Prenatal Ultrasound and X-ray - Potentially Adverse Effects on the CNS

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Abstract

The aim with this thesis was to assess the impact of prenatal ultrasound exposure on psychotic illness, childhood brain tumors (CBT) and school achievement, and to evaluate prenatal X-ray exposure and the risk of CBT.

In a cohort study, children born in Malmö 1973-1978, where prenatal ultrasound was used routinely, were considered exposed (n=13, 212) and children born at hospitals with no use of ultrasound, were considered unexposed (n=357,733). Exposed men had a tendency toward a higher risk of schizophrenia. For other psychoses there were no differences between groups. Other factors related to place of birth might have influenced the results.

In a case control study, children born 1975-1984 with a diagnosis of CBT (n=512), and randomly selected control children (n=524) were included. Exposure data on X-ray and ultrasound from antenatal records was completed with information from the Medical Birth Register. We found no overall increased risk for CBT after prenatal X-ray exposure. When stratifying by histological subgroups, primitive neuroectodermal tumors had the highest risk estimates. For ultrasound exposure, no increased risk for CBT was seen and numbers of examinations or gestational age at exposure had no substantial impact on the results.

In a follow-up of a randomized trial on prenatal ultrasound scanning 1985-87, we assessed the children’s school grades when graduating from primary school (15-16 years of age). We performed analyses according to randomization, ultrasound exposure in the second trimester and exposure at any time during pregnancy. There were no differences in school performance for boys or girls according to randomization or exposure in the second trimester. Boys exposed to ultrasound any time during fetal life had a reduced mean score in physical education and small, non-significant increased risk of poor school performance in general.

Keywords: ultrasound, X-ray, fetus, risk, childhood cancer, schizophrenia

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To Ester, Alice & Peter
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This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Stålberg K, Haglund B, Axelsson O, Cnattingius S, Hultman CM and Kieler H
Prenatal ultrasound scanning and the risk of schizophrenia and other psychoses
_Epidemiology_. 2007 Sep;18(5):577-582

II. Stålberg K, Haglund B, Axelsson O, Cnattingius S, Pfeifer S and Kieler H
Prenatal X-ray exposure and childhood brain tumours, a population based case control study on tumour subtypes.
_Br J Cancer_. 2007 Dec 3;97(11):1583-7

III. Stålberg K, Haglund B, Axelsson O, Cnattingius S, Pfeifer S and Kieler H
Prenatal ultrasound and the risk of childhood brain tumour and its subtypes
_Br J Cancer_. 2008 Apr 1;98(7): 1285-87

IV. Stålberg K, Axelsson O, Haglund B, Hultman CM, Lambe M and Kieler H
Prenatal ultrasound exposure and children’s school performance at age 15-16; follow-up of a randomised controlled trial
_Submitted for publication_

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### Abbreviations

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<td>CBT</td>
<td>Childhood brain tumor</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IRR</td>
<td>Incidence rate ratio</td>
</tr>
<tr>
<td>ISPTA</td>
<td>Spatial peak temporal average intensity</td>
</tr>
<tr>
<td>MBR</td>
<td>Swedish Medical Birth Register</td>
</tr>
<tr>
<td>mGy</td>
<td>Milligray</td>
</tr>
<tr>
<td>mSv</td>
<td>Millisieverts</td>
</tr>
<tr>
<td>NRN</td>
<td>National registration number</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PNET</td>
<td>Primitive neuro ectodermal tumors</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>USW</td>
<td>Ultrasound waves</td>
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</table>
Introduction

When evaluating potentially harmful effects of exposure to environmental factors in fetal life, it is necessary to do so in the context of embryological development. For most organs in the body, it is possible to detect a specific vulnerable gestational period, as was exemplified by the specific malformations caused by thalidomide. For the central nervous system (CNS), the association between exposure and effects is complex; the development of the CNS involves several intricate procedures proceeding from the embryologic period to childhood. Moreover, outcomes such as intellectual impairment, psychiatric disorders or malignancies may not be manifest until decades later. Therefore a life course perspective should be adapted when evaluating interventions in fetal life, particularly when CNS effects are suspected.

The use of prenatal ultrasound scanning has been introduced worldwide over the last 20-30 years. In many countries today, pregnant women are scanned on a routine basis and the energy levels from ultrasound machines have increased several fold. The use of prenatal X-ray for fetal indications has decreased but is still unavoidable in some situations. In these cases, an evidence-based approach that takes into account the risks of radiation is essential. We have good reasons to believe that the CNS is particularly sensitive to biological effects of ultrasound and ionizing radiation compared to other organs. This thesis assesses prenatal ultrasound as a possible risk factor for different CNS outcomes; psychotic disorders, childhood brain tumors (CBT) and school performance, including intellectual and motor skills. The association between prenatal X-ray exposure and CBT is also examined.

Development of the CNS and vulnerable mechanisms

The effects a particular type of exposure causes in the CNS are directly related to the cell types and developmental processes that are affected and the time at which the exposure occurs. External or internal factors can influence the structure and function of the developing brain by modifying one of the basic processes of cell proliferation, cell migration or cell differentiation.
The formation of the brain begins at the end of the third week after conception, by folding of the fetal ectoderm into the neural tube, which later forms the CNS, and the neural crest, which forms most of the peripheral nervous system. The proliferation of neurons reaches its maximum speed between the 10th and the 20th gestational week, with over 200,000 new neurons being created per minute \(^4\). In a study of children exposed to high radiation levels \textit{in utero}, at the atomic bombing in Hiroshima and Nagasaki, the highest risk of forebrain damage and mental retardation occurred at 8-15 gestational weeks. Overall, the risk was five times higher or more during these weeks than in subsequent weeks. Furthermore, no increased risk was observed before gestational week \(^8\).\(^5\) It was previously believed that neuronal proliferation only existed during fetal life and early childhood, but in recent years, neural stem cells have been identified in the adult mammalian brain.\(^6\) The capacity of these cells is still not fully understood.

Neurons can move from the proliferation zone (mainly in the epithelium lining the ventricles of the brain) to their final destination in two essentially different ways. Passive cell displacement, by which new cells “push” previously formed cells further away, or an active process called neuronal migration.\(^7\) Migrating neurons appear especially in cerebral cortex and in subcortical areas and the mechanism of movement is believed to be through guidance by glial fibers.\(^3\) As neurons migrate, intra and extra cellular signals influence gene expression which initiates the process of differentiation.\(^8\) Factors that alter neural proliferation and migration often result in altered differentiation as well, which has been illustrated in X-ray irradiation in rodent models.\(^9\)

When the neurons have reached their final position, they begin to sprout axons and dendrites. Each neuron also requires specific enzymes to produce neurotransmitter(s) and receptors are needed to receive input from presynaptic neighbor neurons. Simultaneously, glia cells differentiate into astrocytes and myelin-producing oligodendrocytes.\(^3\) The myelination process continues long after birth. Later in fetal development, excessive synapses are eliminated and unnecessary axons are withdrawn. Apoptosis of neurons is another mechanism by which functional units are sorted out. It is hypothesized that natural loss of axons of the corpus callosum may be the underlying mechanism of right hand preference and functional brain asymmetries, and that this process could be influenced by prenatal and early postnatal events, especially in men.\(^10\) According to this hypothesis, premature birth could interrupt the normal process of axon loss in the corpus callosum and children born extremely premature have shown an increased prevalence of left-hand preference.\(^11,12\)
Ultrasound; physics and biological effects

In a study on pregnant mice, the effect of ultrasound waves (USW) on neuronal migration within the fetal cerebral cortex was evaluated. The results revealed that, when exposed to USW for a total of 30 min. or longer during the period of migration, a small but statistically significant number of neurons failed to acquire their proper position in the cortex and remained scattered within inappropriate cortical layers and/or in the subjacent white matter. The magnitude of dispersion of labeled neurons was variable but increased systematically with duration of exposure to USW. Even though the significance of these results on humans has been debated, they illustrate a potential biological explanation for the epidemiological findings on prenatal ultrasound and increased prevalence of non right-handedness (including left handedness and mixed handedness) in men.

