Scandinavian Journal of Public Health, 2010; 38: 208–219

STUDY DESIGN ARTICLE

Lifestyle during pregnancy: Neurodevelopmental effects at 5 years of age. The design and implementation of a prospective follow-up study

ULRIK SCHIØLER KESMODEL[1], METTE UNDERBJERG[1,2], TINA RØNDRUP KILBURN[1], LEIV BAKKETEIG[3], ERIK LYKKE MORTENSEN[1,4], NILS INGE LANDRØ[5], DIANA SCHENDEL[6], JACQUELYN BERTRAND[6], JAKOB GROVE[1], SHAHUL EBRAHIM[6] & POUL THORSEN[1,7]

[1]*School of Public Health, Department of Epidemiology, University of Aarhus, Denmark,* [2]*Vejlefjord Children's Neurocenter, Stouby, Denmark,* [3]*Institute of Public Health, Research Unit of Epidemiology, University of Southern Denmark, Denmark,* [4]*Institute of Public Health, University of Copenhagen, Denmark,* [5]*Center for the Study of Human Cognition, Department of Psychology, University of Oslo, Norway,* [6]*Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, and* [7]*Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA*

Abstract

Background: It has been suggested that even mild exposure to alcohol, caffeine, smoking, and poor diet may have adverse long-term neurodevelopmental effects. In addition, there is evidence that timing of high exposures (e.g. binge drinking) can have particularly negative effects. This paper describes the design and implementation of The Lifestyle During Pregnancy Study addressing major methodological challenges for studies in this field. The study examines the effects of lifestyle during pregnancy on offspring neurodevelopment. *Methods:* In 2003, we initiated a prospective follow-up of 1750 mother–child pairs, sampled on the basis of maternal alcohol drinking patterns from The Danish National Birth Cohort (DNBC), a study of 101,042 pregnancies enrolled 1997–2003. Data collection in the DNBC involved four prenatal and postnatal maternal interviews, providing detailed information on maternal alcohol drinking patterns before and during pregnancy, caffeine intake, smoking, diet, and other lifestyle, medical, and sociodemographic factors. *Results:* At the age of 5 years, the children and their mothers participated in a comprehensive assessment of neurobehavioural development focusing on global cognition, specific cognitive functions, and behaviour. Two new tests assessing attention and speed of information processing among children were developed, and data on important potential confounders such as maternal intelligence quotient, vision, and hearing abilities were collected. Efforts were made to standardise procedures and obtain high inter-rater reliability. ***Conclusions:*** **We expect that the study will illuminate the significance or lack of significance of maternal lifestyle during pregnancy and contribute to better understanding the effects of alcohol drinking during pregnancy at low to moderate consumption levels.**

Key Words: *Alcohol, binge drinking, cognition, intelligence, lifestyle during pregnancy, neurobehavioural development, pregnancy*

Introduction

It is well established that exposure to high levels of certain toxic agents during pregnancy can affect the risk of malformations and other life long disabilities among offspring. Alcohol, heavy metals, and certain medications are some of the most widely recognised teratogens. Some modifiable exposures are often referred to as lifestyle factors. Common modifiable lifestyle risk factors include alcohol and caffeine consumption, tobacco smoking, and diet.

Fetal alcohol exposure is the leading known preventable cause of birth defects and developmental disabilities among Western countries [1]. Maternal

Correspondence: Ulrik Schiøler Kesmodel, School of Public Health, Department of Epidemiology, University of Aarhus, Bartholins Alle 2 8000 Aarhus C, Denmark. Tel: +45 89 42 60 70. Fax: +45 86 13 15 80. E-mail: ukes@soci.au.dk

(Accepted 12 November 2009)

DOI: 10.1177/1403494809357093

intake of large daily volumes of alcohol during pregnancy has been shown to be associated with malformations [2], mental retardation, behavioural and psychosocial problems in childhood and adolescence [3], and, in the most severe cases, fetal alcohol syndrome (FAS) [2,4].

Maternal intake of about one drink per day or more has been associated with reduced birthweight and intrauterine growth restriction [5], spontaneous abortion [6], preterm delivery [7], and stillbirth [8]. Furthermore, varying degrees of prenatal alcohol exposure have been found to be associated with dysfunctions in the speed and efficiency of information processing [9] and in deficits in specific cognitive functions such as attention [10–16], learning and memory [17–19], visual perceptual and visual motor skills [16–18], language [16–18], and executive functions [20–22]. However, the results have been inconsistent with numerous studies showing little or no effect of more moderate alcohol intake during pregnancy [23,24], while studies on birth weight [5], preterm delivery [7], and long term neurodevelopmental effects [25,26] have even suggested apparent beneficial effects of low weekly doses of alcohol.

Tobacco smoking during pregnancy has been associated with many short-term adverse pregnancy outcomes [27–29]. Studies have shown an association between smoking during pregnancy and adverse effects on cognitive and behavioural development [30].

Experimental studies among animals have suggested that high doses of caffeine can cause behavioural problems among the offspring [31], although it is unclear whether these high levels of exposure are comparable with the ordinary consumption levels of pregnant women [31]. A single study among humans showed no association between caffeine intake during pregnancy and developmental outcomes at the age of 7 [32].

Prenatal diet may influence fetal development. It is well documented that periconceptional supplementation with folic acid can prevent neural tube defects [33]. Morover, several studies support that long-chain *n*-3 fatty acids, abundant in seafood, may prolong gestation and reduce the risk of preterm delivery [34,35]. It is possible that suboptimal nutrition at vulnerable stages in pregnancy might even affect the risk of diseases that may first appear in adulthood [36].

Several dietary factors have been associated with offspring development. Poor nutrition during brain development may impair cognitive development [37] and supplementation studies with long-chain polyunsaturated fatty acids to very preterm infants have shown significantly enhanced visual acuity, visual recognition, memory, and performance during early infancy [38]. Food-borne environmental toxins have also received great attention in relation to neurodevelopment. Consumption of seafood contaminated with methyl-mercury has been associated with subtle neurodevelopmental deficits [39,40], although some studies have failed to confirm this association [41,42].

