

# Introduction to cohort studies

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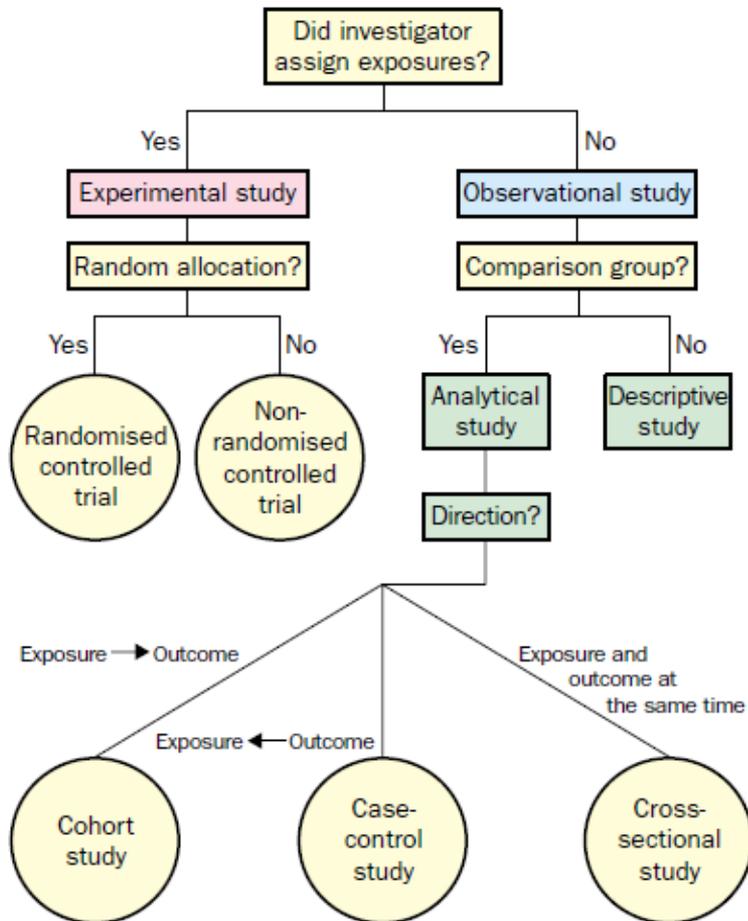
UNIVERSITY OF COPENHAGEN



Hvad hedder det på dansk?

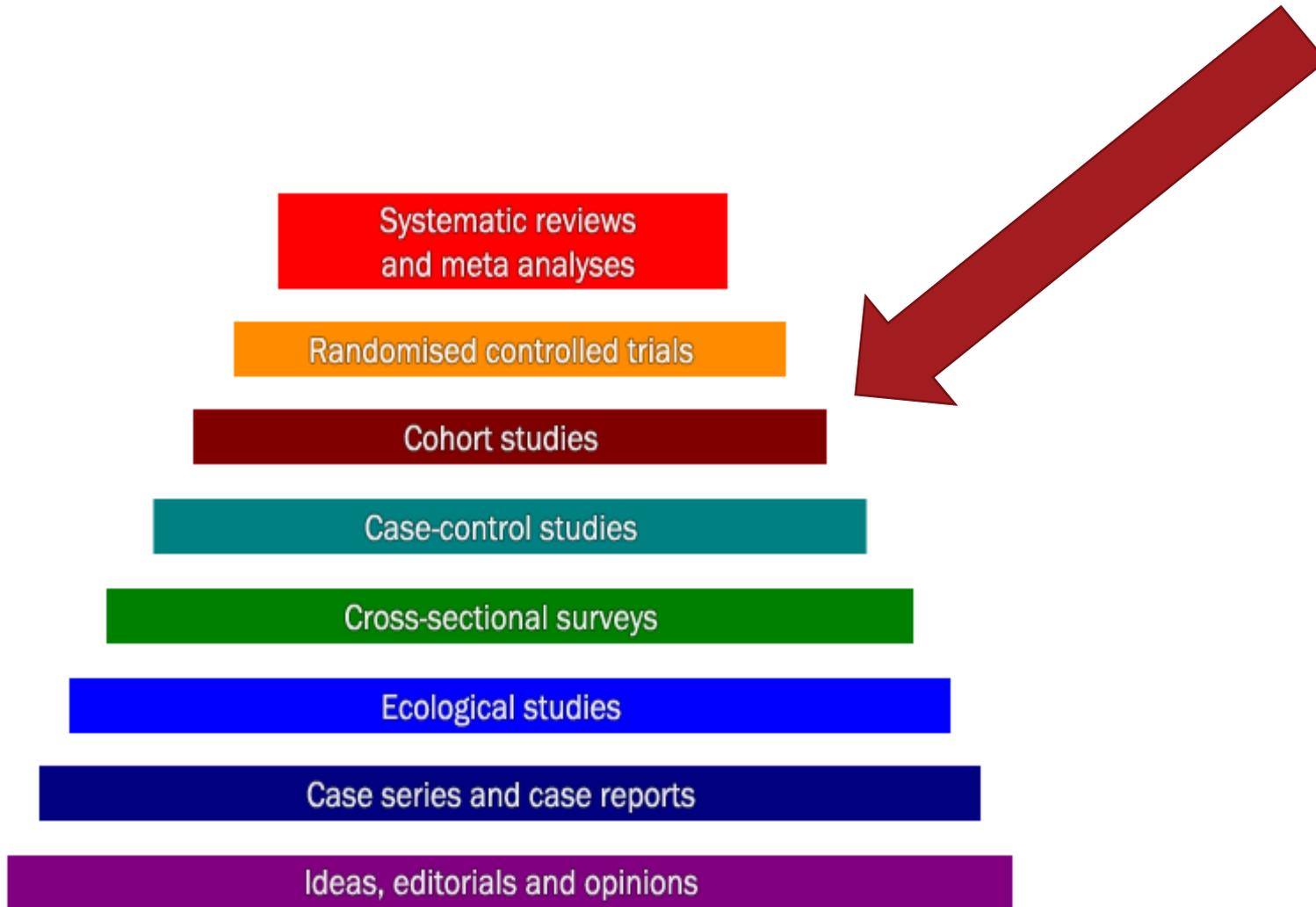
Statistical methods in cohort studies  
- Better taught by statisticians

# The lecture today



- Principles
- Strengths and weaknesses
- Real life examples

# The evidence hierarchy



## Which study design?

### **Abstract**

***Aim***—To study the neurobehavioural effects that diabetes during pregnancy might have on children by school age.

***Methods***—The neurobehavioural function of 57 school age children born to 48, well controlled diabetic mothers was compared with 57 control children matched for age, birth order, and parental socioeconomic status, using several cognitive, behavioural, sensory and motor neurological tests.

***Results***—The IQ scores of the index group children were similar to those of control children ( $117.7 \pm 13.4$  vs  $118.5 \pm 10.1$ ). There were no differences between the groups in various sensory motor functions. However, the index group children performed less well than the controls on indices of fine and gross motor functions, as observed on the Bruininks–Oseretzky test of motor proficiency. The scores of children born to diabetic mothers were higher than controls on the Touwen and Prechtl neurological examination. They also performed worse in the Pollack tapper test which is designed to detect minor neurological deficits, inattention, and hyperactivity. The index children had higher scores on the Conners abbreviated parent–teacher questionnaire which measures hyperactivity and inattention.

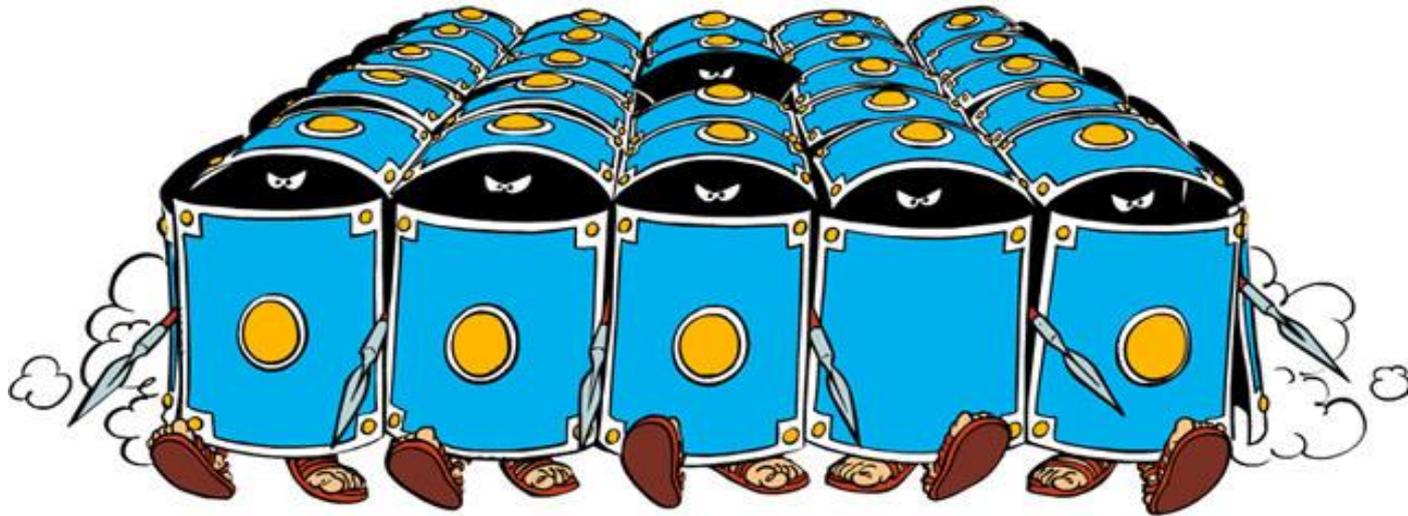
## Abstract

***Aim***—To study the neurobehavioural effects that diabetes during pregnancy might have on children by school age.

***Methods***—The neurobehavioural function of 57 school age children born to 48, well controlled diabetic mothers was compared with 57 control children matched for age, birth order, and parental socioeconomic status, using several cognitive, behavioural, sensory and motor neurological tests.

***Results***—The IQ scores of the index group children were similar to those of control children ( $117.7 \pm 13.4$  vs  $118.5 \pm 10.1$ ). There were no differences between the groups in

# Design of cohort studies



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# Many names....