According to the World Federation for Ultrasound in Medicine and Biology (WFUMB), possible effects of USW on human tissue can be divided into thermal and non-thermal. Temperature increase in mammalians exposed to USW depends on the properties of the ultrasound field parameters and the biological tissue exposed. The important tissue properties in this matter are the acoustic absorption coefficient (i.e. how much heat is deposited), thermal conduction and blood perfusion. The absorption coefficient increases with increasing protein, particularly collagen, content. Body fluids have low absorption and the highest values are seen in bone. In fatty tissue, periostium and bone, the blood perfusion, which has a cooling effect, is low, and these tissues are more susceptible to heating by USW. The amount of ultrasound-induced heating correlates with gestational age and bone development (mineralization and ossification of bone starts in the 12th gestational week). The CNS tissues have a low absorption coefficient, but are encased in the skull or vertebrae and can be heated by conduction. In the guinea pig, 2 minutes of exposure to pulsed ultrasound (similar to pulsed Doppler) in utero resulted in a temperature increase of 4.3 °C in the brain close to bone and 1.1 °C in the midbrain.

The radiation force generated by the ultrasound pulses causes fluid to be pressed away from the transducer (streaming). This effect has been noted in blood, breast cyst fluid and abscesses. In adult soft tissue, streaming forces by diagnostic ultrasound are not believed to cause damage. However, in embryonic tissue the strength of the extra-cellular matrix is not fully established and the human brain is in liquid phase in early gestation. Thus, streaming could possibly cause biological effects. Another non-thermal mechanism is cavitation, which involves formation, oscillation and occasional collapse of gas bubbles in the ultrasound field. This is not believed to affect the fetus to any appreciable extent, since lungs and bowels are not filled with air. There is no scientific evidence that USW can alter chromosomes.
In clinical practice, it is not possible to calculate the acoustic energy for the individual examination. Thermal index (TI) and Mechanical index (MI) on the ultrasound screen give the user a guide of likelihood and magnitude of heating and non-thermal effects. However, these indices are not very precise \(^{14}\) and awareness about how to use them in clinical practice is insufficient.\(^{22}\) Consequently, it is stated that prenatal ultrasound examinations should always be performed according to the ALARA principle (as low as reasonably achievable) e.g. the use of the lowest possible acoustic outputs to achieve a satisfactory examination.\(^{23}\)

**Ionizing radiation**

Radiation can be divided into electromagnetic radiation (energy in form of electro-magnetic waves or photons) and particle radiation (energy in the form of moving subatomic particles e.g. alpha, beta or neutron radiation). Further, radiation is classified into non-ionizing or ionizing, where ionizing radiation has enough energy to ionize atoms or molecules. Ionizing radiation may be emitted by natural decay of some unstable nuclei or following induced excitation of atoms and their nuclei. The photon component of ionizing radiation emitted by the excited nucleus is termed gamma rays and that emitted from machines is termed X-rays. The charged particles emitted from the nucleus are referred to as alpha rays (helium nuclei) and beta rays (electrons).

Absorbed radiation dose is expressed in Gray (Gy) = J/kg or milligray (mGy). The biological effects per unit of absorbed dose vary according to the type of radiation and part of the body exposed. To take these variations into account, a quantity called effective dose is used and is measured in Sievert units (Sv). The numerical values of Gy and Sv are essentially equal.\(^{24}\)

Sources of natural radiation include cosmic rays from outer space and from the surface of the sun, radionuclides in the Earth’s crust, building material, air, water, foods and the human body itself. The worldwide average annual effective radiation dose from natural sources is estimated at approximately 2.4 mSv (with a range of 1-10 mSv depending on location). The comparable annual dose for diagnostic medical examinations is 0.4 mSv/person (0.04-1.0 mSv depending on level of health care).\(^{25}\)

Exposure to ionizing radiation can cause damage or death to living cells, particularly during phases of rapid proliferation e.g. fetal growth. However, in general most organs and tissues of the body are not affected by the loss of even considerable numbers of cells. Radiation-induced damage is usually repaired, but if the repair is not perfect the resulting modification will be transmitted to other cells and may eventually lead to cancer. Tumors induced by radiation do not differ from cancer due to other causes and may not be manifest until decades after exposure, which makes epidemiologic research a
challenge. For example, long-term evaluation of 86,500 survivors of the atomic bombing of Hiroshima and Nagasaki revealed an excess of a few hundred cancer deaths and in the Chernobyl accident 1986, there is only scientific evidence of an increased rate of thyroid cancer. No increased risk of childhood cancer was observed in survivors of the atomic bombings irradiated in utero. They have now passed 60 years of age but since it is believed that the relative risk for cancer increases with decreasing age at exposure, it is important to evaluate their cancer risk henceforth. ²⁵

The literature on risks associated with diagnostic radiology in pregnancy is ambiguous. It has been reported that prenatal exposure to a radiation dose of over 10 mGy may increase the risk of childhood cancer ²⁶. However, it is not clearly understood if a safe lower dose limit of exposure of ionizing radiation exists. Studies on dose-response associations indicate that there is a linear relationship with no lower threshold, but a low dose of exposure is associated with a very low increased risk of cancer. ²⁷,²⁶ The following are approximate fetal doses from common diagnostic procedures: chest X-ray <0.01 mGy; pelvis X-ray 1.1-4 mGy; abdominal CT 8-49 mGy. ²⁴

Potential clinical effects on the CNS

An increased prevalence of non-right handedness in boys exposed to prenatal ultrasound, has been reported in two randomized controlled trials (RCTs) and two cohort studies ¹⁶-¹⁸,²⁸ and there are yet no published contradictory studies. Left-handedness as well as disorders of language, dyslexia, autism and schizophrenia is more common in men than in women. ²⁹ Moreover, patients with schizophrenia have less cerebral asymmetry and a higher prevalence of non right-handedness than healthy individuals. ³⁰ Changes in genes involved in neuronal migration have been found in schizophrenic patients ³¹ as well as in children with dyslexia. ³² If, as animal studies indicate, ¹³ prenatal ultrasound disrupts neuronal migration, this might result in an increased incidence of schizophrenia, dyslexia or impaired intellectual abilities in exposed individuals.

The association between ultrasound exposure and cognitive functions has been evaluated in 8-9 year old children ³³-³⁵ but not previously in older children or teenagers. School grades have not before been used as a measure of outcome and have the advantage of also including physical education, which provides information on motor skills. The association between prenatal ultrasound and schizophrenia has not previously been analyzed.

Childhood brain tumors are severe diseases with high mortality and morbidity and the incidence has increased during the last 20-30 years. ³⁶,³⁷ The increase probably mainly reflects improved diagnosis and reporting ³⁸ but still it is urgent to clarify if changes in the environment, such as introduction of prenatal ultrasound, may have contributed. Only a few risk
factors for CBT are considered to be established: rare genetic disorders and high-dose ionizing radiation. Radiological examinations during pregnancy have been associated with a slightly increased risk of CBT in offspring in some studies but meta-analyses have found no clear associations.

