Epidemiology : Endocrine Disruptors and Human Endometriosis

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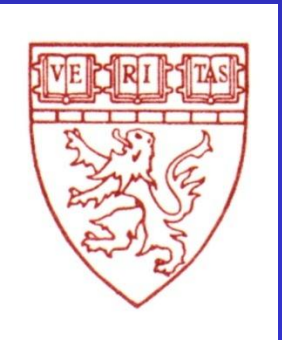
Fondazione Italiana Endometriosi

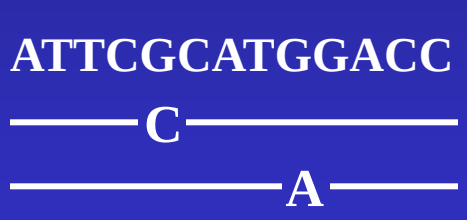


Harvard Medical School
Brigham and Women's Hospital

The Challenge of Complex Disease

Understanding the link between -

DNA sequence (Genotype)



Biology/Disease (Phenotype)

Environment

Sex Steroid Hormones and Endometriosis

- Plaques have estrogen, progesterone, and androgen receptors

- Estrogen → growth
- Androgens → atrophy

- Symptom remediation observed with pharmaceutical achievement of hypoestrogenic / hyperandrogenic milieu

Endocrine Disruptors

"Exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior."

U.S. EPA 1997

Bisphenol-A and Phthalates

- National Health and Nutrition Examination Survey (NHANES)
  - 93% of participants with detectable BPA
  - 75% of participants with at least one of 4 measured phthalate metabolites

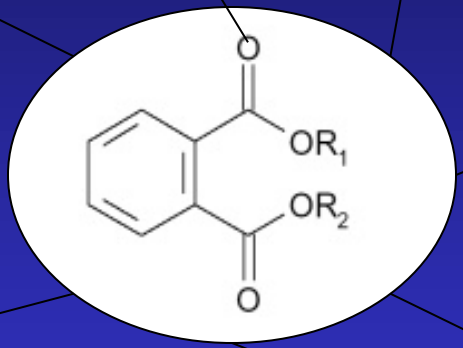
Bisphenol-A

- More than 6 billion pounds produced/year

Used to manufacture:

- Epoxy resins to line metal cans
- Polycarbonate plastic products
- Dental sealants and composites
- Toys and consumer products

How are we exposed to phthalates?



Human Study of Bisphenol-A and Endometriosis

Cobellis et al., 2009, Italy

– 69 fertile women undergoing laparoscopy

– Presented with suspected ovarian cysts, chronic pelvic pain, or dysmenorrhea

– 30 sera of 58 endometriosis cases with BPA

– 0 sera of 11 controls with BPA

Human Studies of Phthalates and Endometriosis

5 human studies published to date

- 4 case-control
- 1 large cross-sectional
- Array of metabolites evaluated
- Inconsistent findings

Human Studies of Phthalates and Endometriosis

Cobellis et al., 2003, Italy

– Fertile women undergoing laparoscopy for suspected ovarian cysts, chronic pelvic pain or dysmenorrhea

– 35 surgically-confirmed endometriosis cases

– 24 control women

– Plasma and peritoneal DEHP and MEHP

– Plasma DEHP, Wilcoxon rank sum p = 0.005

Case-control Studies

Lead Author	Year	Country	Case Number and Descriptor	Control Number and Descriptor	Metabolite Evaluated	P-value / Odds Ratio
Reddy	2006	India	49 infertile laparoscopy confirmed	38 age-matched infertile laparoscopy evaluated 21 age-matched fertile at ligation	4 plasma metabolites	Unadjusted T-test p-values all <0.004 DnBP, BBP, DnOP, DEHP
Itoh	2009	Japan	57 infertile laparoscopy confirmed	80 infertile laparoscopy evaluated	6 creatinine-adjusted urinary metabolites	Adjusted ORs all null 1.57 (0.74-3.30) dichotomized MEHP
Huang	2010	Taiwan	28 laparotomy confirmed	29 laparotomy evaluated	7 creatinine-adjusted urinary metabolites	Unadjusted Wilcoxon Rank-sum p-value <0.05 MnBP, 5oxo-MEHP

Human Studies of Phthalates and Endometriosis

Weuve et al., 2010

- 1227 women in NHANES from 1999-2004
- Aged 20-54
- Cross-sectional analysis
- 6 creatinine-corrected urinary metabolites

- 87 (7%) self-reported endometriosis cases

Human Studies of Phthalates and Endometriosis

Weuve et al., 2010

Adjusted Odds Ratio =

– 1.36 (95% CI = 0.77-2.41)

comparing 4th to lower 3 quartiles of MBP

– 0.44 (95% CI = 0.19-1.02)

comparing 4th to lower 3 quartiles of MEHP

Persistent Organochlorines

- Distributed widely in the environment
- Environmentally persistent (resist degradation)
- Bioaccumulation/biomagnification
- Lipophilic; biologically persistent

Sources:

- Dietary: Fish, meat, dairy products, fats
- Air
- Soil
- Mother-Infant (*in utero*, breast milk)

Dioxins

- Contaminants from production and combustion of chlorinated compounds
- 75 Congeners (i.e. variants, forms)

Sources:

- Agent Orange (herbicides: 2,4,5-T and 2,4-D)
- Burning trash, coal, hospital waste, sewage sludge, forest fires, and car exhaust
- Chlorine bleaching of pulp and paper
- Metal smelting (Al, Mg and Ni production)

Polychlorinated Biphenyls (PCBs)

Sources: paints, plastics (PVC), adhesives, lubricants, sealants (grain silos), carbonless copy paper

- 209 theoretical congeners with varying biologic activities:
  - Estrogenic (*in vitro / in vivo*)
  - Dioxin-like activity
    - Coplanar (non and mono-ortho substituted)

Human Studies of Persistent Organochlorines and Endometriosis

14 human studies published to date

- 12 case-control
- 2 retrospective cohort studies
- Dozens of congeners evaluated
- Largely inconsistent findings

Case-control Studies – Not Statistically Significant

Lead Author	Year	Country	Case Number and Descriptor	Control Number and Descriptor	Congeners Measured
Mayani	1997	Israel	44 infertile laparoscopy confirmed	35 with tubal infertility	TCDD
Lebel	1998	Canada	86 laparoscopy confirmed	70 laparoscopy evaluated	14 PCBs, 11 chlorinated pesticides
Pauwels	2001	Belgium	42 infertile laparoscopy confirmed	27 infertile laparoscopy evaluated	4 PCBs, dioxin-like TEQ
Fierens	2003	Belgium	10 self-reported	132 no report	17 PCDDs, 16 PCBs
De felip	2004	Belgium, Italy	23 nulliparous laparoscopy confirmed	17 nulliparous with benign adnexal mass	17 PCDDs, 12 PCDFs, 12 PCBs
Heilier	2004	Belgium	7 MRI confirmed	10 fertile	11 PCBs
Buck Louis	2005	U.S.	32 laparoscopy confirmed	52 laparoscopy or ligation	62 PCBs
Tsukino	2005	Japan	58 nulliparous stage 2+ infertile	81 nulliparous stage 0 or 1 infertile	8 PCDDs, 10 PCDFs, 4 cPCBs, 36 PCBs, 13 chlorinated pesticides

Case-control Studies – Significant (but unadjusted)

Lead Author	Year	Country	Case Number and Descriptor	Control Number and Descriptor	Congeners Evaluated	P-value / Odds Ratio
Reddy	2006	India	85 infertile laparoscopy confirmed	135 ligation	4 PCBs	unadjusted ANOVA p-value <0.05
Quaranta	2006	Italy	10 nulliparous laparoscopy confirmed	8 nulliparous laparoscopy evaluated	4 PCBs	unadjusted t-test p-value = 0.002
Heilier	2005	Belgium	25 MRI confirmed	25 fertile	17 PCDDs, 12 PCBs	unadjusted OR = 3.2 (95% CI = 1.0-9.9) for 10 pg/g lipid increase in dioxin-like PCBs

Case-control Study – Statistically Significant

Porpora, 2006, Italy

– 40 nulliparous laparoscopy confirmed cases

– 40 nulliparous laparoscopy evaluated controls

– 8 PCBs

– Adjusted Odds Ratio = 5.3 (95% CI = 1.3-23) comparing 3rd vs. 1st tertile of all PCBs combined

Cohort Study of Dioxin (TCDD) and Endometriosis

Eskenazi, 2002, Italy

– 1976 Seveso chemical plant explosion

– Serum samples collected close to exposure

– N = 601 women (aged ≤30 in 1976)

- 19 laparoscopy/ultrasound confirmed cases
- 277 symptom free women
- 305 endometriosis-uncertain women

– Adjusted Rate Ratio = 2.1 (95% CI = 0.5-8.0) comparing >100 ppt to ≤20 ppt

– No dose-response relation

Cohort Study of PCBs and Endometriosis

Hoffman, 2007, U.S.

- 1973 cattle feed contamination
- Serum samples collected 1976-1981
- N = 943 women (aged >10 in 1973)
  - 79 self-reported cases
    - (medical record confirmation of only 37 (47%))
- PBBs, PCBs
- Adjusted Rate Ratio = 1.68 (95% CI = 0.95-2.98) comparing high to low level of all PCBs combined
- Adjusted Rate Ratio = 1.23 (95% CI = 0.52-2.89) when restricted to confirmed endometriosis cases

State of the Evidence

- Strong and consistent *in vitro* and biochemical evidence
- Strong and consistent non-human primate and murine evidence
- BUT, in humans – weak and inconsistent associations

What can we conclude regarding endocrine disruptors and endometriosis?

