replicated. Mindful pregnant women had less depressive symptoms, both during and after pregnancy and higher parasympathetic activity (reflected by RMSSD and HF HRV). This supports earlier findings that techniques enhancing mindfulness during pregnancy reduce anxiety and perceived stress for up to two months after the intervention (Bastani et al., 2005; Beddoe et al., 2009; Vieten & Astin, 2008). The relationship between mindfulness trait and depressive symptoms after pregnancy remained significant after adjustment for prenatal maternal depressive symptoms. Finally, Chapter 5 also provides evidence that more mindful mothers tend to have an offspring with fewer problems in adaptive functioning. Other social-emotional development measures were not associated with mindfulness.

In Chapter 6 it was suggested that pregnant women who engaged in moderate-intensity physical exercise (e.g. running, swimming and cycling) tend to have an improved ANS functioning as shown by an enlarged circadian variation in parasympathetic activity, but also may have an offspring with a lower birth weight compared to pregnant women who do not often exercise. Additionally, and importantly, the results indicated that the negative association between physical exercise and offspring birth weight was mediated through the enlarged circadian variation in parasympathetic activity. The consequences of physical exercise during pregnancy have, for a long time, been a topic of debate, with contradictory results with respect to its association with birth weight. Both positive (Clapp et al., 2000) and negative (Hopkins et al., 2010) associations have been found. Notably, more and more researchers seem to agree that low-intensity or low-to-moderate-intensity physical exercise is not a risk factor for lower birth weight and can even play a protective role during pregnancy in reducing risks of developing preeclampsia, hypertension and gestational diabetes mellitus (Melzer et al., 2010; Schlüssel et al., 2008). This is in line with our findings that light-intensity physical exercise such as walking or yoga is linked to improved ANS functioning, but it is not associated with birth weight. Previous research also found beneficial cardiovascular outcomes associated with physical exercise during pregnancy, such as lower HR during rest, as well as reduced blood pressure and enhanced vagal tone (Melzer et al., 2010). This dissertation complements these findings with evidence pointing to a link between physical exercise during pregnancy and circadian variation in parasympathetic activity, measured by RMSSD HRV and HF HRV.

7.3 Methodological considerations

7.3.1 ANS-related cardiovascular measures

It is often suggested that altered activity in stress response systems such as the ANS and HPA axis might mediate and thus provide insight into the relationship between maternal psychological functioning (e.g. distress or anxiety) during pregnancy and fetal (brain) development and later cognitive, behavioral and social-emotional development of the child (Glover, 2011; Talge et al., 2007; Van den Bergh et al., 2005; Weinstock, 2008). Maternal psychological functioning and associated physiological responses may affect fetal development as it may cause altered blood flow to the uterus (Dipietro, 2012; Mulder et al., 2002; Van den Bergh et al., 2005). Nevertheless, there is a lack of studies investigating ANS activity during pregnancy and its relationship with the psychological functioning of the pregnant woman. Therefore, the main aim of this dissertation was to provide insight in how ANS (re)activity changes during pregnancy and how psychological functioning is associated with the ANS activity.

Existing studies on physiological stress responsiveness during pregnancy are mostly limited to examining HR and BP reactivity. The study described in Chapter 3 complements these studies by presenting evidence for attenuated autonomic responses to stress as pregnancy advances. Consistent with prior research, people with past psychiatric disorders have altered autonomic activity (as indexed by a lower HRV) (Kemp & Quintana, 2013; Kemp et al., 2012, 2010; Licht et al., 2008). The novel contribution made by the study presented in Chapter 4 is that a past anxiety disorder is also linked to reduced HRV during pregnancy. Chapter 5 extends the existing literature by showing how ANS activity changes during pregnancy and how psychological functioning (i.e. mindfulness) is related to these physiological adaptations. Finally, to the best of our knowledge, Chapter 6 presented the most detailed longitudinal study that gives insight into circadian variations in autonomic functioning in pregnancy.

Both PEP and skin conductance measures are often used as non-invasive indices of SNS activity. Both measures are responsive to SNS activity, but PEP and skin conductance responses tend to be uncorrelated, suggesting that the heart and skin might reflect different aspects of SNS activity (Goedhart, Willemsen, & De Geus, 2008). It is believed that maternal psychological functioning and associated changes in ANS activity may affect fetus development through altered blood flow to the uterus (Dipietro, 2012; Mulder et al., 2002; Van den Bergh et al., 2005). Therefore, the research presented in this dissertation made use of impedance cardiography to calculate PEP and assess mechanisms underlying blood pressure change. No previous studies exist that examined SNS activity during pregnancy using PEP.

A mental arithmetic task was used to examine autonomic reactivity during pregnancy. Notably, the stress phases in the task did not lead to significant changes in PEP. This suggests that stress responses elicited with the mental arithmetic task are mainly reflected in a decreased PNS activity, but not in a significant change in SNS activity. The lack of PEP reactivity could also suggest that the mental arithmetic task might be not threatening or stressful enough to activate the sympathetic nervous system. However, a test protocol must be selected with care, as a (too) strong stressor may result into only a minor interindividual variability, making it difficult to reveal differences in PEP reactivity (Monk et al., 2001). It is also possible that it is not the intensity of the stressor, but the type of stressor that can explain the absence of significant PEP responses to stress. Laboratory stressors do not always provoke subjects' affective response. Social interaction stressors such as public speaking tasks are often used to provide a more appropriate social context in which negative emotions can be elicited (Waldstein, Neumann, Burns, & Maier, 1998).

It must be noted that mother's ICG was recorded using four spot electrodes. It cannot be excluded that a band electrode configuration could have resulted in a different outcome. Although both spot and band electrodes tend to be highly correlated for baseline PEP measurements, lower correlations have been reported when assessing PEP responsiveness to a mental arithmetic stressor (McGrath, O'Brien, Hassinger, & Shah, 2005). Furthermore, from our own experience, we learned that placing electrodes in late pregnancy can be challenging given the distended abdomen.

7.3.2 Reactivity and recovery measures

Compared to previous studies on cardiovascular responsiveness (reactivity and recovery) during pregnancy, this dissertation complements the literature by studying a relatively large population of pregnant women in both the first and third pregnancy trimester, using multiple presentations of the stressor and including autonomic measures such as HRV and PEP.

It must be noted that in the present study the same stress task was used at the beginning and the end of pregnancy. Therefore, in theory, any decrease in stress responsiveness across pregnancy can also be contributed to a habituation process. Future study designs should have appropriate control groups to assess this possibility.

The stress task used in this dissertation has five-minute relaxation phases for each participant. As individuals can vary greatly in the speed of physiological recovery from a stressor, five minutes might be not enough to truly measure physiological recovery (de Weerth & Buitelaar, 2005). A better approach could be to observe how long it takes to return to baseline HR and HRV levels after termination of a stressor (Stewart & France, 2001). Such a recovery measure might reveal more inter-individual differences, which might have stronger relationships to the psychological well-being of the pregnant woman.