Study design
Randomized studies or observational studies such as cohort or case control studies are the epidemiological tools used when studying possible adverse effects of prenatal environmental exposures. The choice of study design depends on the accessibility of data on exposure and outcome (e.g. medical records, registers), the incidence or prevalence of the outcome and logistics such as budget for the project.

Randomized controlled study
The RCT is believed to be the golden standard in evaluating outcomes after medical interventions since it minimizes the risk of confounding and bias by indication. However, since it is not ethical to perform an RCT solely to evaluate potentially adverse effects, the only possibility to take advantage of the randomized design, is to use RCTs purposely designed to assess beneficial outcomes. Consequently, the original trial might not be perfectly sized or designed for measuring the adverse effects in the follow-up.

A limitation of the RCT on humans is cross-over between the intended randomized groups which may cause a dilemma in the analyses. Analyzing according to randomization only (the intention-to-treat principle) might dilute possible true differences between exposed and unexposed individuals. On the other hand, analyzing according to exposure might increase the risk of bias. Adjusting for possible confounding factors may to some extent, compensate for this risk.

Cohort study
In a cohort study, individuals are observed over a period of time to determine the frequency of occurrence of disease. The study population is considered with reference to exposure. Advantages of cohort studies include large study populations and the possibility to assess several outcomes.

An open cohort includes a dynamic dimension where subjects may enter or leave the study and contribute a “person-time”. A fundamental term is time at risk i.e. when the outcome might possibly occur. The essential measure of disease occurrence is incidence rate (I) which is defined as new cases over a period / sum of period time (e.g. cases / 100,000 person years).
To measure the association between a disease and an exposure variable, the *incidence rate ratio (IRR)* defined as \( \frac{I_{\text{exposed}}}{I_{\text{unexposed}}} \) is the method of choice. The preferable analysis when follow-up time differs between individuals is the Poisson regression analysis.

**Case control study**

In a case control study, groups of individuals are selected based on whether or not they have the disease of which the etiology is to be studied. The two groups are compared with respect to exposure frequency. An advantage of case control studies is that several exposures for a specific outcome can be assessed at the same time and it is also more feasible and cost effective for studying rare diseases than the cohort design.

When designing a case control study, it is important to remember that the controls should reflect a sample from the source population that gave rise to the cases, e.g. a random sample of individuals from a nation-wide, population based register. A skewed control sampling may give rise to selection bias. Another risk in sampling is a loss of precision and power, and a power calculation should be made in advance.

In case control studies, the association between exposure and outcome is estimated with the odds ratio (OR). OR can be calculated using a two by two table:

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Unexposed</td>
<td>c</td>
<td>d</td>
</tr>
</tbody>
</table>

The odds ratio is the ratio between the odds = \( \frac{a}{c} / \frac{b}{d} \)

When more than one explanatory variable are involved, the OR is usually estimated in a logistic regression model.
Aims

The overall objective of this thesis was to evaluate the long-term consequences of prenatal ultrasound and X-ray exposure on the child’s central nervous system.

The specific aims of the studies included in this thesis were:

1. To study the association between prenatal ultrasound scanning and the incidence of schizophrenia and other psychoses (Study I).

2. To evaluate the association of prenatal X-ray exposure and childhood brain tumors, including tumor subtypes (Study II).

3. To evaluate the association of prenatal ultrasound exposure and childhood brain tumors, including tumor subtypes (Study III).

4. To investigate the association of prenatal ultrasound exposure and school performance in teenagers (Study IV).
Material and methods

The studies in this thesis are based on data from Swedish nation-wide population-based registers (Study I-IV), data from antenatal records (Study II-III) and data from an RCT (Study IV). Linkage between data was possible through the Swedish national registration numbers (NRNs) individually assigned to each Swedish resident at birth.

Registers

The Swedish Medical Birth Register (MBR) contains prospectively collected information on more than 99% of all births in Sweden since 1973 \(^{48}\). The register includes data on maternal demographics, reproductive history and complications during pregnancy, delivery and the neonatal period. The information is provided through antenatal, obstetric and neonatal records, which are filled in by midwives and physicians. Complications during pregnancy and delivery are classified according the Swedish version of the International Classification of Diseases (ICD).

The Hospital Discharge Register includes data on dates of each hospital admission and discharge, and main and secondary diagnoses. The diagnoses are classified and recorded by the treating physician according to the ICD. The register provides nation-wide coverage since 1987 and includes care in psychiatric as well as medical clinics. Data on outpatient care is incomplete, especially regarding psychiatric diagnoses.

The Cancer Register was founded in 1958 and contains information on clinical and histological diagnosis, and date and place of residence at diagnosis. It is updated annually and has reliable information on more than 97% of all patients with cancer \(^{49}\).

The Cause of Death Register contains data on dates and causes of death for Swedish citizens since 1961. Coverage is more than 99.5% and data are updated yearly.
The Swedish Population and Housing Censuses contains information about employment, income, household size and ownership. We used information from the latest census from 1990.

The Education Register provides information on level of education for all Swedish residents since 1985.

The National School Register contains data on individual educational achievements for children graduating from class 9 (15-16 years of age) and data on immigration status of the children and their parents. Public schools (about 95% of all Swedish schools) were included in 1988 and private schools in 1993. Evaluation and statistics are published yearly.

The Swedish school system consists of nine year primary school followed by 2-3 years of elective secondary school to achieve qualification for university studies. The grading system in compulsory school is based on 16 school subjects and can be converted into numeric values (pass=10p, pass with distinction=15p and pass with honors =20p). A student can obtain a maximum summary score of 320p. Qualifying to secondary school requires pass in the core subjects: Swedish, mathematics and English.

Subjects and settings

Table 1. Overview of the papers.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Study design</th>
<th>Subjects</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cohort study</td>
<td>370,945 singletons born 1973-78 at 49 Swedish hospitals with information on ultrasound scanning. Only mothers from the Nordic countries.</td>
<td>IRR of schizophrenia and other psychoses according to ultrasound exposure.</td>
</tr>
<tr>
<td>II</td>
<td>Case control study</td>
<td>Children born in Sweden 1975-84 with a diagnosis of CBT (n=601). Equal number of controls randomly selected from MBR.</td>
<td>OR of CBT and its subtypes according to prenatal X-ray exposure.</td>
</tr>
<tr>
<td>III</td>
<td>Case control study</td>
<td>As in Study II</td>
<td>OR of CBT and its subtypes according to prenatal ultrasound exposure.</td>
</tr>
<tr>
<td>IV</td>
<td>Randomized controlled study</td>
<td>4905 singleton children to women randomized to routine ultrasound scan or no ultrasound 1985-87. Low risk pregnancies.</td>
<td>Differences in means or OR of poor school performance according to randomization and exposure to prenatal ultrasound.</td>
</tr>
</tbody>
</table>
Study I
Study I is a cohort study of individuals born in Sweden 1973-1978. Of 97 Swedish hospitals we included 49 with reliable information on their ultrasound program during the current time period. To increase the homogeneity, we included only singleton children of mothers who had been born in one of the Nordic countries. Of 593,917 singleton live births, we could include 370,945 individuals (62.5%), of whom 190,405 were men and 180,540 were women.

The University Hospital in Malmö was the first hospital in Sweden to introduce ultrasound scanning as part of standard antenatal care. During the study period 90% of all pregnant women living in the city of Malmö have been scanned. Consequently, children born in Malmö to mothers registered as citizens of Malmö were considered exposed to ultrasound. Before 1980, 48 hospitals did not practice ultrasound scanning and children born at any of these hospitals were considered unexposed to ultrasound.

