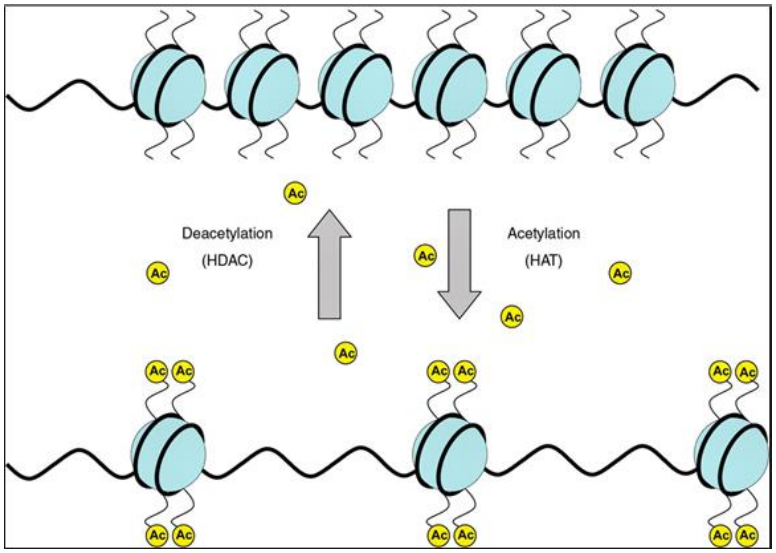
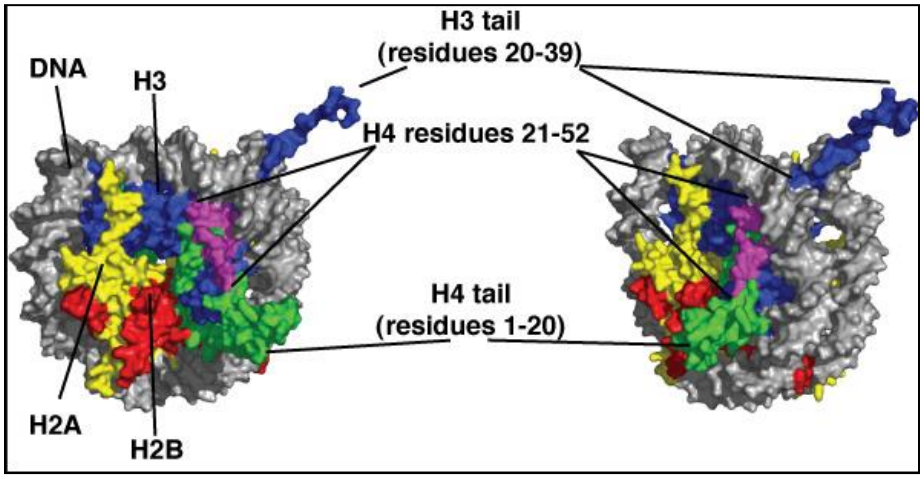
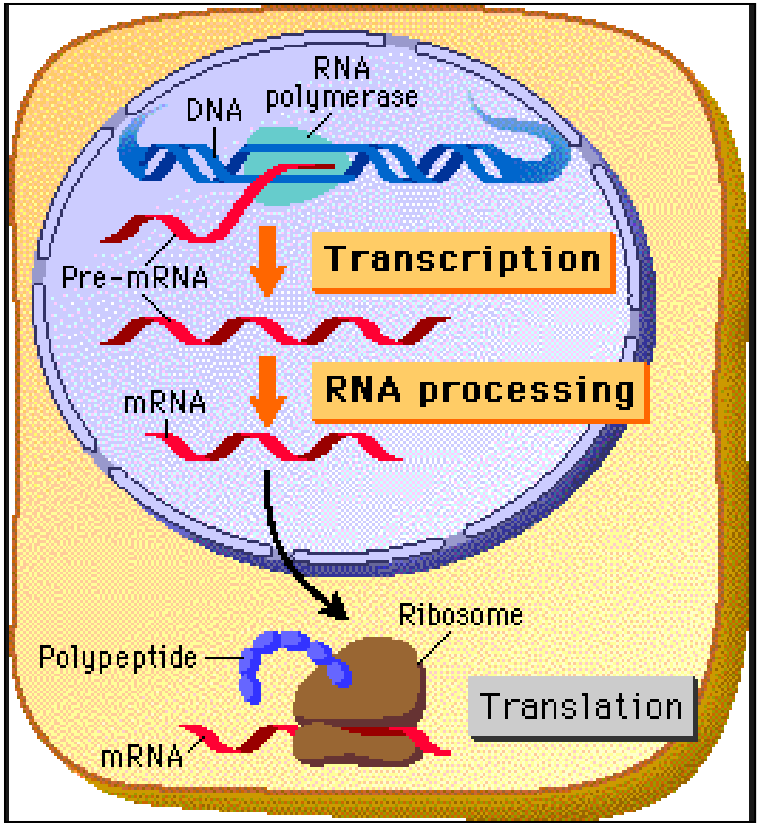
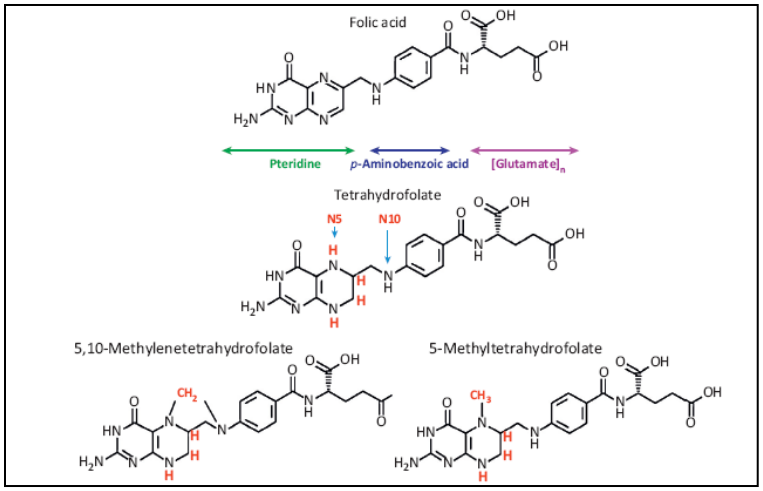
EPIGENOMIC MODIFICATION

