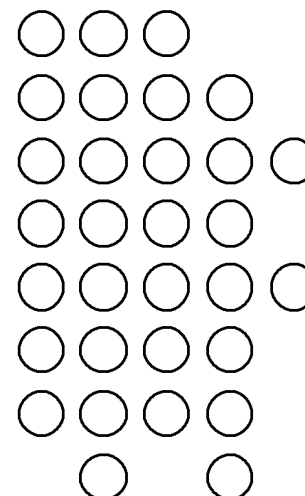
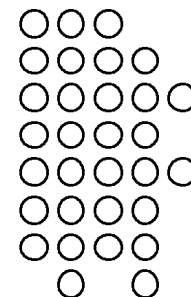


Biomarkers of tobacco smoke exposure: Comparison between smokers, Eclipse, and nonsmokers

Buddy Brown, David Heavner, David Bombick, Joel
Richardson, Riley Davis, Gary Byrd, Victoria Connell,
Michael Morton, and Michael Ogden

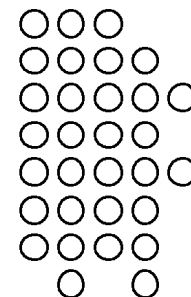
R. J. Reynolds Tobacco Company





INTRODUCTION

- Most of the tobacco smoke constituents to which smokers are exposed during smoking are not unique to tobacco smoke.
- To develop potentially reduced exposure products (PREPs) – as advocated recently by the Institute of Medicine (IOM) – it is critical to understand the baseline difference in biomarker levels between smokers and nonsmokers, if any.
- Only those smoke constituents (and biomarkers) that are demonstrably elevated in smokers of traditional cigarette products are potential target constituents in successful PREP development.

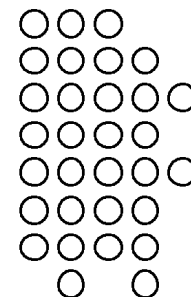


EXPERIMENTAL DESIGN

- A preliminary study was conducted to assess:
 - (a). the state of method development for several biomarkers of potential interest.
 - (b). evaluate the applicability of selective biomarkers in the design of PREPs.
- Participants
 - 10 Smokers
 - 9 Eclipse Smokers
 - 10 Nonsmokers

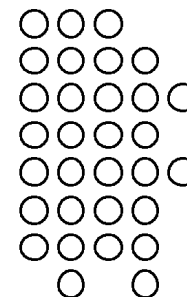
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EXPERIMENTAL DESIGN



- Usual brand of cigarettes maintained by smoker subjects.
- Proven analytical methods.
- Acquired samples included:
 - 24-hour urine collection
 - Morning and evening mouthwash

BIOMARKERS

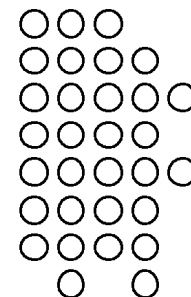


- Biomarkers that are primarily due to cigarette smoke exposure:

<u>Smoke Constituent</u>	<u>Biomarkers</u>	<u>Analysis</u>
Nicotine	Nicotine + 5 nicotine metabolites	LC-MS/MS
NNK	NNAL + NNAL-Gluc	LC-MS/MS

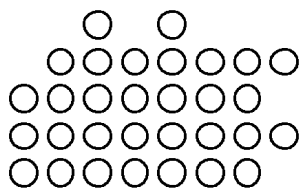
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BIOMARKERS



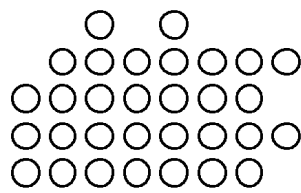
- Biomarkers with significant background interference due to non-tobacco exposures:

<u>Smoke Constituent</u>	<u>Biomarkers</u>	<u>Analysis</u>
Benzene	trans, trans-Muconic Acid	GC-MS
Benzene	S-Phenylmercapturic acid	HPLC-MS
Acrolein	3-Hydroxypropylmercapturic acid	LC-MS/MS
Pyrene	1-Hydroxypyrene	HPLC-Fluorescence
Free radicals	8-OHdG	HPLC-EC
Free Radicals	15-F _{2t} -Isoprostane-M	ELISA or GC/MS
Carbonyls	Carbonyl protein adducts	UV Spectrophotometry