Study II-III
All children born between 1975 and 1984 in Sweden were eligible for this case control study. In the Cancer Register we identified 601 children, up to 15 years of age, diagnosed with CBT (ICD-7 code 193) defined as cases. Controls were randomly selected children from the MBR and frequency matched to cases by gender and year of birth. For 62 of the 601 cases, the NRNs were incomplete or information on hospital of birth was missing, which made it impossible to track their antenatal records. Of the remaining 539 cases, we were able to retrieve antenatal records for 512 (95%). For the 539 control subjects, we found 524 records (97%).

Study IV
This is a follow-up of an RCT where participating mothers were randomized to prenatal ultrasound scanning in the second trimester or no ultrasound examination. The aim was to evaluate the effect of routine ultrasound scanning on obstetric outcomes such as estimation of date of delivery and detection of twins.

From October 1985 to March 1987, all women who were booked for antenatal care at Södersjukhuset, Stockholm, Västerås Central Hospital and Örebro Hospital were asked to participate in the randomized trial. Of 8768 invited women, 1414 were not included because they declined participation, booked too late to have a scan before 19 gestational weeks or already had a scan, or did not intend to have antenatal care at that clinic. 2357 women were not included because they fulfilled one or more of the predetermined indications for a second trimester scan. The remaining
women (n=4997) were randomized to ultrasound scanning around gestational week 15 (n=2482) or no ultrasound examination (n=2511). Four were lost to follow up. Children born from those pregnancies were followed up with a survey in 1995 regarding their general health, growth, vision, hearing, preference of handedness and neurological development.

In Study IV we excluded twins (n=88) and of the remaining 4905 children, 4458 (91%) were identified in the National School Register. Reasons for loss to follow-up were death, emigration, not completing compulsory school or non-matching NRNs between original study and registers.

Measure of exposure and outcome

Study I
In the prenatal ultrasound program in Malmö 1973-1975, examinations were performed at approximately 28 weeks of gestation. Around 50% had additional scans, mostly to confirm measurements. From October 1976 through December 1978, two examinations were performed. For most mothers (95%) the first scan was in gestational week 18 to 20 and the second in week 32. There were no scans performed between these examinations except for patients with bleeding from a suspected placenta previa.

Diagnoses of schizophrenia (ICD-9 codes 295A-295E, 295G, 295W, 295X and ICD-10 code F20) and other psychoses (ICD-9 codes 295F, 296-298 and ICD-10 codes F21-31) were obtained from the Hospital Discharge Register.

Study II-III
From the antenatal records we included data on X-ray and ultrasound exposure and information on pregnancy complications and maternal diseases. Radiological examinations were divided into abdominal (pelvimetry, X-ray for fetal position and others) and non-abdominal examinations. All ultrasound examinations were performed abdominally. Gestational age at exposure was divided into trimesters and was calculated from the date of last menstrual period. Data was extracted blindly with regard to case or control status from both antenatal records and registers.

Risk for CBT was evaluated for all types of tumors combined and according to the following subtypes; low grade astrocytoma, high grade astrocytoma, primitive neuroectodermal tumor (PNET), ependymoma, germ cell tumor, or others (rare and not completely specified tumors).
Study IV

Information on ultrasound exposure and obstetric variables from the RCT were linked with data from the National School Register (school grades), the Education Register (parents’ educational level) and the Population and Housing Census (socio-economic variables).

When evaluating actual ultrasound exposure it turned out that 1.3% of the women assigned to ultrasound scan had no scan, 4.1% assigned to no ultrasound scan had a scan before 19 weeks of gestation and 31% had a scan later in pregnancy. The scans were done by experienced midwives or doctors with linear real-time machines. The intensity output levels $I_{SPTA}$ were approximated to 11-35 mW/cm$^2$. The exposure times were not recorded individually, but the booking interval was 15 minutes.

We assessed school performance by: the mean summary score for all 16 subjects as a continuous variable, mean score in physical education, number of students with poor school performance (defined as mean summary score <160 p, where 160p correspond to pass in all subjects) and number of students not qualified to enter secondary school. The children graduated 2001-2005.

Statistical methods

Study I

Poisson regression analysis was used to estimate the effect of ultrasound exposure on the incidence of schizophrenia and other psychotic disorders. The results are presented as incidence rate ratios (IRRs) with 95% confidence intervals (CIs). The time at risk was calculated from the 12th birthday, but no earlier than 1 January 1987, to the first date of event, death, emigration or end of follow up (31 December 2004). The incidence was calculated as cases per 100,000 person years. The following variables were included as potential confounders in the multivariate regression analyses: maternal age, parity, gestational age at birth, intrauterine growth, Apgar score, mother’s psychiatric care and subjects’ attained age during follow-up. As the preliminary analyses indicated that gestational age, intrauterine growth and Apgar score had practically no impact on the estimates of interest, we excluded these covariates from the analyses in order to avoid problems with missing values.

To evaluate whether unmeasured factors related to hospital level might affect the results, we performed analyses in which tertiary level hospitals were compared with all other hospitals. Apart from Malmö there were seven tertiary level hospitals in Sweden during the study period. We adjusted for county of residence during the follow-up period to control for geographical variation in diagnostic and admittance routines.
Because other factors related to place of birth might affect the risk of being diagnosed with schizophrenia, we compared the incidence of schizophrenia in Malmö and three other cities before (1967-72) and after (1973-78) the introduction of ultrasound scanning in Malmö.

In order to assess the impact of the number and frequency of ultrasound examinations on the risk for schizophrenia, we divided the exposed cohort into two sub-cohorts born 1973 to 1975 (one routine scan) and 1976 to 1978 (two scans).

We included sex as an effect modification term in all analyses and we present the results according to sex.

Study II-III

Logistic regression was performed to evaluate the association between prenatal exposure to diagnostic X-ray or ultrasound respectively, and incidence of CBT. Estimates of ORs and 95% CIs were calculated. The following potential confounders from MBR were included in the adjusted analyses: maternal age at birth, parity, multiple birth, mother’s country of birth (Nordic country or non-Nordic country), hypertension during pregnancy, mode of delivery, breech position, gestational age at birth, birth weight, head circumference at birth and level of hospital.

Study IV

Linear regression was used to compare mean score values and logistic regression to analyze the dichotomous outcomes. Risk estimates are presented as means or ORs with 95% CIs.

Analyses were performed according to randomization group, routine ultrasound exposure in the second trimester and ultrasound exposure at any time during pregnancy. The analyses according to exposure were adjusted for the following factors: maternal age at birth, birth order, maternal smoking, birth weight, gestational age at birth, Apgar score at 5 minutes, parental educational level, ownership of one’s home, household income and mother’s country of birth (Sweden or other country). Additionally we replicated the analyses when excluding all children of mothers born outside Sweden. The results were presented according to sex and we included sex as an effect modification term in all analyses.
Results

Study I

In all, 370,945 individuals were included in the study, of whom 13,212 were exposed to ultrasound. We found a higher incidence of schizophrenia among individuals born at Malmö University Hospital (exposed to ultrasound) when compared with individuals born at all other hospitals. The difference was more pronounced among men (17.2 versus 10.9 cases/100,000 person-years) than women (7.5 versus 6.0 cases/100,000 person-years). The same pattern could not be seen for other psychoses. Among exposed as well as non-exposed children, male sex, high maternal age, preterm birth, and mother’s psychiatric care were associated with high incidence rates of schizophrenia and other psychotic disorders. The estimated crude IRR for schizophrenia when exposed to ultrasound was 1.58 (95% CI = 0.99-2.51) for men and 1.26 (0.62-2.55) for women. The same estimates for other psychotic disorders were 1.12 (0.80-1.58) for men and 0.92 (0.62-1.37) for women.