Many significant limitations are, however, encountered by most studies of long-term developmental outcomes, especially studies of prenatal alcohol exposure. Differences in findings among studies might be due to a variety of methodological problems, including: failure to obtain valid and reliable prospective assessments of drinking patterns during pregnancy [3,43]; a systematic lack of information on low-to-moderate-level alcohol exposure [3,43,44] and pattern (chronic vs. binge) and timing of binges [3,43–46]; failure to adequately control for confounding by parental intellectual abilities [45,47–50], socioeconomic position [3,45,50,51], and other environmental factors [3,45,51]; failure to evaluate a range of developmental outcomes at different ages [47,50] to assess multiple effects that could emerge over time; and generally small sample sizes in studies on neurodevelopment [3,44,52,53], most likely because of the costs of conducting such studies.

In addition, the task of examining young children involves special methodological problems: Although many measures for young children are available for clinical use, there are few theoretically founded tests suitable for pre-school-aged children and few tests that successfully distinguish between different cognitive functions and different aspects of behaviour. Furthermore, the results of tests for young children might be affected substantially by response modus, (i.e. whether motor or verbal skills are required to perform the tasks). Finally, young children are very susceptible to factors associated with test administration and tester effects [45,47,48,50].

The current study was designed as outlined below to address and overcome the challenges and limitations of previous studies of the relation between lifestyle factors during pregnancy and later neurodevelopment of the child. The study is based on one of the largest cohorts of pregnant women available in the world. In this paper we describe the study design, our implementation of important methodological decisions and preliminary results regarding participation as well as inter-rater reliability.

Aim

We wanted to create a study population that would make it possible to examine the relation between maternal lifestyle during pregnancy and offspring

neurodevelopment at the age of 5 years. The primary exposure of interest in The Lifestyle During Pregnancy Study is alcohol. Specifically, we wanted to be able to study the association between average alcohol intake (drinks per week) before and during pregnancy and binge drinking (intake of five or more alcohol containing drinks on a single occasion) at different points in time during pregnancy. In addition, the design of the study and data available through the Danish National Birth Cohort (DNBC) allow for investigation of several other exposures or mitigating factors, including: smoking habits, caffeine intake, and diet – particularly seafood diet.

This study was designed to address and overcome the limitations of previous studies by using prospective data with regard to exposure to risk factors, obtained at two points in pregnancy, including information on different drinking patterns and timing of binge drinking. Thus, we specifically focused our study on the potential effects of very low, weekly (rather than daily) alcohol intake and binge drinking. Furthermore, we used data from a homogenous, generally middle-class population with access to comprehensive healthcare free of charge for the individual (thereby reducing the potential for confounding) and without the stigma associated with alcohol that might exist in some countries [54], possibly reducing the magnitude of information bias associated with self-reported alcohol intake. We also sampled across different alcohol consumption levels with sufficient numbers in each stratum to allow for relevant analyses of the effects of different drinking patterns.

We included theoretically founded tests suitable for young children and, when no relevant tests were available, we developed and validated new tests for use in this study and for subsequent clinical use. In choosing and developing new tests, we wanted to include tests that tapped fundamental cognitive processes as well as distinguished between specific cognitive functions and different aspects of behaviour. Finally, we standardised all possible procedures and performed regular inter-rater comparisons for both groups of examiners (psychologists and physiotherapists) to minimise any potential effects of examiner, examination site, or other systematic bias.

Design

The study is a prospective follow-up study, based on a sample from the DNBC [55] with oversampling of certain alcohol exposure categories.

The Danish National Birth Cohort

During the period 1997–2003, pregnant women in Denmark were invited to participate in the DNBC study at the first antenatal visit by the general practitioner. Enrolment was completed in the autumn of 2003 and the database includes 101,042 pregnancies, corresponding to approximately 60% of those invited and approximately 30% of all pregnant women in Denmark during the enrolment period [55]. For this study, data points of interest from the DNBC study included: two prenatal maternal telephone interviews conducted at week 12 and 30 of gestation concerning maternal health, use of medicines, socioeconomic status, obstetric history, and lifestyle factors (e.g. alcohol, smoking, caffeine, and diet); two postnatal maternal interviews conducted at 6 and 18 months postpartum focusing on family conditions and offspring health and development.

Additional medical and socioeconomic information on DNBC participants can be obtained by linking with Danish computerised registries, using the unique Danish personal identification number.

Information on exposures in the DNBC

Both prenatal interviews provide information on the number of drinks per week of beer, wine, fortified wine, and spirits that each participating pregnant woman consumed at the time of the interview; in addition the first prenatal interview provides information on the number of drinks per week consumed before pregnancy. Such interviews have been shown to yield valid (relative to other methods) and reliable information among pregnant Danish women [56]. The interviews also provide information on binge drinking (intake of five or more drinks on a single occasion) and when during pregnancy each binge episode occurred. This approach has been shown to yield valid (relative to other methods) and reliable information on binge drinking, and it is the only validated method for the collection of data on timing of binge drinking [57,58]. Furthermore, the prenatal interviews provide information on smoking habits before and during pregnancy, intake of caffeine-containing beverages (coffee, tea, cola, and hot chocolate), seafood and iron consumption during pregnancy, and other detailed information on diet.

Outcomes in the lifestyle study

The neurodevelopmental outcomes included measures of cognitive, behavioural, emotional, and social functions and measures of growth at 5 years of age (age span: 60–64 months). The outcomes were

assessed by administration of a neuropsychological test battery and questionnaires on the child's social functioning, behaviour, and health to be answered by parents and staff in the child's day-care centre (Table I).

For all outcomes, we hypothesised that for alcohol consumption at low weekly levels to have any potential long-term adverse effects, a documented effect at higher levels should be present. The decision to include outcomes in this study was based on thorough literature reviews showing negative associations between high daily intake of alcohol and intelligence quotient (IQ) [3], attention [10–16], speed of information processing [9], executive functioning [21,22,59], and motor development [16–18].