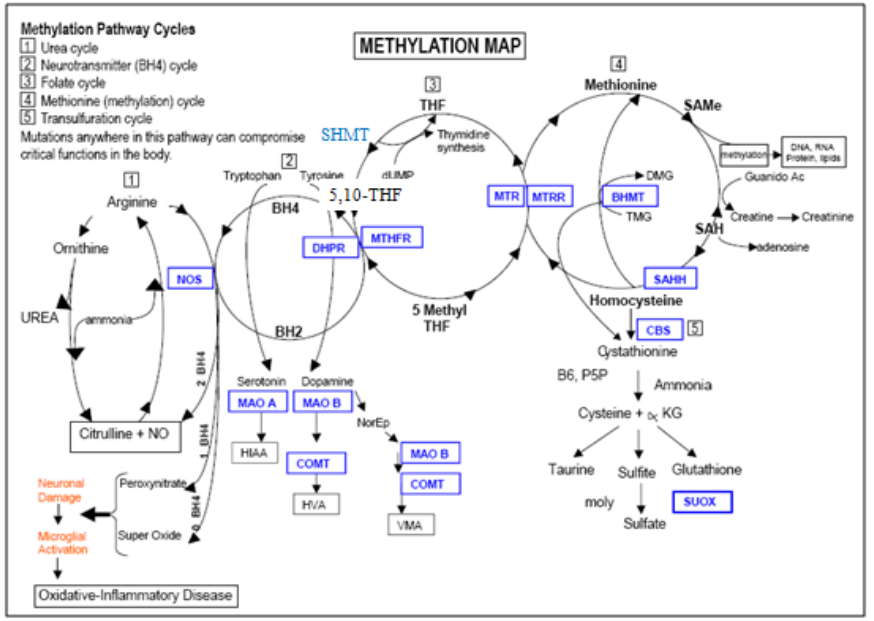


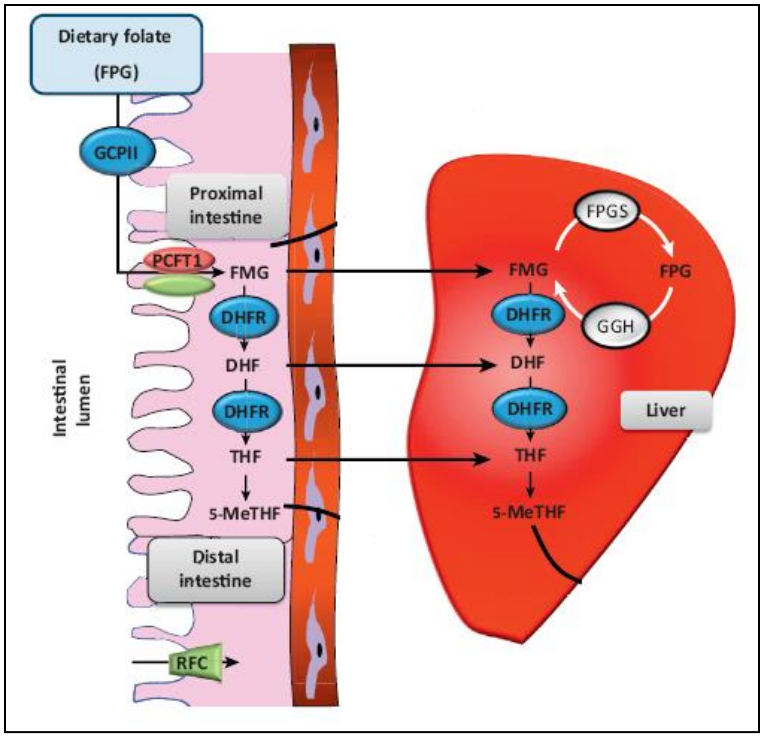


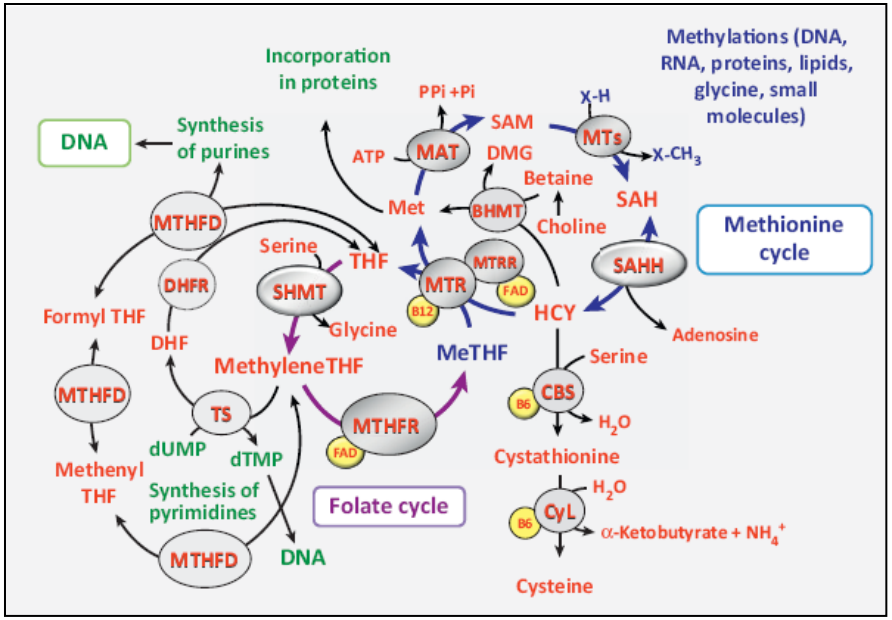


OVERVIEW of the METHYL CYCLE

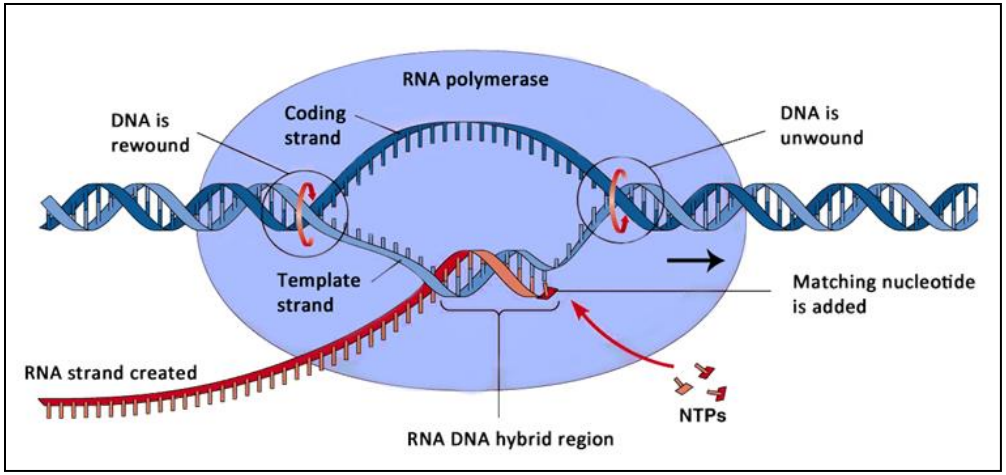


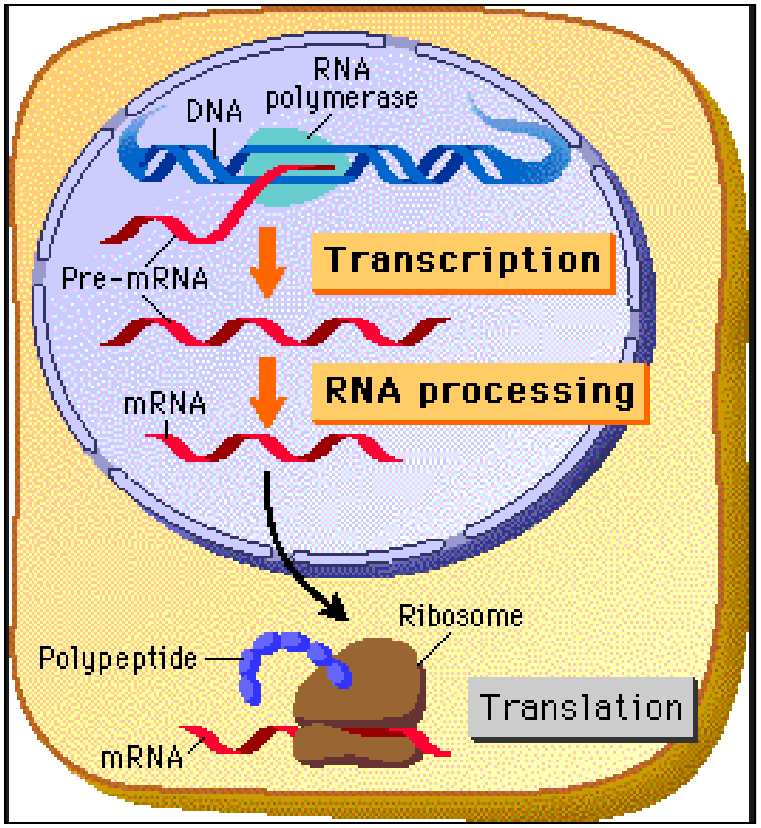


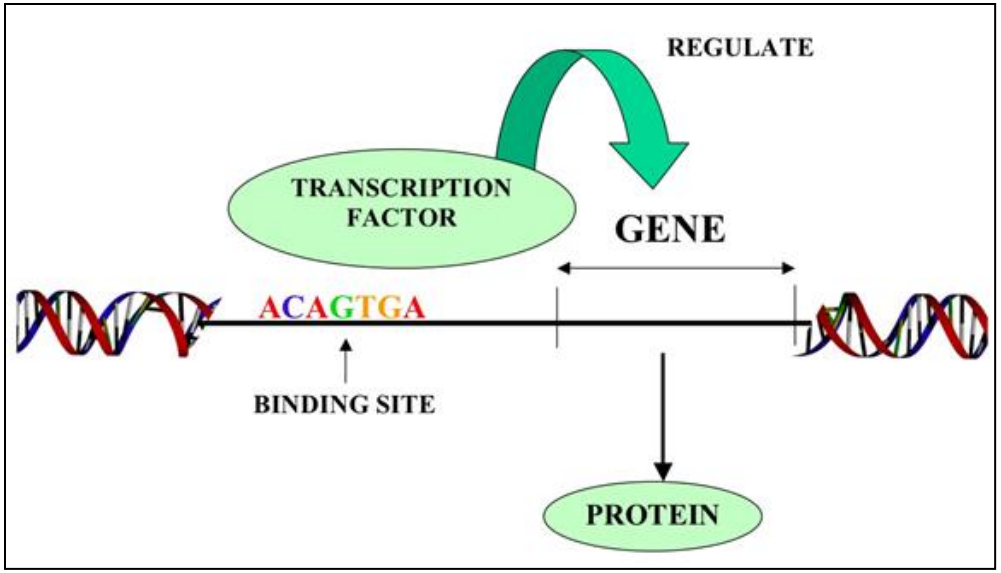




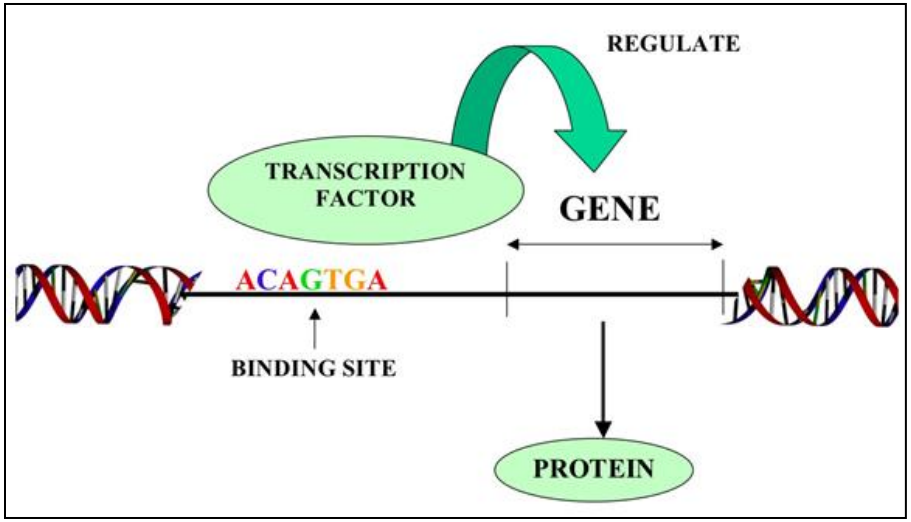
TRANSCRIPTION and TRANSLATION

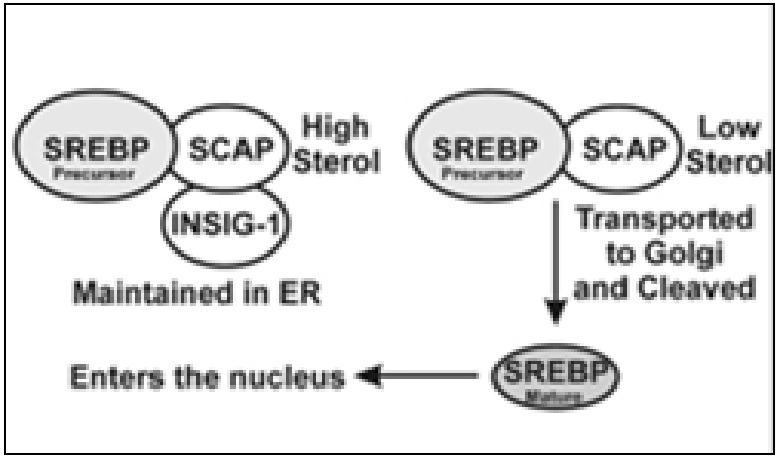




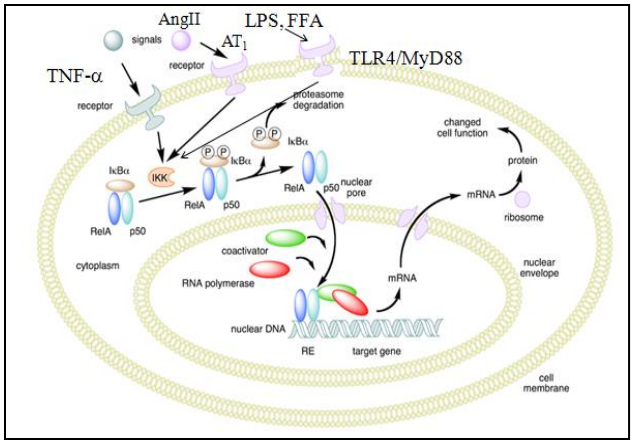


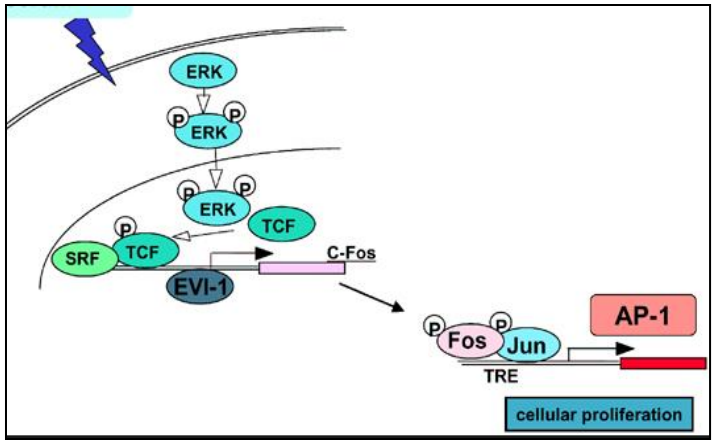
TRANSCRIPTION FACTOR and PROMOTERS

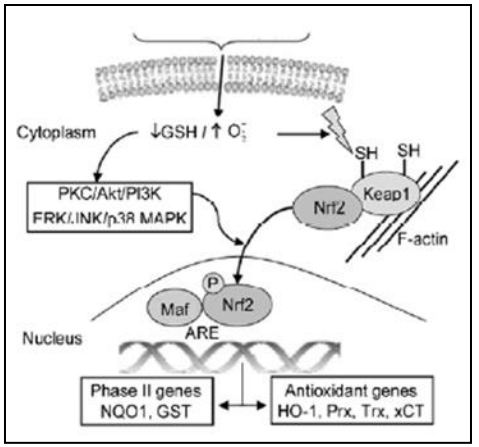




Sterol Regulatory Element Binding Protein







Nuclear Factor Kappa Beta

Activator Protein-1

Nrf2

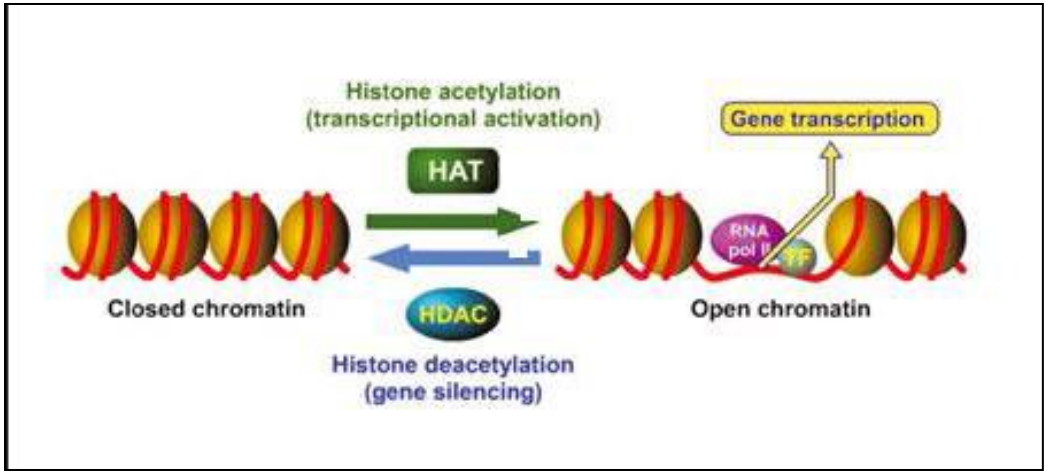
CHANGING EXPRESSION of the HUMAN TEMPLATE

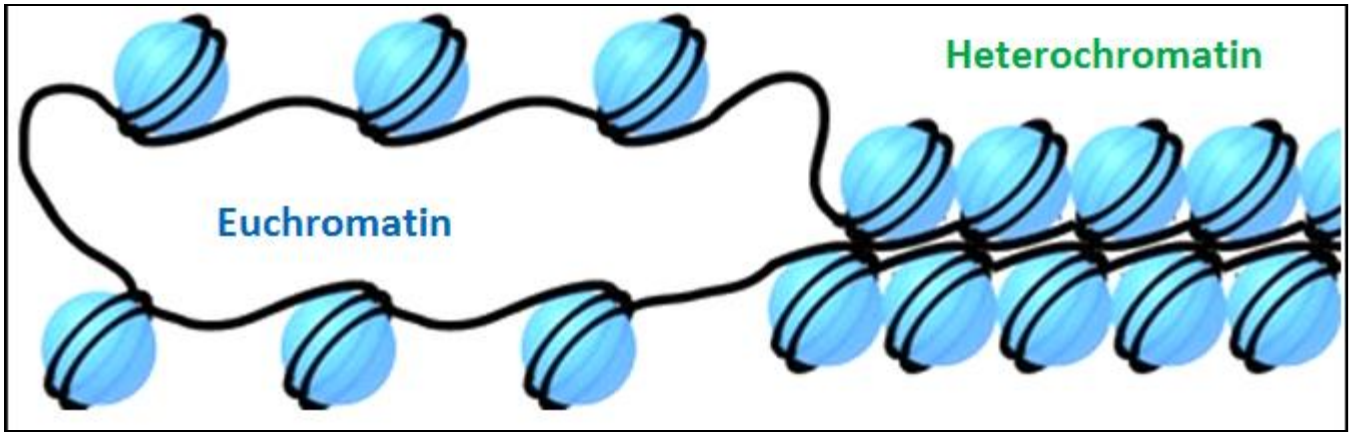
Time Frame	Process	Example
Instantaneous	Post-Translational Modification	pAMPK → pHMG CoA-Reductase
Intermediate	Histone Modification	Turmeric Anti-Inflammation
Lifetime	DNA Methylation	Silencing Oncogenes
Generational	SNIP Experimentation	MTHFR

DNA Methylation critical towards:

- Silencing fetal appropriate (oncogenes) and Imprinting
- X-Inactivation
- Silencing parasitic DNA
- Preparing for life stresses (taking cues from maternal physiology)
- Shielding genes from oxidative damage

HISTONE CODE





DNA METHYLATION

DNA Methyl Transferases transfer CH_3 from SAMe to cytosine within CpG pair

- DNMT3A and DNMT3B – de novo patterns during gestation
- DNMT1 replicates Methylgenome during cell division

mCpGs bind to MBDs (Methyl-CpG Binding Domain Proteins)

MBDs bind to HDACs (Histone Deacetylases)

HDACs remove acetyl groups from lysine within histones $\rightarrow$ imparts (-) charge

Negatively charged histones collapse onto (+) mCpG containing segments of DNA

$\Rightarrow$ Gene Silencing (DNA Polymerase cannot bind promoters)

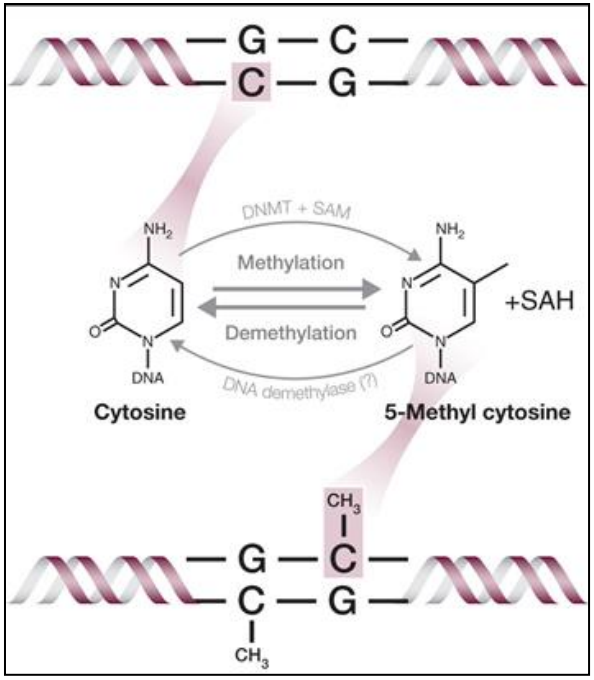
$\Rightarrow$ Gene protection from oxidative attack

DNA METHYLATION

60-90% of mammalian CpGs are methylated

CpG islands - Unmethylated clusters of CpG-enriched DNA

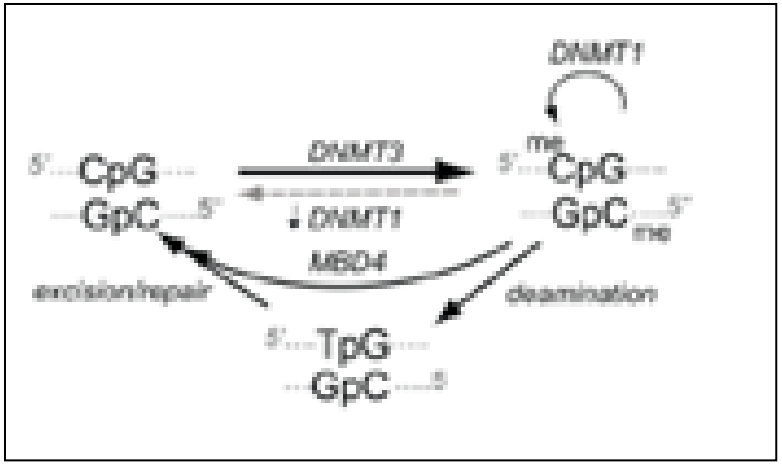
- Reside within gene promoter regions
  - ♦ Unmethylated promoter CpGs ≈ Active gene
  - ♦ Methylated promoter CpGs ≈ Inactive gene



DNA DEMETHYLATION

DNA Demethylation:

- Slow Process
- Neutralized by DNA remethylation
- Methylgenome changes only slowly (decades)



Ten eleven translocation enzymes (TETs)

- Oxidize 5mC → 5-OHmethylcytosine (5hnC) → 5-formylcytosine → 5-carboxylcytosine (5caC), which is decarboxylated to cytosine
- 5acC can be removed by thymine-DNA glycosylase (TDG)

TETs protective factor vs. age and disease-related hypermethylation

DNA METHYLATION

Global DNA Demethylation

- Gametogenesis
- Pre-Implantation

DNA methylation during gestation ≈ anticipated post-natal environment

- Cues from maternal physiology
- Perturbations → Life long effects on offspring

DNA METHYLTRANSFERASES

DNMT3 active during embryogenesis

- Silenced post-embryogenesis CpG methylation at its promoter site
- Like other genes active in fetal development (reactivate $\rightarrow$ oncogenes)

DNMT1

- Copies Methylgenome pattern established by DNMT3 in utero
- Suppresses DNMT3 via promoter CpG methylation

DNMT1 highly regulated:

- Targeted by multiple transcription factors
- Can be methylated, phosphorylated, acetylated, and ubiquinated
- Activated by and dependent upon SAMe
- Inhibited by SAH