Since there was no association between ultrasound exposure and other psychoses, we limit the following presentation to estimated risk of schizophrenia. As we found practically no differences in risks for schizophrenia between the sub-cohorts exposed to one or two routine scans, we present data for the whole cohort.

In Table 2, Model I, individuals born at Malmö University Hospital were compared with those born at all other included hospitals. Among men, we found higher risks of schizophrenia if born in Malmö. For women, the point estimate was slightly increased but the confidence interval did not support an association. In Model II, individuals born in Malmö and seven other tertiary level hospitals were compared with those born at primary and secondary hospitals. The highest risks of schizophrenia were found among men born at the university hospitals in Malmö and Uppsala. For women, the highest risk of schizophrenia was found among those born in Uppsala. Similarly, when adjusting also for county of residence during follow-up (Model III), the highest risk of schizophrenia was found among men born in Malmö (IRR= 1.60; CI = 0.90-2.83) and women born in Uppsala (1.66; 0.89-3.10).

Finally, we compared risk of schizophrenia for singletons born in four cities between 1967 and 1972, before the introduction of ultrasound scanning, with risk of schizophrenia for those born between 1973 and 1978.
Men born in Malmö had the highest ratio of IRRs between cohorts born before and after the introduction of ultrasound scanning.

Table 2. Adjusted incidence rate ratio for schizophrenia according to ultrasound exposure by sex and hospital of birth (n = 357,733).

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRR</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Model I*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malmö</td>
<td>1.55</td>
<td>(0.97-2.46)</td>
</tr>
<tr>
<td>Malmö</td>
<td>1.60</td>
<td>(0.90-2.83)</td>
</tr>
<tr>
<td>Danderyd</td>
<td>1.25</td>
<td>(0.77-2.05)</td>
</tr>
<tr>
<td>Karolinska</td>
<td>1.04</td>
<td>(0.57-1.91)</td>
</tr>
<tr>
<td>Örebro</td>
<td>0.52</td>
<td>(0.25-1.10)</td>
</tr>
<tr>
<td>Sahlgrenska</td>
<td>0.83</td>
<td>(0.42-1.66)</td>
</tr>
<tr>
<td>Södersjukhuset</td>
<td>0.79</td>
<td>(0.42-1.48)</td>
</tr>
<tr>
<td>Uppsala</td>
<td>1.43</td>
<td>(0.86-2.39)</td>
</tr>
<tr>
<td>Umeå</td>
<td>0.39</td>
<td>(0.12-1.32)</td>
</tr>
<tr>
<td>Model II§¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malmö and other hospitals of tertiary level</td>
<td>0.39</td>
<td>(0.12-1.32)</td>
</tr>
<tr>
<td>Other hospitals</td>
<td>1.55</td>
<td>(0.97-2.46)</td>
</tr>
<tr>
<td>Model III§¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malmö</td>
<td>1.55</td>
<td>(0.97-2.46)</td>
</tr>
<tr>
<td>Other hospitals</td>
<td>1.39</td>
<td>(0.63-3.05)</td>
</tr>
</tbody>
</table>

Model II is excluded in this table.

* Malmö in relation to all other hospitals
H Adjusted for maternal age at birth, parity, mother’s hospitalization at a psychiatric clinic and subjects’ attained age during follow up.
I Children born at the University hospital in Malmö, which had ultrasound scanning as part of standard antenatal care.
§ Malmö and other hospitals of tertiary level in relation to all other hospitals
¶ Adjusted for factors in Model I and county of residence during the follow-up period

Study II

Among children with CBT it was more common to be the first born child (p=0.01) and to be born at a primary or secondary level hospital (p=0.04), than for controls. There were no other significant differences in maternal and neonatal characteristics between cases and controls.

Overall, 21.1% (n=108) of the mothers of children diagnosed with CBT were exposed to X-ray during pregnancy compared to 21.2% (n=111) of mothers of children randomly selected as controls. For abdominal X-ray, the exposure frequency was 10.7% (n=55) for cases and 9.4% (n=48) for controls. For both cases and controls, more than 96% of the abdominal examinations were performed in the last trimester. Non-abdominal
examinations included mainly pulmonary X-rays. Only three individuals in each group were exposed to X-ray more than once during the pregnancy.

Median age at diagnosis for all CBTs was 8 years. For subtypes, ependymomas had the lowest median age at diagnosis (4 years) and high grade astrocytomas had the highest (9 years).

In Table 3, the ORs for prenatal X-ray are presented by tumor subtype. Since there were practically no differences between cases and controls in frequency or timing of exposure, analyses are restricted to children of mothers exposed to prenatal X-ray, regardless of numbers of examinations or time of exposure. Being exposed to prenatal abdominal X-ray was not associated with an increased overall risk of brain tumors compared with being unexposed (adjusted OR=1.02, 95% CI= 0.64-1.62). When stratifying according to histological subgroups, we found that PNET had the highest risk estimates (OR=1.88, CI= 0.92-3.83). Low and high grade astrocytomas had no increased ORs. As there were only 44 cases of ependymoma, it was not possible to perform multivariate analyses to adjust for possible confounders and only crude risk estimates are presented. For similar reasons, germ cell tumors (n=17) were included in all cancers and not analyzed separately.

<table>
<thead>
<tr>
<th>Table 3. Odds ratios and 95% confidence intervals for all childhood brain tumors combined and by tumor subtype in relation to abdominal X-ray exposure.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposed (n)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>All Brain tumors&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n=503)</td>
</tr>
<tr>
<td>Astrocytoma low grade</td>
</tr>
<tr>
<td>Astrocytoma high grade</td>
</tr>
<tr>
<td>PNET&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ependymoma&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Adjusted for maternal age, parity, multiple birth, mother born in a Nordic country, gestational age at birth, mode of delivery, breech position, birth weight, birth head circumference, level of hospital and hypertension during pregnancy.

<sup>2</sup> Includes the subtypes in the table, germ cell tumors and other miscellaneous tumors. Only subjects with information on all variables adjusted for are included.

<sup>3</sup> Primitive neuroectodermal tumors

<sup>4</sup> Multivariate analyses could not be performed because of the small number of cases.
Study III

In Table 4, the ORs for prenatal ultrasound are presented by tumor subtype. No increased risk of CBT could be seen after prenatal ultrasound exposure for all tumors together (adjusted OR 1.00, 95% CI= 0.77-1.29) or for the separate subtypes. Ependymomas and germ cell tumors were too rare to be analyzed separately in the adjusted analyses.

When calculating the OR of ultrasound exposure according to trimester of exposure, no significant differences were seen and no increased risk for CBT was observed with increasing numbers of prenatal ultrasound examinations.

Table 4. Odds ratios and 95% confidence intervals for all childhood brain tumors combined and tumor subtype in relation to prenatal ultrasound exposure

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th></th>
<th>Adjusted¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N²</td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>All brain tumors³</td>
<td>503</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma low grade</td>
<td>190</td>
<td>0.96 0.68-1.34</td>
<td>1.02 0.72-1.44</td>
</tr>
<tr>
<td>Astrocytoma high grade</td>
<td>60</td>
<td>0.86 0.50-1.48</td>
<td>1.10 0.62-1.96</td>
</tr>
<tr>
<td>PNET⁴</td>
<td>104</td>
<td>0.85 0.55-1.30</td>
<td>0.85 0.54-1.35</td>
</tr>
<tr>
<td>Ependymoma⁵</td>
<td>42</td>
<td>0.67 0.35-1.29</td>
<td>.</td>
</tr>
<tr>
<td>Germ cell tumors⁵</td>
<td>17</td>
<td>1.07 0.41-2.82</td>
<td>.</td>
</tr>
</tbody>
</table>

1 Adjusted for maternal age, parity, multiple birth, mother born in a Nordic country, gestational age at birth, mode of delivery, breech position, birth weight, birth head circumference, level of hospital, hypertension during pregnancy and maternal smoking
2 Nine subjects were excluded because information on variables adjusted for was missing.
3 Including the subtypes in the table and other miscellaneous tumors (n=90)
4 Primitive neuroectodermal tumors
5 Multivariate analyses could not be performed because of the small number of cases

Study IV

There were no major differences in socio-economic, obstetric or neonatal variables between groups when compared according to randomization or when compared according to actual ultrasound exposure.

