The effect of maternal exposure to psychosocial job strain on pregnancy outcomes and child development

Ann Dyreborg Larsen

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Tutor(s): Ane Marie Thulstrup, Karin Sørig Hougaard, Harald Hannerz, Carsten Obel

Official opponents: Jette Kolding Kristensen, Petter Kristensen, Lone Schmidt.

Correspondence: National Research Centre for the Working Environment, Lersø Parkalle 105, 2100, Copenhagen, Denmark.

E-mail: adl@arbejdsmiljoforskning.dk

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The 4 original papers are

1. Larsen AD, Hannerz H, Obel C, Thulstrup AM, Bonde JP, Hougaard KS: Testing the association between psychosocial job strain and adverse birth outcomes - design and methods. BMC Public Health 2011, 11: 255.
2. Larsen AD, Hannerz H, Juhl M, Obel C, Thulstrup AM, Bonde JP, Hougaard KS: Psychosocial job strain and risk of adverse birth outcomes: a study within the Danish national birth cohort. Occup Environ Med 2013, 70: 845-851.
3. Larsen A, Hannerz H, Thulstrup A, Bonde J, Obel C, Hougaard K: Psychosocial job strain and risk of congenital malformations in offspring-a Danish National cohort study. BJOG 2014, 121: 830-839.
4. Larsen AD, Schlunssen V, Christensen BH, Bonde JP, Obel C, Thulstrup AM, Hannerz H, Hougaard KS: Exposure to psychosocial job strain during pregnancy and odds of asthma and atopic dermatitis among 7-year old children - a prospective cohort study. Scand J Work Environ Health 2014 [Epub ahead of print].

INTRODUCTION

In 2008 a group of dedicated researchers in the fields of epidemiology, occupational medicine, reproductive health and animal research received a grant from the Danish Work Environment Research Foundation. The overall aim for the application was to place reproductive health in relation to the working environment on the agenda of Danish research. The work presented in this thesis is part of the MINERVA project, as it was called, and the intention of this thesis is to fill in some of the gaps in the research of prenatal stress exposure from the working environment and health issues related to children.

Psychological stress at work is a rising problem in Denmark. The Danish National Institute of Public Health have in 1987, 1994, 2000, 2005 and 2010 conducted national representative studies of the status and trends in adults' health and morbidity by use of self-administered questionnaires to elucidate factors not available in national registers. In the report from 2005, nearly one third of the women reported that they had difficulties completing their work tasks and 17% found that they had only limited or no influence on their work tasks. The corresponding numbers for 1987 were 18.3% and 16%, respectively [1]. Due to changes in data collection newer numbers from 2010 are not fully comparable [2]. Psychological stress at work is for women estimated to shorten the life expectancy with half a year, and to reduce the number of years without prolonged disease with two years [3]. Further, psychological stress at work is associated with more than 30,000 hospital admissions each year for both women and men, half a million extra days on sick-leave for women, 500,000 inquiries to general practitioners, 1600 early retirements for women, and an overuse of the healthcare system amounting yearly to more than 850 million DKK covering both women and men [3]. Denmark has the second highest employment rate for women in Europe with more than 70% of the women between 15 and 67 years of age working [4]. Furthermore, many of these women are in the child-bearing age and effects of psychological stress at work may extend beyond the exposed individual and affect pregnancy, birth and health of the child.

Job stress has only been studied little relative to pregnancy. Animal studies have shown that maternal exposure to stressful conditions during pregnancy have adverse effects on the offspring for example in terms of low birthweight [5], development of the nervous system and behaviour in the offspring [6]. Epidemiological studies have shown stress exposure in pregnancy not related to work to be associated with various outcomes e.g. preterm birth, low birthweight [7,8] and congenital malformations [9]. But for many outcomes, studies of the effect of job stress give a very mixed results and a diverging picture (e.g. for preterm birth and birthweight related to gestational age, this will be elaborated in the background section), other outcomes have only been studied very narrowly in regards to job stress (e.g. congenital malformations) or not at all (asthma and atopic dermatitis).

To discuss effects of maternal emotional state is not a new thing, as this quote from a USA public health education poster from 1919 shows: "Don't listen to "Old Wives Tales". No shock can "mark" an unborn baby. No horrible sight can deform him. But Worry, Fear and Anger may affect his mother's blood, which supplies his food. Therefore, She should be Calm, Happy and Sweet-tempered"
(USA public health education poster, 1919)[10].

AIMS OF THIS THESIS

This thesis has two main aims: One; to investigate the role of psychosocial stress at work in relation to birth outcomes and health of the child based on data from the Danish National Birth Cohort (DNBC) and two; to follow Guidelines for Good Epidemiology Practices for Occupational and Environmental Epidemiologic Research [11]. This includes as much transparency as possible, e.g. for each study an extensive protocol should be written, approved by all authors and followed thoroughly when doing the studies and writing the related articles.

The specific aims for the three studies were:
To investigate the association between maternal psychosocial job strain during pregnancy, measured as high demands, and low control and the risk of:

- Having a child born preterm or with low or high birth weight relative to gestational week (paper I + II)
- Congenital malformations in offspring (paper III)
- Asthma and atopic dermatitis in the children (paper IV)

BACKGROUND

Prenatal exposure to stress – naturally and experimentally.

"Natural experiments" with prenatal stress exposure involving natural disasters and terror show effects in the unborn child. Studies on the Canadian ice storm, which knocked out electricity for 45 days showed pregnant women who were affected by the storm had children with lower birthweight, length and head circumference [12] and impaired cognitive and language abilities at 5½ years of age [13]. Studies from the Chernobyl disaster showed exposure to maternal stress was associated with changes in cortisol and testosterone levels in the children, independently of the exposure to biohazards [14]. Timing of stress in pregnancy seems also to be of relevance as pregnant women exposed to stress in relation to the Northridge earthquake in California showed a shorter gestational age at delivery, but only if the women were exposed during the first trimester [15]. Further, women living close to the World Trade Center around the time of the 9/11 attack, showed a slightly shorter length of gestation, but again only for women exposed in their first trimester [16].

Very few experiments with humans have been carried out within this research field for ethical reasons. Monk and colleagues have conducted one, in which pregnant women were asked to carry out a stressful assignment on the computer. Simultaneous with the computer work, both the women's heart rate and the foetal heart rate were monitored. The study showed that the foetal heart rate went up during the assignment, but only in the cases where the woman rated herself as anxious at the same time [17]. So even without knowing of or studying the mechanism of psychosocial stress when pregnant, results points in the direction of the mother's emotional state affecting the unborn child.

Hypothesis of prenatal stress mechanisms

How can the unborn child be affected by its mother's emotional state? Early suggestions went on a decrease in the blood flow via the placenta to the foetus [18], but other studies have found it difficult to replicate these findings [19]. Some studies have shown that psychosocial stress during pregnancy is associated with changes in the diurnal pattern or altered functions of the hypothalamic-pituitary-adrenal axis (the HPA axis) [20] and it is known that glucocorticoids have a range of effects on the developing foetus [21]. The problem with accepting this as the full explanation for the effects of prenatal stress, is as the pregnancy progress, the maternal HPA axis becomes gradually less responsive to stress [22] and only a weak association between maternal emotional state and the woman's cortisol level are seen in the pregnancy [23]. Furthermore, the placenta inactivates a significant percentage of maternal glucocorticoids via 11β-hydroxysteroid dehydrogenase type 2 (11-βHSD2) during transfer to the foetus [24]. Cortisol could still play a role in the foetal programming as exposure to psychosocial stress during pregnancy may cause increased transplacental transfer of maternal cortisol to the foetal compartment or induce alterations in the rate of activation of the barrier enzyme (11β-HSD2) [25]. Studies in rats show a down-regulation of placental 11β-HSD2, when the dams are restrained in the last week of pregnancy [5], and similar results are found in human studies of maternal prenatal anxiety and depression [26]. Other mediators e.g. inflammatory markers have been found to be elevated in studies of psychosocial stress in pregnancy [27], but we still do not know how they influence the function of the placenta and foetal development in general. Work from Bonnin et al. show that increased serotonin exposure in pregnancy was associated with changes in neuronal processes in the child, which can lead to changes in the behaviour of the offspring [28]. They identified an endogenous serotonin biosynthetic pathway in the human placenta, indicative of foetal programming through alterations in the placental serotonin.

Epigenetic changes – meaning changes to the structure of DNA (e.g. adding of a methyl group) – have also been suggested to play a role in the relationship between maternal stress during pregnancy and effects in the children. Studies in mice have shown that exposure to stress during pregnancy causes epigenetic changes in the DNA coding for the receptor that are involved in the binding of cortisol [29]. This is supported by a study in humans where the methylation status of the glucocorticoid receptor gene of adolescent children was influenced by their mother's experience of psychosocial stress, in this case violence from her partner, during pregnancy [30].

From "stress" to "job strain"

Stress; job strain, adverse life events, daily hassles, distress etc. There are no clear consensus regarding the definition and use of the word stress and often it covers several aspects of this condition or state – that is as the stress stimuli, the experience of stress, the general response to stress and the experience of the response to stress.

In this thesis, stress is defined as job strain for several reasons: First we wanted to focus on work settings in relation to stress. Stress expiring from work settings must be presumed preventable opposite some life events as loss of close relatives, which also may affect the unborn child, but is hard to prevent. The focus on work stress therefore has both practical and interventional use and supplements the rather limited knowledge about psychosocial stress at work and effect on the offspring. Secondly, we wanted to use the Danish National Birth Cohort (DNBC), as it is one of the largest birth cohorts in the world with adequate info on maternal lifestyle. The questions reflecting job strain were the data made available for us by the DNBC, when we

wished to look at psychosocial stress at work while pregnant. Thirdly, the job strain model is the most used and cited model on work, stress and disease [31], making the results from the studies somewhat comparable.

The job strain model was suggested by Robert Karasek in 1979 [32]. He wanted to elucidate the problems with monotonous work at assembly lines and the implications for health primarily in regards to cardiovascular diseases. The model operates with two dimensions; job demands and job control. The situation with high demands and low control is defined as high strain. This is the stressful quadrant associated with higher risk of diseases. The quadrant with high demands and high control are referred to as the active quadrant and is in the theory not associated with disease, but with motivation and learning. The quadrant with low demands and low control are defined as the passive quadrant and the quadrant with low demands and high control as the low strain quadrant, the latter often being the one working as a reference, as it is presumed to be a healthy quadrant [32-34]. A presentation of the four quadrants as used in this study can be seen in Figure 3 in a later section. The theory and model have previously been used in the DNBC studies of pelvic pain and late foetal loss [35,36].

OUTCOMES

For the studies in this thesis, several outcomes were selected when studying the effects of exposure to job strain during pregnancy;

Preterm birth

Preterm birth is normally defined as a delivery occurring before 37 completed gestational weeks [37]. In this thesis being born preterm is defined as a delivery after the 22nd and up to 36th completed week of gestation. Pregnancies that terminated before 22 completed gestational weeks were excluded as these per definition count as miscarriages in Denmark [38]. Preterm birth accounts for approximately 5% of all births in Denmark [39] and is strongly associated with perinatal and neonatal mortality and morbidity [40]. Babies born preterm are at a higher risk for chronic pulmonary disease [41], cerebral palsy [42] and other neurological disorders [43-44]. The overall proportion of preterm deliveries seems to be increasing in both Denmark [45] and the USA [46]. A number of lifestyle factors have been associated with increased risk of preterm birth or miscarriages e.g. alcohol [47,48], physical exercise [49], maternal overweight and obesity [50] etc.

To maintain a normal pregnancy a balance between the immune, the endocrine and the nervous systems are needed. It is hypothesized that a disruption of this balance increases the risk of adverse pregnancy and birth outcomes [51]. This is supported by studies on viral or bacterial infections during pregnancy showing elevated risk of preterm deliveries [52,53]. When exposed to psychosocial stress elevated cortisol levels are seen. During pregnancy corticotropin-releasing hormone (CRH), which also serves as a signal for initiation of labour, is released into maternal and foetal circulation from the placenta. Cortisol increases the excretion of CRH, and psychosocial stress has therefore been suggested to induce labour prior to term, with premature birth as the result [54].

Studies of prenatal exposure to stress, unrelated to work, observe a higher relative number of preterm deliveries if the mother is exposed to even moderate psychosocial loads [55-59]. When focusing on an adverse psychosocial work environment and preterm birth the results are a bit more diverging in conclusions even though some do support the findings from generalized stress. Literature search on ((“job stress” OR “work stress” OR “occupational stress” OR “job strain” OR “psychosocial working conditions” OR “work-related psychosocial stress”) AND (“preterm birth” OR “preterm delivery”), NOT “physical stress”) was conducted, resulting in four prospective studies, five case-control studies and two cross-sectional. Due to the limited number of studies, all studies are included in this review of current literature on job stress and risk of preterm birth. A presentation of the studies can be seen in table 1.

All four prospective studies showed no association with job strain on preterm birth [60-63], but the Korean study found higher levels of effort-reward imbalance associated with lower gestational age at birth [63]. Two case-control studies found higher risk of having a preterm birth when exposed to high job strain, but only for black women [64], or combined with low or moderate support [65]. The findings were not supported in a Danish case-control study on job strain [66], neither was it for mentally demanding jobs [67] or on self-perceived work stress [68]. The two cross-sectional studies revealed in the literature search both found an association between job stress and risk of preterm birth; one found job titles grouped in relation to job strain to be associated with preterm delivery [69], the other that job strain was related with preterm birth, but only for women not wanting to stay in the work force [70].