The age of 5 years was chosen based on considerations of stability of cognitive performance: While the reliability of IQ measures increases with age into middle age, IQ from the age of about 5 years is a reliable measure with reliability about 0.8 [60]. IQ in childhood and adolescence predicts adult health and mortality [61], as well as social socioeconomic position [62,63] and social pathology [63], with high IQ being beneficial.

Potential confounders

The prenatal and postnatal telephone interviews included detailed information on most potential confounders, e.g. smoking, diet, socioeconomic position, and medical and obstetric histories. A parent-administered questionnaire included information on the parent's education, current socioeconomic status,

Table I. Final tests and examinations used in The Lifestyle During Pregnancy Study.

Psychological domain	Test instrument used
General intellectual ability (IQ)	WPPSI-R subtests [67]
• verbal IQ	– information
• performance IQ	– vocabulary
	– arithmetic
	– object assembly
	– geometric design
	– block design
Learning/motor skills	Animal house (WPPSI-R) [67]
Attention (visual and auditory)	TEACh-5 (test of everyday attention for 5-year-old children)
(sustained, focused, spatial, control/inhibition)	– balloon hunt
	– barking
	– draw a line
	– hide and seek-V
	– hide and seek-A
Reaction time and speed of information processing	Sternberg task – KVC (Kilburn's version for children)
Prediction of executive function	BRIEF (behaviour rating inventory of executive functions) [68]
• eight subscales/aspects of executive functions	(parent and teacher part)
Social skills, behaviour, including adaptive behaviour	SDQ (strengths and difficulties questionnaire)
	(parent and teacher part) – modified version [69]
General developmental status	Draw a person [70]
Behaviour during test session	Behaviour Checklist (clinical rating)
• 4 subscales	
Maternal general intellectual ability (IQ)	– information (from WAIS) [71,72]
• verbal IQ	– vocabulary (from WAIS) [71,72]
• nonverbal IQ	– Raven (Raven's standard progressive matrices) [73]
Physical examination	
Height, weight, head circumference	
Vision	Østerberg vision board
Hearing	Audiometry
Motor development (fine and gross)	Movement ABC [74]
• ball skills	
• manual dexterity	
• static and dynamic balance	
Other	
Dysmorphology	Photography (full face and profile of child and mother)
Background information on SES, upbringing, disease and medication during childhood, etc.	Parent-administered questionnaire

WAIS, Wechsler adult intelligence scale.

and lifestyle, etc. In addition, the mother was administered a test of intelligence.

Sampling procedure

As opposed to smoking and caffeine intake, high average alcohol intake and binge drinking after the first 4 weeks of pregnancy are relatively rare events [57]. A primary consideration in the development of the sampling design, therefore, was to include women with different alcohol drinking patterns (Table II). The primary analyses were planned to be based on two distinct drinking patterns: average intake among four groups (0, 1–4, 5–8, and 9 or more drinks per week) and binge drinking (yes or no as well as the number and timing of binge episodes). To study the interaction between average alcohol intake and binge drinking, we subsequently defined seven main categories according to average drinking levels during pregnancy (categories 1–5) or prepregnancy drinking levels (categories 6–7). Each of these seven categories was further stratified on the basis of binge drinking patterns: whether or not binge drinking occurred and, if so, during which weeks during the pregnancy the binging episodes occurred. Category 1 is the "unexposed" reference group for the study (no drinking during pregnancy and no binge episodes). Category 7 comprised women who had the highest drinking levels before pregnancy (15 or more drinks per week, i.e. above the maximum intake level recommended by the Danish National Board of Health). This category was established as a separate risk category because of the risk of continued high drinking levels before pregnancy is recognised.

A total of 150 women were selected for the reference group (category 1), while 75 women were selected for each of the other categories. For a few categories, however, fewer than 75 women had reported this drinking pattern in the DNBC, and hence fewer participants were expected (Table II). A total of 1450 women were expected to participate in the initial sample (Table II) based on power calculations and calculations of the predicted number of pregnancies in each alcohol category (Table II), as only approximately 65,000 pregnancies were available from the DNBC when decisions were made as to the number of participants in this study.

Table II. Primary selection procedure in The Lifestyle During Pregnancy Study.

	Average number of drinks/week		*Binge drinking* Weeks of pregnancy[a]					
Categories	Before pregnancy	During pregnancy	1–2	3–4	5–8	9 or more	Expected number of participants[b]	Actual number of participants
1	NA	0	No	No	No	No	150	189
1a	NA	0	Yes	No	No	No	75	114
1b	NA	0	No	Yes	No	No	75	102
1c	NA	0	No	No	Yes	No	75	117
1d	NA	0	No	No	No	Yes	50	96
2	NA	1–4	No	No	No	No	75	108
2a	NA	1–4	Yes	No	No	No	75	117
2b	NA	1–4	No	Yes	No	No	75	119
2c	NA	1–4	No	No	Yes	No	75	105
2d	NA	1–4	No	No	No	Yes	75	121
3a	NA	0	Yes	Yes	Yes	Yes[c]	75	83
3b	NA	1–8	Yes	Yes	Yes	Yes[c]	75	87
			1–2	3–4	5 or more			
4	NA	5–8	No	No	No		75	88
4a	NA	5–8	Yes	No	No		50	12
4b	NA	5–8	No	Yes	No		75	42
4c	NA	5–8	No	No	Yes		75	42
5a	NA	9+	No	No	No		50	15
5b	NA	9+	Yes	Yes	Yes[c]		50	6
6	0	0	No	No	No		50	77
7	15+	0–∞[d]					75	88

NA, not applicable.
[a]Measured from the last menstrual period.
[b]Based on 50% participation in all categories.
[c]Any combination of two or more "yes".
[d]Random sample of women from all subgroups "in pregnancy".

During 2005, an additional group of 300 women who completed a nutritional survey component of the DNBC and who had different intake patterns of seafood and iron and different reported durations of breast feeding were included to ensure sufficient variance with respect to these factors in the final sample for supplemental analyses (Table III).

Mothers and children were excluded if they did not speak Danish, if they had impaired hearing or vision loss that inhibited the performance of the psychological tests or if a child was affected by congenital disorders that might lead to mental retardation (e.g. Downs syndrome, autism, and cerebral palsy).