The mean grade-point summary score for the whole study population was 207. Girls had a higher mean score (218) than boys (196). 15% of the children had poor school performance and 8% were not qualified for secondary school. The only measure for which boys had higher mean score than girls was for physical education.
When comparing the differences in mean summary scores, a small reduction for exposed boys was seen in all the estimates presented. However, the reduction was statistically significant only for physical education (mean difference -0.48 grade points, 95% CI -0.95--(-0.02)). For girls, the same tendency could not be seen. In the analyses of the dichotomous outcomes the highest risk estimates were seen for boys in the adjusted analyses according to exposure (poor school performance OR 1.16, 95% CI 0.90-1.50; not qualified for secondary school OR 1.32, 95% CI 0.94-1.83).

There were practically no differences in results between analyses performed before and after exclusion of children born to non-Swedish women.
Discussion

Epidemiological and statistical considerations

This thesis includes three different epidemiological methods: cohort (Study I), case control (Study II-III) and randomized controlled study (Study IV). In the following section, the three study designs are discussed with emphasis on the application in the current works.

Choice of study design

In general, case control studies are considered preferable when studying rare outcomes. However, if the study population is large enough, the cohort design is superior since it minimizes selection bias. Schizophrenia is a rare disease, with an incidence rate of about 15/100,000 person years, and a large cohort was a necessity to achieve power in Study I. Our cohort included 370,945 individuals and had sufficient power to detect small to moderate increases in IRR. Such a large study population is only manageable if data is obtained from registers, which may introduce some uncertainty on individual exposure. A limitation of cohorts defined by place of birth is that socio-economic conditions as well as health care routines might differ between the settings. In a previous study on intellectual performance in men, using the same cohort, Malmö was equivalent to the rest of the included hospitals when comparing immigration rates and parents’ employment and educational level. However, some degree of residual confounding is still likely in this cohort.

In Study II-III our aims were to analyze the associations of CBT and exposure to prenatal X-ray and ultrasound. Since information on exposure is not included in any nation-wide register, and we were only able to scrutinize a limited number of antenatal records, the most feasible was a case control study design. We chose the birth cohort 1975-1984 since it correlated with a time period when we assumed that 50% of the children were exposed to prenatal ultrasound. With this cohort we calculated for 600 children with CBT and a similar number of controls. By assuming a power of 80%, a two-sided 5% significance level and an abdominal X-ray exposure frequency of 10%, we should be able to detect an OR of CBT of at least 1.7. The corresponding estimate for ultrasound with an exposure frequency of 50% was an OR for all CBT of at least 1.4. However, for the analyses stratified by
tumor subtype, a larger population would have been needed to find statistically significant risks. In Study II, for the subgroup of PNET, we found an OR of 1.9 for abdominal X-ray exposure (105 cases) corresponded to a power of 52%, which is not sufficient.

In Study IV, the power calculation including 2482 exposed and 2511 unexposed individuals from the RCT, demonstrated that we would be able to detect a reasonable decrease in mean grade summary score from 205 (mean score in 2003) to 200.

When assessing beneficial effects of an intervention in an RCT, it is recommended to analyze according to the intention-to-treat principle e.g. by randomization. However, possible adverse effects can only be evaluated if an individual actually has been exposed. In Study IV, only analyzing according to randomization would increase the risk that a true association between ultrasound and school performance would be diluted because of exposure cross-over. On the other hand, analyzing by exposure might increase the risk of accepting a false assumption if confounding factors are not properly controlled for. As none of the two methods has a preference above the other, we performed analyses both according to randomization and actual ultrasound exposure. For boys, the adjusted analyses according to exposure showed slightly increased risk estimates for all outcomes compared to the analyses by randomization.

Confounding and effect modification

A confounding factor is a systematic error that is associated with the exposure and the outcome and is unevenly distributed between the compared groups. By definition it should not be an effect of the exposure. Common ways to prevent confounding in the design phase of a study are restriction, matching and randomization. Since it is difficult to completely avoid confounding in the study design, it must also be dealt with in the data analyses.

In Study I, the exposed cohort was defined as children born at a certain hospital with an ultrasound scanning program. Since immigration rates differ between regions and may influence the risk of schizophrenia, we restricted the population to children of mothers born in the Nordic countries. To control for confounders, we used a stepwise regression model were obstetric and socio-economic variables were included in the first model and county of residence was added in the second model and the results differed only slightly (Table 2).

In Study II-III, we decided to frequency match controls to cases by sex, as the different subtypes are unevenly distributed between boys and girls, and by year of birth, since routines for clinical and histological diagnosis might vary over time. Matching inevitably reduces the possibility to study the impact of the matching factor, thus we could not evaluate whether X-ray or
ultrasound exposure influenced the risk of CBT differently according to sex. In the analyses we used regression models including socio-economic, obstetric and perinatal factors that could affect the association between the exposures (prenatal ultrasound and X-ray) and CBT.

In Study IV, we restricted the study population to singleton children since multiparity might influence ultrasound exposure as well as the intellectual capacity. Based on the original study design, all analyses were restricted to healthy mothers with regular menstruation. Confounding might occur in randomized studies as well, but these imbalances are regarded as more random than systematic, and can hardly be adjusted for. Consequently, the analyses according to randomization are presented as crude estimates and when analyzing according to exposure we additionally performed multivariate analyses.

The situation in which the measured effect of an exposure changes depending on another variable is often referred to as effect modification. According to the literature, boys seem to be more sensitive to prenatal risks and prenatal ultrasound in particular. Thus, we had reason to believe that sex could act as an effect modifier. In Study I and IV we introduced sex as a multiplicative interaction term in the regression models and the results were presented according to sex.

Exposure data

Ultrasound exposure

Some circumstances influence the possibility to conduct observational epidemiological studies on long-term effects of prenatal ultrasound. First of all, there are no national or regional registers including information on ultrasound scans. Consequently, either approximations on exposure must be done on a population level (e.g. follow-up of screening programs as in Study I), or individual data on exposure has to be collected elsewhere, e.g. in antenatal records (Study II-III) or through questionnaires. Another obstacle is identifying an unexposed control group since ultrasound is now usually part of routine antenatal programs.

In Study I, we assumed that all pregnant women in Malmö were exposed to routine scanning and according to data from the scanning program that was true for about 90%. To avoid the risk of misclassification, we included only hospitals with reliable documentation on ultrasound programs. However, some of the women in Malmö did not have an ultrasound, and some of those considered being unexposed may have had a scan in another part of Sweden. This possible error might lead to an underestimation of the association and reduce the possibility of finding an existing relation between exposure and outcome. Since the association of prenatal ultrasound exposure
and schizophrenia in men was of borderline significance this might be of importance.