Human relation may be obscured by epidemiologic design issues

Study Design Issues

- Control selection
  - Diagnostic bias
  - Selection bias
- Case heterogeneity
- Exposure Timing
- Sample size

Nurses' Health Study II

Prospective cohort study

- 116,678 female nurses
- Baseline questionnaire in 1989
- Age range in 1989 = 25 – 42
- Follow-up in 2-year intervals

Endometriosis in NHSII

Prevalence at baseline = 6,203 (5%)

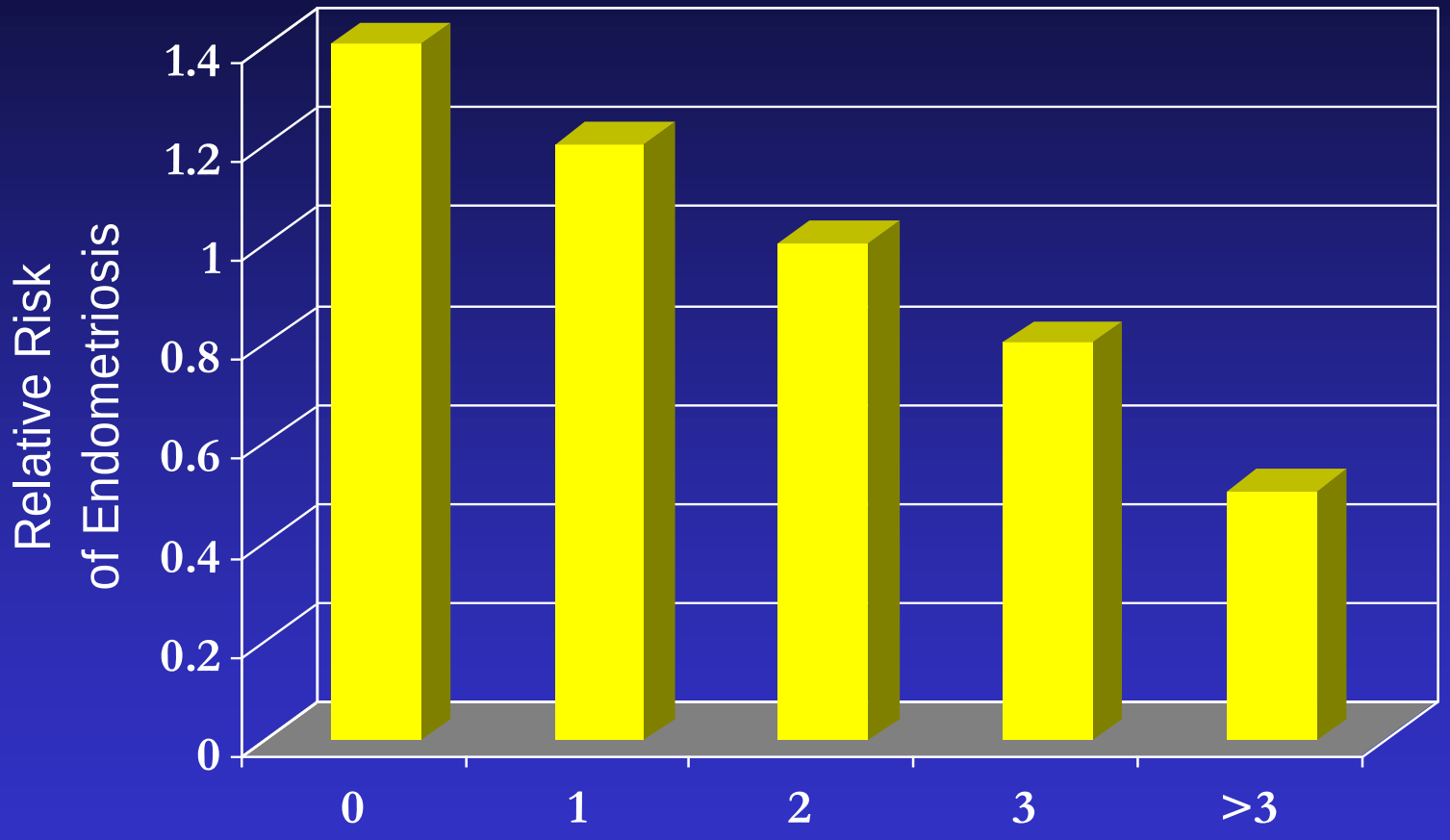
Incidence:

- 2,941 laparoscopically confirmed cases
  - Pain symptoms prompted dx = 77%
  - Infertility work-up prompted dx = 23%

Study Design Issues

- Control selection
  - Diagnostic bias
  - Selection bias
- Case heterogeneity
- Exposure Timing
- Sample size

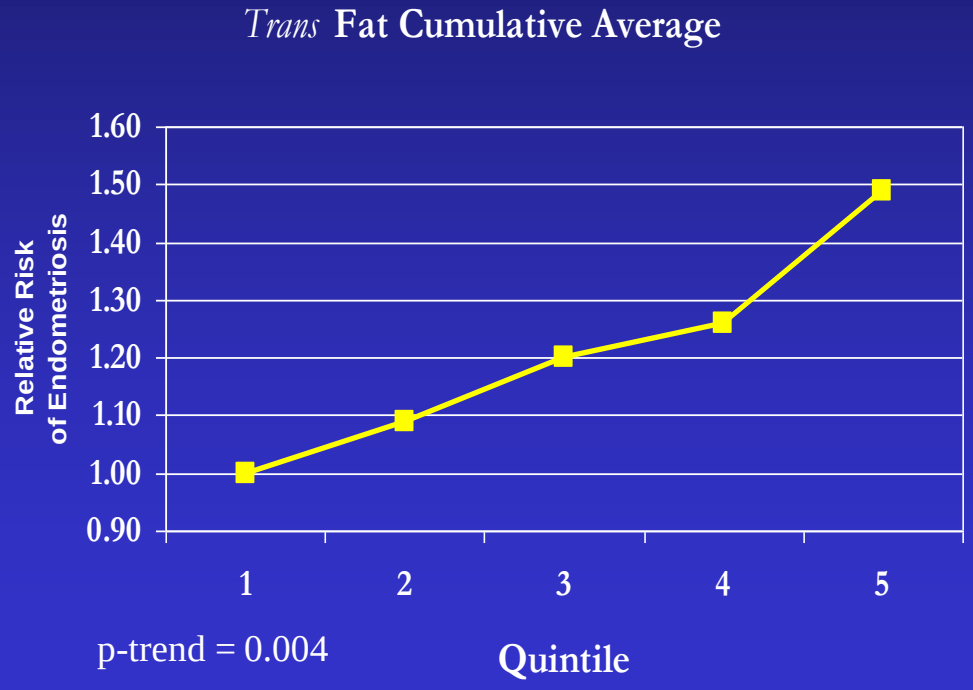
Parity and Endometriosis

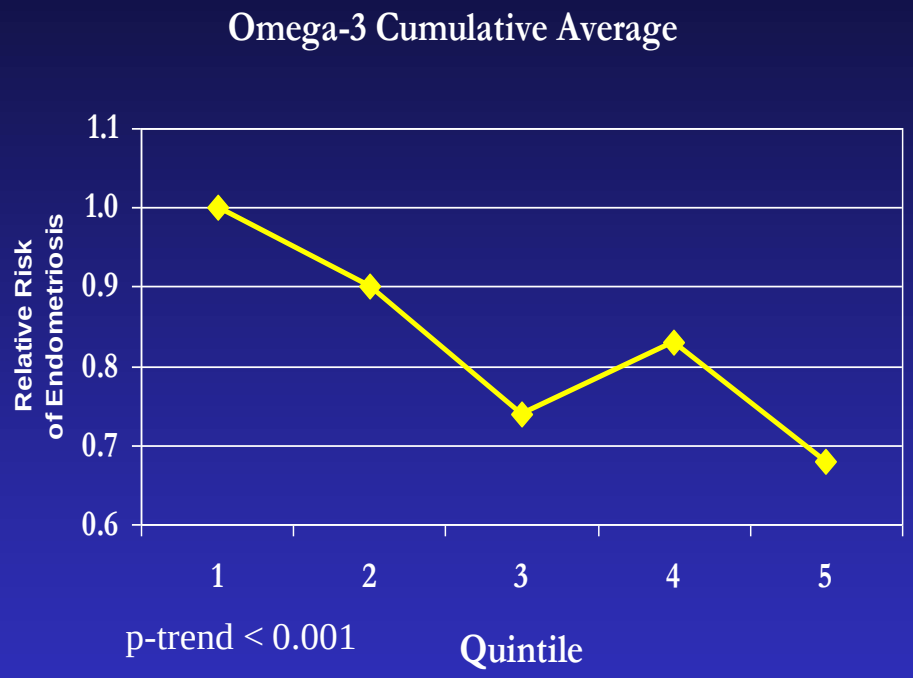


p-trend < 0.001

Missmer et al., Obstet Gynecol 2004

Trans and Omega-3 Fatty Acid Consumption and Endometriosis





Missmer et al., Human Reprod 2010

Study Design Issues

- Control selection
  - Diagnostic bias
  - Selection bias
- Case heterogeneity
- Exposure Timing
- Sample size

Current BMI (kg/m^2) and Endometriosis

	Relative Risk (95% CI)	
	Pain Sx	Infertility Work-up
<19	1.1 (0.8-1.4)	0.7 (0.5-1.2)
19-20.4	1.0 (referent)	1.0 (referent)
20.5-21.9	0.9 (0.7-1.1)	1.0 (0.7-1.4)
22-24.9	1.0 (0.8-1.3)	1.0 (0.7-1.3)
25-29.9 (overwght)	1.1 (0.9-1.3)	0.8 (0.5-1.1)
>29.9 (obese)	1.1 (0.8-1.3)	0.4 (0.2-0.7)
	$p_t = 0.27$	$p_t = 0.008$

Missmer et al., Am J Epidemiol 2004

Cigarette Smoking and Endometriosis

	Relative Risk (95% CI)	
	Pain Sx	Infertility Work-up
Never Smoker	1.0 (referent)	1.0 (referent)
Current Smoker		
1-14 per day	1.1 (0.8-1.4)	0.8 (0.5-1.3)
15-24 per day	1.5 (1.2-1.9)	0.7 (0.4-1.2)
25-34 per day	1.9 (1.3-2.7)	0.4 (0.1-0.7)
35+ per day	1.4 (0.7-2.7)	0.6 (0.1-4.2)
	$p_t < 0.0001$	$p_t = 0.05$

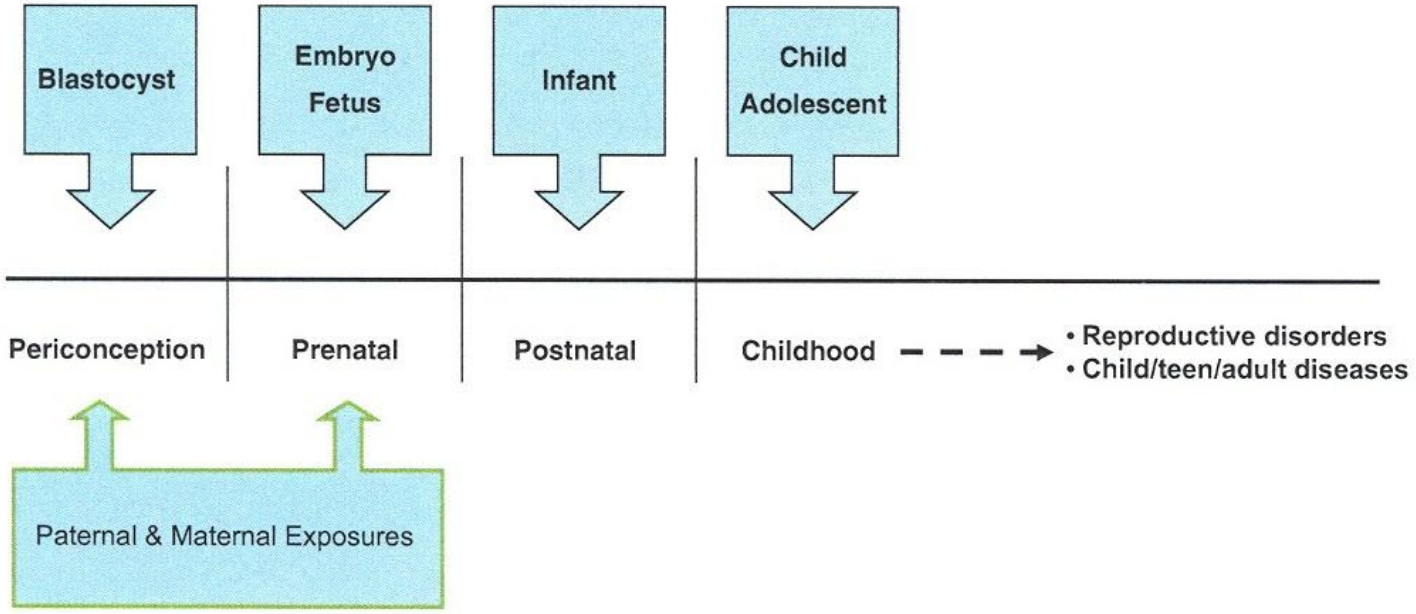
Missmer et al., Am J Epidemiol 2004

Study Design Issues

- Control selection
  - Diagnostic bias
  - Selection bias
- Case heterogeneity
- Exposure Timing
- Sample size

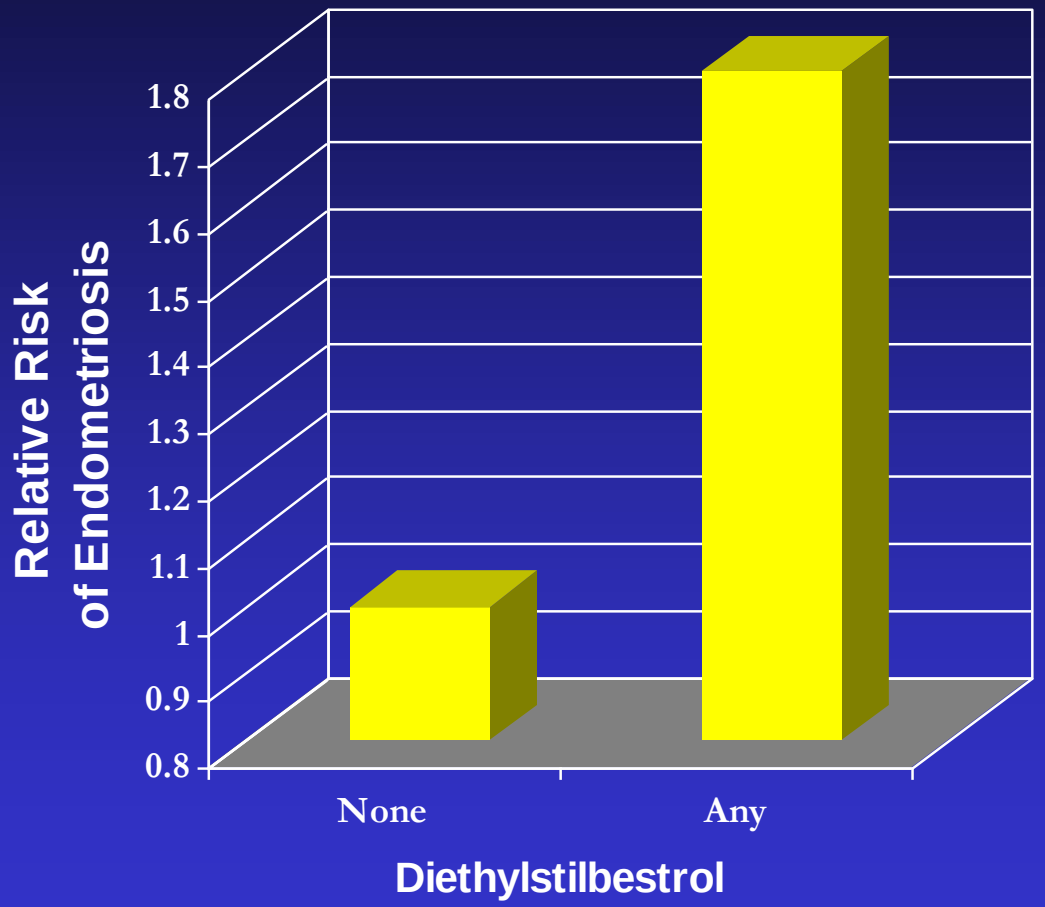
FIGURE 2

Windows of susceptibility to environmental insults [adapted from (253)].



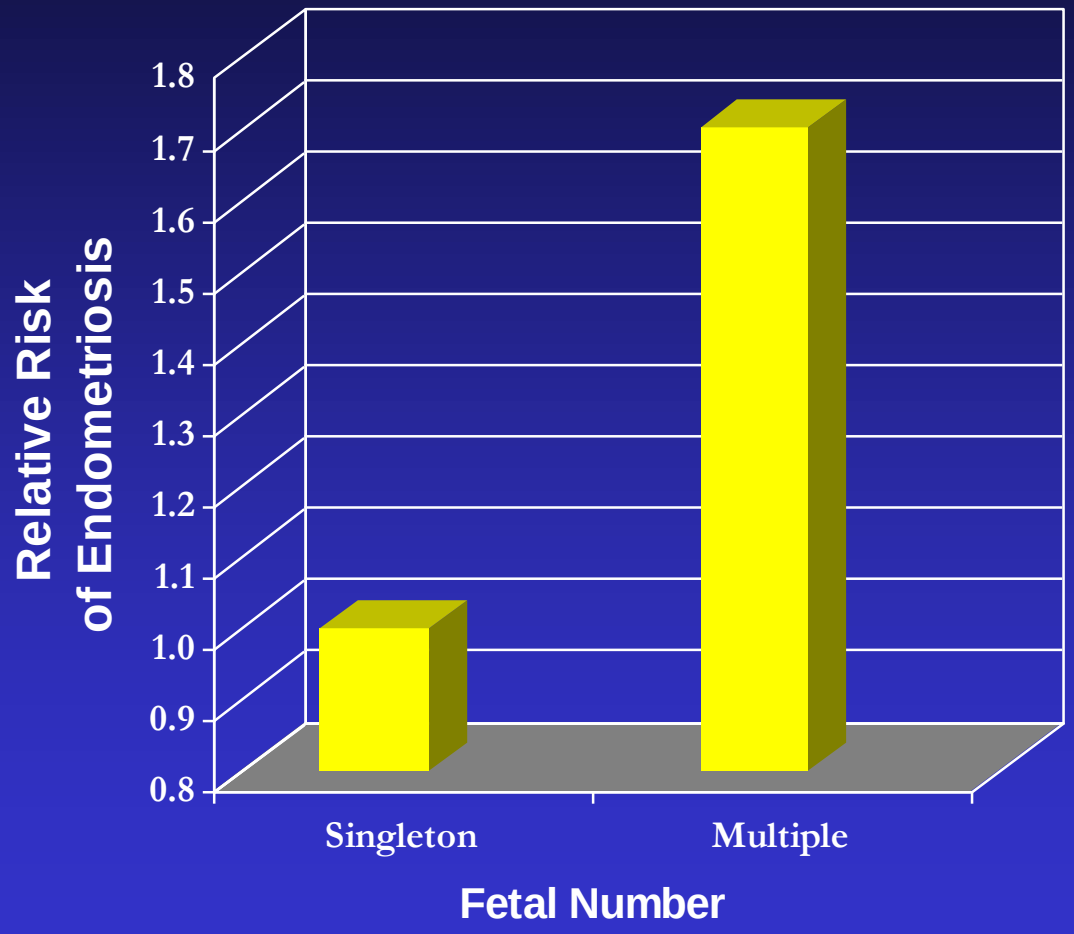
Woodruff. Environmental reproductive health. Fertil Steril 2008.

DES and Endometriosis



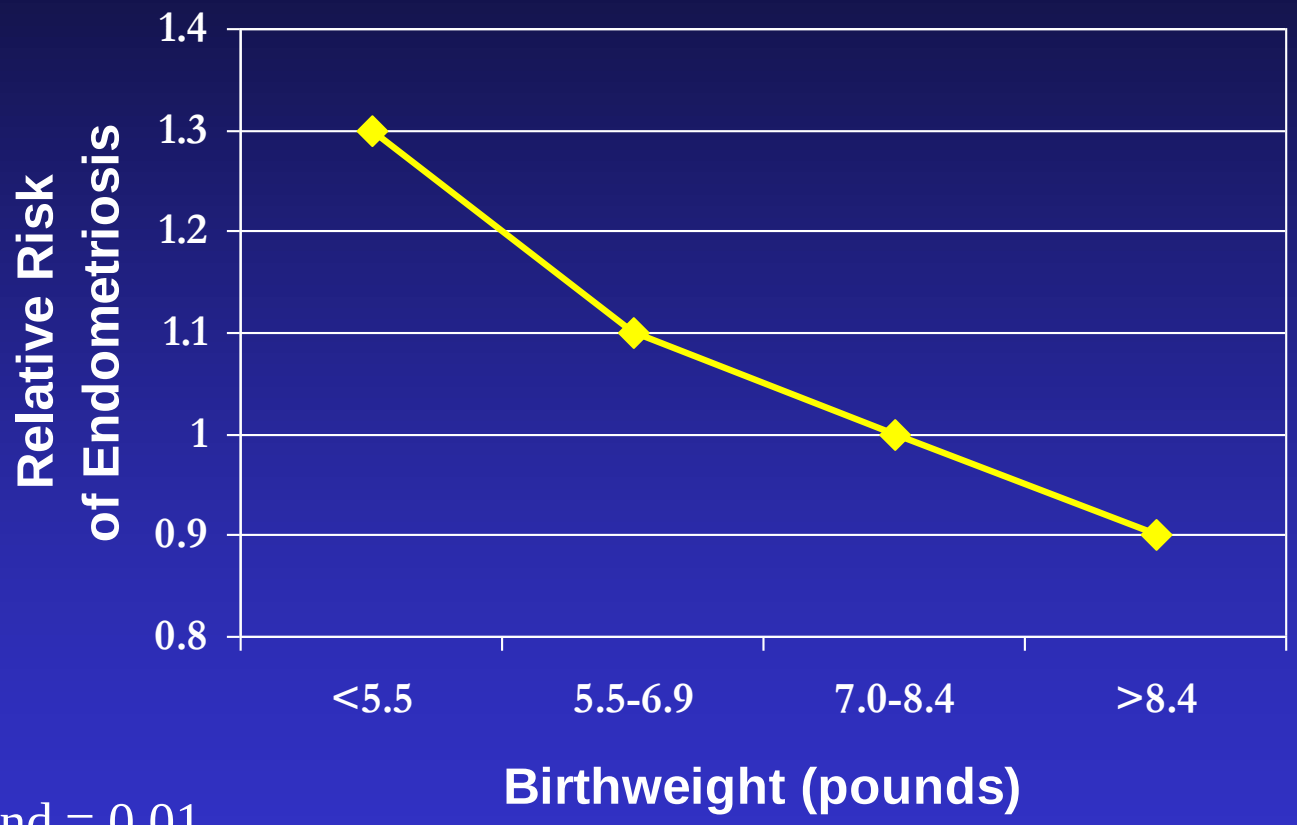
Missmer et al., Fertil Steril 2006

Multiple Gestation and Endometriosis



Missmer et al., Fertil Steril 2006

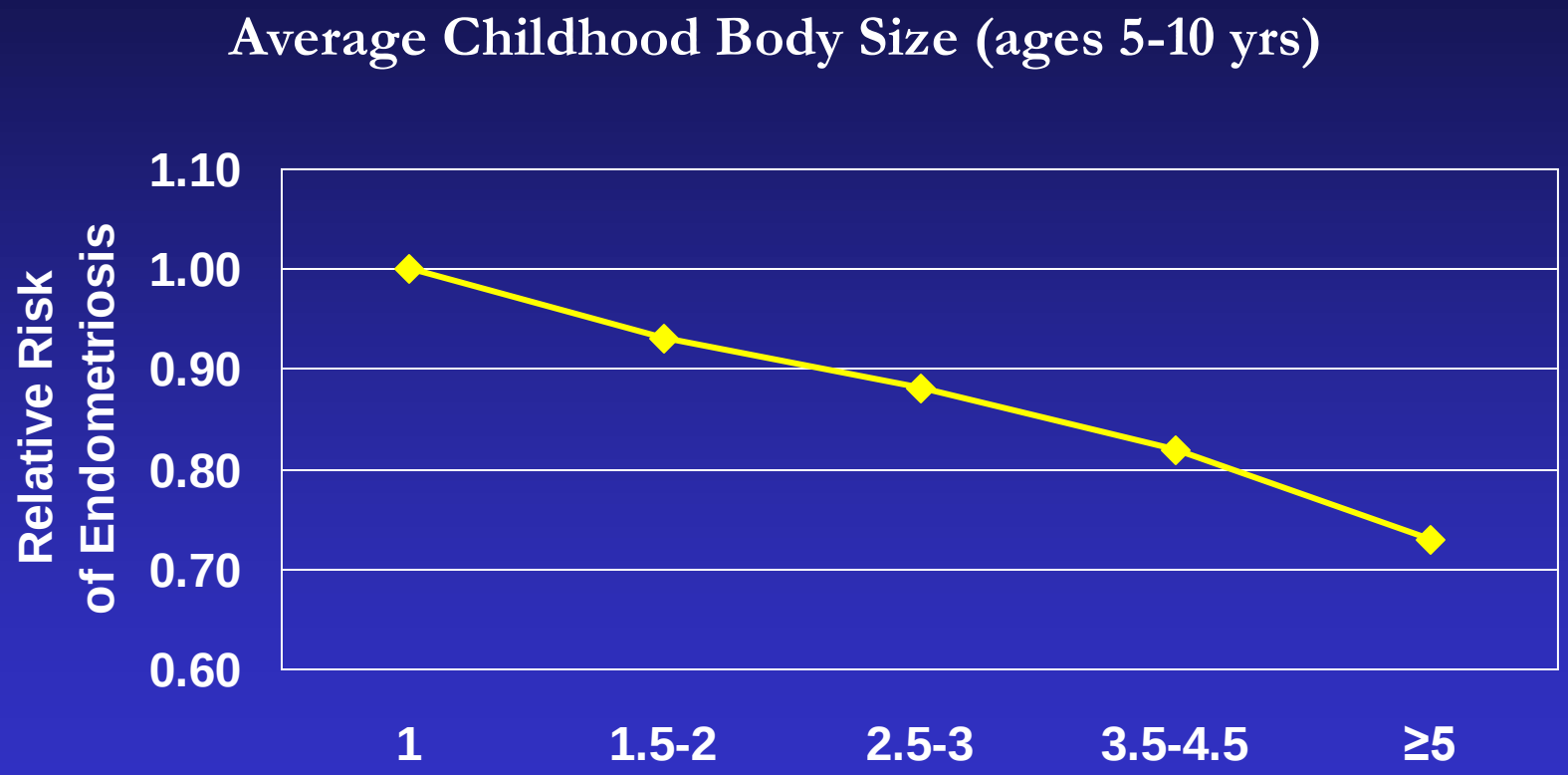
Birthweight and Endometriosis



p-trend = 0.01

Missmer et al., Fertil Steril 2006

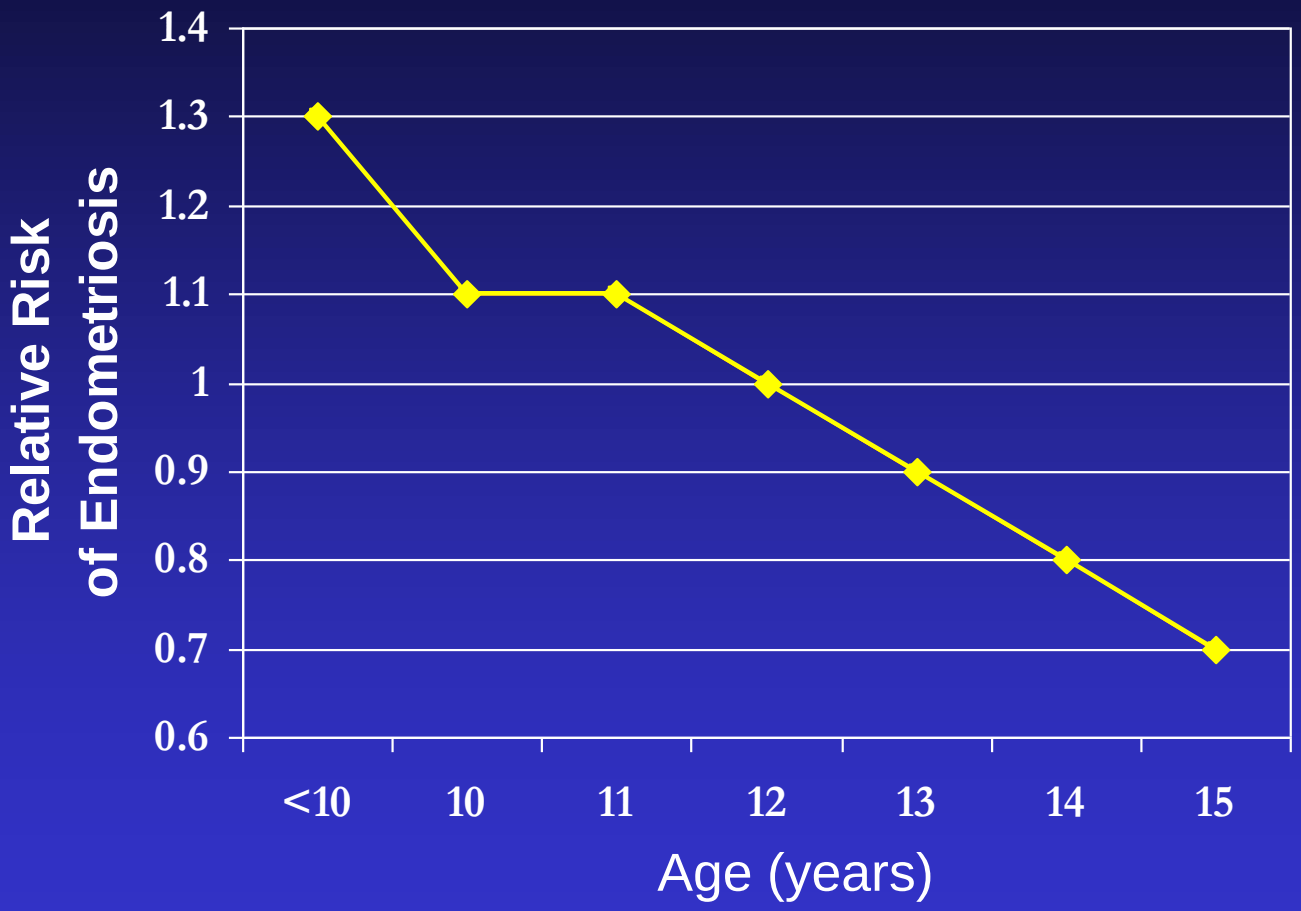
Average Childhood Body Size and Endometriosis



p-trend = 0.0007

Vitonis et al., Human Reprod 2010

Age at Menarche and Endometriosis



p-trend < 0.0001

Missmer et al., Obstet Gynecol 2004

Study Design Issues

- Control selection
  - Diagnostic bias
  - Selection bias
- Case heterogeneity
- Exposure Timing
- Sample size

Minimum Detectable Relative Risk

- Largest Case-control Study
  - N = 86 cases
  - Minimum Odds Ratio = 2.4 to 4.4
- Smallest Case-control Study
  - N = 10 cases
  - Minimum Odds Ratio = 17.2 to 28.9
- Seveso Cohort Study
  - N = 19 cases
  - Minimum Rate Ratio = 6.6 to 14.0

Evidence Summary

Given:

- *In vitro* sex steroid hormone evidence
- Animal study dose-response relations
- Complexity of human study

Additional human study is critical

Future Directions

- Large, collaborative, geographically diverse studies
- Stratified case definition
- Critically considered control selection or comparison group inclusion
- Exposure data collection from potentially critical developmental windows

Future Directions

- Heterogeneity in genetic, biologic, anthropometric, sociologic factors must exist
- Power and analyze for gene-environment interactions

Thank you

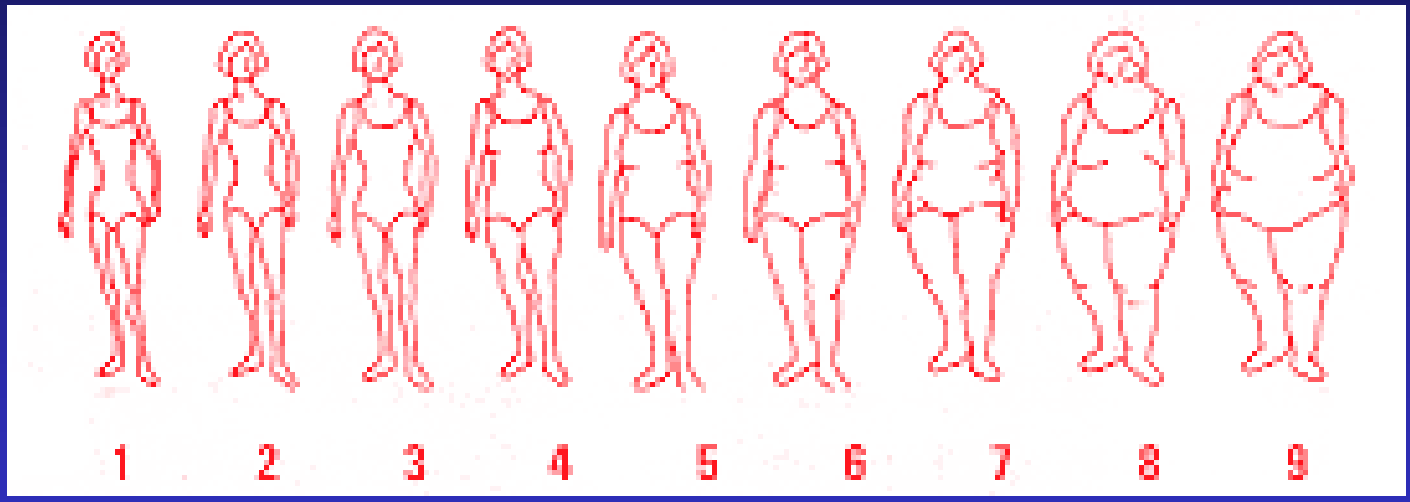
Acknowledgments:

Drs. Hankinson, Spiegelman, Willett – Nurses Health Study, HMS

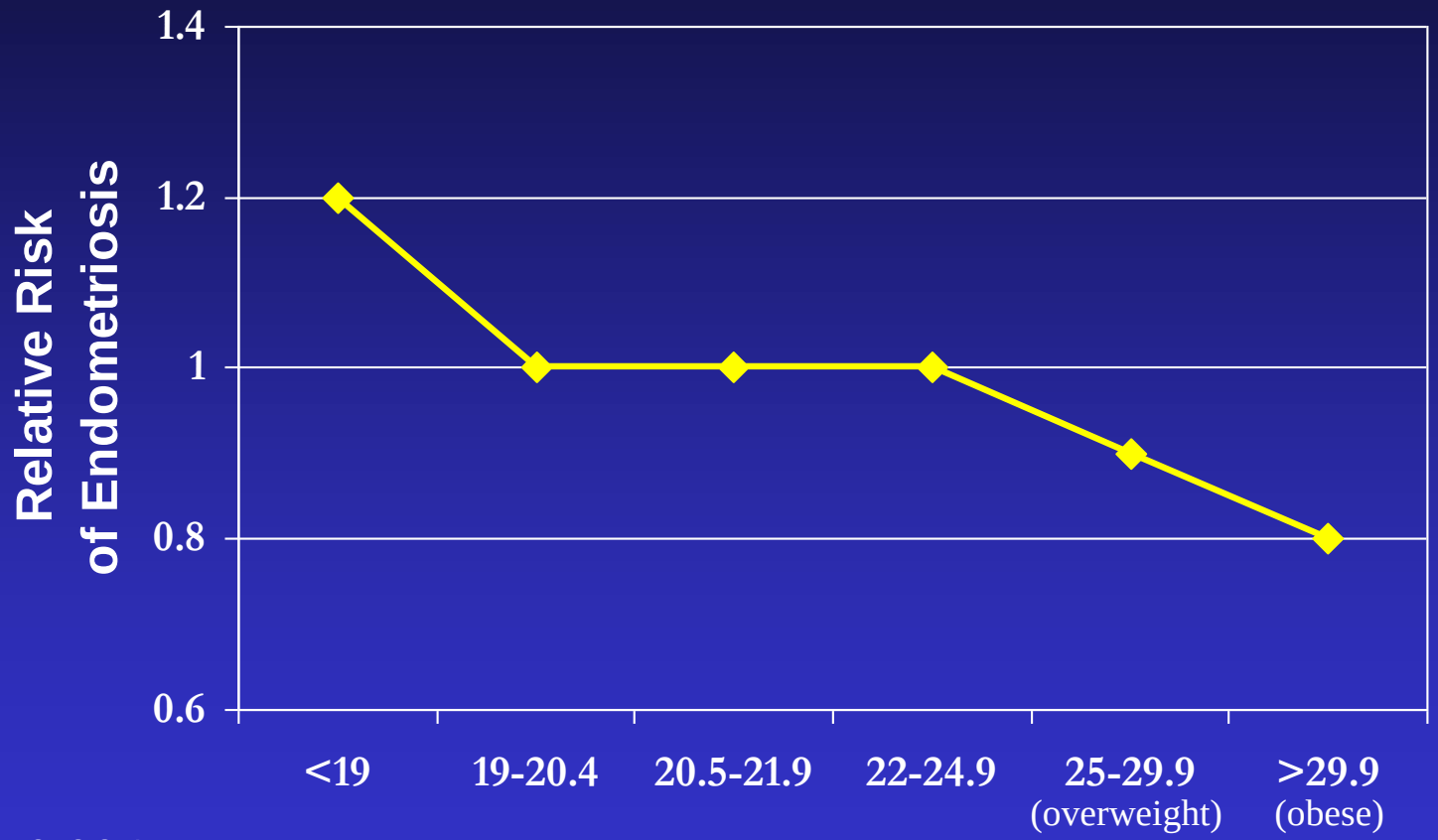
Dr. Hauser – Environmental Health, HSPH

Drs. Hornstein, Barbieri – Center for Infertility and Reproductive Surgery, BWH

Figure Drawing used to Assess Childhood Body Size



BMI at age 18 (kg/m^2) and Endometriosis



p-trend = 0.004

Missmer et al., Am J Epidemiol 2004

Polychlorinated Biphenyls (PCBs)

- Introduced in 1929 for use in electrical transformers and capacitors (production ceased in U.S. in 1977)
- Marketed as Aroclor
- Persistent and bioaccumulate

Source: Swedish Environmental Protection Agency

High Dioxin Exposure Groups

- Vietnam Veterans
  - Agent orange (herbicides)
  - “lowered” testosterone levels
- Seveso, Italy
  - 1976, Runaway chemical reaction and explosion creating a cloud containing dioxins

High PCB Exposure Group

Yu-Cheng, Taiwan

- 1979, contaminated rice oil with PCBs used as heat exchangers
- ≅ 2000 people poisoned
- Chloracne, hyperpigmentation of skin, liver cancer, neurodevelopmental disorders in children

Non-Human Primate Studies of Dioxin and Endometriosis

Rhesus monkeys (*Macaca mulatta*)
Spontaneous endometriosis

Chronic dietary TCDD exposure ≅ 4 years
(0 ppt, 5 ppt, and 25 ppt arms)

Significant dose-dependent increase in incidence and endometriosis severity

Rier et al., Fundam Appl Toxicol 1993

Murine Studies of Dioxin / PCBs and Endometriosis

- Cummings et al.
  - Autotransplantation
  - Dioxin, dioxin-like compounds only
  - Estrogen level, exposure timing
- Bruner-Tran et al.
  - Peritoneal seeding
  - Lifecourse timing
  - Progesterone regulation of MMPs

Adolescent onset Endometriosis

- Only case series reports of sx presentation and tx response exist
- At Children's Hospital Boston
  - mean time from menarche to laparoscopy among cases with intractable pelvic pain = 3.8 years
  - mean age of laparoscopic confirmation (among girls <23 years of age) = 16.1 years

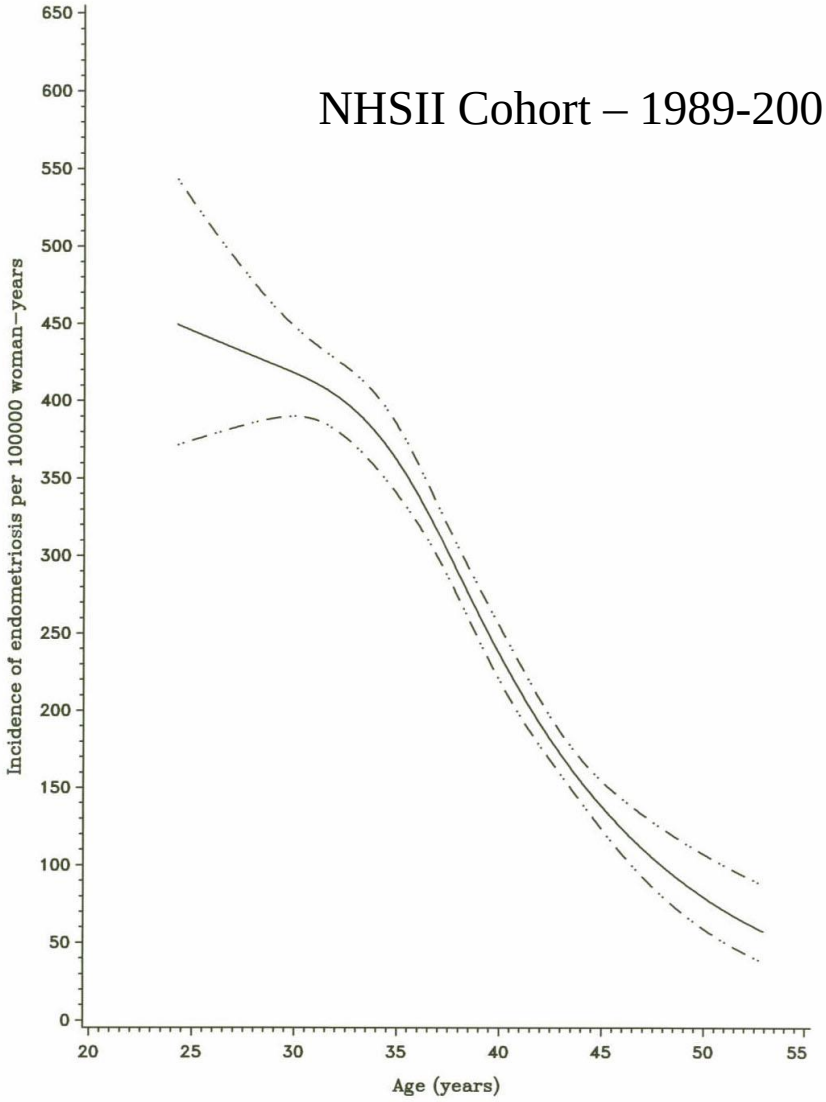
World Health Organization Toxic Equivalency Factors (TEQ) for Humans

Congener	TEQ
Dioxins	
2,3,7,8-TCDD	1
1,2,3,7,8-PentaCDD	1
1,2,3,4,7,8-HexaCDD	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01
OctaCDD	0.0001
Furans	
2,3,7,8-TetraCDF	0.1
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
1,2,3,4,7,8,9-HeptaCDF	0.01
OctaCDF	0.0001
PCBs	
3,3’,4,4’-TetraCB (77)	0.0001
3,3’,4,4’,5-PentaCB (126)	0.1
3,3’,4,4’,5,5’-HexaCB (169)	0.01

GUTS (Growing Up Today Study)

- Children of NHSII participants
  - 9,039 girls enrolled in 1996
  - 5,673 girls enrolled in 2004
  - Age 9-14 at enrollment (born 1982-1995)
- Question about endometriosis added in 2005
- Adolescent onset may signal different etiology – entering the peak years of age-incidence!
- Funded to confirm self-reports and abstract medical record details through 2009 (expect 185 cases)
- Have 2 generations of prospective data

NHSII Cohort – 1989-2001



Diet and Endometriosis

- ~ 12 books / lay articles suggest dietary changes to "prevent" endometriosis

- One human study of diet –
  Hospital-based case-control study
  (n = 504 cases:504 controls)

Green vegetables	OR = 0.3 (95% CI = 0.2-0.5)
Fruit	OR = 0.6 (95% CI = 0.4-0.8)
Red meat	OR = 2.0 (95% CI = 1.4-2.8)

- One animal study of fats –
  Rabbits fed fish oil

series 2 prostaglandins
growth of surgically implanted endometrial lesions

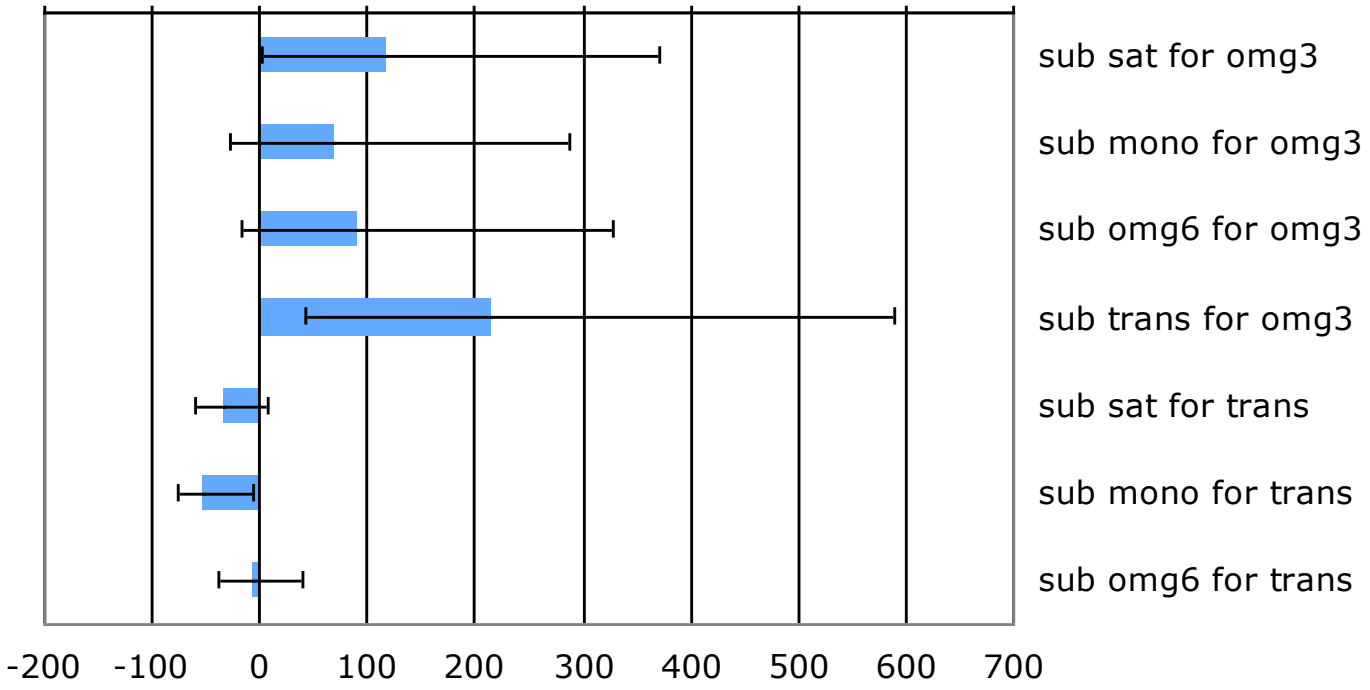
Dietary assessment in the NHSII

- Food Frequency Questionnaires
  - Began in 1991
    - 131 food items
    - Average intake throughout previous year
    - Intake change in past 10 years
    - Collected every 4 years

Fat Consumption and Endometriosis

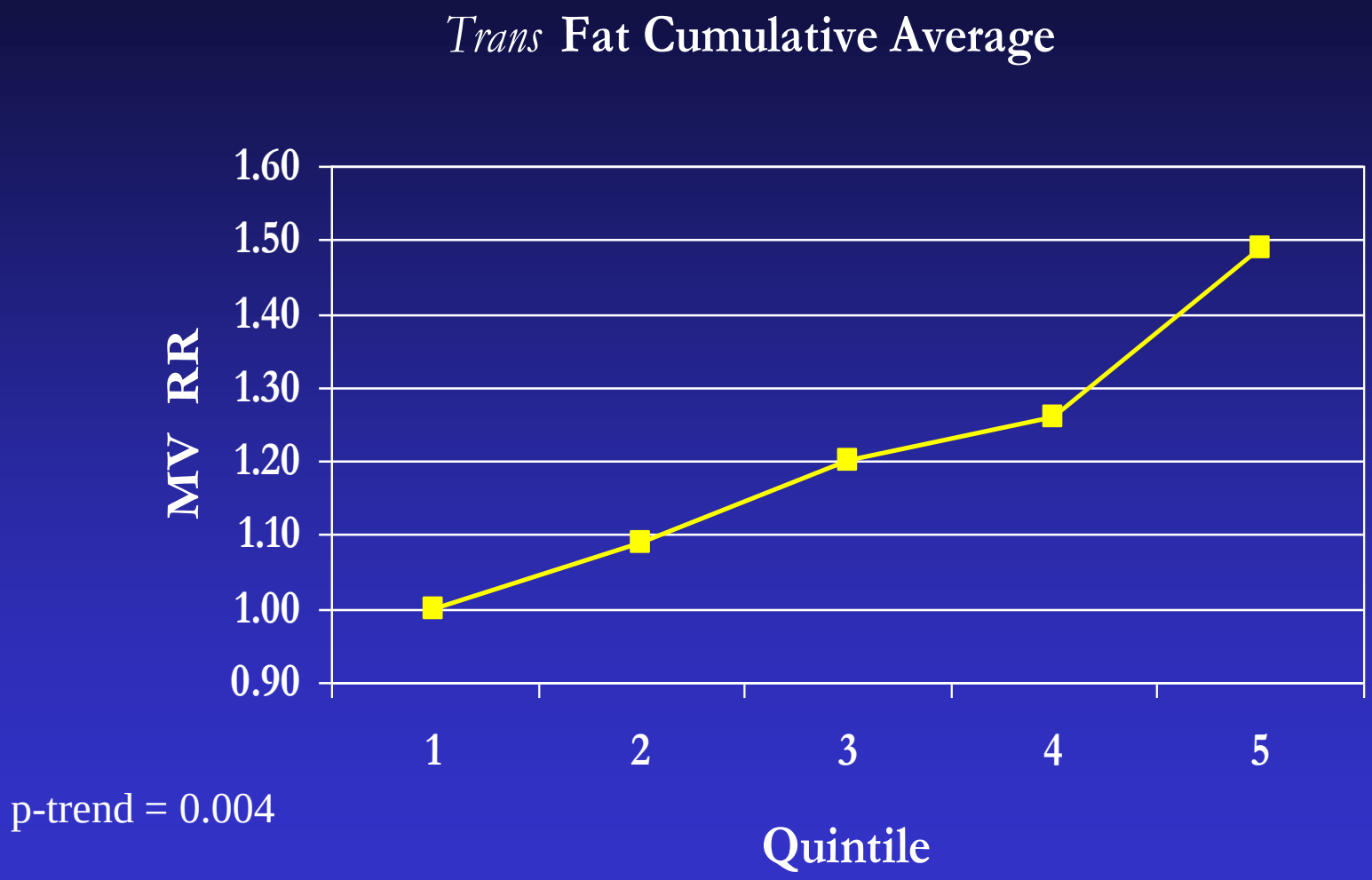
- Series 2 prostaglandins
  - Arachidonic acid
  - Impaired smooth muscle contractility
  - Increased inflammation
- Series 3 prostaglandins
  - α-linolenic acid
  - Improved T-cell function
  - Decreased inflammation
  - Decreased series 2 prostaglandin production

Dietary Fat Intake and the Risk of Endometriosis



Percent Change in Risk of Endometriosis with 95% Confidence Intervals

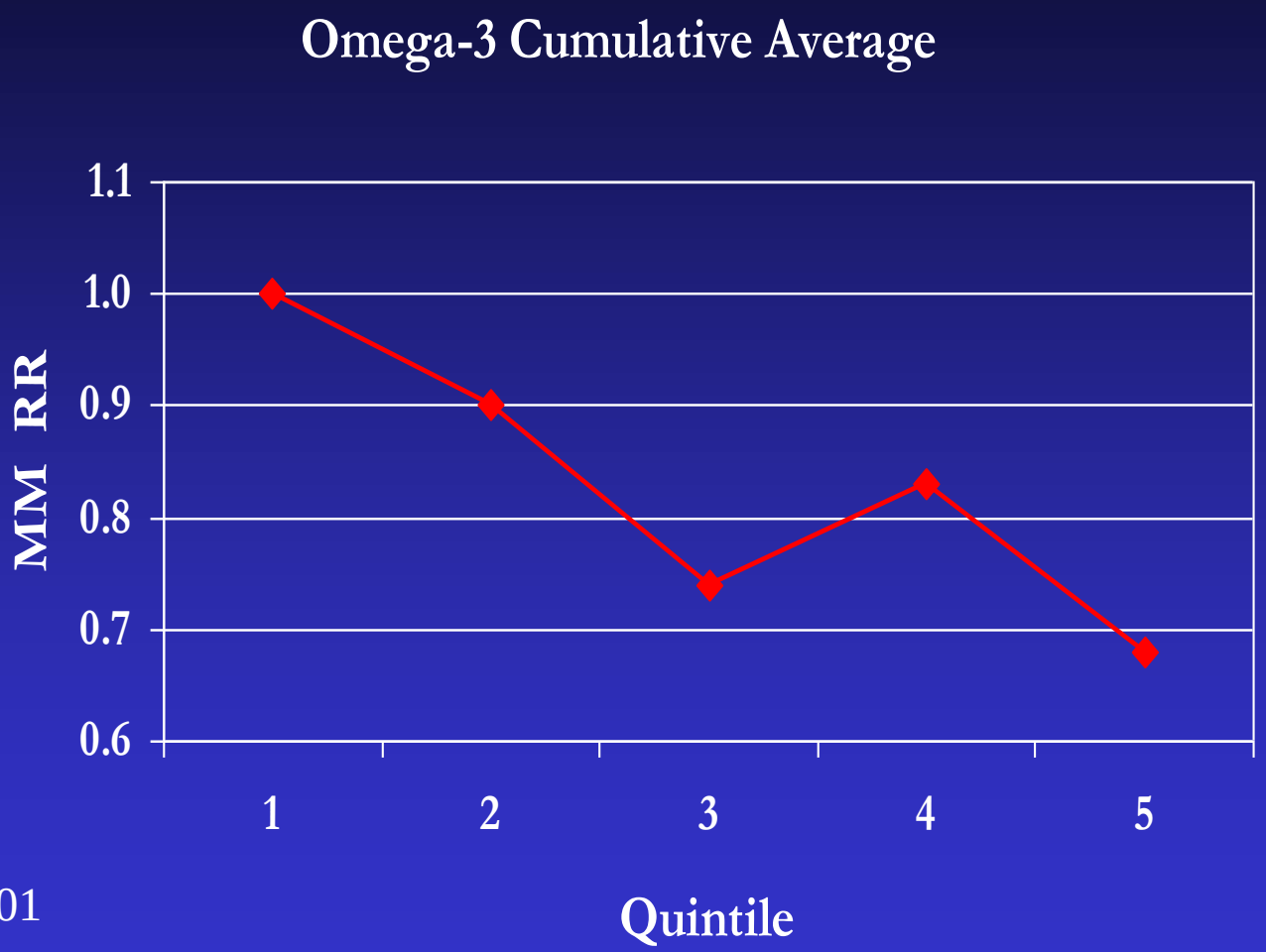
Trans Fat



p-trend = 0.004

Adjusted for age, calendar time, total energy, parity, race, age at menarche, menstrual cycle length, BMI

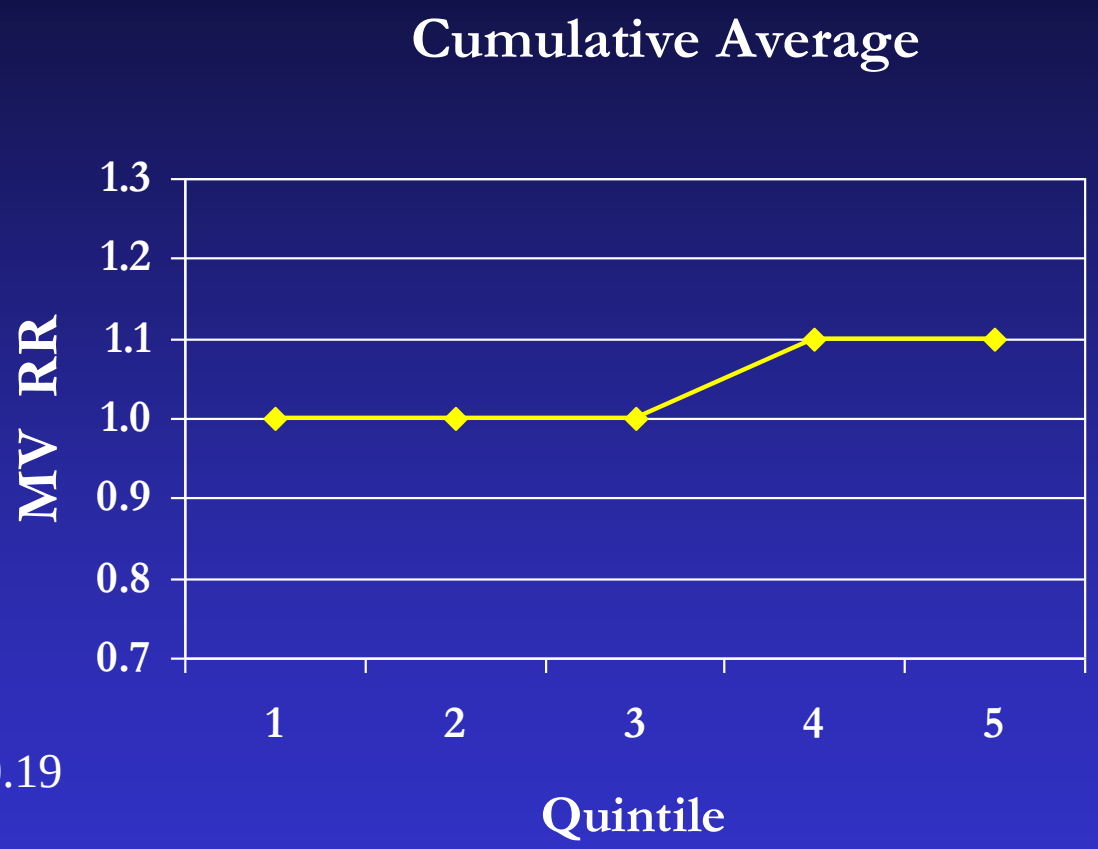
Omega-3



p-trend < 0.001

Adjusted for age, calendar time, total energy, parity, race, age at menarche, menstrual cycle length, BMI

Total Fat



p-trend = 0.19

Adjusted for age, calendar time, total energy, parity, race, age at menarche, menstrual cycle length, BMI

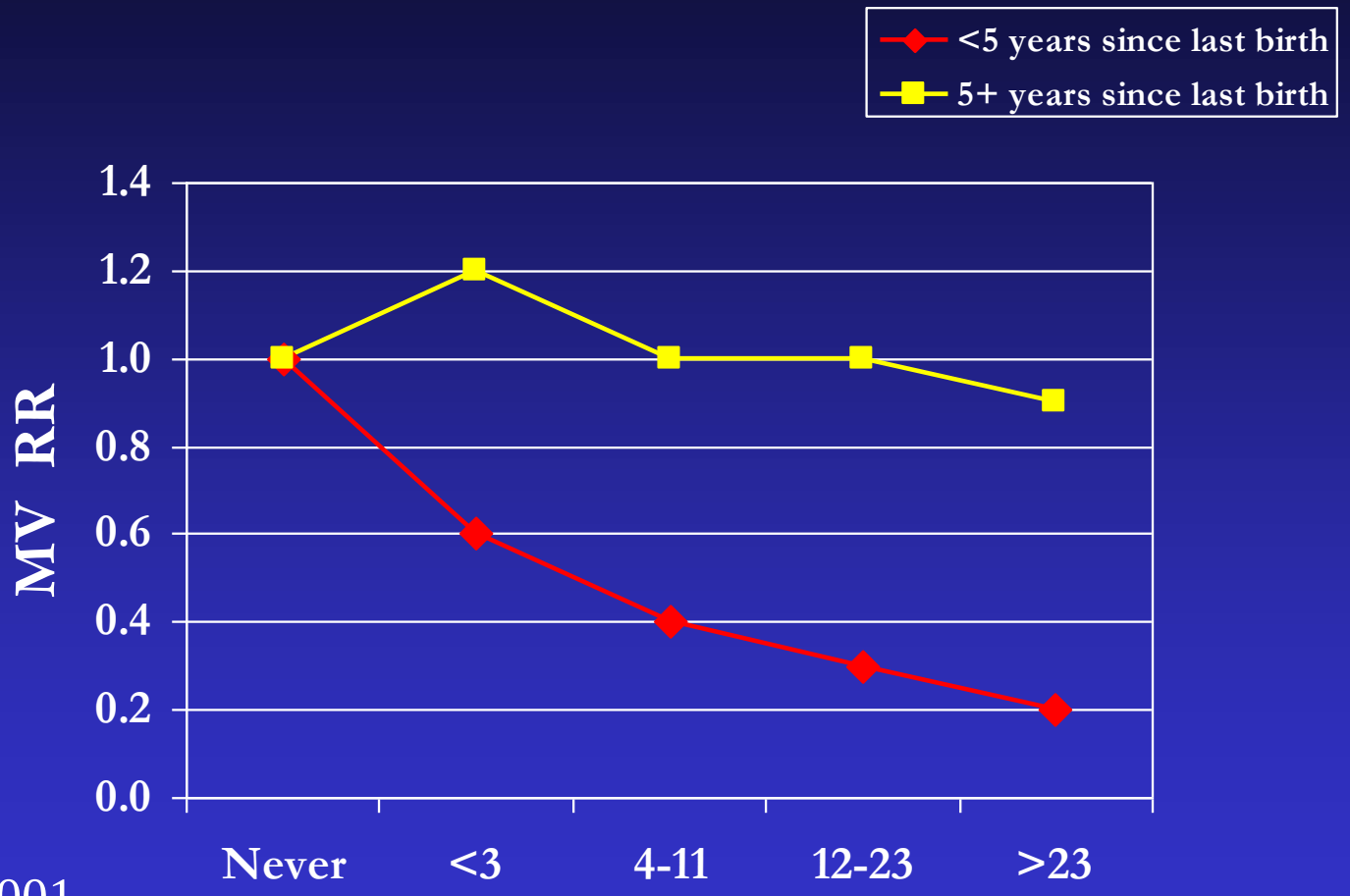
Other Fats/Fatty Acids

Type of Fat	Cumulative Avg. Q5 MV RR (95% CI)	P-trend
Vegetable Fat	0.97 (0.81-1.16)	0.64
Animal Fat	1.17 (0.97-1.40)	0.08
Dairy Fat	0.88 (0.73-1.06)	0.12
Saturated	1.12 (0.94-1.33)	0.12
Palmitic	1.16 (0.97-1.38)	0.07
Myristic	0.96 (0.80-1.15)	0.81
Stearic	1.11 (0.93-1.32)	0.10
Monounsaturated	1.08 (0.90-1.30)	0.37
Oleic	1.07 (0.89-1.29)	0.34
Palmitoleic	1.13 (0.94-1.36)	0.14

Other Fats/Fatty Acids

Type of Fat	Cumulative Avg. Q5 MV RR (95% CI)	P-trend
Polyunsaturated	0.95 (0.80-1.14)	0.60
Omega-3		
Linolenic	0.90 (0.75-1.08)	0.32
EPA	0.89 (0.74-1.08)	0.21
DHA	0.91 (0.76-1.09)	0.19
Omega-6	0.85 (0.70-1.02)	0.22
Linoleic	1.00 (0.84-1.20)	0.90
Arachidonic	1.06 (0.89-1.27)	0.49
Omega-6:Omega-3	1.19 (0.99-1.43)	0.05

Lifetime Lactation (months)

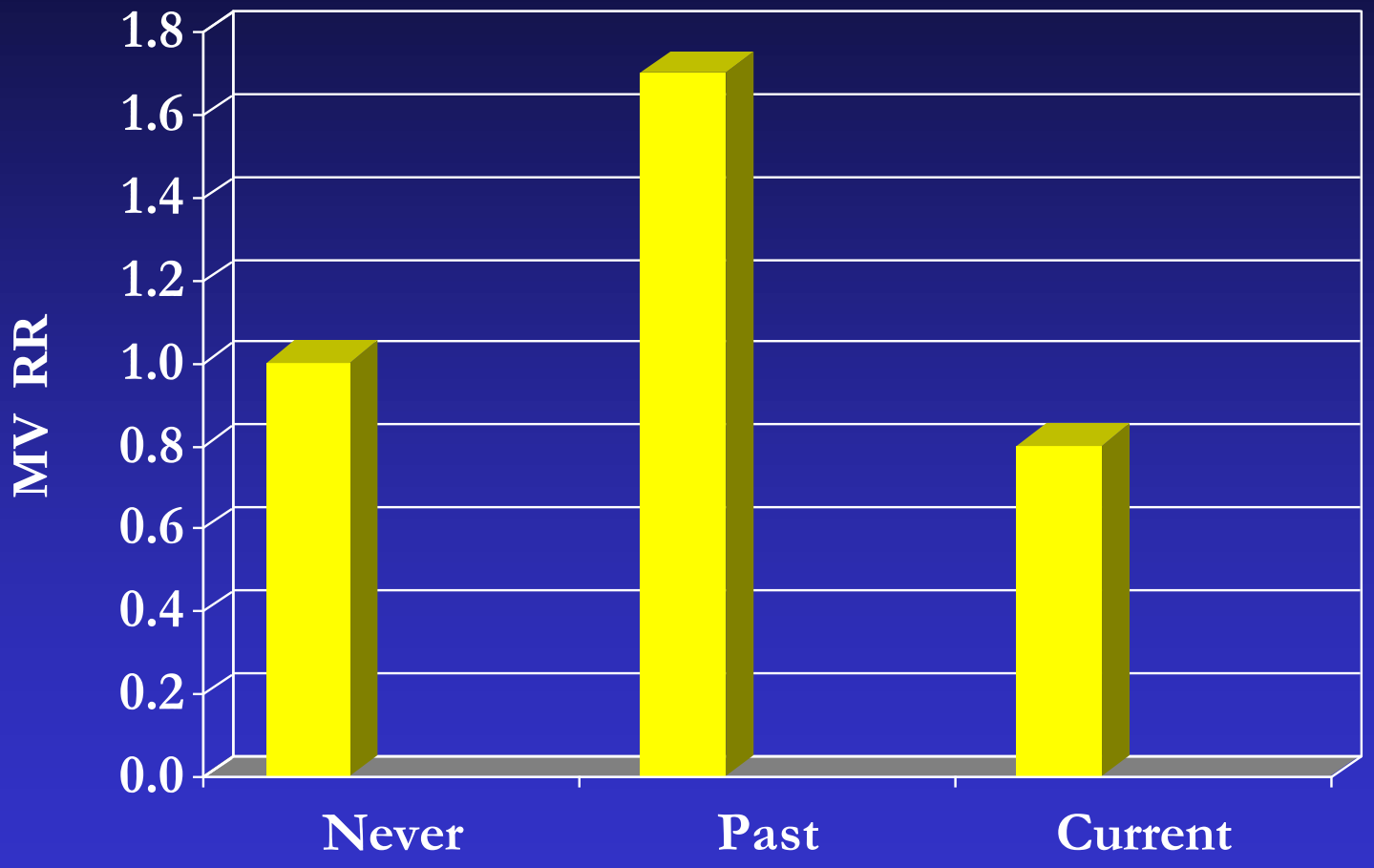


p-trend = 0.001

Adjusted for age, calendar time, parity, age at first birth, time since last birth, BMI at age 18

Missmer et al., Obstet Gynecol 2004

Oral Contraceptive Use



Adjusted for age, calendar time, parity, age at first birth, time since last birth, BMI at age 18

Missmer et al., Obstet Gynecol 2004

Race/Ethnicity

	Cases	IR/1000	MV RR (95% CI)
Caucasian	1603	2.4	1.0 (referent)
Asian	31	2.1	0.8 (0.5-1.1)
African-American	17	1.5	0.6 (0.4-0.9)
Hispanic	17	1.5	0.6 (0.4-1.0)

Adjusted for age, calendar time, parity, BMI at age 18

Uterine Leiomyoma Race/Ethnicity

	Cases	IR/1000	MV RR (95% CI)
Caucasian	2679	8.9	1.0 (referent)
Asian	50	8.0	1.0 (0.8-1.4)
African-American	140	30.6	3.3 (2.7-3.9)
Hispanic	50	11.0	1.2 (0.9-1.6)

Marshall et al., Obstet Gynecol 1997

Cases confirmed by ultrasound or hysterectomy

First Cohort Study

- 17,302 U.K.women
- Enrolled from family planning clinics
- Caucasian, married, 25-39 years old
- Using oral contraceptive, IUD, or diaphragm for a minimum of 5 months
- 313 cases -- identified from hospital records

Vessey et al., Br Med J 1993

Validation Study / Case Definition

- 1994 – Supplementary Questionnaire
- 200 self-reported incident cases
  - 78% reported laparoscopic confirmation
  - 92% responded (n = 184)
- Laparoscopy = 96% confirmed
- No laparoscopy = 54% confirmed
- Laparoscopically confirmed endometriosis
  - Validation results = 61% minimal/mild disease

Validity of weight at age 18

- self-report compared to records from admission to nursing school/college
- recalled vs measured weight r=0.87
- mean BMI recalled weight 21.6
- mean BMI measured weight 22.1
- mean diff recalled weight 1.4 kg under-reported