Biomarkers in Vascular Inflammation (hsCRP, Lp-PLA_2)

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원 규 장

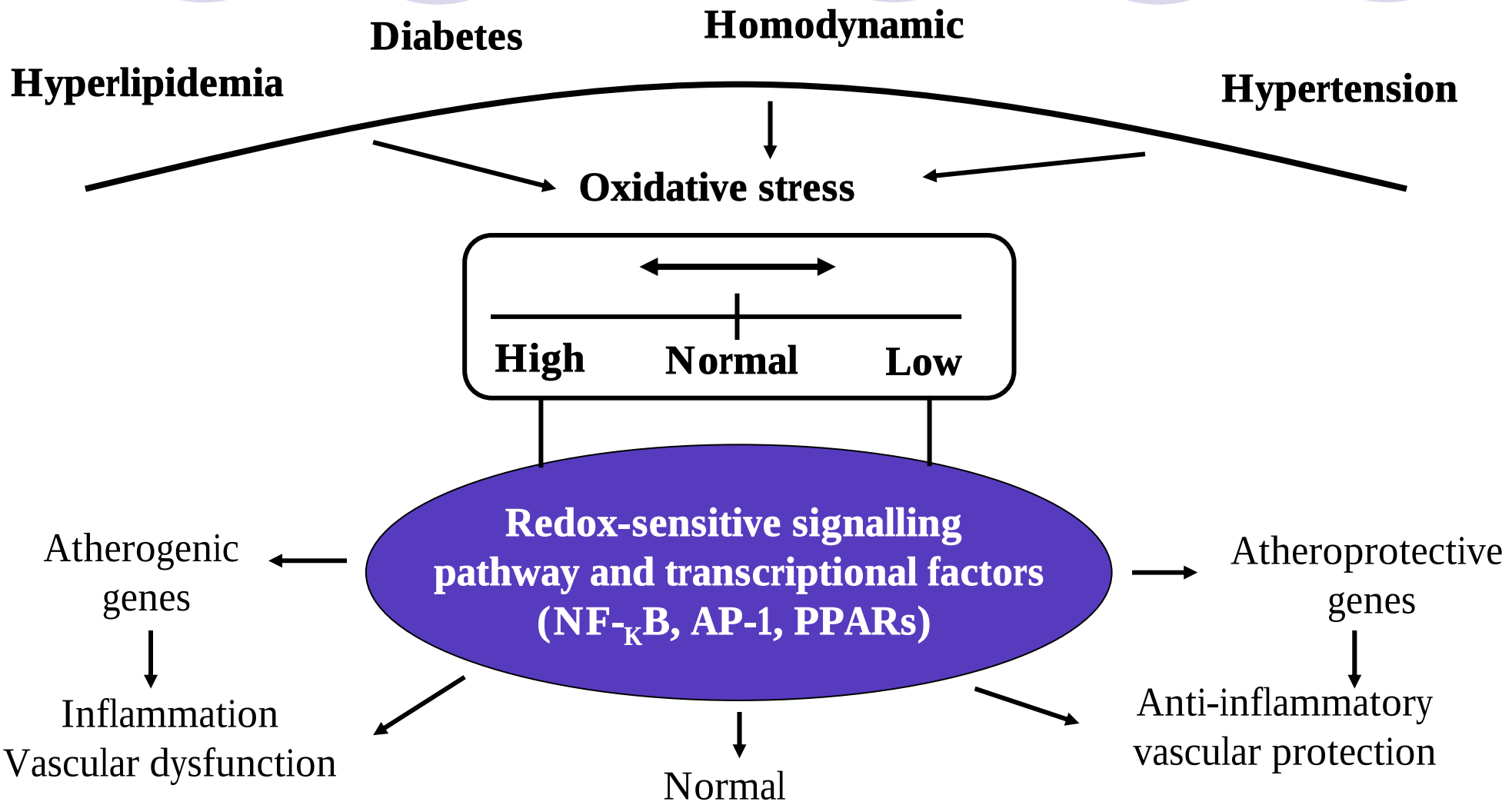
- **Inflammation; Current evidence supports –**

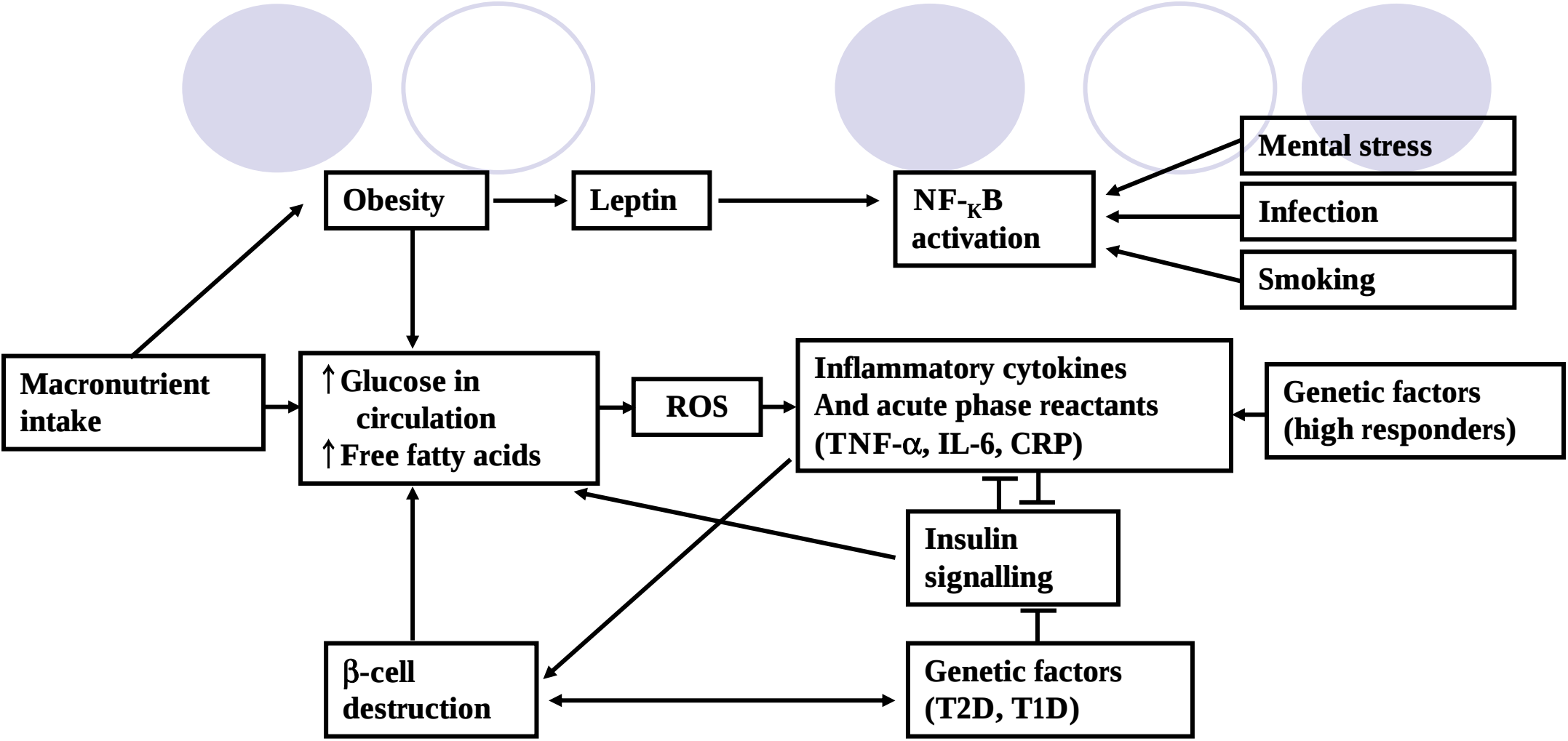
  - **a central role in all phases of the atherosclerosis**

  - from **lesion initiation** through to **progression** and, ultimately, the **thrombotic complications of atherosclerosis**.

May circulating levels of inflammatory biomarkers help identify those at risk of future cardiovascular events ?