Table 1. Overview of studies on work related stress and risk of preterm delivery (PTD)

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Henriksen 1994 [61]	Denmark	3503 singleton pregnancies from Aarhus University Hospital.	Self-administered questionnaire. Job demands, job control, dichotomized at median score and combine to four exposure categories.	Questionnaire at week 16 of gestation for women working more than 30 hours a week	PTD <37 weeks of gestation	No statistical significant association between high job strain and PTD.
Lee 2011 [63]	Korea	310 mother-infant pairs were drawn from the Mothers and Childrens Environmental Health study, recruited at the first trimester of pregnancy	Job strain was measured using Karasek's demand-control questionnaire in a Korean version. Also questions regarding the effort-reward-imbalance model (ERI) was included	Standard questionnaires administered at 20 or fewer weeks of pregnancy	Gestational age obtained from medical records.	The ERI-ratio was found to be inversely related to gestational age at birth
Loomans 2012 [60]	The Nether-lands	7740 pregnant women from the Amsterdam Born Children and Development study Questionnaire two weeks after the first prenatal visit at GP.	Self-administered questionnaire. Used JCQ to assess job strain. Making five clusters of psychosocial stress	Two weeks after the first prenatal care visit	PTD <37 weeks of gestation	The experience of job strain during pregnancy did not seem to be a risk factor for preterm birth
Niedham mer 2009 [62]	Ireland	The Lifeway cohort including 676 singleton pregnancies where the mother was working at the first prenatal care visit	Self-administered questionnaire. “work is a source of stress” + two items on job influence	First prenatal care visit.	PTD = infants born before 37 completed weeks of gestation	Job stress was not associated with any pregnancy outcome.
Case-control Studies						
Brandt 1992 [66]	Denmark	Case-based study with 214,108 women included	Questionnaire including 16 questions about stress-related job characteristics on job demands and control. The questionnaire was tested in a pilot study.	Questionnaire mailed in September 1987 with recall period of 2.5 to 4.5 years	PTD = delivery before the 36th week of gestation	No statistically significant association on job strain and the risk of PTD.
Brett 1997 [64]	USA	421 cases of PTD 612 controls. Hospital-based population from central North Carolina. Cases were selected among women who had a PTD. The next women who delivered normal weight and full-term were selected as control (if same race).	Telephone interviews/ in-person interviews 14 questions from the Framingham Study version of the Job Content Survey instrument.	Interviews were conducted a median of six months after delivery. Two definitions; Exposure to high strain at least one month during pregnancy and exposure to high strain in the 27th week of gestation.	PTD = infants born before 37 completed weeks of gestation	Black women exposed to high strain jobs had an elevated risk of PTD aOR: 1.8 (CI: 1.1-3.1), whereas no association were found for white women.
Croteau 2007 [65]	Canada	1,242 cases of PTD 4,513 controls 20 % random sample from all singleton live births reported to government.	Questionnaire Psychosocial factors evaluated by use of job demand-control support model	Telephone interview when birth certificate information was received. Asked to “the beginning of pregnancy”	PTD = infants born before 37 completed weeks of gestation	High job strain with low or moderate support: OR: 1.3, CI: (1.0-1.5)

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Case-control Studies						
Henrich 2003 [67]	Germany	236 cases of PTD 471 controls Hospital-based population For each PTD mother asked, two non-PTD mothers giving birth immediately after her was controls	Questionnaire "Mentally demanding jobs" vs. "physically demanding jobs". Job satisfaction	Personal interview shortly after delivery. No information on timing of "mental demands"	PTD = infants born before 37 completed weeks of gestation	No differences found when comparing physically and mentally demanding jobs. 21.9% of the women with PTD had low job satisfaction, where only 8.5 % among the term births had low job satisfaction (p=0.001).
Ronda 2010 [68]	Spain	94 hairdressers 138 shop assistants and office workers. All functioning hairdressing salons registered in Alicante were included	Face-face interviews by trained interviewers. Self-perceived work-related stress situation (low, normal, high)	Not measured in relation to pregnancy	PTD = infants born before 37 completed weeks of gestation	No significant differences in PTD - hairdressers vs. shop assistants.
Cross sectional Studies						
Homer 1990 [70]	USA	786 employed women from the National Longitudinal Survey of Labor Market Experience, who prior to the questionnaire had a singleton birth	Questionnaire. "Job Characteristics Scoring System" based on the job demand-control support model. Job titles	Job titles during pregnancy	Preterm, low birthweight = gestation less than 38 weeks and birthweight 2,500 g or under.	Job title alone was not associated with preterm birth. The RR was 8.1 (CI: 1.5-50.2) when combing job title with information on not wanting to stay in the work force.
Meyer 2007 [69]	USA	Register-study 26,408 singleton births where information on occupation and industry were available for the mother	Job titles were coded in relation to Quality of Employment Surveys and the Job Content Questionnaire for specific occupations	Not measured in relation to pregnancy	Delivery before 37 weeks of gestation	High strain jobs and PTD: OR: 1.35, aOR: 1.17 (CI: 1.00-1.36)

Small for gestational age (SGA) and Large for gestational age (LGA)

Small and large for gestational age were included as measurements of intrauterine growth. Birthweight is often used as it is a simple record, easy to determine and often measured quite precisely [37]. For most developed countries, birthweight is by law registered on the birth certificate [37]. Low birthweight however, fails to distinguish between a child born preterm and child born at term but small. This is important as the birthweight is not only a result of foetal growth, but also the length of the gestation. SGA is defined as the 10 % smallest of the babies for each gestational week for each gender within the present study population. LGA is defined as the 10 % largest (heaviest) of the babies for each gestational week for each gender within the present study population. SGA and LGA therefore serves as markers of babies at high risk for health implications independent of gestational age [37].

Low birthweight is a powerful predictor of neonatal survival and has been associated with health problems later in life such as hypertension and type 2 diabetes [71]. Several risk factors for reductions of birthweight are identified e.g. increasing maternal age [72,73], parity (generally women deliver lighter babies in their first pregnancy compared to the following pregnancies)[74,75], cigarette smoking [76] etc. High birthweight is also associated with higher mortality and risk of health problems later in life [37]. Often the reason for having heavier babies is linked to maternal obesity and diabetes, but the pattern is also seen in developing countries where these risk contributors are rare [77].

The maternal HPA-axis has been hypothesized to be involved in intrauterine growth. Foetal overexposure to glucocorticoids have been proposed as the moderating factor for growth [78]. Under normal circumstances, plasma level of glucocorticoids is lower in the foetus compared to the mother, due to the placental enzyme 11-beta-hydroxysteroid dehydrogenase type 2 (11-beta-HSD2). This enzyme catalyses the conversion of cortisol to cortisone, the inert 11-keto form. As a consequence, most maternal cortisol is inactivated in the placenta before it reaches the foetus, although a certain percentage of maternal cortisol reaches the foetus in the active form. Hence, high plasma levels of maternal glucocorticoids may increase foetal exposure. In addition, deficiency of 11-beta-HSD2 may increase access of maternal glucocorticoids to the foetus. Both cases would be expected to potentially retard foetal growth [78]. It is also worth noticing, that placental activity of 11-beta-HSD2 is subject to regulation. In rodents, maternal stress and under-nutrition have both been shown to reduce placental activity of 11-beta-HSD2, thereby allowing for increased access of bioactive glucocorticoids to the foetus [5,79].

Literature search on ((“job stress” OR “work stress” OR “occupational stress” OR “job strain” OR “psychosocial working conditions” OR “work-related psychosocial stress”) AND (“birth weight” OR “low birth weight” OR “infant, small for gestational age” OR “infant, large for gestational age”), NOT (“physical stress”) was conducted. SGA and LGA are not used as often as birthweight in research studies; birthweight is therefore also included in the review. A presentation of the studies can be seen in table 2. The literature search resulted in six prospective studies, two case-control studies and two cross-sectional. All studies found in the literature search are included in the review of job stress and risk of SGA or LGA.

Three prospective studies found effects of job stress on the risk of low birthweight or SGA: one study found an adjusted birthweight difference of 190 grams when exposed to high strain [82], another found high control (job strain includes low control) to be positive related to birthweight [63] and the last saw high levels of job strain in the early pregnancy to be associated with a reduction of birthweight and an increased risk of SGA, but only when the mothers worked 32 or more hours per week [81]. The three other prospective studies found no effect of job strain on birthweight or SGA [60-62]. One case-control study found high job strain to increase the risk of having a term baby with low birthweight [66].

The other case-control study and the two cross-sectional studies reported no association between stressful work and low birthweight [68-70]. No studies included LGA as outcome.

Table 2 Overview of studies on work related stress and risk of changes in birthweight or having a child born SGA/low birth weight

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Henriksen 1994 [61]	Denmark	3503 singleton pregnancies from Aarhus University Hospital.	Self-administered questionnaire. Job demands, job control, dichotomized at median score and combine to four exposure categories.	Questionnaire at week 16 of gestation for women working more than 30 hours a week	SGA – a proxy for intrauterine growth retardation. Defined as birthweight below the 10th percentile of the sex-specific birthweight for gestational age curve for the population.	No statistically significant association between high strain and the risk of having a child born SGA
Lee 2011 [63]	Korea	310 mother-infant pairs were drawn from the Mothers and Childrens' Environmental Health study, recruited at the first trimester of pregnancy	Job strain was measured using Karasek's demand-control questionnaire in a Korean version. Also questions regarding the effort-reward-imbalance model (ERI) was included	Standard questionnaires administered at 20 or fewer weeks of pregnancy	Birthweight on a continuous scale obtained from medical records.	Decision latitude was found to be positive related to birthweight.
Loomans 2012 [60]	The Netherlands	7740 pregnant women from the Amsterdam Born Children and Development study Questionnaire two weeks after the first prenatal visit at GP.	Self-administered questionnaire. Used JCQ to assess job strain. Making five clusters of psychosocial stress	Two weeks after the first prenatal care visit	SGA/LGA the bottom and top 10 % of birth weight standardized for gender, gestational and parity using reference values from the Dutch Perinatal Registration.	The experience of job strain during pregnancy did not seem to be a risk factor for SGA/LGA

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Niedhammer 2009 [62]	Ireland	The Lifeway cohort including 676 singleton pregnancies where the mother were working at the first prenatal care visit	Self-administered questionnaire. "work is a source of stress" + two items on job influence	First prenatal care visit.	Birthweight: ≤3000 g and ≤2500 g. SGA was defined by weight below the 10th percentile for gestational age on the basis of gender-and-parity-specific Scottish standards.	Job stress was not associated with any pregnancy outcome.
Oths 2001 [82]	USA	Prospectively collected data form four public and private clinics serving persons of various socioeconomic levels including 480 black and white women. Age between 20 and 40, receiving early prenatal care, free of chronic diseases.	In depth private interviews of 30 to 60 minutes conducted by trained staff. Job strain questions based on the original Karasek questionnaire.	Interviewed in the first 14 weeks of pregnancy and again after the 28th.	Birthweight on a continuous scale.	Work in high strain jobs was associated with an adjusted difference in birthweight of 190 g (CI: 48-333)
Vrijkotte 2009 [81]	The Netherlands	Prospective data from the Amsterdam Born Children and their Development study, with a final sample size of 7135 women, recruited in Amsterdam at their first antenatal visit	Self-administered validated Dutch version of the Job Content Questionnaire.	Questionnaires mailed 2 weeks after recruitment (which had median of 13 weeks).	Birthweight on a continuous scale in grams and delivery of an SGA child – defined as a birthweight below the 10th percentile for gestational age on the basis of gender- and parity-specific standards.	High levels of job strain during early pregnancy were associated with reduced birthweight and an increased risk of SGA IF the mothers work 32 or more hours per week
Case-control Studies						
Brandt 1992 [66]	Denmark	Case-based study with 214,108 women included	Questionnaire including 16 questions about stress-related job characteristics. The questionnaire was tested in a pilot study.	Questionnaire mailed in September 1987 with recall period of 2.5 to 4.5 years	"light-for-date" birthweight defined as the lowest fifth percentile of gestational age of each sex. "Term low birthweight" defined as weight ≤2,500 g.	High job strain was associated with "term low birthweight" (OR= 1.46, CI: 1.05-2.04). "Light-for-date"-results were not statistically significant.
Ronda 2010 [68]	Spain	94 hairdressers 138 shop assistants and office workers. All functioning hairdressing salons registered in Alicante were included	Face-face interviews by trained interviewers. Self-perceived work-related stress situation (low, normal, high)	Not measured in relation to pregnancy	Low birthweight (less or equal to 2,500 g)	No statistically significant differences related to birthweight among hairdressers compared to shop assistants.
Cross sectional Studies						
Homer 1990 [70]	USA	786 employed women from the National Longitudinal Survey of Labor Market Experience, who prior to the questionnaire had a singleton birth	Questionnaire. "Job Characteristics Scoring System" based on the job demand-control support model. Job titles	Job titles during pregnancy	Low birthweight included in the preterm definition, low birthweight itself, defined as a birthweight at 2,500 g or less and birthweight viewed on a continuous scale.	After adjustments, stressful work was not statistically significantly associated with low birthweight or birthweight differences
Meyer 2007 [69]	USA	26,408 singleton births where information on occupation and industry were available for the mother	Job titles were coded in relation to Quality of Employment Surveys and the JCQ for specific occupations	Not measured in relation to pregnancy	Low birthweight defined as less than 2,500 g, Term low birthweight defined as less than 2,500 g at 37 weeks of gestation or later.	Risks of term- and all low birthweight deliveries were not statistically significantly association with high strain.

Congenital malformations

Malformations are relatively rare. Approximately 5 % of children born in Denmark have a congenital malformation of any kind (varying between 4.2 % and 5.1 % in the years 1994-2005 [83]). Thus, relatively few cases are included in the specific groups of malformations even in the DNBC.

Often the aetiology regarding congenital malformations is unknown, but animal studies have suggested effects of maternal stress. This is seen for example when the animals are restrained during pregnancy [84,85]. Again increased glucocorticosteroids are suggested as a causal factor of malformations. Stressful life-events have been reported to be associated with elevated maternal corticotrophin-releasing hormone (CRH) and corticosteroids levels during pregnancy in humans [86]. A causal link between glucocorticosteroid exposure and congenital malformations is supported by studies on mothers taking corticosteroids during pregnancy, showing increased risk of oral clefts among the offspring [87]. Several other risk factors for congenital malformations related to pregnancy and lifestyle have been identified; maternal age [88], smoking [89,90], alcohol consumption [91], maternal overweight and obesity [92] and maternal diabetes [93-95].

A literature search on job stress and risk of congenital malformations were conducted with the search terms ((“job stress” OR “work stress” OR “occupational stress” OR “job strain” OR “psychosocial working conditions” OR “work-related psychosocial stress”) AND (“congenital abnormality” OR “congenital malformation” OR “infant, small for gestational age” OR “infant, large for gestational age”), NOT (“physical stress”)). Only a single study from Denmark has included job stress as exposure [108]. Therefore to give an overview of the literature in the field, the search were expanded to include other types of maternal exposure e.g.” bereavement” , “stressful life events” and “maternal stress”. The very different exposures should however be kept in mind, when comparing the results presented in *Table 3*.

Three prospective studies and five case-control studies were identified in the literature search. From Denmark, the three register-based cohort studies on bereavement and stressful life events (including bereavement) showed an association to cranial-neural-crest malformations [96], oral cleft [97] and congenital heart defects [98]. The Danish case-control study found no association between job stress and congenital malformation [66], but effects of stressful life events were seen in the other four case-control studies; one study found a higher incidence of malformations when the mother were refused an abortion [99], another found an association between at least one stressful life event (death of close relative, job loss or divorce) and risk of conotruncal hearth defects, neural tube defects and cleft lip [100]. The last two case-control studies both found an association between experiencing stressful life events during pregnancy and having a child with neural tube defects [101,102].

