Methyl Cycle Nutrigenomics

James C. Roberts, M.D. FACC, FAARFM

Relevant financial relationships in the past twelve months by presenter or spouse/partner:

Employment: None
Grant/Research Support: National Institute of Health, Roche Labs, Magnetico, Daiichi-Sankyo, and Relox, Inc.
Consultant: None
Speakers Bureau: Metagenics Midwest
Stock Shareholder: Med Five, Inc
Other: None

Status of FDA devices used for the material being presented
NA/Non-Clinical

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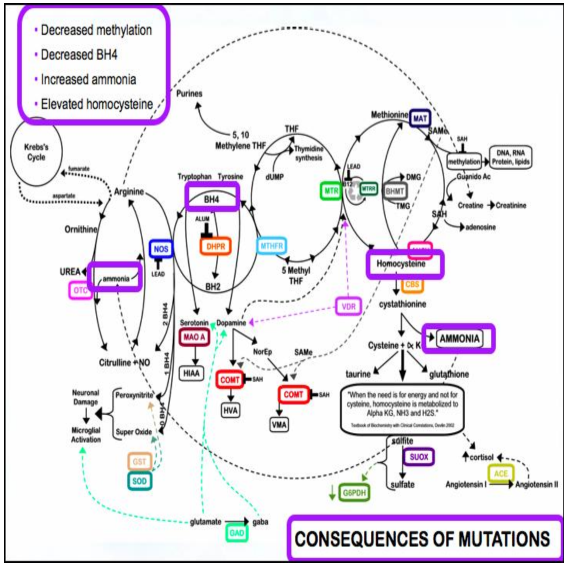
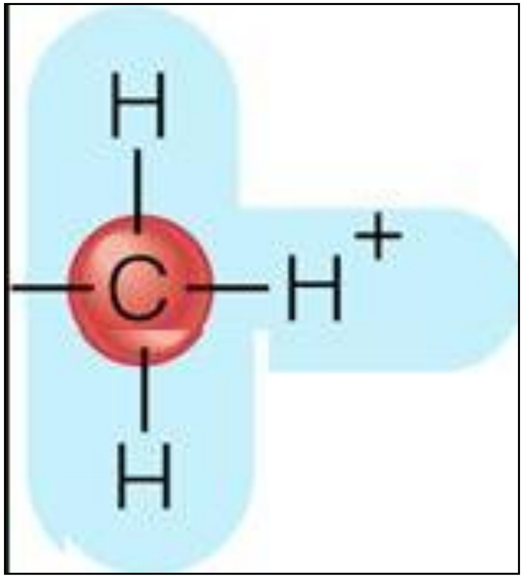
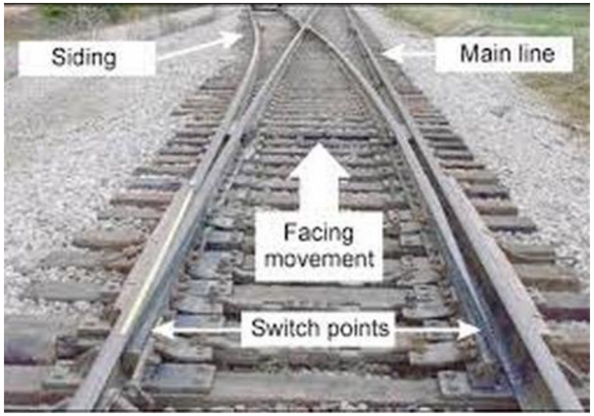
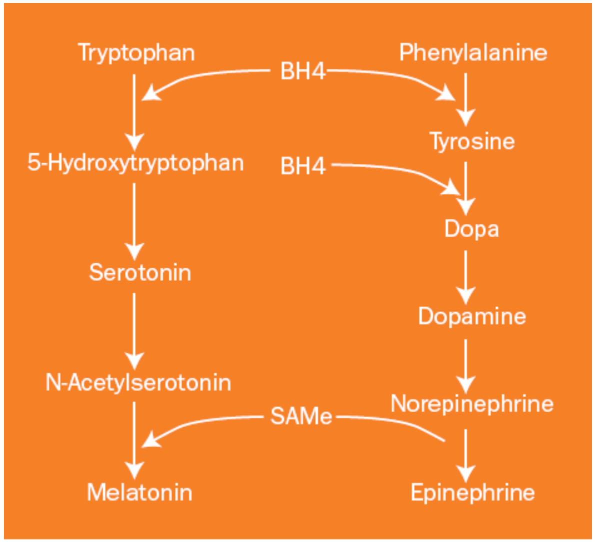
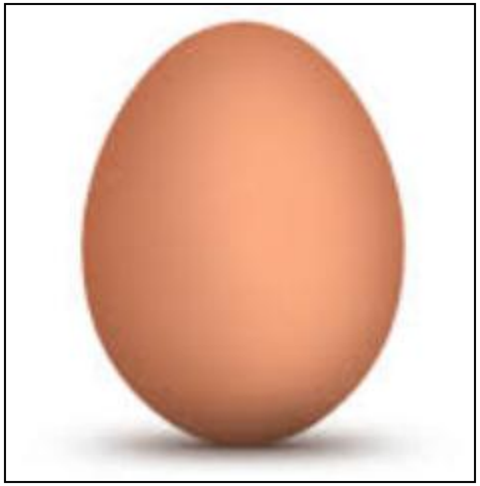
METHYL CYCLE NUTRGENOMICS











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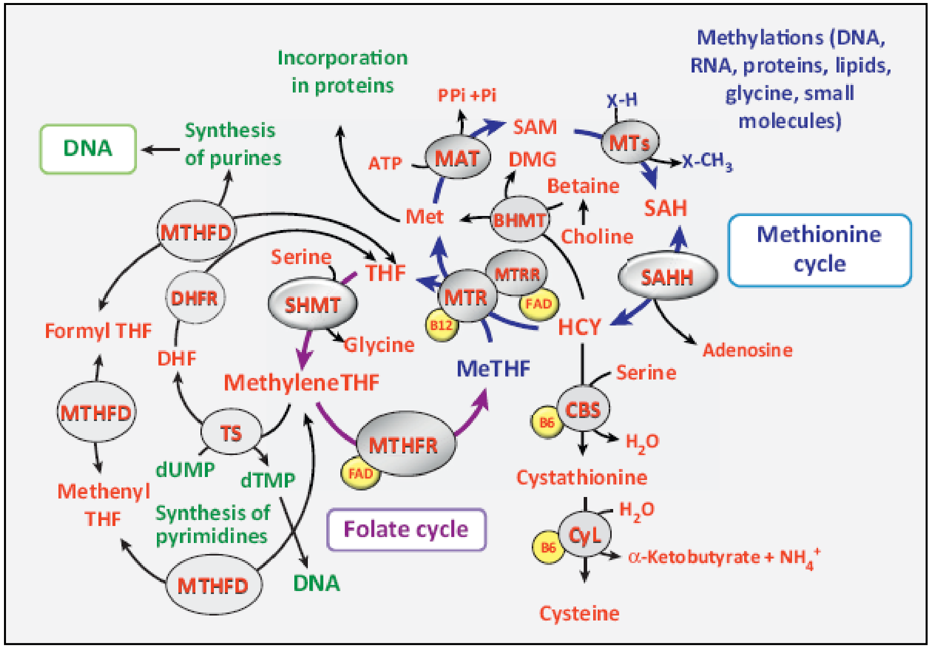

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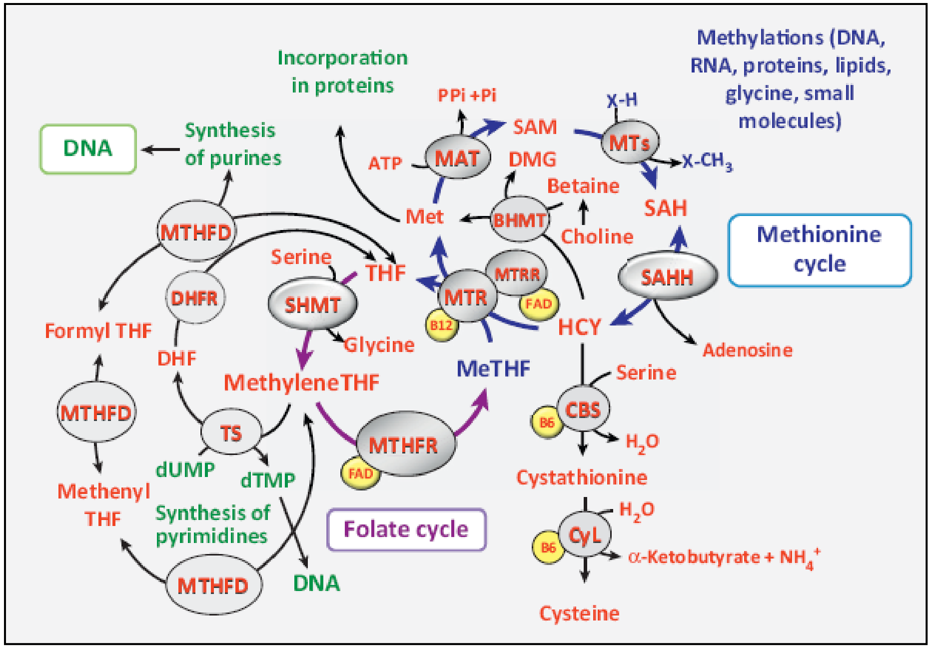
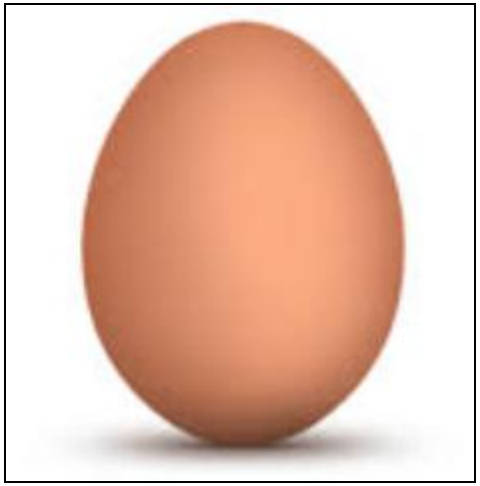




FETAL HYPERTENSIVE PROGRAMMING

♥ Female Wistar Rats

Mate with genomically normal male rats

Between conception and weaning of pups, place dams on:

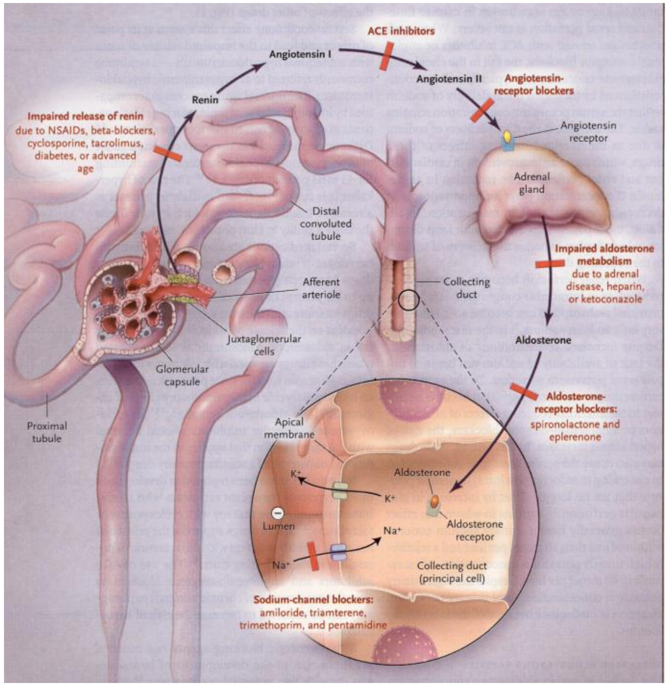
- Standard rat chow (20% protein)
- Low protein (8%) chow (extra calories as carbohydrate)

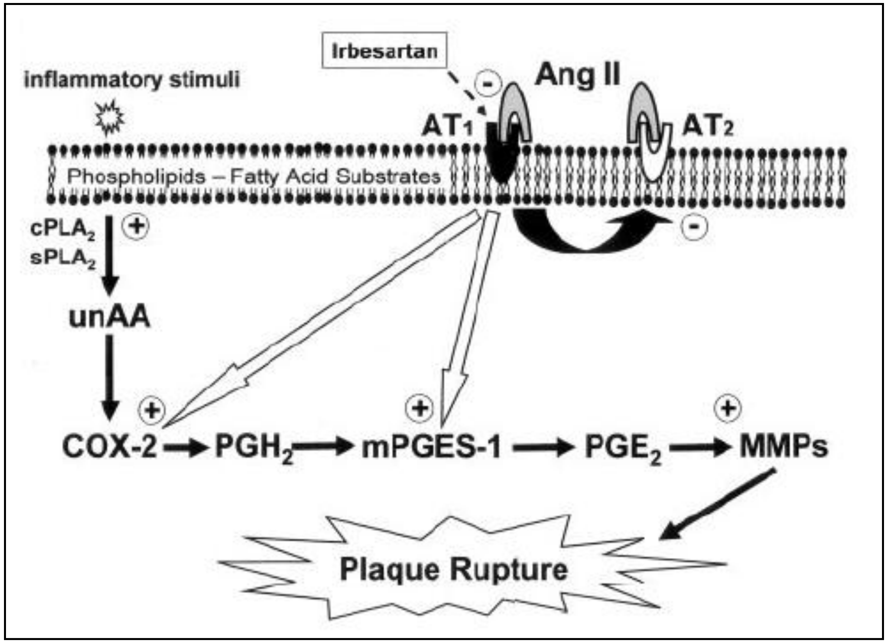
At three weeks pups weaned to standard 20% protein diet

At one or twelve weeks sacrifice pups and evaluate mRNA of RAS proteins:

- Angiotensinogen
- Renin
- ACE
- AT1 and AT2 receptor

FETAL HYPERTENSIVE PROGRAMMING





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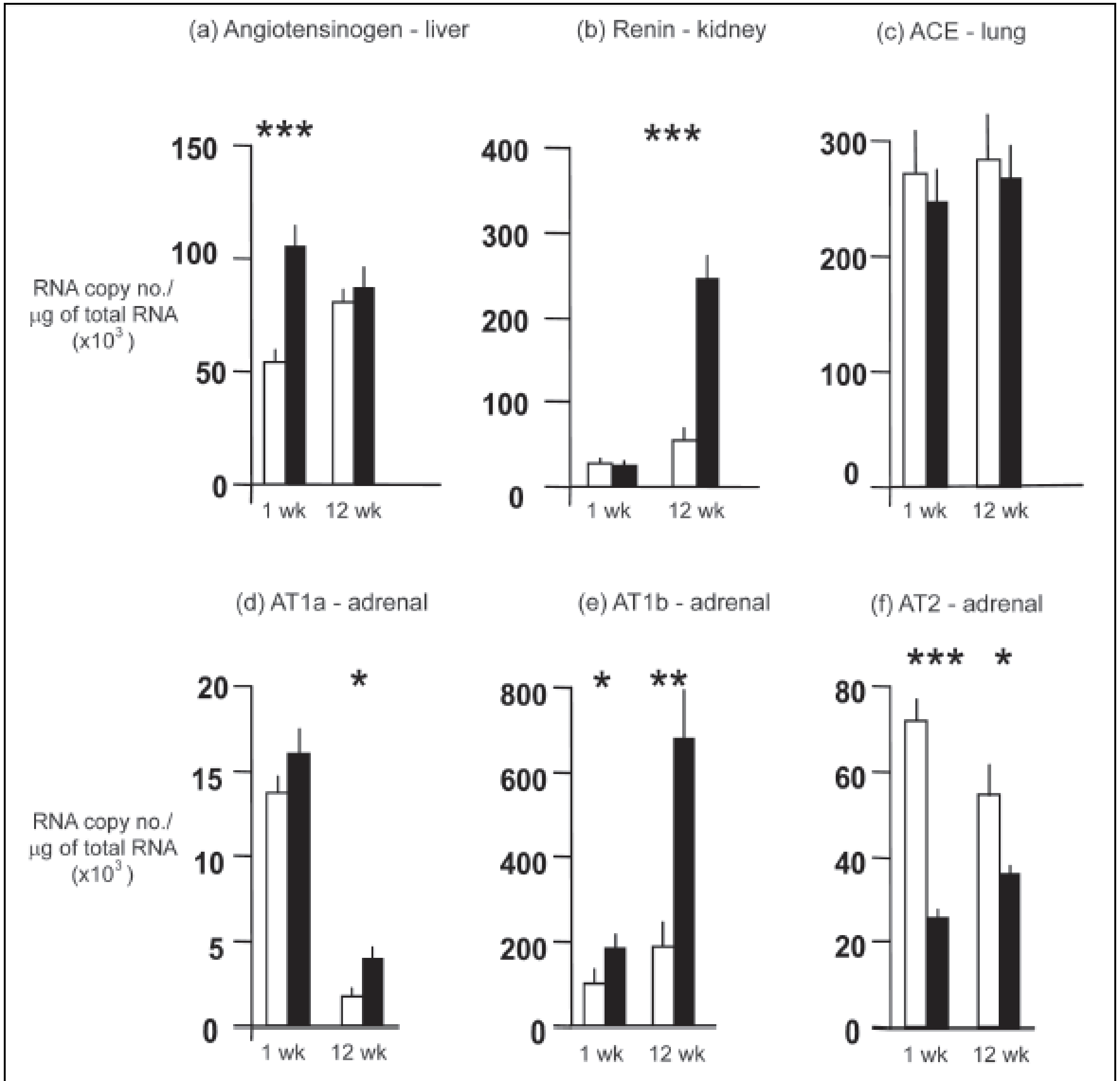
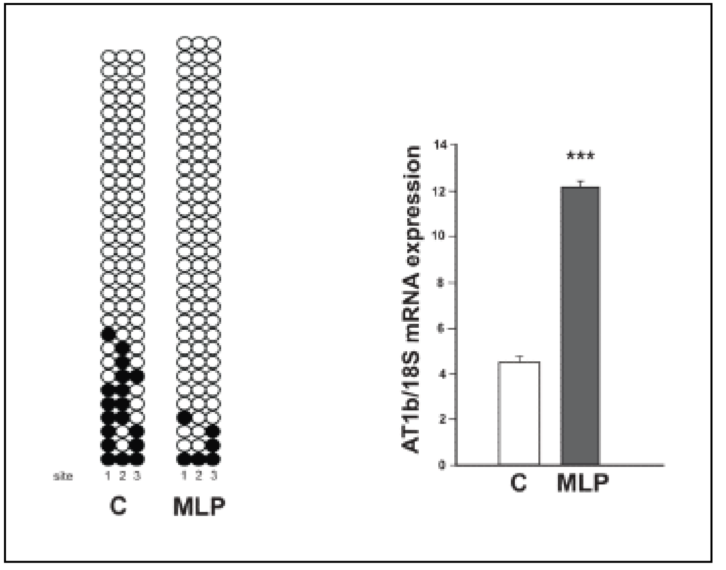


FETAL HYPERTENSIVE PROGRAMMING

AT1 gene

Promoter

Methylation



AT1

mRNA

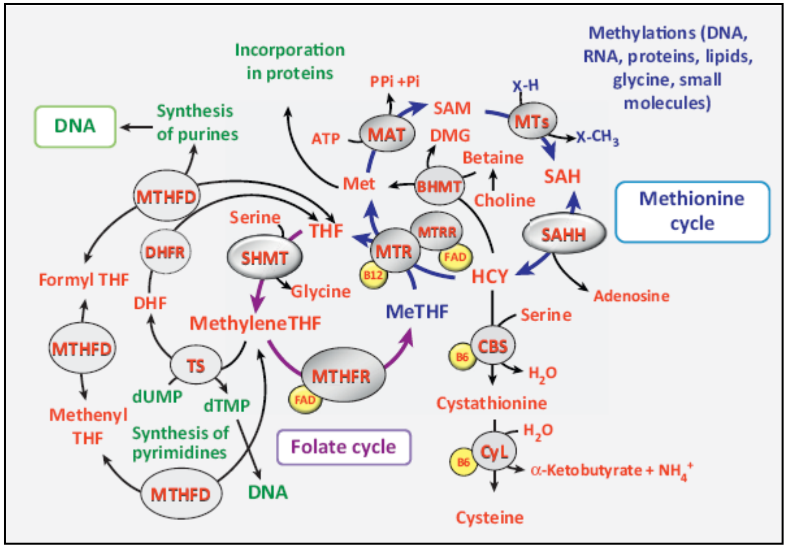
Maternal protein insufficiency →

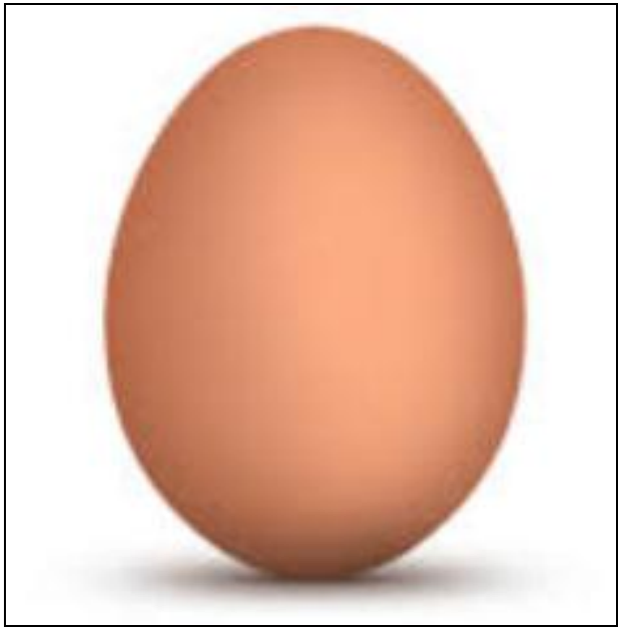
Reduced methylation of RAS gene promoter sites →

Increased transcription of RAS proteins →

Hypertensive phenotype

METHYL CYCLE NUTRGENOMICS

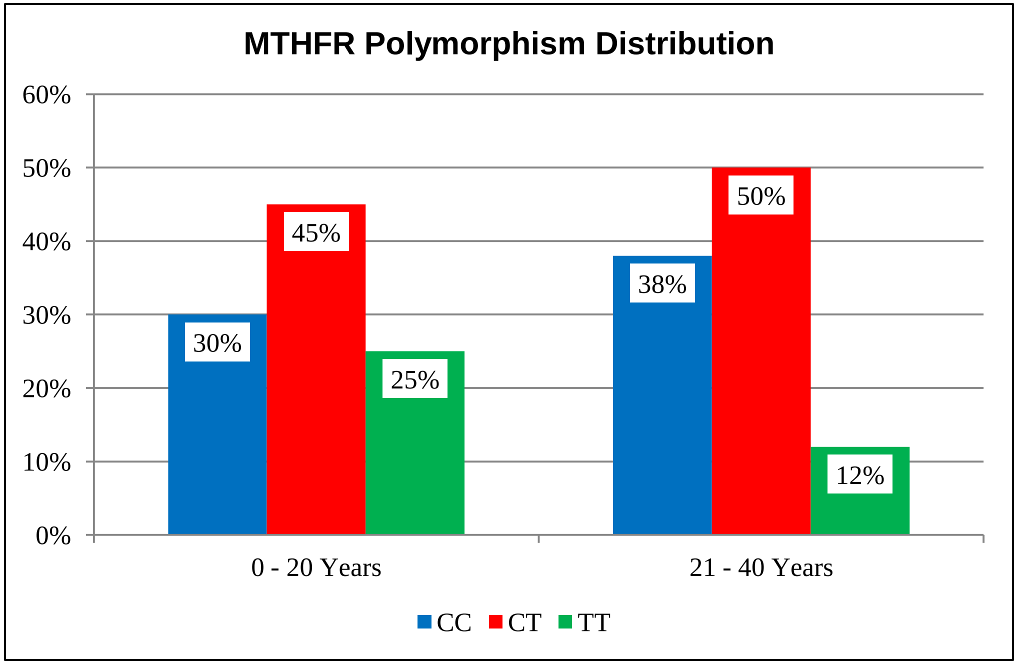




Nutrient (unit)	Whole Egg
Weight	60g
Water (percentage)	65-68.5
Calories (kcal)	70
Protein (g)	6.3
Carbohydrate (g)	0.36
Total fat (g)	4.8
Polyunsaturated fat (g)	1
Monounsaturated fat (g)	1.8
Saturated fat (g)	1.6
Cholesterol (mg)	185
Choline (mg)	126
Vitamin A (IU)	270
Vitamin D (IU)	41
Vitamin E (mg)	0.5

MTHFR 677C→T POLYMORPHISM

- ♥ Genotype 695 Spaniards
  - • All ≤ 40 years of age
  - • No migration in or out of area
    - ♦ ACE I/D – Stable over all age ranges
    - ♦ MTHFR 677C→T (Val→Met)



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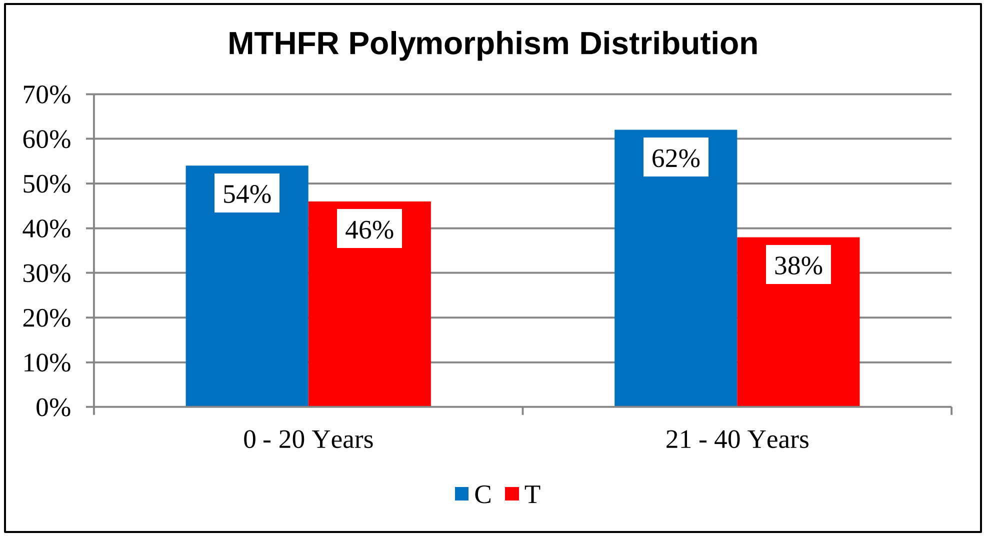

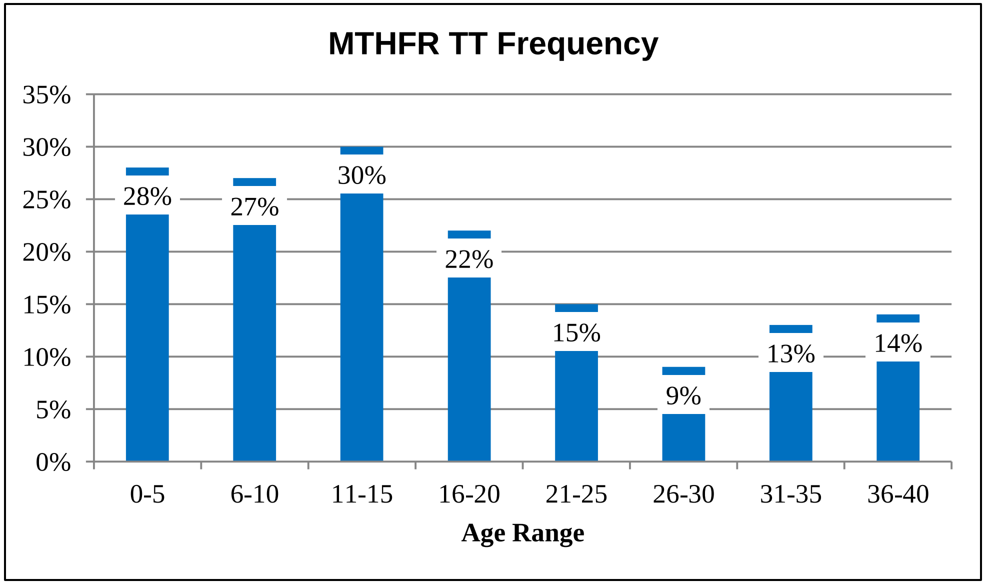

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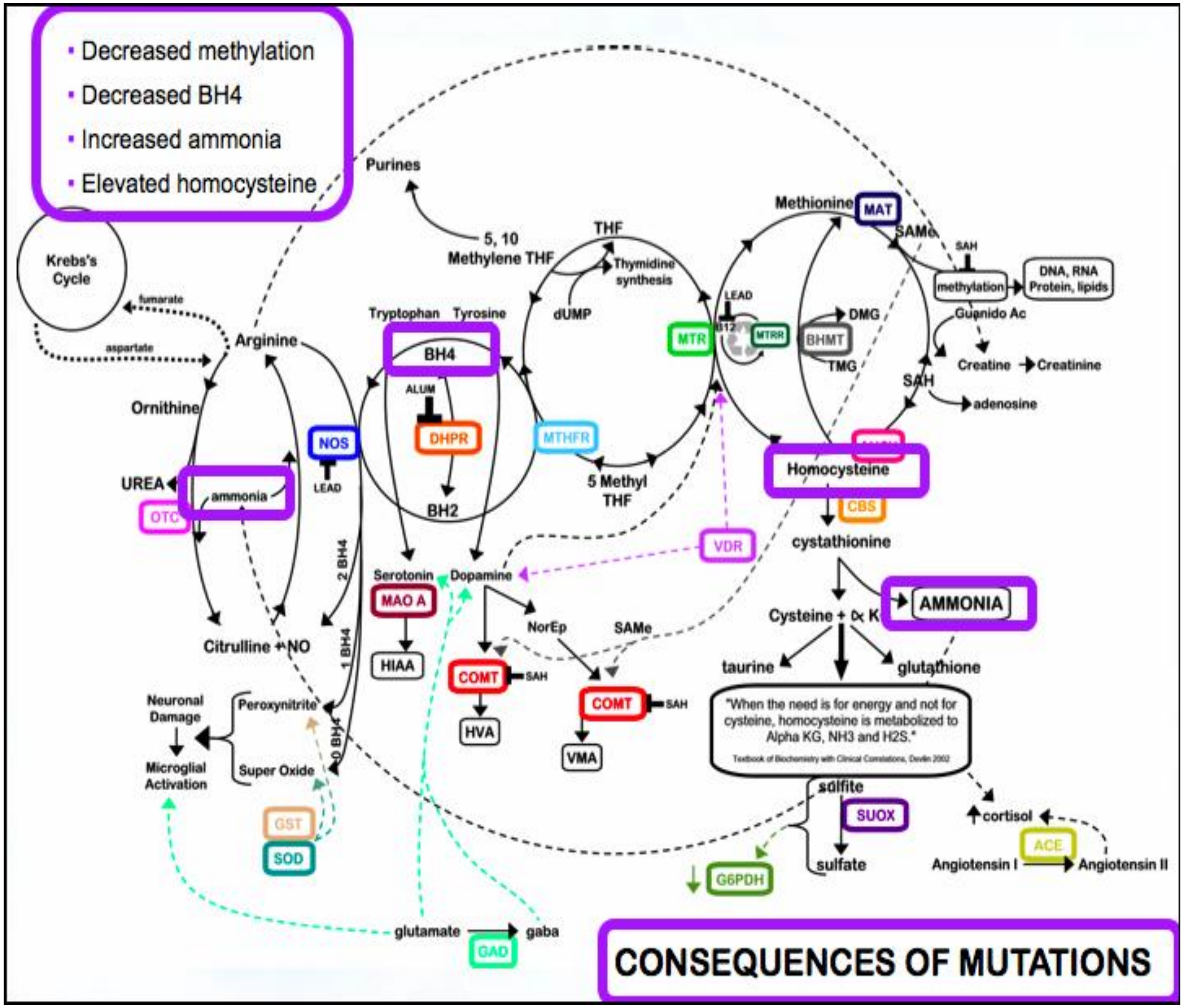
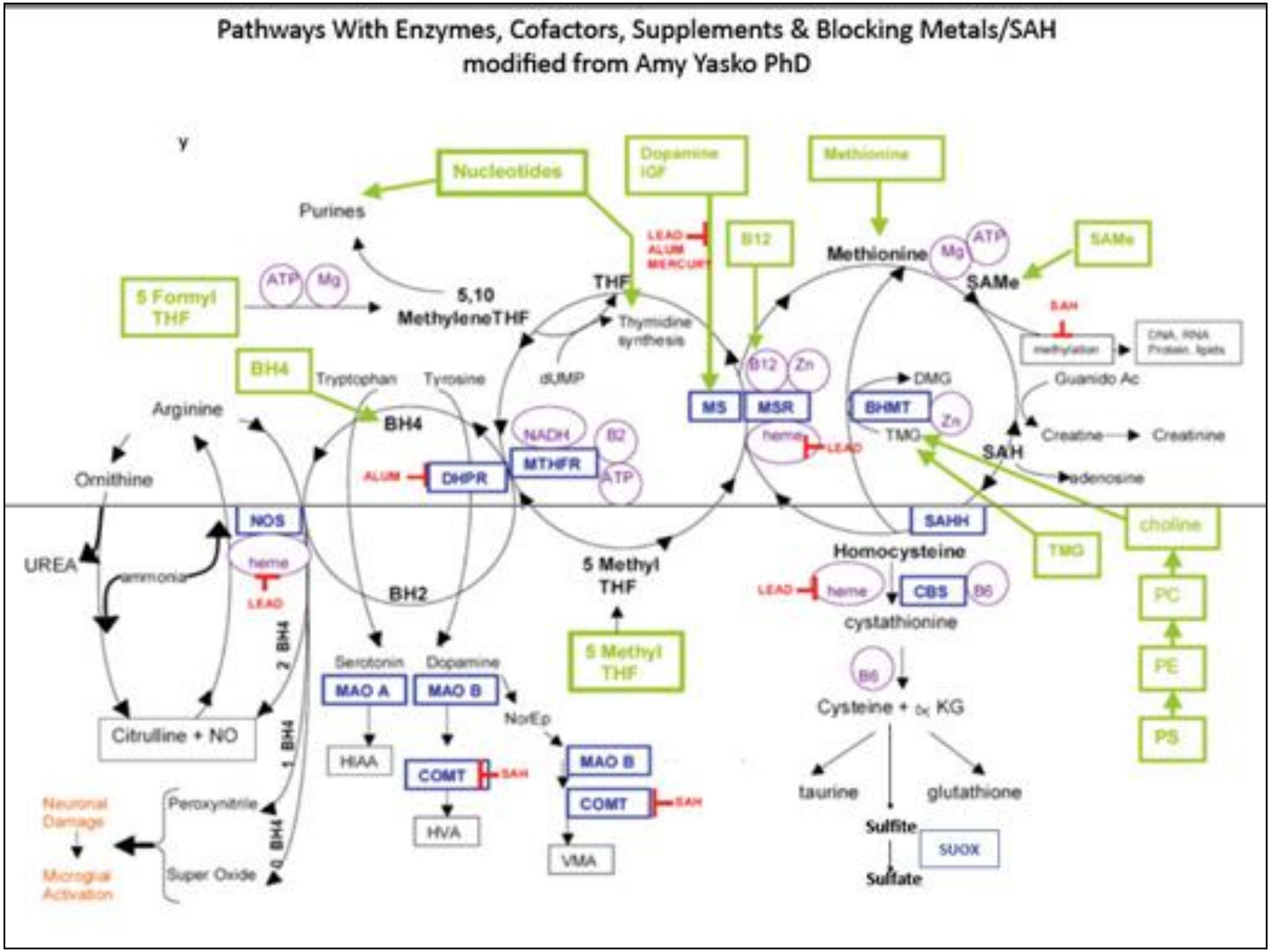


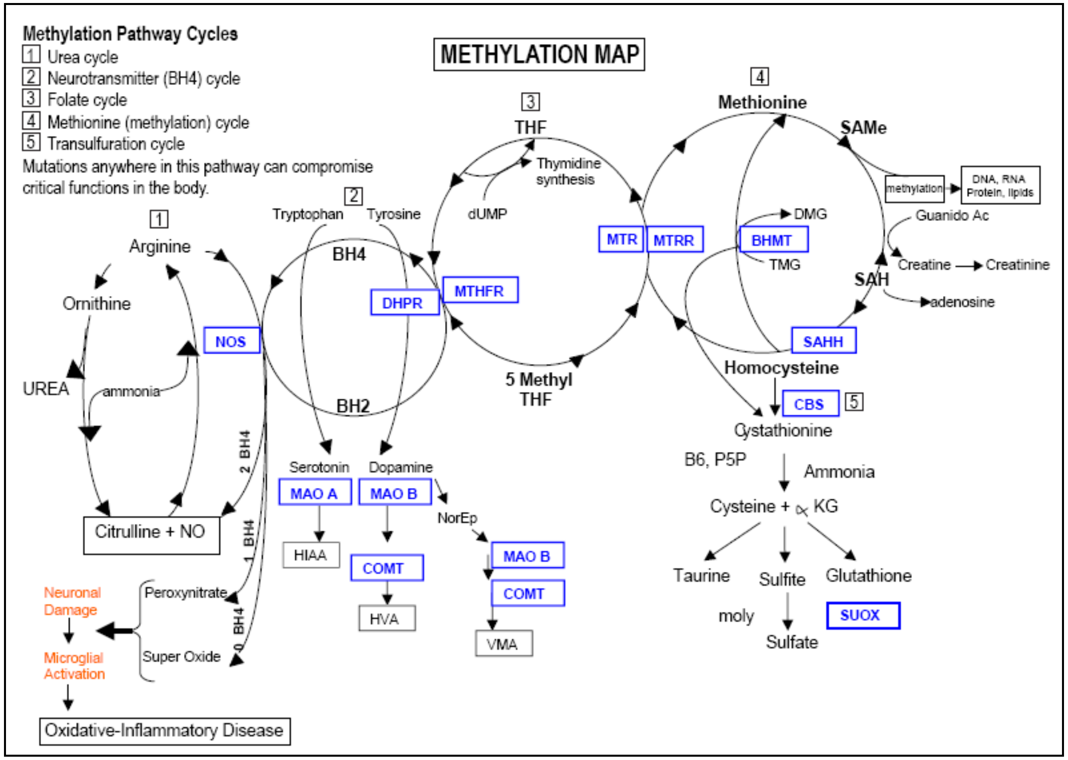
Thanks to Amy Yasko PhD.

METHYL CYCLE NUTRGENOMICS



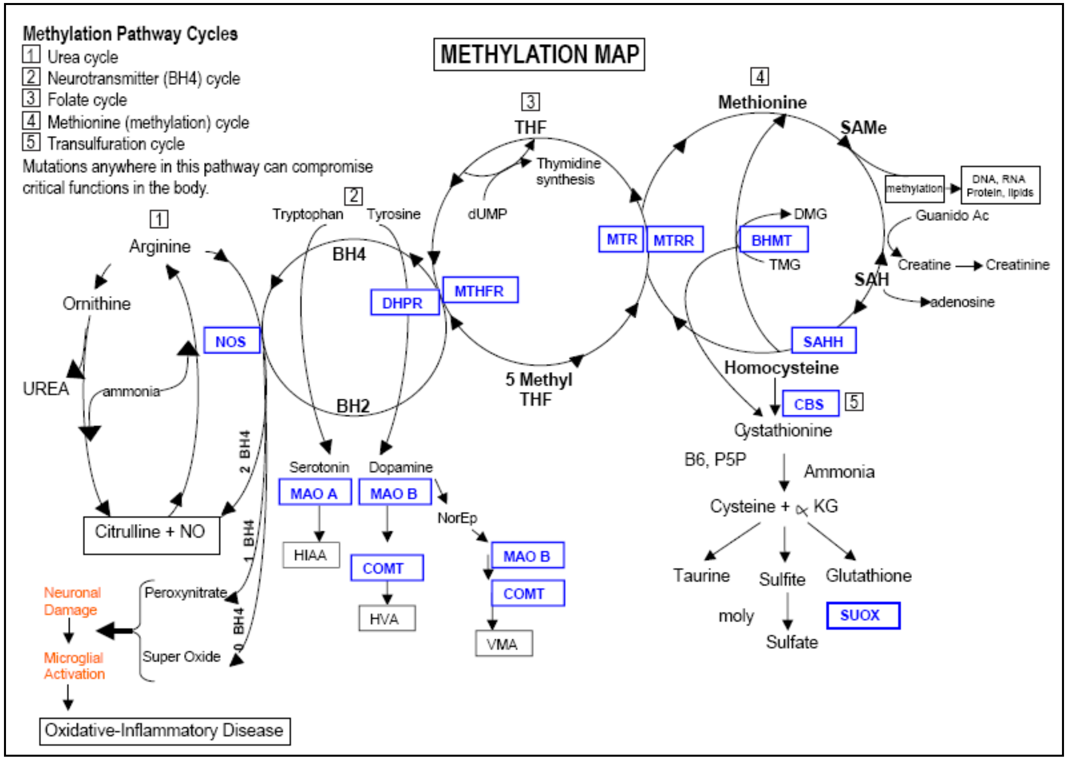
Genomic – Nutritional – Toxicity Interaction ⇒ Phenotype and Overall Health

CAVEATS to METHYL CYCLE NUTRIGENOMIC DISCUSSION



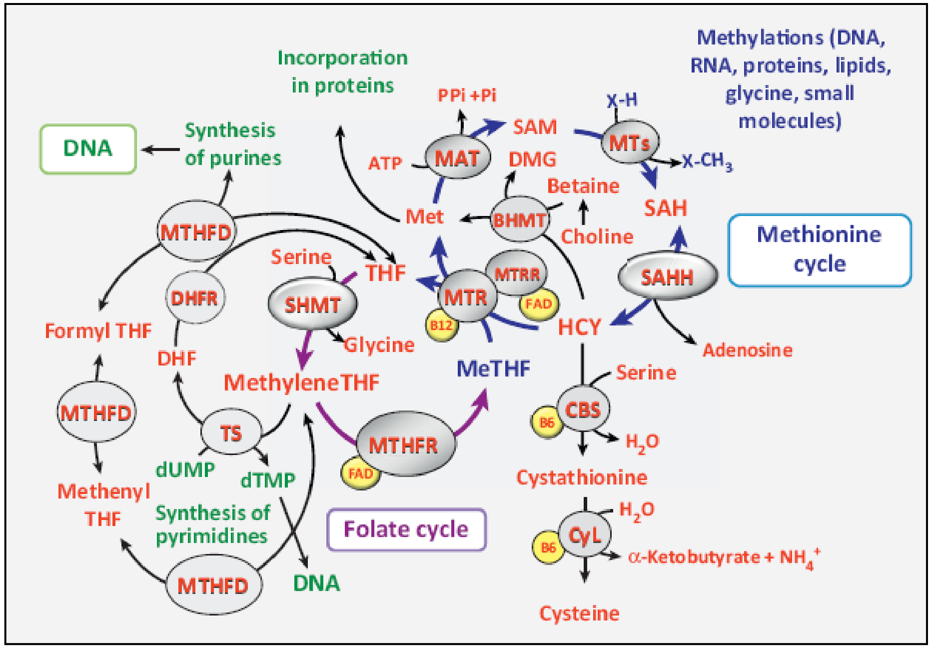
1. Unsettled science (application of clinical experience to basic science)
2. Dr. Yasko's work focuses on kids with autism
    - Pediatric recs often inappropriate for adults with acquired disease states
3. Genotype does not always determine phenotype
4. Acquired factors (toxicity, new health challenge) "brings out" genomic weakness
5. Methyl Cycle Genomic Status is not the sole determinant of health

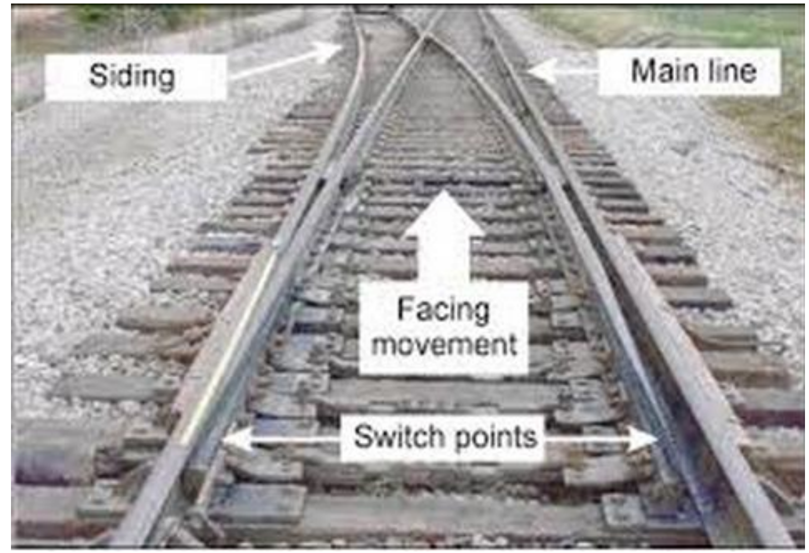
UNDERSTANDING the METHYL CYCLE



1. No need to memorize
2. Methyl Cycle is a perfect system
   - Creation vs. Darwinian Evolution
3. “Defects” response to altered nutrient availability (time period or location)
4. Understand the purpose of “Defect” → Neutralize adverse effects on individual
5. All this must make sense (or its probably wrong)

UNDERSTANDING the METHYL CYCLE

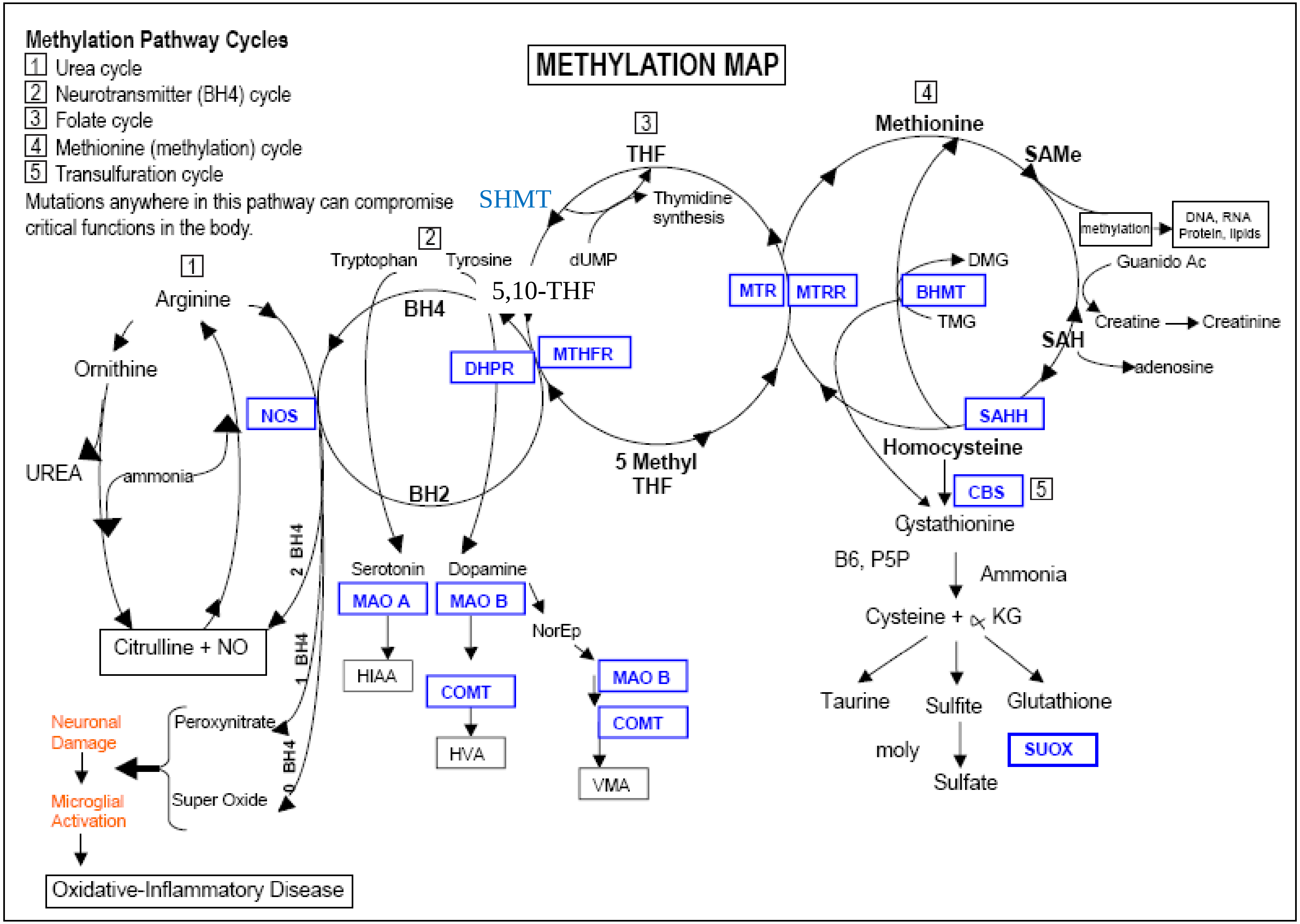




MTHFR C677T:

- Not a health challenge (if diet is adequate in ********** and ******)
- Decreases risk of certain cancers
- Shunts Methyl Groups towards DNA synthesis
  - ♦ Prevalence of MTHFR 677C→T increasing

OVERVIEW of the METHYL CYCLE



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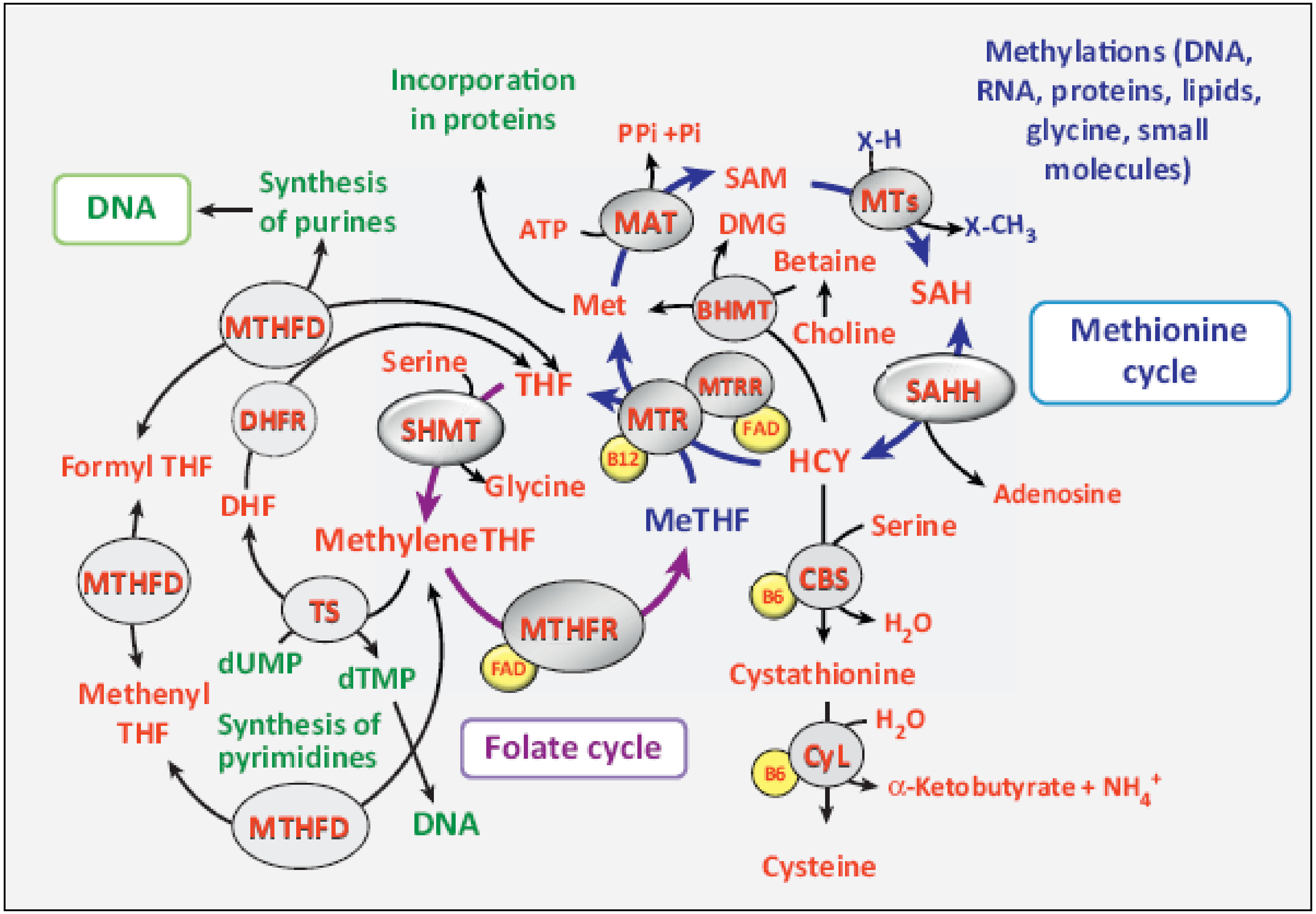