Risk factors for atherosclerosis

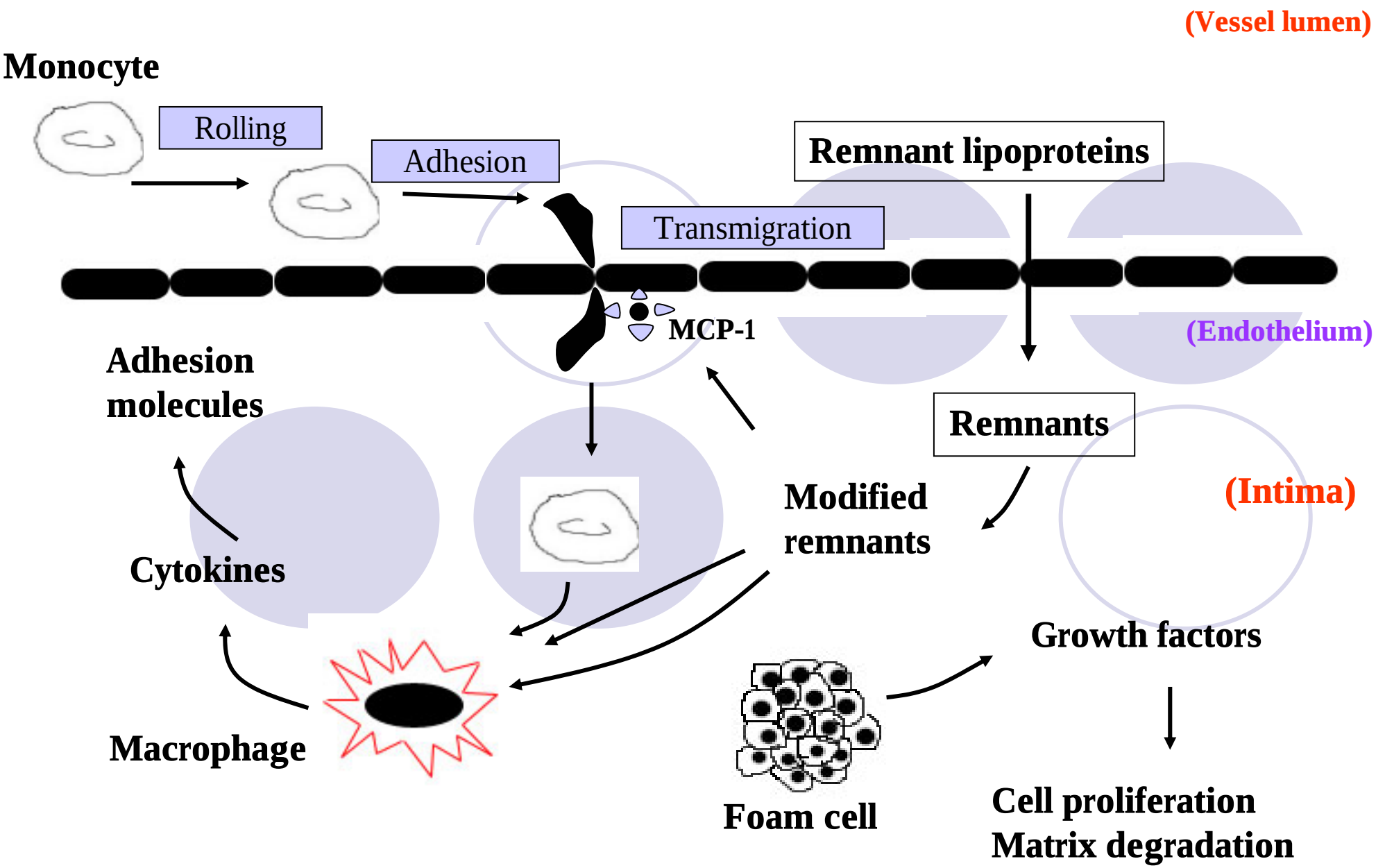




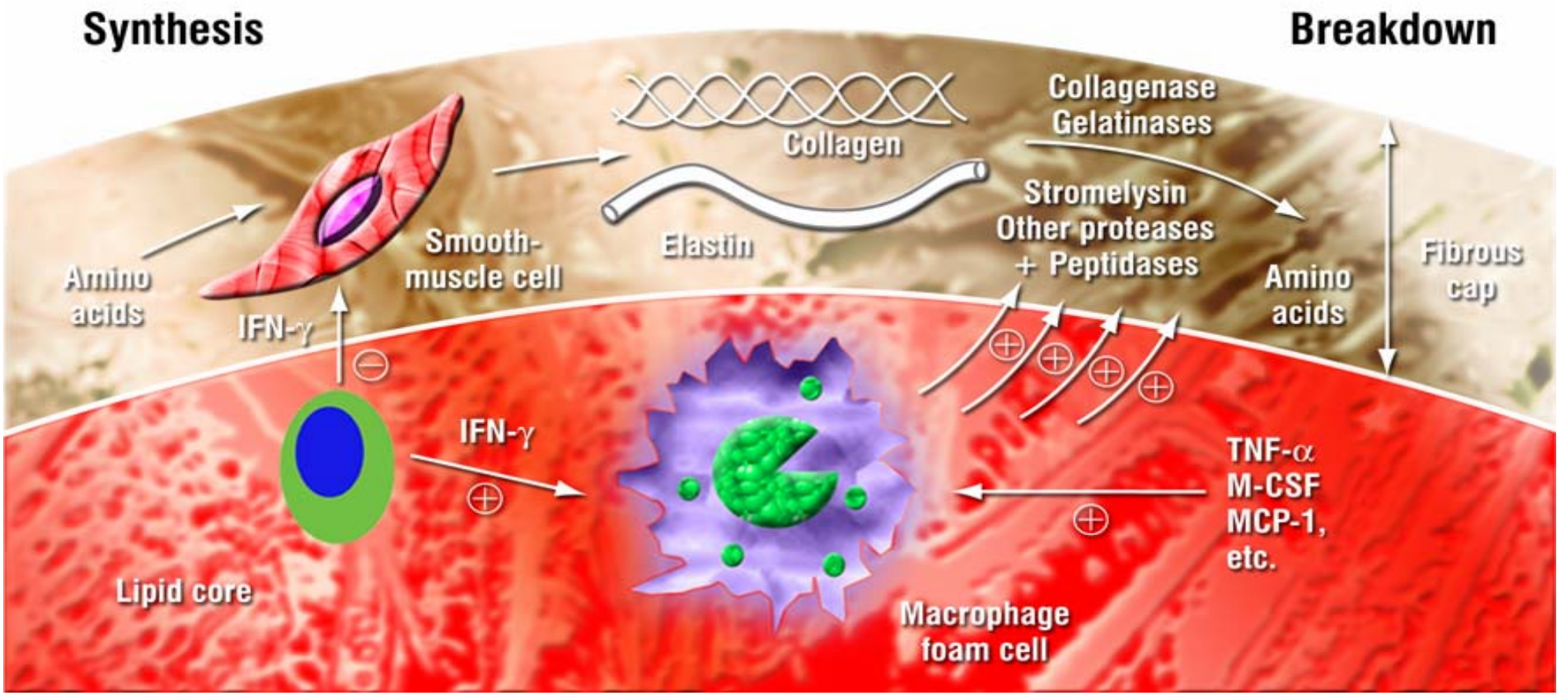
Induction of reactive oxygen species (ROS) and inflammation (NF-$_K$B activation) by macronutrient intake, obesity, free fatty acids, leptin, infection, smoking, mental stress, and genetic factors

(Sjoholm A et al. Lancet 365:610-612, 2005)

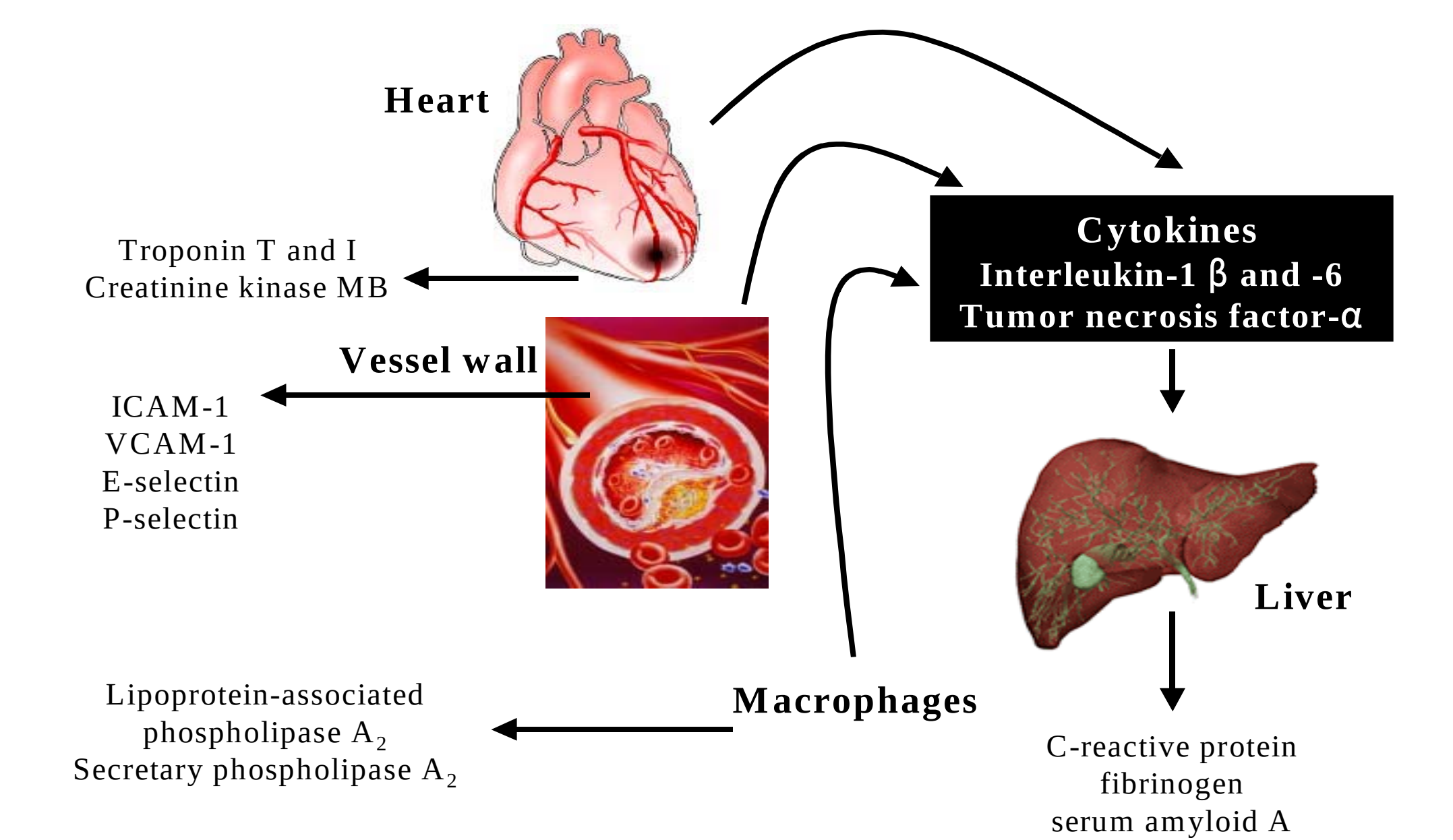
The role of inflammation in atherosclrosis


(Vessel lumen)
Monocyte
Rolling
Adhesion
Transmigration
Remnant lipoproteins
MCP-1
(Endothelium)
Adhesion molecules
Remnants
Modified remnants
(Intima)
Cytokines
Growth factors
Macrophage
Foam cell
Cell proliferation
Matrix degradation

The Synthesis and Breakdown of Atheromatous Plaques



*Adapted from Libby P. Circulation 1995;**91**:2844–2850*



Potential markers of inflammatory activity and their sources

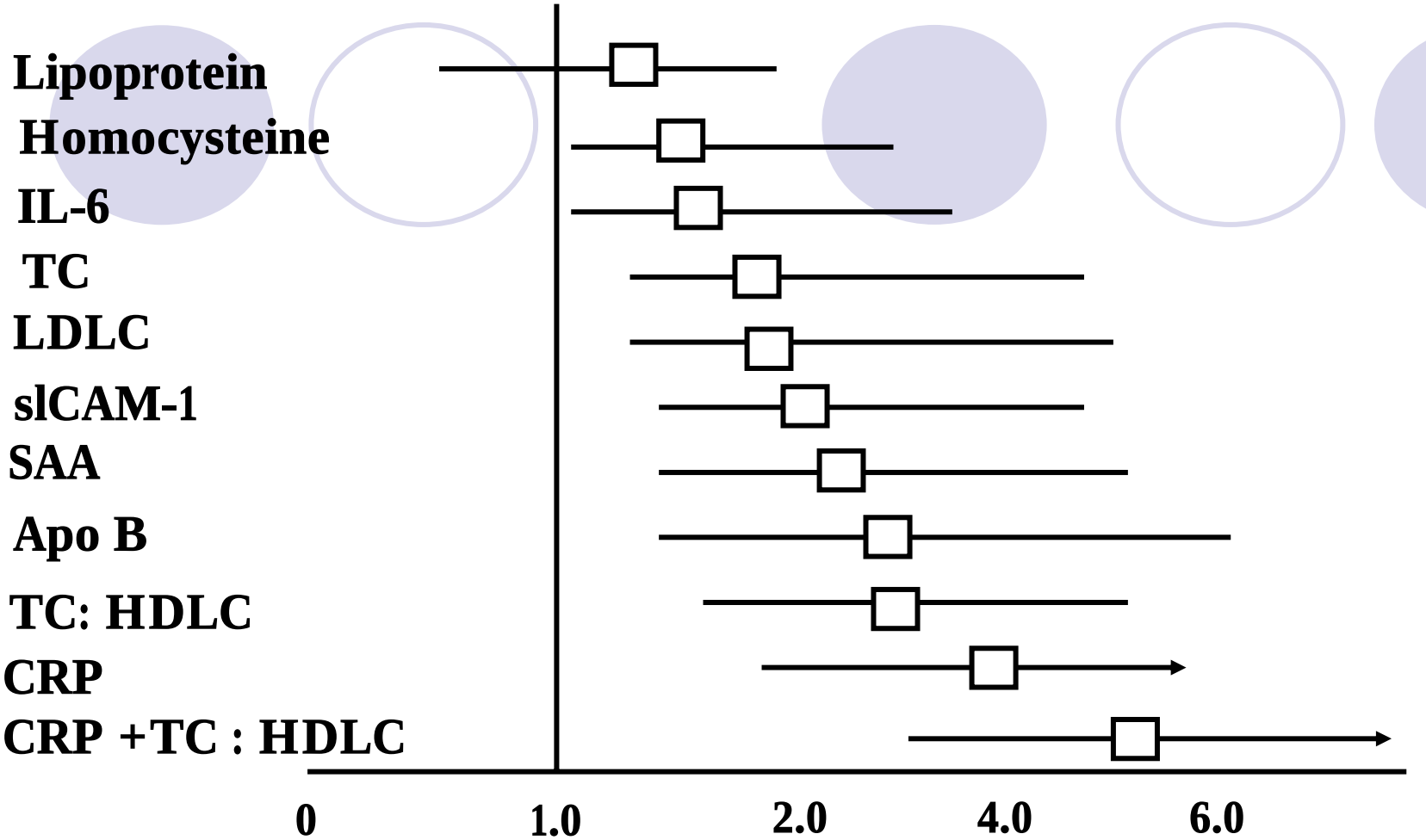
Inflammatory Markers for Consideration as Predictors of Cardiovascular Risk

- Adhesion molecule
- Cytokines
  - Acute-phase reactants
    - Fibrinogen
    - SAA
    - **CRP**
  - **Lp-PLA$_2$**
- WBC count
- Other (eg,erythrocyte sedimentation rate)

Assays of Inflammatory Markers for Potential Clinical Use

Analytic	Stability	Assay Availability	World Health Organization Standards Available?†	Interassay Precision
Soluble adhesion molecules (eg, E-selectin, P-selectin, intracellular adhesion molecule-1, vascular cell adhesion molecule-1)	Unstable	Limited	No	CV<15%
Cytokines (eg, interleukin-1β, -6, -8, and -10 and tumor necrosis factor-α)	Unstable	Few	Yes	CV<15%
Acute-phase reatants				
Fibrinogen	Unstable	Many	Yes CV	CV<8%
SAA	Stable	One	Yes	CV<9%
hs-CRP	Stable	Many	Yes	CV<10%
WBC count	Stable	Many	Yes	CV<3%

(Pearson TA et al. Circulation 107:499-511, 2003)



Comparison of relative risk of future cardiovascular events associated with lipid and inflammatory risk factors

(Blake GJ et al. J Intern Med 252:283-294, 2002)

CRP

Marker of inflammation
Predictor of atherosclerosis
Therapeutic target

The CDC/AHA recommendations for the use of CRP in the diagnosis and management of cardiovascular disease

- **high-sensitivity CRP assay**
  - at least **two measurements**, preferably **2 weeks apart**,
  - cut points for CRP according to approximate tertiles in the adult population
    - **low risk (<1.0 mg/l)**
    - **average risk (1.0–3.0 mg/l)**
    - **high risk (>3.0 mg/l)**

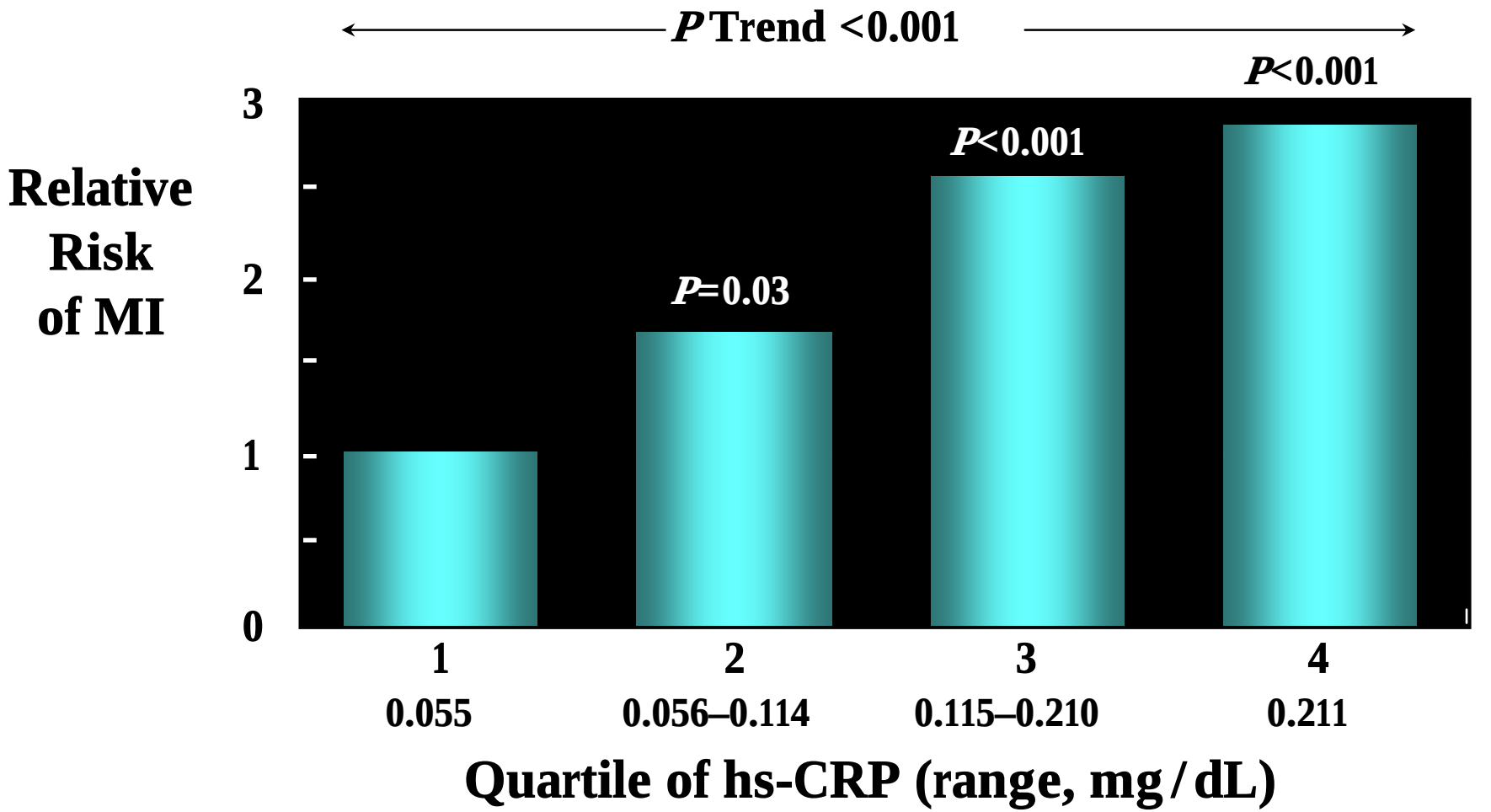
  - **CRP level >10 mg/l**

    presence of a significant acute-phase response

    e.g.) rheumatoid arthritis

- **CRP**
  - **non-specific biochemical marker of inflammation**
    - to assess disease activity in
      - inflammatory conditions ; **rheumatoid arthritis, ankylosing spondylitis**.
      - infections, including **bacterial endocarditis**
      - intercurrent infection in **systemic lupus erythematosus**
  - **stronger predictor of cardiovascular risk**
    - no apparent circadian variability
    - no evidence for seasonal variations
    - long half-life and concentrations appear to be fairly stable over long periods of time in most individuals

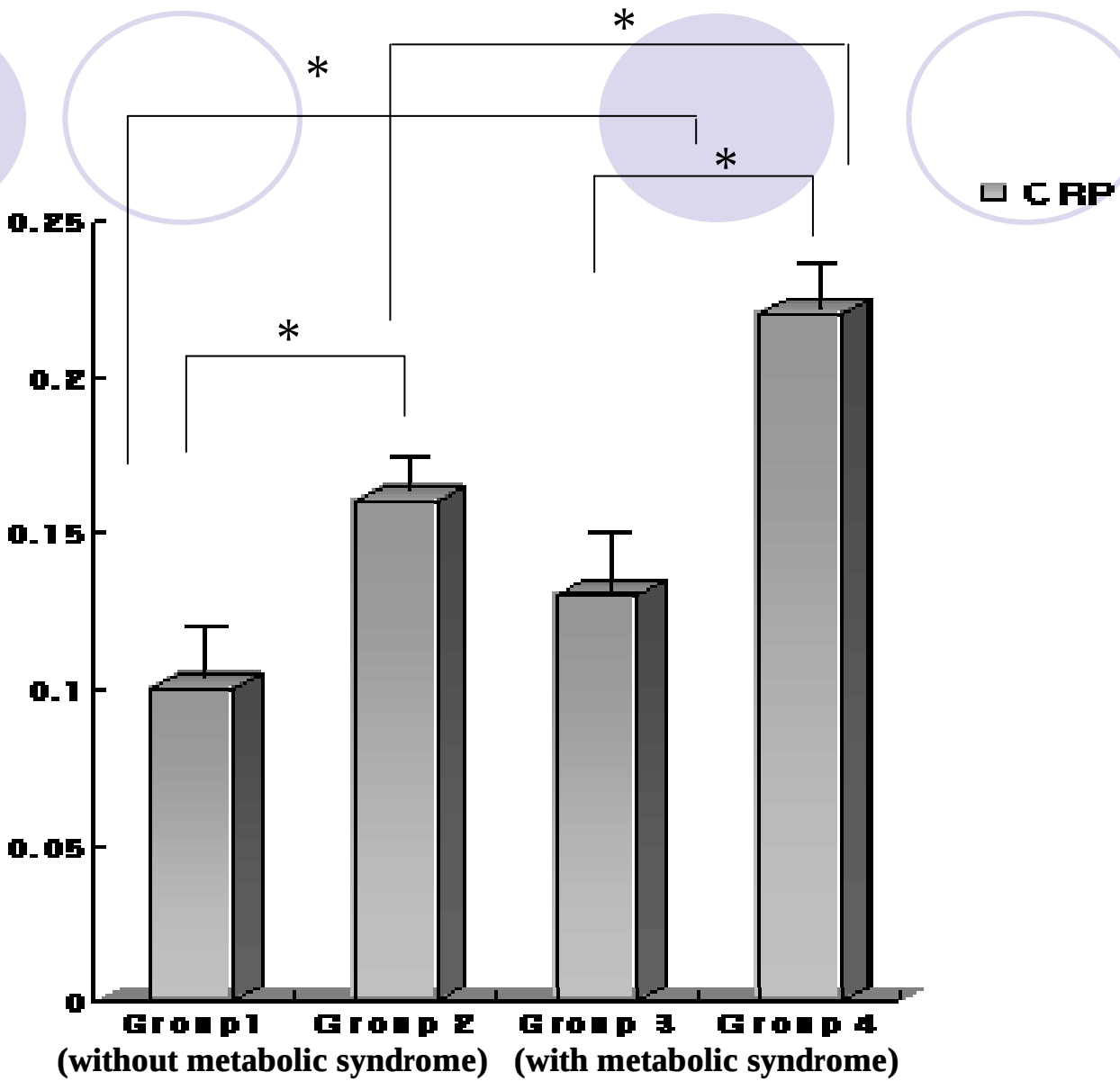
hs-CRP and Risk of Future MI in Apparently Healthy Men