Table 3 Overview of studies on work related stress, bereavement and stressful life events and risk of congenital malformations in offspring

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Hansen 2000 [96]	Denmark	Registry data: 23,859 women, where 3,560 had been exposed to severe life events during pregnancy from 1980 to 1992	Severe life events were defined as death or first hospital admission for cancer or acute myocardial infarction in partners or children	Exposure to severe life events during pregnancy and up to 16 months previously	Malformation, particularly of the cranial neural crest	Higher frequency of cranial-neural-crest malformations when exposed to severe life events (OR= 1.54; CI: 1.05-2.27). Death of older child was associated higher risk of cranial-neural-crest malformations (OR= 4.75, CI: 1.63-13.8).
Ingstrup 2013 [97]	Denmark	Registry data: 1,771,663 children born from 1978 to 2008 in Denmark.	Bereavement - death of a close relative – distinguish between expected or sudden death	Loss of a first-degree relative from 1 year before conception till birth of the child	Cleft (cleft lip, cleft lip and palate and cleft palate)	Maternal bereavement was associated with increased risk of oral cleft (OR =1.28; CI: 1.01; 1.61). Higher risk of oral cleft if mothers lost a relative due to a sudden death (OR=1.76,CI: 1.06; 2.94).
Zhu 2013 [98]	Denmark	Registry data: 1,770,878 singletons born in Denmark from January 1, 1978, to December 31, 2008. 44,820 children were born to bereaved mothers	Bereavement – loss of another child, partner, parent or sibling.	Loss of a first-degree relative 1 year before their last menstrual period until delivery	Congenital heart defects (CHD) identified in Danish Registry of Congenital Heart Disease	Children of bereaved mothers had a slightly higher prevalence of CHD than unexposed children (OR = 1.11, CI: 1.00-1.22). The association was most marked for children of mothers who had lost a child or partner (OR = 1.32, CI: 1.04-1.67).
Case-control Studies						
Brandt 1992 [66]	Denmark	Case-based study with 214,108 women included	Questionnaire including 16 questions about stress-related job characteristics on job demands and control. The questionnaire was tested in a pilot study.	Questionnaire mailed in September 1987 with recall period of 2.5 to 4.5 years	Register information on congenital malformations	No association was found between job stress and congenital malformations.
Blomberg 1980 [99]	Sweden	The children of 1,263 women whose applications for legal abortion in 1960 had been refused were compared with the next children born in the same delivery wards.	Stress was set equal to refusal to legal abortions.	Collected by registers.	Register information on congenital malformations	The incidence of malformations for children of abortion applicants from social class III was 3 % in the refused group against 0.6 % in the control series (P = 0.017)
Carmichael 2000 [100]	USA	Hospital and genetic counseling centers, 1988; 344,214 deliveries and 1989; 208,387deliveries were found eligible for study. 207 cases of conotruncal heart defects, 265 cases of neural tube defects and 662 cases of orofacial cleft from	Stressful life events: Deaths of close relative, job loss or separations /divorces, for themselves or anyone close to them	Interview took place on average 3.7 years after birth.	Interview data on conotruncal heart defects, tube defects, and facial cleft from hospital and genetic counseling centers.	Experiencing at least one stressful event was associated with a prevalence odds ratio of 1.4-1.5 for the delivery of infants with conotruncal heart defects, neural tube defects, and isolated cleft lip with or without palate
Li 2013 [101]	China	Participants included 631 neural tube defect (NTDs) cases and 862 normal controls born between 2002 and 2007	Maternal severe stressful life events during the periconceptional period	Exposure information was collected within 1 week after delivery	Hospital information on anencephaly, spina bifida, and encephalocele	Maternal severe stressful life events was associated with NTDs (OR=4.2; CI: 1.4-12.6), strongest for anencephaly (OR= 4.4; CI: 1.2-15.9).

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Case-control Studies						
Suarez 2003 [102]	Texas/ Mexico	Cases (N = 184): Mexican-American children with neural tube defects born between 1995 to 2000. Control mothers (N = 225): randomly selected from Mexican-American women residing in the same area and delivering normal live births.	Maternal stress; the number of job changes, residential moves, and major injuries. Social support was measured using social integration and perceived emotional support scales	Maternal stress during the year before conception	Hospital information on neural tube defects.	Mothers experiencing one or more stressful life events during the year before conception had increased risks for NTDs (OR = 2.9; CI: 1.8-4.7) compared with mothers experiencing no events. Mothers with low emotional support had an elevated risk compared with those who scored high (OR = 4.6; CI = 2.2-9.7).

Asthma and atopic dermatitis (AD)

Asthma is a chronic, inflammatory lung disease characterized by shortness of breath, wheeze and coughing. With more than 300 million people of all ages affected worldwide, asthma is one of the most prevalent chronic diseases. Recent studies show increasing prevalence of asthma in Western countries [103-105]. Self-reported asthma rose from 5.3 % in 1986 to 11.7 % in 2001 among Danish children [106]. Asthma-patients often require long-term treatment, and especially for smaller children, who more often get infections, the disease can be quite exhausting and cause repeated hospitalization. Furthermore childhood asthma accounts for many lost school days and attenuated school and work performance as well as negative effects on social life [107,108].

Atopic dermatitis (AD) is a chronic, inflammatory skin disorder with a lifetime prevalence of 15-20 % in children and adolescents. More than 80 % of AD in childhood developing before the child turns five years [109]. AD is increasing especially in Western countries for example self-reported AD rose from 17.3 % in 1986 to 27.3 % in 2001 among Danish children [105].

The pathogenesis of asthma, AD and other allergic diseases is complex and still not fully understood. Asthma and particularly AD often have an early onset, suggesting that risk factors very early in life need to be investigated. It is known that most allergic diseases, especially asthma, has a strong genetic component [110], but other perinatal factors have also been suggested as possible risk factors for allergic diseases e.g. low maternal age [111], maternal atopic disposition [112,113], parity [74,75], increased BMI [114], smoking [115], use of paracetamol [116,117] and antibiotics [118], SGA [119], and work environment [120,121]. Lately, also prenatal stress has also been associated with interference with foetal immune development [122,123] and studies have shown that immune development during foetal growth is altered in asthmatic children [124,125]. Stensen et al. has reviewed this topic, and found several studies showing allergen exposure to influence the atopic phenotype and indicate that allergen or toxicant exposure are associated with a sensitization process, which can lead to childhood asthma when hitting critical windows of the development of the immune system [104]. Prenatal stress has been associated with elevated IgE in cord blood and alterations in innate and adaptive immune responses [127,128]. Further, psychosocial stress disturbs the regulation of the HPA-axis which may cause the physiologic immune systems to operate at higher or lower levels and disrupt the normal homeostasis. This imbalance in the immune, metabolic and neural systems may inflict long-term damage in the child, if not terminated [129].

As with the previous presented outcomes, a literature search were conducted with the search terms ((“job stress” OR “work stress” OR “occupational stress” OR “job strain” OR “psychosocial working conditions” OR “work-related psychosocial stress”) AND (“asthma” OR “atopic dermatitis” OR “allergic disease” OR “allergic dermatitis” OR “eczema”), NOT (“physical stress”)). The search gave no results on prenatal exposure to work related stress and asthma and only a single study in regards to AD. The search was therefore expanded to include other types of maternal exposure e.g.” bereavement”, “stressful life events”, “anxiety” and “maternal stress”. The results are presented in *Table 4* and 5.

Three prospective and one cross-sectional study related to asthma were identified. One prospective study showed an association between mothers who had reported anxiety during pregnancy and asthma in the children [130]. The other two cohort studies showed a significantly higher risk of asthma in the children if the mother was bereaved during pregnancy [131], but in one study only for boys [132]. From the cross-sectional study stressful life events experienced during pregnancy were reported to be associated with asthma in the children [133].

The literature search on AD resulted also in three prospective studies and a cross-sectional study. One prospective study had included exposure to stress at work and found a dose-response relationship with higher work stress leading to higher risk of AD [134]. Two other prospective studies on birth cohorts were included; one found an association between maternal stress and ever having had AD [135], the other reported an association between maternal psychological and social stress and childhood eczema, although insignificant [136]. The cross-sectional study found foetal exposure to stressful life events to be positively associated with atopic eczema [133].

Table 4 Overview of studies on anxiety, bereavement and stressful life events and risk of asthma in offspring

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Cookson 2009 [130]	United Kingdom	Based on the Avon Longitudinal Study of Parents and Children. Data on maternal anxiety score were available for 5,810 children.	Maternal anxiety were assessed by use of the validated Crown-Crisp Experiential Index	Anxiety was assessed at 18 and 32 weeks of gestation.	Asthma was defined at age 7½ years as doctor-diagnosed asthma with current symptoms or treatment in the last 12 months.	Children at age 7½ years had higher likelihood of asthma if exposed to anxiety prenatally (OR: 1.64, CI:1.25-2.17)
Fang 2011 [132]	Sweden	Registry-data: followed two samples; ‘younger sample’ = 449,363 children from 2004-2008 and ‘older sample’ = 514,261 from 1997-2002	Maternal bereavement of close relative (older child, spouse, parent or sibling)	Maternal bereavement from one year before pregnancy to child birth found in registers	Asthma event was defined as hospital contact for asthma or at least two dispenses of inhaled corticosteroids or montelukast.	Boys of bereaved mother had significant higher risk of asthma event when bereaved during second trimester (OR: 1.55, CI:1.19-2.02) or if the bereavement was an older child (OR: 1.58, CI:1.11-2.25).
Khashan 2012 [131]	Sweden	Registry-data: all singleton live births in Sweden between 1973 and 2004	Bereavement: loss of spouse or child	The women were defined as exposed if they had loss a child or spouse during or 6 months before pregnancy	Information on asthma was extracted from hospital discharges. Secondary outcome measures as chronic obstructive pulmonary disease were also collected.	Risk of offspring asthma was increased with any prenatal exposure to bereavement (RR: 120, CI: 1.03-1.39). The risk was higher when exposed in pregnancy (RR: 1.43, CI: 1.06-1.92) and also if bereavement was death of spouse (RR: 1.59, CI: 1.10-2.30)
Cross sectional Studies						
de Marco 2012 [133]	Italy	Information from 3854 children from Northern Italy aged 3-14,	Stressful life events were measured by questionnaire asking the mothers if she had experienced any situations of loss or uneasiness.	Exposure to stressful life events during pregnancy	Information on asthma was collected in the questionnaire by use of core questions from international validated questionnaires	Stressful life events were positively associated with asthma (OR: 1.71, CO: 1.02-2.89)

Table 5 Overview of studies on work stress, mental status and stressful life events and risk of AD or atopic eczema in offspring

First author Year	Country	Material	Stress measure	Time of stress measurement	Outcome measure	Findings
Cohort Studies						
Sausenthaler 2009 [136]	Germany	3004 children from a prospective German birth cohort (LISA)	A list of stress factors was made, stress was defined as the presence of two or more factors	Questionnaires were completed shortly after birth	Physician-diagnosed eczema in the first 6 years of life.	Maternal stress factors during pregnancy was positively associated with childhood eczema although insignificant (OR: 1.48, CI:0.95-2.30)
Wang 2013 [134]	Taiwan	The Taiwan Birth Cohort, with 19,381 subjects included in the present study	Work stress was measured through the question "How much stress did you feel when working during pregnancy" by home interviews.	Information on exposure to stress was obtained after birth	Cases of AD were defined as physician-diagnosed AD or by questionnaire asking "Has your child ever had a recurrent itchy rash at least six consecutive half-month periods over the elbow, knees, face, wrists, neck periauricular and eyebrow areas during his/her lifetime"	The risk of AD was found to increase with maternal work stress during pregnancy in a dose-response manner.
Wen 2011 [135]	Taiwan	1264 mother-infant pairs recruited to participate in a birth cohort.	Maternal self-reported mental status (vitality, vigor, happiness, anxiety, discouragement, nervousness, tiredness, exhaustion and working stress) from the "modified Chinese version of Short Form 36 Health Survey".	Questionnaires were completed one month before expected due date	Identification of AD children was based on response to questionnaire when the child was 6 months and 2 years of age. "Did your child ever have physician-diagnosed AD?" and yes to question on itchy rash in locations typical for AD. Futher, IgE was measured.	Elevated IgE and maternal stress during pregnancy were associated with ever having physician-diagnosed AD in 2-year old children.
Cross sectional Studies						
de Marco 2012 [133]	Italy	Information from 3854 children from Northern Italy aged 3-14,	Stressful life events were measured by questionnaire asking the mothers if she had experienced any situations of loss or uneasiness.	Exposure to stressful life events during pregnancy	Information on AD was collected in the questionnaire by use of core questions from international validated questionnaires	Foetal exposure to stressful life events was positively associated with atopic eczema (OR: 1.53, CI: 1.11-2.10)

Good Epidemiological Practices for Occupational and Environmental Epidemiological Research

As stated in the aims of the thesis - the ambition was to maximize and secure the quality of research and integrity of the data used by documenting the methods in a protocol that described the analyses before they were done and to keep transparency in the methods used by e.g. publishing the protocol inclusive all reviewers' remarks, in open access and following good epidemiological practices (GEP) for occupational and environmental epidemiological research [11].

The American Journal of Epidemiology published already in 1981 guidelines for the documentation of observational epidemiological studies [137]. The guidelines were aimed at assisting regulatory agencies in the evaluation of epidemiological studies for use in public health decisions. They did not focus on publication of epidemiological research, but still presented elements which should be defined and documented in relation to background and objectives, study design, study and comparison subjects, data collection procedures, analysis and supporting documentation [137].

The GEP was published by the Chemical Manufacturers Association in 1991, based on an increasing interest in the development and application of more formal practices in epidemiological research for use in the formulation of public health policies. In the GEP detailed requirements concerning every aspect of research were included; requirements concerning personnel and facilities, development of a study protocol, review, approval, study conduct, communication, archiving and quality assurance [11]. The "new" thing for epidemiological researchers was the demand of written and detailed descriptions of routine procedures e.g. coding of variables as done when working in laboratories with standard operating procedures (SOPs).

The journal of Occupational and Environmental Medicine has since 1994 used a panel of medical statisticians in the review process of submitted papers. In addition to genuine errors in design, execution and analysis – many of the submitted papers had problems with inadequate or incomplete reports of essential aspects of their research, calling for a structured approach for documentation of study procedures [138].

Even though the GEP cannot guarantee good epidemiology, it is a framework that can be useful to ensure that all research issues are addressed sufficiently.

