From the womb into the world

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From the Womb into the World

Early life influences on neurocognitive functioning and behaviour in five to six year olds

Vroege invloeden op de cognitieve- en gedragsontwikkeling bij vijf tot zes jarigen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan Tilburg University
op gezag van de rector magnificus,
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1

Introduction

Introduction

'(…)*the same soul governs the two bodies* (…)*the things desired by the mother are often found impressed on the members of the child which the mother carries at the time of the desire* (…)*one will, one supreme desire, one fear that the mother has, or other mental pain, has more power over the child than over the mother*'(…) (Leonardo da Vinci, Quaderni)

From the Womb into the World

The idea that early life influences such as mothers' emotional state during pregnancy may reflect upon the developing foetus while in the womb, is deeply rooted in human beliefs among various cultures and religions since ancient times. After the Dark Ages, questions arose about the extent to which the developing foetus is susceptible and capable to respond to these environmental influences in its early environment and whether there would be any long-term consequences (Ferreira, 1965). Experimental embryologists such as Preyer (1885), who observed and studied sensory and motor development during pregnancy in various species, have acknowledged the importance of early life influences for later behavioural development already in the 19th century (Gottlieb, 1976). Accordingly, in 1867 Whitehead observed that pregnant women with severe symptoms of emotional distress were more likely to have a hyperactive foetus (Van den Bergh, Mulder, Visser, et al., 1989).

A century thereafter, Sontag and his colleagues replicated and expanded this finding in a more scientific setting -the FELS Longitudinal Study- when they found that the hyperactive foetuses of emotionally distressed mothers were more likely to become hyperactive children (Sontag, 1941; Sontag, 1944). As Sontag's focus was not confined to the influence of emotional distress, at a scientific meeting in 1943 he postulated that '*The soundness and adequacy of the body of the newborn infant* (…) *are dependent to an important degree upon the adequacy of the food eaten by his mother during the period of gestation.* (…) *there may of course, be specific differences in brain structure as a result of prenatal nutritional differences. About this we know nothing*' (Sontag, 1944). One year later in November of 1944, a famine struck the German-occupied part of the Netherlands. In cities such as Amsterdam this resulted in adult rations between 400 and 800 kilocalories per day, which led to severe malnutrition in pregnant women. Decades later this horrific event formed the basis for the Dutch

Famine Cohort that studies the effects of prenatal exposure to famine on health and behavioural development in later life (Stein, Susser, Saenger, & Marolla, 1975). Although at first results did not suggest an association between maternal malnutrition during pregnancy and mental retardation and IQ in adolescents (Stein, Susser, Saenger, & Marolla, 1972), in later studies from the same cohort, foetal exposure to famine in early gestation was found to be associated with schizophrenia (Susser et al., 1996) and schizoid personality disorder (Hoek, Susser, Buck, & Lumey, 1996).

Developmental Origins of Health and Disease

More recently, research into a broad range of mostly health related long-term consequences of early life influences has experienced a resurgence (Schlotz & Phillips, 2009), since epidemiologists found associations between the quality of the prenatal environment and the risk of cardiovascular and metabolic diseases in later life, which formed the basis for the Developmental Origins of Health and Disease (DOHaD) hypothesis (Barker, 2003; Barker, Osmond, Winter, Margetts, & Simmonds, 1989). This hypothesis proposes that human health and development have their origin in early life; in the womb (Gluckman & Hanson, 2004, 2010). In the current dissertation we deliberately term this process developmental *re*programming, because we presume that programming by means of early environmental cues in order to shape an organisms development is a fundamental part of the trajectory in typical development (Van den Bergh, 2007). Evidence for health related short-term consequences of developmental reprogramming by means of early life influences is accumulating. Foetal exposure to maternal psychosocial stress (Littleton, Bye, Buck, & Amacker, 2010), micronutrient deficiencies (Christian, 2010) and substances such as caffeine (Vik, Bakketeig, Trygg, Lund-Larsen, & Jacobsen, 2003) have been linked to an increased risk for adverse perinatal outcomes such as low birth weight, shorter gestational age and growth retardation.

Developmental Origins of Behaviour, Health and Disease

In humans, critical stages in brain development regarding brain growth (e.g. neurogenesis, neuronal proliferation) and connectional specificity (e.g. neuronal migration, differentiation and synaptogenesis) occur during pregnancy and at birth all gross anatomical structures are present (Gazzaniga, Ivry, & Mangun, 2002). Therefore the brain has been suggested to be particularly susceptible to potential reprogramming effects of early life influences (Räikkönen, Seckl, Pesonen, Simons, & Van den Bergh, 2011; Van den Bergh, 2011). Moreover, suboptimal brain development is associated with adverse long-term neurodevelopmental outcomes in terms of problem behaviour and impaired cognitive functioning (Castellanos & Tannock, 2002). For this reason, and in order to reintegrate early brain and behavioural development within the existing DOHaD hypothesis, Van den Bergh (2011) has proposed to extend the DOHaD hypothesis into the Developmental Origins of *Behaviour*, Health and Disease (DOBHaD) hypothesis. This extension not only emphasises the importance of potential early life reprogramming effects on offspring's neurodevelopmental outcomes, it also provides a conceptual framework for research into early life influences on specific long-term outcomes in terms of behaviour and cognitive functioning.

The majority of results from previous research have indicated adverse effects of reprogramming by means of early life influences on long-term neurodevelopmental outcomes (Räikkönen et al., 2011; Van den Bergh, Mulder, Mennes & Glover, 2005a; Weinstock, 2008). Recent findings underline that long-term consequences of reprogramming also depend on the context in later life. This is nicely illustrated in a recent study by Daskalakis, Oitzl, Schächinger, Champagne, and de Kloet (2012), in which the effects of early life adversity and later life stress exposure on psychosis susceptibility in rodents was investigated. Adult offspring that were not licked and groomed extensively as a pup, and had been isolated during the post-weaning period, were more susceptible to the effects of an acute stressor in later life. In addition, if a mismatch between the early life environment and the later social environment occurred, psychosis susceptibility was increased. In sum, these results emphasise that the long-term consequences of reprogramming by early life influences depend on their interaction with an individuals' exposure to environmental adversities in later life.

The child's sex as a moderator?

Previous studies have suggested sex differences in the developmental reprogramming effects of early life influences (Sandman & Davis, 2012). Results from animal studies have indicated sex differences in reprogramming influences of antenatal maternal stress or anxiety (Weinstock, 2001) and intrauterine caffeine exposure (Fisher & Guillet, 1997; Hughes & Beveridge, 1986; Hughes & Beveridge, 1991). In humans, higher levels of antenatal anxiety or stress during pregnancy have been associated with alterations in cognitive functioning, such as a slower and more variable response speed (Van den Bergh et al., 2006), a higher percentage of errors on an encoding task (Van den Bergh et al., 2005b), and with more hyperactivity/inattention problems (O'Connor, Heron, Golding, Beveridge, & Glover, 2002; Rodriguez & Bohlin, 2005) in boys. Higher levels of maternal anxiety during pregnancy have been related to HPA-axis functioning (altered cortisol day-time profile) in both sexes, which was associated with self-reported depressive symptoms in adolescent girls (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). Hence, developmental reprogramming effects of early life influences seem to increase vulnerability in both male and female offspring, but boys and girls seem to be affected in different ways (Coe, Lulbach, & Schneider, 2002).

Methodological shortcomings in previous studies

In addition, findings from previous research are not entirely unequivocal, which could most likely be ascribed to methodological differences between studies (DiPietro, 2012). First, despite the growing body of literature into a broad range of neurodevelopmental outcomes, until now relatively few studies have focussed on cognitive functioning (e.g. Barr & Streissguth, 1991; de Rooij, Wouters, Yonker, Painter, & Roseboom, 2010; Henrichs et al., 2011; Van den Bergh et al., 2005b), especially by means of tasks that asses basic information processing capacities which form the basis for the execution of more complex higher order cognitive functions. Second, most studies that examined the effects of early life influences on children's behavioural development were based on maternal reports of child behaviour (Bekkhus, Skjothaug, Nordhagen, & Borge, 2010; Chiu, Gau, Tsai, Soong, & Shang, 2009; Gale et al., 2008; Hibbeln et al., 2007; Kohlboeck et al., 2011; Krabbendam, Bakker, Hornstra, & van Os, 2007; O'Connor et al., 2002), which provide a valuable source of information about the child's behaviour in the home environment (DiPietro, 2012). However, considerable debate in literature exists

about inconsistencies in reports on child behaviour among different informants (Briggs-Gowan, Carter, & Schwab-Stone, 1996), due to inherent differences in experiences that these informants share with the children; for example the home environment versus the classroom (Achenbach, McConaughy, & Howell, 1987; Najman et al., 2000). Third, maternal reports of their child's behaviour bare the risk for maternal perceptual bias (Kroes, Veerman, & De Bruyn, 2003; Najman et al., 2000; Van der Toorn et al., 2010), especially among emotionally distressed women who tend to perceive their child's behaviour more negatively (DiPietro, 2012). Fourth, there seems a lack of research into the long-term effects of early life influences on neurodevelopmental outcomes in children aged five to six (Talge, Neal, & Glover, 2007). This is remarkable, because of the important shift in brain development that occurs in children between three and seven years of age (Sameroff & Haith, 1996). Furthermore, children attend primary school at this age where they will be put in situations with high attentional demands where specific cognitive functions will be challenged for the first time. Therefore, still more prospective research is warranted into early life influences on children's information processing using cognitive tasks with objective outcome measures (DiPietro, 2012; Schlotz & Phillips, 2009; Van den Bergh, 2011).

Early life influences

The aim of the current thesis is to investigate developmental reprogramming effects of specific early influences during pregnancy on children's birth outcomes, neurocognitive functioning and behaviour. In capturing the essence of a long line of research, Plagemann (2012) has stated six early influences that have the potential to adversely affect optimal human development during sensitive periods in the prenatal and postnatal period, including '*infection or inadequate immune challenges,*' '*cardiovascular challenges or disorders*', '*exposure to drugs or disrupting medication*', '*maternal distress'*, *'quantitative or qualitative malnutrition'* and exposure to '*environmental toxins'* (p. 271). The potential reprogramming effects of core elements of the latter three of those early influences on children's birth outcomes, neurocognitive functioning and behaviour will be subject to the different chapters in this dissertation.

These selected early life influences have in common that they are not only highly prevalent in the general population, but also in women in the childbearing age. First, research has revealed that substantial numbers of pregnant women experience '*maternal distress'* or negative emotions. Reports on the prevalence

of anxiety ranged from 0.2 to 25 percent (Andersson et al., 2003; Heron, O'Connor, Evans, Golding, & Glover, 2004; Ross & McLean, 2006; Yali & Lobel, 1999). Second, foetal exposure to *'environmental toxins'* such as the substance caffeine is common, as 75 to 93 percent of pregnant women have reported to consume caffeine, via caffeinated drinks like coffee, tea, and soft drinks on a daily basis. (Frary, Johnson, & Wang, 2005; Kaiser, 2008). Third, although nowadays severe *'quantitative or qualitative malnutrition'* during pregnancy is unlikely in modern civilized countries, deficiencies or an imbalance in micronutrients such as long-chain polyunsaturated fatty acids in pregnant women are not uncommon (Cetin, Berti, & Calabrese, 2010). To sum up, the developmental reprogramming effects of maternal negative emotions, caffeine intake and fatty acid concentrations during pregnancy on children's birth outcomes, neurocognitive functioning and behaviour, will be addressed in the following chapters of this thesis. In addition, moderation of these associations by the child's sex will be examined. The research questions that will be addressed in the following chapters are:

1. Are psychosocial stress and negative emotions during pregnancy related to adverse birth outcomes? *(Chapter 3)*

2. Is there an association between maternal anxiety during pregnancy and children's neurocognitive functioning and behaviour at the age of five to six? *(Chapters 4 and 5)*

3. Is there an association between caffeine intake during pregnancy and the risk of problem behaviour in five to six year old children? *(Chapter 6)*

4. Is there an association between maternal long-chain polyunsaturated fatty acid status during early pregnancy and children's risk of problem behaviour at age five to six years? *(Chapter 7)*

Thesis outline

In *chapter 2*, the methods that were used to answer the research questions are described. In *chapters 3 to 7,* we will examine the research questions as presented above. Finally, a general discussion and conclusion are presented in *chapter 8*.

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2

Methods

METHODS

The Amsterdam Born Children and their Development Study

The current research project is embedded in a large community based multi-ethnic birth cohort situated in Amsterdam; the Amsterdam Born Children and their Development Study (ABCD-study). The main goal of the ABCD-study is to examine a broad range of factors during pregnancy and in early life that are potentially related to the child's health and development at birth and in later life. Special attention is paid to ethnic differences, both in explanatory factors (lifestyle and psychosocial conditions), and in outcomes (health and development of the child). Extensive information about the cohort and details regarding data collection is provided elsewhere (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2011). For more information on current data collection, output and collaboration visit: www.abcd-studie.nl.

Procedure

Pregnancy and infancy

Pregnant women from Amsterdam were approached for participation between January 2003 and March 2004 when they first visited an obstetric care provider. An overview of all phases of the ABCD-study is provided in figure 3. All pregnant women (12,373), which is approximately 99% of the target population, received a questionnaire covering socio-demographic, obstetric, life-style and psychosocial conditions, which was filled out by 8266 women (67%). In addition, 4389 women (53%) agreed to participate in the ABCD biomarker study, for which an extra blood sample was taken during routine blood collection for screening purposes following the first antenatal check-up. These data were completed with information on pregnancy outcomes from Youth Health Care Registration and the Dutch Perinatal Registration. Three months after delivery, during the second phase of the study, another questionnaire was sent to the mothers who had given permission to follow the health status of their child for further research (n = 6735). The infancy questionnaire consisted of questions concerning the course of pregnancy and delivery, questions about health, development and growth of the baby, and questions about lifestyle of the mother during and after pregnancy. With 5131 women returning the infancy questionnaire the response rate was 76%.

Early life influences

The different constructs that were used to examine maternal negative emotions during pregnancy were included in the pregnancy questionnaire.

Anxiety

Maternal state-anxiety was measured using the Dutch version of the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, & Lushene, 1970; van der Ploeg, Defares, & Spielberger, 1980). This self-report questionnaire is often used to assess anxiety during pregnancy and the postnatal period (Austin, Tully, & Parker, 2007). In this dissertation, only the state-anxiety subscale consisting of 20 items scored on a four point scale (0= rarely or none of the time, 1= some or a little of the time, 2= occasionally or a moderate amount of the time, 3= most or all of the time) was used. A higher score indicates a higher level of experienced anxiety. The state-anxiety scale was found to be a valid (Spielberger, 1975) and reliable (Cronbach's alpha = 0.94) measure of anxiety that is experienced temporarily or transiently.

Depressive symptoms

Depressive symptoms were assessed using the validated Dutch version (Hanewald, 1987) of the 20-item Centre for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977), which evaluates the frequency of depressive symptoms experienced during the preceding week. Each item was scored on a four point scale (0= rarely or none of the time, 1= some or a little of the time, 2= occasionally or a moderate amount of the time, 3= most or all of the time), resulting in a total score that ranged from zero to sixty. Internal consistency (Cronbach's alpha) of the CES-D scale was 0.90.

Pregnancy-related anxieties

Pregnancy anxieties were assessed using an abbreviated 10-item version (Huizink, Mulder, Robles de Medina, Visser, & Buitelaar, 2004) of the Pregnancy Related Anxiety Questionnaire (PRAQ) (Van den Bergh, 1990). Each item was scored on a four point scale (0= rarely or none of the time, 1= some or a little of the time, 2= occasionally or a moderate amount of the time, 3= most or all of the time). Three factors can be distinguished with regard to 'fear of giving birth', 'fear of bearing a physically or mentally handicapped child' and 'concern about one's

appearance'. Internal consistencies (Cronbach's alpha) of the scales were 0.77, 0.86 and 0.77, respectively.

Work stress

To assess work stress (job strain), a Dutch version (Houtman et al., 1998) of the Job Content Questionnaire (JCQ) (Karasek et al., 1998) was included in the pregnancy questionnaire. It consists of two subscales: job demands and job control. The job demands subscale consists of 25 items (scored on four point scale) that focused on work pace, mental workload and physical workload. The job control subscale consists of 11 items (scored on four point scale) (Cronbach's alpha 0.85 and 0.92, respectively). Job strain is a combination of high job demands and low job control. Women in the category 'low job strain' had reported low job demand with moderate or high job control. 'High job strain' consisted of women who reported high job demand with low or moderate job control. All other women were assumed to experience 'moderate job strain'.

Parenting stress

Parenting stress was assessed using a Dutch adaptation (Groenendaal & Gerrits, 1996) of the 20-item Parenting Daily Hassles scale (Crnic & Greenberg, 1990). Mothers rated the occurrence of typical everyday events in parenting and mother–child interactions on a four point scale (0= never or rarely, 1= sometimes, 2= a lot and 3= constantly). Internal consistency (Cronbach's alpha) of the scale was 0.85.

Maternal caffeine intake

Information on mother's dietary caffeine intake during pregnancy was obtained from items in the pregnancy questionnaire. Pregnant women were asked whether they drank coffee, tea or cola in the past week. Additionally, they were asked about the amount and type of coffee, tea and cola (caffeinated, decaffeinated, or both) they consumed. Total caffeine intake per day was calculated using the Dutch Food Composition Database which contains data on the nutritional composition and caffeine content of food and beverages.

Maternal long-chain polyunsaturated fatty acid status

From each pregnant woman that participated in the biomarker study, one blood sample was taken in a 10-mL EDTA(K2) evacuated tube (Vacutainer; Becton

Dickinson BV, Alphen aan de Rijn, The Netherlands) and sent to the Regional Laboratory of Amsterdam for processing.

Transport was by courier or by overnight mail in special envelopes, enabling processing within 28 hours of sampling. A previous study of our group showed that this delay did not compromise the validity of measured biomarkers (van Eijsden, van der Wal, Hornstra, & Bonsel, 2005). At the laboratory, plasma was prepared by centrifugation (1600 x g for 10 min at room temperature) and stored as 1-mL aliquots at - 80 °C until analysis. Fatty acid analysis was performed at the Analytic Biochemical Laboratory (ABL, Assen, the Netherlands). In short, after the addition of an internal standard (1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine) and 10-heptadecenoic acid (17:1) to check for carryover of free fatty acids during the isolation procedure, plasma lipid extracts were prepared by a modified Folch extraction method (Hoving, Jansen, Volmer, Van Doormaal, & Muskiet, 1988) after which phospholipids were isolated by solid-phase extraction on aminopropyl-silica columns (500 mg/3 mL; Varian, Palo Alto, CA) (Kaluzny, Duncan, Merritt, & Epps, 1985). Phospholipids were then hydrolyzed, and the resulting fatty acids were methylated with boron trifluoride-methanol (Morrison & Smith, 1964). Finally, the fatty acid methyl esters were separated and quantified by capillary gas chromatography with flame ionization detection (HP5890 series II; Hewlett-Packard, Palo Alto, CA) with the use of a polar and a nonpolar column (BPx70 and BP1, respectively; SGE Analytical Science Pty. Ltd, Ringwood, Victoria, Australia). The oven temperature was programmed to begin at 160 °C for 4 min and then to increase to 200 °C by 6.0 °C/min. After 3 min, the temperature was further increased to 260 °C at a rate of 7 °C/min and kept constant for 2.34 min. The injector temperature was kept at 250 °C and the detector temperature at 300 °C. Absolute amounts of fatty acids (in mg/L plasma) were quantified on the basis of recovery from the internal standard and calculated in relative values (percentage of total fatty acids). In the current dissertation, the early life influence of the following fatty acids will be examined: eicosapentaenoic acid (EPA; 20:5n3), docosahexaenoic acid (DHA; 22:6n3), arachidonic acid (AA; 20:4n6).

Age five to six

Questionnaires and health check

In 2008, when the children were five years old, the third phase of the ABCD-study started. The addresses of 6161 of the 6735 mothers (92%) who gave permission for follow-up of their child were retrieved from the Youth Health Care registry; attrition in this follow-up number was largely due to untraceable changes in address or migration. Around two weeks after their child's fifth birthday, mothers received a questionnaire, including an informed consent sheet for granting permission for the age five health check.

The age five health check took place at the children's primary school (located all over Amsterdam) or at alternative locations such as Science Centre NEMO and the ARTIS zoo for children who had moved out of the city. Half an hour before school started, a fasting blood sample was taken from the children by means of a small finger prick. Thereafter a small breakfast was provided to the children before the school started. During the school day children were picked up from their classroom to take part in the second part of the health check; the physical measurements and cognitive testing. The physical measurements included anthropometric measurements (height, weight, waist and hip circumference), body composition measures (fat mass and fat-free mass) by bioelectrical impedance analysis, blood pressure in supine and sitting positions and cardiovascular function measures (heart rate, heart rate variability and pre-ejection period) in supine and sitting positions (van Dijk, van Eijsden, Stronks, Gemke, & Vrijkotte, 2010).

Problem behaviour

In addition to items about the child's health, medical conditions, family socio-demographics, maternal lifestyle and psychosocial conditions, the five-year questionnaire contained items from the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997), a short behavioural screening questionnaire which is used to measure children's risk for problem behaviour in this thesis. This questionnaire consists of 25 items, which are divided in 5 subscales: emotional symptoms, conduct problems, hyperactivity/inattention problems, peer relationship problems and pro-social behaviour. All items (without pro-social behaviour items) added together form a total difficulties score that represents children's overall problem behaviour. The questionnaire for the mother was

accompanied by a questionnaire about the child's behaviour (SDQ) and school performance, to be filled out by the child's teacher. Two weeks before the health check was scheduled mothers received a notifying letter and an additional food frequency questionnaire in order to assess their child's dietary habits and energy intake.

Cognitive testing

During the health check children's cognitive functioning was tested by using four tasks from a standardized, sensitive, neurocognitive test battery the *Amsterdam Neuropsychological Tasks* (ANT). The ANT program incorporates a number of well-founded tasks and task manipulations that are adjusted and suitable for the assessment of (pre)school aged children (De Sonneville, Visser, & Licht, 1999). The two ANT tasks described in this thesis were developed to evaluate processing speed, which forms the basis for the execution of more complex higher order cognitive functions. Furthermore, the ANT program has been successfully used in a wide diversity of healthy and clinical child populations (De Sonneville et al., 1999; Groot, De Sonneville, Stins, & Boomsma, 2004; Swaab-Barneveld et al., 2000). In the current study, in order to assess children's cognitive functioning two reaction time tasks were administered. The tasks were presented in a predefined and fixed order. Children were tested individually by a trained researcher or trained research assistant, in a quiet room predominantly in the morning. All tasks were presented on a laptop and responses were made by clicking the mouse buttons. First, a verbal instruction was given by the researcher or research assistant, with an example of the task displayed on the screen. Thereafter, the child was given a practice run to become familiar with the task stimuli and response mechanism. The test trial was started if the researcher felt confident that the child understood the task. For an overview of the outcome parameters see table 1.

Task (1). The first task, baseline speed (BS), is a measure of the child's processing speed and intensity of attention, involving minimal cognitive effort. The child was required to respond to a stimulus (white cross changing into a white square, see figure 1) as quickly as possible, using the non-dominant hand in part 1 and the dominant hand in part 2. There were 32 trials for each hand. Signal duration was variable until response, and a response was considered valid when made between 150 to 4000 milliseconds after stimulus appearance. A variable (random) inter stimulus interval was used ranging from 500 to 2500 milliseconds. Main outcome measures of the BS task are response speed as defined by the

mean reaction time and response speed stability as defined by the within-subject standard deviation of the reaction time.

Figure 1. Schematic representation of baseline speed reaction time task.

Task (2). The second task, response organization objects (ROO), is a measure of inherent response inhibition and response flexibility (see figure 2). The first part is the compatible part, in which the child was required to respond to a random laterally presented object (a red ball) on the computer screen. On the ball's appearance to the right side of a white fixation cross, the child should respond by clicking on the right mouse button using the right forefinger, on its appearance to the left, by clicking on the left mouse button by the left forefinger. The second part is the incompatible part, in which the reaction pattern is reversed. The child is now shown a white ball on the computer screen. On its appearance to the left

Figure 2. Schematic representation of compatible and incompatible parts of the response organization objects task.

of a white fixation cross, the child should respond by clicking on the right mouse button using the right forefinger, on its appearance to the right, by clicking on the left mouse button by the left forefinger. Main outcome measures of the ROO task are response speed as defined by mean reaction time per part, response speed

Table 1. Overview of cognitive outcome parameters at age five to six.

Task 1: Baseline speed (BS)		
Dominant hand (32 trials)	Mean RT	Mean reaction time in milliseconds
	SD(RT)	Within subject standard deviation of reaction time
	Omissions	Number of ignored stimuli (no valid response between 150-4000 milliseconds)
	Premature responses	Number of responses made before 150 milliseconds
Non-dominant hand (32 trials)	Mean RT	Mean reaction time in milliseconds
	SD(RT)	Within subject standard deviation of reaction time
	Omissions	Number of ignored stimuli (no valid response between 150-4000 milliseconds)
	Premature responses	Number of responses made before 150 milliseconds
Task 2: Response organization objects (ROO)		
Compatible part 1 (30 trials)	Mean RT	Mean reaction time in milliseconds
	SD(RT)	Within subject standard deviation of reaction time
	Errors	Number of errors
	Omissions	Number of ignored stimuli (no response between 200-6000 milliseconds)
	Premature responses	Number of responses made before 200 milliseconds
Incompatible part 2 (30 trials)	Mean RT	Mean reaction time in milliseconds
	SD(RT)	Within subject standard deviation of reaction time
	Errors	Number of errors
	Omissions	Number of ignored stimuli (no response between 200-6000 milliseconds)
	Premature responses	Number of responses made before 200 milliseconds

stability as defined by the within-subject standard deviation of the reaction time per part.

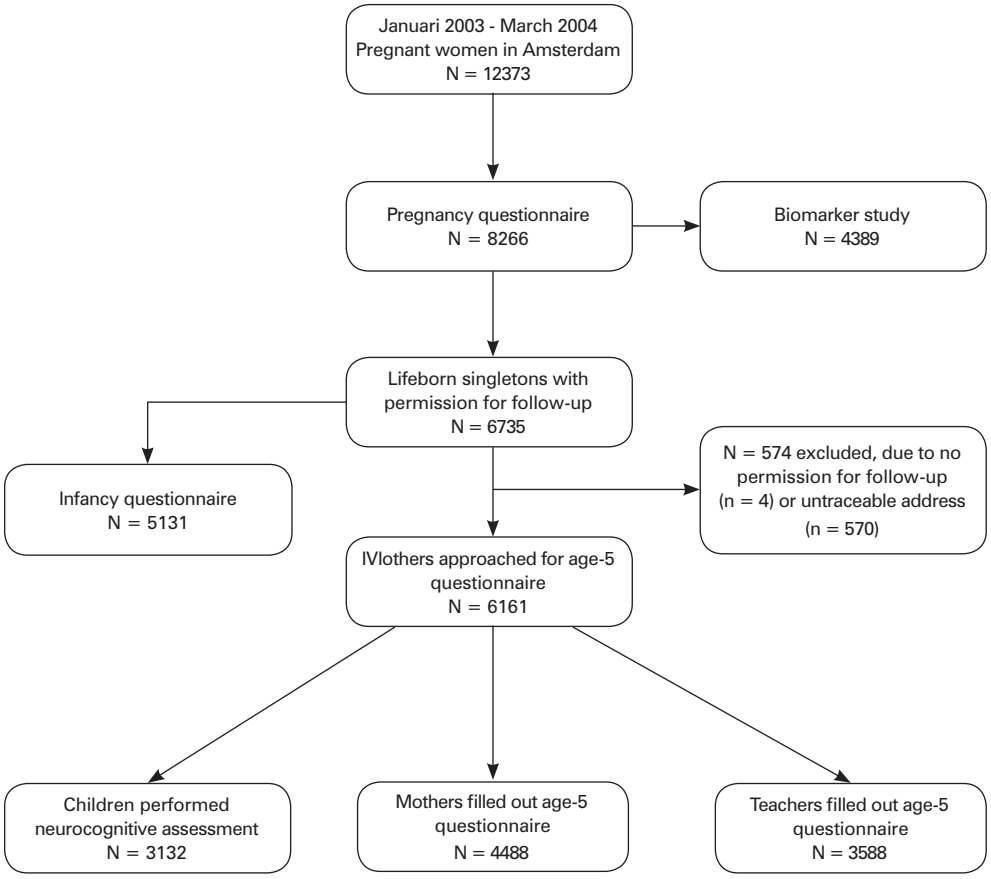

Figure 3. Flow chart of assessments and participants in the phases of the ABCD-study.

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3

Psychosocial stress during pregnancy is related to adverse birth outcomes: results from a large multi-ethnic community-based birth cohort

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Abstract

Background: Prevalence rates of psychosocial stress during pregnancy are substantial. Evidence for associations between psychosocial stress and birth outcomes is inconsistent. This study aims to identify and characterise different clusters of pregnant women, each with a distinct pattern of psychosocial stress, and investigate whether birth outcomes differ between these clusters.

Methods: Latent class analysis was performed on data of 7,740 pregnant women (Amsterdam Born Children and their Development, ABCD study). Included constructs were depressive symptoms, state anxiety, job strain, pregnancy-related anxiety and parenting stress.

Results: Five clusters of women with distinct patterns of psychosocial stress were objectively identified. Babies born from women in the cluster characterised as 'high depression & high anxiety, moderate job strain' (13%) had a lower birth weight, and those in the 'high depression & high anxiety, not employed' cluster (15%) had an increased risk of preterm birth.

Conclusion: Babies from pregnant women reporting both high levels of anxiety and depressive symptoms are at highest risk for adverse birth outcomes.

Key words: Psychosocial stress; pregnancy; gestational age; birth weight; latent class analysis

Introduction

About 25% of pregnant women experience some form of psychosocial stress (Yali & Lobel, 1999). From a public health perspective it is important to identify those who suffer from psychosocial stress during pregnancy, because psychosocial factors (besides biomedical risk factors) might, in part, be accountable for pregnancy complications and adverse obstetric outcomes (Littleton, Radecki Breitkopf, & Berenson, 2007). Elevated levels of anxiety and depressive symptoms are reported to be related to obstetric complications and adverse pregnancy outcomes, like preterm birth (Alder, Fink, Bitzer, Hösli, & Holzgreve, 2007). Accordingly, in a recent meta-analytic review, psychosocial stress during pregnancy was found to be weakly related to neonatal weight and the risk for low birth weight (Littleton, Bye, Buck, & Amacker, 2010). In contrast, in a meta-analysis of 50 studies, no relation was found between anxiety symptoms during pregnancy and adverse perinatal outcomes (Littleton et al., 2007).

Although the experience of severe job strain during pregnancy was found to be related to adverse birth outcomes (Brandt & Nielsen, 1992; Chen et al., 2000; Oths, Dunn, & Palmer, 2001; Vrijkotte, van der Wal, van Eijsden, & Bonsel, 2009), these findings are not unequivocal among comparable studies (Henriksen, Hedegaard, & Secher, 1994; Klebanoff, Shiono, & Rhoads, 1990). Feelings of pregnancy-specific stress were directly associated with preterm delivery and indirectly with low birth weight (Lobel et al., 2008). However, it is unclear whether stress specifically related to the parenting role (parenting stress) in women who have additional children, is related to adverse birth outcomes.

The fact that findings and effect sizes vary among studies is probably due to differences in study design, such as which measure of psychosocial stress was used, and the pregnancy trimester in which these measures were administered. Furthermore, potential confounding factors and biomedical risk factors that might affect birth outcomes are not always taken into account (for reviews see: Alder et al., 2007; Littleton et al., 2007). Previous results from our prospective longitudinal community based birth cohort, also show that lifestyle factors (e.g. smoking) largely confounded the association between depression and major pregnancy outcomes (Goedhart, van der Wal, Cuijpers, & Bonsel, 2009).

In an attempt to elucidate inconsistent findings from previous research, we investigated the potential influence of latent clusters of psychosocial stress during pregnancy on adverse birth outcomes. Moreover, we apply a *person-*

oriented approach that incorporates multiple validated psychosocial stress constructs (anxiety and depressive symptoms, pregnancy-related anxieties, parenting stress and work-related stress) to objectively identify and characterise clusters of women with distinct latent patterns of psychosocial stress (von Eye, Bogat, & Rhodes, 2006). Second, we investigate whether different associations with birth outcomes exist between women in different clusters taking potentially confounding factors into account.

Methods

Participants

Between January 2003 and March 2004, all pregnant women (N= 12,373; 99% of target population) living in Amsterdam, were approached to participate in the Amsterdam Born Children and their Development (ABCD) study during their first prenatal visit to an obstetric care provider. Two weeks later, a pregnancy questionnaire that covered sociodemographic characteristics, obstetric history and psychosocial conditions was sent to their address. 8,266 women filled out the questionnaire (67% response rate) at an average of 16 weeks' gestation (IQR 14-18 weeks) and complete data (i.e., five psychosocial stress questionnaires) were available for 7,740 women (93.6%). All live born singletons with data on gestational age (N= 7,391), preterm birth (N= 7,391), birth weight and birth size (N= 7,385) were included. Further details about this cohort have been described (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2011). Approval of the study was obtained from the Central Committee on Research Involving Human Subjects in the Netherlands, the medical ethics review committees of the participating hospitals, and the Registration Committee of the Municipality of Amsterdam.

Materials

The pregnancy questionnaire included five validated Dutch translations of widely used questionnaires:

Depressive symptoms

Depressive symptoms were assessed using the validated Dutch version (Hanewald, 1987) of the 20-item Centre for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977), which evaluates the frequency of depressive

symptoms experienced during the preceding week. Two categories were defined: no depressive symptoms (<16) and depressive symptoms (≥16). In the present sample the internal consistency (Cronbach's alpha) of the CES-D scale was 0.90.

Anxiety

Anxiety was assessed using the Dutch version (van der Ploeg, Defares, & Spielberger, 1980) of the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, & Lushene, 1970). The 20 items regarding state anxiety (transient or temporarily experienced anxiety) were included in our questionnaire. A cut-off score of 44 delineated the low-average from the high anxiety group (Mennes, Stiers, Lagae, & Van den Bergh, 2006). Internal consistency (Cronbach's alpha) of the scale was 0.94.

Pregnancy-related anxieties

Pregnancy anxieties were assessed using an abbreviated 10-item version (Huizink, Mulder, Robles de Medina, Visser, & Buitelaar, 2004) of the Pregnancy-Related Anxiety Questionnaire (PRAQ) (Van den Bergh, 1990). Three factors that can be distinguished are 'fear of giving birth', 'fear of bearing a physically or mentally handicapped child' and 'concern about one's appearance'. Because cut-offs were not available for this instrument, we used the 90th percentile in all *three* subscales to identify women scoring high on pregnancy anxiety. Internal consistency (Cronbach's alpha) of the scales was 0.77, 0.86 and 0.77, respectively.

Work stress

To assess work stress (job strain) a Dutch version (Houtman et al., 1998) of the Job Content Questionnaire (JCQ) (Karasek et al., 1998) was used. It consists of 2 subscales: job demands and job control (Cronbach's alpha 0.85 and 0.92, respectively). In accordance with the JCQ guidelines, we divided the job demand score into: low (<50th percentile), moderate (50th-90th percentile) and high (>90th percentile). Similarly, we divided the job control score into high (>50th percentile), moderate (10th-50th percentile), and low (<10th percentile). Next, also in accordance with the JCQ guidelines, the categorical variable of interest, i.e. job strain, could be determined. One of the four categories consisted of non-employed women. Women in the category 'low job strain' had reported low job demand with moderate or high job control. 'High job strain' consisted of women

who reported high job demand with low or moderate job control. All other women fell into the 'moderate job strain' category.

Parenting stress

Parenting stress was assessed using a Dutch adaptation (Groenendaal & Gerrits, 1996) of the 20-item Parenting Daily Hassles (PDH) scale (Crnic & Greenberg, 1990). The parents rated the occurrence of typical everyday events in parenting and parent-child interactions. Because cut-offs were not available for this instrument, we used the 90th percentile to identify the women scoring high on parenting stress. The 'no parenting stress' category also included the pregnant women with no previous children. Internal consistency (Cronbach's alpha) was 0.85.

Maternal characteristics and birth outcomes

Data sources and definitions of the maternal characteristics have been described previously (van Dijk, van Eijsden, Stronks, Gemke, & Vrijkotte, 2010). These are: maternal age (continuous), educational level (continuous; years of education after primary school; proxy for socioeconomic status), ethnicity (categorical), living with a partner (categorical), smoking (discrete), alcohol consumption (discrete), obesity and parity (dichotomous). Information on birth outcomes was available from Youth Health Care Registration and the Dutch Perinatal Registration (PRN; www.perinatreg.nl). Gestational age was dichotomized into at term and preterm (gestational age < 37 weeks). To define 'birth size', the bottom and top 10% of birth weight, standardized for gender, gestational age and parity using reference values from the PRN, were labelled small-for-gestational-age and large-for-gestational-age, respectively. The middle 80% was labelled appropriate-for-gestational-age (Visser, Eilers, Elferink-Stinkens, Merkus, & Wit, 2009).

Statistical analysis

A latent class analysis (Vermunt, 2005) was performed to identify and describe clusters of women with similar patterns of psychosocial stress (Latent GOLD 4.5; Statistical Innovations, Boston, USA). In subsequent analyses, maternal characteristics and birth outcomes were compared among women belonging to the different clusters using ANOVA with post-hoc Tukey's tests, and Chi-square tests. Subsequently, associations between cluster membership and birth outcomes were tested in multiple poisson regression (gestational age), linear

regression (birth weight) and logistic regression models (preterm and small-for-gestational age birth), adjusted for maternal age, educational level, ethnicity, smoking, alcohol consumption, hypertension, pre-pregnancy BMI, parity, foetal sex and gestational age (when analyzing birth weight). The first cluster was used as the reference category.

Results

Descriptive analyses

Table 1 presents the sociodemographic characteristics of the study sample. Results from the latent class analysis suggested that the optimal number of clusters was either five or six (table 2). This assumption was based on the substantial decrease in the log likelihood ratio; L^2 between the 4- and 5-cluster model (L^2 difference=131). Despite the additional decrease in L^2 between the 5- and 6-cluster model (L^2 difference=203), the subsequent analysis concentrates on the 5-cluster solution because it was the most comprehensive and parsimonious. When cases were classified into these five clusters, misclassification is estimated at 15.2%, which can be considered satisfactory (Vermunt, 2005). Classification certainty ranged from acceptable to high as mean posterior assignment probabilities were 93%, 84%, 90%, 91%, and 83% respectively. The local independence assumption was tested and inspection of bivariate residuals suggested that indicators were roughly unrelated within each latent class.

Table 1. Characteristics of the study population (n=7,440).

	Mean/Percentage	SD	Interquartile range	
			Lower	Upper
Age (years)	30.8	5.2	24.8	36.8
Education after primary school (years)	8.7	4	2.7	14.7
Ethnicity (%)(n)	(7740)			
Dutch	63.5 (4914)			
Surinamese	5.5 (423)			
Antillean	1.1 (89)			
Turkish	3.9 (304)			
Moroccan	6.7 (516)			
Ghanaian	2.2 (170)			
Other non-western country	8.3 (641)			
Other western country	8.8 (683)			
Living with partner (% yes)	86.1			
Smoking (%)(n)	(7732)			
No smoking	90.5 (6995)			
1-5 cigarettes/day	6.1 (470)			
≥ 6 cigarettes/day	3.5 (267)			
Alcohol (%)(n)	(7737)			
No alcohol	78.9 (6101)			
< 1 glass/day	20.5 (1586)			
1-3 glasses/day	0.6 (50)			
Obesity (BMI ≥ 30) (% yes)	6.1			
Primiparous (% yes)	56.1			
CES-D total score	13	9	2	24
STAI total score	38	10	24	52
PRAQ: fear of handicapped child	12	3	9	15
PRAQ: fear of giving birth	9	2	6	12
PRAQ: concerns about appearance	9	2	7	11
Job strain (%)(n)	(7740)			
No job	35.7 (2766)			
Low job strain	11.1 (859)			
Moderate job strain	45.1 (3490)			
High job strain	8.1 (625)			
PDH total score (multiparous 43.9%)	36	8	25	47

	Mean/Percentage	SD	Interquartile range	
			Lower	Upper
Gestational age (weeks)	39.7	2.4	37.7	41.7
Preterm birth (<37 weeks) (% yes)	6.3			
Birth weight (g)	3419	615	2739	4099
Size at birth (%)(n)	(7740)			
Large for gestational age	9.5 (734)			
Appropriate for gestational age	81.2 (6284)			
Small for gestational age	9.3 (722)			

Table 2. Summary of model fit indices of the Latent Class Analysis.

				Information Criteria				Bootstrap	Bootstrap
	DF	L2	p-value	BIC	AIC	AIC3	CAIC	L2-difference	p-value
2-Cluster model	238	2144	<0.001	13	1668	1430	-225		
3-Cluster model	230	948	<0.001	-1111	488	258	-1341	1196	<0.001
4-Cluster model	222	631	<0.001	-1375	187	-35	-1579	317	<0.001
5-Cluster model	214	500	<0.001	-1517	72	-142	-1631	131	<0.001
6-Cluster model	206	297	<0.001	-1548	-115	-321	-1754	203	<0.001

DF = Degree of freedom, L2 = Log-Likelihood ratio, p-value = Bootstrap estimate of the p-value associated with the L2, BIC = Bayesian Information Criterion, AIC(3) = Akaike Information Criterion (3), CAIC = Consistent Akaike Information Criterion, Bootstrap L2-difference = L2 difference after adding a cluster Bootstrap p-value = Bootstrap estimate of the p-value associated with the L2 difference.

Characterisation of clusters

Table 3 shows the distribution of women over the five clusters. In cluster 1, few women reported either high depressive symptoms or high levels of state anxiety. Also, pregnancy-related anxieties were not frequently reported by these women; they generally reported moderate job strain and most of them did not report parenting stress.

Table 3. Probability and characterization of cluster membership (n=7,440).

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**R^2**
Cluster size	n=3869	n=1116	n=967	n=818	n=670	
%	52	15	13	11	9	
Depressive symptoms (%)						0.70
No depressive symptoms	94	4	10	88	91	
Depressive symptoms	6	96	90	12	9	
Job strain (%)						0.47
No job	17	94	3	89	32	
Low job strain	14	5	6	8	17	
Moderate job strain	61	1	67	3	47	
High job strain	9	0	24	0	4	
Fear of giving birth (%)						0.39
No fear of giving birth	95	97	95	100	32	
Fear of giving birth	5	3	5	0	68	
Fear of handicapped child (%)						0.15
No fear of handicapped child	95	91	93	75	52	
Fear of handicapped child	5	9	7	25	48	
Concerns about appearance (%)						0.43
No concerns about appearance	94	94	93	95	16	
Concerns about appearance (%)	6	6	7	5	84	
State anxiety (%)						0.73
Low state anxiety	97	8	6	86	92	
High state anxiety	3	92	94	14	8	
Parenting stress (%)						0.05
No parenting stress	94	74	84	88	92	
Parenting stress	6	26	16	12	8	

R^2 = the amount of the variance of each indicator explained by the 5-cluster model

Cluster 2 consists of women who scored high on depressive symptoms and reported high levels of state anxiety. However, these women did not report high levels of pregnancy- related anxieties. Most of the women in cluster 2 are unemployed and this cluster contains the highest percentage of women that experienced parenting stress. Women in cluster 3 also reported a high number of depressive symptoms and scored high on state anxiety; however, compared to women in cluster 2, most of them are employed and reported moderate job strain. Similar to women in cluster 2, women in cluster 3 did not have high levels of pregnancy-related anxiety. This cluster does not show a discriminate percentage of women who experienced parenting stress. Women in cluster 4 scored low on depressive symptoms, and on state anxiety and pregnancy anxieties. They are similar to the women in cluster 1, except that most of the women in cluster 4 are unemployed. Women in cluster 5 seem more anxious about their pregnancy as they frequently reported concerns about their appearance and about giving birth. They scored low on depressive symptoms and on state anxiety. Cluster 5 does not show a discriminate distribution of employment and job strain. The clustering explained the variance of most constructs by minimally 5% (parenting stress) to maximally 73% (state anxiety).

Associations between cluster membership and maternal characteristics

Associations between cluster membership and other maternal characteristics during pregnancy, such as smoking, alcohol use, educational level and ethnicity are presented in Table 4.

Table 4. Maternal characteristics and birth outcomes per cluster (n=7,440).

	1 Low depression & low anxiety, moderate job strain		2 Depressed & anxious, not employed		3 Depressed & anxious, moderate job strain		4 Low depression & low anxiety, not employed		5 Low depression & low anxiety, with pregnancy anxieties	
	n=3869		n=1116		n=967		n=818		n=670	
	mean/percentage	SD								
Age (years)	31.4 bcde	4.7	28.7 ace	6.0	30.8 abde	5.2	28.1 ace	5.8	32.2 abcd	p<0.01
Education after primary school (years)	9.7 bcde	3.7	6.2 ace	4.1	8.9 abd	3.6	6.2 ace	4.0	8.9 abd	p<0.01
Ethnicity (%)	bcde		acde		abde		abce		bcd	p<0.01
Dutch	73.5		33.7		66		28.9		67.9	
Surinamese	3.5		8.8		8.5		8.1		7.6	
Antillean	0.8		1.8		1.9		2		0.7	
Turkish	2		12.2		2.4		8.5		0.8	
Moroccan	4.6		14.5		4.3		13.9		5.6	
Ghanaian	0.9		4		1.6		10.1		3.3	
Other non-western country	5.5		18.1		5.4		20.2		5.8	
Other western country	9.2		6.9		10		8.3		8.1	
Living with partner (% yes)	90.7 bcde		73.5 ace		82.7 abde		77.1 ace		87.7 abcd	p<0.01
Smoking (%)	bce		ade		ae		b		abc	p<0.01
No smoking	92.9		84.6		86		90.3		90.4	
1-5 cigarettes/day	4.8		8.6		9.4		5.9		5.6	
≥ 6 cigarettes/day	2.3		6.8		4.6		3.8		4	

	1 **Low depression & low anxiety, moderate job strain**		2 **Depressed & anxious, not employed**		3 **Depressed & anxious, moderate job strain**		4 **Low depression & low anxiety, not employed**		5 **Low depression & low anxiety, with pregnancy anxieties**	
	n=3869 mean/percentage	SD	n=1116		n=967		n=818		n=670	
Alcohol (%)	bde		ace		bd		ace		abd	p<0.01
No alcohol	75.1		90.3		77.4		87.9		80.4	
< 1 glass/day	24.2		8.8		22.2		11.5		19.3	
1-3 glasses/day	0.7		1		0.4		0.6		0.3	
Obesity (BMI ≥ 30) (% yes)	4.6 bcd		9.9 ace		7.6 ab		9.4 ae		5.8 bd	p<0.01
Primiparous (% yes)	63.9 bcde		42 ace		57.9 abde		40.6 ace		33.4 abcd	p<0.01
Gestational age (weeks)	39.7 bc	2.7	39.3 a	3.2	39.4 a	3.2	39.4	2.9	39.5	p<0.01
Preterm birth (<37 weeks) (% yes)	5.7 b		8.4 ad		7.2		5.5 b		6.1	p=0.01
Birth weight (g)	3451 bc	581	3333 ae	685	3359 ae	654	3382	631	3455 bc	p<0.01
Size at birth (%)	b		ace		b				b	p<0.01
Small for gestational age	8.3		13		10		11		7.5	
Appropriate for gestational age	81.7		77.9		82.2		81.2		82.6	
Large for gestational age	10		9.1		7.9		7.8		10	

a Significantly different from Cluster 1 (p<0.05), b Significantly different from Cluster 2 (p<0.05), c Significantly different from Cluster 3 (p<0.05), d Significantly different from Cluster 4 (p<0.05), e Significantly different from Cluster 5 (p<0.05).

Most women in cluster 1 (low depression & low anxiety, moderate job strain) are of Dutch origin, highly educated, live with their partner, do not smoke and do not drink alcohol. Furthermore, for most of them it was their first pregnancy. The ethnic background of women in cluster 2 (high depression & high anxiety, not employed) is more diverse, almost 10% of them are obese and the rate of unemployment is high. All women in cluster 3 (high depression & high anxiety, moderate job strain) reported to have a job and 24% of them reported high levels of job strain (Table 3). Women in cluster 4 (low depression & anxiety, not employed) are relatively young and for 59% of them this is not their first pregnancy. Comparable to cluster 2 (high depression & high anxiety, not employed), cluster 4 (low depression & low anxiety, not working) includes women from various ethnic backgrounds, however only 1% of these women reported to have a job. Women in cluster 5 (low depression & low anxiety, high pregnancy anxieties) are relatively old and highly educated.

Associations between cluster membership and birth outcomes

The unadjusted associations between cluster membership and perinatal outcomes (i.e. gestational age, preterm birth, birth weight, size at birth) are presented in Table 4; the adjusted associations are shown in Table 5. There was a significant difference in mean gestational age and birth weight between the clusters in the unadjusted models (both $p<0.01$). As compared to babies of women in the 'low depression & low anxiety, moderate job strain' cluster (1; the reference cluster), babies from women in the 'high depression & high anxiety, not employed' and 'high depression & high anxiety, moderate job strain' clusters (2 and 3) had a lower gestational age. After adjustment for confounders, these differences attenuated to the null.

As compared to the reference cluster, babies from women in the 'high depression & high anxiety, not employed' and 'high depression & high anxiety, moderate job strain' clusters (2 and 3) had a lower birth weight. After adjustment for confounders, the difference in birth weight in cluster 3 remained significant ($p=0.02$). Although the difference in this continuous outcome variable is small, it is an important result that the differences between the clinically-driven, categorized equivalents, (i.e., preterm birth and small for gestational age birth) are significant ($p=0.01$ and $p<0.01$, respectively). However, after adjustment for confounders, only the increased risk for preterm birth remained significant in women from the 'high depression & high anxiety, not employed' cluster (2) ($p=0.02$).

Table 5. Adjusted associations of birth outcomes by cluster.

	1 Low depression & low anxiety, moderate job strain	2 Depressed & anxious, not employed	3 Depressed & anxious, moderate job strain	4 Low depression & low anxiety, not employed	5 Low depression & low anxiety, with pregnancy anxieties
Gestational age (weeks) [a]*					
Estimated marginal means (SE)	38.2 (0.8)	38.0 (0.9)	38.1 (0.8)	38.0 (0.8)	38.4 (0.8)
Birth weight (g) [b]					
β (95%CI)	*Reference*	-27 (-60;7)	-39 (-72;-6)	-6 (-56;44)	11 (-29;51)
Preterm birth [c]					
OR (95%CI)	*Reference*	1.4 (1.0;1.9)	1.2 (0.9;1.7)	1.0 (0.6;1.7)	1.1 (0.7;1.6)
Small for gestational age birth [c]					
OR (95%CI)	*Reference*	1.1 (0.9;1.4)	1.1 (0.8;1.4)	1.0 (0.7;1.4)	0.7 (0.5;1.0)

All models (a=multivariate poisson; b=linear; c=logistic regression models) are adjusted for maternal age, educational level, ethnicity, smoking, alcohol consumption, hypertension, pre-pregnancy BMI, parity, foetal sex and gestational age (when analysing birth weight). Mean/modal values were used when calculating the estimated marginal means of gestational age.* Gestational age in none of the clusters was significantly different from gestational age in cluster 1.

Discussion

Based on five validated questionnaires addressing psychosocial stress, five distinct clusters of pregnant women were objectively identified by means of a latent class analysis. Babies born from women in the cluster characterised as 'high depression & high anxiety, moderate job strain' had a significantly lower birth weight compared to babies from women in the 'low depression & low anxiety, moderate job strain' (reference) cluster. Those in the 'high depression & high anxiety, not employed' cluster had an increased risk of preterm birth.

The current study provides insight into the inconclusive results from previous studies on the relation between psychosocial stress during pregnancy and adverse birth outcomes (Littleton et al., 2010; Littleton et al., 2007). Our results indicate that women who experience both high levels of anxiety as well as depressive symptoms are particularly at risk for adverse birth outcomes; this is in accordance with conclusions from a previous review, where both constructs

were identified as risk factors that contributed independently to adverse obstetric, foetal and neonatal outcome (Alder et al., 2007). At first sight, effect sizes in the present study may seem small, but the clinically-driven cut-off for preterm birth was significantly different between clusters. In all clusters, mean gestational age was 39 weeks, but preterm birth, an important indicator for health-related adversities in later life (Barker, 2004a, 2004b, 2004c), still ranged from 5.5-8.4% over the five clusters. Such small differences may have a large impact on public health when extrapolated to a larger population (Rose, 1992).

Based on our findings, maternal employment status and the experience of job strain during pregnancy did not seem to be a discriminatory risk factor for negative birth outcomes, which corroborates the finding that the experience of job strain by itself was not associated with adverse pregnancy outcomes (Mutambudzi, Meyer, Warren, & Reisine, 2011). However, earlier results from our cohort showed an association between high levels of job strain during pregnancy and offspring's lower birth weight (Vrijkotte et al., 2009). Presumably, the experience of high levels of job strain combined with other psychosocial risk factors such as depressive symptoms and high levels of state anxiety (cluster 3) might increase the odds for adverse pregnancy outcomes. In contrast to another study (Lobel et al., 2008), we found that pregnancy-specific anxieties in the absence of other feelings of psychosocial stress (cluster 5) were not related to adverse birth outcomes. Hence, these pregnancy-related worries might indicate a healthy concern about the development of the unborn child (Leifer, 1977, 1980), which confirms the idea that pregnancy-related anxieties are distinct from general anxiety (Huizink et al., 2004). Parenting stress was most frequently reported by women in cluster 2, who were most often unemployed and, therefore, more likely to spend extended time at home with their children. The amount of explained variance in parenting daily hassles by cluster allocation was low (5%) and was not associated with negative birth outcomes. To our knowledge, no previous studies have reported on an effect of parenting stress on birth outcomes. It is important to note that, in the present study, because assessment of psychosocial stress was at a subclinical level, associations with birth outcomes might be an underestimation in certain groups of women who suffer from (diagnosed) mood disorders (Alder et al., 2007).

Strengths & limitations

We have demonstrated in a large cohort that the application of a *person-oriented* approach that appreciates inter-individual differences in psychosocial stress patterns is useful to identify clusters of women with unique latent patterns of psychosocial stress. It is also shown that these clusters can subsequently be used to investigate associations with birth outcomes. Furthermore, analyses were conducted in a large multi-ethnic community-based sample which is a clear advantage in terms of statistical power; moreover, only validated and commonly used questionnaires were used as indicators to assess various constructs of psychosocial stress. Pregnancy outcomes were available through record linkage with information from Youth Health Care Registration and the Dutch Perinatal Registration, eliminating any potential recall bias. In addition, analyses between cluster membership and birth outcomes were adjusted for a large number of *a priori* selected potentially confounding factors.

The study also has some methodological limitations. First, because psychosocial stress was examined at one occasion during gestation, we were unable to examine patterns and chronicity over the course of pregnancy. Second, assessment of depressive symptoms during pregnancy using a questionnaire is complicated because physical complaints (e.g. fatigue) are associated with both pregnancy and depression. However, Kabir, Sheeder, and Stevens-Simon (2008) showed that removing the somatic items from the CES-D did not improve the psychometric properties or its predictive capacity (Kabir et al., 2008). Third, selective non-response poses a threat to study validity. However, an anonymous non-response analysis using national perinatal registry data has revealed that although selective non-response was present in our cohort, selection bias was acceptably low and did not influence main study outcomes (Tromp, van Eijsden, Ravelli, & Bonsel, 2009).

Implications

Results of the current study are informative and valuable to different groups of public health professionals. First, for researchers our results provide insight into the inconsistent findings from previous studies. The presence of both elevated levels of anxiety and depressive symptoms seem to be related to adverse pregnancy outcomes, independent of biomedical risk factors, other types of psychosocial stress and maternal characteristics. Secondly, results from the current study have identified and characterised pregnant women at risk for adverse birth outcomes;

our findings strengthen the argument that pregnant women should be screened for the presence of anxiety and depressive symptoms early in gestation during routine antenatal care (Lynn, Alderdice, Crealey, & McElnay, 2011). Addressing the needs of these women at risk (representing almost 30% of the women in our sample) by means of support from public health professionals may enhance the prevention of long-term negative outcomes for both mothers and their offspring. In the future, we aim to investigate whether the identified clusters are differentially related to long-term offspring's (mental) health outcomes in this large multi-ethnic birth cohort.

Key-points

Prevalence rates of psychosocial stress during pregnancy are substantial, and evidence for associations with birth outcomes is inconsistent.

Different clusters of pregnant women, each with a distinct pattern of psychosocial stress can be identified using latent class analysis. Significant differences in adverse birth outcomes exist between these clusters; babies from pregnant women reporting both high levels of anxiety and depressive symptoms were most at risk.

Addressing the needs of these women (i.e. 28% of the women in our cohort) by means of support from public health professionals may enhance the prevention of long-term negative outcomes for both mothers and their offspring.

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4

High levels of antenatal maternal anxiety are associated with altered cognitive control in five year old children

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Abstract

This longitudinal prospective study examined the relation between maternal anxiety during pregnancy and specific aspects of children's cognitive functioning at age five. Antenatal maternal state-anxiety was measured around the 16th week of pregnancy. Children's neurocognitive functioning was examined using a simple reaction time (RT) task, and a choice RT task. Multiple regression analyses in the total sample ($N = 922$) showed that antenatal anxiety was positively related to children's intra-individual variability in RT in the simple task. In a subsample ($n = 100$) of women with state-anxiety scores above the 90th percentile, antenatal anxiety was positively associated with mean RT and intra-individual variability in RT in the incompatible trials of the choice RT task. In addition, in this subsample of highly anxious mothers we found a significant positive association in boys but not in girls, between prenatal maternal anxiety and intra-individual variability in RT in the simple task.

Introduction

There is increasing evidence for an association between antenatal exposure to maternal anxiety and alterations in neurodevelopment in both animals and humans (Räikkönen, Seckl, Pesonen, Simons & Van den Bergh, 2011; Van den Bergh, Mulder, Mennes, & Glover, 2005a). Although the mediating mechanisms through which this antenatal influence occurs remain unclear, an established significant correlation between glucocorticoid levels (e.g., cortisol) in the maternal and foetal compartments points to a potential role of glucocorticoids (Gitau, Fisk, Teixeira, Cameron, & Glover, 2001). If maternal cortisol is insufficiently converted into inactive cortisone in the placenta, exposure to high levels of cortisol may influence important foetal neuronal developmental processes, such as proliferation, migration and differentiation of neurons that occur in the first and second trimester of pregnancy (Räikkönen et al., 2011).

Until now, developmental outcomes after prenatal exposure to maternal anxiety have often been assessed by using behavioural scales completed by parents. In addition, aspects of infant and toddler motor development -i.e., fine and gross motor control, posture, and cognitive development- were in some studies tested using the Bayley Scales of infant development (Brouwers, van Baar, & Pop, 2001; Buitelaar, et al., 2003; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002; Van den Bergh, 1990, 1992). Others have examined school performance (Niederhofer & Reiter, 2004) and language development (Laplante, Brunet, Schmitz, Ciampi & King, 2008). However, these objective evaluations of development are relatively crude, and they do not assess specific cognitive processes and functions that might be affected by antenatal exposure to maternal anxiety.

Long-term evidence for an association between antenatal anxiety and specific cognitive functions in the offspring is accumulating. High levels of antenatal anxiety have been related to specific impairments in cognitive control that last into adolescence (Mennes, Stiers, Lagae, & Van den Bergh, 2006; Van den Bergh et al., 2005b, 2006). This finding has been strengthened by measuring brain functioning with event related potentials (ERP) and fMRI during cognitive tasks, where altered patterns of activity were found in areas that are hypothesized to be connected with the prefrontal cortex in adolescents of mothers scoring high on state anxiety at 12-22 weeks of pregnancy (Mennes, 2008; Mennes, Van den Bergh, Lagae, & Stiers, 2009).

On the other hand, other studies have found no associations between antenatal maternal stress with scores on the Bayley Scales (Van den Bergh, 1990, 1992), performance IQ in the (pre)school age (Laplante et al., 2008), vocabulary development in middle childhood (Whitehouse et al., 2010), working memory (Mennes et al., 2006, 2009) and exogenous cognitive control in adolescents (Van den Bergh et al., 2005b). Furthermore, mild stress during gestation was found to enhance learning performance (Fujioka et al., 2001) and improve cognitive functioning (Hougaard et al., 2005) in rat offspring. In humans, modest but favourable effects of maternal anxiety and nonspecific stress (within normal limits) on child development have also been reported (DiPietro, Novak, Costigan, Atella, & Reusing, 2006; Laplante et al., 2008). It could be possible that mild levels of stress during pregnancy have a beneficial influence on early brain maturation processes and alter HPA-axis functioning, thereby programming the offspring to adapt to mild stressful circumstances later in life (DiPietro et al., 2006). At least these results plea for a view in which the variability in results is stressed and more attention is paid to a profile of cognitive functioning in which some cognitive functions are affected and others remain unaffected.

The present study aims to extend current knowledge about antenatal developmental origins of cognitive functioning. To achieve this we used a standardized, sensitive, neurocognitive test battery (the Amsterdam Neuropsychological Tasks; ANT), which incorporates a number of well-founded tasks and task manipulations that are adjusted for the assessment of (pre)school children (De Sonneville, Visser, & Licht, 1999). The ANT has been found useful to assess basic attention and information-processing functions in a wide diversity of healthy and clinical child populations (De Sonneville et al., 1999; Groot, De Sonneville, Stins, & Boomsma, 2004; Slaats-Willemse, Swaab-Barneveld, De Sonneville, van der Meulen, & Buitelaar, 2003; Swaab-Barneveld et al., 2000). Both of the selected tasks are related to reaction time (RT): one is a simple RT task and the other is a more complex, choice RT task. To investigate children's neurocognitive functioning, we have evaluated their overall level of processing speed (mean RT) and intra-individual variability in processing speed. In the literature there is an increasing recognition that these measures of task performance reflect distinct aspects of cognitive and brain functioning. Whereas mean RT indexes overall performance efficiency, intra-individual variability in RT reflects the stability or inconsistency of performance across stimulus trials and time, which has been found to be related more strongly to individual differences

in intelligence (Jensen, 1992; Walhovd & Fjell, 2007), cognitive impairments that accompany aging (Hultsch, MacDonald, & Dixon, 2002; Li, Lindenberger, & Sikstrom, 2001), and certain neurological disorders (Bruhn & Parsons, 1977). Furthermore, intra-individual variability in RT during a continuous performance task in adolescent boys has been found to be affected by antenatal maternal anxiety. Boys of mothers who were highly anxious during the 12th to 22nd week of pregnancy showed significantly more variability in their performance near the end of a long and tedious task (Van den Bergh et al., 2006). Accordingly, the use of both measures (mean RT and SD(RT)) in the present study serves to facilitate our ability to detect subtle effects of maternal antenatal anxiety on children's cognitive functioning.

Based on previous studies, we hypothesized that there is an association between antenatal maternal anxiety and specific aspects of children's neurocognitive functioning at age 5 years. We expected high levels of antenatal anxiety to be related to alterations in the children's cognitive functioning such as longer RT and higher intra-individual variability in RT (i.e., a more variable performance) (Mennes et al., 2009; Van den Bergh et al., 2005b; Van den Bergh et al., 2006). Previous research has suggested sex specific effects of maternal anxiety on some cognitive tasks (i.e., sustained attention tasks) indicating long-term cognitive impairments in boys, but not in girls (Van den Bergh et al., 2006). Therefore, we hypothesized that the influence of prenatal maternal anxiety on RT and intra-individual variability in RT would be stronger in boys than in girls.

Methods

The present study is part of the Amsterdam Born Children and their Development (ABCD) study (www.abcd-study.nl), a community-based prospective cohort study that examines the relationship of maternal lifestyle and psychosocial determinants during pregnancy, to multiple aspects of development and health of the child.

Participants

Between January 2003 and March 2004, pregnant women in Amsterdam were asked to participate in the ABCD study during their first prenatal visit to an obstetric care provider. All together 12,373 women were approached; by estimate 99% of the target population (van Eijsden, Vrijkotte, Gemke & van der Wal, 2011). A questionnaire covering socio-demographic characteristics, obstetric history,

lifestyle, and psychosocial conditions was sent to the women's home addresses and the women were requested to return it to the Public Health Service by prepaid mail. Of these 12,373 approached women, 8,266 women filled in the pregnancy questionnaire (response rate 67%) and subsequently 7863 women gave birth to viable singleton infants and 132 women gave birth to viable multiples. The remaining mothers either experienced a miscarriage or fetal death (n=92) or were lost to follow-up (n=179) with no registered birth and no information on miscarriage or fetal death available from the care provider. Of this group, 7,050 (85%), women gave informed consent and permission for follow-up. At the current phase of the study, 6,554 mothers (93% of the follow-up group) were eligible for the fifth-year follow-up measurement (health as well as cognitive assessment) of their child (Phase III, 2008-2010). The presented cognitive data are from the first 952 mother-child pairs, who have participated in the third wave of the study. This sample of mothers and children is relatively random as we have visited the children at their primary schools across the city.

Mothers in the current sample did not differ from mothers in the follow-up group that did not participate, in smoking habits during pregnancy and the number of children they had given birth to before this pregnancy. However, mothers in the sample were on average 2 years older (p <.01), were more often employed (82.9 % vs. 70.1%, p <.01) and were more often highly educated compared to women not included in the sample (23.3% vs. 23.2% for low education, 39.4% vs. 38.3% for middle education, 39.3% vs. 38.5% for higher education, p <.01). More mothers in the sample reported to have consumed alcohol during their pregnancy than mothers who were not included (28% vs. 20.6% p <.001). Table 1 gives the demographic characteristics of the participating mothers and children. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands, the Medical Ethical Committees of the participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

Table 1. Descriptive statistics of mother and child characteristics.

Maternal characteristics during pregnancy	Antenatal maternal anxiety	
	Total group *N* = 922 *Mean* (*SD*) (IQR)	**Highly anxious subsample** *n* = 100 *Mean* (*SD*) (IQR)
Age (years)	32.2 (4.3) (30 – 35)	30.8 (5.2) (27 – 35)
Employed (%)	82.0	64.0
Education following primary school (%)		
0 – 5 years	23.3	32.3
6 – 10 years	39.3	29.3
11 years or more	37.3	38.4
Ethnicity (%)		
Dutch	77.0	49.0
Nulliparous (%)	57.0	41.0
Cohabiting (%)	91.0	90.0
STAI	36 (9.5) (30 – 41)	54.7 (5.7) (51 – 57)
STAI completed (gestational week)	16.4 (4.3) (14 – 18)	16.6 (4.5) (13.3 – 18)
Child characteristics at age 5 years		
Sex (boy %)	50.0	52.0
Age (years)	5.4 (0.2) (5.2 – 5.5)	5.4 (0.2) (5.3 – 5.5)
Birth weight corrected for gestational age (grams)	3475.7 (342.6) (3338.7 – 3698.5)	3391.2 (367.9) (3228 – 3643.1)

Note. SD = standard deviation ; IQR = interquartile range, STAI = State-Trait Anxiety Inventory.

Measures

Antenatal maternal anxiety

Antenatal maternal anxiety was measured using the Dutch version of the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, & Lushene, 1970; Van der Ploeg, Defares, & Spielberger, 1980). This self-report questionnaire is often used to assess anxiety during pregnancy and the postnatal period (Austin, Tully, & Parker, 2007). In the present study, only the State-Anxiety subscale consisting of 20 items (score range 1-4) was used. A higher score indicates a higher level of experienced anxiety. The State-Anxiety scale was found to be a valid (Spielberger, 1975) and reliable (Cronbach's alpha .91) measure of anxiety experienced temporarily or transiently (Van der Ploeg et al., 1980).

Neurocognitive assessment

The children's neurocognitive outcome was assessed using a computerised assessment program; the ANT. The psychometric properties of this battery have been found satisfactory (e.g., test-retest correlations range from 0.70 to 0.85) (Koekoek, De Sonneville, Wolfs, Licht, & Geelen, 2008). Children were individually tested predominantly in the morning or early afternoon during school days in a quiet room by trained investigators. The ANT was presented on a laptop and responses to task stimuli had to be made using the mouse. Before starting the task, the investigator gave a verbal task instruction while showing the child an example of the task on the computer screen. Thereafter, the child was given a practice run to become familiar with the task stimuli and response mode. When the investigator felt sure that the child understood the task demands the test trial was started. The present study evaluated neurocognitive functioning using several outcome parameters from two ANT tasks. The descriptive statistics of the main outcome parameters and the errors, omissions and premature responses made by the children are shown in table 2.

Simple RT task

The simple RT task is a measure of an individual's processing speed that requires minimal cognitive effort. The child is required to respond as quickly as possible when a white fixation cross in the centre of the computer screen changes into a white square. In the first part of the test, responses were made through a mouse click with the non-preferred hand, in the second part responses were made with the preferred hand. There are 32 trials for each hand. Signal duration is variable until response, and a response is considered valid when made between 150 to 4000 milliseconds after stimulus appearance. A variable (random) inter stimulus interval was used ranging from 500 to 2500 milliseconds. The main outcome parameters are mean RT and intra-individual variability in RT; the within-subject standard deviation of reaction time: SD(RT).

Choice RT task

The second task is a more complex, choice RT task. The task consists of two parts. During the first, compatible part (1), children are required to respond to laterally (at random) presented objects (red balls) on a computer screen. When a red ball appears on the left side of a white fixation cross, children have to respond by means of a mouse click with their left forefinger on the left mouse button. In case

a red ball appears on the right side of the fixation cross, a correct response is made by a click on the right mouse button with the right forefinger. The second, incompatible part (2) of the task is more complex, because the reaction pattern is reversed. An incompatibility exists between the correct (opposing) response mode with respect to the stimulus which evokes the compatible response mode. When a white ball appears on the left side of the fixation cross, the child is required to respond by means of a mouse click with the right forefinger on the right mouse button. Consequently, when a white ball appears on the right side of the fixation cross, a correct response is made by means of a left forefinger mouse click on the left mouse button. There are 30 trials per part in which signal duration is variable until the child responds. Children make a valid response when they click the right mouse button 200 to 6000 milliseconds after the stimulus appears on the screen. The post-response interval in this task is constant, 1200 milliseconds after a response a new stimulus appears. The main outcome parameters are mean RT and intra-individual variability in RT; the within-subject standard deviation of reaction time: SD(RT) per part.

Data analysis

Analysis of missing data for the main predictor maternal anxiety and the most important outcome parameters of the two cognitive tasks revealed that less than 5% of data were missing in all parameters. Prior to analysis, 9 children were excluded from further analysis due to the presence of congenital malformations, severe medical problems, behavioural problems or neurological conditions that may have altered their test performance. After evaluation of z-scores ($z < -3$ and $z > 3$ for mean RT and SD(RT) in both tasks) based on age-appropriate norm values (De Sonneville, 2005) and after reading the investigators' comments on assessment circumstances, 21 children (with high or low z-scores, who were not measured appropriately, refused to continue with the assessment, or were not able to perform accurately) were removed from the sample.

The association between antenatal maternal anxiety and the child's cognitive functioning was investigated using univariate and multiple regression analyses (SPSS, version 17.0). First, bivariate correlations (not reported) were conducted to investigate the associations between variables and to assess the influence of potential covariates for each analysis. Sex, birth weight corrected for gestational age, parity, maternal educational level as a proxy measure for socio-economic status, antenatal maternal smoking, alcohol use, ethnicity and postnatal

maternal anxiety were considered as potential covariates. Centered second order polynomials for the independent variable were added to the univariate regression models to investigate the existence of non-linear associations between maternal anxiety and cognitive outcome. Fourteen hierarchical multiple regressions were performed in which covariates that were significantly related to the outcome parameter were entered in the first step and the predictor (maternal anxiety) was entered in the second step. To test whether the child's sex was moderating the relation between prenatal anxiety and neurocognitive outcome, an interaction term (Sex × Anxiety) was also included. Results are shown in table 3.

Table 2. Descriptive statistics of the main outcome parameters.

	Total group *N* = 922 *Mean* (range)	**Highly anxious subsample** *n* = 100 *Mean* (range)
Outcome parameter (milliseconds)		
Simple RT task		
Mean RT	607 (377 – 1877)	604 (379 – 933)
SD(RT)	300 (66 – 944)	309 (95 – 735)
Omissions	0.2 (0 – 20)	0.3 (0 – 14)
Premature responses	2.6 (0 – 43)	2.6 (0 – 16)
Choice RT task		
Mean RT1	720 (354 – 1762)	712 (354 – 1541)
SD(RT)1	289 (61 – 1438)	311 (93 – 1438)
Mean RT2	1095 (485 – 2408)	1087 (485 – 1895)
SD(RT)2	454 (82 – 1492)	479 (84 – 1117)
Errors (part 1)	1.5 (0 – 28)	2.0 (0 – 20)
Omissions (part 1)	0.0 (0 – 2)	0.1 (0 – 2)
Premature responses (part 1)	1.1 (0 – 11)	1.2 (0 – 7)
Errors (part 2)	2.9 (0 – 30)	3.5 (0 – 30)
Premature responses (part 2)	0.3 (0 – 15)	0.4 (0 – 15)
Omissions (part 2)	0.1 (0 – 9)	0.1 (0 – 3)

Note. Mean RT = mean reaction time; SD(RT) = within subject standard deviation of reaction time; Mean RT1 = mean reaction time part 1; SD(RT)1 = within subject standard deviation of reaction time part 1; Mean RT2 = mean reaction time part 2; SD(RT)2 = within subject standard deviation of reaction time part 2. Omissions = number of omissions; premature responses = number of premature responses; errors (part 1) = number of errors part 1; omissions (part 1) = number of omissions part 1; premature responses (part 1) = number of premature responses part 1; errors (part 2) = number of errors part 2; premature responses (part 2) = number of premature responses part 2; omissions (part 2) = number of omissions part 2.

Table 3. Multiple regression analyses investigating the association between antenatal maternal anxiety and the child's cognitive outcomes in the total group and in the highly anxious subsample.

		Total group $N = 922$		
Outcome parameter		R^2	ΔR^2	β
Simple RT task				
Mean RT	Covariates[2,3]	.03***		
	STAI	.03***	.00	.05
SD(RT)	Covariates[3]	.01**		
	STAI	.02*	.01*	.08*
Choice RT task				
Mean RT1	Covariates[2,3]	.01*		
	STAI	.01*	.00	.01
SD(RT)1	Covariates[1,2,5]	.03***		
	STAI	.03***	.00	.06
Mean RT2	Covariates [a]			
	STAI	.00	.00	.04
SD(RT)2	Covariates[5]	.01*		
	STAI	.01*	.00	.06
	STAI (quadratic)	.02*	.01*	.08*
		Highly anxious subsample $n = 100$		
Outcome parameter		R^2	ΔR^2	β
Simple RT task				
Mean RT	Covariates[2,3]	.01		
	STAI	.03	.02	.14
SD(RT)	Boys			
	Covariates[3]	.09*		
	STAI	.18*	.09*	.31*
	Girls			
	Covariates[3]	.00		
	STAI	.03	.02	-.16
Choice RT task				
Mean RT1	Covariates[2,3]	.01		
	STAI	.02	.00	.07
SD(RT)1	Boys			
	Covariates[2,5]	.12		
	STAI	.15	.03	.21
	Girls			
	Covariates[2,5]	.07		
	STAI	.12	.05	-.23

		Highly anxious subsample $n = 100$		
Outcome parameter		R^2	ΔR^2	β
Mean RT2	Covariates [a]			
	STAI	.05*	.05*	.23*
SD(RT)2	Covariates[5]	.00		
	STAI	.05*	.05*	.23*

Note. R^2 = increment in explained variance for all predictors in the model; ΔR^2 = explained variance for specific predictor; β = standardized beta; STAI = State-Trait Anxiety Inventory. Covariates included: gender[1] birth weight corrected for gestational age[2], parity[3], maternal educational level[4], maternal smoking[5], maternal alcohol consumption[6], postnatal anxiety[7]. Mean RT = mean reaction time; SD(RT) = within subject standard deviation of reaction time; mean RT1 = mean reaction time part 1; SD(RT)1 = within subject standard deviation of reaction time part 1; mean RT2 = mean reaction time part 2; SD(RT)2 = within subject standard deviation of reaction time part 2. [a)] no covariates included; univariate regression. $p < .05$; * $p < .01$; ** $p < .001$***.

Results

Regression analyses and non-linear associations in the total group

Univariate regression analyses showed a significant positive association between maternal anxiety and SD(RT) in the simple RT task $F(1, 915) = 4.68$, $\beta = .07$, $p < .05$, in the compatible $F(1, 892) = 4.95$, $\beta = .07$, $p < .05$, and incompatible $F(1, 889) = 4.47$, $\beta = .07$, $p < .05$, part of the choice RT task. Higher levels of antenatal maternal anxiety were related to more intra-individual variability in their children. No significant associations were found between antenatal maternal anxiety and mean RT in the simple and choice RT task. Interactions between maternal anxiety and the child's sex were tested but no significant interactions were found in the total group.

Multiple regression analysis adjusted for birth weight corrected for gestational age and parity showed that maternal anxiety did not remain related to the child's mean RT, in the simple RT task. The child's SD(RT) remained positively related to antenatal maternal anxiety after adjusting for parity. Higher levels of maternal anxiety were associated with more intra-individual variability in RT than lower levels of maternal anxiety. Analyses revealed no significant associations between antenatal anxiety and the child's mean RT and SD(RT) in the compatible and incompatible trials of the choice RT task after the child's sex, birth weight corrected for gestational age, parity and maternal smoking were entered in the regression models. The children's SD(RT) in the incompatible part of the choice RT task showed a significant ($p < .05$) non-linear relation with antenatal anxiety, which indicated that higher levels of anxiety were related to a stronger than linear increase in SD(RT). To investigate this relation in depth, we have conducted supplementary analyses in a group of highly anxious women who had a state-anxiety score above the 90th percentile ($M = 54.7$, $SD = 5.7$; $n = 100$).

Supplementary analyses in the highly anxious subgroup

We found a positive association between antenatal anxiety and the child's mean RT and SD(RT), in the incompatible trials (part 2) of the choice RT task. Higher anxiety levels reported by a mother during pregnancy were related to longer mean RTs and larger variability in the RTs of the children. No significant associations were found between antenatal maternal anxiety and children's mean RT in the simple task and on the compatible trials of the choice RT task. We found a significant interaction of antenatal anxiety and the child's sex in SD(RT) on the

simple task and on the compatible part of the choice RT task. Stratified analyses for boys and girls revealed that antenatal anxiety was positively related to the SD(RT) on the simple RT task in boys, but not in girls. Stratified analyses on the compatible trials of the choice RT task showed an association between antenatal anxiety and SD(RT) in boys (not in girls), which did not remain significant when adjusted for the child's birth weight corrected for gestational age and maternal smoking during pregnancy.

Discussion

In the current study antenatal maternal state-anxiety was positively related to the child's intra-individual variability in performance in a simple RT task. Children of highly anxious pregnant mothers were more variable in their performance than children of less anxious women. These results corroborate findings in previous studies (Mennes et al., 2006; 2009; Van den Bergh et al., 2005b; Van den Bergh et al., 2006), although it should be noted that the amount of explained variance in intra-individual variability in RT in the simple RT task by antenatal anxiety was relatively low (1%). Moreover, no associations were found between antenatal anxiety and the children's processing speed (i.e., mean RT) in both the simple and the choice RT task. As such, the latter results in the total group are in accordance with previous studies in humans that have not found significant associations between antenatal maternal anxiety and outcomes in the offspring (Laplante et al., 2008; Mennes et al., 2006; Van den Bergh et al., 2005b; Whitehouse et al., 2010). A possible explanation for the fact that in the total group we did not find associations is that the degree of anxiety experienced by the women was relatively low. Their mean state anxiety score was 36 (SD = 9.5) which is equal to the 50th percentile in a Dutch female reference population (Van der Ploeg et al., 1980).

As the exact nature of the relationship between antenatal anxiety and cognitive development is not unambiguous, we have tested linear as well as non-linear associations. Our study is the first that enables such a test in a large sample of women with varying levels of anxiety using objective measures to examine specific aspects of children's cognitive functioning. However, as none of the second order polynomials in the total sample showed a U-shaped curve we can conclude that our data do not provide empirical support for a favourable

influence of exposure to moderate levels of antenatal anxiety (DiPietro et al., 2006; Laplante et al., 2008).

We did find a significant non-linear association (indicating a stronger than linear increase) between antenatal anxiety and the children's intra-individual variability in RT in the incompatible part of the choice RT task (part 2). This finding suggested a threshold for the hypothesized programming effects of antenatal anxiety. Visual inspection of the data revealed that mean state-anxiety scores higher than 50 were associated with a greater than linear increase in the children's intra-individual variability in RT. This finding suggests that the programming effect of antenatal anxiety becomes stronger when reported anxiety levels rise.

Subsequent analyses in a highly anxious subsample showed a positive linear association between antenatal anxiety and the children's mean RT and intra-individual variability in RT in the incompatible part of the choice RT task (part 2), that explained 5% of the variance in cognitive functioning. In this subsample of women with state-anxiety scores between 49 and 78, higher levels of antenatal anxiety were associated with longer RT's and more intra-individual variability in RT. These results in a subgroup of highly anxious women provided evidence in support of our hypothesis that high levels of antenatal anxiety are related to alterations in specific aspects of children's cognitive functioning (Mennes et al., 2006; 2009; Van den Bergh et al., 2005b; Van den Bergh et al., 2006).

In accordance with earlier findings (Van den Bergh et al., 2006) we found that the child's sex was a moderator in the relation between antenatal anxiety and intra-individual variability in the simple RT task, but we have found this moderating effect only in the highly anxious subsample. In boys, we found a significant positive association between prenatal maternal anxiety and intra-individual variability in RT in the simple task, but no significant associations were found in girls. Our results strengthen the idea of sex specific programming effects of antenatal maternal anxiety, with heightened vulnerability for developing impairments in male offspring on specific cognitive tasks.

When interpreting the results of the current study, it is important to consider the magnitude of the effects that were found. Findings in the total sample did only weakly confirm our hypothesis that antenatal anxiety is related to children's cognitive functioning at age five. However, results in the highly anxious subsample showed that antenatal anxiety explained up to 9% of the variance in intra-individual variability in RT in boys. These results suggest that only high levels of antenatal anxiety have a profound long-term influence on

children's cognitive functioning as measured with neurocognitive tasks, which confirms our hypothesis and is in accordance with previous findings (Mennes et al., 2006, 2009; Van den Bergh et al., 2005b; Van den Bergh et al., 2006).

In previous research several underlying mechanisms that explain the relation between antenatal anxiety and children's neurodevelopment have been proposed, such as programming of neurodevelopmental pathways and of the HPA axis in the offspring by maternal stress hormones that act in concert with other factors. It is believed that "*the disturbance of the particular developmental processes taking place in specific brain layers and areas at the time of antenatal maternal stress hormone release, in interaction with the genetic susceptibility of the offspring and mediated by later pre- and postnatal environmental factors, will determine the way in which cognitive, motor, arousal and emotional structure-function relationships are altered*" (Van den Bergh et al., 2005a, p. 254).

More specifically, current results revealed that the children's intra-individual variability in RT was most strongly related to antenatal maternal anxiety. Although, it remains unclear why variability in performance in particular was found to be related to antenatal maternal anxiety, similar results in a previous study have been ascribed to be mediated by the medial prefrontal cortex and related subcortical areas (Van den Bergh et al., 2006). Furthermore, our findings are consistent with previous studies showing that inconsistency in performance predicts intellectual ability (Jensen, 1992) and cognitive impairments accompanying aging (Hultsch et al., 2002) independent of the level of performance. Although the precise functional meaning of inconsistency in cognitive performance remains to be elucidated, it has been suggested on the basis of theoretical considerations, empirical research, and mathematical modelling that this aspect of performance may reflect neural "noise" in underlying neurobiological mechanisms (Li & Lindenberger, 1999; Li et al., 2001), especially those mediating higher-order cognition or 'executive' functioning (Walhovd & Fjell, 2007). Regardless of the exact interpretation, the present data indicate that intra-individual variability in RT is also sensitive to detect long-term effects of maternal antenatal anxiety on specific aspects of children's neurocognitive functioning.

To our knowledge, this is the first longitudinal prospective study that investigated the influence of maternal antenatal anxiety on 5-year-old children's cognitive functioning using sensitive, computerized neurocognitive tasks in a large community-based birth cohort. In addition, we used an established measurement of antenatal anxiety (STAI; State-anxiety scale), our sample covered

the whole range of anxiety scores and although the mean state anxiety score was only situated at 50th percentile in the total group, our sample also included enough women with a high level of anxiety to form a subgroup of highly anxious women. Moreover, we collected a large amount of demographic information, which allowed us to control for many potentially confounding factors.

In addition it is important to address some limitations of the study. First, women in our follow-up sample were on average 2 years older, more often employed and were more often highly educated compared to women not included in this sample. Therefore caution is warranted in the interpretation and generalization of the results. Despite this, a recent investigation of selective attrition in a British birth cohort has revealed that the validity of regression models is only marginally affected by selective attrition in large samples (Wolke et al., 2009). Therefore, we feel quite certain that although our analyses were conducted on a subsample within our cohort, selection criteria are not likely to result in biased outcomes. Second, anxiety was measured during pregnancy only on one occasion around the 16th week of gestation. Therefore, we were unable to investigate whether there are specifically sensitive or critical periods in pregnancy during which the foetus is more sensitive for programming effects of maternal anxiety. Moreover, we could not examine whether chronically experienced anxiety during pregnancy would have yielded different results. Third, we did not use endocrine (e.g. cortisol, (nor)adrenaline) or physiological measures (e.g., heart rate variability) and we were therefore unable to test potential underlying biological mechanisms that might explain the association between maternal anxiety and the child's altered cognitive function. Finally, data concerning pregnancy and birth complications of the mothers were not taken into account.

Despite these limitations, our study was the first that has examined the association between antenatal maternal anxiety and specific aspects of cognitive functioning in pre-school aged offspring in a large community based birth cohort. As such it has strengthened previous findings and emphasised that especially high levels of maternal anxiety during pregnancy negatively affect specific aspects of cognitive functioning in their offspring years later. More research is warranted into the nature of this relation and its underlying mechanisms, taking into account the child's sex as a potential moderator.

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5

Antenatal maternal anxiety is associated with problem behaviour at age five

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Abstract

Background: Developmental programming by maternal stress during pregnancy is found to influence behavioural development in the offspring.

Aim: To prospectively investigate the association between antenatal maternal anxiety and children's behaviour rated by their mothers and teachers.

Methods: In a large, community based birth-cohort antenatal maternal state-anxiety (M = 36.7, SD = 9.8) was measured using the State Trait Anxiety Inventory around the 16th week of gestation. Five years later, 3446 mothers and 3520 teachers have evaluated 3758 children's overall problem behaviour, emotional symptoms, conduct problems, hyperactivity/inattention problems, peer relationship problems and pro-social behaviour.

Results: Hierarchical multiple regression analysis using a large number of potential covariates revealed that children of mothers who reported higher levels of anxiety during their pregnancy showed more overall problem behaviour, hyperactivity/inattention problems, emotional symptoms, peer relationship problems, conduct problems and showed less pro-social behaviour when mothers rated their child's behaviour. When teachers rated child behaviour, children showed more overall problem behaviour and less pro-social behaviour that was related to antenatal anxiety. The child's sex moderated the association between antenatal anxiety with overall problem behaviour and hyperactivity/inattention problems when reported by the mother. In boys, exposure to antenatal anxiety was associated with a stronger increase in overall problem behaviour compared to girls. Furthermore, antenatal anxiety was significantly related to an increase in hyperactivity/inattention problems in boys, while this was not the case in girls.

Conclusions: Exposure to antenatal maternal anxiety is associated with children's problem behaviour, with different outcome patterns for both sexes. Nevertheless, effect sizes in this study were small.

Keywords: Antenatal, Anxiety, Child, Sex, Preschool age, Problem behaviour, Mother, Teacher, Cross-informant discrepancies, ABCD study

Introduction

The idea that the basis for a good health and development in later life is formed in the very early stages of development has a long history (Ferreira, 1965). Recently, programming influences of maternal stress during pregnancy on long-term behavioural and cognitive development of the offspring have received increased interest (for reviews see: Talge, Neal, & Glover, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005; Weinstock, 2008).

In prospective studies that were focussed on long-term behavioural outcome, evidence was found for an association between antenatal maternal distress and a higher prevalence of problem behaviour in the offspring. For example, pre-school aged children (47 months old) of mothers who scored in the top 15% of the scale used to measure anxiety at 32 weeks gestation, were more than twice as likely to have behavioural problems. In the same cohort, high levels of antenatal anxiety in late gestation were related to a twofold increase in overall problem behaviour at 81 months of age (O'Connor, Heron, Golding, Beveridge, & Glover, 2002; O'Connor, Heron, Golding, & Glover, 2003). In addition, anxiety in early gestation (12 to 22 weeks) was related to hyperactivity, externalizing problems and self-reported anxiety in 8 and 9 year olds (Van den Bergh & Marcoen, 2004). In line with these findings, exposure to stress during pregnancy (strongest effects in 10th week of gestation) was found to be associated with symptoms of ADHD particularly in boys that were assessed at the age of 7 (Rodriguez & Bohlin, 2005). Antenatal programming of offspring behaviour has even been shown to persist well into adolescence. Antenatal maternal anxiety in early pregnancy (12 to 22 weeks) was associated with depressive symptoms in girls at the age of 15 (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008) and mothers' antenatal depression significantly predicted antisocial behaviour in their offspring at age 16 (Hay, Pawlby, Waters, Perra, & Sharp, 2010).

So far, most of these previous studies that have investigated the association between antenatal anxiety and child behaviour are based on maternal reports (O'Connor et al., 2002; O'Connor et al., 2003) or composite scores (mother + teacher) (Rodriguez & Bohlin, 2005; Van den Bergh & Marcoen, 2004) of child behaviour. However, considerable debate in literature exists about inconsistencies in reports on child behaviour among different informants (Briggs-Gowan, Carter & Schwab-Stone, 1996). These disparities between informants might be due (at least partially) to inherent differences in experiences that these

informants share with the children; for example the home environment versus the classroom (Achenbach, McConaughy & Howell, 1987). In addition, evidence is accumulating for the influence of parental psychopathology on cross-informant discrepancies (Fergusson, Horwood, & Lynskey, 1993; Kroes, Veerman, & De Bruyn, 2003; Najman et al., 2000; van der Toorn et al., 2010). To sum up, although evidence concerning the association between maternal negative emotions during pregnancy with long-term behavioural outcome is accumulating, these findings were based on maternal ratings of child behaviour. Therefore, the aim of the present study was to investigate the association between antenatal maternal anxiety and problem behaviour in children at age five using both maternal as well as the child's primary school teacher's ratings of child behaviour.

In addition, we aimed to examine the moderating role of the child's sex in the association between antenatal anxiety and children's problem behaviour. Results from animal studies have indicated sex differences in the programming effects of antenatal maternal stress or anxiety (Weinstock, 2001). In humans, antenatal anxiety or stress in early gestation (12 to 22 weeks, 16 weeks and 10 weeks respectively) was associated with cognitive impairments (Loomans et al., 2012; Mennes, Van den Bergh, Lagae, & Stiers, 2009; Mennes, Stiers, Lagae, & Van den Bergh, 2006), ADHD symptoms and externalizing problems (Rodriguez & Bohlin, 2005; Van den Bergh & Marcoen, 2004) in boys and with more emotional symptoms, conduct problems (O'Connor et al., 2002; O'Connor et al., 2003) and self-reported depressive symptoms (Van den Bergh et al, 2008) in girls. Hence, both male and female offspring seem at risk for these antenatal programming effects, although in each sex these effects seem to be represented in different outcomes.

In sum, the first aim of the current study was to investigate the relation between antenatal maternal anxiety and children's behaviour at age five. An important addition to the existing body of literature was the use of mother as well as teacher reports on child behaviour. Furthermore, because of our large community based, non-clinical sample, we were able to test the moderating effect of the child's sex. We expected higher levels of antenatal maternal anxiety to be associated with more overall problem behaviour and more externalising problems in boys and with more internalising problems (emotional symptoms) in girls.

Methods

Sample

The current study is part of the Amsterdam Born Children and their Development (ABCD) study, a large community based birth cohort. Extensive information about the cohort and procedures regarding data collection is provided elsewhere (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2011). In short, between January 2003 and March 2004, 12373 pregnant women (99% of target population) were approached to participate in the study via their obstetric care provider and a questionnaire covering socio-demographic, obstetric, life-style and psychosocial conditions was sent to them. Currently, 6161 of the 6735 mothers (92%) who gave permission for follow-up of their child were approached for the 5th-year measurement of their child (phase III, 2008-2010). Attrition in this follow-up number is due to withdrawal, infant or maternal death and loss-to-follow-up as a result of unknown current address or emigration.

Prior to analyses, 128 mothers were excluded from further analysis due to the presence of a severe medical condition (e.g. (pre-)pregnancy diabetes, cancer), or the use of medication (corticosteroids, antidepressants, anti-anxiety drugs, antipsychotics) during pregnancy. One hundred and eighty-seven children that were born premature (GA < 33 weeks), had a low birth weight (< 2500 gram), or suffered from obstetric complications, cancer, congenital malformations and syndromes related to the central nervous system, were removed from the sample. Sixteen questionnaires were not filled in by the child's birth mother; therefore these reports were not included in the analysis. After these a priori exclusions the sample consisted of 3758 children; 3446 mothers and 3520 teachers have evaluated the children's behaviour. All participating mothers gave their written informed consent. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

Participants

Demographic characteristics about the participating mothers and children are presented in Table 1. Attrition analysis on key variables revealed that mothers who filled in the pregnancy questionnaire and rated their child's behaviour at age five were somewhat older ($F(1,8264) = 311.42$, $p < .001$), more often highly

educated ($\chi^2_2 = 531.1$, $p < .001$), had a Dutch or Western background ($\chi^2_5 = 583.1$, $p < .001$) and were less anxious ($F(1,7763) = 202.89$, $p < .001$) compared to mothers who did not fill in the 5-years questionnaire.

Measurements

Antenatal maternal state-anxiety

Antenatal maternal state-anxiety was measured using the Dutch version of the State Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, & Lushene, 1970; Van der Ploeg, Defares, & Spielberger, 1980) around the 16th week of gestation. This self-report questionnaire is often used to assess anxiety during pregnancy and the postnatal period (Austin, Tully, & Parker, 2007). The state-anxiety scale of the questionnaire consisted of 20 items scored 1-4; a higher score represents a higher level of experienced anxiety. The state-anxiety scale was found to be a valid (Spielberger, 1975) and reliable measure of temporarily or transient experienced anxiety (Van der Ploeg et al., 1980). In this study, state-anxiety scores ranged from 20 to 78 and internal consistency (Cronbach's alpha) was .94.

Behavioural assessment

Children's behaviour was reported by their mothers and primary school teachers using the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997). The SDQ is a short screening questionnaire suitable for 4 to 16 year olds. The questionnaire consisted of 25 items, with positive and negative statements, which are divided in 5 scales: emotional symptoms, conduct problems, hyperactivity/ inattention problems, peer relationship problems and pro-social behaviour. All items (without pro-social behaviour items) added together form the total difficulties score that represents children's overall problem behaviour. The SDQ has satisfying psychometric characteristics comparable to those of the CBCL (van Widenfelt, Goedhart, Treffers, & Goodman, 2003).

Table 1. Descriptive statistics of predictor, dependent variables and mother and child characteristics.

Maternal characteristics during pregnancy	*Mean* (*SD)*	
Age (years)	31.8 (4.6)	
Education following primary school (%)		
0 – 5 years	13.8	
6 – 10 years	35.8	
11 years or more	50.5	
Ethnicity (%)		
Dutch	75.5	
Turkish	2.6	
Moroccan	4.0	
Surinamese	3.5	
Other western countries	6.6	
Non-western countries	7.8	
Smoking (%)	8.0	
Alcohol consumption (%)	26.8	
Nulliparous (%)	56.7	
STAI score	36.7 (9.8)	
STAI completed (gestational week)	16.3 (4.1)	
Child characteristics at age 5 years		
Gender (boy %)	50.3	
Age (years)	5.1 (0.13)	
Birth weight (grams)	3530.3 (467.8)	
	SDQ mother	**SDQ teacher**
Overall problem behaviour	5.1 (4)	5.2 (4.7)
Hyperactivity/ inattention	2.4 (2.1)	2.3 (2.6)
Emotional symptoms	0.9 (1.3)***	1.2 (1.6)
Peer relationship problems	0.8 (1.2)***	1.0 (1.4)
Conduct problems	1.0 (1.2)***	0.8 (1.3)
Pro-social behaviour	8.0 (1.8)***	7.6 (2.2)

Note. SDQ = Strengths and Difficulties Questionnaire; STAI = State-Trait Anxiety Inventory.*$p < .05$; **$p < .01$; ***$p < .001$.

Data analysis

The association between antenatal maternal anxiety and the child's problem behaviour was investigated using (multiple) regression in SPSS (version 17.0). Table 2 gives an overview of the bivariate correlations between the independent variable (antenatal anxiety), the outcome parameters (child behaviour) and potential covariates. Risk factors (potential covariates) were chosen on a theoretical basis (literature) in the first place. Thereafter, we have tested whether a potential

covariate was significantly related to the outcome variable and therefore might influence the association between antenatal anxiety and children's problem behaviour. When a covariate was significantly related to the outcome variable of interest, it was included in the multiple regression analyses.

We assessed the potential influence of the child's birth weight corrected for gestational age. This variable was derived from a regression model with the children's gestational age as the predictor and their birth weight as the dependent variable. Unstandardised predicted residuals were saved and these values represent the children's birth weight accounted for their gestational age. Parity (nulliparous, primiparous, multiparous), maternal ethnicity (Dutch, Turkish, Moroccan, Surinamese, other West-European countries, other non West-European countries), maternal educational level (low, middle, high), maternal smoking during pregnancy (0= no/1= yes), maternal alcohol consumption during pregnancy (0= no/1= yes), maternal current emotional distress (total score of the Depression-Anxiety-Stress (DASS-21) questionnaire (Lovibond & Lovibond, 1995) when the child reached his or her fifth birthday), parental self-reported history of psychopathology (0=no/1=yes). Hierarchical multiple regressions with significant covariates included were performed and reported for overall problem behaviour first, followed by analyses in the behavioural subscales (Table 3).

To investigate whether the child's sex moderated the effect of antenatal anxiety on child behaviour, interaction terms between maternal anxiety and the child's sex were computed and tested in univariate regressions. When an interaction effect reached significance ($p < .05$) subsequent (multiple regression) analyses were stratified for the child's sex.

Table 2. Bivariate correlations between predictor, potential covariates and dependent variables.

	SDQ Mother (N = 3446)					
	Ov prob	Hyp/inatt	Emo symp	Cond prob	Peer prob	Pro-social
BW_GA	.00	.00	-.00	.01	.00	-.01
Parity	-.01	-.06**	-.08***	.09***	.05**	-.05**
Mat age	-.20***	-.17***	-.11***	-.08***	-.16***	.00
Mat edu	-.21	-.17***	-.07***	-.11***	.21***	.02
Smoking	.08***	.09***	-.00	.05**	.04*	.01
Alcohol	-.09***	-.06***	-.07***	-.03	-.12***	.02
Cur distr	.32***	.22***	.24***	.20***	.20***	-.10***
Par psych	.10***	.07***	.11***	.03	.05**	-.01
Anxiety	.28***	.19***	.15***	.18***	.24***	-.11***
	SDQ Teacher (N = 3520)					
	Ov prob	Hyp/inatt	Emo symp	Cond prob	Peer prob	Pro-social
BW_GA	-.03	-.03	.01	-.03	-.03	.01
Parity	-.01	-.04*	-.03	.04*	.03	-.01
Mat age	-.05**	-.07***	-.00	-.04*	.01	.04*
Mat edu	-.12***	-.11***	-.03	-.08***	-.08***	.04*
Smoking	.05**	.06***	.03	.02	.01	-.02
Alcohol	-.06***	-.04*	-.06***	-.02	-.04*	.00
Cur distr	.09***	.07***	.06**	.05**	.05**	-.06**
Par psych	.02	.01	.04*	.00	.00	-.01
Anxiety	.10***	.08***	.06**	.06**	.07***	-.06**

Note. Potential covariates and predictor: BW_GA = birth weight corrected for gestational age, Mat age = maternal age, Parity = nulliparous= 0, multiparous = 1, Mat edu = maternal educational level (low, middle, high), Smoking during pregnancy: no =0/yes =1, Alcohol during pregnancy: no =0/yes =1, Cur distr = current maternal distress, Par psych = parental self-reported history of psychopathology, Anxiety= antenatal anxiety during pregnancy. Dependent variables: Ov prob= overall problem behaviour, Hyp/inatt= hyperactivity/inattention problems, Emo symp= emotional symptoms, Cond prob= conduct problems, Peer prob= peer relationship problems, Pro-social= pro-social behaviour. SDQ= Strengths and Difficulties Questionnaire. *p < .05; **p < .01; ***p < .001.

Results

Cross-informant agreement

Bivariate correlations between mother and teacher ratings were r= .40 (overall problem score), r= .29 (emotional symptoms), r= .29 (conduct problems), r= .43 (hyperactivity/inattention), r= .32 (peer relationship problems), and r= .23 (pro-social behaviour) (all p's< .01).

Antenatal anxiety and children's behaviour rated by mother

Analyses revealed a significant interaction between antenatal anxiety and the child's sex F(1, 4372)= 4,34, p= .04, in children's overall problem behaviour when child behaviour was rated by the mother. In boys, antenatal maternal anxiety was positively associated with the child's overall problem behaviour F(1, 1748)= 163.2, p= .00. In girls, prenatal anxiety also showed a significant positive relation with overall problem behaviour F(1, 1724)= 128.0, p= .00, which was slightly weaker than in boys (see Fig. 1). After the addition of covariates (Table 3), antenatal maternal anxiety remained positively related to overall problem behaviour in boys and girls with stronger association in boys than in girls. Analyses revealed a significant interaction between antenatal anxiety and the child's sex F(1, 3473)= 4.70, p= .03 in children's hyperactivity/inattention problems. Univariate analysis showed that antenatal maternal anxiety was positively related to symptoms of hyperactivity and inattention in boys F(1, 1749)= 77.51, p= .00, and in girls F(1, 1724)= 46.33, p= .00 (see Fig. 2). However, after controlling for significant covariates, antenatal anxiety remained significantly related to hyperactivity/inattention problems in boys, but not in girls. Univariate regression revealed a positive association between antenatal anxiety and children's emotional symptoms F(1, 3475)= 76.55, p= .00. After controlling for relevant covariates, antenatal anxiety remained positive but weakly related to children's emotional symptoms. Antenatal anxiety was positively related to peer relationship problems F(1, 3475)= 215.25, p=. 00, in an unadjusted analysis. After covarying significant confounders, antenatal anxiety remained significantly related to peer relationship problems. A univariate regression revealed a positive relation between antenatal anxiety and children's conduct problems F(1, 3474)= 121.32, p= .00, which remained significant after controlling for significant covariates. Antenatal anxiety was negatively related to pro-social behaviour F(1, 3467)= 39.59, p= .00 in an unadjusted analysis, after covarying significant covariates this negative association remained significant.

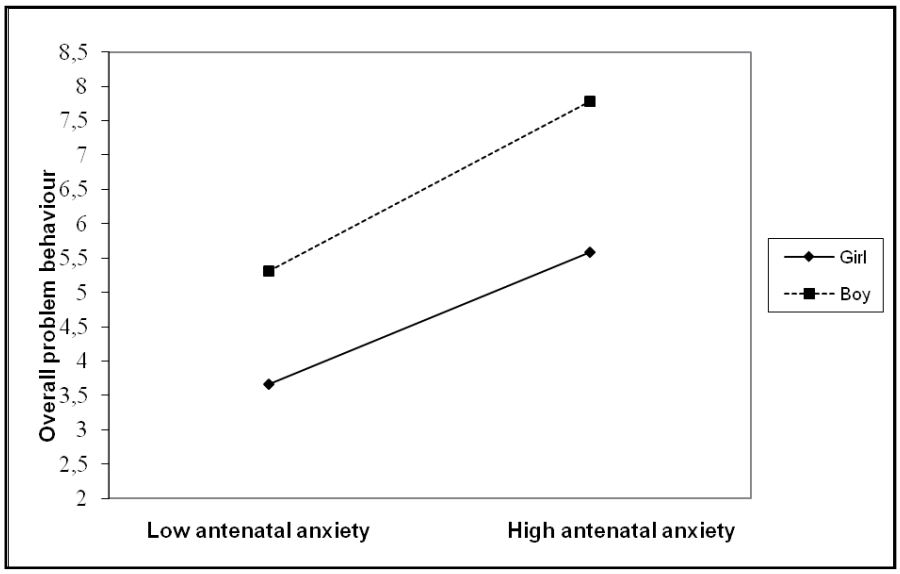

Figure 1. Sex moderates the association between antenatal anxiety and children's overall problem behaviour. Boys show a stronger increase in overall problem behaviour related to antenatal anxiety compared to girls.

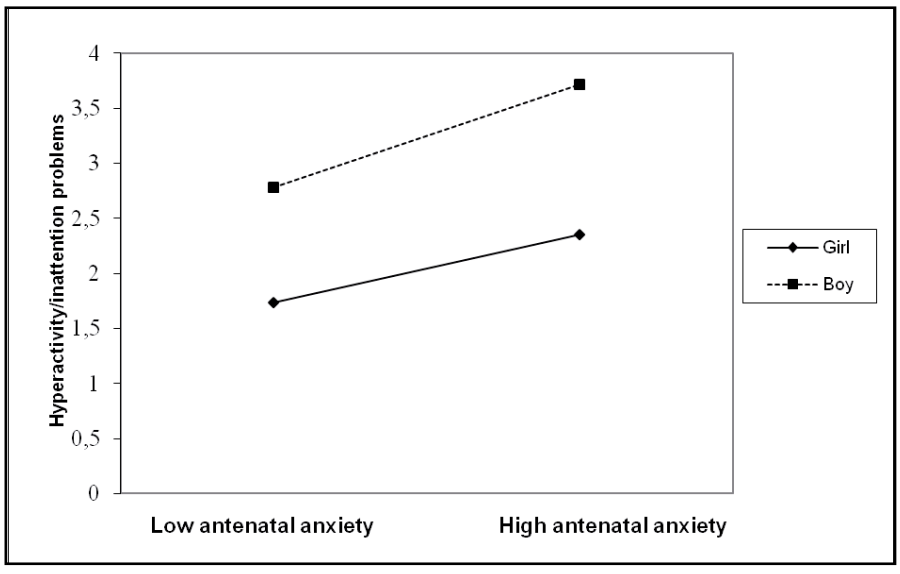

Figure 2. Sex moderates the association between antenatal anxiety and children's hyperactivity/inattention problems. Boys show a stronger increase in hyperactivity/inattention problems related to antenatal anxiety compared to girls.

Antenatal anxiety and children's behaviour rated by teacher

Univariate analysis showed significant positive relations between antenatal anxiety and children's overall problem behaviour F(1, 3252)= 33.92, p= .00, hyperactivity/ inattention problems F(1, 3254)= 21.23, p= .00, emotional symptoms F(1, 3253)= 9.73, p= .002, peer relationship problems F(1, 3254)=18, p= .001 and conduct problems F(1, 3253)= 12.01, p= .001. A significant negative association was found between antenatal anxiety and children's pro-social behaviour F(1, 3253)= 10.04, p= .002. After controlling for significant covariates (Table 3) antenatal anxiety remained positively related to children's overall problem behaviour and a negative association with pro-social behaviour was found.

Table 3. Hierarchical multiple regression between antenatal anxiety and child problem behaviour reported by mothers and teachers.

	n	β	*F model*	R^2	ΔR^2
Mother					
Overall problem behaviour					
Boys	1654	.13***	25.23***	.18***[3,4,5,6,7,8,9]	.01***
Girls	1639	.10***	26.20***	.17***[3,4,5,6,7,8,9]	.01***
Hyperactivity/ inattention					
Boys	1668	.09***	13.28***	.11***[2,3,4,5,6,7,8,9]	.01***
Girls	1639	.05	10.30***	.09*[2,3,4,5,6,7,8,9]	.00
Emotional symptoms	3277	.05*	18.21***	.08***[2,3,4,5,7,8,9]	.01*
Peer relationship problems	3307	.11***	38.83***	.15***[2,3,4,5,7,8,9]	.01***
Conduct problems	3308	09***	18.58***	.07***[2,3,4,5,6,8,9]	.01***
Pro-social behaviour	3335	-.07***	8.84***	.03***[2,4,8]	.01***
Teacher					
Overall problem behaviour	2895	.04*	8.95***	.04***[3,4,5,6,7,8]	.01*
Hyperactivity/ inattention	2897	.03	7.05***	.03***[2,3,4,5,6,7,8]	.00
Emotional symptoms	2868	.02	6.19***	.01***[5,7,8,9]	.00
Peer relationship problems	2897	.03	5.44***	.02***[,4,5,7,8]	.00
Conduct problems	2897	.02	4.34***	.02***[2,3,4,5,8]	.00
Pro-social behaviour	3242	-.04*	3.87**	.01**[3,5,6]	.01*

Note. β = standardized beta for antenatal anxiety only; R^2 = explained variance when all predictors are included in the model; ΔR^2 = explained variance for antenatal anxiety. Covariates included: birth weight corrected for gestational age[1], parity[2], maternal age[3], maternal ethnicity[4], maternal educational level[5], maternal smoking[6], maternal alcohol consumption[7], current maternal distress[8], parental self-reported history of psychopathology[9]. *$p < .05$; **$p < .01$; ***$p < .001$.

Discussion

Results in the current study provided support for the hypothesis that antenatal anxiety is related to children's problem behaviour and are in accordance with a foetal programming perspective (Seckl, 2001). Current results corroborate findings from previous comparable studies, which have reported adverse effects of antenatal anxiety on child behaviour as they provided modest support for the hypothesis that antenatal anxiety is related to children's problem behaviour. Results are in line with results from previous comparable studies, which have reported adverse effects of antenatal anxiety on child behaviour (de Bruijn, van Bakel, & van Baar, 2009; Hay et al., 2010; Loomans et al., 2012; Mennes et al., 2009; O'Connor et al., 2002; O'Connor et al., 2003; Rodriguez & Bohlin, 2005; Van den Bergh & Marcoen, 2004; Van den Bergh et al., 2008). Children of mothers who reported higher levels of anxiety during their pregnancy showed more overall problem behaviour, hyperactivity/inattention problems, emotional symptoms, peer relationship problems, conduct problems and showed less pro-social behaviour when mothers had rated their child's behaviour. When child behaviour was rated by their primary school teachers, children showed more overall problem behaviour and less pro-social behaviour in relation with antenatal anxiety.

We found that the child's sex moderated the relation between antenatal anxiety with overall problem behaviour and hyperactivity/inattention problems in children when reported by their mother. As could be expected (Bongers, Koot, Van der Ende, & Verhulst, 2003) and becomes clear from Figs. 1 and 2, baseline rates for overall problem behaviour and hyperactivity and inattention problems were higher for boys than for girls. However, Figs. 1 and 2 also show that the lines which represent boys have steeper slopes than the lines that represent girls. In other words, the lines are not parallel which suggests a moderating role of the child's sex that was confirmed by the finding of significant interaction effects in the regression models. Thus, with higher levels of antenatal anxiety, the positive association between antenatal anxiety and overall problem behaviour became stronger in boys than in girls. Furthermore, antenatal anxiety was significantly associated with hyperactivity/inattention problems in boys, while this was not the case in girls. Hence, our study corroborates the idea of sex differences in programming effects of antenatal anxiety on child behaviour in general (Weinstock, 2001) and it has provided evidence in support of our hypothesis that

boys would have more overall and externalising behaviour problems (Kroes et al., 2003; O'Connor et al., 2002; O'Connor et al., 2003). On the other hand our results did not confirm our hypothesis for more emotional problems in girls born to mothers who reported higher levels of anxiety in their pregnancy (O'Connor et al., 2002; O'Connor, et al., 2003; Van den Bergh et al., 2008).

A great strength of the current study was the fact that we have evaluated maternal as well as teacher reports on child behaviour separately. Cross-informant correlations were weak to moderate, which is a common finding (Achenbach, 1998). Mothers reported their children to have more hyperactivity/inattention problems, conduct problems and to show more pro-social behaviour compared to teachers. Teachers reported more overall problem behaviour, emotional symptoms and peer-relationship problems.

Remarkably we found that evidence for an independent association between antenatal maternal anxiety and children's problem behaviour was most profound when mothers had reported on their child's behaviour. Literature on cross-informant discrepancies poses several possible explanations for this finding. First, as was mentioned in the introduction, mothers and teachers observe children in different circumstances where children might actually behave differently. Furthermore, mothers have known their child for a longer period of time compared to the teacher, whereas teachers in turn might be more able to view the child's behaviour in comparison with peers (Briggs-Gowan et al, 1996). Second, the idea that mothers tend to over report problem behaviour (positive bias) has been studied extensively and has been linked to maternal psychopathology. Especially, maternal internalising symptomatology (such as anxiety and depression) affects their reporting of children's problem behaviour (Fergusson et al., 1993; Kroes et al., 2003; Najman et al., 2000; van der Toorn et al., 2010). Findings in the present study corroborate this idea as analyses showed that parental history of self reported psychopathology was only positively associated with maternal reports on child behaviour. Thus, when parents had a self-reported history of psychopathology, mothers viewed their children's behaviour as more problematic. Teachers' evaluations of children's behaviour (except for emotional symptoms) were not related to parental self reported history psychopathology. So far, previous research that was solely based on maternal reports on child behaviour, did not take into account this potential influence of parental history of psychopathology. However, current results indicate that this factor influences the association under investigation and the fact that we statistically controlled for

the influence of this variable might explain the more modest results found in this study compared to others.

Another strength of the current study is that we were able to statistically control for a large number of prenatal, postnatal and sociodemographic potential risk factors in an attempt to identify the independent influence of antenatal maternal anxiety on child behaviour. The choice for these covariates was primarily based on previous studies and literature. Although to date several theoretical models (e.g. Schlotz & Phillips, 2009) aim to explain the association between antenatal anxiety and children's neurodevelopment, no model specifies the strengths and directions of the associations between all variables involved. Therefore, results from this study that were obtained by using statistical control for confounding factors need to be interpreted with caution. Furthermore, the small amount of variance in children's problem behaviour that was independently explained by antenatal maternal anxiety needs to be taken into account while interpreting the results.

Finally, a number of important limitations need to be considered. First, our large prospective, community based, non-clinical sample is a clear advantage in terms of statistical power, unfortunately sample attrition was not completely random. Women who were younger, less well educated, who did not have a Dutch or western background, and were more anxious during their pregnancies, were less likely to participate in the follow-up measurements of their child. However, a recent investigation of selective attrition in a British birth cohort has revealed that the validity of regression models is only marginally affected by selective attrition in large samples (Wolke et al., 2009). Second, we did not use endocrine (e.g. cortisol) or physiological measures (e.g., heart rate variability) and we were therefore unable to test potential underlying mechanisms that might explain the association between maternal anxiety and children's behavioural development. Furthermore we were unable to rule out potential genetic factors that might affect the association between antenatal anxiety and child behaviour problems.

A possible explanation for the fact that we did not find strong associations could have been that the degree of antenatal anxiety experienced by the mothers was relatively low. Their mean state anxiety score was 36.7 (SD=9.8) which is equal to decile 5 in a Dutch female norm population (van der Ploeg et al., 1980). However, post-hoc analyses in a subsample of highly anxious mothers (mean state-anxiety scores above the 90th percentile) did not reveal stronger independent associations between antenatal maternal anxiety and child

behaviour. Alternatively, mothers and teachers in the current study have reported relatively low levels of problem behaviour in children compared to normative data (sample split by age band and child's sex) from a British national survey (Meltzer, Gatward, Goodman, & Ford, 2000). This low prevalence of problem behaviour poses an alternative explanation for the modest associations that were found. Furthermore, we measured stress at only one occasion around the 16th week of gestation. Therefore, we were unable to investigate whether there are specifically sensitive or critical periods in pregnancy during which the foetus is more sensitive for programming effects of maternal anxiety. Hence, it is possible that our findings underestimated the association under investigation and would have been stronger when examined in other periods during pregnancy.

Despite these limitations, the current study contributed to the existing body of literature by replicating and strengthening earlier findings and revealing that the inclusion of multiple informants on child behaviour is of great importance. To conclude, more research taking sex differences in the effects of antenatal distress on behavioural development into account is warranted in large, community based birth cohorts, where child behaviour is assessed by multiple informants.

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6

Caffeine intake during pregnancy and risk of problem behaviour in 5 to 6 year old children

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Abstract

Background and objective: Human studies that have investigated the association between caffeine intake during pregnancy and offspring's behavioural outcomes are scant and inconclusive. We prospectively investigated the association between maternal caffeine intake during pregnancy and children's problem behaviour at age 5 to 6 years. Mediation by fetal growth restriction and gestational age as well as effect modification by the child's gender and maternal smoking was tested.

Methods: In a community based multiethnic birth cohort, dietary caffeine intake (coffee, caffeinated tea, and cola) was measured (maternal self-report, n = 8202) around the 16th week of gestation. At age 5, children's overall problem behaviour, emotional problems, conduct problems, hyperactivity/inattention problems, peer relationship problems, and pro-social behaviour were rated by both mother and teacher (n = 3439) with the Strengths and Difficulties Questionnaire. Analyses were adjusted for maternal age, ethnicity, cohabitant status, education, smoking and alcohol consumption during pregnancy, child's gender, family size, and prenatal maternal anxiety.

Results: Caffeine intake was not associated with a higher risk for behaviour problems or with suboptimal pro-social behaviour. No evidence was found for mediation by fetal growth restriction or gestational age, nor for effect modification by the child's gender.

Conclusions: Results did not provide evidence for developmental programming influences of intrauterine exposure to caffeine on offspring's problem behaviour at age 5. Present results give no indication to advise pregnant women to reduce their caffeine intake to prevent behaviour problems in their children.

Introduction

Caffeinated drinks like coffee, tea and soft drinks are frequently consumed throughout the world (Nehlig & Debry, 1994; Sobotka, 1989). Moderate amounts of caffeine act as a central nervous system stimulant (Christian & Brent, 2001) by blocking adenosine receptors that inhibit neuronal activity of cholinergic, glutamatergic and GABAergic neurons in the brain (Porkka-Heiskanen & Kalinchuk, 2011; Soellner, Grandys, & Nuñez, 2009). Daily caffeine intake is common among 75% - 93% of pregnant women (Frary, Johnson, & Wang, 2005; Kaiser, 2008), which has raised concerns about its potential influence on offspring's neurodevelopment, because caffeine reaches the foetal brain by crossing the placenta (Mose et al., 2008) and foetal blood-brain barrier (Tanaka, Nakazawa, Arima, & Iwasaki, 1984). Moreover, caffeine metabolism during gestation is slowed down in the mother and has an extended half-life in the foetus (Soellner et al., 2009), therefore its potential programming influence on the developing foetal brain may be lengthened.

Evidence for an association between prenatal exposure to caffeine and alterations in foetal brain development with persistent alterations in offspring's brain and behaviour in later life comes mainly from animal studies (Anderson & Hughes, 2008; Nakamoto, Roy, Gottschalk, Yazdani, & Rossowska, 1991; Sinton, Valatx, & Jouvet, 1981; Soellner et al., 2009). Caffeine ingestion during pregnancy is associated with a reduction of fetal cerebral weight (Tanaka, Nakazawa, & Arima, 1983), long-term biochemical alterations in the brain, heightened locomotive activity (Nakamoto et al., 1991), increased emotional reactivity, impulsivity (Anderson & Hughes, 2008), and impaired cognitive functioning (Soellner et al., 2009) in rodent offspring. Human studies that have investigated gestational caffeine consumption and (long-term) neurodevelopmental and behavioural outcomes in offspring are scant and results are inconclusive. Prenatal caffeine exposure was related to altered neuromuscular development, reflex functioning, heightened arousal, and irritability in newborns (Jacobson, 1984), neural tube defects (i.e. spina bifida) (Schmidt et al., 2009), hyperactivity in 18 month olds (Bekkhus, Skjothaug, Nordhagen, & Borge, 2010) and social problems in middle childhood (Chiu, Gau, Tsai, Soong, & Shang, 2009). Conversely, no associations were found with mental and motor development at 8 months (Streissguth, Barr, Martin, & Herman, 1980), IQ and attention at age 7 (Barr & Streissguth, 1991), and a clinically verified hyperkinetic disorder and attention-deficit hyperactivity

disorder (Linnet et al., 2009). The fact that findings vary among studies is most likely due to differences in study design, such as behavioural reports that were solely based on maternal ratings, limited control for important confounding factors, and retrospective information on caffeine intake.

Caffeine intake during pregnancy might also affect offspring's neurodevelopment and subsequent behavioural outcomes indirectly via foetal growth restriction and gestational age because it decreases placental blood flow and foetal heart rate (Kirkinen, Koivula, Vuori, & Puukka, 1983), which may alter foetal growth. In turn, foetal growth restriction (Grunau, Whitfield, & Fay, 2004; Hack et al., 2004; Mick, Biederman, Prince, Fischer, & Faraone, 2002; Wiles et al., 2006) and gestational age (Rice, Jones, & Thapar, 2007) have been linked to an increased risk for problem behaviour in offspring. Results from animal studies have indicated gender differences in the programming effects of intrauterine caffeine exposure with a heightened susceptibility for adverse developmental outcomes in male offspring (Fisher & Guillet, 1997; Hughes & Beveridge, 1986; Hughes & Beveridge, 1991). In humans, evidence for effect modification by the child's gender is lacking, although one study has reported an increased risk for foetal growth retardation in boys, related to high caffeine intake in the third trimester (Vik, Bakketeig, Trygg, Lund-Larsen, & Jacobsen, 2003). Tobacco smoking induces the CYP1A2 liver enzyme which accelerates caffeine metabolism (Rasmussen, Kyvik, & Brøsen, 2002). Hence, smoking could moderate the association between caffeine intake and offspring's neurodevelopmental outcomes.

The aim of the current study was to prospectively investigate the association between prenatal maternal dietary caffeine intake and children's problem behaviour in a large multiethnic, community-based birth cohort. We were able to take into account a large number of potential confounding factors, and we included mothers' as well as teachers' ratings on multiple dimensions of children's behaviour. Mediation by fetal growth restriction and gestational age as well as effect modification by prenatal smoking and the child's gender were taken into account.

Methods

Design

The current study is part of the Amsterdam Born Children and their Development (ABCD) study, a large multi-ethnic community based birth cohort. Extensive information about the cohort and procedures regarding data collection is provided elsewhere (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2011). In short, pregnant women from Amsterdam were approached for their participation between January 2003 and March 2004 during their first visit with an obstetric care provider. All women (12,373 i.e. approximately 99% of target population) received a questionnaire covering socio-demographic, obstetric, life-style and psychosocial conditions, which was filled out by 8266 women (67%). These data were completed with information on pregnancy outcome from Youth Health Care Registration and the Dutch Perinatal Registration. Currently, 6161 of the 6735 mothers (92%) who gave permission for follow-up of their child were eligible for the 5th-year measurement of their child. Attrition in this follow-up number is due to withdrawal, infant or maternal death and loss-to-follow-up as a result of unknown current address or emigration. To be included in the current study, complete data on both maternal caffeine intake and children's behavioural assessment (both mother and teacher reports) had to be available. Additional information about inclusion criteria is provided in figure 1. All participating mothers gave their written informed consent. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in The Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

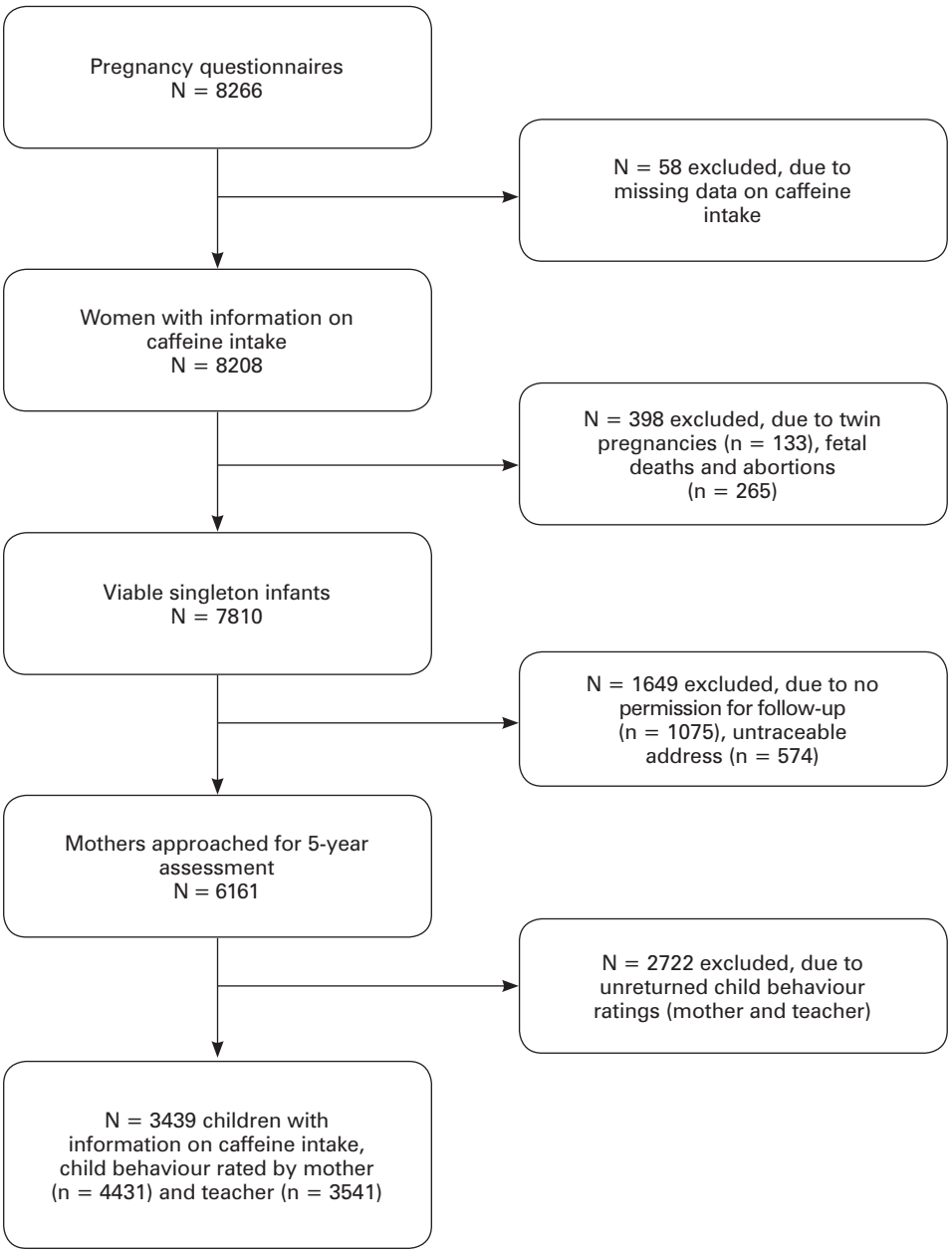

Figure 1. Flow chart of participants included for analysis.

Maternal caffeine intake

Information on women's dietary caffeine intake during pregnancy was obtained from items in the pregnancy questionnaire that was filled in during the 16th week of gestation (interquartile range, 14-18 weeks). Pregnant women were asked whether they drank coffee, tea and cola in the past week. In addition, they were asked about the amount and type of coffee, tea and cola (caffeinated, decaffeinated, both, or herbal tea) they consumed. Total caffeine intake per day was calculated using the Dutch Food Composition Database (NEVO online version 2011/3.0) that contains data on the nutritional composition and caffeine content of food and beverages. The type of coffee, tea or cola (a regular coffee or tea contains 125 ml, a regular cola 150 ml) determined the total caffeine intake in milligrams per day (one regular coffee = 85 mg, decaffeinated coffee = 3 mg, both regular and decaffeinated coffee = 44 mg, regular tea = 45 mg, regular cola = 35 mg, decaffeinated cola = 0 mg, regular and decaffeinated cola = 17 mg, no cola, coffee, tea, only herbal tea = 0 mg). To explore the influence of high doses of caffeine, total caffeine intake was categorized in 4 groups (I = 0–85 mg/d, II = 86–255 mg/d, III = 256–425 mg/d, IV = ≥425 mg/day), that correspond to the number of cups of coffee per day (I: 0–1, II: 2–3, III: 4–5, IV: >5 cups).

Children's problem behaviour

Children's problem behaviour was reported by their mothers and primary school teachers using the Strengths and Difficulties Questionnaire (SDQ), a short behavioural screening questionnaire suitable for 4 to 16 year olds (Goodman, 1997). This questionnaire consists of 25 items, which are divided in 5 subscales: emotional symptoms, conduct problems, hyperactivity/inattention problems, peer relationship problems and pro-social behaviour. All items (without pro-social behaviour items) added together form a total difficulties score that represents children's overall problem behaviour. Behavioural outcomes were dichotomized ("no behaviour problems" or "at risk for problem behaviour") (Schmeck et al., 2001). Children with SDQ (subscale) scores by both mother and teacher below the 83rd percentile were not considered to be at risk for problem behaviour. In accordance, children with a score above the 83rd percentile reported by either mother or teacher were also not considered to be at risk for problem behaviour. Only children with a mean (subscale) score above the 83rd percentile reported both by their mother and their teacher were considered to be at risk for behaviour problems. For pro-social behaviour, children with SDQ (subscale) scores by both

mother and teacher above the 17th percentile were not considered to show suboptimal pro-social behaviour. Children with a score below the 17th percentile reported by either mother or teacher were also not considered to be at risk for suboptimal pro-social behaviour. Only children with a score below the 17th percentile reported both by their mother and their teacher were considered to be at risk for suboptimal pro-social behaviour. The reliability and validity of the SDQ have been established in a Dutch population with satisfactory psychometric characteristics comparable to those of the Child Behaviour Checklist (van Widenfelt, Goedhart, Treffers, & Goodman, 2003).

Data analysis

Descriptive statistics were used to explore the association between maternal characteristics and caffeine intake; statistical differences were tested with analysis of variance for continuous variables and χ^2 tests for categorical variables (Table 1). The association between maternal prenatal caffeine intake and problem behaviour was analyzed by multiple logistic regression analysis (Table 2). Potential covariates were selected a priori on a theoretical basis and were included in the regression model at once by using a forced-entry method. First, associations were tested in a crude (unadjusted) model and subsequently maternal age (years), ethnicity (Dutch, Surinamese, Mediterranean, and others), maternal education (years after primary school), maternal state anxiety (low/high), cohabitant status (yes/no), smoking (yes/no), alcohol (yes/no), child's gender, family size (child plus brothers or sisters) were added to the unadjusted model. Thereafter, in the third step, birth weight standardized for gender, gestational age, and parity based on the most recent Dutch reference values (Visser, Eilers, Elferink-Stinkens, Merkus, & Wit, 2009), and gestational age (based on ultrasound, when unavailable (<10%) on the first day of the last menstrual period) were added to examine potential mediation. Interaction terms with the child's gender and maternal smoking were added to the fully adjusted models to investigate effect modification. Analyses were conducted by using SPSS 17.0 (SPSS Inc, Chicago, IL).

Results

Subject characteristics

Attrition analysis on key variables revealed that mothers who filled in the pregnancy questionnaire and rated their child's behaviour at age 5 were somewhat older ($F [1, 7808] = 338.14$, $P < .001$), more often highly educated ($F [1, 7736] = 539.50$, $P < .001$), had a Dutch background ($\chi^2 [3] = 529.9$, $P < .001$), were less anxious ($F [1, 7678] = 136.38$, $P < .001$), had fewer premature ($\chi^2 [1] = 7.8$, $P < .01$) and heavier babies ($F [1, 7755] = 52.13$, $P < .001$), and fewer babies that were small for gestational age ($\chi^2 [1] = 11.2$, $P < .01$) in comparison with mothers in the nonresponse group who gave birth to a viable singleton infant ($n = 4371$). Mothers in the response group had taken more caffeine during pregnancy (mean = 174.9 mg, SD = 131.0) in comparison with nonresponders (mean = 144.5, SD = 125.2), $F (1, 7808) = 108.97$, $P < .001$.

Demographic characteristics about the participating mothers and children are presented in Table 1. The mean age of the mothers in this sample was 31.9 (SD = 4.5) years. Almost 77% of the mothers were Dutch, 3% were Surinamese, 6% were either Turkish or Moroccan, and 14% had another ethnical background. Dutch mothers consumed more caffeine compared with non-Dutch mothers (most women who consumed no or little caffeine were non-Dutch). Compared with mothers in the reference group (0–85 mg/d caffeine), mothers who consumed more caffeine tended to be older, worked more during pregnancy, and were more highly educated. They were more frequently smokers and alcohol consumers. The sample consisted of 8.5% children ($n = 293$) who were small for gestational age and 4.5% ($n = 156$) who were born premature. The mean gestational age of the children was 39.9 (SD = 1.6) weeks, and the mean birth weight was 3485.6 g (SD = 540.7). The children's mean age at the time of the behavioural assessment was 5.1 years (SD = 0.15). The mean SDQ scores by both mother and teacher are presented in Table 1, and the prevalence of problem behaviour in children is reported in Table 2. Bivariate correlations between mother and teacher behaviour ratings were $r = 0.44$ (hyperactivity/inattention problems), $r = 0.28$ (emotional symptoms), $r = 0.30$ (conduct problems), $r = 0.32$ (peer relationship problems), $r = 0.22$ (pro-social behaviour), and $r = 0.40$ (overall problem behaviour), which compares with parent-teacher agreement on behavioural/emotional problems in general (Achenbach, McConaughy & Howell, 1987).

Table 1. Demographic characteristics of 3439 women and their children according to caffeine intake.

Maternal characteristics	N	Caffeine intake (mg/day) 0 – 85 [a] n = 963	86 - 255 n = 1.614	256 - 425 n = 719	> 425 n = 143
Mean (SD) age (years)	3.439	31.1 (5.0)	31.9 (4.4)***	32.8 (3.8)***	33.1 (4.2)***
Nulliparous	1.972	59.9	55.9	56.7	59.4
Ethnic background					
Dutch	2.630	59.1	80.1***	88.2***	93.7***
Surinamese	117	6.2	2.8***	1.5***	0.7***
Mediterranean	202	10.3	5.1***	2.5***	2.1***
Other	490	24.4	12.0***	7.8***	3.5***
Mean (SD) education (years)	3.425	9.1 (3.9)	10.0 (3.6)***	10.6 (3.1)***	10.1 (3.6)**
Living with partner	3.120	89.7	90.6	93.0	91.6
High levels of anxiety	256	9.3	7.3	5.4**	7.7
Alcohol consumption	911	16.2	25.7***	39.2 ***	41.3***
Smoking	285	5.8	6.8	12.8***	19.6***
Mean (SD) gestational age (weeks)	3.417	39.7 (1.7)	39.9 (1.6)*	40.0 (1.7)**	40.0 (1.4)
Child characteristics					
Preterm birth	156	5.9	4.1*	4.3	2.1
Mean (SD) standardized birth weight	3.415	1.01 (0.1)	1.01 (0.1)	1.00 (0.1)	1.01 (0.1)
Small for gestational age [b]	293	10.1	7.0**	10.5	7.1
Female	1.694	50.2	48.4	50.9	44.8
Siblings					
1	2.406	75.0	75.0*	71.3**	79.6
2	694	19.3	21.3*	25.3**	19.0
3 or more	132	5.7	3.7*	3.4**	1.5

Mean (SD) SDQ scores	N	Mother	N	Teacher
Overall problem behaviour	3439	5.2 (4.0) ***	3439	5.3 (4.7)
Hyperactivity/ inattention	3439	2.4 (2.2) ***	3439	2.3 (2.6)
Emotional symptoms	3439	1.0 (1.3) ***	3439	1.2 (1.6)
Conduct problems	3439	1.0 (1.2) ***	3439	0.8 (1.3)
Peer relationship problems	3439	0.8 (1.2) ***	3439	1.0 (1.4)
Pro-social behaviour	3431	8.0 (1.8) ***	3437	7.6 (2.2)

Values are numbers (percentages) unless stated otherwise.

[a] Reference group.

[b] Small for gestational age (birth weight < 10th percentile for gestational age).

* $p < .05$ ** $p < .01$ *** $p < .001$ (significantly different from reference group).

Association between caffeine intake during pregnancy and children's problem behaviour

Table 2 shows that prenatal caffeine intake was not associated with a higher risk for hyperactivity/inattention problems, emotional symptoms, conduct problems, peer relationship problems, overall problem behaviour, or suboptimal pro-social behaviour in the adjusted models.

Furthermore, no evidence was found for mediation by fetal growth restriction and gestational age, because no consistent associations were found between caffeine intake and these perinatal outcomes (Table 1). Moreover, fetal growth restriction and gestational age were not related to children's problem behaviour, with the exception of hyperactivity/inattention problems (Table 3). Children with hyperactivity/inattention problems were more often born preterm, had a lower standardized birth weight, and a shorter gestational age, but these associations did not depend on the level of caffeine intake. We did not find evidence for effect modification by the child's gender (tests for interaction, all p's $> .05$). However, maternal smoking during pregnancy moderated the association between caffeine intake and peer relationship problems (test for interaction, $p = .02$). Caffeine intake >425 mg/d compared with an intake of 0–85 mg/d increased the risk for offspring's peer relationship problems in women who smoked, whereas an inverse trend was found in women who did not smoke (Table 4).

Table 2. Risk of problem behaviour in five year old children according to maternal caffeine intake during pregnancy.

	Problem behaviour [a] (%)	**Crude model** Odds ratio (95% CI)	**Model 1**[b] Odds ratio (95% CI)	**Model 1**[c] Odds ratio (95% CI)
Overall problem behaviour	n = 224			
I. 0 – 85 (mg/day) [d]	6.9	-	-	-
II. 86 – 255 (mg/day)	7.4	0.95 (0.68 - 1.32)	1.04 (0.74 - 1.48)	1.05 (0.74 - 1.50)
III. 256 – 425 (mg/day)	4.7	0.70 (0.45 - 1.08)	0.85 (0.53 - 1.36)	0.86 (0.54 - 1.37)
IV. > 425 (mg/day)	6.3	0.97 (0.47 - 1.99)	1.04 (0.49 - 2.21)	1.04 (0.49 - 2.22)
Hyperactivity/ inattention	n = 257			
I. 0 – 85 (mg/day) [d]	8.0	-	-	-
II. 86 – 255 (mg/day)	7.6	0.90 (0.66 - 1.22)	0.93 (0.67 - 1.29)	0.94 (0.68 - 1.31)
III. 256 – 425 (mg/day)	6.4	0.77 (0.52 - 1.14)	0.88 (0.57 - 1.34)	0.87 (0.57 - 1.33)
IV. > 425 (mg/day)	8.4	1.10 (0.58 - 2.09)	1.08 (0.55 - 2.12)	1.08 (0.55 - 2.12)
Emotional symptoms	n = 147			
I. 0 – 85 (mg/day) [d]	5.2	-	-	-
II. 86 – 255 (mg/day)	3.7	0.70 (0.47 - 1.05)	0.75 (0.50 - 1.13)	0.74 (0.49 - 1.12)
III. 256 – 425 (mg/day)	4.2	0.80 (0.50 - 1.30)	0.94 (0.57 - 1.57)	0.93 (0.56 - 1.54)
IV. > 425 (mg/day)	4.9	0.85 (0.36 - 2.04)	1.02 (0.42 - 2.51)	1.02 (0.42 - 2.51)
Conduct problems	n = 109			
I. 0 – 85 (mg/day) [d]	3.2	-	-	-
II. 86 – 255 (mg/day)	3.5	0.86 (0.53 - 1.39)	0.91 (0.55 - 1.50)	0.93 (0.56 - 1.53)
III. 256 – 425 (mg/day)	2.2	0.62 (0.32 - 1.17)	0.67 (0.34 - 1.34)	0.67 (0.34 - 1.35)
IV. > 425 (mg/day)	3.2	1.12 (0.43 - 2.94)	1.14 (0.42 - 3.11)	1.16 (0.42 - 3.16)

	Problem behaviour [a] (%)	Crude model Odds ratio (95% CI)	Model 1[b] Odds ratio (95% CI)	Model 1[c] Odds ratio (95% CI)
Peer relationship problems	n = 205			
I. 0 – 85 (mg/day) [d]	8.0	-	-	-
II. 86 – 255 (mg/day)	5.8	0.73 (0.52 - 1.01)	0.86 (0.61 - 1.22)	0.85 (0.60 - 1.21)
III. 256 – 425 (mg/day)	4.0	0.47 (0.29 - 0.75)**	0.64 (0.39 - 1.05)	0.64 (0.39 - 1.05)
IV. > 425 (mg/day)	3.5	0.47 (0.19 - 1.19)	0.66 (0.25 - 1.68)	0.65 (0.25 - 1.67)
Pro-social behaviour	n = 64			
I. 0 – 85 (mg/day) [d]	2.0	-	-	-
II. 86 – 255 (mg/day)	1.9	0.84 (0.44 - 1.59)	0.89 (0.46 - 1.73)	0.85 (0.44 - 1.66)
III. 256 – 425 (mg/day)	1.9	0.97 (0.45 - 2.06)	1.17 (0.52 - 2.63)	1.09 (0.49 - 2.45)
IV. > 425 (mg/day)	0.7	0.40 (0.05 - 3.03)	0.45 (0.06 - 3.54)	0.45 (0.06 - 3.54)

[a] Percentage of children at risk of problem behaviour.
[b] Adjusted for maternal age, ethnicity, maternal education, maternal anxiety, cohabitant status, smoking, alcohol, child's gender, family size.
[c] Additionally adjusted for standardized birth weight and gestational age (potential mediators).
[d] Reference group.
* $p < .05$ ** $p < .01$ *** $p < .001$ (significantly different from reference group).

Table 3. Association between children's problem behaviour and perinatal adversities.

	Gestational age (weeks)				Preterm birth [a]				Standardized birth weight [b]				SGA [c] (n = 293)			
Problem behaviour	Yes		No		Yes		No		Yes		No		Yes		No	
	n	M (SD)	n	M (SD)	n	%	n	%	n	M (SD)	n	M (SD)	n	%	n	%
Overall problem behaviour	224	39.7 (1.7)	3193	39.9 (1.6)	13	5.8	143	4.5	224	1.00 (0.12)	3191	1.01 (0.13)	19	8.5	274	8.6
Hyperactivity/ inattention	255	39.7 (2.1)*	3162	39.9 (1.6)	18	7.1*	138	4.4	255	0.99 (0.12)**	3160	1.01 (0.13)	20	7.8	273	8.6
Emotional symptoms	145	40.0 (1.7)	3272	39.9 (1.6)	7	4.8	149	4.8	145	1.01 (0.14)	3270	1.01 (0.13)	18	12.4	275	8.4
Conduct problems	109	39.7 (1.6)	3308	39.9 (1.7)	7	6.4	149	4.5	109	1.00 (0.12)	3306	1.01 (0.13)	8	7.3	285	8.6
Peer relationship problems	204	39.8 (1.8)	3213	39.9 (1.6)	8	3.9	148	4.6	204	1.00 (0.13)	3211	1.01 (0.13)	23	11.3	270	8.4
Pro-social behaviour	63	40.2 (1.5)	3354	39.9 (1.7)	2	3.2	154	4.6	63	0.99 (0.11)	3352	1.01 (0.13)	63	9.5	287	8.6

M, mean; SD, standard deviation; SGA, small for gestational age.

[a] Delivery between 24 and 36.6 weeks of gestation.

[b] Birth weight standardized for gender, pregnancy duration and parity.

[c] Small for gestational age (birth weight < 10th percentile for gestational age).

* $p < .05$ ** $p < .01$ *** $p < .001$.

Table 4. Risk for peer relationship problems according to maternal caffeine intake during pregnancy stratified for maternal smoking status.

	Peer relationship problems	Crude model Odds ratio (95% CI)	Model 1[a] Odds ratio (95% CI)	Model 1[b] Odds ratio (95% CI)
Smokers	n = 14			
I. 0 – 85 (mg/day)[c]	1	-	-	-
II. 86 – 255 (mg/day)	6	2.76 (0.31 - 24.25)	3.64 (0.37 - 36.28)	6.50 (0.56 - 75.43)
III. 256 – 425 (mg/day)	3	1.22 (0.11 - 13.77)	1.97 (0.15 - 26.60)	2.53 (0.17 - 38.13)
IV. > 425 (mg/day)	4	10.10 (1.07 - 95.67)*	18.69 (1.64 - 212.72)*	54.73 (3.48 - 860.32) **
Non smokers	n = 191			
I. 0 – 85 (mg/day)[c]	76	-	-	-
II. 86 – 255 (mg/day)	88	0.70 (0.50 - 0.98)*	0.81 (0.57 - 1.16)	0.81 (0.56 - 1.15)
III. 256 – 425 (mg/day)	26	0.47 (0.29 - 0.77)**	0.62 (0.37 - 1.04)	0.61 (0.37 - 1.03)
IV. > 425 (mg/day)	1	0.11 (0.02 - 0.77)*	0.14 (0.02 - 1.04)	0.14 (0.02 - 1.03)

CI, 95% confidence interval.

[a] Adjusted for maternal age, ethnicity, maternal education, maternal anxiety, cohabitant status, smoking, alcohol, child's gender, family size.

[b] Additionally adjusted for standardized birth weight and gestational age (potential mediators).

[c] Reference group.

* $p < .05$ ** $p < .01$ *** $p < .001$ (significantly different from reference group).

Discussion

Prenatal maternal dietary caffeine intake was not associated with a higher risk for hyperactivity/inattention problems, emotional symptoms, conduct problems, peer relationship problems suboptimal pro-social behaviour, and overall problem behaviour in their five year old offspring. Consequently, no evidence was found for mediation by fetal growth restriction and gestational age. The child's gender did not modify the association between intrauterine caffeine intake and children's problem behaviour. Maternal smoking during pregnancy moderated the association between caffeine intake and peer relationship problems.

A number of this study's limitations need to be addressed. First, attrition analysis on key variables revealed that mothers who filled in the pregnancy questionnaire and rated their child's behaviour differed from mothers in the non-response group. This may have resulted in an underestimation of the prevalence of behavioural problems, as children of non-responding women might be more prone to develop problem behaviour, since low socioeconomic status is associated with behavioural difficulties (McLoyd, 1998). However the prevalence of problem behaviour in the response group (e.g. hyperactivity/inattention; 7.5%) is in line with prevalence rates from previous studies that varied between 3% and 10% (Alloway, Elliott, & Holmes, 2010; Faraone, Doyle, Mick, & Biederman, 2001). Furthermore, mean SDQ (subscale) scores on problem behaviour by mothers and teachers in our sample were somewhat lower, whereas scores on pro-social behaviour were slightly higher compared with scores from a Dutch norm population that consisted of older children (van Widenfelt et al., 2003). Second, caffeine intake was measured by self-report, which is considered to be the most valid measure of antenatal caffeine exposure (Grosso, Triche, Benowitz, & Bracken, 2008) by use of the best available estimates of caffeine content from coffee, tea and soft drinks. Multiple assessments of caffeine consumption would have given insight in potential sensitive or critical periods in pregnancy during which the foetus might be more susceptible to potential programming effects of caffeine intake. Although intake levels remained fairly stable after the first trimester in a large observational study (CARE study, 2008), it is known that caffeine half-life is extended during the last trimester of pregnancy, which could lead to a decreased caffeine intake and hence overestimation of caffeine intake over the course of pregnancy in the current study. Third, no information about caffeine intake via chocolate, energy drinks and medication was available, which

may have lead to an underestimation of caffeine intake. We do not expect this to lessen the validity of our findings, because it is known that caffeine ingestion in pregnant women stems mainly from coffee and tea (Knight, Knight, Mitchell, & Zepp, 2004). Individual differences in preparation and portion size may have also induced unaccounted variability in estimated caffeine content (Bracken, Grosso, Hellenbrand, Belanger, & Leaderer, 2002). Furthermore, no data on caffeine metabolism were available. Fourth, nausea is a common symptom in the first trimester of healthy pregnancies (Brent, Christian, & Diener, 2011). Nausea (n = 1.586 women reported nausea) did reduce caffeine intake significantly in our sample, nevertheless findings (not presented) in a subsample of only non-nauseous women did not differ from results shown in table 2.

A major strength of the current study is that we assessed multiple domains of children's problem behaviour in the (pre)school age, by using a validated questionnaire with good psychometric properties (van Widenfelt et al., 2003), filled in by both mother and teacher, because children tend to behave differently in their home and school environment (Najman et al., 2000). Previous studies were solely based on maternal reports of child behaviour and hence are at risk for a maternal bias (overrated problem behaviour) (Kroes, Veerman, & De Bruyn, 2003). Furthermore, by using multiple informants on children's behaviour we have implemented an accurate as well as conservative approach to identify potential problem behaviour. In addition, most studies considered 3 or more cups of coffee per day (>255 mg/d) as high intake (Peck, Leviton, & Cowan, 2010). Because a relatively large group of women in our study reported to consume comparable or even higher amounts of caffeine, we were able to fully explore the effect of high doses of caffeine. Current analyses were conducted in a large, community based, multi-ethnic birth cohort, which is a clear advantage in terms of statistical power and generalisabilty. In addition, we were able to control for a large number of potential confounding factors such as maternal ethnic background, which appeared to be an important confounder, because non-Dutch women drank significantly less coffee compared with Dutch women (Table 1). In addition, ethnic differences in reports on offspring's mental health problems have been found. However, neither statistical control for confounding by ethnic background nor analyses within a sample that consisted of only Dutch women (n = 2630) led to different results (data not shown).

Current findings are in accordance with previous studies that have reported no association between prenatal caffeine consumption and neurodevelopmental

outcomes in the offspring. No long-term neurobehavioural consequences assessed in the first seven years of life that were related to prenatal maternal caffeine consumption were found in a large cohort (Barr & Streissguth, 1991; Streissguth et al., 1980). Caffeine intake examined during a similar period early in pregnancy (16th week) was not associated with hyperkinetic disorder and attention-deficit hyperactivity disorder in children in another prospective cohort study (Linnet et al., 2009). In contrast, some studies did find evidence for an increased risk for neurodevelopmental adversities. However, the explained variance in inattention/hyperactivity by prenatal caffeine intake was very low (Bekkhus et al., 2010). An increased risk for social problems related to retrospectively assessed coffee consumption (no information on quantity and caffeine content was taken into account) during pregnancy was found. However, the number of women that had reported to consume coffee on a regular basis was very low (n = 19) (Chiu et al., 2009). We did not find evidence for mediation by gestational age and fetal growth restriction. As such, the current study did not replicate previous studies that have found significant associations between high caffeine intake and shorter gestational age and fetal growth restriction (Bakker et al., 2010; CARE study, 2008; Vik et al., 2003). Fetal growth restriction and gestational age were not related to children's problem behaviour except for hyperactivity/inattention problems, which is in line with findings from a previous study (Mick et al., 2002). The child's gender did not moderate the association between prenatal caffeine intake and children's problem behaviour, which is not in accordance with findings in animal studies (Fisher & Guillet, 1997; Hughes & Beveridge, 1986; Hughes & Beveridge, 1991). However, in human studies the child's gender has not been reported to moderate the association up until now. Interpretation of the effect modification by maternal smoking could only be based on findings in the crude model as the ratio of cases to the number of predictors in the adjusted models was too small. Therefore, this association should be interpreted with caution, because confounding by, for example, socioeconomic status or ethnic background might be present.

To conclude, this study has provided insight in to what extent caffeine consumption during pregnancy contributes to the development of problem behaviour. Our results did not provide evidence to advise pregnant women to reduce their caffeine intake in order to prevent problem behaviour in their children.

What's known on this subject:
In humans, evidence for an association between maternal caffeine intake during pregnancy and alterations in foetal brain development with persistent alterations in offspring's brain and behaviour in later life is inconclusive.

What this study adds:
Prenatal caffeine intake is not associated with a higher risk for behaviour problems in young children. Results do not provide evidence to advise pregnant women to reduce their caffeine intake in order to prevent problem behaviour in their children.

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7

Maternal long-chain polyunsaturated fatty acid status during early pregnancy and children's risk of problem behaviour at age 5 to 6 years

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Abstract

Objective: Long-chain polyunsaturated fatty acids (LCPUFA) eicosapentaenoic acid (EPA; omega-3), docosahexaenoic acid (DHA; omega-3) and arachidonic acid (AA; omega-6) play an important role in neurodevelopment. We prospectively investigated the association between maternal LCPUFA status and ratio during pregnancy and children's risk of problem behaviour at age five.

Design: Maternal LCPUFA status in plasma phospholipids during pregnancy (M= 13.3, SD= 3 weeks) was available for 4336 women. Children's behaviour was rated by mother (n= 2502) and teacher (n= 2061).

Results: Multivariate logistic regression analyses showed that higher concentrations of omega-3 fatty acids DHA (OR: 0.75 [95% CI, 0.56 – 0.99]; p= .05) and EPA (OR: 0.35 [95% CI, 0.11 – 1.08]; p= .07) decreased children's risk for emotional symptoms. A higher omega-6 to omega-3 ratio increased the risk for emotional symptoms (OR: 1.66 [95% CI, 0.97 – 2.86]; p= .07) and hyperactivity/inattention problems (OR: 1.39 [95% CI, 0.95 – 2.05]; p= .07). No evidence was found for mediation by preterm birth and being small for gestational age. The child's sex and infant feeding did not modify the associations.

Conclusions: Our results suggest long-term developmental programming influences of maternal LCPUFA status during pregnancy and stress the importance of an adequate and balanced supply of fatty acids in pregnant women to ensure optimal foetal brain development and subsequent long-term behavioural outcomes.

Introduction

Essential fatty acids and particularly their long-chain polyunsaturated derivatives eicosapentaenoic acid (EPA; omega-3), docosahexaenoic acid (DHA; omega-3) and arachidonic acid (AA; omega-6) play an important role in neurodevelopmental processes such as neurogenesis, cell proliferation, membrane functioning and, potentially, myelination (Georgieff, 2007). To enable optimal foetal brain development, both a sufficient and balanced supply of omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFA) via maternal circulation (i.e. placental transfer) are crucial (Campbell, Gordon, & Dutta-Roy, 1996; Gibson, Muhlhausler, & Makrides, 2011). Hence, deficiencies in maternal LCPUFA status, or an imbalance in the omega-6 to omega-3 LCPUFA ratio during gestation might affect foetal brain development and influence subsequent long-term behavioural outcomes.

Although a number of studies indeed suggest beneficial effects of maternal omega-3 and omega-6 LCPUFA during pregnancy on offspring's long-term neurodevelopmental outcomes, they are compromised by methodological shortcomings and results remain inconclusive. First, in two studies maternal omega-3 LCPUFA status was represented by maternal fish consumption, therefore findings could be the result of a nutritious diet or a healthy lifestyle in general (Gale et al., 2008; Hibbeln et al., 2007). In order to capture maternal LCPUFA status in late gestation, previous studies have assessed maternal venous (Cheruku, Montgomery-Downs, Farkas, Thoman, & Lammi-Keefe, 2002) or umbilical cord blood at birth, (Kohlboeck et al., 2011; Krabbendam, Bakker, Hornstra, & van Os, 2007) leaving the potential influence of maternal LCPUFA status during early gestation unstudied. However, there is evidence that LCPUFA are involved in early neurodevelopmental processes which take place in early stages of pregnancy (Georgieff, 2007; Innis, 2007). Second, although a balanced supply of omega-3 and omega-6 LCPUFA is known to be important (Simopoulos, 2002), human studies that have investigated the long term influence of the omega-6 to omega-3 LCPUFA ratio on neurodevelopmental outcomes are lacking. A study by Cheruku et al. (2002) showed that a higher ratio of total omega-6 to omega-3 fatty acids in maternal venous blood at delivery was related to altered sleep patterns in newborns, which is suggestive of central nervous system vulnerability. This might be the result of LCPUFA precursors linoleic acid (LA; omega-6) and alpha-linolenic acid (ALA; omega-3) that compete over the same enzymes for

conversion into their long-chain polyunsaturated derivatives. Hence, a higher LA (omega-6) status, which is typical in Western diets, results in a lower omega-3 (DHA and EPA) status which potentially influences early neurodevelopmental processes (Gibson, et al., 2011). Third, in previous studies children's behaviour was solely rated by their mothers or fathers and setting-specific behaviour problems (e.g. home vs. school) and cross-informant discrepancies between parents and teachers were not taken into account (Achenbach, 2011). Fourth, the potential role of infant feeding (breast milk vs. formula) in the association between maternal LCPUFA status and children's neurodevelopmental outcome remains unclear (Tozzi et al., 2012).

In an attempt to address these issues we are the first study that prospectively investigated the association between maternal LCPUFA status (EPA; omega-3, DHA; omega-3 and AA; omega-6) and the omega-6 to omega-3 ratio during early pregnancy and children's risk of problem behaviour at age five to six years in a large multiethnic, community based birth cohort. We were able to take into account a large number of potentially confounding factors and we included mothers' as well as teachers' ratings on multiple dimensions of children's behaviour. Mediation by preterm birth, being born small for gestational age (SGA) (Al, van Houwelingen, & Hornstra, 2000; van Eijsden, Hornstra, van der Wal, Vrijkotte, & Bonsel, 2008) and effect modification by type of infant feeding were also examined.

Methods

Study design and participants

The current study is part of the Amsterdam Born Children and their Development (ABCD) study. Extensive information about the cohort and procedures regarding data collection is provided elsewhere (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2010). In short, pregnant women living in Amsterdam were approached for their participation between January 2003 and March 2004 during their first visit with an obstetric care provider. All women (12373) received a questionnaire covering sociodemographic, obstetric, life-style and psychosocial conditions, which was filled out by 8266 of them (67%). Of those respondents, 53% (n = 4389) participated voluntarily in the biomarker study, in which an additional blood sample was taken during routine blood collection for prenatal screening

purposes. To be included in the current study, complete data on both maternal fatty acid status and children's behavioural assessment had to be available. Additional information about inclusion criteria is provided in figure 1. All participating mothers gave their written informed consent. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in The Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

Maternal LCPUFA status

Maternal LCPUFA concentrations in plasma phospholipids were determined with biochemical analyses that were described in detail elsewhere (van Eijsden, et al., 2008). In short, phospholipids isolated from plasma were saponified and the resulting fatty acids were methylated and measured by capillary gas chromatography with flame ionisation detection. The absolute amounts of omega-6 AA, omega-3 DHA, and omega-3 EPA (in mg/L plasma) were quantified on the basis of the recovery of an internal standard and expressed in a relative value (percentage of total amount of phospholipids-associated fatty acids).

Children's risk of problem behaviour

Children's problem behaviour was reported by their mothers and primary school teachers using the Strengths and Difficulties Questionnaire (SDQ), a short behavioural screening questionnaire suitable for 4 to 16 year olds (Goodman et al., 1997). The SDQ consists of 25 items, which are divided in 5 subscales: emotional symptoms, conduct problems, hyperactivity/inattention problems, peer relationship problems and pro-social behaviour. All items (without pro-social behaviour items) added together form a total difficulties score that represents children's overall problem behaviour. The reliability and validity of the SDQ have been established in a Dutch population with satisfactory psychometric characteristics (van Widenfelt, Goedhart, Treffers, & Goodman, 2003). Inter-rater reliability between mothers and teachers was calculated using Cohen's kappa coefficients for hyperactivity/inattention problems (K= 0.28), conduct problems (K= 0.21), emotional symptoms (K= 0.15), peer relation problems (K= 0.24), pro-social behaviour (K= 0.09) and overall problem behaviour (K= 0.28) (all p's < .001). Based on these coefficients mother and teacher agreement on children's problem behaviour was considered to be slight to fair (Landis & Koch, 1977). Therefore, we chose to identify children to be at risk for problem behaviour

only when both mother and teacher ratings were consistent. Because no valid norm scores for a Dutch population of young children are available (Mieloo et al., 2012) and in accordance with previous work, (Loomans et al., 2012) behavioural outcomes were dichotomized ("no behaviour problems" or "at risk for behaviour problems") using the 83rd percentile as a cut-off (optimal sensitivity and specificity) (Schmeck et al., 2001). Children with SDQ (subscale) scores by both mother and teacher below the 83rd percentile were not considered to be at risk for problem behaviour. In accordance, children with a score above the 83rd percentile reported by either mother or teacher were also not considered to be at risk for problem behaviour. Only children with a mean (subscale) score above the 83rd percentile reported both by their mother and their teacher were considered to be at risk for behaviour problems. For pro-social behaviour, children with SDQ (subscale) scores by both mother and teacher above the 17th percentile were not considered to show suboptimal pro-social behaviour. Children with a score below the 17th percentile reported by either mother or teacher were also not considered to be at risk for suboptimal pro-social behaviour. Only children with a score below the 17th percentile reported both by their mother and their teacher were considered to be at risk for suboptimal pro-social behaviour.

Covariates, mediators and moderators

Theoretically based a priori selected potential covariates were: self reported maternal ethnicity defined by country of birth (the Netherlands, other Western country, other non-Western country) (van Eijsden, Hornstra, van der Wal, & Bonsel, 2009), maternal age (years), parity (0, >1), pre-pregnancy BMI (kg/m^2) based on self-reported height and weight, smoking (no, stopped since pregnant, yes) and alcohol consumption (no, stopped since pregnant, yes), maternal state-anxiety (Spielberger, Gorsuch, & Lushene, 1970), maternal education (years after primary school) and child's sex, and child's age (years). Birth weight (grams) and gestational age (weeks) were available from Youth Health Care Registration and the Dutch Perinatal Registration (PRN, www.perinatreg.nl). Information on infant feeding (formula fed, 1-3 months of exclusive breastfeeding, >3 months of exclusive breast feeding), was obtained by combining data from two questionnaires (during infancy and when child was five years old) and information available from the Youth Health Care Registration.

Statistical analyses

The association between maternal LCPUFA status and children's risk of problem behaviour was first examined using a logistic regression model (crude model) that was adjusted for gestational age at blood sampling to account for changes in LCPUFA status during pregnancy (Al et al., 1995). Second, multivariate logistic regression analyses were conducted that included covariates (model 1). If maternal LCPUFA status remained significantly related to children's problem behaviour after full adjustment in the multivariate model, mediation by preterm birth (< 37 weeks of gestation) and being small for gestational age (birth weight < 10th percentile for gestational age standardized for sex and parity) (Visser, Eilers, Elferink-Stinkens, Merkus, & Wit, 2009) was tested by adding these variables to the adjusted model one by one (i.e. in absence of the other mediator). A 10% attenuation of effect size in the association between LCPUFA status and problem behaviour caused by the mediator was considered as a threshold for mediation (Szklo & Nieto, 2000). In addition, interaction terms with the child's sex and infant feeding were added to the fully adjusted models to investigate effect modification. Associations were considered significant at $p < 0.05$. All analyses were conducted with IBM SPSS version 19.

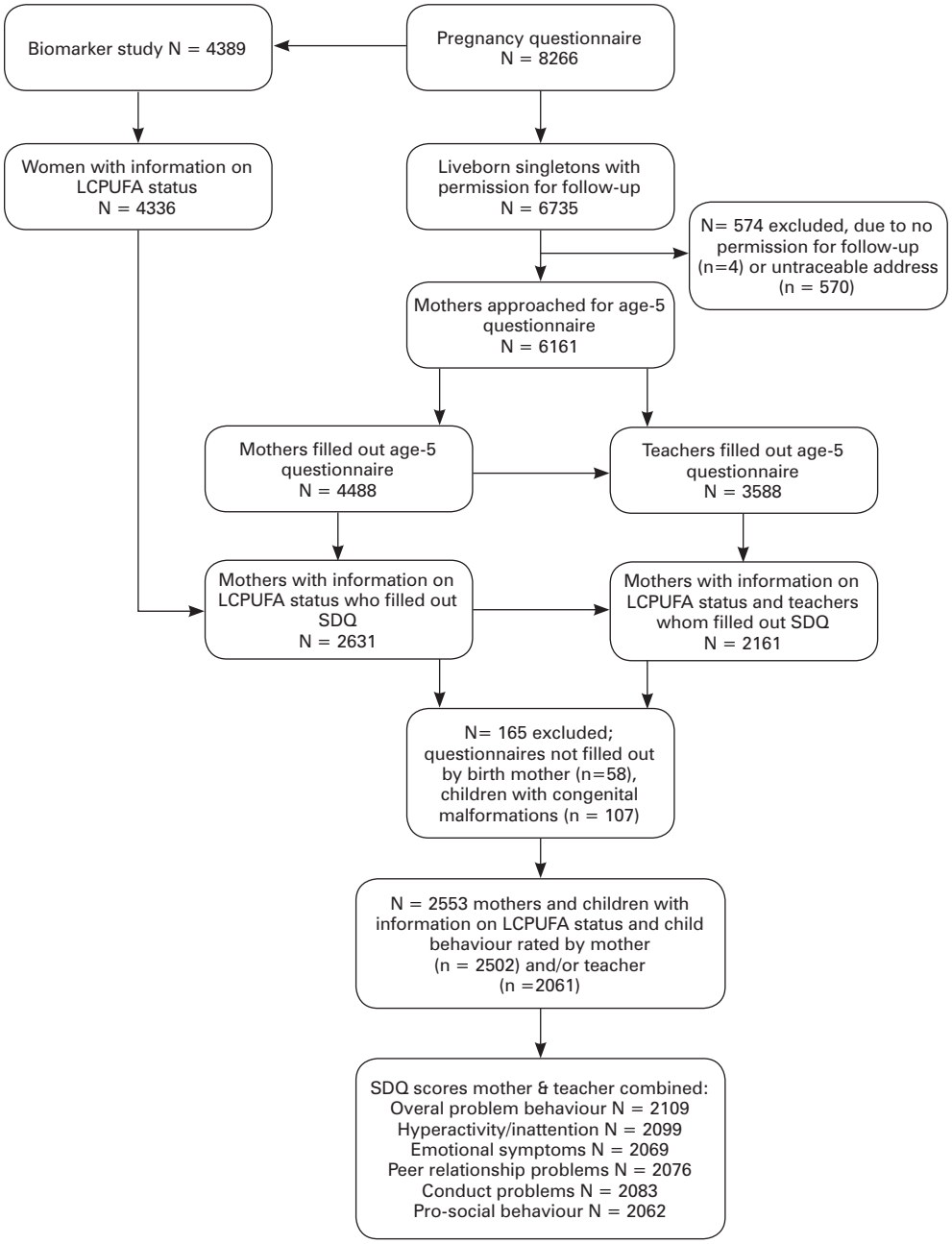

Figure 1. Participants included for analysis.

Results

Demographic characteristics about the participating mothers and children are presented in table 1. Attrition analysis on key variables revealed that mothers for whom both fatty acid results were available and who rated their child's behaviour at age five (n = 2553) were on average 1.85 years older ($p < 0.001$), more often highly educated (51% vs. 31%; $p < 0.001$), more often had a Dutch background (77% vs. 55%; $p = 0.001$), less often gave preterm birth (5% vs. 6%; $p = 0.043$), and more often breastfed their child (72% vs. 28%; $p = 0.032$), compared to mothers in the non-response group (n = 1783). No differences were found in the number of small for gestational age babies (11% vs. 12%; $p = 0.520$). Mothers in the response group had higher concentrations of EPA (0.65% vs. 0.60%; $p = 0.001$), lower concentrations of AA (9.21% vs. 9.44%; $p < 0.001$), and a lower omega-6 to omega-3 ratio (1.79 vs. 1.88%; $p < 0.001$) compared to non-responders, DHA concentrations did not differ (4.81% vs. 4.77%; $p = 0.390$).

Association between maternal LCPUFA status and children's risk of problem behaviour

Analyses in the crude model revealed that higher concentrations of omega-6 AA increased the risk for peer relationship problems. A higher omega-6 to omega-3 ratio increased the risk for hyperactivity/inattention problems and peer relationship problems. After adjustment for potential confounders higher concentrations of omega-3 DHA significantly decreased the risk for children's emotional symptoms. When adopting a slightly less conservative approach ($p < 0.10$), higher concentrations of omega-3 DHA decreased the risk for children's emotional symptoms and peer relationship problems. Higher concentrations of omega-3 EPA decreased the risk for children's overall problem behaviour and emotional symptoms and higher concentrations of omega-6 AA increased the risk for overall problem behaviour, hyperactivity/inattention problems in the crude model. After adjustment for potential confounders omega-3 EPA decreased the risk for children's emotional symptoms and a higher omega-6 to omega-3 ratio increased the risk for emotional symptoms and hyperactivity/inattention problems. Addition of potential confounders in the adjusted models reduced the number of significant associations between maternal LCPUFA concentrations and children's problem behaviour, which was mainly attributable to the influence of the child's sex, maternal state-anxiety and maternal pre-pregnancy BMI. The

child's sex or infant feeding did not modify any of the associations between maternal LCPUFA status and children's risk of problem behaviour.

Mediation by small for gestational age and preterm birth

Maternal DHA and EPA concentrations and omega-6 to omega-3 ratio remained significantly related to children's emotional symptoms in the adjusted model. Subsequently we examined whether these associations could be mediated by preterm birth or small for gestational age. We found some evidence for associations between maternal LCPUFA concentrations with preterm birth and being small for gestational age. Higher maternal DHA concentrations decreased the risk of being small for gestational age (OR: 0.86 [95% CI, 0.79 – 0.99]; p= .04). Higher AA concentrations increased the risk of being small for gestational age (OR: 1.12 [95% CI, 1.04 – 1.21]; p= <.01) and preterm birth (OR: 1.22 [95% CI, 1.09 – 1.37]; p= <.01). A higher omega-6 to omega-3 ratio increased both the risk of small for gestational age (OR: 1.57 [95% CI, 1.27 – 1.94]; p= <.01) and preterm birth (OR: 1.58 [95% CI, 1.16 – 2.17]; p= <.01). No associations were found between preterm birth, small for gestational age and children's risk of problem behaviour. In accordance, no evidence was found for mediation by preterm birth and small for gestational age as they did not attenuate the influence of maternal LCPUFA status on children's risk of problem behaviour in the adjusted models (Table 2).

Table 1. Mother and child characteristics in the study population (N= 2553).

Maternal characteristics				
Age (years)	2553	31.6 ± 4.4		
Pregravid BMI kg/m^2	2553	22.9 ± 3.7		
Education following primary school (years)	2543	9.9 ± 3.5		
Country of birth (%)	2553			
Netherlands	1970	77.2		
Other Western country	175	6.9		
Other non-Western country	408	16.0		
Parity (% nullipara)	1490	58.4		
State anxiety (STAI score)	2535	36.8 ± 9.9		
Alcohol consumption during pregnancy (%)	2552			
No	668	26.2		
Stopped drinking since pregnant	1163	45.6		
Yes	721	28.2		
Smoking during pregnancy (%)	2553			
No	1907	74.7		
Stopped smoking since pregnant	419	16.4		
Yes	227	8.9		
Exclusive breast-feeding (%)	2521			
No	416	16.5		
< 1 month	167	6.6		
1-3 months	689	27.3		
> 3 months	1249	49.5		
Relative fatty acid concentration (% of total fatty acids)				
Total fatty acids (mg/L)	2553	1466.6 ± 241.8		
EPA (20:5n-3) (%)	2553	0.7 ± 0.4		
DHA (22:6n-3) (%)	2553	4.8 ± 1.1		
AA (20:4n-6) (%)	2553	9.2 ± 1.6		
Omega-6 to omega-3 ratio	2553	1.8 ± 0.5		
Child characteristics				
Gender (% male)	1237	48.5		
Age (years)	2553	5.2 ± 0.3		
Gestational age at blood sampling (weeks)	2553	13.3 ± 3.0		
Gestational age at birth (weeks)	2550	39.9 ± 1.7		
Birth weight (grams)	2542	3486.3 ± 544.3		
Preterm birth (%)	118	4.6		
Small for gestational age (%)	293	11.5		
Children's problem behaviour (SDQ)		**Mother**		**Teacher**
Overall problem behaviour	2501	5.1 ± 4.0	2060	5.2 ± 4.6
Hyperactivity/inattention problems	2501	2.4 ± 2.2	2061	2.3 ± 2.6
Emotional symptoms	2502	0.9 ± 1.3	2061	1.2 ± 1.6
Peer relationship problems	2502	0.8 ± 1.2	2061	1.0 ± 1.4
Conduct problems	2502	1.0 ± 1.2	2060	0.8 ± 1.3
Pro-social behaviour	2497	8.0 ± 1.8	2061	7.6 ± 2.2

BMI= Body Mass Index, STAI= State-Trait Anxiety Inventory, SDQ= Strengths and Difficulties Questionnaire, AA= arachidonic acid, EPA= eicosapentaenoic acid, DHA= docosahexaenoic acid, Omega-6 to omega-3 ratio = AA/(DHA+EPA), Preterm birth (< 37 weeks of gestation), Small for gestational age (birth weight < 10th percentile for gestational age standardised for sex and parity).

Table 2. Association between maternal fatty acid concentrations in plasma phospholipids during pregnancy and children's risk of problem behaviour.

				DHA (22:6n-3)		
	%	n	Crude model	Model 1	Model 1+ SGA†	Model 1+ Preterm birth†
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ov prob	5.1	2109	0.93 (0.79 - 1.10)	1.02 (0.86 - 1.22)	1.02 (0.86 - 1.22)	1.02 (0.86 - 1.23)
Hyp/inatt	4.3	2099	0.87 (0.72 - 1.04)	0.90 (0.74 - 1.10)	0.91 (0.74 - 1.10)	0.91 (0.74 - 1.10)
Emo symp	1.9	2069	0.78 (0.59 - 1.03) *	0.75 (0.56 - 0.99) **	0.76 (0.57 - 1.01) *	0.75 (0.56 - 1.00) *
Peer prob	2.9	2076	0.80 (0.64 - 1.01) *	0.85 (0.67 - 1.08)	0.85 (0.67 - 1.08)	0.86 (0.67 - 1.09)
Cond prob	2.4	2083	0.97 (0.76 - 1.23)	1.02 (0.79 - 1.31)	1.02 (0.79 - 1.31)	1.02 (0.79 - 1.31)
Pro-soc	1.4	2062	1.12 (0.83 - 1.52)	1.09 (0.81 - 1.49)	1.10 (0.81 - 1.49)	1.09 (0.80 - 1.48)
				EPA (20:5n-3)		
	%		Crude model	Model 1	Model 1+ SGA†	Model 1+ Preterm birth†
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ov prob	5.1	2109	0.63 (0.37 - 1.07) *	0.79 (0.48 - 1.31)	0.79 (0.48 - 1.30)	0.80 (0.48 - 1.31)
Hyp/inatt	4.3	2099	0.67 (0.38 - 1.16)	0.74 (0.42 - 1.29)	0.73 (0.42 - 1.29)	0.74 (0.42 - 1.29)
Emo symp	1.9	2069	0.35 (0.12 - 1.03) *	0.35 (0.11 - 1.08) *	0.37 (0.12 - 1.12) *	0.35 (0.11 - 1.09) *
Peer prob	2.9	2076	0.53 (0.25 - 1·12)	0.71 (0.36 - 1.40)	0.71 (0.36 - 1.40)	0.71 (0.36 - 1.40)
Cond prob	2.4	2083	0.49 (0.21 - 1.16)	0.53 (0.23 - 1.25)	0.53 (0.22 - 1.24)	0.53 (0.23 - 1.25)
Pro-soc	1.4	2062	0.81 (0.34 - 1.94)	0.72 (0.30 - 1.74)	0.73 (0.30 - 1.76)	0.72 (0.30 - 1.73)
				AA (20:4n-6)		
	%		Crude model	Model 1	Model 1+ SGA†	Model 1+ Preterm birth†
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ov prob	5.1	2109	1.12 (1.00 - 1.25) *	1.05 (0.94 - 1.19)	1.05 (0.94 - 1.19)	1.05 (0.93 - 1.19)
Hyp/inatt	4.3	2099	1.13 (1.00 - 1.27) *	1.09 (0.96 - 1.25)	1.10 (0.96 - 1.25)	1.09 (0.96 - 1.25)
Emo symp	1.9	2069	1.09 (0.91 - 1.30)	1.10 (0.90 - 1.34)	1.10 (0.90 - 1.34)	1.10 (0.90 - 1.34)
Peer prob	2.9	2076	1.17 (1.02 - 1.36) **	1.07 (0.92 - 1.24)	1.07 (0.92 - 1.24)	1.07 (0.92 - 1.24)
Cond prob	2.4	2083	1.12 (0.95 - 1.32)	1.08 (0.90 - 1.29)	1.08 (0.90 - 1.29)	1.08 (0.90 - 1.29)
Pro-soc	1.4	2062	0.92 (0.74 - 1.14)	0.92 (0.72 - 1.16)	0.92 (0.73 - 1.17)	0.92 (0.72 - 1.16)

Omega-6 to omega-3 ratio						
	%		Crude model	Model 1	Model 1+ SGA†	Model 1+ Preterm birth†
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ov prob	5.1	2109	1.29 (0.94 - 1.77)	1.00 (0.69 - 1.44)	1.00 (0.69 - 1.44)	1.00 (0.69 - 1.43)
Hyp/inatt	4.3	2099	1.52 (1.09 - 2.12) **	1.39 (0.95 - 2.05) *	1.39 (0.94 - 2.03) *	1.38 (0.94 - 2.03)
Emo symp	1.9	2069	1.51 (0.92 - 2.47)	1.66 (0.97 - 2.86) *	1.63 (0.95 - 2.81) *	1.66 (0.96 - 2.86) *
Peer prob	2.9	2076	1.69 (1.13 - 2.53) **	1.32 (0.84 - 2.08)	1.32 (0.84 - 2.09)	1.31 (0.83 - 2.06)
Cond prob	2.4	2083	1.18 (0.74 - 1.89)	1.01 (0.59 - 1.72)	1.01 (0.60 - 1.71)	1.01 (0.60 - 1.72)
Pro-soc	1.4	2062	0.65 (0.33 - 1.29)	0.65 (0.32 - 1.33)	0.65 (0.32 - 1.33)	0.66 (0.32 - 1.34)

Ov prob= overall problem behaviour, hyp/inatt= hyperactivity inattention problems, emo symp= emotional symptoms, peer prob= peer relationship problems, cond prob= conduct problems, pro-soc= pro-social behaviour, AA= arachidonic acid, EPA= eicosapentaenoic acid, DHA= docosahexaenoic acid, Omega-6 to omega-3 ratio = AA/(DHA+EPA), SDQ= Strengths and Difficulties Questionnaire. Crude model is adjusted for: gestational age at blood sampling. Model 1 is adjusted for: gestational age at blood sampling, maternal ethnicity, maternal age, parity, pre-pregnancy BMI, smoking, alcohol consumption, maternal state-anxiety, maternal education, birth weight, gestational age, child's sex and age. OR per 1 unit increase in LCPUFA concentration is presented. † Mediators: SGA (birth weight < 10th percentile for gestational age standardized for sex and parity) and preterm birth (< 37 weeks of gestation), were added to the adjusted regression model separate of one another. * $p < .10$ ** $p < .05$

Discussion

Our results indicate that maternal LCPUFA status during early pregnancy is associated with children's emotional symptoms and hyperactivity/inattention problems at age five to six. Higher maternal concentrations of omega-3 fatty acids DHA and EPA decreased the risk for children's emotional symptoms. A higher omega-6 to omega-3 ratio increased the risk for emotional symptoms and hyperactivity/inattention problems. No evidence was found for mediation by preterm birth and being small for gestational age. The child's sex and infant feeding did not modify these associations.

A number of this study's limitations need to be addressed. First, attrition analysis on key variables revealed that mothers for whom both fatty acid results were available and who rated their child's behaviour at age five differed from mothers in the non-response group. This may have resulted in an underestimation of the prevalence of problem behaviour, as children of non-responding women might be more prone to develop problem behaviour, since low socioeconomic status is associated with problem behaviour (McLoyd, 1998). Second, compared to scores from a Dutch population (van Widenfelt, et al., 2003) SDQ scores on problem behaviour by mothers and teachers in our sample were somewhat lower. In addition, we chose to identify children at risk for behaviour problems only when both mother and teacher ratings were consistent. Therefore, current estimates of the association between maternal LCPUFA status and children's risk of behaviour problems may be quite conservative in comparison to previous work. Third, we were not able to take the potential influence of the children's LCPUFA status at age five into account. However, Krabbendam et al., (2007) have found no association between children's current LCPUFA status and problem behaviour. Fourth, due to the non-invasive design of the present study, maternal LCPUFA status was determined at one occasion during pregnancy. Therefore we were unable to investigate sensitive or critical periods in pregnancy during which the foetus might be more susceptible to potential programming effects of maternal LCPUFA status. Nevertheless, we do not expect this limitation to lessen the validity of our findings, because maternal fatty acid concentrations remain relatively stable over the course of pregnancy (Al, et al., 1995). Moreover, current results add to the existing literature as they emphasize the importance of an adequate and balanced maternal LCPUFA status in early gestation (Georgieff, 2007; Innis, 2007).

Our results strengthen and elucidate previous findings that suggested a beneficial influence of a higher gestational omega-3 fatty acid status on children's neurodevelopmental outcomes and behavioural problems (Gale, et al., 2008; Hibbeln, et al., 2007; Kohlboeck, et al., 2011; Krabbendam, et al., 2007). Furthermore, we found no associations attributable to a higher AA status in the adjusted models, which is in accordance with findings from a previous study that found no evidence for an association between AA status and internalizing and externalizing problem behaviour in seven year olds (Krabbendam, et al., 2007). On the other hand, Kohlboeck et al., (2011) showed that higher AA concentrations in cord blood were associated with fewer emotional symptoms. The fact that findings differ between studies could be due to differences in sample size, timing and type of the fatty acid assessment (i.e. blood sample in early pregnancy vs. cord or venous blood at delivery) or report on offspring's problem behaviour. Although current findings do not suggest an influence of a higher AA status, a higher omega-6 to omega-3 ratio increased the risk for emotional symptoms and hyperactivity/inattention problems. This is in line with a previous study that linked a higher relative omega-6 to omega-3 ratio to behaviour regulation problems and altered sleep patterns in neonates (Cheruku, et al., 2002). Although findings seem to concur, given the observational nature of our study, it remains unclear whether the association between a higher omega-6 to omega-3 ratio and increased behavioural problems could be merely attributed to a higher AA status or to relatively low EPA or DHA concentrations (Gibson, Muhlhausler, & Makrides, 2011).

Being born small for gestational age or before 37 weeks of gestation did not attenuate the associations between maternal LCPUFA status and children's behaviour problems. However, it must be noted that in the current study preterm births were less prevalent compared to the number in the Dutch population (4.6 % vs. 7.7 %), therefore these results must be interpreted cautiously and need to be replicated. Our results did not suggest effect modification by type of infant feeding (exclusive breast feeding vs. formula). In accordance, findings from previous studies that examined the association between being fed breast milk vs. formula in infancy and later cognitive development are inconsistent. Possibly, inconsistencies among studies could be attributed to confounding by parental education (Tozzi, et al., 2012), or children's genetic variations in LCPUFA metabolism that moderate effects of breastfeeding (e.g. LCPUFA) on children's cognitive development (Caspi et al., 2007). In order to preclude bias due to

formula fortification with LCPUFA's in preterm babies, we repeated analyses in a subsample in which preterm infants (n= 118) were excluded. Again no evidence for effect modification by type of infant feeding was found.

Current analyses were conducted in a large, community based, multi-ethnic birth cohort, which is a clear advantage in terms of statistical power. In addition, we were able to control for a large number of potentially confounding factors. A major strength of the current study was that plasma phospholipid fatty acid concentrations during early gestation were obtained instead of estimates of fatty acid status represented by maternal fish consumption, or LCPUFA status at birth that were used in previous studies. In addition, the potential influence of a higher omega-6 to omega-3 ratio was examined, which clearly adds to the existing literature because it is a characteristic of modern Western dietary habits, and according to current findings it has an adverse influence on long-term behavioural outcomes. Another strength of the current study is that we assessed multiple domains of children's problem behaviour, using a validated questionnaire with good psychometric properties (van Widenfelt, et al., 2003), filled in by both mother and the child's primary school teacher. Incorporating multiple informants that rated children's behaviour in different circumstances (home vs. school) clearly provides a more objective assessment of children's behaviour. Hence, it is crucial that future studies include multiple informants to preclude the risk of maternal bias.

In conclusion, results from the present study are suggestive of long-term developmental programming influences of maternal LCPUFA status during early pregnancy on offspring's problem behaviour at age five to six years. Our results stress the importance of an adequate and balanced supply of dietary fatty acids in pregnant women to ensure optimal foetal brain development and subsequent long-term behavioural outcomes.

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8

General discussion

General discussion

The aim of the current dissertation was to investigate early life influences on children's birth outcomes, neurocognitive functioning using objective cognitive tasks and children's behaviour rated by multiple informants. In this general discussion we will first summarize our main findings and provide answers to our research questions. Second, we will address methodological considerations that should be taken into account when interpreting current findings. Third, we will present a number of potential underlying mechanisms and mediators that could explain the associations between early life influences and neurodevelopmental outcomes. Fourth, directions for future research and implications of our findings for public health practice will be considered followed by a final conclusion.

The research questions that were addressed in this dissertation are:

1. Are psychosocial stress and negative emotions during pregnancy related to adverse birth outcomes? (Chapter 3)

2. Is there an association between maternal anxiety during pregnancy and children's neurocognitive functioning and behaviour at the age of five to six? (Chapters 4 and 5)

3. Is there an association between caffeine intake during pregnancy and the risk of problem behaviour in five to six year old children? (Chapter 6)

4. Is there an association between maternal long-chain polyunsaturated fatty acid status during early pregnancy and children's risk of problem behaviour at age five to six years? (Chapter 7)

Main findings

Early life influences

In chapter 3, our findings clearly indicated that children of women who experience both high levels of anxiety as well as depressive symptoms in early gestation (almost 30% of the women in our sample), are particularly at risk for lower birth weight and preterm birth independently of biomedical risk factors, other types of psychosocial stress and maternal characteristics.

Results presented in chapter 4 suggested that there indeed is an association between higher levels of maternal anxiety during pregnancy and alterations in children's neurocognitive functioning at the age of five to six. Children of highly anxious pregnant mothers were more variable in their performance than children of less anxious women, but no associations were found between antenatal anxiety and the children's mean reaction time in both the simple and the choice reaction time task. Examination of nonlinear associations revealed a significant nonlinear association between antenatal anxiety and the children's variability in reaction time in the incompatible part (i.e. incompatible stimulus-response mode) of the choice reaction time task. Visual inspection of the data showed that higher levels of maternal anxiety were related to a stronger than linear increase in children's variability in reaction time. This finding suggested that the reprogramming effects of antenatal anxiety become stronger when reported anxiety levels rise. Subsequent analyses in a highly anxious subsample showed that higher levels of antenatal anxiety were more strongly associated with longer reaction times and more intra-individual variability in reaction time in the incompatible part of the choice reaction time task. The child's sex moderated the relation between antenatal anxiety and intra-individual variability in the simple reaction time task in the highly anxious subsample. Boys performed less stable on the simple reaction time task, but no significant associations were found in girls.

In addition, higher levels of maternal anxiety were related to more overall problem behaviour, hyperactivity/inattention problems, emotional symptoms, peer relationship problems, conduct problems and less pro-social behaviour when mothers had rated their child's behaviour. When child behaviour was rated by their primary school teachers, maternal anxiety during pregnancy was related to more overall problem behaviour and less pro-social behaviour (chapter 5). Child's sex moderated the association between antenatal maternal anxiety and

children's problem behaviour. Boys showed more overall problem behaviour and hyperactivity/inattention problems compared to girls.

The main conclusion in chapter 6 was that maternal dietary caffeine intake during pregnancy was not associated with a higher risk for hyperactivity/inattention problems, emotional symptoms, conduct problems, peer relationship problems, suboptimal pro-social behaviour, and overall problem behaviour in their five to six year old children. In addition, no evidence was found for moderation by the child's sex or for mediation by foetal growth restriction and gestational age. Given the fact that a relatively large group of women consumed considerable quantities of caffeine (n= 862 > 4 cups), we were able to fully explore the effect of high doses of caffeine intake, nevertheless our findings did not provide evidence for a dose-response effect of intrauterine caffeine exposure.

In the final chapter we found evidence for an association between maternal long-chain polyunsaturated fatty acid (LCPUFA) status during early pregnancy and offspring's problem behaviour at age five to six years. Higher maternal concentrations of omega-3 fatty acids DHA and EPA decreased the risk for children's emotional symptoms. A higher omega-6 to omega-3 ratio increased the risk for emotional symptoms and hyperactivity/inattention problems. No evidence was found for mediation by preterm birth and being small for gestational age. The child's sex and infant feeding did not modify these associations.

The aforementioned results provide evidence for developmental reprogramming of children's birth outcomes, cognitive functioning and behaviour by maternal negative emotions and fatty acid status. Despite the fact that effect sizes in our studies were modest they are of significance for a number of reasons. First, it is known that adverse birth outcomes (chapter 3), are associated with an increased risk of negative physical as well as mental health outcomes in later life. Low birth weight has been identified as a risk factor for developing hypertension, insulin resistance, type 2 diabetes and cardiovascular disease in later life (Barker et al., 1993; Rich-Edwards et al., 1997). In addition, children that were born preterm or with a (very) low birth weight more often have emotional and behavioural regulation problems (Clark, Woodward, Horwood, & Moor, 2008; Spittle et al., 2009). Second, even a minor impairment in information processing (chapter 4) could have a substantial impact on a child's functioning in daily life, primarily in the school situation (Biederman et al., 2004) where attentional demands are high. In addition, children that display problematic behaviour (chapter 5 and 7) such as hyperactivity and attention problems are socially less competent (Abikoff et

al., 2004), more likely to be rejected by their peers (Bagwell, Molina, Pelham, & Hoza, 2001) and more often achieve poorly at school (DeShazo Barry, Lyman, & Klinger, 2002).

The child's sex as a moderator?

Previous studies have suggested sex differences in the developmental reprogramming effects of early life influences in a wide range of developmental domains such as cognitive functioning and problem behaviour (Sandman & Davis, 2012). Our results have only provided evidence for a moderating role of the child's sex in the association between maternal anxiety during pregnancy and children's cognitive functioning and behaviour. No evidence for sex differences in children's behaviour were found that could be ascribed to the influence of prenatal caffeine intake and fatty acid concentrations. Although both male and female offspring are presumed to be at risk for developmental reprogramming by early life influences (Coe, Lulbach, & Schneider, 2002), our findings with regard to prenatal anxiety suggested a heightened vulnerability in boys. Boys that were born to highly anxious mothers performed less stable on the simple reaction time task, and showed more overall problem behaviour and hyperactivity/inattention problems compared to girls. These findings corroborate results from previous studies that have found alterations in cognitive functioning (Van den Bergh et al., 2005a; Van den Bergh et al., 2006) and an increased risk for hyperactivity/inattention problems (O'Connor, Heron, Golding, Beveridge, & Glover, 2002; Rodriguez & Bohlin, 2005) in boys that were born to highly anxious mothers. However, our findings did not show an increased risk for emotional symptoms in girls born to mothers who reported higher levels of anxiety during pregnancy. This is not in accordance with a previous study that showed higher levels of maternal anxiety during pregnancy to be related to offspring's HPA-axis functioning (altered cortisol day-time profile) in both sexes, which was associated with self-reported depressive symptoms in adolescent girls only (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). It is possible that mothers and teachers in our study found it more difficult to observe emotional symptoms as opposed to externalizing behaviour problems in the children, which is a common shortcoming in behavioural reports especially in young children (Clarke-Stewart, Allhusen, McDowell, Thelen, & Call, 2003).

Multiple informants

In comparison with previous studies that were predominantly based on maternal ratings of child behaviour (e.g. Bekkhus, Skjothaug, Nordhagen, & Borge, 2010; Chiu, Gau, Tsai, Soong, & Shang, 2009; Gale et al., 2008; Hibbeln et al., 2007; Kohlboeck et al., 2011; Krabbendam, Bakker, Hornstra, & van Os, 2007; O'Connor et al., 2002), results presented in this dissertation provide a more objective picture because the child's primary school teacher rated the child's behaviour as well. Cross-informant agreement on child behaviour in our studies was modest which is in line with reports on parent-teacher agreement in the literature (Mieloo et al., 2012), and could be ascribed to inherent differences in experiences that mothers and teachers share with the children, at home or at school (Achenbach, McConaughy & Howell, 1987).

In chapter 5, results based on both mother as well as teacher ratings provide evidence for developmental reprogramming of children's behaviour due to maternal anxiety during pregnancy, however associations were most profound when mothers had reported on their child's behaviour. Despite the fact that, in chapter 6, a composite score based on maternal and teacher ratings is presented, preliminary (unpublished) analyses showed a consistent pattern of no associations between maternal caffeine intake during pregnancy and children's problem behaviour when rated by mothers and teachers separately. This consensus strengthens our finding that intrauterine caffeine exposure is not associated with children's problem behaviour at age five to six.

Results in chapter 7 indicate that maternal LCPUFA status during pregnancy is associated with children's behavioural problems based on a combination of maternal and teacher reports on child behaviour. Although child behaviour was assessed in different circumstances (home vs. school), it is clear that current findings are in need of replication and further study. Preliminary results from our research group at least provide some concurring evidence suggesting that omega-3 fatty acids DHA and EPA are associated with enhanced information processing at age of five to six (Emmerik, 2012).

Methodological considerations

The current research project is embedded in a large population based multi-ethnic birth cohort; the Amsterdam Born Children and their Development Study (ABCD-study). The main goal of the ABCD-study is to examine a broad range of factors during pregnancy and in early life that are potentially related to the

child's health and development at birth and in later life. A major strength of conducting research within a large birth cohort is the inclusion of high numbers of participants which provides a clear advantage with regard to the statistical power in subsequent analyses. Furthermore, due to the main goal of the ABCD-study, a large variety of data concerning pre-, peri- and postnatal health related factors have been collected that provide the opportunity to statistically control for potentially confounding factors. It is important to note that due to the observational design of the ABCD-study no causal inferences with regard to any of the associations presented in this dissertation can or should be made.

Timing of early life influences

Due to the large scale and non-invasive design of the ABCD-study, the early life influences addressed in this dissertation were assessed at one occasion during gestation, therefore we were unable to examine patterns and chronicity over the course of pregnancy and depict sensitive or critical periods in pregnancy during which the foetus is more sensitive to reprogramming effects of early life influences. The early life influences described in this dissertation were all assessed during the first and second trimester in pregnancy which have been suggested to be important periods with regard to foetal reprogramming due to the ongoing rapid development of organs and structures (de Rooij, Wouters, Yonker, Painter, & Roseboom, 2010; Georgieff, 2007; Godfrey & Barker, 2001; Innis, 2007; Rodriguez & Bohlin, 2005; Van den Bergh & Marcoen, 2004; Van den Bergh et al., 2008). Others have found influences in late gestation to be most influential (O'Connor et al., 2002). These differences in susceptibility to reprogramming effects of early life influences over the course of pregnancy that are reflected in various adverse long-term outcomes, may be indicative of different underlying mechanisms of developmental reprogramming that operate across gestation (Van den Bergh, Mulder, Mennes, & Glover, 2005b). Previous research has revealed that the experience of emotional distress is higher in early and late pregnancy and somewhat lower in mid pregnancy (Jomeen, 2004; Lubin, Gardner, & Roth, 1975). Intake levels of caffeine (CARE study group, 2008) remain fairly stable over the course of pregnancy. In addition, previous work has indicated that fatty acid status in early pregnancy predicts fatty acid concentrations in late pregnancy quite well (Al et al., 1995). Therefore we do not expect the fact that we were unable to evaluate effects of early life influences over the course of pregnancy to lessen the validity of findings presented in this dissertation.

Assessment of early life influences

Maternal state- anxiety (Spielberger, Gorsuch, & Lushene, 1970) (chapter 4 & 5) and other constructs of emotional distress (chapter 3) were examined by means of widely used questionnaires (Van den Bergh, 1990; Crnic & Greenberg, 1990; Karasek et al., 1998; Radloff, 1977; Spielberger et al., 1970). Although the studies in this dissertation suggest adverse influences of (subclinical) symptoms of emotional distress on children's birth outcomes, behaviour and cognitive performance, it is possible that these reprogramming effects might be more pronounced in pregnant women who suffer from (diagnosed) mood disorders (Alder, Fink, Bitzer, Hösli, & Holzgreve, 2007).

Caffeine intake was measured by self-report, which is considered to be the most valid measure of antenatal caffeine exposure (Grosso, Triche, Benowitz, & Bracken, 2008) by use of the best available estimates of caffeine content from coffee, tea, and soft drinks. Although it is known that caffeine ingestion in pregnant women stems mainly from coffee and tea (Knight, Knight, Mitchell, & Zepp, 2004), individual differences in preparation and portion size may have induced unaccounted variability in estimated caffeine content (Bracken et al., 2002). Furthermore, no information on caffeine intake via medication was obtained, which could have lead to an underestimation of caffeine exposure in some women.

A major strength of the study presented in chapter 7 was that plasma phospholipid fatty acid concentrations during early gestation were obtained instead of estimates of fatty acid status represented by for example maternal fish consumption, or LCPUFA status in cord blood at birth that were used in previous studies (Gale et al., 2008; Hibbeln et al., 2007). In addition, the potential influence of a higher omega-6 to omega-3 ratio was examined, which clearly adds to the existing literature because it is a characteristic of modern Western dietary habits (Gibson, Muhlhausler, & Makrides, 2011).

Children's cognitive functioning and behaviour

One of the aims and a strong point of the research presented in the current dissertation is the fact that children's neurocognitive functioning was examined using two sensitive, objective, computerized reaction time tasks that have been found suitable for the assessment of (pre)school aged children (De Sonneville, Visser, & Licht, 1999). In order to reduce the burden for parents and children and hence enhance participation, children were tested during school days, with

limited possibilities for fully standardised test circumstances, which for some children may have resulted in less optimal test circumstances. For example in some schools the testing room was situated at a location near the school's playground resulting in more noise during the assessments, which may have interfered with the children's performance. Furthermore, although children were tested predominantly in the morning, for some children testing took place in the early afternoon when they might have felt tired from a morning at school. Unfortunately the exact time at which children were assessed was not recorded; hence we could not examine time-of-day effects on children's performance in our study. Nevertheless, we do not believe that this variation in the time at which children were assessed poses a threat to the validity of our findings, as previous work revealed no time-of-day effects on similar simple reaction time tasks and tasks that required set-shifting and the inhibition of prepotent responses (van der Heijden, De Sonneville, & Althaus, 2010).

Another strength of the current study is that we assessed multiple domains of children's problem behaviour based on ratings by both mother and the child's primary school teacher. In general, the psychometric properties of the Strengths and Difficulties Questionnaire (Goodman, 1997) were found to be satisfactory (van Widenfelt, Goedhart, Treffers, & Goodman, 2003). However, when evaluated in a sample of younger children aged five to six years, validity and reliability of the total difficulties score was found to be adequate, but the subscales were found to be less reliable and hence unsuitable for screening purposes. Nevertheless, the aim of the studies presented in this thesis was merely to identify children at risk for behavioural problems as opposed to screening for psychopathology, and therefore we do not question the validity of our findings based on subscale scores (Mieloo et al., 2012).

Selection bias and non-response

The ABCD-study was cleverly designed to establish the highest possible response rates. For example, pregnant women were invited to participate in the study via their obstetric care providers during routine antenatal care. Furthermore maternal blood samples were collected in conjunction with routine blood collection for screening purposes. Children were examined at their primary schools in order to reduce the burden for parents and children as much as possible. As a result, the response rate of the ABCD-study is adequate in comparison to other national and international birth cohorts (van Eijsden, Vrijkotte, Gemke, & van der Wal, 2011).

Nevertheless, it is important to address selective non-response which might pose a threat to the study's validity. Anonymised record linkage of data from our cohort with national registry data indicated that although selective non-response was present in the ABCD-study, selection bias was acceptably low and did not influence the main study questions related to obstetric outcome (Tromp, van Eijsden, Ravelli, & Bonsel, 2009).

Selection bias due to attrition in the studies in this dissertation was addressed in the discussion sections of the chapters. In short, mothers for whom data on early life influences were available and who rated their child's behaviour were somewhat older, more often employed and more highly educated compared to women who did not participate in the follow-up measurements of their child. Therefore caution is warranted in the interpretation and generalization of the results. However, a recent investigation of selective attrition in a British birth cohort has revealed that the validity of regression models is only marginally affected by selective attrition in large samples (Wolke et al., 2009). Analyses in order to investigate the association between maternal anxiety during pregnancy and children's cognitive functioning (chapter 4) were conducted in a subgroup of 922 women and children from our cohort. Although selection bias might pose a threat to this study's validity, to date no other study has investigated reprogramming effects by prenatal anxiety on children's cognitive functioning at age five to six with objective cognitive tasks in such a large sample of mothers and children. Hence, we believe that the presented associations are adequate representations of the true associations under investigation.

Potential underlying mechanisms and alternative explanations

Our findings, except with regard to the influence of caffeine intake during pregnancy, have provided evidence for developmental reprogramming effects of exposure to maternal negative emotions and fatty acid status during pregnancy on offspring's birth outcomes, cognitive functioning and behaviour. Although research in animals has identified potential underlying mechanisms through which early life influences may reprogram offspring's neurodevelopment, in humans these mediating mechanisms remain largely unclear (Schlotz & Phillips, 2009; Van den Bergh, 2011). In the following paragraphs several potential underlying mechanisms and alternative explanations with regard to our findings will be discussed. It is important to note that the proposed underlying mechanisms and alternative explanations for developmental reprogramming do not take place in

isolation, are not mutually exclusive and may even be correlated (Harris & Seckl, 2011; Räikkönen, Seckl, Pesonen, Simons, & Van den Bergh, 2011).

Potential underlying mechanisms

Overexposure to glucocorticoids

Based on studies in animals it is hypothesized that endogenous glucocorticoids play a crucial role in normal foetal development, and more specifically also in foetal brain development (Harris & Seckl, 2011). However, when women experience negative emotions such as anxiety and stress during pregnancy this may amplify activity in their hypothalamic-pituitary-adrenal (HPA) axis (Talge, Neal, & Glover, 2007), which may result in elevated glucocorticoid levels (e.g. cortisol the main hormonal mediator of stress) (Meaney, Szyf, & Seckl, 2007). These maternal glucocorticoids are able to cross the placenta, and indeed there is evidence of a strong correlation between cortisol in the maternal and foetal compartments (Gitau, Fisk, & Glover, 2001; Sarkar, Bergman, Fisk, O'Connor, & Glover, 2007). In the placenta, the 11β-hydroxysteroid dehydrogenase type 2 enzyme (11β-HSD2) converts about 80 to 90 percent of maternal cortisol into inactive cortisone (Watterberg, 2004) which protects the foetus from excessive exposure (Edwards, Benediktsson, Lindsay, & Seckl, 1993). However, several factors, such as the experience of stress (Mairesse et al., 2007), maternal protein deficiency (Langley-Evans, Gardner, & Jackson, 1996), consumption of glycyrrhizin (an elementary component of liquorice) (Räikkönen et al., 2011) and stress induced release of catecholamines in the first trimester of pregnancy (e.g. adrenalin) (Sarkar et al., 2001) down regulate 11β-HSD2 activity which may result in foetal overexposure to glucocorticoids. Moreover, high levels of cortisol and adrenaline caused by maternal stress affect maternal vessel tone which may elicit reductions in the blood flow to the foetus, resulting in a reduced supply of nutrients and oxygen to the foetus. It is hypothesized that these sources of foetal stress elicit an increased release of coricotropin-releasing hormone in the placenta which may also increase levels of cortisol (Huizink, Mulder, & Buitelaar, 2004). During specific sensitive periods in utero this excessive exposure to glucocorticoids might bring on alterations in the homeostasis of the foetal HPA-axis setting with long lasting consequences for its future neurodevelopment (for reviews see: Räikkönen, et al., 2011; Van den Bergh, Mulder, Mennes, & Glover, 2005b; Van den Bergh, 2011; Weinstock, 2008).

Caffeine as a neurodevelopmental modulator

Results in our study did not suggest developmental reprogramming effects of maternal caffeine intake during pregnancy on offspring's problem behaviour measured with questionnaires at age five to six. Nevertheless, it remains possible that excessive exposure to caffeine in utero affects children's long-term neurodevelopmental outcome, but that more sensitive neurocognitive assessment techniques are needed to detect such associations. Hence, caffeine might act as a subtle modifying factor which is soluble in fat (Brent, Christian, & Diener, 2011), known to cross the placenta (Tanaka, Nakazawa, & Arima, 1983) and foetal blood-brain barrier (Mose et al., 2008). Intrauterine exposure to caffeine may lead to vasoconstriction and hypoxia in the foetoplacental arteries which may alter foetal growth (Kirkinen, Jouppila, Koivula, Vuori, & Puukka, 1983) and reduce foetal cerebral weight and protein content (Tanaka, et al., 1983). Caffeine may also affect neuronal growth and neuronal interconnections during gestation and the neonatal period (Brent et al., 2011).

Long-chain polyunsaturated fatty acids and brain development

Essential fatty acids such as linoleic acid (LA) and alpha-linolenic acid (ALA) are found as structural elements in all mammalian cell membranes (Al, van Houwelingen, & Hornstra, 2000). Their long-chain polyunsaturated derivatives (LCPUFA) eicosapentaenoic acid (EPA; omega-3), docosahexaenoic acid (DHA; omega-3) and arachidonic acid (AA; omega-6) play an important role in neurodevelopmental processes such as neurogenesis, cell proliferation, synaptogenesis, membrane functioning and potentially myelination (Georgieff, 2007). Although a foetus is able to synthesize LCPUFA's to a certain extent, it is largely dependent on supply from its mother via placental transfer (Campbell, Gordon, & Dutta-Roy, 1996). Hence, deficiencies or an imbalance in maternal LCPUFA status, during gestation may alter foetal brain development and affect subsequent long-term cognitive and behavioural outcomes in the offspring.

Epigenetic reprogramming

Early life influences may also reprogram offspring's neurodevelopment by means of eliciting alterations in foetal chromatin structure through DNA methylation, histone modification and RNA interference. These alterations in foetal chromatin structure may induce modifications in gene expression that result in phenotypic changes, without changing the actual DNA sequence, a

process called epigenetic reprogramming (Van den Bergh, 2011). Few human studies have investigated epigenetic modifications due to prenatal influences and their long-term consequences in humans. Oberlander et al. (2008) have found a link between maternal depression in the third trimester and methylation of the NR3C1 gene 1_F promoter in DNA from human cord blood. In the same study, a higher methylation status of the NR3C1 gene was related to an altered stress response reactivity when infants were three months old. Recent work by Hompes et al. (in press) showed that maternal emotional state and cortisol levels during pregnancy are associated with the methylation state at different loci of the NR3C1 gene, analyzed in genomic DNA from cord blood. Together these studies provide evidence for epigenetic reprogramming due to early life influences. Hence it is possible that the early life influences addressed in this dissertation have elicited such epigenetic changes which resulted in impaired cognitive performance and behavioural problems in the offspring.

Possible alternative explanations for adverse neurodevelopmental outcomes

Genomic based inheritance

In addition to factors that exert their reprogramming influence during the period of foetal development, genetic predispositions cannot be ruled out. Although genetic effects in terms of genomic based inheritance could never be solely accountable for the origin of behavioural problems and cognitive impairments (Meaney, 2010), the increased risk for problem behaviour and altered cognitive functioning we have found can without doubt partially be ascribed to a genetic predisposition. Results from a recent study conducted with a research design which separated maternally provided inherited factors from prenatal influences, suggested that associations between prenatal stress and certain offspring outcomes do indeed arise from inherited factors. The link between prenatal stress and offspring's attention deficit hyperactivity disorder was only present in biologically related mother-offspring pairs and therefore seemed to be attributable to inherited factors (Rice et al., 2010). Hence, when interpreting findings from the current thesis, it is important to take into account that the alterations in cognitive functioning and behavioural problems in the children can be partially ascribed to a genetic predisposition inherited from their parents.

Other micronutrients

Although it is known that a wide spectrum of micronutrients during pregnancy may have long-lasting effects on offspring's physical health (Godfrey & Barker, 2001), as well as cognitive functioning and behaviour (Monk, Georgieff, & Osterholm, 2013; Schlotz & Phillips, 2009), in the current study we did not take into account the potential influence of other micronutrients. Despite the fact that long-chain polyunsaturated fatty acids are essential components in brain development, it is possible that the alterations in cognitive functioning and increased risk for problem behaviour we found could be attributed to deficiencies in other nutrients relevant to brain development, such as iron, zinc, selenium, iodine, folate and vitamin A (Georgieff, 2007; Monk et al., 2013).

Maternal infections during gestation

Foetal exposure to maternal bacterial or viral infections during pregnancy, as a result of stress induced immune dysregulation (Coussons-Read, Okun, & Nettles, 2007), could be an alternative explanation for the increased prevalence in problem behaviour and altered cognitive functioning in our studies. Prospective observational studies in humans have found evidence for an association between maternal prenatal infection and enhanced risk of neurodevelopmental brain disorders such as schizophrenia (Brown & Susser, 2008). It is proposed that foetal exposure to pro-inflammatory cytokines and other inflammation markers may alter normal foetal brain development with potential long-term consequences (Feldon & Meyer, 2012).

Directions for future research

The results presented in the current dissertation have replicated previous findings as well as extended current knowledge concerning the contribution of three early life influences on the origin of offspring's birth outcomes, alterations in cognitive functioning and problem behaviour. Nevertheless, much more remains unclear and therefore our work also provides an impetus for future studies. In order to substantially extend and improve current knowledge about the effects of early life influences on long-term neurocognitive and behavioural outcomes, we argue for a distinction between the longitudinal follow-up of large birth cohorts and small-scale studies in both humans and animals aimed at unravelling potential underlying mechanisms.

First, research in prospective, large, population based, multi-ethnic birth cohorts should be aimed at identifying subpopulations at heightened risk for developmental reprogramming effects of early life influences. It is known that long-term consequences of reprogramming by early life influences depend on their interaction with an individuals' exposure to environmental adversities in later life. Ideally studies are designed to investigate the influence of multiple early life influences that occur in the context of one another, and are highly prevalent in the general population (e.g. prenatal stress and malnutrition). In addition, these studies should attempt to follow-up their participants and their living conditions well into adulthood in order to investigate whether reprogramming effects persist or disappear at older age in specific contexts. Findings from large cohort studies are fairly generalizable to the general population and should therefore be aimed at examining early life influences that are relevant to public health professionals and policy makers in order to enhance public health. On the other hand, cohort studies need to be designed cost efficiently and hence some concessions with regard to the measurement of predictors and outcomes are inevitable. At least, only validated questionnaires filled out by multiple informants (e.g. self-report, parental report) with optimal psychometric properties should be administered. These data should ideally be completed with information on clinical diagnoses from medical records.

Second, in addition to large cohort studies, there is a clear need for studies in both animals and humans that are aimed at examining underlying mechanisms of developmental reprogramming by early life influences. Sex differences in developmental reprogramming by means of early life influences need to be investigated thoroughly in future studies as well. In addition, biological markers of early life influences such as stress hormones, immune parameters, micronutrient concentrations, and physiological measures of the autonomic nervous system should be assessed at multiple time points across gestation, in order to detect specific sensitive periods and the effects of chronic exposure on long-term neurodevelopmental outcomes. Furthermore, especially studies in humans that address gene-environment interactions of early life influences during pregnancy and epigenetic studies are warranted in order to reveal mechanisms that lead to adverse long-term neurodevelopmental outcomes. Furthermore, research is warranted in clinical populations in order to determine whether reprogramming effects of for example maternal negative emotions are stronger or more prevalent in women with a diagnosed mood disorder. At the same time, interventions

targeted at coping with these negative emotions in order to prevent adverse long-term neurodevelopmental outcomes should be tested in these clinical samples, with randomized controlled trials (e.g. Bogaerts et al., 2012).