$\Rightarrow$ SAMe:SAH determines efficiency/fidelity of DNA methylation

EPIGENOMIC DRIFT

Identical twins have identical methyl genomic patterns at birth

- Disparity with age related to differing adult environments/physiologies

Epigenomic drift age-related:

- Age prediction within five years
- Process accelerated by abnormalities in SAMe:SAH
  - ♣ Why high homocysteine associated with diverse disease states
- DNA global hypomethylation ≈ frailty and loss of physiologic function
- Global hypomethylation predicts 7-yr decline in health status
- Patterns associated with specific disease states
  - ♣ NPTX2 un silenced in PD and pancreatic cancer
- Caloric restriction ≈ maintenance of Methylgenome

DNA METHYLATION

Drosophila melanogaster has a single DNMT:

- Over expression increases life span and resistance to oxidative stress
- Under expression decreases lifespan

Honeybees: DNA of workers and queens is the same

Royal jelly fed to "Queen selected" larvae

- Larger, functional ovaries, and longer lived
- Altered 5mC content vs. worker bees

Treat larvae with siRNAs for DNMT3 $\rightarrow$

- Queen phenotype and mC pattern

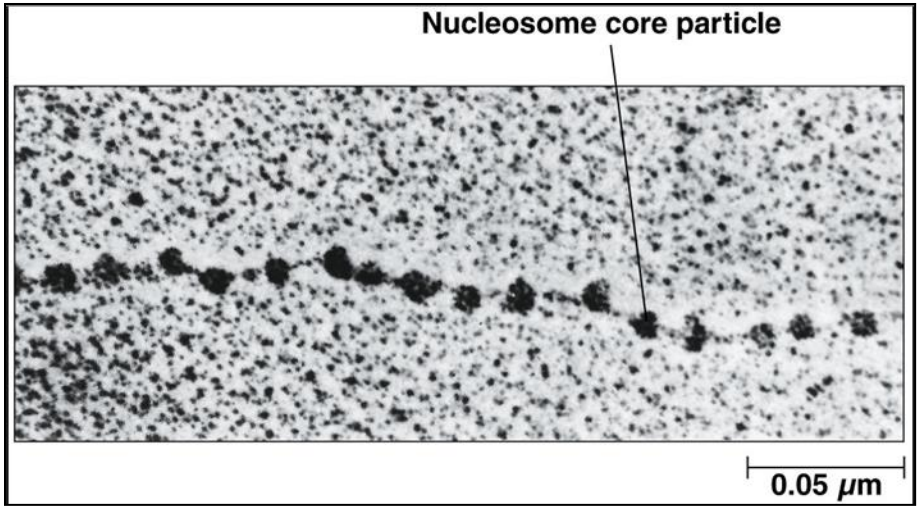
OP-1 protein is human cartilage growth factor

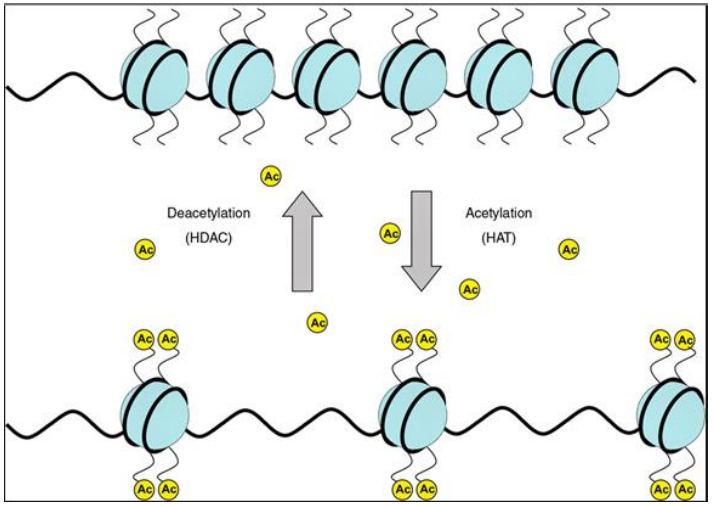
- Four fold loss with aging
- Methylation of CpGs within promoter

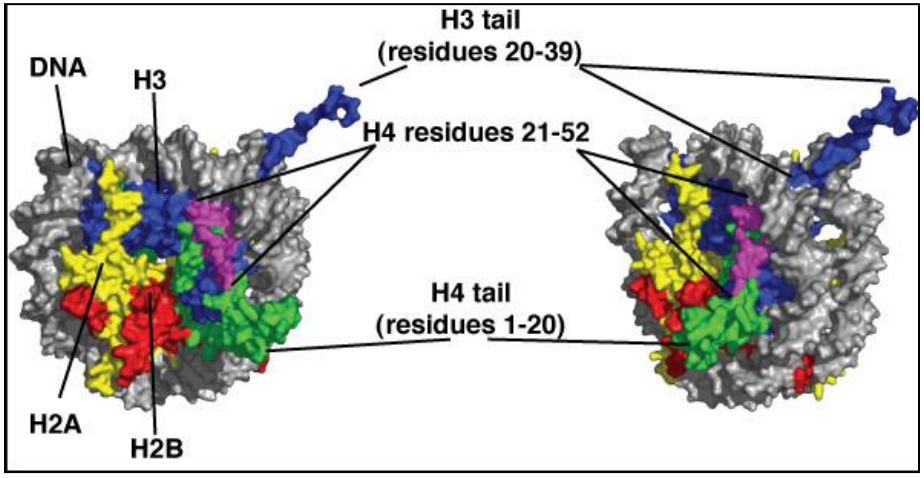
Procaine demethylates DNA

- Inhibits growth of breast cancer cells
- Demethylates hypermethylated CpG islands
- Reactivates previously silenced (RARBeta2) tumor suppressor genes
- Beneficial effects (hydralazine) in human cervical cancer trial

NUCLEOSOME

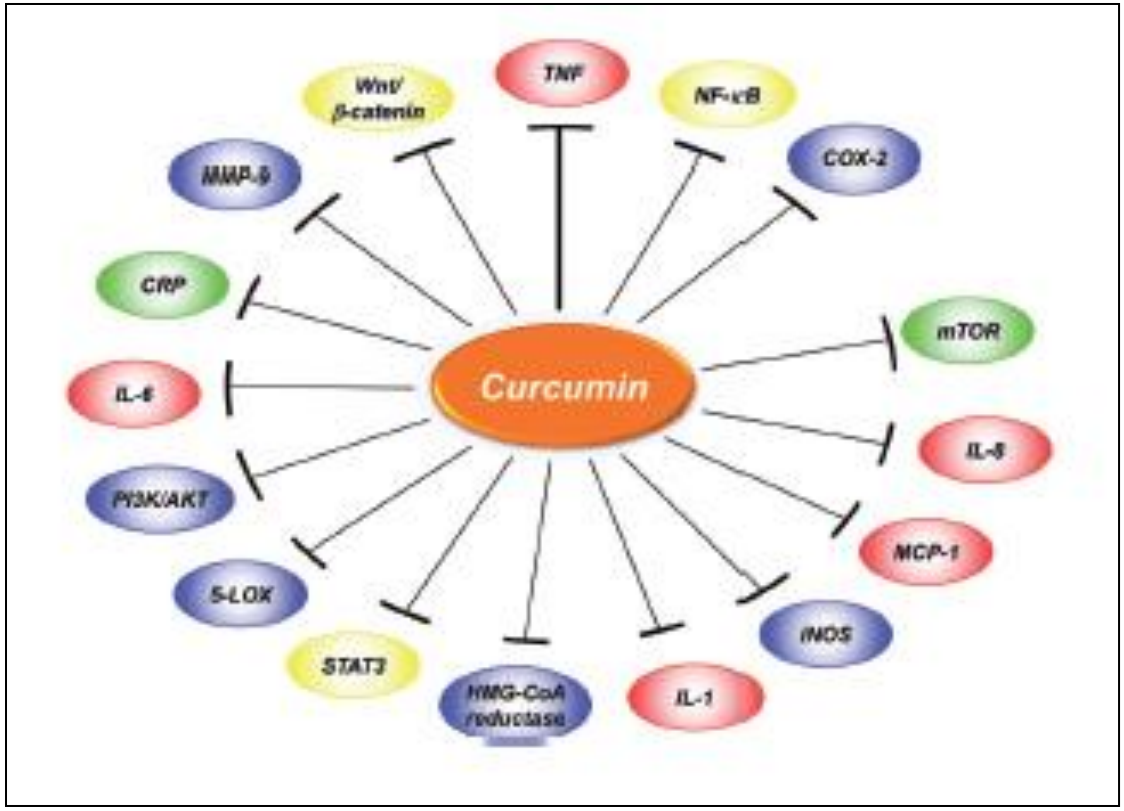






147 bp of DNA
Octomeric core of histone proteins
Two H3-H4 dimers
surrounded by
two H2A-H2B dimers

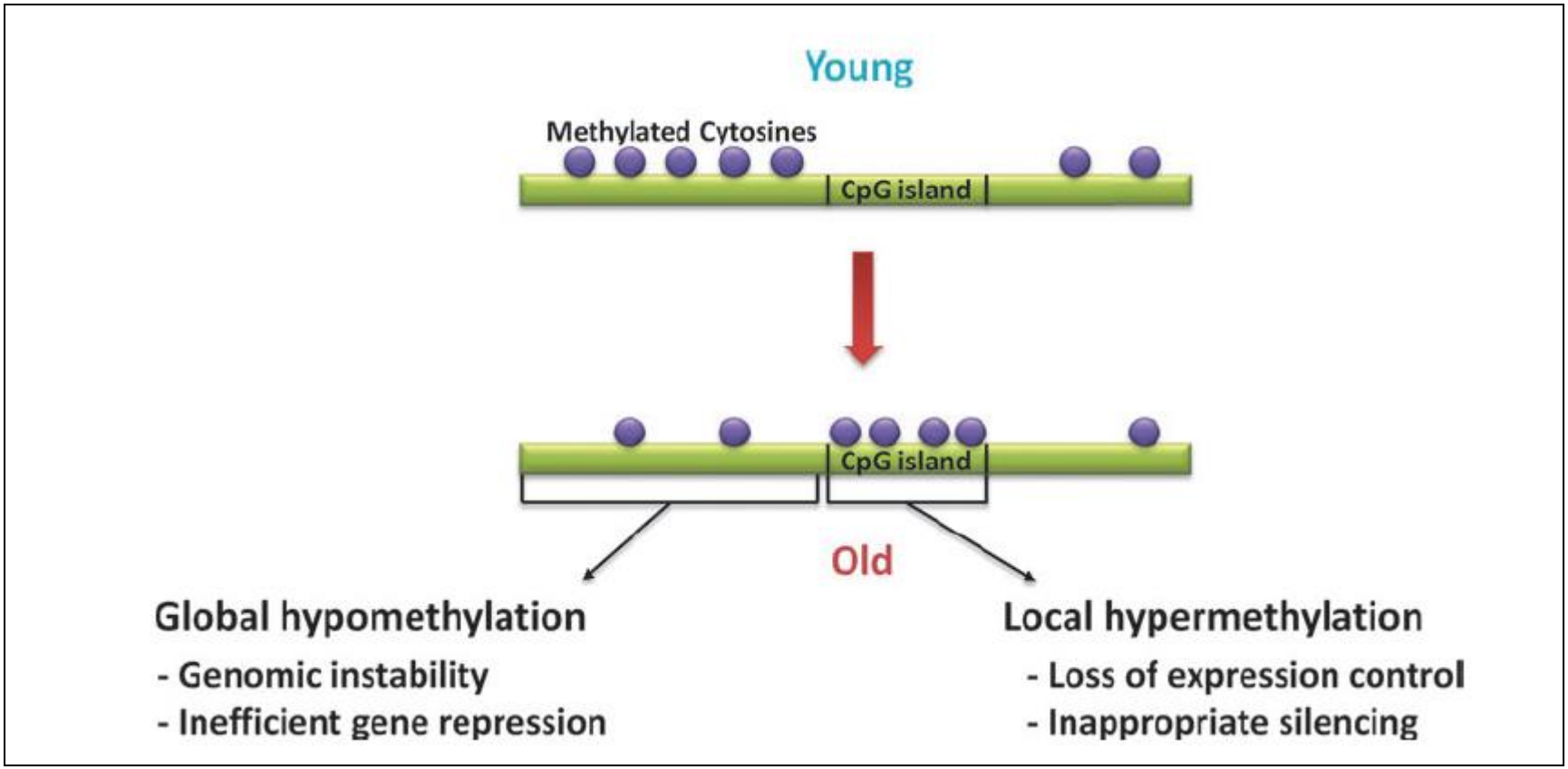
TURMERIC is a HISTONE DEACETYLASE



Inactivates threat response genes

Inappropriately activated by “pseudo-infection” cues

DISTORTION of the METHYLGENOME



Cancer and age-related illness associated with:

- DNA global hypomethylation → Activation of oncogenes
- Promoter hypermethylation → Silencing of tumor-suppressive genes

HYPOMETHYLATION LEADS TO HYPERMETHYLATION?

I was for methylation before I was against it

HYPOMETHYLATION LEADS TO HYPERMETHYLATION?

DNMT3 involved in de novo DNA methylation

- Active during and inactive post-embryogenesis
- Methylation of its promoter suppresses its transcription

DNMT1 maintains birth pattern → senescence

- Binds methylated DNA at cell division → Methylgenome replication

SAMe:SAH insufficiency (low methionine, folate, choline, oxidative stress, etc.) →

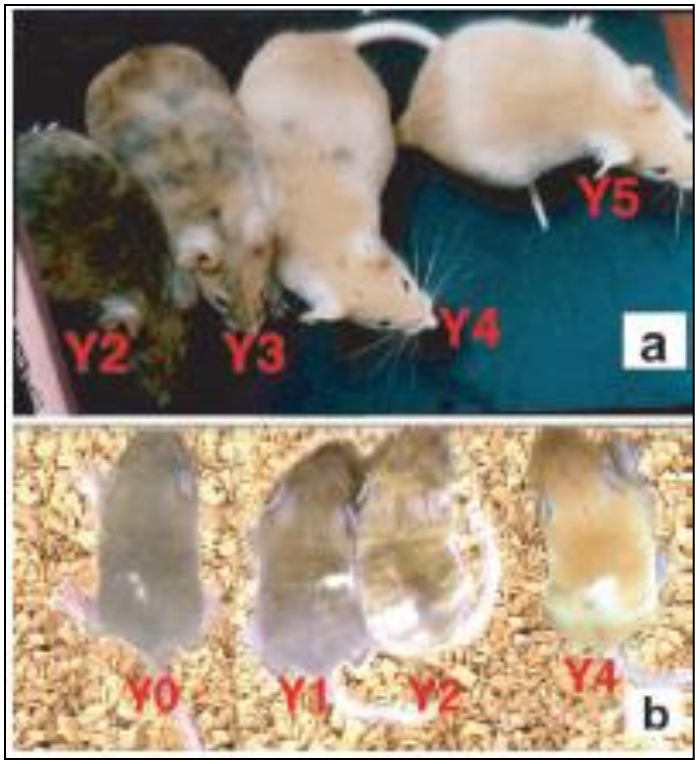
DNMT1 fails to methylate promoter of (life long) repressed genes →

- Oncogene activation
- Inflammatory gene up regulation

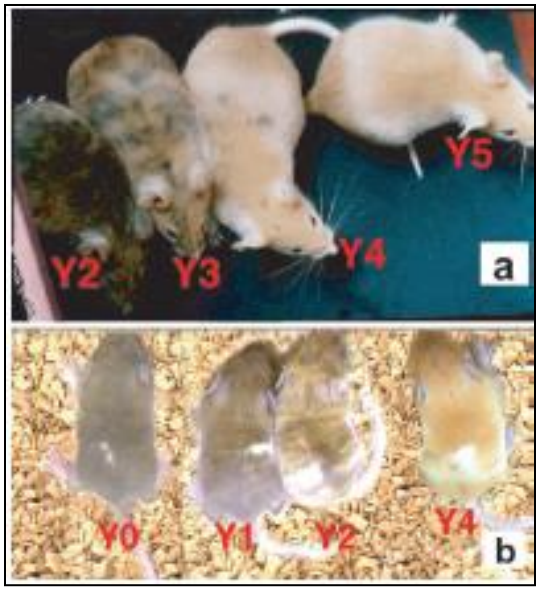
DNMT1 fails to methylate DNMT3 promoter → DNMT3 transcription →
Inappropriate methylation of beneficial genes →
"Silencing" of anti-cancer and anti-inflammatory genes ⇒