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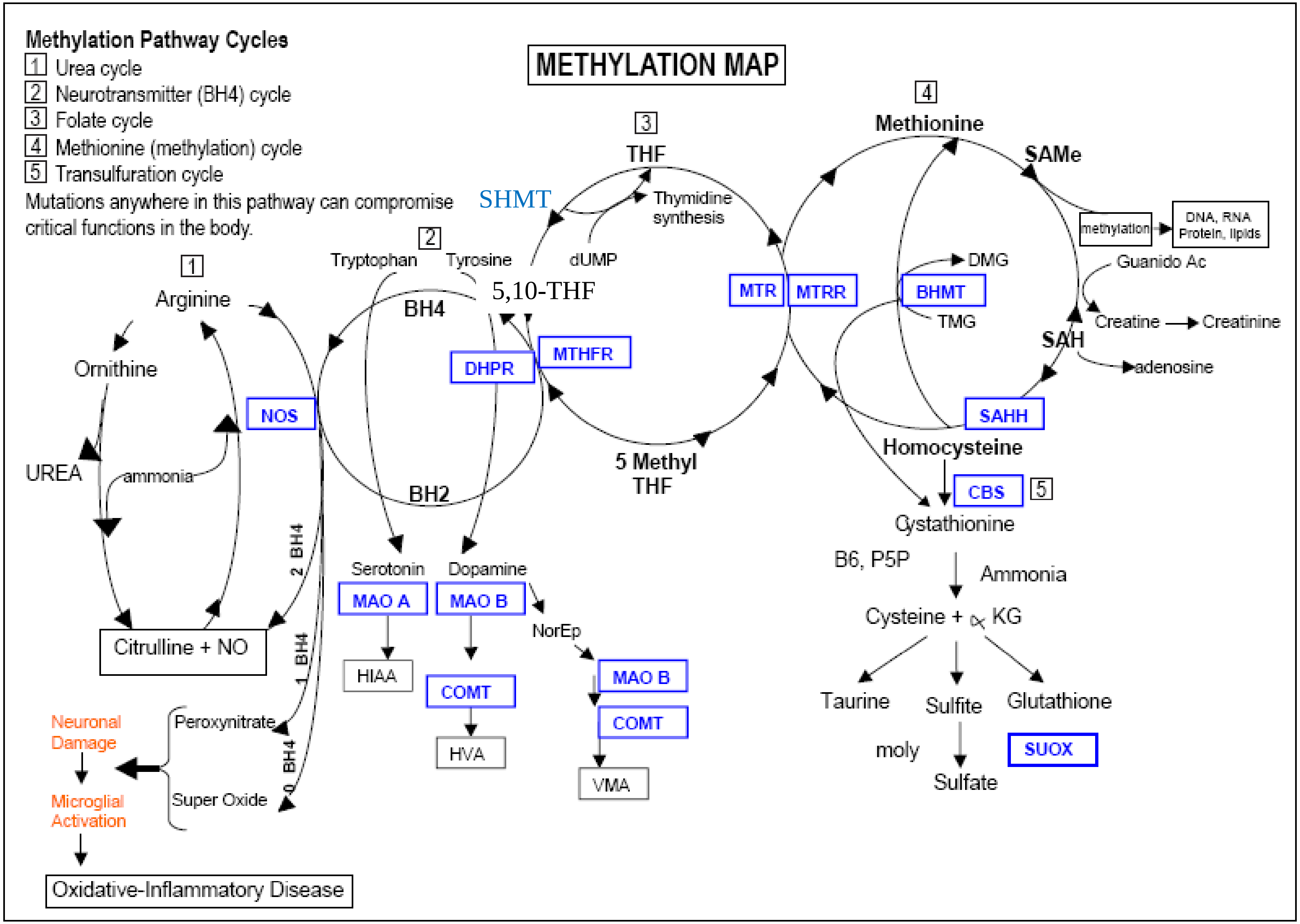
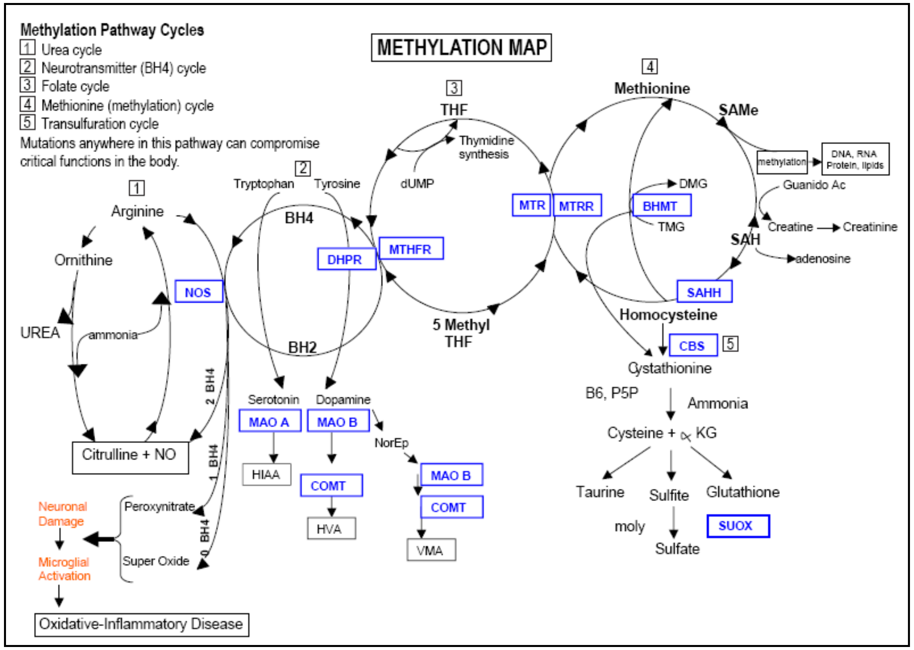


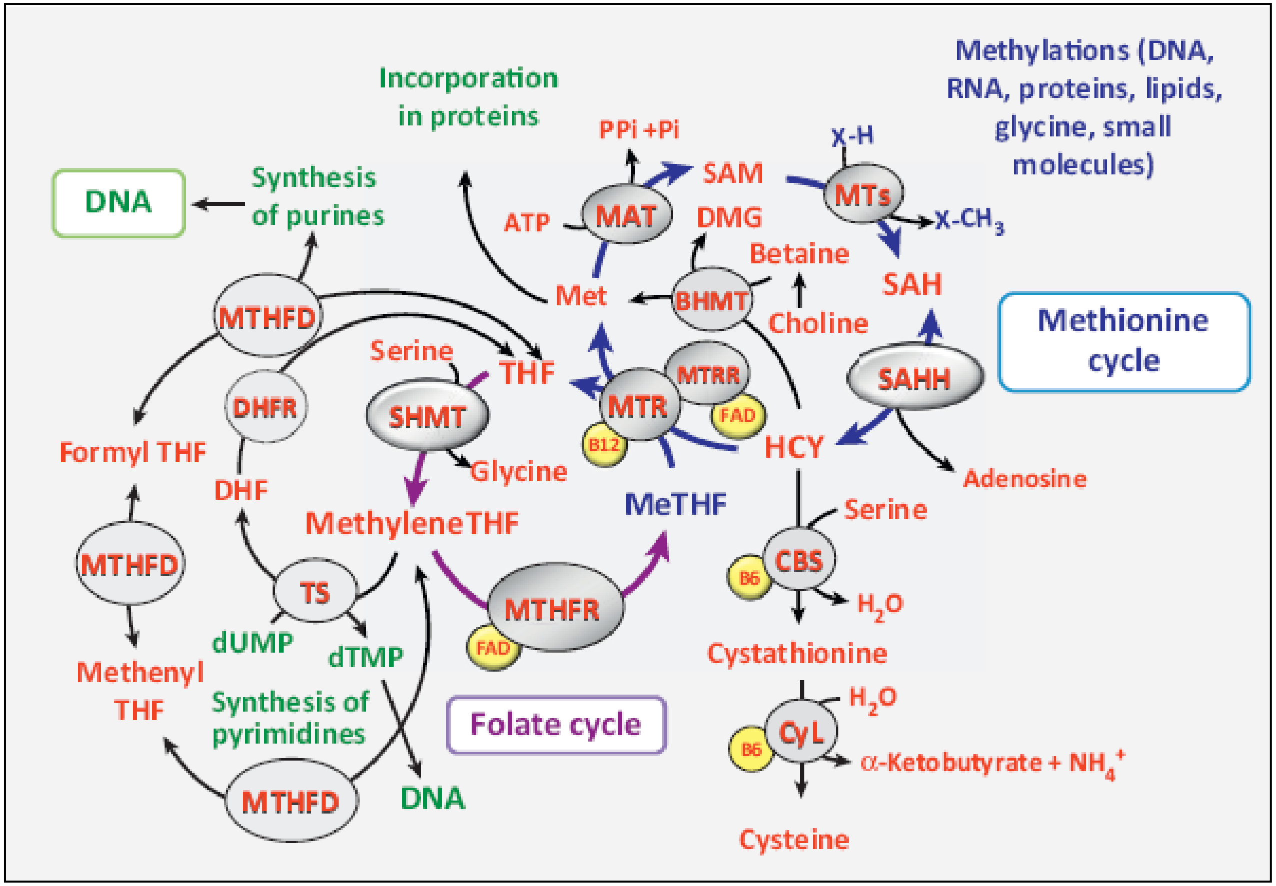
FUNCTIONS of the METHYL CYCLE



Maintain (current health status) appropriate levels of:

- Pyrimidine and purine bases for DNA and RNA synthesis
- Antioxidant/Detox molecules glutathione, cysteine, taurine, & sulfate
- BH4 (tetrahydrobiopterin)
- Transferable methyl groups ≈ High SAMe:SAH

FUNCTIONS of the METHYL CYCLE



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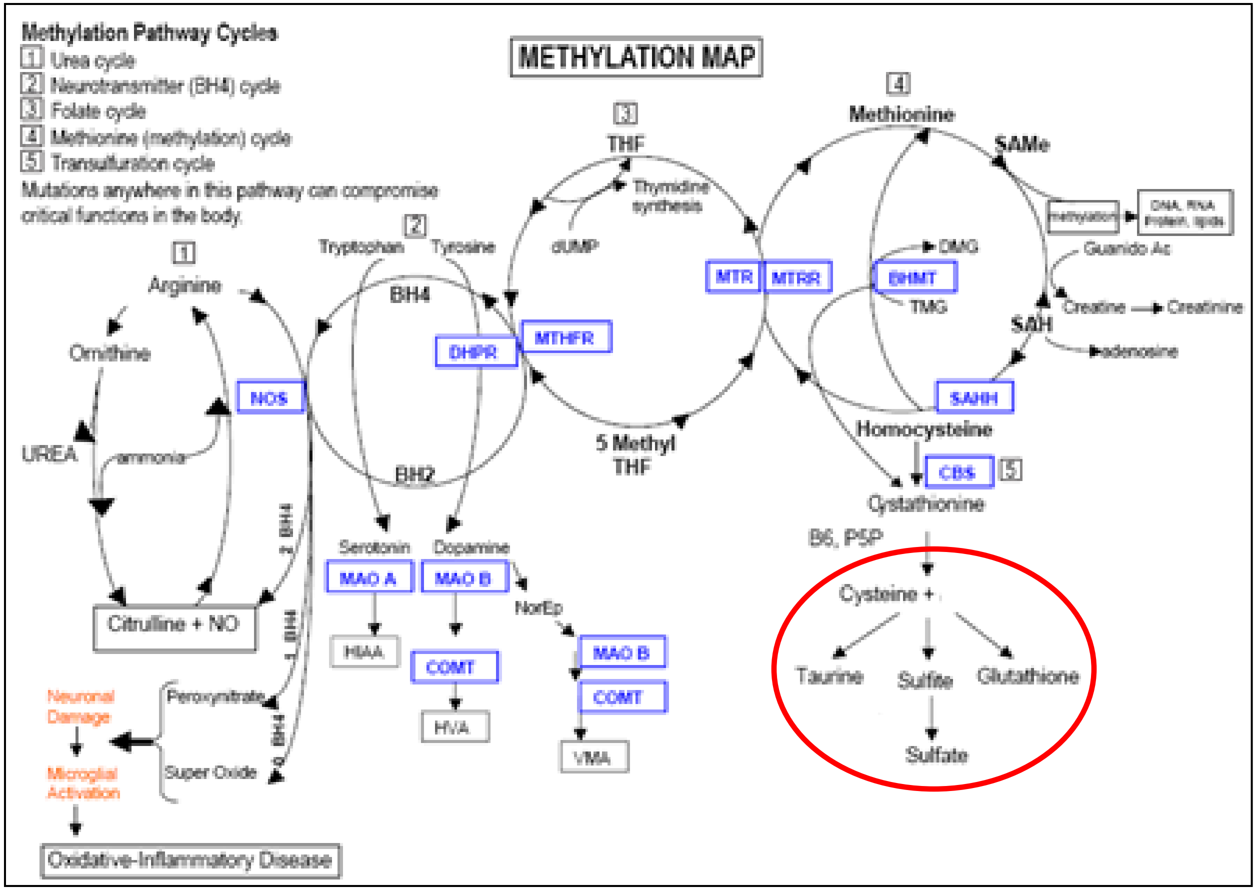

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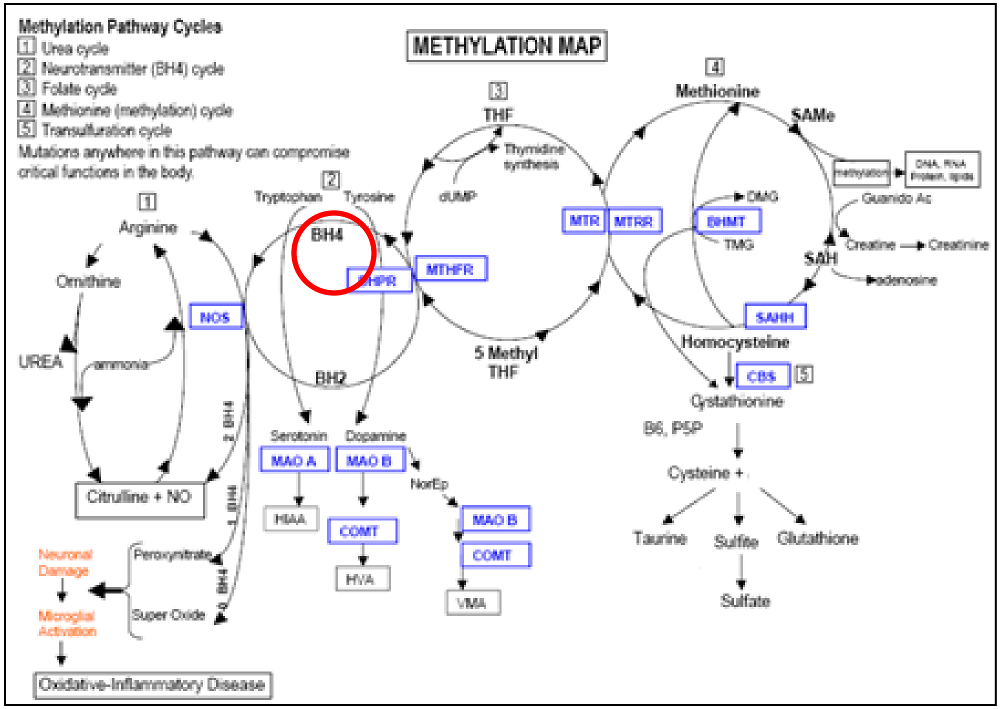

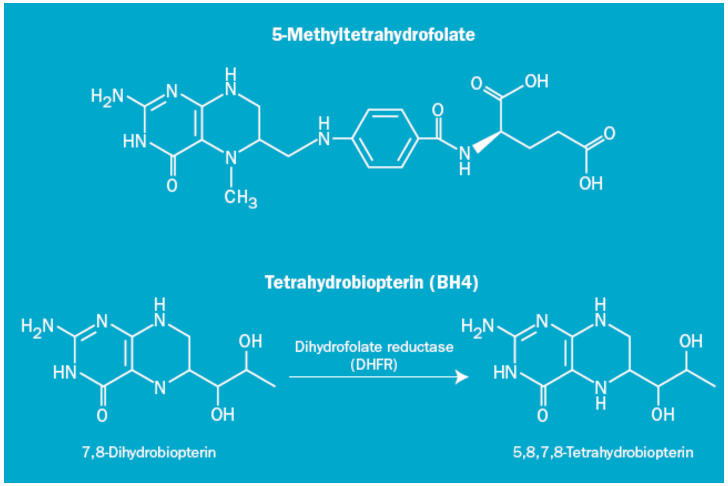

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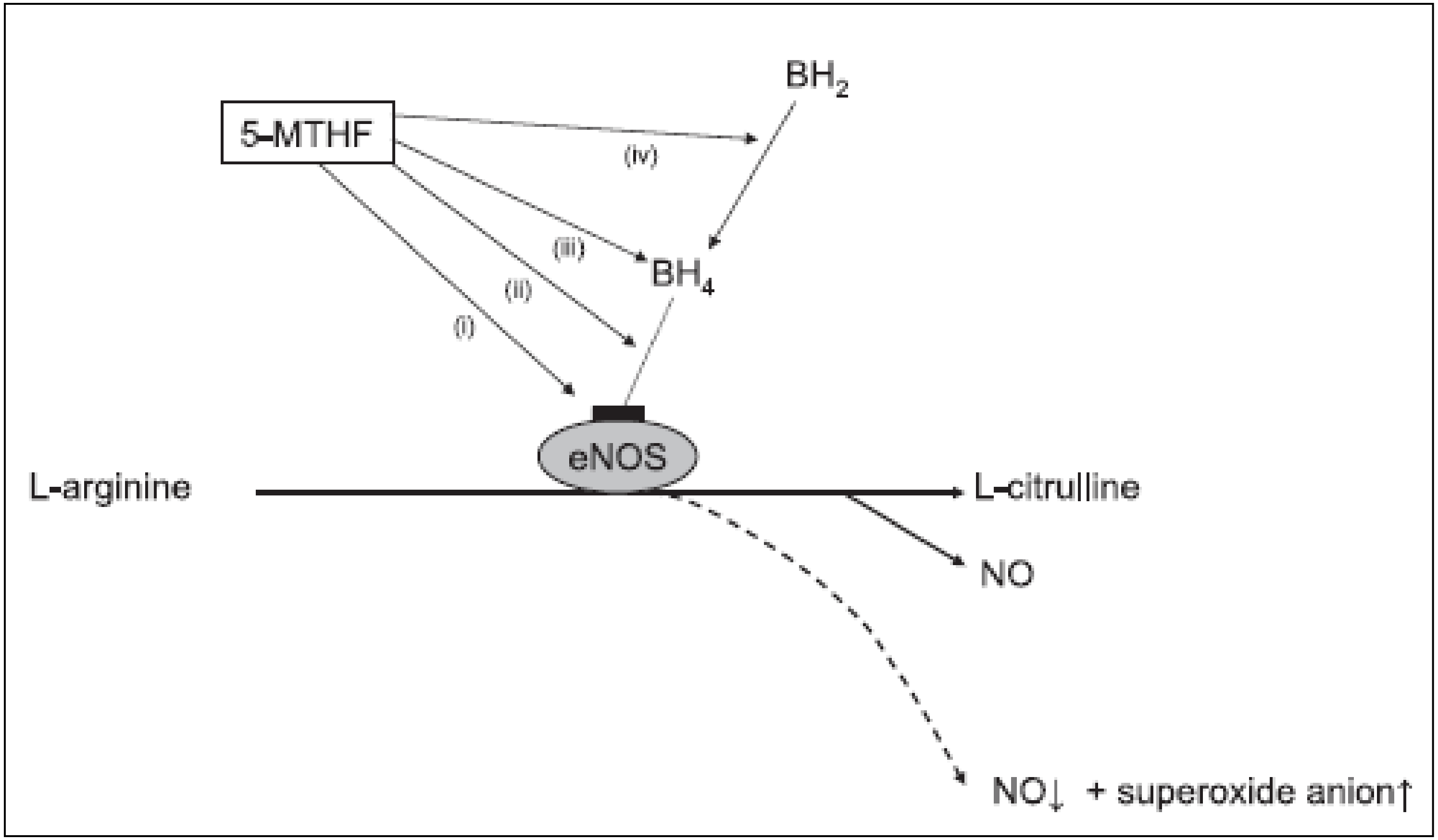
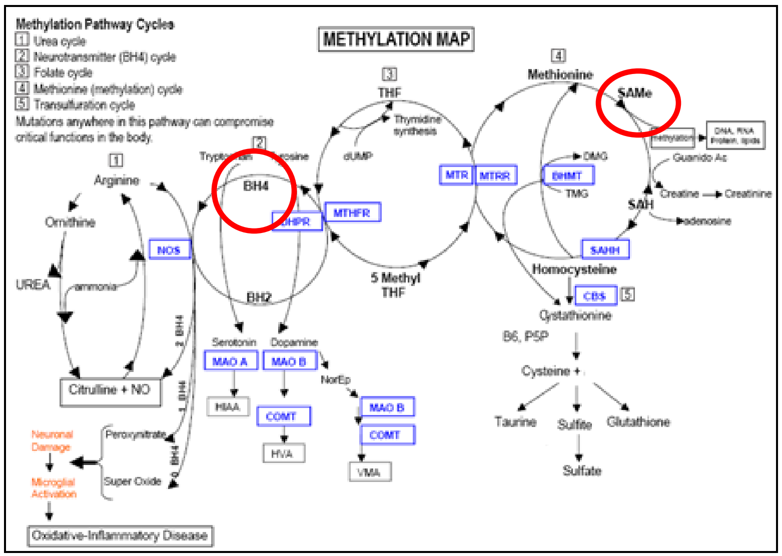
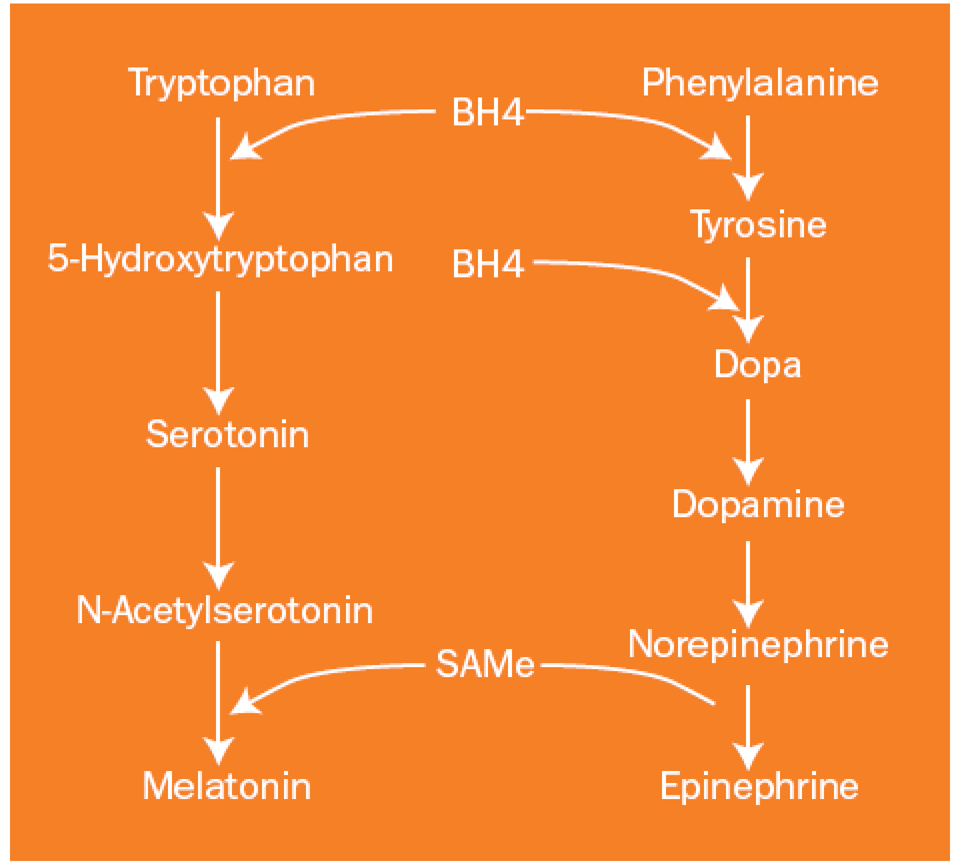
FOLATES (5-METHYL FOLATE) SUPPORT BH4



FOLATES (5-METHYL FOLATE) SUPPORT BH4 and SAMe





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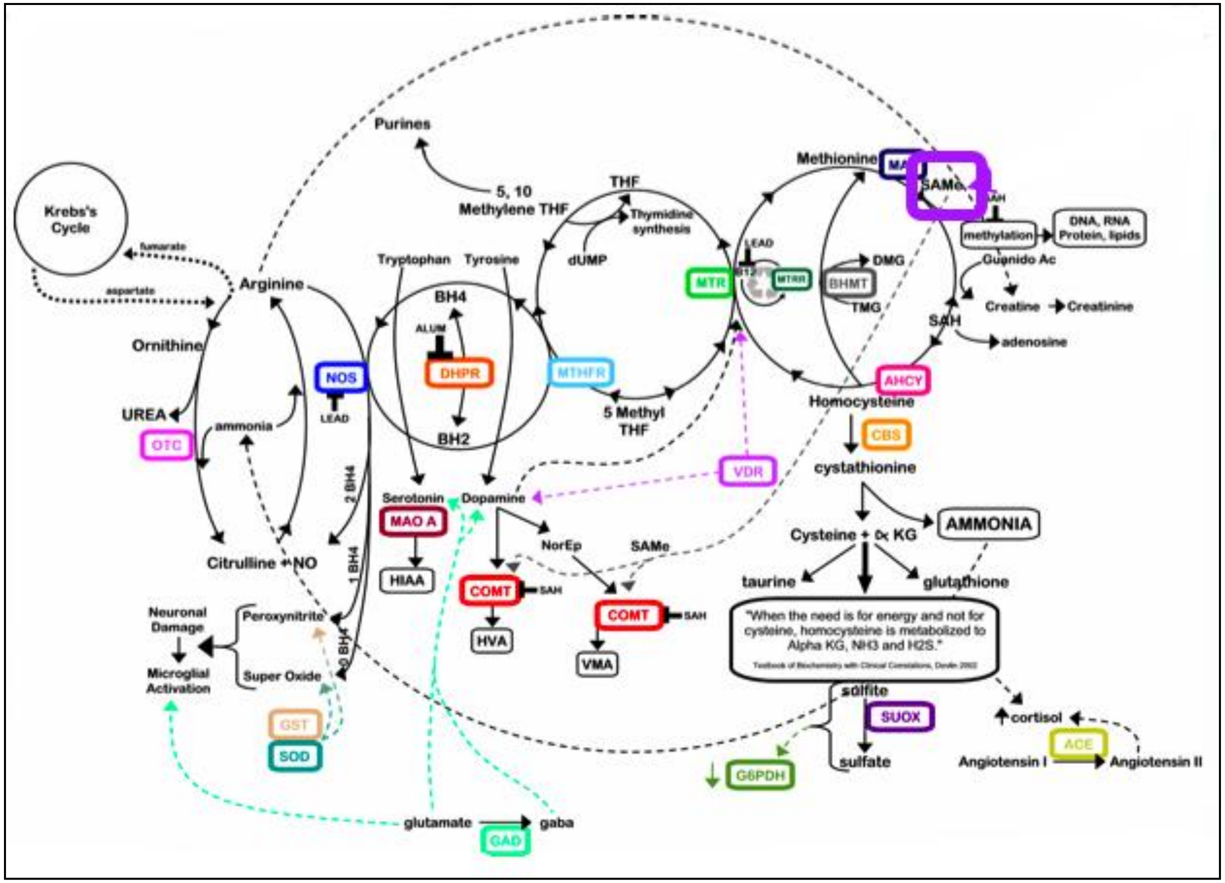

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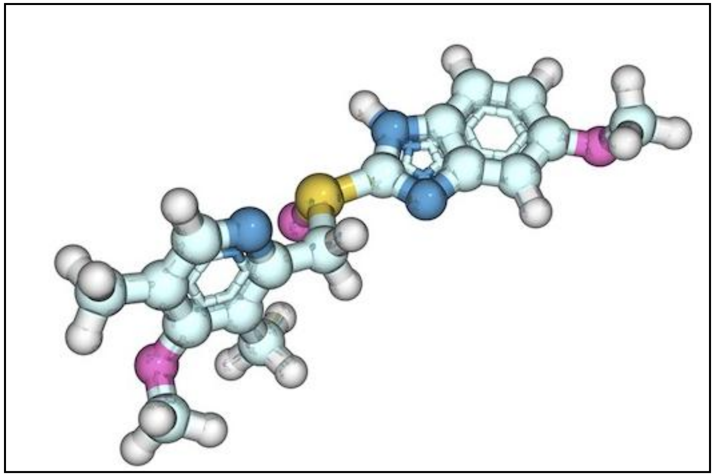
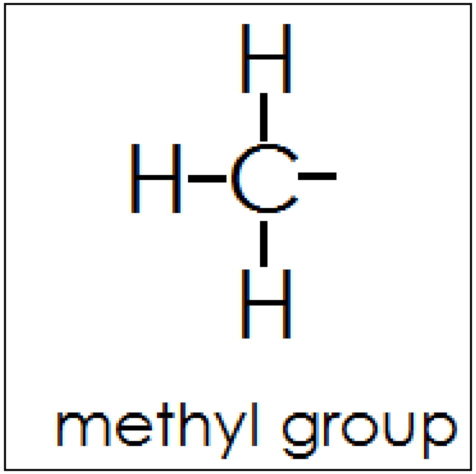
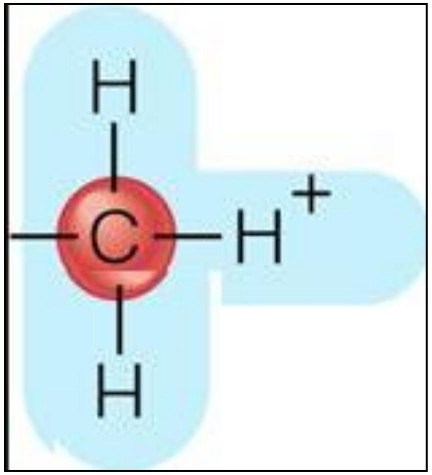
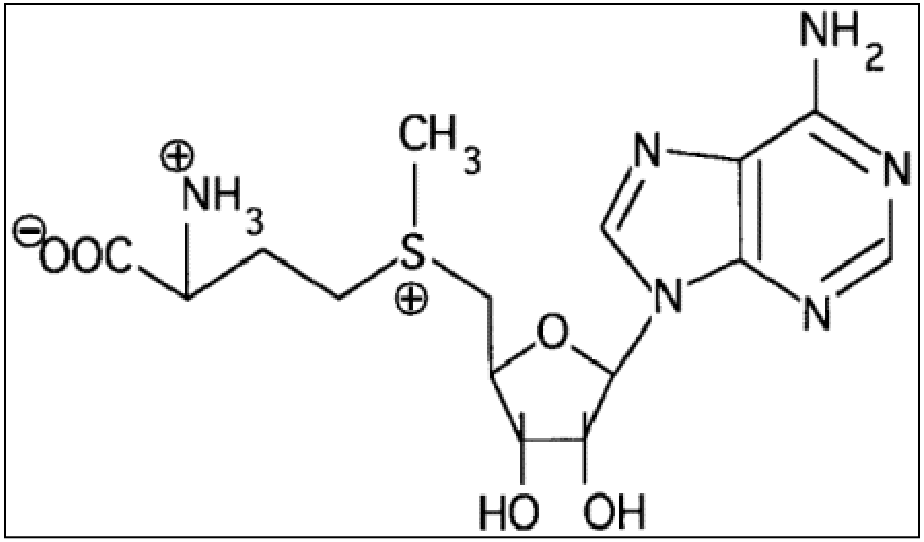
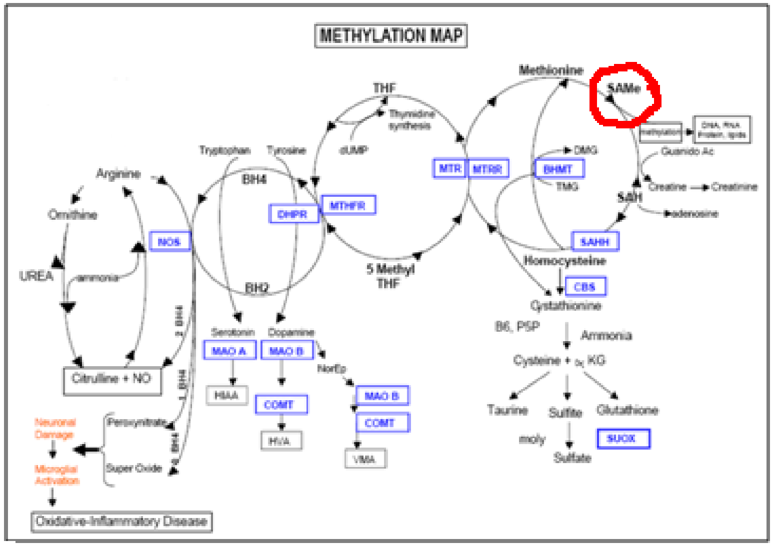
TRANSFERABLE METHYL GROUPS







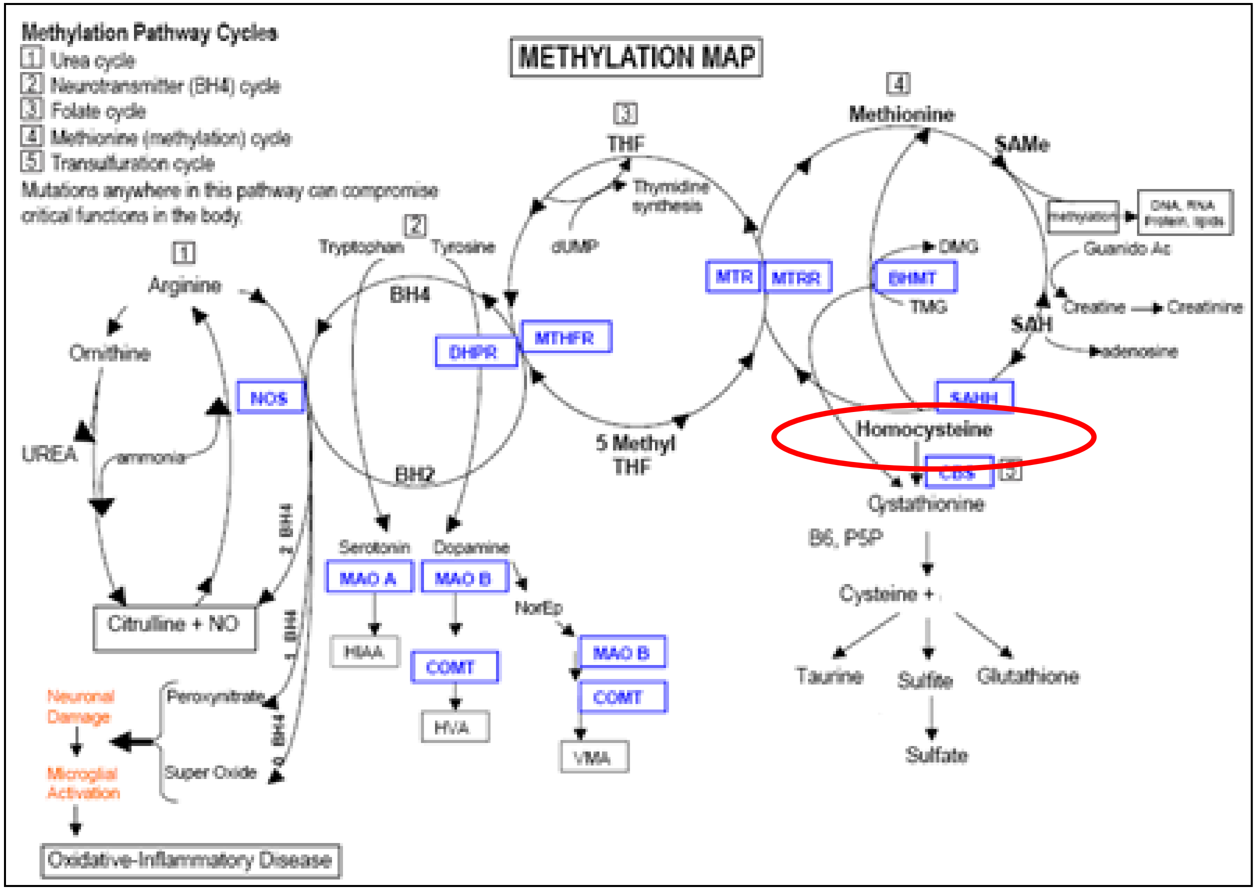




SAMe METHYL TRANSFER REACTIONS

Enzyme	Substrate and Effect
DNA Methyl Transferases	Alters DNA Transcription (Bookmarking)
Synthetic Reactions	Generation of Carnitine
Protein Methyl Transferases (PRMT)	Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)
Catechol-*O*-Methyl Transferase COMT	Inactivates Catecholamines
	Methylates 2-OH and 4-OH Estrogens
	Metabolizes Bioflavonoids
PEMT Phosphatidylethanolamine N-Methyl Transferase	Generation of Phosphatidylcholine
GAMT Guanidinoacetate N-Methyl Transferase	Generation of Creatine
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF

FUNCTIONS of the METHYL CYCLE



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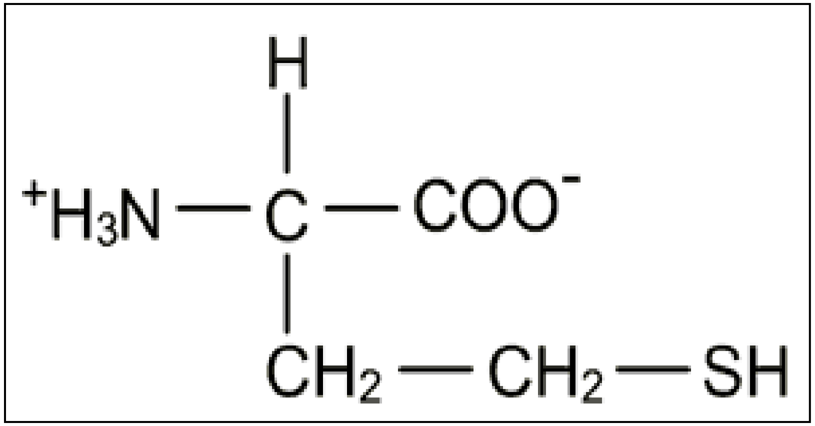
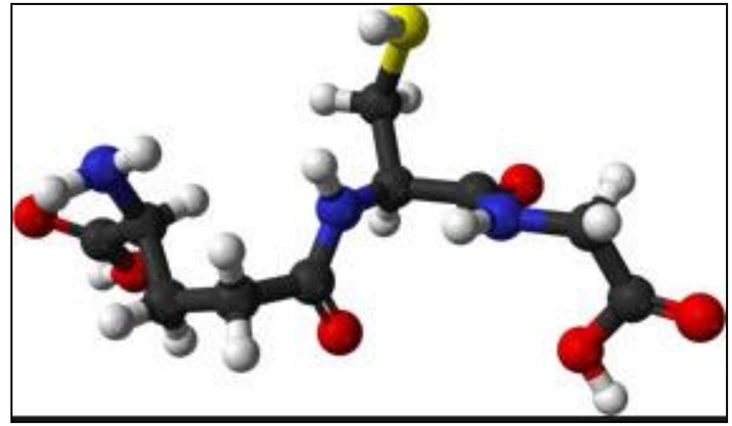
HOMOCYSTEINE





Oxidative stress → NF-κB translocation, pAMPK → AMPK ⇒ HMG Co-A Reductase, endothelial dysfunction, mitochondrial failure → apoptosis

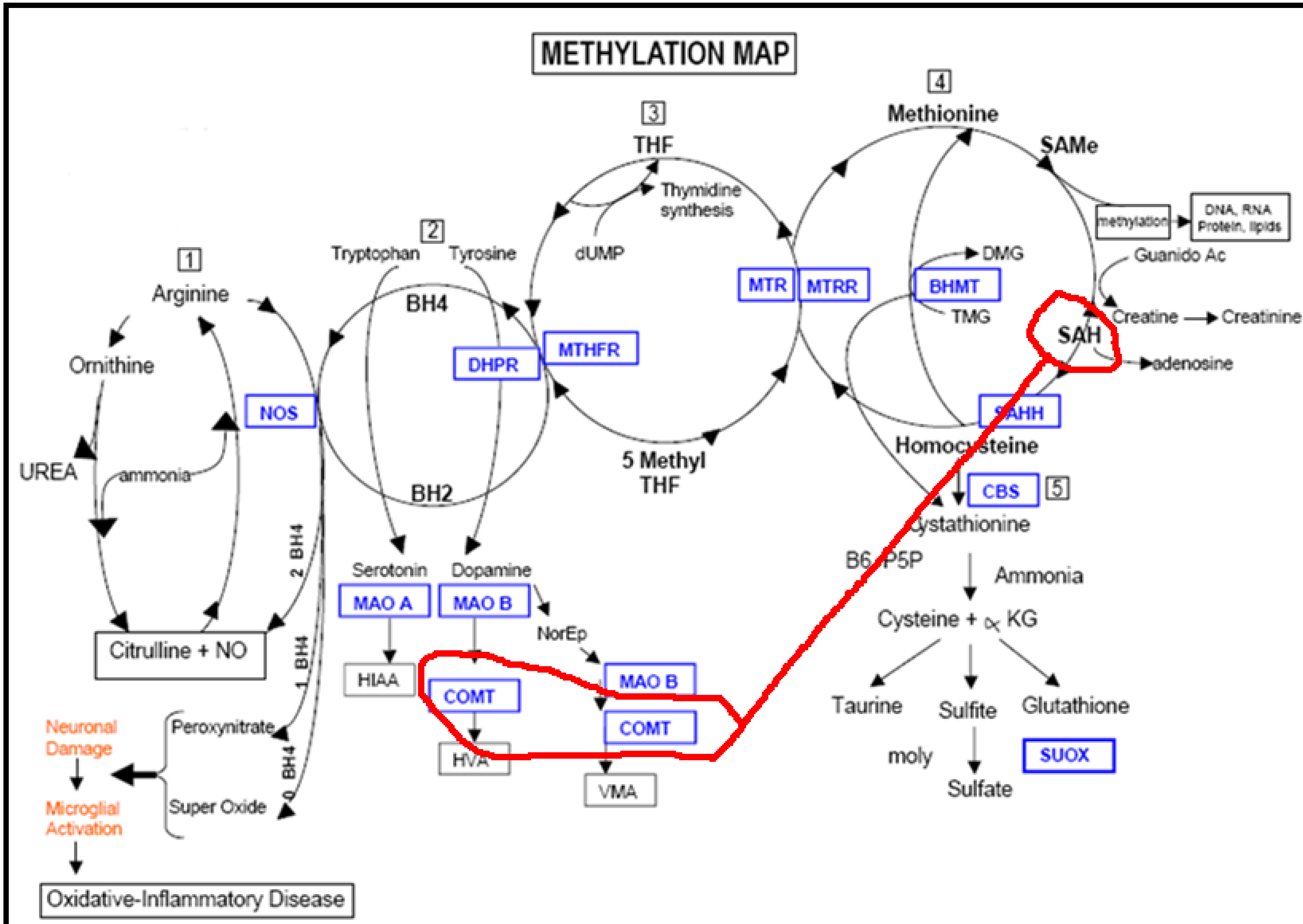
Homocysteine (Hcy) excess → build up of S-Adenosylhomocysteine (SAH)

SAH build up inhibits COMT → Impaired catecholamine and catachol estrogen metabolism

SAH build up inhibits DNMT → Accelerated aging and malignancy

Contributes to multiple disease states (Autism to Alzheimer’s)

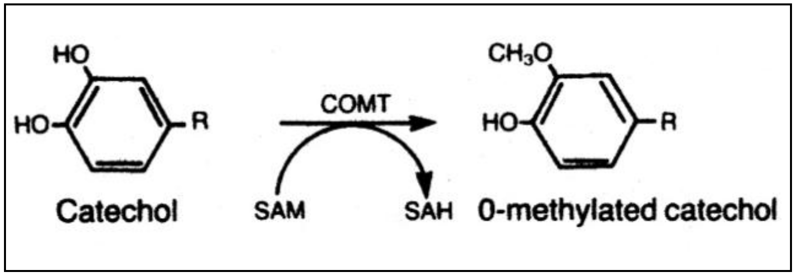
SAH INHIBITS COMT



SAH INHIBITS COMT

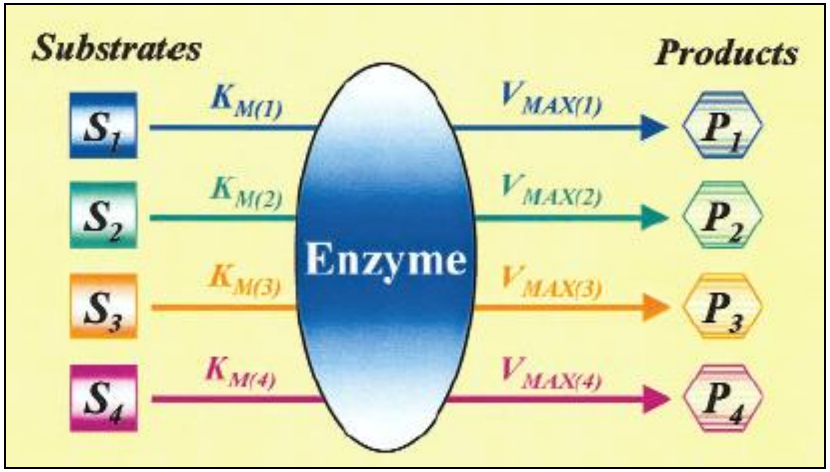
COMT (Catechol-*O*-Methyl Transferase):

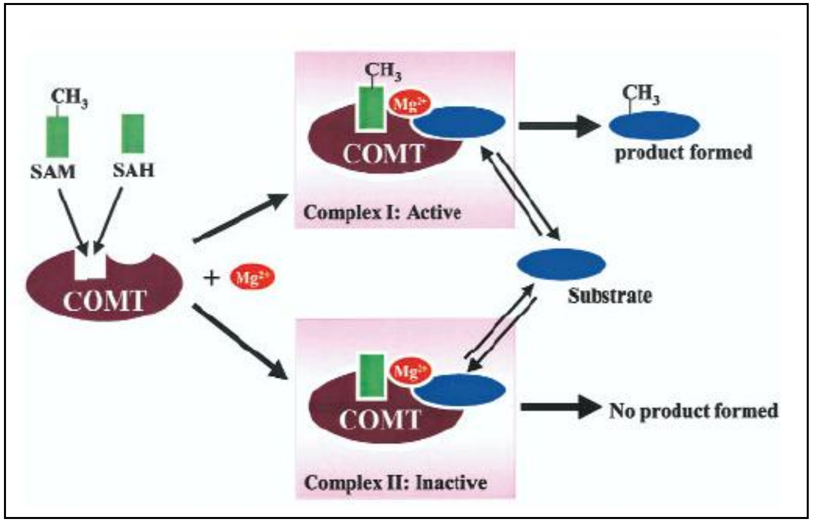
- High capacity
- Low specificity



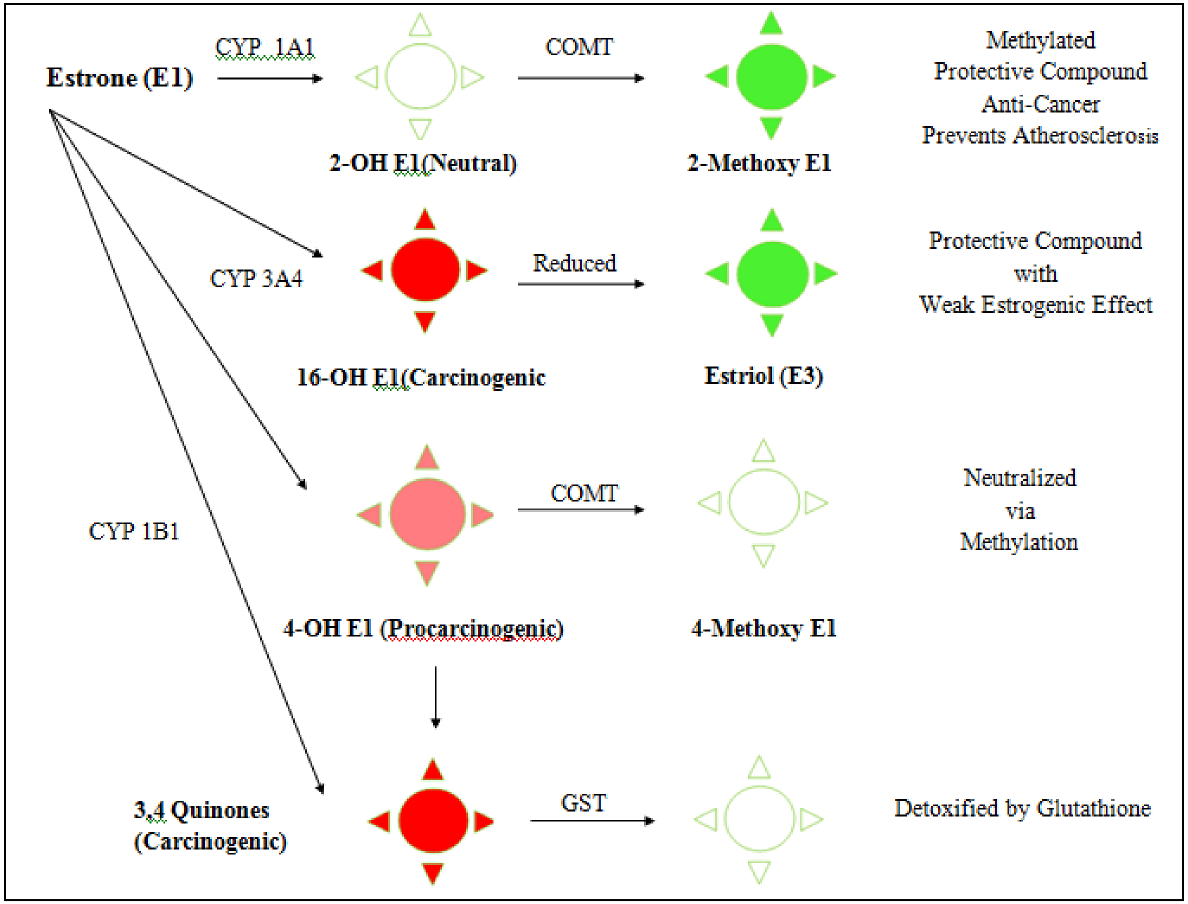
COMT *O* –Methylates Catechols:

- Catecholamines
- Catechol (OH)-Estradiol and Estrone
- Catechol bearing Bioflavonoids (ECGC and Quercetin)





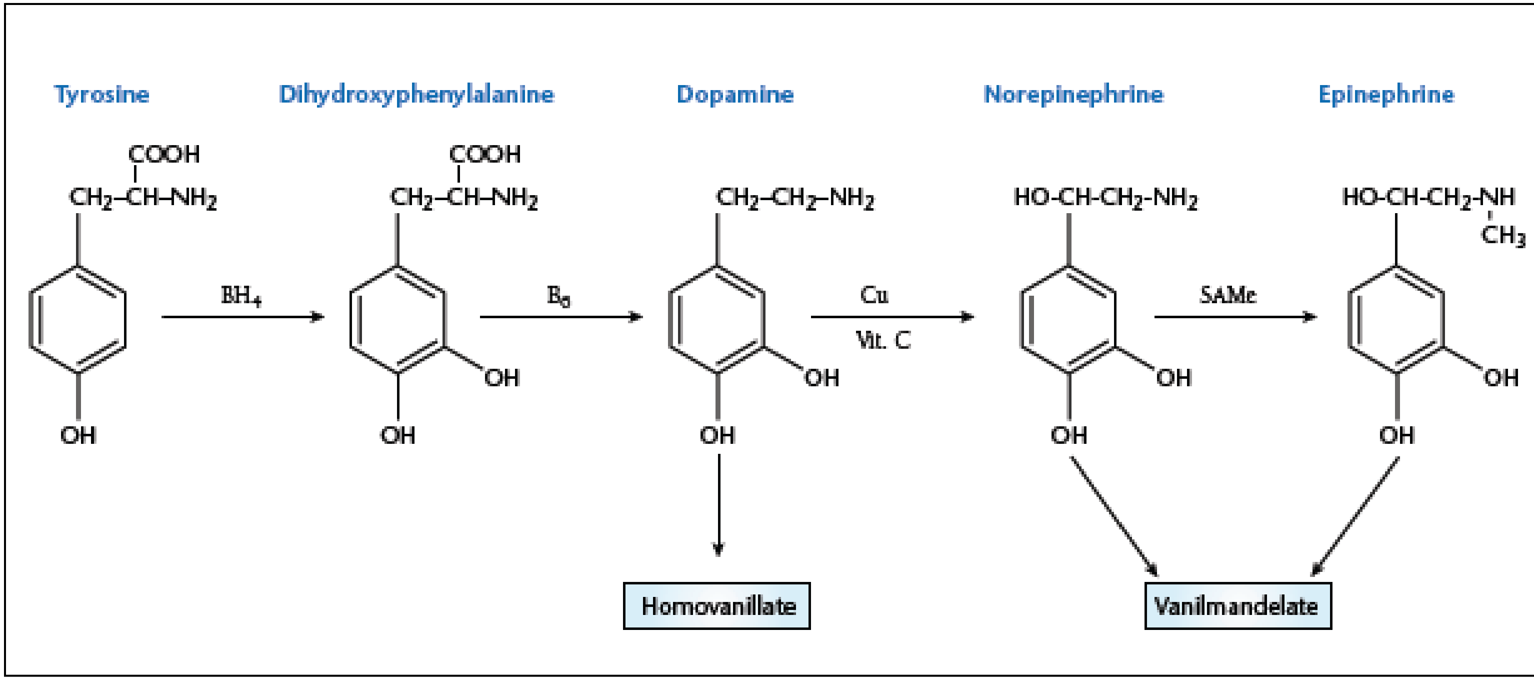
IMPAIRED ESTROGEN METABOLISM

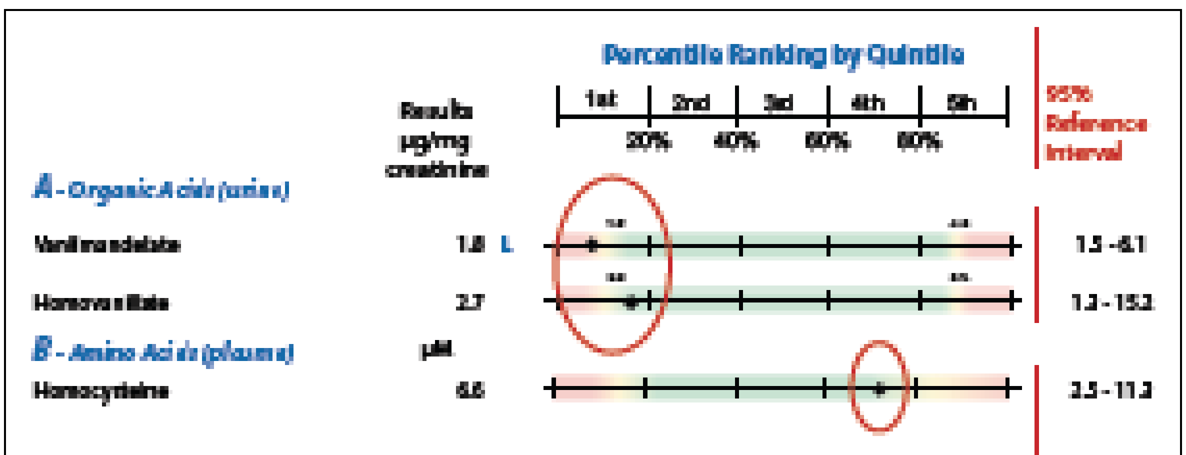


Impaired conversion of 2 and 4-OH into Methoxy Estrogens

(Impaired neutralization of 16-OH and 3,4 Quinones)