Another challenge when analyzing potentially adverse effects of a diagnostic tool or a treatment is to be able to separate it from effects deriving from the cause of the intervention (confounding by indication). In the specific situation of prenatal ultrasound, examinations outside routine programs are most often caused by suspicion of fetal growth disturbances, multiparity or other maternal and obstetric conditions that might affect fetal cerebral functions. Study III includes both routine scanning and clinically indicated examinations, making indication bias a possibility. This is compensated for, to some extent, in the adjusted regression models and it is reassuring that numbers and time of exposure did not change the risk estimates significantly. The original trial in Study IV consisted of women with low risk pregnancies and regular menstruation that should reduce the need for additional ultrasounds. However, we know that at least one third of women randomized to no ultrasound underwent a scan later in pregnancy and this proportion can be even higher since scans might have been performed out of record. To minimize bias we adjusted for obstetric confounders in analyses according to exposure.

One should bear in mind that all these studies assessed ultrasound exposure in the 1970 and 1980s, with average intensity output levels for ultrasound machines of around 20 mW/cm² ($I_{SPTA}$)\textsuperscript{33,59}. This is very low compared to the maximum limit of 720 mW/cm² set by the USA’s Food and Drug Administration (FDA) in 1993.\textsuperscript{60} Presumably most fetal ultrasound examinations do not involve maximum energy levels but still the outputs are probably ten times higher today than 20-30 years ago.\textsuperscript{2} Further, the intensities for ultrasound machines are based on the manufacturer’s data and high discrepancies have been found between declared and measured $I_{SPTA}$ outputs.\textsuperscript{61} Consequently there are uncertainties concerning the ultrasound energy fetuses are exposed to. The suspicion of much higher energy exposures nowadays in combination with more frequent scans and use of Doppler ultrasound means that these results can not unconditionally be valid for the exposure of today.

When discussing potential side effects it should be emphasized that the progress in prenatal ultrasound has increased the possibility to detect and manage complicated pregnancies. When ultrasound examinations are done on proper indications, benefits for the fetus usually outweigh potential risks.

X-ray exposure

In Study II, we had an X-ray exposure rate of 21% which is a high frequency compared to similar studies from other countries \textsuperscript{62,63} and mirrors the routines in Sweden 20-30 years ago. During the last two decades ultrasound has taken over the position as the examination of choice for determining
fetal status (position, multiple births etc.), and antenatal pelvimetry is rarely performed. Nevertheless, abdominal radiation in pregnancy still occurs especially for maternal indications. It is worth considering that the abdominal X-ray examinations in Study I consisted mainly of pelvimetry with relatively low radiation doses (1.1-4 mGy) compared to abdominal CT (8-49 mGy).\textsuperscript{24} It is important to have reliable facts on fetal risks when subjecting a pregnant woman to diagnostic imaging. Withholding an indicated radiological examination, which may cause delay in diagnosis and treatment, often involves greater risks for the mother and indirectly for the fetus than the potential long-term harmful effects of radiation.

Findings and implications

Prenatal ultrasound exposure and cognitive function

Table 5. Studies on schizophrenia, intellectual performance and handedness in boys/men according to ultrasound exposure

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Study design</th>
<th>Analyzed groups</th>
<th>N</th>
<th>IRR/ OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia, Study I</td>
<td>Cohort study</td>
<td>Exposed/unexposed cohort\textsuperscript{1}</td>
<td>370945</td>
<td>1.55</td>
<td>0.97-2.46</td>
</tr>
<tr>
<td>Subnormal intellectual performance\textsuperscript{64}</td>
<td>Cohort study</td>
<td>Exposed/unexposed cohort\textsuperscript{1}</td>
<td>167059</td>
<td>1.28</td>
<td>1.18-1.38</td>
</tr>
<tr>
<td>Non-right handedness\textsuperscript{65}</td>
<td>Meta-analysis of two RCTs</td>
<td>Randomization</td>
<td>2882</td>
<td>1.26</td>
<td>1.03-1.24</td>
</tr>
<tr>
<td></td>
<td>Exposed/unexposed cohort\textsuperscript{1}</td>
<td>Randomization</td>
<td>2882</td>
<td>1.34</td>
<td>1.10-1.65</td>
</tr>
<tr>
<td>Poor school performance, Study IV</td>
<td>RCT</td>
<td>Exposure\textsuperscript{1}</td>
<td>2289</td>
<td>1.16</td>
<td>0.90-1.50</td>
</tr>
<tr>
<td></td>
<td>Randomization</td>
<td>Exposure\textsuperscript{1}</td>
<td>2289</td>
<td>1.01</td>
<td>0.77-1.34</td>
</tr>
<tr>
<td>Not qualified for secondary school, Study IV</td>
<td>RCT</td>
<td>Randomization</td>
<td>2289</td>
<td>1.32</td>
<td>0.94-1.83</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Adjusted for socio-economic and obstetric confounding factors

Table 5 presents selected findings for boys from Study I and IV and two related studies. The conclusions from the separate studies were cautious but when looking at the summarizing table, a pattern might be discernible. Consistently, all analyses according to exposure resulted in an increased risk for exposed boys and neither of these studies revealed any increased risk for exposed girls (the cohort study on subnormal intellectual performance included boys only). The question is whether this tendency is caused by a

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true biological effect on the male fetal brain or whether it can be explained by other factors or by chance.

If hypothesizing that these minor differences do reflect a true effect of ultrasound on the fetal brain, what are the plausible underlying mechanisms? There are good reasons to believe that vulnerability for schizophrenia and other psychiatric disorders may be shaped over the life span by both genetic constitution and environmental factors. Early hazards in utero and deviant fetal growth have a relatively consistent but modest risk-increasing effect for later development of schizophrenia.\textsuperscript{66-68} Thus, ultrasound and other prenatal and environmental events may be the elements that ‘turn on’ susceptibility genes in predisposed individuals.\textsuperscript{69}

An alternative or perhaps overlapping model, strengthened by experiments in mice\textsuperscript{13 5} is that ultrasound could affect the migration of neurons and disrupt the normal process of cerebral lateralization. Patients with schizophrenia have an increased prevalence of non right-handedness as well as other signs of defective lateralization\textsuperscript{30} and genes involved in cerebral migration have been associated with the pathogenesis of schizophrenia.\textsuperscript{31,70}

Left-handedness is known to be more prevalent in both extremely intelligent people and individuals with impaired cognitive functions. This may by explained by three types of mechanisms for developing left-handedness; pathological, natural and learned.\textsuperscript{71} If ultrasound is a risk factor for pathological left-handedness\textsuperscript{16-18,28} it could theoretically also affect intellectual capacity. Two case-control studies have reported an association between ultrasound and dyslexia and delayed speech.\textsuperscript{72,73} However, follow-up of three RCTs on prenatal ultrasound\textsuperscript{33,34} and Doppler ultrasound\textsuperscript{35} exposure and neurological and intellectual performance in children 8-9 years of age, have not demonstrated any increased risks. Even though the findings in younger children were negative, longer follow-up might be important to assess complex cerebral functions. Potential effects of ultrasound could either be temporary and decline with age or the brain might in some way be abnormally 'set up' as a result of an early insult and exhibit functional impairment later in life. Study IV and the study on intellectual performance in Table 2\textsuperscript{64} are the only studies assessing cognitive functions later in life.