COHORT STUDY

follow-up study  
prospective study  
longitudinal study

# The cohort study

An observational design

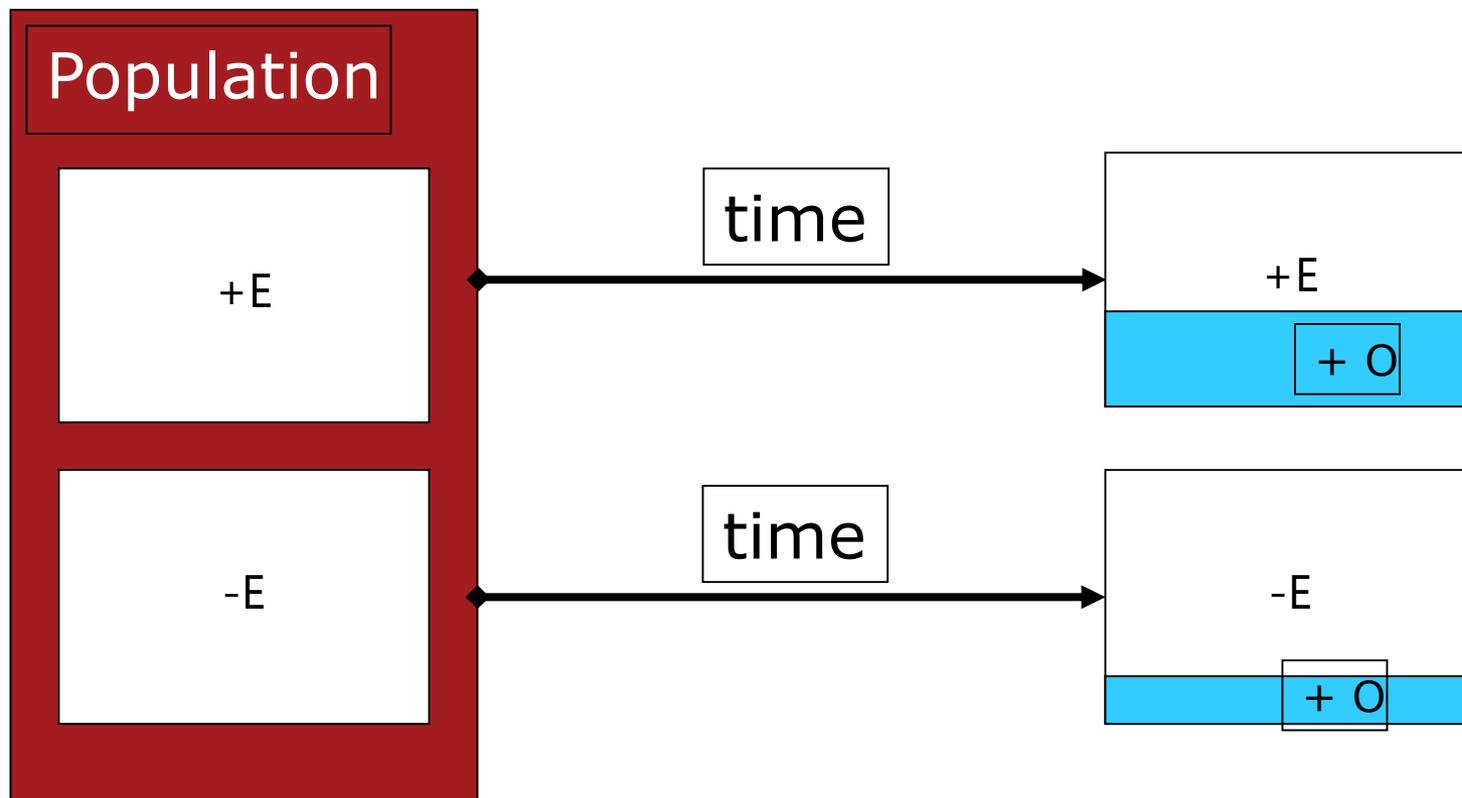
Primary identification of the study base

Exposed and un-exposed individuals are followed over time

Incidence of outcome (health, disease, behaviour etc.) in different exposure groups are compared

At least two time points of measurement

# The model



# Types of cohort studies

Prospective cohort

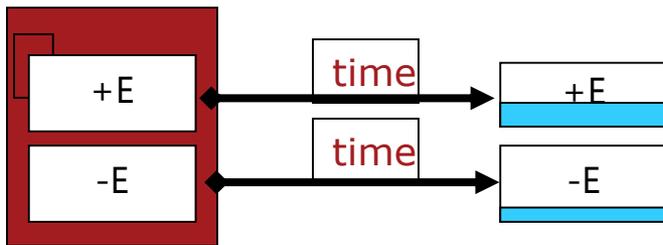
Historical cohort

Ambi-directional cohort

Nested case-control study in cohort

# Prospective vs. Historical cohort

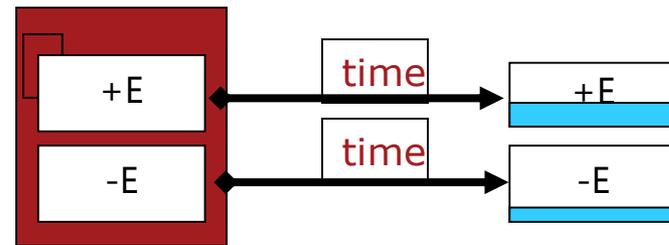
Prospective cohort



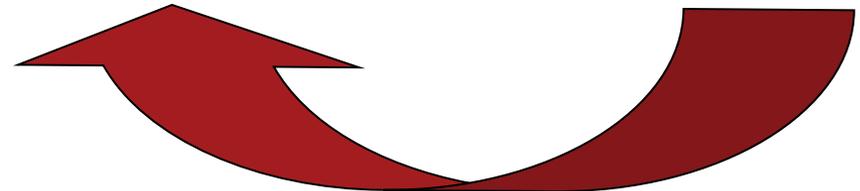
researcher



Historical cohort



researcher



# Take care



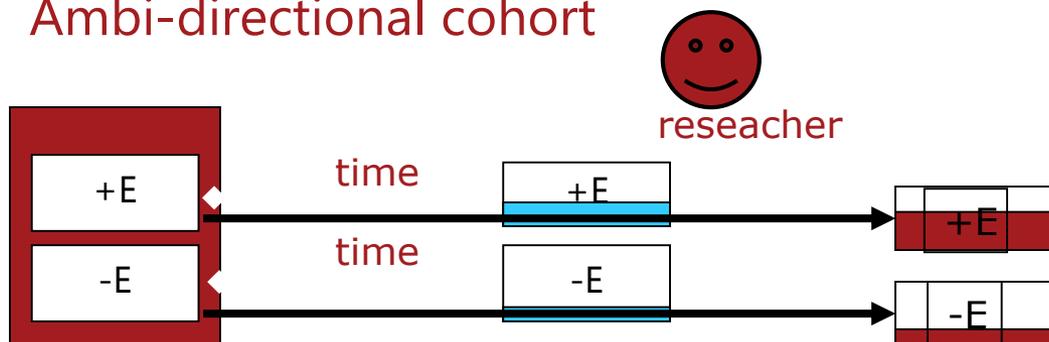
When are data collected retrospectively?

Always think about the time in relation to the outcome!

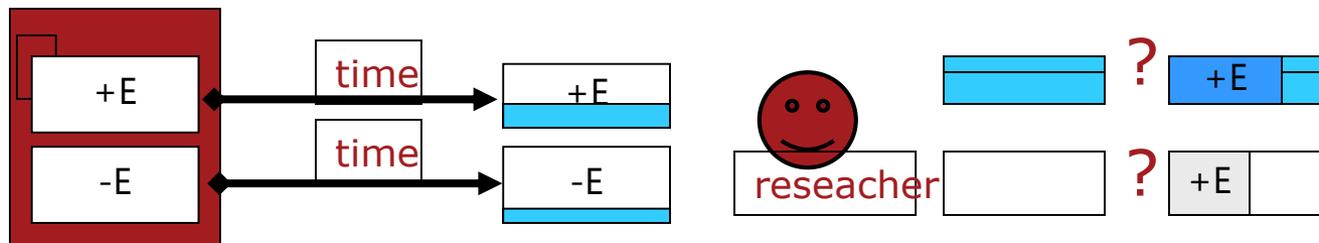
DO NOT use the concept/term retrospective cohort

# Other cohort designs

## Ambi-directional cohort



## Nested case-control study in cohort



# Types of cohort studies

Prospective cohort

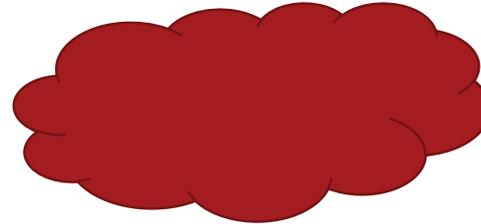
Historical cohort

Ambi-directional cohort

Nested case-control study in cohort

# Types of cohort studies

Cohort studies



Nested case-control study in cohort

# Some famous cohort studies that changes the world



## British doctors: 50 years' observations

- Questionnaire sent in 1951 to doctors resident in the UK and registered with an address in the BMA → 34,439 participants
- Follow-up for mortality until 2001
- In 2001:
  - 3192 censored (17 struck off the medical register for misconduct; 467 requested to exit; 2459 living abroad in 1971; 249 untraced)
  - 5902 alive in 2001 (administrative censoring)
  - 25346 had the event before 2001