IMPAIRED CATECHOLAMINE BREAKDOWN





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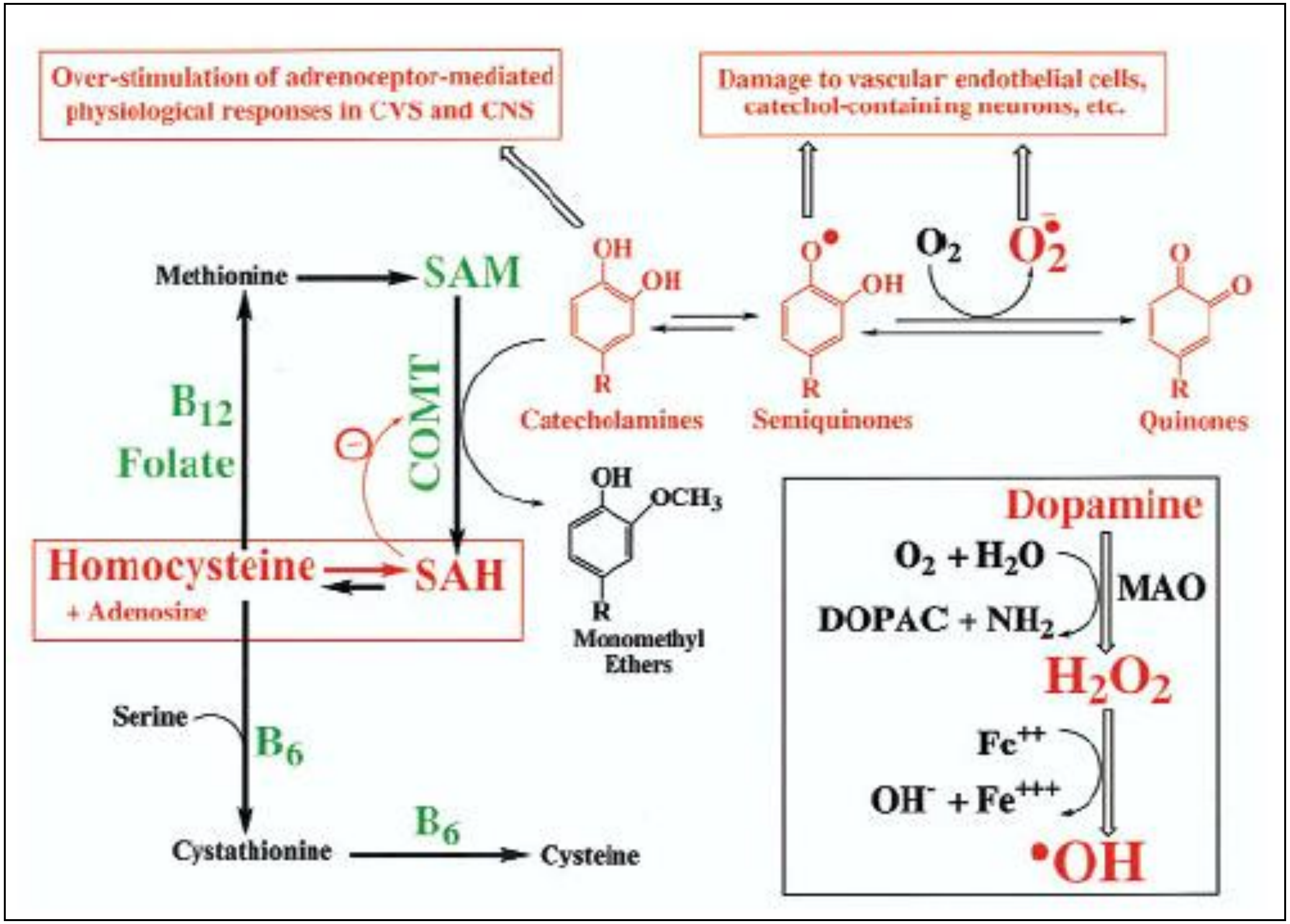
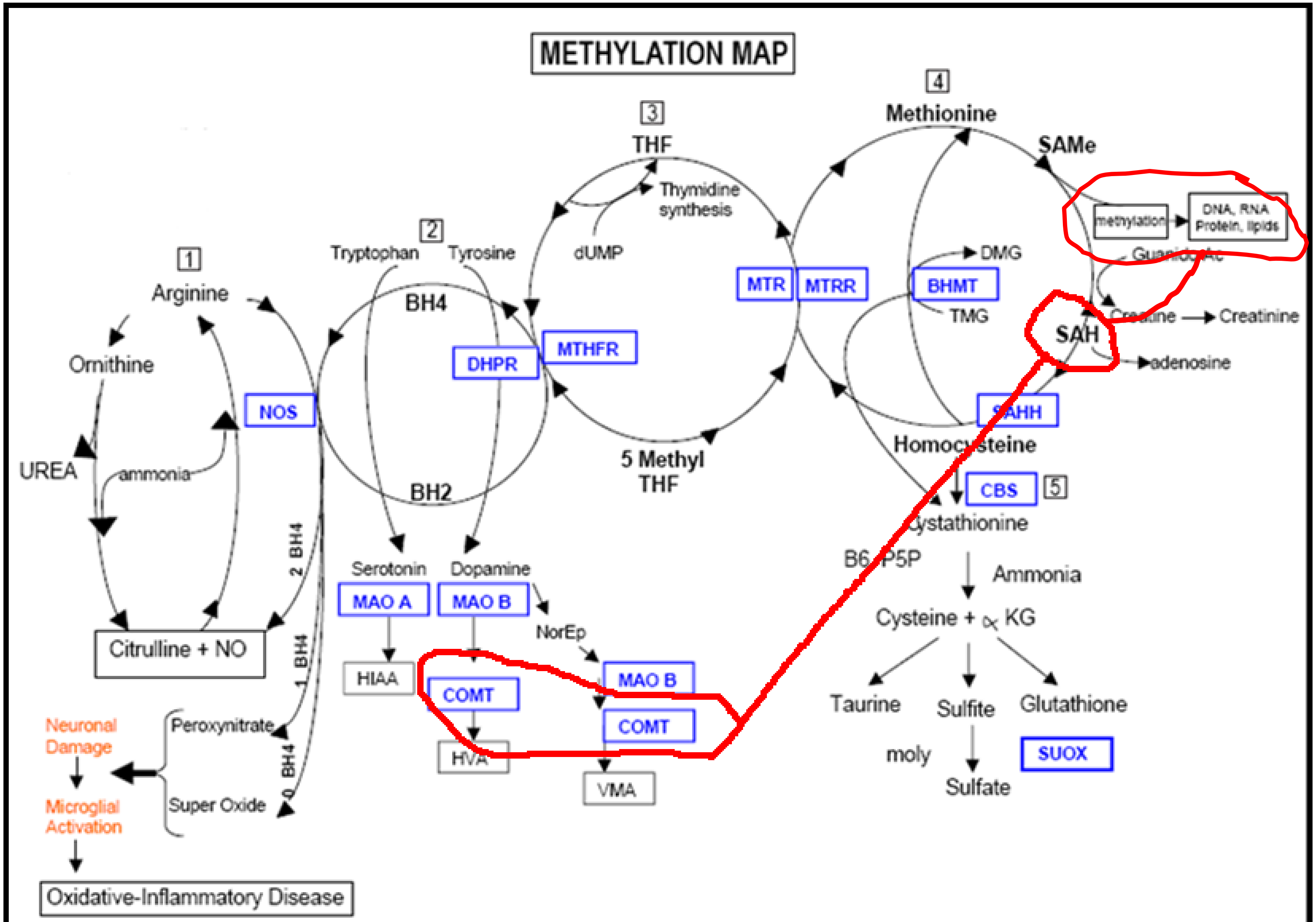
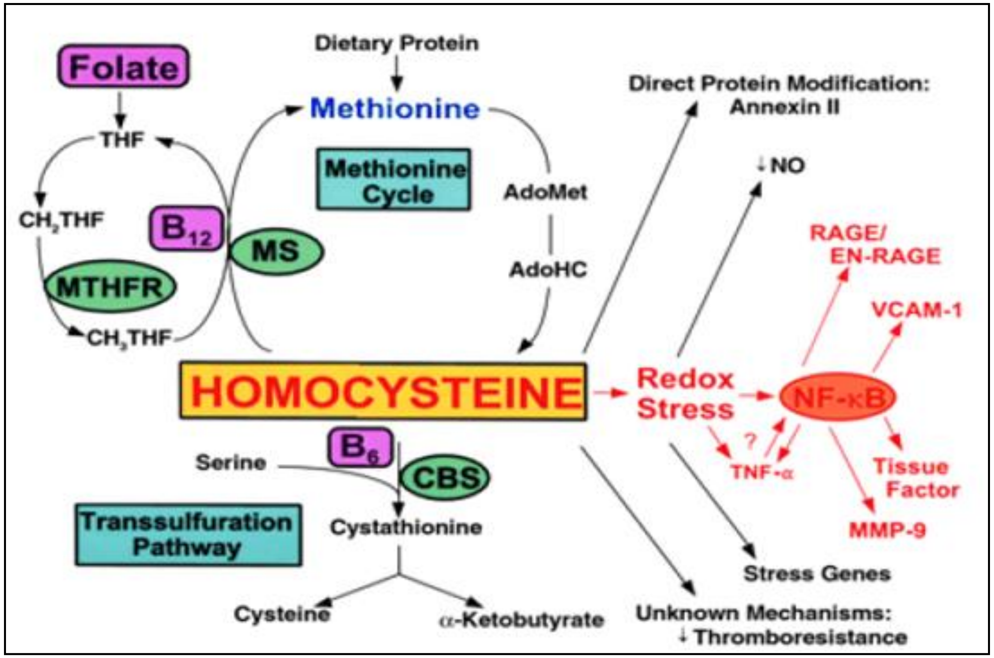
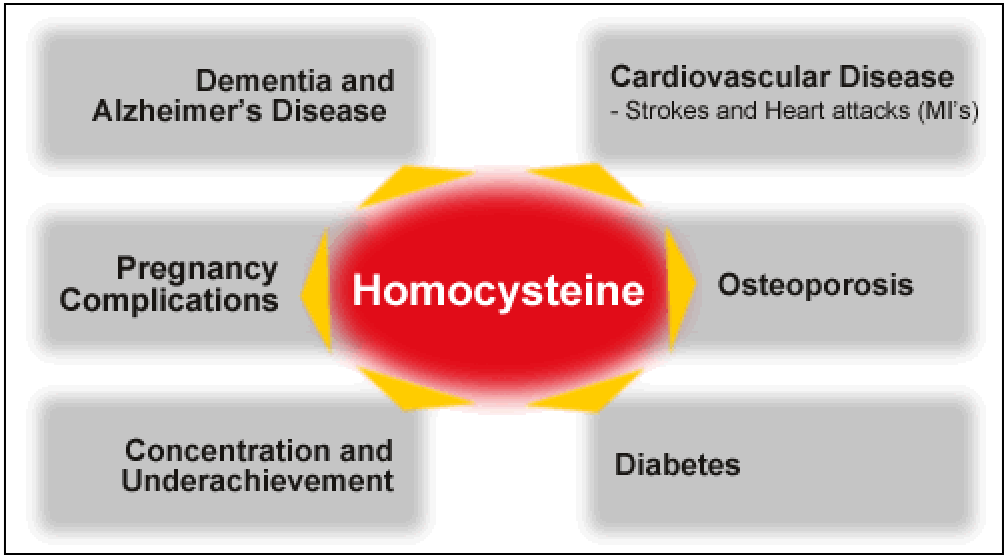


SAH INHIBITS METHYL TRANSFER BIOLOGY



HOMOCYSTEINE





METHYL CYCLE INTERMEDIATES and PRODUCTS

Methyl Donors	Methyl Taxis	Co-Factors	Products
Serine (Glycine)	THF	P-5-P	SAMe
Methionine	5,10-THF (CH2)	Riboflavin	Glutathione
TMG (Choline)	5-Methyl-THF (CH3)	Methyl-B12	Taurine
	Methenyl-THF (=CH)	Zinc	Cysteine
	Formyl-THF (CHO)		BH4
			DNA

Modifying Factors:

- Oxidative stress
- Inflammatory stress
- Renal status
- Nutritional status

ELEVATED HOMOCYSTEINE

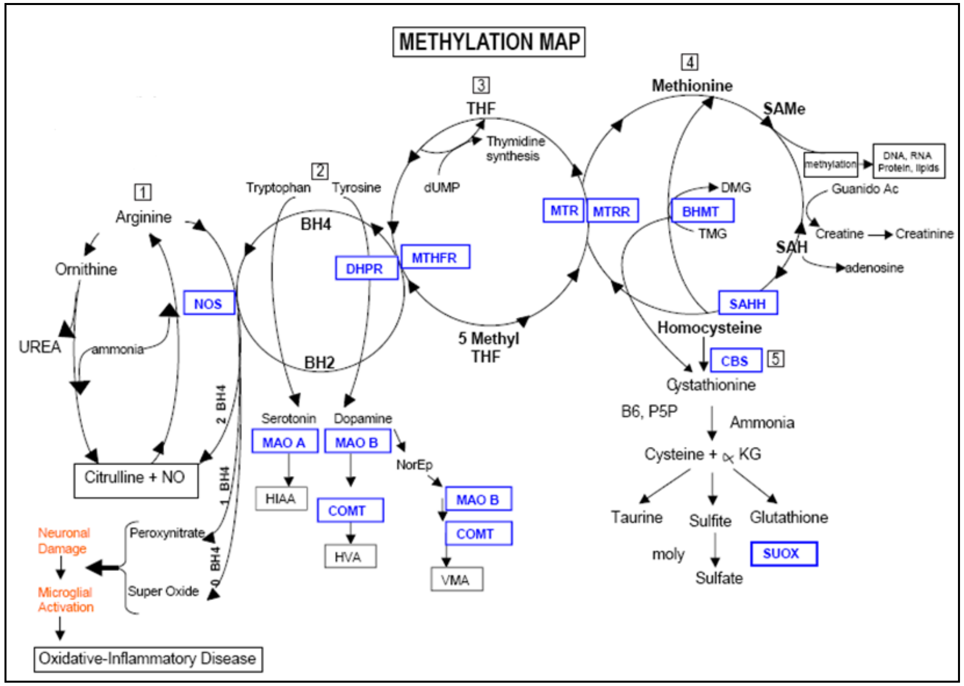


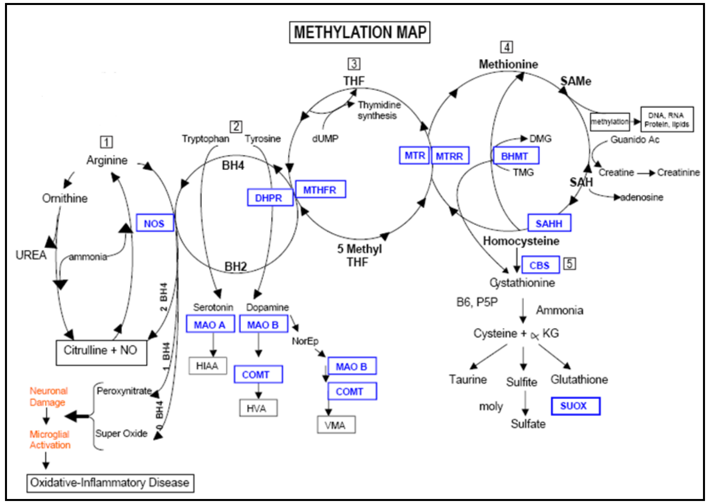
TABLE 6.7 — DIFFERENTIATING B_{12}, FOLATE, AND B_6 DEFICIENCY

Scenario	Markers	Potential Deficiency
1	Homocysteine ↑ with no other test result	Either B_{12}, folate or B_6, or methyl donor compounds
2	Methylmalonate ↑ with Homocysteine and FIGLU normal	B_{12}
3	Homocysteine ↑ with normal methylmalonate	B_6 or folate
4	Both Homocysteine ↑ and methylmalonate ↑	B_{12}, folate, and B_6
5	Homocysteine ↑ with MMA and FIGLU normal	B_6 and possible methyl donor compounds
6	HCys, MMA and FIGLU all ↑	B_6, B_{12}, folate and methyl donor compounds

Oxidative and Inflammatory Stress – Cause and Consequence

Renal Insufficiency

HOMOCYSTEINE REDUCTION



Folic Acid (less effective if MTHFR 677C→T) → 5-Methyl Folate

B12 (less effective if MTRR +) → Methyl-B12

B6 (Pyridoxal-5 Phosphate, P-5-P, more effective)

Riboflavin if MTHFR CT or TT

TMG and Zinc to support BHMT

Serine (Glycine) to support SHMT and CBS

Resolve oxidative stress and improve kidney function

Decrease dietary methionine (if excessive)

Eliminate/neutralize methyl thieves (Alcohol, Niacin, Estradiol, Fibrates, Diuretics)

Decrease Hcy generation (Creatine and Phosphatidylcholine)

NAC, Fish Oil, Danshensu, & Estradiol

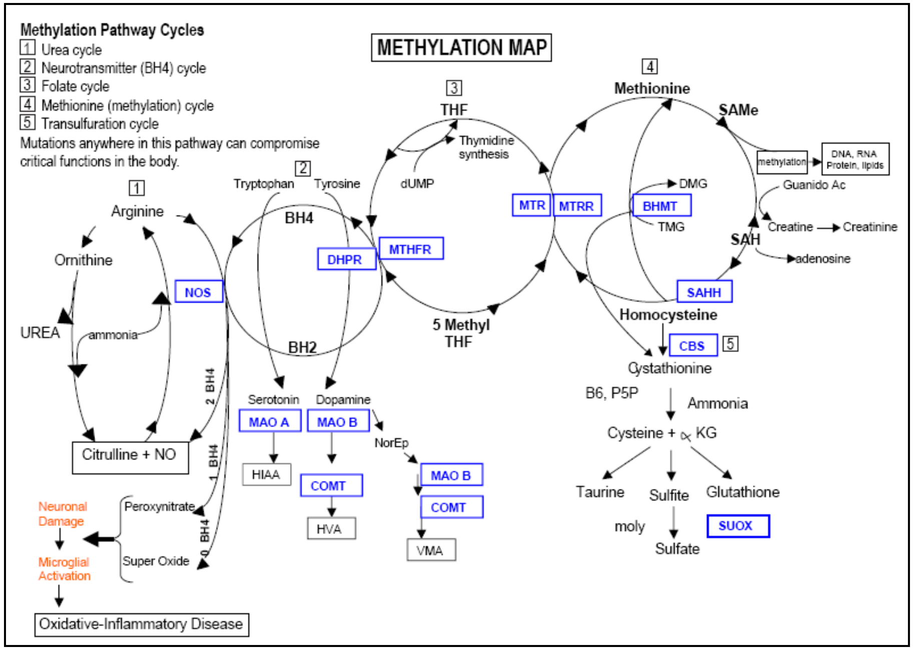
HOMOCYSTEINE REDUCTION TRIALS

<u>Reduction in Hcy achieved but no beneficial effect on outcome</u>

Large populations vs. subgroups with higher Hcy values
Folic Acid (less effective vs. methyl-folate if MTHFR CT/TT); riboflavin ignored
B12 low dose (less effective if MTRR +/- and ineffective if +/+) → Methyl-B12
B6 low dose (Pyridoxal-5 Phosphate, P-5-P, more effective)
BHMT pathway ignored (Zinc and TMG or Phosphatidylcholine)
Genomic variants not considered
Methyl thieving drug therapy not considered
Methionine intake not considered
Oxidative and inflammatory stress not considered
No attempt to decrease Hcy production (Creatine and Phosphatidylcholine)
Consequences of high Hcy not measured, such as impaired/low:

- SAMe:SAH
- Glutathione
- DNA Methylation
- Methylation of catecholamine and estrogen molecules
- BH4

FUNCTIONS of the METHYL CYCLE



Maintain (current health status) appropriate levels of:

- Pyrimidine and purine bases for DNA and RNA synthesis
- Antioxidant/Detox molecules glutathione, cysteine, taurine, & sulfate
- BH4 (tetrahydrobiopterin)
- Transferable methyl groups ≈ High SAM:SAH

♦ Genomic – Nutritional – Toxicity Interaction

HOMOCYSTEINE vs. S-ADENOSYLHOMOCYSTEINE

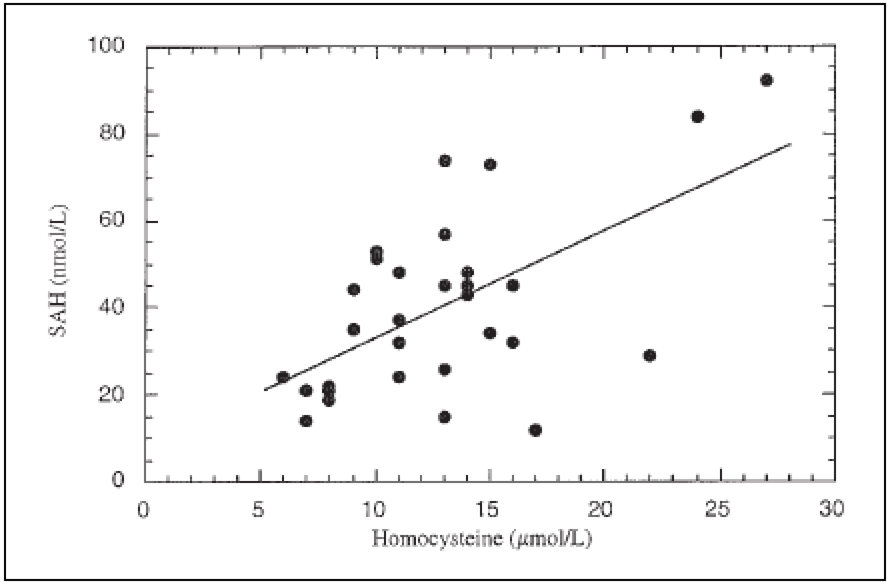
♥ 60 subjects:

- 30 with known CADz (15 s/p MI and 21 s/p revascularization)
- 30 age and gender matched healthy controls

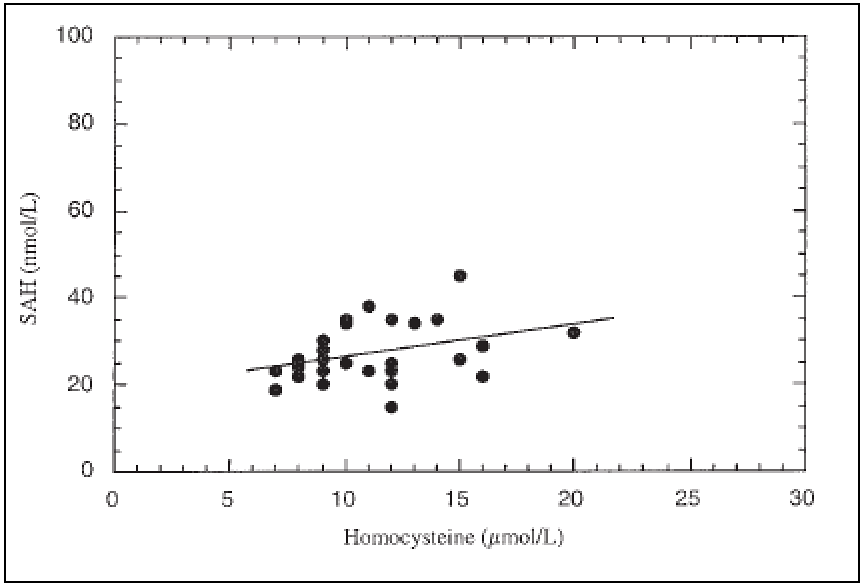
	CADz	Control Subjects	Units
Age	64	61	Years
Folate	34.9	32.4	nmol/l (10^{-9})
B12	292	310	pmol/l (10^{-12})
Homocysteine	12.8	11.0	umol/l (10^{-6})
SAMe	122	104	nmol/l (10^{-9})
SAH	43	27	nmol/l (10^{-9})
SAMe:SAH	2.8	3.9	
Creatinine	110	97	umol/l (10^{-6})
Creatinine	1.24	1.1	mg/dl

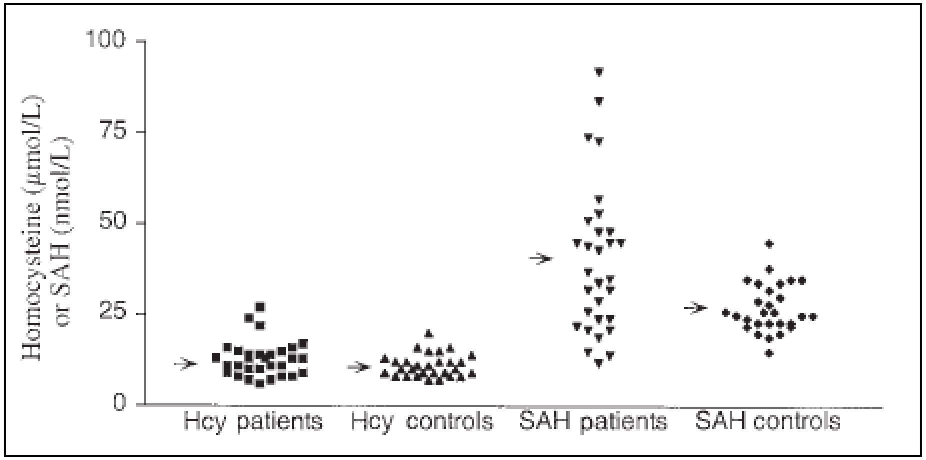
HOMOCYSTEINE VS. S-ADENOSYLHOMOCYSTEINE

Coronary Disease Patients



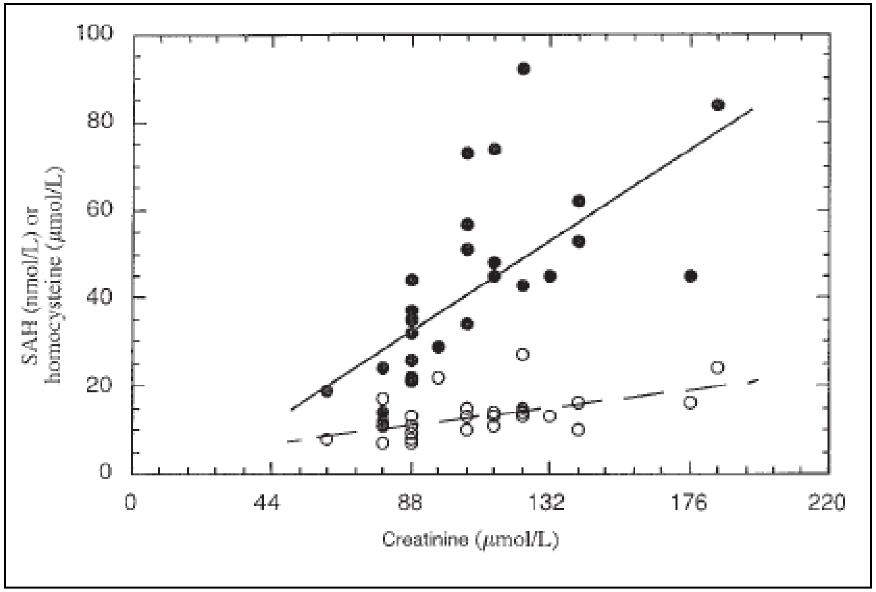
Healthy Controls



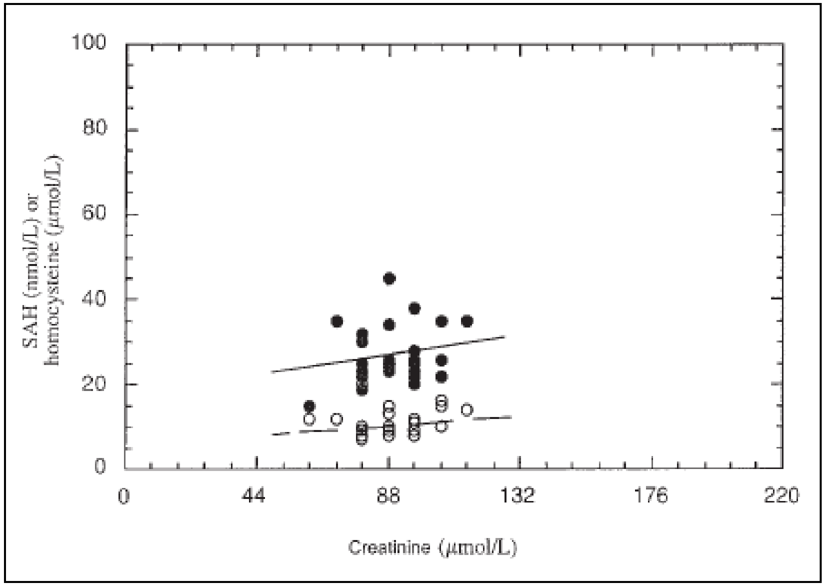


HOMOCYSTEINE VS. S-ADENOSYLHOMOCYSTEINE

Coronary Disease Patients



Healthy Controls



O = Homocysteine

• = S-Adenosylhomocysteine

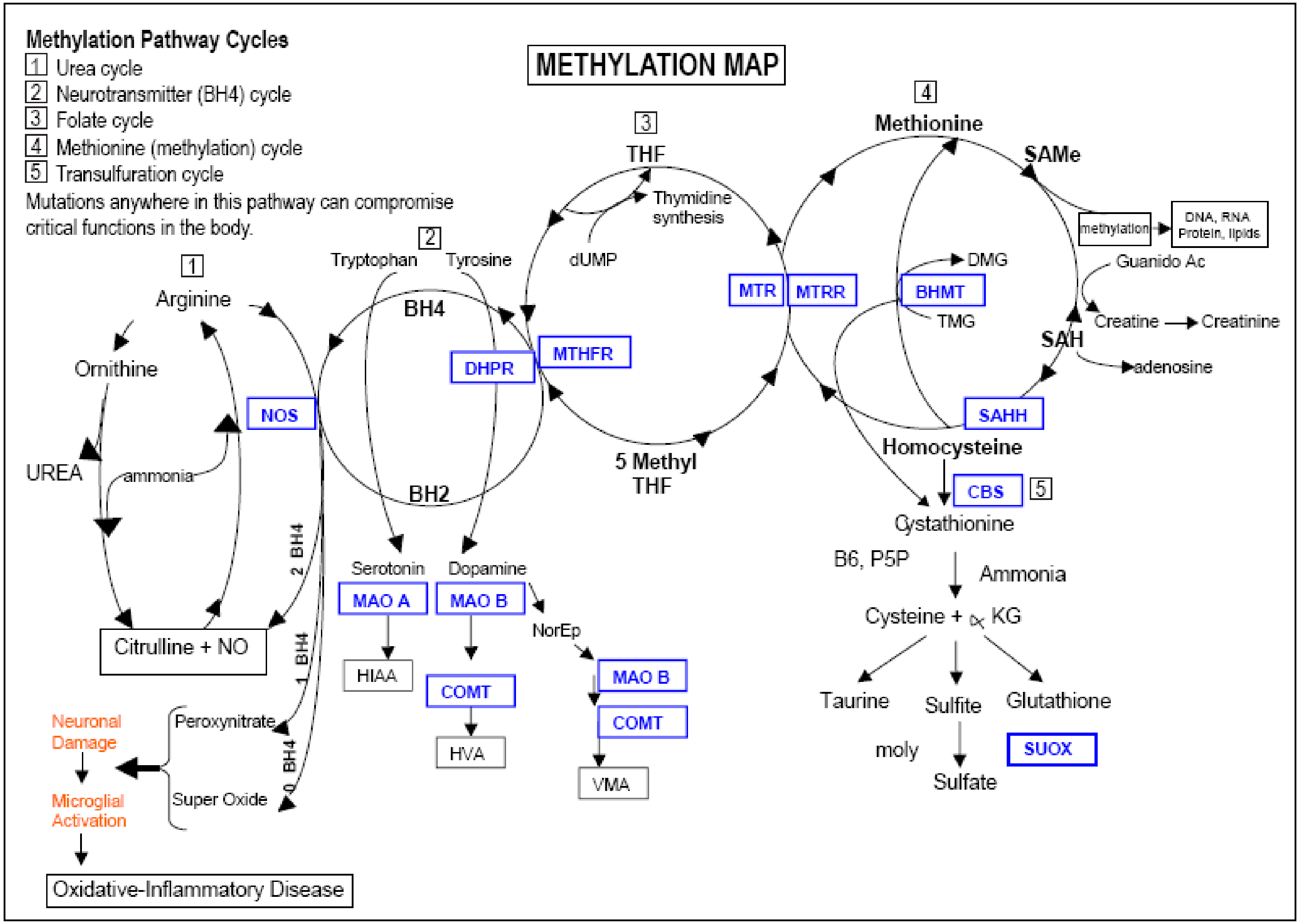
HOMOCYSTEINE VS. S-ADENOSYLHOMOCYSTEINE

	CADz	Control Subjects
Homocysteine	12.8	11.0
SAM	122	104
SAH	43	27
Creatinine	1.24	1.1

	ACEI		β-Blocker		Diuretic	
	Yes	No	Yes	No	Yes	No
Creatinine	1.35	1.24	1.24	1.35	1.35	1.24
Hcy	14.0	11.8	11.6	13.8	14.7	12.4
SAH	46	35	36	43	47	38

Nutrigenomic Report for an 53 Year Old Marathon Runner		
Methylation Panel Abnormalities for Genes with Characterized SNPs		
Gene Name	**Variation**	**Finding**
COMT	V158M	OK
COMT	H62H	OK
COMT	61	OK
VDR	Taq	Homozygous (+/+)
VDR	Fok	OK
MAO A	R297R	OK
ACAT	102	OK
ACE	Del16	Homozygous (+/+)
MTHFR	C677T	Homozygous (+/+)
MTHFR	3	OK
MTHFR	A1298C	OK
MTR	A2756G	OK
MTRR	A66G	Heterozygous (+/-)
MTRR	H595Y	OK
MTRR	K350A	OK
MTRR	R415T	OK
MTRR	S257T	OK
MTRR	11	Heterozygous (+/-)
BHMT	1	OK
BHMT	2	Heterozygous (+/-)
BHMT	4	Heterozygous (+/-)
BHMT	8	Heterozygous (+/-)
AHCY	1	OK
AHCY	2	OK
AHCY	19	OK
CBS	C699T	OK
CBS	A360A	Heterozygous (+/-)
SUOX	S370S	Heterozygous (+/-)
SHMT	C1420T	OK
NOS	D298E	Heterozygous (+/-)

Methyl Cycle with analysis ($550)
Multiple Genes without analysis ($98)



VDR Taq	SHMT	DHFR	ACAT	CYP1A2	GSTP1
VDR Bsm	AHCY	GNMT	ACE	CYP1B1	PON1
MTHFD	FOLR	PEMT	MTHFS	SOD	GAD1

HELP with UDERSTANDING and INTERPRETATION

Multiple Genes without analysis result →

Report generator website

- Yasko Methylation Report
- Detoxification Enzyme report

Support website

- Entire (Multiple Genes without analysis) report

Hack the genes website

- Brief functional significance of variant alleles

Initial website (Methyl Cycle with analysis)

Website of Fellowship instructor

HELP with UDERSTANDING and INTERPRETATION

Organic Acid Analysis ($170 with commercial insurance and covered by Medicare)

- AAs, B Vitamins, FAs, and RBC Minerals
- Oxidative Stress and Inflammatory markers
- Glucose, Fatty acid, and Krebs Cycle function
- RBC metals and 2 Organic Pollutant markers ⇒

24 hour urine nutritional minerals ($65)

Eastern Labs Methyl Cycle Intermediates ($350)

- SAMe, SAH, THF, methyl-folate, folinic acid,
- Oxidized and reduced glutathione

Central Labs Methyl Cycle ($155)

- SAMe, SAH, Hcy, methionine, cystathionine, cysteine

Southern Labs ($95)

- Four point salivary cortisol and morning DHEAs
- Urine neurotransmitters

NUTRIGENOMIC ANALYSIS

Metabolic Analysis Markers

All biomarkers reported in mmol/mol creatinine unless otherwise noted.

Malabsorption and Dysbiosis Markers

Malabsorption Markers	Result	Reference Range
Indoleacetic Acid (IAA)	2.1	<= 4.2
Phenylacetic Acid (PAA)	0.78	<= 0.12

Bacterial Dysbiosis Markers	Result	Reference Range
Dihydroxyphenylpropionic Acid (DHPPA)	1.5	<= 5.3
3-Hydroxyphenylacetic Acid	9.0	<= 8.1
4-Hydroxyphenylacetic Acid	15	<= 29
Benzoic Acid	>0.71	<= 0.05
Hippuric Acid	<dl	<= 603

Yeast / Fungal Dysbiosis Markers	Result	Reference Range
Arabinose	70	<= 96
Citramalic Acid	2.4	<= 5.8
Tartaric Acid	<dl	<= 15

Cellular Energy & Mitochondrial Metabolites

Carbohydrate Metabolism	Result	Reference Range
Lactic Acid	17.5	1.9-19.8
Pyruvic Acid	18	7-32
β-OH-Butyric Acid (BHBA)	1.0	<= 2.8

Energy Metabolism	Result	Reference Range
Citric Acid	67	40-520
Cis-Aconitic Acid	9	10-36
Isocitric Acid	20	22-65
α-Ketoglutaric Acid (AKG)	14	4-52
Succinic Acid	<dl	0.4-4.6
Malic Acid	0.9	<= 3.0
β-OH-β-Methylglutaric Acid (HMG)	8	<= 15

Fatty Acid Metabolism	Result	Reference Range
Adipic Acid	1.3	<= 2.8
Suberic Acid	0.8	<= 2.1

Creatinine Concentration

	Result	Reference Range
Creatinine *	6.9	3.1-19.5 mmol/L

Neurotransmitter Metabolites

	Result	Reference Range
Vanilmandelic Acid	1.0	0.4-3.6
Homovanillic Acid	1.7	1.2-5.3
5-OH-indoleacetic Acid	6.6	3.8-12.1
3-Methyl-4-OH-phenylglycol	0.22	0.02-0.22
Kynurenic Acid	5.2	<= 7.1
Quinolinic Acid	4.0	<= 9.1
Kynurenic / Quinolinic Ratio	1.30	>= 0.44

Vitamin Markers

	Result	Reference Range
α-Ketoadipic Acid	0.7	<= 1.7
α-Ketoisovaleric Acid	0.31	<= 0.97
α-Ketoisocaproic Acid	0.56	<= 0.89
α-Keto-β-Methylvaleric Acid	0.5	<= 2.1
Formiminoglutamic Acid (FIGlu)	1.5	<= 1.5
Glutaric Acid	0.25	<= 0.51
Isovalerylglycine	1.3	<= 3.7
Methylmalonic Acid	0.9	<= 1.9
Xanthurenic Acid	0.34	<= 0.96
3-Hydroxypropionic Acid	7	5-22
3-Hydroxyisovaleric Acid	17	<= 29

Toxin & Detoxification Markers

	Result	Reference Range
α-Ketophenylacetic Acid (from Styrene)	0.28	<= 0.46
α-Hydroxyisobutyric Acid (from MTBE)	4.4	<= 6.7
Orotic Acid	0.44	0.33-1.01
Pyroglutamic Acid	24	16-34

Tyrosine Metabolism

	Result	Reference Range
Homogentisic Acid	4	<= 19
2-Hydroxyphenylacetic Acid	0.65	<= 0.78

Metabolic Analysis Reference Ranges are Age Specific

NUTRIGENOMIC ANALYSIS

Amino Acids (Urine FMV)

All biomarkers reported in micromol/gm creatinine unless otherwise noted.

Nutritionally Essential Amino Acids

Amino Acid	Result	Reference Range
Arginine	14	10-64
Histidine	744	271-993
Isoleucine	40	17-52
Leucine	66	25-77
Lysine	56	34-226
Methionine	37	26-69
Phenylalanine	51	22-61
Taurine	272	80-545
Threonine	139	52-192
Tryptophan	79	23-88
Valine	23	10-53

Nonessential Protein Amino Acids

Amino Acid	Result	Reference Range
Alanine	243	103-392
Asparagine	72	37-134
Aspartic Acid	55	27-74
Cysteine	40	10-70
Cystine	29	23-68
γ-Aminobutyric Acid	6	<= 23
Glutamic Acid	26	3-15
Glutamine	288	153-483
Proline	4	2-14
Tyrosine	70	28-113

Creatinine Concentration

	Result	Reference Range
Creatinine ◆	6.8	3.1-19.5 mmol/L

Amino Acid Reference Ranges are Age Specific

The performance characteristics of all assays have been verified by Genova Diagnostics, Inc. Unless otherwise noted with ◆, the assay has not been cleared by the U.S. Food and Drug Administration.

Intermediary Metabolites

B Vitamin Markers	Result	Reference Range
α-Aminoadipic Acid	43	11-73
α-Amino-N-butyric Acid	21	9-49
β-Aminoisobutyric Acid	112	19-163
Cystathionine	<dl	6-29
3-Methylhistidine	286	134-302

Urea Cycle Markers	Result	Reference Range
Ammonia	36.3	12.0-41.0 mmol/g creatinine
Citrulline	39	9-40
Ornithine	9	3-16
Urea ◆	363	150-380 mmol/g creatinine

Glycine/Serine Metabolites	Result	Reference Range
Glycine	1,095	434-1,688
Serine	98	135-426
Ethanolamine	197	156-422
Phosphoethanolamine	13	14-50
Phosphoserine	36	28-64
Sarcosine	34	<= 41

Dietary Peptide Related Markers

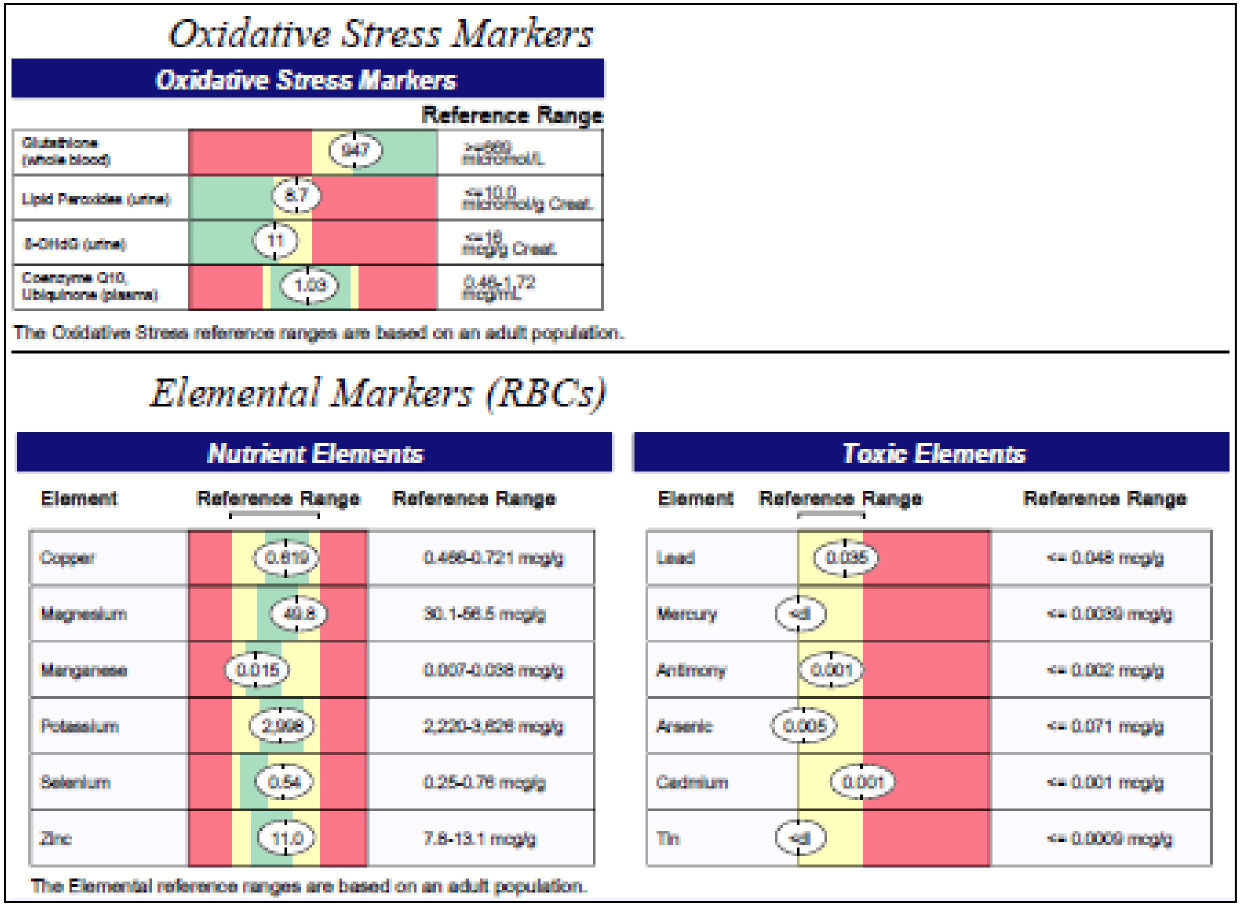
	Result	Reference Range
Anserine (dipeptide)	5	8-118
Carnosine (dipeptide)	28	12-120
1-Methylhistidine	487	83-1,008
β-Alanine	23	<= 17

Markers for Urine Representativeness

	Result	Reference Range
Glutamine/Glutamate	11	>= 12
Ammonia	36.3	12.0-41.0 mmol/g creatinine
Arginine/Ornithine	1.6	>= 1.0

	Result	Ref Range
Urine Representativeness Index	9	5 - 10

NUTRIGENOMIC ANALYSIS



Oxidative Stress Markers

Oxidative Stress Markers	Result	Reference Range
Glutathione (whole blood)	947	>=669 micromol/L
Lipid Peroxides (urine)	8.7	<=10.0 micromol/g Creat.
8-OHdG (urine)	11	<=16 mcg/g Creat.
Coenzyme Q10, Ubiquinone (plasma)	1.03	0.46-1.72 mcg/mL

The Oxidative Stress reference ranges are based on an adult population.

Elemental Markers (RBCs)

Nutrient Elements

Element	Reference Range	Reference Range
Copper	0.819	0.466-0.721 mcg/g
Magnesium	49.8	30.1-56.5 mcg/g
Manganese	0.015	0.007-0.038 mcg/g
Potassium	2,998	2,220-3,626 mcg/g
Selenium	0.54	0.25-0.76 mcg/g
Zinc	11.0	7.8-13.1 mcg/g

Toxic Elements

Element	Reference Range	Reference Range
Lead	0.035	<= 0.048 mcg/g
Mercury	<dl	<= 0.0039 mcg/g
Antimony	0.001	<= 0.002 mcg/g
Arsenic	0.005	<= 0.071 mcg/g
Cadmium	0.001	<= 0.001 mcg/g
Tin	<dl	<= 0.0009 mcg/g

The Elemental reference ranges are based on an adult population.

Metals - Provocative challenge +/- porphyrin analysis
Organic Pollutants - Urine levels of major pollutants ($126)
Reproductive hormone analysis

HELP with UDERSTANDING and INTERPRETATION

Genomic data

Nutritional adequacy

Toxic burden

Hormone status

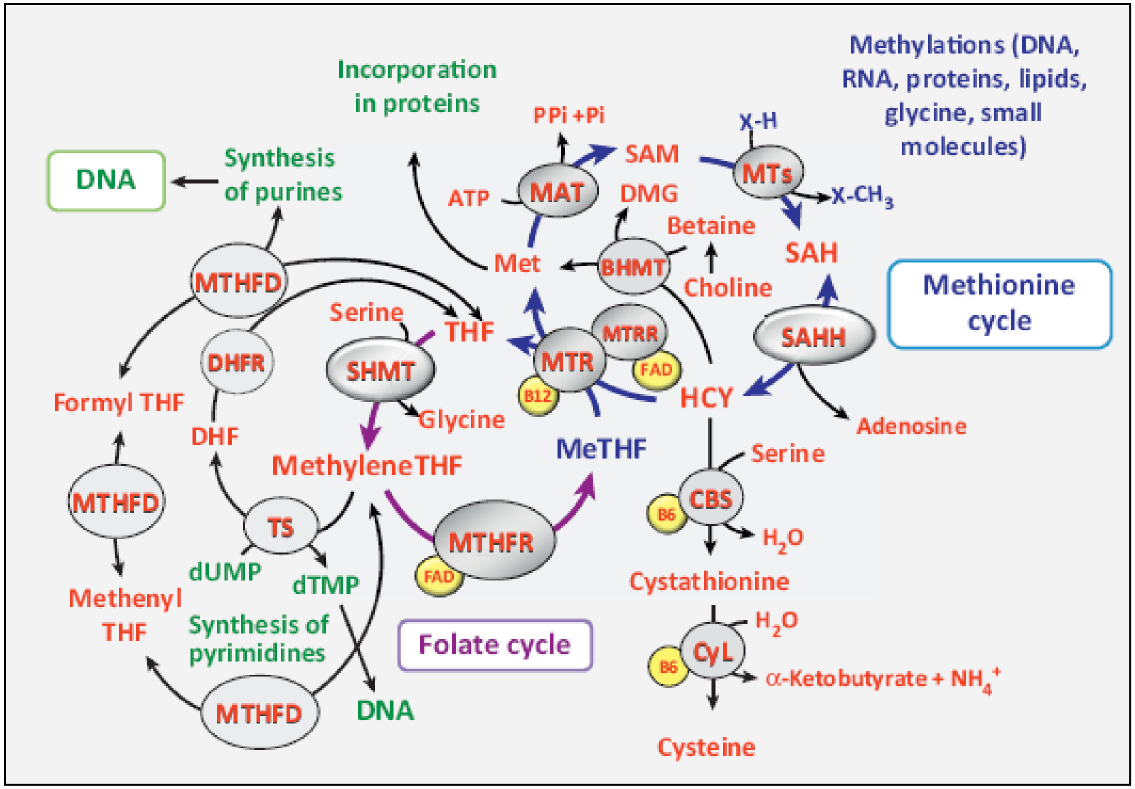
Effects on Methyl Cycle performance

- SAMe:SAH
- Glutathione, taurine, cysteine – overall redox status
- Neurotransmitter levels
- Estradiol/Estrone methylation
- BH4
- DNA Methylation

$\Rightarrow$ Study the individual genes

MTHFR

5,10-Methylene Tetrahydrofolate Reductase



Shunts CH_3:

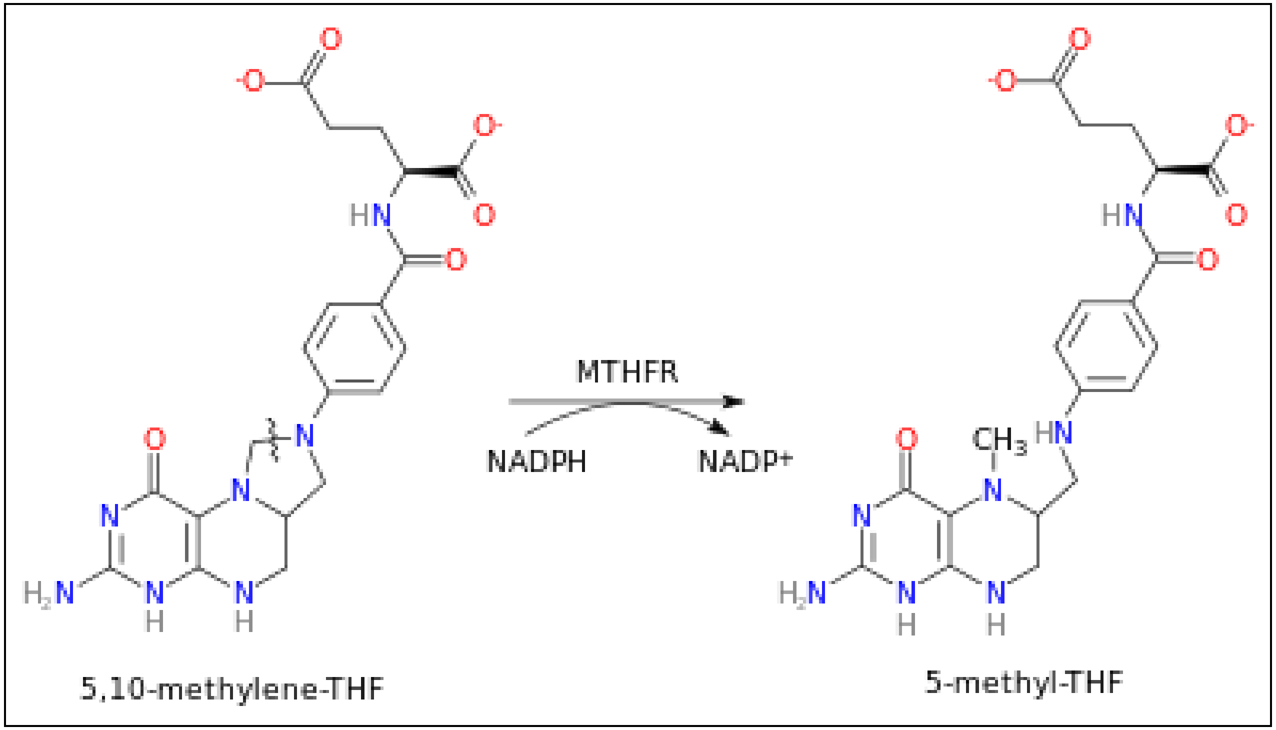
- Away from DNA synthesis
- Towards DNA Methylation, Methylation, & Antioxidant/Detox molecule synthesis

5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR)

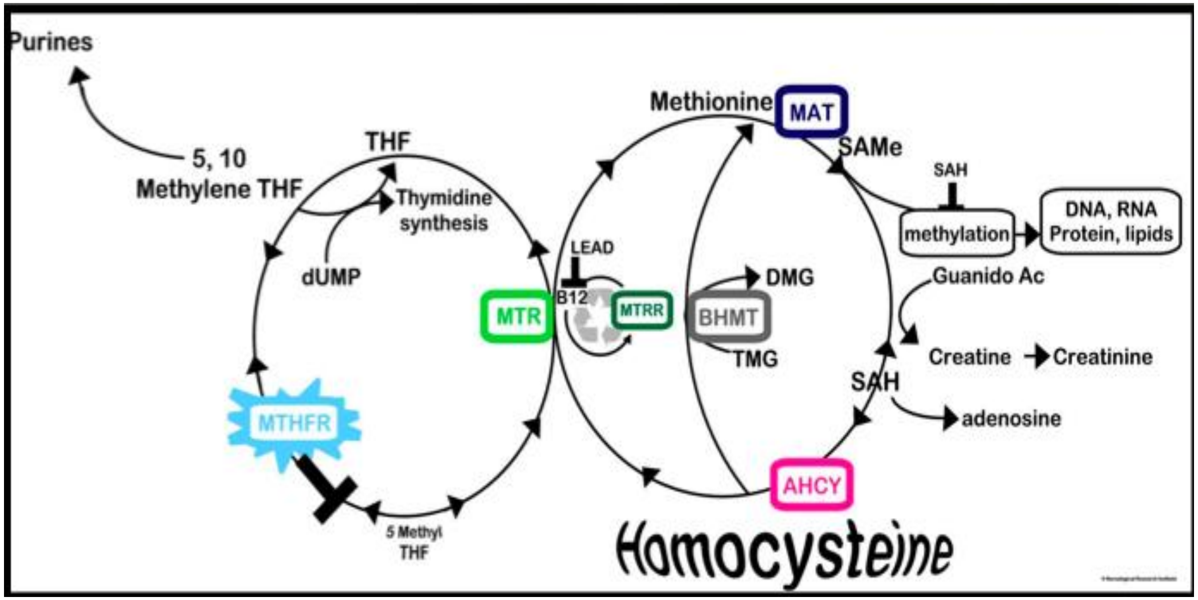
5,10-Methylene-THF ⟶ 5-Methyl-THF (5-Methylfolate)

FAD

Riboflavin



5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR 677C→T)



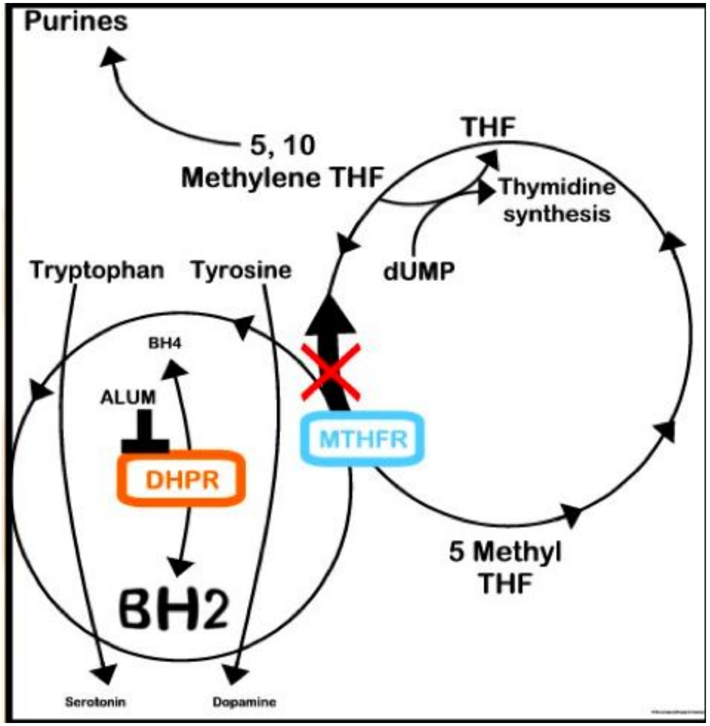
5,10-Methylene-THF —X→ 5-Methyl-THF (5-Methylfolate)

MTHFR TT enzyme efficiency 25% MTHFR CC

Indirect Remethylation of Homocysteine Compromised → Elevated Homocysteine & SAH

Reduced Regeneration of SAMe, decrease in SAMe:SAH → Impaired Methylation
Reduced Generation of Detox/Antioxidant Molecules
Reduced Recycling of BH4

5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR 1298A→C)