Test battery and pilot testing

The overall purpose of the test battery was to enable a thorough and theoretically relevant assessment of each child's neurodevelopmental status, including general intelligence, fundamental specific cognitive functions, and functioning in specific behavioural domains. Specifically, we wanted measures of verbal and nonverbal intelligence (IQ), measures of sustained and focused attention, and measures of speed of information processing, executive functions, learning skills, fine and gross motor development, social skills and general behavioural development. Furthermore, height, weight, and head circumference were measured, photographs were taken to allow for subsequent measurement of (dysmorphic) facial features, and on-site screening of vision and hearing abilities was conducted. The final test battery was selected after an extensive pilot phase. Three of the key issues were the relatively limited time that a 5-year-old child is able to collaborate on mentally demanding tasks, the limited number of available tests and questionnaires, suitable for young Danish children, and theoretical relevance in relation to prenatal alcohol exposure and other lifestyle factors. Table I presents an overview of the final test battery.

With regard to attention and speed of information processing, we were unable to locate theoretically founded tests for young children. Therefore, a new test of attention (TEACh-5) and a children's version of Sternberg's speed of information processing paradigm were developed. In developing both tests, it

Table III. Additional selection procedure in The Lifestyle During Pregnancy Study.

		Number of women before additional selection[a]	Number of women after additional selection
Fish[b]	Group 1	32	121
	Group 2	137	305
	Group 3	239	239
	Group 4	146	294
	Group 5	55	148
Iron from foods[c]	First decentile	127	258
	Second decentile	129	256
	Third decentile	146	146
	Fourth decentile	123	123
	Fifth decentile	150	150
	Sixth decentile	120	120
	Seventh decentile	128	128
	Eighth decentile	122	122
	Ninth decentile	110	241
	Tenth decentile	113	222
Iron from supplements	0 mg	124	234
	0.1–13 mg	48	86
	14 mg	44	87
	14.1–49 mg	421	421
	≥50 mg	631	938
Breastfeeding	≤1 month	124	229
	2–3 months	143	244
	4–6 months	253	253
	7–9 months	313	452
	≥10 months	376	529

[a]Numbers do not add up to 1450 because of missing information for each variable.
[b]Group 1 (never eating fish), Group 2 (eating fish each month or less), Group 3 (hot meal each month, sandwich each week), Group 4 (hot meal and sandwich each week, low frequency), and Group 5 (hot meal and sandwich each week, high frequency).
[c]The estimated intake in mg/day (energy-adjusted).

was paramount that the tests require as little verbal skill as possible and making it possible to control for potential confounding of test results by individual differences in motor function.

A total of six pilot studies were performed of which four were mainly related to the development of the TEACh-5 and the Sternberg method. The main pilot involved 25 mother–child pairs and was used to test enrolment procedures, to estimate participation rate, to ensure comprehensibility of questionnaires, and to decide on the final content and order of the test elements.

Data collection

Selected mothers were invited to participate in the study by letter approximately 8–12 weeks before their child's fifth birthday (2003–08). The mother/parents could return a slip (a) agreeing to participate, (b) asking for more information by telephone, or (c) declining to participate. Those agreeing to participate were contacted by telephone and provided more detailed project information, and an appointment for testing the child was made. The parent-administered questionnaires (Table I) were mailed to the participants before the day of the testing, and, if permission was obtained from the parents, questionnaires were subsequently mailed to the child's day-care centre. Further oral information was given on the day of testing before the consent form was signed. A maximum of two reminders were mailed to the parents and/or day-care institutions if they did not respond to the initial letter.

The assessments were conducted in a controlled setting at four regional sites (Copenhagen, Odense, Aarhus, and Aalborg). The test session lasted approximately 3 hours, which, based on the pilot studies, was the maximum length of time that 5-year olds can be tested. All psychological tests were administered by one of 10 psychologists with special training in neuropsychological testing and study procedures. Assessment of motor development was conducted by 30 trained physiotherapists (this part of the study was stopped in February 2006 due to lack of funding). At the time of the test session, psychologists and physiotherapists were blinded to exposure status and to any information regarding the child's development in general. The mothers completed adult IQ tests, while the child was being tested by the physiotherapist or, from February 2006, at the end of the test session.

The total cost of the data collection was estimated at US$ 4,723,537.

Standardisation of procedures and inter-rater reliability

Given the study size and the inherent need to involve several psychologists and physiotherapists in the data collection, special attention was paid to methodological issues, such as inter-rater reliability and standardised test procedures.

Psychologists

During the pilot projects, the psychologists observed and supervised each other to ensure a uniform and standardised administration of all test procedures and a consistent scoring of all tests. This at-site supervision procedure was repeated every 6 months and whenever a new psychologist began working with the project. In addition, test manuals were continuously supplemented with additional guidelines for test procedures and scoring criteria (copies available from the authors).

Approximately every 6 months, each psychologist blindly rescored a number of subtests administered by other psychologists. This procedure included the vocabulary and the geometric design subtests of the Wechsler preschool and primary scale of intelligence – revised (WPPSI-R), and the vocabulary subtest from the Wechsler adult intelligence scale (WAIS). Subsequently, identified discrepancies were discussed, the correct scoring was agreed upon, and scoring criteria were specified. Typically, there was approximately 97–97.5% agreement before discussion, and about two-thirds of the discrepancies were deemed to be unavoidable because of ambiguous scoring criteria in published manuals.

A successful attempt was made to allocate testers evenly across alcohol exposure categories (Table IV). Preliminary analyses of the distribution of test scores among psychologists demonstrated small but detectable differences on IQ scores and on some TEACh-5 subtests of attention, but no tester differences with regard to the Sternberg speed of information processing test. However, no systematic pattern of tester differences was observed and, because tester status was not associated with exposure categories, tester effects per se should not lead to confounding.