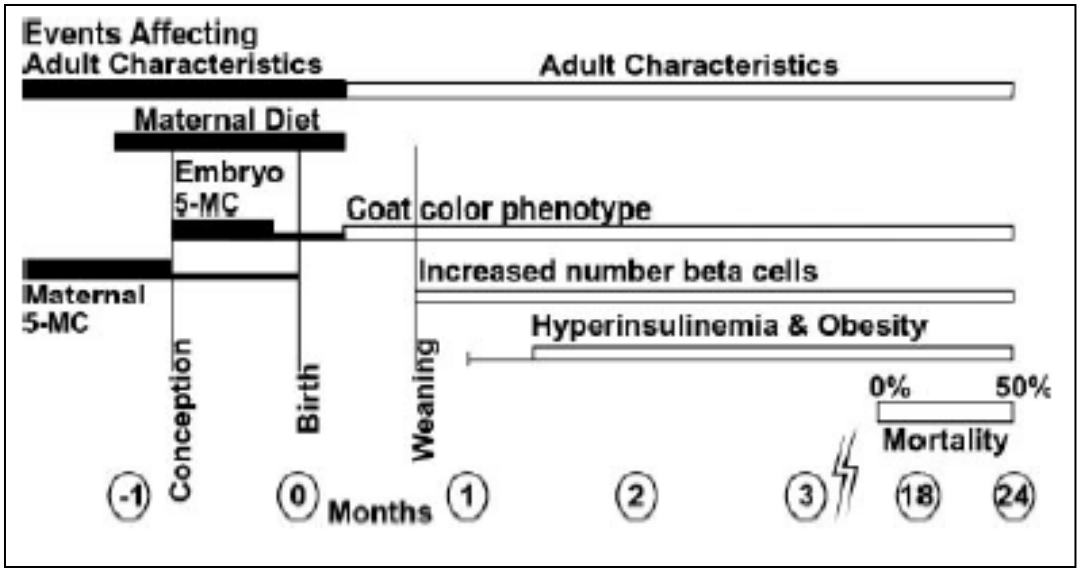
Distortion of Methylgenome (degenerative disease and malignancy)

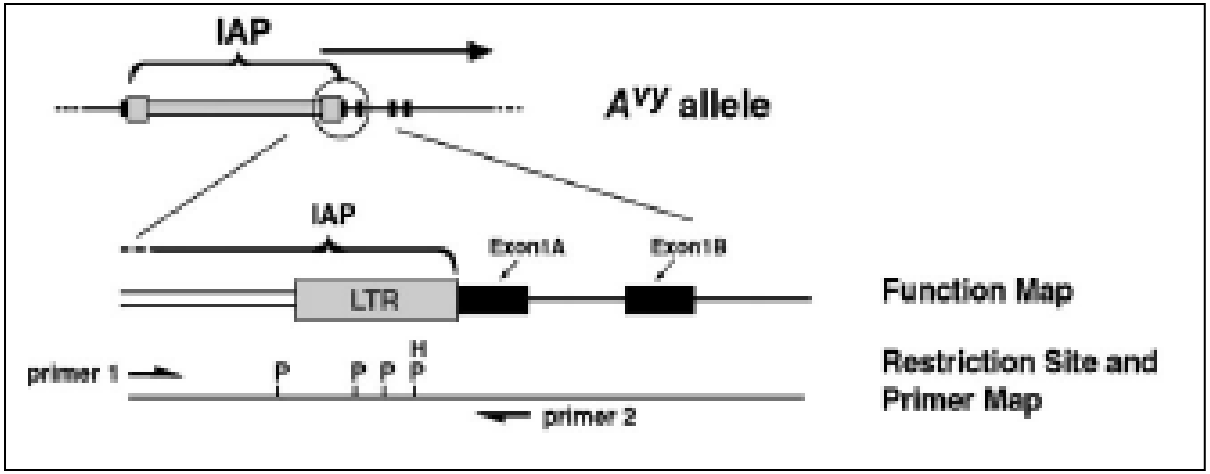
VIABLE YELLOW AGOUTI MOUSE (Avy/a)



VIABLE YELLOW AGOUTI MOUSE (Avy/a)







AGOUTI MOUSE

♥ Female Viable Yellow Agouti mice (Avy/a) mated with (a/a) males

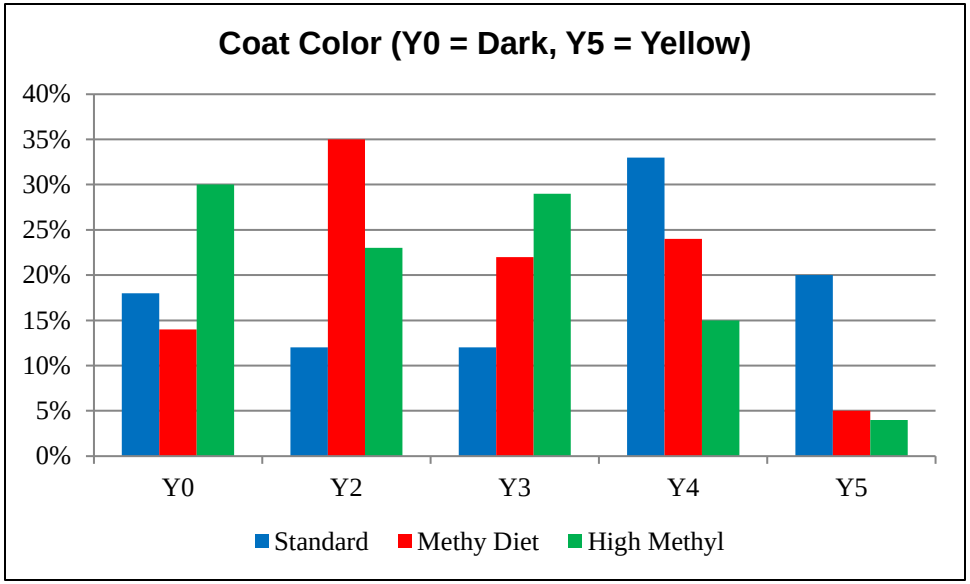
Provide to dams at conception → weaning:

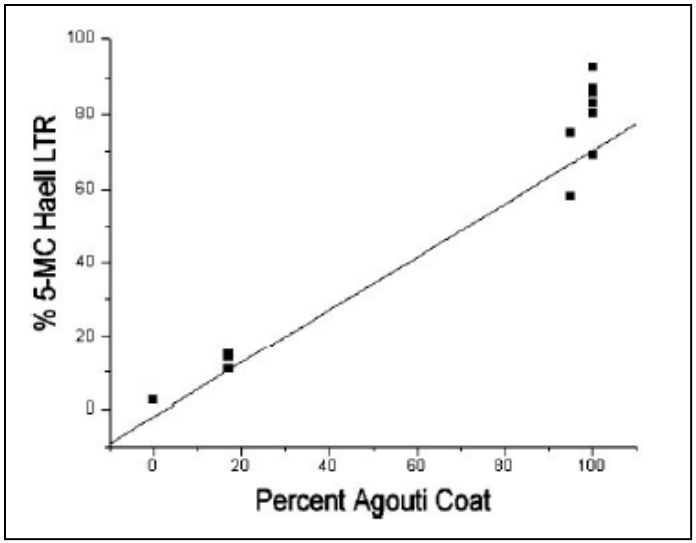
- Standard chow ad lib
- Chow with methylation support
- High methyl support chow

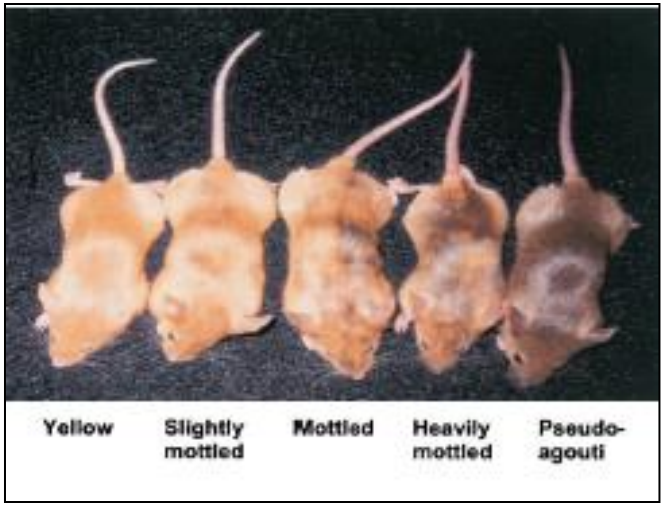
Diet Component	Methyl Support	High Methyl Support
Choline (gm/kg)	5	15
Betaine (gm/kg)	5	15
Folic acid (mg/kg)	5	15
B12 (mg/kg)	0.5	1.5
Methionine (g/kg)	-	7.5
Zinc (mg/kg)	-	150

Evaluate Avy/a offspring for Agouti phenotype

AGOUTI MOUSE







GENISTEIN and AGOUTI EXPRESSION

♥ Female (a/a) mice

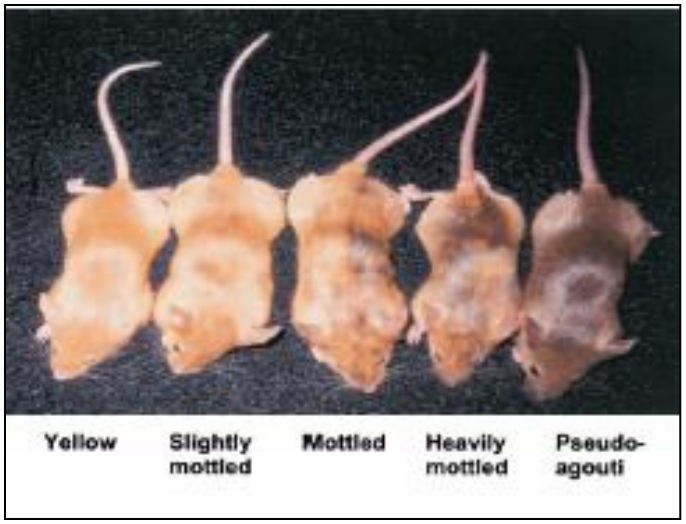
Two weeks pre mating with male Avy/a mice → weaning of the pups

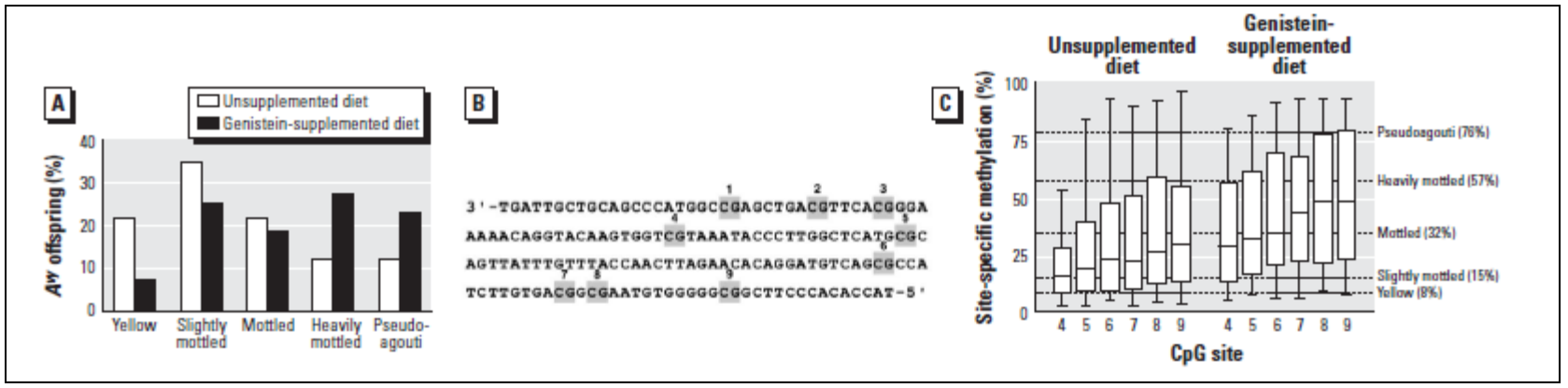
Place on:

- Standard chow
- Genistein supplemented chow (250 mg/kg chow)

At 21 days of life evaluate (Avy/a) pups for agouti expression

GENISTEIN and AGOUTI EXPRESSION





ORGANIC POLLUTANTS and AGOUTI EXPRESSION

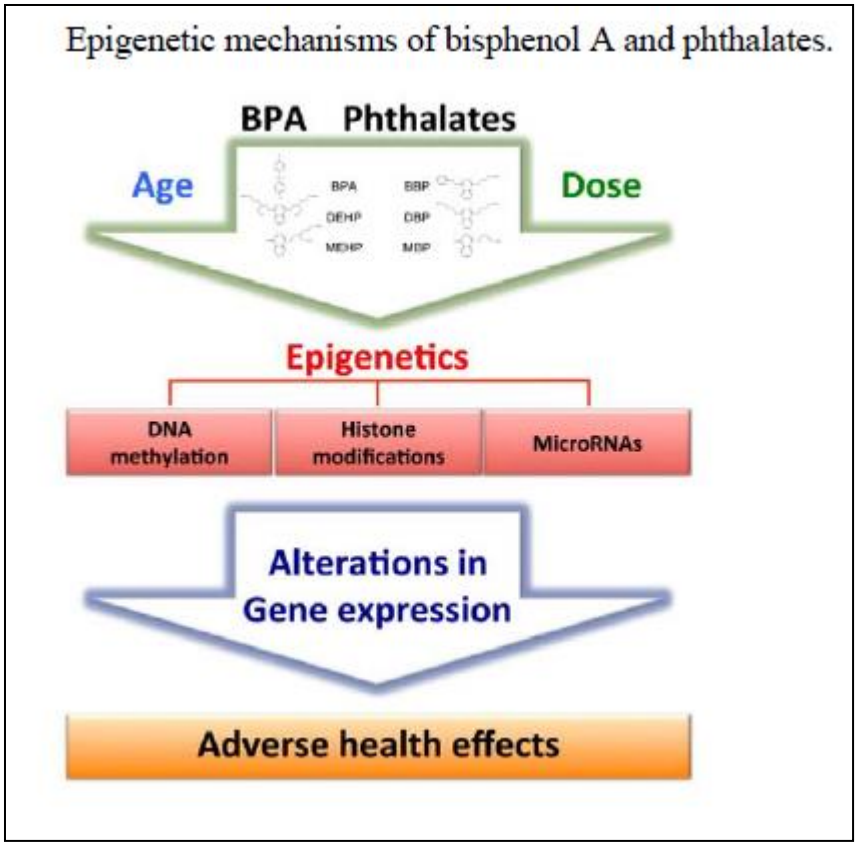
Persistent Organic Pollutants

Ligate estrogen receptor

Persistent

Cross placenta → Bioconcentration

Epigenomic effect



ORGANIC POLLUTANTS and AGOUTI EXPRESSION

♥ Female (a/a) mice

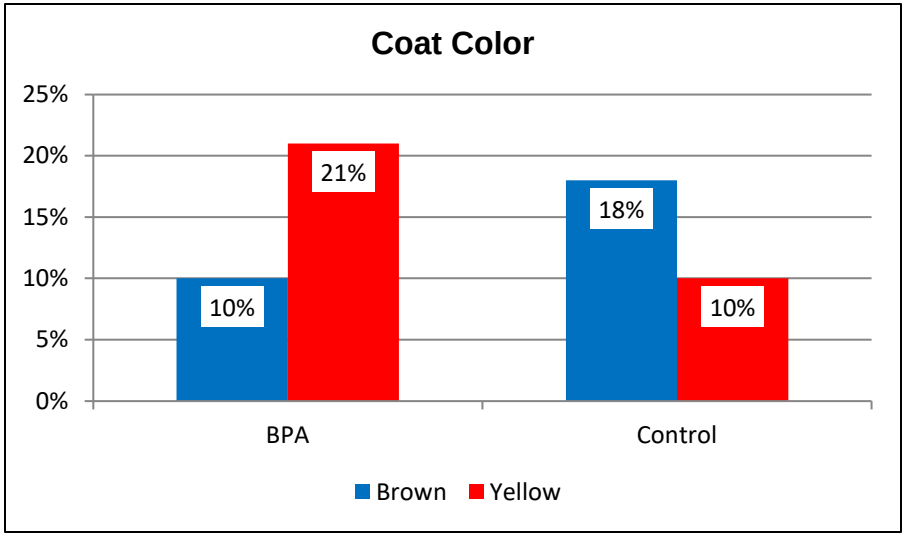
Place on:

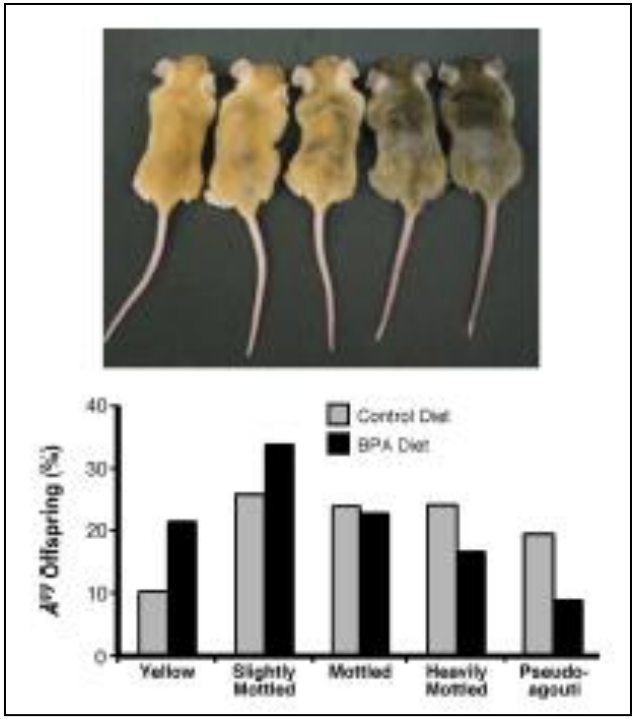
- Standard (phytoestrogen-free) chow
- Chow with BPA 50 mg/kg
- Chow with BPA + methyl supplements
- Chow with BPA + genistein 250 mg/kg

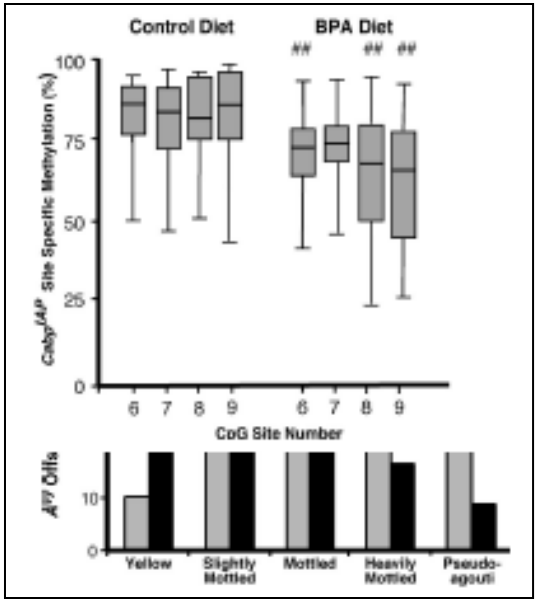
Two weeks pre-mating with (Avy/a) male mice → weaning of pups

Evaluate pups for Agouti phenotype at 10 weeks

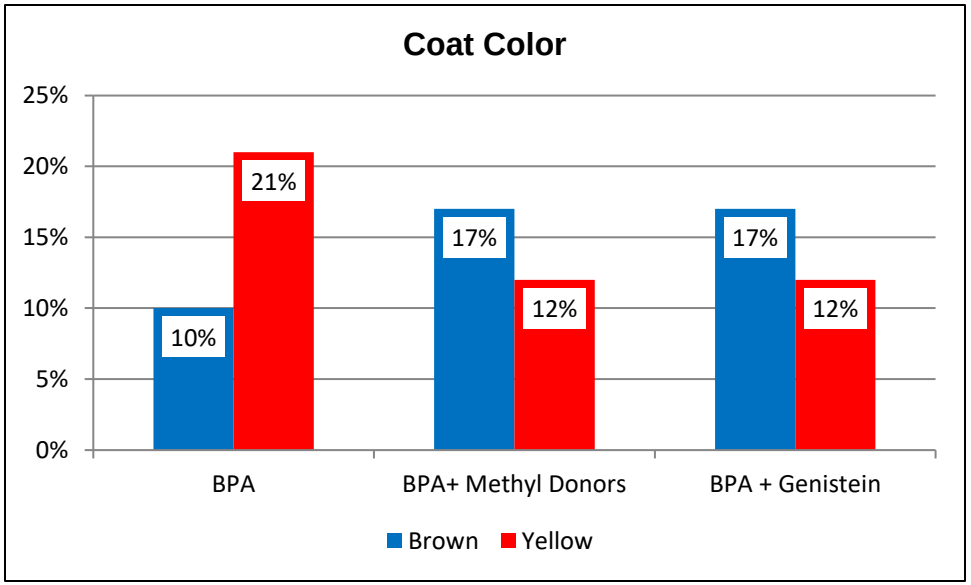
ORGANIC POLLUTANTS and AGOUTI EXPRESSION

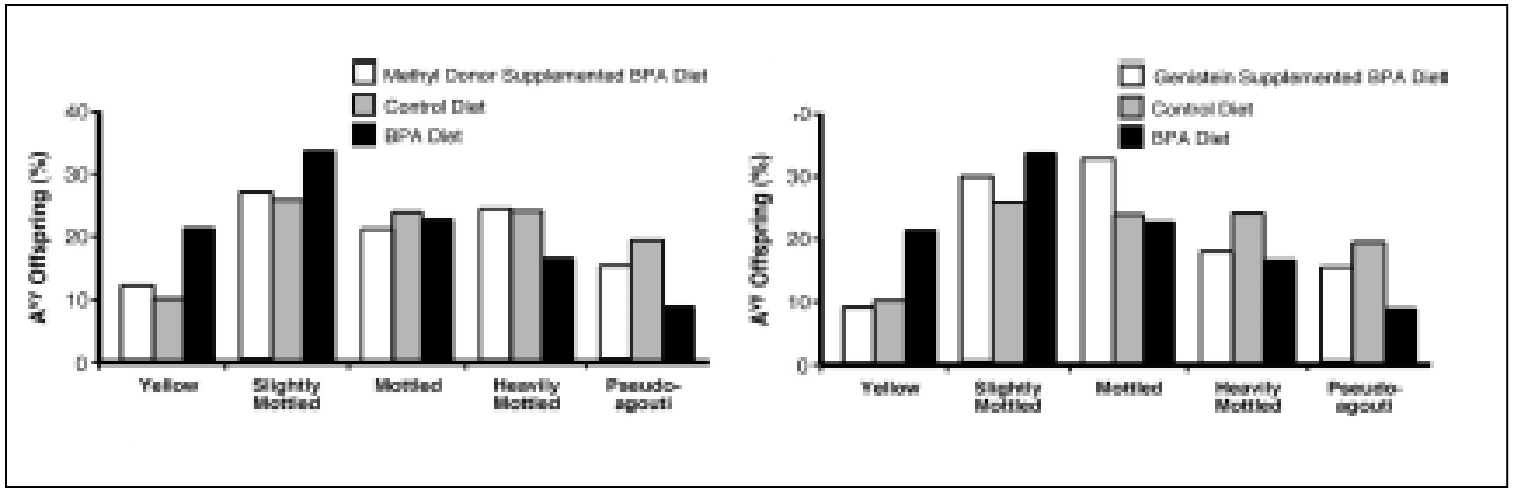






ORGANIC POLLUTANTS and AGOUTI EXPRESSION





MATERNAL DIET and ADAPTIVE SATIETY

♥C57BL/6J mice

Offer ad lib high fat (highly palatable) diet → Increased caloric intake and DIO

- Progeny predisposed to DIO

Provide to healthy male and female mice ad lib consumption of:

- Standard chow (10% fat)
- HFD (60% calories from fat)
  - ♣ 83% females and 80% males → Hyperphagia and DIO

Switch HFD/DIO females to standard diet →

- Reduced caloric intake
- 12% weight loss over 8 months; still overweight (25 vs. 21 gm.)
- Still insulin insensitive, and hyperlipidemic

MATERNAL DIET and ADAPTIVE SATIETY

Cross HFD/DIO females with lean, standard chow males

Prior to conception → weaning place HFD/DIO females on standard chow diet

At weaning, place pups on HFD →

- 80% male pups developed DIO with IR and hyperlipidemia
- 57% female pups developed DIO

43% female pups resistant to HFD induced DIO

- Weight and caloric intake similar to females on standard diet
- Hyperphagic response to high fat diet (expected) did not occur
- Glucose tolerance and lipid status nearly normal

Diet change in HFD/DIO mice during pregnancy and lactation →

- Epigenomic protection vs. DIO
- Positive effect on satiety mechanism

ESTROGEN RECEPTOR GENE METHYLATION

Estrogen ligation of ER receptor → altered expression of multiple genes

Methylation (of CpG islands within) ER gene promoter → inactivates ER receptor

Estrogen ligation of promoter methylated ER → physiologic effect attenuated/lost

ER receptor present in arterial wall

- ER activity less in vessels with atherosclerosis

ER receptor negative breast cancer ≈ ER promoter is methylated

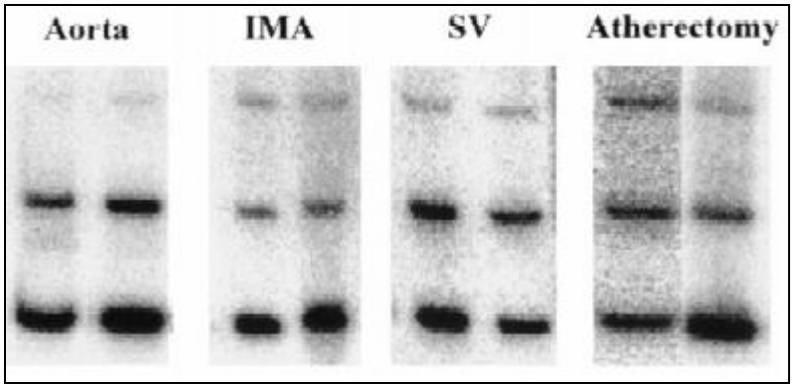
ER receptor promoter methylation:

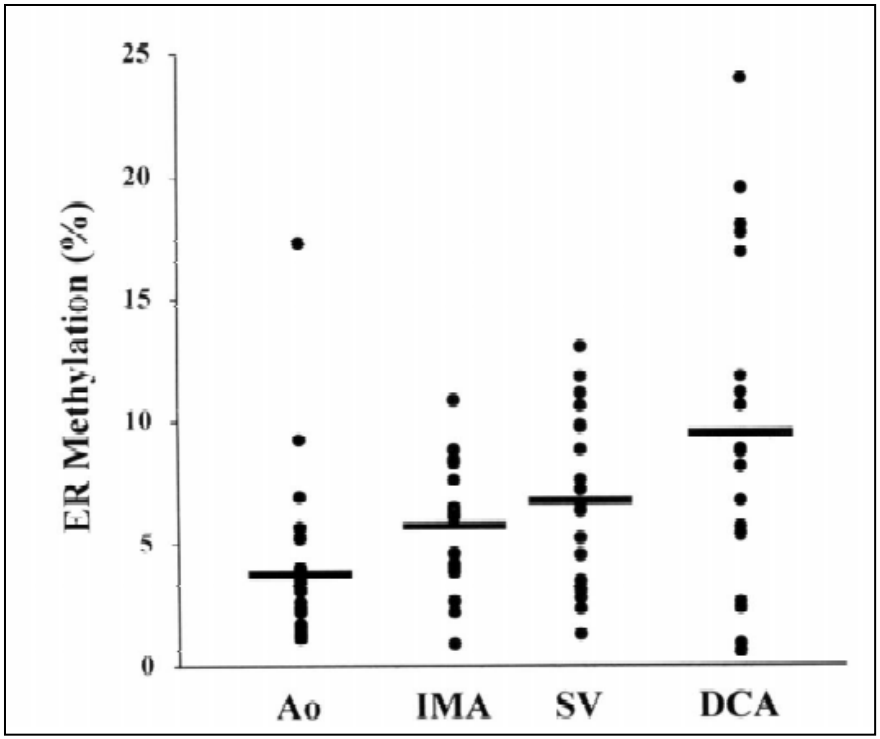
- Increases with age
- Increases with age in normal colonic mucosa
- Universally present in colonic neoplasms

Is ER receptor methylation a link between aging and CV Dz and malignancy?