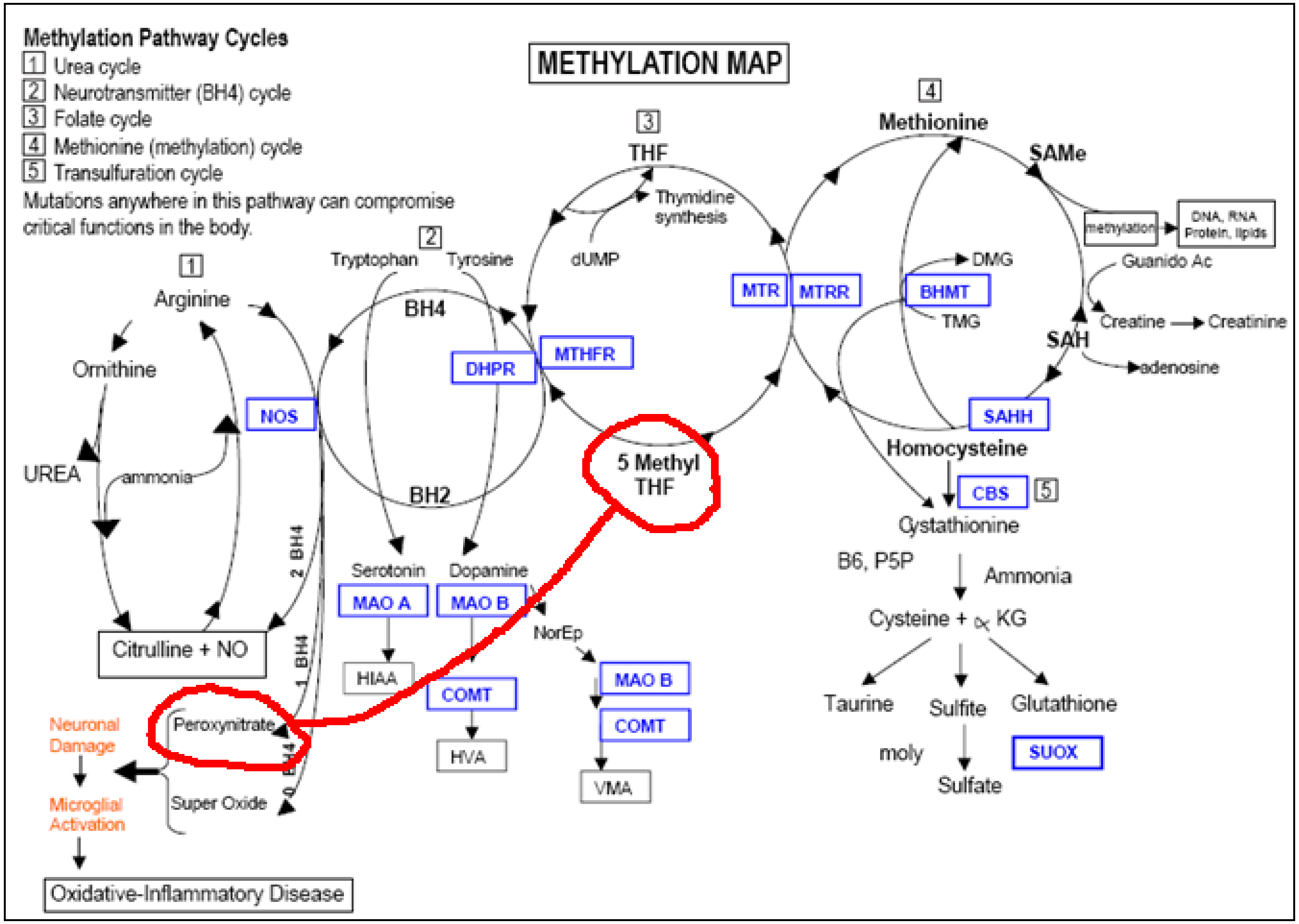
5-Methyl-THF + BH2 —X→ 5,10-Methylene-THF + BH4

Mild impairment in 5-Methyl-THF production

Reduced Recycling of BH4

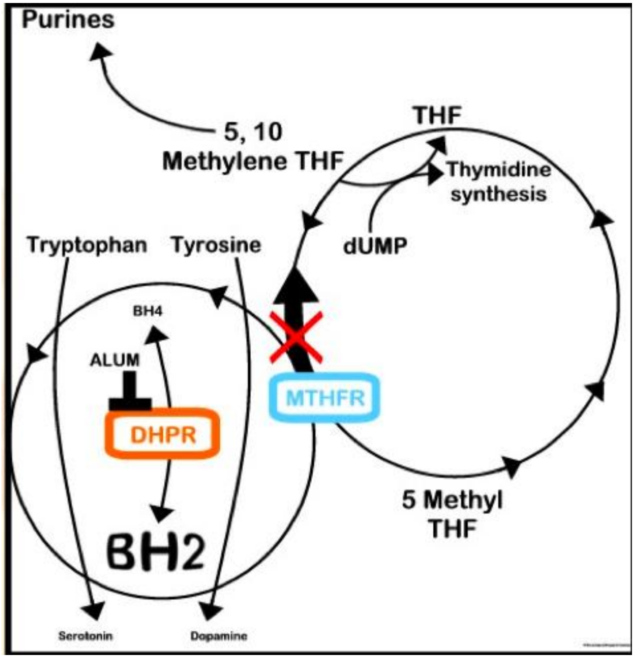
- Direct effect (Dr. Yasko)
- Indirect effect
- Reduced neutralization of Peroxynitrite

5-METHYL FOLATE



5-Methyl Folate Neutralizes Peroxynitrite

5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (A1298C)



Compromises "backward" conversion of

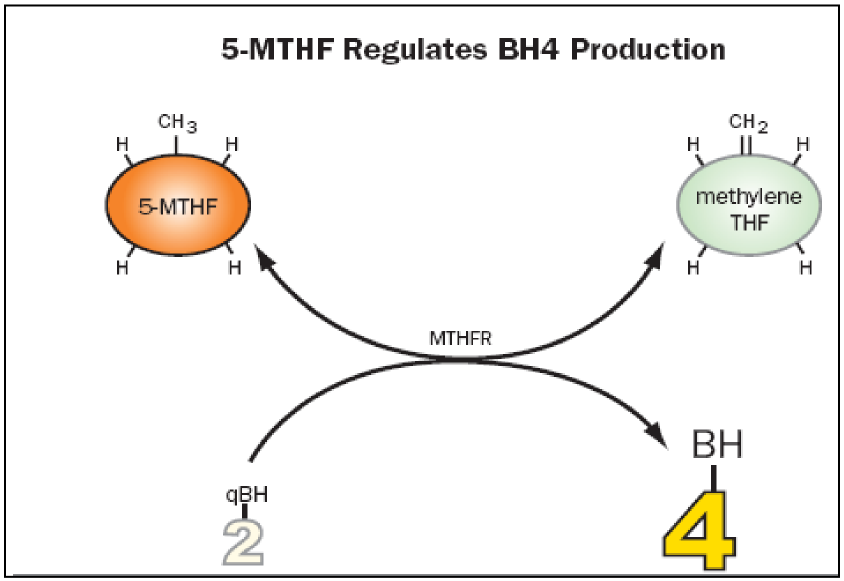
5-Methyl Folate (5-Methyl THF) ⟶ 5,10-Methylene Tetrahydrofolate

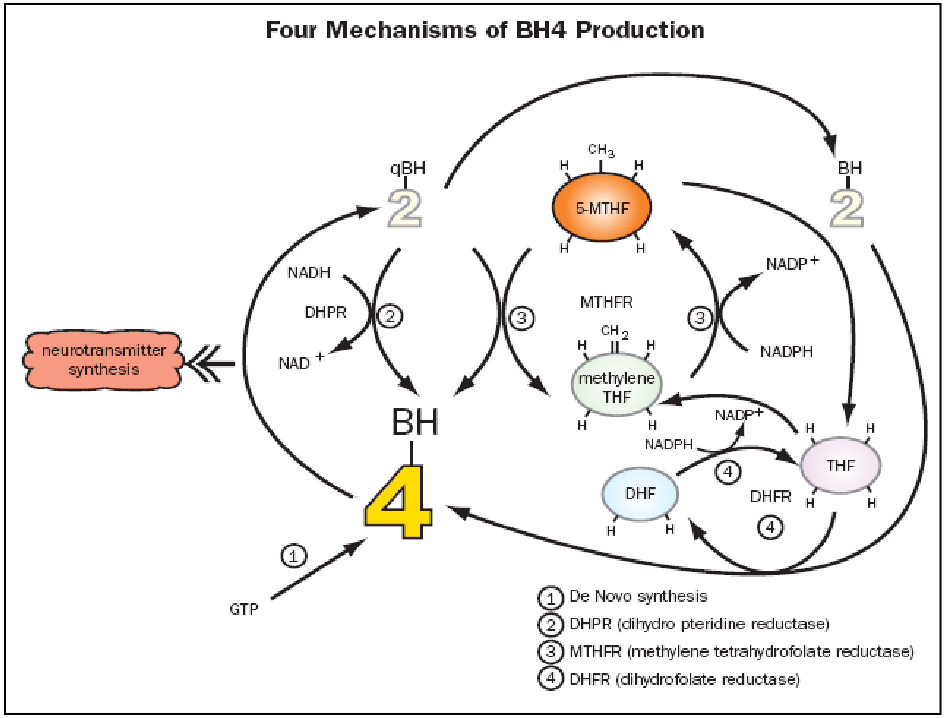
MTHFR

5-Methyl Folate + BH2 ⟶ 5,10-Methylene THF + BH4

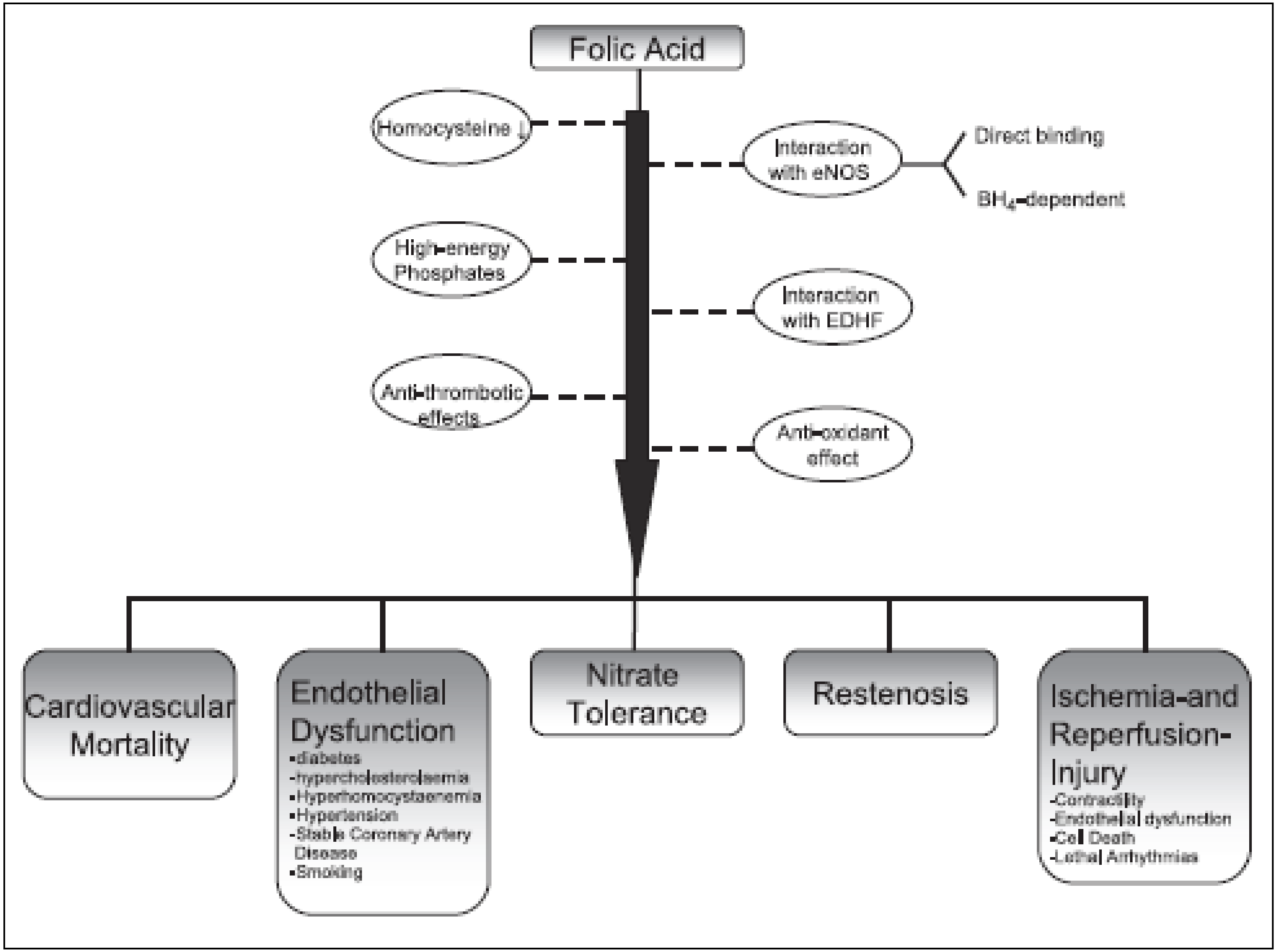
(MTHFR A1298C aggravates CBS up regulation induced BH4 depletion)

MTHFR and BH4 REGENERATION



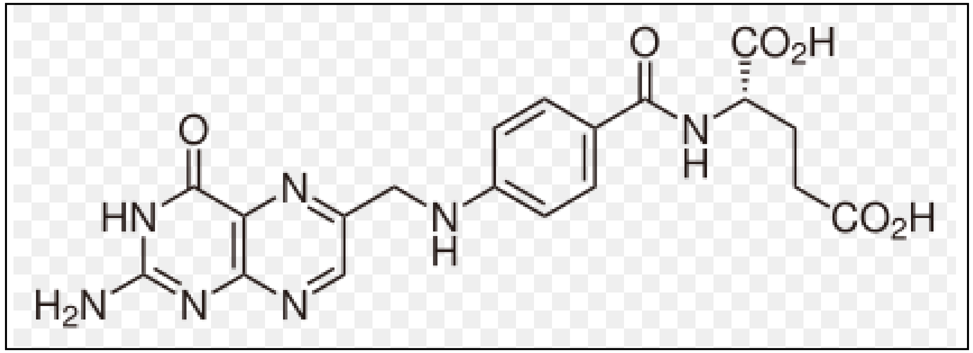


METHYL-FOLATE SUPPORT BH4 (TETRAHYDROBIOPTERIN)

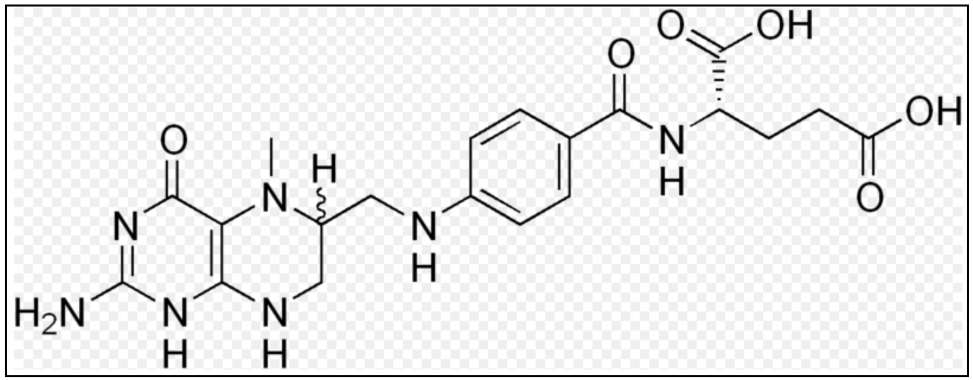


FOLATES SUPPORT BH4 (TETRAHYDROBIOPTERIN)

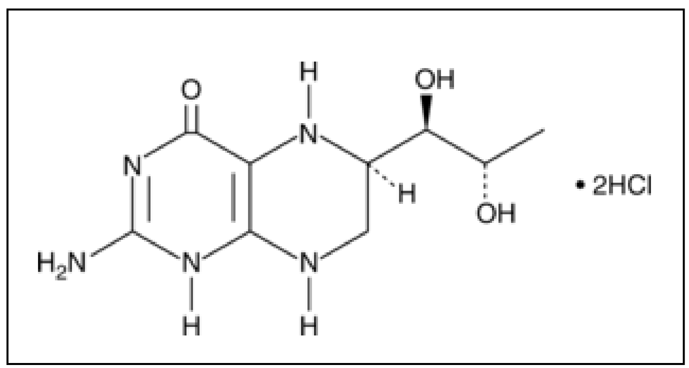
Folic Acid



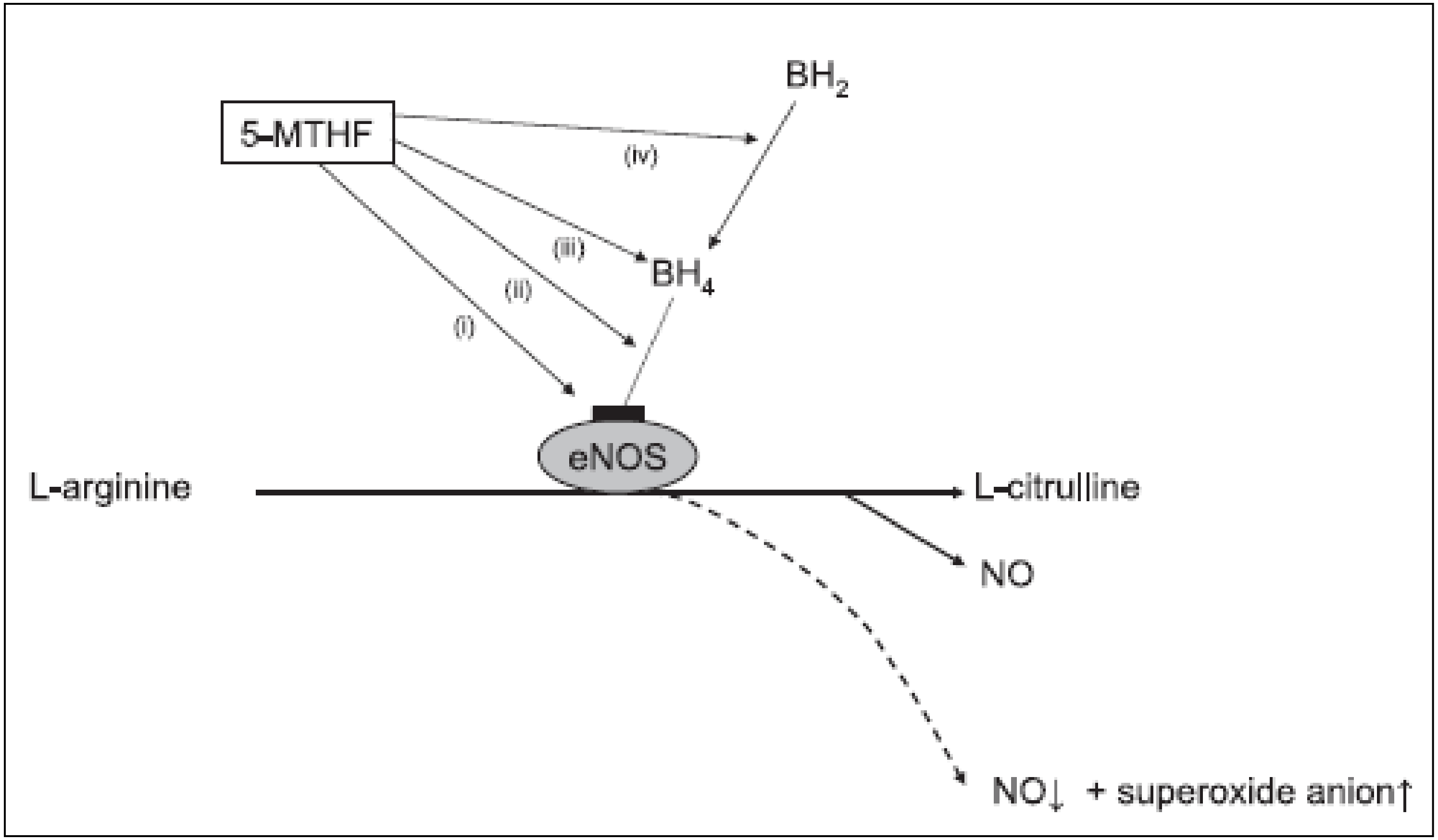
Methyl Folate



BH4



METHYL-FOLATE SUPPORT BH4



MTHFR and the RDA for FOLATE

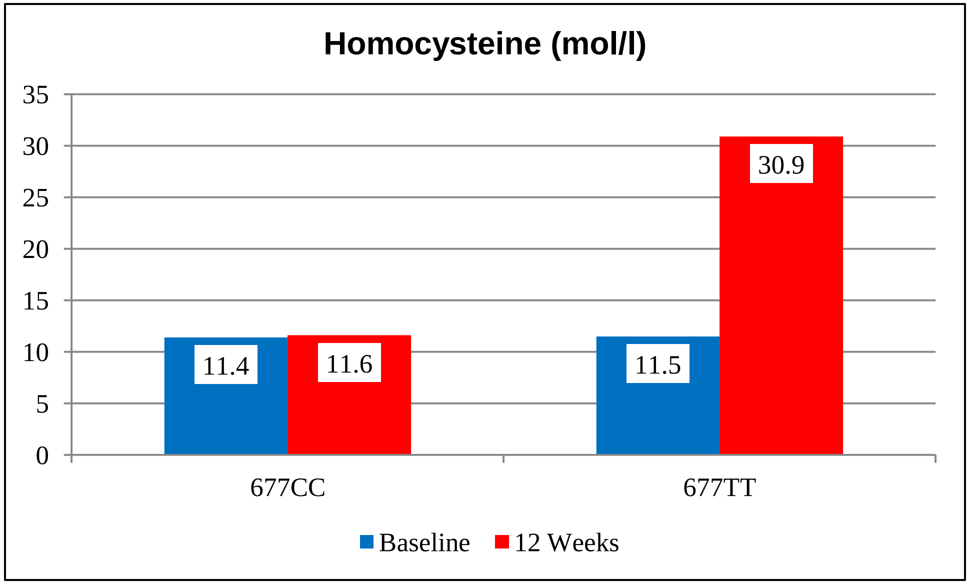
♥ 60 healthy 18-55 year old Mexican-American men:

- ½ MTHFR CC
- ½ MTHFR TT

Baseline measurements

Usual diet → Diet with RDA (438 mcg/day) folic acid

Repeat studies at 12 weeks



RIBOFLAVIN – FOLATE MTHFR INTERACTION

♥ 126 healthy 20-63 year old residents of South Wales
- 1/3rd MTHFR CC
- 1/3rd MTHFR CT
- 1/3rd MTHFR TT

Table 1. Riboflavin status, homocysteine, and folate in all participants at baseline, stratified by genotype.[a]

		MTHFR genotype			
	All participants	CC	CT	TT	*P*
Plasma riboflavin, nmol/L	10.9 (7.4)	11.4 (7.2)	11.5 (8.5)	9.9 (6.4)	0.80
Plasma FMN, nmol/L	4.9 (3.0)	4.6 (2.4)	5.1 (3.2)	4.9 (3.4)	0.83
Plasma FAD, nmol/L	30.9 (5.3)	31.6 (5.1)	31.4 (4.7)	29.7 (5.9)	0.24
EGRAC[b]	1.44 (0.18)	1.41 (0.17)	1.47 (0.21)	1.43 (0.15)	0.36
Plasma tHcy, μmol/L	10.2 (4.2)	8.8 (2.4)	9.3 (2.5)	12.5 (5.7)	0.001
Plasma folate, nmol/L	17.7 (7.7)	20.6 (8.0)	17.2 (6.9)	15.2 (7.4)	0.004
Plasma B_{12}, nmol/L	266 (104)	281 (113)	262 (100)	256 (101)	0.58
Plasma B_6, nmol/L	40.9 (18.3)	39.7 (17.1)	42.2 (19.0)	40.9 (19.2)	0.88

[a] Data are presented as the mean (SD).
[b] Lower EGRAC values are indicative of better riboflavin status.

EGRAC - Erythrocyte glutathione reductase activation coefficient
- Glutathione reductase is FAD dependent
- Record any increase in enzyme activity with exogenous riboflavin
- High value reflects riboflavin insufficiency
- EGRAC > 1.4 ≈ biochemical ariboflavinosis
- 52% suboptimal riboflavin nutriture

RIBOFLAVIN – FOLATE MTHFR INTERACTION

♥ 126 healthy 20-63 year old residents of South Wales:

Table 3. Pearson correlation coefficients for measures of riboflavin status at baseline.

	Log riboflavin	Log FMN	FAD	Log EGRAC
Log plasma FMN	0.30[a]			
Plasma FAD	−0.08	0.04		
Log EGRAC	−0.48[a]	−0.14	−0.11	
Log plasma tHcy	−0.21[b]	−0.03	−0.10	0.36[a]
Plasma folate	0.27[c]	0.18	0.06	−0.39[a]
Age	0.31[a]	−0.12	0.00	−0.46[a]
Creatinine	0.07	0.02	−0.08	−0.07
Log vitamin B_{12}	0.18[b]	−0.07	0.13	−0.38[a]
Log vitamin B_6	0.15	0.14	0.08	−0.16

[a–c] Significant: [a] $P < 0.001$; [b] $P < 0.05$; [c] $P < 0.01$.

Low riboflavin (high EGRAC) ≈ Higher homocysteine values

- Homocysteine 2.6 umol/l > in 1st vs. 4th riboflavin quartile
- Homocysteine 4.2 umol/l > in 4th vs. 1st EGRAC quartile
- Linkage confined to CT and TT genotypes

Low riboflavin ≈ Higher homocysteine values (in TTs more than CTs)

RIBOFLAVIN – FOLATE MTHFR INTERACTION

♥ 126 healthy (20-63) year old residents of South Wales:

- 1/3rd MTHFR CC
- 1/3rd MTHFR CT
- 1/3rd MTHFR TT

Baseline measurements

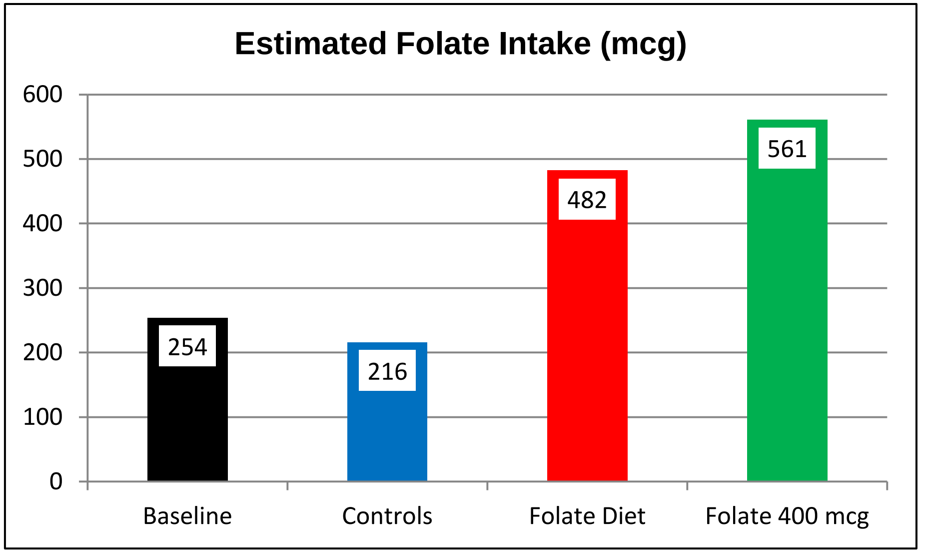
Randomize to receive over four months:

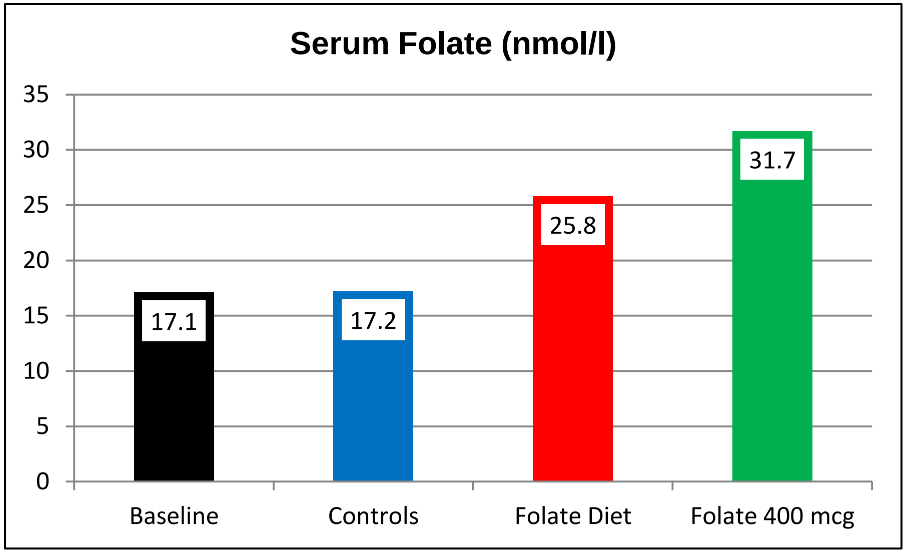
- Usual diet (avoid folate fortified foods) + placebo
- Usual diet (encourage folate rich foods ≈ 400 mcg folate/day)
- Usual diet (avoid folate fortified foods) + 400 mcg/day folic acid

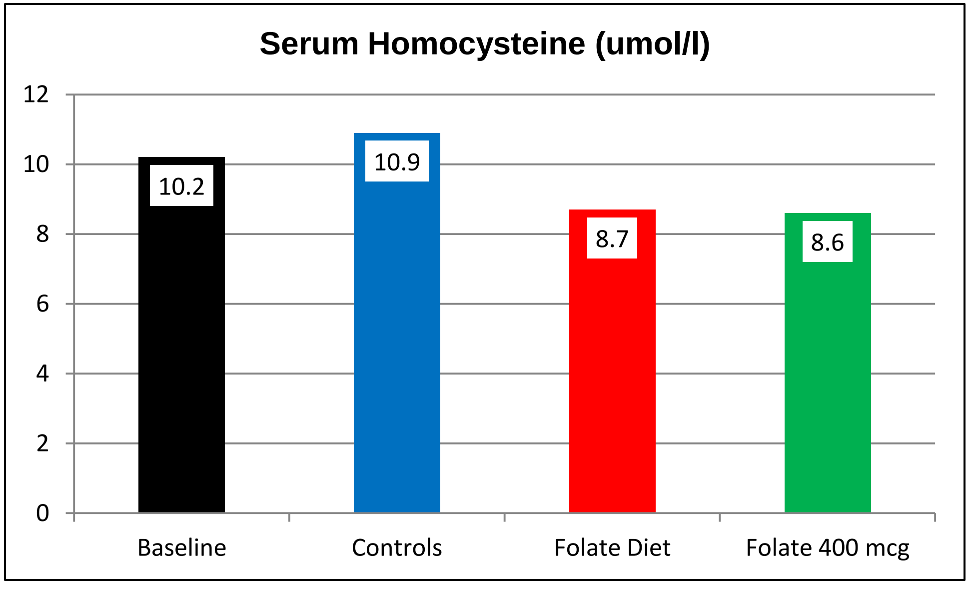
Repeat baseline measurements

Groups one and three double-blind

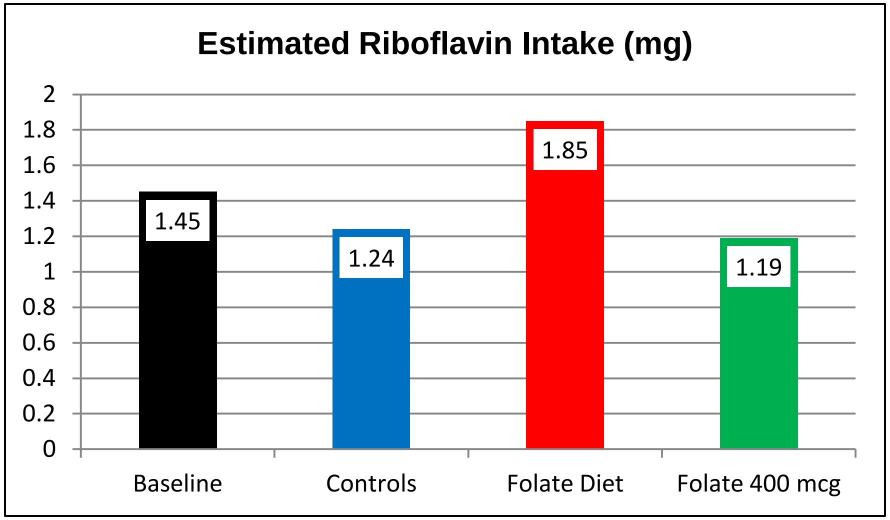
RIBOFLAVIN – FOLATE MTHFR INTERACTION

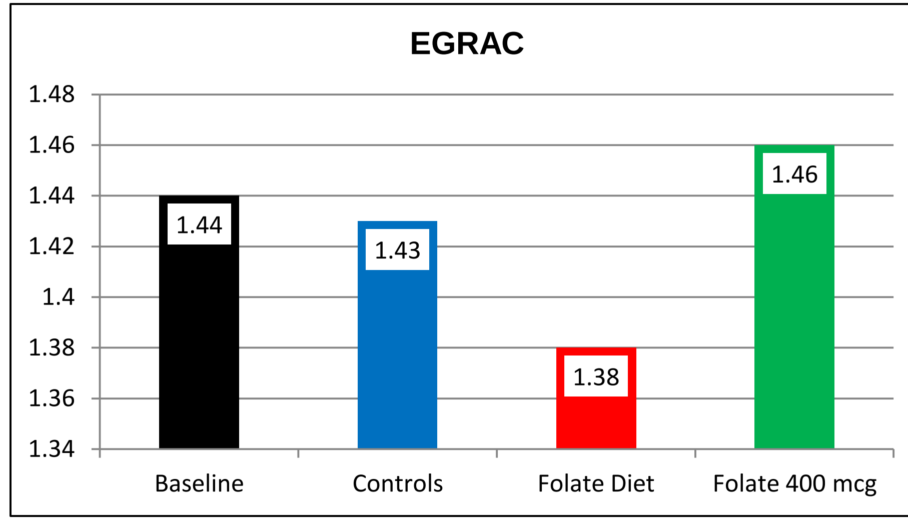


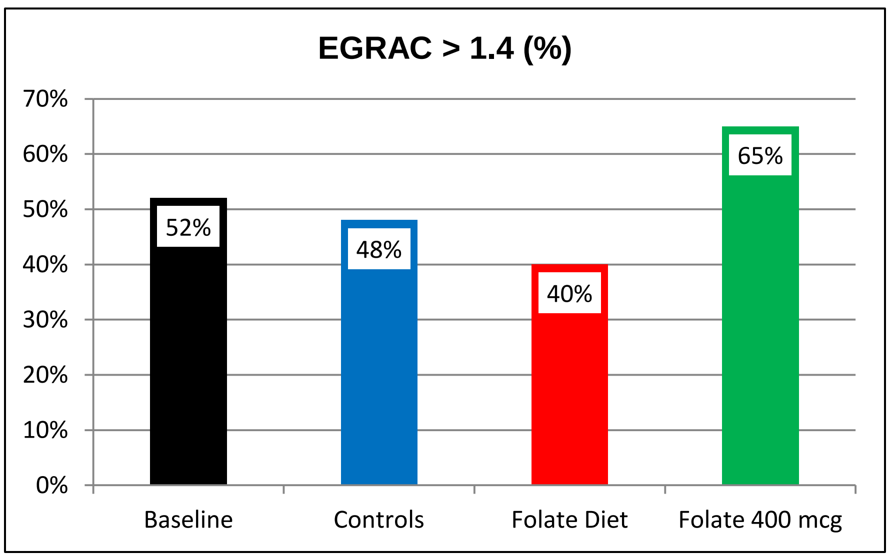




RIBOFLAVIN – FOLATE MTHFR INTERACTION





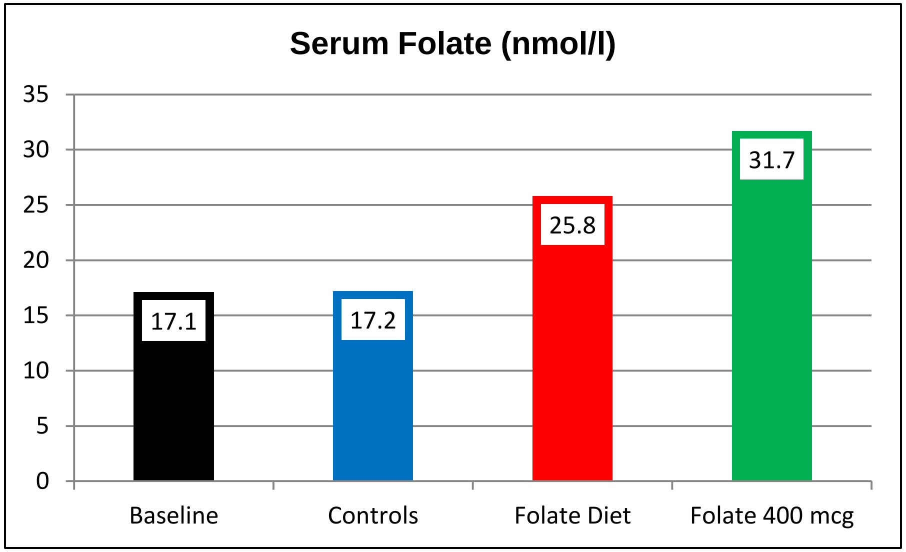


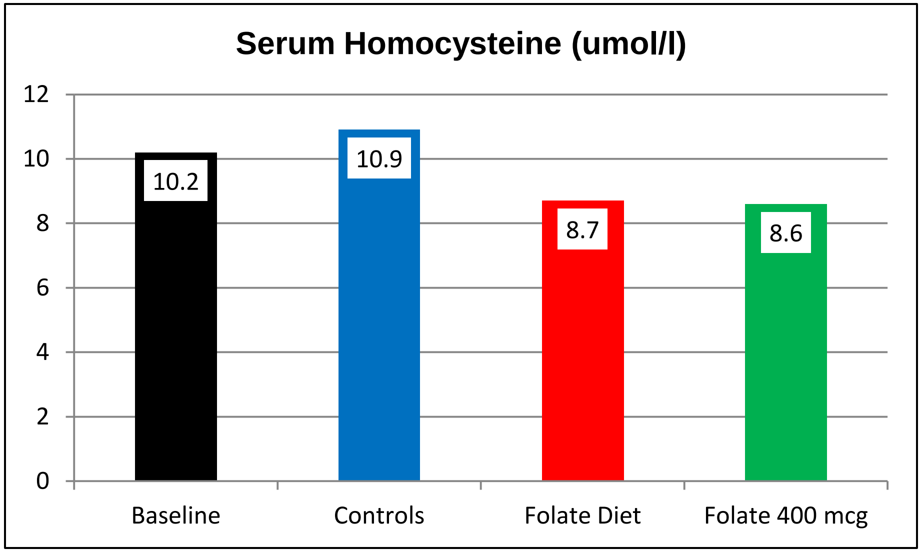
Low folate diet ≈ low riboflavin diet

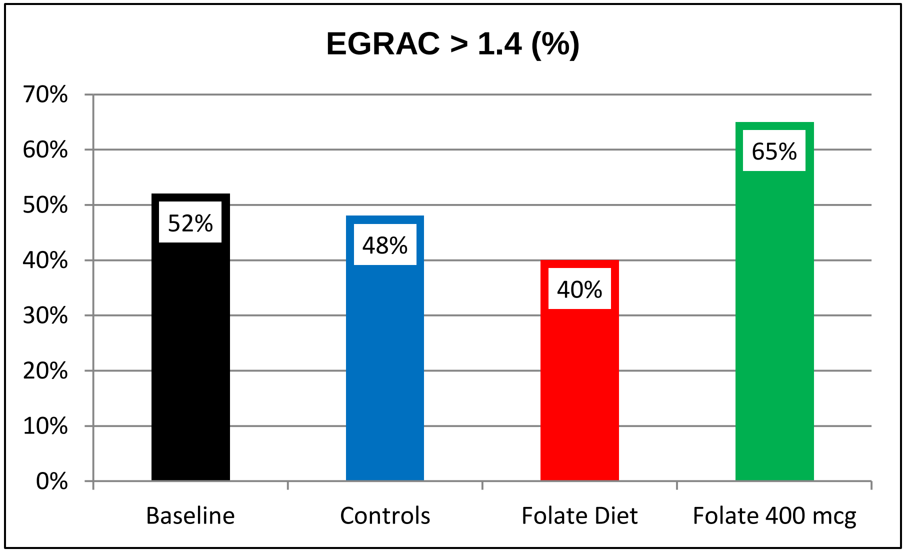
Folate alone “drains” riboflavin

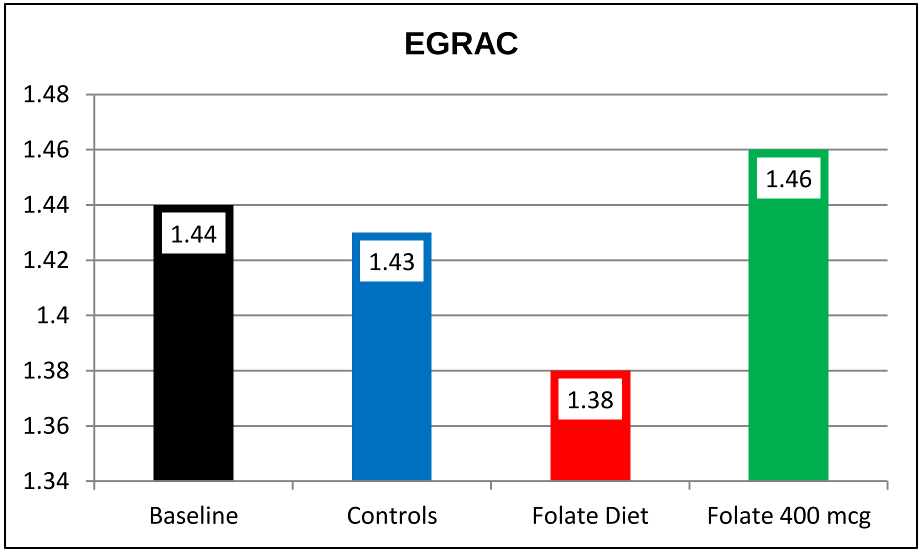
$$\text{Folate} \xrightarrow[\text{MTHFR}]{\text{Riboflavin}} \text{Methyl-Folate}$$

RIBOFLAVIN – FOLATE MTHFR INTERACTION









RIBOFLAVIN – FOLATE INTERACTION in IRELAND

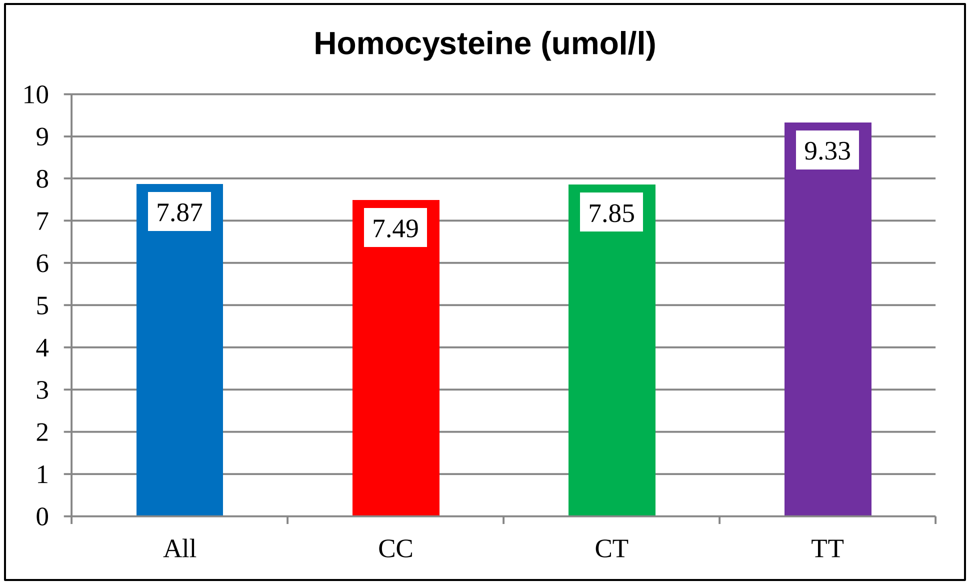
♥ 286 healthy (16-63) year old residents of Ireland:

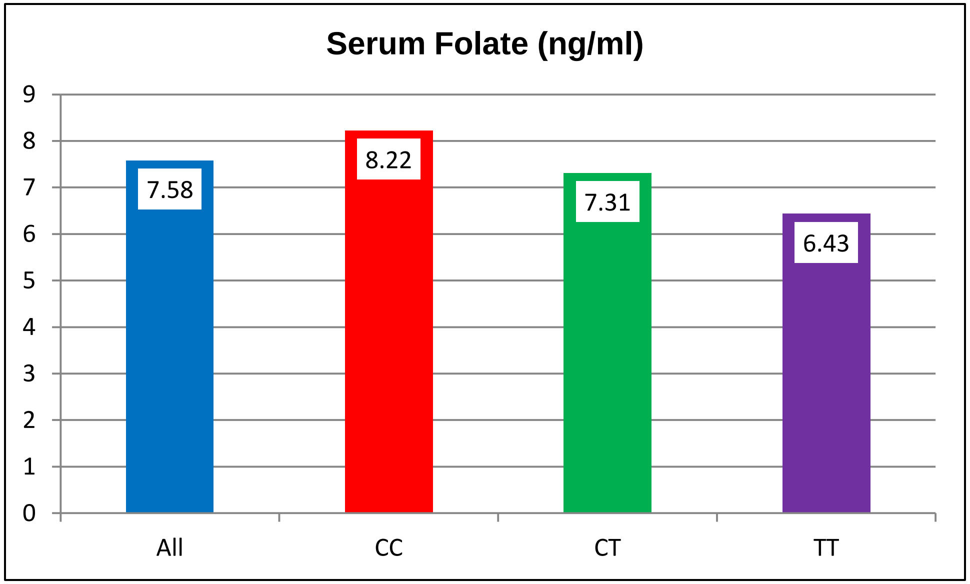
- 43% MTHFR CC
- 45% MTHFR CT
- 12% MTHFR TT

Similar levels of riboflavin, B12, and B6 within all three groups

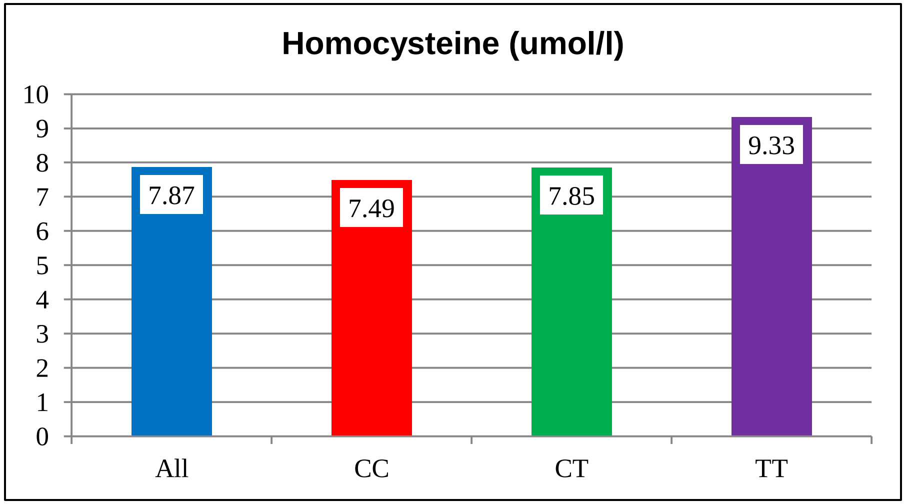
28% subjects riboflavin deficient

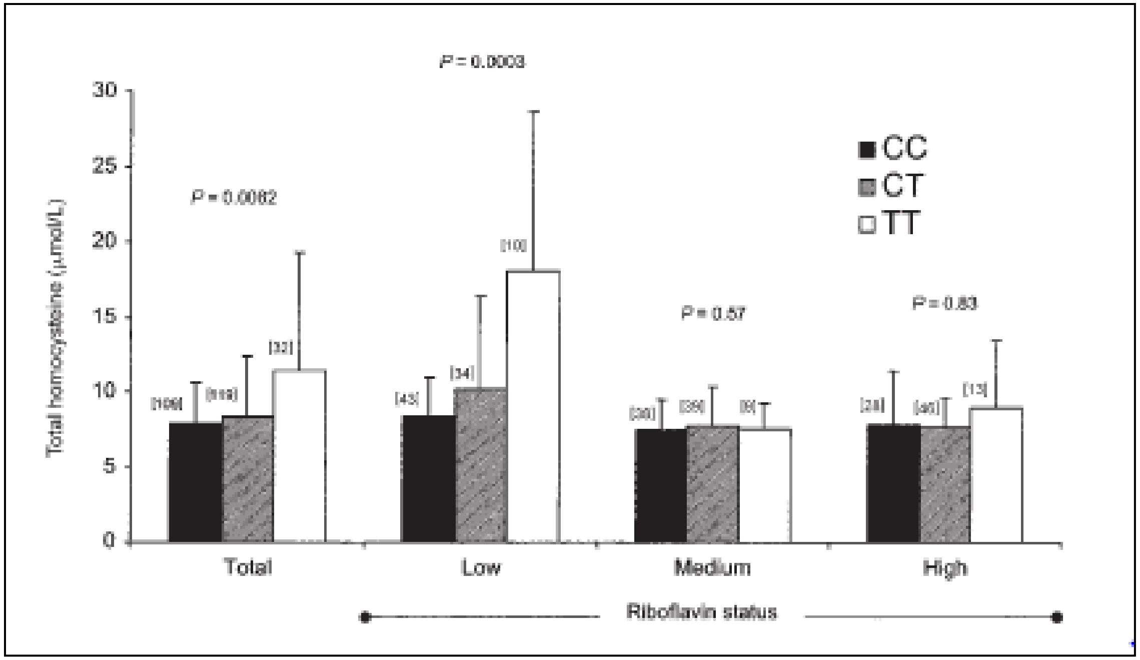
RIBOFLAVIN – FOLATE INTERACTION in IRELAND





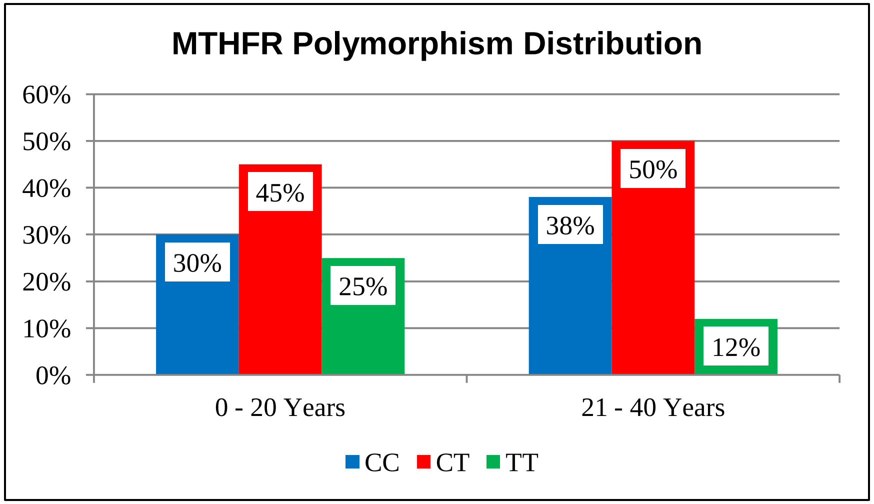
RIBOFLAVIN – FOLATE INTERACTION in IRELAND

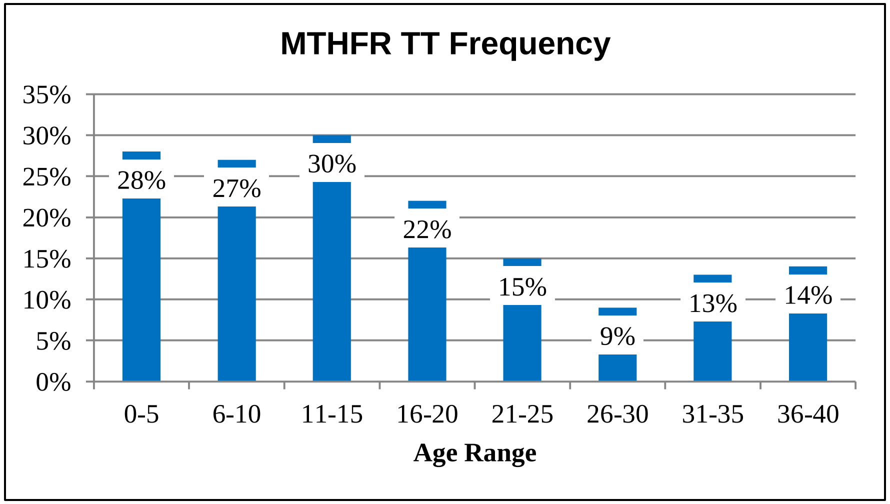




MTHFR 677C→T POLYMORPHISM

- ♥ Genotype 695 Spaniards
  - • All ≤ 40 years of age
  - • No migration in or out of area
    - ♦ ACE I/D – Stable over all age ranges
    - ♦ MTHFR 677C→ T





MTHFR 677C→T and MISCARRIAGE

♥ 35 y/o healthy Spanish woman

- Two stillbirths (28 and 26 weeks)
- Three miscarriages (9, 10, and 11 weeks)

Metabolic and thrombophilia work up negative (nl insulin, CRP, and fibrinogen)

Impaired fibrinolytic response (10 min. venous occlusion test)

♣ No shortening of the euglobulin clot lysis time

♣ Abnormally high PAI-1 (plasminogen activator inhibiter-1) activity

B12 wnl but folate 4.3 (5-28) and MTHFR TT → Homocysteine 46 umol/l

Treat with 15 mg folate and 500 mg B6 500 mg for four weeks:

- Homocysteine falls to 9 with normalized fibrinolytic response

Conceives, B vits continued ⇒ Delivery at 32 weeks (C-section d/t metrorrhagia)

MTHFR 677C→T and MISCARRIAGE

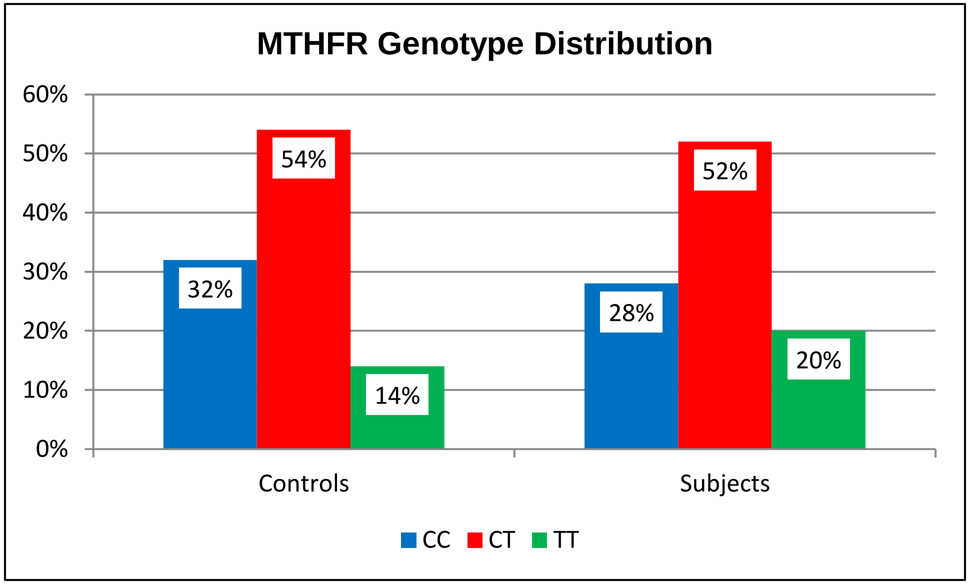
♥ 100 consecutive nulliparous with recurrent early miscarriages

- ≥ three episode of fetal loss
- Unknown etiology (1998)

♦ Age-matched healthy controls without history of fetal loss

Homocysteine levels:

- Median levels similar
- Greater range (5^{th}-95^{th} percentile) of 3-29 subjects vs. 3-12 controls
- Folate low in 15% subjects

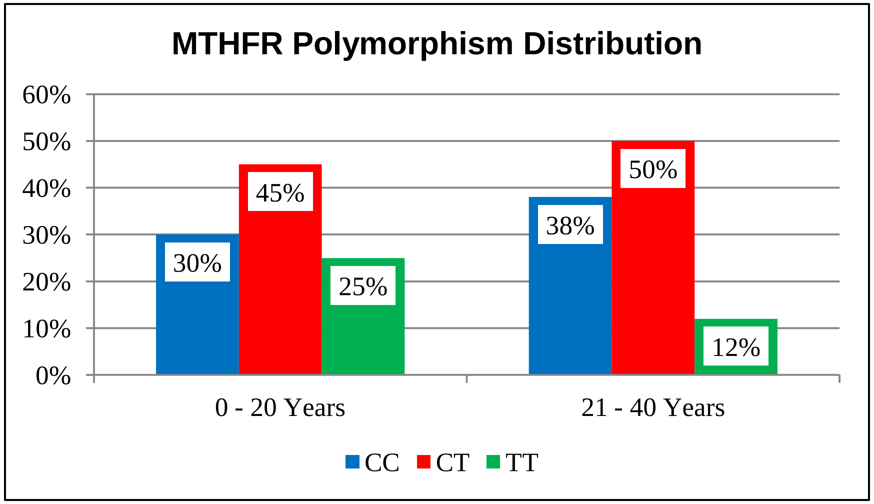


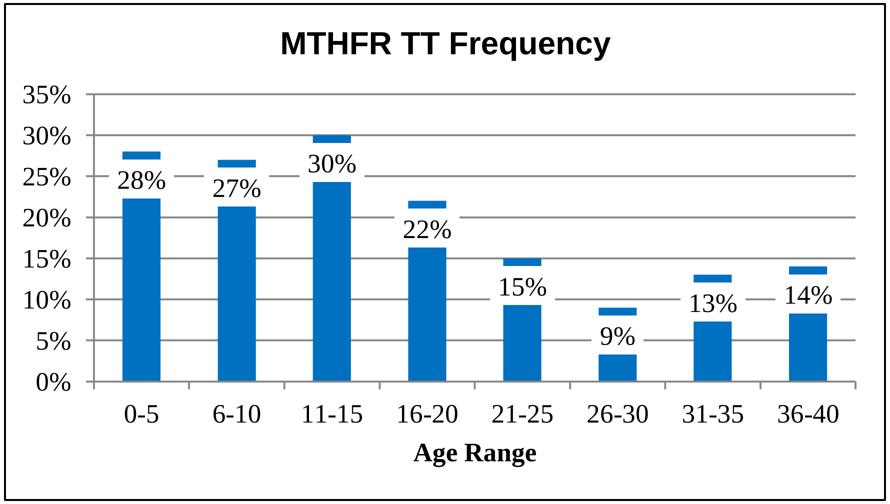
Highest homocysteine values:

- Low folate
- MTHFR TT

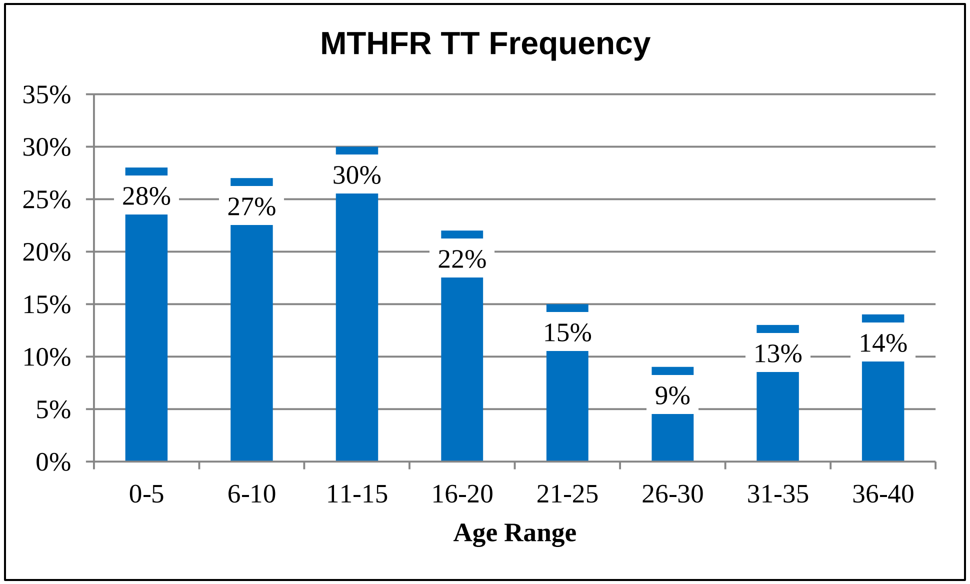
MTHFR 677C→T POLYMORPHISM

- ♥ Genotype 695 Spaniards
  - • All ≤ 40 years of age
  - • No migration in or out of area
    - ♦ ACE I/D – Stable over all age ranges
    - ♦ MTHFR 677C→ T





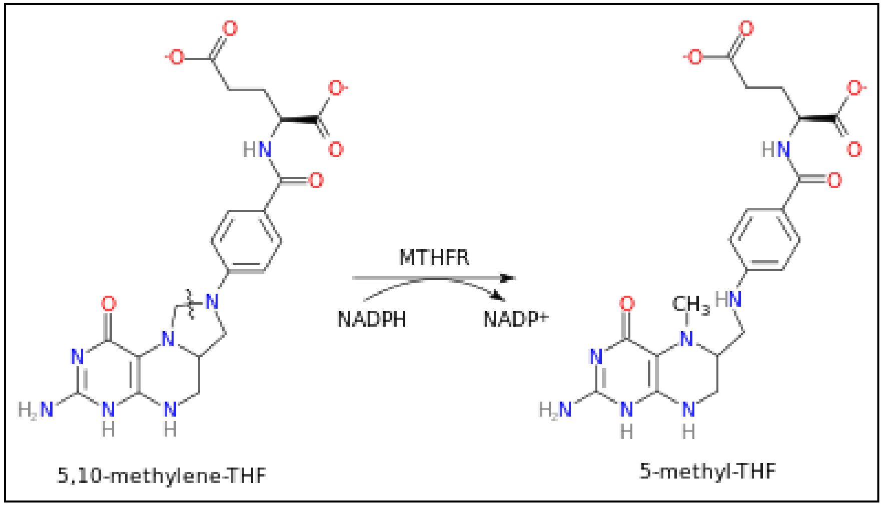
MTHFR 677C→T POLYMORPHISM

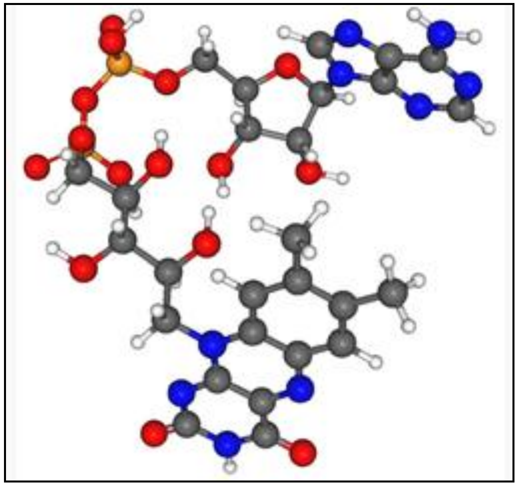


Folate Supplementation During Pregnancy (%)	
1976	3%
1977	10%
1982	35%
1986	55%

MTHFR C677T POLYMORPHISM

5,10-Methylene-THF ⟶ 5-Methyl-THF (5-Methylfolate)
FAD



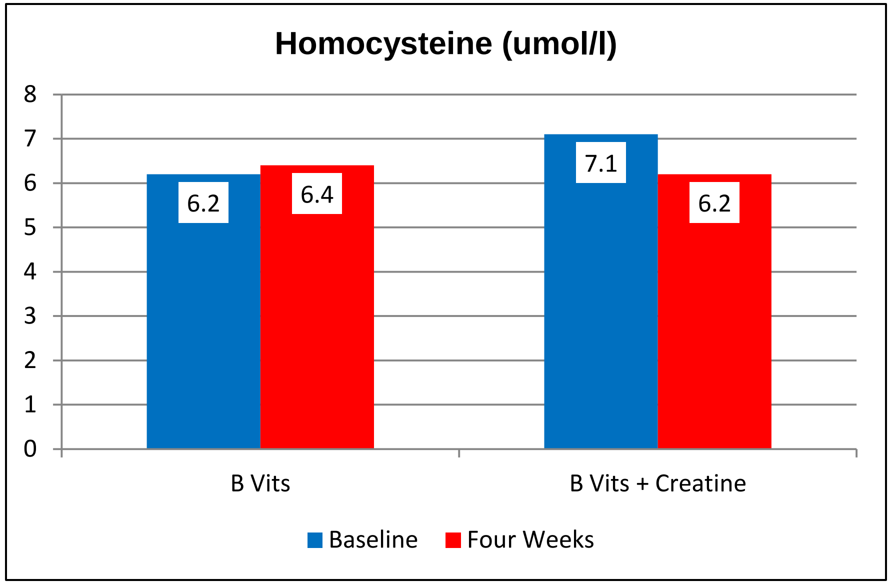


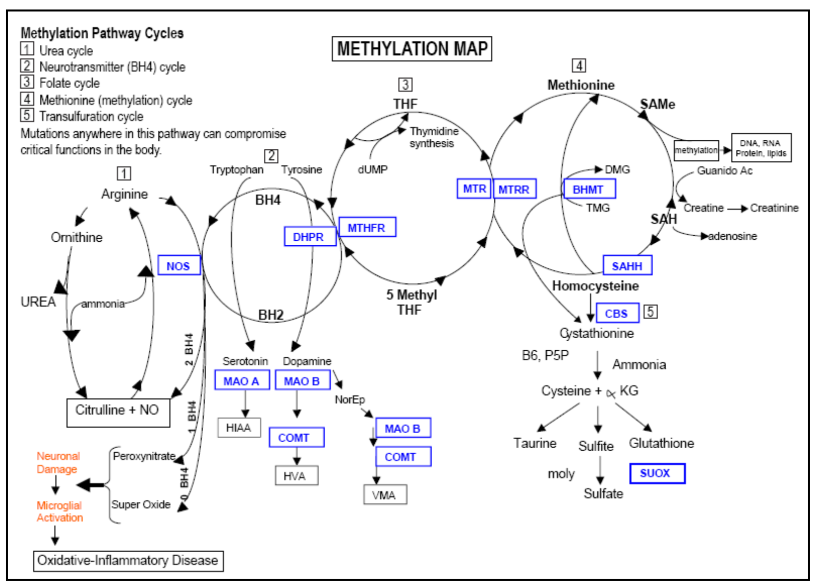
Impaired binding of MTHFR to FAD (Flavin Adenine Dinucleotide):

Conditionally increased requirement for:

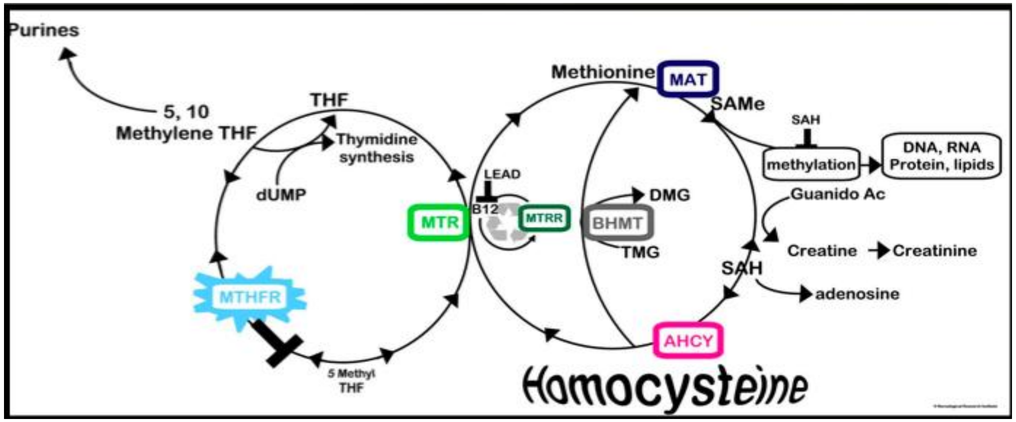
- Riboflavin
- Folate → 5,10-Methylene-THF
- Bypass C677T with 5-Methylfolate

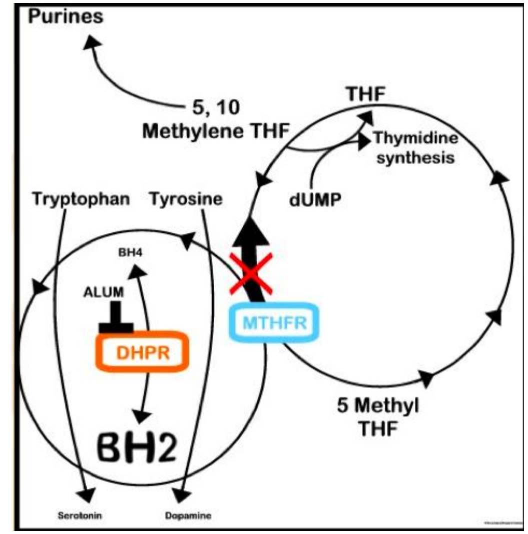
CREATINE to DECREASE HOMOCYSTEINE





5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR C677T and/or A1298C)





Management:

High dose Folic Acid (≥ 5 mg/day)

- Concern regarding build up of Unmetabolized Folic Acid?

5-Methylfolate 400 to 15,000 mcg/day

Riboflavin 50-100 mg/day +/- Creatine 5 gm/day (677TT)

Caveats with 5-Methylfolate supplementation:

- CBS up regulation and/or BHMT down regulation – Watch Sulfate status
- COMT down regulation – Watch for mood swings

MTHFR C677T and COLORECTAL MALIGNANCY

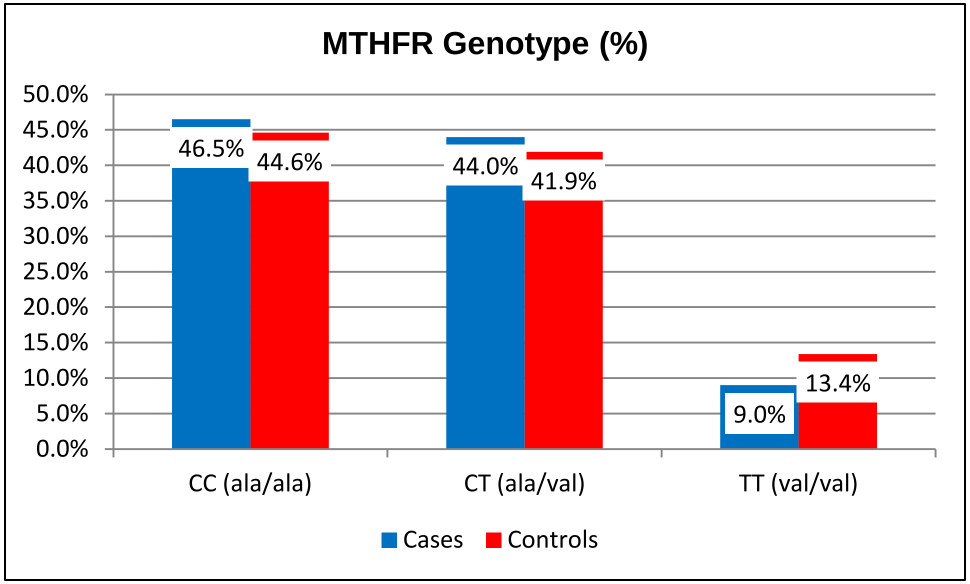
- ♥ 18,025 US Health Professionals (lab analysis in ‘93-’94)
  - 144 with colorectal malignancy dxed (‘86-’94)
  - 627 control participants

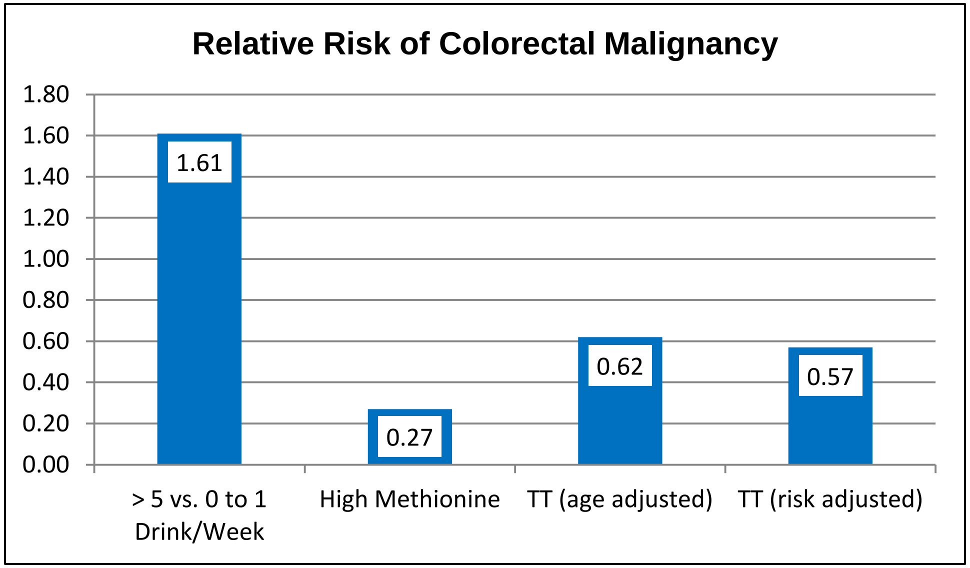
Record:

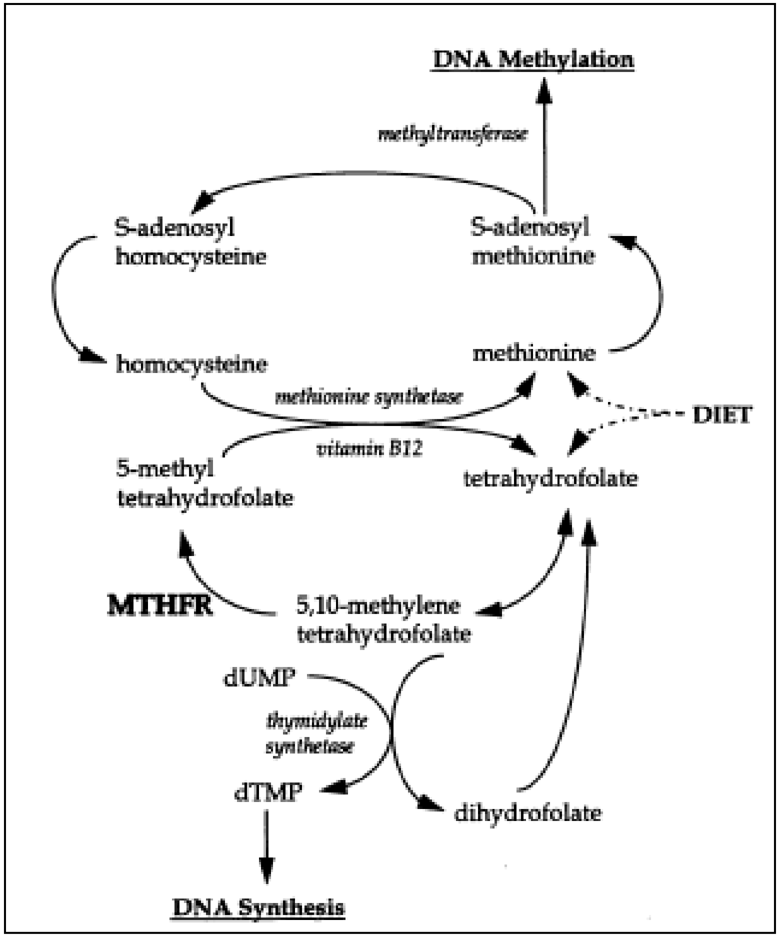
- MTHFR genomic status
  - ♥ 677CC (ala/ala)
  - ♦ 677CT (ala/val)
  - ♣ 677TT (val/val)
- Dietary folate and methionine intake
- Alcohol intake

Correlate with risk of colorectal malignancy

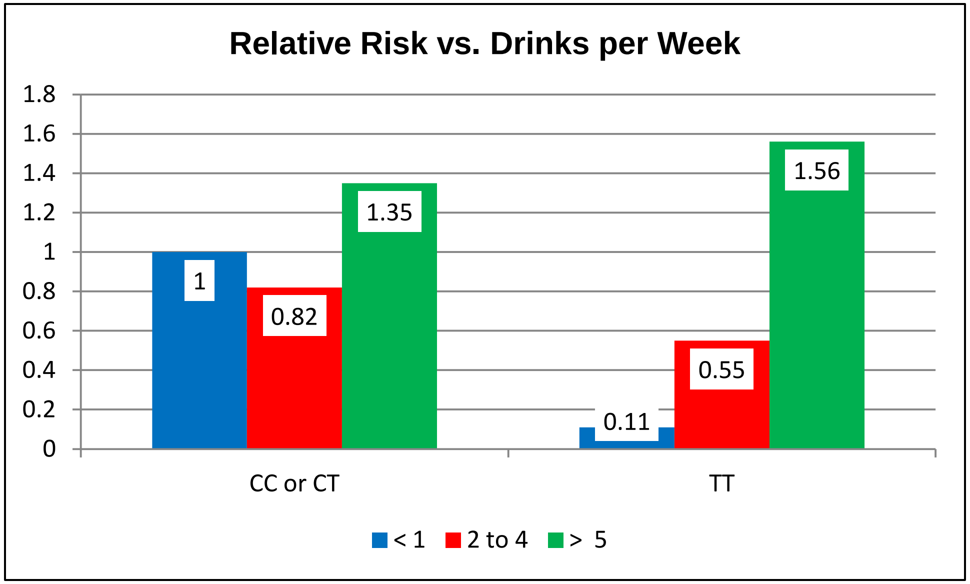
MTHFR C677T and COLORECTAL MALIGNANCY

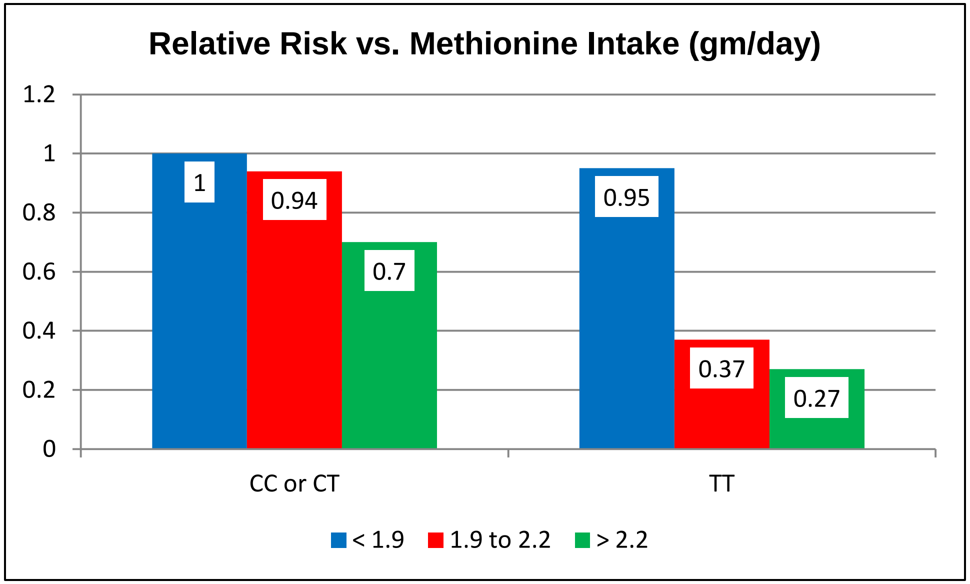


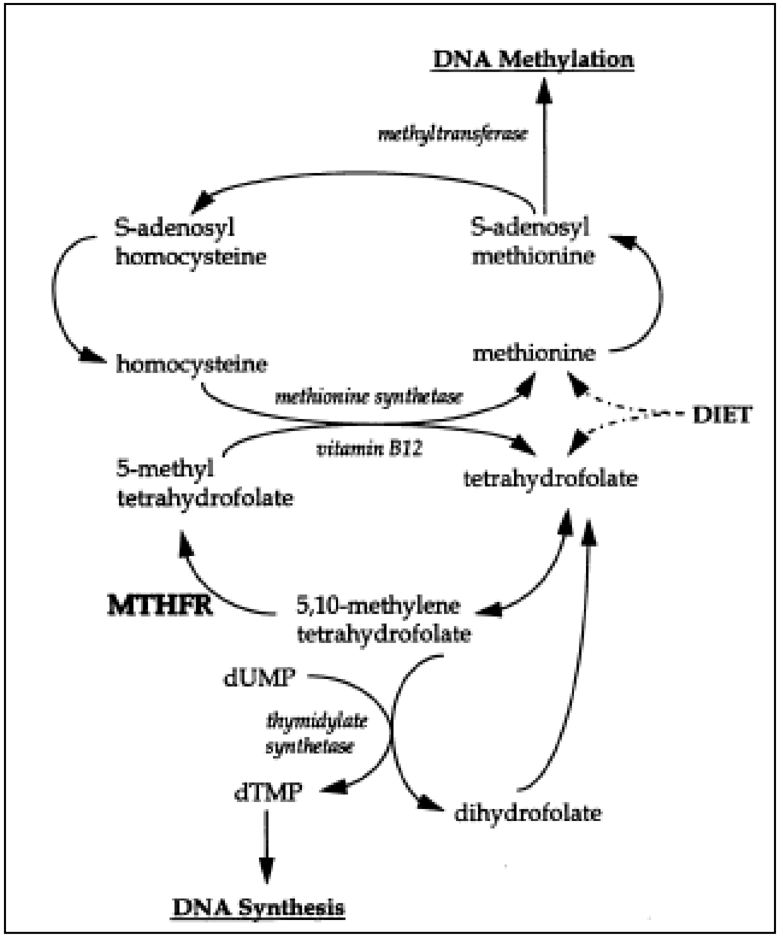




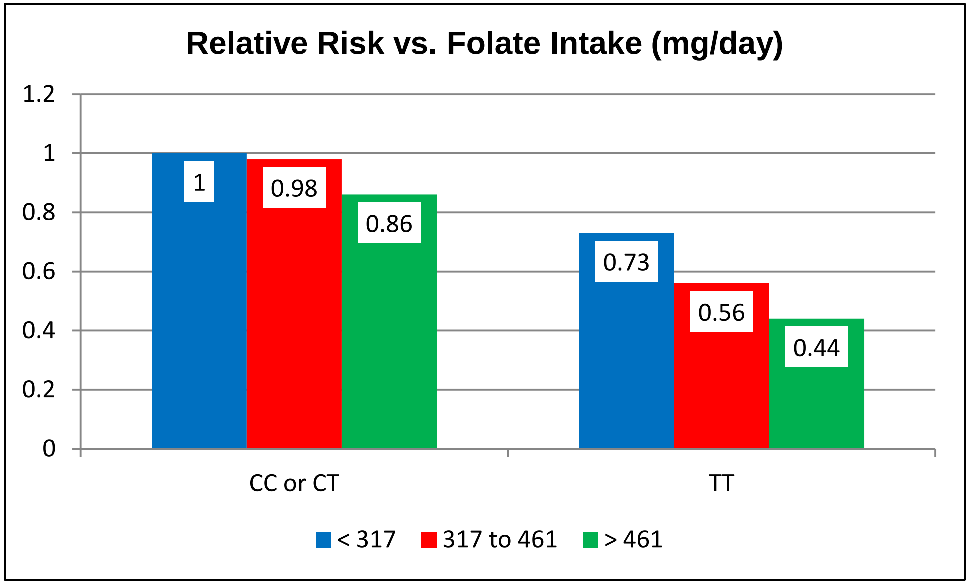
MTHFR C677T and COLORECTAL MALIGNANCY

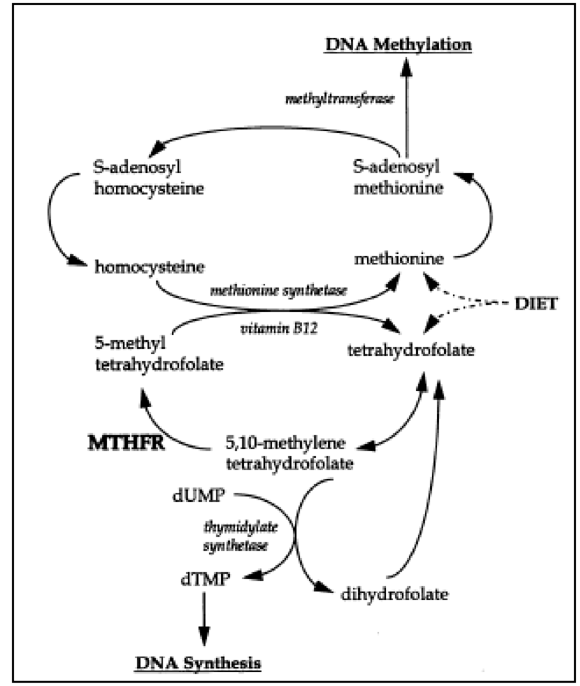


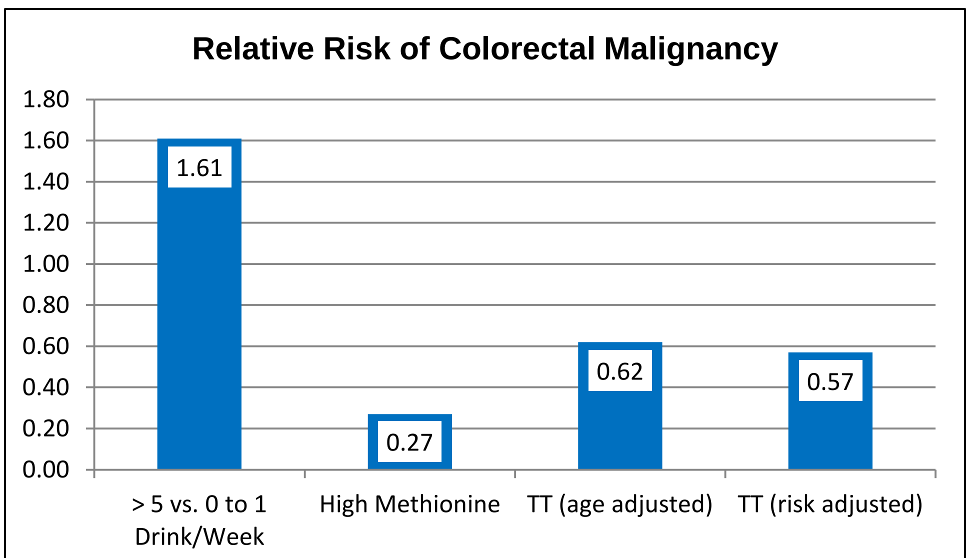


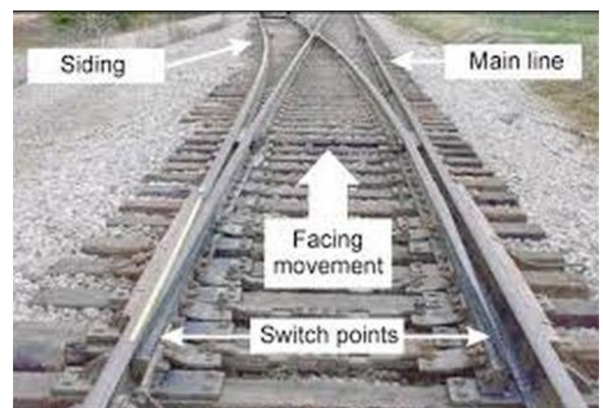


MTHFR C677T and COLORECTAL MALIGNANCY

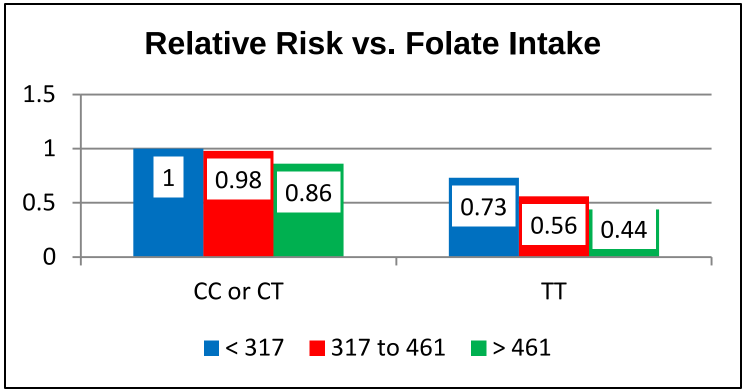


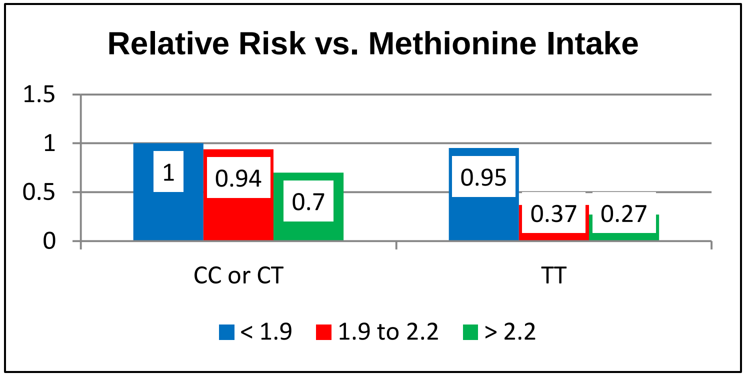


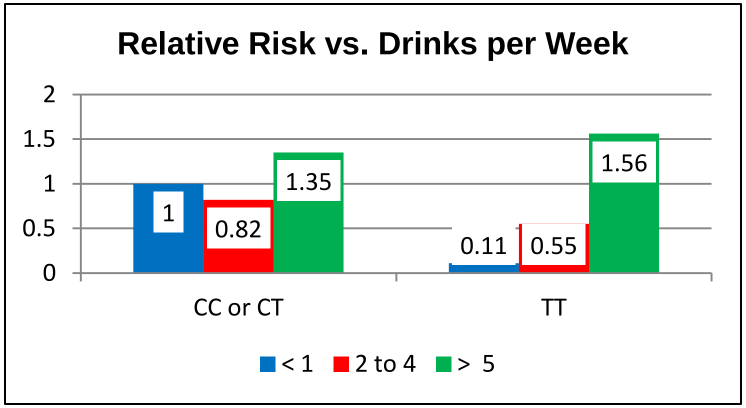


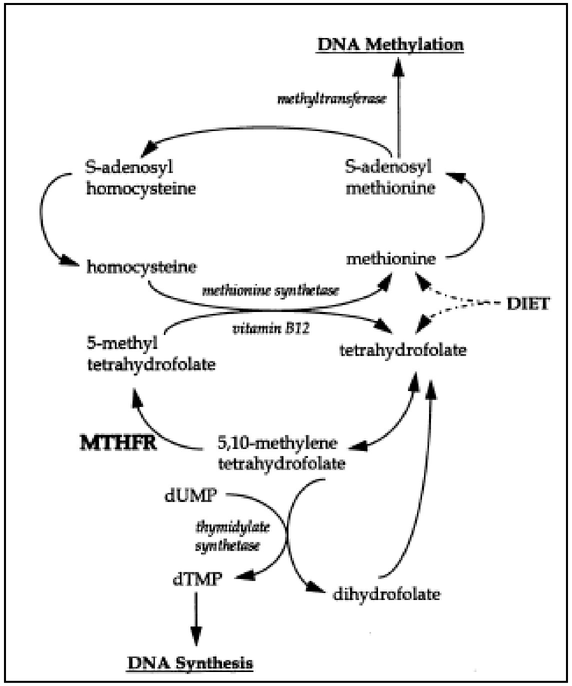


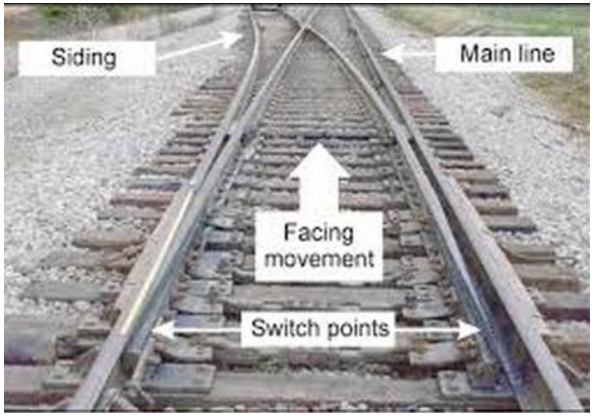
MTHFR C677T and COLORECTAL MALIGNANCY





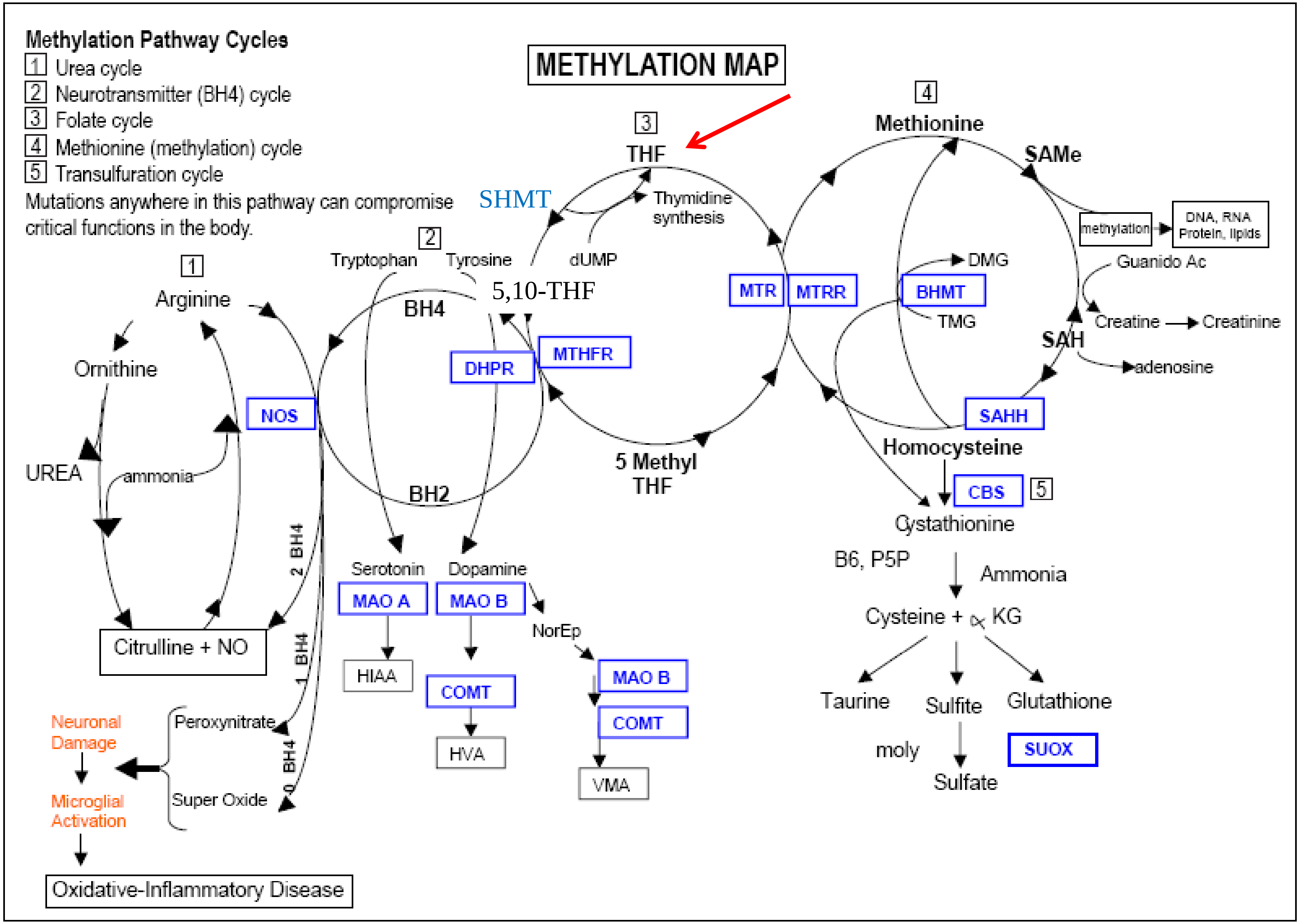




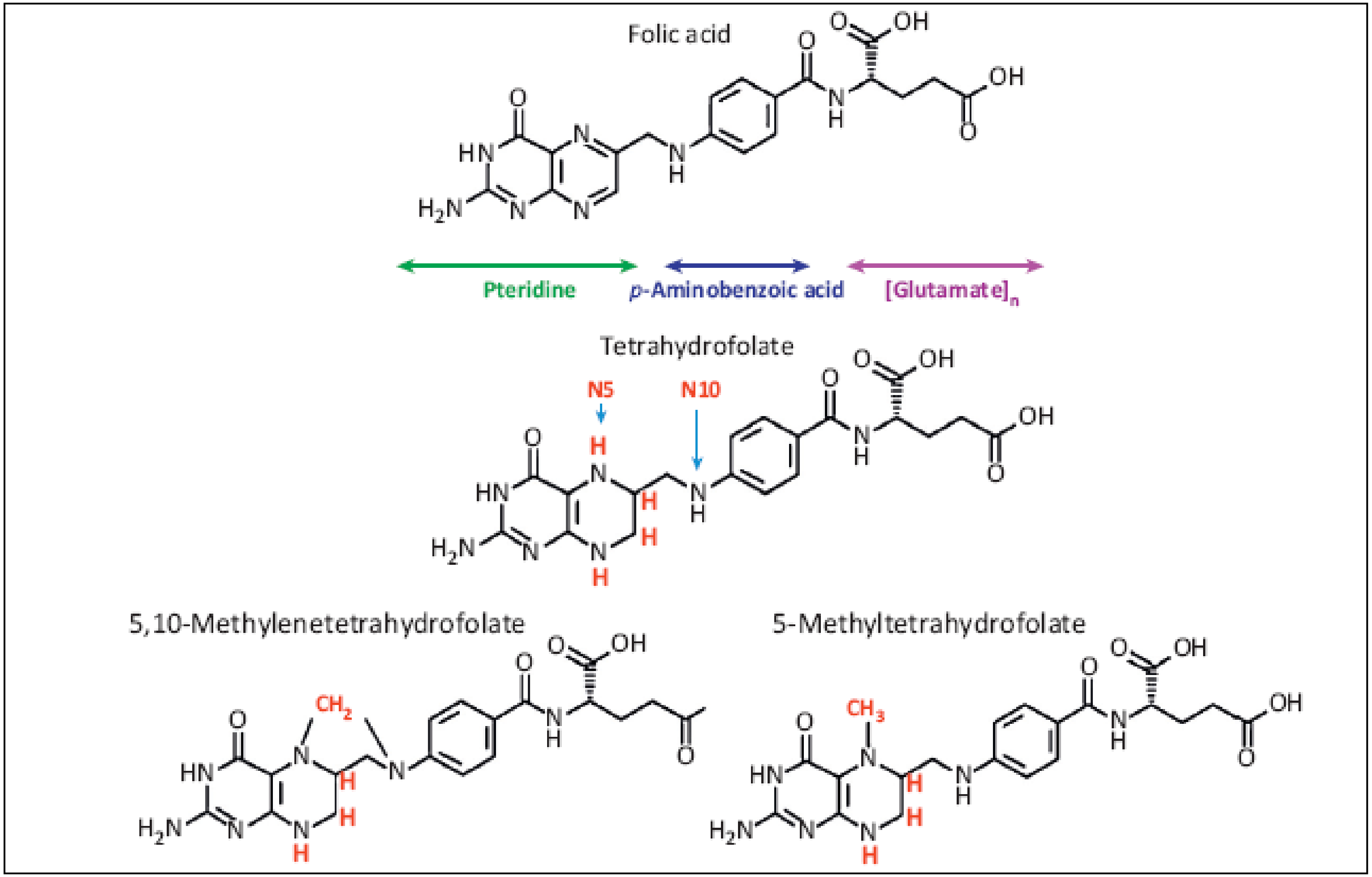


MTHFR C677T and COLORECTAL MALIGNANCY

PRIMING the METHYL CYCLE



REDUCED FOLATES



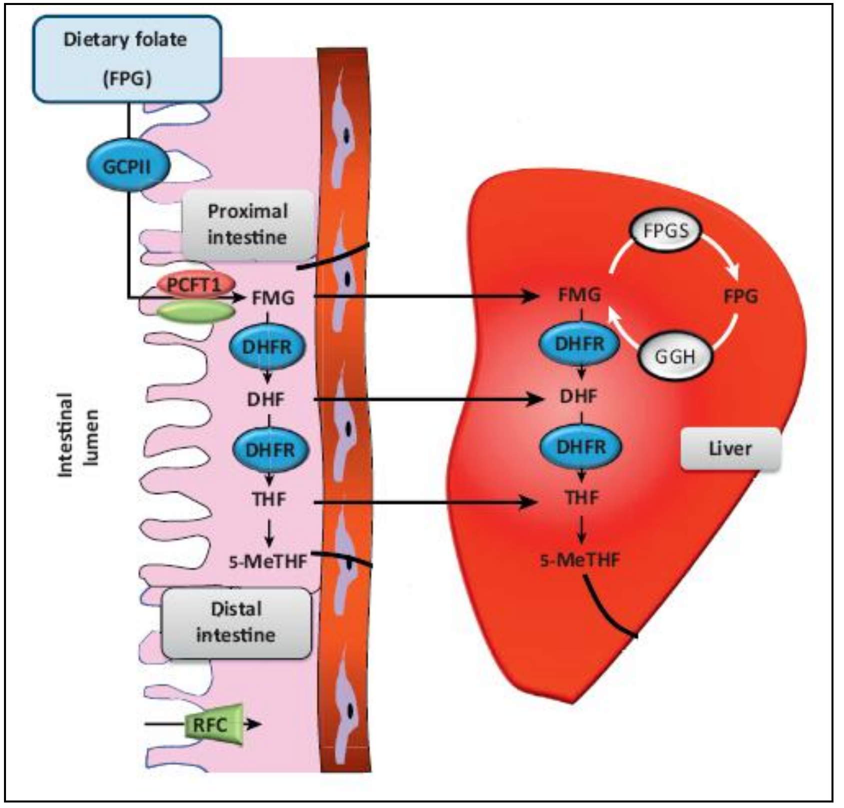
FOLATE ABSORPTION

Dietary folate is a polyglutamate

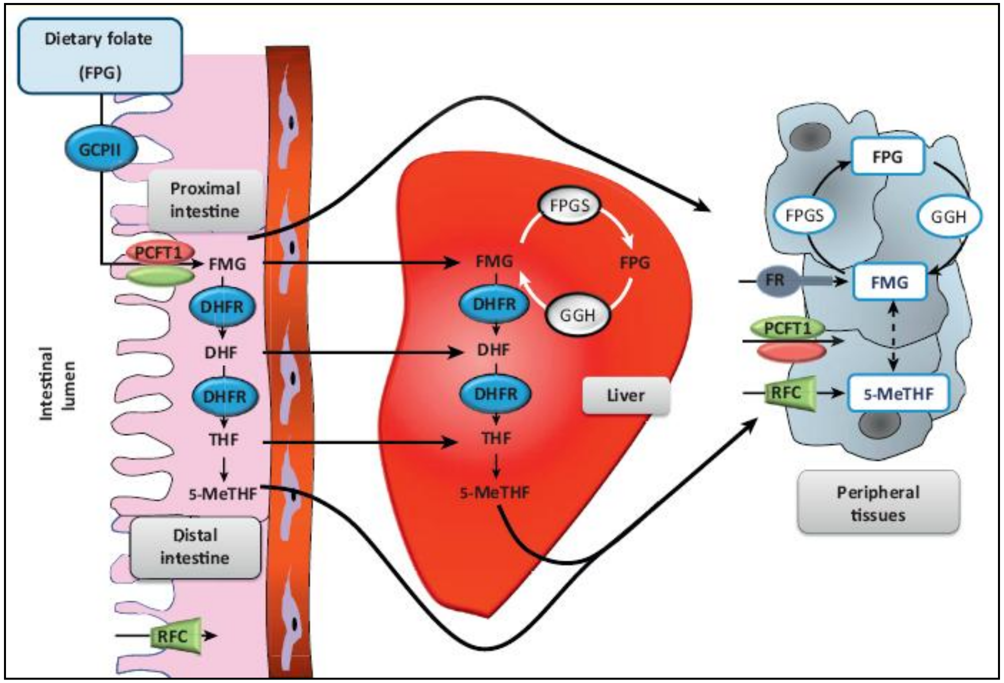
FPG ⟶ FMG
Glutamate carboxypeptidase II (GPCII)

FMG ⟶ FMG
Proton-coupled folate transporter (PCFT1)
acidic pH

FMG ⟶ FMG
Reduced folate carrier (RFC)
neutral pH of distal GI tract



FOLATE STORAGE and TRANSPORT

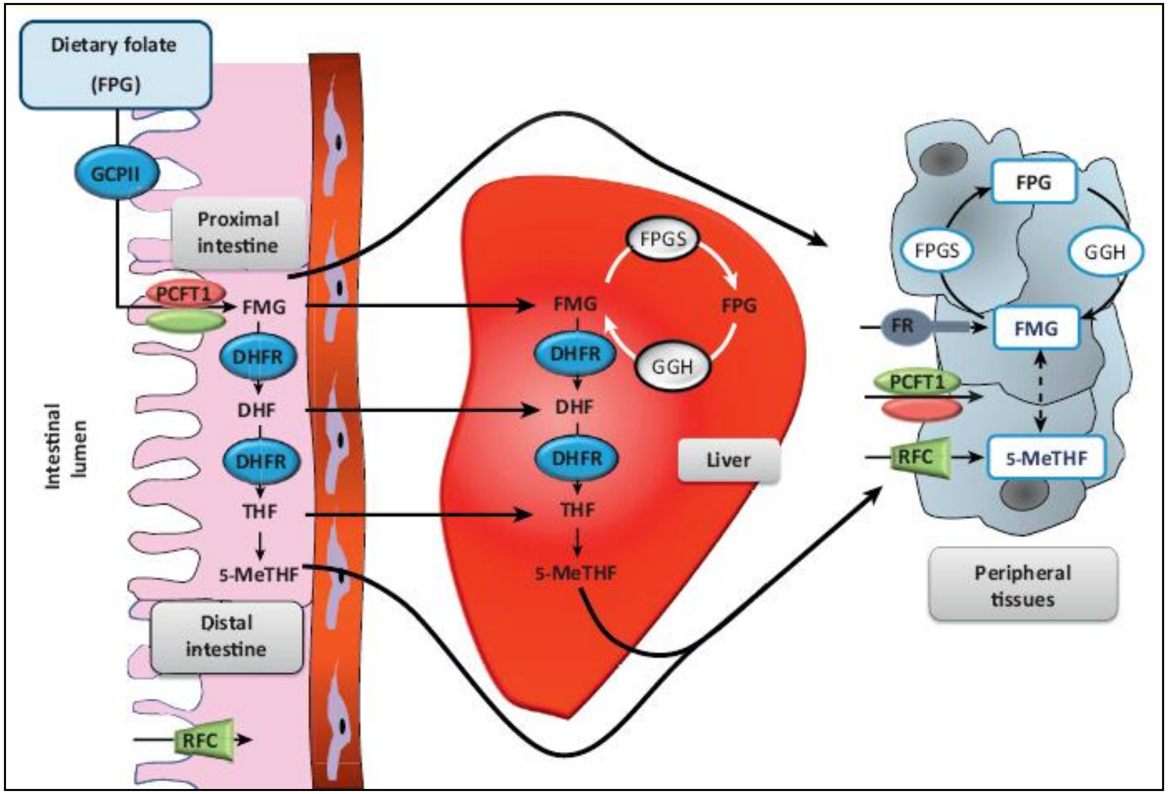


Folate stored (primarily In liver) as FPG

FMG ⟶ FPG
Folylpoly-gamma-glutamate synthetase (FPGS)

FPG ⟶ FMG
Gamma-glutamyl hydrolase (GGH)

PERIPHERAL FOLATE UPTAKE

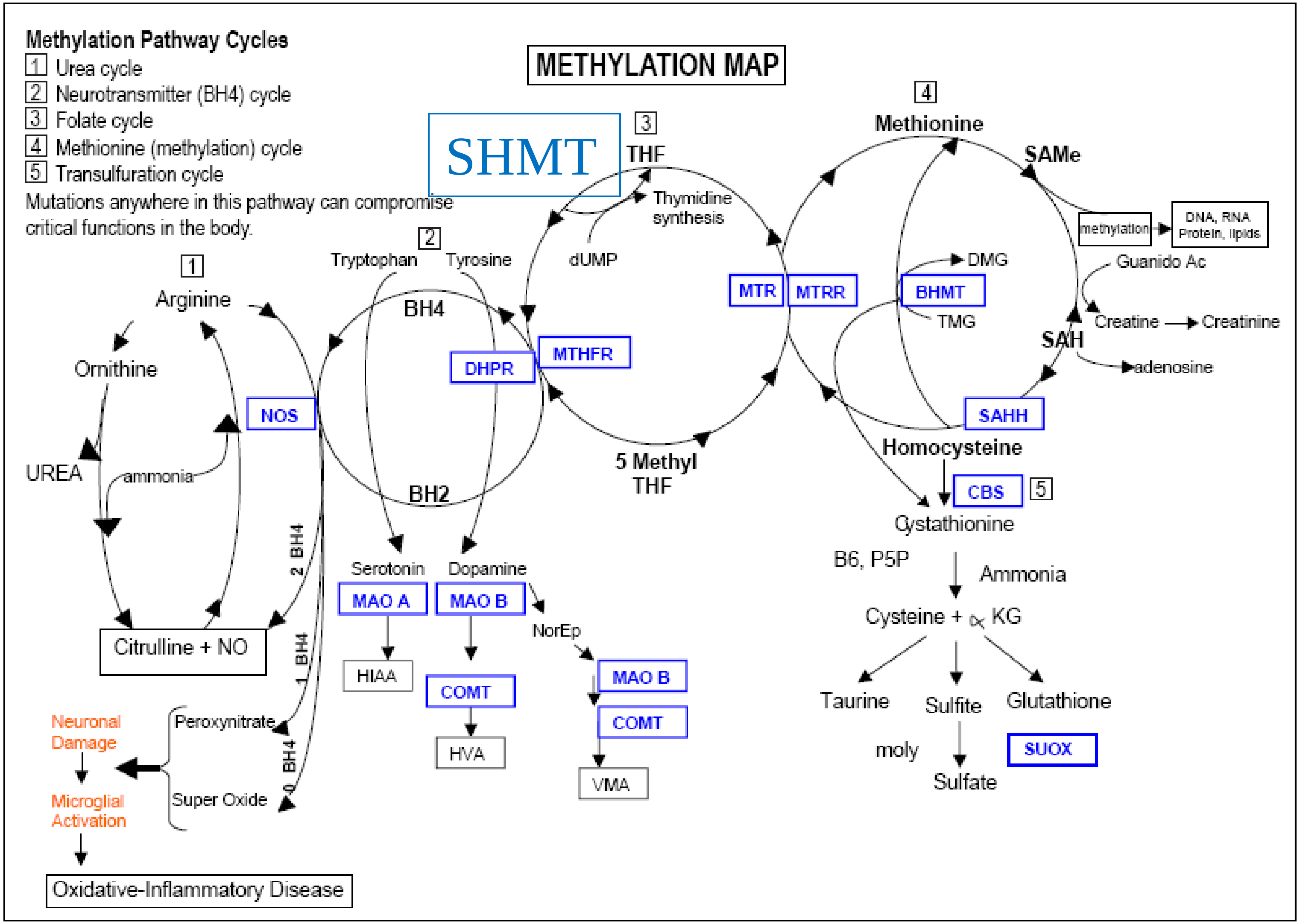


Reduced folate receptor

Folate receptors α, β, and γ

5-methyl folate > reduced folates > folic acid

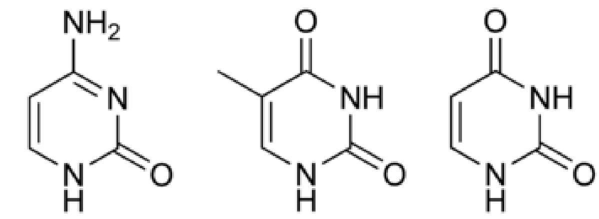
SERINE HYDROXY METHYL TRANSFERASE (SHMT)