Physiotherapists

Before the beginning of the project, the physiotherapists participated in an introductory whole daylong meeting, allowing for in depth discussions of the project procedures and training in administration and scoring of the movement ABC test. Every 6 months, all the procedures of the movement ABC were demonstrated and discussed, using volunteers

Table IV. Distribution of testers across alcohol sampling categories, based on initial sampling.

	Psychologist, numbered according to time of entering the project										
Categories	1	2	3	4	5	6	7	8	9	10	11
1	39	23	16	29	18	0	18	21	7	1	2
1a	19	22	12	23	3	1	6	17	1	1	1
1b	11	15	13	21	3	0	15	10	5	0	2
1c	19	18	10	23	10	1	11	10	4	0	3
1d	24	24	14	11	7	0	7	3	2	1	0
2	11	19	7	15	12	1	17	11	5	1	0
2a	18	18	5	30	8	1	12	12	4	1	1
2b	20	14	9	27	11	1	17	10	2	0	0
2c	14	19	6	18	9	0	13	10	0	0	2
2d	18	21	7	22	12	2	8	16	4	0	0
3a	16	13	8	12	5	1	6	10	4	0	2
3b	9	13	5	20	8	0	14	8	4	1	0
4	15	13	7	12	4	2	10	11	3	1	1
4a	2	0	3	4	0	0	0	2	0	0	0
4b	4	7	6	8	3	0	4	4	0	0	1
4c	10	5	1	9	6	0	6	3	0	0	0
5a	1	1	2	5	2	0	1	3	0	0	0
5b	1	0	2	1	0	0	0	0	0	1	0
6	12	14	7	14	5	0	5	9	2	1	0
7	12	14	8	15	9	0	11	11	2	1	2
Total	275	273	148	319	135	10	181	181	49	10	17

p-value (chi-squared test) for the distribution of testers across alcohol sampling categories = 0.242.

and video recordings. Where the manual appeared unclear, additional guidelines for the different procedures were outlined. There were no substantial or significant differences in the distribution of mother–child pairs tested by each physiotherapist across the alcohol exposure categories.

Participation

Initially, we expected a participation rate of about 50% and consequently sampled 3000 mother–child pairs from the DNBC. In 2005, an additional 600 pairs were sampled to include 300 mother-child pairs with different intake of seafood diet and iron and different duration of breast feeding. A total of 3292 mother–child pairs have been invited to participate, with a consent rate of 57.0%. For various reasons 1.9% of those who initially agreed to participate failed to do so (e.g. because of illness or lack of motivation). Consent and participation rates have been almost equally distributed across alcohol intake categories.

Tests, examinations, and questionnaires

Of all the mother–child pairs invited to participate, 50.8% returned the questionnaire and participated in the test session, 0.2% only participated in the test session, whereas 4.3% only returned the questionnaires. Of the participating mothers, 95.3% gave permission for the questionnaires to be sent to their child's day-care centre: 93.4% of the centres returned the questionnaires after 1–2 reminders. Of the children participating in the test session, 83.5% completed all 13 psychological subtests (subtests from WPPSI-R, TEACH-5 and draw-a-person), except the speed of information processing test (completed by 76.6% of the children). For the remaining children, single subtests were missing due to e.g. motivational factors, lack of understanding test premises, or inability to complete the subtests. Of the mothers to the participating children, 99.0% completed all three adult IQ test elements.

The overall participation in the DNBC has been estimated at approximately 30% [55]. While a strong selection is likely in the DNBC [64], and further selection has taken place in this study, the differential participation in the DNBC seems to cause only little if any bias in studies within the DNBC [64].

Power calculations

Each individual outcome will be analysed in appropriate regression models taking into account the sample design (including timing of binge drinking within the predefined week groups). The power calculations shown below relate to these subanalyses. The information derived through the subanalyses is used to build a large mixed-model analysis of variance

Table V. The smallest detectable differences for continuous outcomes (IQ) and smallest detectable risk ratio for categorised outcomes with α-level = 0.05, power = 80%. Both calculations based on expected numbers at study initiation and calculations based on the actual participation are shown. The Lifestyle During Pregnancy Study.

Average alcohol intake				Binge drinking			
Average alcohol intake (drinks/week)	*N*	Continuous outcomes (IQ)[a]	Categorised outcomes (RR)[b]	Binge drinking	*N*	Continuous outcomes (IQ)[a]	Categorised outcomes (RR)[b]
0[c]	500[d]	Ref	Ref	No[e]	350[d]	Ref	Ref
1–4	412	2.8	1.6	Yes	975	2.7	1.7
5–8	312	3.0	1.7				
9+	100	4.6	2.0				
0[c]	701[f]	Ref	Ref	No[e]	400[f]	Ref	Ref
1–4	613	2.3	1.5	Yes	1162	2.4	1.6
5–8	226	3.2	1.7				
9+	21	9.3	3.5				

[a]Based on smallest detectable difference in IQ from reference IQ of 100 (SD = 15).
[b]Based on an expected proportion of cognitive and behavioural deficits among the unexposed of 10%.
[c]Alcohol intake categories included from Table II: 0 (1 − 1d + 3a); 1–4 (2 − 2d + ½X3b); 5–8 (4 − 4c + ½X3b); 9+ (5a − 5b).
[d]Expected numbers at study initiation.
[e]Alcohol intake categories included from Table II: no (1 + 2 + 4 + 5a); yes (1a − 1d + 2a − 2d + 3a − 3b + 4a − 4c + 5b).
[f]Actual numbers at the end of the study.
IQ, intelligence quotient; Ref, reference category, RR, risk ratio.

model, which takes care of multiple testing issues by analysing all outcomes simultaneously. Conducting a power calculation on the combined model is impractical. However, it is not unreasonable to assume that the combined power of analysing all outcomes in one model will give a power no less than the weakest of the subanalyses, and possibly more powerful.

Based on the groups proposed for follow-up (Table II), we operated with two levels of analyses: Primary analyses included analyses of overall average intake among four groups: 0, 1–4, 5–8, and 9 or more drinks per week, and binge drinking (yes or no) (Table V). Assuming a standard deviation of 15, we calculated the smallest detectable differences for continuous outcomes (e.g. IQ) at a power of 80% and an α-level of 0.05 and, with the basic assumption of disease proportion of 10% among the reference group, we derived the smallest detectable relative risk for dichotomous outcomes (Table V).

