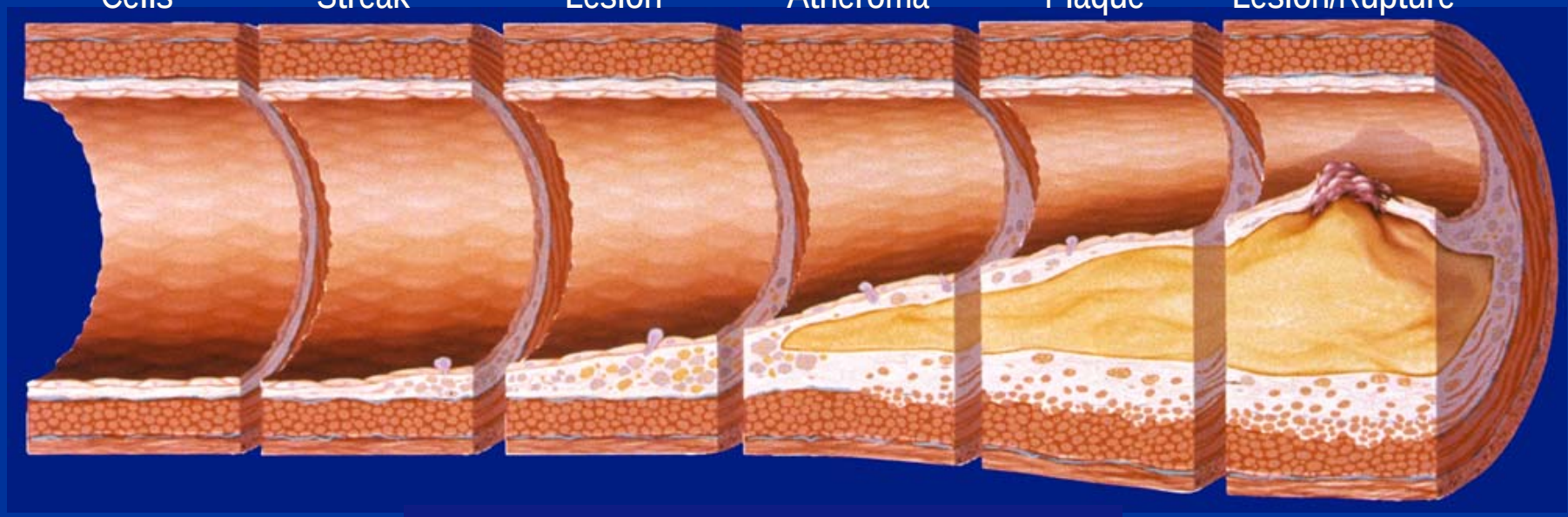
Recent Trial of Antioxidant therapy

고려대학교
구로병원 심혈관센터

서 홍 석

Atherosclerosis Timeline



Endothelial Dysfunction

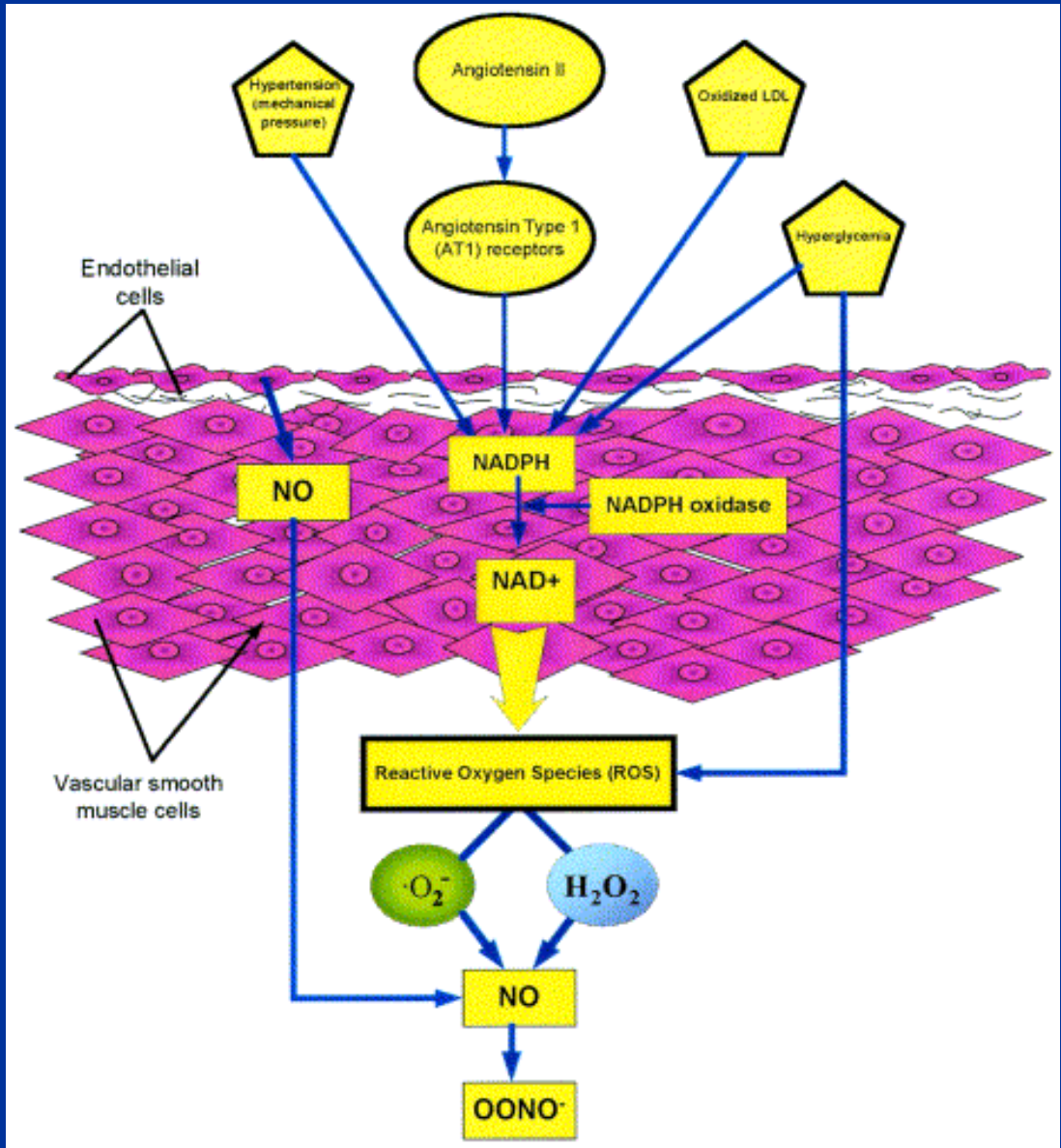
From first decade	From third decade	From fourth decade	
Growth mainly by lipid accumulation		Smooth muscle and collagen	Thrombosis, hematoma

Circulation. 1995;92:1355-1374.

Definition of oxidation

- Removal of electrons from a molecule
- Instigate tissue damage by modifying a number of molecular species, such as lipids, protein,and nucleic acid.

Formation of ROS at Vascular Wall



AJC2003;91(suppl)30H

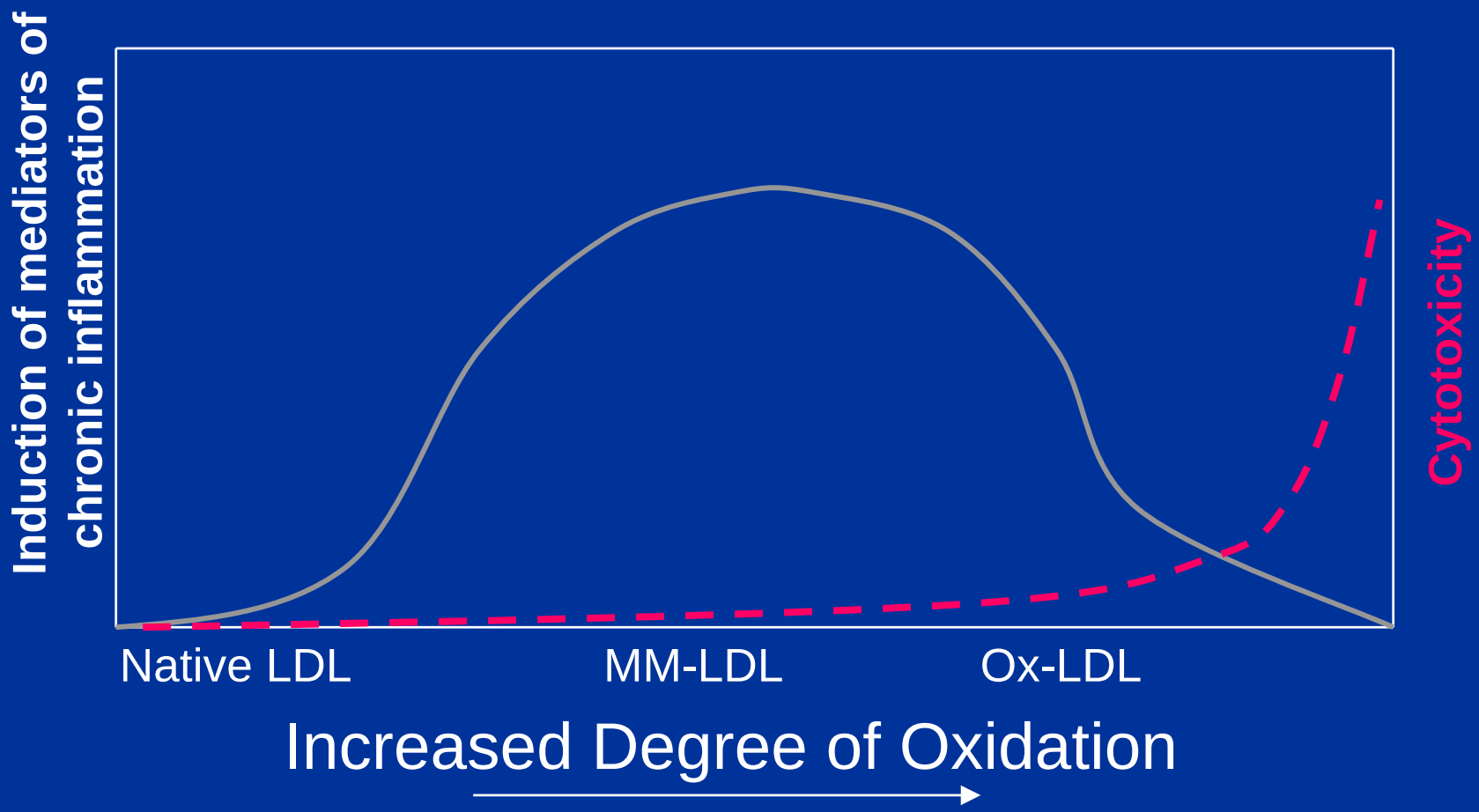
Physical and chemical properties of ox-LDL

- Increased negative charge and density
- Decreased uptake via LDL receptor
- Increased uptake via scavenger receptor
- Decreased vitamin E and antioxidants
- Decreased polyunsaturated fatty acids
- Decreased phosphatidylcholine and increased lyso phosphatidylcholine
- Increased cholesterol oxidation products
- Increased fatty acid oxidation products
- Fragmentation of apo B
- Loss of amino acids(histidine, lysine, & proline)

Pro-atherogenic effects of ox-LDL

- Degraded at a faster rate than native LDL by Mϕ leading to lipid accumulation
- Chemotatic to monocytes, SMCs, & T-cells: inducing T-cell activation & monocyte diferentiation
- Inhibits Mϕ mobility: trapping of Mϕ in the artery
- Cytotoxic to cells
- Inhibits EDRF
- Enhances monocytes adhesion to endothelium
- Induces expression of MCP-I and G-MCF
- Inhibits migration of endothelial cells
- Induces expression of adhesion molecules on endothelium
- Induces IL-1 synthesis & secretion by Mϕ