SERINE HYDROXY METHYL TRANSFERASE (SHMT)

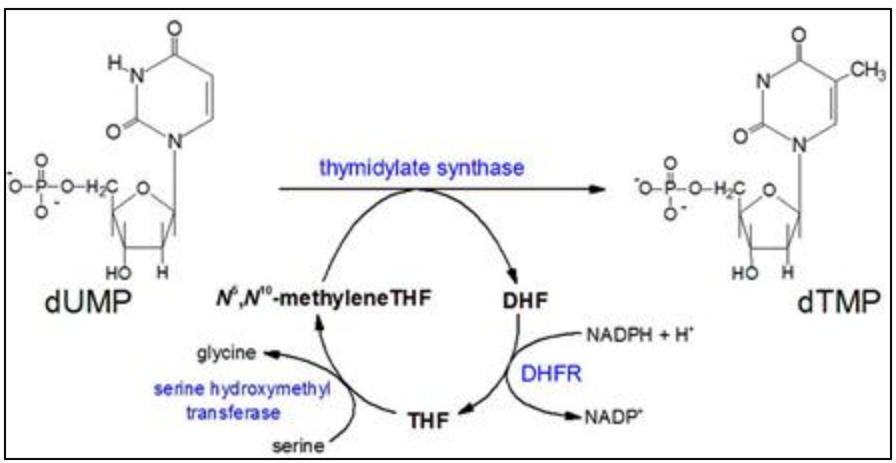
THF + Serine ⟶ 5,10-MethyleneTHF + Glycine
P-5-P

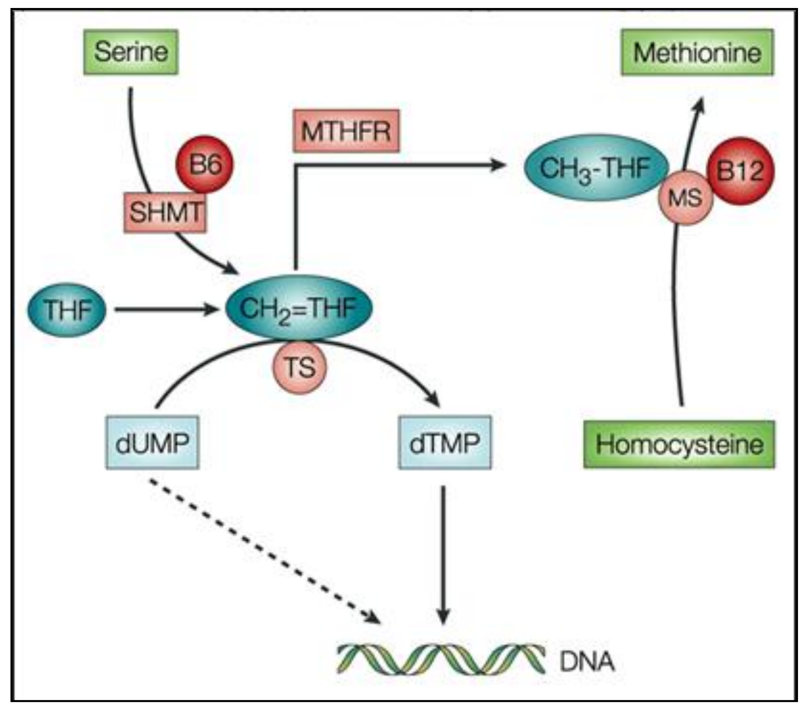
5,10-MethyleneTHF + dUMP ⟶ DHF + dTMP
Thymidine Synthase



Cytosine (**C**) Thymine (**T**) Uracil (**U**)

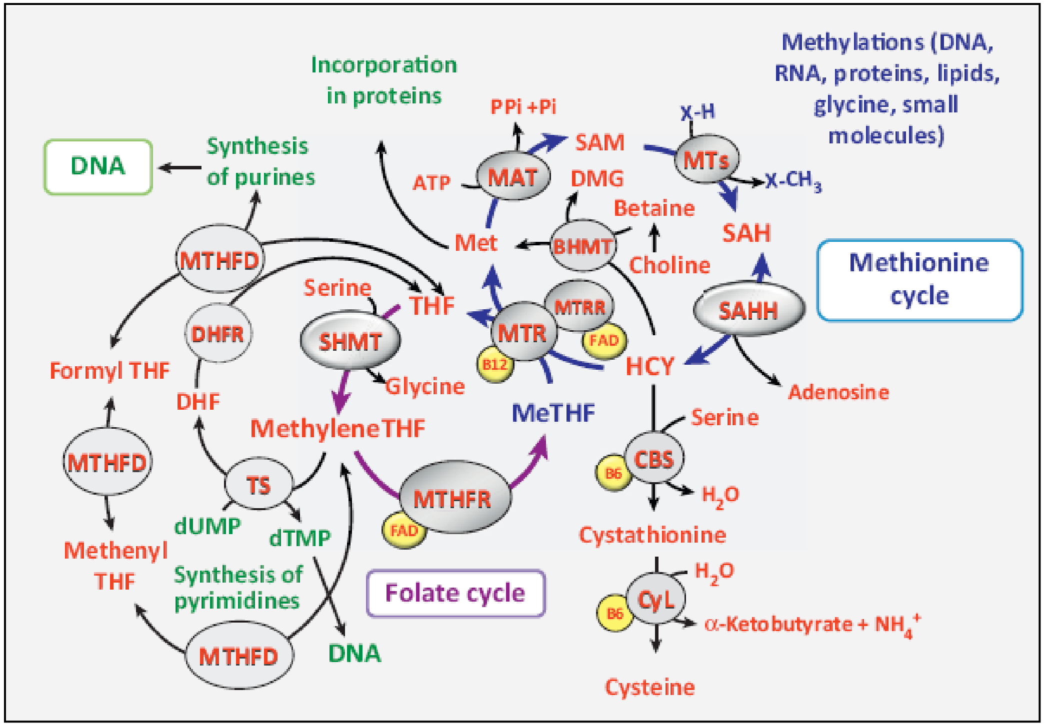
SHMT C1420T ≈ Down regulation





Treatment: Folinic Acid 4-800 mcg/day
Methyl Folate 4-800 mcg/day

DNA SYNTHESIS



THF → 5,10-MethyleneTHF → Pyrimidines → DHF → THF
SHMT Thymidine Synthase

THF → 5,10-MethyleneTHF → Methenyl THF → Formyl THF → Purines + THF
SHMT MTHFD MTHFD

THF → 5,10-MethyleneTHF → 5-Methyl THF
SHMT MTHFR

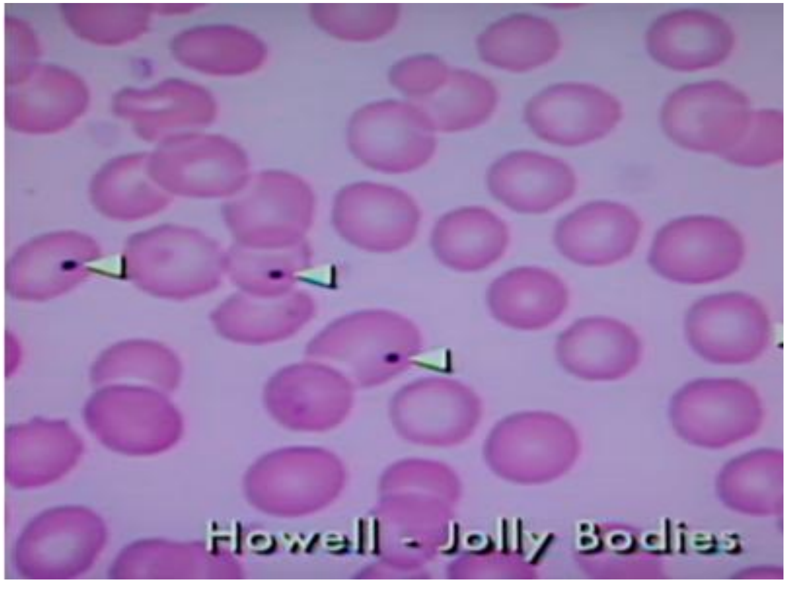
SPONATANEOUS DNA DAMAGE and FOLATE STATUS

♥ 122 subjects s/p splenectomy (not for malignancy)

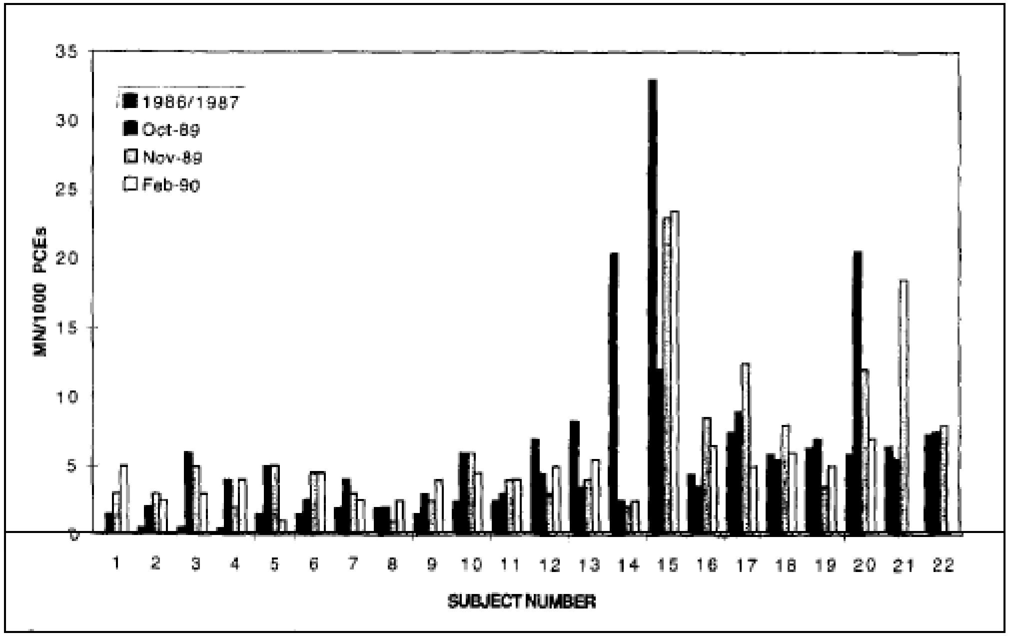
Select 22 with highest and lowest values for micronucleated RBCs

Repeat assessment over time
(‘87 – ’90)

Correlate with nutritional status



SPONATANEOUS DNA DAMAGE and FOLATE STATUS



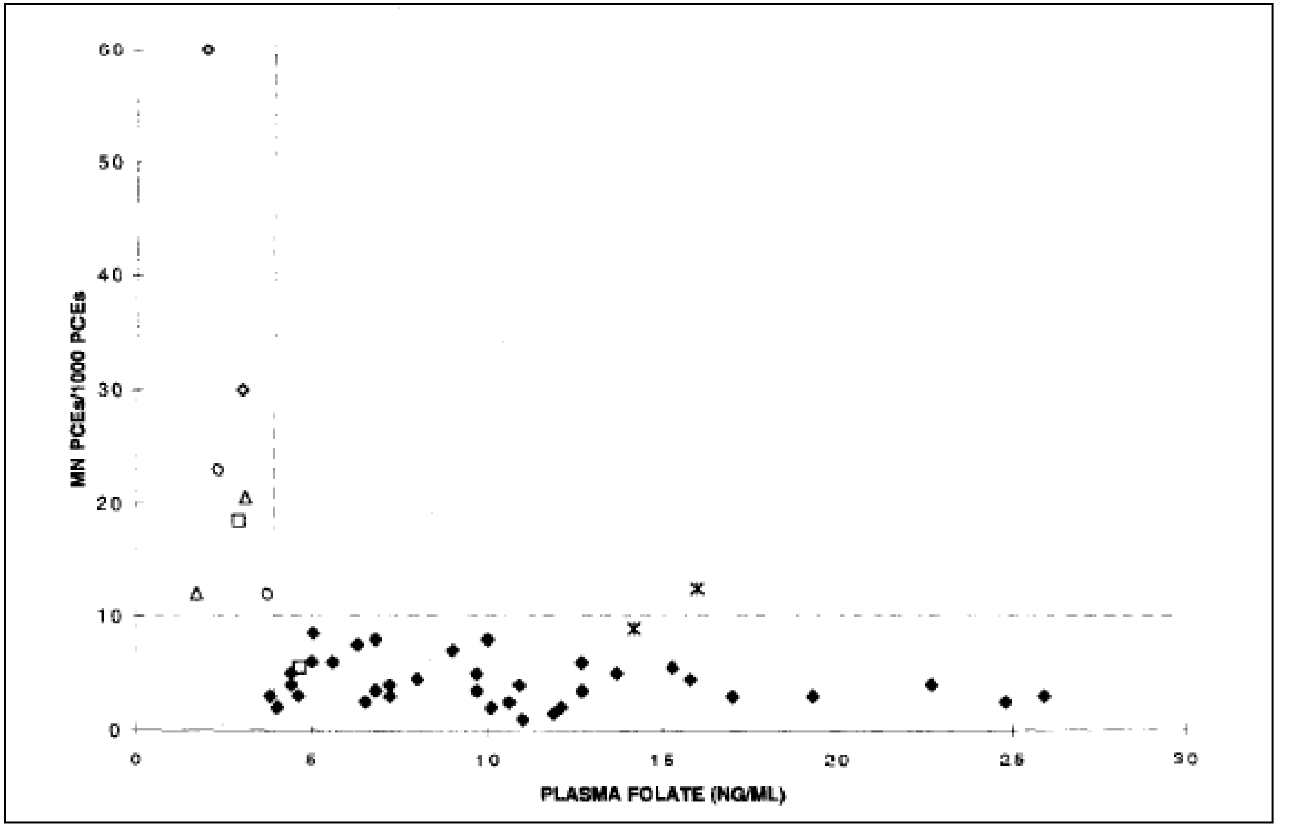
Individuals with low micronucleus formation stayed low

High micronucleus formation showed greatest variability

Cause is not genomic and Cause is not a constant

Micronucleus formation inversely related to folate and B12 status

SPONATANEOUS DNA DAMAGE and FOLATE STATUS



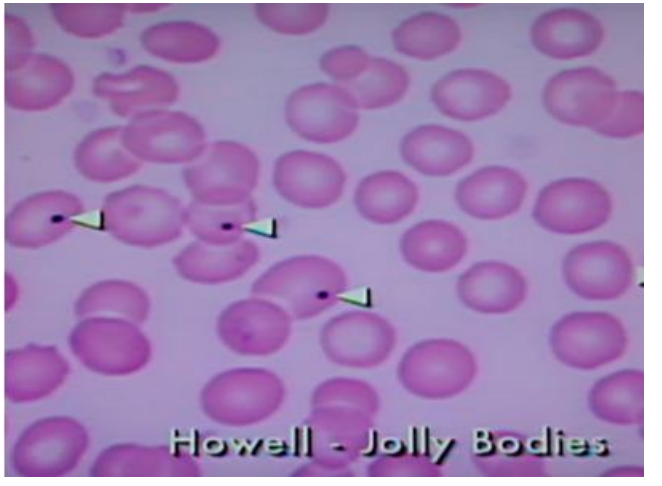
URACIL DNA MISINCORPORATION

♥ 122 subjects s/p splenectomy (not for malignancy)

Select 22 with highest and lowest values for micronucleated RBCs

Measure at baseline:

- RBC and plasma folate
- Uracil in DNA
- Micronucleated RBCs

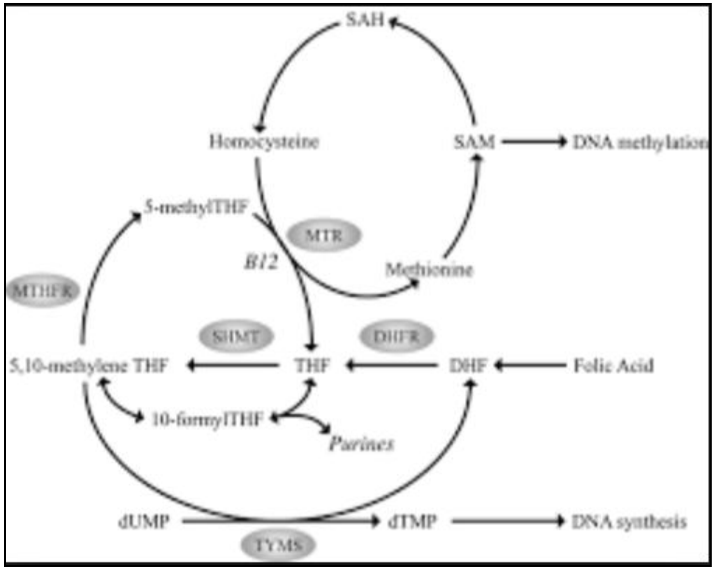


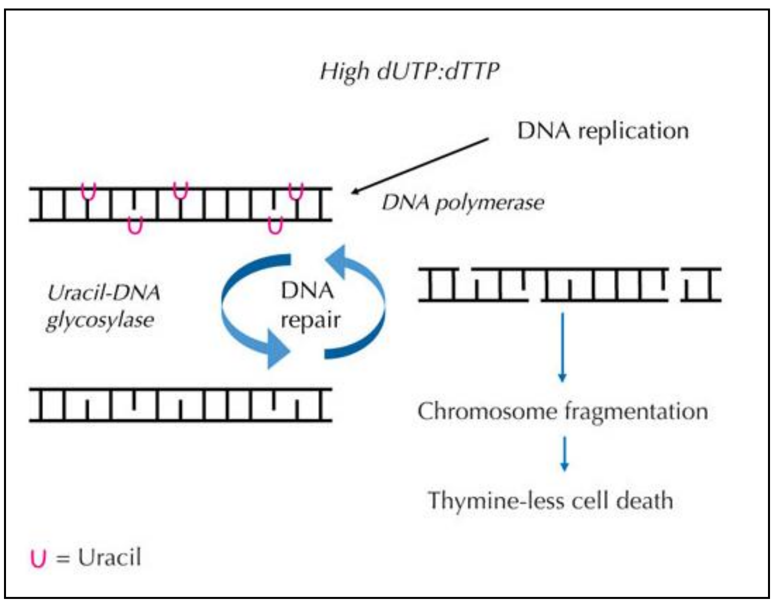
Treat all with 5 mg/day folic acid

Repeat baseline measures at one week

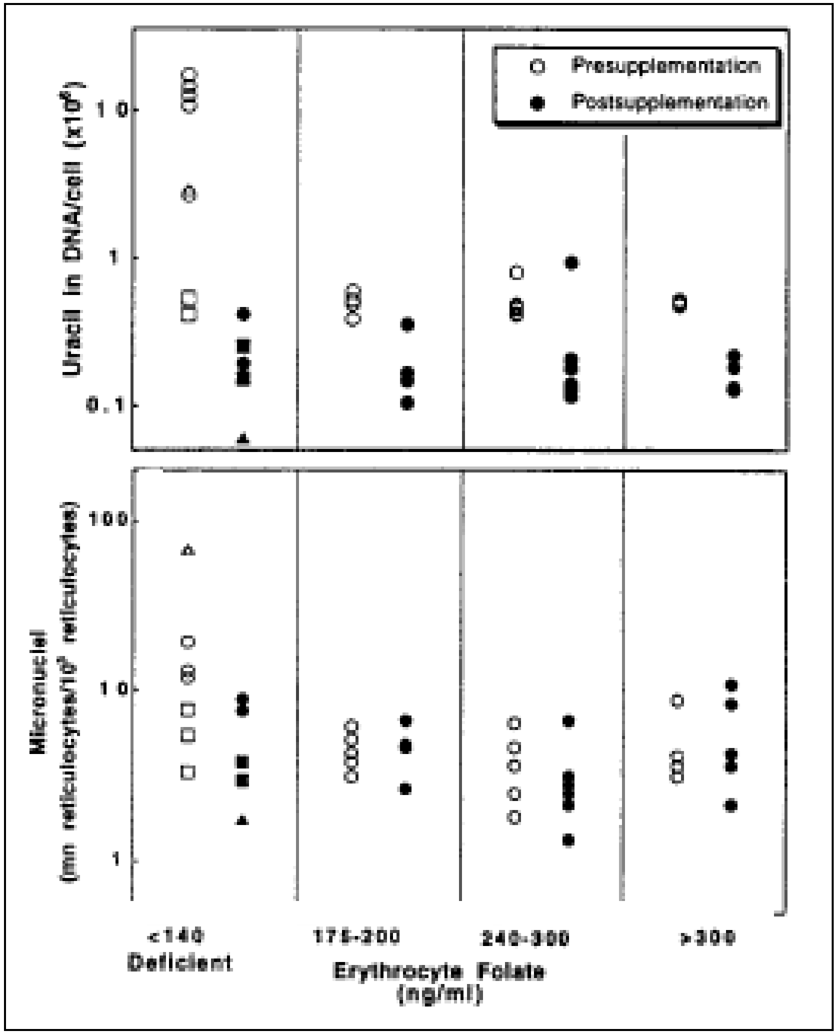
♥ Crohn's Disease patient s/p splenectomy
Treat with folinic acid 25 mg/day followed by folic acid at 5 mg/day

URACIL DNA MISINCORPORATION

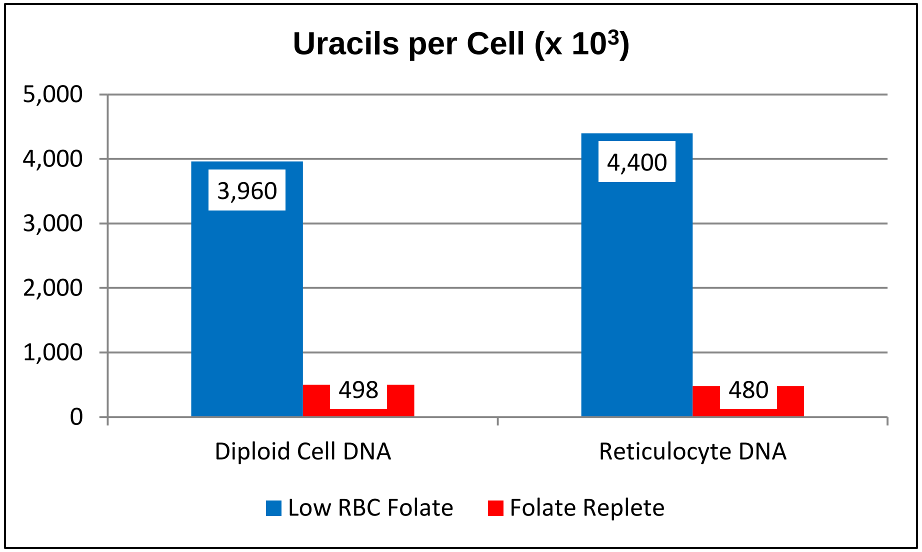




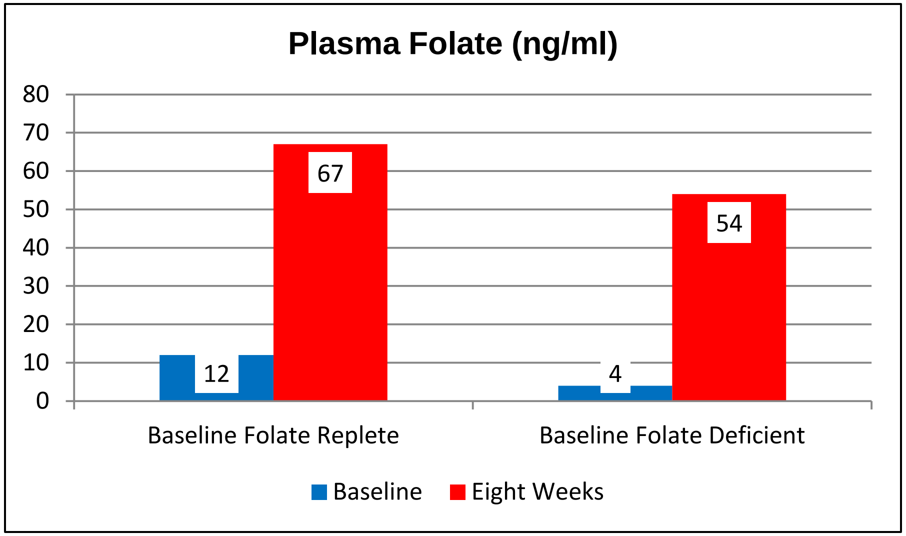
URACIL DNA MISINCORPORATION

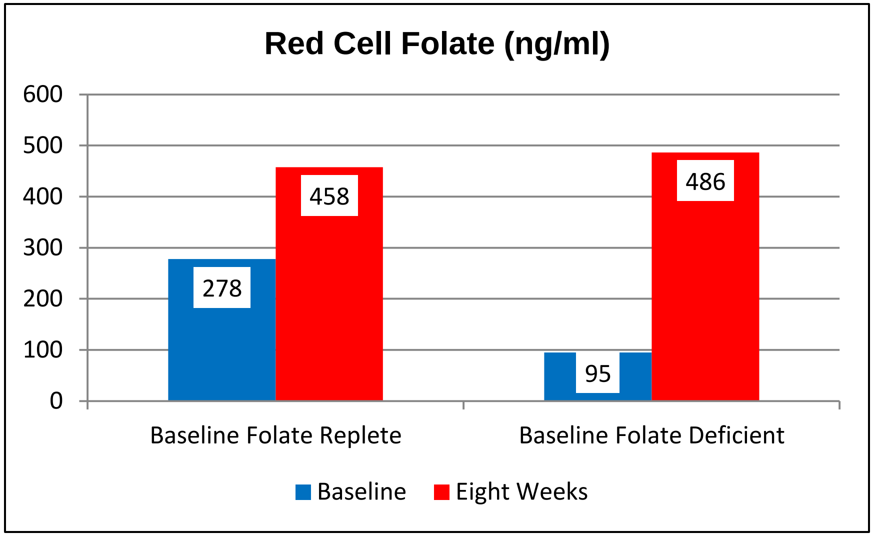


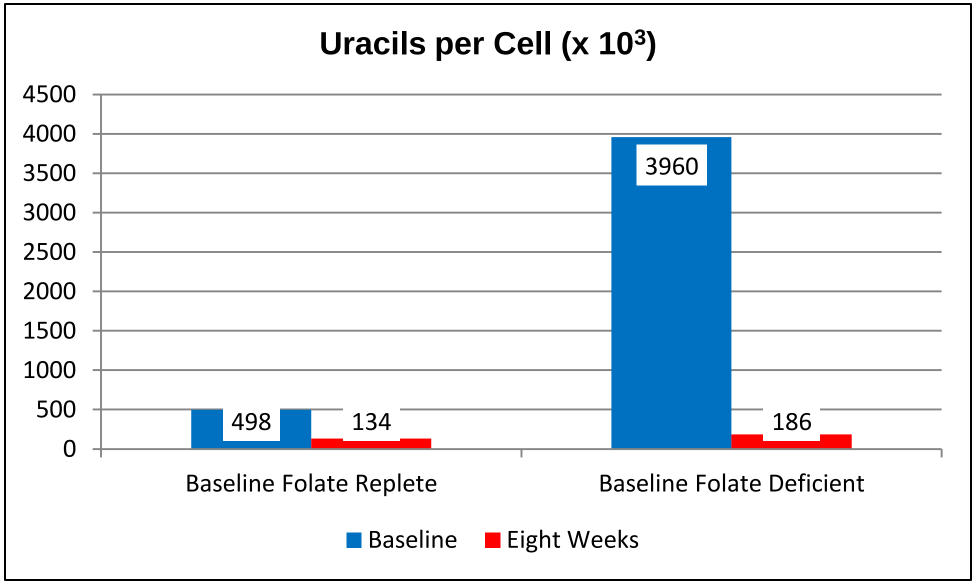
o RBC Folate
□ Low RBC, Borderline Plasma
Δ Crohn's Patient



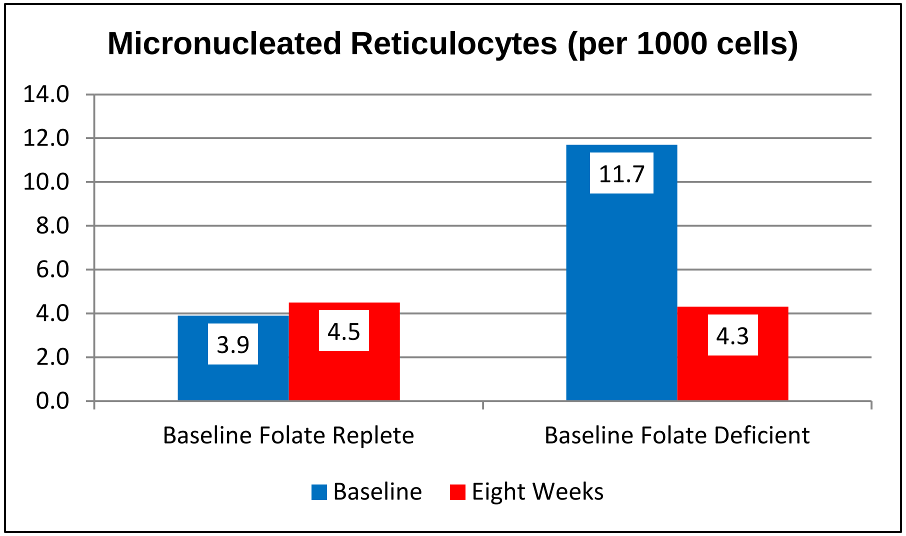
URACIL DNA MISINCORPORATION

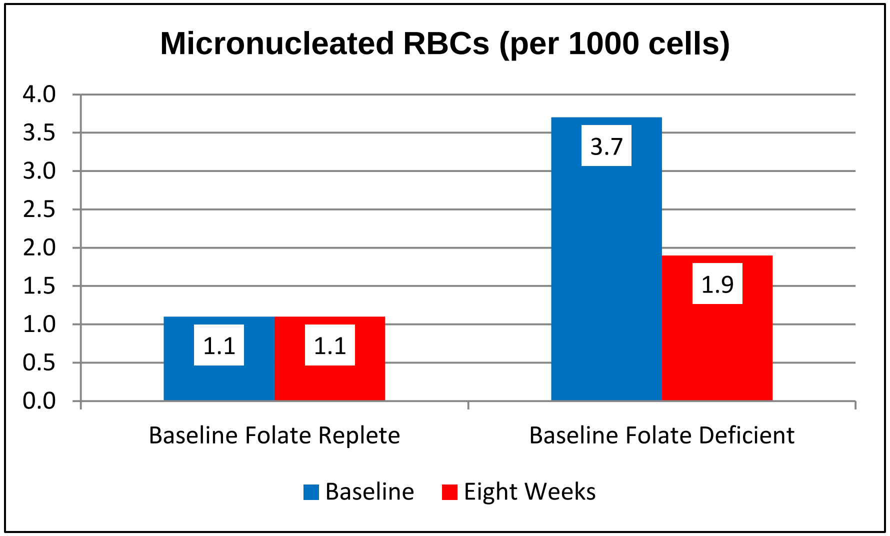


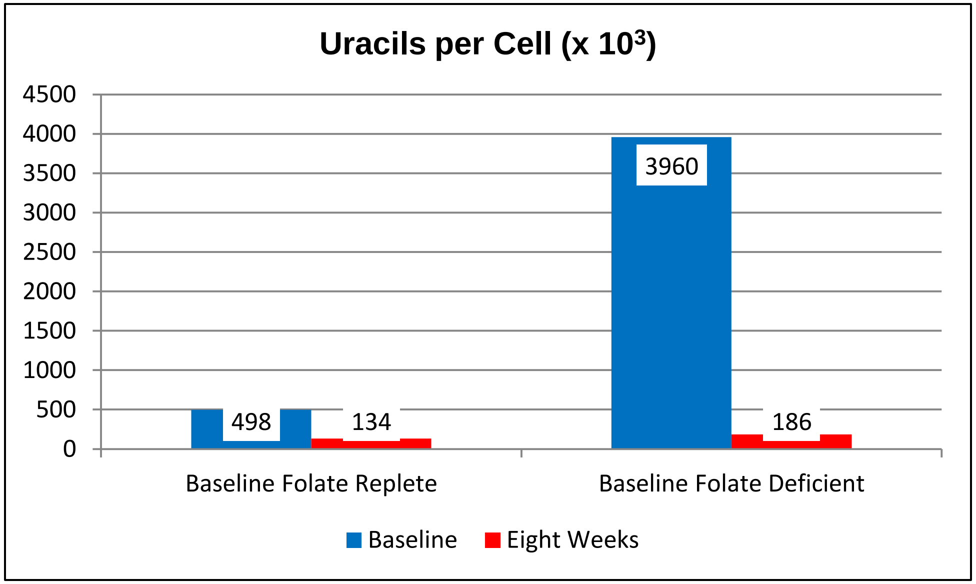




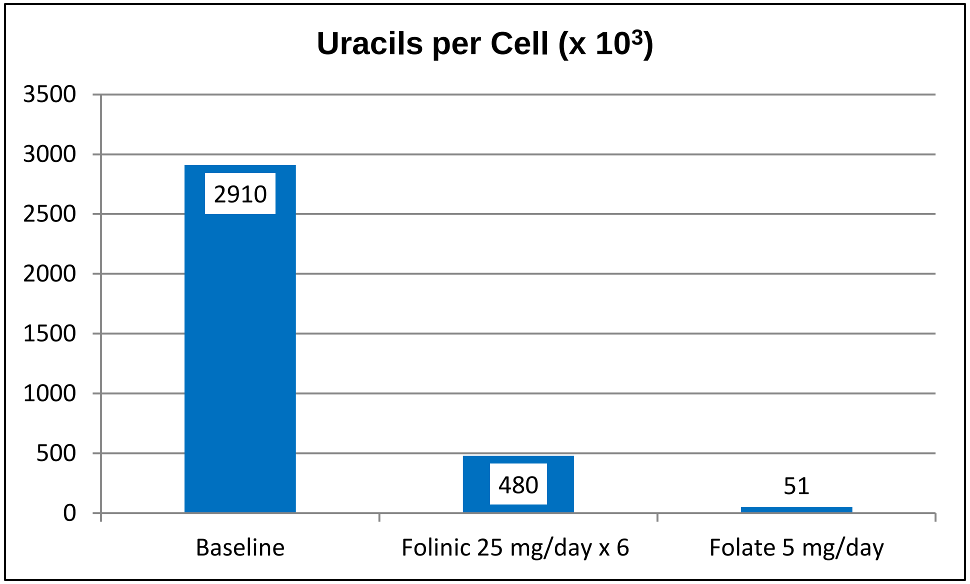
URACIL DNA MISINCORPORATION

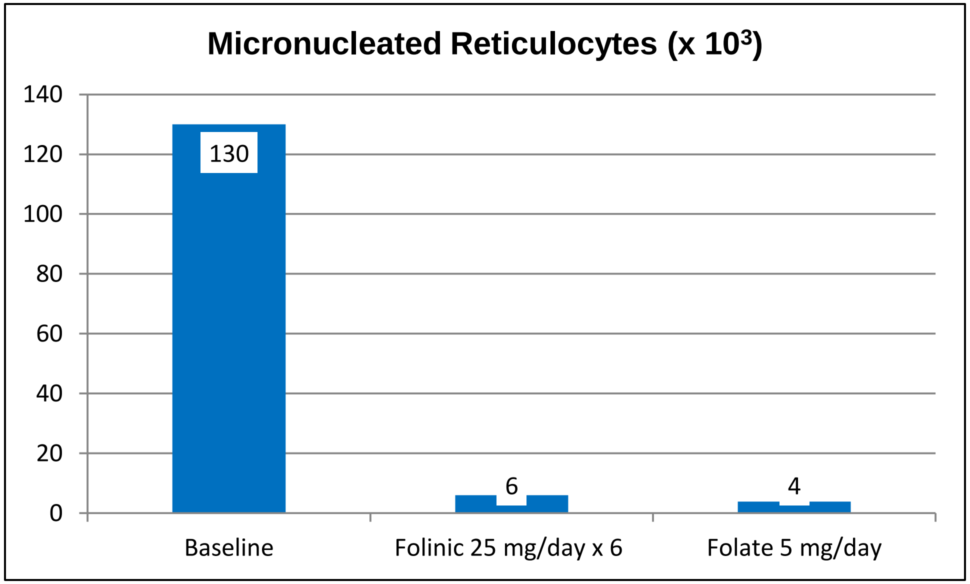




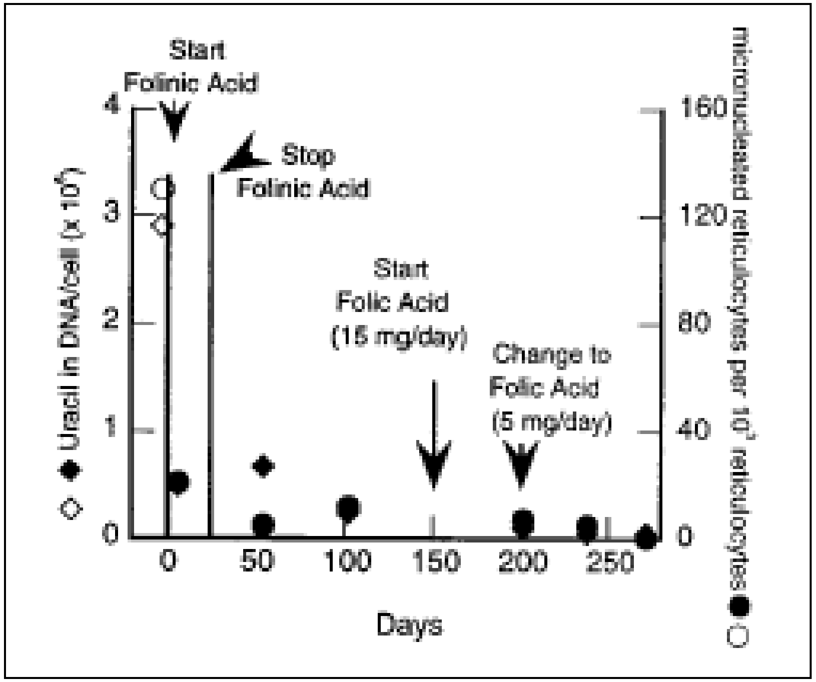


URACIL DNA MISINCORPORATION



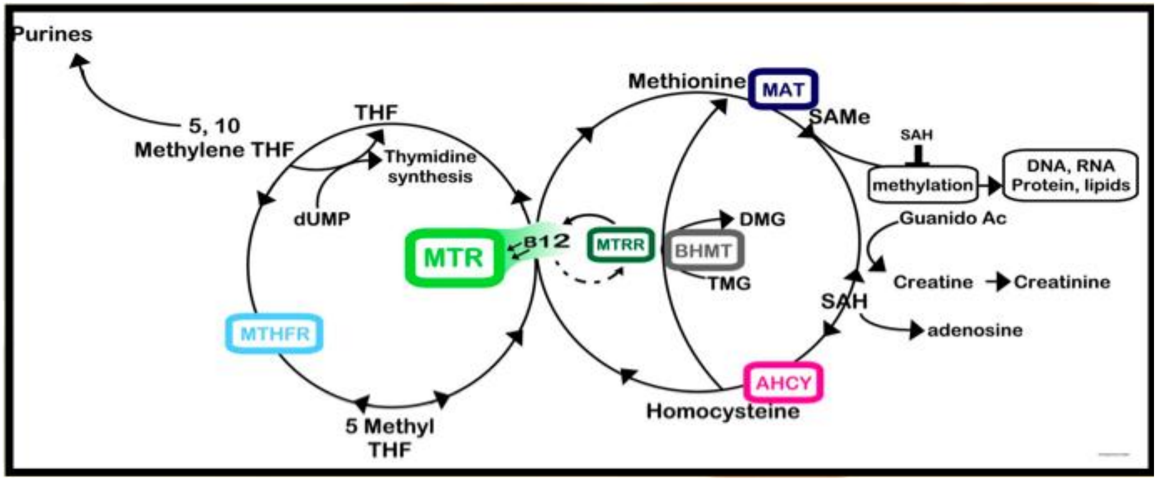


Crohn's Disease Patient



METHIONINE SYNTHASE (MTR)

5-Methyl Folate + Homocysteine ⟶ Methionine + THF
Methyl-B12 (from MTRR)



<u>Alcohol and Mercury inhibit MTR → Low SAMe:</u>

- Low Phosphatidylcholine → Membrane dysfunction
- Impaired fatty acid oxidation (PGC1-alpha)
- Oxidative stress
- Oncogene activation

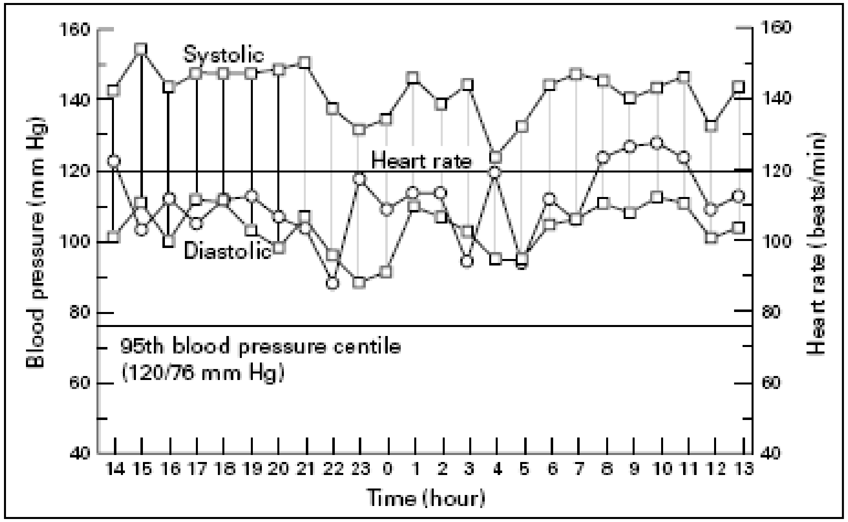
<u>Treatment</u>

- Methyl-Folate and Methyl-B12
- SAMe (and/or other measures to ↑ SAMe:SAH)

MERCURY and AUTONOMIC DYSFUNCTION

♥ 11 year old girl presents with:

- ♦ Irritability, weakness, and ataxia
- ♦ BP 160/120 and HR 120 BPM



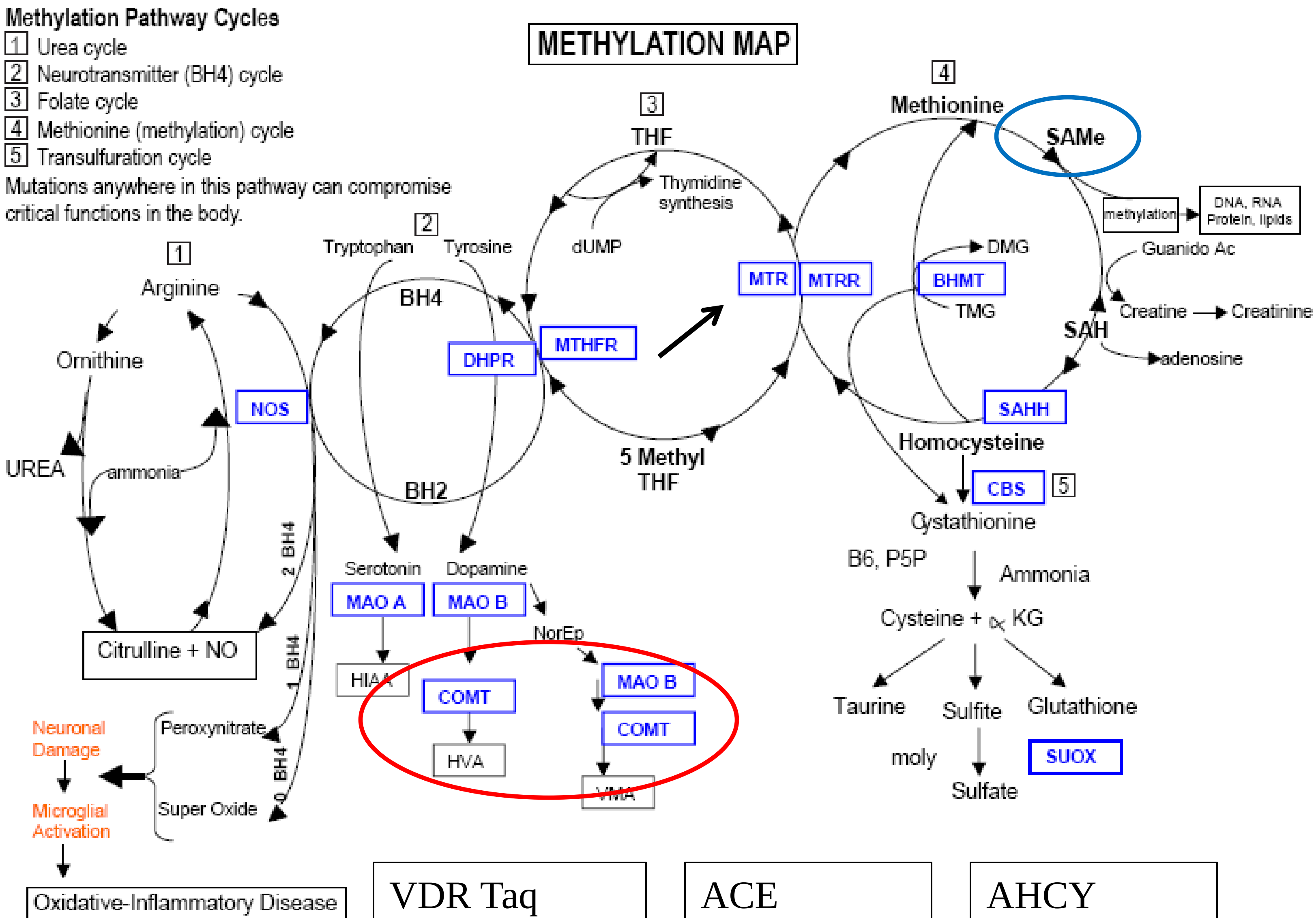
Thyroid chemistries normal

Negative toxin screen

Renal function normal

Catecholamine levels elevated - but no adrenal mass by CT

- ● Blood and urine Hg levels elevated
- ● Full resolution of signs and symptoms with Penicillamine Hg chelation



VDR Taq

ACE

AHCY

VDR Fok

SHMT

ACAT

MERCURY and AUTONOMIC DYSFUNCTION

COMT (Catecholamine-O-Methyl Transferase) metabolizes catecholamines

SAMe (S-Adenosyl-Methionine), a cofactor for COMT, is generated via MTR

Mercury inhibits MTR (Methionine Synthase)

COMT function is compromised

Catecholamines accumulate and cause CV signs and symptoms and oxidative stress

Acrodynia, or “pink disease” includes mental changes such as insomnia and irritability, pain in the extremities, skin lesions, profuse sweating, anorexia, hypertension, tachycardia.

Kawasaki Syndrome – SNIP in IP_3R (Inositol 1,4,5 triphosphate receptor)

Mercury associated with hypertension, arrhythmia, autonomic dysfunction, and CV Dz

B VITAMINS and S-ADENOSYLHOMOCYSTEINE

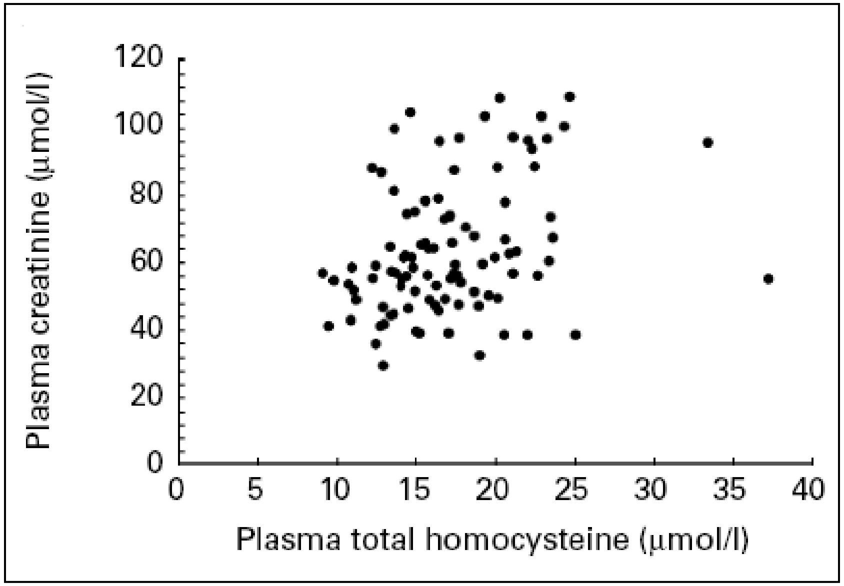
♥ 276 mature (≥ 65 years) New Zealanders

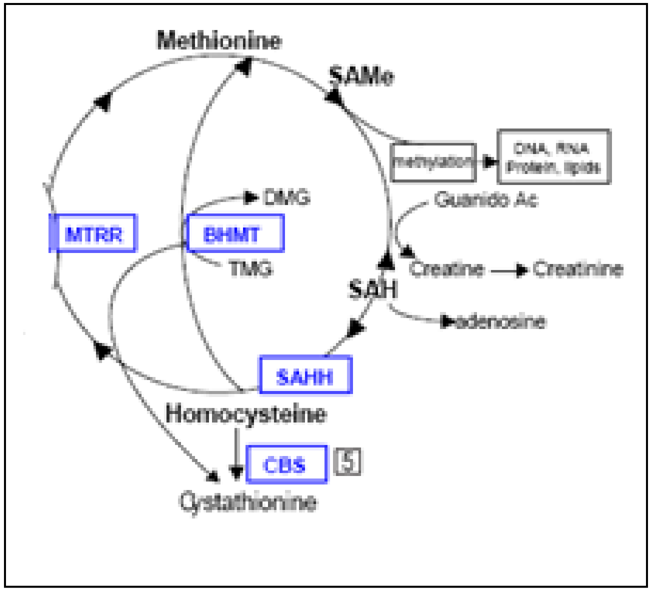
- Overall good health
- None with CV disease
- Homocysteine > 13 umol/l

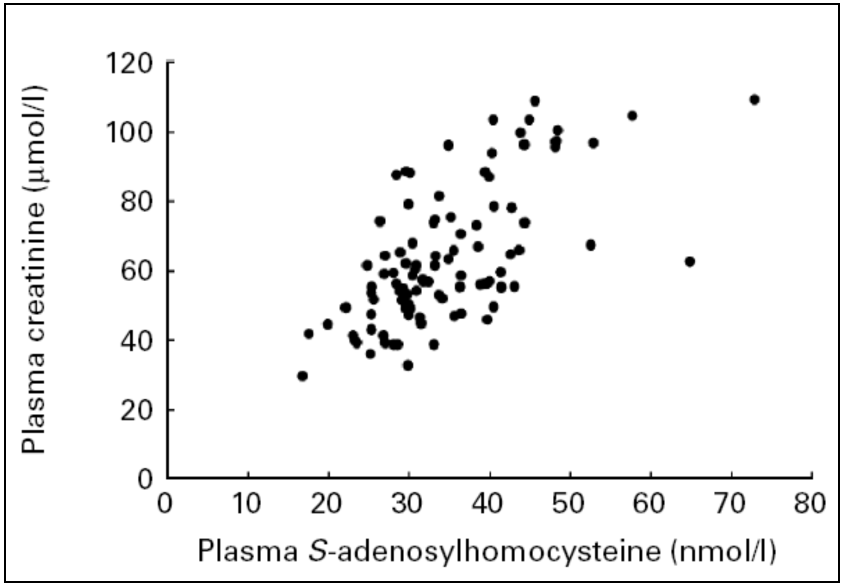
Evaluate relationships between:

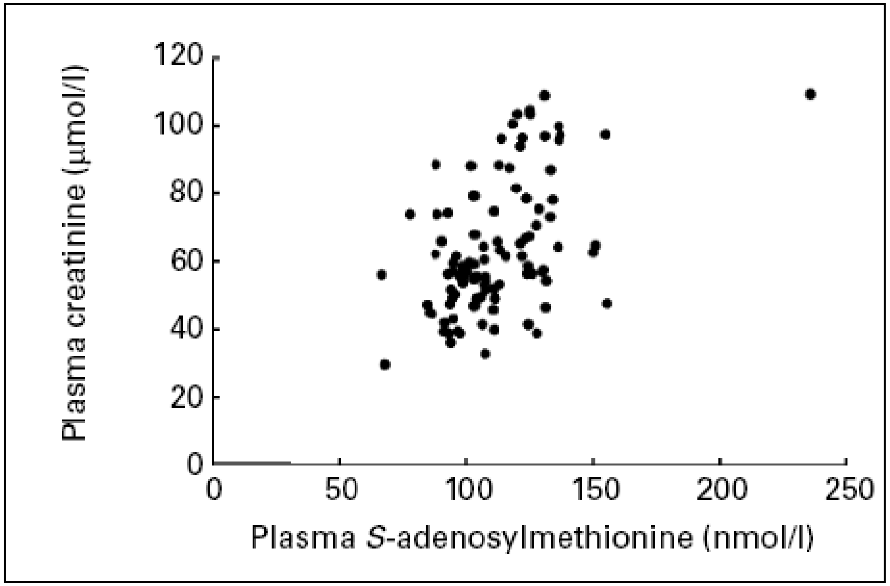
- Homocysteine
- Creatinine
- Folic acid
- S-Adenosylmethionine (SAMe)
- S-Adenosylhomocysteine (SAH)

