NIEHS Superfund Research Program

Health Effects and Geochemistry of Arsenic and Manganese

Columbia University

Columbia University

Associations of glutathione and arsenic methylation in Bangladesh

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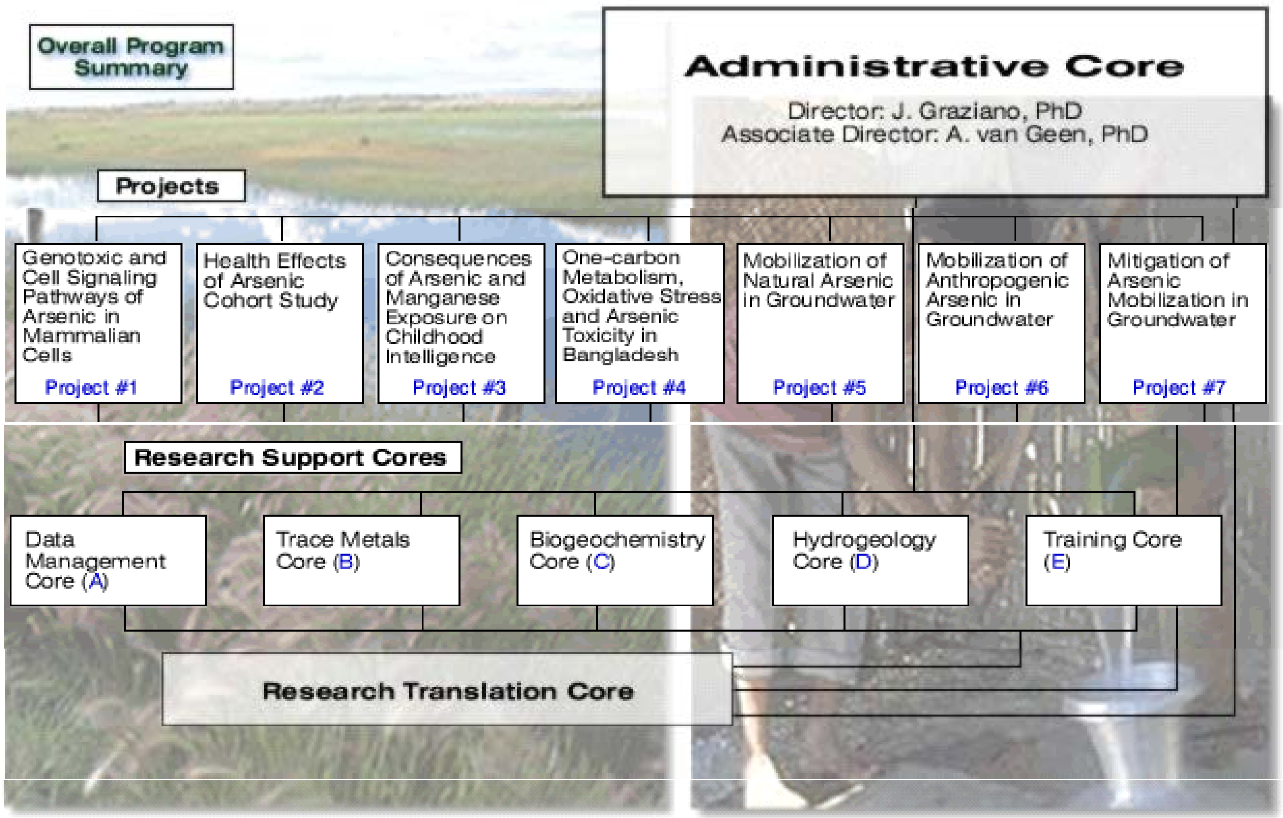
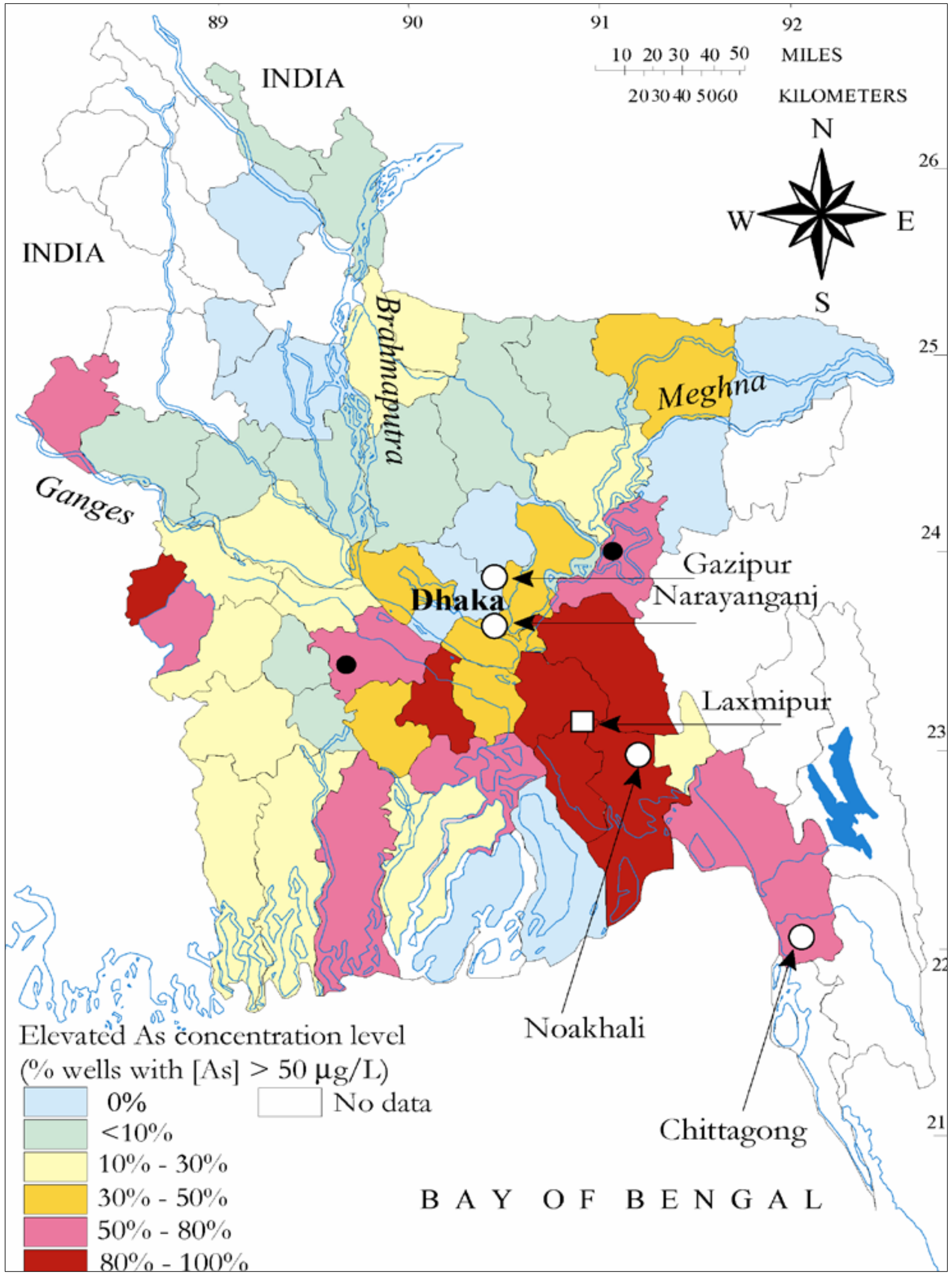


http://superfund.ciesin.columbia.edu



89
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MILES
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KILOMETERS
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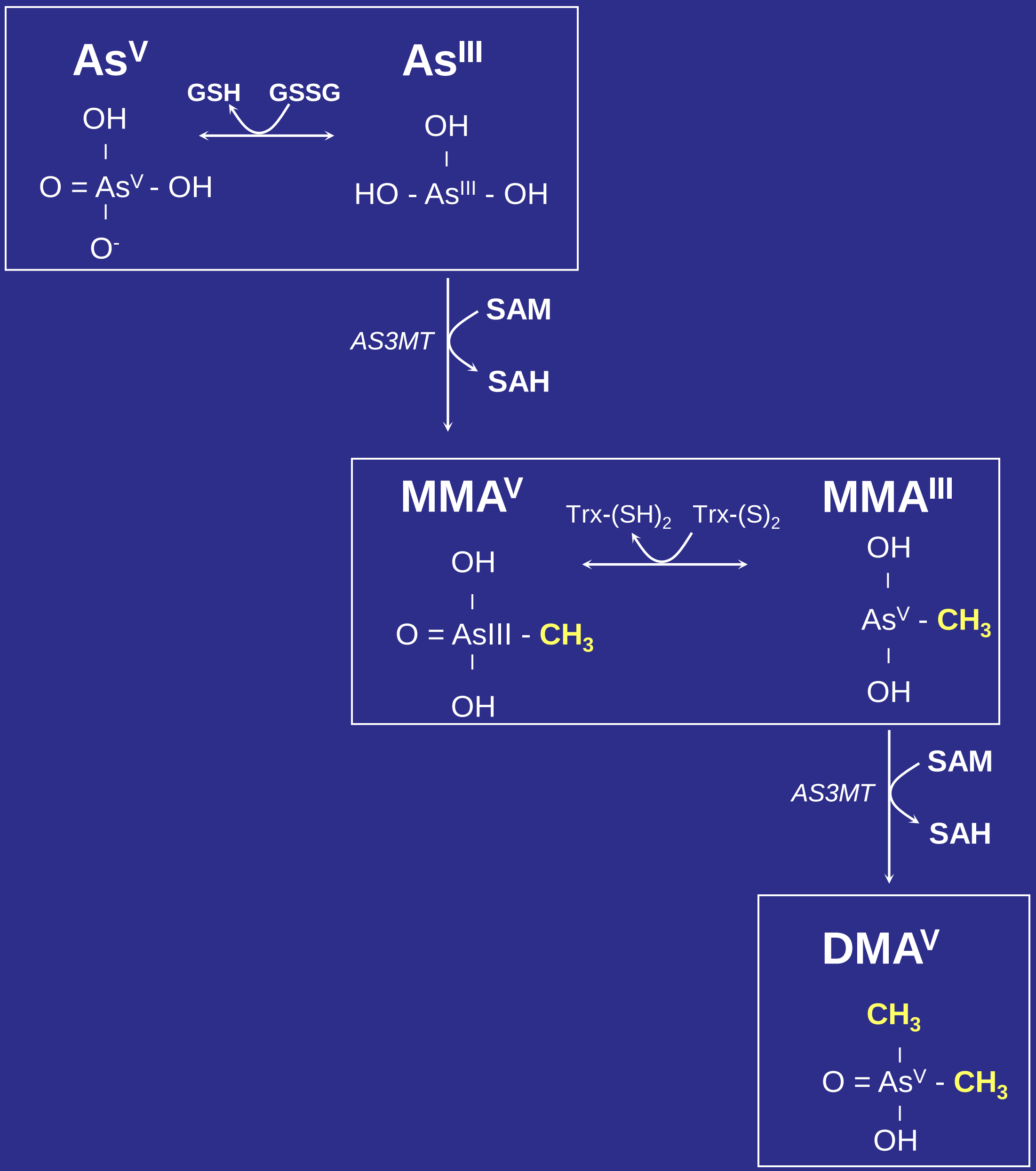
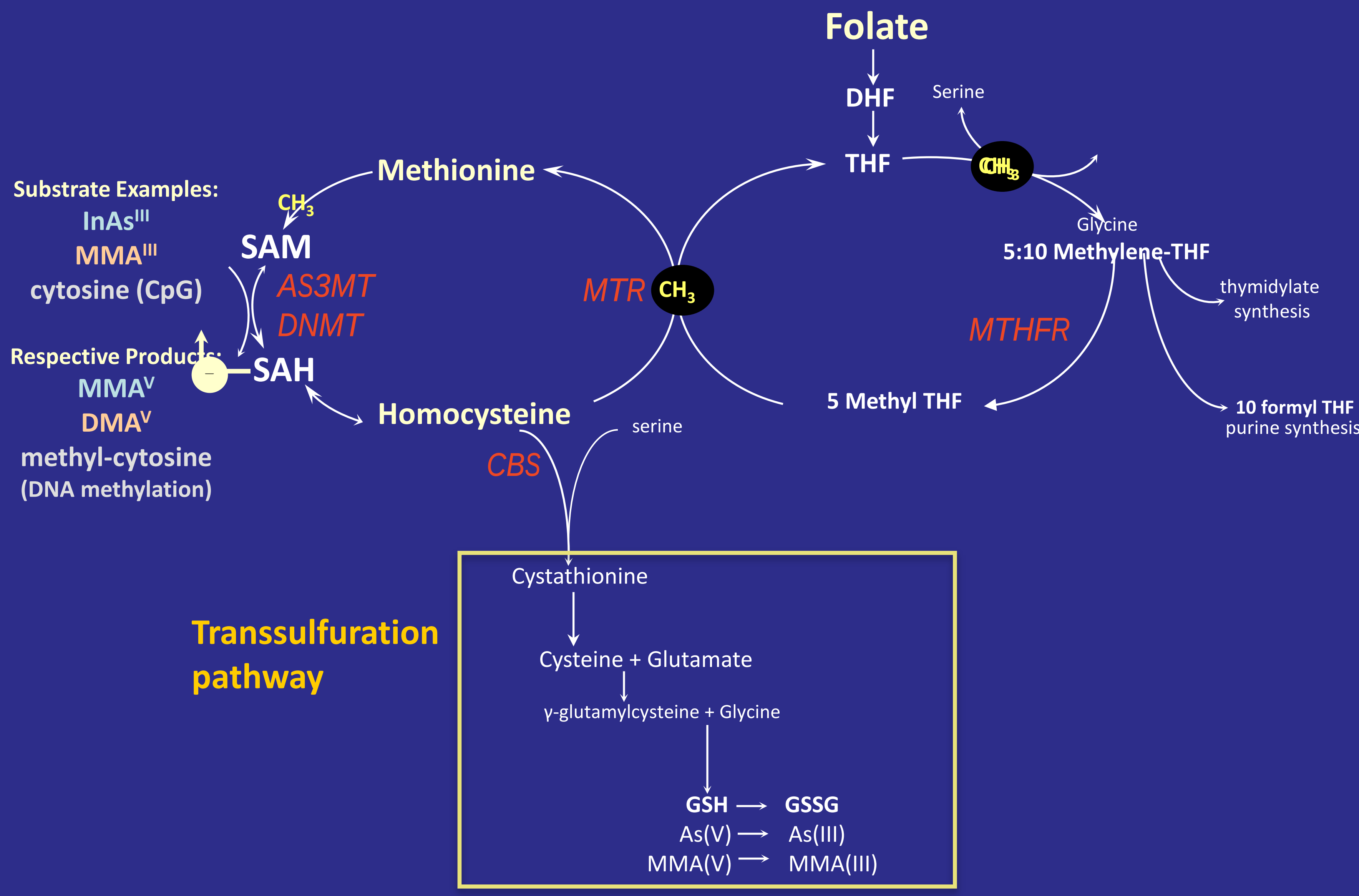
Ganges
Gazipur
Narayanganj
Dhaka
Laxmipur
Noakhali
Chittagong
BAY OF BENGAL
Elevated As concentration level
(% wells with [As] > 50 µg/L)
0%
No data
<10%
10% - 30%
30% - 50%
50% - 80%
80% - 100%

Arsenic is methylated in the body



Arsenic is methylated via one-carbon metabolism

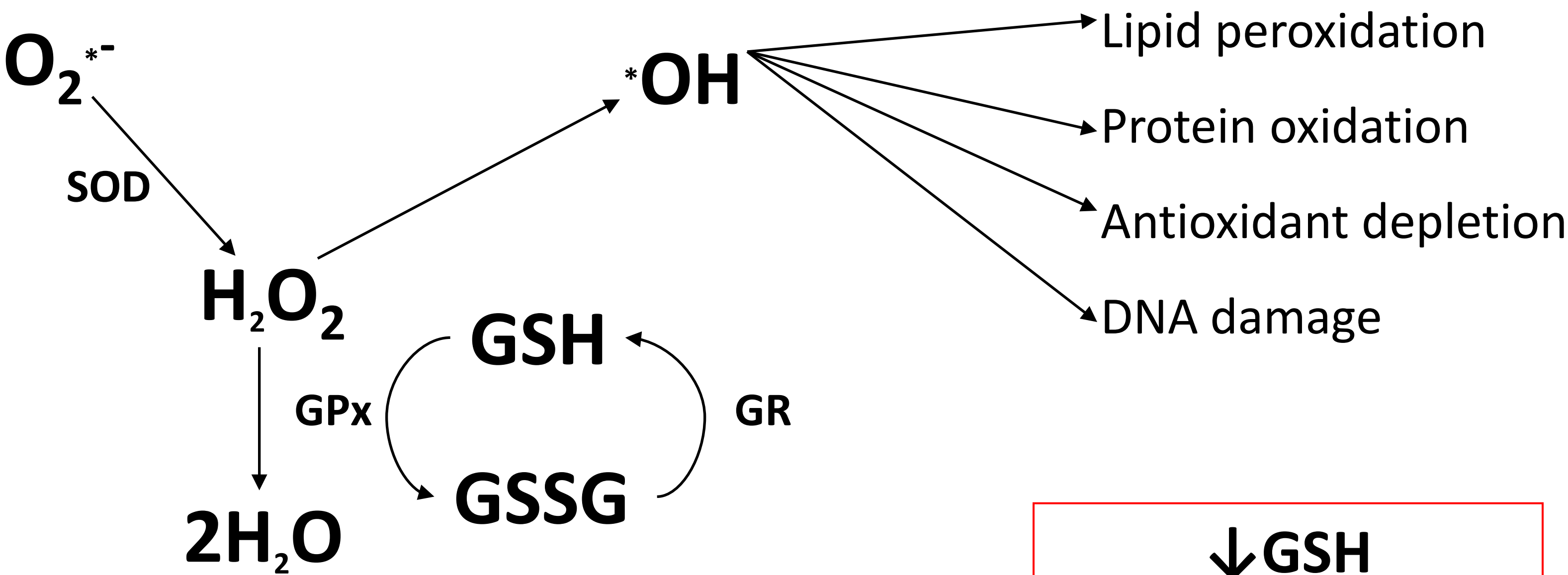


Oxidative stress may decrease arsenic methylation in two ways:

1. Upregulation of GSH production leads to decreased SAM production

2. Changes in redox inhibit methyltransferase enzymes

Glutathione and oxidative stress



↓GSH

↑GSSG

↓GSH/GSSG

One-carbon metabolism under pro-oxidant conditions

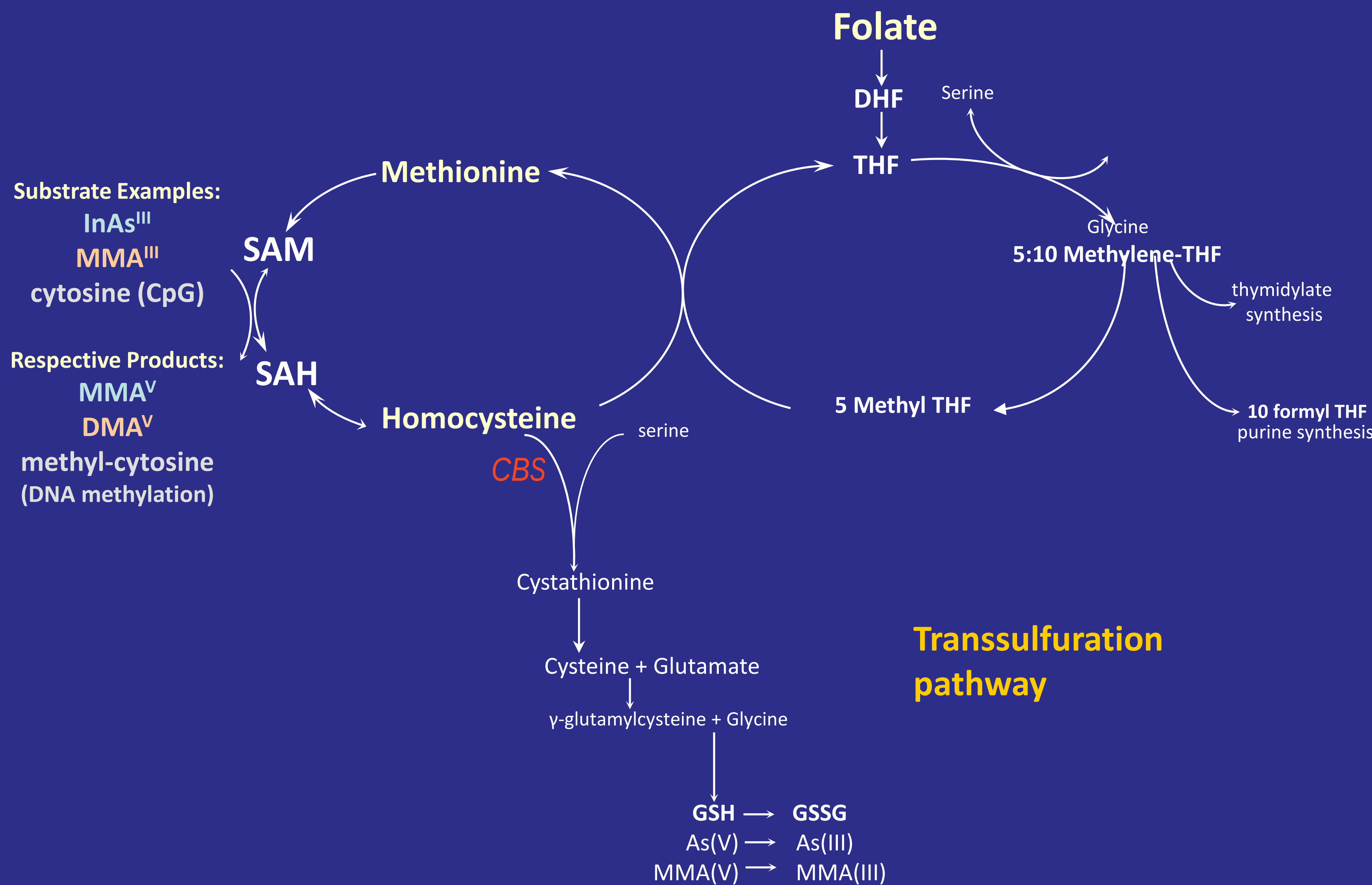


Figure courtesy of Dr. Mary Gamble

Redox, E (mV) calculation, and interpretation

Redox state = energetic force for electron transfer; measures ability of compound to donate or receive electrons (*reduction potential*)

Nernst equation:

E (mV) = E_0(-RT/*n*F) * ln([reductant]/[oxidant])

E (mV) = -264 – 30 * log([**GSH**]^2/[**GSSG**])

E_0 = standard reduction state at pH 7 (-264 mV for GSH)

R = gas constant

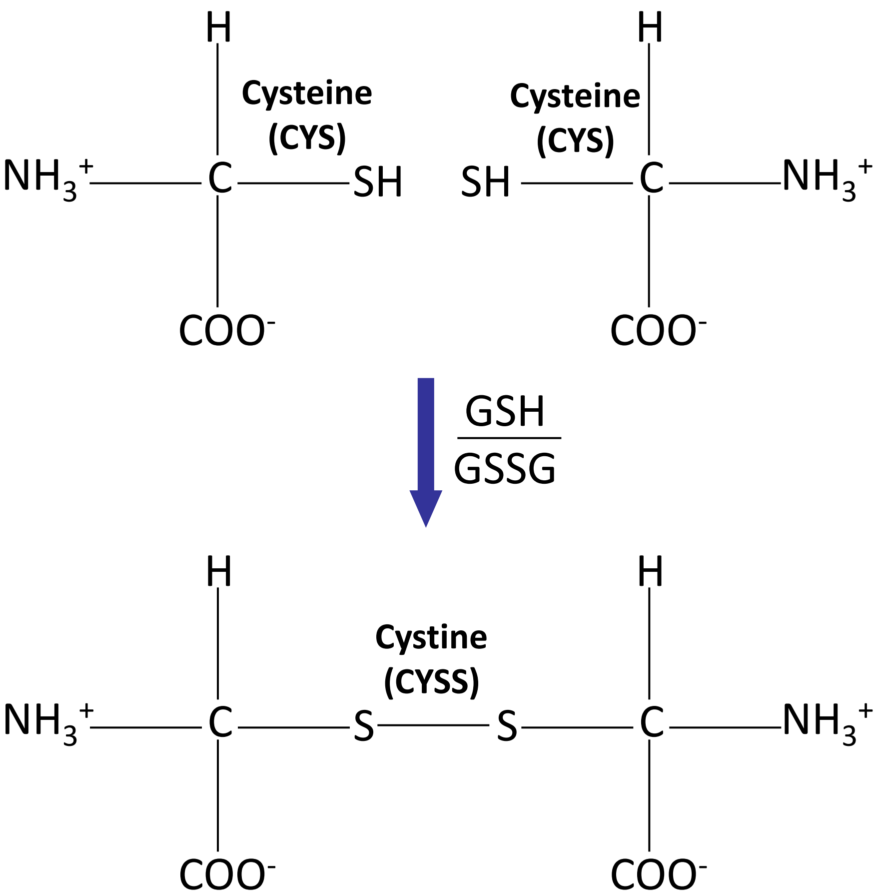
T = temperature in Kelvin

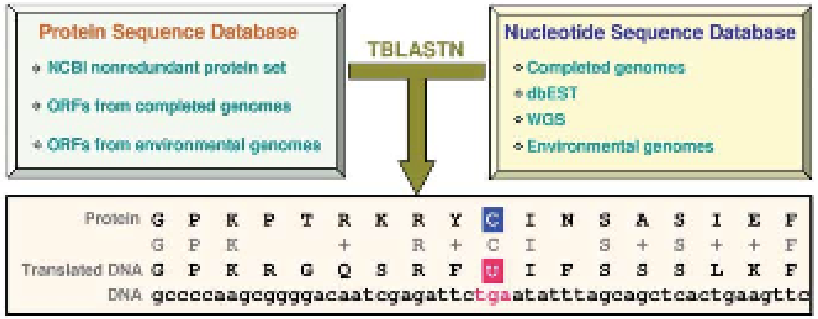
n = number of transferred electrons

F = Faraday's constant

(Brewer, 2010)

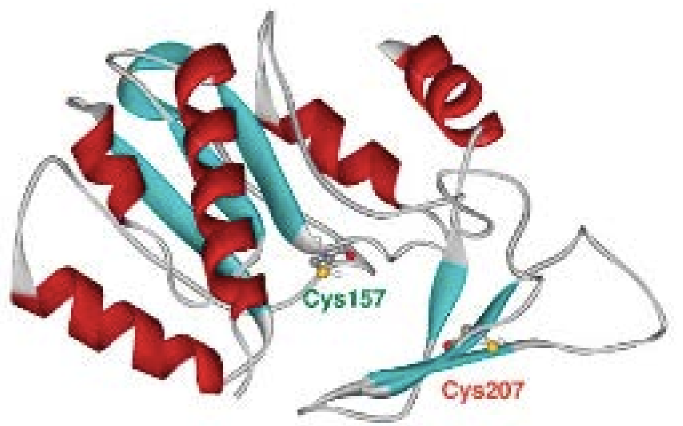
Intracellular redox state influences enzyme activity





SAM-dependent methyltransferases

AS3MT



Fomenko et al., 2008

Hypothesis: Increased oxidative stress is associated with decreased arsenic methylation.

- H1. Decreased GSH is associated with decreased As methylation, which may be mediated by a decrease in SAM.

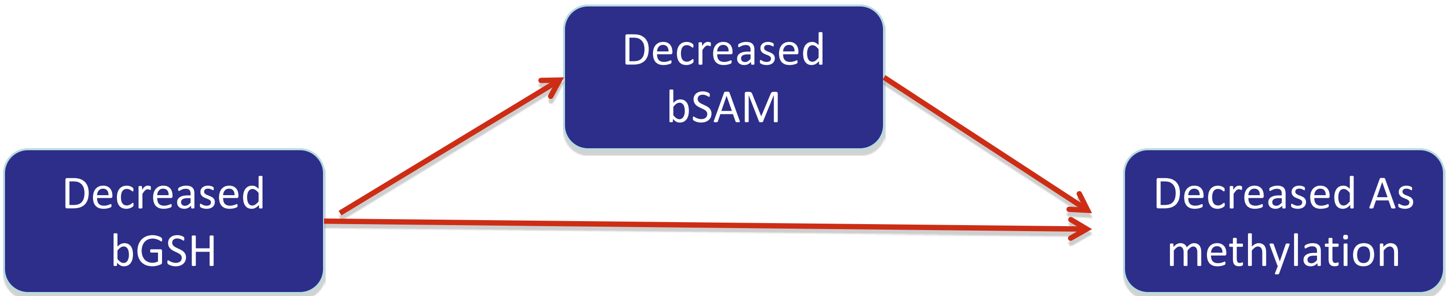
- H2. Increased GSSG and Eh(bGSH) (*indicators of a more oxidized intracellular redox environment*) are associated with decreased As methylation.

Folate and Oxidative Stress (FOX) Study

- Cross-sectional study of 379 arsenic-exposed adults in Araihazar, Bangladesh

- Primary focus: examine dose-response relationships between arsenic exposure and oxidative stress

H1: Increased GSH production under conditions of oxidative stress leads to decreased SAM, which leads to decreased As methylation.



Is decreased GSH associated with decreased SAM? **YES,** **overall and folate-sufficient**

Outcome	Predictor	Folate-sufficient (n=266)		Folate-deficient (n=112)	
		B ± SE	*p*	B ± SE	*p*
bSAM	bGSH, unadjusted	0.042±0.012	0.0007	0.010 ±0.025	0.68
	bGSH, full*	0.037 ±0.014	0.0078	0.0086 ±0.026	0.74

betas for bGSH represent 100-unit change in bGSH

*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betelnut use (log), BMI (log), vitamin B-12 (log), and television ownership

H1: Increased GSH production under conditions of oxidative stress leads to decreased SAM, which leads to decreased As methylation.

- Is decreased GSH associated with decreased As methylation? **NO**
- Is decreased SAM associated with decreased As methylation? **NO**

Outcome	Predictor	Folate-sufficient (n=266)		Folate-deficient (n=112)	
		B ± SE	*p*	B ± SE	*p*
%uInAs	bSAM*	-1.39±0.95	0.15	-1.79 ±1.54	0.25
	bGSH*	-0.093 ±0.20	0.65	-0.34 ±0.39	0.39
%uMMA	bSAM*	0.19 ±0.74	0.80	1.19±1.33	0.39
	bGSH*	0.17 ± 0.16	0.27	0.21± 0.34	0.54
%uDMA	bSAM*	1.20 ±1.28	0.35	0.59±2.21	0.79
	bGSH*	-0.08 ±0.28	0.77	0.14±0.56	0.81

betas for bGSH represent 100-unit change in bGSH

*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betelnut use (log), BMI (log), vitamin B-12 (log), and television ownership

H2. Oxidative stress is associated with decreased As methylation due to a more oxidized intracellular redox environment.

1. Is increased bGSSG associated with decreased As methylation?

Outcome[a]	Folate-sufficient (n=266)		Folate-deficient (n=112)	
	B ± SE	*p*	B ± SE	*p*
%uInAs*	0.073±0.76	0.92	1.91 ±1.12	0.09
%uMMA*	-0.20 ±0.59	0.74	1.61±0.97	0.09
%uDMA*	0.12 ±1.02	0.90	-3.53±1.58	0.03

2. Is a more positive Eh(bGSH) associated with decreased As methylation?

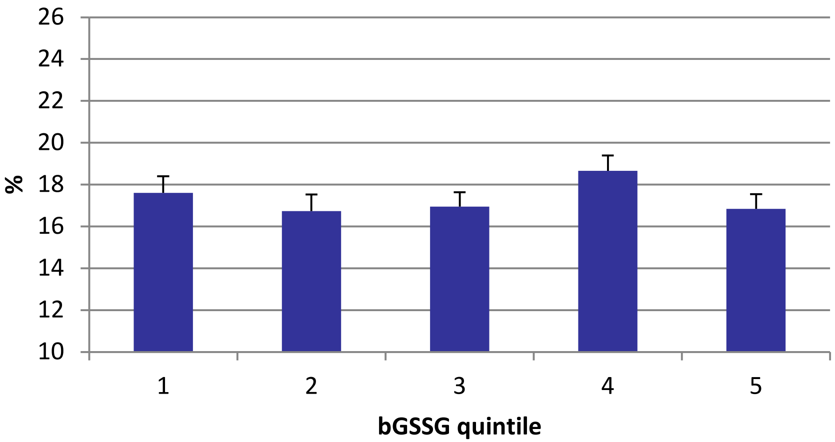
Outcome	Folate-sufficient (n=266)		Folate-deficient (n=112)	
	B ± SE	*p*	B ± SE	*p*
%uInAs*	0.010±0.027	0.71	0.08 ±0.05	0.10
%uMMA*	-0.018 ±0.021	0.39	0.031±0.044	0.48
%uDMA*	0.008 ±0.037	0.83	-0.11±0.07	0.12

a. log bGSSG

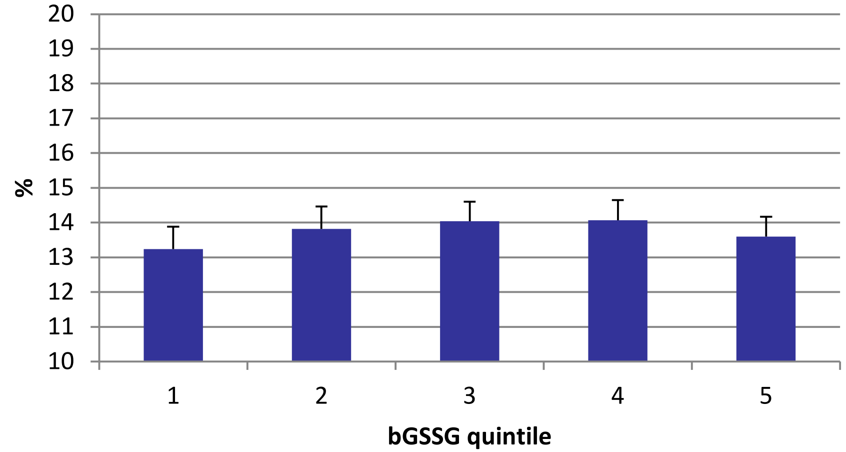
*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betelnut use, BMI (log), vitamin B-12 (log), and television ownership

Percent InAs, MMA, and DMA by quintile of bGSSG, in folate sufficient

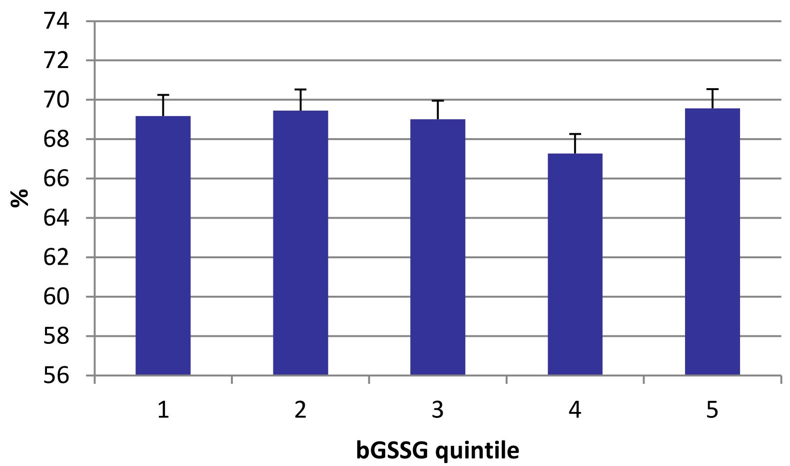
%InAs



%MMA

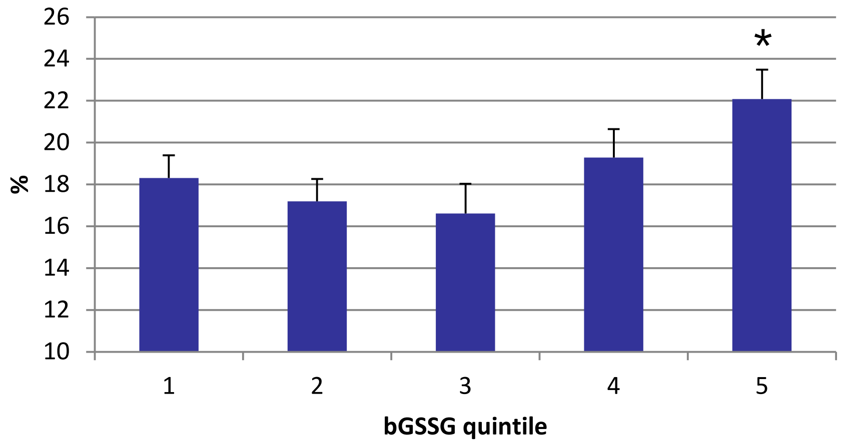


%DMA



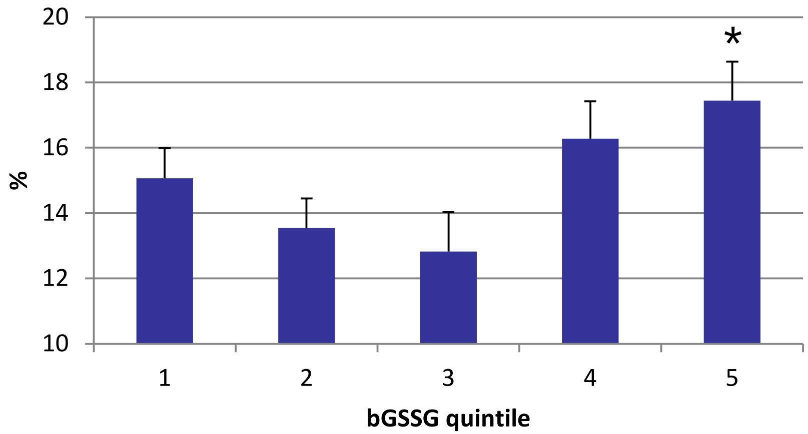
Percent InAs, MMA, and DMA by quintile of bGSSG, in folate deficient

%InAs



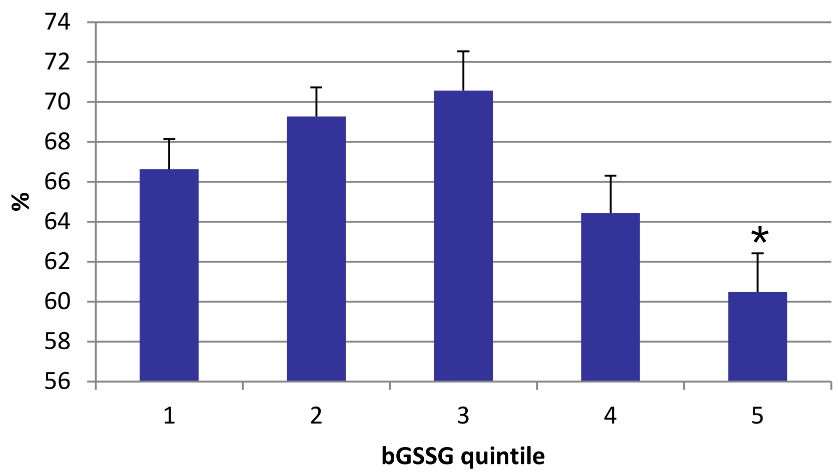
*p<0.05, compared to quintiles 1, 2, and 3