Change in the biological activities of LDL with increasing degrees of oxidation



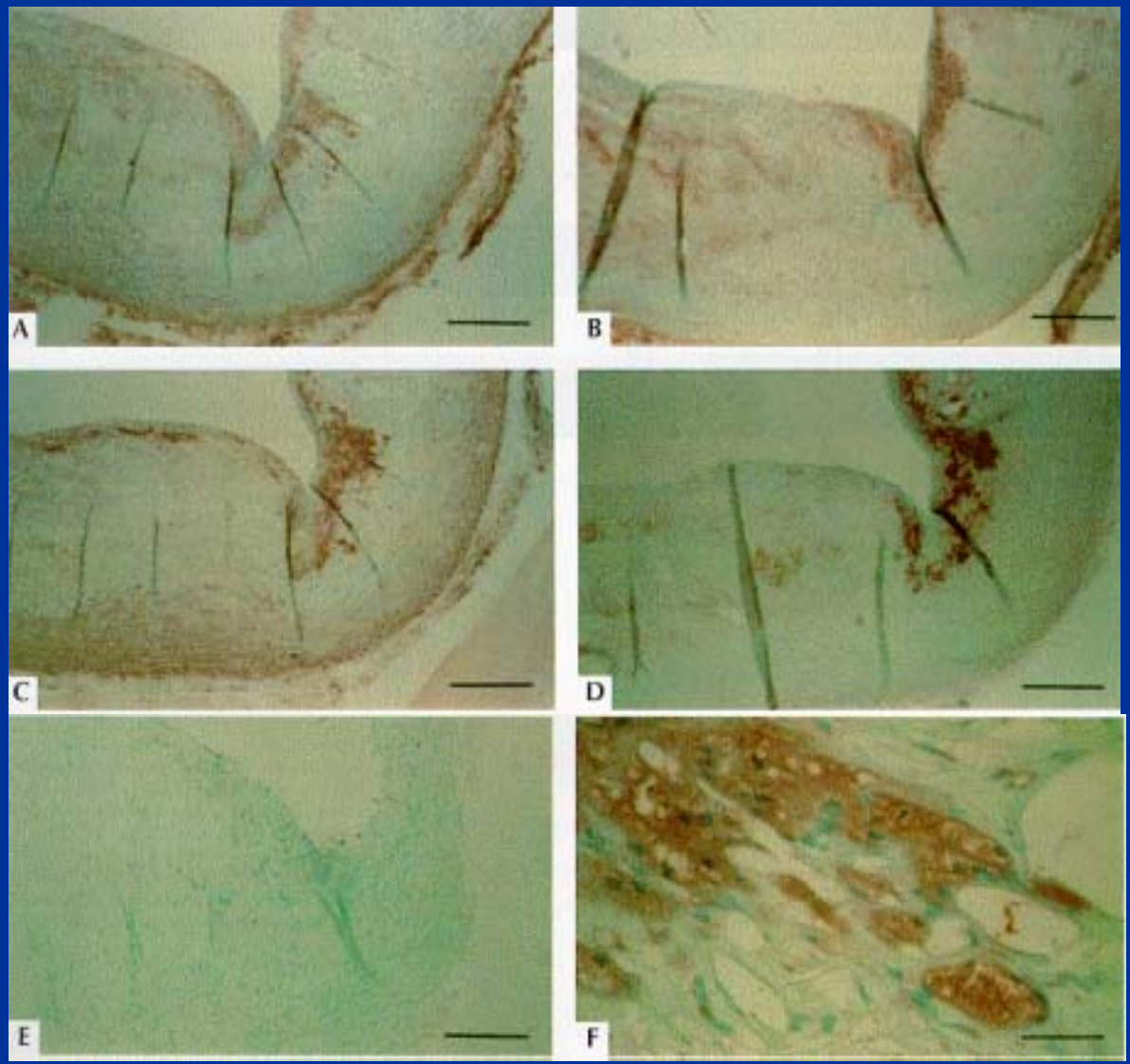
Lipid auto-oxidation hypothesis

- Crawford & Blankenhorn(1991)
  hypoxia(intimal thickening), high oxygen demand
  $\rightarrow$ oxygen radical production & modification
  $\rightarrow$ atherosclerosis
- Lack of evidence at Pulm vasculature,
  cyanotic heart disease,
  A-V shunt & fistula
- Witzum(1994),Steinberg, Parthasarathy
  - LDL modification causes chemotaxis of Mϕ

Evidence for the presence of oxidized LDL in atherosclerotic lesions

- LDL isolated from atherosclerotic lesions resembles oxLDL.
- Epitopes characteristic of oxLDL are present in atherosclerotic lesions.
- Atherosclerotic lesions contain IGs which recognize oxLDL.
- Serum contains autoantibodies against oxLDL
- Antioxidant treatment reduces atherogenesis in experimental animals.

oxLDL in Atherosclerotic lesion



Antioxidant defense systems.

Intracellular	Membrane	Extracellular
SOD	Vitamin E	Ceruloplasmin
Catalase	β -Carotene	Transferrin
Peroxidase	Ubiquinol-10	Lactoferrin
DT-diaphorase		Albumin
Glutathione (reduced)		Haptoglobin
Metal binding proteins		Ascorbic acid
proteolytic systems		uric acid
DNA repair systems		Vitamin E
Ascorbic acid		

Selected characteristics of antioxidant vitamins

Vitamin	Dietary source	Biochemical properties
Vitamin E	Vegetable fats, seed oils, Margarine, nuts, seeds, Wheat germ	Chief lipid soluble antioxidant vitamin in cell membranes and circulting lipoproteins. α-tocopherol accounts for 90% vitamin E.
β -Carotene	Carrots, dark green vegetables	A lipid soluble antioxidant carried with vitamin E in the fatty core of LDL particle. A precusor of retinol(vitamin A)
Vitamin C	Vegetables, citrus fruit, Fruit juices, nuts	Chief water-soluble antioxidant. It can regenerate vitamin E from its oxidized state back to its active state

Effects antioxidants on experimental atherosclerosis

Antioxidant	Experimental system	↓in lesion area,%
Probucol	WHHL rabbit	50-60
Probucol	Cholesterol-fed rabbit	75
Butylated hydroxytoluene	Cholesterol-fed rabbit	70
Diphenylene-diamine	Cholesterol-fed rabbit	71
Vitamine E	Cholesterol-fed macaque	Changes in carotid stenosis:35

Observational Studies of Antioxidants

- Beneficial effects of consuming antioxidant supplements
- Limitation:

1. they tend to rely on subjective data such as patients' 24-hour recall of dietary intake.
2. Those who enter such studies are often 'health-conscious'.
3. Diets contain many substances, and it is difficult to know which specific nutrient in the diet or supplements is the one that led to a positive outcome.

Observational Studies of Antioxidants

Rotterdam study

- **Purpose**: relationship between cardiovascular events and dietary intake of β-Carotene, vitamin C, and vitamin E
- **Subjects**: 4,802 people ages 55-95 with no history of MI
- **Protocol**: intake of these vitamins for 4 yrs, using comuterized food-frequency questionnaires
- **Results**:high intake of β-Carotene protected against MI, and more pronounced in current & former smokers. No beneficial effect of vitamin C or vitamin E.

Am J Clin Nutr 1999;69:261-266

Observational Studies of Antioxidants

Iowa Women's Health study

- **Purpose**: test for an inverse relationship between mortality due to CAD and intake of antioxidant vitamins from food sources and supplements.
- **Subjects**: 34,486 postmenopausal women ages 55-69 with no history of CVD for 7 yrs
- **Protocol**: estimation of intake of vitamins A, E, C, using food-frequency questionnaires with five 24-hour dietary-recall interview. Validation by plasma level of β-Carotene(r=0.30), and α-tocopherol(vitamin E: r=0.41)
- **Results**:high intake of vitamin E seemed to protect against death from CAD. No beneficial effect of vitamin A and vitamin C.

N Eng J Med 1996;334:1156-1162

Observational Studies of Antioxidants

Established Population for Epidemiologic Study of the Elderly(EPESE)

- **Purpose**: Effects of vitamin E and vitamin C supplement on all-cause mortality and risk of death from CAD.
- **Subjects & Duration** : 11,178 people ages 67-105 for 8-9 yrs
- **Results**: Vitamin E was associated with a decrease in all-cause mortality, especially due to death from CAD. Beneficial effects was enhanced by vitamin C.

Am J Clin Nutr 1996;64:190-196

Observational Studies of Antioxidants

Finnish Study

- **Purpose**: Effects of dietary intake of carotene, vitamin C and vitamin E on coronary mortality.
- **Subjects & Duration** : 5,133 people ages 30-69, free of heart disease, for 14 yrs
- **Results**: Vitamin E consumption was linked to a reduction in death from CAD in both men and women. Carotene and vitamin C appeared to impact coronary protection to women only.

Am J Epidemiol 1994;139:1180-1189

Observational Studies of Antioxidants

Health Professionals Follow-up Study

- **Purpose**: Effects of antioxidant on prevention of CAD.
- **Subjects & Duration** : 39,910 male heath professionals ages 40-75, no CAD, for over 4 yrs with dietary questionnaire.
- **Results**: Increased intake of vitamin E was associated with a lower risk of CAD.

N Eng J Med 1993;328:1450-1456

Observational Studies of Antioxidants

Nurses' Health Study

- **Purpose**: To measure any cardioprotective effects of dietary antioxidant intake.
- **Subjects & Duration** : 87,245 female nurses ages 34-59, no CVD, no cancer for 8 yrs through dietary questionnaire.
- **Results**: Increased intake of vitamin E was associated with a lower risk of CHD especially if antioxidant supplement form.

N Eng J Med 1993;328:1444-1449

Observational Studies of Antioxidants

National Health and Nutrition Examination Survey(NHANES I)

- **Purpose**: association of normal dietary intake and supplement intake of vitamin C with all-cause mortality and CVD.
- **Subjects**: 117,348 adults ages 25-74.
- **Results**: Dietary consumption and supplement intake of vitamin C were inversely associated with all-cause mortality and CVD in men, but not in women.
- **Limitation**: similar to those of Health Professionals Follow-up Study and Nurses' Health Study:

  (lack of uniform screening procedure to determine CVD, lack of independent validation of vitamin intake.)

Epidemiol 1992;3:194-202

Observational Studies of Antioxidants

Scottish Heart Health Study

- **Purpose**: Effects of dietary and supplementary intake of vitamin C, β-Carotene, and vitamin E on CAD.
- **Subjects**: 4,036 men and 3,833 women ages 40-59 with no history of heart disease.
- **Results**: Vitamin C and β-Carotene reduced CAD in men only. Vitamin E ingestion did not seem to confer any significant protection against CHD in men or women.

  No antioxidant had any effect on all-cause mortality.

Am J Epidemiol 1999;150:1073-1080

Conclusion from observational studies of antioxidants

- increased intake of antioxidant was associated with a reduced risk of CVD.
- Consider studies as preliminary observation.
  - inconsistencies among studies
  - difficulty accounting for confounding variables
  - a reliance on food questionnaires
  - a lack of validation of vitamin intake with objective laboratory evaluations
- Need randomized controlled trials(RCT)

Interventional Studies of Antioxidants

- A few interventional clinical trials have shown a protective effects of antioxidant vitamins, most have not.
- Reasons for Mixed result:
  - different doses
  - different demographic characteristics
  - age
  - duration

Interventional Studies that showed neither harm nor benefit

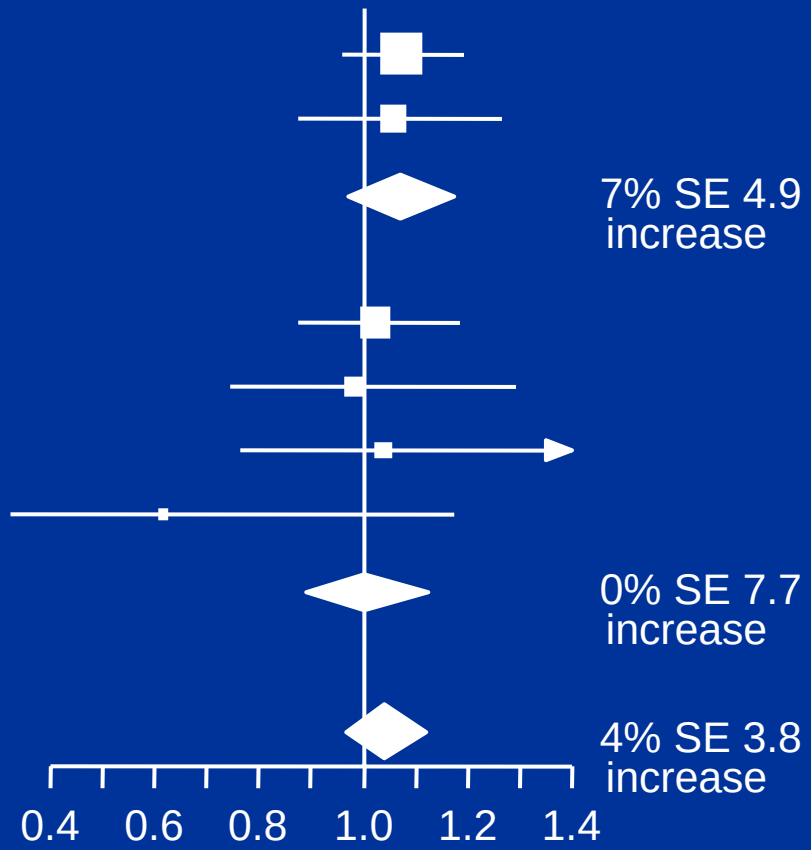
Heart Protection Study(HPS)

- **Purpose**: relationship of antioxidant supplement to major coronary events, fatal or nonfatal vascular events, cancer, and other major morbidity.
- **Subjects & Protocol**: 20,536 adults ages 40-80 with CAD, other occlusive arterial disease, or diabetes. Vitamin E 600mg/d, vitamin C 250mg/d and β-Carotene 20mg/d or placebo for 5 yrs
- **Results**: antioxidant had any neither harm nor benefit effect.

Lancet 2002;360:23-33

VITAMIN: CAUSE-SPECIFIC MORTALITY

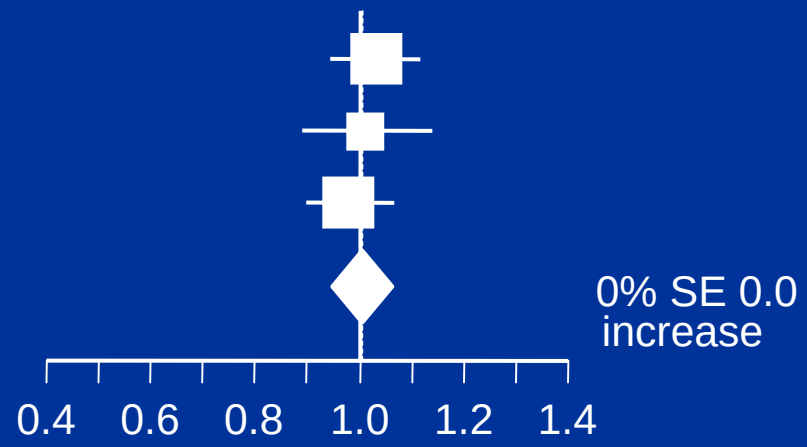
Cause of death	VITAMIN (10269)	PLACEBO (10267)
CHD	661	617
Other vascular	234	222
ALL VASCULAR	895 (8.7%)	839 (8.2%)
Neoplastic	348	341
Respiratory	101	103
Other medical	85	82
Non-medical	14	23
ALL NON-VASCULAR	548 (5.3%)	549 (5.3%)
ALL CAUSES	1443 (14.1%)	1388 (13.5%)



Lancet 2002;360:23-33

VITAMIN: MAJOR VASCULAR EVENTS

Vascular event	VITAMIN (10269)	PLACEBO (10267)
Total CHD	1089	1059
Total stroke	537	532
Revascularisation	1044	1067
ANY OF ABOVE	2327 (22.7%)	2321 (22.6%)



Lancet 2002;360:23-33

Interventional Studies that showed neither harm nor benefit

Primary Prevention Project(PPP)

- **Purpose**: Effects of low-dose aspirin(100mg/d) or vitamin E (300mg/d) on coronary events
- **Subjects**: 4,495 people, mean age 64 with at least 1 risk factor for CAD.
- **Results**: Early stopped trial(3.6yrs) due to proven beneficial effect of aspirin. No effect with vitamin E.

Lancet 2001;357:89-95

Interventional Studies that showed neither harm nor benefit

Heart Outcomes Prevention Evaluation (HOPE) study

- **Purpose**: Effects of either ACEI(ramilpril 10mg/d) or placebo and either vitamin E (400IU/d) or placebo on MI, stroke, or death from CV causes.
- **Subjects**: 2,545 women and 6,996 men age 55 or older with a history of CAD or diabetes in addition to 1 other risk factor for atherosclerosis.
- **Results**: At 4.5 yrs, vitamin E had no apparent effect on cardiovascular outcome.

N Engl J Med 2000;342:154-160

Interventional Studies that showed neither harm nor benefit

Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarcto Miocardico (GISSI) study

- **Antioxidant**: omega-3 PUFA(1g/d), vitamin E (300mg/d), both or neither for 3-5 yrs.
- **Subjects**: 11,324 patients survived from MI with 3 months.
- **Results**: Vitamin E supplementation showed no benefit, whereas omega-3 PUFA supplementation seemed beneficial.

Lancet 1999;354:447-455

Interventional Studies that showed neither harm nor benefit

Women' Health Study(WHS)

- **Intervention**: Effect of aspirin(100mg/d) or vitamin E (600IU/d), β-Carotene (50mg alternate day) on prevention of cancer and CVD
- **Subjects**: 39,876 women age 45 or older
- **Results**: Early stopped trial(2.1yrs) due to concerns of harmful effects of β-Carotene. Additional 2 year-follow-up showed β-Carotene was associated with neither harm nor benefit.

J Natl Cancer Inst 1999;91:2102-2106

Interventional Studies that showed neither harm nor benefit

Physicians' Health Study(PHS)

- **Intervention**: Effect of β-Carotene (50mg alternate day) on primary prevention of CVD and cancer
- **Subjects**: 22,071 male physicians age 40-84
- **Results**: β-Carotene was associated with neither harm nor benefit.

N Engl J Med 1996;334:1145-1149

Interventional Studies that showed possible deleterious effects

Beta Carotene and Retinal Efficacy Trial (CARET)

- **Intervention**: either combination of β-Carotene
- (30mg daily) and vitamin A(25,000 IU daily) or placebo
- **Subjects**: 18,314 people at high risk of lung cancer
- **Results**: combination of β-Carotene and vitamin A was associated with 28% higher incidence of lung cancer and 17% more deaths than placebo group.

J Natl Cancer Inst 1996;88:1550-1559

Interventional Studies that showed possible deleterious effects

Alpha Tocopherol Beta Carotene Cancer Prevention study(ATBC)

- **Intervention**: α-tocopherol(50mg daily) or β-Carotene (20mg daily) or a combination of vitamins or placebo.
- **Subjects**: 29,133 male smokers age 50-69
- **Results**: total mortality rate was 8% higher in the group taking β-Carotene, primarily due to more deaths from lung cancer.

N Engl J Med 1994;330:1029-1035

Interventional Studies that showed possible deleterious effects

HDL Atherosclerosis Treatment Study(HAT

- **Intervention**: simvastatin + niacin, antioxidants(vitamin E 800 IU, β-Carotene 25mg, selenium 100μg daily), simvastatin + niacin + antioxidant, placebo for 3 yrs.
- **Subjects**: 160 men under age 63 and women under age 70 with CAD: low HDL, LDL 145mg/dl ↓, TG 400mg/dl↓
- **Results**: Simvastatin + niacin led to a significant benefit. Antioxidants fail to show any substantial benefit. Antioxidants seemed to diminish the beneficial effect of simvastatin + niacin. Antioxidants blunted niacin-induced elevation of HDL_2.

Arteriolscler Thromb Vasc Biol 2001;21:1320-1326

Interventional Studies that showed possible benefit

The Cambridge Heart Antioxidant Study (CHAOS)

- **Intervention**: vitamin E 800 IU or 400 IU for 1.4 yrs.
- **Subjects**: 2002 patients with angiographically proven CAD
- **Results**: While fewer nonfatal MI in those taking α-tocopherol, the number of cardiovascular deaths was not reduced.
- **Limitation**: no randomization: first 546 pt were given 800 IU, newly recruited subjects were given 400 IU.

Lancet 1996;347:781-786

Interventional Studies that showed possible benefit

Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study

- **Intervention**: d-alpha-tocopherol 136 IU daily or slow-release vitamin C 250mg daily or a combination of two for over 3 yrs.
- **Subjects**: 520 men and postmenopausal women age 45-69
- **Results**: Progression of carotid atherosclerosis was reduced only in smoker men who took both vitamins.

J Intern Med 2000;248:377-286

Interventional Studies that showed possible benefit

Chinese Nutrition Intervention Trials

- **Intervention**: Retinol 5,000 IU + zinc 22.5mg,
  Riboflavin 3.2mg + niacin 40mg,
  Ascorbic acid 120mg + molybdenum 30μg
  β-Carotene 15mg + selenium 50μg +
  α-tocopherol 30mg.
- **Subjects**: 29,548 people age 40-69
- **Results**: Total mortality was significantly lower in those receiving β-Carotene + selenium + α-tocopherol due to lower rates of cancer(especially stomach cancer) from 1-2 yrs after start of supplementation.
- **Limitation**: no screening for nutritional status. Not known which constituent is beneficial.

J Natl Cancer Inst 1993;85:1483-1492

Meta-analysis or RCT

7 RCT of vitamin E,

8 RCT of β-Carotene,

vitamin E & β-Carotene

Lancet 2003;361:2017-2023

Meta-analysis of RCT

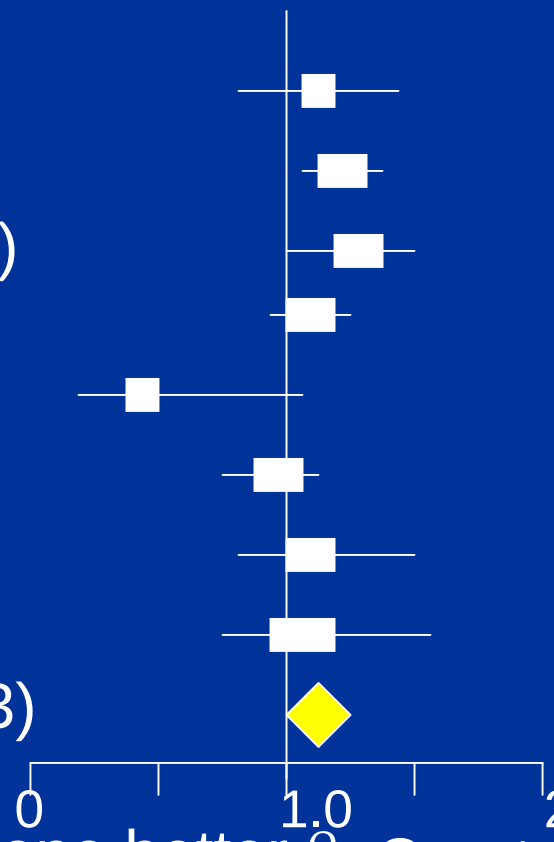
- dose:
  Vitamin E : 20-800 IU
  β-Carotene: 15-50mg
- follow-up range: 1.4 ~ 12.0 years
- patients involved(at least 1,000 subject each trial)
  Vitamin E : 81,788
  β-Carotene: 138,113

: -no beneficial effect on cardiovascular mortality and morbidity in the long term.

- Taking β-Carotene led to a small but significant increase in all-cause mortality (7.4% vs 7.0%, p=0.003) and a slight increase in cardiovascular death(3.4% vs 3.1% p=0.003). Therefore, some antioxidant vitamins, notably β-Carotene, may be harmful.

Lancet 2003;361:2017-2023

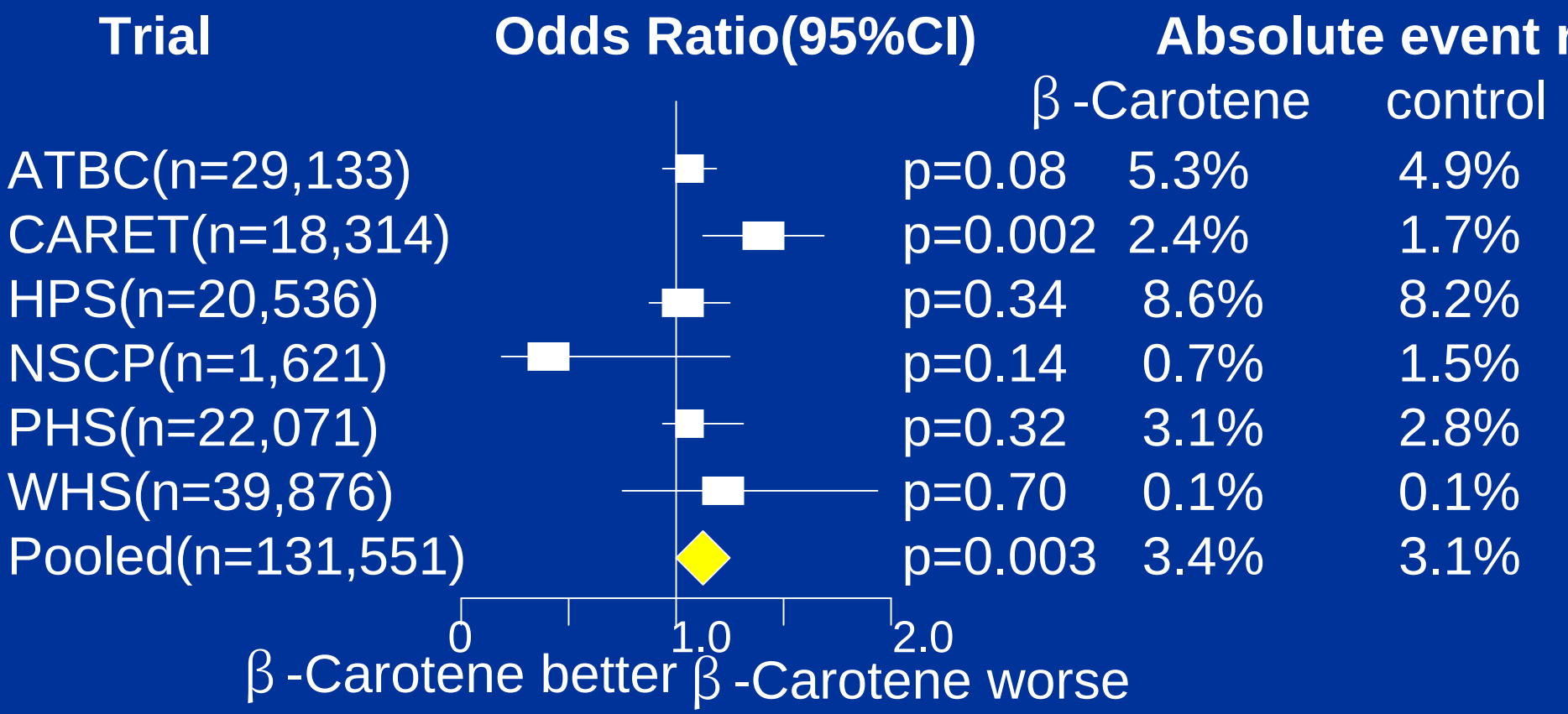
Odds ratios(95% CI) of all-cause mortality for individuals treated with β-Carotene or control therapy



Trial	Odds Ratio(95%CI)	Absolute event r		
			β-Carotene	control
AREDS(n=4,757)		p=0.54	10.6%	10.1%
ATBC(n=29,133)		p=0.02	12.7%	11.8%
CARET(n=18,314)		p=0.02	4.9%	4.1%
HPS(n=20,536)		p=0.25	14.1%	13.5%
NSCP(n=1,621)		p=0.06	1.3%	2.6%
PHS(n=22,071)		p=0.80	8.9%	8.8%
SCP(n=1,805)		p=0.66	8.7%	8.1%
WHS(n=39,876)		p=0.71	0.30%	0.28%
Pooled(n=138,113)		p=0.003	7.4%	7.0%

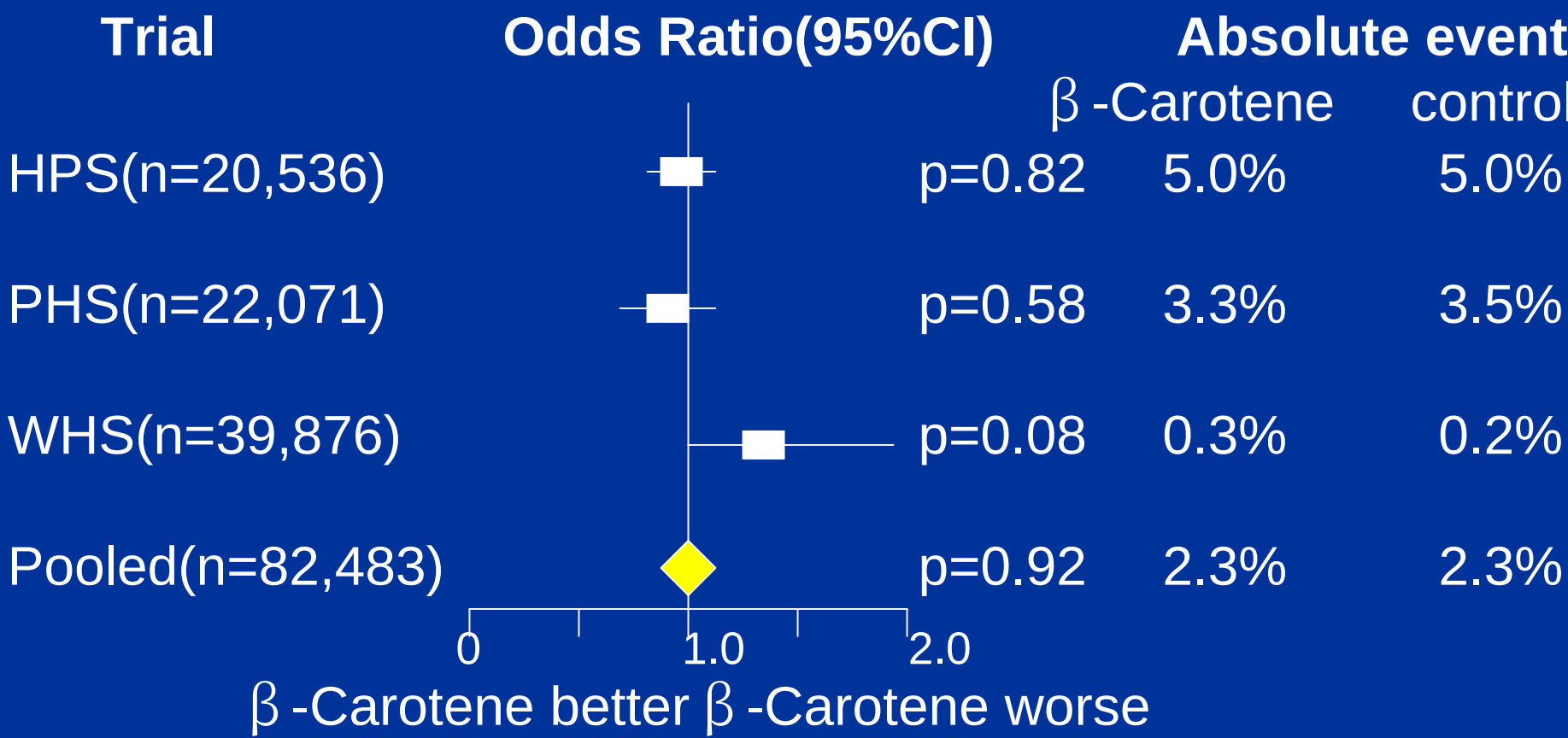
Lancet 2003;361:2017-2023

Odds ratios(95% CI) of cardiovascular death for individuals treated with β-Carotene or control therapy



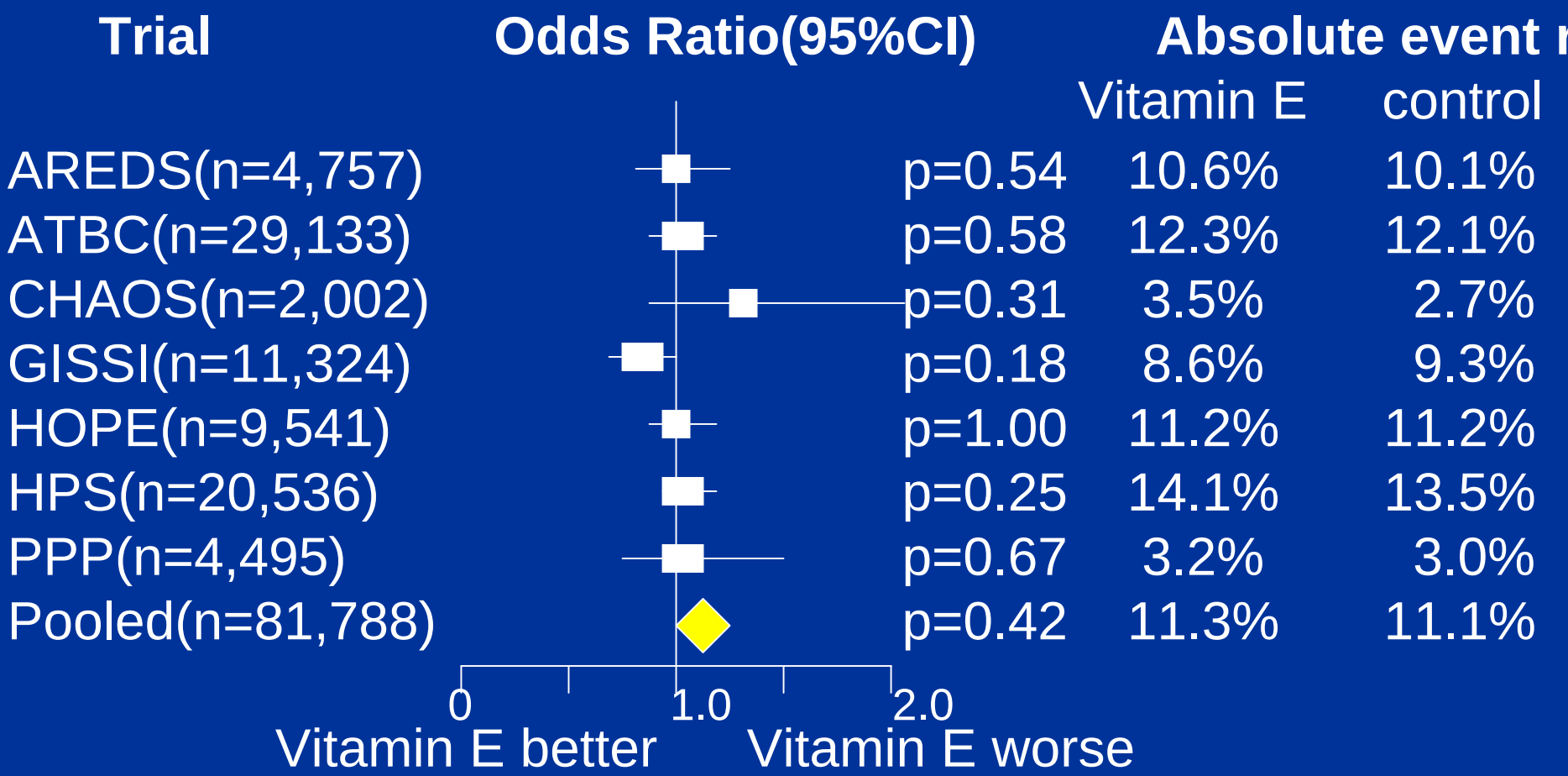
Lancet 2003;361:2017-2023

Odds ratios(95% CI) of cerebrovascular accidents for individuals treated with β-Carotene or control therapy



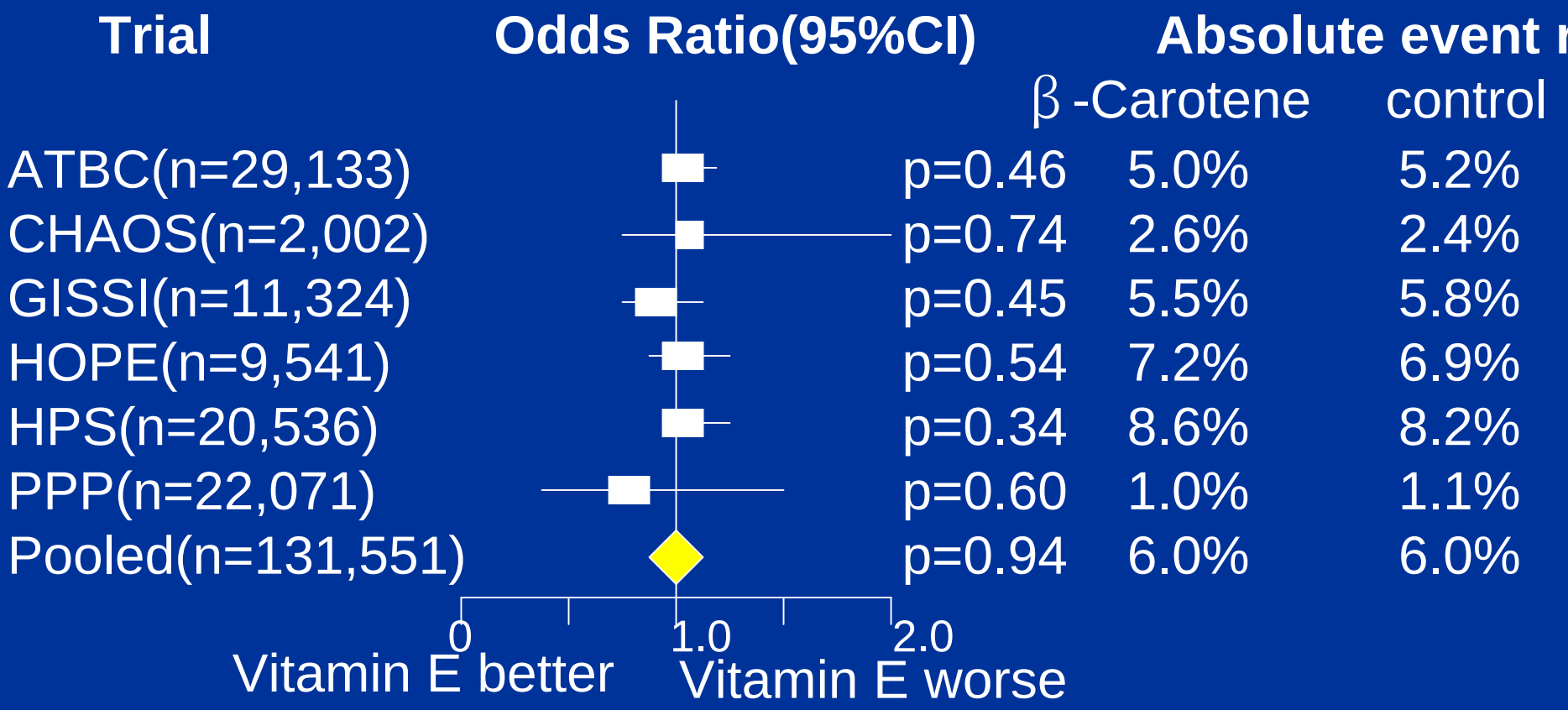
Lancet 2003;361:2017-2023

Odds ratios(95% CI) of all-cause mortality for individuals treated with vitamin E or control therapy



Lancet 2003;361:2017-2023

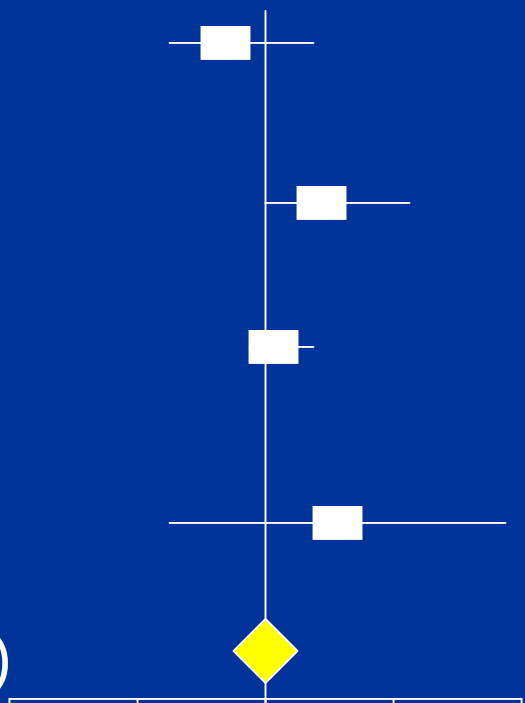
Odds ratios(95% CI) of cardiovascular death for individuals treated with vitamin E or control therapy



Lancet 2003;361:2017-2023

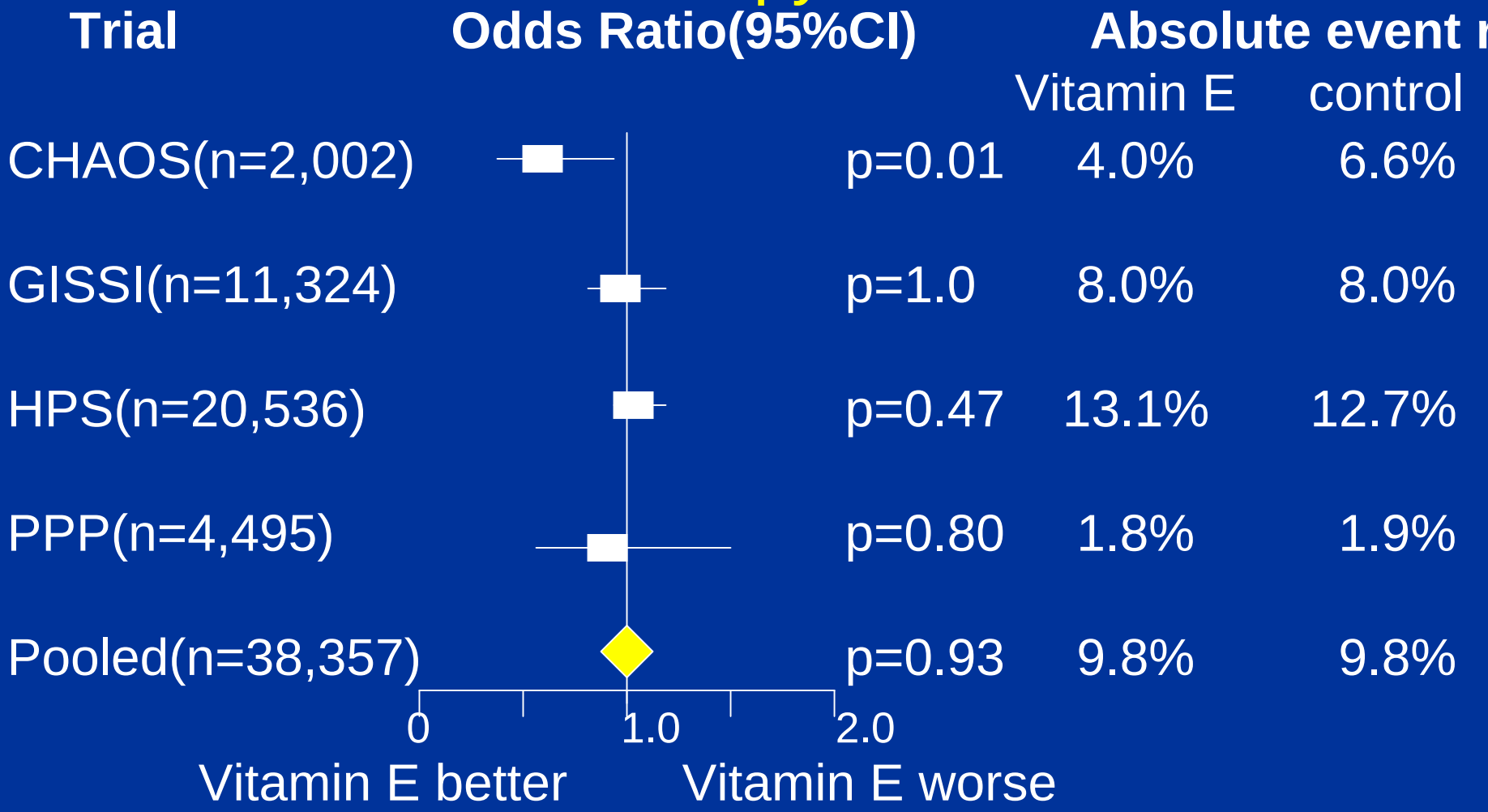
Odds ratios(95% CI) of all-cause stroke for individuals treated with vitamin E or control therapy

Trial	Odds Ratio(95%CI)	Absolute event r	
		Vitamin E	control
GISSI(n=11,324)	p=0.37	1.5%	1.7%
HOPE(n=9541)	p=0.12	4.4%	3.8%
HPS(n=20,536)	p=0.82	5.0%	5.0%
PPP(n=4,495)	p=0.50	1.0%	0.8%
Pooled(n=45,896)	p=0.70	3.6%	3.5%



Lancet 2003;361:2017-2023

Odds ratios(95% CI) of combined endpoint of cardiovascular death or no-fatal MI for individuals treated with vitamin E or control therapy



Lancet 2003;361:2017-2023

Possible mechanism for the increased risk of death associated with β-Carotene

- Poor inhibitor of in vivo LDL-oxidation
- Cigarette smoke destabilizes β-carotene molecule resulting in abnormal signal transduction and up-regulation of growth factors associated with tumorogenesis.
- Adverse effects on lipids

Lancet 2003;361:2017-2023

Possible mechanism for the lack of clinical efficacy with vitamin E

- Blunt the HDL-raising effects by niacin and simvastatin.
- inhibitor of in vivo LDL-oxidation at high serum level(2,000mg/d).
- Timing of antioxidant treatment:

  More effective in inhibiting the early stages of atherosclerosis than in preventing sequelae in the advanced stages

Lancet 2003;361:2017-2023

What should we tell our patients?

Shortcomings worth noting for clinical trials

- Most were done in people with symptoms, and it may be easier to prevent the onset of disease than to reverse an established atherosclerotic lesion.
- Clinical trials generally do not include measures of oxidation.
- The failure of vitamin E or β-Carotene to reduce the incidence of cardiovascular end points does not necessarily mean that other antioxidants yet to be tried would not be successful as therapeutic agents, because oxidation of compounds can occur via alternate pathways that are not inhibited by vitamin E or β-Carotene.

What should we tell our patients?

- Most did not prove beneficial effects of taking antioxidants.
- Harmful effects of antioxidants
  - subjects at high risk of developing lung cancer(smoking, asbestos)
  - attenuated cardiovascular benefit of simvastatin or niacin with concurrent use of antioxidants
- Need further investigation

What should we tell our patients?

- Concern about overuse of antioxidant
  - vitamin C: bloating, diarrhea,
    hemolysis in G6PD deficiency,
    serious cardiac arrhythmia in
    iron overload
  - vitamin A: osteoporosis, teratogenecity
  - vitamin E: worsen retinitis pigmentosa,
    increase risk of hemorrhagic stroke

감사합니다.