ESTROGEN RECEPTOR GENE METHYLATION

♥Tissue specimens from men and women undergoing CABG or DCI

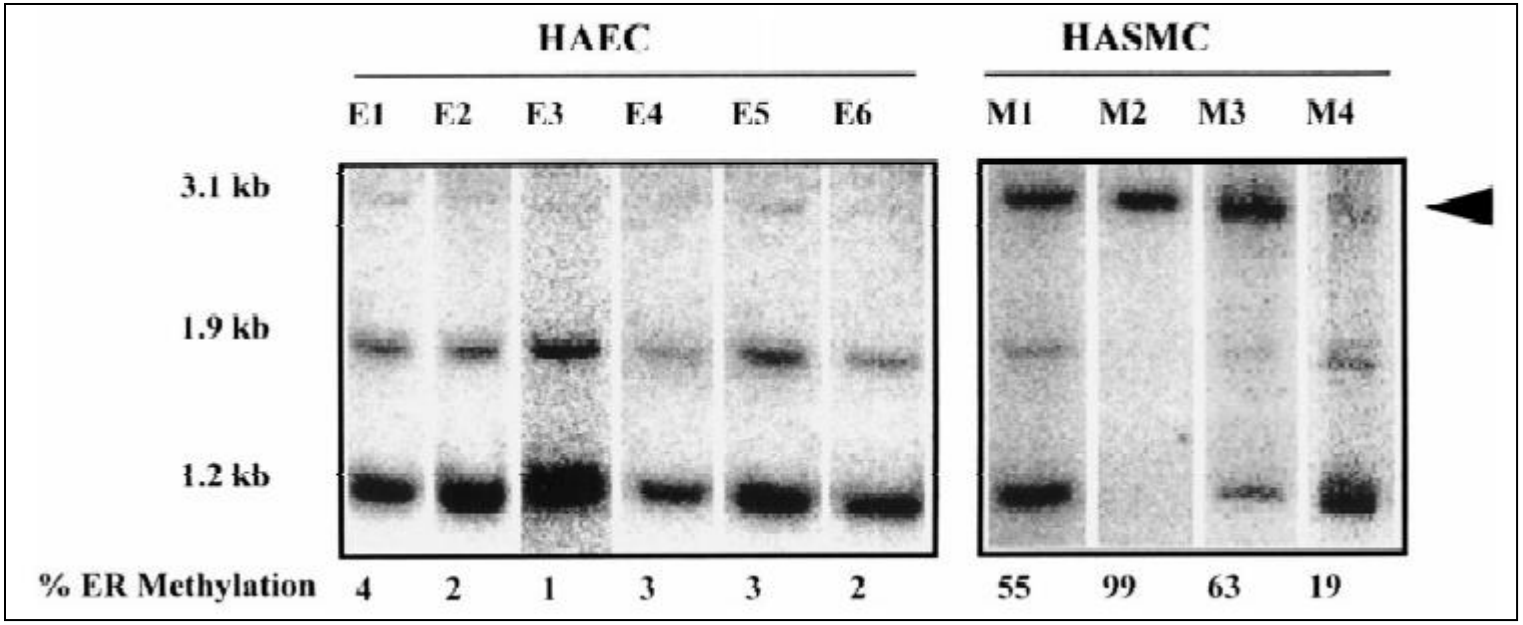




ER promoter methylation (gene inactivating)

- Increases with age in men and women
- More prominent in plaque vs. aortic tissue

ESTROGEN RECEPTOR GENE METHYLATION



ER methylation in VSMCs →

- Loss of estrogen growth inhibition effect
- Loss of benefit from ERT
- Increased risk for CADz

ESTROGEN, HOMOCYSTEINE, and DNA METHYLATION

♥ 13 healthy post-menopausal women
- None on HRT
- None taking B vitamins

Baseline studies

Randomize to receive over eight weeks:
- CEE 0.625 mg/day
- Placebo

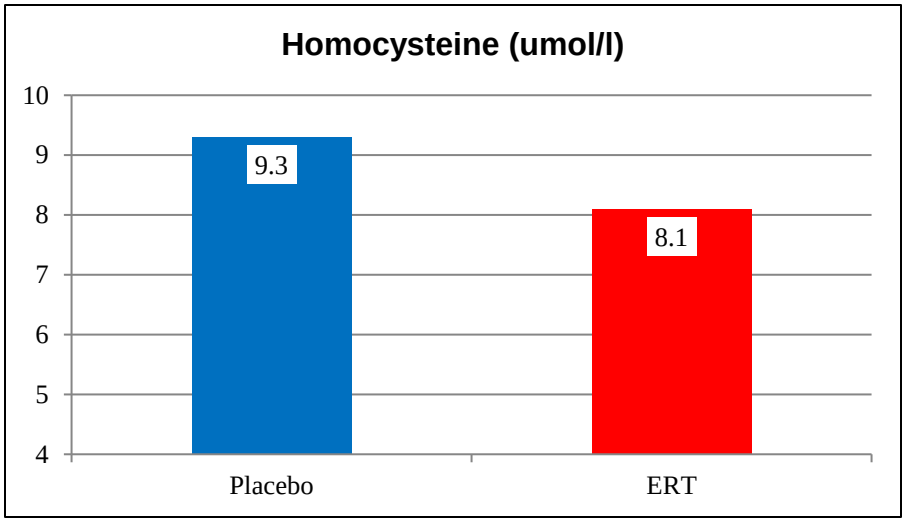
Repeat baseline measurements

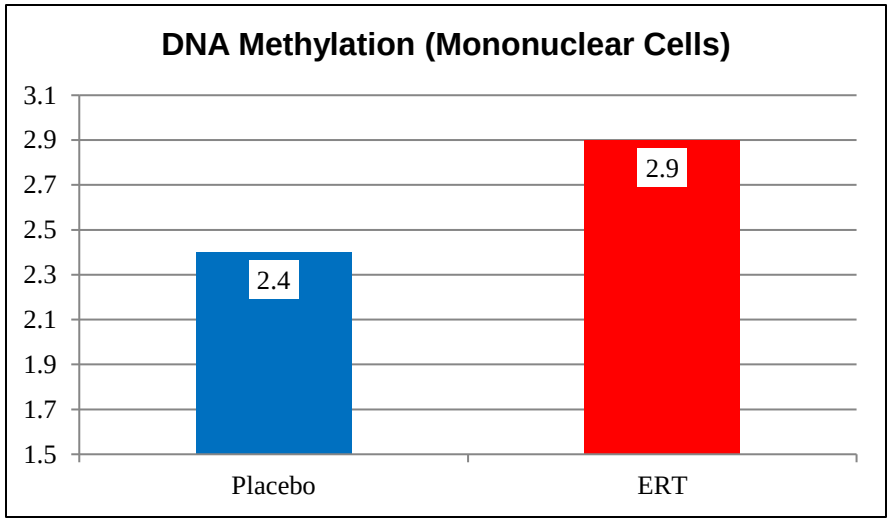
After four-week washout cross-over to other treatment

Double blind protocol followed

ESTROGEN, HOMOCYSTEINE, and DNA METHYLATION

	Placebo	ERT
Folate (nmol/l)	10.8	11.8
B12 (pmol/l)	420	395
B6 (nmol/l)	54	33



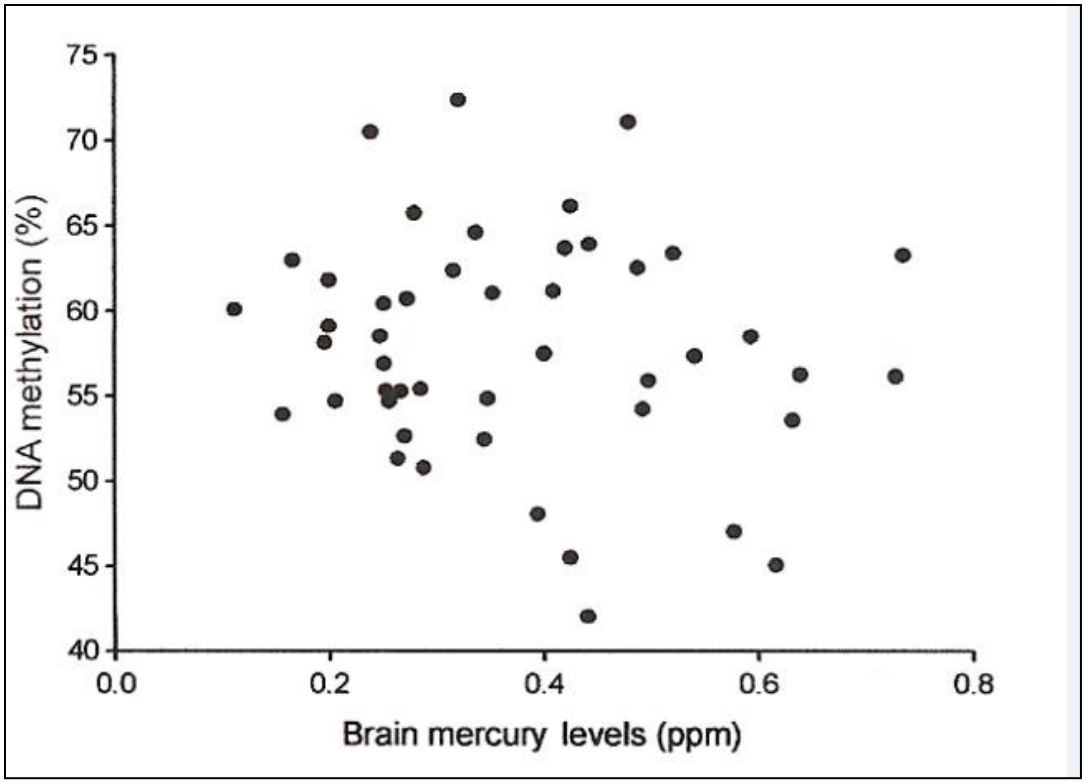


MERCURY and POLAR BEAR DNA METHYLATION

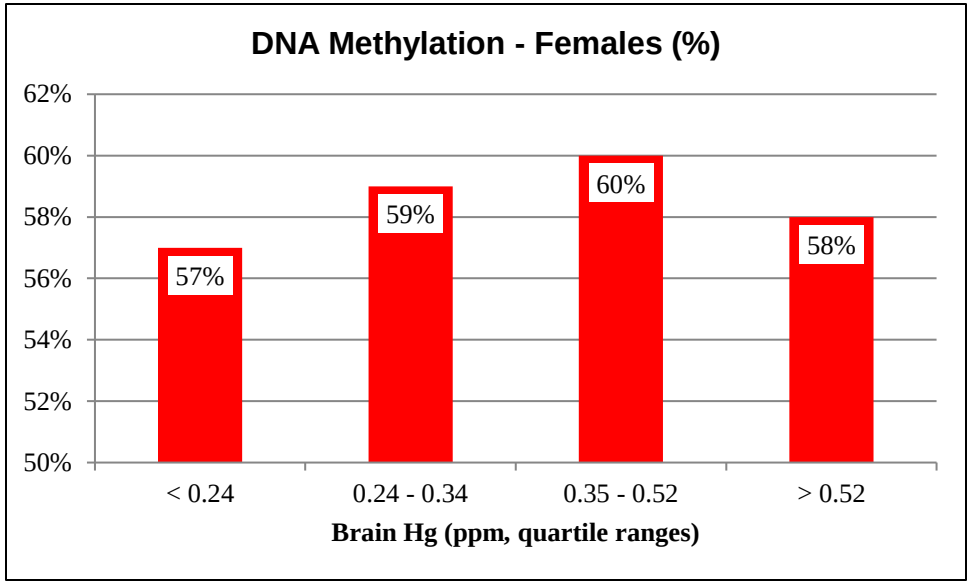
♥ 47 Polar Bears harvested by Greenland Inuit subsistence hunters

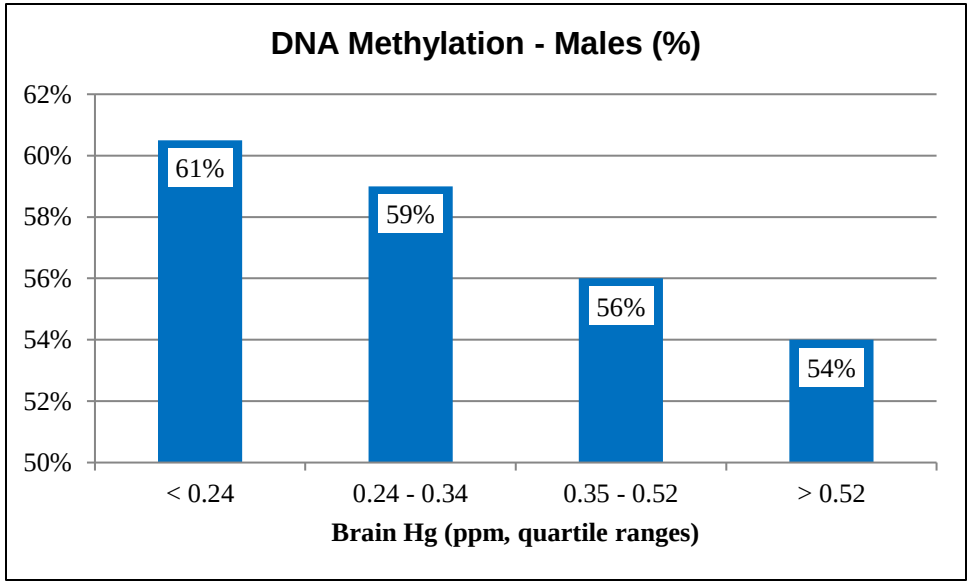
Analyze Medulla oblongata levels of:

- Mercury
- DNA methylation



MERCURY and POLAR BEAR DNA METHYLATION





FOLIC ACID and DNA METHYLATION

♥ 31 patients with colorectal adenoma by colonoscopy

- No history (or family history) of colorectal malignancy
- No B12 deficiency
- No inflammatory bowel disease

Baseline measurements

- Lab studies
- Morphologically normal colonic mucosa

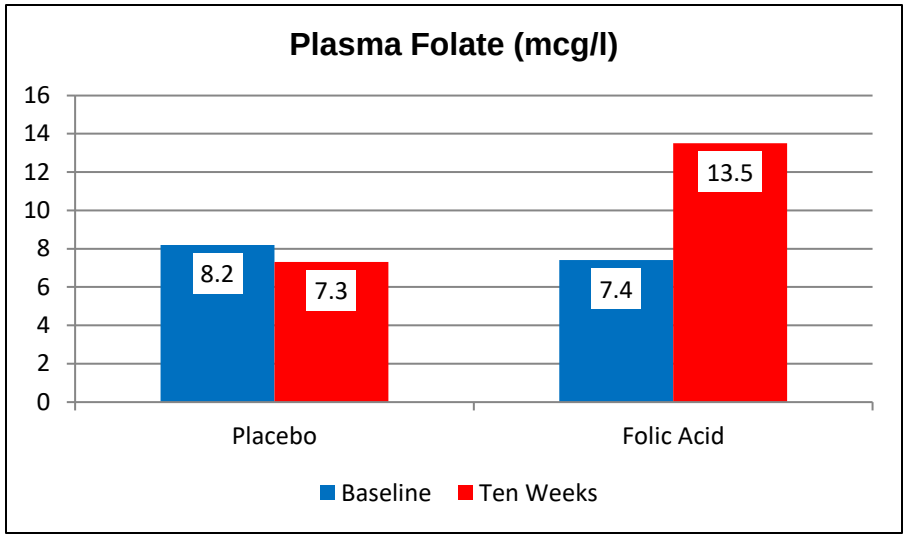
Randomize to receive over 16 weeks:

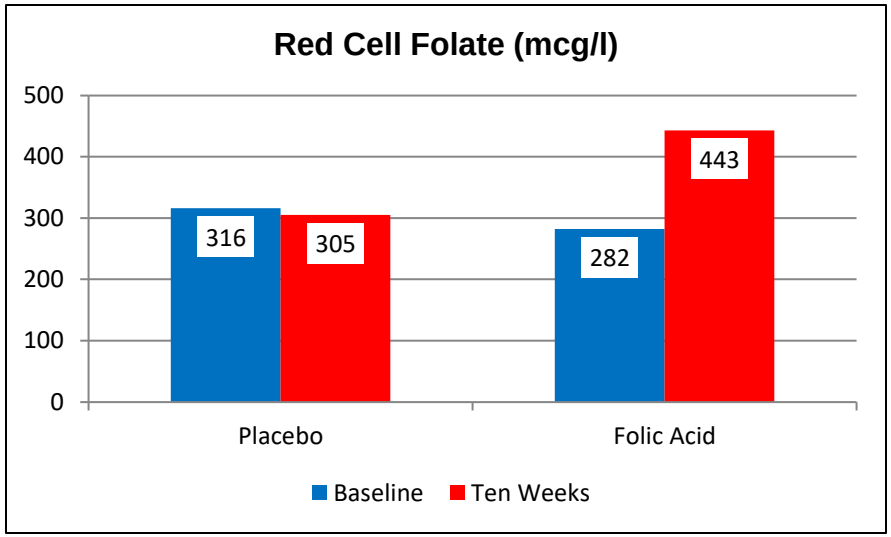
- Folic Acid 400 mcg/day
- Placebo

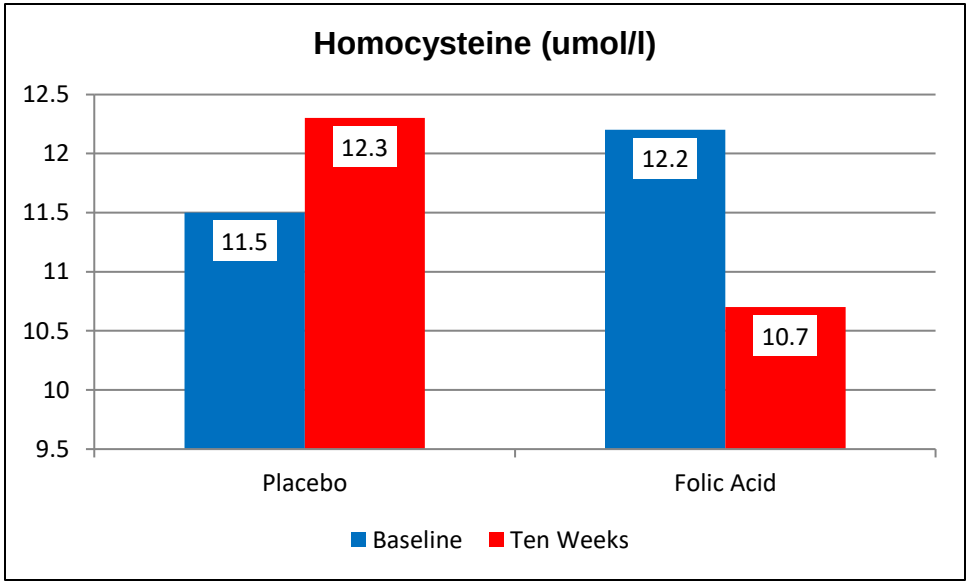
Repeat baseline measurements

- Rectal vs. colonic biopsy

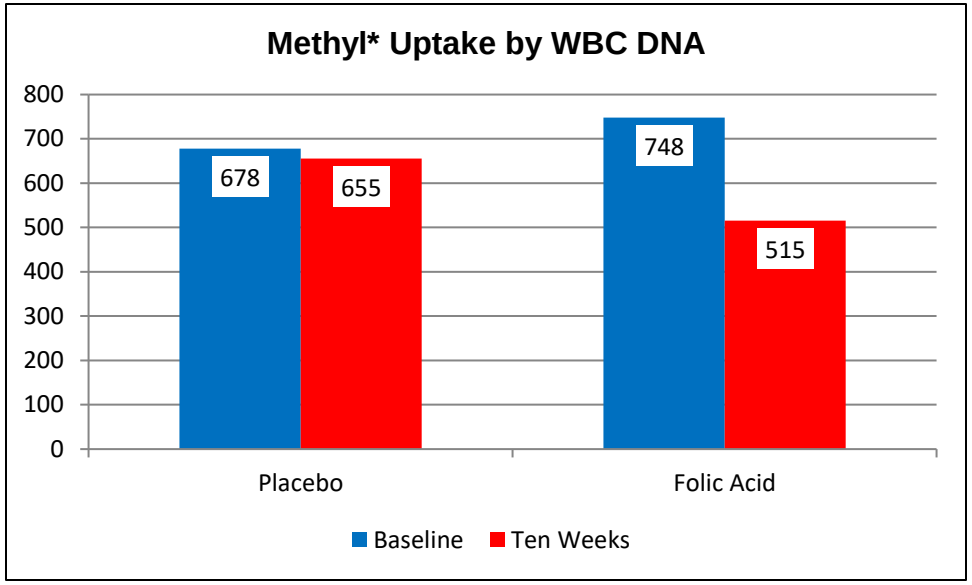
FOLIC ACID and DNA METHYLATION

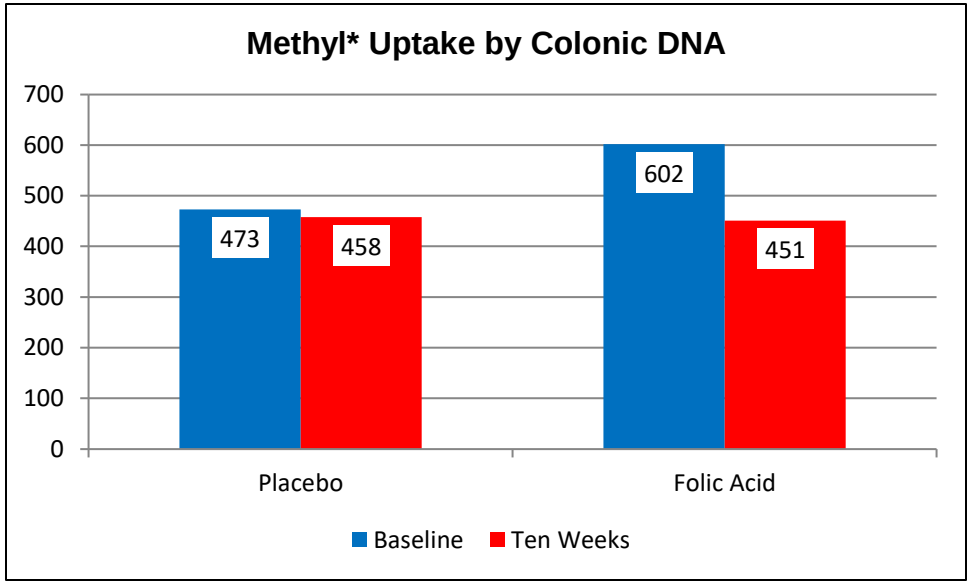






FOLIC ACID and DNA METHYLATION

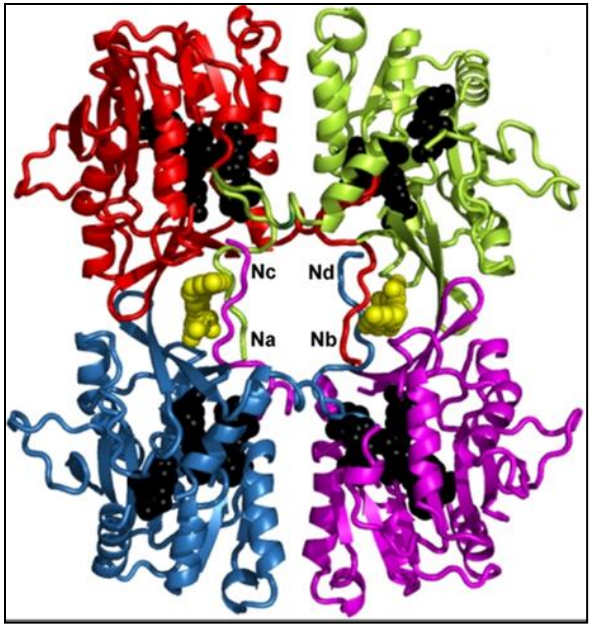


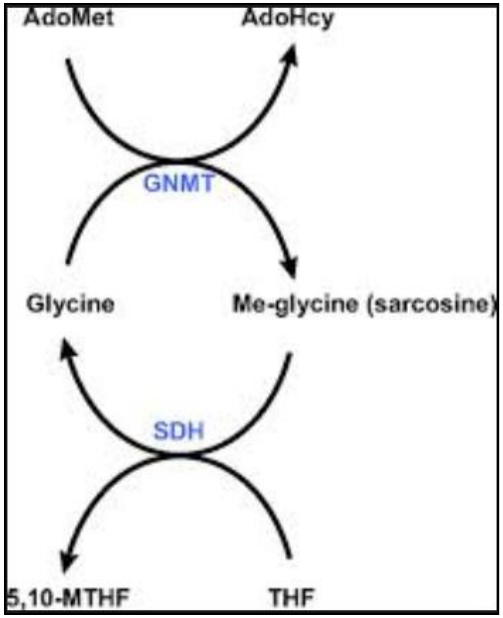


SAMe METHYL TRANSFER REACTIONS

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
COMT	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

GLYCINE N-METHYL TRANSFERASE



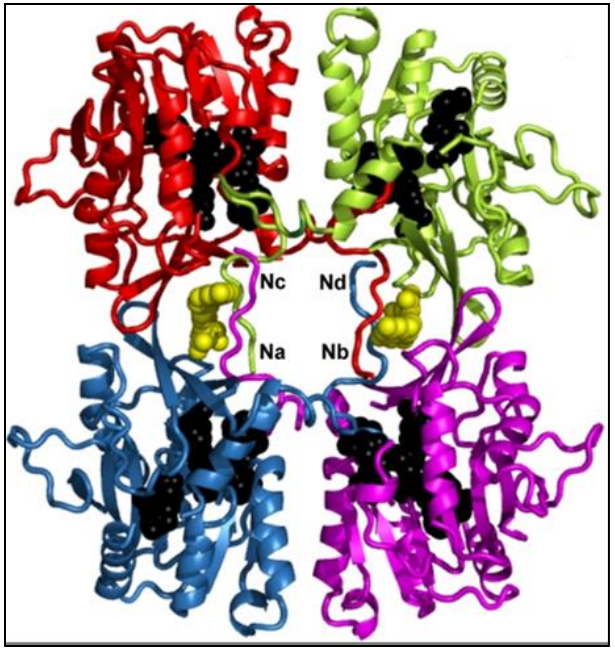


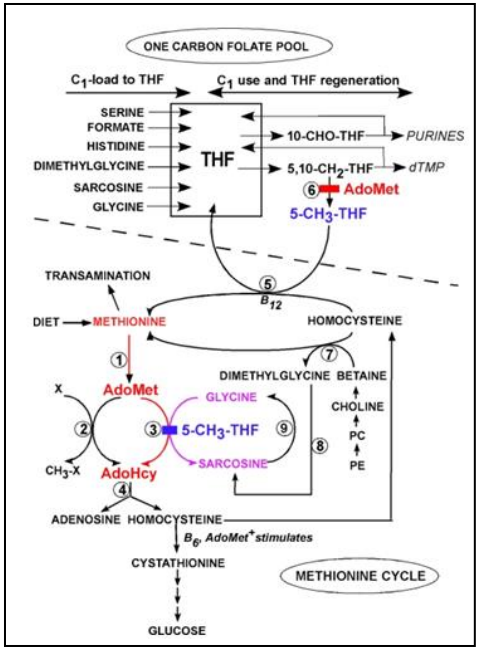
SAMe + Glycine $\xrightarrow[\text{GNMT}]{}$ Sarcosine (Methylglycine) + SAH

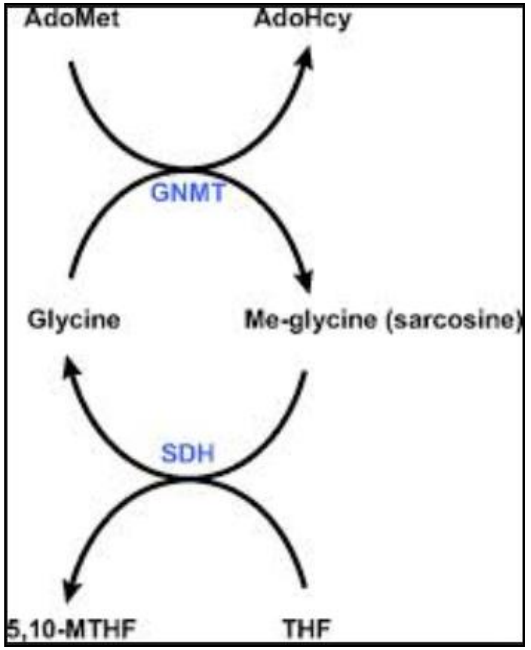
Sarcosine + THF $\xrightarrow[\text{Sarcosine Dehydrogenase}]{}$ Glycine + 5,10-Methylene THF

SAMe blow off valve $\Rightarrow$ Shuttles CH_3 away from SAMe and towards Methyl-folate/DNA synthesis

GLYCINE N-METHYL TRANSFERASE







Not directly stimulated by high SAMe

Negatively regulated (inhibited) by Methyl-folate

High SAMe inhibits MTHFR $\rightarrow$ Low Methyl-folate $\rightarrow$ Disinhibiton of GNMT

$\Rightarrow$ Shift from SAMe reformation to 5,10-Methylene THF

SAMe METHYL TRANSFER REACTIONS

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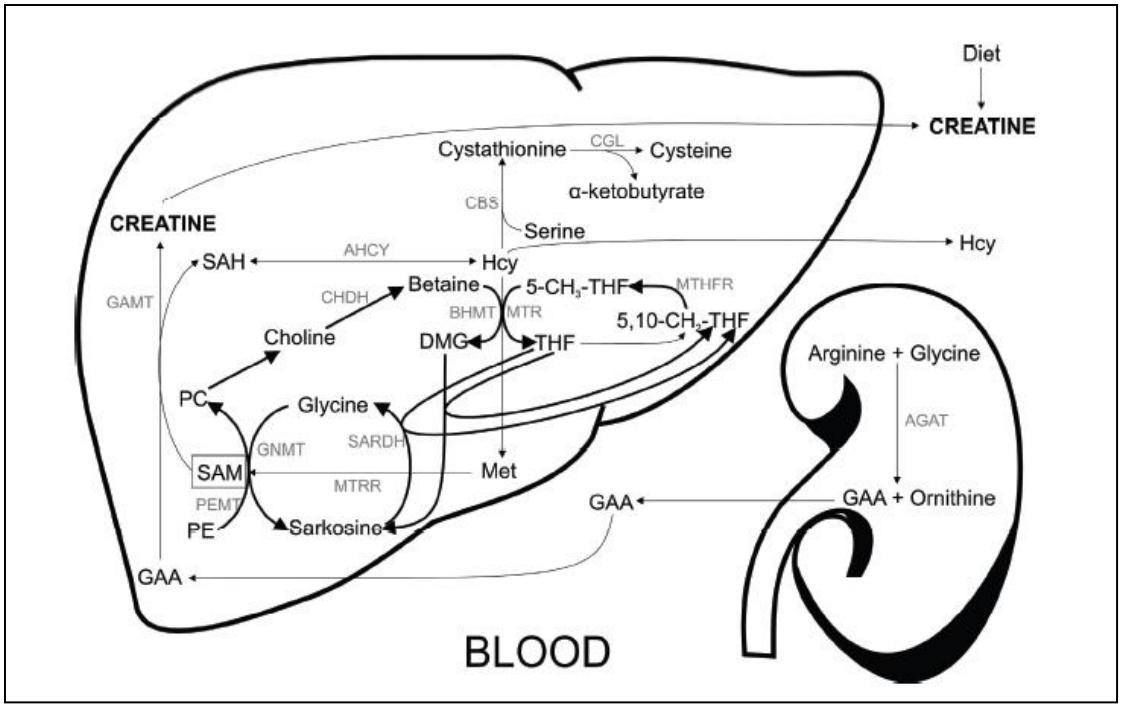
GAMT Guanidinoacetate N-Methyl Transferase	Generation of Creatine
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF

GUANIDINOACETATE N-METHYL TRANSFERASE (GAMT)

GAMT

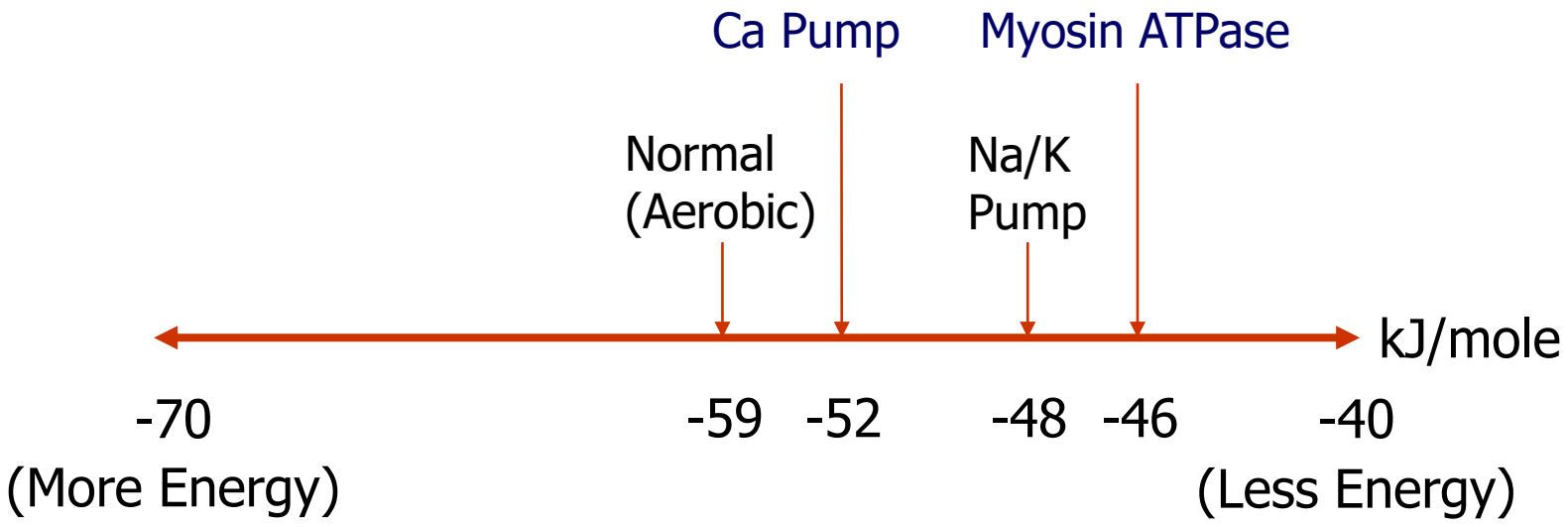
GAA + SAMe ⟶ Creatine + SAH

GAMT
Stimulated by GAA
Not inhibited by Creatine



Arginine Glycine Amidino Transferase (AGAT)
Amidino group of arginine is transferred to glycine, forming guanidinoacetate (GAA) & ornithine
(Inhibited by Creatine)

A High [ATP] is the Driving Force Underlying all Cellular Functions



As [ATP] falls, one by one, cellular functional mechanisms become depressed.

