Cigarette smoke yield and carcinogen biomarkers

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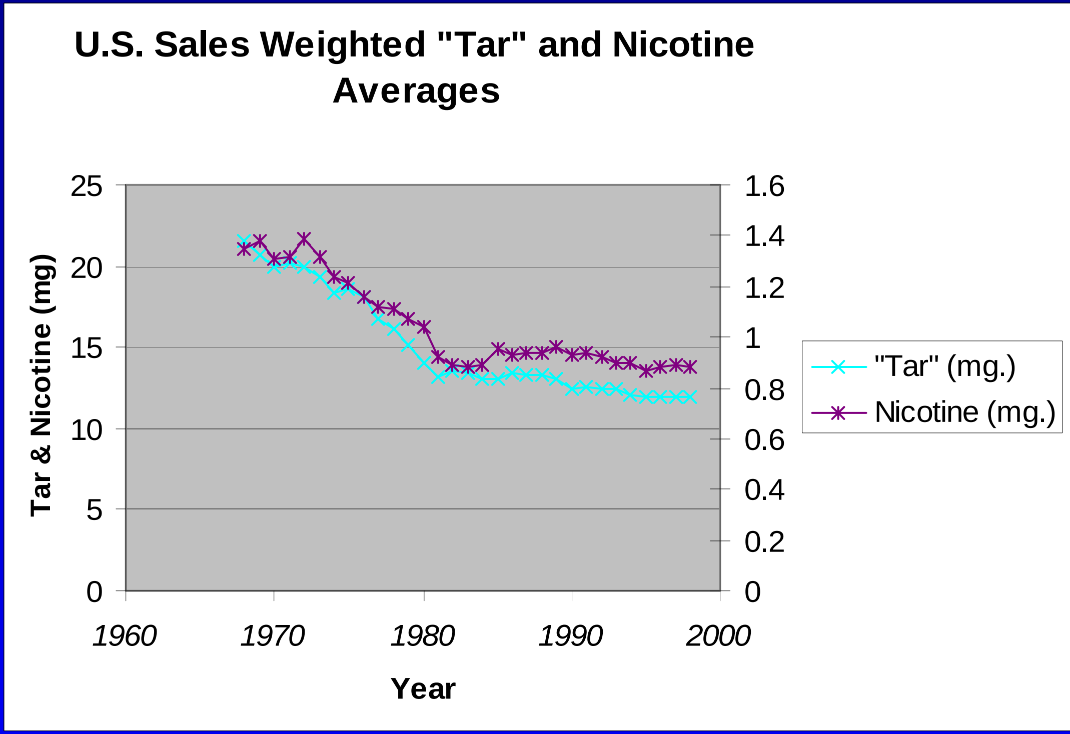
Objective:

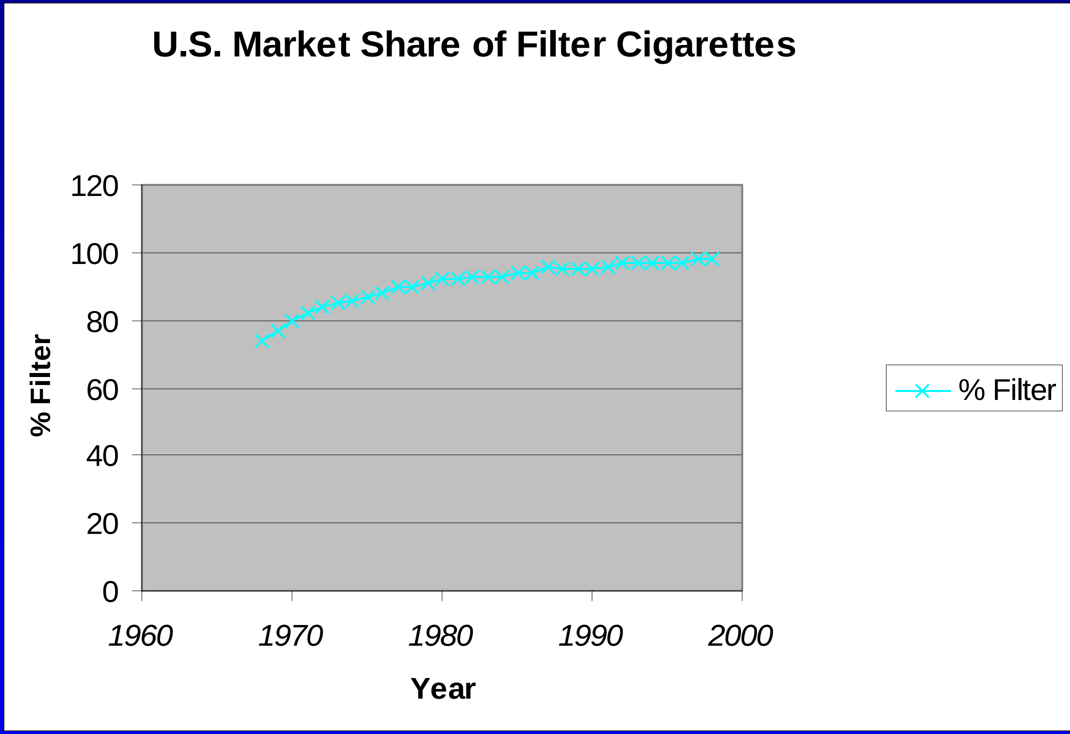
Determine whether biological exposure to tobacco smoke toxins varies by cigarette yield.

Background

- The sales-weighted FTC machine-measured “tar” and nicotine yields of cigarettes have declined substantially since 1950.
- There is conflicting data on the effect of harm reduction (e.g. lung cancer) and yield reduction.
- Some data indicate increased age-specific lung cancer death rates from 1950’s-1980s (Thun et al. Tobacco Control 2001 Suppl 1; i4-11).

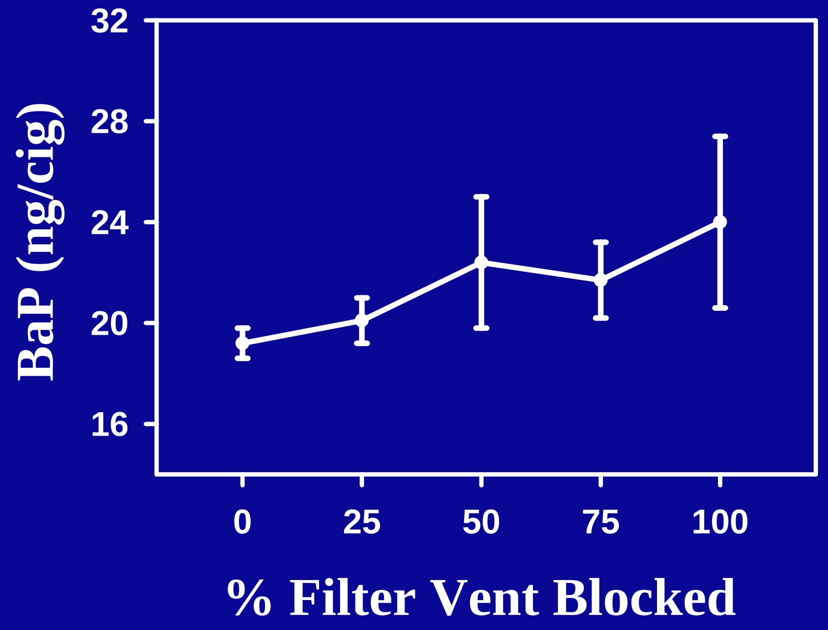

U.S. Sales Weighted "Tar" and Nicotine Averages
25
20
15
10
5
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Tar & Nicotine (mg)
1960
1970
1980
1990
2000
Year
"Tar" (mg.)
Nicotine (mg.)

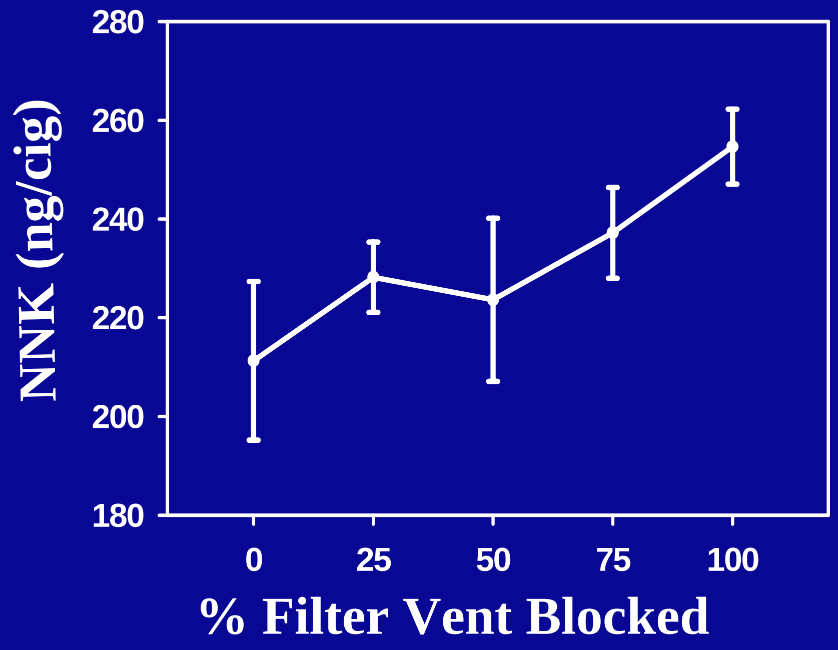
Source: FTC


U.S. Market Share of Filter Cigarettes
120
100
80
60
40
20
0
% Filter
1960
1970
1980
1990
2000
Year
% Filter

Source: FTC

Effects of Blocking Cigarette Filter Vents on Yields of Benzo(a)pyrene and NNK in Mainstream Smoke





BaP: n=3; NNK: n=5

Melikian et al, 2002

Problems with data on cigarette yield and cancer rates/risk

There is limited epidemiologic data on cigarette yield and cancer risk.

More data available on effects of cigarette filter than yield,although this too is limited.

Measuring compensatory behaviors including possible increase in cigarettes/day. Epi Studies adjust for cpd based on most recent cigarette smoked.

Temporal changes and measurement of confounders across time.

Methods

- The study is a community-based study of healthy adult non-Hispanic black and white current smokers with similar socioeconomic backgrounds from lower Westchester County, NY.
- Plasma and urinary cotinine, and SCN, were measured in nearly all subjects. NNAL+NNAL-Gluc assays were performed for 161 (46%) randomly selected subjects.
- Analytic methods:
- Urinary NNAL and NNAL-Gluc assessed by GC-TEA after purification by HPLC

1. Plasma and urinary cotinine assessed by ELISA
2. Plasma thiocyanate measured spectrophoto-metrically

Details of the analytic methods are described in:

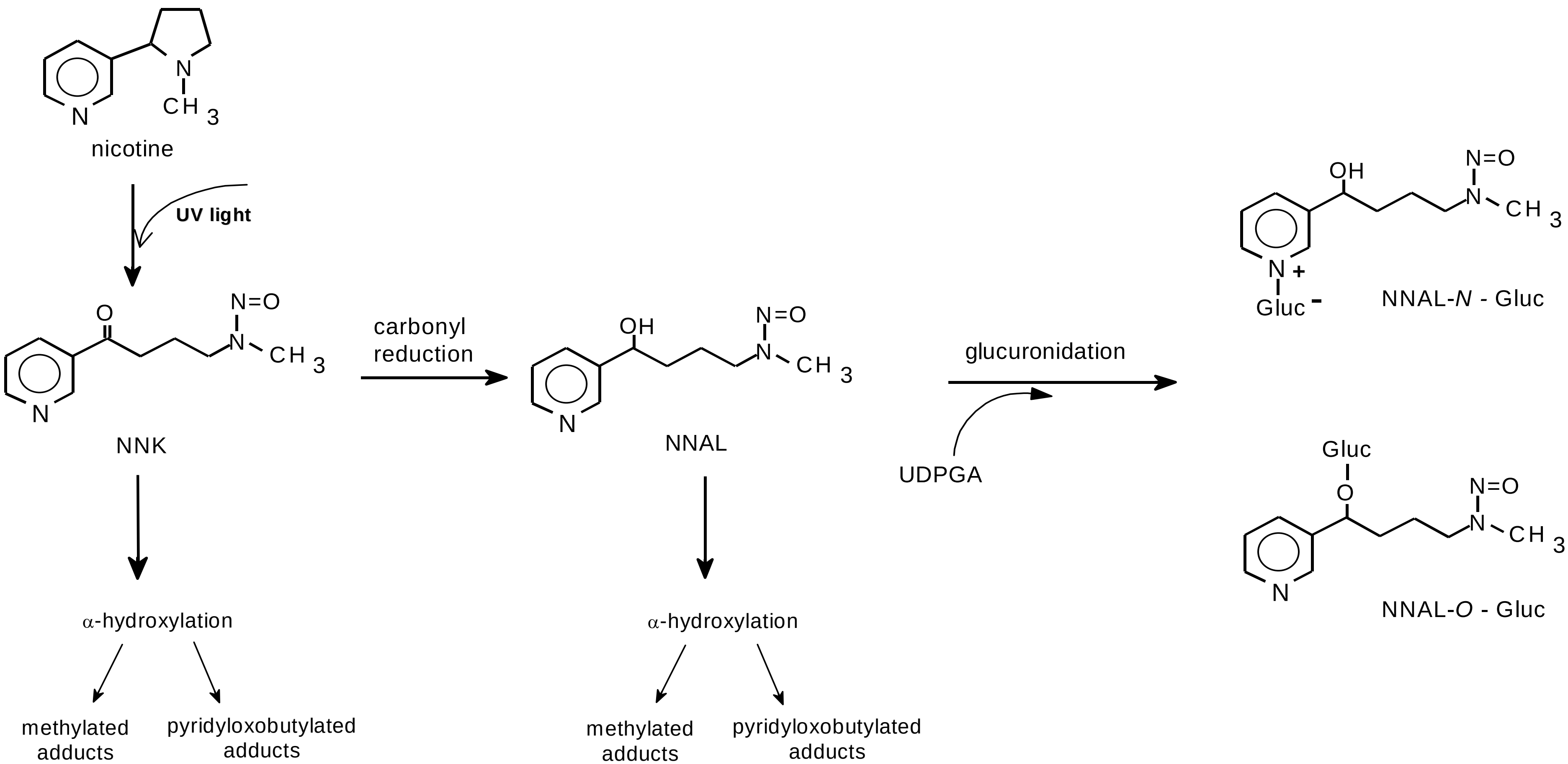
Richie et al. Differences in the urinary metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in black and white smokers. Cancer Epidemiol Biomarkers Prev. 1997;6:783-90.

Subject smoking characteristics

	Men			Women		
	Black (n=28)	White (n=47)	P-value	Black (n=41)	White (n=46)	P-value
Age started	15.5± 2.4	16.0± 3.5	0.41	17.0± 5.4	16.0± 4.1	0.34
Cigarettes per day	16.7 ± 8.9	23.7 ± 11.9	<0.01	14.0 ± 7.9	22.0 ± 10.3	<0.01
Years of smoking	18.7 ± 8.3	17.8 ± 10.9	0.72	17.8 ± 7.6	15.2 ± 9.6	0.15
Mean FTC nicotine (mg/cig)	1.2 ± 0.15	1.0 ± 0.27	<0.01	1.2 ± 0.22	0.87 ± 0.25	<0.01
(mg/day)	19.9 ± 11.2	24.4 ± 13.4	0.15	17.1 ± 11.2	20.1 ± 12.8	<0.01
Mean FTC tar content (mg/cig)	15.9 ± 1.9	13.2 ± 4.0	<0.01	15.6 ± 3.0	10.8 ± 3.7	<0.01
(mg/day)	268 ± 150	320 ± 198	0.23	223 ± 144	251 ± 171	0.41
Menthol (%)	78.6	17.0	<0.01	82.9	15.2	<0.01
Cigarette size (%)						
70-85 mm	71.4	87.3	N.S.	56.1	64.4	N.S.
100-120 mm	28.6	12.7		43.9	35.6	

N.S. Not significant.

Schematic of NNK metabolism to NNAL & its glucuronides



Correlation between total daily nicotine intake and total daily FTC “tar” intake with NNAL levels *(pmol/mg creat.)* in men

Daily nicotine intake			Daily FTC “tar” intake		
NNAL	NNAL-Gluc	TNNAL	NNAL	NNAL_Gluc	TNNAL
0.29	0.22	0.25	0.28	0.21	0.23

$p < 0.05$ for all comparisons.

Correlation between total daily nicotine intake and total daily FTC "tar" intake with NNAL levels *(pmol/mg creat.)* in women

Daily nicotine intake			Daily FTC "tar" intake		
NNAL	NNAL-Gluc	TNNAL	NNAL	NNAL_Gluc	TNNAL
0.31	0.32	0.36	0.30	0.32	0.36

$p < 0.01$ for all comparisons.

Total NNAL levels by FTC cigarette nicotine yield

	NNAL + NNAL-Gluc levels *(pmol/mg creat.)* *							
	Men				Women			
FTC nicotine yield	N	NNAL	NNAL-G	TNNAL	N	NNAL	NNAL-G	TNNAL
Low *(≤ 1.0 mg)*	24	0.79	1.89	2.66	38	0.72	2.02	2.74
Medium *(1.1-1.2 mg)*	41	0.71	2.29	3.00	34	1.25	3.52	4.77
High *(≥ 1.3 mg)*	10	0.54	1.60	2.13	15	1.47	3.43	4.90

* Adjusted for age, race and cigarettes per day.

Total NNAL levels by cigarette type

	NNAL + NNAL-Gluc levels *(pmol/mg creat.)* *							
	Men				Women			
Cigarette type	N	NNAL	NNAL-G	TNNAL	N	NNAL	NNAL-G	TNNAL
Ultralight *(≤ 6.5 mg)*	1	0.76	1.6	2.46	6	0.65	2.0	2.59
Light *(>6.5-14.5 mg)*	23	0.86	2.0	2.93	35	0.73	2.3	3.01
Regular *(≥ 14.5 mg)*	50	0.65	2.1	2.74	46	1.35	3.4	4.76

* Adjusted for age, race and cigarettes per day.

Total NNAL levels in smokers of menthol vs. 'plain' cigarettes

TNNAL	NNAL+NNAL-Gluc levels *(pmol/mg creat.)*	
	Men	Women
Menthol	2.63	3.59
"Plain"	2.86	4.19

* Differences in means are not significant. Adjusted for age, race, cigarettes per day, and "tar" yield.

Urinary total NNAL, 1-HOP, and cotinine per CPD

	n	Mean (95% CI)		
		Total NNAL (pmol/mg creatinine/CPD)	1-HOP (pmol/mg creatinine/CPD)	Total cotinine (pmol/mg creatinine/CPD)
Regular	23	0.093 (0.077-0.109)	0.085 (0.064-0.105)	1.28 (0.975-1.58)
Light	58	0.106 (0.096-0.117)	0.061 (0.050-0.072)	1.11 (0.974-1.24)
Ultralight	34	0.106 (0.083-0.130)	0.069 (0.054-0.085)	1.18 (0.942-1.42)

Source: Hecht SS et al. Cancer Epidemiology Biomarkers & Prevention 2005;14:693.

Table 3. Exposure to tobacco-specific nitrosamines and PAHs while smoking usual brand and light cigarettes

		Week 1 (usual brand)	Week 2 (light)	Week 3 (usual brand)	Overall, *P*
Total NNAL (pmol/mg creatinine)					
	Mean	2.3	2.0	2.1	0.54
	SD	1.6	1.6	1.9	
	95% CI	(1.4-3.2)	(1.0-3.0)	(0.8-3.3)	
Free NNAL (pmol/mg creatinine)					
	Mean	1.1	1.1	1.0	0.81
	SD	0.8	0.9	0.9	
	95% CI	(0.7-1.6)	(0.6-1.6)	(0.4-1.6)	
1-Hydroxypyrene (pmol/mg creatinine)					
	Mean	1.7	2.1	1.9	0.72
	SD	0.9	2.8	1.5	
	95% CI	(1.2-2.1)	(0.6-3.6)	(1.1-2.7)	
1-Naphthol and 2-naphthol (pmol/mg creatinine)					
	Mean	181.5	166.4	178.6	0.56
	SD	88.2	70.8	86.9	
	95% CI	(134.5-228.6)	(128.6-204.1)	(128.4-228.8)	
Sum of hydroxyfluorenes (pmol/mg creatinine)					
	Mean	32.3	30.1	35.4	0.98
	SD	11.8	20.7	25.2	
	95% CI	(26.0-38.6)	(18.6-41.5)	(21.4-49.4)	

Source: Benowitz et al. CEBP 2005; 14:1736-1783.

Summary of carcinogen biomarker data

- Cigarette FTC yield among smokers of filter cigarettes is unrelated to urinary carcinogen biomarker levels.

- Cigarette type (e.g. ultralight, light, regular) is unrelated to urinary carcinogen biomarker levels in men. There is a modest association in women.

- Marketed cigarette flavoring agent (e.g. menthol) is unrelated to urinary carcinogen biomarker levels.

Second objective:

Determine whether biological exposure to tobacco-induced oxidative stress varies by cigarette yield.

Components of tobacco smoke

- 4,800 compounds
- 122 biologically active compounds in condensate (IARC).
- Particulate phase carcinogens include PAH, N-Nitrosamines, Aromatic Amines, Metals.
- Suggested gas phase carcinogens include NO, benzene, formaldehyde, acetaldehyde, others.

Source: Hoffmann et al. 2001; Chem. Res. Toxicol., 14: 767-790.

Free radicals in tobacco smoke

- Gas phase components: 10^{14} C and O radicals per puff. NO is also abundant.
- Particulate phase: quinones, semiquinones (less reactive)
- Tobacco smoke is sometimes considered the litmus test for the validity of biological markers to oxidative damage

Evidence for tobacco-induced oxidative stress

- Smokers have reduced circulating levels of ascorbic acid, carotene and other micronutrients vs. nonsmokers. Association is independent, although partly due to dietary intake differences.
- Diet rich in fruits and vegetables associated with decreased cancer incidence.
- Smokers have increased levels of 8-OHdG and isoprostanes.

Protein glutathiolation as a marker of oxidative stress

Significance:

Direct evidence for a role of oxidative stress in cancer is lacking in part because biological markers are not responsive to the subtle and chronic changes in overall oxidative stress levels, and provide information only on specific forms of oxidative damage (e.g. 8-OHdG). The difference between biomarkers of oxidative **stress** and oxidative **damage**, while perhaps not widely recognized, is important since recent concepts on oxidative stress have emphasized the regulation of key cellular activities through pathways that do not involve direct and irreversible damage of macromolecules.

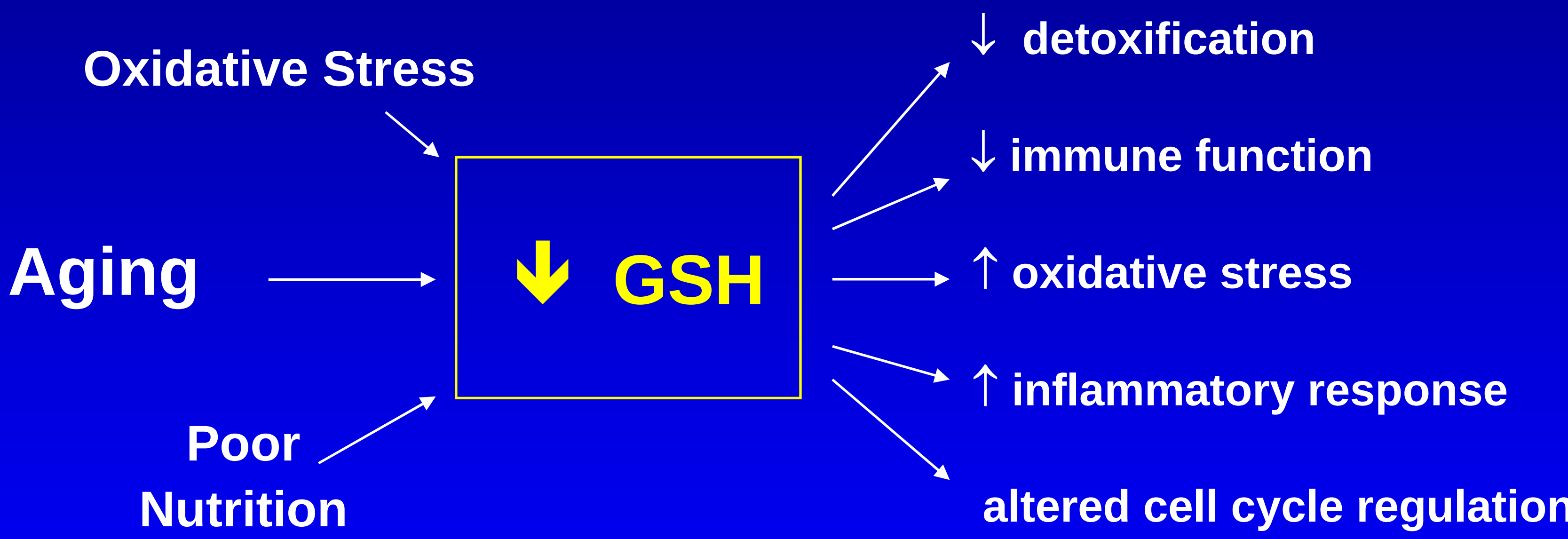
Glutathione (GSH)

- **Ubiquitous tripeptide (γ-Glu-Cys-Gly)**

- **Most abundant antioxidant in animal tissues**

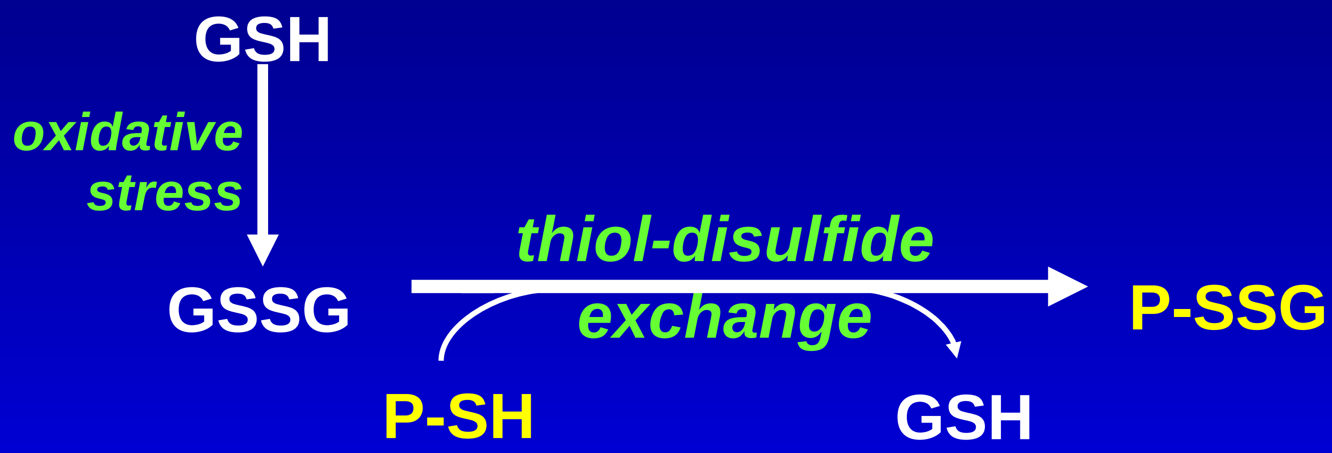
- **Functions:**
  - Major intracellular redox buffer
  - Detoxification of xenobiotics and endobiotics
  - Preservation of protein structure
  - Maintenance of immune function
  - Regulation of protein function

Role of Glutathione in Carcinogenesis



Protein Glutathiolation

(or "glutathionylation")



- The reversible covalent addition of glutathione to cysteine residues on the target proteins.
- Enhanced in oxidative stress & aging

Proteins Regulated by Glutathiolation

- *Ubiquitin conjugating enzymes*
- *PKC isozymes*
- *NF-κB*
- *c-jun*
- *alpha-ketoglutarate dehydrogenase*
- *cAMP-dependent protein kinase*
- *GSTs*
- *Mitochondrial Complex-1*
- *betaA1/betaA3-crystallins*
- *carbonic anhydrase III*
- *creatine kinase*
- *NADP+-dependent isocitrate dehydrogenase*
- *endoplasmic reticulum calcium (Ca(2+)) ATPase (SERCA)*
- *glyeraldehyde-3-phosphate dehydrogenase*
- *S100A1 and S100B (acidic calcium binding proteins)*
- *hemoglobin*
- *actin*
- *nucleoside diphosphate kinase B*
- *protein phosphatase 2A*
- *Thioredoxin*
- *thioredoxin peroxidase II*
- *thiosephosphate isomerase*
- *tyrosine hydroxylase*
- *1-Cys peroxiredoxin*
- *carbonic anhydrase III*
- *aconitrate hydrase*

Protein Glutathiolation in Different Tissues of the Rat

Tissue	Protein-bound Glutathione μmol GSH/g tissue	Protein-bound Glutathione nmol GSH/mg protein
Red Cell	40 – 300	350 – 2600
Plasma	1 –10	8 – 80
Colon	82	387
Heart	74	450
Kidney	90	620
Liver	210	1100
Oral mucosa	50	270

Enhanced Protein Glutathiolation in Tumor Tissues

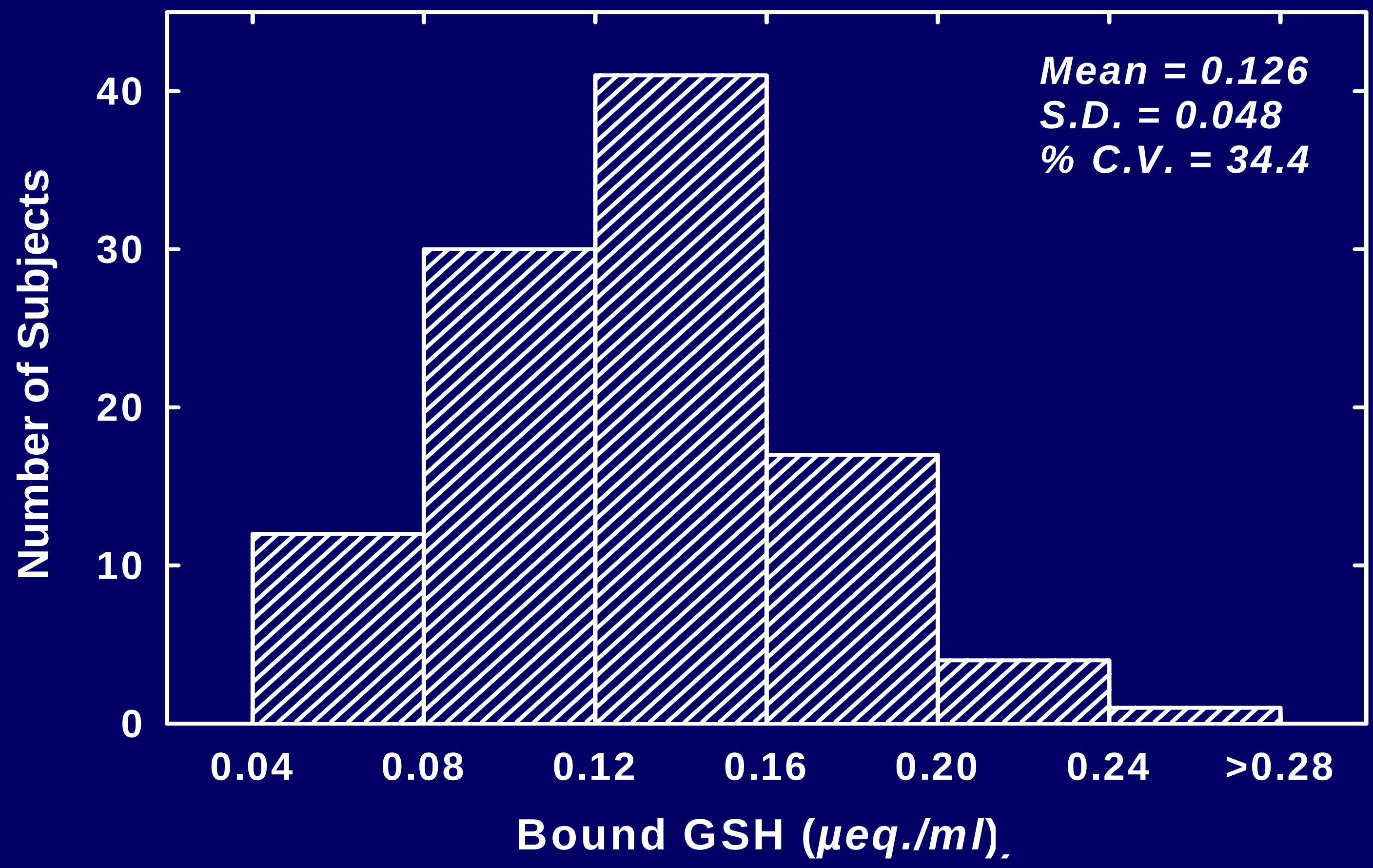
Laboratory animal models:

- Tongue (4-NQO, rat) - 50-70% ↑
- Colon (azoxymethane, rat) - 50-100% ↑
- Liver (LEC rat) - 2-3-fold ↑

Human tumors:

- Lung SCC - 2-3-fold ↑
- Oral SCC - 2-fold ↑
- Mammary - 50% ↑
- Prostate - 30% ↑
- Colon - 2-fold ↑

Protein Glutathiolation in human blood



Kleinman et al., Biochem. Pharm. 65: 741 (2003)

Increased Protein Glutathiolation in Smokers

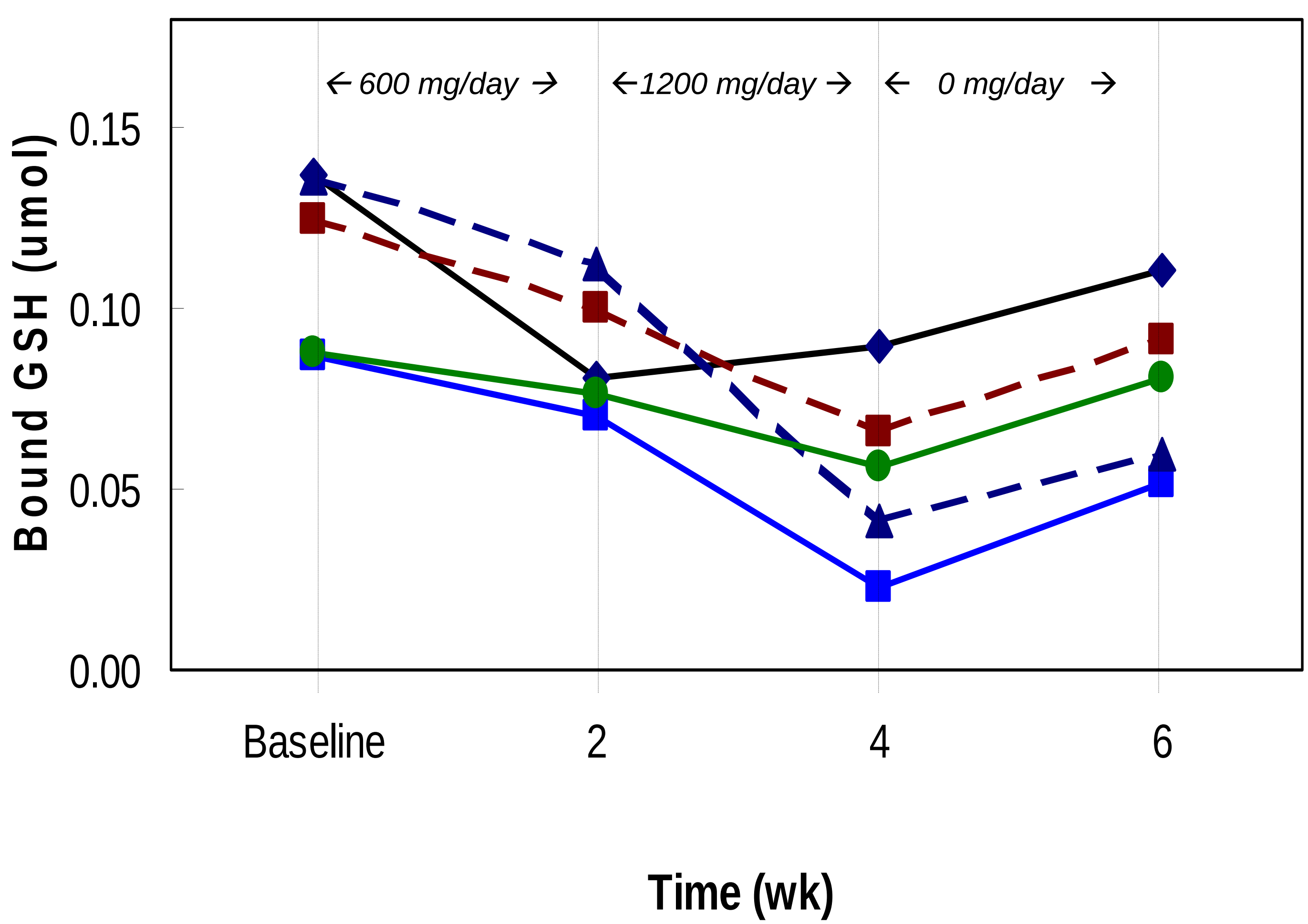
Correlation of GSSP with Levels of Smoking:

Cigarettes per day	Cotintne *(ng/ml)*	Thiocyanate *(nmol/ml)*
r = 0.39 *	r = 0.38 *	r = 0.40 *

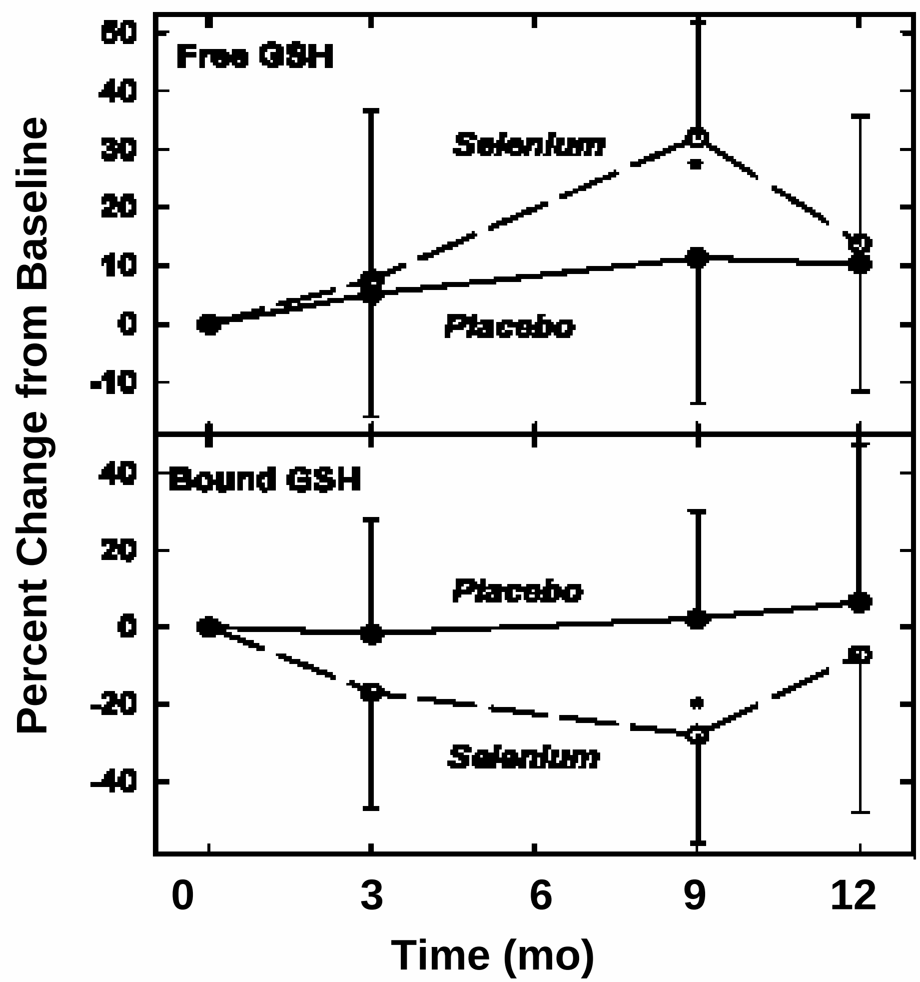
* *P<0.01*

Muscat et al., Free Radicals Biol. Med. In press.

Effect of N-Acetylcysteine (NAC) on Blood GSSP Levels in Healthy Adult Males



Effect of Selenium-Yeast Supplementation on Blood GSH and GSSP Status



El-Bayoumy et al., CEBP 11:1459 (2003).

Correlation between total daily nicotine intake and total daily FTC “tar” intake with GSH levels *(µmol/ml)* in 112 male smokers

Daily nicotine intake			Daily FTC “tar” intake		
BGSH	BGSH/Hb	Bound/free	BGSH	BGSH/Hb	Bound/Free
0.06	0.03	0.01	0.07	0.04	0.03

p = n.s. for all values. Unit for Hg is grams.

Correlation between total daily nicotine intake and total daily FTC "tar" intake with GSH levels *(μmol/ml)* in 103 female smokers

Daily nicotine intake			Daily FTC "tar" intake		
BGSH	BGSH/Hb	Bound/free	BGSH	BGSH/Hb	Bound/Free
0.15	0.09	0.11	0.15	0.07	0.13

p = n.s. for all values. Unit for Hg is grams.

Total BGSH *(μmol/ml)* levels by FTC cigarette nicotine yield

	Men				Women			
FTC nicotine yield	N	BGSH	BGSH/Hb	BGSH/GSH	N	BGSH	BGSH/Hb	BGSH/GSH
Low *(≤1.0 mg)*	35	0.16	7.1	1.5	50	0.15	7.7	1.4
Medium *(1.1-1.2 mg)*	58	0.16	7.3	1.6	36	0.22	9.7	1.6
High *(≥1.3 mg)*	17	0.16	7.1	1.5	16	0.18	9.6	1.7

* Adjusted for age, race and cigarettes per day.

Total BGSH (*μmol/ml*) levels by cigarette classification

		Men			Women	
FTC nicotine yield	N	BGSH	BGSH/GSH	N	BGSH	BGSH/GSH
Ultralight *(≤ 6.5 mg)*	1	0.17	0.10	9	0.19	0.16
Light *(>6.5-14.5 mg)*	30	0.16	0.16	42	0.14	0.14
Regular *(≥ 14.5 mg)*	81	0.16	0.15	51	0.18	0.16

Adjusted for age, race and cigarettes per day.

Total BGSH levels *(μmol/ml)* in smokers of menthol vs. ‘plain’ cigarettes

BGSH	Men	Women
Menthol	0.16	0.20
“Plain”	0.16	0.15

* Differences in means are not significant. Adjusted for age, race, and cigarettes per day.

Summary of protein glutathiolation biomarker data

- Cigarette FTC yield among smokers of filter cigarettes is unrelated to urinary carcinogen biomarker levels.
- Cigarette type (e.g. ultralight, light, regular) is unrelated to blood BGSH levels in men and in women.
- Marketed cigarette flavoring agent (e.g. menthol) is unrelated to urinary carcinogen biomarker levels.

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