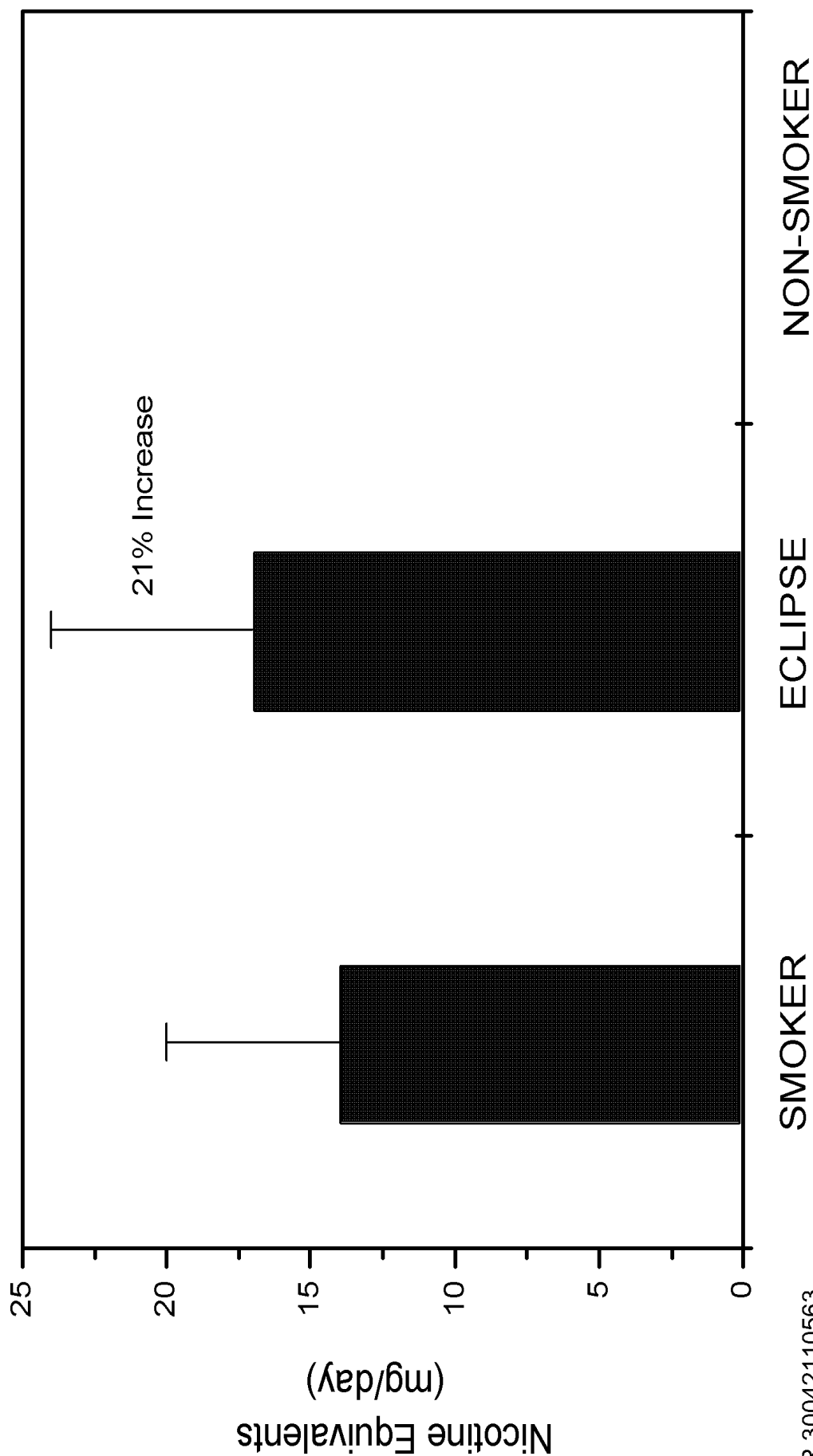


Nicotine & Nicotine Metabolites

- Nicotine & nicotine metabolites are increased in smokers as compared to nonsmokers.

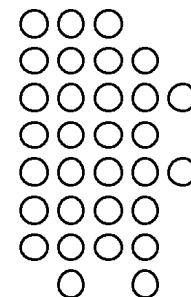


NICOTINE EQUIVALENTS



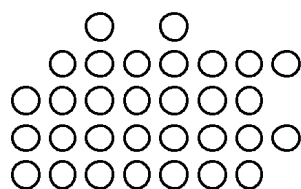
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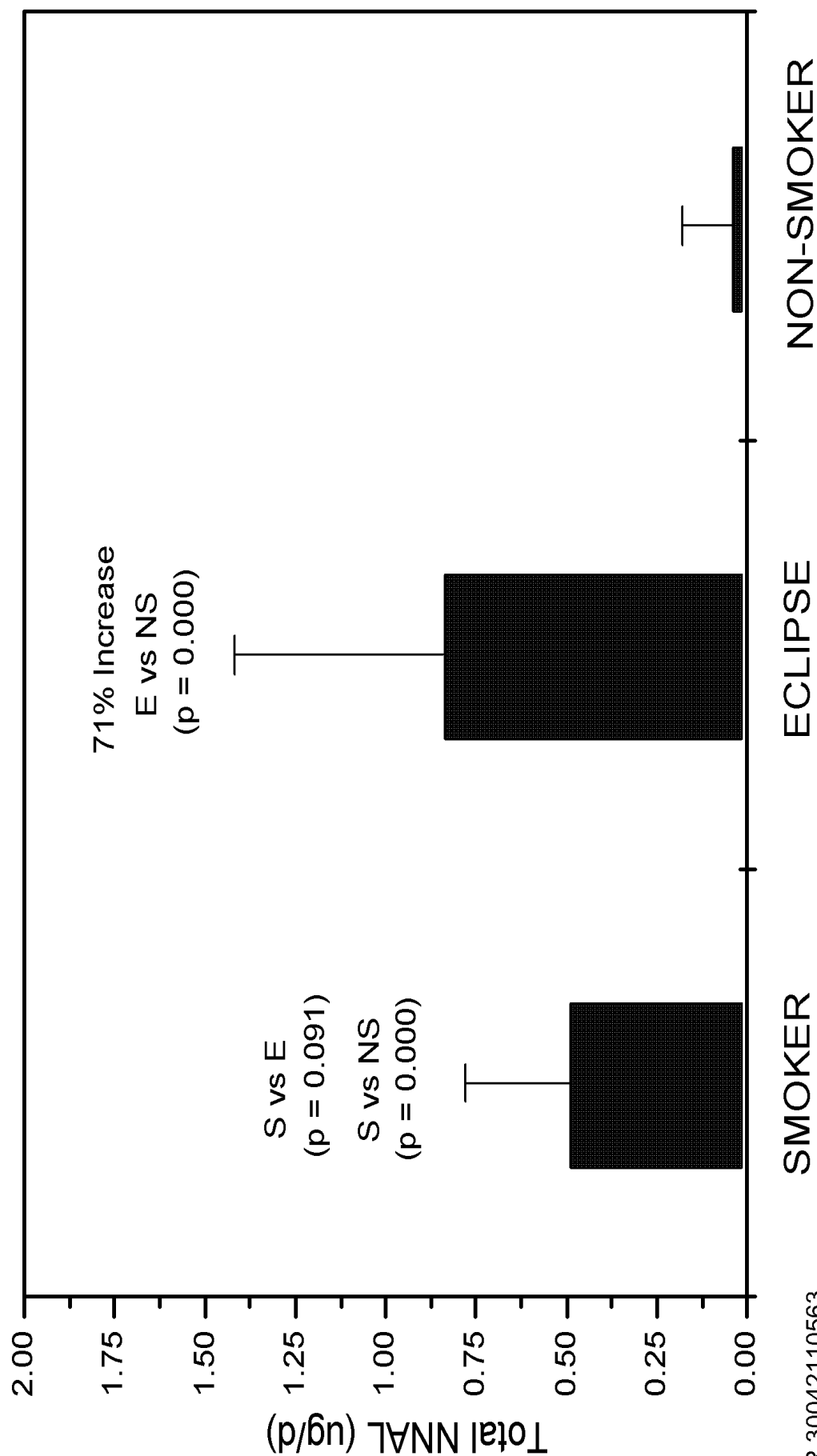


NNK: NNAL + NNAL-Gluc

- NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is a tobacco-specific nitrosamine found in cigarette smoke. NNK is a suspected lung carcinogen.
- In humans, NNK is metabolized to primarily NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and its glucuronides. NNAL is also a suspected lung carcinogen.
- NNAL is a specific biomarker for tobacco smoke.

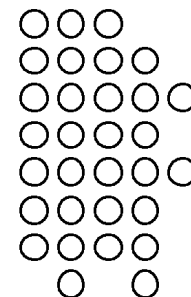


TOTAL NNAL



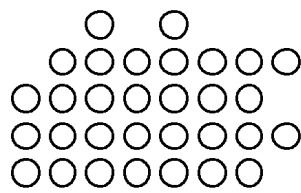
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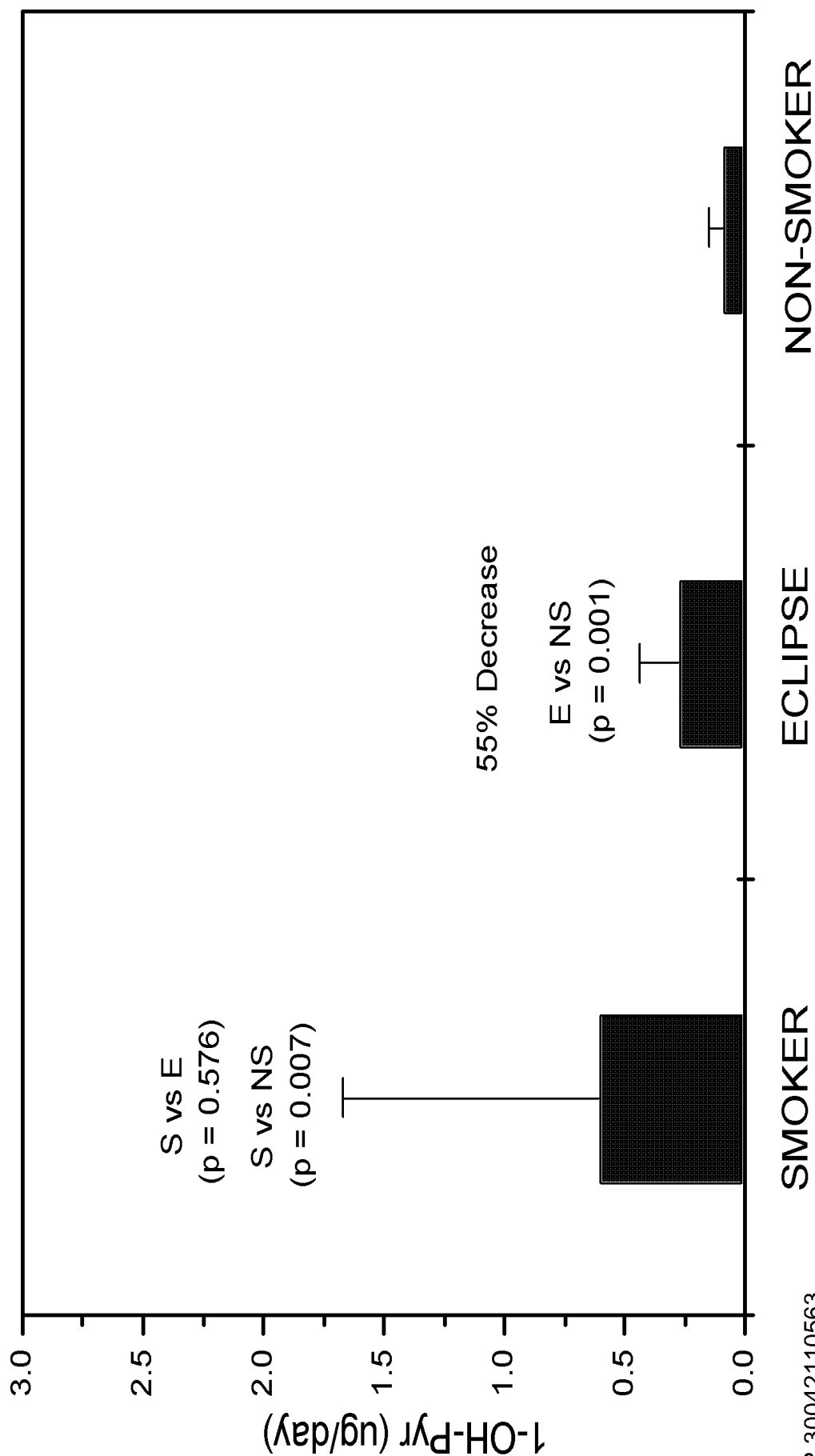


PAHs: 1-Hydroxypyrene

- PAHs are environmental contaminants.
- Tobacco constituents are pyrolyzed to form PAH smoke compounds at temperatures between 450° – 600° C.
- Pyrene is a PAH present in MS smoke at levels between 50 – 270 ng/cigarette, and is used as a marker compound for PAH exposure.
- 1-hydroxypyrene (1-OHP) and/or its glucuronide (1-OHP-gluc) the most extensively studied.
- In non-occupationally exposed persons, levels of urinary 1-OHP have been reported to be significantly higher in smokers compared to nonsmokers.

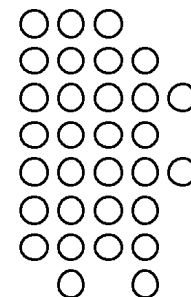


1-HYDROXYPYRENE



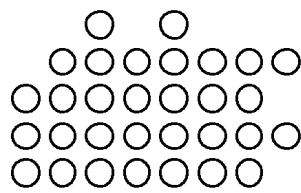
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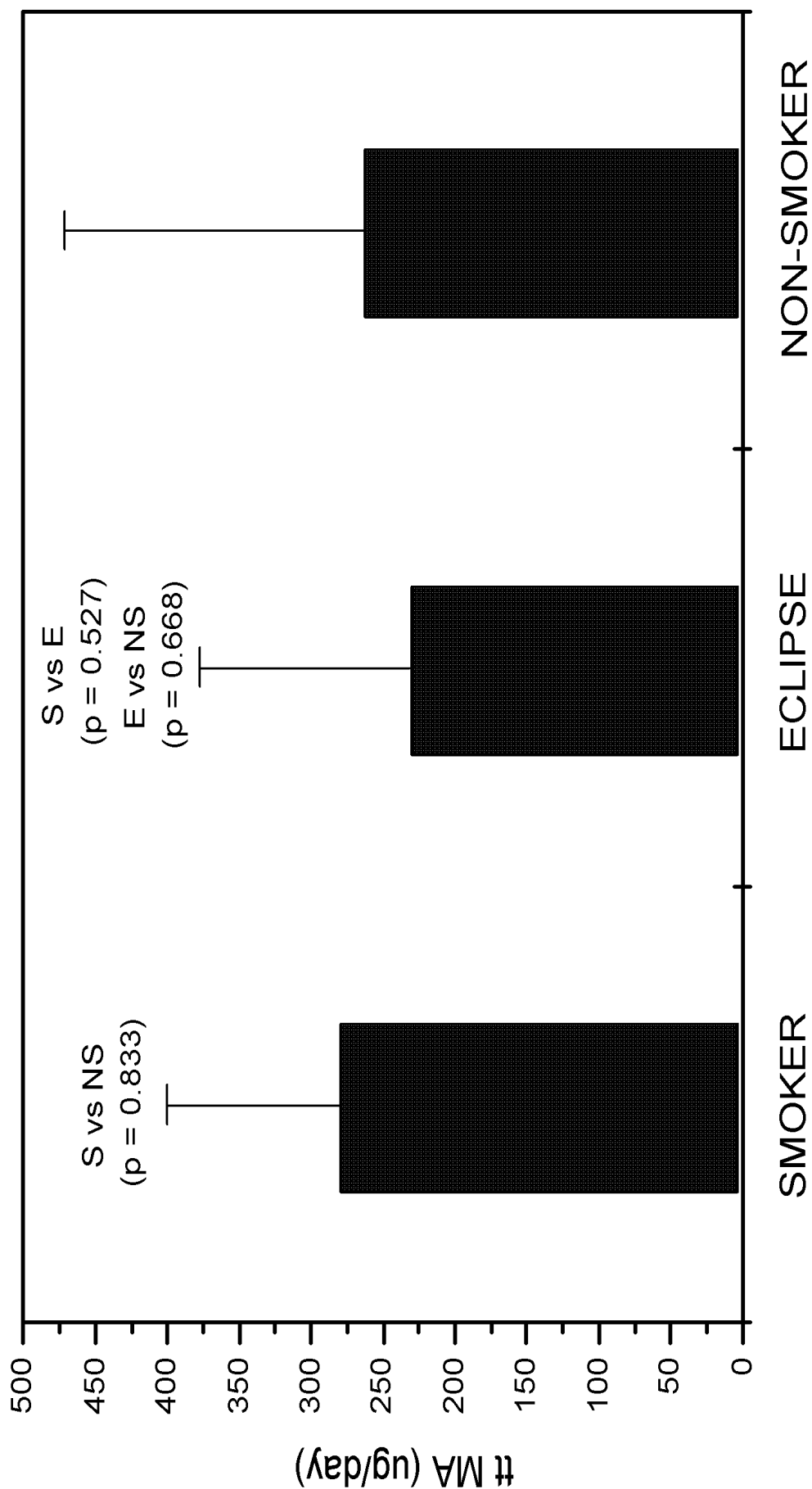


Benzene: t,t'-MA & S-PMA

- Benzene, an aromatic hydrocarbon, is a volatile organic compound (VOC) in mainstream, sidestream, and environmental tobacco smoke.
- Benzene is formed within the tobacco rod as a result of combustion during the smoking process.
- Benzene is a known human carcinogen whose primary site of action is bone marrow.
- A major source of exposure is automobile exhaust; food does not appear to be a significant source of exposure.
- In non-occupationally exposed persons smokers have higher mean values (about 2x's) of SPMA compared with nonsmokers as reported in the literature.
- Trans, trans-Muconic acid is a metabolite of both benzene and sorbic acid, a food preservative.

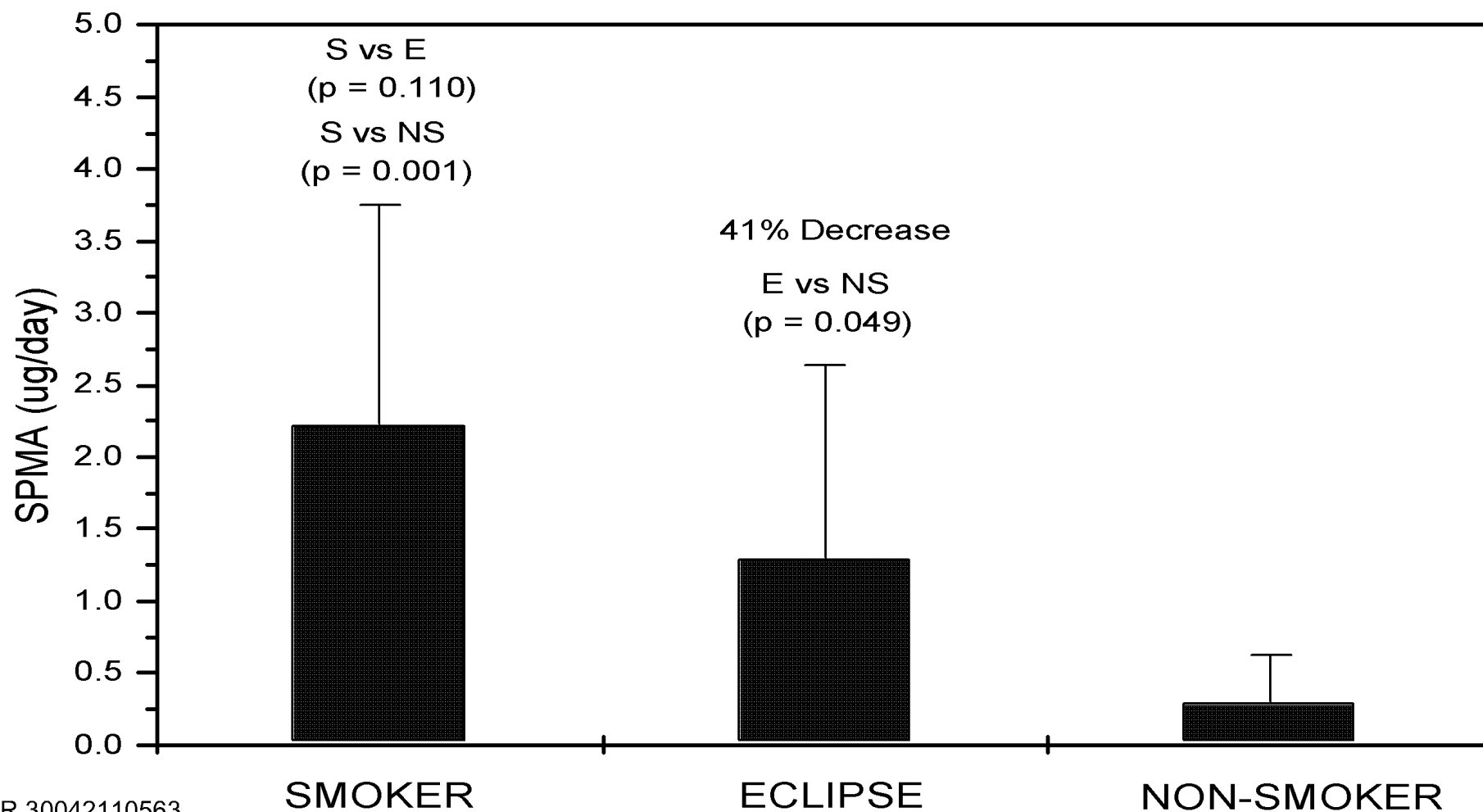
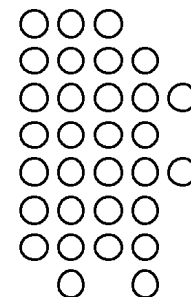


T,t-Muconic Acid



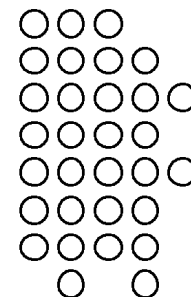
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S-PHENYLMERCAPTURIC ACID (S-PMA)