It is important to note that the stress task was not performed at the same time of the day for each subject. On the one hand, given the existence of circadian rhythms of HR and HRV, it may have been better to study ANS activity during specific time frames. On the other hand, there exists evidence that comparable HPA axis and HR responses to stress can be measured in the morning and afternoon (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004).

There is good evidence that increased reactivity during pregnancy may amplify the effect of underlying mental disorders or cardiovascular risk factors (Magnussen et al., 2009). In non-pregnant humans there is a growing belief that both exaggerated and diminished reactivity to stress may indicate greater cardiovascular disease risk (Lovallo, 2011). Such a quadratic relationship between stress reactivity and any adverse outcomes or measure of psychological functioning was not examined in Chapter 3.

7.3.3 24-hour ECG and ICG recording

An essential strength of this dissertation is the inclusion of a longitudinal study that gives insight into circadian variations in autonomic functioning in pregnancy, based on 24-hour ambulatory ECG and ICG recordings. Short-term ECG and ICG recordings can provide relevant data on ANS activity under specific controlled conditions. However, 24-hour ambulatory recordings provide a better view on individuals' ANS activity in normal day-to-day activities, increasing the ecological validity of research conducted in this dissertation. Moreover, 24-hour recordings allow the analysis and comparison of sleep and circadian rhythm data. The main challenge compared to short-term laboratory recordings, is that participants are required to wear a monitor without attendance, increasing the risk of losing data if something goes wrong during the recording. Common problems experienced in the context of this dissertation were a loose or dried electrode, a wire dysfunction, subjects not wanting to wear the monitory device anymore after a while, subjects forgetting to switch on the device after they took a shower, accidently switching off the device and batteries dying sooner than expected.

There only exist two previous studies on 24-hour recordings of HRV during pregnancy (Ekholm et al., 1997; P. K. Stein et al., 1999). However, these studies were based on much smaller samples (i.e. respectively 16 and 20 pregnant women) and/or only focused on one pregnancy trimester and did not include PEP as a measure of sympathetic activity, limiting the knowledge about circadian ANS functioning during pregnancy. This dissertation showed that circadian rhythms of HR, HRV and PEP persist in all three trimesters of pregnancy.

Since diurnal changes in cardiovascular parameters (e.g. HRV) are the result of the influence of both external stimuli (e.g. physical movements) and endogenous homeostatic control mechanisms, the analyses related to the circadian variation in ANS activity were controlled for physical movements as registered by the VU-AMS. It is important to note that the accelerometer in the VU-AMS was one-dimensional. Three-dimensional accelerometers might provide more details about the physical movements, but measurements by one-dimensional and three-dimensional accelerometers tend to be strongly correlated (Welk, Blair, Wood, Jones, & Thompson, 2000). At the time of writing an updated VU-AMS has been released, which enables the registration of motion along three axes.

7.3.4 Longitudinal design

As described in Chapter 1 and confirmed by the studies presented in Chapters 3, 5 and 6, pregnancy is a time of marked physiological changes (e.g. decrease of parasympathetic activity). The prospective longitudinal design is an important strength of the research presented in this dissertation. There are only few studies investigating pregnant women across different pregnancy trimesters. In contrast to the design used throughout this dissertation, existing reports often use different participant samples in different stages of pregnancy (Kuo et al., 2000; McCubbin et al., 1996). However, such a design could result into misleading results, as it is known that there is a wide inter-individual variation in cardiovascular stress reactivity (de Weerth & Buitelaar, 2005; Monk et al., 2001; Piha, Puukka, & Seppänen, 1991; Woisetschläger et al., 2000). Compared to previous studies, we studied a relatively large population of pregnant women and included data from the three pregnancy trimesters. Most prior studies have focused on the second or third trimester only.

The longitudinal nature of the study and the fact that most pregnant women voluntary participated, may explain the biased sample with a higher education in comparison with the general population of pregnant women. Furthermore, as with many longitudinal studies some subjects in the PELS study stopped participating as the study advanced. This is certainly not surprising in a pregnant population as pregnancy is a time of many changes and pregnant women are not always aware of what exactly they are able to manage as the pregnancy advances.

An important strength in this dissertation was the use of multilevel statistical methods, allowing to use data from multiple phases (cfr. mental arithmetic task) and pregnancy trimesters in one model. Additionally, multilevel models can incorporate both continuous and categorical predictors.

7.3.5 Anxiety symptoms versus anxiety disorders

In studying the psychological functioning of pregnant women and its relationship to ANS functioning a key focus in this dissertation was on anxiety. An important strength of this dissertation is that both anxiety symptoms and anxiety disorders were examined in its association with ANS functioning. While previous research predominantly focused on current anxiety disorders and found associations with reduced parasympathetic activity, a novel contribution made in this dissertation is the result that pregnant women with a past anxiety disorder also have reduced parasympathetic activity. Importantly, this finding remained significant after controlling for confounding factors including state anxiety.

No distinction was made between various types of anxiety disorders. In a non-pregnant population humans with a specific anxiety or social phobia had greater cardiovascular responses than controls, while patients with post-traumatic stress disorder or panic disorders had lower reactivity compared to controls (Cuthbert et al., 2003). Hence, results may vary between different types of anxiety disorders.

Additionally, assessment of general anxiety during pregnancy may underestimate anxiety specifically related to pregnancy (e.g. worrying about the fetus' health). There exists evidence that pregnancy anxiety rather than general anxiety is a predictor of birth outcome (Huizink, Mulder, Robles de Medina, Visser, & Buitelaar, 2004). Therefore, some of the associations found between anxiety and ANS might have been more pronounced when specifically pregnancy anxiety was measured.

7.3.6 Positive characteristics of psychological functioning and ANS activity

There are numerous studies that have focused on the effects of negative emotions (anxiety and depression) during pregnancy. However, very few studies have investigated the possibility of enhancing psychological characteristics such as mindfulness to counteract the effects of these negative emotional states. The results in Chapter 5 linked mindfulness to less depressive symptoms during and after pregnancy, better maternal physiological adaptations during pregnancy and better social-emotional development of the offspring. Interventional randomized controlled trials need to be conducted to examine the effect of psychological interventions on positive factors such as mindfulness in pregnancy. There is the potential for significant mental and physiological benefits for both mothers and their children.

Chapter 6 provided evidence that moderate-intensity physical exercise during pregnancy (e.g. running, swimming and cycling) may improve ANS functioning, as it was associated with enlarged circadian variation in parasympathetic activity. Additionally, it was shown that circadian variation in ANS activity mediated the association found between moderate-intensity physical exercise and reduced birth weight. An important methodological consideration hereby is the physical exercise measure used in this dissertation. As mentioned in Chapter 2 the present studies were part of a larger study (cfr. PELS) in which pregnant women needed to fill out various questionnaires. Therefore, it was decided to use a relatively short questionnaire for the physical exercise assessment. Information regarding the type, intensity, frequency, and duration of the physical exercise was not collected. However, short questionnaires to assess physical exercise have been reported to be fairly reliable and reasonably valid (Wendel-Vos et al., 2003).