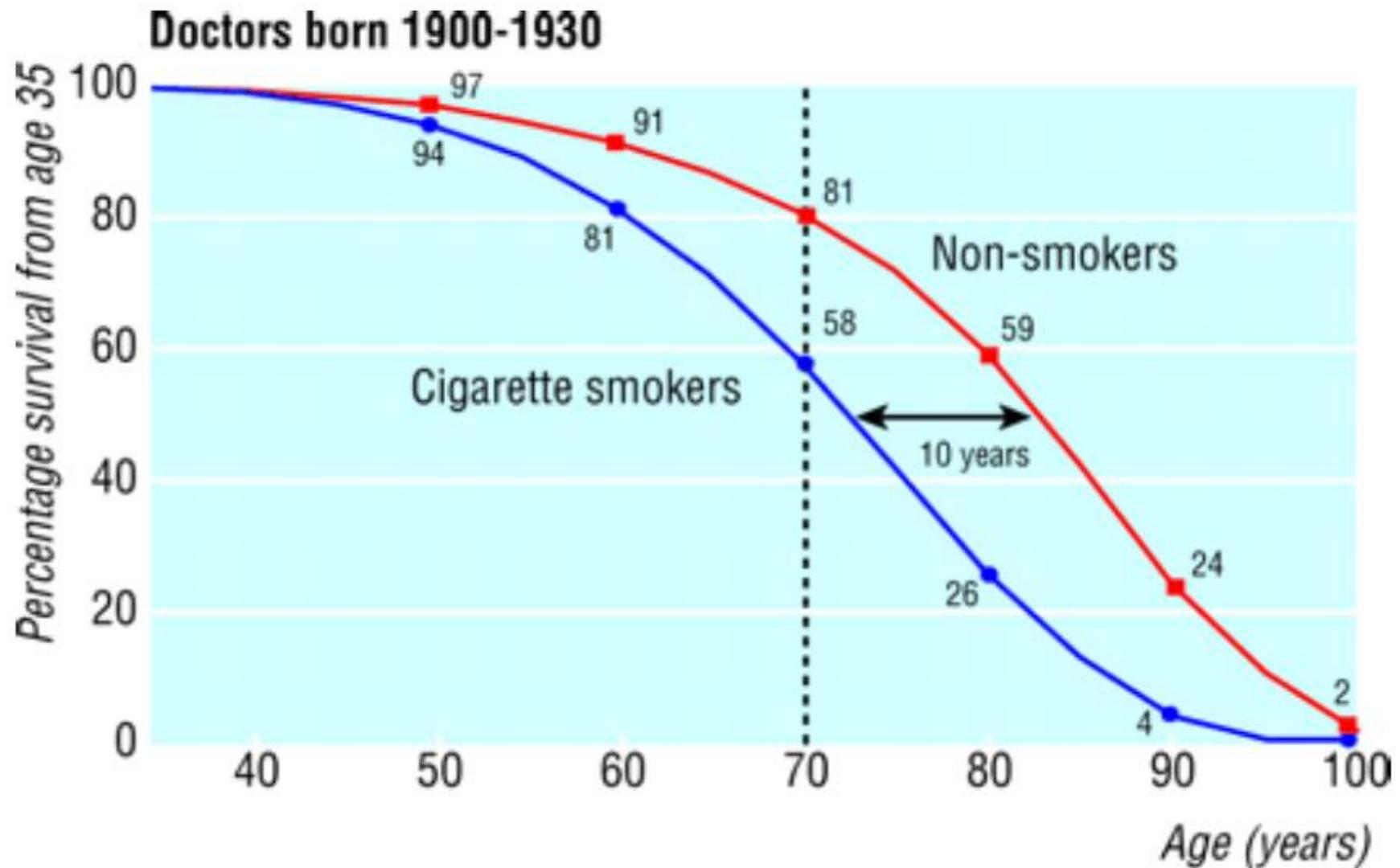
# SIR RICHARD DOLL

(October 28, 1912. TO July 24, 2005)



- Epidemiologist
- Activist
- Researcher
- Public health lobbyist

# Survival smokers vs. non-smokers



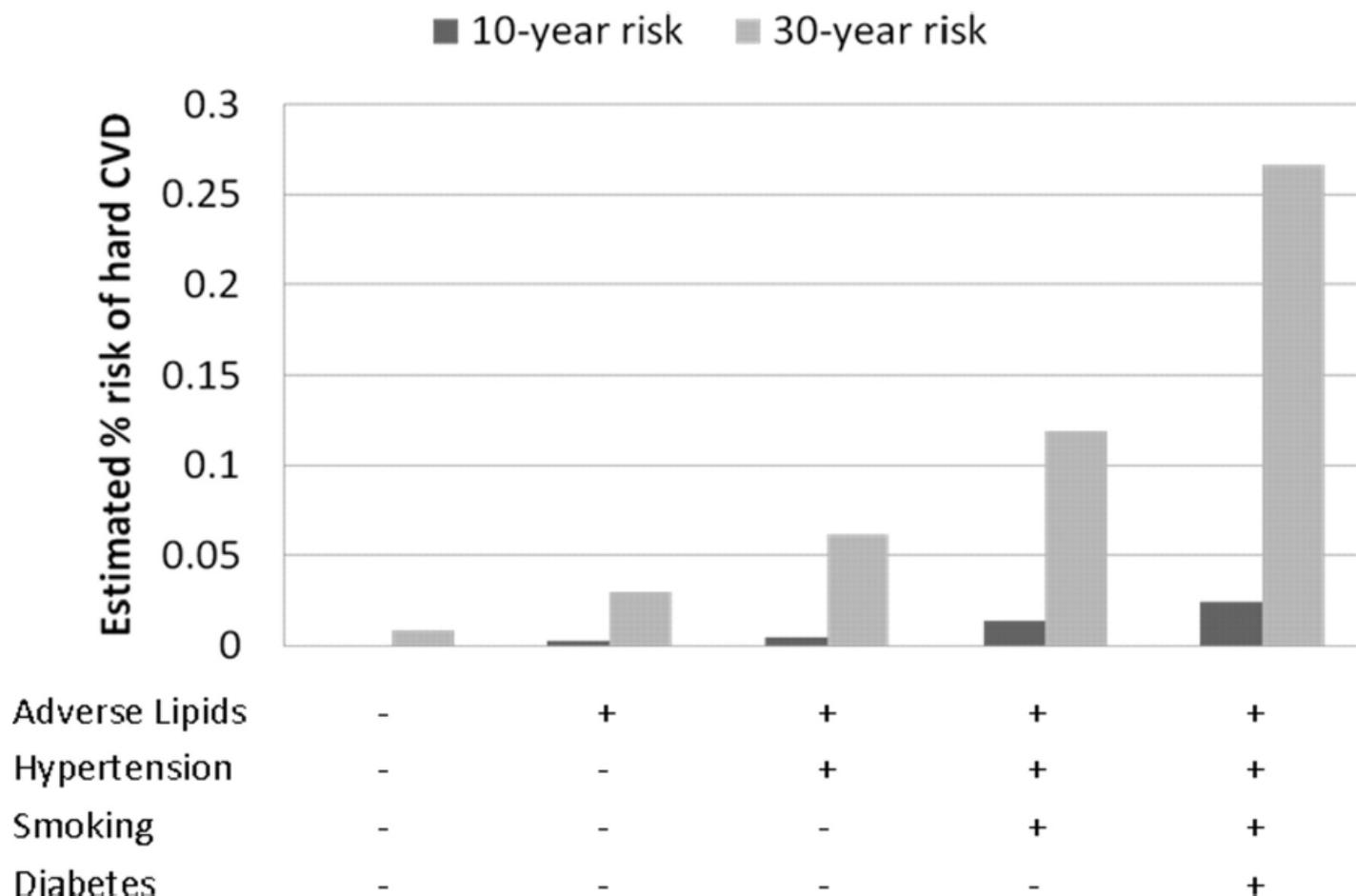
# Framingham Heart Study

- In 1948 sample of 2/3 of the adult population of Framingham, Massachusetts, 30 to 62 years of age → 5209 individuals enrolled
- Cardiovascular risk factors (blood pressure, diabetes, obesity, etc.)
- Medical visit every 2 years
- Follow-up from multiple sources
- Offspring cohort (offspring and their spouses recruited in 1971 → 5124 individuals
- Third generation cohort in 2005 → 4095 individuals

## Thirty-year risk of cardiovascular disease

- First visit of the offspring cohort.  
5124 individuals: 20-59 years, with no CVD or cancer at baseline not lost to F-U → 4680.  
Complete exposure information : 4506
- CVD: coronary death, myocardial infarction and stroke

Figure 3. Ten- vs 30-year risk of hard CVD for 25-year-old women with different risk profiles.



Pencina M J et al. Circulation 2009;119:3078-3084



# EPIC

Established by IARC, 1993-1998

## European Prospective Investigation into Cancer and nutrition

### EPIC cohort

	Subjects included	
	Questionnaire	Q+Blood
France	72 996	20 725
Italy	47 749	47 725
Spain	41 440	39 579
UK	87 940	43 138
Netherlands	40 072	36 318
Greece	28 572	28 500
Germany	53 094	50 679
Sweden	53 830	53 755
Denmark	57 054	56 131
Norway	37 231	9 197
<b>All</b>	<b>519 978</b>	<b>385 747</b>



Fig. 1 Collaborating centres and cohort subjects.

# UK biobank

## 500,000 40-69 year olds from 2006-2010

The screenshot shows a web browser displaying the UK Biobank news page. The browser's address bar shows the URL <http://www.ukbiobank.ac.uk/news/>. The page has a navigation menu on the left with items: Home, About UK Biobank, Participants, Scientists, Our news, Resources, Approved Research, and Published papers. The main content area is titled "News" and features five news items, each with a "Read More" link. A "Tag Cloud" on the right lists various topics such as adiposity, ageing, Association, biomarkers, BMI, Brain, cancer, cardiovascular, disease, cognition, cognitive function, COPD, Dementia, depression, diabetes, diet, disease, environment, epidemiology, exercise, featured, genes, Genetic, genetics, GWAS, health, heritability, Hypertension, inflammation, lifestyle, Machine Learning, Mendelian, randomization, mental health, mortality, MRI, obesity, osteoporosis, physical, activity, prediction, risk factors, risk, prediction, sleep, smoking, stroke, and vision. The Windows taskbar at the bottom shows the time as 10:13 on 19-06-2018.