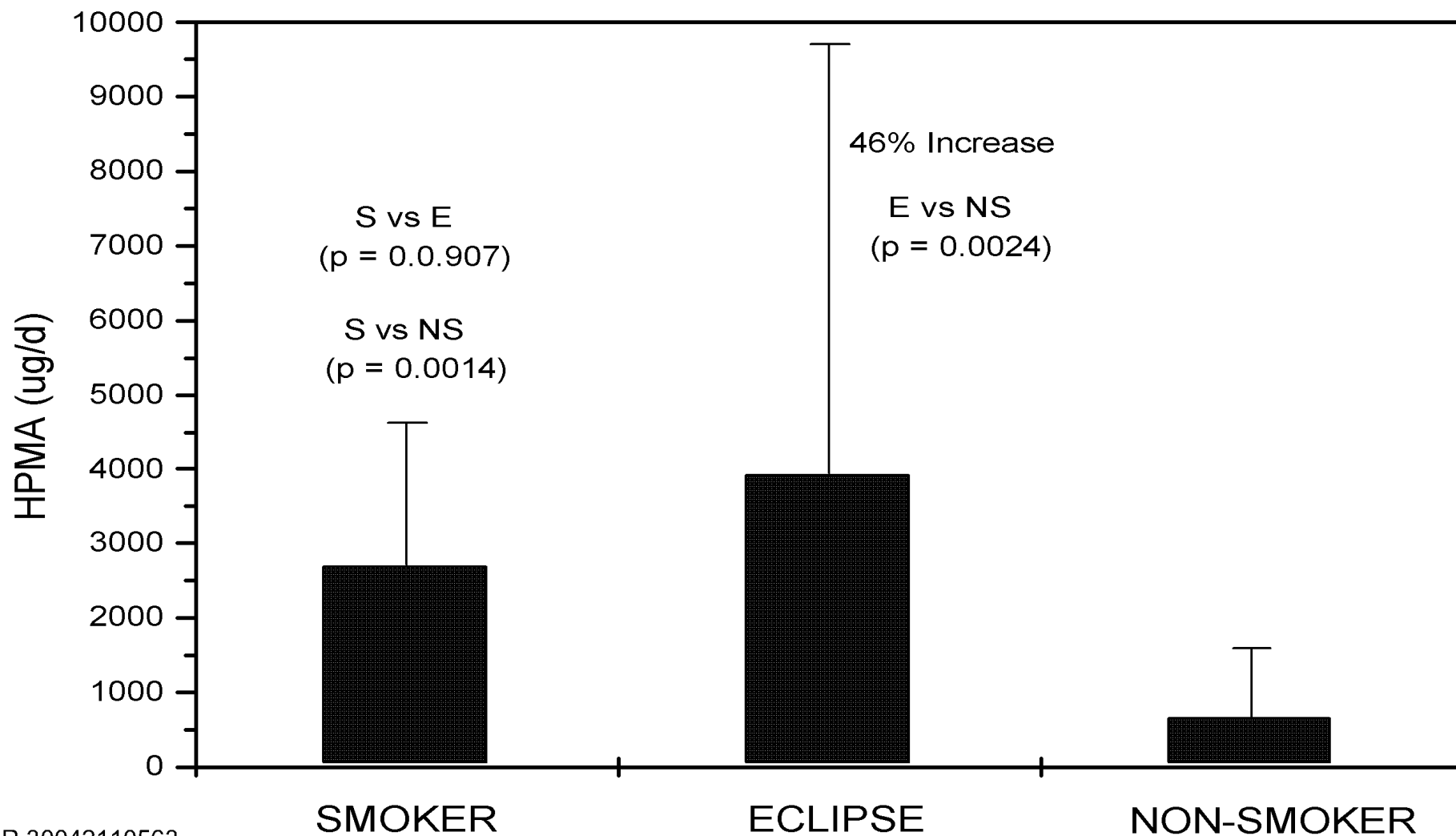
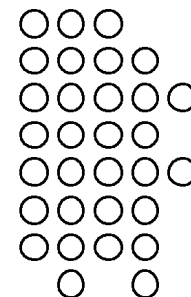
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Acrolein: 3-Hydroxypropylmercapturic Acid

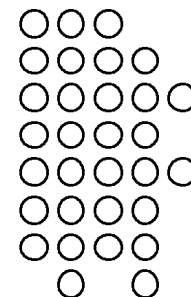


- Acrolein is formed during partial combustion of organic material (e.g. in forest fires, urban fires, exhaust emissions, and tobacco smoke).
- Acrolein is associated with pulmonary irritation and inflammation due to its cytotoxicity and ability to incur oxidative damage.
- In pulmonary exposures, chronic pulmonary irritation, inflammation, and sustained oxidative stress may ultimately contribute to pulmonary pathologies including chronic bronchitis, COPD, emphysema, and pulmonary cancers.
- In a recent study (Urban and Scherer: CORESTA 2002), smokers showed an ~ 3 X level of 3-HPMA compared to non-ETS exposed nonsmokers. There was also a good correlation between cotinine levels and 3-HPMA in smokers.

3-HYDROXYPROPYLMERCAPTURIC ACID (3-HPMA)



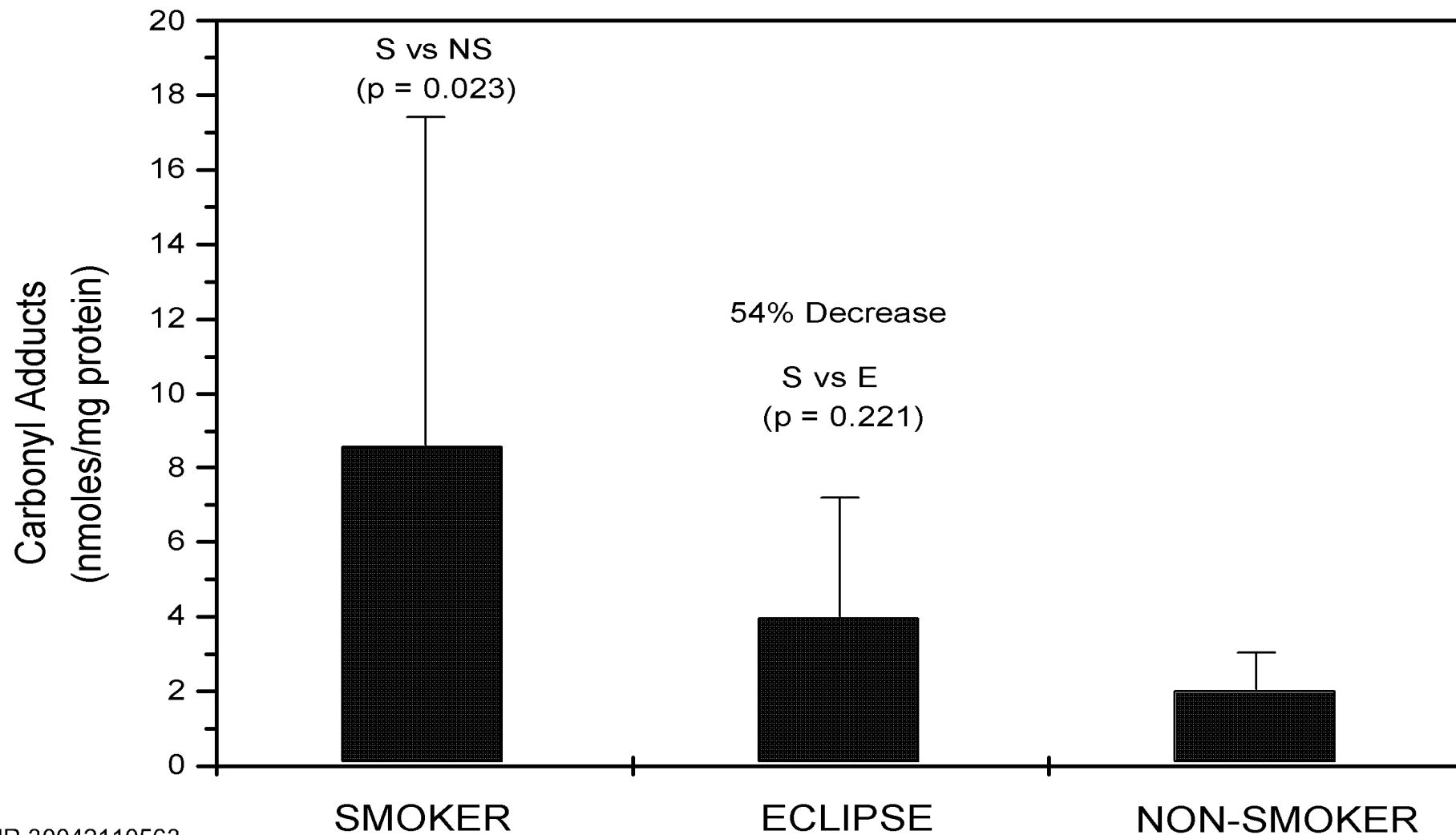
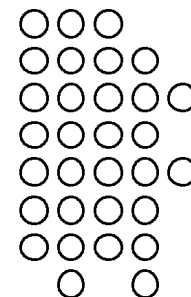
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Carbonyl Protein Adducts