%MMA



*p<0.05, compared to quintiles 1, 2, and 3

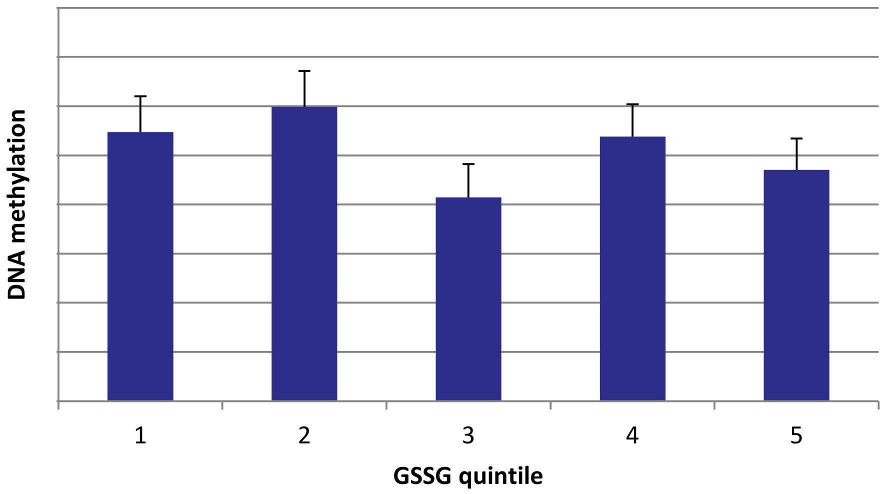
%DMA



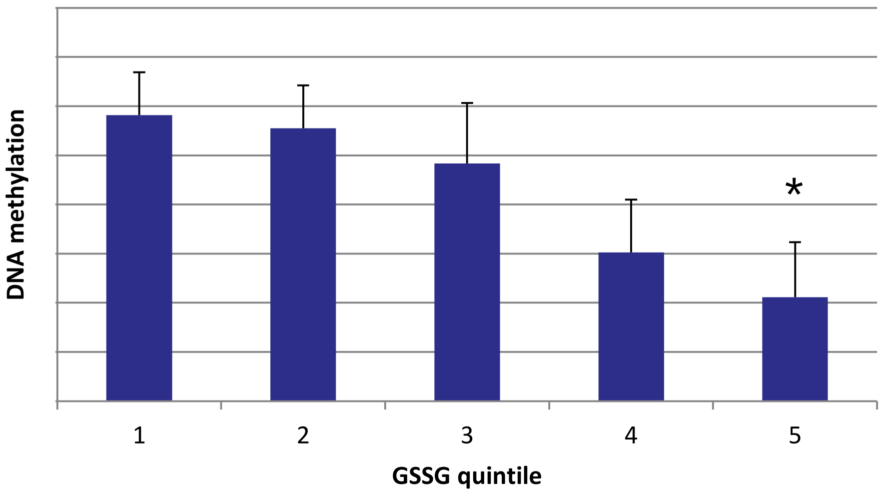
*p<0.05, compared to quintiles 1, 2, 3, and 4

Global DNA methylation by quintile of blood GSSG, by folate nutritional status

Folate sufficient



Folate deficient



*p<0.05, compared to quintiles 1 and 2

Conclusion and future directions

- Increased bGSSG associated with decreased As methylation capacity in folate deficient
  - Mechanism: Inhibition of methyltransferases or other metabolic changes?
- Opportunities for intervention
  - Antioxidant supplementation
  - Folate supplementation

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  - David Santiago
  - Tiffany Sanchez

Thank you!

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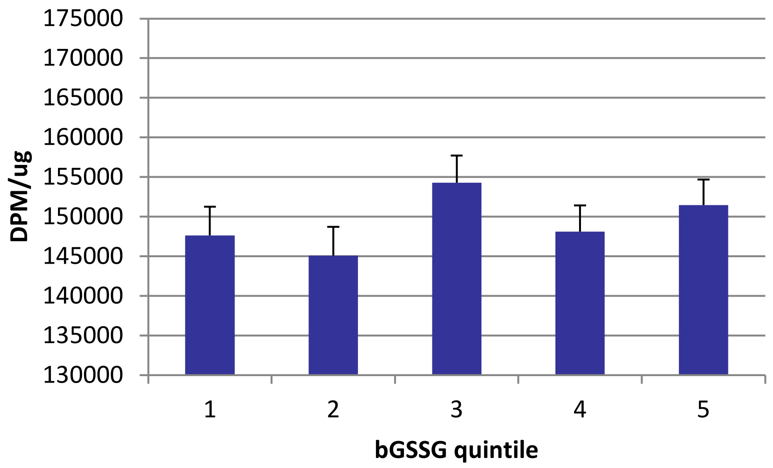
  - Jagota
  - David Santiago
  - Tiffany Sanchez

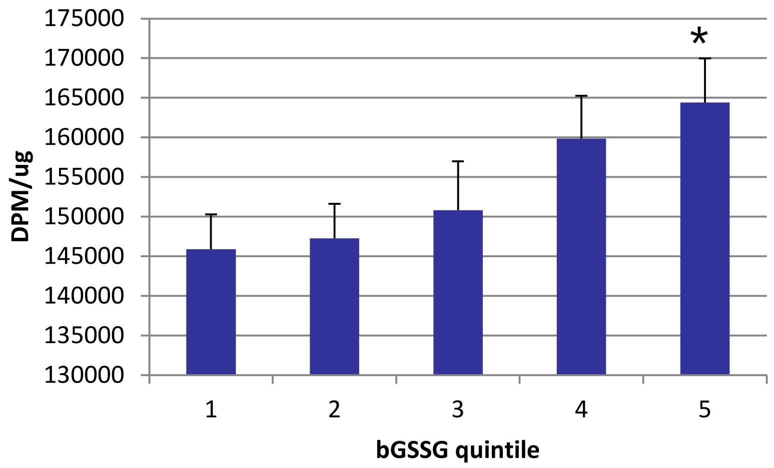
[3’]-methyl incorporation (DPM) by quintile of bGSSG, by folate nutritional status

**Increased DPM = Decreased global DNA methylation*

Folate sufficient



Folate deficient

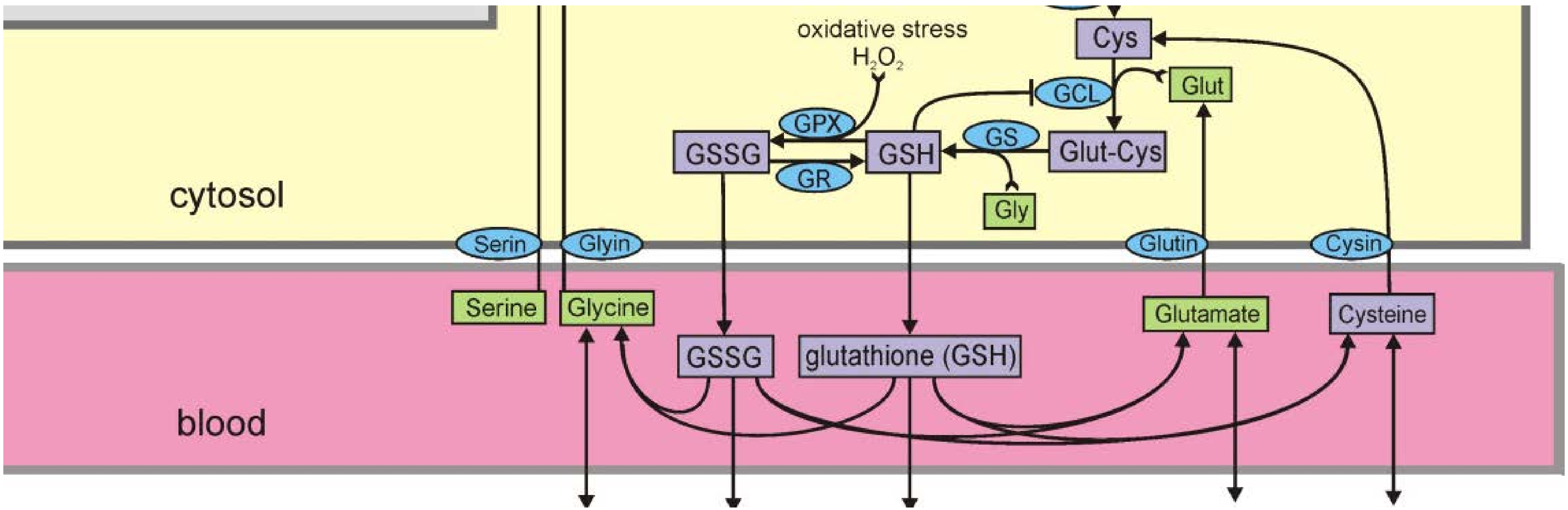


*p<0.05, compared to quintiles 1 and 2

Demographic and clinical data of subjects in the current study

Baseline variables	Folate-deficient (<9 nmol/L) (n=100)	Folate-sufficient (≥9 nmol/L) (n=222)	Group difference	Overall (n=322)
Blood GSH (uM)	505.2 ± 149.9	484.5 ± 185.7	0.35[2]	491 ± 173
Blood GSSG (uM)	34.4 ± 19.0	38.6 ± 18.4	0.019[2]	37.2 ± 18.6
Eh bGSH:GSSG (mV)	-200.6 ± 11.7	-196.6 ± 13.9	0.034[2]	-197.9 ± 13.4
Plasma GSH (uM)	2.5 ± 0.71	2.6 ± 0.72	0.40[2]	2.6 ± 0.72
Plasma GSSG (uM)	2.06 ± 0.66	2.16 ± 0.57	0.052[2]	2.13 ± 0.60
Eh pGSH:GSSG (mV)	-98.4 ± 6.7	-98.3 ± 7.4	0.97[2]	-98.3 ± 7.2
Plasma Cys (uM)	3.55 ± 2.27	3.89 ± 2.57	0.26[2]	3.78 ± 2.49
Plasma CySS (uM)	53.1 ± 14.7	57.5 ± 13.1	0.0037[2]	56.2 ± 13.4
Eh pCys:CySS (mV)	-46.8 ± 15.8	-47.6 ± 17.3	0.61[2]	-47.2 ± 16.8
Blood SAM (uM)	1.35 ± 0.54	1.23 ± 0.47	0.038[2]	1.27 ± 0.50
Blood SAH (uM)	0.33 ± 0.15	0.30 ± 0.17	0.012[2]	0.31± 0.17

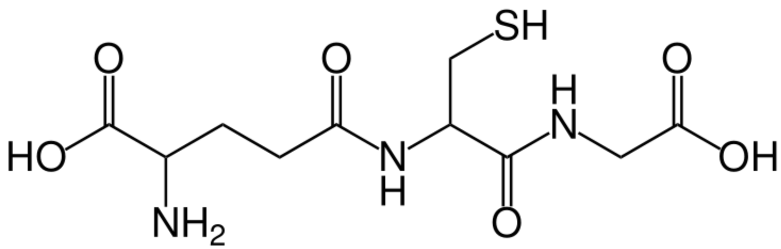
1. Mean ± SD (all such values); 2. P-values determined by Wilcoxon's rank sum test; 3. P-values determined by chi-square test

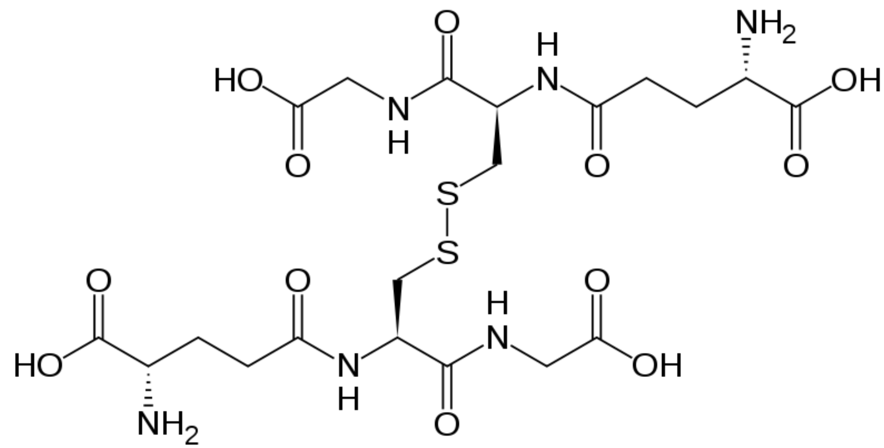

oxidative stress
H_2O_2
Cys
GCL
Glut
GPX
GSSG
GSH
GS
Glut-Cys
GR
Gly
cytosol
Serin
Glyin
Glutin
Cysin
Serine
Glycine
GSSG
glutathione (GSH)
Glutamate
Cysteine
blood

E for various cell processes

E(mV)	Process
-165	Necrosis
-185	G0/differentiated G1
-195	Dephosphorylation threshold of phosphoproteins on serine residues
-205 to <-260	Proliferation

Glutathione





- L-cysteine, L-glutamic acid, and glycine
- Thiol (SH) group acts as proton donor
- Intracellular – high (1-10 mM, liver 5-10 mM)
  - GSH
  - GSSG: < 1% of GSH
  - 3 major reservoirs: ~90% in the cytosol, ~10% in the mitochondria, small percentage in the ER
- Extracellular – lower (1-10 uM)
- GSH/GSSG usually > 10

Methods

- Glutathione processing:
  - Blood collected and immediately transferred to Eppindorf tubes containing either
    - 5% perchloric acid (PCA), 0.1 M boric acid and γ-glutamyl glutamate as internal standard (for whole blood GSH), or
    - 100 mM serine borate, 60 units heparin, 0.75 mg bathophenanthrolene, 4.5 mg iodoacetic acid, and γ-glutamyl glutamate
  - Samples centrifuged for 1 min.; 200 µl of supernatant transferred into Eppendorf tubes containing an equal volume of 10% PCA, 0.2M boric acid
  - Samples sent to Columbia for derivatization and HPLC analysis
  - Measure Cys/CySS, GSH/GSSG