B VITAMINS and S-ADENOSYLHOMOCYSTEINE



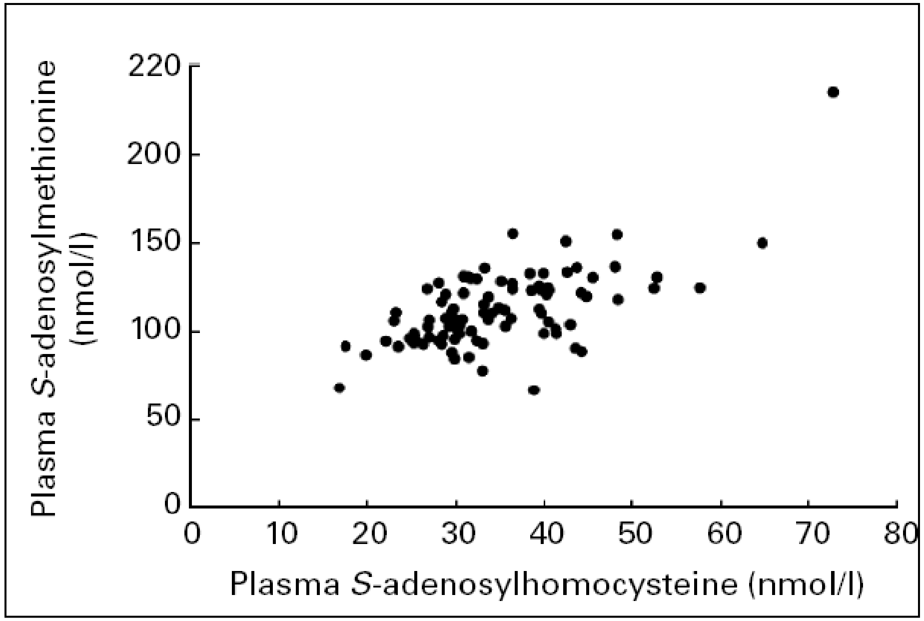






B VITAMINS and S-ADENOSYLHOMOCYSTEINE

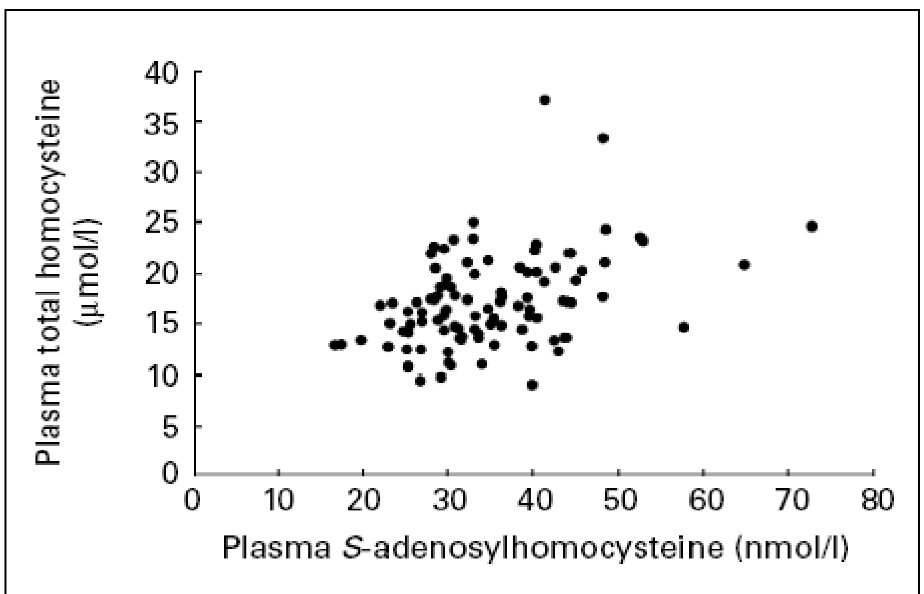
SAMe



SAH

Hcy

(umol/l)

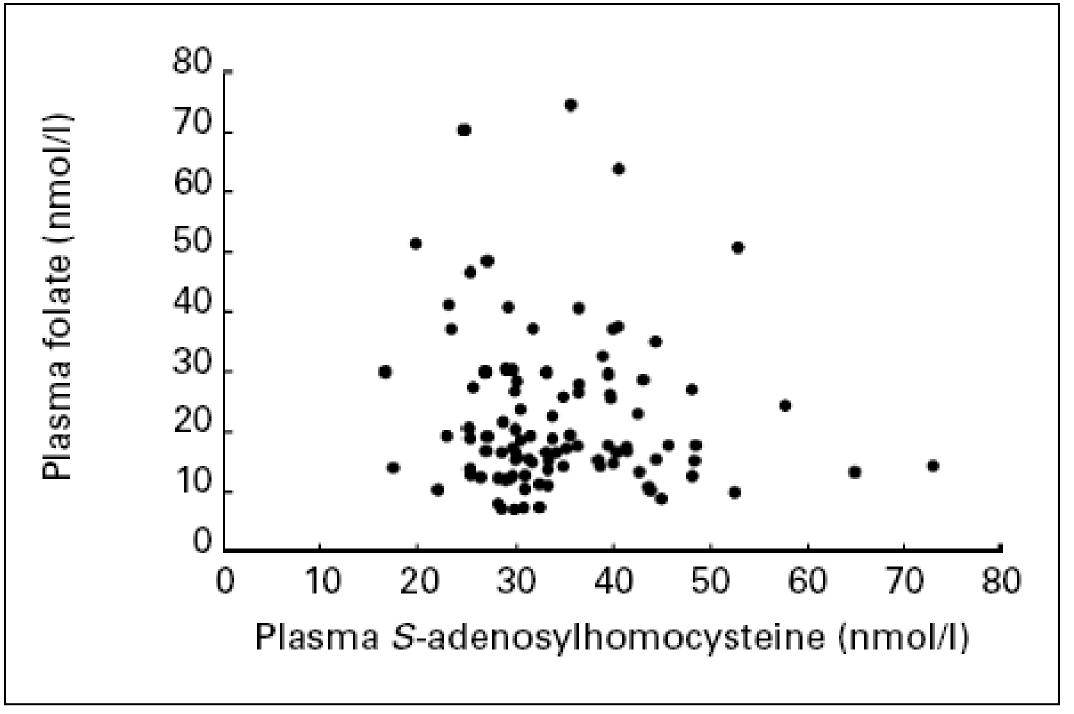


SAH

(nmol/l)

B VITAMINS and S-ADENOSYLHOMOCYSTEINE

Folate



SAH

- Homocysteine inversely related to serum Folate
- S-Adenosylhomocysteine not related to Folate

B VITAMINS and S-ADENOSYLHOMOCYSTEINE

♥ 276 mature (≥ 65 years) New Zealanders

- Overall good health
- None with CV disease
- Homocysteine > 13 umol/l

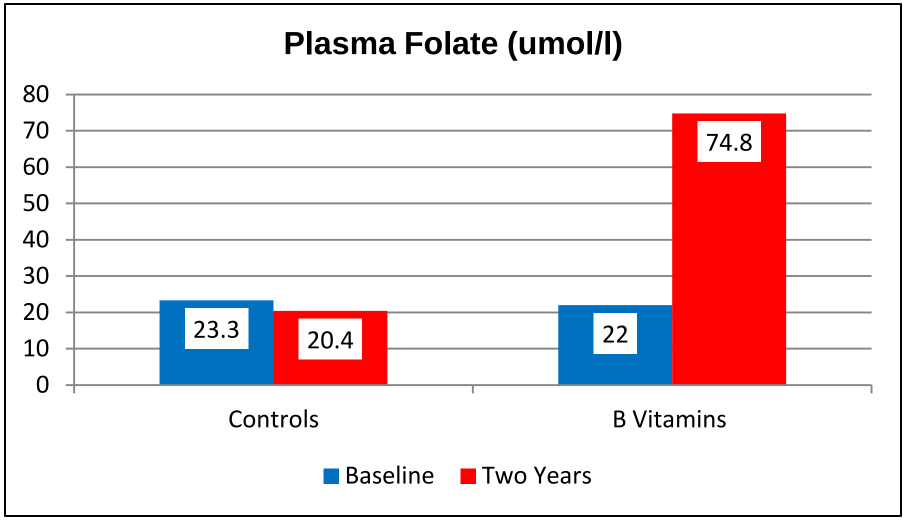
Baseline measurements

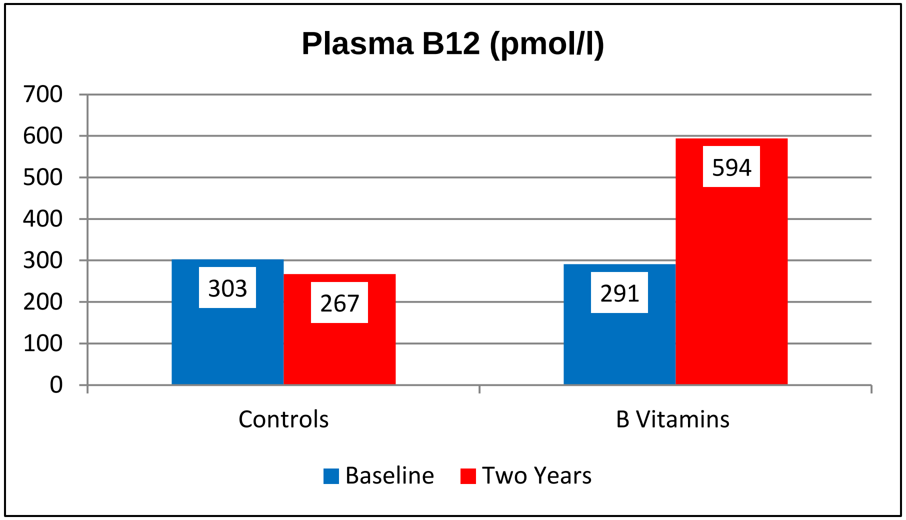
Randomize to receive over two years:

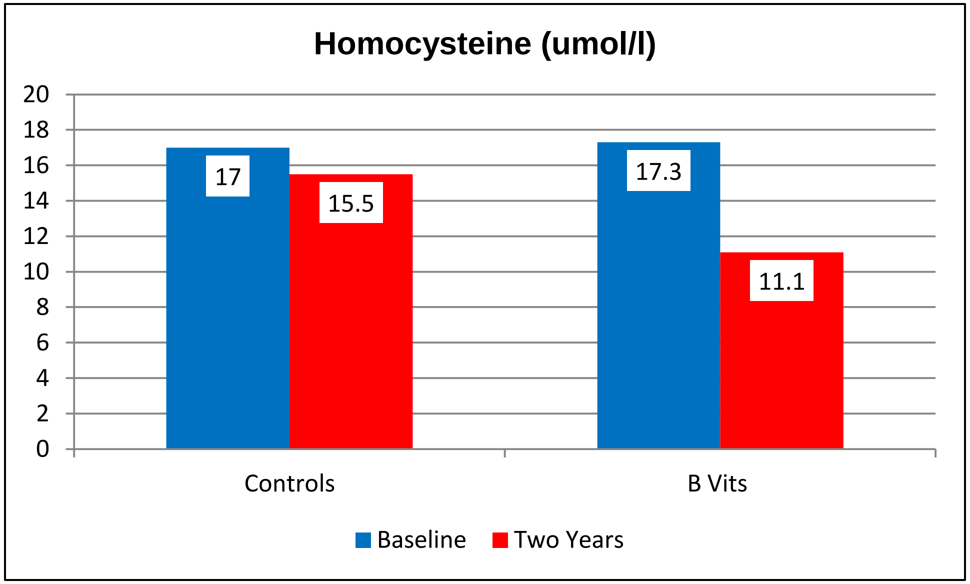
- Methyl-Folate 1 mg, B12 500 mcg, and B6 10 mg
- Placebo

Repeat baseline measurements

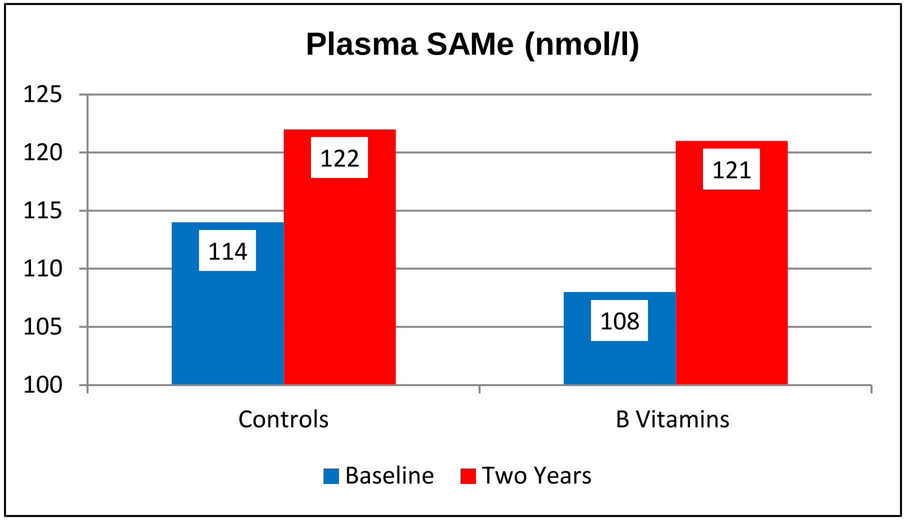
B VITAMINS and S-ADENOSYLHOMOCYSTEINE

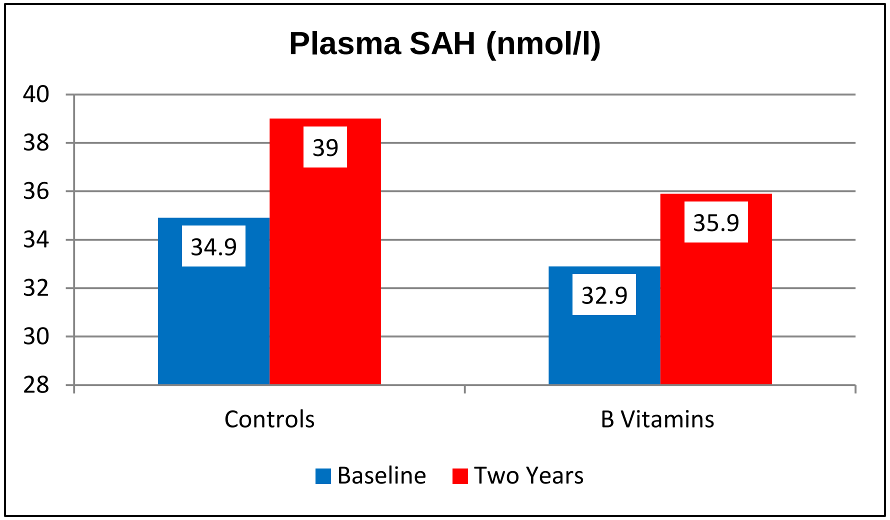


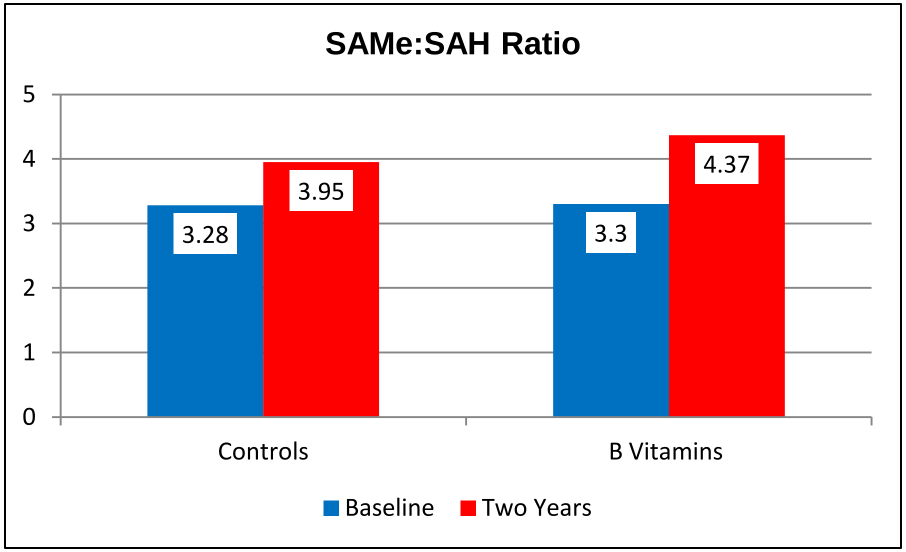




B VITAMINS and S-ADENOSYLHOMOCYSTEINE







Homocysteine Reduction
(Folate, B12, and B6 alone)

Not sufficient to

Lower S-Adenosylhomocysteine

Increase SAMe:SAH

B 12 and S-ADENOSYLHOMOCYSTEINE CONTROL

♥ 149 elderly (mean age 76 years) Americans

- Overall good health
- None with diagnosed B12 deficiency
- None on IM or high dose oral B12 (lower dose or multi OK)

Factor	Mean	Abnormal	% Abnormal
Folic Acid	46.3 nmol/l	< 13.6 nmol/l	2%
B12	365 pmol/l	< 258 pmol/l	26%
MMA	272 nmol/l	> 271 nmol/l	30%
Creatinine	1.1 mg/dl	> 1.23	21%
Hcy	10.7 umol/l	> 13.7 umol/l	13%
SAH	35 nmol/l	> 26 nmol/l	64%
SAM	118 nmol/l	71 – 168 nmol/l	10% high 10% low
SAMe:SAH	4.0	< 4.4	68%

B 12 and S-ADENOSYLHOMOCYSTEINE CONTROL

♥ 149 elderly (mean age 76 years) Americans

- ♦ 45/149 with MMA > 271 nmol/l → B12 1000mcg/day (cyanocobalamin)

- ♦ 104/149 with MMA ≤ 271 randomized to receive:
    - B12 25 mcg/day
    - B12 100 mcg/day
    - Placebo

Repeat baseline measurements at three months

1000 mcg/day – open format

Lower doses – double blind format

B 12 and S-ADENOSYLHOMOCYSTEINE CONTROL

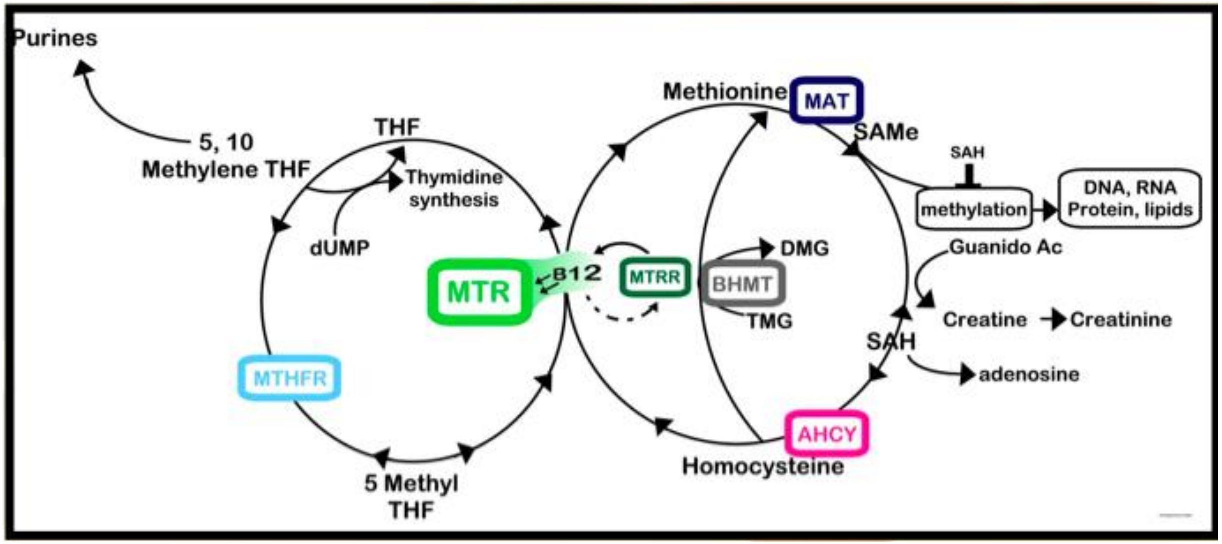
Factor	100 mcg/day			1000 mcg/day	
	Baseline	3 Months		Baseline	3 Months
B12 (>258)	364	424		312	831
MMA (< 271)	199	187		434	240
Hcy (< 13.9)	9.0	8.2		12.7	9.6
SAMe	95	113		153	159
SAH (< 26)	28	28		43	37
SAMe:SAH	4.9	5.3		4.1	5.1

B 12 and S-ADENOSYLHOMOCYSTEINE CONTROL

Subjects receiving 1000 mcg/day B12					
Factor	Creatinine > 1.2 mg/dl			Creatine ≤ 1.2 mg/dl	
No. (mean)	21/45 (mean 1.8)			24/45 (mean 0.98)	
	Baseline	3 Months		Baseline	3 Months
B12 (>258)	326	926		300	756
MMA (< 271)	458	256		443	227
Hcy (< 13.9)	16.4	11.4		11.4	8.3
SAMe	189	176		119	146
SAH (< 26)	63	46		36	29
SAMe:SAH	3.4	4.1		3.9	5.8

METHIONINE SYNTHASE (MTR)

5-Methyl Folate + Homocysteine ⟶ Methionine + THF
Methyl-B12 (from MTRR)



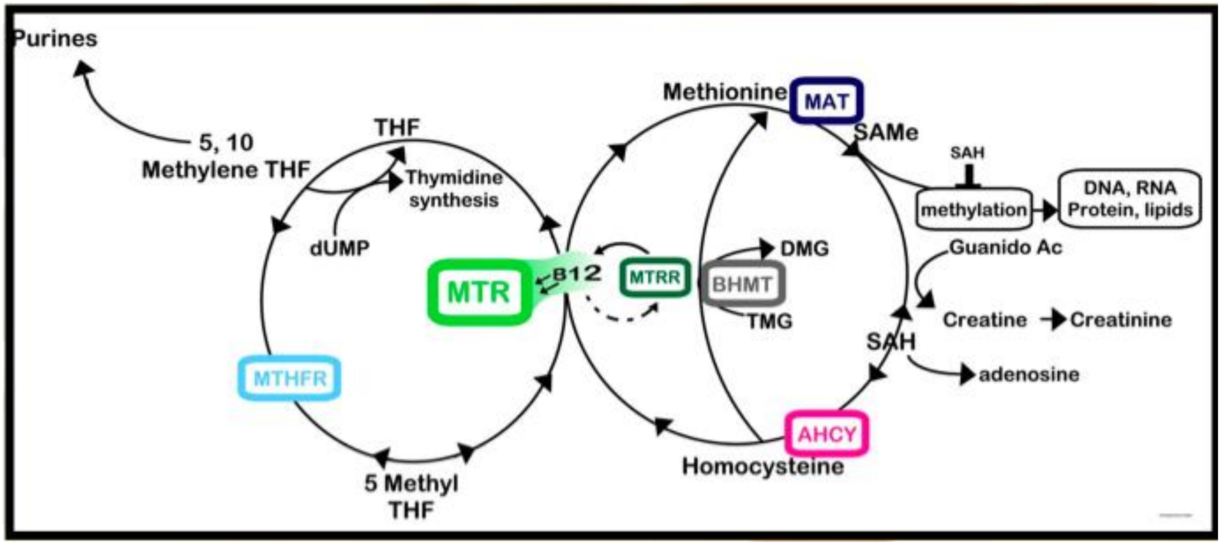
Function Dependent on Methyl-Folate and Methyl-B12

MTR sensitive to Mercury and Alcohol

Inhibited by SAMe and Oxidative Stress

METHIONINE SYNTHASE (MTR)

5-Methyl Folate + Homocysteine ——→ Methionine + THF
Methyl-B12 (from MTRR)



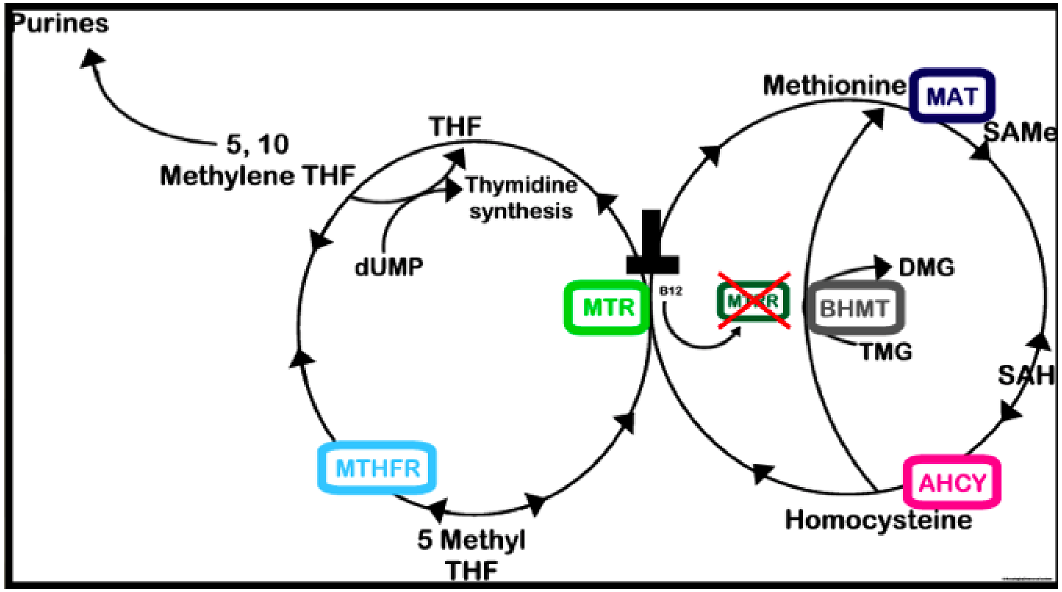
MTR A2756G is an Up Regulation

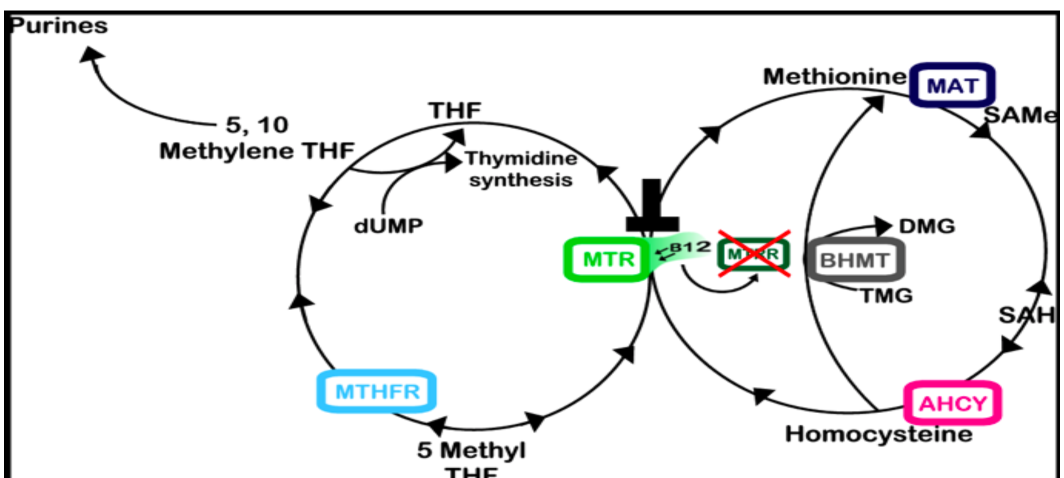
MTR is "Always On" → Methyl-Folate and Methyl-B12 Depletion

Treatment: Methyl-Folate and Methyl-B12 Supplementation

METHIONINE SYNTHASE REDUCTASE (MTRR)

B12 + CH3 (SAMe) ⟶ Methyl-B12





MTRR abnormalities
are all
Down Regulations

Treatment:

- High Dose B12
- Methyl-B12

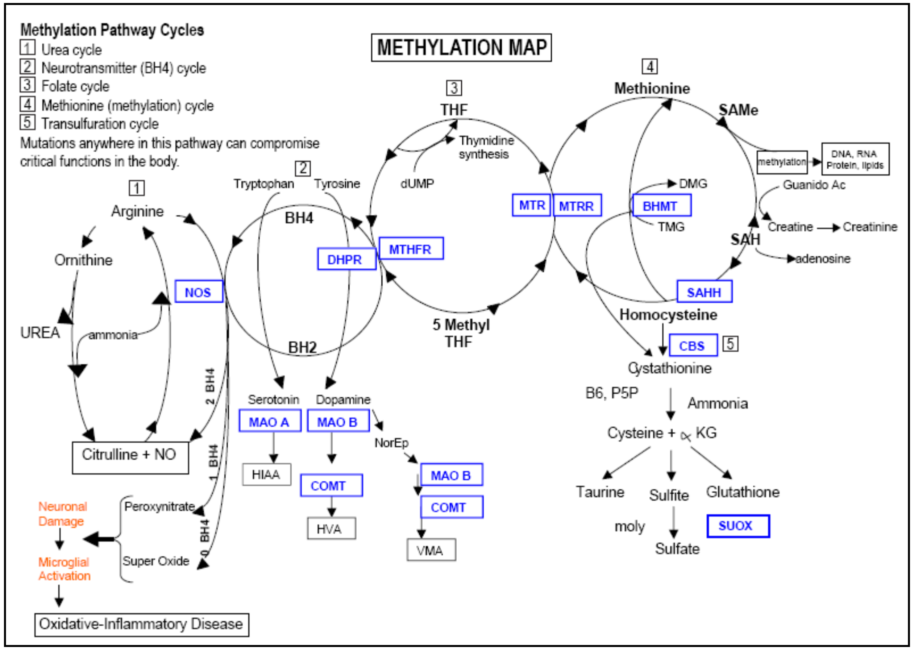
Caveats:

- CBS/BHMT
- COMT

MTR Up Regulation
MTRR Down Regulation

→ Pronounced
Methyl-B12 Deficiency

FUNCTIONS of the METHYL CYCLE

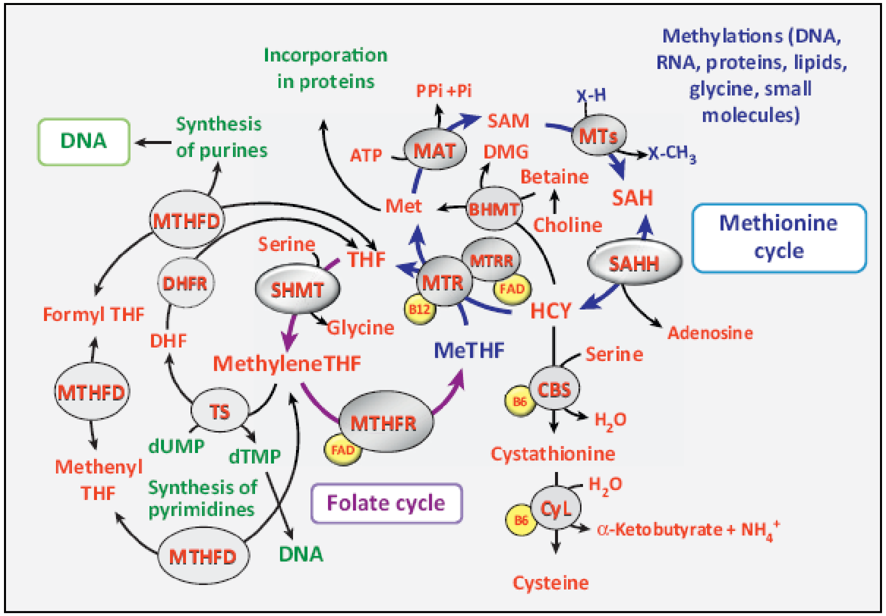


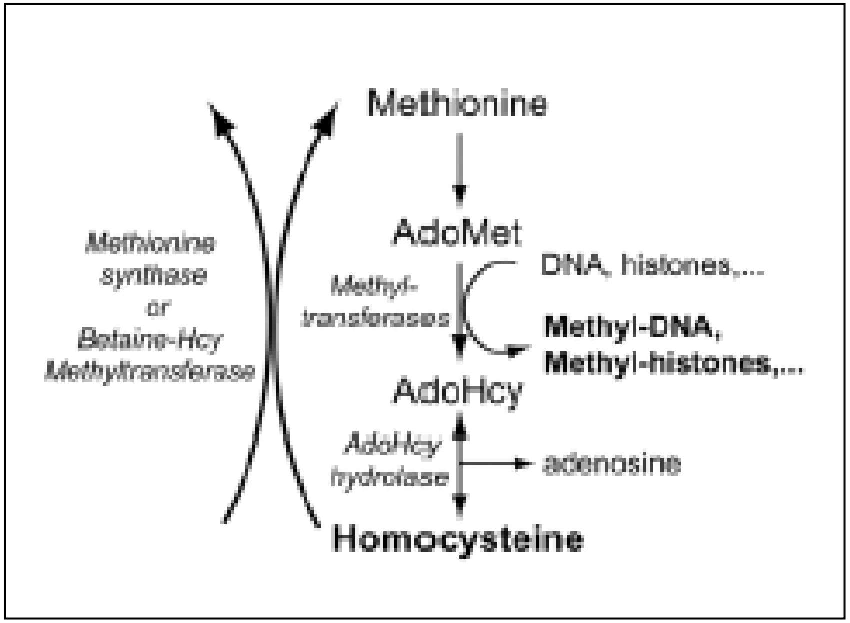
Maintain (current health status) appropriate levels of:

- Pyrimidine and purine bases for DNA and RNA synthesis
- Antioxidant and detox molecules glutathione, cysteine, and taurine
- BH4 (tetrahydrobiopterin) ⇒
- Transferable methyl groups ≈ High SAMe:SAH

METHIONINE ADENOSYL TRANSFERASE (MAT)

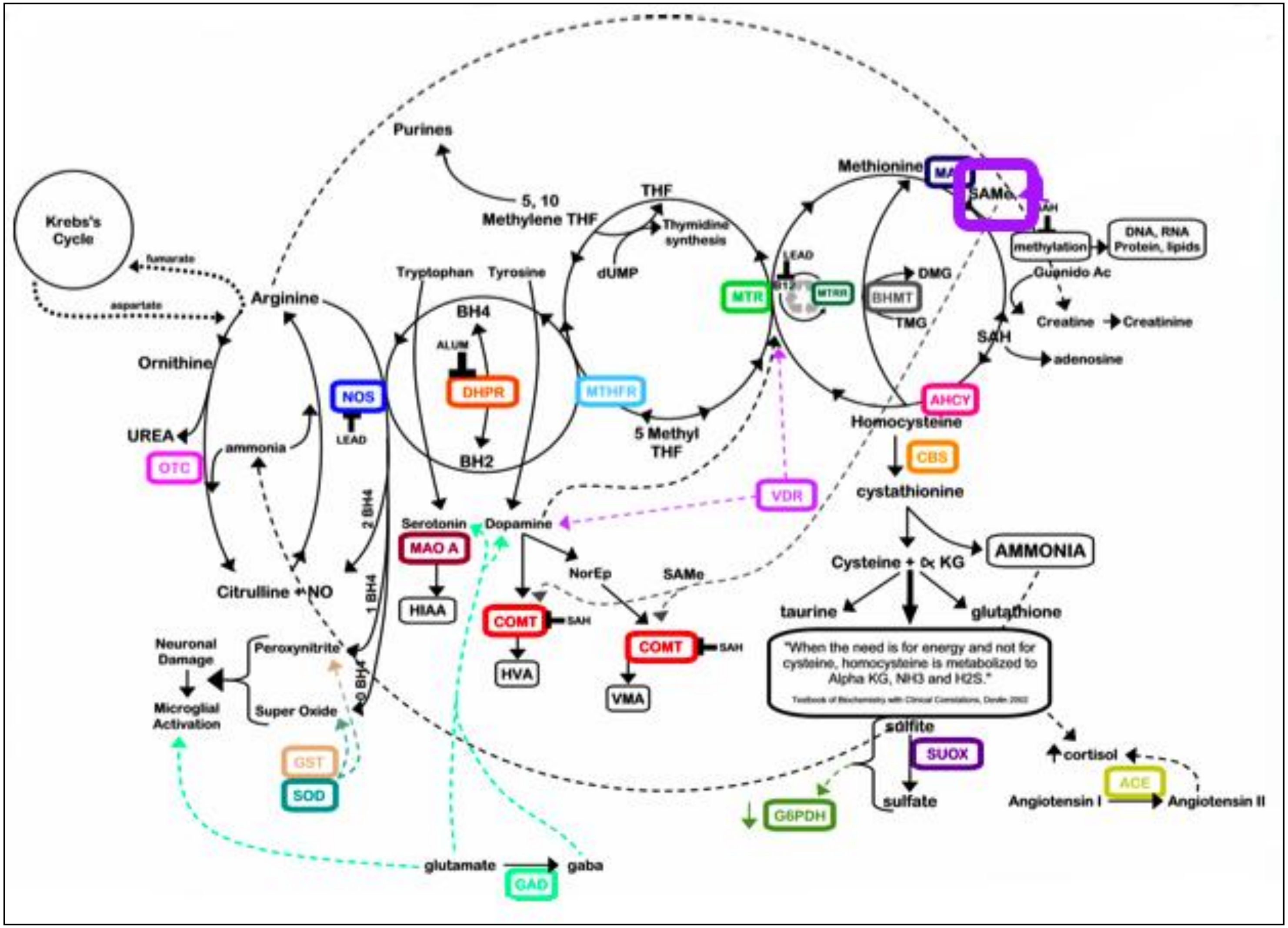
Methionine + ATP → SAMe





Inhibited by SAMe

S-ADENOSYL METHIONINE (SAMe)



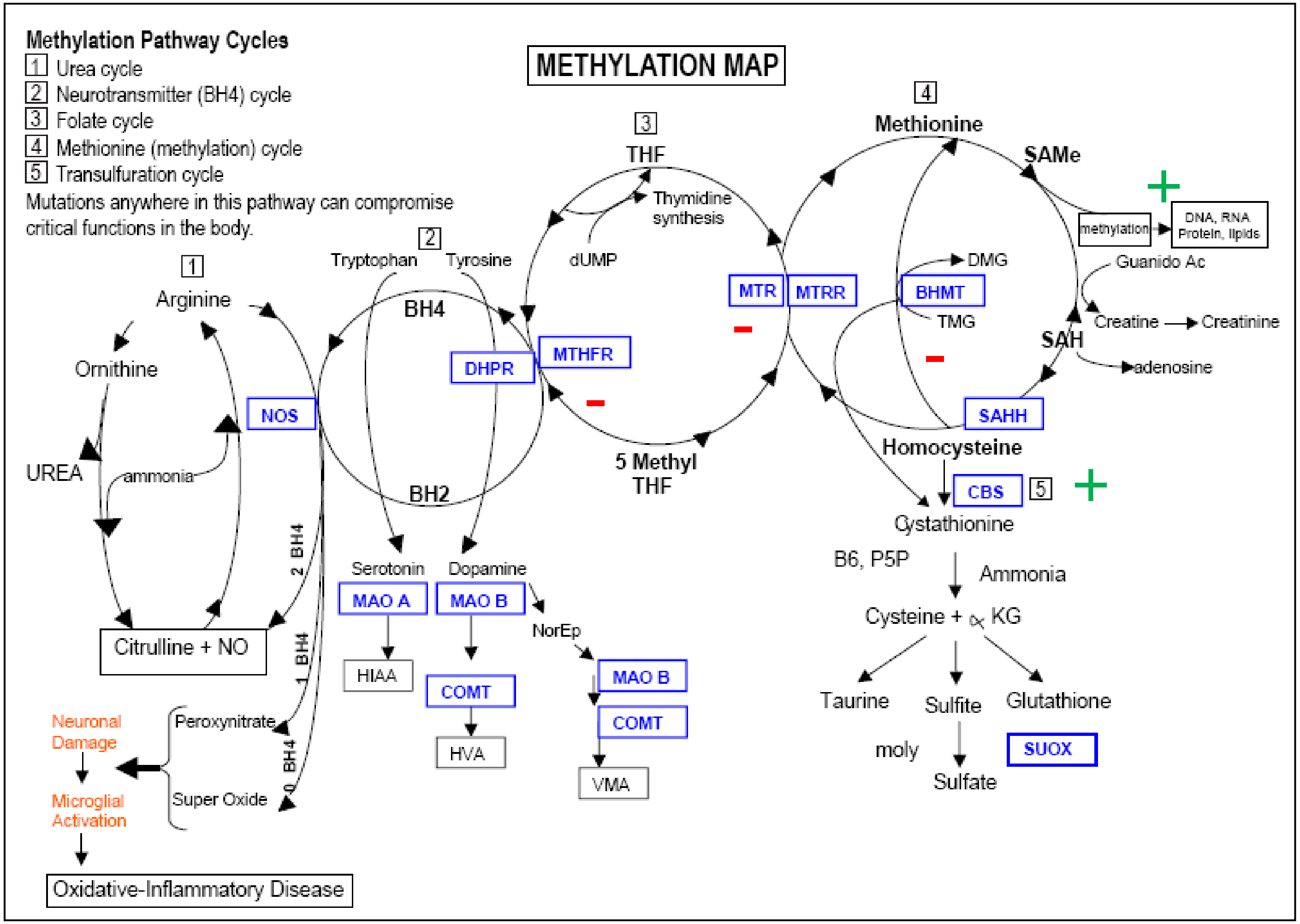
SAMe METHYL TRANSFER REACTIONS

Enzyme	Substrate and Effect
DNA Methyl Transferases	Alters DNA Transcription (Bookmarking)
Synthetic Reactions	Generation of Carnitine
Protein Methyl Transferases (PRMT)	Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)
Catechol-*O*-Methyl Transferase	Inactivates Catecholamines
	Methylates 2-OH and 4-OH Estrogens
COMT	Metabolizes Bioflavonoids
PEMT Phosphatidylethanolamine N-Methyl Transferase	Generation of Phosphatidylcholine
GAMT Guanidinacetate N-Methyl Transferase	Generation of Creatine
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF

S-ADENOSYL METHIONINE (SAMe)

Enzyme	Substrate and Effect
DNA Methyl Transferases	Alters DNA Transcription (Bookmarking)
Synthetic Reactions	Generation of Carnitine
Protein Methyl Transferases (PRMT)	Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)
Catechol-*O*-Methyl Transferase COMT	Inactivates Catecholamines
	Methylates 2-OH and 4-OH Estrogens
	Metabolizes Bioflavonoids
↑ Cystathione β Synthase (CBS)	Hcy → Glutathione and ∉ SAMe
↓ BetaineHcy MethylTransferase	Hcy → Glutathione ∉ SAMe
↓ Methionine Synthase (MTR)	Hcy → Glutathione and ∉ SAMe
↓ MTHFR	THF → DNA and ∉Methyl-Folate

SAMe and METHYL CYCLE ENZYME KINETICS



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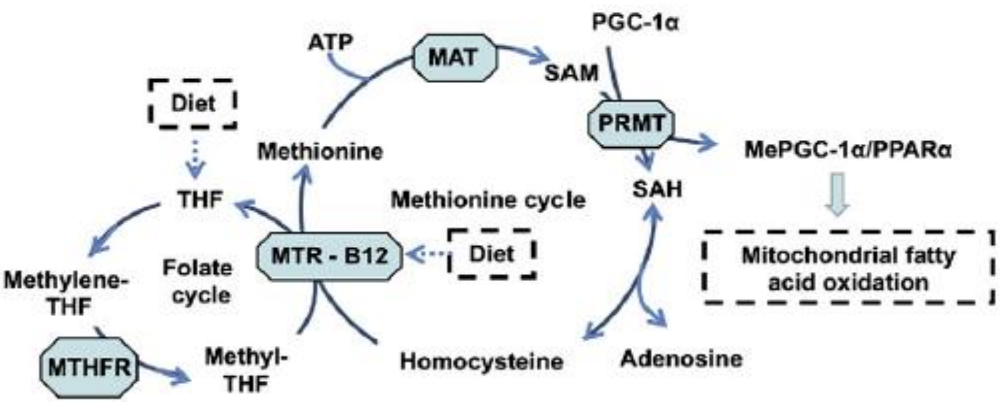
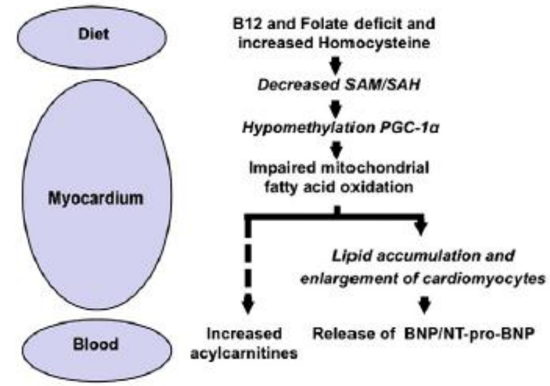
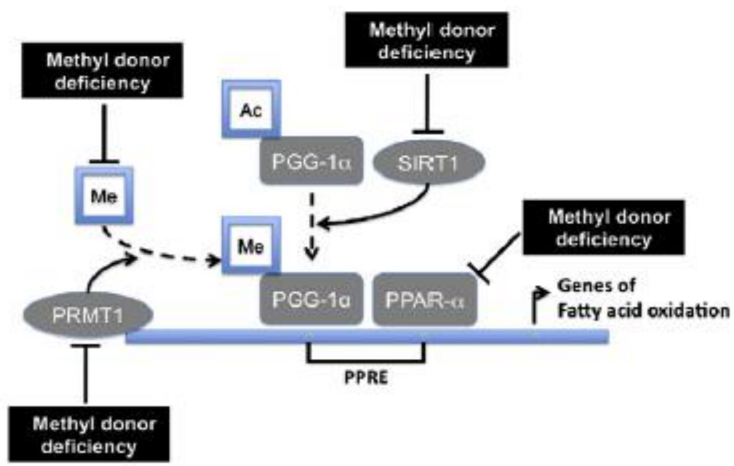
PEMT Phosphatidylethanolamine N-Methyl Transferase	Generation of Phosphatidylcholine
GAMT Guanidinacetate N-Methyl Transferase	Generation of Creatine
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF

SAMe and POST-TRANSLATIONAL ENZYME MODIFICATION







SAMe + PGC-1α $\rightarrow$ MePGC-1α
PRMT

SAMe $\rightarrow$ HDAC SIRT

AcPGC-1α $\rightarrow$ PGC-1α
SIRT1

MePGC-1$\rightarrow$ PPARα, ERα, ERRα & HNF-4α

Protein Arginine Methyl Transferase
Histone Deacetylase
Peroxisome Proliferator-Activated Receptor-Gamma Co-Activator-1

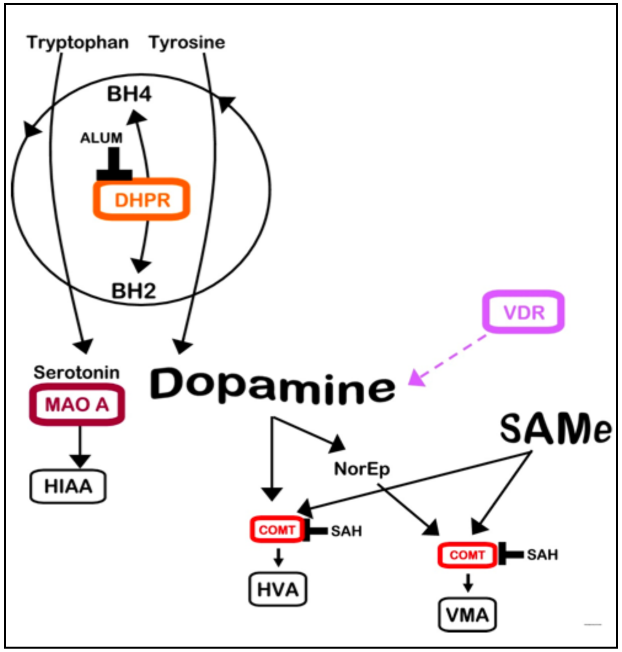
$\rightarrow$ Fatty Acid Oxidation $\Rightarrow$ ATP

S-ADENOSYL METHIONINE (SAMe)

Enzyme	Substrate and Effect
DNA Methyl Transferases	Alters DNA Transcription (Bookmarking)
Synthetic Reactions	Generation of Carnitine
Protein Methyl Transferases (PRMT)	Alters Enzyme Activity (PGC-1α $\rightarrow$ PPARα $\rightarrow$ FA Oxidation)
Catechol-*O*-Methyl Transferase COMT	Inactivates Catecholamines
	Methylates 2-OH and 4-OH Estrogens
	Metabolizes Bioflavonoids
$\uparrow$ Cystathione β Synthase (CBS)	Hcy $\rightarrow$ Glutathione and $\notin$ SAMe
$\downarrow$ BetaineHcy MethylTransferase	Hcy $\rightarrow$ Glutathione $\notin$ SAMe
$\downarrow$ Methionine Synthase (MTR)	Hcy $\rightarrow$ Glutathione and $\notin$ SAMe
$\downarrow$ MTHFR	THF $\rightarrow$ DNA and $\notin$Methyl-Folate

CATECHOL-O-METHYL TRANSFERASE (COMT)

Substrate + CH3 ⟶ Methylated Substrate



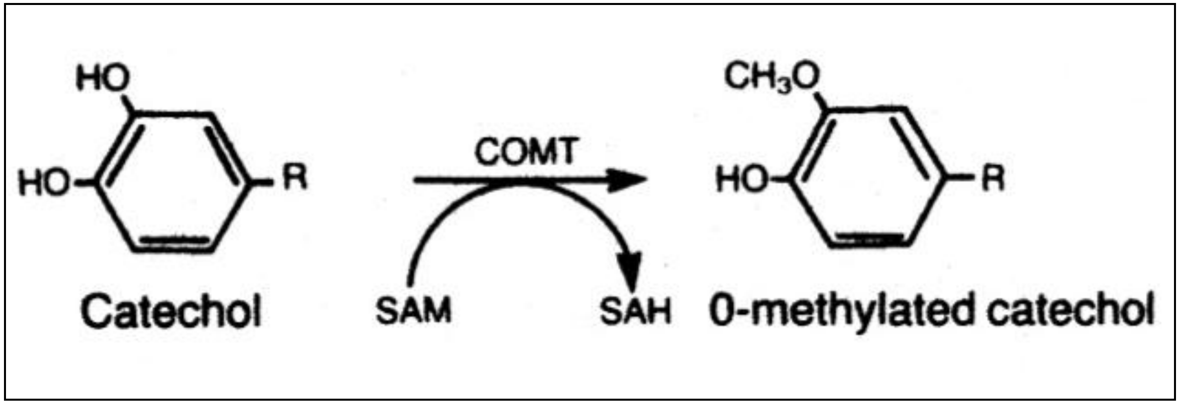
COMT V158M and COMT H62H
are
Down Regulations

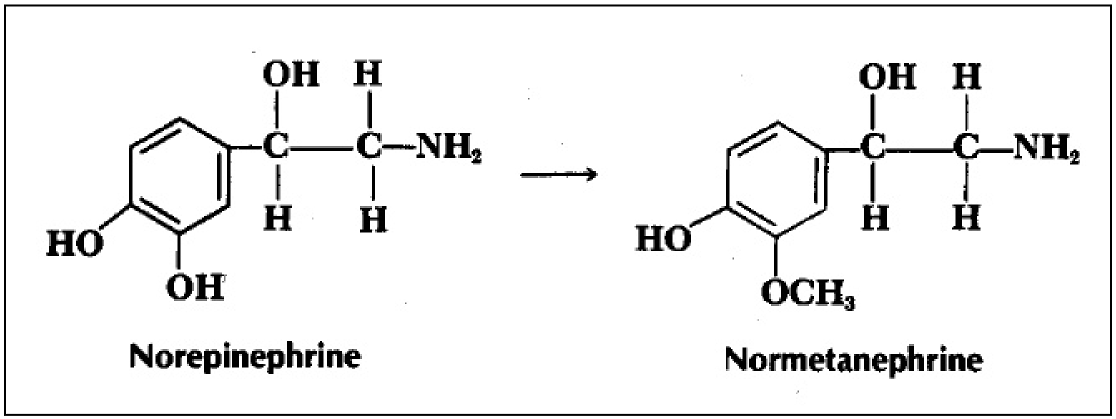
Catecholamine and Catechol Estrogen Metabolism
Compromised vs. Wild Type

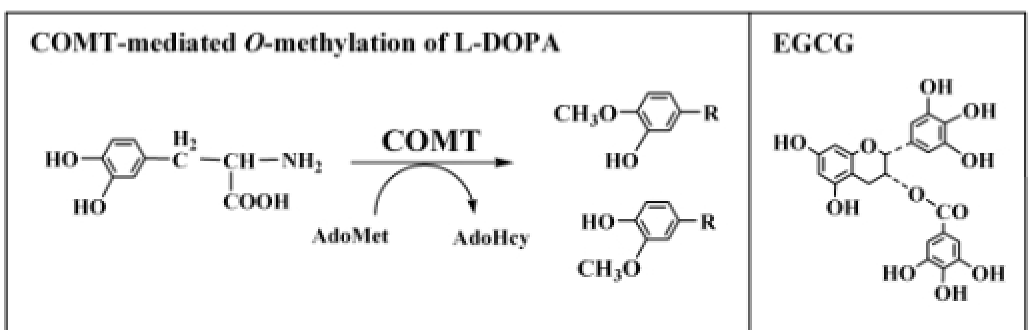
COMT 61 is an Up Regulation
Catechol Metabolism is Increased vs. Wild Type

COMT V158M (+/+) COMT H62H (+/+) COMT 61 (-/-)	Highest catecholamine levels Lower tolerance to methyl group donors Greatest susceptibility to mood swings
COMT V158M (-/-) COMT H62H (-/-) COMT 61 (+/+)	Lowest catecholamine levels Greater tolerance to methyl group donors Lower susceptibility to mood swings

CATECHOL-O-METHYL TRANSFERASE (COMT)







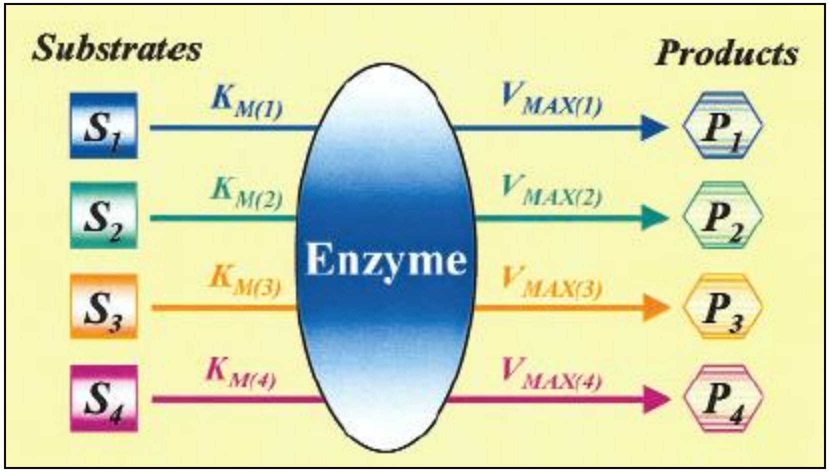
SAH NON-COMPETITIVELY INHIBITS COMT

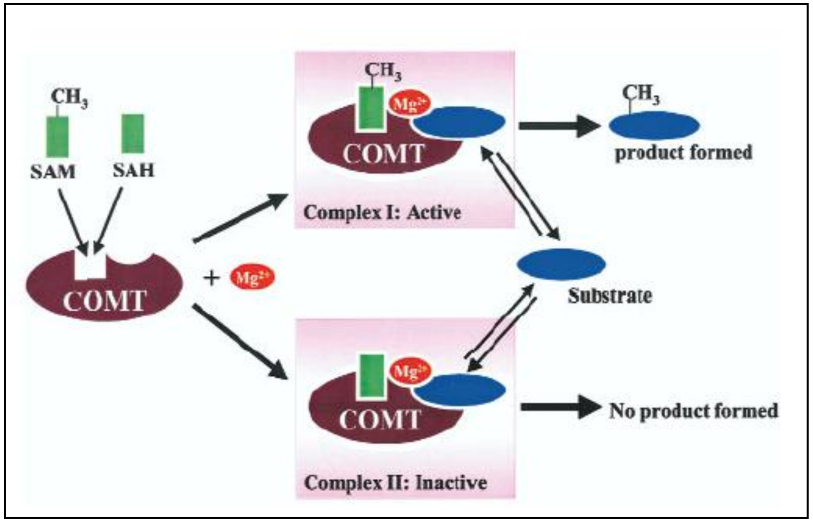
COMT (Catechol-*O*-Methyl Transferase):

- High capacity
- Low specificity

COMT *O* –Methylates Catechols:

- Catecholamines
- Catechol (OH)-Estradiol and Estrone
- Catechol bearing Bioflavonoids (ECGC and Quercetin)