METHODS

Data sources:

The Danish National Birth Cohort

All analyses in this thesis are based on information and data from the Danish National Birth Cohort (DNBC). The cohort was established in 1994 and baseline information from the enrolled 100,418 pregnancies was gathered from 1996 to 2002. At their first antenatal visit pregnant women were invited to participate in the cohort by their general practitioner (GP). About 50 % of the GPs agreed to invite the women and gave information on the study and approximately 60 % of the invited pregnant women chose to participate in the DNBC [139]. To contribute the women had to be pregnant, have intentions of carrying the pregnancy to term, reside in Denmark and speak Danish sufficiently well to participate in telephone interviews.

When enrolled the women were asked to participate in two telephone interviews during pregnancy at approximately 12-14 and 30-32 weeks of gestation and two after birth when the child was six and 18 months old. They were also asked to provide two blood samples during pregnancy and one cord blood sample at birth. The first two interviews contain information on lifestyle factors during pregnancy e.g. smoking, diseases, weight gain etc. They also included information on exposures, type of work, course of pregnancy and background information on both mother and father. The postnatal interviews included information on the child's development, environmental exposures etc. Afterwards the same women and children were asked to participate in a follow-up at age seven including questions on the child's development both mentally and physically. For an overview of the participation and structure of the DNBC, please see Figure 1.

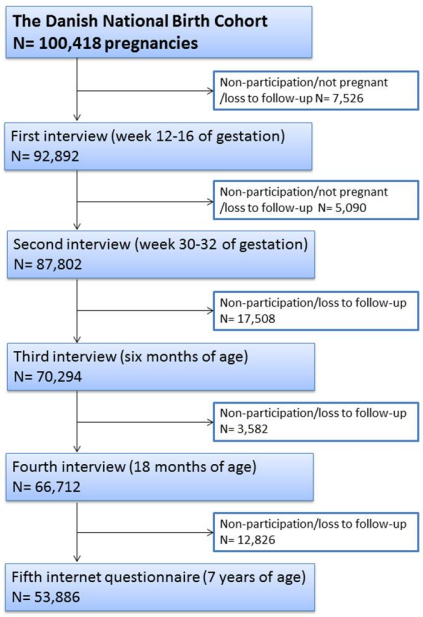

Figure 1 Flowchart for the Danish National Birth Cohort

For the analyses in this thesis, data from all of the interviews, but the third, were used; exposure to psychosocial job strain was assessed based on answers in the first interview. Information on many covariates is likewise extracted from the first

interview. The second interview provided information on use of folic acid, painkillers and antibiotics. From the fourth interview we have information on atopic dermatitis and the fifth provided information on atopic dermatitis and asthma.

As previously mentioned 100,418 pregnancies were included in the cohort, however in this thesis the selection of data depended on the outcomes and intended analyses. Figure 2 will give an overview of the criteria used for exclusion and the exact number of participants in the final dataset for each study.

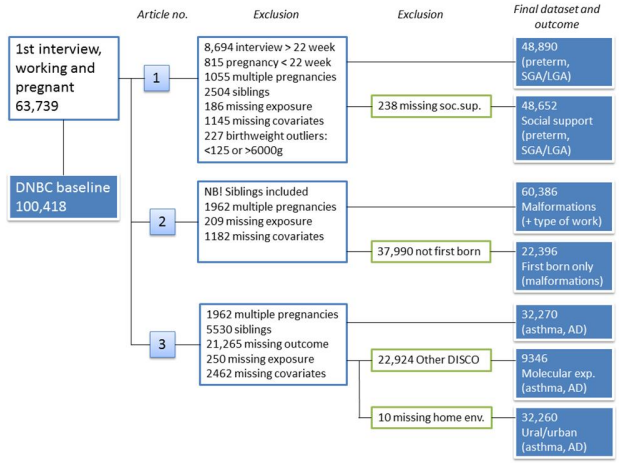

Figure 2 Overview of the criteria used for exclusion and the exact number of participants in the final dataset for each of the three studies.

The Danish Medical Birth Registry

Denmark and the other Nordic countries have a long history of collecting information in registries on many aspects of life e.g. birth, deaths, migration and disease [140]. The Danish Civil Registration System (CRS) was established in 1968 and includes all persons alive and living in Denmark [141]. When registered in the CRS all persons are assigned a unique personal identification number, the "Centrale Person Register" (Danish for CRS) -number (CPR), which is used in all registers allowing linkage between all national registers and to the DNBC data.

The Danish Medical Birth Registry (MBR) was established in 1973 and includes all live births and stillbirths of women residing permanently in Denmark [142]. Before 1996 birth data were notified by the attending midwife and a civil notification to the Danish Civil Registration System (CRS) was made along with a medical notification for the MBR. In 1996 the MBR ceased to be an independent registry. After 1997 the midwives report data electronically to the Danish National Patient Registry (NPR) and the MBR is now a highly specialized extraction from the NPR. From MBR information on birth weight, gestational age, parity, maternal age at birth and multiple births were extracted.

Information on congenital malformation at the time of birth is included in the MBR with withdraw on specific diagnoses from NPR [143]. Congenital malformation at birth is recorded as a secondary-diagnosis to the primary diagnosis given at birth by use of the International Classification of Diseases ICD-10 codes.

Operationalization of exposure

The DNBC includes information on the women's exposure to psychosocial work environment in terms of self-reported information from the first telephone interview where the women were asked: "Do you have too many tasks at your work?" and "Do you have the opportunity to influence your tasks and working conditions?" with the response categories: often, sometimes and seldom. These questions were interpreted as dimensions of demands and control, and hereafter used as a proxy for the dimensions of the job strain model by Karasek [34], as described previously. Based on their answers, the women were divided into the four job strain categories. To maximise contrast in exposure, the high strain group was defined by those who answered 'often' to high demands and 'seldom' to the question relating to control. The reference group was the low strain group, defined as those who answered 'sometimes' or 'seldom' to high demands and 'often' or 'sometimes' to high control. The active quadrant was defined by the answers "often" to demands and "often" or "sometimes" to control, whereas the passive group was defined as those answering "seldom" or "sometimes" to the demand and "seldom" to the control dimension. It is illustrated in Figure 3. This grouping of answers to the demand and control questions were used in all studies included in this thesis.

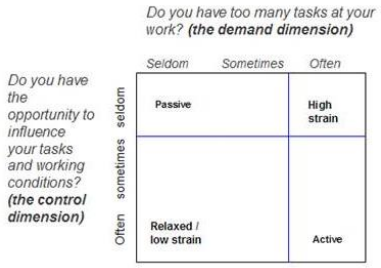

Figure 3 Illustration of the grouping of strain categories used in this study in accordance to the Job Strain Model by Karasek [32]

Overview of studies

Preterm birth SGA/LGA (Paper I and II)

The hypothesis for this study: Exposure to high job strain during pregnancy increases the risk of giving birth to a child preterm and having a child with lower birth weight for gestational age.

Study population and design

For this study a peer-reviewed and published protocol provides a detailed description of methods, design, study population, exposure, outcomes, covariates and planned statistics prior to conduction of the analyses (paper I or[144]).

As presented in Figure 2, the final study population included 48,890 pregnancies when studying preterm birth and SGA/LGA. The women should be pregnant and working at the time of the first interview, which resulted in 63,739 pregnancies. To avoid recall bias the women needed to have answered the questions regarding psychosocial work environment before they knew the outcome of the pregnancy. This was especially relevant for preterm birth. Therefore, 8,694 pregnancies were excluded as the first interview was carried out later than week 22 of gestation. Next, 815 pregnancies were excluded because they per definition were miscarriages (ended before 22 completed weeks of gestation). Only singleton pregnancies (n=53,175) were included, as lowered birth weight and preterm birth could arise from different causes in singletons and multitons. The woman was only allowed to contribute with her first pregnancy in the cohort (n=50,671) to avoid over-representation of gene material. 186 cases missed exposure information and 1,145 missed information on covariates. 227 children were excluded as outliers in birthweight (<125 g or >6000 g) based on established growth charts [145].

Operationalization of outcome, definition of covariates etc.
Gestational age (GA) is defined as the number of days since conception. As the moment of conception is rarely known, the GA is normally calculated as the number of days from "the first day of the last normal menstrual period" (LMP) to the day of birth. This way of measuring gestational age includes uncertainties specifically in regards to LMP in terms of recollection of last menstrual period, ovulation variation or irregular bleedings [146]. Nowadays all women in Denmark are offered the opportunity to have an ultrasound examination early in pregnancy. This was first fully introduced for all women after 2004 and therefore not available for all women in the studied cohort. Data on GA for use in this thesis is extracted from the Danish Medical Birth Register and can therefore be a combination of either calculated GA or ultrasound-based GA.

Preterm birth was defined as a delivery after 22 and up to 36 completed weeks of gestation. SGA was defined as the 10 % smallest babies at each gestational week for each sex within the DNBC study population. For gestational weeks with less than 10 children in each group (week 22-24), SGA was equal to the lowest birth weight in the group. Similarly for LGA, just using the 90th percentile or above. In the published protocol [144], we failed to exclude outliers in birth weight when calculating SGA and LGA. 227 children were excluded in the actual analysis due to unlikely measures in birth weight (< 125 g or > 6,000 g) based on established growth charts [165].

The analysis was adjusted for several covariates (the protocol includes a detailed description):

- Maternal age, due to elevated risk of low birth weight when mother is older 35 years [72,73].
- BMI as maternal overweight and obesity are known risk factors for preterm delivery [50].
- Parity, as women often deliver lighter babies in their first pregnancy compared to following [74,75].
- Gestational age at interview to crudely control for differences due to the healthy worker effect.
- Exercise due to its beneficial effects on adverse health outcomes such as gestational diabetes, and thereby an indirect protection against LGA [147].
- Smoking habits, as cigarette smoking is causatively related to low birth weight and SGA [76].
- Alcohol consumption as it increases the risk of adverse birth outcomes [47,48].
- Coffee consumption as a high caffeine intake has been associated with reduced birth weight [148].
- Type of work (manual versus non-manual), as women with manual work might have a different risk of adverse pregnancy outcomes than those performing non-manual work.
- Maternal serious disease of epilepsy and diabetes as both has been associated with increased risk of congenital malformations, obstetric complications and neonatal morbidity [93-95].
- Parental height (combined into a continuous variable) as we assume that the probability of a child being SGA or LGA at birth depends on the length of child, which again depends of how tall the parents are.

The distribution of the covariates on the four strain groups can be seen in paper I.

Statistical analysis
A multinomial logistic regression model was used to estimate the ORs with 95 % CI for being either; 1) full term and normal weight for gestational age, 2) preterm 3) full term but SGA or 4) full term but LGA as a function of job-strain (high strain, active and passive versus low strain). The analysis was conducted with the procedure proc logistic in the computer package SAS version 9.1. A likelihood ratio tested the overall null-hypothesis, which stated that the outcome vector is independent of job-strain, this was also done the separate endpoints.

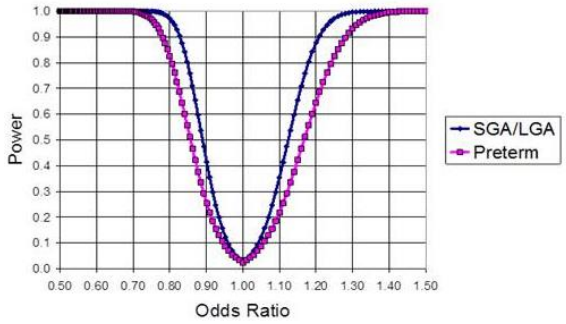

Figure 4 Power curves for preterm birth and SGA/LGA by focusing on the contrast between high strain and low strain

Figure 4 depicts the power curves for preterm birth and SGA/LGA with focus on the contrast in the high strain versus low strain group based on the whole study population with preterm birth defined as 5 % and SGA/LGA as 10 %. If an OR of 1.3 or higher is considered clinically significant, the analysis will have a 99.6 % possibility of identifying a statistically significant association between job strain and SGA. The same holds for the outcome LGA, since the prevalence of LGA, by definition, is the same as it is for SGA. For preterm birth, the corresponding power is 93.2 %.
For explorative purposes, the question "Do you get any help from colleagues when you have troubles in the work?" with the response categories: often, sometimes and seldom was included in an additional analysis, to study social support in relation to the job strain model. Low social support has been hypothesized to alleviate the effect of job strain (iso-strain-model) as introduced by Johnson and Hall [149,150].

Congenital malformations (Paper III)
The hypothesis for this study: Exposure to high job strain during pregnancy increases the risk of having a child with a congenital malformation.

Study population and design
A study protocol was developed, dated and signed prior to the data analyses. It described all methods, study design and defining exposure, outcomes and covariates (can be presented at request).
In this study 60,386 pregnancies from the DNBC were included. We needed the women to be pregnant and working at the time of the baseline interview which results in 63,739 pregnancies. Only singleton pregnancies (n=61,777) were included, as twins and triples may have different causes for developing congenital malformation. 209 pregnancies were excluded due to missing response to the exposure questions and 1,182 pregnancies were excluded due to missing information on covariates.

During the period of data collection (1996-2002) a woman could contribute with more than one pregnancy leaving us with 57,211 unique women giving birth to 60,386 children whereof 22,396 were first-borns (parity 1) and 3,175 had a sibling in the cohort. Correlation-coefficients and variance inflation factors relative to congenital malformations showed no correlation among siblings. Among all malformations the sibling correlation-coefficient was 0.014, for musculoskeletal malformations 0.003 and for malformations in the circulatory system -0.009. Thus the intra-class correlation was negligible and siblings were therefore included in the study population.

Operationalization of outcome, definition of covariates etc.
From the Danish Medical Birth Register (MBR) we extracted information on congenital malformations. The MBR catalogues congenital malformation according to the international Statistical Classification of Disease and Health Related Problems (ICD-10). The "Q-codes" defines all malformations in ICD-10, including: Congenital malformations of the nervous system (Q00-Q07), eye, ear, face and neck (Q10-Q18), the circulatory system (Q20-Q28), the respiratory system (Q30-Q34), cleft lip and cleft palate (Q35-Q37), the digestive system (Q38-Q45), genital organs (Q50-Q56), the urinary system (Q60-Q64), the musculoskeletal system (Q65-Q79), other congenital malformations (Q80-Q89) and chromosomal abnormalities (Q90-Q99).

Based on power calculations (presented in the next section) the study included malformations in the circulatory system (N=582), the musculoskeletal system (N=1,555), and any system (all malformations Q00-Q99, N=3,059).

The analysis was adjusted for several covariates:

- Maternal age, women above 35 years have an elevated risk of giving birth to children with congenital malformations, e.g. hypospadias [88].
- BMI, maternal overweight is a known risk factor for children with congenital malformations [92].
- Parity, most epidemiological studies on congenital malformations include parity.
- Gestational age at interview to crudely control for the healthy worker effect.
- Smoking habits, smoking is positive associated with congenital malformations [89,90].
- Alcohol consumption during pregnancy increases the risk of congenital malformations, e.g. the foetal alcohol syndrome [91].
- Type of work (manual versus non-manual), as women with manual work might have a different risk of adverse pregnancy outcomes than those performing non-manual work.
- Maternal serious disease of epilepsy and diabetes as both has been associated with increased risk of congenital malformations, obstetric complications and neonatal morbidity [93-95].

The distribution of the covariates on the four strain groups can be seen in the protocol (can be presented at request).

Statistical analysis
A logistic regression model was used to estimate prevalence ORs with 95 % CI for having a child with malformations in the circulatory system, malformations in the musculoskeletal system or any type of malformation as functions of job strain (high strain, active and passive versus low strain).

For testing of robustness of results, two supplementary analyses were completed:
a) as siblings were included in the study population, a similar logistic regression analysis were conducted including only first-born children (parity =1, n=22,396).
b) an analysis with stratification on manual work, to investigate if women with manual work might come out with a different risk than those performing non-manual work.
The interpretation of the additional analyses was nested. If the main null-hypothesis (malformations independent of strain) turned out to be significant, the analyses will be a hypothesis-test, where results can be interpreted. Otherwise the results from the analyses are hypothesis-generating.

Power calculation focused on the contrast between the high strain and the low strain groups, which were of primary interest, see Figure 5. As seen, the analyses had an 87 % possibility of identifying a statistically significant association between job-strain and musculoskeletal malformations if an OR of 1.3 or higher is considered clinically significant. The figure also presents the power curve for malformations in the nervous system, where it is apparent that the power is insufficient for a meaningful interpretation of the results. In the power calculations the women contributes with all her pregnancies.

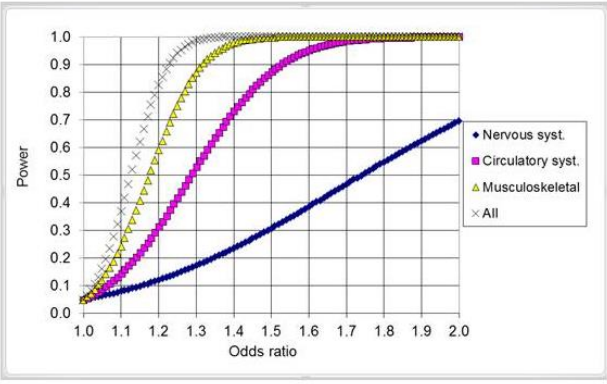

Figure 5 Power curves for congenital malformations in any organ system as well as the nervous, circulatory and musculoskeletal system by focusing on the contrast between high strain and low strain.

Asthma and atopic dermatitis (Paper IV)
The hypothesis for this study: Exposure to high job strain during pregnancy increases the risk of self-reported asthma and/or ever atopic dermatitis when studied at age 7 years.

Study population and design
A protocol was designed with information on data analysis, methods, study design and exposure, outcomes and covariates. The protocol was dated and signed prior to the analyses (can be presented at request).

32,104 pregnancies were included in the study. 3.519 women agreed to participate in the study, but did not respond to the first interview; 33.160 women were not pregnant or not working at the time of the first interview, leaving us with 63.739 women. Twin or triplet pregnancies were excluded (N=1.962) in order to avoid dependency between participants. A woman could only participate with one pregnancy and child to avoid over-representation of genes; siblings were therefore excluded (5.530). 21.265 women whom had not responded to the 5th questionnaire with data on asthma and AD were excluded. 250 had not responded to questions regarding exposure to job strain and

2.462 were missing on information regarding covariates. These were excluded.

Operationalization of outcome, definition of covariates etc.
The International Study on Asthma and Allergies in Childhood (ISAAC) which includes 156 research centres in 56 countries has developed a standardized questionnaire on asthma and allergy in order to compare prevalence and severity in epidemiological studies of allergic diseases [151]. The core questions from ISAAC are included in the DNBC and used to form the outcome measurements in this study [152].

Asthma in the child at age 7 was confirmed if the mother responded with yes to one or more of the three questions: "Has your child experienced wheezing or whistling in the last 12 months?", "Has your child ever had asthma?" and "Has your child ever been diagnosed with asthma by a doctor?" . Information on ever AD was defined as parental report at 18 months of atopic dermatitis ever OR itchy rash in the locations known to be typical for atopic dermatitis (around eyes, ears, on the neck, elbow, knees and front and back of legs) AND/OR report of a persistently itchy rash in the locations known to be typical for AD. Based on the study population of 32.270 pregnancies, 4.214 (13 %) children have had asthma symptoms at age 7 years and 20 % of the children could be defined as having ever AD at age 7 years.

The analysis was adjusted for several covariates:

- Maternal age, lower maternal age is associated with higher risk of having a child with asthma [111].
- BMI, maternal obesity is associated with increased risk of asthma and wheezing in the offspring [114].
- Parity, generally women deliver lighter babies in the first compared to following pregnancies and lighter babies might have a higher risk of respiratory diseases [74,75].
- Gestational age at interview, to crudely control for differences due to the healthy worker effect.
- Smoking habits, as studies have shown an association between foetal exposure to maternal smoking with childhood asthma and related atopic illnesses [115].
- Alcohol intake during pregnancy, increases risk for the child developing atopic dermatitis during the first seven years of age [154].
- Maternal atopic disposition, known to increase the risk of allergic diseases in the child [112,113].
- Folic Acid, folate intake in pregnancy can heighten the risk of childhood asthma [157].
- Paracetamol, studies have shown an association between maternal use of paracetamol and asthma among their children [116,117].
- Antibiotics, increases risk of asthma [118].
- Furry animal ownership during pregnancy, some studies indicate an association between ownership of furry pets during the first two years of life and reduced likelihood of becoming sensitized [158]
- Small for gestational age, neonatal size in term children is associated with asthma at age 7 [119]
- Sex of the child, before puberty, the prevalence of asthma and wheeze is higher in boys than girls [159]

The distribution of the covariates on the four strain groups can be seen in the study protocol (can be presented at request).

Statistical analysis
Multinomial logistic regression models were used to estimate odds ratios (ORs) with 95 % confidence intervals (CI) for having asthma and/or AD, having asthma alone or AD alone at seven years of age compared to the group with neither asthma nor AD by the four job-strain groups (high strain, active and passive versus low strain). Analyses were conducted in the Statistical package SAS version 9.3 with the procedure 'proc logistic'. A likelihood ratio was used to test the overall null-hypothesis in the multinomial logistic regression, assuming that the outcome is independent of job-strain. The null-hypothesis was rejected if $p \leq 0.05$.

Two supplementary analyses were made; an analysis to estimate the influence of asthmogen exposure at work during pregnancy on the association between psychosocial job strain and the risk of developing asthma and/or AD. Based on the job exposure matrix developed by Kennedy et al. [120,160], with some modifications three subgroups related to airborne asthmogen exposure was constructed. The three subgroups were; exposure to i) high molecular weight agents (e.g. veterinarians, gardeners and bakers), ii) low molecular weight agents (e.g. cooks, cleaners, hairdressers, dentistry, manufacturing of dusty products) and iii) mixed environments (e.g. health care professionals). Other professions were not included in this exploratory analysis. Furthermore, to crudely check the impact of microbial burden according to the "hygiene hypothesis" [161], an additional exploratory analysis was made. Higher living standards with cleaner homes expose children to a lower amount of microbial components [162]. This should be particularly important in the first years as the innate immune system is not challenged to suppress the allergenic Th2 immune phenotype. The consequence is a dominance of the Th2 phenotype and thereby a higher risk of developing allergic diseases later on [163]. The women were in the first questionnaire asked if they lived on a farm with animals with 2.105 women confirming this.

Power calculations focused on the contrast between high strain and low strain as presented in Figure 6 and Figure 7 for asthma and AD, respectively. In relation to asthma (Figure 6) with odds ratios of 1.3 or higher, the analysis had a 99.8 % probability of identifying a statistically significant association between high strain and asthma. The corresponding numbers were 98.7 % for asthma with no AD and 80.6 % for AD with no asthma.

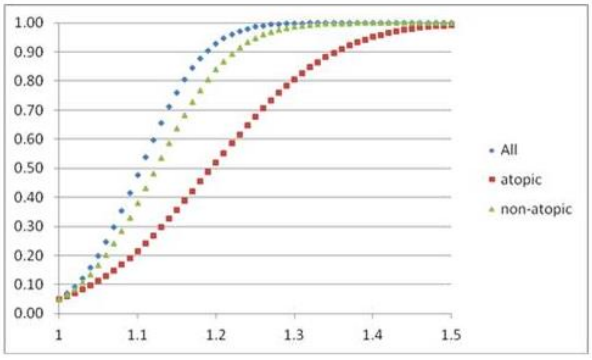

Figure 6 Power figure on the possibility of identifying a statistically significant association between high strain and asthma compared to low strain

Regarding AD (figure 7); the analysis had a 99.99 % probability of identifying a statistically significant association between high

strain and AD compared with low strain with an OR of 1.3 or higher.

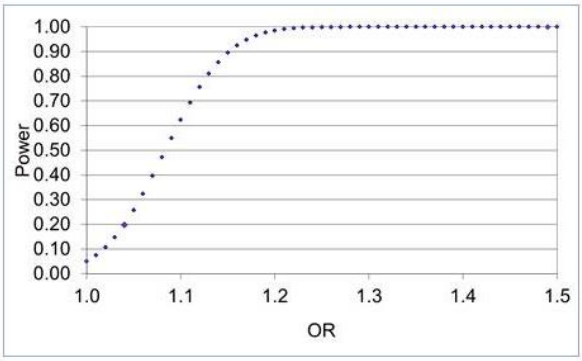

Figure 7 Power figure on the possibility of identifying a statistically significant association between high strain and atopic dermatitis compared to low strain.

Ethics and permissions

Statens Serum Institut (the State Serum Institute) gave permission for use of all data, and a permission from the Danish Data Protection Agency for usage and storage of the data were obtained. Permission from the Scientific Ethical Committee was exempted as none of the studies included risk for or contact with the study population.

SUMMARY OF RESULTS

This section presents a summary of the findings for each of the outcomes in the three studies. A more detailed presentation is available in paper I-IV.

The role of job strain on preterm birth (Paper I and II)

In the sub-dataset of DNBC used for the study on exposure to job strain during pregnancy and risk of having a child born preterm including 48,890 pregnancies, 162 pregnancies ended with preterm birth in the high strain group (4.8 %), 199 in the passive group (4.6 %), 558 in the active group (5.0 %) and 1456 in the low strain group (4.8 %). No effect of high strain was seen on the odds of preterm birth neither for crude (OR = 1.00, 95 % CI: 0.84-1.18) or adjusted data (OR = 0.98, 95 % CI: 0.82-1.16) when compared to low strain.

Analysis of the influence of social support, showed a non-statistically significant tendency of the less social support you experience combined with exposure to high strain the higher risk of preterm birth: Social support; often (OR = 0.92, 95 % CI: 0.74-1.15, sometimes (OR = 1.11, 95 % CI: 0.77-1.59) and seldom (OR=1.39, 95 % CI: 0.86-2.23).

Foetal growth (Paper I and II)

Within this same sub-dataset of DNBC, the risk of having a child born small or large for gestational age was studied as a proxy for foetal growth. In the high strain group 327 (9.7 %) children were born SGA and 268 born LGA (8.0 %), the corresponding numbers for the other strain groups were; passive: SGA 400 (9.3 %), LGA 402 (9.4 %), active: SGA 935 (8.4 %), LGA 1085 (9.8 %) and low strain SGA 2750 (9.1 %) and LGA 2897 (9.5 %), respectively. The reasons for the percentage varying from the 10 % the definition included is due to low numbers (= less than 10) of weekly births especially in the early gestational weeks, where SGA is defined as the (one) smallest child for each week and LGA as the (one) largest child for each week.

The role of job strain on SGA (Paper I and II)

The multinomial logistic regression analysis showed no effect of high strain on SGA in crude (OR = 1.07, 95 % CI: 0.94-1.20) or adjusted analyses (OR = 1.01, 95 % CI: 0.89-1.14) when compared to low strain. A relationship between active style and reduced risk of SGA appeared to be statistically significant according to the 95 % CI (OR = 0.92, 95 % CI: 0.85-0.99 and aOR = 0.90, 95 % CI: 0.83-0.98) when compared to low strain. These results should, however, be interpreted with care as the overall p value of the relationship between SGA and job strain was insignificant (p = 0.09). No clear picture was seen when introducing social support in the analysis.

The role of job strain on LGA (Paper I and II)

An effect of high strain on LGA was seen in both crude and adjusted analyses (OR = 0.83, 95 % CI: 0.73-0.96 and aOR = 0.81, 95 % CI: 0.70-0.92) when comparing to the low strain group. The same effect was seen in the analysis with stratification for social support in the group with often social support (aOR = 0.75, 95 % CI: 0.63-0.90). ORs indicate higher risk of LGA with decreasing amount of social support: sometimes (aOR = 0.79, 95 % CI: 0.60-1.04) and seldom (aOR = 1.09, 95 % CI: 0.76-1.57).

The role of job strain on congenital malformations (Paper III)

The sub-dataset of DNBC used for the study on exposure to job strain during pregnancy and risk of having a child born with malformations included 60,386 pregnancies. In the high strain group 199 children (4.9 %) were born with any type of malformations. When looking at specific congenital malformations 89 children (2.2 %) had musculoskeletal malformations and 41 children (1 %) had malformations in the circulatory system among women experiencing high strain during pregnancy.

Logistic regression testing showed no effect of high strain on the odds of having a child with congenital malformations of any kind (aOR = 0.99, 95 % CI: 0.85-1.15) or for musculoskeletal (aOR = 0.88, 95 % CI: 0.71-1.10) or circulatory malformations (aOR = 1.04, 95 % CI: 0.75-1.44) compared to the low strain group. Supplementary analyses on first-borns only, showed no effect (any malformation, high strain aOR = 1.00, 95 % CI: 0.77-1.29) and similar remarks can be made to the test of manual (any malformation, high strain aOR = 0.89, 95 % CI: 0.73-1.10) and non-manual work (any malformation, high strain aOR = 1.14, 95 % CI: 0.91-1.42) - no effect of high strain on the risk of congenital malformations.

The role of job strain on asthma (Paper IV)

The dataset for studying the effect of exposure to job strain during pregnancy and risk of asthma in seven year old children included 32,270 pregnancies. In the high strain group 87 children (4.3 %) have had asthma and AD and 196 children (9.7 %) have had asthma without AD at the age of 7 years.