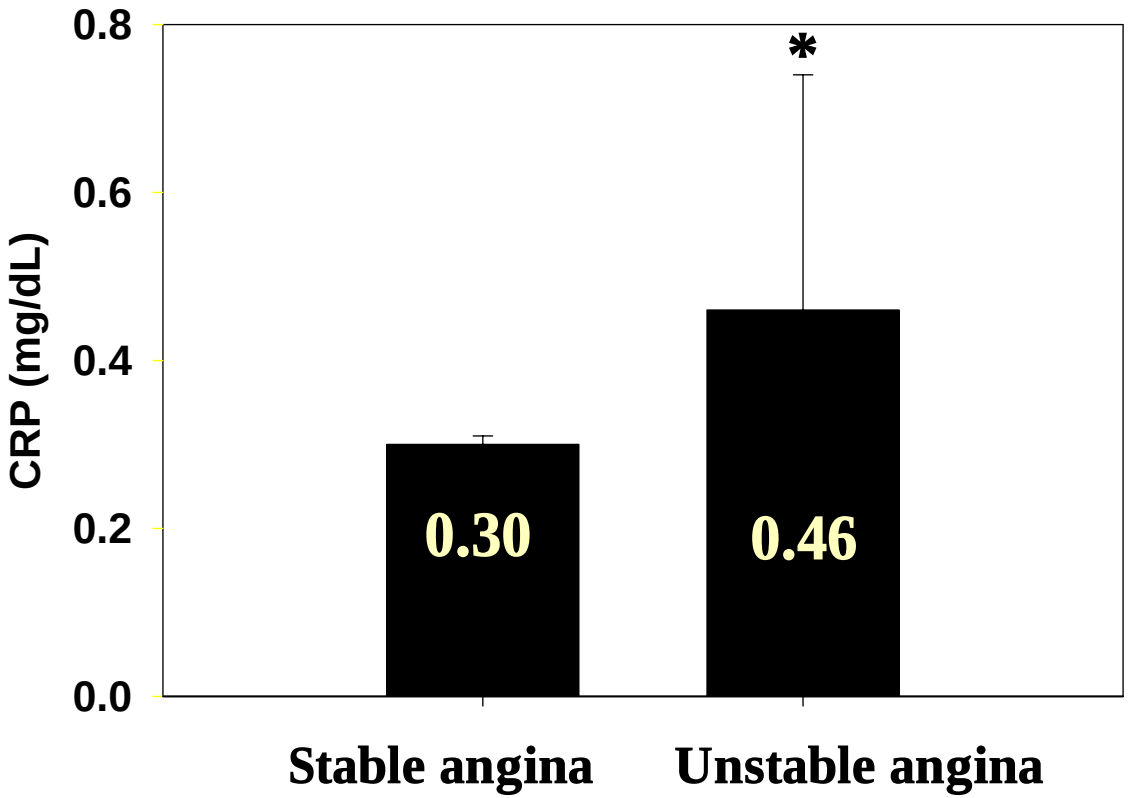
Ridker PM et al. N Engl J Med 1997;336:973-979.

Patient Characteristics and Conditions Associated With Increased or Decreased Levels of hs-CRP

Increased Levels	Decreased Levels
Elevated blood pressure	
Elevated body mass index	Moderate alcohol Consumption
Cigarette smoking	Increased activity/endurance Exercise
Metabolic syndrome/diabetes mellitus	Weight loss
Low HDL/high triglycerides	Medications
Estrogen/progestogen hormone use	Statins
Chronic infections (gingivitis, bronchitis)	Fibrates
Chronic inflammation (rheumatoid arthritis)	Niacin



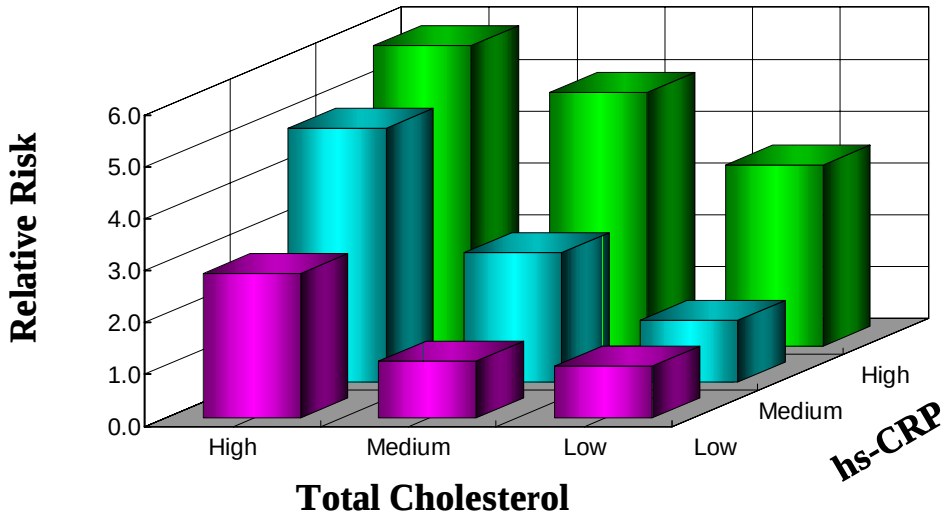
CRP level between each groups

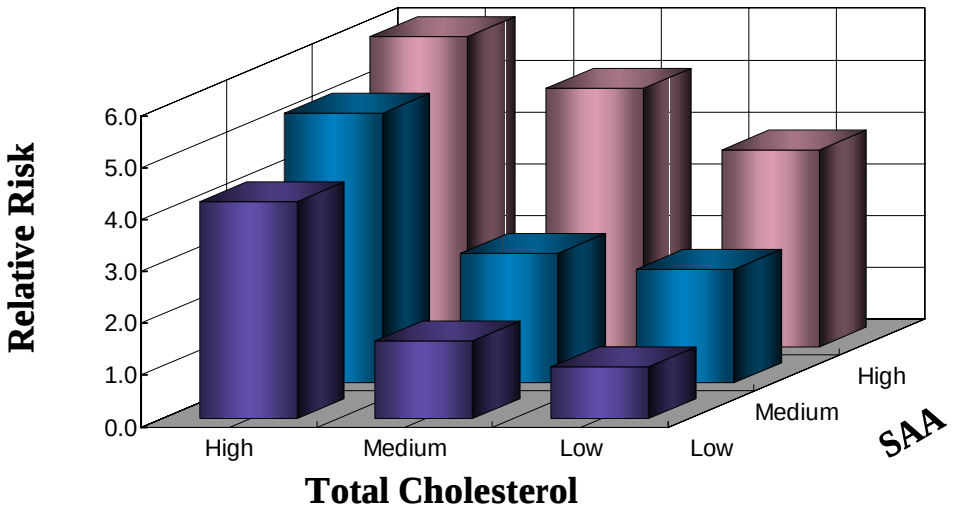


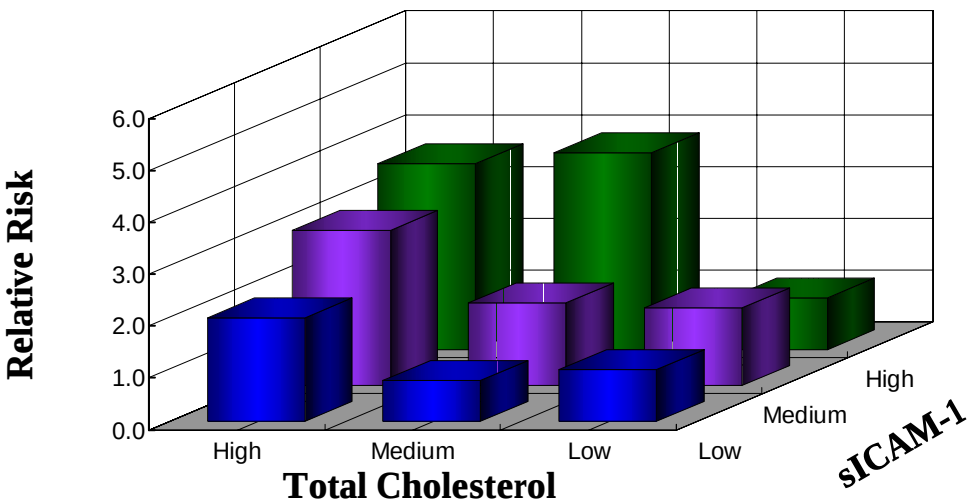
CRP levels in patients with different disease groups

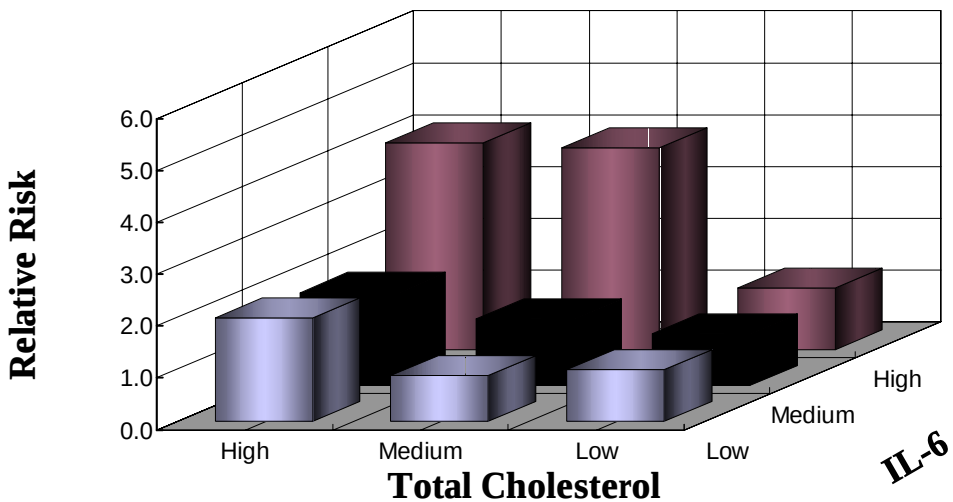
*** p<0.05 compared to stable angina.**

Markers of Inflammation in the Prediction of Cardiovascular Disease in Women









Ridker PM et al. N Engl J Med 2000;342:836-43.