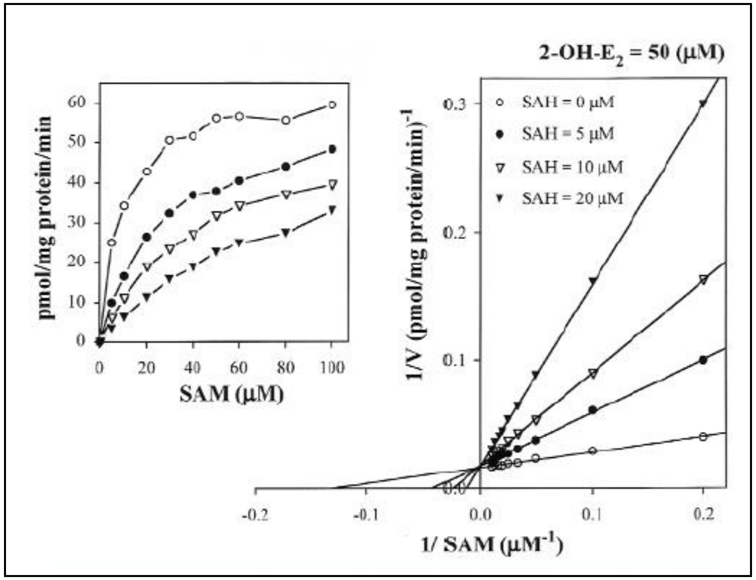


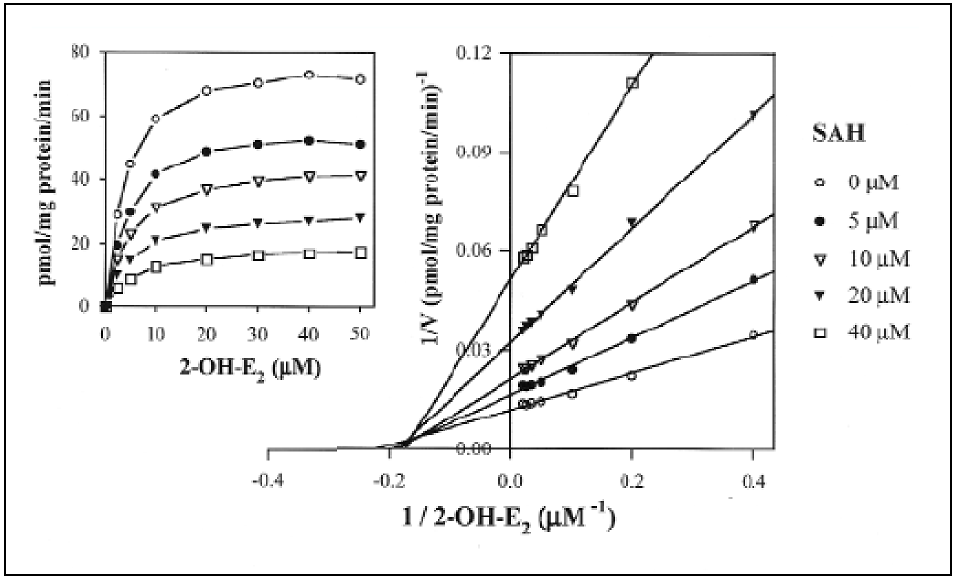


K_M for SAM for COMT (24 uM)
K_I for SAH (4 uM)

IMPAIRED ESTROGEN METABOLISM

Measure 2-OH → 2-Methyoxy Estradiol conversion by COMT

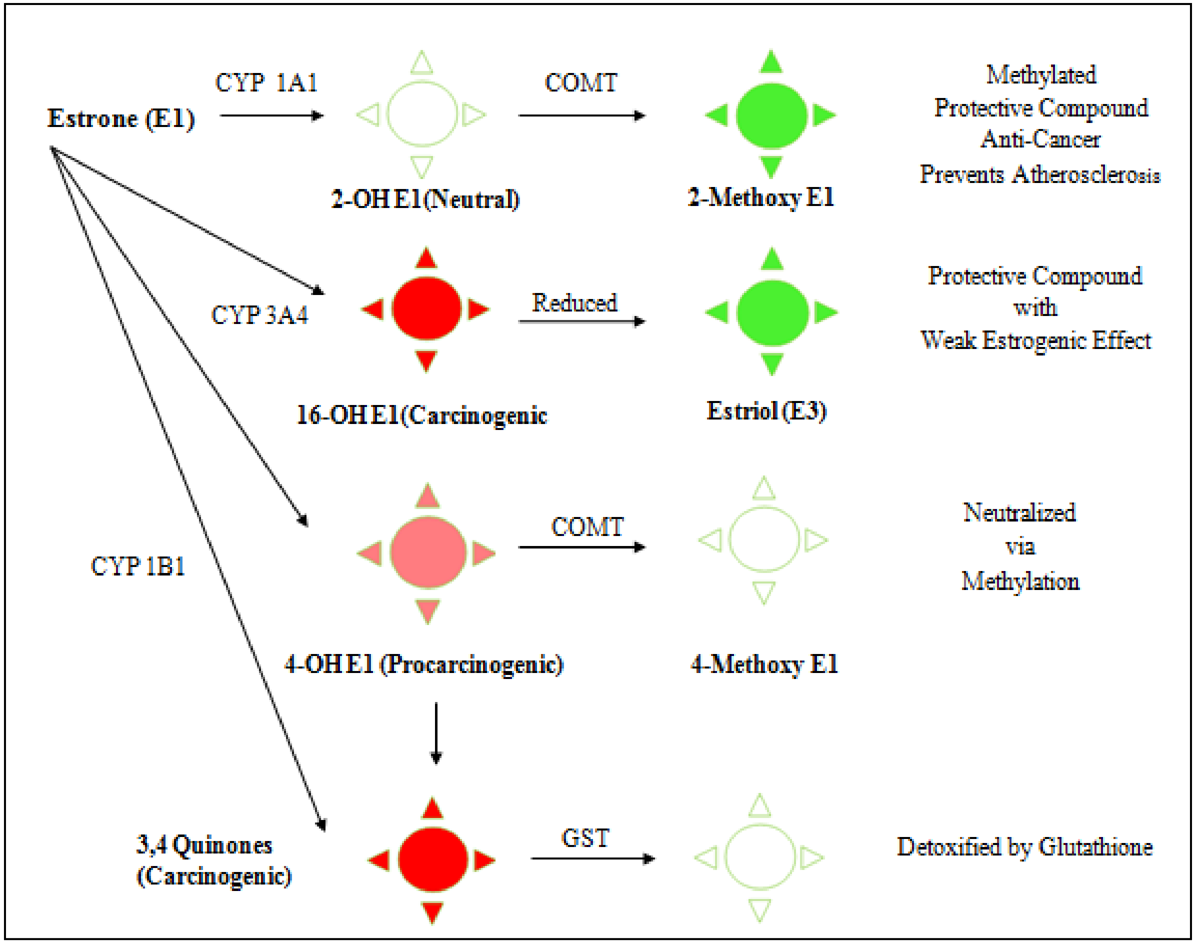




Fixed [Estradiol]
Increasing [SAMe]
Increasing [SAH]

Fixed [SAMe]
Increasing [Estradiol]
Increasing [SAH]

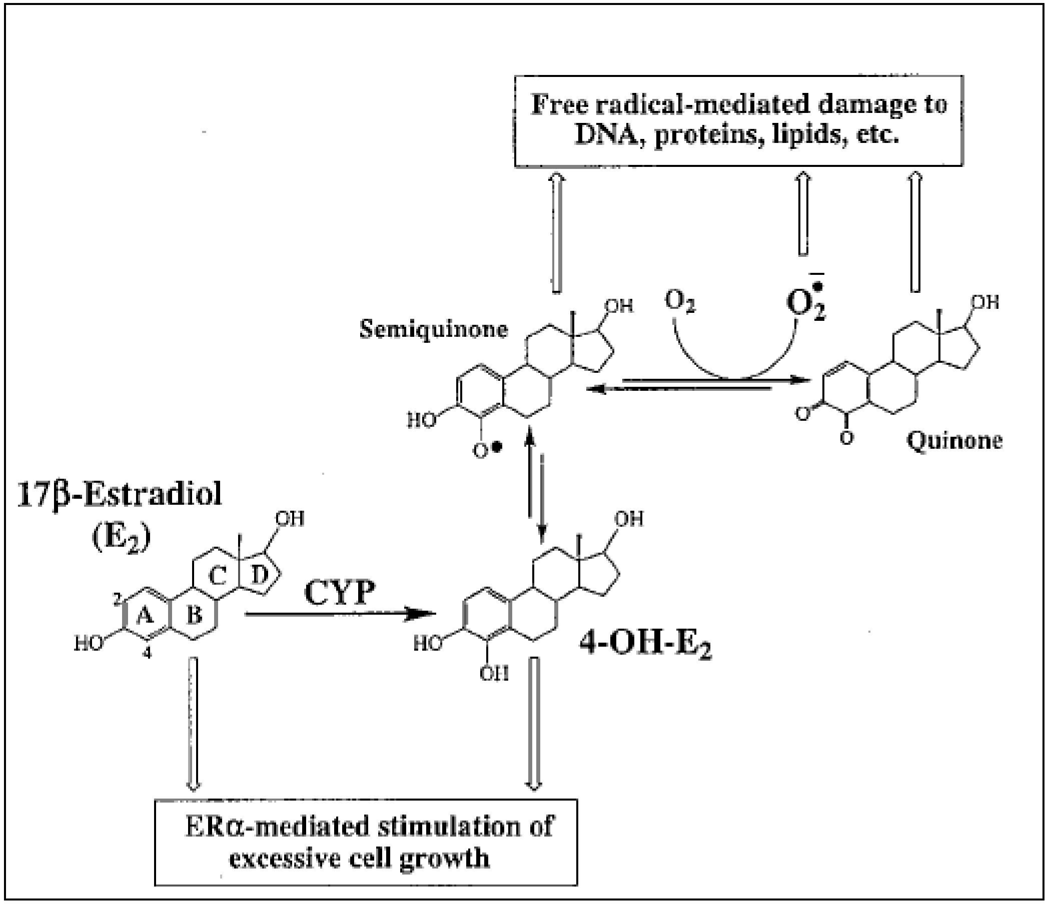
IMPAIRED ESTROGEN METABOLISM



Impaired conversion of 2 and 4-OH into 2 and 4-Methoxy Estrogens

Impaired neutralization of 16-OH and 3,4 Quinone Estrogens

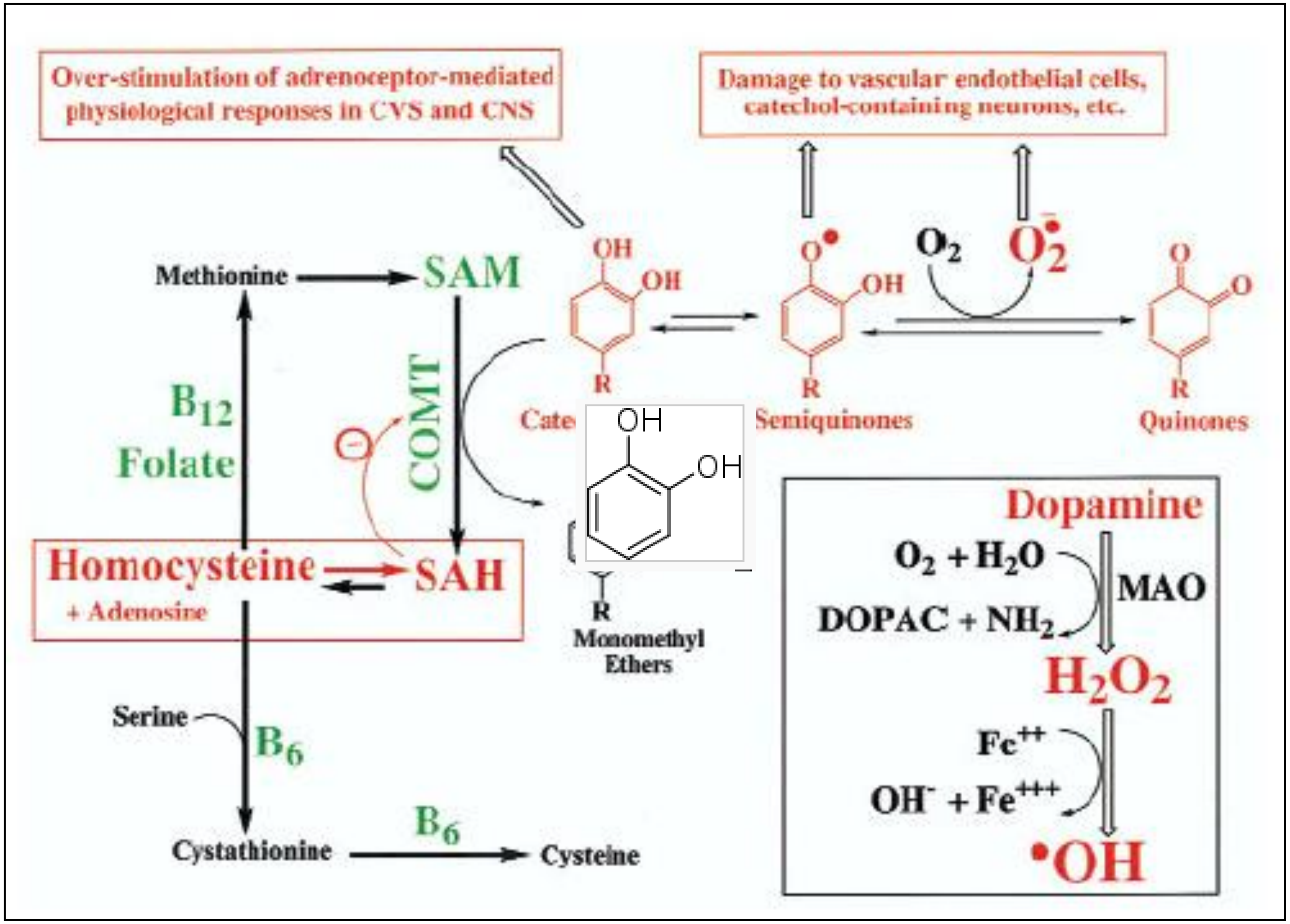
IMPAIRED ESTROGEN METABOLISM



4-OH Estrogen Redox Cycling

Oxidized 4-OH species form adducts with DNA

IMPAIRED CATECHOLAMINE BREAKDOWN



COMT and CV SURGERY RISK

♥ 260 subjects undergoing CABG

Genotype for COMT:

- 28% wild type VV (HH)
- 47% VM (HL)
- 25% MM (LL)

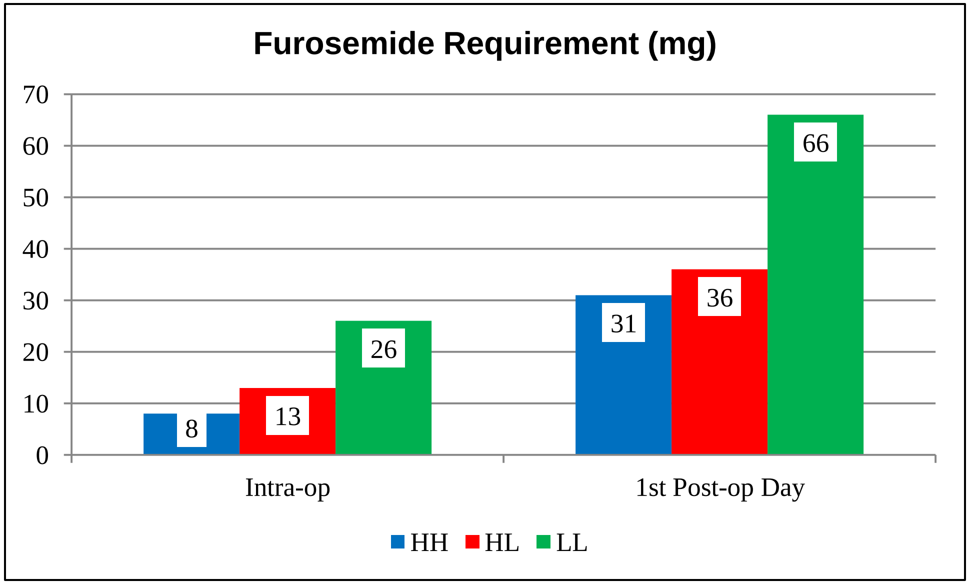
No significant differences in:

- Prior CV history
- Risk factors
- Pre-op meds

Carry out CABG; no difference in:

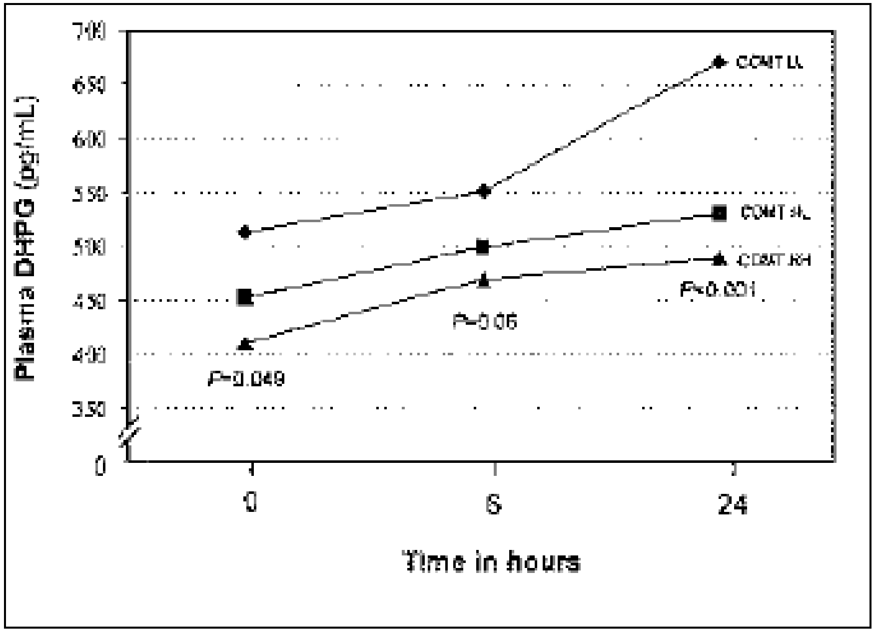
- Length of surgery
- Intra-op and peri-op treatments (except furosemide requirement)

COMT and CV SURGERY RISK



Increased diuretic need with COMT low function

COMT and CV SURGERY RISK



LL

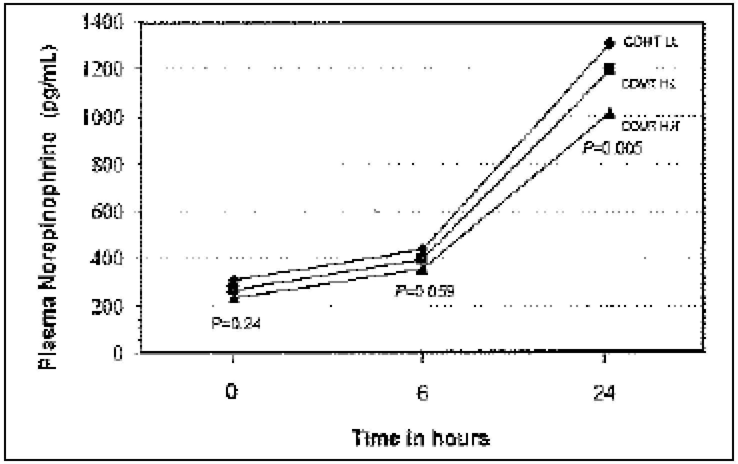
HL

HH

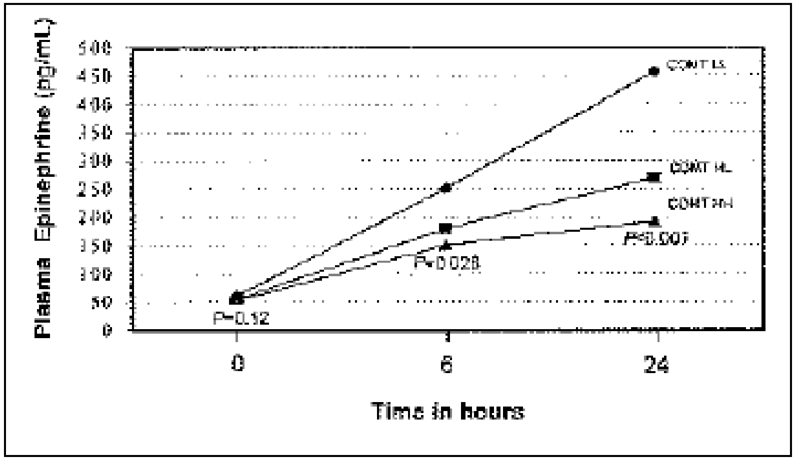
DHPG (3,4-dihydroxyphenylglycol)

Formed by deamination of epinephrine and norepinephrine by MAO

COMT and CV SURGERY RISK



LL
HL
HH



Norepinephrine (endogenous)

Epinephrine (endogenous)

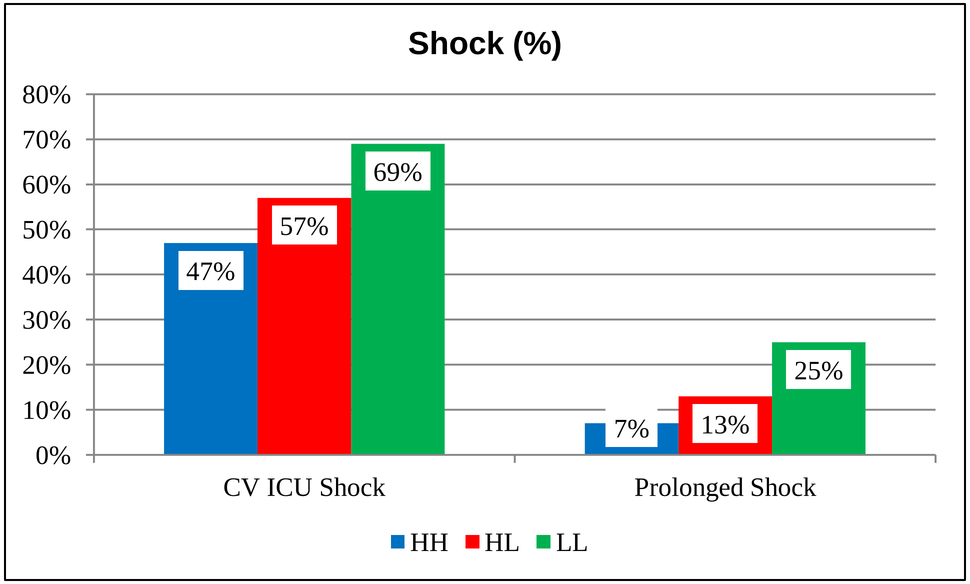
CABG → Sympathetic outpouring

Dysfunctional COMT → Persistent high catecholamine levels →

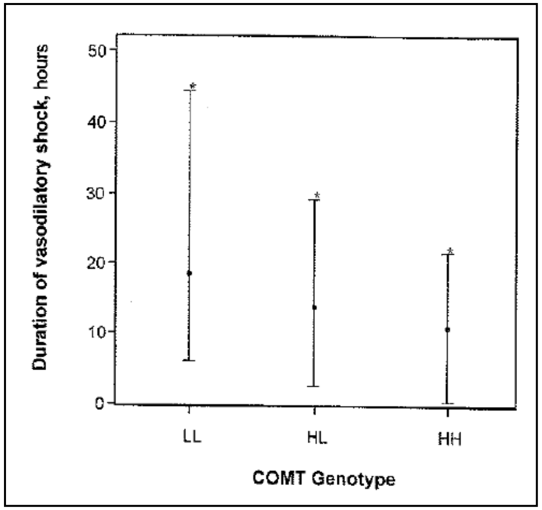
Desensitization of alpha-adrenergic receptors:

- Vascular wall
- Kidney

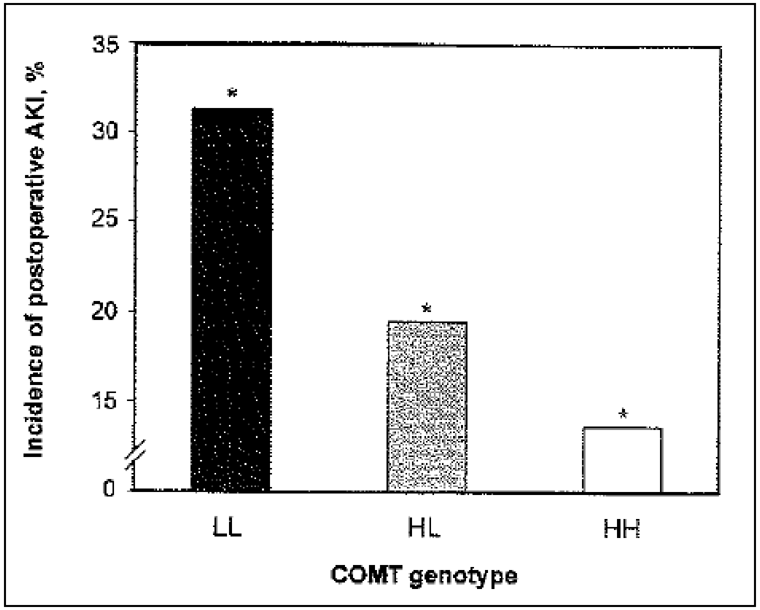
COMT and CV SURGERY RISK



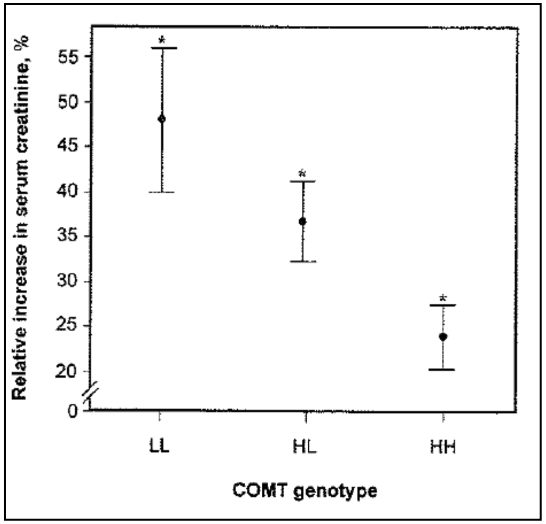
Duration of Shock



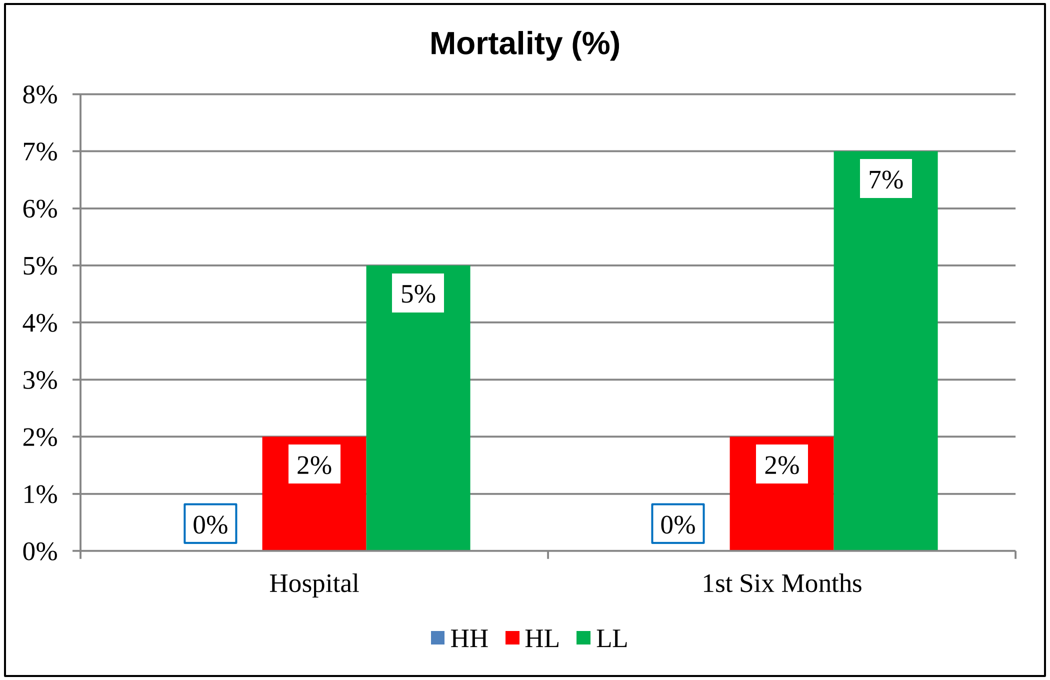
Post-op AKI



Relative Increase in Creatinine



COMT and CV SURGERY RISK



COMT and FUTURE CORONARY RISK

♥ 792 Finnish men (45-64 year-old) in overall good health

Genotype for COMT:

- 28% wild type VV (HH)
- 47% VM (HL)
- 25% MM (LL)

Measure Homocysteine

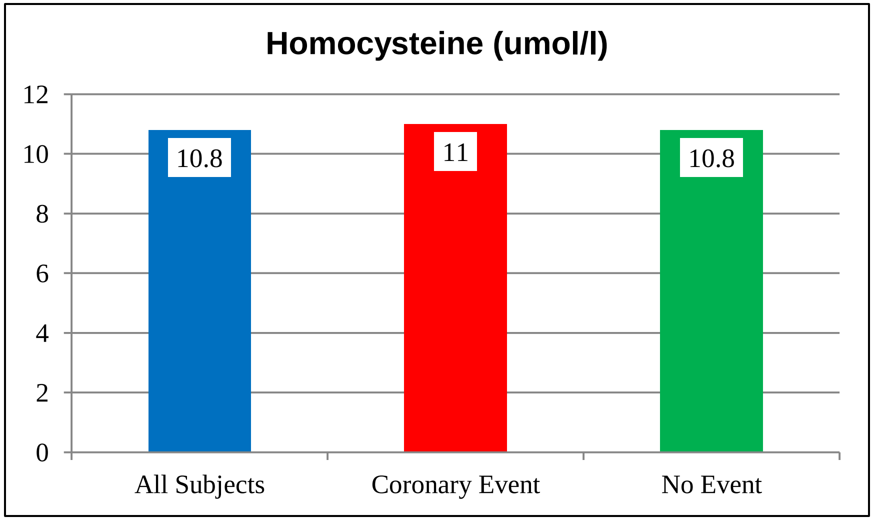
Record coronary events over 9.3 year follow-up period

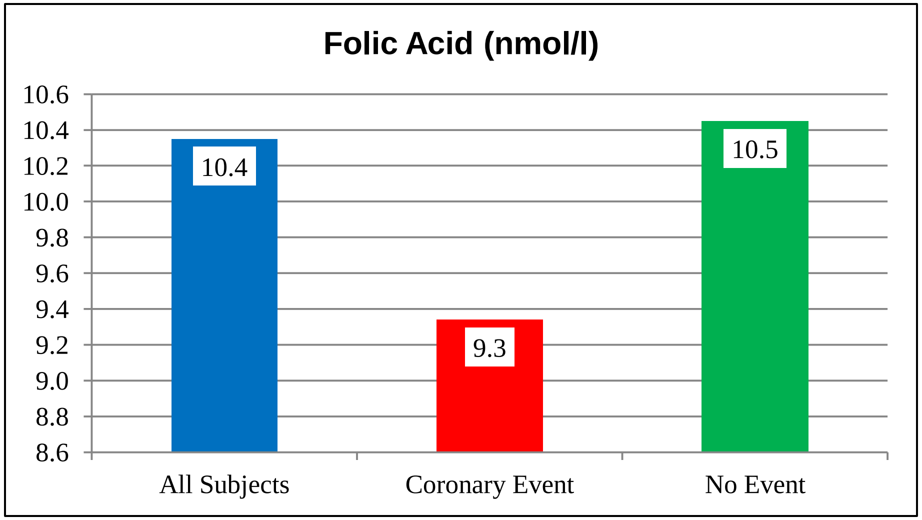
- 69 event
- 43 infarctions
- 17 possible infarctions
- 9 unstable angina

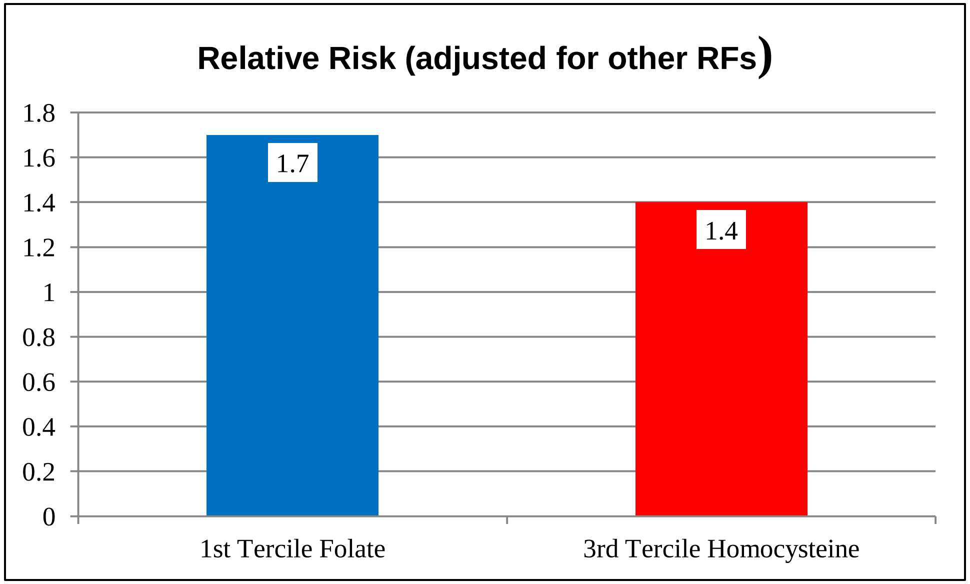
CV risk increased in relation to SBP, lipids, and FMH CADz

Look at CV risk in relation to Homocystine, Folate, and COMT Genotype

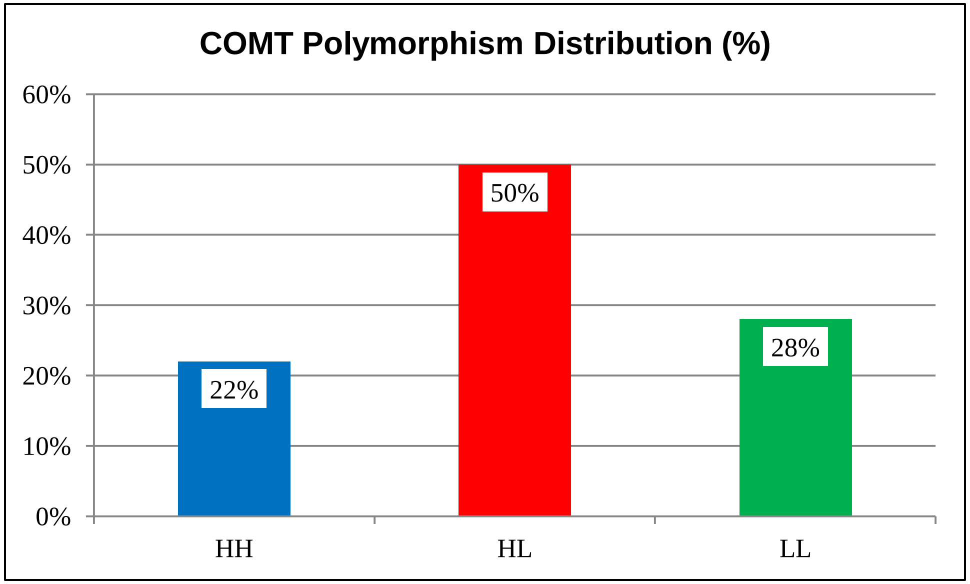
COMT and FUTURE CORONARY RISK

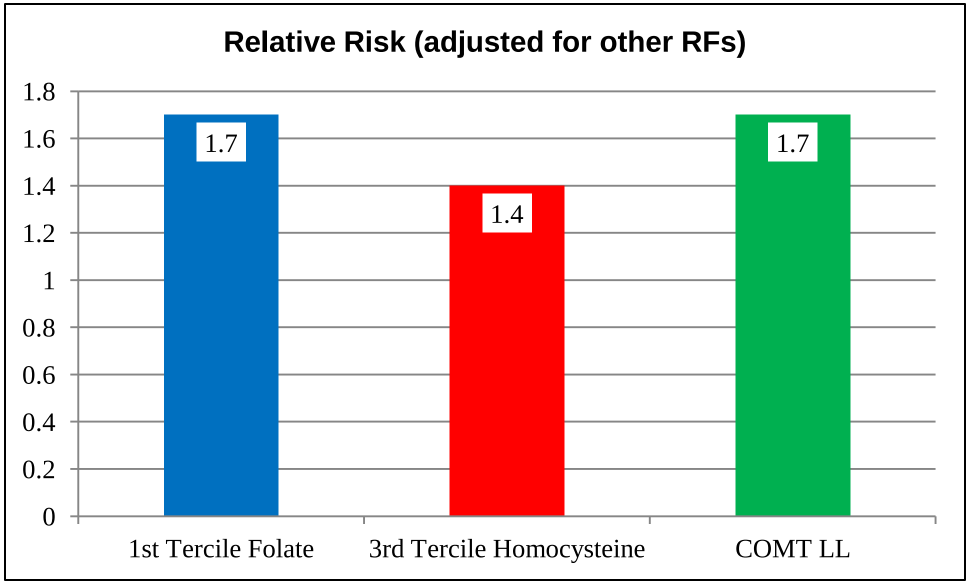




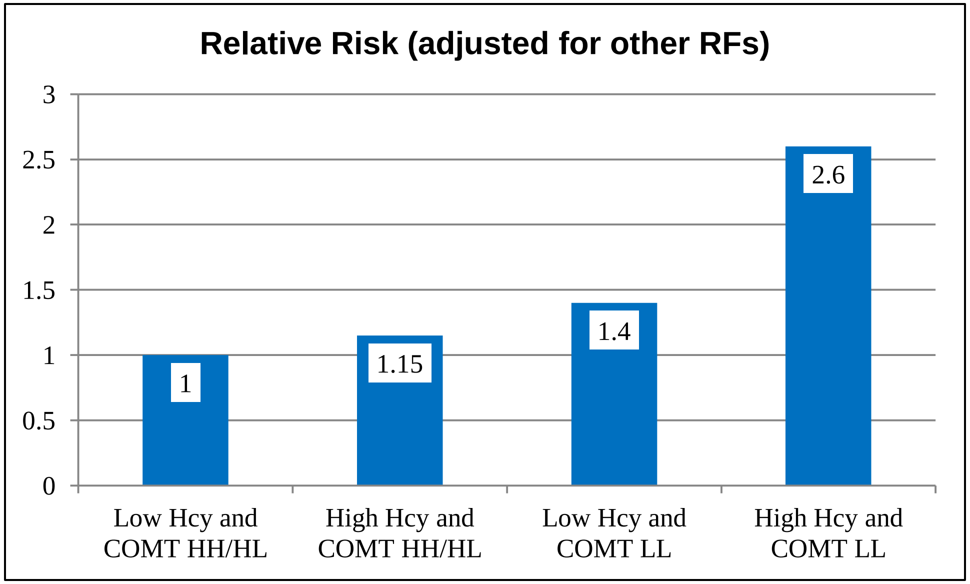


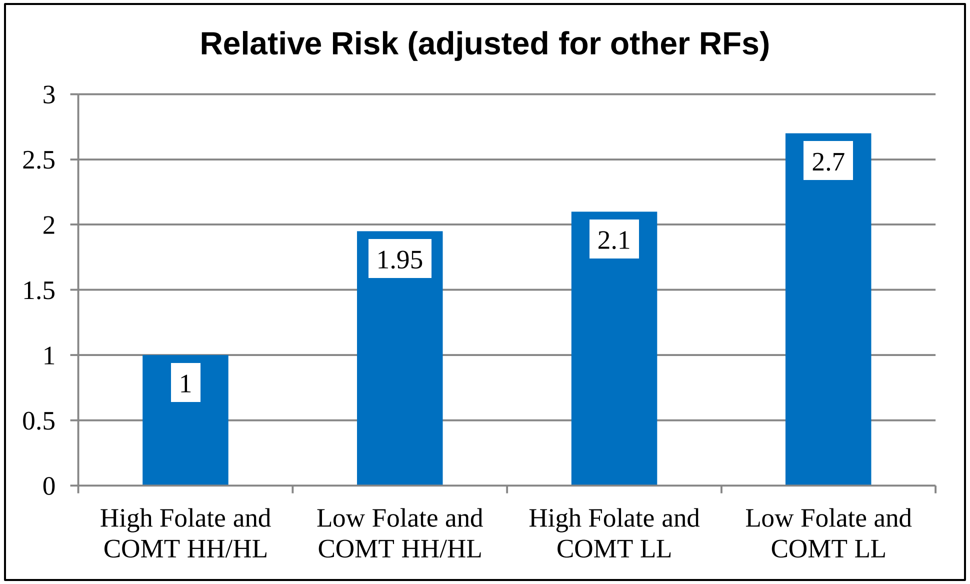
COMT and FUTURE CORONARY RISK



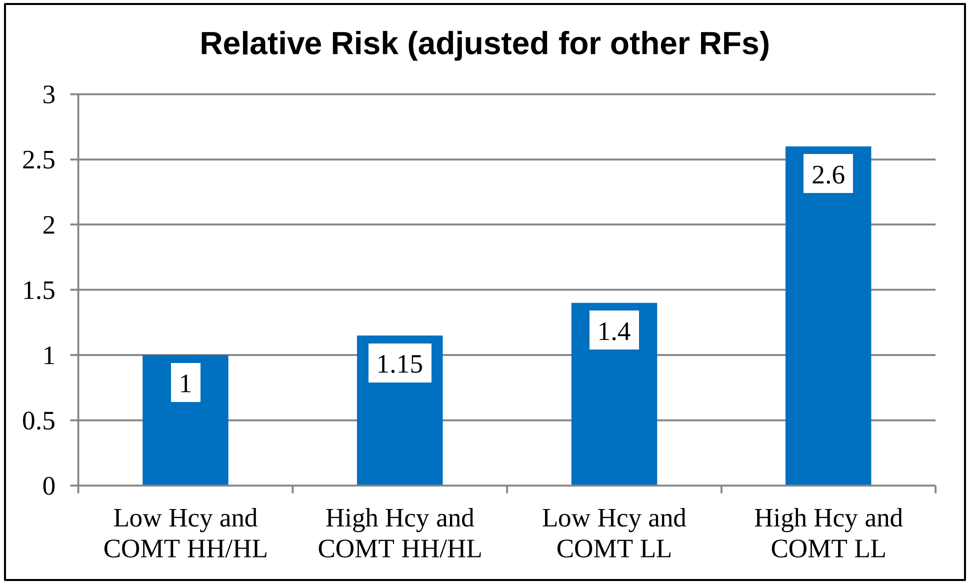


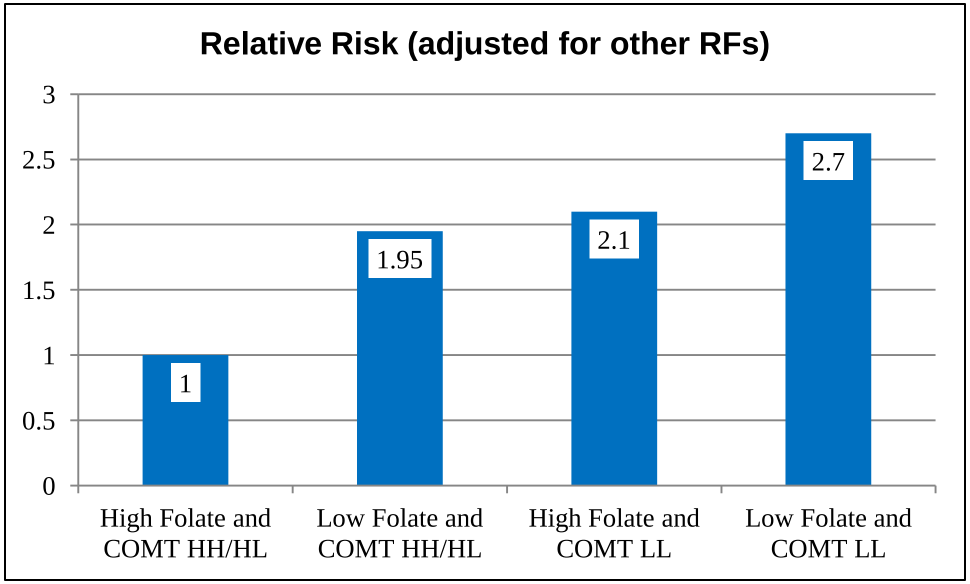
COMT and FUTURE CORONARY RISK

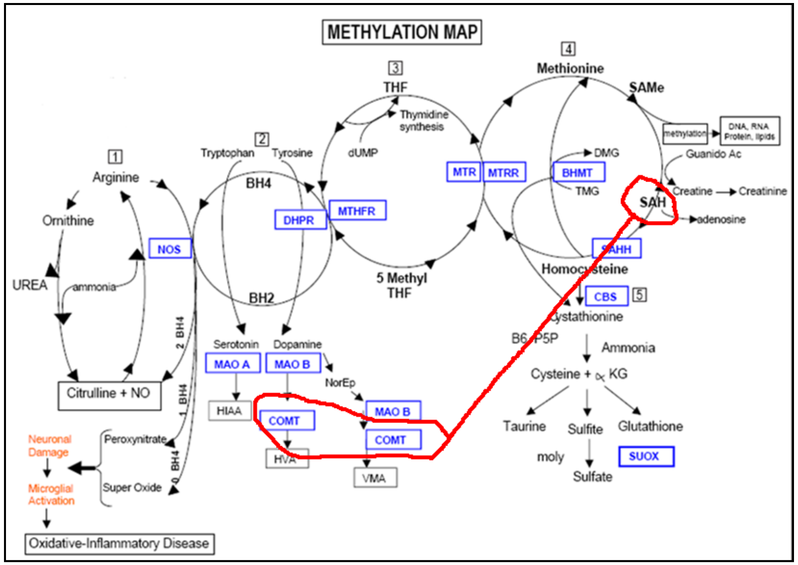




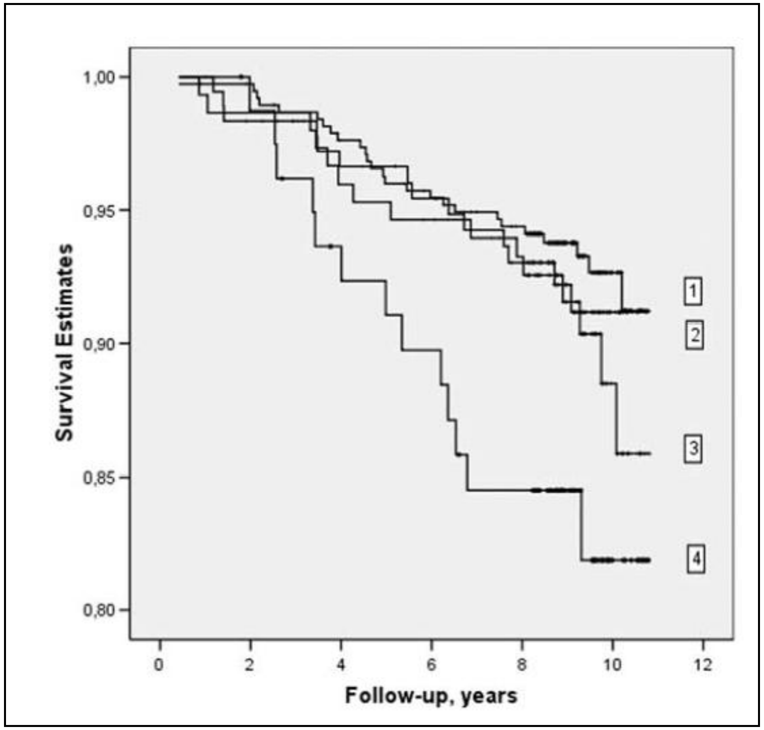
COMT and FUTURE CORONARY RISK







COMT and FUTURE CORONARY RISK



Five Year Survival

Low Hcy (<11.3) and COMT HH/HL
High Hcy (≥11.3) and COMT HH/HL

Low Hcy and COMT LL

High Hcy and COMT LL

Low folate → High homocysteine → High SAH → COMT Inhibition

Low folate → Lower risk if COMT functional

Low folate → Major risk if COMT dysfunctional

COMT and COFFEE CV RISK

♥ 773 (42-60) year-old Finnish men in overall good health

Genotype for COMT:

- 22% wild type VV (HH)
- 49% VM (HL)
- 29% MM (LL)

Record standard risk factors

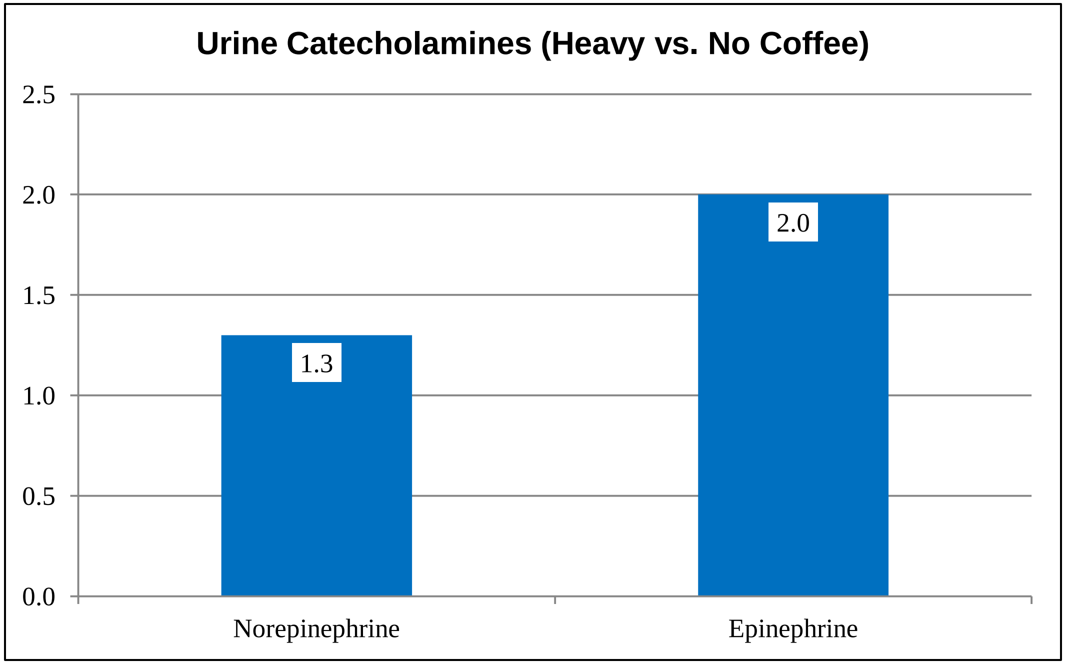
Assess coffee consumption

Record coronary events over 13 year follow-up period

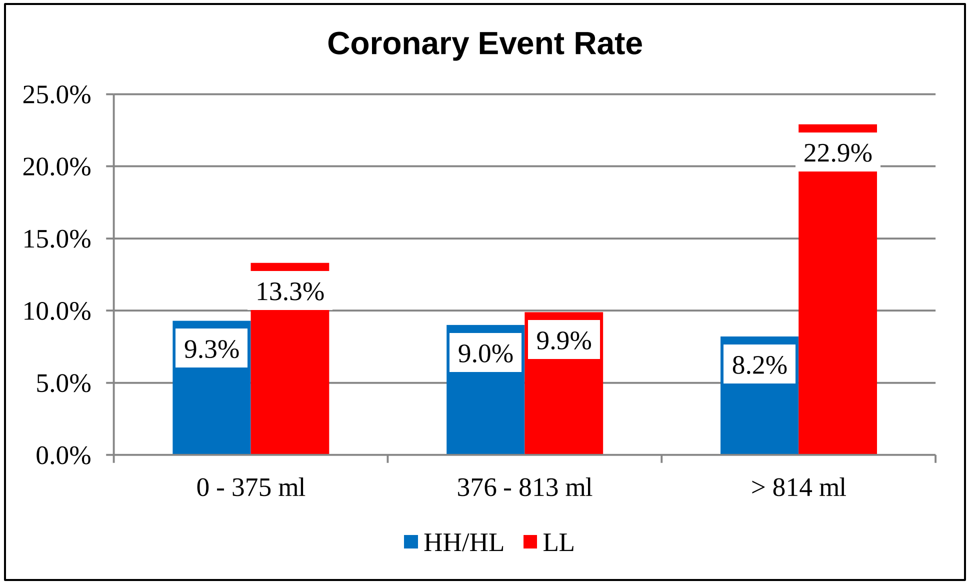
- 78 events

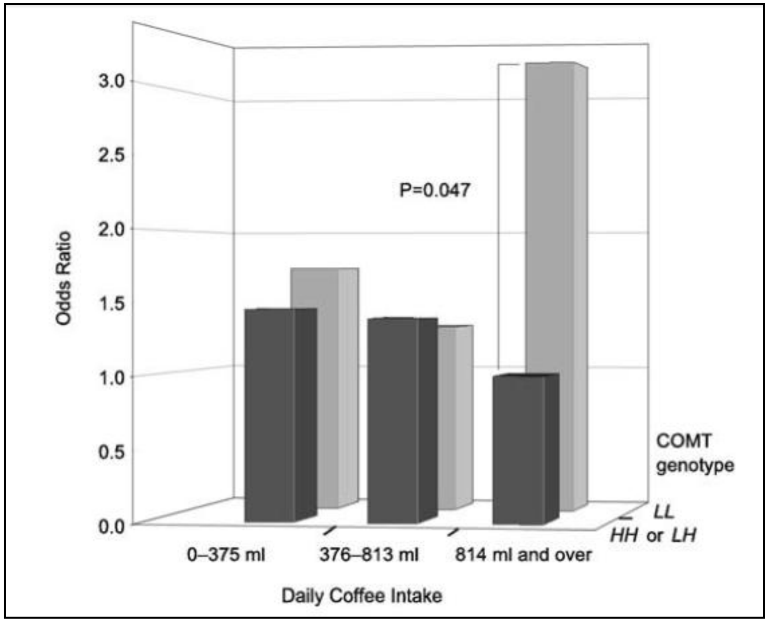
Relate coffee associated risk (RF adjusted) with COMT status

COMT and COFFEE CV RISK



COMT and COFFEE CV RISK

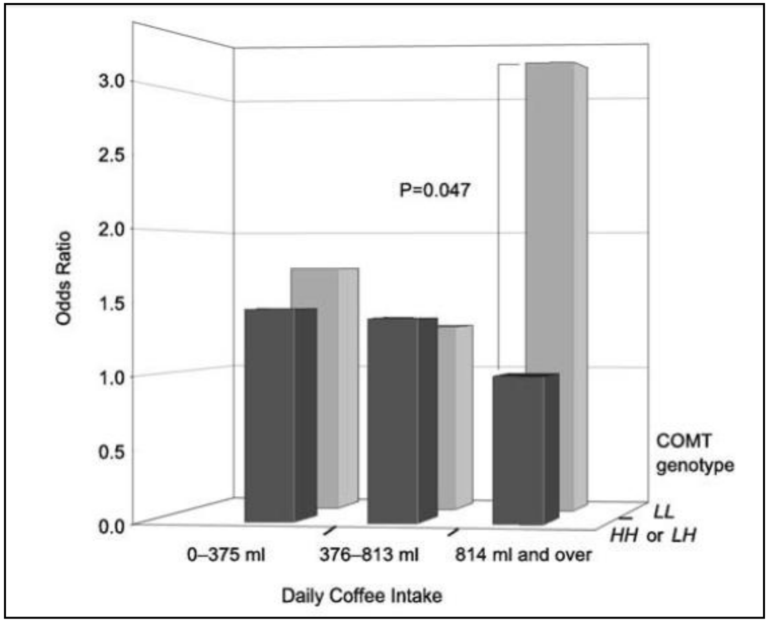




COFFEE and CV RISK

Coffee provides bioflavonoids and caffeine
Caffeine → Norepinephrine and epinephrine → Methylated by COMT → SAH
Caffeic acid → Methylated by COMT → SAH
Caffeine inhibits adenosine metabolism → SAH

Not a major issue if COMT functional (COMT HH/HL and/or SAH (Hcy) low
Major issue if COMT dysfunctional (COMT LL, high SAH, high substrate burden)



SAMe METHYL TRANSFER REACTIONS

Enzyme	Substrate and Effect
DNA Methyl Transferases	Alters DNA Transcription (Bookmarking)
Synthetic Reactions	Generation of Carnitine
Protein Methyl Transferases (PRMT)	Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)
Catechol-*O*-Methyl Transferase COMT	Inactivates Catecholamines
	Methylates 2-OH and 4-OH Estrogens
	Metabolizes Bioflavonoids
PEMT Phosphatidylethanolamine N-Methyl Transferase	Generation of Phosphatidylcholine
GAMT Guanidinoacetate N-Methyl Transferase	Generation of Creatine
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF