ASSESSMENT OF TOBACCO SMOKE-MEDIATED ATHEROSCLEROSIS IN MOUSE MODEL

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1. **Models of human atherosclerosis**
2. **Description of model used in our studies**
3. **End points**
4. **Measurement of atherosclerotic lesion**
5. **Smoke studies**
6. **Strengths and Weaknesses of the model**
7. **Information needed for reduced risk assessment**

ANIMAL MODELS OF ATHEROSCLEROSIS

Two types-Diet-induced

-Genetically manipulated

Lipid or lipoprotein disorders

Develop Hyperlipidemia

Current Usage--genetically manipulated mice

Most extensively used models:

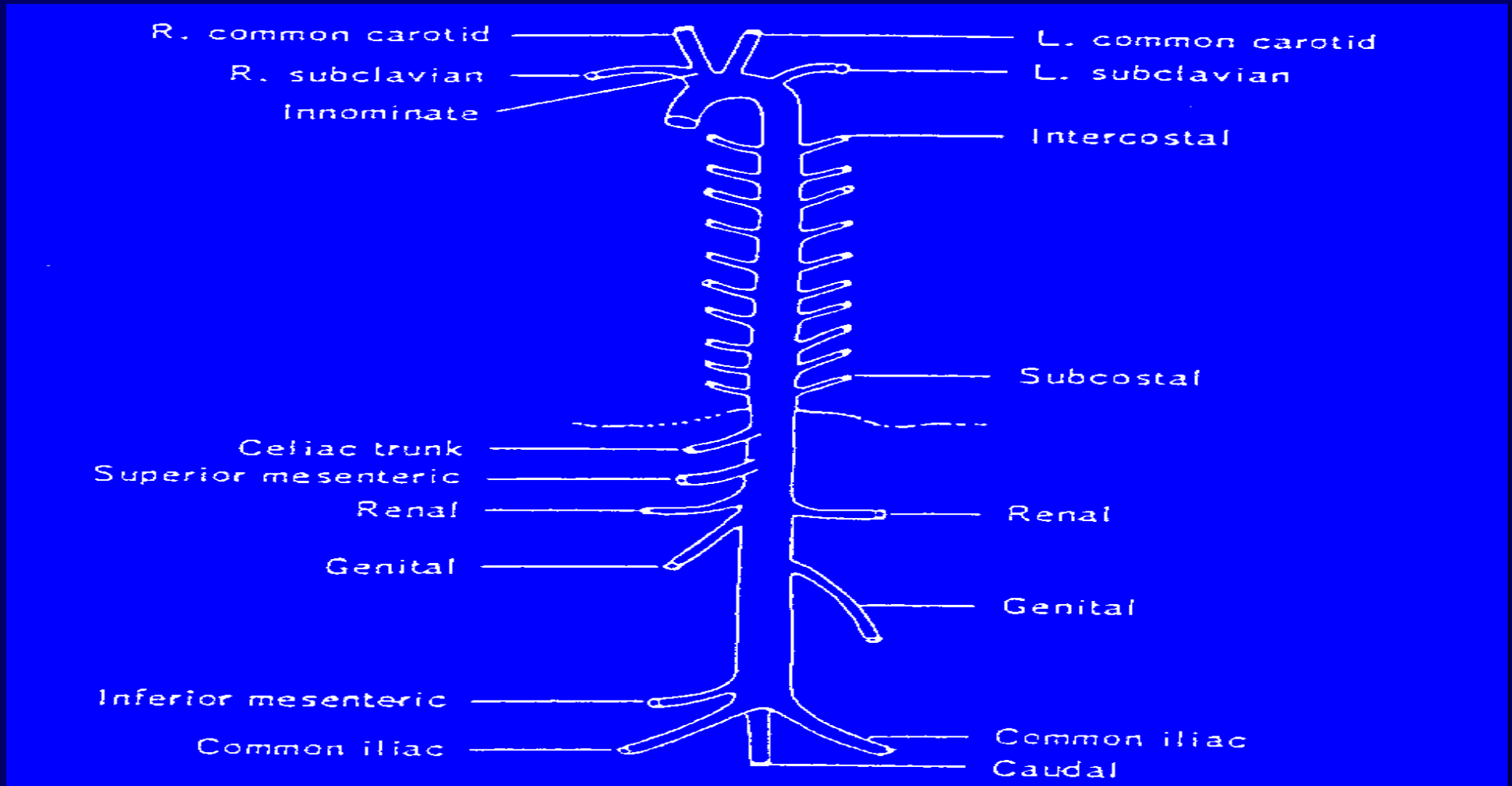
apolipoprotein E or LDL-receptor- deficient mice

DIETS

- Regular mouse chow
- High saturated fat/cholesterol diet

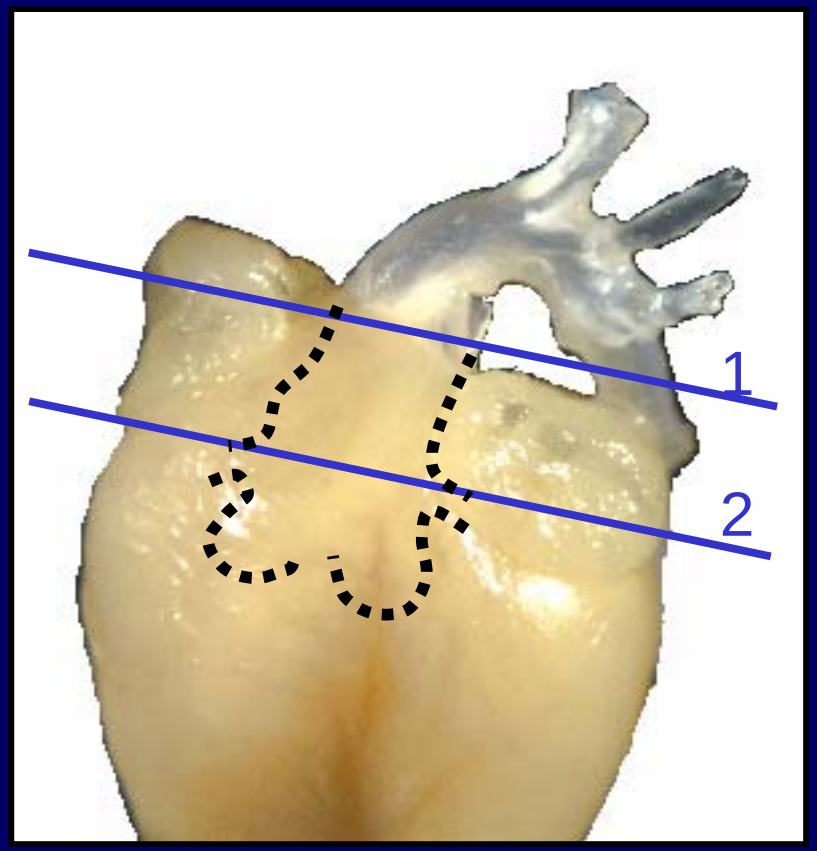
  21% fat, 0.5-1.5% cholesterol

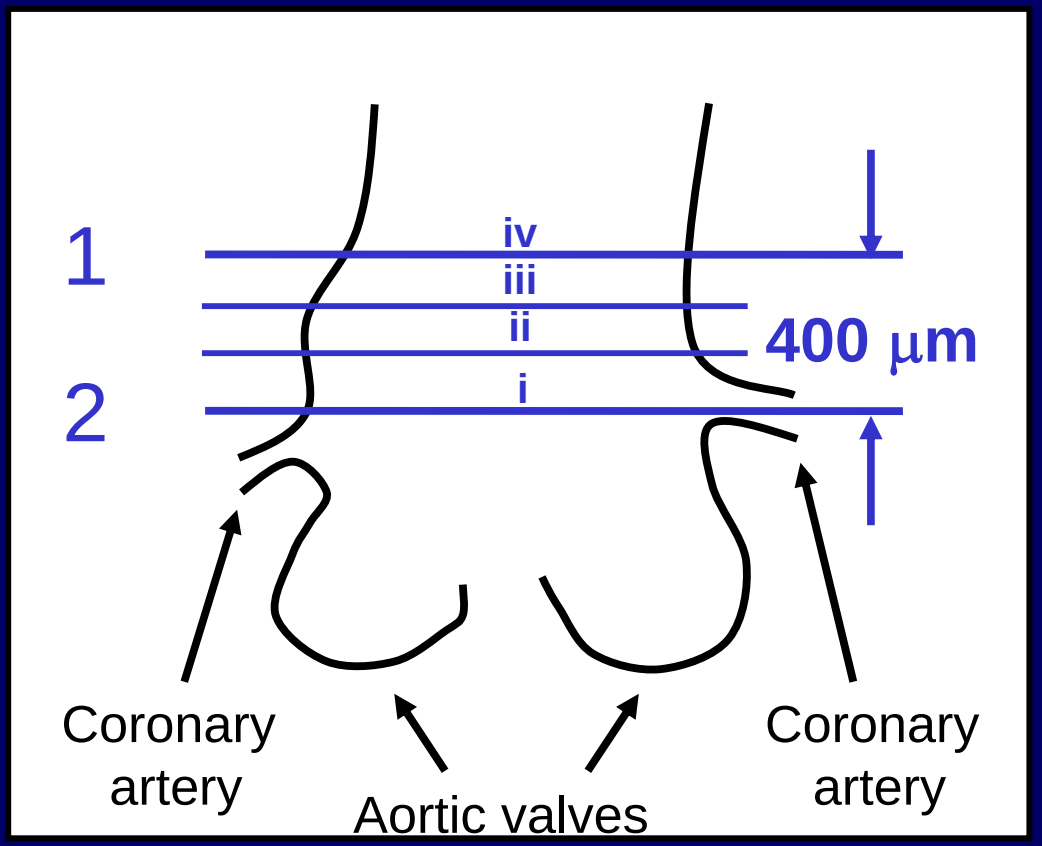
MOUSE AORTA



Drawing of the main arteries branching from the aorta.

Lesion Size Determination: Aortic Root

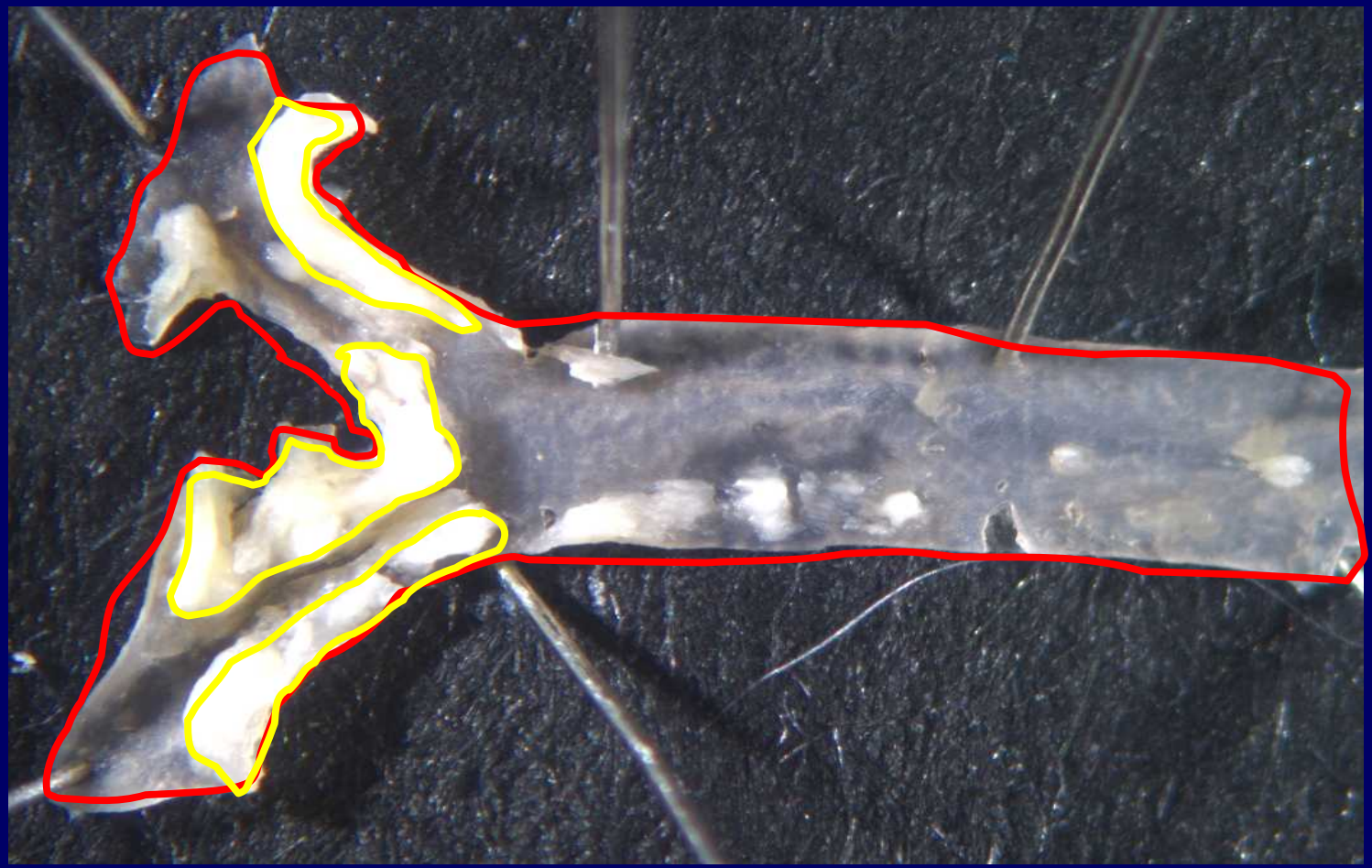




INTIMAL LESION AREA MEASUREMENT

- Aorta cleaned, opened and pinned
- Lesions traced under dissecting microscope
- Images captured with a digital camera for analysis by appropriate programs
- Planar measurements of the area

Quantification of Atherosclerotic Lesions En Face



ANIMAL MODEL USED IN OUR STUDIES

C57Bl-apolipoprotein E-knock out mice

LDLreceptor-deficient mice

Commercially available from

Jackson Laboratories, Maine

CHARACTERSTICS

- ApoE-deficiency induces hypercholestrolemia because the LDL/VLDL are not cleared.
- Animals develop atherosclerotic lesions on the intimal surface of the aorta which are formed all through the aortic tree.
- Time course dependent on diet.

END POINTS

- **lesion area**
- **plasma cholesterol**
- **aortic tissue cholesterol**
- **aortic constriction and relaxation ?**

SMOKE STUDIES

EXPERIMENTAL

- ApoE-deficient mice -- Two Groups-*Sham controls (*SH*) and Smoke-exposed (*SM*)*
- Exposures are in a whole-body exposure chamber to sidestream smoke from 1R4F cigarettes.
- Exposures are for 4 hrs/day, 5 days /week.
- Animals were sacrificed and plasma lipids and aorta examined.

University of Kentucky Research Cigarettes

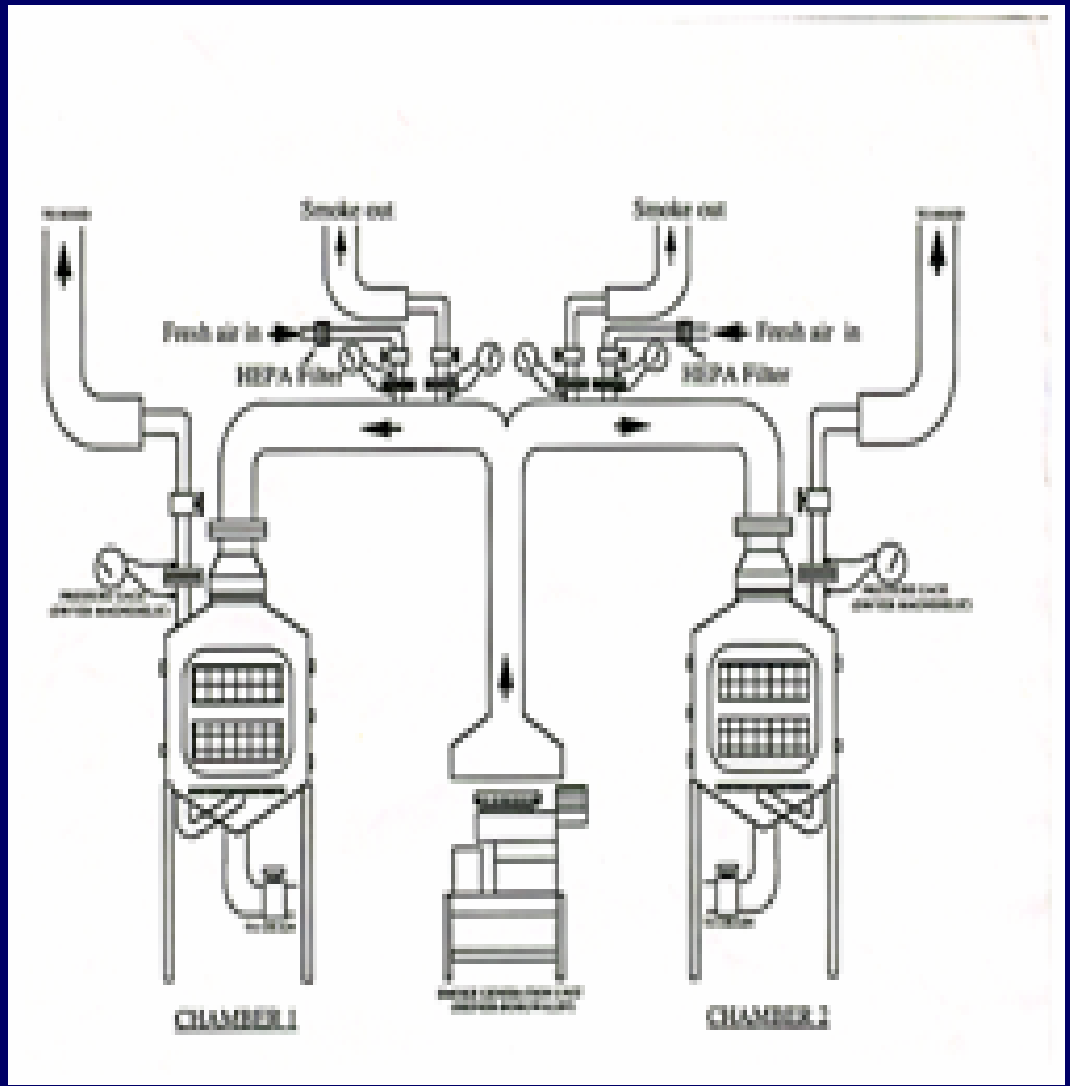
UK

WHOLE-BODY SMOKE EXPOSURE SYSTEM

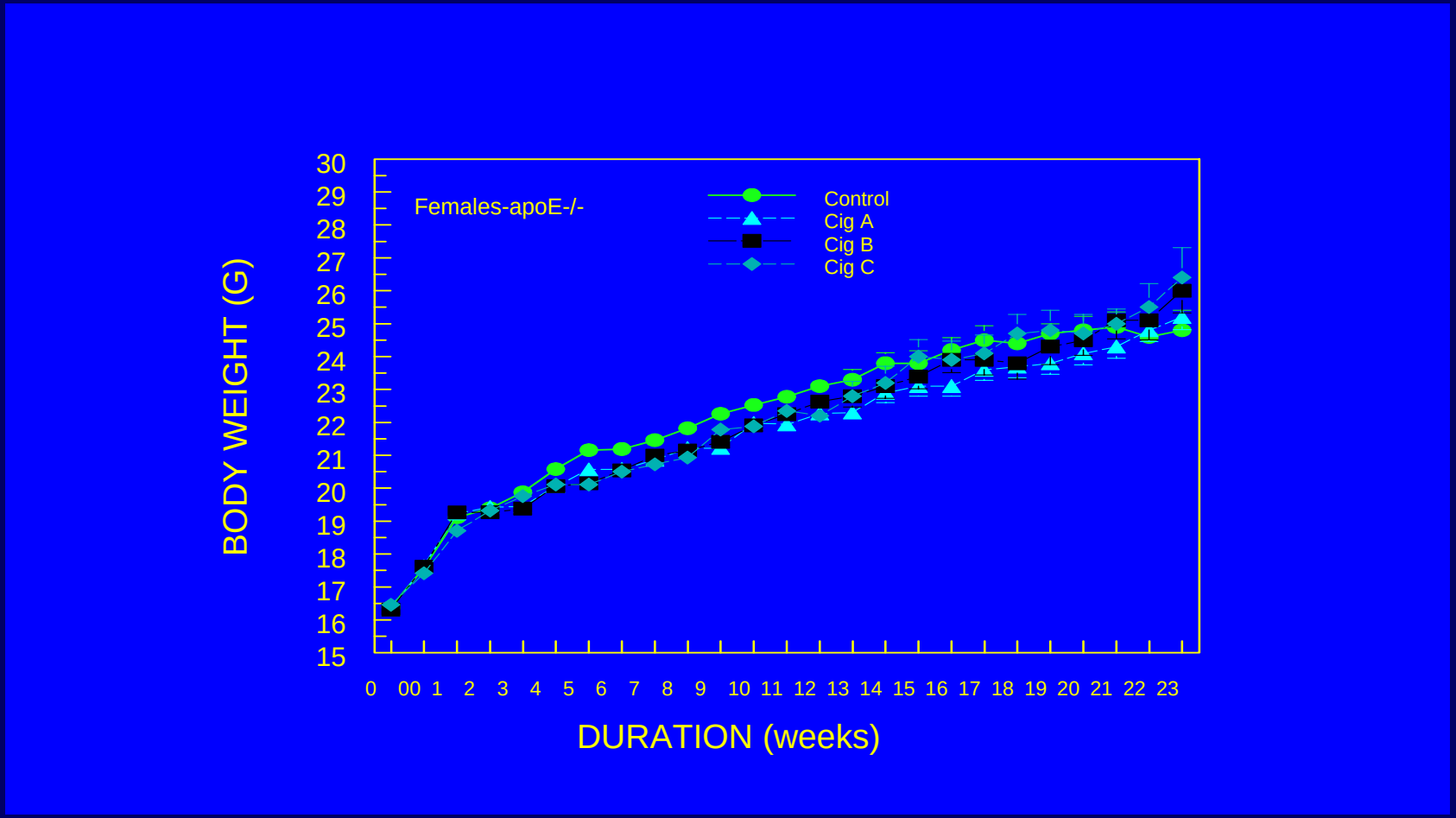
WHOLE-BODY CHAMBER FOR EXPOSURE OF RODENTS TO CIGARETTE SMOKE

Total Particulate Matter

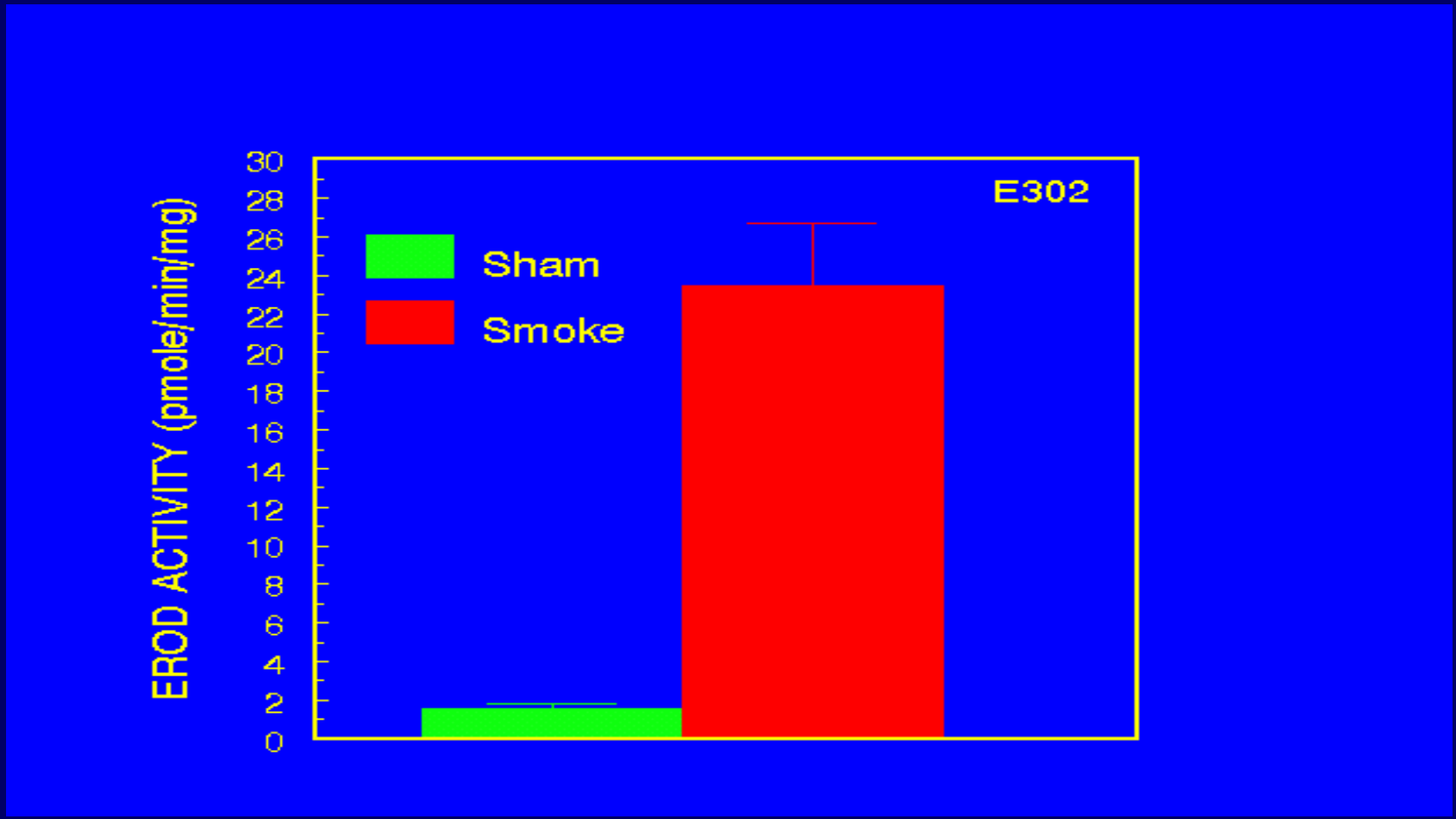
32 ± 4 mg/m^3



Body Weights of ApoE$^{-/-}$ Mice



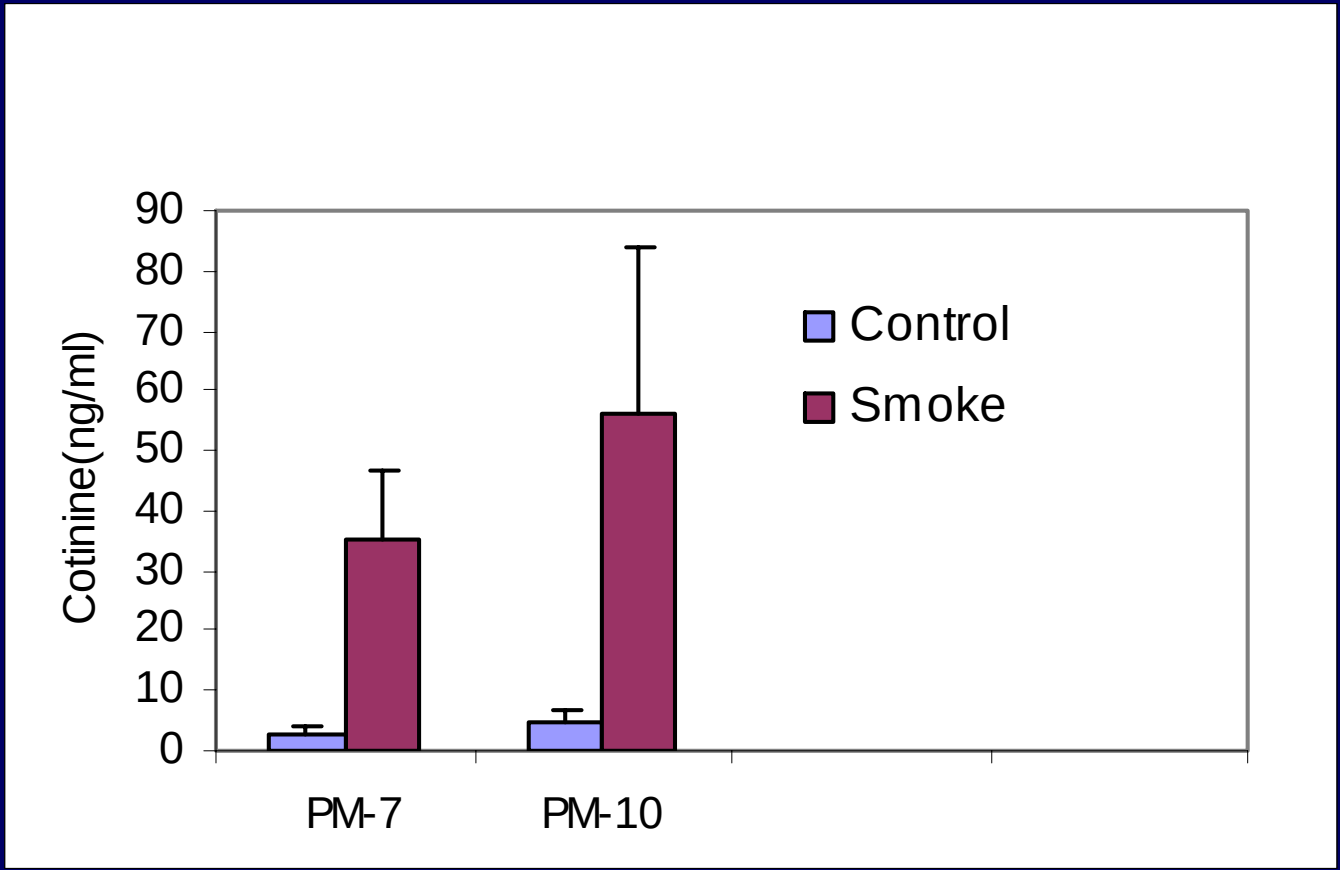
Lung Ethoxyresorufin-O-deethylase Activity



CYP1A1-PROTEIN LEVELS IN SMOKE-EXPOSED ApoE$^{-/-}$ MICE LUNGS

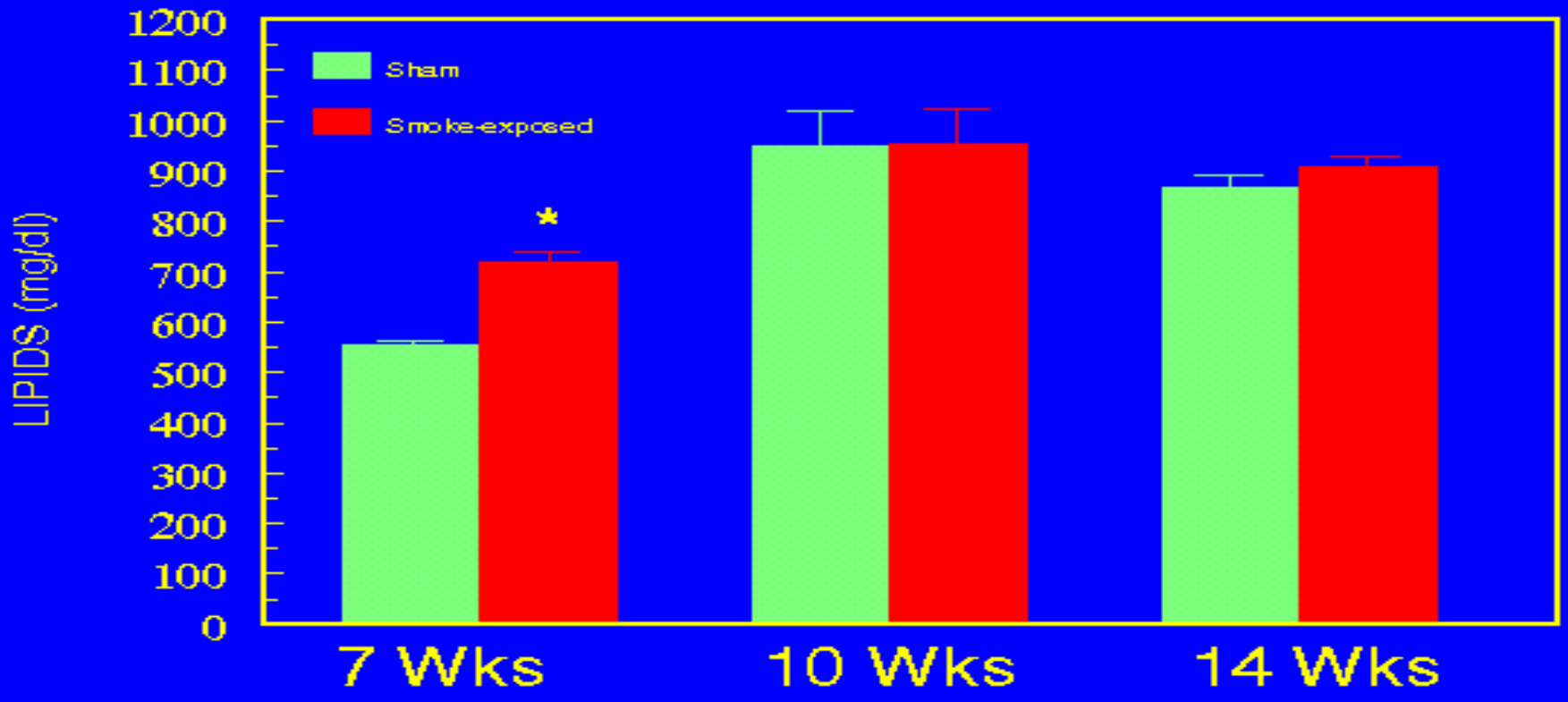
URINARY COTININE

CONTROL vs SMOKE apoE-/- mice



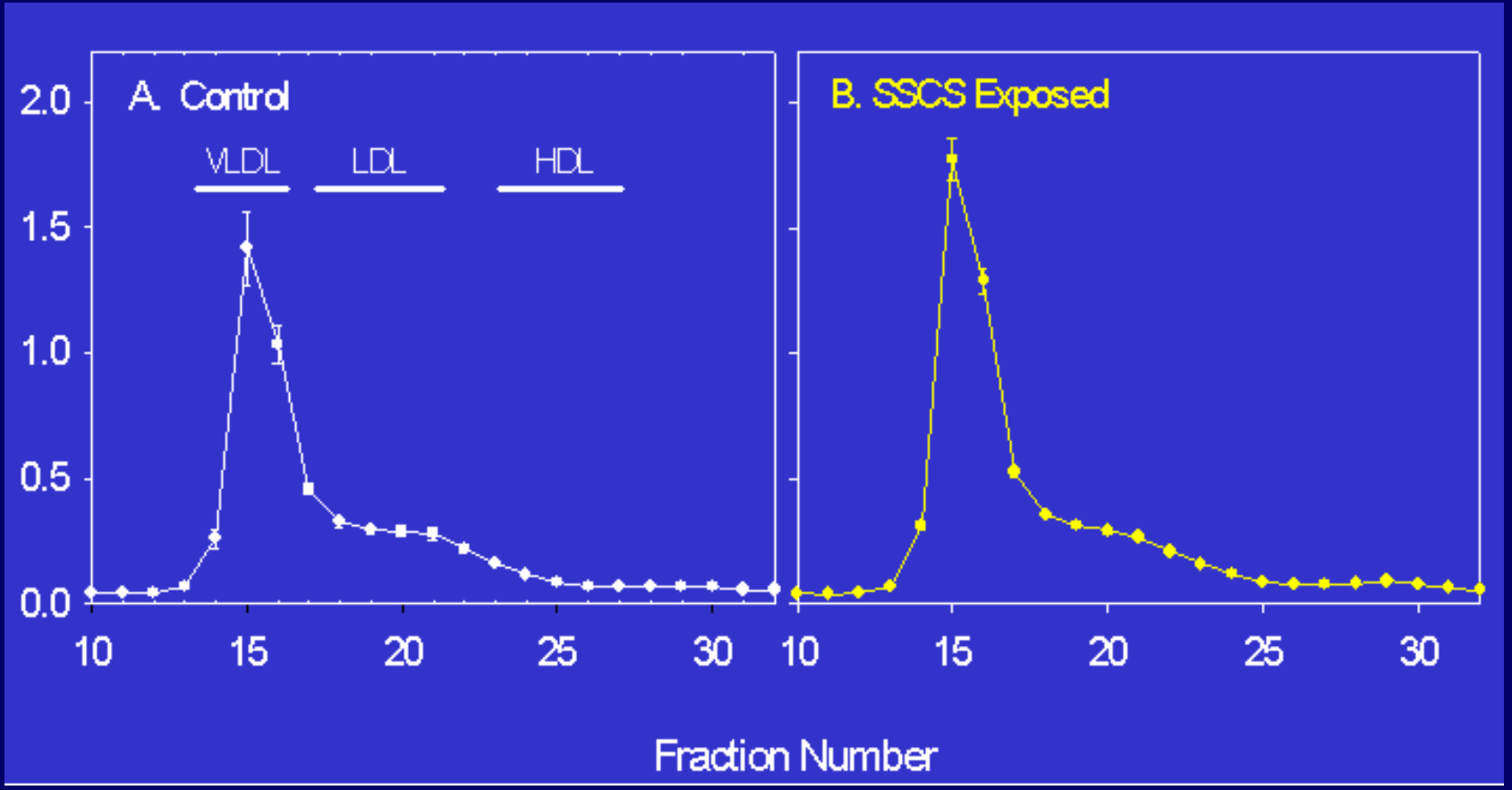
PLASMA CHOLESTEROL IN ApoE$^{-/-}$ MICE

Sham vs Smoke-exposed



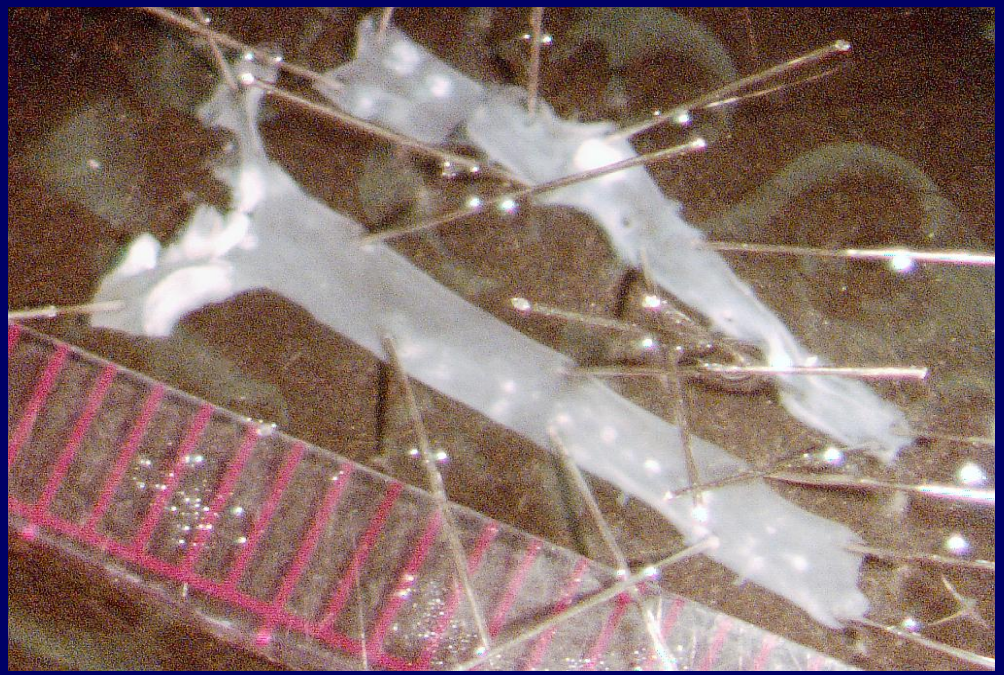
DISTRIBUTION OF LIPOPROTIEN-CHOLESTEROL IN PLASMA

ApoE-/-Mice: Control and Smoke-Exposed

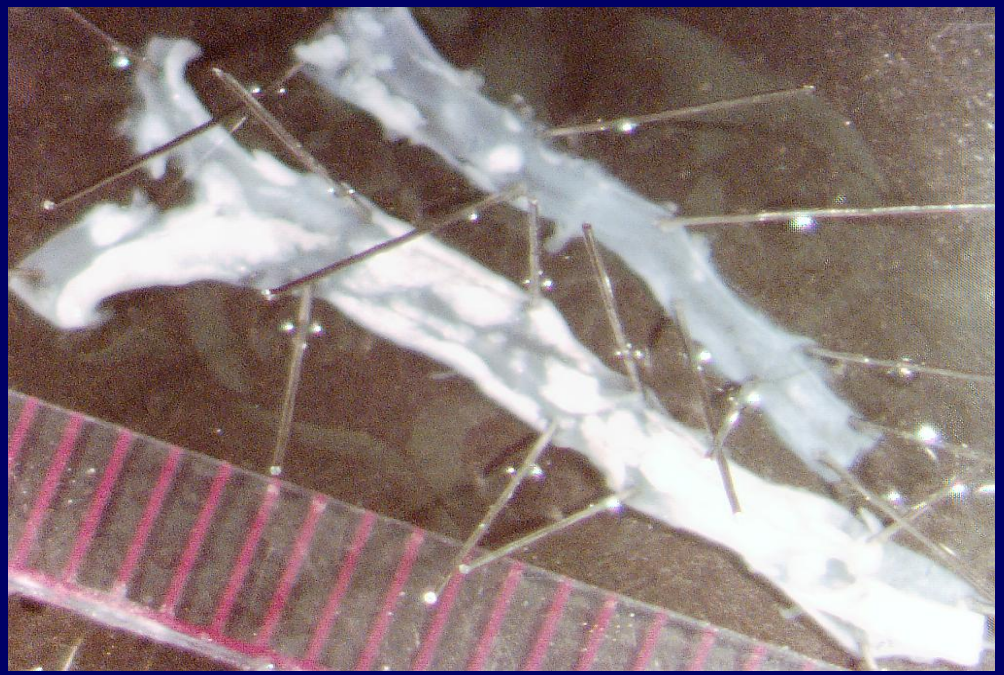


En Face Comparison of Aortic Lesions

apoE$^{-/-}$ Study



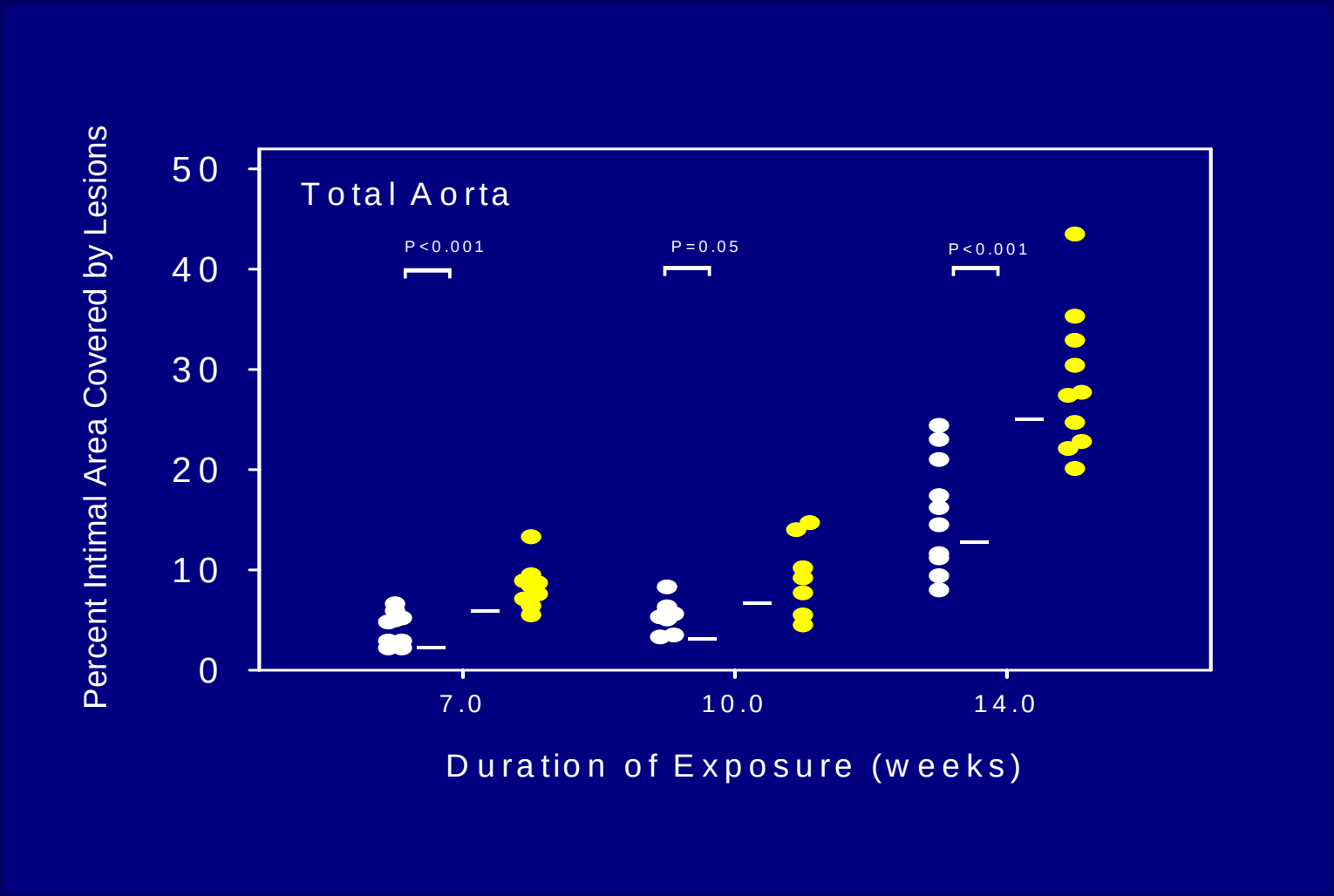
Control Mouse Aorta



Smoke-Exposed Mouse Aorta

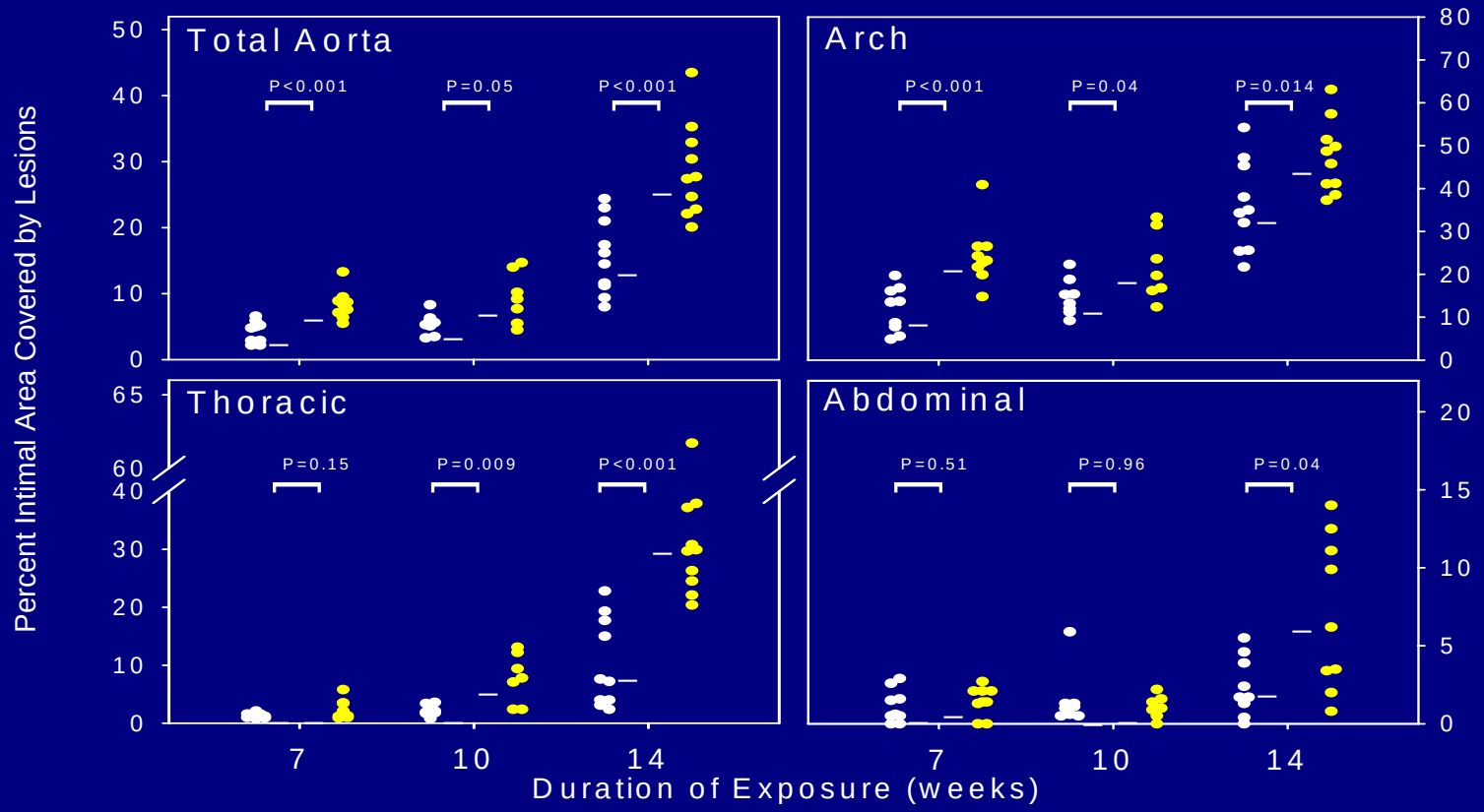
TOTAL AORIC INTIMAL AREA COVERED BY LESIONS

Sham vs Smoke-exposed ApoE$^{-/-}$ Mice



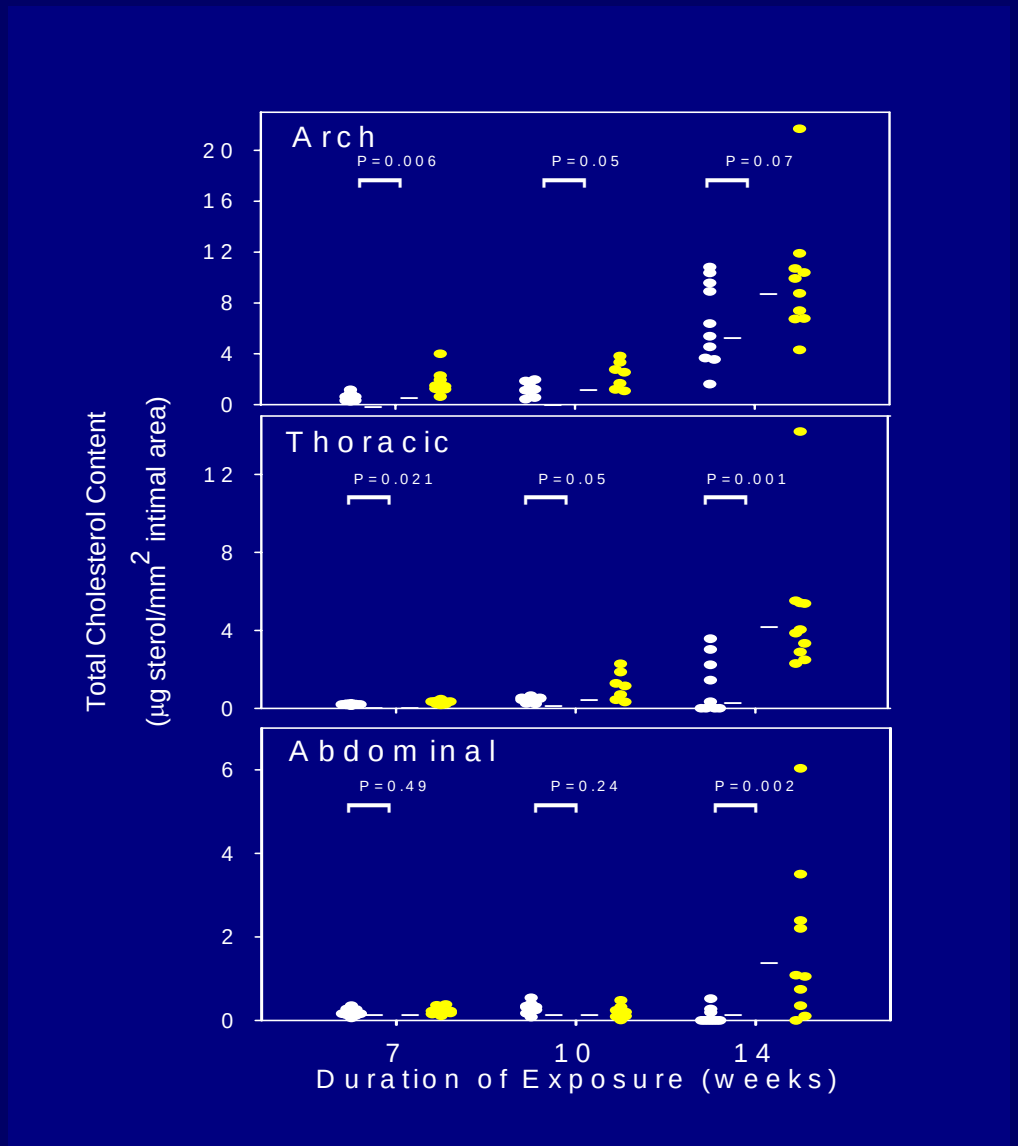
ATHEROSCLEROTIC LESION AREA

Sham vs Smoke-exposed ApoE $^{-/-}$ Mice

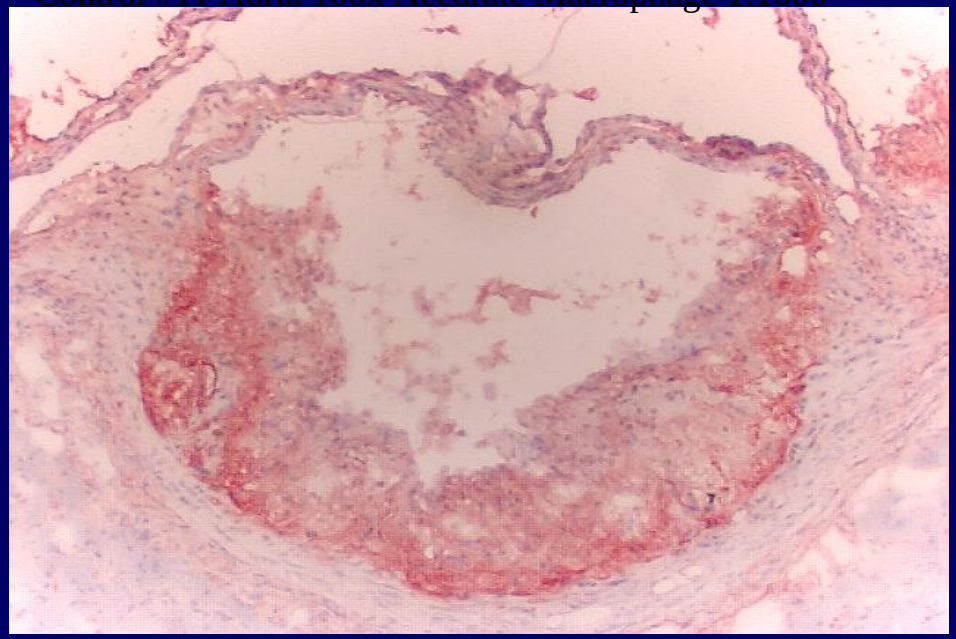


AORTIC TISSUE CHOLESTEROL CONTENT

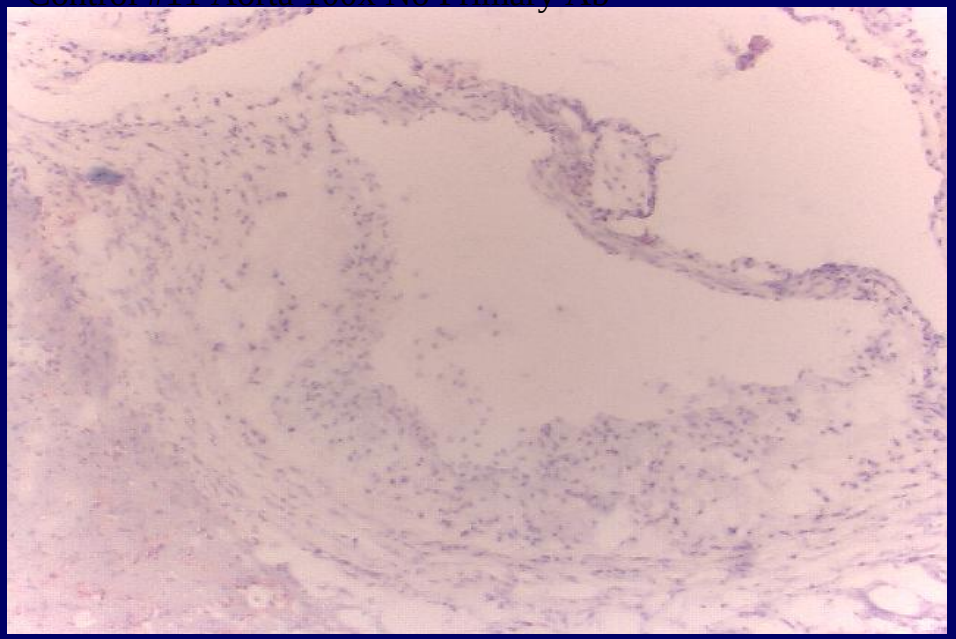
Sham vs Smoke-exposed



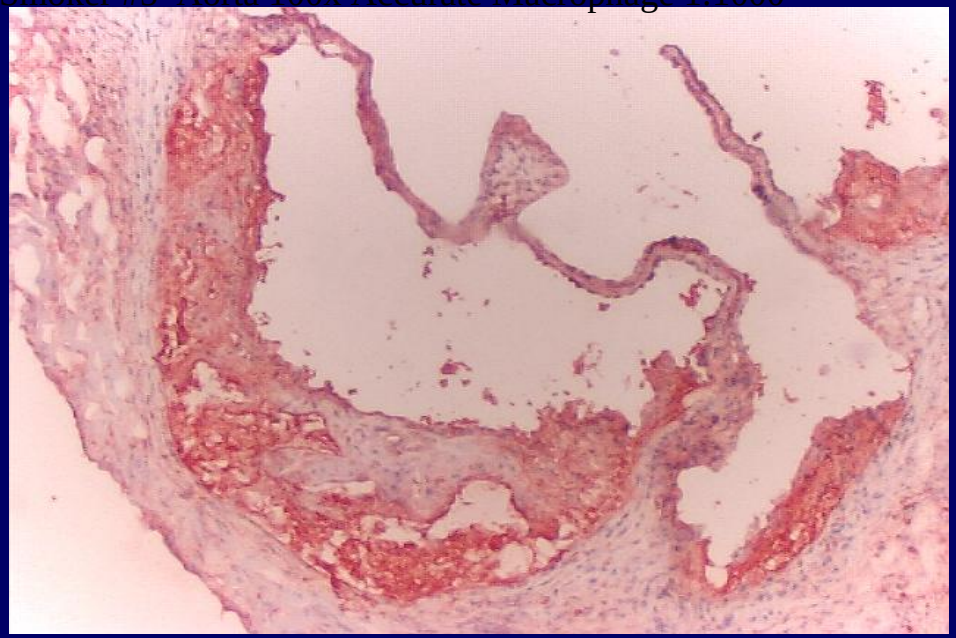
Control #11 Aorta 100x Accurate Macrophage 1:1000



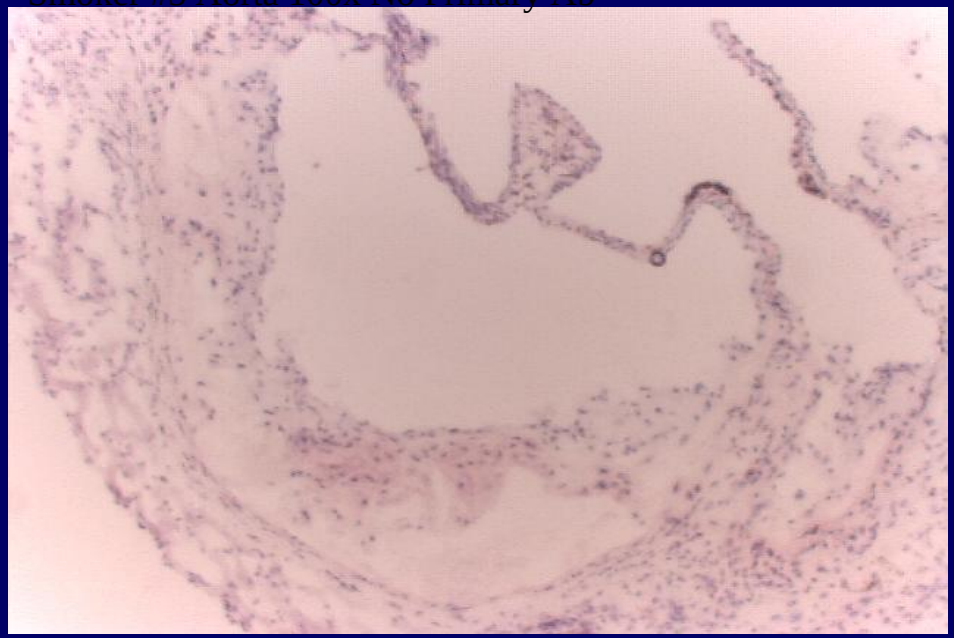
Control #11 Aorta 100x No Primary Ab



Smoker #5 Aorta 100x Accurate Macrophage 1:1000



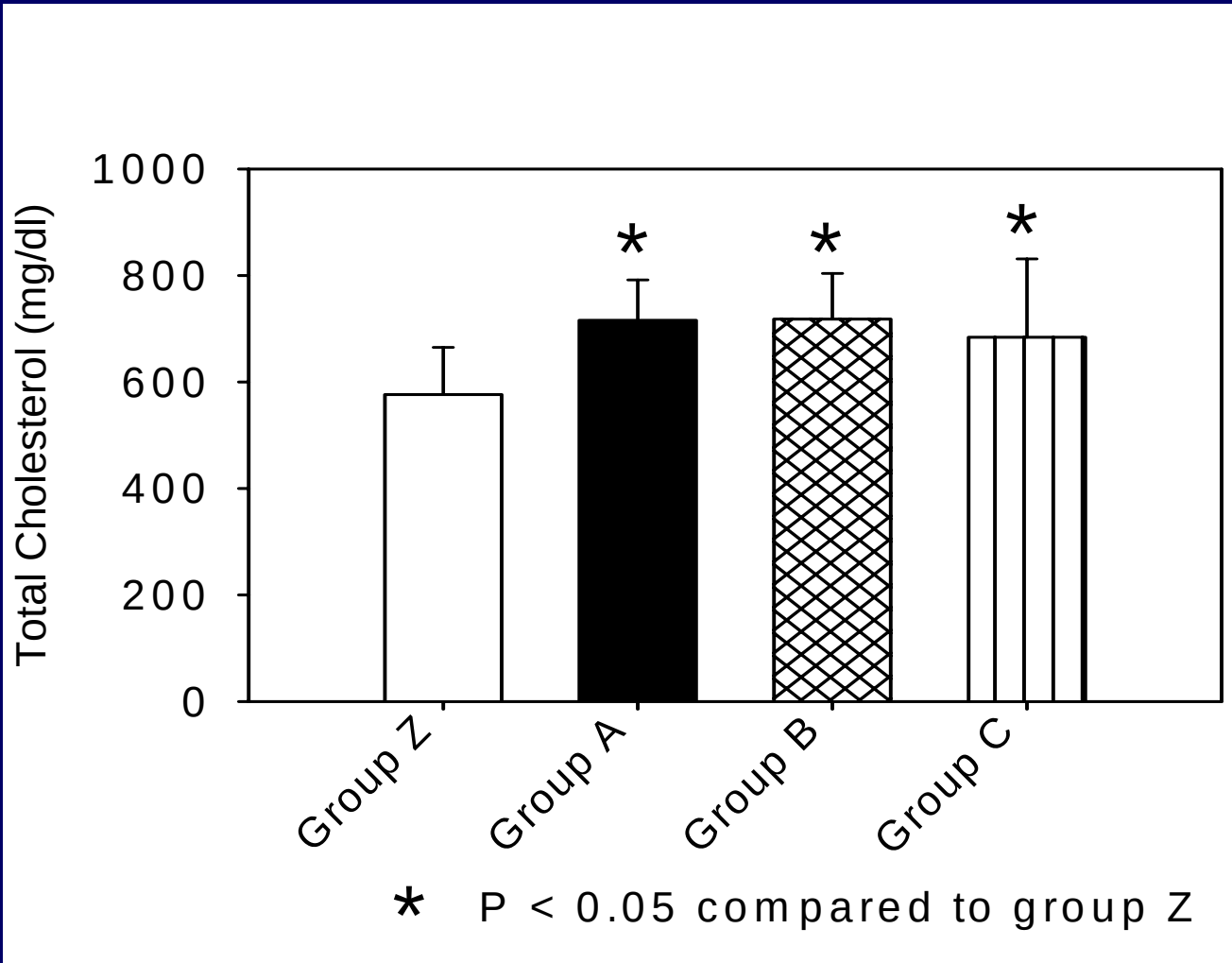
Smoker #5 Aorta 100x No Primary Ab



CIGARETTE MODIFICATION

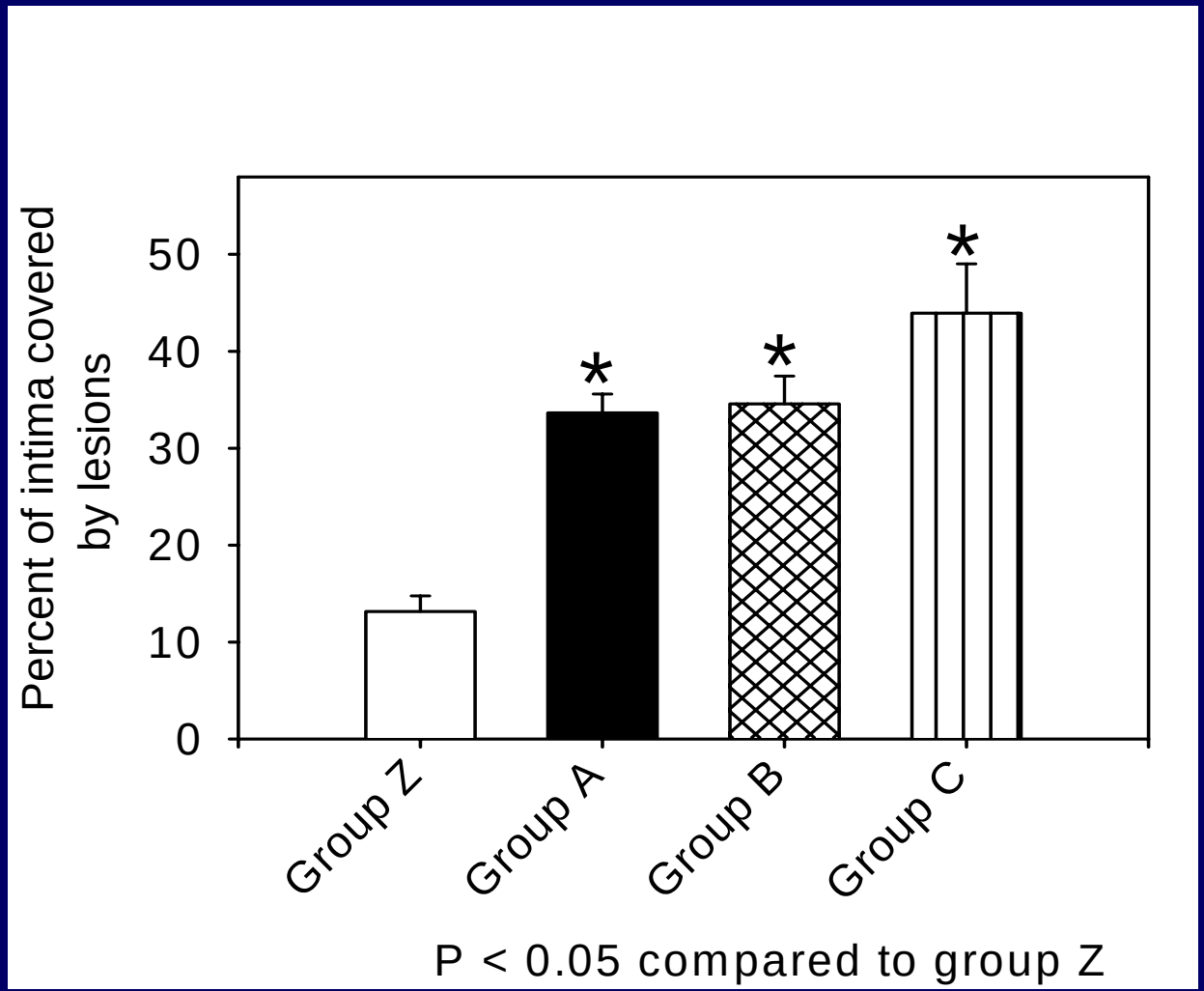
Plasma Cholesterol of ApoE$^{-/-}$ Mice

Smoke Exposure-12 wks



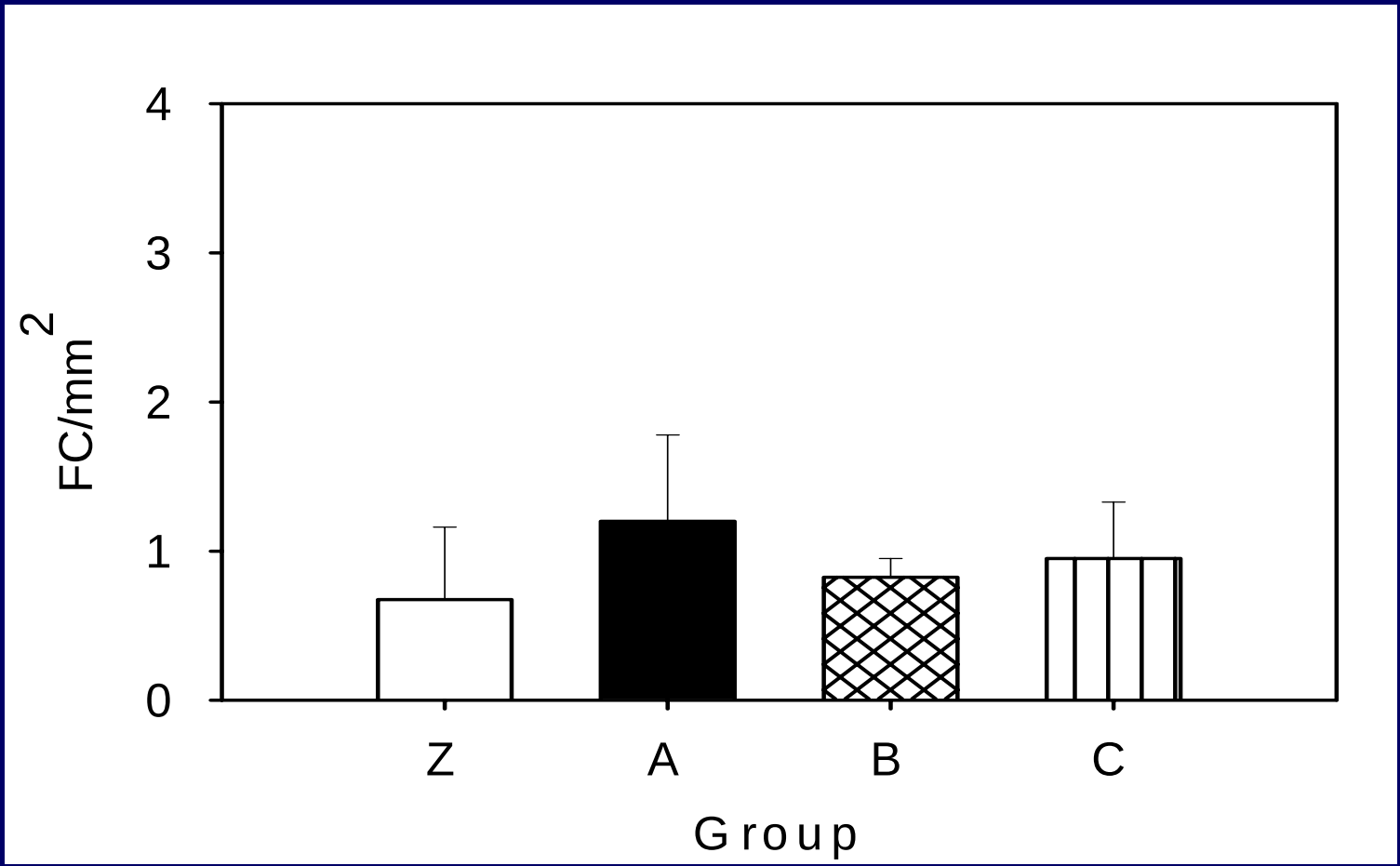
Atherosclerotic Lesions in ApoE$^{-/-}$ Mice

Smoke Exposure-12 wks



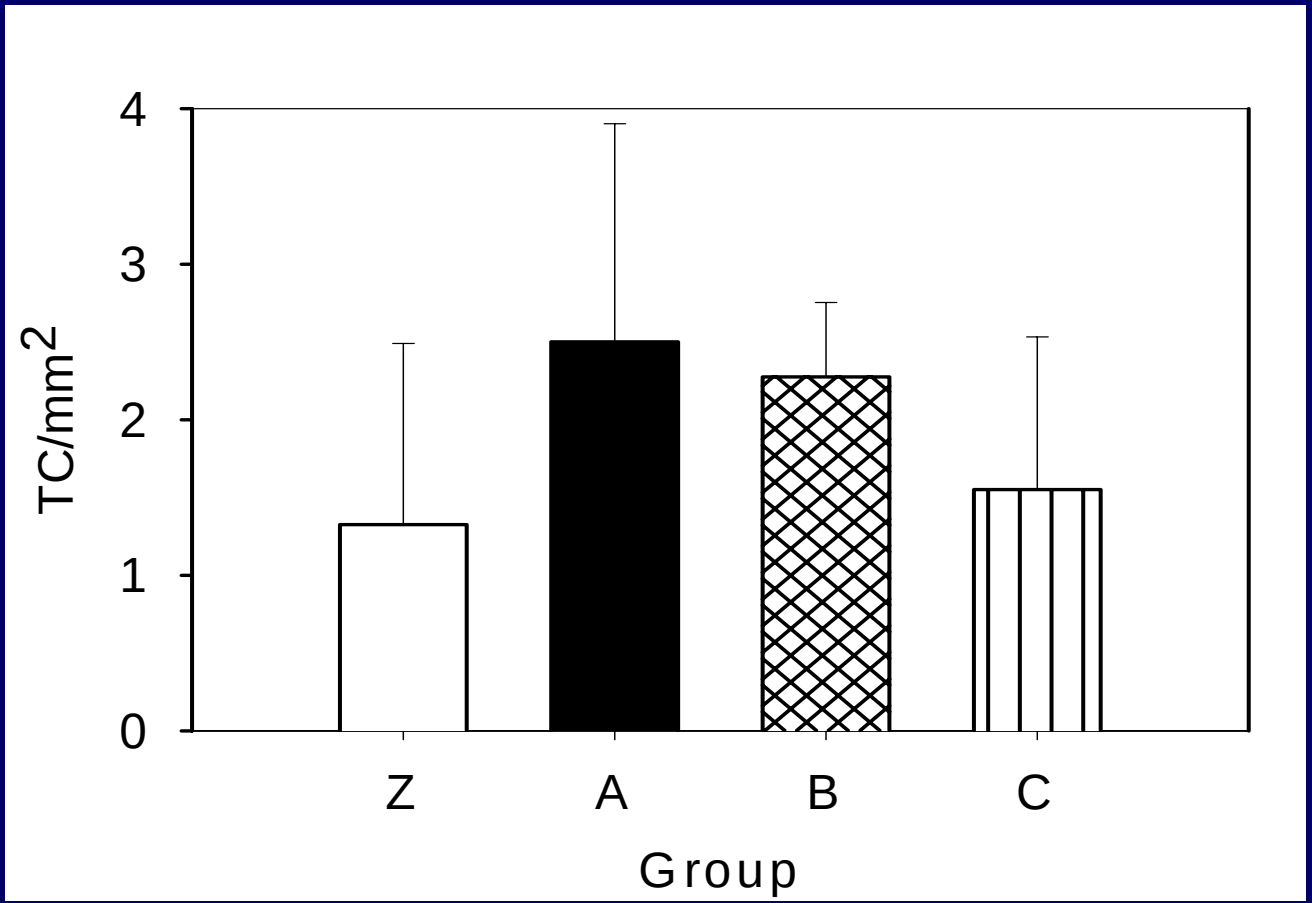
Free Cholesterol in ApoE$^{-/-}$ Mice Vessels

Smoke Exposure-12 wks



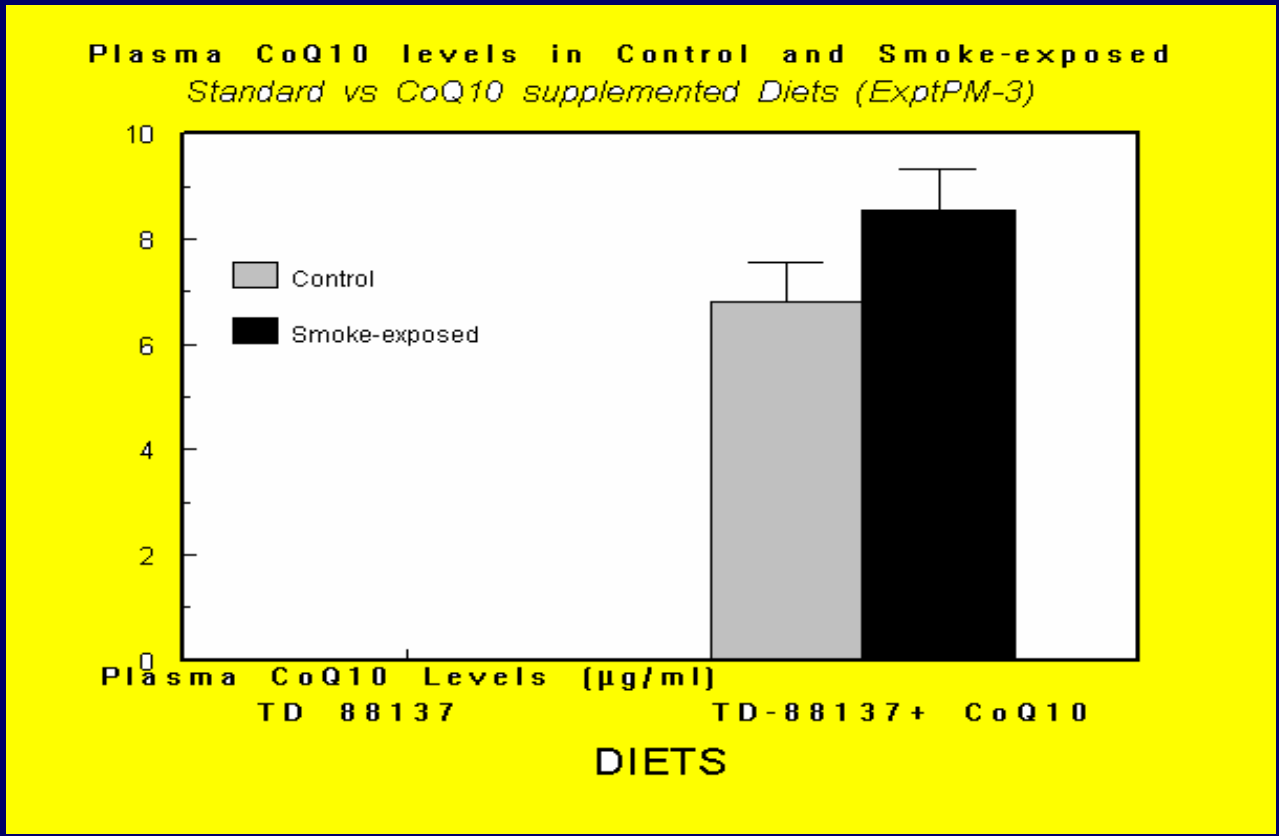
Total Cholesterol in ApoE$^{-/-}$ Mice Vessels

Smoke Exposure-12 wks

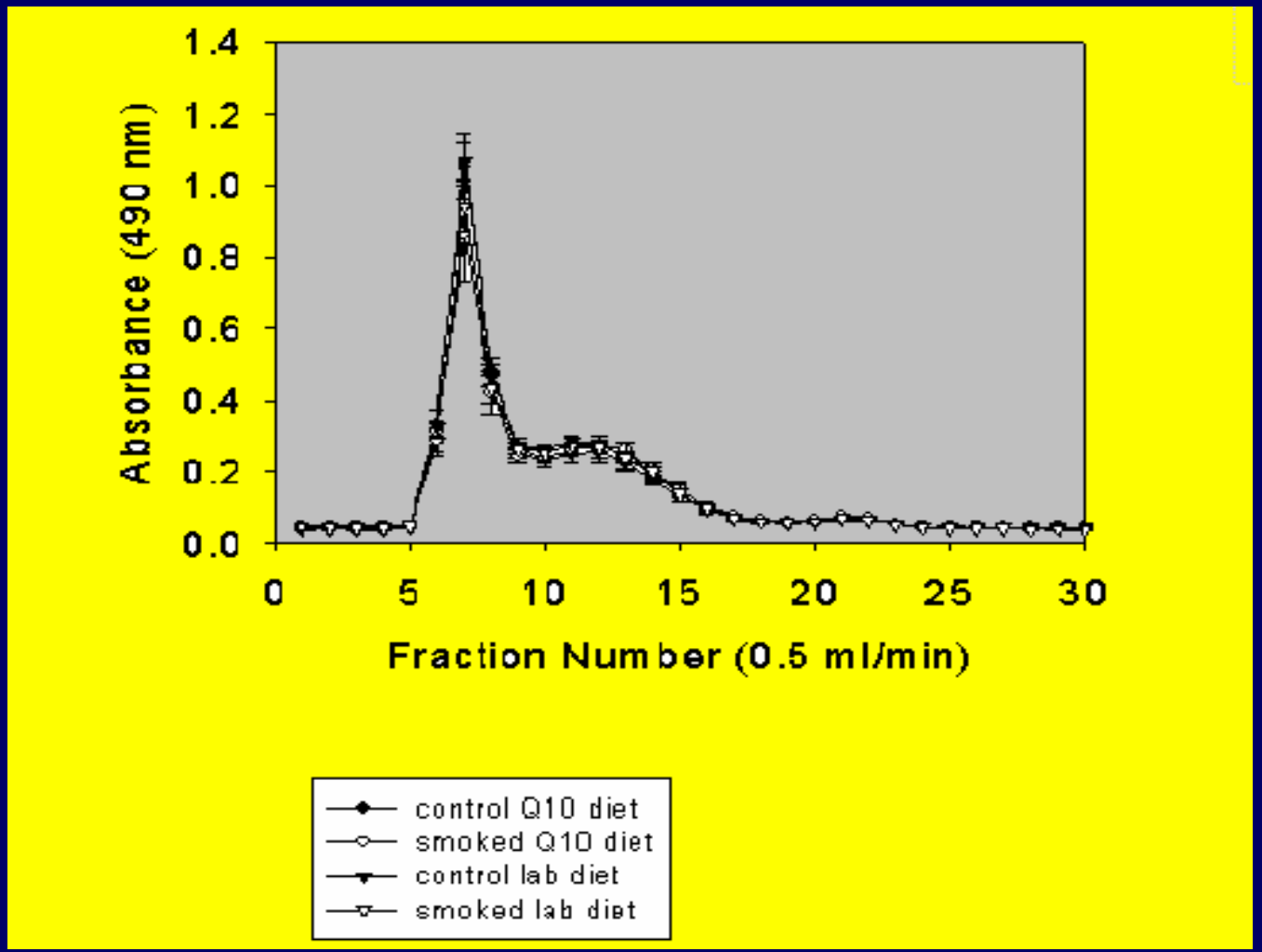


DIETARY INTERVENTION

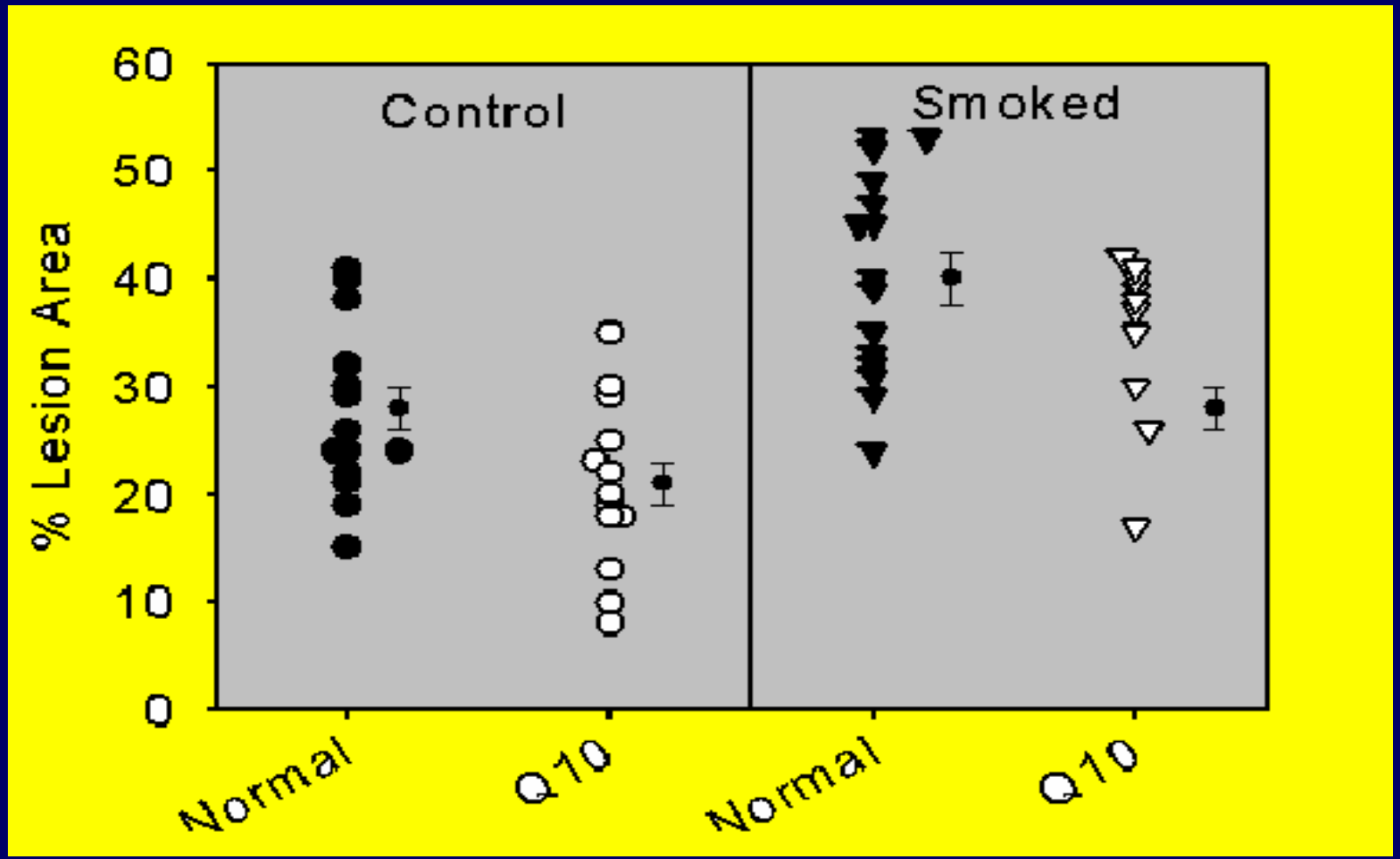
DIETARY CoQ10 INTERVENTION



CHOLESTEROL DISTRIBUTION



Dietary CoQ10 and Atherosclerosis

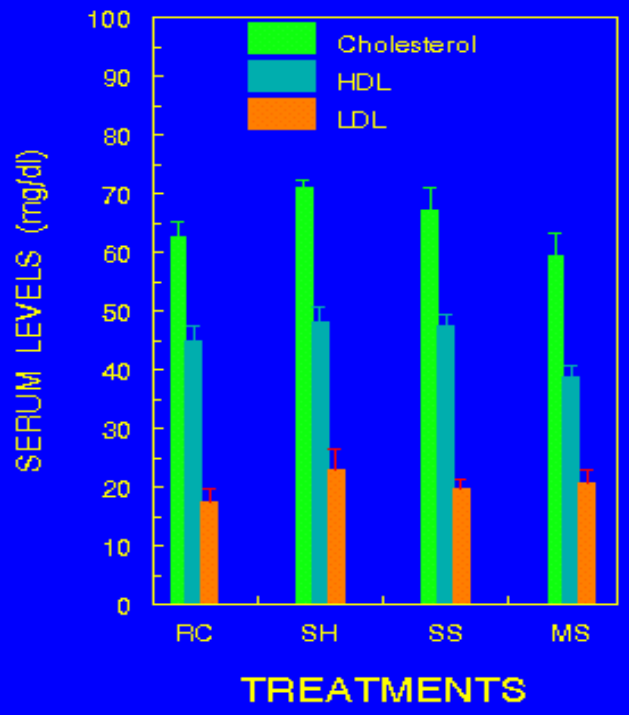


NOSE-ONLY vs WHOLE-BODY

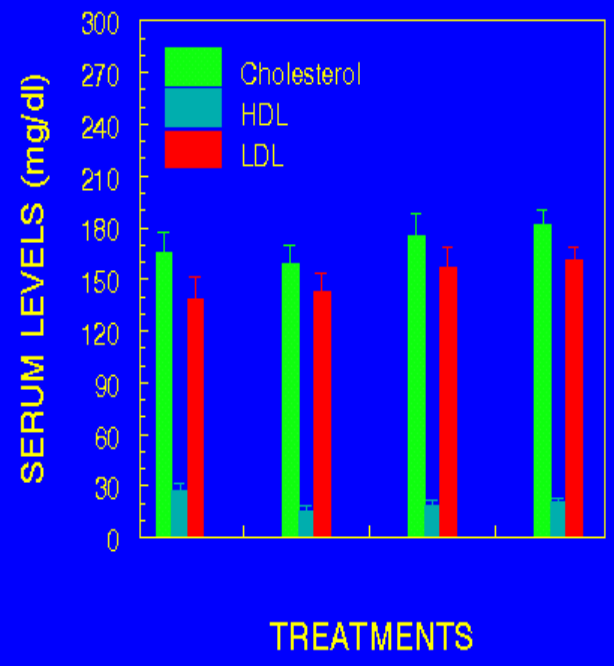
Diet and Cholesterol Distribution

C57BL Mice-Standard and High Cholesterol Diet

STANDARD

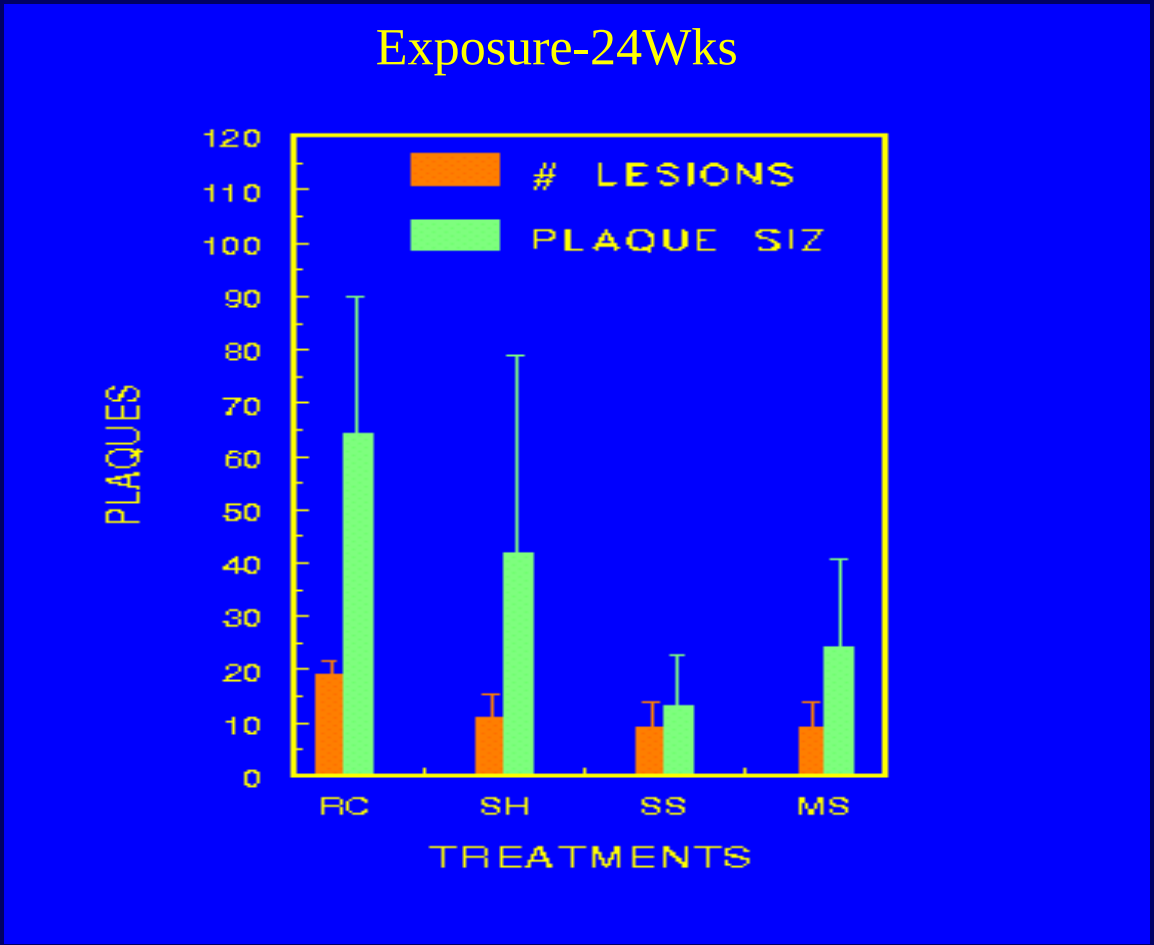


HIGH CHOLESTEROL



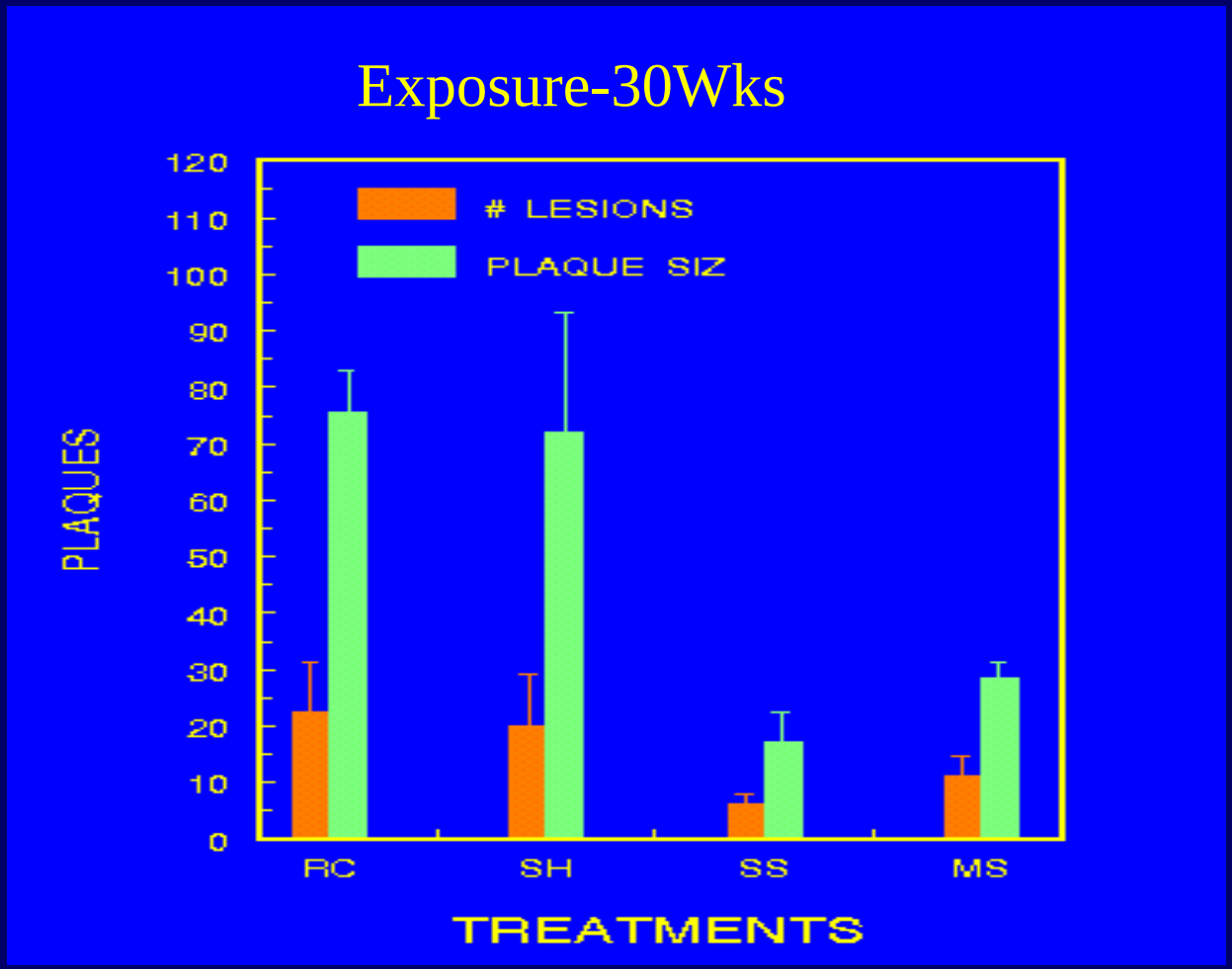
SMOKE EXPOSURE AND ATHEROSCLEROSIS

C57Bl Mice-High Cholesterol Diet



SMOKE EXPOSURE AND ATHEROSCLEROSIS

C57Bl Mice-High Cholesterol Diet



STRENGTHS

- Relevant disease end point
- Quantitative
- Reproducible
- Large data base available

WEAKNESSES

- Thrombosis
- Sites of lesion formation
- Labor-intensive and expensive
- Dose-response not yet established

WHAT IS NEEDED TO STANDARDIZE AND EVALUATE THE ASSAY?

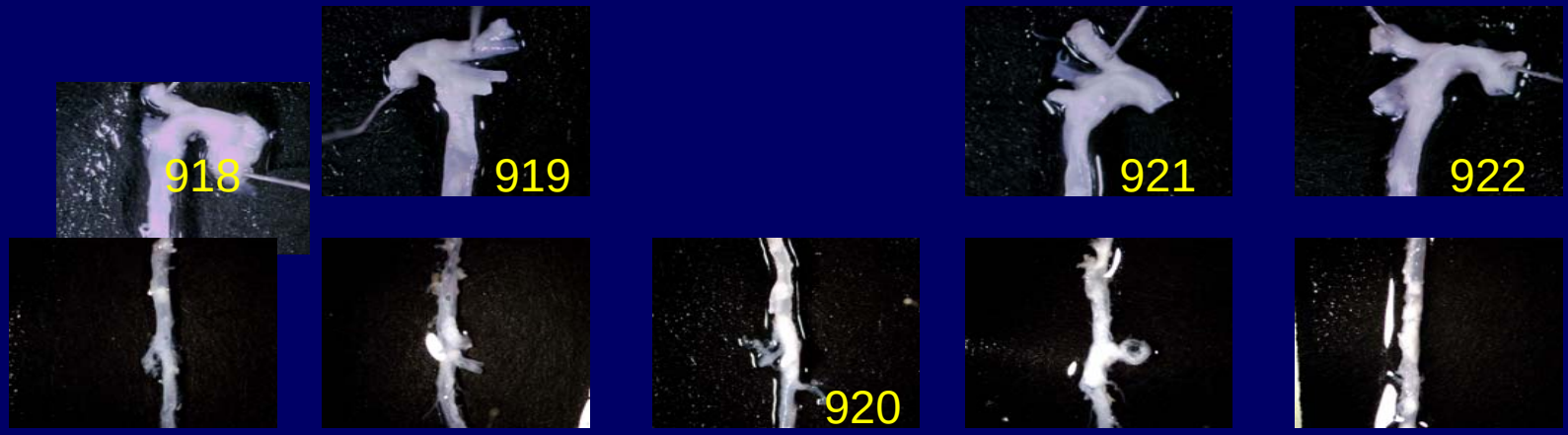
- Dose response --smoke particulates conc.vs disease
  --urinary/plasma cotinine vs disease
- Longer exposures --plaque composition and rupture
- Standard vs Western Diet
- Plaque area vs vessel cholesterol
- Whole vs gas phase smoke

Important points Committee needed to be addressed

- Description of the model
- Animal disease endpoints and relevance to human physiology and disease
- Method of exposure and relevance to human exposures
- Reproducibility
- Practicality (sample size/expense/study duration)
- Dose-response
- Significance of observed change in biological response
- Uncertainties and shortcomings
- Overall validity for evaluating claims of reduced risk
- How are doses selected?
- How many animals need to be used?
- How should data be reported/ normalized?

Control ApoE-/-

Saline



Ang II

Smoke ApoE-/-

Saline

Ang II