Study IV reports potentially adverse effects of prenatal ultrasound exposure as evaluated by school grades as an outcome, and comparisons with previous studies should be regarded with caution. The Swedish grading scale consists of three levels only, which makes it a rather crude instrument for measuring possible minor effects that ultrasound might have on the brain. It is likely that school grades reflect not only intellectual and for physical education, motor, skills but also mirror features such as personality and social adaptability.
Prenatal ultrasound and childhood cancer

No increased risk for CBT after prenatal ultrasound exposure was observed in Study III when analyzing the overall risk of CBT or for the separate subtypes. Trimester of exposure and number of ultrasound examinations had no impact on risks. This strengthens the results from earlier studies on prenatal ultrasound and childhood cancer.\textsuperscript{42,62,63,74,75}

Ultrasound can damage biological tissue by heating, cavitation or streaming. Whether any of these mechanisms may be carcinogenic is not known, but experimental studies on tissue cultures have shown that ultrasound with intensities used for prenatal scanning can damage cell membranes.\textsuperscript{76} In some of the first reports on potential hazards by ultrasound, chromosomal damages, including sister chromatid exchanges, were described.\textsuperscript{77} However, repeated studies have not been able to confirm these results.

To summarize, it is reassuring that no significant carcinogenic effect of prenatal ultrasound has been reported in this thesis, which is in accordance with the literature.

X-ray exposure and childhood brain tumors

In two previous case control studies on CBT subtypes, no increased risk after prenatal X-ray could be detected for all CBTs or for PNET specifically.\textsuperscript{62,63} In these studies, retrospective interviews on exposure data were used and the risk of recall bias could not be excluded. In both studies less than 5% of the study population was exposed to prenatal X-ray, while we had an exposure rate of 21%. The present study is, to our knowledge, the first study on X-ray and CBT subtypes with prospectively collected information from antenatal records.

In Study II, we did not see any overall risk increase of CBT after prenatal X-ray exposure but there was a slightly increased risk for the PNET subgroup. PNET tumors are presumed to arise from undifferentiated neural stem cells and little is known regarding the molecular genetic events initiating carcinogenesis.\textsuperscript{78} The most important finding in our results might not be the clinical implication, but that it gives rise to the theory that prenatal or neonatal radiation might affect the various neural cell types differently. Hypothetically, neural stem cells could be extra sensitive during their differentiation and DNA damage caused by radiation could initiate malignant transformation, leading to the development of embryonic CNS tumors such as PNET. Further, risks associated with fetal diagnostic radiation could probably be extrapolated to the early childhood period, since the brain still develops and grows rapidly during the first years of life.
Conclusions

From the studies of which this thesis consists, the following conclusions could be drawn:

- No significant associations between prenatal ultrasound exposure and schizophrenia or other psychoses were found. Exposed men tended to have a higher incidence of schizophrenia, but other factors related to place of birth might have influenced the results (Study I).

- We found no overall increased risk for childhood brain tumors after prenatal abdominal X-ray exposure. Risk estimates varied between the different histological subtypes and there was an almost doubled, but not statistically significant increased risk for primitive neuroectodermal tumors (PNET) (Study II).

- We found no increased risk for childhood brain tumors after prenatal ultrasound exposure when analyzing all brain tumors together or for the separate subtypes: astrocytomas (low and high grade), PNET, ependymomas or germ cell tumors. Trimester of exposure and number of ultrasound examinations had no impact on risks (Study III).

- Second trimester ultrasound scanning does not seem to affect school performance in teenagers. Boys exposed to ultrasound at any time during gestation had lower mean grades in physical education and a tendency towards lower school grades in general (Study IV).
Aims for the future

Knowledge of the etiology of CBT is limited. High dose radiation is an established risk factor, but studies on diagnostic X-ray and CBT are ambiguous. In Study II, children exposed to prenatal X-ray had an almost doubled, but not significantly increased, risk for PNET. In order to increase power enough to be able to reject or strengthen this finding, a larger study population would be needed, either including a larger birth cohort or pooling data from the other Nordic countries.

Previous reports have shown an association between use of oral contraceptives before conception, narcotics and penthrane during delivery and CBT.\textsuperscript{79} Maternal virus infections such as influenza have been associated with increased risk of CBT \textsuperscript{80-82} and in animal studies influenza RNA has been able to cross the placental barrier and has been found in the brain of the mouse fetus.\textsuperscript{83} When collecting data for Study II-III, information on medications and infections during pregnancy was recorded and we intend to use this data to analyze the association between drug exposure \textit{in utero} and fetal infections and CBT.

There is a need for continuous assessment of the potentially adverse effects of prenatal ultrasound of today. Results from older studies, involving low ultrasound energy outputs, may not be valid in the future, and additional outcomes need to be evaluated. Epilepsy, migration disorders, autism and attention disorders have been associated with disturbances in neuronal migration and thus might be associated with prenatal ultrasound exposure. At present, case control studies seem to be the only alternative to study the impact of prenatal ultrasound on such rare outcomes, and the study design is complex since few pregnant women are unexposed today and ultrasound exposure might be confounded by other obstetric factors. It would be desirable if ultrasound examinations could be included in a nation-wide register to facilitate future studies.
Svensk sammanfattning (Summary in Swedish)

I Sverige genomgår ungefär 97% av alla gravida kvinnor rutinmässig ultraljudsundersökning och i genomsnitt görs drygt två undersökningar per graviditet. Fördelar med rutinmässig ultraljudsundersökning är en förbättrad uppskattning av graviditetslängd och tidigare upptäckande av tvillinggravider och fostermissbildningar. För riskgravider kan det finnas indikationer för ytterligare undersökningar. När man beslutar om en undersökning eller behandling under graviditeten måste troliga fördelar ställas mot tänkbara nackdelar för mor och barn.

Tidigare studier rörande eventuella risker med ultraljudsanvändning under graviditeten har visat att exponerade pojkar kan ha en något ökad förekomst av icke-högerhänthet senare i livet. Man inte sett några säkerställda risker med avseende på fostertillväxt, cancerrisk, samt intellektuell och neurologisk utveckling. Än så länge är dock eventuella risker med prenatalt ultraljud relativt sparsamt studerat.

Röntgenundersökning under graviditeten var vanligt i Sverige för 20-30 år sedan, men idag är ultraljud förstahandsalternativ vid fosterövervakning. Man vet att höga doser joniserande strålning under fosterlivet innebär en ökad risk för uppkomst av cancer i barndomen. Om låga stråldoser som ges vid enstaka röntgenundersökning under graviditeten ökar cancerriskerna för barnet är dock omdiskuterat och kunskapen om riskmönstret för olika tumörtyper är bristfällig.

Målet med denna avhandling var att utvärdera sambandet mellan ultraljudsexponering under fosterlivet och barnets risk för:

1) framtida schizofreni och annan psykossjukdom
2) hjärntumör i barndomen
3) försämrad prestation i skolan i årskurs 9

Samt att:
4) bedöma risken med röntgenexponering i fosterlivet med avseende på hjärntumör i barndomen.
Studie I

Studie II-III

Inget samband sågs mellan ultraljudsexponering i fosterlivet och insjuknande i hjärntumör, inte heller för de olika tumörtyperna. Antal ultraljud eller tidpunkten för undersökningen påverkade inte resultaten nämnvärt.

Studie IV

Eftersom det visade sig att ca 30% av de kvinnor som enligt randomiseringen inte skulle genomgå ultraljud, ändå genomgått minst en undersökning, valde vi att jämföra skolresultat enligt tre uppdelningar: 1)
enligt den ursprungliga randomiseringen 2) enligt faktisk exponering för rutinultraljud samt 3) enligt ultraljudsexponering under hela graviditeten.

Vi såg inga ökade risker för försämrad skolprestation för de pojkar och flickor som randomiserats till eller genomgått rutinultraljud. Pojkar som varit undersökta med ultraljud någon gång under graviditeten hade signifikant lägre betyg i Idrott, samt en tendens till lägre skolbetyg generellt än icke ultraljudsundersökta pojkar.
Tack! (Acknowledgements)


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References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)

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