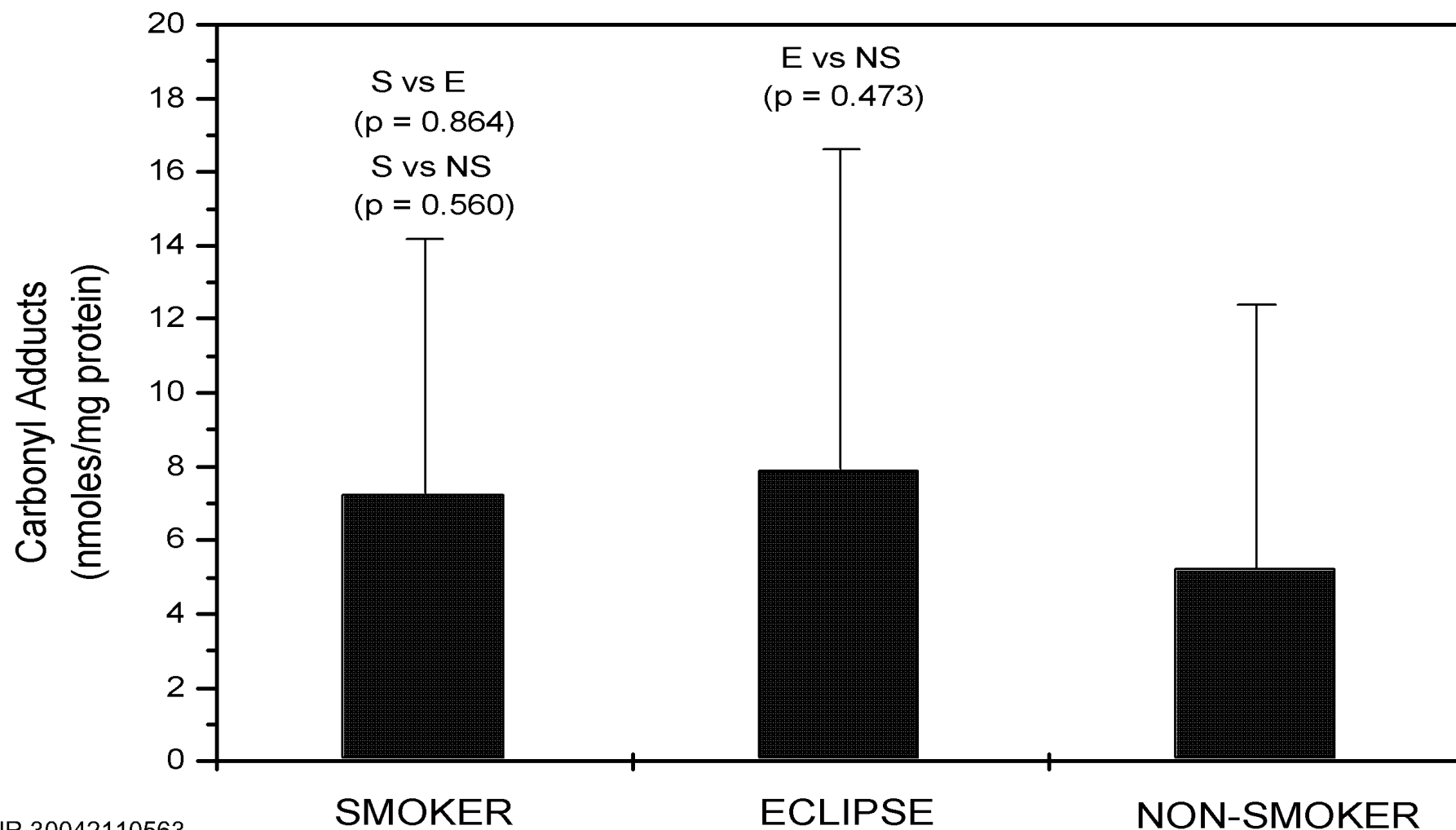
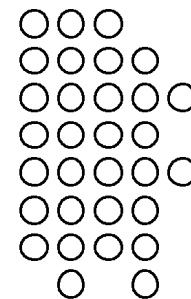
- Reactive aldehydes (present in tobacco smoke or formed within the body by oxidative reactions) result in the formation of carbonyl adducts on proteins.
- Increased carbonyl protein adducts are associated with increased oxidative stress and damage. Increased and sustained oxidative stress can result in chronic pulmonary irritation and inflammation.
- Other lifestyle factors (e.g., high alcohol consumption, chewing tobacco, and diets high in broiled or fried foods) could also impact the formation of carbonyl protein adducts.

CARBONYL PROTEIN ADDUCTS (AM MOUTHWASH)

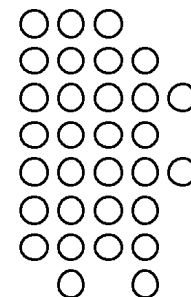


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CARBONYL PROTEIN ADDUCTS (PM MOUTHWASH)



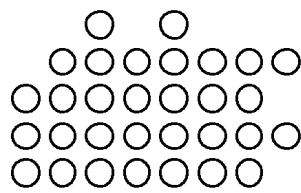
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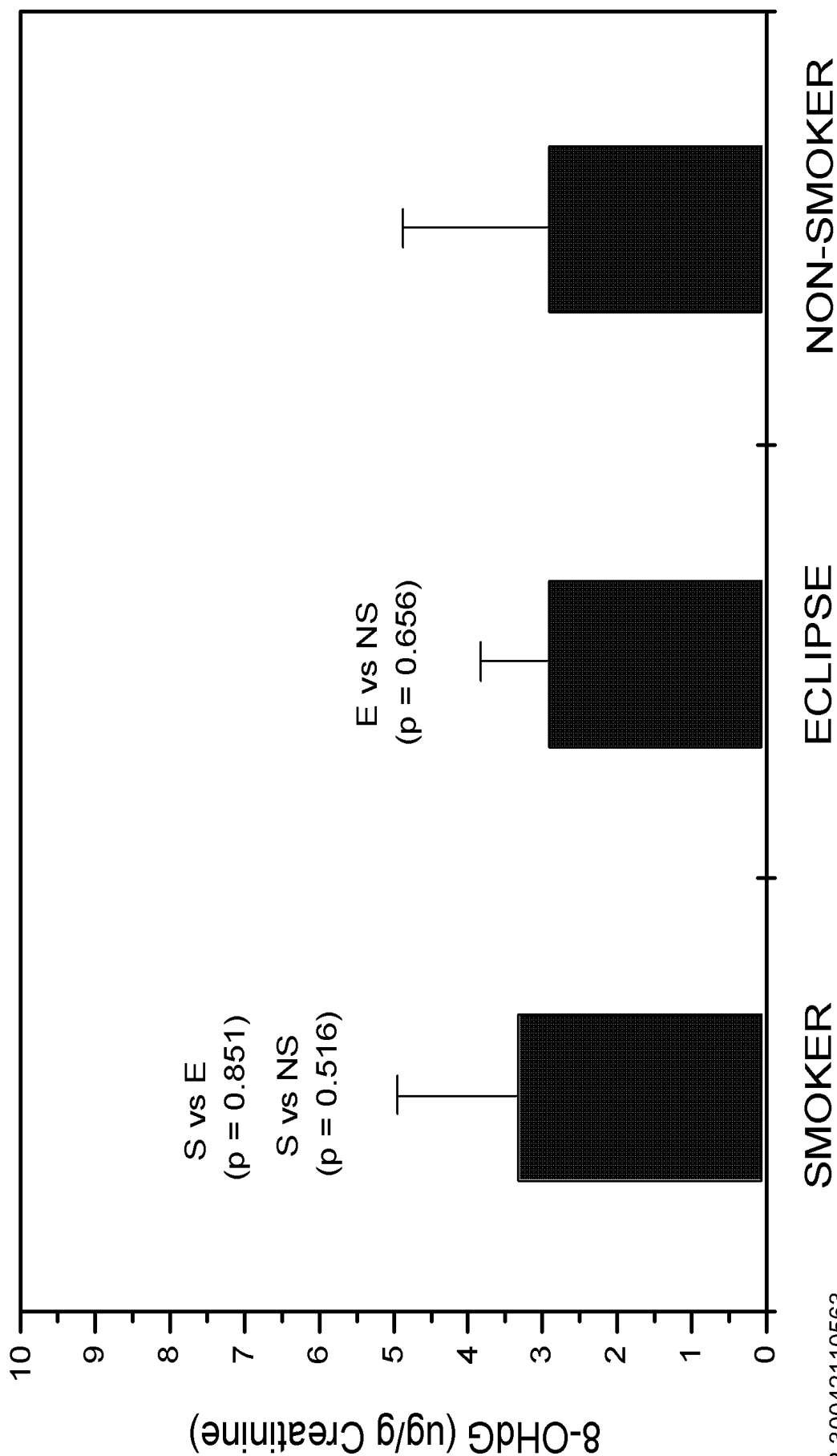
Oxidative Stress: 8-OHdG

- Cigarette smoke contains free radicals and may contribute to oxidative stress.
- Oxidative DNA damage can be assessed from the urinary excretion of the DNA-repair product 8-hydroxydeoxyguanosine (8-OHdG) and from the extracellular matrix of the mouth and pharynx obtained by mouthwash samples.
- The chemical basis for the effects of free radicals on DNA is probably due to oxidation and chemical modification of the bases. Such point mutations, if transcribed and translated (and not corrected) will lead to modified protein structure, which may also lead to altered functionality and disease.
- Diet-controlled studies conducted by RJRT have shown no statistically significant differences in urinary 8-OHdG between smokers and nonsmokers.

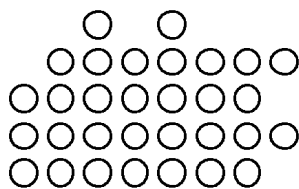
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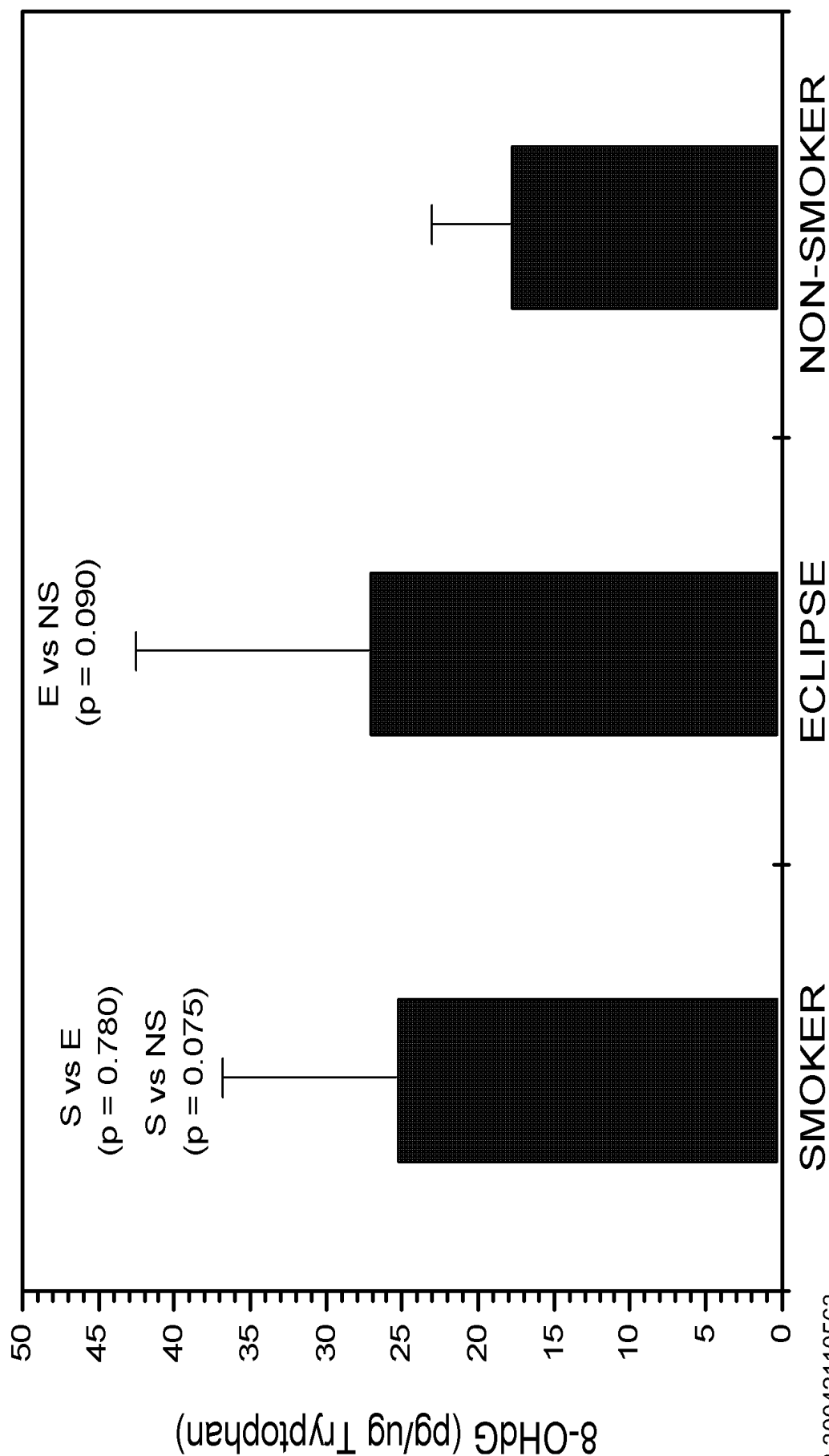
URINARY 8-OHdG



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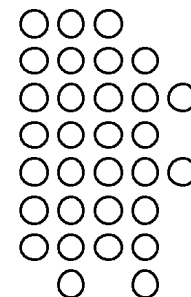
8-OHdG (MOUTHWASH)



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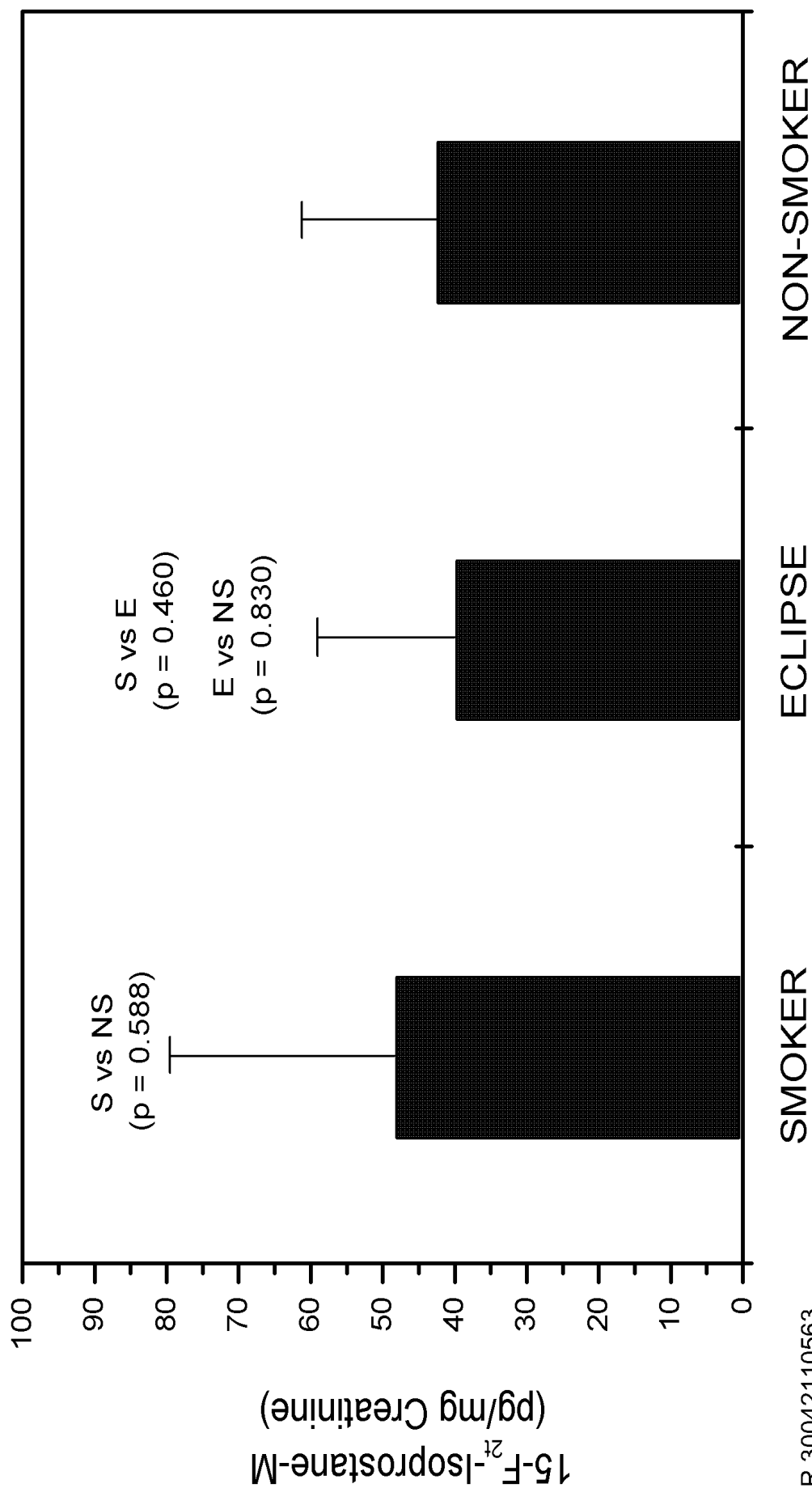
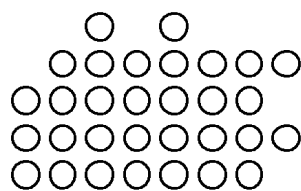
Oxidative Stress: Isoprostanes, 15-F_{2t}-Isoprostane-M



- F₂-isoprostanes are prostaglandin isomers produced by cyclooxygenase-independent free-radical peroxidation of arachidonic acid.
- Isoprostanes are sensitive & specific indicators of lipid peroxidation.
- Reilly et al., observed that levels in smokers were 122.5 ± 10.8 pmol/mmol creatinine and in nonsmokers were 63.7 ± 5.0 pmol/mmol creatinine; p<0.005.

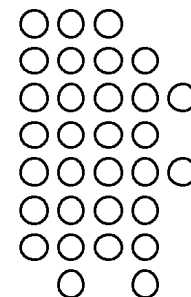
15-F_{2t}-ISOPROSTANE

(ELISA METHOD)



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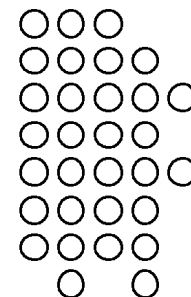
CONCLUSIONS

- Biomarkers significantly different between smokers and nonsmokers:

urinary nicotine and 5 nicotine metabolites
free and total NNAL
urinary 1-hydroxypyrene
urinary S-phenylmercapturic acid
urinary hydroxypropylmercapturic acid
carbonyl protein adducts in mouthwash

- Biomarkers not significantly different between smokers and nonsmokers:

urinary 8-OHdG
8-OHdG as determined in mouthwash (trend in smokers)
urinary t,t'-muconic acid
urinary 8-iso PGF₂ a isoprostane (ELISA)



CONCLUSIONS

- Some biomarkers are more sensitive than others in differentiating smokers and nonsmokers.
- Of the biomarkers not significantly different in this study, some may differ if studied in a greater number of subjects or in a different study design (for example, controlled diet).
- To develop PREPS, it is critical to establish baseline values for biomarkers in smokers and nonsmokers.