No statistically significant associations between high strain and asthma with or without AD were seen (asthma + AD; aOR = 1.11, 95 % CI: 0.88-1.40, asthma alone; aOR = 1.08, 95 % CI: 0.92-1.27) compared to low strain, although ORs were elevated in both crude and adjusted analyses. Working in the active group defined by high demands and high control were associated with higher risk of asthma in both crude (OR = 1.14, 95 % CI: 1.03-1.25) and adjusted analyses (aOR = 1.13, 95 % CI: 1.03-1.24).

Analyses in the subgroup with the 9,346 women working with either high molecular, low molecular or mixed exposure showed no effect of high strain in any of the groups. The active

group showed generally elevated ORs in regards to asthma with or without AD, and also a significantly higher risk of asthma and AD when exposed to low molecular airborne asthmogens during pregnancy (aOR = 1.63, 95 % CI: 1.02-2.62). The analysis was however severely underpowered, and interpretation should be done with care.

A similar picture is drawn for the analysis of risk of asthma with or without AD when exposed to job strain during pregnancy – stratified for home environment (rural with animals or urban). No effect of high strain was seen in any of the groups on asthma with AD or asthma alone. Again the active group showed generally elevated ORs in regards to asthma either with or without AD. A statistically significant association between working in the active group and living in urban areas appeared for asthma without AD (aOR = 1.11, 95 % CI: 1.01-1.23) compared to the low strain group.

The role of job strain on atopic dermatitis (Paper IV)

In the same dataset as for the asthma analyses we studied the effect of job strain on atopic dermatitis (AD) in 32,270 children. In the high strain group 87 children (4.3 %) have had AD together with asthma and 356 children (17.6 %) have had AD alone at the age of 7 years. In both crude and adjusted analyses high strain was statistically significantly associated with AD without asthma (OR = 1.14, 95 % CI: 1.01-1.29, aOR = 1.15, 95 % CI: 1.02-1.31). Working in the active group was also associated with increased risk or AD (OR = 1.08, 95 % CI: 1.00-1.16), but the association disappeared when adjusting for several covariates including maternal atopic disposition.

In the subgroup with the 9,346 women working with either high molecular, low molecular or mixed exposure analyses showed effects of high strain on AD when working with exposure to low molecular asthmogens (aOR = 1.53, 95 % CI: 1.07-2.18). Results from the active group are reported in the previous section under asthma. In the analyses of AD when exposed to job strain during pregnancy – stratified for home environment (rural with animals or urban), a statistically significant association was seen between high strain and AD when living in a rural home environment (aOR = 1.69, 95 % CI: 1.06-2.71). A similar, but not statistically significant tendency was seen for the urban home environment (aOR = 1.11, 95 % CI: 0.98-1.27).

The main results from the three studies on exposure to high strain and risk of preterm birth, SGA, LGA, congenital malformations, asthma and AD relative to our hypotheses for each outcome are summarized in Table 6.

Hypothesis	Hypothesis confirmed	Hypothesis not confirmed
Prenatal high strain exposure Preterm birth		The study found no association between high strain and preterm birth aOR = 0.98, 95 % CI: 0.82,1.16
Prenatal high strain exposure SGA		The study found no association between high strain and SGA aOR = 1.01, 95 % CI: 0.89-1.14
Prenatal high strain exposure LGA	(The study found an association between high strain and LGA aOR= 0.81, 95% CI: 0.70-0.92*)	
Prenatal high strain exposure Congenital malformations		The study found no association between high strain and congenital malformations aORall = 0.99, 95 % CI: 0.85-1-15
Prenatal high strain exposure Asthma		The study found no association between high strain and asthma aOR = 1.08, 95 % CI: 0.92-1.27
Prenatal high strain exposure Atopic dermatitis	The study found an association between high strain and AD aOR = 1.15, 95 % CI: 1.02-1.31	

Table 6 Presentation of main results from the studies on the exposure to high strain and risk of preterm birth, SGA, LGA, congenital malformations, asthma and AD
** The brackets mean that no hypothesis was described prior to the statistical analysis regarding job strain and LGA.*

GOOD EPIDEMIOLOGICAL PRACTICES - RESULTS

The main results from the work with good epidemiological practice are the three protocols. The guidelines include eight points useful as a structured approach for documentation of the study procedures [11]. The following section presents how the points from GEP were addressed:

1. Organization and personnel: In the application for enrolment to Aarhus University (AU) the roles and responsibilities for both the organizations (AU and the National Research Centre for the Working Environment) and the individuals (PhD student and four supervisors) were described, as this is required by AU.
2. Facilities, resource commitment, and contractors: GEP suggest this point to include "adequate physical facilities", "sufficient resources" etc. As the work related to this thesis does not require special facilities, this point was not addressed.
3. Protocol: The main results of the GEP method were the protocols. By writing protocols for each study and having them approved by all authors before beginning statistical analyses, an attempt of a structured approach for documentation of study procedures was initiated
4. Review and approval: The protocol for the first study on preterm birth, SGA and LGA was peer-reviewed and published open access in BMC Public Health [144], the rest of the protocols were not published in journals. All protocols are approved by all authors by mail, signed and dated by at least three.
5. Study conduct: The studies have been conducted in accordance with the protocols. When small changes were made, they were described and motivated in the articles. Danish Data Protection Agency approved storage, handling and linking of data. GEP suggest all completed studies to be summarized in a final report. This is interpreted as papers (avoiding publication bias) and a final report that is this thesis.
6. Communication: Use of DNBC obliged the investigators to inform DNBC of results and to make the results available for the participants. The MINERVA group has conducted two information meetings, where results from all of the groups were presented in Danish. Also a homepage was established: www.minervanet.dk.
7. Archiving: Study protocols, articles, this thesis, statistical programs, correspondence, documentation of right to use and store data etc. can be presented at request.
8. Quality assurance: The GEP recommends that written procedures should be established to ensure the quality of data. We think that we have accomplished that by writing and following the protocols.

DISCUSSION

When focusing on maternal high job strain during pregnancy no associations were found to preterm birth or SGA. Women exposed to high strain during their pregnancy had lower risk of having a LGA child than women exposed to low strain. No effects were seen in relation to congenital malformations when exposed to high strain, and neither was there association for asthma in seven year old children. High strain exposure during pregnancy was associated with approximately 15 % higher odds of atopic dermatitis in the children relative to maternal low strain.

Methodological discussion

Selection bias

Selection bias might occur if the association between exposure and outcome differs in those participating in the study and those eligible but not participating [164]. In the case of DNBC this may

occur with non-participation at the time of the inclusion/interviews or by incomplete follow-up. The latter is quite unlikely in the studies included in this thesis as most endpoints rely on register data by use of the civil registration numbers, which must be assumed to include nearly everyone (a few could be missing due to incomplete information on migration or death). Selection bias due to incomplete follow-up on outcomes is therefore expected to be a negligible for paper I-III.

As stated in the method section, only 50 % of the GPs agreed to invite women to the DNBC and approximately 60% of the invited pregnant women chose to participate in the DNBC [139]. Selection bias could therefore occur both due to the GP and due to the individual women. The GP could introduce selection bias if he or she rejected to participate in the inviting-process due to socio-economical reason for example if the medical practices of non-participating GPs were geographically placed in areas with low socio-economic status, low socio-economic status could become under-represented in the DNBC. The GP could also introduce selection bias if he or she made their own selection of participant e.g. by not inviting women highly burdened by other issues e.g. stress in the home environment resulting in under-representation of this group. Studies of the DNBC show that participants have higher socio-economic resources in terms of education, occupation and income compared with the general Danish population [165].

On the individual level the women could decline to participate in the DNBC due to issues that could be related to stress. If the women exposed to the highest job strain did not have the resources and energy to participate in the DNBC, it would give an under-representation of the most exposed group. The same reasoning holds for women that agreed to participate in the DNBC, but subsequently declined one or more interviews.

If selection bias should have been avoided, the study should have been based on population-based registries and involving no contact to participants (this can be done with permission from the e.g. the Danish Data Protection Agency, the Danish National Board of Health etc.). But registries would not have provided us with the information we needed that had to be obtained by self-report on both exposure to job strain and data on covariates e.g. consumption of alcohol during pregnancy.

Regarding outcome measures; selection bias in regards to outcomes drawn from registers are as said previously expected to be negligible as they are based on personal identification numbers [142]. Concerning asthma and AD, selection into the cohort is not expected to introduce bias as register data indicate that associations e.g. for childhood asthma are not biased by non-participation [166,167]. It cannot be ruled out if the availability of ultrasound examinations and terminations of pregnancies due to malformations could have affected the participation rate in the first questionnaire, as some women may choose an abortion based on information on malformations. But as most of these ultrasound examinations are performed quite early in pregnancy (median week 15 of gestation), we expect most women to have answered the exposure questions before knowing the outcome of their pregnancies.

Information bias

Information bias may occur if the exposure or outcome measures are subject to misclassifications – this can be either differential (vary between the exposed groups) or non-differential (be the same in all groups) [164]. Differential misclassification can bias the observed estimate either towards or away from the null value, whereas the non-differential misclassification tends to bias the effect estimate towards the null value [138,168].

Misclassification of exposure

The participating women were only asked once during pregnancy about their exposure to psychosocial work environment. It is therefore not known whether the exposure was at the same level throughout pregnancy or if it changed. This is not only relevant for the women that reported high strain in early pregnancy, but were in fact less strained later on as they may experience effects of this, but also for the women reporting low strain, but might have been highly strained later. This implies a risk of differential misclassification, as some types of jobs might entail risk for higher stress towards the end of pregnancy compared to others e.g. jobs where some of the work needs to be handed over to a substitute or finished before maternity leave. Misclassification could also occur due to the women's interpretation of the questions if some women answered based on "right now". For the latter, we assume potential misclassification to be non-differential.

Without discussion the job strain variable used here is the potential Achilles' heel in the thesis. When the questionnaires were developed for the DNBC the main purpose was not to give a complete overview of the women's psychosocial work environment. The DNBC was constructed to get information on maternal health, job exposures, life style etc. to enable study of how the time from conception to birth could have importance for the child's health conditions [139]. The psychosocial work environment was just a little piece of the puzzle. I believe that all users of DNBC data would have liked to have just one or two questions more included with reference to their own research.

The inclusion and use of only two items versus the full Job Content Questionnaire (JCQ) developed by Karasek [169] may however, have precluded detection of the full spectrum of job strain. As far as known, no studies have looked at the validity of using global questions in regards to Karasek's model. Comparisons of single-items measure on stress vs. fully validated multi-item instruments have been done by Littman et al. [170]. In the study 218 adults living in Washington State in the US were selected to evaluate a 3-month test-retest reliability and inter-method reliability of stress questions. Littman and colleagues assessed the stress questions by comparing two single-item measures on stress with three fully validated multi-item instruments on perceived stress, daily hassles and life events which assessed the same underlying constructs as the single-item measures. The two single-item measures that were constructed in the study were considered reliable at measuring stress with validity similar to longer questionnaires.

The demand and control variables were combined to form the job strain variables as suggested by Karasek [33]. But not all agree on this way of handling demand and control. Some find that the analysis of the effect of job strain should be done by including the main effects (job demands and job control) in the model and testing for interaction between those main effects [171]. For the studies in this thesis, it was hypothesized that women experiencing high strain (as defined by high demands and low control) had higher risk of having children with the different outcomes compared to the women exposed to low strain (low demands, high control), no hypothesis was constructed for the main effects of demands and control. Further on, the interaction-method has an important logical shortcoming when applied to the studies in this thesis; freedom from interaction effects on the

logarithmic scale implies interaction effects on an ordinary scale and vice versa.

Information on exposure to psychosocial work environment is collected as self-reported data in the first interview. This precluded inclusion of more objective measures associated with stress response e.g. blood or urine samples for cortisol [172], even though cohort studies combined with objective measures are called for [173]. The exposure data used in this thesis is however, collected prospectively, relatively early in pregnancy and before the women knew the outcome of the pregnancy. Also, objective measures are not always easy to interpret; a Danish study has measured cortisol levels in pregnant women. Women who had experienced more than one stressful life event had 27% higher evening cortisol concentrations in late pregnancy. In early pregnancy women who had experienced stressful life events, did not have higher evening cortisol levels, but tended to have a blunted morning HPA response. This indicates that the response to stress is dependent on the stage of pregnancy [174]. Further, some researchers find subjective measures of stress to be the best predictor of adverse birth outcomes e.g. in relation to birthweight [175].

The non-associations found between high job strain and preterm birth, SGA and congenital malformations could reflect a true absence of effect i.e. that maternal stress does not biologically interfere with these outcomes. It could also reflect that the preventive measures taken at the woman's work, e.g. sick-leave, replacement are sufficient to avoid effects of job strain. But it is also possible, that due to selection, the non-effects reflect "a healthy worker effect", that the high strain group includes the women who are best at handling stress. The women who are not able to cope with stress have left the group, sent home, not participating etc. To crudely control for a healthy worker effect gestational age at interview were included as a covariate. The women should be working at the time of the initial interview to be included in the study. To require that the women should be working e.g. the tenth week of the pregnancy may however differ from requiring that the woman should be working at the 19th week of gestation. Therefore "gestational age at interview" is included to crudely control for potential differences.

At last, there is also the possibility that the questions included in the studies for this thesis, are not sufficient enough to measure job stress. We did see effect on LGA and AD, suggesting that high job strain during pregnancy has an effect on the unborn child. We therefore assume that the exposure variable to some extent do measure job strain, knowing the risk of selection bias, misclassification and healthy worker effect.

Misclassification of outcomes

Gestational age/preterm birth

GA was withdrawn from the MBR for use of determining preterm birth and also in regards to SGA and LGA. The GA registered in MBR is based on information from midwives after birth. GA could either be determined by calculation of the number of days from the last menstrual period or by use of ultrasound examinations. Both methods have their own drawbacks; the calculation method with irregular cycles, bleedings, trouble remembering last menstrual period and the ultrasound examination with measurements errors (the measurements are made by hand by the ultrasonographer). The use of ultra sound examinations in pregnancy has increased in the years where baseline data were collected for the DNBC [176], clinical maturity of the child at birth could therefore be introducing bias if GA is primarily based on calculations for the early cohort and mostly of ultrasound examinations in the late cohort. The recording of gestational age was validated during 1996-2002 where researchers compared self-reported gestational age data from the DNBC with ultrasound measurements from medical records in the county of Vejle and with the gestational age reported to NPR. In the study they concluded that self-reported GA in the DNBC are in reasonably good concordance with data from the NPR and with ultrasound-estimated GA, but statistical differences do exist [177]. GA determined by ultrasound is up to 2-3 days shorter than those based on calculations [177]. Bias could be introduced if the GA is based on ultrasound examinations early in pregnancy and high job strain affect the growth of the child early in pregnancy. In this way the foetus would be assessed to be younger (e.g. 10 weeks), even if it is not (e.g. 12 weeks) – implying date of birth being sooner than expected, resulting in higher reporting of preterm births – which were actually term births [178]. Despite the many possibilities we do not expect bias due to determination or reporting of GA to have high impact on this study.

SGA/LGA

SGA was used as a measure of foetal growth, but there are problems using SGA instead of low birthweight. SGA is widely used and defined as the lowest 10 % of babies born in a given week for each gender therefore the prevalence of SGA will always be 10 % and cannot be lowered by preventive measures. Also SGA defines all small children (the lowest 10 %) as growth restricted, but not all small children are growth restricted, some are just small due to natural causes. Also some children not included among the lowest 10 % could be growth restricted too [179]. In relation to job strain, we found no association to SGA but found a lower risk of having LGA children in women exposed to high strain. It may be that the whole weight curve is shifted to the left i.e. in general the birthweight is affected, but nothing that can be seen among the lowest 10 % - it could affect the normal growth but not necessarily enough to lower the child's birthweight to be in the lowest 10 %.

When using the multinomial logistic regression method in the first study, the children were divided into four exposure groups when looking at preterm birth, SGA and LGA; 1) full term and normal weight for gestational age, 2) preterm 3) full term but SGA or 4) full term but LGA. We are aware that some children will in addition to being born preterm also be defined as SGA or LGA. However as only 5 % of all cases are preterm and 10 % of all cases are SGA, a total of 0.5 % of the cases are both preterm and SGA. The expected number of preterm*SGA in the high strain group is then less than the number of parameters in the model. A further division of the preterm category is therefore not feasible.
An advantage of this outcome categorisation which also was used in the study of asthma and/or AD is a clean reference outcome (full term and normal weight for gestational age or no asthma and no AD) with which the other types of cases can be contrasted. Multinomial logistic regression models is not widely used, even though it has some clear advantages; when testing multiple outcomes in one test, the risk of mass-significance decreases and the power of the study increases.

Congenital malformations

As with the information on birth weight, we collected this information from the MBR and rely on the information given to the register at the time of birth. The issue with malformation is that the ones reported to the MBR is the malformation found at birth,

but some malformations e.g. heart malformations may be detected later on, and is therefore not included in the study.

The study on congenital malformations did not reveal any associations to job strain. But as we only got information from congenital malformations detected at birth, we cannot rule out the possibility of association to malformations detected later in life. At the same time, there might be differences in the timing of detection of eventual malformations; it may be that women experiencing high strain also have less energy and resources after birth, being less sensitive to react to signs from the child etc.

Asthma and AD

When defining children as asthmatic or having AD the "diagnosis" is based on reports from the mothers. There were no objective diagnoses from physicians. Differential misclassification can therefore occur if the reports from the mothers can be affected by their exposure to job strain during pregnancy. Mothers who are stressed while pregnant, might also be stressed after the birth, which could affect their care and parenting of the child [180] and studies have shown, that exposure to stress in early childhood is associated with an atopic immune profile in the children predisposed to asthma [181].

The questions used in the DNBC in regards to asthma are standardized and validated questions from the International Study of Asthma and Allergies in Childhood (ISAAC), which have been developed and used in more than 155 collaborating centres in 56 countries [151]. One study in the DNBC compared three ways of measuring asthma, being self-reports from the mothers, ICD-10 codes from physicians when hospitalized appearing in registers and data on asthma medication from the Prescription Registry. The three ways of detecting asthma resulted in three different prevalences, with the highest among asthma defined by use of medication (32%), then self-reports (12%) and finally hospital-diagnosis (7%). Further on, they found a substantial non-overlap between the methods [182]. It is therefore rather difficult to determine which way is the most correct. The mothers could over-report or under-report, the medication been given to children with "asthma-like" symptoms, but not having asthma, and the hospital may only see the worst cases, leaving many less severe cases out of the equation.

Similarly, the diagnoses for AD children were not confirmed by a physician. AD was defined as a combination of maternal reports of AD and of eczema in places typically for AD. The questions used has been validated in a clinical study and evaluated to be suitable for large scale epidemiological studies [183].

Confounding, residual confounding etc.

A general strength of self-reported prospective cohort data is the possibility to include an extensive number of covariates which reflect the lifestyle of participants in the analyses. In the DNBC the women have reported a wide range of lifestyle factors as smoking and use of alcohol, parents' heights and use of medication during pregnancy etc.

Women with missing values on covariates were excluded from the study. It can be discussed, if this is the best way to handle missing values. There could be selection bias if women with e.g. high consumption of alcohol do not reply to the alcohol consumption questions. These women could have been in the high strain group, but is excluded due to missing information. The normal procedure of assigning the mean value of the group for the explicit covariate is not applicable here. It would be as wrong to give a heavy drinker a mean value – as it would be to give a non-drinker a mean value. Furthermore, the dataset is so large, that it can be justified to exclude the women with missing values. Flowcharts included in the protocols describe the inclusion/exclusion criteria thoroughly.

A confounder is a factor associated to the exposure but also affecting the outcome. The covariates included in the analyses for this thesis are mostly treated as confounders, adjusting for them in the analyses. All covariates are chosen a priori based on the literature in the field - based on mainly their relationship to the outcomes. They are all described in the related protocols. Even though several covariates were included, we cannot rule out that some are missing, or should have been treated differently, e.g. as a mediating factor.

Socio-economic status (SES) was not included as a covariate. This was done to avoid over-adjustment. Your level of education, income etc. is a strong determinant for the job you have. Your job may be strongly correlated to the exposure to job strain. This could for example be in terms on influence (our proxy for control in the job strain model), as control is often high in certain types of jobs that is only available with a higher education and therefore higher income. If SES was included in the model, some of the exposure could therefore be adjusted for. The covariate "manual work" was however included to crudely adjust for blue-collar vs. white-collar job positions and thereby social class. Adjustments were also not made for ethnicity or race; even though for example black women have a higher risk of early preterm births than other ethnic groups even after adjustment for medical, psychosocial and behavioural risk factors [184]. The reason for not adjusting for ethnicity is that for enrolment in the DNBC all women had to speak Danish sufficiently well to participate in telephone interviews and the cohort is therefore quite homogenous in ethnicity. As a consequence, the reported results may apply only to ethnical Danish women and women with a high degree of integration in the Danish society.

For the analyses on preterm birth, SGA, LGA and congenital malformations, the included covariates were based on information obtained during pregnancy. For asthma and AD also postnatal factors were available from the postnatal interviews. Information on breastfeeding was available from the 3rd and 4th questionnaires, but only approximately 1 % of the women responded that they never breastfed their child. Control for breastfeeding would therefore have had to be extremely skewed to affect the outcomes and was therefore not include in the analyses. Furthermore, the results from studies on breastfeeding and allergic diseases are a bit diverging. Some find a protective effect of breastfeeding on asthma [185,186] and others an increased risk of AD after breastfeeding [187,188]. Similarly, day-care attendance is positively associated with asthma, but early day-care seems to protect against asthma later in life (reviewed in [189]). Questions on day-care were included in the DNBC interviews, but with more than 90 % of the children attending day-care, it was not relevant to include this variable in the analyses.

All covariates were selected a priori based on previous literature. Both crude and adjusted risk estimates are presented, so the reader can assess any effects of the confounders. Significance tests on covariates were however not conducted. Irrespective of whether the test would be statistically "significant" or not, a difference in the distribution of a potential confounder between the strain groups could confound the results exactly as much as a large difference would do [190]. Furthermore, not only the magnitude of the difference, but also the size of the study material

and to some extent the statistical methods chosen, would influence whether covariates were significant or not.

Good epidemiological practices – pros and cons

With a study population of more than 100,000 pregnancies and 30,000 variables, 7 years of follow-up and the possibility to link to all sorts of additional registries, researchers are obliged to treat such data with respect. To follow good epidemiological practices seems to be the least we can do, not least to avoid fishing expeditions and uncontrolled exploration of the data. I have learned that ambitions and reality are not always easy to combine. Even though many researchers can see the benefits of defining and describing – and agreeing on – analyses before their execution, such procedures also represent barriers, obstacles and inflexibility

For all of the studies in this thesis, a protocol outlined the theory, hypotheses, and statistical models before any analyses were initiated. This means that for every study a very detailed description of the study, background, research questions, aims, full information on all variables and their definitions and use, how analyses should be handled and executed, presented and to some extent interpreted, were included. All authors have read and commented on this, the protocol has been thoroughly discussed until everybody agreed – implicating that only small changes could be made to the actual analyses – and only if it was clearly commented where the analyses in the study varied from the analyses described in the protocol. Even though you can say, that this work is always done in relation to writing up a manuscript, very seldom are all authors involved in selecting and defining even the smallest of covariates, how data should be presented etc. So protocol writing can be quite time consuming and include a bit more work for everybody involved.

The GEP recommends the protocol should be reviewed and accepted by a person outside the research group. This was initially interpreted as the protocol should go through peer-review and be published in an international journal – preferable with open-access for accessibility. The protocol for the first study is therefore published in BMC Public Health with open-access [144]. Several issues needed to be discussed in relation to this; first – it was not easy to find a journal that accepted to publish study protocols. Secondly – even when a willing journal was identified, it was not easy to get it through proper peer-review, which was a very important issue. Thirdly – when writing the article based on the protocol, it was time consuming to figure out new ways of describing the study, background etc. so that we could not be accused for plagiarism even though we were describing the same things. Fourthly - it is quite expensive to publish open access (10.000 - 15.000 DKK) and last – a peer revision takes time, time where you are not able to work on the main articles because you need the protocol to be accepted first. Based on this experience, only the first protocol was submitted to a journal and published in the open literature. The rest of the protocols were discussed, read and approved by all authors, and signed and dated by three authors. In this way, the text from the protocol could be re-used in the final manuscripts without risk of accusation of plagiarism, and the spared time could be used for the actual analyses and study and publication hereof. The signed protocols would still be available upon request. Strict adhesion to the principles of GEP may however imply both increased costs and time.

To my knowledge, no journals requires use of GEP (as yet), but many international biomedical journals now requires STROBE check lists to be filled out and submitted together with the manuscripts [191]. STROBE stands for STrengthening the Reporting of OBservational studies in Epidemiology and represents international collaboration between researchers in epidemiology, methodology, and statistics together with journals editors [192]. STROBE and GEP shares many similarities as to the report of what was planned (and not planned), done, found, and interpreted. The difference is that STROBE is a tool for reporting and publishing data, whereas GEP should be included already when the project is planned. Application of GEP are therefore suggested to lead to better designed and executed studies, but also to more rigorous reporting of results in biomedical journals, presenting valuable research for use by public health authorities [11].

Main findings in light of other studies

Preterm birth

As presented in paper II, high job strain during pregnancy was not associated with increased risk of preterm birth. This finding is consistent with the four other comparable prospective studies on job strain and preterm birth, which showed no effect of high strain either [60-63]. Three case-control studies on job strain, mentally demanding jobs and self-perceived job stress support these null-findings [66-68].

In addition to job strain, one of the prospective studies included effort-reward imbalance as a way of measuring job stress. Higher levels of effort-reward imbalance were associated with lower gestational age at birth [63]. These findings suggest that the two models (job strain model and effort-reward imbalance model) could be detecting components of job stress contributing differently to the mechanisms underlying gestational age at birth. Job strain was associated with preterm birth in two case-control studies; in one case-control study the effects were observed in black women only [64]. As previously mentioned ethnicity is not included as a covariate in this thesis. To participate in the DNBC all women needed to speak Danish well enough to be able to answer the telephone interviews and we must also assume that the Danish population is not as heterogeneous as the American. So even if the variable were included, it would not be certain, that the same effects would appear in the studies included in this thesis. In the other case-control study an association between high strain and preterm birth were also seen, but only when combined with low or moderate support [65]. The sub-analysis on social support presented in paper II lends some small supports for this. Thus, the risk of preterm birth seemed to increase in the high strain group the lower the level of social support, although the results are statistically insignificant.

Only the two cross-sectional studies found an association between job stress and risk of preterm birth. In one job strain was assessed by job titles [69], which differ from the subjective exposure assessment included in this thesis. When doing cross-sectional studies, recall bias offer a potential explanation of the differences in findings relative to the prospective studies In addition, one prospective study found that women with the same job titles reported very differently on job demands and job control [61], and job titles may therefore not be a valid instrument alone to assess job strain. The other cross-sectional study showed that job strain was related with preterm birth, but only for women not wanting to stay in the work force during their pregnancy [70]. In the sub-group of the DNBC on which the present study on preterm birth is based, one of the inclusion criteria was that the woman was working at the time of the baseline interview. Furthermore, more than 90 % of the women worked between 30 and

40 hours per week (fulltime). No information about "wanting to stay in the work force" was available, so this could not be adjusted for. As there are big differences in the legislation regarding pregnancy and maternity leave in the US, where the cross-sectional study was carried out, and in Denmark with the DNBC, it might not be an issue here.

SGA/LGA

The results from the first study on SGA and LGA showed no association between exposure to high job strain and risk of SGA, but high strain seems to be protective against LGA, indicating some effect of job strain on birthweight.

The results from the study are as diverging as the results from the review (Table 2). Three large prospective studies [60-62], a case-control study [68] and two cross-sectional studies [69,70] found no association between job stress and low birthweight or SGA, which support our findings. Three other prospective studies [63,80,81] and a case-control study[66] found effects of job stress on birthweight or SGA, which to some extent support the finding on LGA. No studies have included LGA as an outcome measure.

The majority of the studies included job strain as the exposure variable. One study found high control to be positively associated with birthweight, as high strain includes low control it should result in lower birthweight. In this study job strain is included as suggested by Karasek [32], with the combination of demands and control. Information on control alone is therefore not included. But should it only be control which was responsible for effects of birthweight, the passive quadrant (low control, low demands), should also have been significantly associated with SGA/LGA. This was not the case.

One study only found effects of job strain on the outcomes if the mothers worked 32 or more hours per week [81]. As mentioned in the previous section, most of the women included in this analysis worked full-time. It is therefore not possible to distinguish between them according to working hours. It is however plausible, that women working more hours per week are also at higher risk for work-family conflicts [193].

In the review of literature concerning job stress and risk of low birthweight or SGA, no distinction was made between the two outcomes, but as described in previous sections, low birthweight and SGA is not the same, as the latter has taken gestational age into account. For the six prospective studies which are the most comparable, there seems to be a pattern of outcomes and associations. If the study had included birthweight, an association was reported [63,80,81], if the study had included SGA no associations were reported [60-62].

Congenital malformations

Results from the study on congenital malformations showed no association to any kind of malformation or specifically to malformations in the circulatory or musculoskeletal systems. Unfortunately, there was not power to observe other types of malformations. Furthermore, only a single study included job stress as the exposure [66], making it difficult to compare the studies. The study which included job stress was a Danish case-based study with use of questionnaires for exposure and register information for endpoints. The findings of no association between job stress and congenital malformations support the findings presented in this thesis.

All three prospective studies found in the literature search, were Danish register-based studies on bereavement finding associations to cranial-neural-crest malformations [96], oral cleft [97] and congenital heart defects [98]. This means that the exposure was objectively measured, as they are collected from the registers based on information on partner, child, and parents' deaths. The discussion could therefore be on objective and subjective measures of stress. Even though many researchers can see the benefits of objective measures to avoid bias, is it also known that subjective measures are sometimes the best way of measuring exposure when it comes to birth outcomes [175]. Further on, bereavement is a massive stressor, with an acute and long-term effect, whereas job strain often is addressed as chronic mild stress. One of the bereavement studies showed that the association was even more marked, if the mothers had lost first-degree relatives e.g. a child or partner compared to mothers who lost a sibling or a parent, indicating that intensity also plays a role [98]. Results from animal studies support this, as the amount of stress experienced by the mice has to be quite high to induce malformations in offspring [84,194]. Power calculations made it possible for us to look at malformations in the circulatory or musculoskeletal systems. Most of the previous studies looked at neural tube defects [100-102], cranial-neural-crest [96] or cleft lip [97,100]. Only a single study included congenital heart defects [98]. No studies included malformations in the musculoskeletal system.

All in all, it is a bit difficult to compare the results from this thesis with the available literature. It is clear however, that if you want to look at specific malformations you either need an objective measure of exposure e.g. bereavement, which can be detected in registers and thereby give you the opportunity to have very large cohorts or to do case-control studies, which makes it possible to identify enough cases to give the power to interpret the studies.

Asthma and Atopic Dermatitis

The main findings from the studies on exposure to high strain and risk of asthma and AD in the children, showed no association to asthma both with and without AD, but we did see an association to AD alone. As with congenital malformations, the number of previous studies which had included job stress as exposure measure is limited – for asthma none and for AD a single one.

The single prospective study on work stress and AD found in the review of the AD literature supported the findings in paper IV [134]. So did the other studies on maternal stress [135], maternal psychological and social stress [136] and the cross-sectional study on stressful life event [133].

For asthma, one cohort study showed an association between mothers experiencing anxiety during their pregnancy and asthma in their children [130]. The problem with this exposure is the difficulties in assessing whether the anxiety is only related to the pregnancy or if it affects the life after birth. Anxiety might also be so different from high strain in so many aspects, that it is too difficult to compare. The two other cohort studies on asthma both included bereavement as exposure [131,132], and the cross-sectional study on life events also included bereavements [133] and as previous discussed, bereavement is a massive stressor which like anxiety might differ so much in impact, that it might not be comparable with job strain.

PRACTICAL IMPLICATIONS

For many of the investigated outcomes, maternal job strain exposure did not seem to have high impact on the health of the child.

For LGA and AD it did show some impact. Not high impact, but some.

In the Danish work environment recommendations for women being pregnant or breastfeeding a number of risk factors are listed in order to keep the women safe when she works; Physical strain (vibration, noise, heat, radiation, prolonged standing and walking etc.) , infectious exposure (childhood diseases, hepatitis, q-fever etc.) and chemical exposure (carcinogenic substances, endocrine disruptors, organic solvents, pesticides etc.). If the woman is exposed to any of the risk factors her employer is obliged to make changes in the working environment or in the planning of the work situations. If that is not possible the pregnant or breastfeeding woman should be replaced or sent home [195].

Psychological or psychosocial factors are only briefly mentioned in the end of the section of physical strain; "If there is uncertainty as to whether the pregnant woman or her unborn child is sufficiently protected from the physical strain at work, the women should contact her GP for a comprehensive risk assessment that in addition to physical factors could include long working hours, recurring overtime, long commuting, shift work (including work at night) or stressful work situation" (translation from Danish) [195].

More research is needed involving exposure to job strain during pregnancy and e.g. risk of allergic diseases, but if the findings from this study can be found in other studies, the recommendations might need rewriting.

CONCLUSION

The studies included in this thesis aim to contribute to the diverging or very limited literature in the fields of maternal exposure to job strain and risk of preterm birth, SGA, LGA, congenital malformations, asthma and atopic dermatitis. The work was based on the large and prospective Danish National Birth Cohort.
All studies have been conducted in line with Guidelines for Good Epidemiology Practices for Occupational and Environmental Epidemiologic Research protocols was therefore developed for each study.

It must be made clear, that all studies have been done from a public health perspective. All studies are made from an observational view, and not in a controlled experimental environment. All kinds of interventions take place during the pregnancy, planned as well as incidental, from colleagues, managers and the employing company to relieve some of the strain the woman might experience. Other interventions might originate from the woman or the health care system as a consequence of the repeated health examinations offered during the pregnancy in Denmark. From a public health perspective the primary interest is not if job strain has an effect per se but if an effect of job strain resides after preventive measures have been taken, i.e. does the preventive system work, and are the measures taken sufficient to protect the woman.

No associations were found between exposure to high strain (high demands, low control) during pregnancy and preterm birth, SGA, congenital malformations and asthma in the children. A protective effect on LGA was observed, suggesting an impact on the birthweight although it was not seen among the SGA children. An association between high strain exposure and ever AD in seven-year-old children was observed, with 15 % higher odds of AD when compared to women exposed to low strain during their pregnancy. The effects of high strain found were modest, but even modest effects might be relevant with high prevalence as with AD (lifetime prevalence of 15-20 %). But as this study is the only one of its kind, other studies are needed to replicate the findings.

PERSPECTIVES

The studies included in this thesis add to the rather limited knowledge of prenatal exposure to work-related stress end health implications for the child. Results from the analyses indicate that instead of focusing on "hard endpoint" as birthweight and preterm birth a shift towards "functional endpoints" could be relevant, therefore future research areas could be on e.g. risk of overweight and cognitive behavioural disorders in the children when the mothers are exposed to work-related stress.

During the latest years a rising focus has been on the overweight and obesity of children. Numbers from Denmark show an increase in overweight among boys age 15 years from 5 % in the early 1970s to 16 % in the late 1990s [196]. Overweight is an important issue as it is related to increased risk of cardiovascular diseases and type-2-diabetes [197]. High intake of calories and low exercise levels can only explain some of the rise. A programming effect of the regulation of the appetite already in the foetus is suggested to have an impact also [198], and it could be explained by changes in the regulation in the release of insulin and leptin by prenatal impact of the HPA-axis [199]. The research in this field is limited and could benefit of prospective long-term studies [200]. The DNBC have included questions regarding the child's weight and height as well as parental anthropometry in all questionnaires including the 11-year follow-up, where it would be possible to access data from in summer 2014.

Cognitive behavioural disorders including attention deficit hyperactivity disorder (ADHD) are the most common childhood psychiatric disorders [201]. Disorders like ADHD have a strong genetic component [202], but also early environmental exposures seem to have an impact [203,204]. Both animal [205] and epidemiological studies [206,207] indicate that stress exposure during pregnancy is associated with cognitive behavioural disorders in the child. In the DNBC questions regarding cognitive development have been included by use of the Strengths and Difficulties Questionnaire (SDQ), which is a brief 25 item behavioural screening instrument developed to assess e.g. ADHD in children aged 4-17 years [208]. The use of SDQ in the DNCB has been validated and has generally shown to have good psychometric properties [209,210].

As stated previously, the DNBC was not planned to focus specifically on job strain, and the use of only two questions to elucidate the whole area of job strain as suggested by Karasek is questionable. It would therefore be very informative in relation to the studies included in this thesis if it was possible to validate single items on job strain vs. full Job Content Questionnaire (JCQ). In the study by Littman and colleagues they compared self-constructed single-item measures (similar to the two questions included in the DNBC on job strain) with fully validated multi-item instruments (similar to the JCQ) [170]. It would require either a cohort where the both types of questions were asked or two comparable cohorts, where one have used single-items and the other the full JCQ - the latter being the most probable study as it would not require establishment of a new cohort and it is expected that no studies have included both already. An obvious choice of comparable material to validate stress exposure could be the Norwegian Mother and Child Cohort study (MoBa), which is a large cohort including more than 90.000 women, with three questionnaires during pregnancy and one when the child is 3, 5,

7, 8 and 12 years old [211]. MoBa have included questions useful in assessment of the psychosocial work environment e.g. stressful work ("Jeg har et stressende eller masete arbeid"), high demands ("Arbeitet mitt krever stor arbeidsinnsats") or control ("Jeg har muligheten for selv å bestemme, hvordan arbeidet skal utføres"). A big advance of using the Norwegian cohort is the possibility to link to health registers similar to the Danish registers, which includes information on birthweight etc., making it possible to include LGA. Further, the MoBa questions regarding allergic diseases at age 7 have also been included, which opens for the possibility for test of the results found in this thesis in regards to atopic dermatis.

Not only the studies included in this thesis would benefit of a validation of the exposure variable, but also the studies in the DNBC using the same variables [35,36] would be strengthened if the validation process showed the same results as in Littman's; that the two single-item measures were considered reliable at measuring stress with a validity similar to longer questionnaires [170].

LIST OF ABBREVIATIONS

11-βHSD2	11β-hydroxysteroid dehydrogenase type 2
AD	Atopic dermatitis
aOR	Adjusted odds ratio
AU	Aarhus University
BMI	Body Mass Index
CI	Confidence interval
CRH	Corticotropin-releasing hormone
CRS	The Danish Civil Registration System
DISCO	Danish version of the International Standard of Classification of Occupation
DNBC	The Danish National Birth Cohort
GA	Gestational age
GEP	Good Epidemiology Practices for Occupational and Environmental Epidemiologic Research
GP	General practitioner
HPA axis	The hypothalamic-pituitary-adrenal axis
ICD-10	International Classification of Diseases version 10
IgE	Immunoglobulin E
LGA	Large for Gestational Age
LMP	Last menstrual period
MBR	The Danish Medical Birth Registry
NPR	The Danish National Patient Registry
OR	Odds ratio
PTD	Preterm delivery
PTSD	Post-traumatic stress disorder
RR	Relative Risk
SAS	Statistical Analytical Software
SES	Socio-economic status
SGA	Small for Gestational Age

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SUMMARY

Psychological stress at work is a rising problem in Denmark. Nearly one third of the women reported in 2005 that they had difficulties completing their work tasks, and 17 % found that they had only limited or no influence on their work tasks. The corresponding numbers for 1987 were 18.3 % and 16 %, respectively. Work-related stress shortens the life expectancy and reduces the number of years without prolonged disease. For the society work-related stress amounts to more than 30,000 hospital admissions each year, half a million extra days on sick-leave for women, 500,000 contacts to general practitioners, 1600 early retirements for women, and an overuse of the healthcare system. With the second highest employment rate in Europe for women – and many of them in the childbearing age – effects of psychological stress at work may extend beyond the exposed individual and affect pregnancy, birth and health of the child. Few studies on job stress relative to pregnancy have been carried out, but both animal and epidemiological studies have shown effect of exposure to stressful conditions during pregnancy and adverse effects on the offspring.

The specific aims for the three studies included in this thesis were to investigate the association between maternal psychosocial job strain during pregnancy, measured as high demands and low control and the risk of:

- Having a child born preterm or with low or high birth weight relative to gestational week (paper I + II)
- Congenital malformations in offspring (paper III)
- Asthma and atopic dermatitis in the children (paper IV)

Furthermore, it was also the ambition to maximize and secure the quality of research and integrity of the data used by documenting the methods in a protocol that described the analyses before they were done and to keep transparency in the methods used following good epidemiological practices (GEP) for occupational and environmental epidemiological research.

All analyses in this thesis are based on information and data from the Danish National Birth Cohort (DNBC). The cohort was established in 1994 and baseline information from the enrolled 100,418 pregnancies was gathered from 1996 to 2002. At their first antenatal visit pregnant women were invited to participate in the cohort by their general practitioner. To contribute the women had to be pregnant, have intentions of carrying the pregnancy to term, reside in Denmark and speak Danish sufficiently well to participate in telephone interviews.

When enrolled the women were asked to participate in two telephone interviews during pregnancy at approximately 12-14 and 30-32 weeks of gestation and two after birth when the child was six and 18 months old and a follow-up questionnaire at age 7 years. Exposure to work-related stress was assessed based on information from the first interview on two questions regarding job control and job demands. These questions were interpreted as dimensions of demands and control, and hereafter used as a proxy for the dimensions of the job strain model by Karasek. Based on their answers, the women were divided into the four job strain categories; high strain, active, passive and low strain. Gestational age at birth, birthweight and congenital malformations were extracted from the Danish Medical Birth Register. The outcome variable on asthma and atopic dermatitis were based on maternal self-reports from the fourth (child 18 months) and fifth (child 7 years old) interviews/questionnaires.

All studies in the thesis were based on protocols describing methods, analyses etc. prior to handling. No associations were found between exposure to high strain (high demands, low control) during pregnancy and preterm birth, small for gestational age, congenital malformations and asthma in the children when compared to women exposed to low strain (low demands, high control). A protective effect on large for gestational age was observed when exposed to high strain, suggesting an impact on the birthweight although it was not seen among the small for gestational age children. An association between high strain

exposure and ever atopic dermatitis in seven-year-old children was observed, with 15 % higher odds of atopic dermatitis when compared to women exposed to low strain during their pregnancy. The effects of high strain found were modest, but even modest effects might be relevant with high prevalence as with atopic dermatitis (lifetime prevalence of 15-20 %). The studies included in this thesis add to the rather limited knowledge of prenatal exposure to work-related stress end health implications for the child.

In the Danish work environment recommendations for women being pregnant or breastfeeding work-related stress is only mentioned very briefly. Findings from this thesis do not support rewriting in regards to risk of preterm birth, low birth weight or malformations. Results did show effects in regards to allergic diseases, but as the study is the only one of its kind, other studies are needed to replicate the findings if to reconsider the recommendations for pregnant women.

Future research could benefit of validation of the use of two single-item measures of job strain compared to fully validated multi-item instruments. A shift toward more "functional endpoints" as overweight and cognitive behavioural disorders in the children could be of relevance.

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