As discussed in Section 1.4 mindfulness and physical exercise are related to negative emotions such as depression and anxiety. Although no clear contraindication was found in the studies described in this dissertation, future studies need to confirm that the associations found between autonomic functioning and maternal anxiety can not completely explained by the association between autonomic functioning and mindfulness or physical exercise (and vice versa).

7.3.7 Child outcomes

The main aim of this dissertation was to provide insight in how ANS activity changes during pregnancy and how psychological functioning is associated with the ANS activity. Secondary, it was also examined how maternal psychological functioning is related to offspring birth weight and psychophysiological development.

The measure for social-emotional development, which was associated with mindfulness, was derived from the ASQ-SE filled out by the mothers. The results showed only a significant positive relationship between mindfulness and the subscale 'adaptive functioning'. It is not certain whether this finding truly reflects a very specific altered social-emotional development in adaptive functioning or whether other subscales of the ASQ-SE were not sufficiently sensitive in this dissertation's infant sample. Although the questionnaire was designed for 3 to 8 months old infants, many of the subscales are likely to be more suitable to children at the upper end of this age range. Our participants were 4 months old and their data on communication and interaction with others, for example, may have had limited power to discriminate the influence of maternal mindfulness.

Chapter 4 studied the association maternal past anxiety disorder and offspring's ANS functioning, and the association between offspring's ANS functioning and fearfulness. The fearfulness measure was based on the Lab-TAB, which is a leading observational measure of childhood temperament. The strength of this measure is the observational nature of it, avoiding bias in parental perceptions.

7.4 Underlying mechanisms and explanations

7.4.1 Maternal anxiety and autonomic functioning

The links found between maternal anxiety and autonomic functioning are supported by various theories integrating psychological and autonomic functioning. Below two popular models are discussed and related to results published in this dissertation.

The model of neurovisceral integration in emotion regulation and dysregulation describes a functional framework that integrates affective regulation, attentional regulation, and ANS functioning (Friedman & Thayer, 1998a; Thayer & Lane, 2000). In this model the central autonomic network (CAN) receives and integrates visceral, humoral, and environmental information and coordinates autonomic, endocrine, and behavioral responses to environmental challenges. A failure to adaptively respond to environmental demands may enhance feelings of anxiety and thus may increase the risk for anxiety problems (Thayer & Lane, 2000). The interplay of sympathetic and parasympathetic outputs of the CAN at the sino-atrial node produces the HRV that is characteristic of a healthy and adaptive response style. A decrease in HRV implies a decrease in autonomic flexibility, and therefore reflects a diminished ability of an individual to respond to environmental demands (Thayer & Lane, 2000). Thus, this model predicts that (pregnant) women with anxiety problems are characterized by lower HRV, supporting the results in Chapter 3 and 4.

Another integrative model of autonomic functioning (Beauchaine, 2001) is based on Gray's motivational theory (Gray, 1987). According to this theory emotional behavior is controlled by at least two neurological systems: a behavioral activation system (BAS) and a behavioral inhibition system (BIS) (Fowles, 1980). BAS is responsible for maximizing rewards (approach behavior) and for minimizing punishments in situations where behavioral responses are required (active avoidance). In contrast, the BIS is activated when conflict occurs between competing motivational goals. BIS activation leads to arousal and experience of anxiety, which in turn causes inhibition of ongoing behaviors and the initiation of information processing for resolution of the conflict (Beauchaine, 2001). An under-responsive BIS has been linked to disorders of disinhibition, while an over-responsive BIS has been linked to internalizing disorders (Beauchaine, 2001). According to the integrative model of autonomic functioning, the BAS and BIS fall under SNS control. The model views behavior and emotions as the outcome of the combination of both the motivational component, contributed by the BIS and the BAS, and the regulational component, contributed by the PNS. Common psychopathologies are associated with specific patterns of activity in the motivational component and the PNS. For example, anxiety is linked to atypical high inhibition (BIS) and low parasympathetic activity (Beauchaine, 2001), which provides an explanation of the findings in Chapter 3 and 4.

7.4.2 Maternal mindfulness and autonomic functioning

To understand and explain the links found between maternal mindfulness and autonomic functioning it is important to consider fundamental components and major outcomes of mindfulness.

Attention is often proposed as a fundamental component of mindfulness (Shapiro, Carlson, Astin, & Freedman, 2006). Theoretically, mindfulness enhances different aspects of attentional abilities, including the capacity to attend for long periods of time to one object, the ability to shift the focus of attention between objects or mental sets at will (switching), and the ability to inhibit secondary elaborative processing of thoughts, feelings and sensations (cognitive inhibition) (Shapiro et al., 2006).

As described in Section 7.4.1 the model of neurovisceral integration in emotion regulation describes a functional framework that integrates attentional regulation with affective regulation and ANS functioning (Friedman & Thayer, 1998a; Thayer & Lane, 2000). A relative reduction in vagally mediated HRV is associated with the psychological symptoms of poor attentional control, linking self-regulation to autonomic flexibility (Thayer & Lane, 2000). Furthermore, it is often shown that mindfulness entails an ability to reduce distractive and ruminative thoughts and behaviors (Deyo, Wilson, Ong, & Koopman, 2009; Jain et al., 2007; Shapiro et al., 2006). According to the perseverative cognition hypothesis (Brosschot, Gerin, & Thayer, 2006), worrying prolong physiological activation beyond the presence of actual stressful situations. Whereas acute physiological changes in response to an actual stressor are useful in enabling a person's behavioral adaptability to stress (i.e. fight or flight), the prolongation of this physiological activation caused by perseverative thoughts explains how stress can yield adverse effects on bodily systems and health (Brosschot et al., 2006). Neurobiologically speaking, perseverative cognition represents a failure of potentially adaptive inhibitory neural processes. Such autonomic inflexibility is associated with reduced parasympathetic activity (Brosschot et al., 2006; Brosschot, Van Dijk, & Thayer, 2007). Hence, both the integrative theory on self-regulation of attention and the perseverative cognition hypothesis support the positive associations between mindfulness and autonomic functioning.

7.4.3 Child outcomes

Maternal stress physiology as mediator

Maternal stress physiology (i.e. HPA axis and ANS functioning) plays a key role in understanding how maternal psychological and autonomic functioning during pregnancy might be associated with fetus development and health.

The HPA axis is often proposed as a key physiological mechanism how maternal distress mediates its effect on fetal development and health. Stress activates the HPA axis releasing corticotrophin-releasing hormone (CRH) from the hypothalamus, which stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary and subsequently the release of cortisol from the adrenal cortex. Hypothalamic CRH production is suppressed by cortisol through a negative feedback loop, but placental CRH production is stimulated by cortisol. Therefore, placental CRH can reach the pituitary gland and stimulate the production of ACTH and hence also cortisol (Mulder et al., 2002). Placental CRH enters the fetal circulation via the umbilical vein and stimulates the fetal HPA axis to produce (fetal) cortisol. Maternal cortisol that has passed through the placenta may participate in the feed-forward loop between the placenta and the fetal pituitary-adrenal axis, causing even more production of fetal cortisol. Importantly, in the placenta, the 11β-HSD2 enzyme converts cortisol into its inactive metabolite cortisone, protecting the fetus from excess exposure to the glucocorticoid (Mulder et al., 2002). However, maternal stress and anxiety during pregnancy have been found to down-regulate placental 11β-HSD2 activity, resulting in increased exposure of the placenta and fetus to cortisol (O'Donnell et al., 2012). During pregnancy, cortisol plays an important role in fetal development (e.g. by promoting lung maturation). However, excess levels of cortisol (e.g. caused by maternal stress) have been associated with adverse fetal outcomes (Mulder et al., 2002; Räikkönen et al., 2011).

The ANS itself also plays a key role in understanding how maternal stress can affect the fetus (Mulder et al., 2002). Activation of the SNS may contribute to vasoconstriction (Teixeira et al., 1999), which is thought to alter uteroplacental blood flow, causing subsequent oxygen and nutrition reduction to the fetus and thereby affecting fetal growth (Copper et al., 1996; McCubbin et al., 1996) and nervous system development (Sjöström et al., 1997). Much research on how maternal stress can affect the fetus emphasizes SNS's role, but this dissertation also highlights the importance of the PNS. Reduced parasympathetic activity is associated with dysregulation of various allostatic systems, including glucose regulation, HPA axis function and inflammatory processes (Thayer & Sternberg, 2006), all of which may modulate the programming of fetal development (Lupien et al., 2009; Matthews & Phillips, 2010; Meyer et al., 2006; Räikkönen et al., 2011; Van den Bergh, 2011; Van den Bergh et al., 2005; Young, 2002). Moreover, since the PNS is sometimes referred to as the 'vagal brake' that provides inhibitory control of heart rate and antagonizes sympathetic activity, a lack of this vagal regulation might indirectly affect fetal development by not compensating the sympathetic activity and its influences.

Heritability of psychological and autonomic functioning and epigenetics

Associations found between maternal psychological functioning (i.e. both anxiety and mindfulness) and offspring psychological functioning (i.e. fearfulness and adaptive functioning) could also (partially) explained by heritability of psychological functioning. There exists evidence that mothers have certain genes that make her more or less likely to become anxious or depressed and these genes may be passed on to her child, who in turn might be more or less prone to emotional or behavioral problems. For example, it is suggested that anxiety sensitivity, which is often proposed as a key risk factor for anxiety problems and the development of an anxiety disorder, may have a heritable component (M. B. Stein, Jang, & Livesley, 1999). To the best of our knowledge, there are no studies published on the potential heritability of mindfulness. However, there is evidence that points to genetic contributions (alongside environmental contributions) to individual differences in emotion regulation (Canli, Ferri, & Duman, 2009), which is considered a key mechanism of mindfulness (Arch & Craske, 2006). A variety of genes influence vulnerability to anxiety disorders. These include serotonin-transporter linked polymorphic region (5-HTTLPR), Catechol-O-methyltransferase (COMT), and brain-derived neurotrophic factor (BDNF) gene variants (Lee & Park, 2011). Interestingly, it has been reported that the combination of a BDNF V/V genotype and early life stress predicts changes in brain structure that are associated with higher anxiety, but also lower HRV (Gatt et al., 2009).

Besides heritability of psychological functioning, it is also possible that offspring autonomic functioning is genetically determined. In laboratory studies a genetic role of HRV is found in twin and family studies during rest (Boomsma, van Baal, & Orlebeke, 1990; Singh et al., 1999) and stress (Boomsma et al., 1990). Additionally, ambulatory recordings over a longer period of time showed that HRV measures are to a large extent determined by additive genetic factors (Kupper et al., 2004).

Alterations in epigenetic modifications have also been proposed as an underlying mechanism that explains how maternal psychological functioning can be associated with adverse child outcomes (Reynolds, Jacobsen, & Drake, 2013; Van den Bergh, 2011). The term ‘epigenetic’ is used to describe alterations in gene expression (i.e. potentially heritable changes in the absence of changes in the DNA sequence). DNA methylation is a primary mechanism for underlying epigenetic changes (Reynolds et al., 2013). Increased DNA methylation is associated with gene silencing, while decreased methylation is related to gene activation. Prenatal exposure to maternal anxiety is associated with altered DNA methylation (Hunter & McEwen, 2013). Therefore, the psychological functioning during pregnancy might trigger altered patterns of gene expression, in which commonly expressed genes are silenced, while genes that normally are silenced become expressed.

7.5 Future research

Various methodological suggestions for future research were already included in Section 7.3. Other directions for future research are presented below.

Previous research on the mediating mechanisms by which maternal psychological functioning is associated with offspring health and development, has mainly focused on the HPA axis. In contrast, this dissertation studied cardiovascular measures (i.e. HR, HRV and BP), providing more insight into how the parasympathetic activity is associated with psychological functioning and how it evolves during pregnancy. In order to even further unravel and understand the stress-related mechanisms responsible for fetal programming, future studies need to combine both HPA-axis and various cardiovascular measures (e.g. HR, HRV, PEP and BP). For example, in non-pregnant humans it has been shown that habituation processes to psychosocial stressors tend to be different in the HPA-axis (measured via ACTH and cortisol responses) and in the sympathetic nervous system (measured via HR and catecholamine responses) (Schommer, Hellhammer, & Kirschbaum, 2003).

The research sample used in this dissertation was drawn from a healthy population, both mentally and physically. In particular, all subjects had no psychiatric disorder, nor any pregnancy-related disorder such as gestational hypertension, pre-eclampsia, or gestational diabetes at the time of recordings. To the best of our knowledge, there are no studies about the autonomic functioning of pregnant woman with a psychiatric disorder so far. Studies with men and non-pregnant women have linked various types of anxiety disorders (i.e. panic disorder, social phobia, generalized anxiety disorder) to significantly reduced basal parasympathetic activity, as indicated by various HRV measures (Blom et al., 2010; Cohen et al., 2000; Friedman & Thayer, 1998b; Licht et al., 2009; Thayer et al., 1996; Yeragani et al., 1991). Therefore, more research is required to study the autonomic functioning in pregnant women with current psychiatric disorders. Although this dissertation provided strong evidence that mindfulness can contribute to more beneficial physiological adaptations during pregnancy, no conclusions could be drawn on the development of pregnancy-related disorders such as gestational hypertension, pre-eclampsia, or gestational diabetes. Future studies that study both healthy pregnant women and women with pregnancy-related disorders, could examine whether psychological functioning (i.e. both anxiety and mindfulness) is also related the development of pregnancy-related disorders.

A novel contribution made in this dissertation is the result that maternal more mindful pregnant women had less depressive symptoms, both during and after pregnancy, higher basal parasympathetic activity, a less decline in basal parasympathetic activity over the course of pregnancy, and an offspring with less problems in adaptive functioning. Therefore, interventional randomized controlled trials need to be conducted to examine the effect of psychological interventions on positive factors such as mindfulness in pregnancy. There is the potential for significant mental and physiological benefits for both mothers and their children. Another promising method to reduce distress and its effects, but currently not investigated in a pregnant population, is HRV biofeedback. The basic idea behind this relatively new method is that individuals regularly monitor their heart rate as it varies with breathing and relaxation, aiming to achieve large HR variation in phase with breathing (Lehrer, 2007). HRV biofeedback has support in a wide range of conditions including posttraumatic stress disorder, major depressive disorder, asthma, fibromyalgia, hypertension and heart failure (Olsson, 2010). Moreover, a recent review links HRV biofeedback to favorable physiology, including higher HRV (Wheat & Larkin, 2010). In the future, more research is required to examine whether HRV biofeedback may improve maternal and offspring's autonomic and psychological functioning and how it could be synergistically combined with mindfulness-based interventions.

Another essential finding in the present dissertation is the result that mothers with a high level of trait anxiety tend to have less dampened stress reactivity in late pregnancy. Therefore, it is important to offer anxiety-reducing intervention programs to pregnant women with anxiety problems. Additionally, psychosocial factors, particularly social support, may also diminish physiological response to acute stressors (Christian & Stoney, 2006). It is suggested that the neuropeptide oxytocin may play an important role as an underlying biological mechanism for stress-protective effects of positive social interactions. Nevertheless, although social support in pregnancy, especially partner support, is a strong predictor of maternal mental health as well as birth outcomes (Feldman, Dunkel-Schetter, Sandman, & Wadhwa, 2000; Stapleton et al., 2012), studies on factors that reveal factors benefiting the adaptation of the stress response during pregnancy are lacking (Christian, 2012).

The main aim of this dissertation was to provide insight into how maternal ANS activity changes during pregnancy and how psychological functioning is associated with the ANS activity. An investigation of child outcomes such as offspring birth weight and psychophysiological development was only a secondary goal in the present study. It is essential that larger studies on the child outcomes need to take into account the three-hit model of vulnerability and resilience (Daskalakis, Bagot, Parker, Vinkers, & de Kloet, 2013). This model emphasizes that any child outcome (e.g. infants autonomic or psychological functioning) needs to be interpreted as the result of three so called hits: genetic predispositions (hit-1), the interactions of these genetic predispositions with the early-life environment (hit-2) that program phenotypes with differential susceptibility to later-life challenges and influences (hit-3). Therefore, future studies need to integrate genetic data with both prenatal and postnatal measurements. Importantly, to avoid a biased measurement, the assessment of a child outcome and the postnatal environment ideally is conducted from various perspectives (i.e. mothers, fathers, gynecologists and midwifes and researchers). Due to time restrictions we were only able to collect data from the children until they were 10 months old. It is important to follow up the infants in later ages as well. This should allow examining whether our results remain the same over time or whether they change if the children reach more milestones in their development. For example, previous research reported that in the neonatal period infant ANS activities were lower in premature infants compared to a reference full-term group, but at ages 2-3 and 6-7 years, premature infants had recovered and had similar ANS activity as the full-term group (De Rogalski Landrot et al., 2007). Hence, although it is possible that the differences in ANS activity we found between infants from women with and without a past anxiety disorder remain or even become larger as the child gets older, it might also occur that the differences in ANS activity are no longer visible.

7.6 Implications for public health

The present dissertation showed that trait anxiety and past anxiety disorders have strong associations with respectively altered maternal and offspring ANS functioning. Therefore, obstetric medicine needs to pay more attention to the (past) mental status of pregnant women, which is still too often neglected (Glover, 2013). Gynecologists and midwifes may have a key role in this process as they are the first who come into contact with the pregnant women. It is advised to assess past mental disorders using an interview, e.g. the Mini-International Neuropsychiatric Interview 6.0 (Sheehan & Lecrubier, 2010). The State-Trait Anxiety Inventory, trait form (Spielberger et al., 1970) and the Edinburgh Postnatal Depression Scale (Cox et al., 1987) were recently identified as the currently best available measures for anxiety and depression during pregnancy. Both questionnaires had a good reliability and validity (Nast et al., 2013). It is recommended to use such stress-related scales already at the beginning of pregnancy by health professionals. Proper mental screening and follow-up of pregnant women could help in giving suitable psychological advice, e.g. following an intervention program to reduce anxiety feelings.

Interestingly, the results indicated that mindfulness might be an essential mental resource for mothers in limiting the risk for both prenatal and postpartum depression or emotional distress. Furthermore, mindfulness was also associated with potentially more beneficial cardiovascular and autonomic changes during pregnancy. As described in Section 7.5, there is a need for interventional trials examining the effect of mindfulness-based intervention programs on the autonomic function. Nevertheless, previous research has shown that mindful yoga and meditation can reduce anxiety and stress during pregnancy (Bastani et al., 2005; Beddoe et al., 2009; Vieten & Astin, 2008). Therefore, given the positive associations found between mindfulness and autonomic functioning, it can be recommended to offer mindfulness training to pregnant women.

It was suggested that regular physical exercise during pregnancy might be beneficial for pregnant women, as it has the potential to enhance circadian variation in ANS activity, but at the same time it might have an adverse birth outcome, as both regular maternal physical exercise during pregnancy and circadian variations in ANS activity were negatively associated with offspring birth weight. Evidence was provided that regular light-intensity physical exercise such as walking or yoga may improve ANS functioning, but not affect birth weight. In contrast, regular moderate-intensity physical exercise including running and swimming was not only related to enhanced circadian variation in ANS activity but also to reduced offspring birth weight. Consequently, advice regarding the optimal level of physical exercise during pregnancy probably always needs to be personalized on the basis of personal health status (e.g. obesity) and risk factors for both low birth weight and fetal macrosomia. The results in the present study suggest that ANS monitoring has the potential to play a guiding role for health professionals in determining the optimal exercise level.

Screening of infant's autonomic functioning might also be useful, as infant's fearfulness was associated with infant's HRV. We were unable to determine whether reduced HRV came before the development of fearfulness or not, making it difficult to infer causation in this association. Nevertheless, reduced HRV in infancy may be an early risk marker for the development of psychological abnormalities, such as fearfulness, in later life. Such behavioral characteristics have been linked to internalizing problems and psychiatric diagnoses (Degnan et al., 2010; Rettew & McKee, 2005).

7.7 General conclusion

In this longitudinal study about prenatal early life stress we were able to have a closer look at the basal ANS functioning and ANS reactivity throughout pregnancy in a moderate to large sample. Moreover, we found strong associations between psychological functioning and the ANS activity in women over the course of pregnancy. In general, the results show that anxiety plays a negative role in ANS activity and reactivity, while mindfulness was linked to beneficial ANS activity and less depressive symptomatology. Moreover, regular physical exercise during pregnancy was linked to improved circadian variation in ANS activity.

As the fetus is dependent on its mother via the placenta, it is reasonable to presume that maternal psychophysiology has an influence on fetal growth, health and development. Prenatal early life experiences partially determine children's future development. The results in the present study indicated that mothers with a past anxiety disorder had an offspring with a more fearful temperament and the autonomic functioning of these mothers and their offspring were positively related to each other. Furthermore, maternal mindfulness was linked to better offspring adaptive functioning. Finally, it was shown that, although regular moderate-intensity physical exercise during pregnancy may be beneficial for the ANS functioning of a pregnant women, it was associated with reduced offspring birth weight.

The above findings suggest that a proper screening of the autonomic and psychological functioning over the course of pregnancy could support health professionals in giving suitable advice, e.g. following an intervention program to reduce anxiety feelings or to enhance mindfulness, or optimizing the level of physical exercise.

Follow-up studies are essential to examine whether the associations found with child outcomes remain visible at later ages. Furthermore, as recommended for all fetal programming studies it is important to combine genetic data with both prenatal and postnatal measurements.

In conclusion, this dissertation delivered a small but fundamental piece in the complex puzzle of ANS functioning during pregnancy by studying how it changes throughout pregnancy, examining the link with psychological functioning and assessing the influence on the offspring's psychophysiological development. Future research should provide more insight into these multifaceted relationships, allowing for a better understanding of the link between maternal and their offspring's health and well-being.

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Summary

Since the middle of the 20th century, when the concepts underpinning psychological stress were first defined, there has been an explosion of interest in how stress influences health and disease. Stress may affect health directly, by activating specific physiological responses, or indirectly through its influence on health-related behaviors, e.g. alcohol use or smoking. Typical physiological stress responses include elevated heart rate (HR) and reduced heart rate variability (HRV), which both reflect alterations in autonomic nervous system (ANS) activity. The ANS is a critical means by which organisms ensure their homeostasis (the regulation of their internal state to maintain optimal function). It has two distinct branches, namely the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Both divisions are essential in the regulation of the cardiovascular system. In a healthy body, a balance exists between parasympathetic and sympathetic activity, which is associated with high HRV. The oscillations seen in cardiovascular parameters, such as heart rate, are an epiphenomenon of cardiovascular control mechanisms governed by central nervous system (CNS) centres and mediated via the ANS. The brain continuously disturbs cardiovascular function by small amounts and then measures the result through baroreceptors and other sensors. This allows cardiovascular control mechanisms to continuously calibrate their control of the system. Therefore, high HRV reflects healthy cardiovascular control in a steady state. During physical or mental stress, the system requires an alteration from the steady state and the control mechanisms diminish, resulting in reduced HRV. In practice, these changes of state are often achieved by alterations in the relative balance between the activities of the PNS and SNS.

It is well known that the ANS plays a key role in the psychological and physical wellbeing of non-pregnant populations. Studies suggest the ANS acts as a final common pathway in many disorders where negative affective states have been linked to physical health disorders. For example, both anxiety and depression are

associated with reduced HRV. Very little is known about the role that ANS activity plays during pregnancy in the development of health disorders. This is particularly important given the well-known associations between pregnancy and psychological disorders. The evidence that does exist suggests that stress response systems such as the ANS and the hypothalamic-pituitary-adrenal (HPA) axis mediate the effects of psychological problems during pregnancy on the health of mothers and their offspring. This includes effects on fetal (brain) development and later cognitive, behavioral and social-emotional development of the child. In this way, effects on ANS and HPA function during pregnancy could be important means by which the health of mothers has a lifelong influence on the health of their offspring (the developmental origins of health and disease hypothesis).

The main goal of this dissertation was to determine how the psychological functioning of pregnant women influences their ANS and how this is linked to outcomes for them and their children. We collected both psychological and physiological data on 190 pregnant women and their partners. In all three pregnancy trimesters, the mothers wore a heart monitor for 24 hours, allowing us to record both an electrocardiogram (ECG) and impedance cardiogram (ICG). Measures of sympathetic and parasympathetic activity were calculated from the ECG and ICG. All participating women filled out questionnaires on their psychological functioning at home and at work. The mothers returned with their offspring when they were between two and four months of age and again when they were between nine and ten months of age. On both occasions, we collected a variety of psychophysiological data on both mothers and their children.

In the first and third pregnancy trimesters, each mother undertook a 25-minute stress task consisting of five testing phases, lasting for five minutes each. Stress was induced in the second and fourth phase, with the remainder being relaxation phases. In chapter 3, results showed that stress responsiveness, reflected by measures of ANS activity at rest and during stress, decreased over the course of pregnancy. However, anxious women did not show this expected decrease of stress responses through pregnancy. Moreover, the study in chapter 4 showed that pregnant women with previous but not current anxiety disorder had lower parasympathetic activity across the whole stress task compared to women who never had a mental disorder. Interestingly, we also found that the children of mothers with a past anxiety disorder had lower parasympathetic activity 2-4 months after birth in comparison to children from ‘healthy’ mothers. Therefore, it appears that anxiety disorders in women, even those that may no longer be clinically active, can influence how women adapt physiologically to pregnancy and may impact on the health of their children. This raises the question of how such psychological disorders might best be managed during pregnancy where traditional therapeutic options may be dismissed due to unwanted effects on the developing child.

Psychopharmaceuticals can be used to good effect to reduce maternal prenatal stress, anxiety or depression and the elevated blood pressure levels that often accompany these conditions. However, their use during pregnancy is often avoided for fear of unwanted harmful effects on the fetus. An alternative approach to management of these conditions that does not carry such risks should be preferred. In chapter 5, mindfulness of mothers was studied as a psychological trait that holds promise as a potential candidate for manipulation to benefit maternal health without resort to pharmaceutical intervention. Interventions to influence mindfulness in mothers are possible and could be tested in randomized controlled trials but before this is done, the influence of mindfulness on maternal and fetal outcomes needs to be understood. The work carried out in the context of this dissertation showed that more mindful pregnant women had not only less emotional distress, both during and after pregnancy, but also higher cardiac parasympathetic activity, indicating potential psychological and physiological benefits. Over the course of pregnancy, all mothers experienced significant decreases in ANS activity between the first and third pregnancy trimester. However, mindfulness in pregnancy was associated with ANS changes that are likely to be adaptive and with better social-emotional offspring development. Thus, the results of this dissertation support the concept that interventions to increase mindfulness during pregnancy might improve maternal and offspring health and highlights the need for clinical trials of such interventions.

In addition to studies on the resting and stressed function of the ANS, this dissertation also describes investigations into the homeostatic variation in ANS control over the course of the day. These circadian variations have received much attention in the ANS literature as indicators of health status but there is very little data on their use as indicators of health status in pregnancy. Chapter 6 demonstrates our work in this area. The results suggest that regular physical exercise during pregnancy may be beneficial for pregnant women, as it has the potential to enhance circadian variation in ANS activity, a characteristic that has been deemed to reflect healthy ANS function. On the other hand, we also found evidence that regular physical exercise might negatively influence birth outcome because moderate-intensity regular maternal physical exercise during pregnancy and circadian variations in ANS activity were negatively associated with offspring birth weight. These findings are somewhat exploratory, coming as they do from one of the first investigations of their kind in pregnant women. Therefore, there is much work to be done before any alteration should be made to the advice given to women. However, the findings are provocative and interesting and suggest that the interactions between health behaviors such as exercise, and autonomic function are worthy of greater scientific attention.

In summary, this dissertation shows that maternal psychological functioning is associated with alterations in ANS activity during pregnancy and with psychological and physical wellbeing of the offspring. Follow-up studies are essential to examine whether the child outcomes persist later in life and to determine where interventions might be beneficial. However, even before interventions are considered, the results of this dissertation suggest that measures of autonomic and psychological functioning over the course of pregnancy hold potential as possible predictors of the health of mothers and their children.

Samenvatting

De negatieve gevolgen van stress tijdens de zwangerschap op de gezondheid van moeder en kind kregen de laatste decennia veel aandacht in de media en in wetenschappelijk onderzoek. Er is voldoende bewijs dat stress zowel direct als indirect de gezondheid beïnvloedt. Indirecte effecten van stress ontstaan vaak door veranderingen in ons gedrag, bijvoorbeeld beginnen roken of drinken om stress te verminderen of te ontwijken. Directe invloeden van stress omvatten de fysiologische reacties op stress zoals een verhoogd hartritme of een verlaagde hartritmevariabiliteit (HRV). Zulke fysiologische reacties zijn uitdrukkingen van veranderingen in de activiteit van ons autonoom zenuwstelsel. Het autonoom zenuwstelsel regelt de basale processen in onze organen die nodig zijn voor het normaal functioneren van het lichaam. Het autonoom zenuwstelsel bestaat uit twee takken (regelsystemen), namelijk het parasympathische en sympathische zenuwstelsel. Beide takken hebben een belangrijke rol in de regulatie van het cardiovasculair systeem. In een gezond lichaam is het autonome zenuwstelsel in balans en is er voortdurend zowel sympathische als parasympathische activiteit. Een goede balans tussen beide takken wijst op een goede regulatie en wordt gekenmerkt door een hoge hartritmevariabiliteit. De activiteiten in het autonoom zenuwstelsel worden soms vergeleken met het besturen van een auto. De parasympathische tak komt overeen met de rem en de sympathische tak stemt overeen met het gaspedaal. In rustige situaties, zoals bijvoorbeeld bij het slapen, heeft het parasympathische deel de overhand (remmen van het systeem); terwijl bij fysieke of mentale belasting het sympathische deel overheerst (opvoeren (gas) van het systeem).

In niet-zwangere populaties werd reeds uitgebreid aangetoond dat veranderingen in activiteiten van het autonoom zenuwstelsel een belangrijke indicator vormen van het fysiek en mentaal welzijn. Zo blijkt dat angst, depressie en hartziekten sterk geassocieerd zijn met een lagere hartritmevariabiliteit. Er is echter veel minder geweten over de activiteit van het autonoom zenuwstelsel tijdens de zwangerschap en de associatie met het psychologisch functioneren van de zwangere vrouw. In het verleden lag de focus in stressonderzoek bij zwangere vrouwen op een ander stress-systeem (de HPA-as met cortisol als eindproduct). Het is geweten dat negatieve emoties tijdens de zwangerschap via stress-systemen een invloed hebben op de (foetale) hersenontwikkeling en later op de cognitieve, sociaal-emotionele en gedragsmatige ontwikkeling van het kind. Zo zouden ook ziektes die in de volwassenheid ontstaan, hun oorsprong hebben tijdens de foetale en vroege postnatale ontwikkeling.

Het doel van dit proefschrift was om het verband te bestuderen tussen activiteit van het autonoom zenuwstelsel en het psychologisch functioneren van de zwangere vrouw. We verzamelden psychologische en fysiologische data van 190 zwangere vrouwen en hun partners. De moeders droegen in elk van de drie zwangerschapstrimesters 24 uur lang een hartmonitor. Met behulp van de hartmonitor was het mogelijk om een electrocardiogram (ECG) en een impedantiecardiogram (ICG) op te nemen, waaruit in een latere fase de sympathische en parasympathische activiteit kon worden berekend. Alle deelnemende vrouwen vulden vragenlijsten in over hun psychologisch functioneren thuis en op hun werk. Twee tot vier maanden, en 9 tot 10 maanden na de geboorte kwamen de moeders met hun kind naar het babylab op de universiteit. Op beide meetmomenten werden tal van psychofysiologische gegevens over zowel moeder als kind verzameld.

Tijdens het eerste en derde trimester van de zwangerschap namen de vrouwen ook deel aan een stresstaak. Deze taak bestond uit vijf fasen van telkens vijf minuten waarin drie fasen met rustige muziek en beelden werden afgewisseld met fasen bestaande uit moeilijke rekenoefeningen. In hoofdstuk 3 werd aangetoond dat de parasympathische reactie op de stresstaak daalt over de zwangerschap heen. In het begin reageren zwangere vrouwen heviger op stress dan later in de zwangerschap. Moeders die meer angst rapporteerden vertoonden echter aan het einde van de zwangerschap meer reactiviteit dan vrouwen die minder angstig zijn. Het lijkt alsof de moeder hiermee schadelijke invloeden op het kind wil tegengaan, maar dat angstige moeders hier minder goed in slagen. Daarnaast werd in hoofdstuk 4 bewezen dat de parasympathische activiteit over de hele taak lager was bij vrouwen die ooit in hun leven een angststoornis hadden in vergelijking met vrouwen die nooit een stoornis hadden. De kinderen van vrouwen die rapporteerden ooit een angststoornis gehad te hebben, hadden 2 tot 4 maanden na de geboorte ook een lagere parasympathische activiteit in vergelijking met kinderen van ‘gezonde’ moeders. Lagere parasympathische activiteit bij het kind op 2 tot 4 maanden werd eveneens geassosieerd met een hogere kans op angstig gedrag op 9 tot 10 maanden. Zowel bij moeder als kind, werd er een verband gevonden tussen angst en een lage parasympathische activiteit.

Angst heeft dus een eerder negatieve invloed op het autonoom zenuwstelsel tijdens de zwangerschap. Het is niet altijd aanvaardbaar om tijdens de zwangerschap medicatie te geven. Het is dan ook aangewezen om te opteren voor alternatieve methodes om negatieve emoties te verminderen. Eerder werd al aangetoond dat mindfulness en beweging zowel de balans in het autonoom zenuwstelsel verbeteren als negatieve emoties verminderen. De resultaten in hoofdstuk 5 tonen dat vrouwen die meer mindful zijn, minder negatieve emoties rapporteren zowel tijdens als na de zwangerschap. Er werd vastgesteld dat tijdens de zwangerschap het hartritme van de vrouwen steeg, terwijl parasympathische en sympathische activiteit daalden. Het is opmerkelijk dat bij vrouwen met een hogere graad van mindfulness de parasympathische en sympathische activiteit minder daalden. Ook hadden hun kinderen minder sociaal-emotionele problemen dan kinderen van moeders die minder mindfulness toonden. Mindfulness houdt dus verband met veranderingen in het autonoom zenuwstelsel waarvan verondersteld wordt dat ze zowel de gezondheid van de moeder als van het kind positief beïnvloeden. Met de 24-uurs metingen tijdens de zwangerschap kon het circadiaan hartritme worden bestudeerd. Het circadiaan ritme is te vergelijken met onze biologische klok. Zo varieert het parasympathische zenuwstelsel tijdens dag (hogere activiteit) en nacht (lagere activiteit). In hoofdstuk 6 werd het verband tussen beweging, het circadiaan hartritme en het geboortegewicht van het kind onderzocht. Indien een moeder tijdens de zwangerschap regelmatig middelzware beweging had, had dit een positieve invloed op haar circadiaan hartritme (i.e. groter verschil in parasympathische activiteit tussen dag en nacht). Het grotere verschil in parasympathische activiteit tussen dag en nacht, bleek echter negatief voor het geboortegewicht voor van het kind. Fysieke beweging tijdens de zwangerschap zou dus het circadiaan hartritme van de zwangere vrouw verbeteren, maar leidt mogelijk ook tot een lager geboortegewicht van het kind.

In het algemeen kunnen we besluiten dat het psychologisch functioneren een verband heeft met de werking van het autonoom zenuwstelsel tijdens de zwangerschap en dit zowel moeder als kind op mentaal en fysiek vlak kan beïnvloeden. Meer onderzoek is vereist om de resultaten te bevestigen en te bestudereren of de gevonden verbanden bij kinderen ook op latere leeftijden waarneembaar zijn. Op basis van de resultaten van dit proefschrift is het aangeraden om in de praktijk zowel het fysiologisch als psychologisch functioneren van zwangere vrouwen te screenen. Nu worden zwangere vrouwen lichamelijk goed opgevolgd, maar wordt er vaak minder rekening gehouden met hun mentale gezondheid. Een opvolging van de balans in het autonoom zenuwstelsel en de nodige aandacht voor emoties tijdens de zwangerschap moet het welzijn van zowel moeder en kind ten goede komen.

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Dankwoord

"The best part of life's journey...
is who you get to share it with."

Vele vrienden en collega's hebben het me voorgedaan: de jarenlange (lijdens)weg van preliminaire data tot finaal resultaat, met als uiteindelijke bewijs een zelfgeschreven boekje en een papier waarop de titel van doctor staat geprint. Ten opzichte van het huzarenwerk dat dit proefschrift inhoudt, leek het schrijven van dit dankwoord in eerste instantie iets dat niet teveel moeite zou kosten. Helaas is dit deel volgens mij het meest gelezen stuk uit ieders proefschrift waardoor de druk om er voldoende tijd in te steken toenam. Hier gaan we dan...

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Sinds 2011 heb ik ook een aanstelling als lector psychologie binnen de opleiding medical management assistant van de PXL en de opleiding revalidatiewetenschappen en kinesitherapie aan de UHasselt. Ondertussen is mijn opdracht aan de UHasselt uitgebreid met een onderzoekstraject. Alle collega's en vooral Joeri, Eddy, Marleen en Lise, bedankt voor jullie interesse en flexibiliteit! Ik ben blij dat ik met jullie mag samenwerken.

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In de afgelopen jaren heb ik toch wel geleerd dat tijd een erg rekbaar begrip is. Alles is relatief en dit bleek ook in het aantal werkuren die er in een werkdag zitten en het aantal werkdagen in een week. Nu worden alle activiteiten niet meer uitgesteld tot na de deadline van het doctoraat en is er terug plaats voor een sociaal leven met:

Elke & Jonas (& Romi), Els & Tom en Ine; wat vind ik het gezellig elke keer met jullie bij te praten en te horen waar jullie mee bezig zijn. 11 april staat al maanden vrij gepland in jullie agenda waardoor ik me zeer vereerd voel! Hartelijk dank voor jullie begrip, "bewondering", bemoedigende woorden en e-mails! Annelies & Bart (& Stan), Bert & Sophie (& Laura), Bram & Laura, Geert I., Kris & Isabel, Peter, Piet(er) & Eveline, Sofie, Sven & Femke, wat kijk ik uit naar onze volgende "date"! Babbelen met jullie over mijn onderzoek en ons leven in het algemeen, doet me altijd deugd. Ook al zijn er ondertussen heel wat mensen uit Leuven verhuisd naar een vaster stekje, toch hoop ik dat onze vaste jaarlijkse hoogtepunten blijven bestaan en gecombineerd worden met tussendoor voldoende uitjes en etentjes! Karel & Véronique (& Wout en Stan), nu is het kwartet inderdaad volledig! Fijn dat ook jullie de weg hebben teruggevonden naar de gezellige Maaskant waar we onze fijne ontmoetingen - nu helemaal zonder 'doctoraatsstress' - zeker moeten voortzetten! Rob, een vaste waarde doorheen heel mijn psychologie-carrière, bedankt voor de prachtige fotomomenten & ontspannende etentjes als afleiding!

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– Marijke Braeken
Leut, 11 maart 2014

"Tevreden over
de wegen die
we kozen.

Gelukkig met
de wegen die
we gaan.

Nieuwsgierig naar
de wegen die
nog volgen."

Biography

Marijke A.K.A. Braeken was born on the 27th of April 1982 in Leut (Belgium), as the middle child of three children. She is married to Geert J.Y.J. Monsieur, PhD. After she graduated from high school at the Stedelijke Humaniora in Dilsen-Stokkem (Belgium), she obtained her Master in Clinical Psychology cum laude in 2007 at the KU Leuven. Furthermore, she received her certificate of teaching competence in 2008 and completed her Master criminology cum laude in 2010 at the KU Leuven. While a student in the Master criminology program, she started working at Tilburg University as a lecturer. In September 2009 she became a PhD student at the department of Developmental Psychology. From February until April 2012 she was a visiting researcher at University of Sydney.

In 2011 Marijke started working as a lecturer in clinical psychology at both PXL (department of Business) and Hasselt University (faculty of Medicine and Life Sciences). Since recently, she additionally is employed as a (postdoctoral) researcher at Hasselt University.