- Analysis of the Cholesterol and Recurrent Events (CARE) study

  increased risk of recurrent events among patients with elevated CRP or serum amyloid A

- Thrombogenic Risk Factor (THROMBO) study
  - **CRP was a predictor of risk** in univariate analyses, but this association was not significant after adjustment for important predictors of prognosis, such as left ventricular ejection fraction and presence of pulmonary congestion

- Women's Health Study
  - measurement of CRP in addition to lipid levels may improve identification of individuals at risk for cardiovascular events
  - **CRP** may be an even stronger predictor than LDL-C level and may provide additional prognostic information to that conveyed by the Framingham risk score

Summary of findings from a prospective studies investigating C-reactive protein (CRP) as a marker of future cardiovascular risk among apparently healthy individuals

Study	End point	Relative risk (95% CI)
Ridker et al.	CVD	3.6(2.5-5.2)
Danesh et al.	CHD	2.13(1.38-3.28)
Roivainen et al.	CHD	3.56(1.93-6.57)
Tracy et al.	MI	2.67(1.04-6.81)
Ridker et al.	MI	2.9(1.8-4.6)
Albert et al.	SCD	2.78(1.35-5.72)
Ridker et al.	Stroke	1.9(1.1-3.3)
Rost et al.	Strode	1.9(1.1-3.3)
Lowe et al.	IHD	2.73(1.60-4.67)
Ridker et al.	PVD	2.2(1.1-4.8)

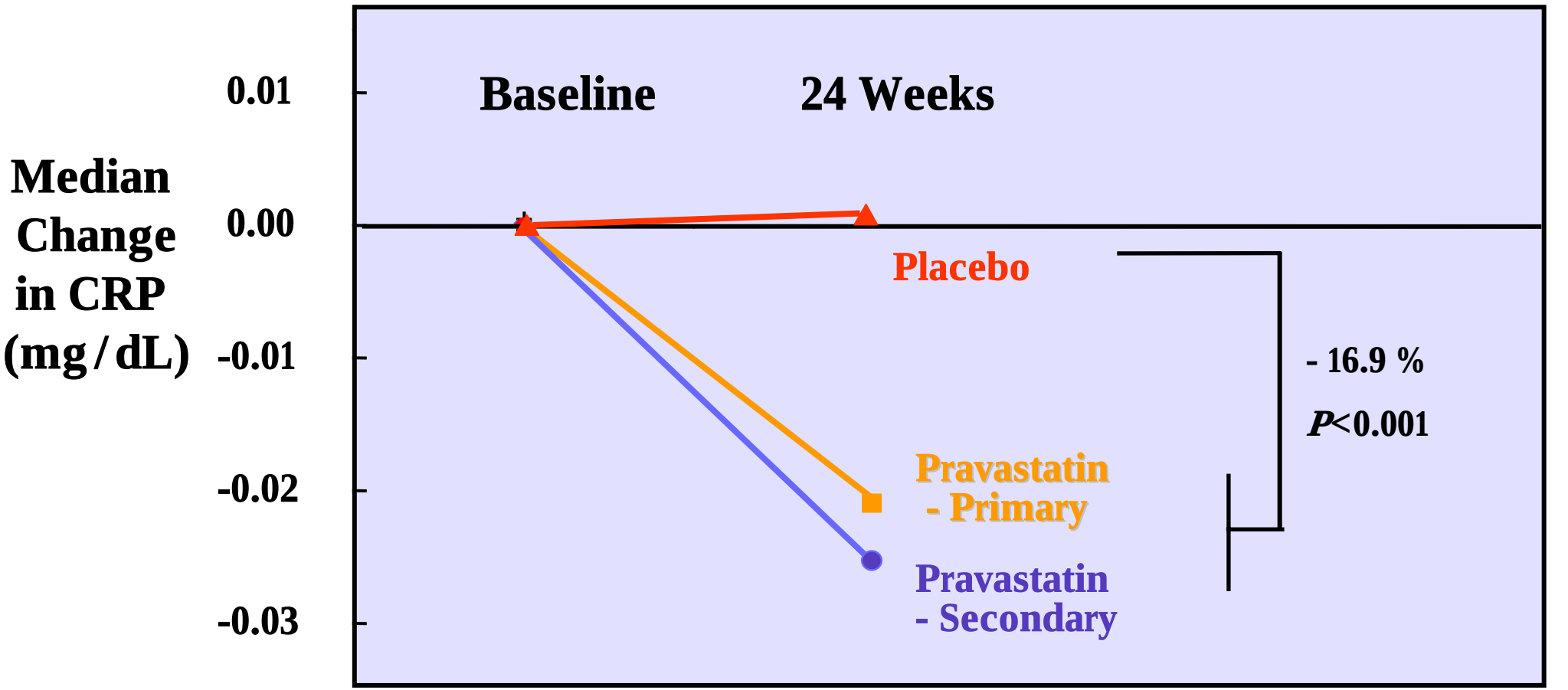
CRP: a therapeutic target for cardiovascular risk reduction
- HMG CoA Reductase Inhibition and CRP

- Do statins reduce hs-CRP?
- Is any effect of statin therapy on hs-CRP dependent or independent of statin induced effects on lipids ?
- Do potential anti-inflammatory effects of statins help to explain the utility of these agents in acute ischemia ?

Design of Key Statin Trials

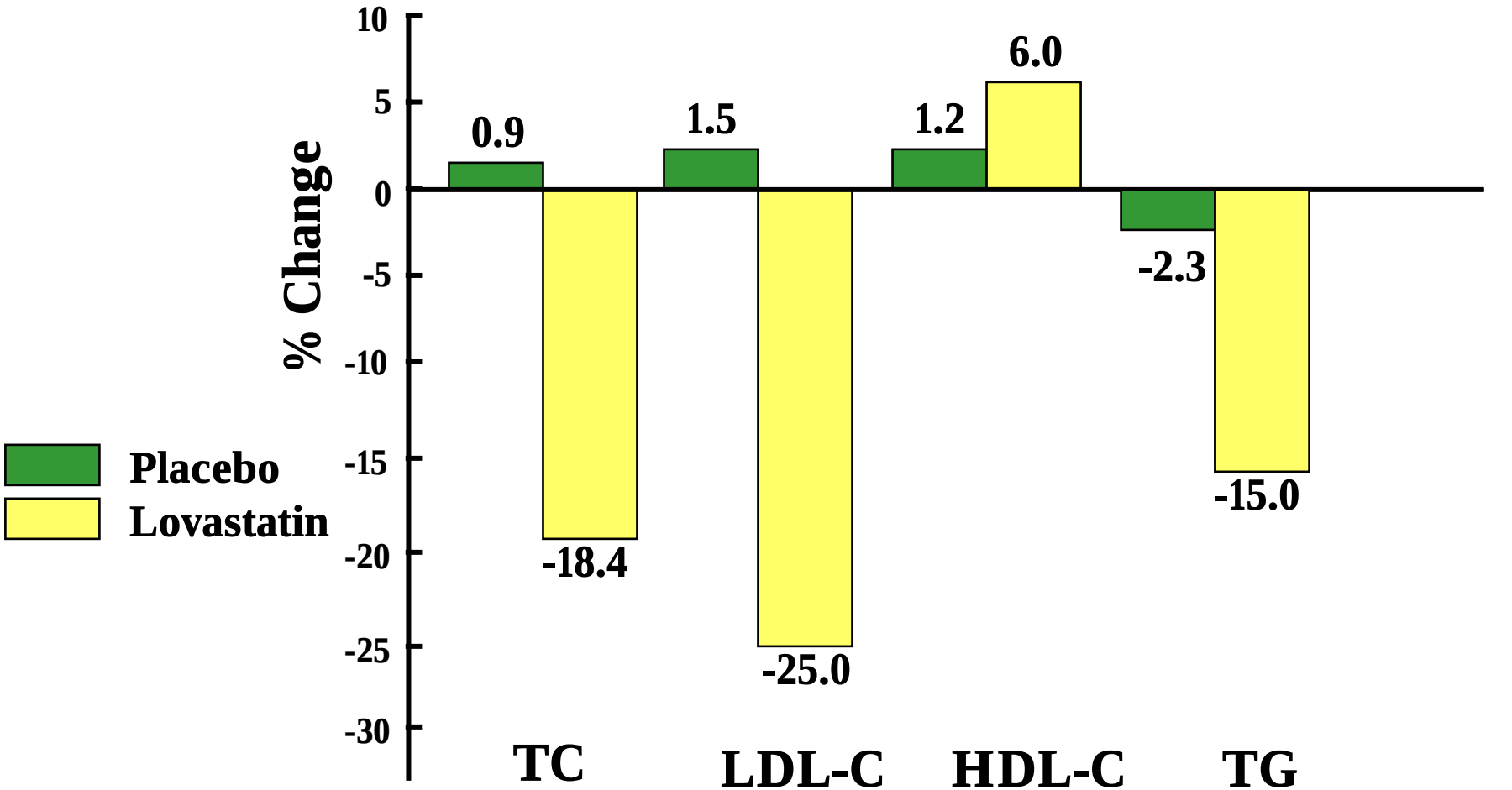
Study	Statin	Existing CHD	Patients	Cholesterol	Follow-up (years)
4S	simvastatin 20 mg od	Yes	4444 male and female, aged 35–70	Raised Mean LDL-C 4.87 mmol/L, 188 mg/dL	5.4
WOSCOPS	pravastatin 40 mg od	No MI, angina (5%)	6595 male, aged 45–64	Raised Mean LDL-C 4.97 mmol/L, 192 mg/dL	4.9
CARE	pravastatin 40 mg od	Yes	4159 male and female, aged 21–75	Average Mean LDL-C 3.59 mmol/L, 139 mg/dL	5.0
LIPID	pravastatin 40 mg od	Yes	9014 male and female, aged 31–75	Average Mean LDL-C 3.80 mmol/L, 147 mg/dL	6.1
AFCAPS/ TexCAPS	lovastatin 40 mg od	No	6605 male and female, aged 45–73	Average Mean LDL-C 3.89 mmol/L, 150 mg/dL	5.2
HPS	simvastatin 40 mg od	Yes	20536 male and female, aged 40–80	Low/average Mean LDL-C 3.4 mmol/L, 130 mg/dL	5.0
ASCOT-LLA	atorvastatin 10 mg od	In some patients	10305 male and female, aged 40–79	Low/average Mean LDL-C 3.4 mmol/L, 130 mg/dL	3.3

Pravastatin Inflammation CRP Evaluation (PRINCE): n=2,884



Albert, Danielson, Rifai, Ridker for the PRINCE Investigators. JAMA 2001;286:64-70.

AFCAPS/TexCAPS - Lipoprotein reduction after 1yr



Reduction in acute coronary events among subgroups in the Air Force / Texas Coronary Atherosclerosis Prevention Study grouped according to median levels of low-density lipoprotein cholesterol (LDL-C) (149 mg / dl) and C-reactive protein (CRP) (1.6 mg / l)

Subgroup	Rate of events		Relative risk	*p* value
	Lovastatin	Placebo	Reduction(%)	
LDL-C>median	0.029	0.053	47	0.001
LDL-C<median, CRP<median	0.025	0.022	-	0.74
LDL-C < median, CRP > median	0.029	0.051	42	0.04
LDL-C>median, CRP > median	0.038	0.055	32	NA

(Ridker et al. N Engl J Med 344:1959-1965, 2001)

Table 1. Baseline clinical characteristics and angiographic findings

	Patients (n=36)
Age, years	61±9
Male (%)	25(69)
Hypertension (%)	12(33)
Diabetes mellitus (%)	10(28)
Current smoking (%)	11(31)
Multivessel disease (%)	13(36)
Dilated coronary artery	
LAD/LCX/RCA	19(53)/7(19)/10(28)
Target lesion before angioplasty	
Reference diameter, mm	*3.1±0.5*
Minimal luminal diameter, mm	*0.7±0.4*
Diameter stenosis, %	*77±13*
Lesion length, mm	*15±6.4*
Target lesion after angioplasty	
Balloon PTCA/Stent (%)	*19(53)/17(47)*
Minimal luminal diameter, mm	*2.8±0.5*
Diameter stenosis, %	*6±8*

LAD:left anterior descending coronary artery, LCX:left circumflex artery, RCA:right coronary artery, PTCA:percutaneous transluminal coronary angiopalsty

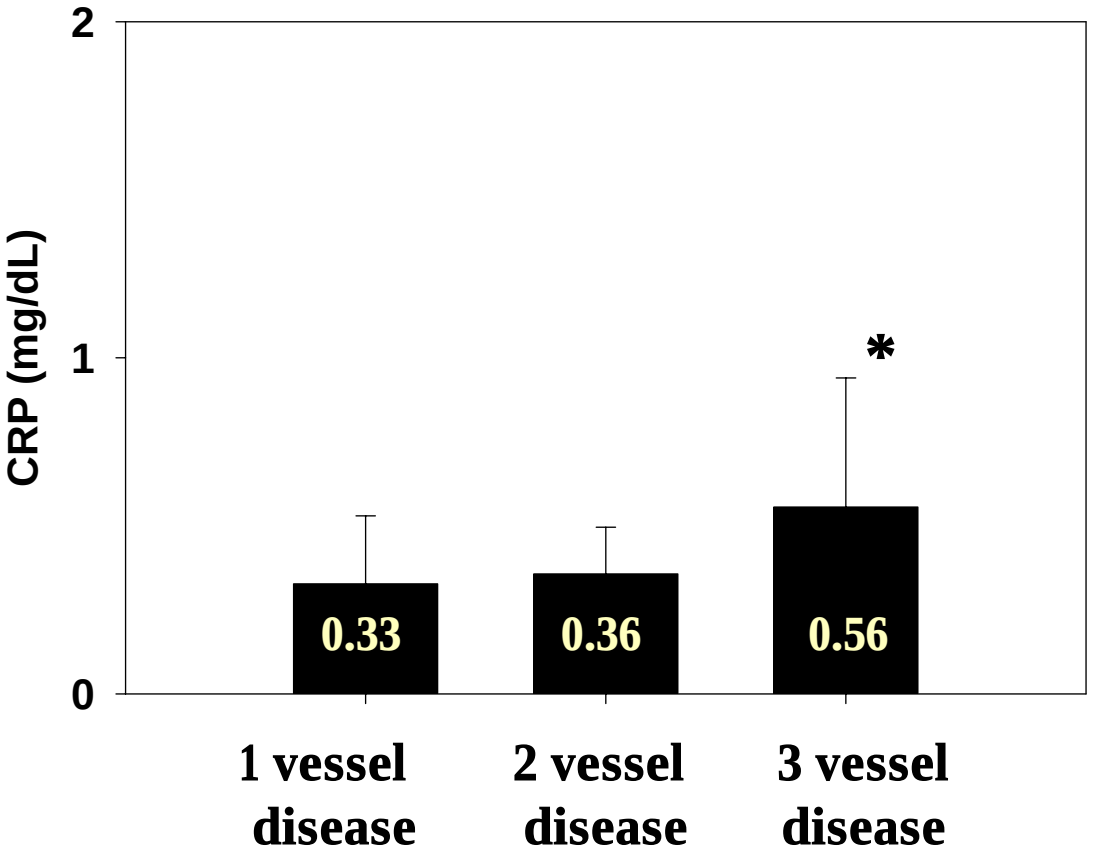


Fig. 1. CRP levels in patients with different number of stenosed coronary vessels. * p<0.05 compared to the 1 vessel disease

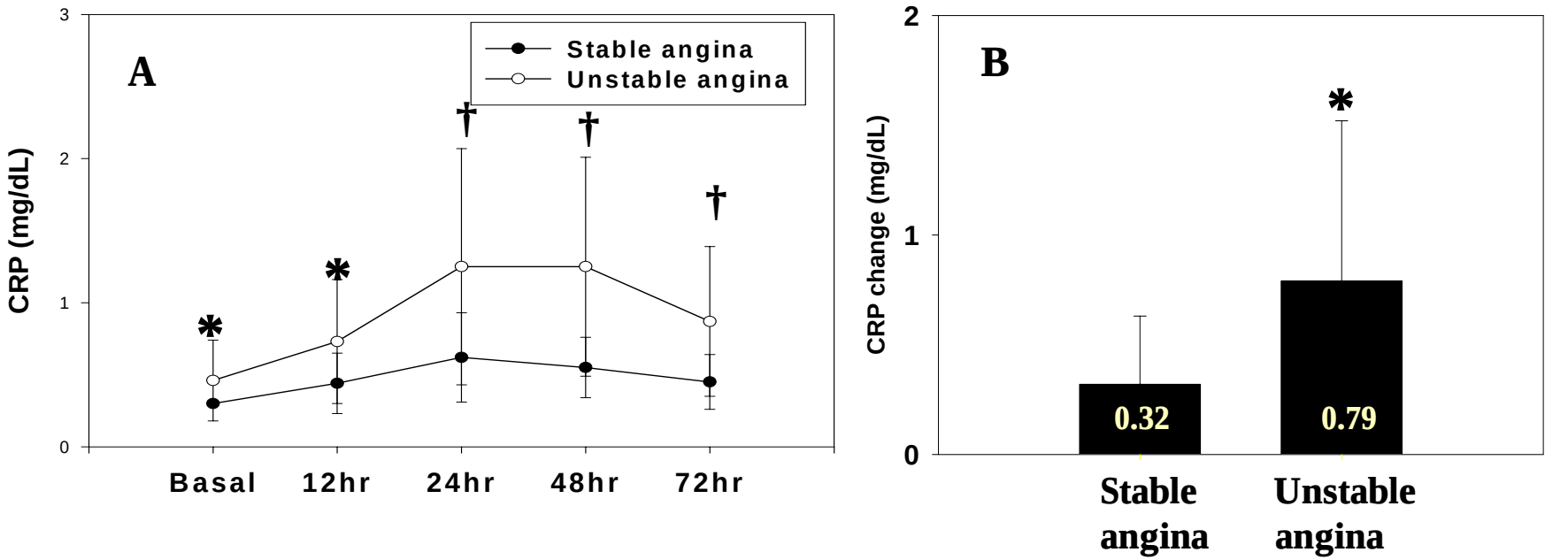


Fig. 4. Sequential change of CRP levels(A) and amount of change of CRP(B) after coronary intervention. CRP change = CRP_{24hr}- CRP_{basal}, *p<0.05, † p<0.01 compared to stable angina.

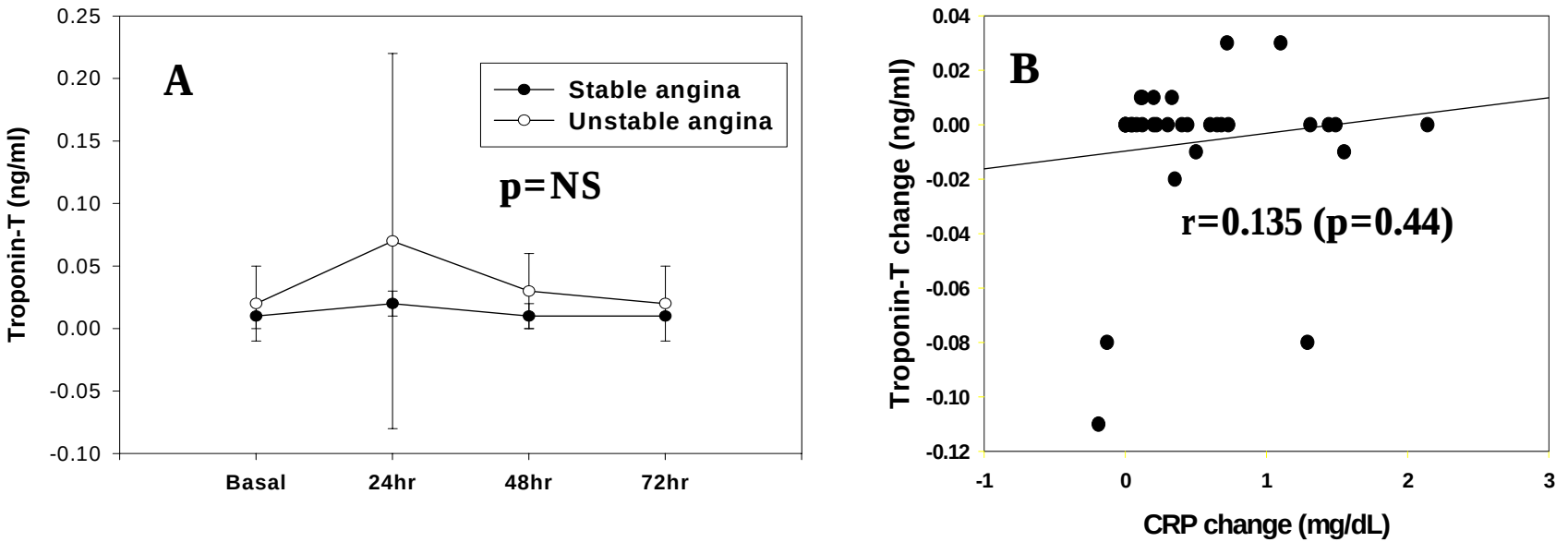


Fig. 5. Sequential change of troponin-T levels(A) and correlation of troponin-t change and CRP change 24 hour after coronary intervention (B). CRP change = CRP_{24hr}- CRP_{basal}, Trotonin-T change = $Troponin\text{-}T_{24hr}$- $Troponin\text{-}T_{basal}$.

Summary

- **Inflammatory processes play a pivotal role** in the initiation, progression and complications **of atherosclerosis**.
- A variety of **plasma markers** have been shown to **predict future cardiovascular risk**.
- Powerful evidence that **elevated CRP concentrations predict future cardiovascular events**.
- **CRP** adds **predictive value** to that afforded by standard lipid screening.
- **CRP** identify individuals without overtly elevated LDL-C **who are at high risk for future events**, and the **benefits of interventions**, such as statin therapy
- Large-scale prospective studies

  If proven, screening for low-grade inflammation using **CRP as a biomarker** may prove an **important tool** for **identifying** individuals at **increased risk who would benefit most from targeted preventive interventions**.

Lp-PLA_2

(Lipoprotein-associated phospholipase A_2)

- **Lipoprotein-associated phopholipase(Lp-PLA_2)**

  ① Member of phopholipase A_2 superfamily
  (hydrolyze phospholipid)

  ② Marker of inflammation(Critical role in atherogenesis)

- **Lp-PLA_2 inhibition → antiatherogenic effects**

- **Major role for inflammation in pathophysiology of cardiovascular event(vulnerable plaque)**

  → LP-PLA_2 level in plasma is **an independent predictor of coronary events**

Biology

- **Two circulating PLA_2**

  ① Lp-PLA_2

  : platelet activating factor acetylhydrolase(PAF-AH)

  50 kD Ca2+ independent phospholipase

  ② Secretary PLA_2

  : 14 kD Ca2+ dependent enzyme

  may destabilize LDL particles & enhances retention

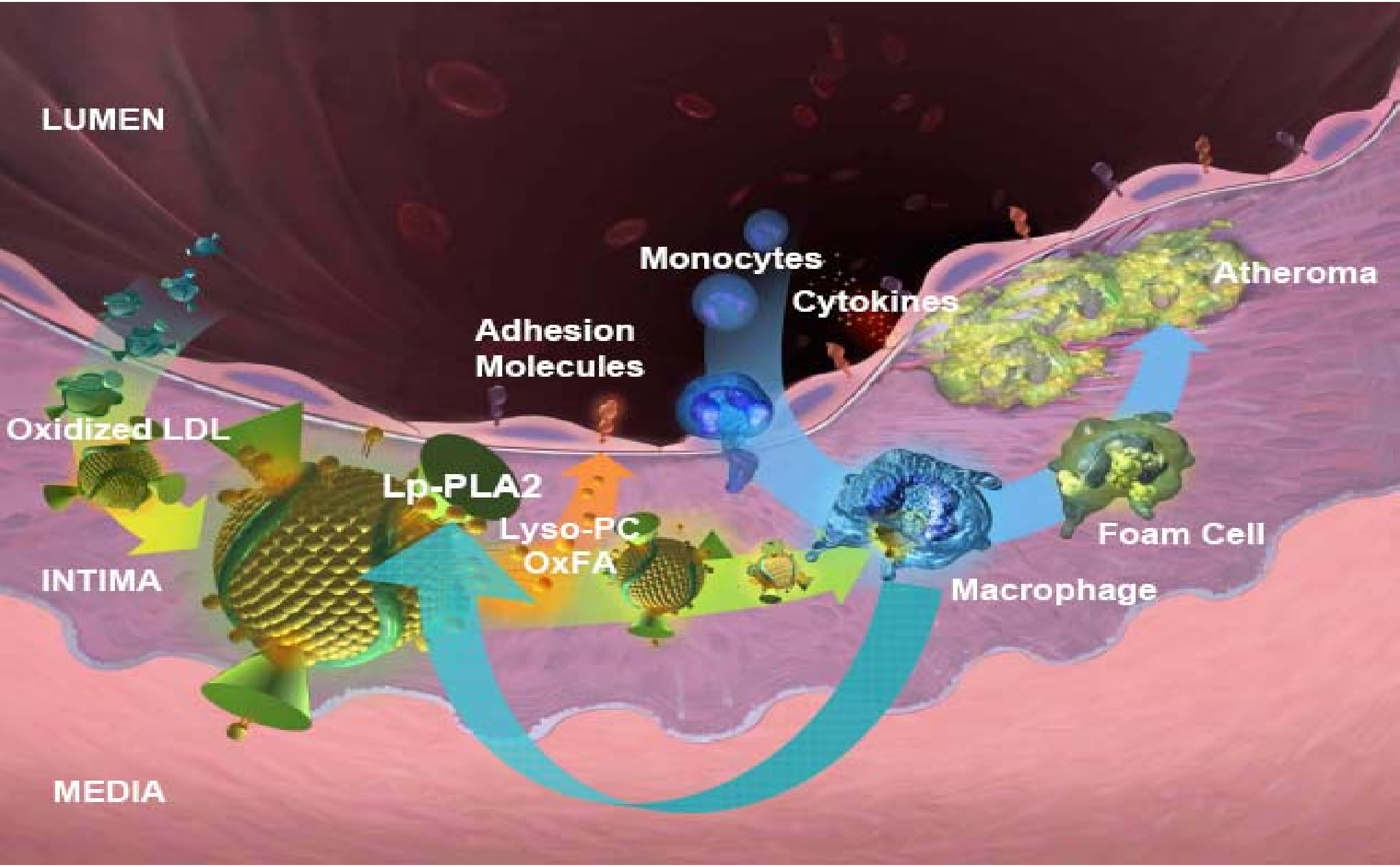
  lipoprotein

- **Lp-PLA_2 is predominantly pro-atherogenic or anti-atherogenic is controversial.**

Biology

- **OxLDL--$_{\text{Lp-PLA2(hydrolysis)}}$**
  → proinflammatory and atherogenic by-product
  1) **lysophosphatidylcholine(LysoPC) &**
  2) oxidized fatty acids(OxFA)

- **LysoPC : critical role of atherogenesis**
  ① chemoattractant for monocyte
  ② impairs endothelial function
  ③ cause cell death by disrupting plasma membrane
  ④ induces apoptosis in smooth muscle cells and macrophages


LUMEN
Monocytes
Cytokines
Atheroma
Adhesion
Molecules
Oxidized LDL
Lp-PLA2
Lyso-PC
OxFA
Foam Cell
INTIMA
Macrophage
MEDIA

Modulation of Lp-PLA_2

- **Inhibition of Lp-PLA_2**

  ① Antiatherogenic effects

  ② Abolishing OxLDL-induced chemoattraction for monocytes and attenuating its ability to induce the apoptotic death of monocytes

- **Agent with ability to specifically inhibit the enzymatic activity of Lp-PLA_2**

  → agents for modification of Lp-PLA_2 and treatment of atherosclerosis

- **In clinical study, statins and fibrates, both agents decrease cardiovascular events and reduce plasma Lp-PLA_2 level**

Medical genetics of Lp-PLA_2

- **Gene for Lp-PLA2(PLA2G7) has 12 exons & located on chromosome 6p21.2 to 12**

- **Some variant noted mainly in certain ethnic group**

  ① Val279Phe variant is common in Japanese, Turks-reduced levels of Lp-PLA2 but higher prevalence of cardiovascular disease

  ② Ala279Phe variant in whites, results in reduced affinity of Lp-PLA2 for exogenous PAF

Epidemiological and clinical studies showing an association between Lp-PLA$_2$ (mass or activity) and cardiovascular events or other endpoints

Author	Population	Endpoints (yrs)	F-up (yrs)	Cases	Controls	Risk
Packard	Hypercholesterolemic men					RR/ISD
Brilakis	CAD	Cardiac events	5	580	1160	1.18
Iribarren	Young adults	Cardiac events	4	382	122	1.27
Koenig	Healthy men	CAC	-	272	862	1.25
Caslake	CAD, MI	Coronary events	14	97	837	1.21
		CAD	-	94	54	-
						HR
Ballantyne	Healthy M/F	Coronary events	6	608	740	1.15
				(LDL<130)		2.08
Ballantyne	Healthy M/F	Stroke	6	223	766	1.97
Blankenberg	CAD	Presence of CAD	-	496	276	1.8
Khusiyenova	CAD	Presence of CAD	-	312	476	1.84
Winkler	Type 2 diabetes	CAD	-	23	66	2.09
Oei	Elderly	Cor event/stroke	7/6	308/110	1822	1.96/1.95
Blake	Healthy women	CV events	3	123	123	1.17*

RR/1SD= Risk ratio/1 Standard Deviation; HR= Hazard ratio from comparison of highest to lowest tertile, quartile or quintile. *indicates a non-significant association. F-up= follow-up.

Lp-PLA_2 as a marker of cardiovascular risk in clinical study

TABLE 2. Plasma Levels of Lp-PLA_2 and the Risk of Cardiovascular Events in Primary Prevention Population[69–74]

Study	Design	Cases/Noncases	End Point	Follow-Up, y	Lp-PLA_2 Assay	Lp-PLA_2 Cases vs Noncases	Adjusted HR (95% CI)
WOSCOPS[69]	Nested case-control	580/1160	CHD death, MI, revascularization	5	Mass	Higher	1.18 (1.05–1.33; P=0.005)*
WHS[70]	Nested case-control	123/123	CHD death, MI, stroke	3	Mass	Higher	1.17 (0.45–3.05; NS)†
ARIC[71,72]	Case cohort	608/740	CHD death, MI, revascularization	6	Mass	Higher	1.15 (0.81–1.63; NS)‡ 2.08 (1.20–3.62; P<0.05)¶‡
		194/766	Ischemic stroke	6	Mass	Higher	1.93 (1.14–3.27; P=0.015)‡
MONICA[73]	Cohort	97/837	CHD death, MI	14	Mass	Higher	1.21 (1.01–1.45; P=0.04)*
Rotterdam[74]	Case cohort	308/1822	CHD death, MI	7	Activity	Higher	1.96 (1.25–3.09; P=0.02)†
		110/1822	Ischemic stroke	6	Activity	Higher	1.95 (1.02–3.73; P=0.04)†

ARIC indicates the Atherosclerosis Risk In Communities study; CHD, coronary heart disease; CRP, C-reactive protein; HR, hazard ratio adjusted for age, smoking, diabetes mellitus, gender, systolic blood pressure, LDL cholesterol (or total cholesterol/HDL or non-HDL cholesterol), high-sensitivity CRP and other variables; MI, myocardial infarction; MONICA, the MONItoring of trends and determinants in CArdiovascular disease in men in Augsburg survey; WHS, the Women's Health Study; WOSCOPS, the West Of Scotland Coronary Prevention Study.

*Increase of 1 SD.