Subsequent secondary analyses were based on the stratified sample. Assuming scenarios in which the analyses would have to be adjusted for differences over strata, the power calculations were repeated (data not shown). These calculations indicated only a very modest loss of power for the moderate strata differences we expect to see. All power calculations were done by simulation.

Ethics

The study was approved by the DNBC Board of Directors, the DNBC Steering Committee, the relevant regional ethics committees in Denmark, the Danish Data Protection Agency, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board.

Following the tests, each mother/parents and her/their child were offered an oral presentation of the psychologists 'and the physiotherapists' overall impression, if they wished. Naturally, most children were expected to perform within the normal range on the neuropsychological tests. But, in the few cases in which the psychologist felt the child warranted special assistance, the mother/parents were informed of this and advised to seek professional assistance as provided by the applicable county administration. In most of these cases, the parents were already aware of the problem.

Although a 3-hour test session may be considered long for 5-year old children, few participants expressed that it was too long or strenuous and only in a few cases were tests abandoned due to exhaustion of the child.

The parents received DKK 400–500 or a book worth approximately that amount for participating and the children received a voucher of DKK 100 for a toy shop. Travelling expenses were covered with appropriate documentation. These incentives were chosen based on the feedback from the pilot studies.

Perspectives

We hope that our study of the potential long-term effects of low-dose weekly alcohol intake and binge

drinking during pregnancy on offspring cognitive and behavioural development of offspring will move this field of research forward. Most importantly, the following methodological issues have been addressed:

Prospective data were obtained for alcohol and other exposures.
Reliable information was obtained for low levels of exposure to alcohol.
For alcohol use, pattern and timing of intake were accounted for.
Diet, environmental factors and maternal intelligence were taken into account as potential confounders.
A range of outcome measures were administered.
Theoretically appropriate measures of fundamental cognitive processes were included in addition to standardised clinical measures.
Several modalities were used for assessments.
Extensive pilot testing ensured that the test battery was appropriate for 5 year-old children in terms of the length of the test session and comprehensibility, and ensured their ability to give a good performance on all measures.
The overall sample size was large enough to allow sufficient power in the analyses.

Little is known about the potential long-term effects of low-dose weekly alcohol intake and binge drinking during pregnancy on the cognitive and behavioural development of offspring. This also applies to the potential long-term effects on neurodevelopment of prenatal exposure to tobacco smoking, caffeine, and diet. Together with similar studies of maternal behaviour and lifestyle during pregnancy, the results of this project will contribute to the evaluation of long-term developmental effects of prenatal exposure to alcohol and other lifestyle factors.

In the development of the test battery, we developed new tests of attention and information processing speed. These tests have the potential to improve studies of neurocognitive development and could be a basis for earlier and more precise diagnosis of children with cognitive dysfunction, including deficits of attention.

It will also be possible to use the study as a basis for research into relevant biomarkers. The action of alcohol (ethanol) on neurodevelopment might be moderated by the host's (mostly the mother's) capacity to detoxify alcohol, which is primarily determined by the level of enzyme efficiency (e.g. alcohol dehydrogenase [ADH], and aldehyde dehydrogenase [ALDH]). The efficiency of ADH and ALDH most likely depends on the genetic composition coding for the ADH and ALDH genes [65,66], and it is possible to evaluate the potential influence of genetic components such as single nucleotide polymorphisms by measuring these factors in blood samples taken from all mothers and children in the DNBC study [55], including those enrolled in the lifestyle study.

The DNBC cohort offers a unique possibility to evaluate associations between multiple prenatal and perinatal factors and the long-term development of offspring. The DNBC is administered by the Danish National Board of Health and is open to researchers on application. Also, the lifestyle study is likely to have global public health implications. Thus, the results will add important and unique information regarding issues about maternal alcohol consumption during pregnancy and any later effects on children who have experienced prenatal alcohol exposure at low and moderate levels, which is an issue of worldwide public health interest.

Conflicts of interest

The authors have no conflicts of interest to declare.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Acknowledgements

This study was supported financially by the Danish National Board of Health, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, and Lundbeck Foundation, Denmark. We gratefully acknowledge the participation and enthusiasm of the children and their parents in this study as well as the Danish National Birth Cohort.

The Danish National Research Foundation has established The Danish Epidemiology Science Centre that initiated and created the Danish National Birth Cohort. The cohort is furthermore a result of a major grant from this Foundation. Additional support for the Danish National Birth Cohort is obtained from the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, theAugustinus Foundation, and the Health Foundation

References

[1] Abel EL, Sokol RJ. Incidence of fetal alcohol syndrome and economic impact of FAS- related anomalies. Drug Alcohol Depend 1987;19(1):51–70.

[2] American Academy of Pediatrics Committee on Substance Abuse. American Academy of Pediatrics Committee on Substance Abuse and Committee on Children with Disabilities: fetal alcohol syndrome and fetal alcohol effects [see comments]. Pediatrics 1993;91(5):1004–6.

[3] Mattson SN, Riley EP, Gramling L, Delis DC, Jone KL. Heavy prenatal alcohol exposure with or without physical features of fetal alcohol syndrome leads to IQ deficits. J Pediatr 1997;131(5):718–21.

[4] Spohr HL, Willms J, Steinhausen HC. Prenatal alcohol exposure and long-term developmental consequences. Lancet 1993;341(8850):907–10.

[5] Passaro KT, Little RE, Savitz DA, Noss J. The effect of maternal drinking before conception and in early pregnancy on infant birthweight. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Epidemiology 1996;7(4):377–83.

[6] Windham GC, Fenster L, Swan SH. Moderate maternal and paternal alcohol consumption and the risk of spontaneous abortion. Epidemiology 1992;3(4):364–70.

[7] Kesmodel U, Olsen SF, Secher NJ. Does alcohol increase the risk of preterm delivery? Epidemiology 2000;11(5):512–18.

[8] Kesmodel U, Wisborg K, Olsen SF, Hensriksen TB, Secher NJ. Moderate alcohol intake during pregnancy and the risk of stillbirth and death in the first year of life. Am J Epidemiol 2002;155(4):305–12.

[9] Burden MJ, Jacobson SW, Jacobson JL. Relation of prenatal alcohol exposure to cognitive processing speed and efficiency in childhood. Alcohol Clin Exp Res 2005;29(8):1473–83.

[10] Streissguth AP, Barr HM, Sampson PD, Parrish Johnson JC, Kirchner GL, Martin DC. Attention, distraction and reaction time at age 7 years and prenatal alcohol exposure. Neurobehav Toxicol Teratol 1986;8(6):717–25.

[11] Streissguth AP, Martin DC, Barr HM, Sandman BM, Kirchner GL, Darby BL. Intrauterine alcohol and nicotine exposure: attention and reaction time in 4-year-old children. Develop Psychol 1984;20(4):533–41.

[12] Streissguth AP, Sampson PD, Olson HC, Bookstein FL, Barr HM, Scott M. Maternal drinking during pregnancy: attention and short-term memory in 14-year-old offspring – a longitudinal prospective study. Alcohol Clin Exp Res 1994;18(1):202–18.

[13] Shaywitz SE, Cohen DJ, Shaywitz BA. Behavior and learning difficulties in children of normal intelligence born to alcoholic mothers. J Pediatr 1980;96(6):978–82.

[14] Brown RT, Coles CD, Smith IE, Platzman KA, Silverstein J, Erickson S, Falek A. Effects of prenatal alcohol exposure at school age. II. Attention and behavior. Neurotoxicol Teratol 1991;13(4):369–76.

[15] Coles CD, Platzman KA, Lynch ME, Freides D. Auditory and visual sustained attention in adolescents prenatally exposed to alcohol. Alcohol Clin Exp Res 2002;26(2):263–71.

[16] Korkman M, Autti-Rämö I, Koivulehto H, Granström ML. Neuropsychological effects at early school age of fetal alcohol exposure of varying duration. Child Neuropsychol 1998; 4(3):199–212.

[17] Mattson SN, Riley EP. A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. Alcohol Clin Exp Res 1998; 22(2):279–94.

[18] Mattson SN, Schoenfeld AM, Riley EP. Teratogenic effects of alcohol on brain and behavior. Alcohol Res Health 2001;25(3):185–91.

[19] Olson HC, Streissguth AP, Sampson PD, Barr HM, Bookstein FL, Thiede K. Association of prenatal alcohol exposure with behavioral and learning problems in early adolescence. J Am Acad Child and Adolesc Psychiatry 1997;36(9):1187–94.

[20] Kodituwakku PW, Kalberg W, May PA. The effects of prenatal alcohol exposure on executive functioning. Alcohol Res Health 2001;25(3):192–8.

[21] Connor PD, Sampson PD, Bookstein FL, Barr HM, Streissguth AP. Direct and indirect effects of prenatal alcohol damage on executive function. Dev Neuropsychol 2000;18(3):331–54.

[22] Noland JS, Singer LT, Arendt RE, Minnes S, Short EJ, Bearer CF. Executive functioning in preschool-age children prenatally exposed to alcohol, cocaine, and marijuana. Alcohol Clin Exp Res 2003;27(4):647–56.

[23] Kesmodel U. Alkohol og graviditet. [Alcohol in pregnancy]. Ugeskr Laeger 1999;161(36):4989–94.

[24] Gray R, Henderson J. Report to the Department of Health – review of the fetal effects of prenatal alcohol exposure. Oxford: National Perinatal Epidemiology Unit; 2006.

[25] Kelly Y, Sacker A, Gray R, Kelly J, Wolke D, Quigley MA. Light drinking in pregnancy, a risk for behavioural problems and cognitive deficits at 3 years of age? Int J Epidemiol 2008;38(1):129–40.

[26] Testa M, Quigley BM, Eiden RD. The effects of prenatal alcohol exposure on infant mental development: a meta-analytical review. Alcohol Alcohol 2008;38(4):295–304.

[27] Secker-Walker RH, Vacek PM, Flynn BS, Mead PB. Smoking in pregnancy, exhaled carbon monoxide, and birth weight. Obstet Gynecol 1997;89:648–53.

[28] Wisborg K, Henriksen TB, Hedegaard M, Secher NJ. Smoking during pregnancy and preterm birth. Br J Obstet Gynaecol 1996;103(8):800–5.

[29] Wisborg K, Kesmodel U, Henriksen TB, Olsen SF, Secher NJ. Exposure to tobacco smoke in utero and the risk of stillbirth and death in the first year of life. Am J Epidemiol 2001;154(4):322–7.

[30] Weitzman M, Gortmaker S, Sobol A. Maternal smoking and behavior problems of children. Pediatrics 1992; 90(3):342–9.

[31] Christian MS, Brent RL. Teratogen update: evaluation of the reproductive and developmental risks of caffeine. Teratology 2001;64(1):51–78.

[32] Barr HM, Streissguth AP. Caffeine use during pregnancy and child outcome: a 7-year prospective study. Neurotoxicol Teratol 1991;13(4):441–8.

[33] Lumley J, Watson L, Watson M, Bower C, et al. Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. Cochrane Database Syst Rev 2001;(3):CD001056.

[34] Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324(7335):447.

[35] Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in high risk pregnancies. Fish Oil Trials In Pregnancy (FOTIP) team. BJOG 2000;107((3):382–95.

[36] Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. Lancet 1998;351((9097):173–7.

[37] Lucas A. Programming by early nutrition: an experimental approach. J Nutr 1998;128(2 Suppl):401S–6S.
[38] Kretchmer N, Beard JL, Carlson S. The role of nutrition in the development of normal cognition. Am J Clin Nutr 1996;63(6):997S–1001S.
[39] Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 1997;19((6):417–28.
[40] Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, et al. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J Pediatr 2000;136(5):599–605.
[41] Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. JAMA 1998;280(8):701–7.
[42] Davidson PW, Myers GJ, Cox C, Wilding GE, Shamlaye CF, Huang LS. Methylmercury and neurodevelopment: longitudinal analysis of the Seychelles child development cohort. Neurotoxicol Teratol 2006;28(5):529–35.
[43] Nordberg L, Rydelius PA, Zetterstrom R. Parental alcoholism and early child development. Acta Paediatr Suppl 1994;404:14–8.
[44] Nulman I, Rovet J, Kennedy D, Wasson C, Gladstone J, Fried S, Koren G. Binge alcohol consumption by non-alcohol-dependent women during pregnancy affects child behaviour, but not general intellectual functioning; a prospective controlled study. Arch Women Mental Health 2004;7(3):173–81.
[45] Streissguth AP, Barr HM, Martin DC. Maternal alcohol use and neonatal habituation assessed with the Brazelton scale. Child Dev 1983;54(5):1109–18.
[46] Bailey BN, Delaney-Black V, Covington CY, Ager J, Janisse J, Hannigan JH, Sokol RJ. Prenatal exposure to binge drinking and cognitive behavioral outcomes at age 7 years. Am J Obstet Gynecol 2004;191(3):1037–43.
[47] Parry GJ, Ogston SA. EUROMAC. A European concerted action: maternal alcohol consumption and its relation to the outcome of pregnancy and child development at 18 months. Results – child development at age 18 months. Int J Epidemiol 1992;21(Suppl 1):S72–8.
[48] Olsen J. Effects of moderate alcohol consumption during pregnancy on child development at 18 and 42 months. Alcohol Clin Exp Res 1994;18(5):1109–13.
[49] Forrest F, Florey CD, Taylor D, McPherson F, Young JA. Reported social alcohol consumption during pregnancy and infants' development at 18 months [see comments]. BMJ 1991;303(6793):22–6.
[50] Sood B, Delaney-Black V, Covington C, Nordstrom-Klee B, Ager J, Templin T. Prenatal alcohol exposure and childhood behavior at age 6 to 7 years: I. dose-response effect. Pediatrics 2001;108(2):E34.
[51] Goldschmidt L, Richardson GA, Cornelius MD, Day NL. Prenatal marijuana and alcohol exposure and academic achievement at age 10. Neurotoxicol Teratol 2004;26(4): 521–32.
[52] Aronson M, Hagberg B. Neuropsychological disorders in children exposed to alcohol during pregnancy: a follow-up study of 24 children to alcoholic mothers in Göteborg, Sweden. Alcohol Clin Exp Res 1998;22(2):321–4.
[53] Korkman M, Kettunen S, Autti-Ramo I. Neurocognitive impairment in early adolescence following prenatal alcohol exposure of varying duration. Neuropsychol Dev Cogn Sect C Child Neuropsychol 2003;9(2):117–28.
[54] Kesmodel U, Kesmodel PS. Drinking during pregnancy: attitudes and knowledge among pregnant Danish women, 1998. Alcohol Clin Exp Res 2002;26(10):1553–60.
[55] Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM. The Danish National Birth Cohort – its background, structure and aim. Scand J Public Health 2001;29(4):300–7.
[56] Kesmodel U, Olsen SF. Self-reported alcohol intake in pregnancy: comparison between four methods. J Epidemiol Community Health 2001;55(10):738–45.
[57] Kesmodel U. Binge drinking in pregnancy – frequency and methodology. Am J Epidemiol 2001;154(8):777–82.
[58] Kesmodel U, Frydenberg M. Binge drinking during pregnancy – is it possible to obtain valid information on a weekly basis? Am J Epidemiol 2004;159(8):803–8.
[59] Roemer FJ, Rowland DY. Long-term developmental outcomes of method of delivery. Early Hum Dev 1994; 39(1):1–14.
[60] Schuerger JM, Witt AC. The temporal stability of individually tested intelligence. J Clin Psychol 1989;45(2):294–302.
[61] Batty GD, Deary IJ, Gottfredson LS. Premorbid (early life) IQ and later mortality risk: systematic review. Ann Epidemiol 2007;17(4):278–88.
[62] McCall RB. Childhood IQ's as predictors of adult educational and occupational status. Science 1977; 197(4302):482–3.
[63] Gottfredson LS. g: highly general and highly practical. In: Sternberg RJ, Grigorenko EL, editors. The general factor of intelligence: how general is it? Mahwah, New Jersey: Erlbaum; 2002. pp. 331–80.
[64] Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? Epidemiology 2006;17:413–18.
[65] Chen HJ, Tian H, Edenberg HJ. Natual haplotypes in the regulatory sequences of affect human alcohol dehydrogenase 1C (ADH1C) gene expression. Hum Mutat 2005;25:150–5.
[66] Choi IG, Kee BS, Son HG, Ham BJ, Yang BH, Kim SH. Genetic polymorphisms of alcohol and aldehyde dehydrogenase, dopamine, and serotonin transporters in familial and non-familial alcoholism. Eur Neuropsychopharmacol 2005.
[67] Wechsler D. Manual for the Wechsler preschool and primary scale of intelligence – Revised UK edition. Sidcup, Kent: The Psychological Corporation; 1990.
[68] Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior rating inventory of executive function: professional manual. Odessa, Florida: Psychological Assessment Resources Inc; 2000.
[69] Goodman R. The strengths and difficulties questionnaire: a research note. J Child Psychol Psychiatry 1997;38(5): 581–6.
[70] Naglieri J. Manual for draw a person: a quantitative scoring system. New York: The Psychological Corporation; 1988.
[71] Wechsler D. Manual for the Wechsler adult intelligence scale. New York: The Psychological Corporation; 1955.
[72] Kasper J. WAIS: Retningslinjer for testprocedure og scoring. København: Dansk Psykologisk Forlag; 1990.
[73] Raven J, Raven J, Court J. Manual for Raven's progressive matrices and vocabulary scales. Oxford: Oxford Psychologists Press Ltd; 1998.
[74] Henderson S, Sugden D. Movement assessment battery for children, 2nd edn. København: Dansk Psykologisk Forlag; 2001.