Because agreement concerning children's behaviour problems among informants (e.g. mothers and teachers) seems to be moderate, it is difficult to reveal developmental reprogramming effects by early life influences on children's neurodevelopmental outcomes based on these assessments. Therefore we would like to emphasize the need for more studies that evaluate children's neurodevelopmental outcomes by means of sensitive objective assessment tools. Neurocognitive functioning and information processing should be measured with objective cognitive tasks and event related potentials which has been done in a few studies (e.g. Mennes, Van den Bergh, Lagae, & Stiers, 2009; Otte, Braeken, & Van den Bergh, 2011). In order to investigate which brain areas are affected by early life influences years later, functional magnetic resonance imaging (fMRI) that measures activity related changes in blood flow (e.g. Buss, Davis, Muftuler, Head, & Sandman, 2010; Mennes, 2008), or the less invasive near-infrared spectroscopy (NIRS) which detects changes in blood haemoglobin concentrations associated with neural activity could be used.

Implications for public health policy

Although the effect sizes of long-term associations of maternal negative emotions and fatty acid status on children's cognitive functioning and behaviour in the studies presented in this dissertation are modest, as a consequence of their relatively high prevalence in the study population as well as in the general population (Andersson et al., 2003; Frary, Johnson, & Wang, 2005; Heron, O'Connor, Evans, Golding, & Glover, 2004; Kaiser, 2008; Ross & McLean, 2006; Yali & Lobel, 1999), they might adversely affect a relatively large group of pregnant women and their children. Therefore, and despite the fact that the exact underlying mechanisms remain unclear, our findings also have implications for public health practice. First, our results plea for creating increased awareness with regard to maintaining a healthy lifestyle before and during pregnancy in women in the childbearing age. Preconception counselling offered to women in childbearing age could be a first step to reduce the prevalence of risk factors for adverse pregnancy outcomes (Van Der Pal-de Bruin et al., 2008), as it is of crucial importance that pregnant women are aware of and acknowledge the potential

influence their health and lifestyle during pregnancy have on their children's long-term physical and mental health.

In addition to creating awareness, tailored screening and interventions may be warranted to improve maternal health and well being during pregnancy, delivery and the postpartum in order to prevent adverse outcomes in terms of offspring's long-term health and development. Given the high prevalence of negative emotions (almost 30%) in our cohort and in the general population, we argue for the implementation of a short standardized screening tool for symptoms of anxiety and depression during routine prenatal care. This would help obstetric care providers to identify women at risk and decide whether to refer them to an expert for counselling, or cognitive-behavioural therapy in order to reduce their distress (Facchinetti, Tarabusi, & Volpe, 2004). Moreover, the experience of psychosocial problems often does not occur in isolation (Joseph et al., 2009), and many pregnant women who suffer from emotional distress also tend to keep smoking (Rodriguez, Bohlin, & Lindmark, 2000), eat less healthy, and experience a burden of other socioeconomic disadvantages (Misra, Guyer, & Allston, 2003). Therefore deliberate screening for emotional distress might also signal or identify women at risk for other disadvantages.

Although we did not find evidence for an association between maternal caffeine intake and children's problem behaviour measured with questionnaires, we would like to emphasize that these results need to be replicated in future studies, with more sensitive objective cognitive tasks. Until then we would recommend pregnant women to follow their governments' or obstetric care providers' advice with regard to caffeine intake, as it has been related to adverse birth outcomes such as foetal growth retardation (Vik, Bakketeig, Trygg, Lund-Larsen, & Jacobsen, 2003). Currently, in the Netherlands pregnant women are recommended to restrict their daily dietary caffeine intake substantially during pregnancy. The Netherlands Nutrition Centre (i.e. Voedingscentrum) recommends pregnant women to limit their caffeine intake to 100mg per day which is roughly equivalent to one cup of coffee per day.

Current results based on maternal ratings of children's behaviour suggest that omega-3 fatty acids (DHA and EPA) protect children from developing problem behaviour, whereas a higher omega-6 to omega-3 ratio seems to increase the risk for problem behaviour. We argue that more research needs to be conducted preferably using sensitive, objective cognitive tasks, to see whether our findings are robust. The question whether or not to advise pregnant women to take fatty

acid supplements remains difficult to answer based on our results. However, previous randomized controlled trials that examined the effects of omega-3 (DHA) supplementation during pregnancy on offspring's visual acuity (Smithers, Gibson, & Makrides, 2011), cognitive and language development (Makrides et al., 2010), did not find evidence for a beneficial influence. Moreover, first more research needs to be conducted in order to rule out potential harmful effects of LCPUFA's such as their antithrombotic and immune suppressive actions (Rogers, Valentine, & Keim, 2013). For now, with regard to our findings and because deficiencies or an imbalance in micronutrient status (e.g. fatty acids) are not uncommon in Western modernized countries (Cetin, Berti, & Calabrese, 2010), we would argue for the incorporation of dietary advice as a part of routine prenatal care. At least until more is known about the optimal composition and dosage of fatty acid supplements to support routine supplementation (Rogers et al., 2013).

General conclusion

The aetiology of children's neurocognitive functioning and behaviour is particularly complex and exceptionally difficult to unravel. The studies presented in this dissertation contribute to this challenge at least to a small extent by emphasizing the importance of experiences and exposures in the prenatal period for later neurodevelopmental outcomes. Children of women who were very anxious or felt depressed during pregnancy not only seemed to have a more difficult start in life reflected in more adverse birth outcomes, they also seemed to have more behavioural difficulties and less optimal cognitive performance at age five to six. Interestingly, boys were more susceptible to the influence of maternal negative emotions during pregnancy compared to girls. In addition to these psychosocial factors, omega-3 fatty acids seemed to protect children from developing problem behaviour, whereas a higher omega-6 to omega-3 ratio seemed to increase the risk for problem behaviour. In contrast, caffeine intake during pregnancy did not affect children's behaviour at the age of five to six. Our findings have several implications for public health practice. Preconception counselling that increases awareness concerning the influence of women's perinatal health and lifestyle on children's physical and mental health in later life should be offered to women in the childbearing age. In addition, dietary advice and a short screening for psychosocial distress should be incorporated in routine prenatal care. Future research needs to study highly prevalent early life influences that exert their influence in the context of one another, unravel underlying mechanisms

and strive to assess neurodevelopmental outcomes using sensitive, objective neurocognitive tasks and neurophysiological measures.

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Summary

In *chapter 1* we introduced the idea that early life influences such as mothers' emotional state and dietary habits during pregnancy may reflect upon the developing foetus while in the womb. Developmental programming by means of early environmental cues plays a fundamental role in typical development, therefore we have deliberately termed alterations in the typical developmental trajectory due to adverse environmental influences developmental *re*programming. Studies in animals and humans have indeed suggested that early foetal experiences have consequences for its health and development at birth and in later life. Moreover, during pregnancy and in the first years of life critical stages in brain development occur, therefore the brain has been suggested to be particularly susceptible to potential reprogramming effects by early life influences during these periods. Accordingly, previous research has found adverse effects of early life influences on offspring's long-term cognitive functioning and behaviour. However, large prospective studies that used sensitive, objective tasks to examine children's cognitive functioning and included multiple informants to report on children's behaviour at the age of five to six were lacking.

Therefore, the aim of the current thesis was to investigate developmental reprogramming effects of three prenatal influences on birth outcomes, neurocognitive functioning and behaviour in five to six year old children with specific attention for sex differences. We selected three early life influences that are highly prevalent in pregnant women; the experience of negative emotions, caffeine intake and an imbalanced fatty acid status. This led to the formulation of four research questions that were addressed in the chapters of this dissertation.

1. Are psychosocial stress and negative emotions during pregnancy related to adverse birth outcomes? (Chapter 3)
2. Is there an association between maternal anxiety during pregnancy and children's neurocognitive functioning and behaviour at the age of five to six? (Chapters 4 & 5)
3. Is there an association between caffeine intake during pregnancy and the risk of problem behaviour at the age of five to six? (Chapter 6)
4. Is there an association between maternal long-chain polyunsaturated fatty acid status during early pregnancy and children's risk of problem behaviour at the age of five to six? (Chapter 7)

In *chapter 2* the procedures and methods with regard to the data collection were described. Studies in this dissertation are embedded in a large community based multi-ethnic birth cohort situated in Amsterdam; the Amsterdam Born Children and their Development Study (ABCD-study). The main goal of the ABCD-study is to examine a broad range of factors during pregnancy and in early life that are potentially related to the child's health and development at birth and in later life. Pregnant women received a questionnaire covering sociodemographic, obstetric and psychosocial conditions at their first visit with an obstetric care provider (N= 12,373 sent; n= 8266 returned). An extra blood sample was taken during routine blood collection for prenatal screening purposes (n= 4389). Pregnancy outcomes were obtained through Youth Health Care of the Public Health Service Amsterdam, and from the Dutch Perinatal Registry. Three months after delivery, mothers received a questionnaire concerning the course of pregnancy and delivery, the health and development of their baby, and questions about their own lifestyle (n= 5131 returned). When the children were five years old, mothers received three questionnaires (n= 6161 sent). The first questionnaire covered the child's health, medical conditions, family socio-demographics, and children's problem behaviour (n= 4488 returned). The second was a food frequency questionnaire (n= 2851 returned). The third, which was addressed to the child's teacher concerned school performance and problem behaviour (n= 3588 returned). Furthermore, children were invited for a health check at school (n= 3321) where they took part in cognitive testing (n= 3132).

In *chapter 3*, our first aim was to objectively identify distinct clusters of pregnant women by means of a latent class analysis based on five constructs of psychosocial stress (i.e. anxiety and depressive symptoms, pregnancy-related anxieties, parenting stress and work-related stress). Thereafter, we investigated whether associations with birth outcomes existed between women in different clusters taking potentially confounding factors into account. Interestingly, our results clearly indicated that children of pregnant women who experience both high levels of anxiety as well as depressive symptoms in early gestation (30%), are particularly at risk for lower birth weight and pre-term birth independently of biomedical risk factors, other types of psychosocial stress and maternal characteristics.

Results presented in *chapter 4* suggested that there indeed is an association between maternal anxiety during pregnancy and alterations in children's neurocognitive functioning at the age of five to six. Children of anxious pregnant mothers were more variable in their performance than children of less anxious women in a simple reaction time task. No associations were found between antenatal anxiety and the children's mean reaction time in both the simple and the choice reaction time task. Subsequent analyses in a highly anxious subsample showed that higher levels of antenatal anxiety were more strongly associated with longer reaction times and more intra-individual variability in reaction time in the incompatible part of the choice reaction time task. The child's sex moderated the relation between antenatal anxiety and intra-individual variability in the simple reaction time task. Boys showed more intra-individual variability in reaction time in the simple task, but no significant association was found in girls.

In *chapter 5*, evidence was provided in support of our hypothesis that antenatal anxiety is related to children's problem behaviour. Children of mothers who reported higher levels of anxiety during their pregnancy showed more overall problem behaviour, hyperactivity/inattention problems, emotional symptoms, peer relationship problems, conduct problems and showed less pro-social behaviour when mothers had rated their child's behaviour. When child behaviour was rated by their primary school teacher, maternal anxiety during pregnancy was related to more overall problem behaviour and less pro-social behaviour. We found that the child's sex moderated the relation between antenatal anxiety with overall problem behaviour and hyperactivity/inattention problems in children when reported by their mother. Higher levels of antenatal anxiety were more strongly related to overall problem behaviour in boys than in girls. Furthermore, antenatal anxiety was significantly associated with hyperactivity/inattention problems in boys, while this was not the case in girls.

The main conclusion in *chapter 6* was that maternal dietary caffeine intake during early pregnancy was not associated with a higher risk for hyperactivity/inattention problems, emotional symptoms, conduct problems, peer relationship problems, suboptimal pro-social behaviour, and overall problem behaviour measured with a questionnaire in their five to six year old children. Furthermore, no evidence was found for mediation by foetal growth restriction and gestational age. The

child's sex did not moderate the association between prenatal caffeine intake and children's problem behaviour.

In *chapter 7* we found evidence for long-term developmental reprogramming influences of maternal long-chain poly unsaturated fatty acid (LCPUFA) status during early pregnancy on offspring's problem behaviour at age five to six years. Higher maternal concentrations of omega-3 fatty acids DHA and EPA decreased the risk for children's emotional symptoms. A higher omega-6 to omega-3 ratio increased the risk for emotional symptoms and hyperactivity/inattention problems. No evidence was found for mediation by preterm birth and being small for gestational age. The child's sex and infant feeding did not modify these associations.

Chapter 8 started with a summary of the main findings from the previous chapters followed by a discussion of methodological considerations. Strong points were that our studies were conducted within a large community based multi-ethnic birth cohort, a clear advantage in terms of statistical power and external validity. Furthermore, prenatal fatty acid concentrations were derived from maternal blood, multiple informants reported on child behaviour and we used sensitive objective neurocognitive tasks to measure cognitive functioning. Specific attention was paid to sex differences in the studied associations. However, we were unable to investigate potential underlying mechanisms or depict sensitive or critical periods in pregnancy during which the foetus is more sensitive to reprogramming effects of early life influences. Subsequently, we proposed several potential underlying mechanisms and alternative explanations for our findings such as excessive exposure to glucocorticoids and epigenetic reprogramming.

Based on our results we argued that future research is warranted in two directions. First, we recommended longitudinal research in prospective birth cohorts aimed at identifying subpopulations at risk for developmental reprogramming effects of early life influences that take an individual's postnatal context into account. Second, there is a clear need for studies in both animals and humans that are aimed at examining underlying mechanisms of developmental reprogramming by early life influences. Moreover, biological and physiological markers of early life influences should be measured at multiple time points across gestation, and neurodevelopmental outcomes need to be examined by means of sensitive, objective assessment tools. Specific attention should be

paid to possible sex differences in reprogramming due to early life influences. Furthermore, studies are warranted in clinically diagnosed populations to determine dose-response effects and to test interventions targeted at reducing the risk of exposure to adverse early life influences in order to prevent adverse long-term neurodevelopmental outcomes.

Our findings also have implications for public health policy and practice. First, we argued for creating increased awareness with regard to maintaining a healthy lifestyle among women in the childbearing age. Preconception counselling could be a first step to reduce the prevalence of risk factors for adverse pregnancy outcomes, long-term cognitive impairments and problem behaviour. In addition we recommended a short screening for symptoms of anxiety and depression and dietary advice to be incorporated in routine prenatal care.

In conclusion, the studies presented in this dissertation provide evidence in support of developmental reprogramming influences by maternal negative emotions and fatty acid status during pregnancy on children's perinatal outcomes and cognitive functioning and behaviour in five to six year olds.

Samenvatting

In *hoofdstuk 1* introduceerden we het idee dat invloeden in het vroege leven zoals de gemoedstoestand en de levensstijl van moeder tijdens de zwangerschap van invloed zijn op de foetus in de baarmoeder. Elke foetus is voor zijn ontwikkeling afhankelijk van informatie uit zijn omgeving, maar negatieve omgevingsinvloeden kunnen ervoor zorgen dat belangrijke organen zich anders of minder goed kunnen ontwikkelen, met mogelijk negatieve gevolgen voor de gezondheid en ontwikkeling in het latere leven. Dit laatste proces wordt foetale herprogrammering genoemd, omdat het ontwikkelingstraject wordt afgestemd op informatie uit de omgeving en daardoor als het ware opnieuw geprogrammeerd wordt. Wetenschappelijke studies bij dieren en mensen hebben laten zien dat de gevolgen van herprogrammering van het normale ontwikkelingstraject door negatieve invloeden uit de prenatale omgeving op latere leeftijd nog meetbaar zijn. Bovendien is bekend dat bij mensen tijdens de prenatale levensperiode een heel belangrijk en cruciaal deel van de ontwikkeling van de hersenen plaatsvindt. Daarom lijken de hersenen tijdens deze periode extra gevoelig voor herprogrammering door invloeden uit de omgeving. Dit idee wordt ondersteund door resultaten uit wetenschappelijk onderzoek waaruit bleek dat blootstelling aan bepaalde negatieve omgevingsinvloeden, zoals stress tijdens de prenatale levensfase samenhangt met een minder goede cognitieve- en gedragsontwikkeling bij kinderen. Er is echter nog onvoldoende onderzoek gedaan naar de gevolgen van herprogrammering door vroege invloeden in grote steekproeven, met sensitieve en objectieve maten voor cognitief functioneren en waarin zowel moeders als leerkrachten het gedrag van kinderen beoordeelden.

Het doel van de studies die in dit proefschrift zijn gebundeld was daarom om te onderzoeken wat de invloed van veelvoorkomende omgevingsinvloeden tijdens de zwangerschap is op geboorte-uitkomsten, het cognitief functioneren en de gedragsontwikkeling bij kinderen van vijf tot zes jaar oud waarbij specifiek aandacht werd besteed aan verschillen tussen jongens en meisjes. We onderzochten de invloed van drie vroege factoren die veel voorkomen bij zwangere vrouwen; het ervaren van negatieve emoties, cafeïne inname en de concentratie van omega-3 en omega-6 meervoudig onverzadigde vetzuren in hun bloed. De volgende onderzoeksvragen kwamen aan bod in de hoofdstukken van dit proefschrift:

1. Zijn psychosociale stress en negatieve emoties bij de moeder tijdens de zwangerschap gerelateerd aan geboorte-uitkomsten? (Hoofdstuk 3)
2. Is er een verband tussen angst bij de moeder tijdens de zwangerschap en de cognitieve- en gedragsontwikkeling van haar vijf tot zes jarig kind? (Hoofdstuk 4 & 5)
3. Is er een verband tussen cafeïne inname bij moeder tijdens de zwangerschap en het risico op gedragsproblemen bij haar vijf tot zes jarig kind? (Hoofdstuk 6)
4. Is er een verband tussen de omega-3 en omega-6 meervoudig onverzadigde vetzuurstatus bij de moeder tijdens de zwangerschap en het risico op gedragsproblemen bij haar vijf tot zes jarig kind? (Hoofdstuk 7)

In *hoofdstuk 2* beschreven we de procedures en onderzoeksmethoden die zijn gebruikt om antwoord te kunnen geven op de onderzoeksvragen. De studies in dit proefschrift maken deel uit van een grootschalig, longitudinaal, multi-etnisch geboortecohort; *the Amsterdam Born Children and their Development Study (ABCD-studie)*. Het belangrijkste doel van de ABCD-studie is het onderzoeken van factoren in het vroege leven (tijdens de zwangerschap en op de jonge kinderleeftijd) die een mogelijke verklaring vormen voor latere gezondheid en gezondheidsverschillen. Zwangere vrouwen werd tijdens de eerste prenatale screening bij de verloskundige, gynaecoloog of huisarts gevraagd of ze een vragenlijst wilden invullen (N= 12,373 verstuurd; n= 8266 ontvangen) en of ze extra bloed wilden afstaan voor onderzoek (n= 4389). De vragenlijst bevatte vragen over sociaaldemografische achtergrond, leefstijl, voeding en over psychosociale omstandigheden. Drie maanden na de geboorte van hun kind kregen de vrouwen een vragenlijst thuisgestuurd met vragen over de bevalling, de gezondheid, groei en ontwikkeling van het kind, en over hun leefgewoonten en psychosociale gezondheid tijdens en na de zwangerschap (n= 5131 ontvangen). Zwangerschapsuitkomsten zoals zwangerschapsduur en geboortegewicht, zijn verkregen via Jeugdgezondheidszorg van de Gemeente Amsterdam en Perinatale Registratie Nederland. Toen de kinderen vijf jaar oud waren ontvingen moeders drie vragenlijsten (n= 6161 verstuurd): één vragenlijst met vragen over de gezondheid, ontwikkeling, opvoeding en het gedrag van hun kind (n= 4488 ontvangen), één vragenlijst die vragen bevatte over de eetgewoonten van het kind (n= 2851 ontvangen) en één vragenlijst voor de leerkracht met vragen over

schoolprestaties en het gedrag van het kind op school (n= 3588 ontvangen). Daarnaast werden kinderen op school uitgebreid fysiek onderzocht (n= 3321) en namen zij deel aan cognitieve testen (n= 3132).

In *hoofdstuk 3* is gekeken of het ervaren van psychosociale stress en negatieve emoties tijdens de zwangerschap van invloed is op geboorte-uitkomsten. Eerst zijn met behulp van een clusteranalyse, op basis van vijf psychosociale stress constructen (namelijk angst, depressieve symptomen, zwangerschapsspecifieke angst, opvoedingsstress en werkgerelateerde stress), clusters vrouwen geïdentificeerd met een vergelijkbaar psychosociaal stress profiel. Vervolgens hebben we onderzocht of er verschillen in geboorte-uitkomsten bestaan tussen vrouwen uit verschillende clusters, waarbij rekening is gehouden met andere variabelen die van invloed kunnen zijn op geboorte-uitkomsten. Onze resultaten lieten zien dat kinderen van vrouwen die zich zowel angstig als depressief voelden tijdens hun zwangerschap (30% van onderzoekspopulatie), een verhoogd risico hadden te vroeg, of met een laag geboortegewicht geboren te worden.

Uit de resultaten die zijn gepresenteerd in *hoofdstuk 4* bleek dat er inderdaad een verband is tussen angstgevoelens bij moeder tijdens de zwangerschap en het cognitief functioneren van haar vijf tot zes jaar oude kind. In eerste instantie werd er geen effect van angst gevonden op de reactiesnelheid van de kinderen in een eenvoudige en complexe reactietijd taak. Wel lieten kinderen van angstige moeders minder stabiele prestaties zien in vergelijking met kinderen van minder angstige moeders tijdens de eenvoudige reactietijd taak. Analyses in een subgroep van heel angstige vrouwen toonden aan dat hoe meer angst een moeder had ervaren, hoe sterker het verband was met verminderde reactiesnelheid en stabiliteit bij de kinderen, met name wanneer de complexiteit van de taak toenam. Jongetjes bleken meer kwetsbaar voor de invloed van angst in vergelijking met meisjes, zij lieten ook een minder stabiele prestatie zien in de eenvoudige taakconditie.

Angst bij de moeder tijdens de zwangerschap is ook van invloed op het ontwikkelen van gedragsproblemen bij kinderen zo bleek uit de resultaten in *hoofdstuk 5.* Kinderen van moeders die tijdens de zwangerschap angstig waren, lieten meer algemene gedragsproblemen, hyperactiviteit- en aandachtsproblemen, emotionele symptomen, problemen met leeftijdsgenoten, problemen met gezag

en minder prosociaal gedrag zien, wanneer hun gedrag werd beoordeeld door hun moeder. Wanneer de leerkracht het gedrag van de kinderen beoordeelde was angst bij de moeder tijdens de zwangerschap gerelateerd aan een toename in algemeen probleem gedrag en minder prosociaal gedrag. Het geslacht van het kind was van invloed op de relatie tussen angst bij de moeder tijdens de zwangerschap en algemene gedragsproblemen en hyperactiviteit- en aandachtsproblemen. Het verband tussen angst tijdens de zwangerschap en algemene gedragsproblemen was sterker bij jongetjes dan bij meisjes. Bovendien bleek angst tijdens de zwangerschap wel gerelateerd aan hyperactiviteit- en aandachtsproblemen bij jongetjes, maar niet bij meisjes.

De belangrijkste conclusie uit *hoofdstuk 6* was dat cafeïne inname bij moeder tijdens de zwangerschap niet zorgt voor een verhoogd risico op algemene gedragsproblemen, hyperactiviteit- en aandachtsproblemen, emotionele symptomen, problemen met leeftijdsgenoten, problemen met gezag en minder prosociaal gedrag. Daarnaast vonden we geen aanwijzingen voor mediatie via foetale groeivertraging en zwangerschapsduur. Ook vonden we geen verschil in resultaten tussen jongetjes en meisjes.

In *hoofdstuk 7* vonden we aanwijzingen voor de invloed van meervoudig onverzadigde vetzuren in het bloed van moeder tijdens de zwangerschap op gedragsproblemen bij haar kind. Hogere concentraties van omega-3 vetzuren docosahexaeenzuur (DHA) en eicosapentaeenzuur (EPA) verminderden het risico op emotionele problemen. Een hogere omega-6 /omega-3 ratio bleek daarentegen samen te hangen met een verhoogd risico op emotionele problemen en hyperactiviteit- en aandachtsproblemen. We vonden geen aanwijzigen voor mediatie via vroeggeboorte of foetale groeivertraging. Ook vonden we geen verschillen in de relatie tussen vetzuurstatus tijdens de zwangerschap en probleemgedrag tussen jongetjes en meisjes en tussen kinderen die borstvoeding of flesvoeding kregen.

In *hoofdstuk 8* vatten we de belangrijkste bevindingen uit de voorgaande hoofdstukken samen. Daarna bespraken we methodologisch sterke en zwakke punten van de studies. Het feit dat de studies zijn uitgevoerd in een grote multi-etnische steekproef is een voordeel als het gaat om de externe validiteit van de resultaten. Verder werd de vetzuurstatus van de moeder vastgesteld door

middel van bloedanalyses, werd aan zowel moeder als leerkracht gevraagd om het gedrag van de kinderen te beoordelen en werd het cognitief functioneren van de kinderen onderzocht met objectieve, sensitieve taken. Daarentegen was het gezien onze onderzoeksopzet niet mogelijk om onderliggende mechanismen van herprogrammering door vroege invloeden te onderzoeken en vast te stellen of er specifieke perioden tijdens de zwangerschap zijn waarbinnen de foetus extra gevoelig is voor invloeden uit zijn omgeving.

Op basis van de huidige resultaten beargumenteerden we dat vervolgonderzoek nodig is twee richtingen. Enerzijds is meer onderzoek nodig in grote prospectieve studies, met als doel het identificeren van zwangere vrouwen met een verhoogd risico op negatieve vroege invloeden die schadelijke gevolgen kunnen hebben voor de mentale en fysieke ontwikkeling van haar kind. Daarnaast zijn studies bij zowel mensen als dieren nodig die zich richten op het onderzoeken van de onderliggende mechanismen die een verklaring kunnen vormen voor herprogrammering door vroege invloeden. Daarbij zou speciaal aandacht besteed moeten worden aan mogelijk geslachtsspecifieke effecten. Ook zouden deze studies biologische en fysiologische maten van vroege omgevingsinvloeden moeten onderzoeken op meerdere tijdstippen in de zwangerschap. Daarnaast zouden deze studies gebruik moeten maken van sensitieve en objectieve maten om het cognitief functioneren van kinderen te meten. Onderzoek bij zwangere vrouwen die zijn gediagnosticeerd met een stemmingsstoornis, zou moeten uitwijzen of lange termijn effecten door herprogrammering sterker zijn in deze groepen. Bovendien zouden interventies onderzocht moeten worden die gericht zijn op het terugdringen van negatieve vroege invloeden om schadelijke gevolgen voor kinderen op de lange termijn te voorkomen.

De resultaten van de studies uit dit proefschrift hebben ook implicaties voor de publieke gezondheidszorg. Ten eerste pleiten onze resultaten voor het versterken van het bewustzijn als het gaat om het nastreven van een gezonde levensstijl onder zwangere vrouwen. Het aanbieden van preconceptie zorg zou kunnen bijdragen aan het terugdringen van negatieve vroege invloeden die een risico vormen voor geboorte-uitkomsten en cognitieve- en gedragsontwikkeling op de lange termijn. Daarnaast zouden een dieetadvies en een korte screening voor symptomen van angst en depressie onderdeel moeten gaan uitmaken van het zorgpakket tijdens de zwangerschap.

Concluderend kunnen we stellen dat de studies die zijn gebundeld in dit proefschrift laten zien dat het ervaren van negatieve emoties tijdens de zwangerschap en het hebben van een minder gunstige vetzuurstatus tijdens de zwangerschap samenhangen met minder goede geboorte-uitkomsten, meer gedragsproblemen en minder goede cognitieve ontwikkeling.

Abbreviations

AA	Arachidonic Acid
ABCD	Amsterdam Born Children and their Development
ADHD	Attention Deficit Hyperactivity Disorder
ALA	Alpha Linolenic Acid
ANS	Autonomic Nervous System
ANT	Amsterdam Neuropsychological Tasks
BMI	Body Mass Index
BS	Baseline Speed
CES-D	Centre for Epidemiological Studies Depression Scale
DASS-21	Depression-Anxiety-Stress Scale
DHA	Docosahexaenoic Acid
DOHaD	Developmental Origins of Health and Disease
DOBHaD	Developmental Origins of Behaviour Health and Disease
EPA	Eicosapentaenoic Acid
ERP	Event related potentials
fMRI	functional Magnetic Resonance Imaging
HPA-axis	Hypothalamic Pituitary Adrenal axis
IQ	Intelligence Quotient
JCQ	Job Content Questionnaire
LA	Linoleic Acid
LCPUFA	Long-chain polyunsaturated fatty acids
NIRS	Near-infrared spectroscopy
PDH	Parenting Daily Hassles
PRN	(*Perinatale Registratie Nederland*) Dutch Perinatal Registration
PRAQ	Pregnancy-Related Anxiety Questionnaire
ROO	Response Organization Objects
RT	Reaction Time
SD(RT)	Standard deviation of reaction time
SDQ	Strengths and Difficulties Questionnaire
SGA	Small for Gestational Age
STAI	State-Trait Anxiety Inventory
YHC	Youth Health Care
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2 enzyme

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About the author

Eva Margarita Loomans, daughter of Theo Loomans and Angela Loomans-Szabò, was born on the 12th of November 1983 in Zaanstad. Eva grew up with her younger brother Rob in Hilversum. After graduating in 2001 Eva took a gap year during which she worked as a sailing instructor on Curaçao and travelled through Central America. In 2002 she moved to Groningen where she studied law for one year. In 2003 she started studying psychology and obtained her Bachelor in Psychology in 2007. In August 2008 Eva graduated as a Master of Science in Neuropsychology. In September 2008 she started as a PhD. Student at the department of Developmental Psychology at Tilburg University. In August 2011 Eva became project coordinator for the Amsterdam Born Children and their Development study at the Public Health Service in Amsterdam. In August 2013 Eva started as a researcher at the Department of Epidemiology, Documentation and Health Promotion at the Public Health Service in Amsterdam.

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