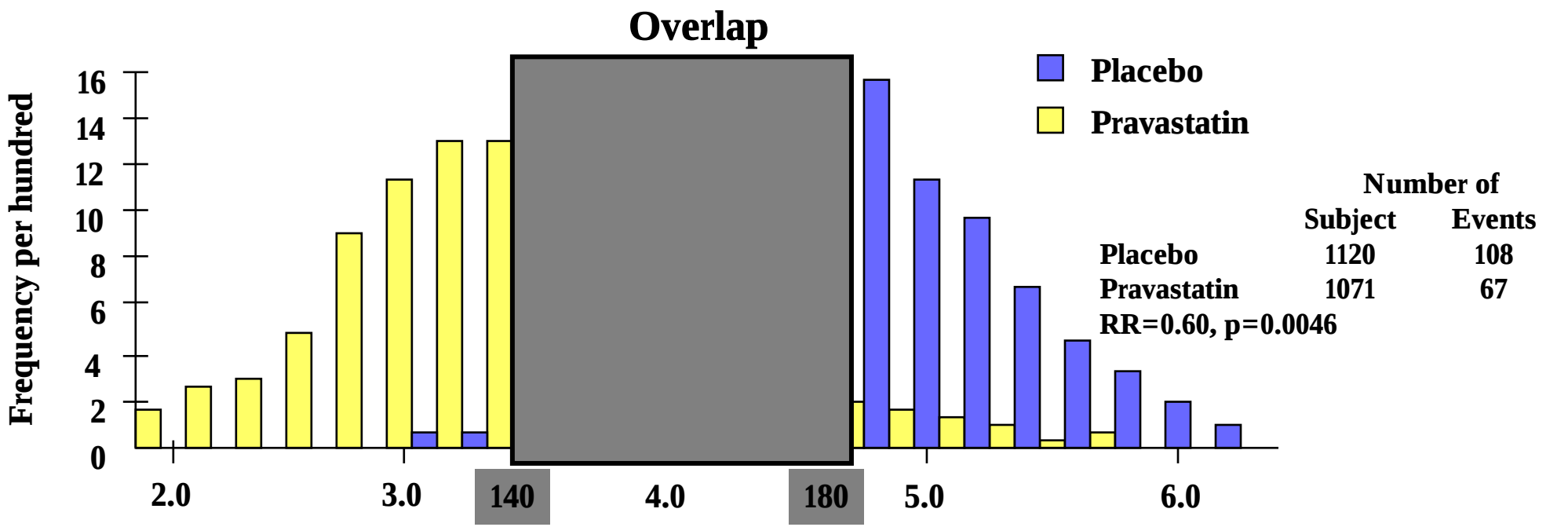
†With the lowest quartile as the reference.

‡With the lowest tertile as the reference.

¶In population with baseline LDL<130 mg/dL.

The West of Scotland Coronary Prevention Study (WOSCOPS) - LDL decrease and risk reduction

- Primary prevention tiral designed to evaluate the use of pravastatin in 6,595 hypercholesterolemic men
- 580-cardiac event / 1160-non case
- Inflammatory marker : Lp-PLA_2, CRP, white-cell count, fibrinogen
- **Lp-PLA_2 is novel risk factor that predicts risk of inflammation and were not affected by smoking**

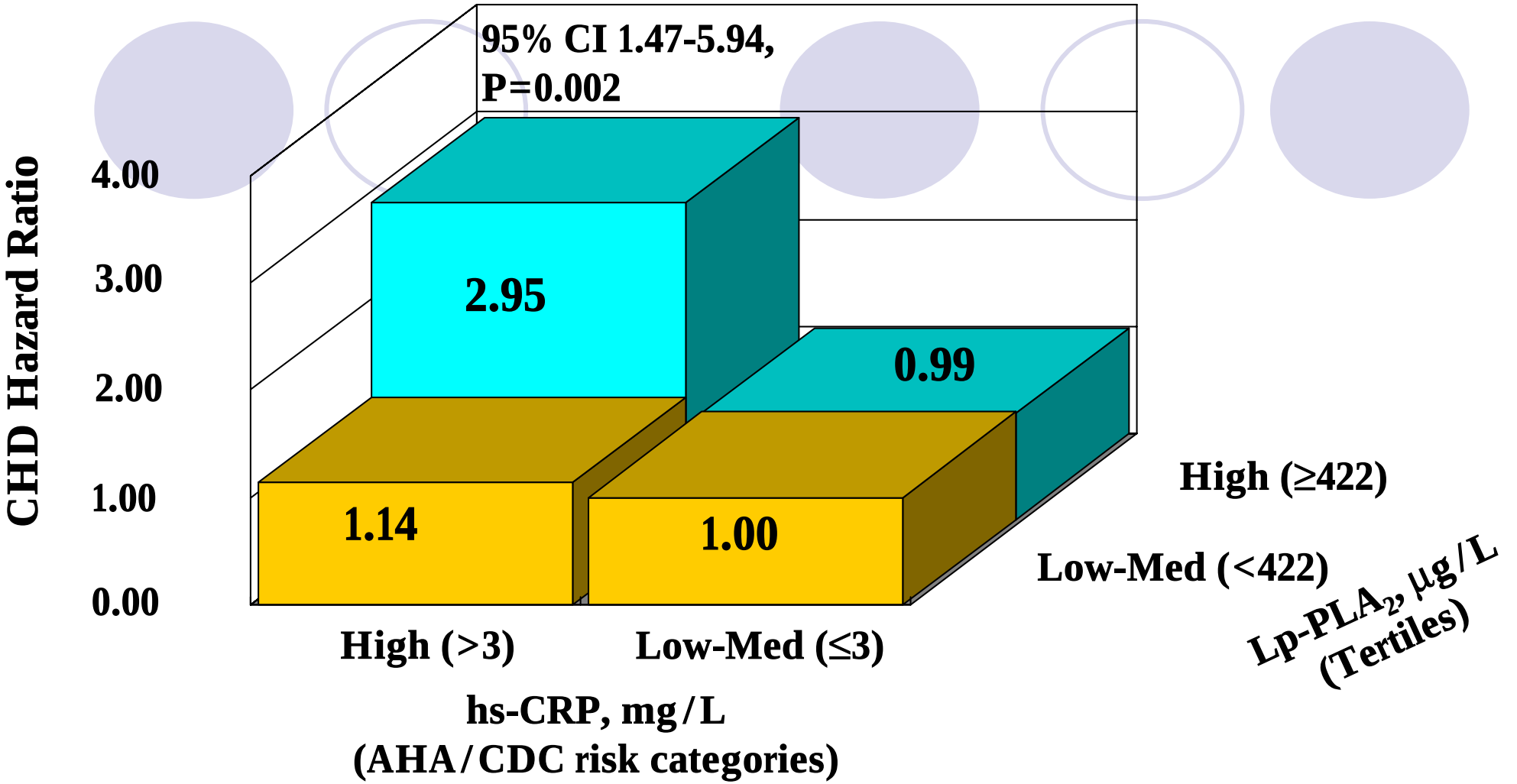


(New Engl J Med 2000;343:1148-1155)

The Atherosclerosis Risk In Communities(ARIC) study

- Support hypothesis that **Lp-PLA$_2$ is independently associated with CHD**
- Prospective , case-cohort study designed to evaluated atherosclerosis over a period of 6 years in 12,819 healthy middle-aged men and women
- 608 patients-coronary event / 740 non
- Mean levels of Lp-PLA$_2$ and CRP were higher in patients who had experienced a CHD
- In patients with LDL levels below median(<130mg / dL), **both Lp-PLA$_2$ and CRP were associated with CHD**
- Both suggesting inflammatory marker & complementary in identifying high-risk individuals

(Circulation. 2004;109:837-842)



Association of Lp-PLA$_2$ and hs-CRP with incident CHD in patients with low LDL-C (<130 mg/dL). CHD risk in individuals with elevations in both Lp-PLA$_2$ (highest tertile) and hs-CRP (high-risk category as defined in the AHA/CDC guidelines[5]) was 3 times greater than in individuals with low to medium levels (first and second tertiles) of Lp-PLA$_2$ and hs-CRP.

(Circulation. 2004;109:837-842)

The monitoring trends and determinants in cardiovascular disease (MONICA) -Augsburg Cohort study

- Relationship between Lp-PLA_2 levels and risk of coronary events was evaluated in 934 healthy men who were followed for 14 years(1984-1998)
- 97 men suffered a coronary event and mean baseline levels of LP-PLA_2 were significantly higher(295ug/L vs 263ug/L)
- 1 SD increase in Lp-PLA_2 mass was associated with a 37% increase in risk of coronary events
- **Combination of high Lp-PLA_2(290.8ug/L) and high CRP(>3mg/L) was associated with increased risk for future coronary event**

(Circulation 2004;110:1903-1908)

Correlation between Lp-PLA$_2$, CRP and other cardiovascular risk factors in the MONICA/KORA Cohort study 1984-1998 (n=934)

	Pearson Correlation Coefficient(*p*)	
Risk Factor	**Lp-PLA$_2$**	**CRP**
Age	0.12(0.0001)	0.15(<0.0001)
TC	0.30(<0.0001)	0.07(0.03)
HDL-C	0.09(0.005)	-0.12(0.00002)
TC/HDL-C	0.07(0.04)	0.13(<0.0001)
Systolic blood pressure	0.02(0.50)	0.11(0.00007)
BMI	-0.06(0.05)	0.21(<0.00001)
CRP	0.06(0.06)	1.00

(Koenig W et al. Circulation 1903-1908,2004)

The Rotterdam study

- Population-based follow-up study in 7983 subjects aged 55 years and over.
- Case-cohort study including 308 coronary heart disease cases and random sample of 1822 subjects.
- **Evidence for an association between Lp-PLA_2 and coronary heart disease independent of other risk factors**

(Circulation, 2004, in press)

Lp-PLA_2 activity in ACS and stable angina

- Increase in both men and women with acute coronary syndrome and stable angina compared with control
- **Lp-PLA_2 levels were diminished in patients with being treated with ACE-inhibitor therapy**

- **No** correlation was found between Lp-PLA_2 activity and levels of common markers of inflammation

(J Lipid Res. 2003;44:1381-1387)

Lp-PLA_2 as a risk predictor for stroke

- CRP has been reported to identify individuals at increased risk for stroke
- ARIC study relation between Lp-PLA_2, CRP, traditional risk factor and stroke over 6 years was examined
- **Mean LP-PLA_2 and CRP levels were higher in the 223 cases(422ug/L and 3.75mg/L)** than 766 noncases(372ug/L and 3.04mg/L)

(Stroke, 2003;34:623-631)

Lp-PLA_2 in diabetic subjects

- Data on Lp-PLA_2 in subjects with diabetes is limited

- Type 2 diabetes patients, increasing Lp-PLA_2 activity was significantly associated with a positive history of CAD
- 8wks of therapy with fluvastatin decreased Lp-PLA_2 activity by about 23%

- **Lp-PLA_2 measurement may be useful in predicting CAD in diabetic subjects**, further studies are needed to confirm this association

(J Clin Endocrinol Metab. 2004;89:1153-1159)

Summary

- **Inflammatory processes play a pivotal role** in the initiation, progression and complications **of atherosclerosis**.
- A variety of **plasma markers** have been shown to **predict future cardiovascular risk**.
- **Elevated Lp-PLA$_2$ predict future cardiovascular events independent of CRP**.
- **CRP and Lp-PLA$_2$ may be addictive in their ability to predict risk of coronary heart disease.**

감사합니다