You Are In **News**

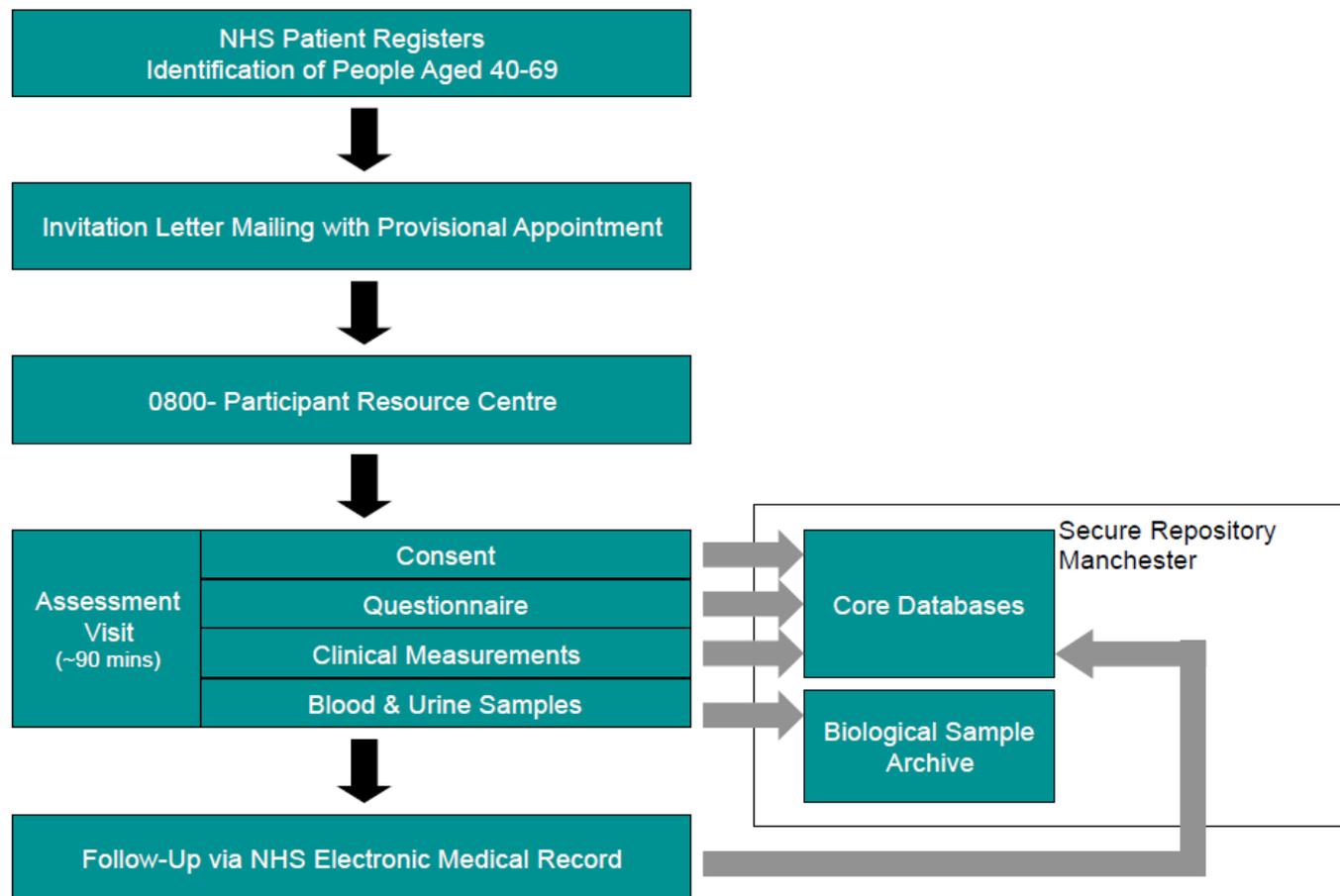
### News

- Frailty in middle aged linked to higher mortality**  
[Read More →](#)
- 25,000 participants scanned in the UK Biobank imaging study**  
[Read More →](#)
- Education linked to higher risk of short-sightedness**  
[Read More →](#)
- Researchers identify genetic variants that may predict glaucoma risk**  
[Read More →](#)
- Improving heart health could prevent frailty in old age**  
[Read More →](#)

### Tag Cloud

adiposity ageing Association  
biomarkers BMI Brain **cancer**  
cardiovascular cardiovascular  
disease **cognition** cognitive function  
COPD Dementia depression  
**diabetes** diet disease  
environment epidemiology exercise  
**featured** genes Genetic  
**genetics** GWAS health  
heritability Hypertension inflammation  
lifestyle Machine Learning Mendelian  
randomization mental health mortality  
MRI **obesity** osteoporosis **physical**  
activity prediction risk factors risk  
prediction sleep smoking stroke vision

# Design of Main Study



# Representativity in a cohort study

- Depends on the research question
- Interaction between exposure of interest and a factor that is uneven distributed



# Data sources in cohort studies

## **Exposures**

### Questionnaires

- Specific information
- Potentials for bias

### Registries

- non-specific
- often no selection, cheap
- minimal bias

### Clinical test

- Points of observation

## **Outcomes**

### Self reports

- Issues about validity

### Registries

- e.g. causes of death
- Hospitalized diseases

### Journal files

- Specific informations

# The 'second wave' birth cohorts

- Oldest:
- ALSPAC 1990-91
- 56 cohorts,
- more than 500,000 children
- Great opportunities:
- Large numbers
- Large variation

402 P. S. Larsen

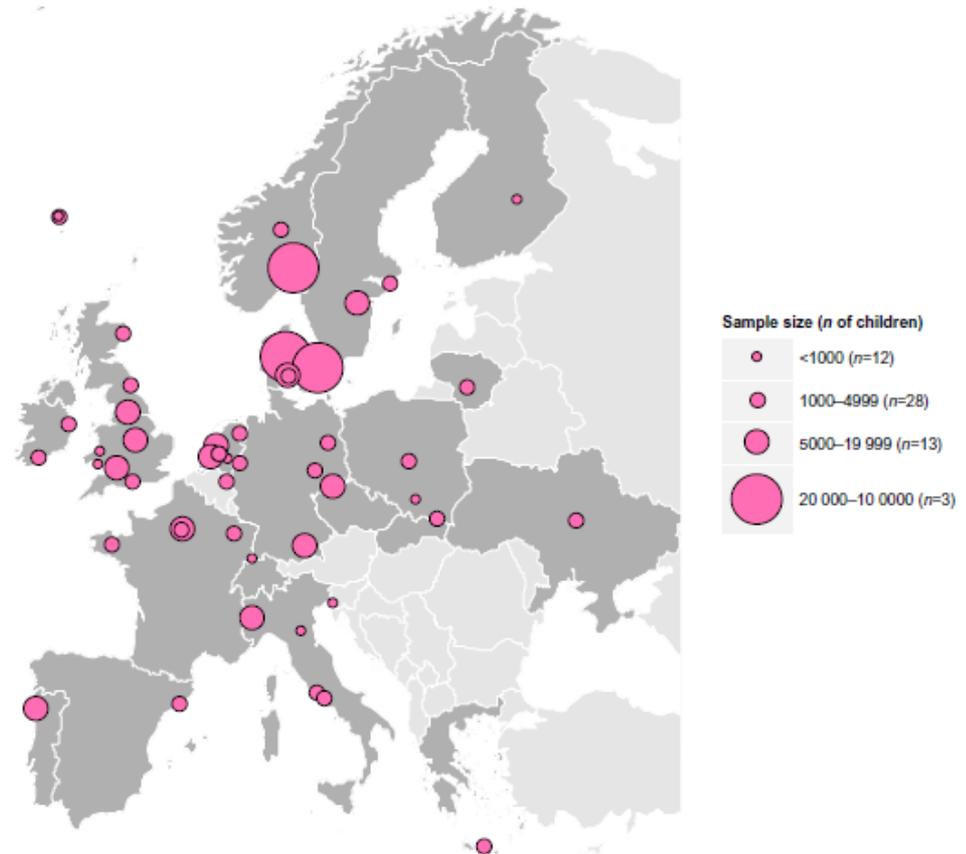
*Paediatric and Perinatal Epidemiology*, 2013, 27, 393-414

Figure 2. Location and sample size (No. of children) of included European pregnancy and birth cohorts.

## EPIDEMIOLOGY

## When an Entire Country Is a Cohort

Denmark has gathered more data on its citizens than any other country. Now scientists are pushing to make this vast array of statistics even more useful

For years, any woman who got an abortion had to accept more than the loss of her fetus: For some unknown reason, she also faced an elevated risk for breast cancer. At least that was what several small case-control studies had suggested before Mads Melbye, an epidemiologist at the Statens Serum Institute in Copenhagen, undertook the largest effort ever to explore the link. He and his colleagues obtained records on 400,000 women in Denmark's national Abortion Register, then checked how many of the same women were listed in the Danish Cancer Register. Their foray into the two databases led to a surprising result: As they reported in *The New England Journal of Medicine* in 1997, there appears to be no connection between abortion and breast cancer.

Their success underscores the value of a trove of data the Danish government has accumulated on its citizenry, which today totals about 5 million people. Other Scandinavian countries have created powerful database systems, but Denmark has earned a preeminent reputation for possessing the most complete and interwoven collection of statistics touching on almost every aspect of life. The Danish government has compiled nearly 200 databases, some begun in the 1930s, on everything from medical records to socioeconomic data on jobs and salaries. What makes the databases a plum research tool is the fact that they can all be linked by a 10-

digit personal identification number, the CPR, that is linked to the national death register, to grave. And this system allows for information that would be impossible to obtain in the United States.



Beauty in a woman's face is a medical reality.

But Melbye can extract information from the database. The being specific about existing data is hampered by the fact that, with more than

by the Danish hospitals, their databases are so complete that Denmark is a giant natural experiment. It won't allow its premises data to be collected in a cumbersome and expensive way. Statistics Denmark

### The Epidemiologist's Dream: Denmark

If the planners of a U.S. study of children's health could work in an ideal world, it might be Denmark. Epidemiologists there finished enrolling a cohort of 100,000 pregnant women into a mother-and-child research project last September and expect to finish collecting data from the children over the next year. The entire survey—which is large for this country of 70,000 annual births—is to be completed in 2005 for about \$15 million, a tiny fraction of what the cost would be in the United States.

The Danes didn't design their Better Health for Mother and Child cohort study to answer specific questions or conduct long-term follow-up, as the Americans plan to do (see main text). Instead, they aim to create a databank that generations of researchers can mine and use as a starting point for studies of how medications, infections, nutrition, and even psychological factors affect pregnancy and child health.

Physicians have recruited volunteers among women making their first pregnancy visit. Participants give two blood samples during pregnancy and cord blood when the baby is born. The samples are saved for later use, including possibly for genetic studies. The mothers also answer a detailed questionnaire concerning nutrition; in an 18-month follow-up, they give information on their health and environmental exposures. The public health system is funding the study, with support from private and public foundations.

"Because the Danish population is probably the world's best registered, Denmark is the ideal place for such studies," says epidemiologist Mads Melbye, a steering group member from Statens Serum Institute



Ready subjects. Denmark's 18-month-long birth cohort survey will collect data from mothers and newborns for a new database.

in Copenhagen. Each citizen has a personal identification number that can be used to track data in centralized health care records, disease registries, and a population registry. Even centralized school records may be used. "It's an epidemiologist's dream," says Mark Klebanoff of the U.S. National Institute of Child Health and Human Development, who says tracking subjects is one of the costliest aspects of long-term U.S. studies.

Norway, which has a system like Denmark's, is launching a mother-child study that will pool data with the Danish group's. Both benefit from streamlined management. It's difficult to get things done with too many decision-makers, says Melbye: "Running such a large study has taught us many things, but the chief lesson is that it is essential to put a very small group of people in charge."

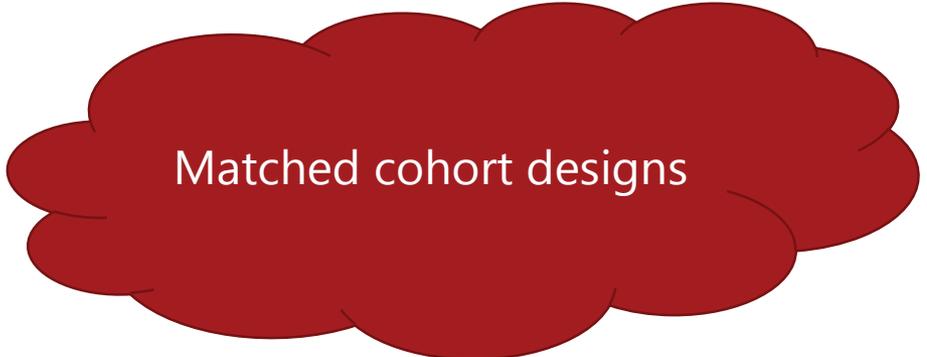
Results are already beginning to trickle out of the Danish study. For example, one group published an article in *The Lancet* last November that disproved the existing consensus view that a fever early in pregnancy increases the risk for miscarriage. That's just the beginning: Denmark's scientific ethics committee has so far given the green light to more than 70 research protocols based on the mother-child study.

—LONE FRANK

Lone Frank is a science writer in Copenhagen.

# Design and interpretations of cohort studies (1)

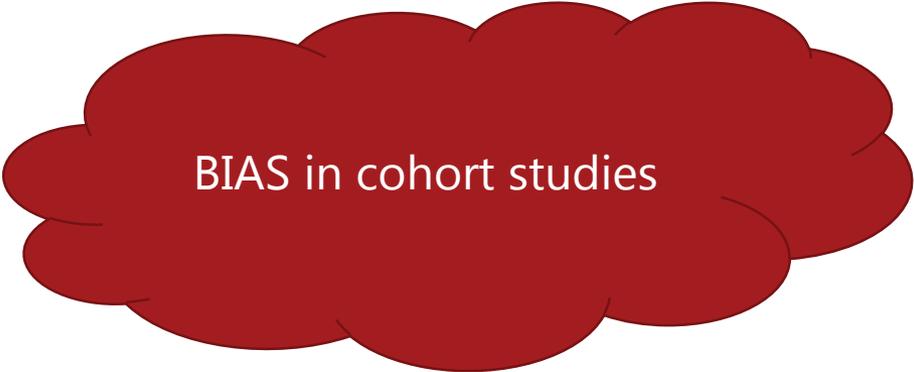
- Are the exposed and the un-exposed group comparable (otherwise)?
- How is the exposure determined?
  - 'objectively', proxy, presumed, self report
- Balanced collection of exposure?



Matched cohort designs

# Design and interpretations of cohort studies (2)

- How is the follow-up?
- Is attrition (non-response) correlated with exposure status?
- Is follow-up affected by outcome?
- Is follow-up time appropriate (latency time, induction time)?



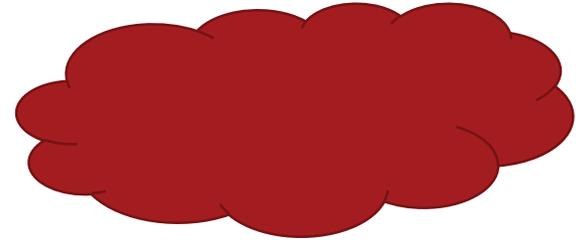
BIAS in cohort studies

# Design and interpretations of cohort studies (3)

- How is outcome defined?
- Balanced collection of outcome information?
- Non-participation at baseline and 'representativity':  
Not very important for internal validity, however,  
may be for generalisability (external validity)

# Can we draw causal inferences from cohort studies?

- This is a key question ...



# Pro et contra

## **Advantages**

Time relation between exposure and outcome

More outcome could be investigated (important for public health!)

Rare exposures

## **Disadvantages**

Large samples sizes for rare outcomes

Time consuming

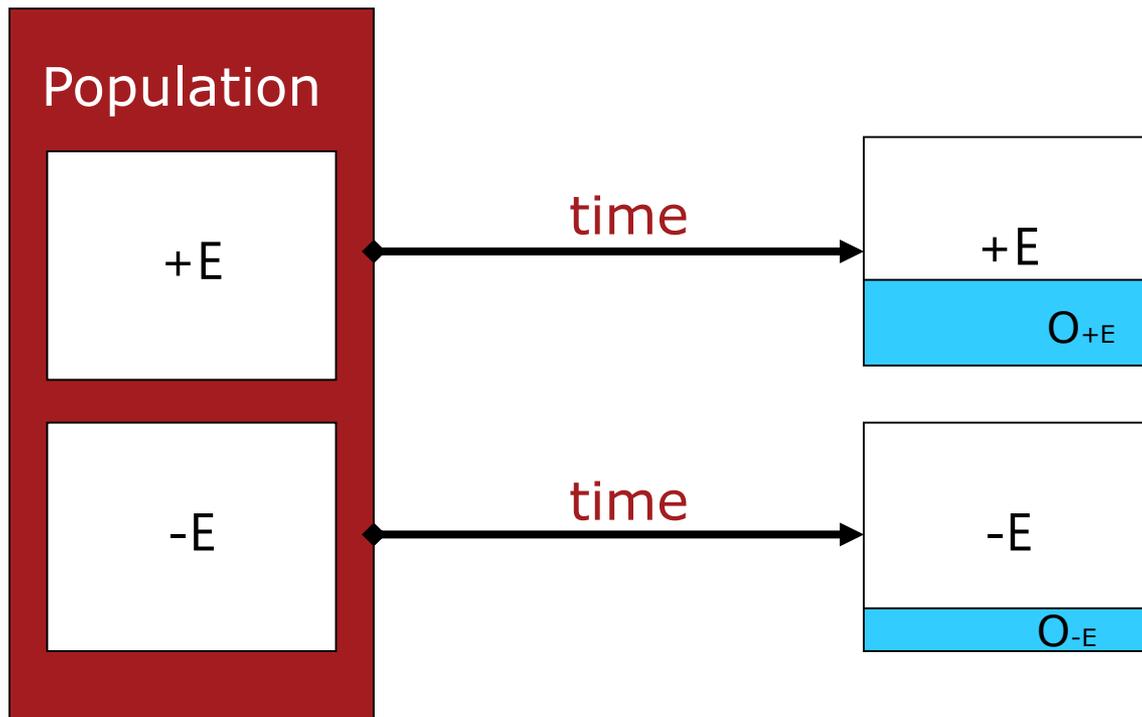
Loss of follow-up

# Frequency measures in cohort studies

cumulated incidence proportion (=risk) = new events until the time t / number of persons under risk

IR = incidence rate = new events / cumulated risk time

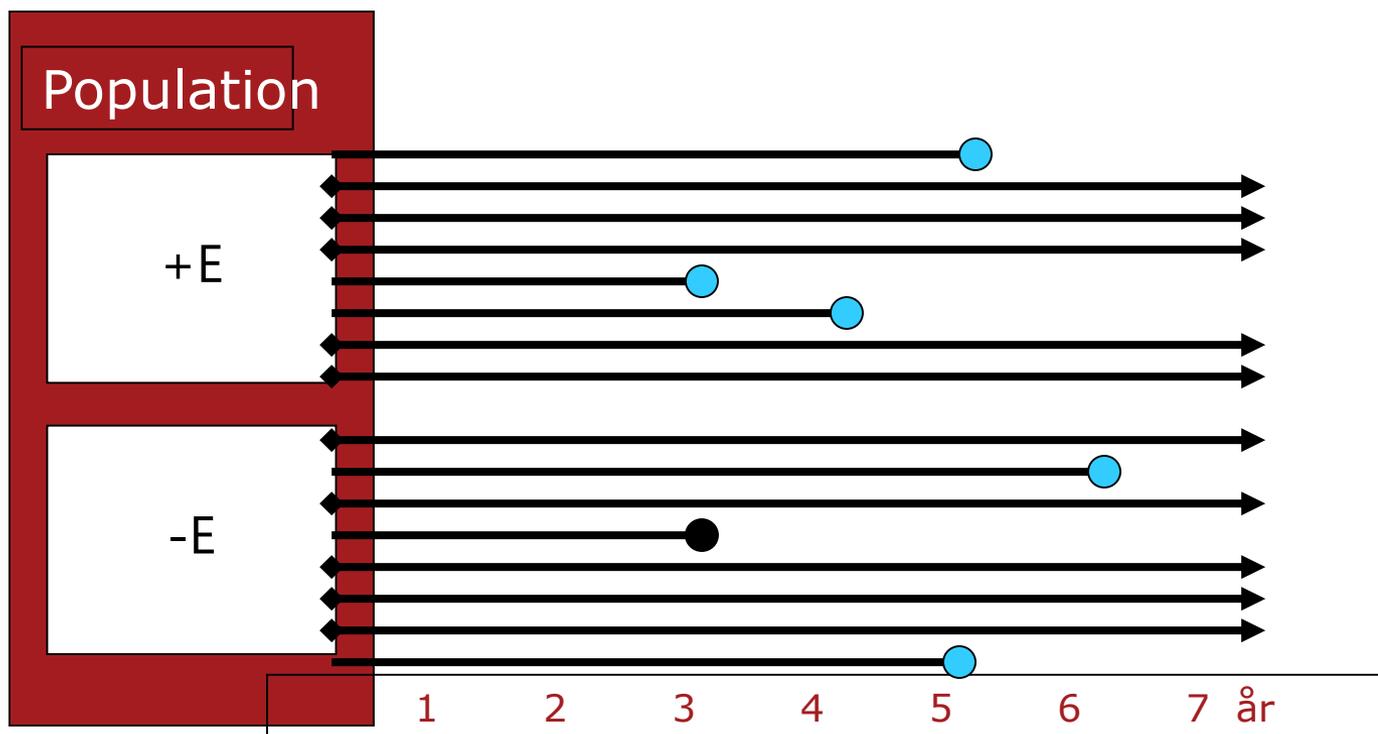
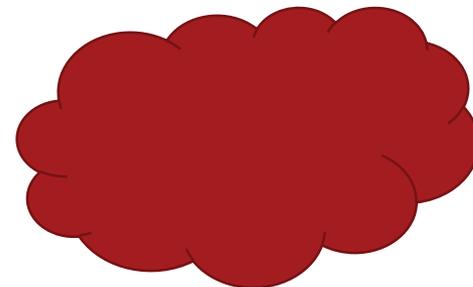
Hazards = probability/time unit



# Association measures in cohort studies

Relative measures  
Risk ratio, Rate ratio, Hazard Ratio

Risk difference, Rate difference



# Comparison of risk ratio and risk difference

Disease	Risk of death in smokers per 100,000	Risk of death in never smokers per 100,000	Risk ratio	Risk difference (per 100,000)
Lung cancer	104	10	10.4	94
IHD*	565	413	1.37	152

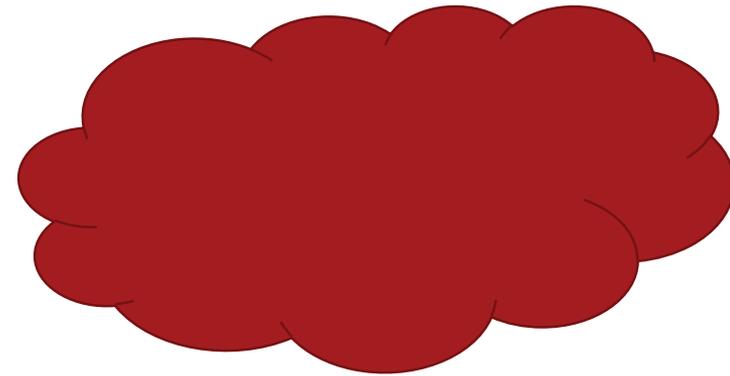
\*Ischaemic heart disease

**Risk ratio** for lung cancer is greater than for IHD. Smokers are 10 times more likely to have lung cancer than never-smokers. Smokers are 1.37 times more likely to have IHD than never-smokers.

**BUT:** IHD is a more common cause of death than lung cancer, so the **risk difference** is greater for IHD than lung cancer. For every 100,000 smokers, there will be 94 extra lung cancer deaths and 152 extra IHD deaths per year.

# 'Famous Danish Cohorts

- HPBS (Østerbro and Herlev Undersøgelserne)
- Kost, Kræft, Helbred
- Rigshospitalskohorten
- Projekt Metropolit
- ... and more – to fill in





Faculty of Health Sciences



# the Danish National Birth Cohort



# Rationales for the DNBC (& others BC studies)

Unresolved health problems in childhood in need to be studied prospectively

childhood cancers, asthma/allergy, mental health problems, obesity etc.

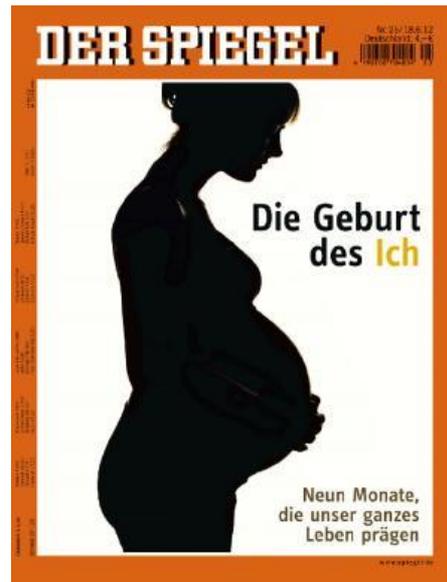
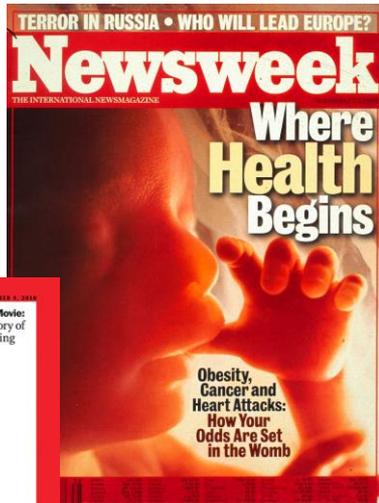
The increased focus on Developmental Origin of Health and Disease (Life-course perspectives)

Early life and pregnancy are dangerous life phases

High mortality and morbidity for mother and child, preterm birth, congenital anomalies, IUGR, etc



# DOHaD Developmental Origin of Health and Disease



Early life and health in youth, adolescence, and adulthood

18-year follow-up

- Mental health
- Musculo-skeletal health

... and it gets only worse .....



# The basic idea

- To create a **data resource**
- with **prospectively** collected data
- on **fetuses/children** and their mothers
- And aiming at **long-term follow-up**
- to be **used by all** researchers
- with a goal of **disease prevention**
- for **etiologic research** in important health problems





# Danish National Birth Cohort

9 months,  
lasting throughout  
life....

**9 måneder**  
som varer hele livet...

Du vil snart opdage det; Når din graviditet begynder at blive synlig, så vil du også begynde at få gode råd. Hvor du end kommer, er der mennesker, som vil fortælle dig, hvad du bør gøre i graviditeten, og hvad du absolut ikke bør gøre. Du vil få råd fra både familie, veninder og sundhedspersonale. Og du kan meget vel komme ud for, at nogle af rådene er modstridende...



Bedre sundhed for mor og barn  
..... Forskning i forebyggelse

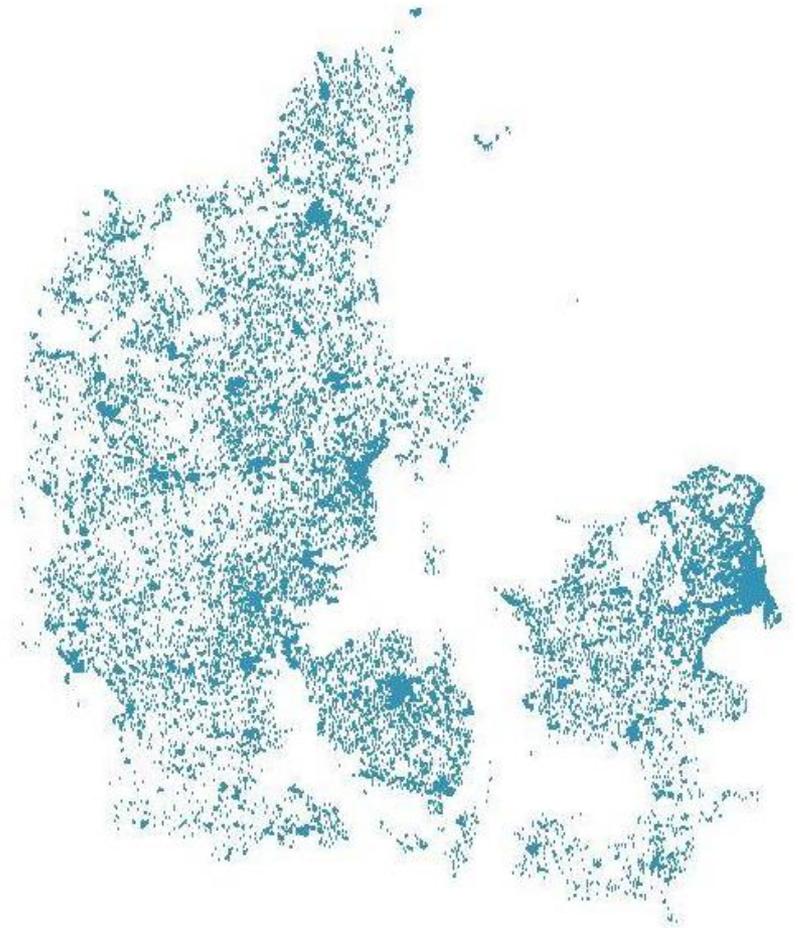
- 100.000 conceptions (via the mother)
- Recruited 1996-2002
- Followed through life
- Data collections several times during pregnancy, and at ½, 1½, 7, 11 and 18 years (so far)
- All contacts to health care systems via register linkages



# One dot for each participating woman...

The many participants' dots clearly outline the shape of Denmark.

The DNBC truly is a nation-wide cohort.





## DNBC: Data as of 2019

Recruited: **100,418 pregnancies** in  
**91,661 women**

CATI I	pregnancy week 12	90,165
CATI II	pregnancy week 30)	87,802
Food-Frequency Questionnaire (week 24)		70,183

Maternal blood sample, 1st trimester	98,000	approx
Maternal blood sample, week 25	80,000	approx
Umbilical blood taken at birth	60,000	approx

**96,986 children** born

CATI III	age 6 months	70,296
CATI IV	age 18 months	65,264
Postal survey, parent, age 7 years		60,250
Web-based questionnaires, parent and child, age 11		approx. 54%

**Continuous register-based follow-up** in hospital register  
Possibilities to link to other population registers



# Medication during pregnancy: Paracetamol (acetoaminophen)

- No adverse pregnancy outcomes, but preeclampsia Int J Epidemiol. 2009 Jun;38(3):706. J Matern Fetal Neonatal Med. 2010 May;23(5):371. Am J Obstet Gynecol. 2008;198(2):178
- Cryptorchidism Epidemiology. 2010 Nov;21(6):779-85
- ADHD like behaviour JAMA Pediatr. 2014 Apr;168(4):313-20
- Asthma Rebordosa C et al. Int J Epidemiol. 2008 Jun;37(3):583-90

**Table 4** Relative risks (RR) for physician-diagnosed asthma/bronchitis and wheezing at 18 months according to pre-natal exposure to paracetamol and/or aspirin

		18-months-old population (N= 66445)		
APAP <sup>a</sup>	AAS <sup>a</sup>	n (%)	Doctor-diagnosed asthma RR <sup>b</sup> (95% CI)	Wheezing ever up to 18-months-old RR <sup>b</sup> (95% CI)
<b>1st trimester</b>				
No	No	43 840 (66.0)	1 (ref)	1 (ref)
Yes	No	18 960 (28.5)	1.15 (1.10–1.19)	1.11 (1.08–1.14)
No	Yes	2 617 (3.9)	0.94 (0.84–1.04)	1.04 (0.96–1.12)
Yes	Yes	1 028 (1.6)	1.08 (0.94–1.25)	1.15 (1.04–1.28)
<b>2nd trimester</b>				
No	No	50 326 (75.7)	1 (ref)	1 (ref)
Yes	No	14 727 (22.2)	1.13 (1.08–1.18)	1.09 (1.05–1.12)
No	Yes	1 085 (1.6)	0.99 (0.85–1.16)	1.02 (0.91–1.14)
Yes	Yes	307 (0.5)	1.24 (0.97–1.58)	1.17 (0.97–1.41)
<b>3rd trimester</b>				
No	No	46 154 (69.5)	1 (ref)	1 (ref)
Yes	No	19 109 (28.8)	1.17 (1.13–1.22)	1.10 (1.06–1.13)
No	Yes	783 (1.2)	0.92 (0.77–1.10)	1.05 (0.92–1.19)
Yes	Yes	399 (0.6)	1.10 (0.89–1.36)	1.21 (1.04–1.40)
<b>Ever</b>				
No	No	27 878 (42.0)	1 (ref)	1 (ref)
Yes	No	33 556 (50.5)	1.19 (1.14–1.24)	1.15 (1.11–1.18)
No	Yes	2 251 (3.4)	1.00 (0.89–1.12)	1.10 (1.02–1.20)
Yes	Yes	2 760 (4.2)	1.06 (0.96–1.17)	1.11 (1.03–1.19)

<sup>a</sup>APAP stands for paracetamol and AAS for acetylsalicylic acid (aspirin).

<sup>b</sup>Adjusted by parental asthma, gender of the child, social class, gestational age, breastfeeding, tobacco exposure during pregnancy and antibiotic use during pregnancy.

# Emerging scientific questions: The Q-fever fear epidemic



**Peder Philipp**  
Formand,  
Dansk Kvæg



## Alvor – men ikke panik

Dansk Kvæg tager q-feber meget alvorligt. For når en sygdom kan smitte fra dyr til mennesker, skal det tages med dybeste alvor. Men det drejer sig om at finde den rette balance, så vi ikke bagatelliserer, og på den anden side heller ikke overreagerer. Desværre ved vi alt for lidt. Både i Danmark og i Holland og Australien, som er de lande, der skulle vide mest om q-feber hos mennesker og dyr. Derfor

## 'Dansk kvæg, 2008' Hej chef – jeg er gravid

**tema** Har du mistanke om q-feber, kan gravide ansatte sandsynligvis få barseldagpenge fra kommunen.

En melding om lykkelige omstændigheder hos kvindelige ansatte kan give hovedbrud, især hvis du har mistanke om q-feber i besætningen. For som nævnt i Sundhedsstyrelsens anbefalinger, bør gravide ikke opholde sig i kvægbesætninger med q-feber eller under mistanke for q-feber.

Men hvordan er hun – og du, så stillet? Britta Sejr Nielsen fra Landscentret, Ret & Regnskab forklarer, at den gravide i følge barsellovens § 6 har ret til barseldagpenge fra kommunen allerede før fireugers dagen før fødslen, hvis arbejdets særlige karakter medfører risiko for fostret. Den gravide skal ikke sygemeldes. Det er dog en forudsætning, at risikofaktorerne ikke umiddelbart kan fjernes, og at den gravide ikke kan flyttes til anden passende beskæftigelse virksomheden.

**"DET ER MIN VURDERING,** at der i tilfælde af q-feber, som udgangspunkt er ret til barsels-



Hvis kvindelige ansatte i besætninger, der er under mistanke for q-feber, bliver gravide, kan de sandsyn-

# No excess risk of adverse pregnancy outcomes among women with serological markers of previous infection with *Coxiella burnetii*: evidence from the Danish National Birth Cohort

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<http://www.biomedcentral.com/1471-2334/13/87>

## Abstract

**Background:** Q fever caused by *Coxiella burnetii* is transmitted to humans by inhalation of aerosols from animal birth products. Q fever in pregnancy is suspected to be a potential cause of fetal and maternal morbidity and fetal mortality but the pathogenesis is poorly understood, and even in Q fever endemic areas, the magnitude of a potential association is not established.

We aimed to examine if presence of antibodies to *C. burnetii* during pregnancy or seroconversion were associated with adverse pregnancy outcomes.

**Methods:** The Danish National Birth Cohort collected blood samples and interview data from 100,418 pregnant women (1996–2002). We sampled 397 pregnant women with occupational or domestic exposure to cattle or sheep and a random sample of 459 women with no animal exposure. Outcome measures were spontaneous abortion, preterm birth, birth weight and Small for Gestational Age (SGA).

Blood samples collected in pregnancy were screened for antibodies against *C. burnetii* by enzyme-linked immunosorbent assay (ELISA). Samples positive for IgG or IgM antibodies in the ELISA were confirmed by immunofluorescence antibody test (IFA).

**Results:** Among the 856 women, 169 (19.7%) women were IFA positive; 147 (87%) of these had occupational or domestic contact with livestock (IFA cutoff  $\geq 1:128$ ).

# The nested case-control study

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PLoS one

## Presence of Antibodies Against *Coxiella burnetii* and Risk of Spontaneous Abortion: A Nested Case-Control Study

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### Abstract

**Background and Aims:** Q fever is a bacterial zoonosis caused by infection with *Coxiella burnetii*. It is well established that Q fever causes fetal loss in small ruminants. The suspicion has been raised that pregnant women may also experience adverse pregnancy outcome when the infection is acquired or reactivated during pregnancy. The purpose of this study was to assess the potential association between serologic markers of infection with *C. burnetii* and spontaneous abortion.

**Methods:** A nested case-control study within the Danish National Birth Cohort, a cohort of 100,418 pregnancies recruited from 1996–2002. Women were recruited in first trimester of pregnancy and followed prospectively. Median gestational age at enrolment was 8 weeks (25 and 75 percentiles: 7 weeks; 10 weeks). During pregnancy, a blood sample was collected at gestational week 6–12 and stored in a bio bank. For this study, a case sample of 218 pregnancies was drawn randomly among the pregnancies in the cohort which ended with a miscarriage before 22 gestational weeks, and a reference group of 482 pregnancies was selected in a random fashion among all pregnancies in the cohort. From these pregnancies, serum samples were screened for antibodies against *C. burnetii* in a commercial enzyme-linked immunosorbent assay (ELISA). Samples that proved IgG or IgM antibody positive were subsequently confirmatory tested by an immunofluorescence (IFA) test.

**Results:** Among cases, 11 (5%) were *C. burnetii* positive in ELISA of which one was confirmed in the IFA assay compared to 29 (6%) ELISA positive and 3 IFA confirmed in the random sample.

**Conclusions:** We found no evidence of a higher prevalence of *C. burnetii* antibodies in serum samples from women who later miscarried and the present study does not indicate a major association between Q fever infection and spontaneous abortion in humans. Very early first trimester abortions were, however, not included in the study.



# Other examples.....

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## SHORT COMMUNICATION

# Maternal vegetarianism and neurodevelopment of children enrolled in The Danish National Birth Cohort

**Table 2** Impaired neurodevelopment according to maternal vegetarianism. The Danish National Birth Cohort, 1996–2002

	Head circumference (cm)			Age at sitting (months)			Age at walking (months)			Total SDQ score*		
	n (77 968)	Mean	95% CI	n (55 185)	HR	95% CI	n (55 158)	HR	95% CI	n (47 176)	Mean	95% CI
Non-vegetarians	77 026	35.50	35.48 to 35.53	54 553	1.00	Ref.	54 553	1.00	Ref.	46 574	1.63	1.62 to 1.64
Pesco-vegetarians	780	-0.10	-0.21 to 0.02	483	1.08	1.00 to 1.17	483	1.17	1.08 to 1.28	468	-0.10	-0.17 to -0.03
Lacto-ovo-vegetarians	193	-0.11	-0.34 to 0.12	137	1.01	0.89 to 1.14	137	1.16	0.99 to 1.34	123	-0.09	-0.21 to 0.04
Vegans	19	-0.17	-0.86 to 0.52	12	0.95	0.64 to 1.42	12	1.25	0.86 to 1.81	11	-0.11	-0.45 to 0.24

Adjusted for maternal age at birth, occupational status, smoking, alcohol consumption and eating disorders.

\*Total SDQ (Strengths and Difficulties Questionnaire) scores ranging from 0 to 40 (pro-social scale excluded).

HR, hazard ratio; HR <1 indicates delayed sitting/walking status.

Due to lack of proportional hazards, the cox regression analyses for age at sitting were stratified on age at birth, occupational status, smoking and eating disorders. The analyses for age at walking were stratified on all these variables.

# Pooling of cohort data

## Ambient air pollution and low birthweight: a European cohort study (ESCAPE)

Marie Pedersen, Lise Giorgis-Allemand, Claire Bernard, Inmaculada Aguilera, Anne-Marie Nybo Andersen, Ferran Ballester, Rob M J Beelen, Leda Chatzi, Marta Cirach, Asta Danileviciute, Audrius Dedele, Manon van Eijsden, Marisa Estarlich, Ana Fernández-Somoano, Mariana F Fernández, Francesco Forastiere, Ulrike Gehring, Regina Grazuleviciene, Olena Gruzjeva, Barbara Heude, Gerard Hoek, Kees de Hoogh, Edith H van den Hooven, Siri E Häberg, Vincent W V Jaddoe, Claudia Klumper, Michal Korek, Ursula Krämer, Aitana Lerchundi, Johanna Lepeule, Per Nafstad, Wenche Nystad, Evidiki Patelarou, Daniela Porta, Dirkje Postma, Ole Raaschou-Nielsen, Peter Rudnai, Jordi Sunyer, Euripides Stephanou, Mette Sørensen, Elisabeth Thiering, Derek Tuffnell, Mihály J Varró, Tanja G M Vrijkotte, Alet Wijga, Michael Wilhelm, John Wright, Mark J Nieuwenhuijsen, Goran Pershagen, Bert Brunekreef, Manolis Kogevinas\*, Rémy Slama\*

### Summary

**Background** Ambient air pollution has been associated with restricted fetal growth, which is linked with adverse respiratory health in childhood. We assessed the effect of maternal exposure to low concentrations of ambient air pollution on birthweight.

**Methods** We pooled data from 14 population-based mother-child cohort studies in 12 European countries. Overall, the study population included 74 178 women who had singleton deliveries between Feb 11, 1994, and June 2, 2011, and for whom information about infant birthweight, gestational age, and sex was available. The primary outcome of interest was low birthweight at term (weight <2500 g at birth after 37 weeks of gestation). Mean concentrations of particulate matter with an aerodynamic diameter of less than 2.5  $\mu\text{m}$  ( $\text{PM}_{2.5}$ ), less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ), and between 2.5  $\mu\text{m}$  and 10  $\mu\text{m}$  during pregnancy were estimated at maternal home addresses with temporally adjusted land-use regression models, as was  $\text{PM}_{2.5}$  absorbance and concentrations of nitrogen dioxide ( $\text{NO}_2$ ) and nitrogen oxides. We also investigated traffic density on the nearest road and total traffic load. We calculated pooled effect estimates with random-effects models.

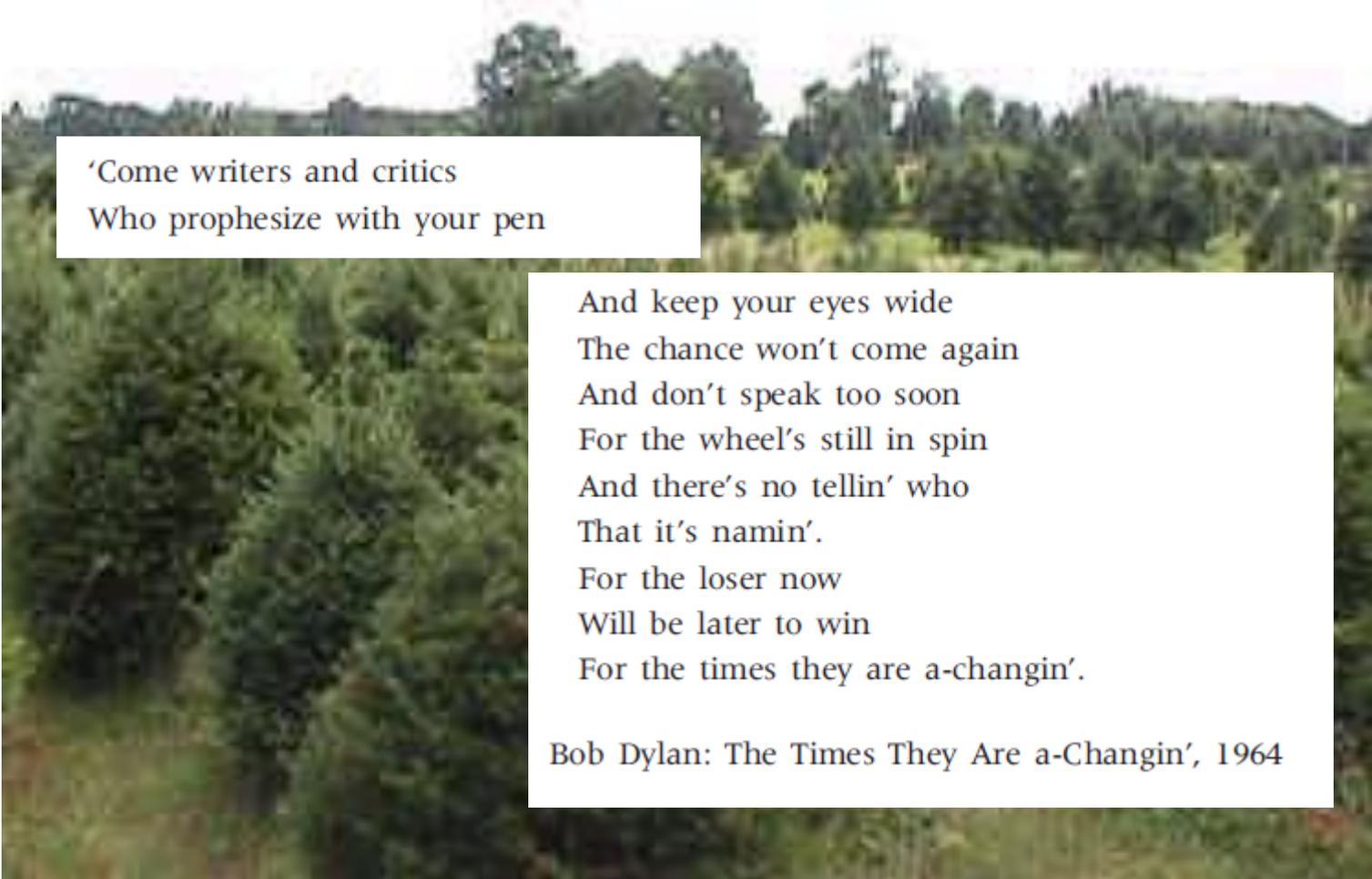
**Findings** A 5  $\mu\text{g}/\text{m}^3$  increase in concentration of  $\text{PM}_{2.5}$  during pregnancy was associated with an increased risk of low birthweight at term (adjusted odds ratio [OR] 1.18, 95% CI 1.06–1.33). An increased risk was also recorded for pregnancy concentrations lower than the present European Union annual  $\text{PM}_{2.5}$  limit of 25  $\mu\text{g}/\text{m}^3$  (OR for 5  $\mu\text{g}/\text{m}^3$  increase in participants exposed to concentrations of less than 20  $\mu\text{g}/\text{m}^3$  1.41, 95% CI 1.20–1.65).  $\text{PM}_{10}$  (OR for 10  $\mu\text{g}/\text{m}^3$  increase 1.16, 95% CI 1.00–1.35),  $\text{NO}_2$  (OR for 10  $\mu\text{g}/\text{m}^3$  increase 1.09, 1.00–1.19), and traffic density on nearest street (OR for increase of 5000 vehicles per day 1.06, 1.01–1.11) were also associated with increased risk of low birthweight at term. The population attributable risk estimated for a reduction in  $\text{PM}_{2.5}$  concentration to 10  $\mu\text{g}/\text{m}^3$  during pregnancy corresponded to a decrease of 22% (95% CI 8–33%) in cases of low birthweight at term.

**Interpretation** Exposure to ambient air pollutants and traffic during pregnancy is associated with restricted fetal growth. A substantial proportion of cases of low birthweight at term could be prevented in Europe if urban air pollution was reduced.

**Funding** The European Union.



# Life-course cohort studies: 'The times are a-changing'



'Come writers and critics  
Who prophesize with your pen

And keep your eyes wide  
The chance won't come again  
And don't speak too soon  
For the wheel's still in spin  
And there's no tellin' who  
That it's namin'.  
For the loser now  
Will be later to win  
For the times they are a-changin'.

Bob Dylan: The Times They Are a-Changin', 1964



# A PROspective study with RETROspective datacollection

Anne-Marie Nybo Andersen, Pernille Vastrup, Jan Wohlfahrt, Per Kragh Andersen, Jørn Olsen, Mads Melbye

## Summary

**Background** Hyperthermia acts as a teratogen in some animals where it can induce resorption of the fetus and fetal death. Fever during pregnancy, especially in the period of embryogenesis, is also suspected as being a risk factor for fetal death in human beings. We did a large cohort study in Denmark to investigate this possibility.

**Methods** We interviewed 24 040 women who were recruited in the first half of pregnancy to the Danish National Birth Cohort Study, and obtained information on the number of fever incidents during the first 16 weeks of pregnancy. For each fever episode, the highest measured body temperature, duration of incident, and gestational age were recorded. Outcomes of pregnancies were identified through linkage with the Civil Registration System and the National Discharge Registry. Cox's regression with time-dependent variables was used to estimate the relative risk of fetal death, taking delayed entry into account.

**Findings** 1145 pregnancies resulted in a miscarriage or stillbirth (4.8%). During the first 16 pregnancy weeks 18.5% of the women experienced at least one episode of fever. However, we found no association between fever in pregnancy and fetal death before or after adjustment for known risk factors of fetal death (relative risk 0.95 [95% CI 0.80–1.13]). This finding was consistent irrespective of measured maximum temperature, duration and number of fever incidents, or the gestational time of the fever incident, and was observed for fetal death in all three trimesters of pregnancy.

**Interpretation** We found no evidence that fever in the first 16 weeks of pregnancy is associated with the risk of fetal death in clinically recognised pregnancies.

*Lancet* 2002; **360**: 1552–56. Published online Oct 22, 2002  
<http://image.thelancet.com/extras/01art12058web.pdf>

## Introduction

Results of several studies in guineapigs, rats, mice, sheep, and monkeys have shown that hyperthermia in pregnancy is associated with resorption of the embryo, fetal deaths, and potentially lethal malformations such as central-nervous-system defects, abdominal-wall defects, and cardiovascular malformations.<sup>1–6</sup> Hyperthermia interferes with protein synthesis via heat-shock proteins, inducing cell death in the S-phase of the cell cycle by apoptosis and delay of mitotic activity in M-phase cells, and causing vascular disruption and placental infarction. All these mechanisms can lead directly to death of the embryo or to severe and lethal malformations. Furthermore, heat-induced increased uterine contractility can lead to expulsion of the fetus at a non-viable stage of gestation. The possible mechanisms of hyperthermia-induced fetal loss differ according to gestational time of exposure.<sup>7</sup>

The results of animal experiments suggest that a similar effect might occur in human beings.<sup>7–9</sup> Some researchers have found maternal exposure to heat (endogenous [eg, fever] and exogenous [eg, hot tubs and saunas]) to be associated with neural-tube defects,<sup>10–14</sup> whereas others have reported no such association.<sup>15,16</sup> Fever and upper-respiratory-tract infection, but not sauna bathing or high workplace temperature, were found to be associated with cardiovascular malformations in a case-control study.<sup>17</sup> However, few studies in human beings have addressed fever as a risk factor for fetal death. In a case-control study by Kline and colleagues, fever was associated with miscarriage of normal-karyotype fetuses (odds ratio 2.96 [95% CI 1.99–4.41]), but not of abnormal-karyotype fetuses.<sup>18</sup> This finding was taken as evidence that fever was a risk factor for miscarriage rather than a consequence of it.

A recent study, based on women who made inquiries to a teratology information programme, showed no association between maternal fever and miscarriage, but women with fever had a significantly increased risk of stillbirth.<sup>19</sup> A study from the Collaborative Perinatal Project found no association between fever in the first trimester and late intrauterine death, but no data were