ATP… A Renewable Energy Source

When oxygen, calories and co-factors are available…

$$ATP \longrightarrow Work + ADP + P_i \Longrightarrow ADP + P_i + energy \longrightarrow More\ ATP$$

When oxygen is not available (as in heart disease and/or exercise)…

$$ATP \longrightarrow Work + ADP + P_i \Longrightarrow ADP + P_i + \text{no energy} \longrightarrow \text{no more ATP}$$

$$PCr + ADP \longrightarrow Cr + ATP$$

$$ADP + ADP \longrightarrow ATP + AMP$$

$$AMP \longrightarrow Adenosine + Pi$$

Adenosine diffuses out of the cell and is lost

When oxygen is re-supplied…

Oxidative Phosphorylation + Pi + no more ADP $\rightarrow$ No ATP

CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

♥ Male Sprague-Dawley rats (250-300 gm.)

Ad lib chow and water intake

Baseline measurements

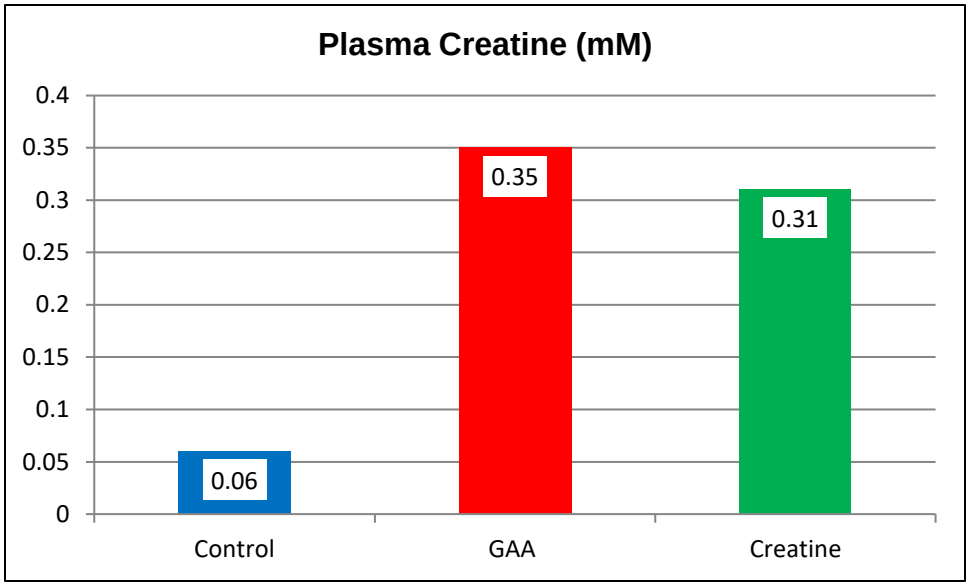
Supplement chow with:

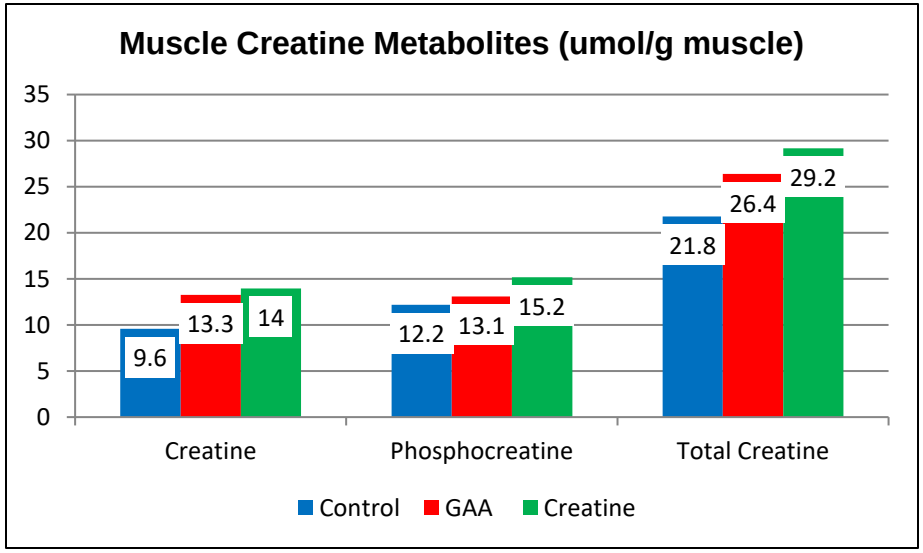
- Creatine monohydrate 0.4%
- Guanidinoacetate 0.36%

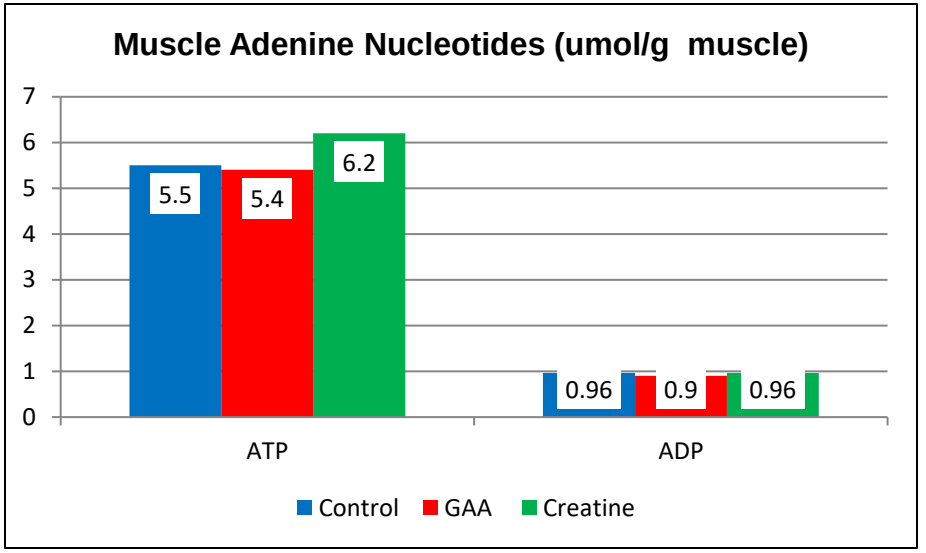
Sacrifice at two weeks and evaluate:

- Plasma creatine
- Muscle creatine metabolites

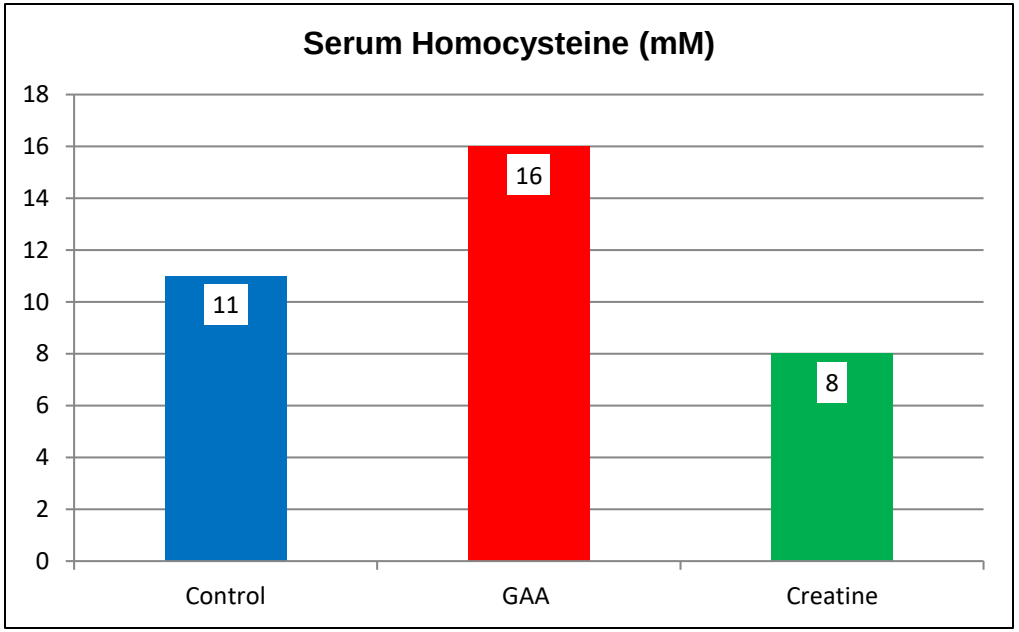
CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

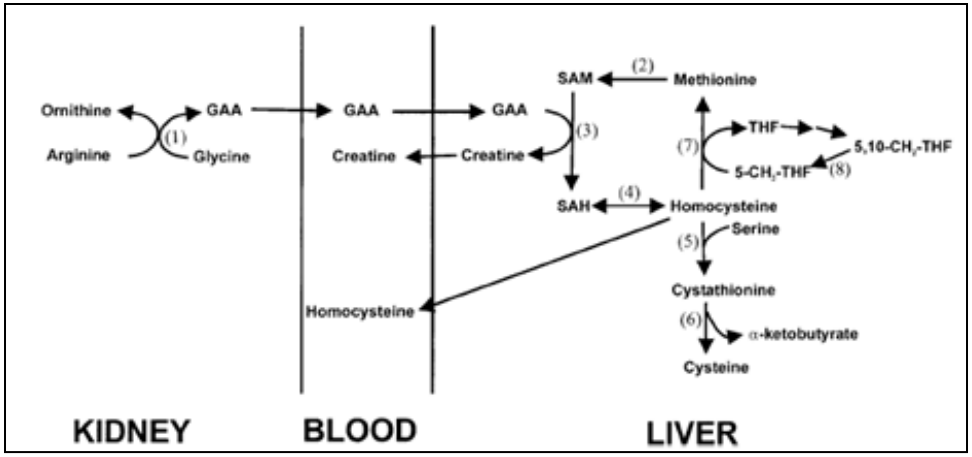






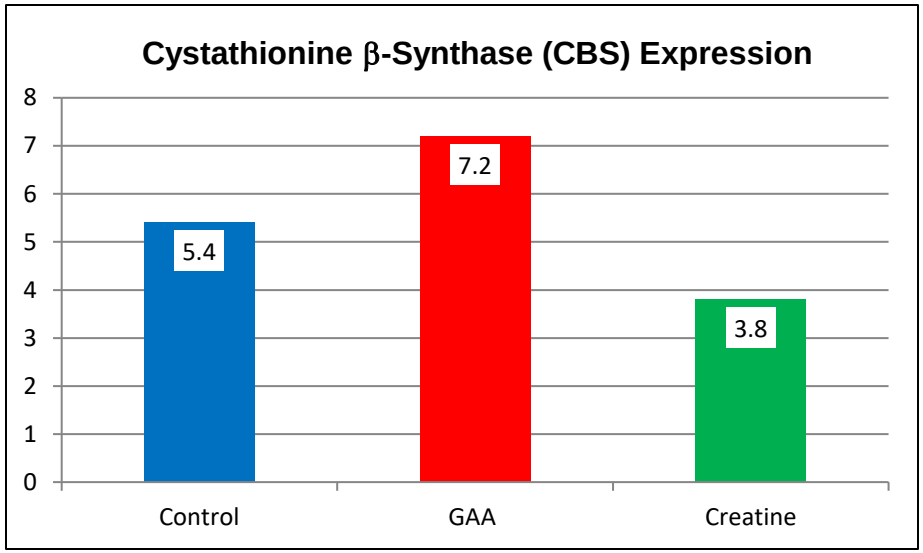
CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

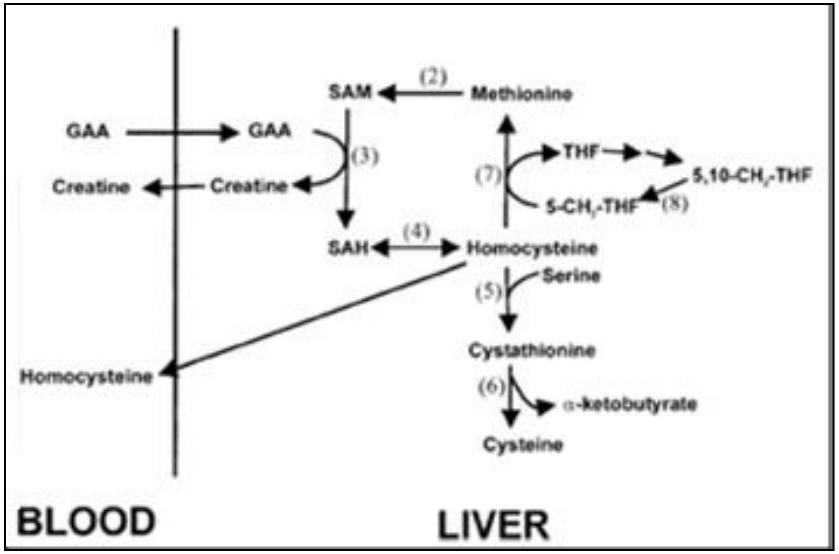


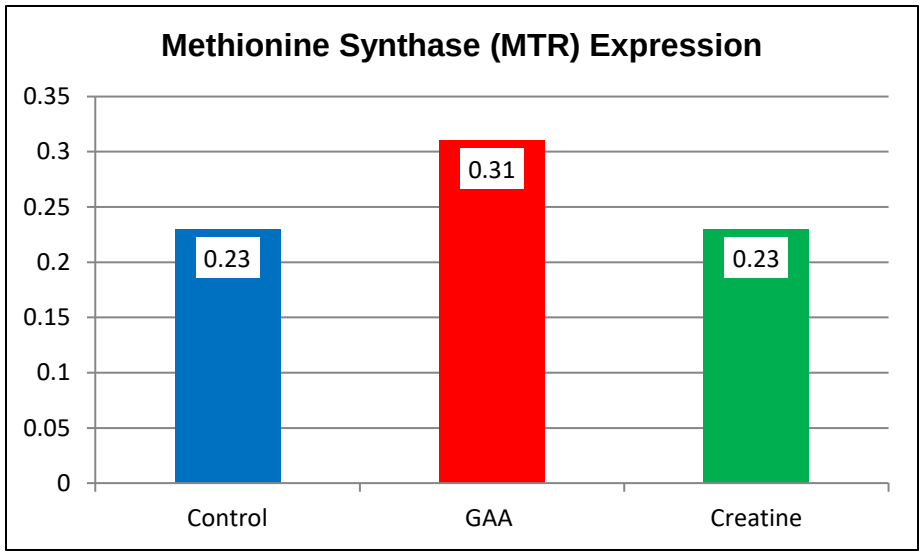


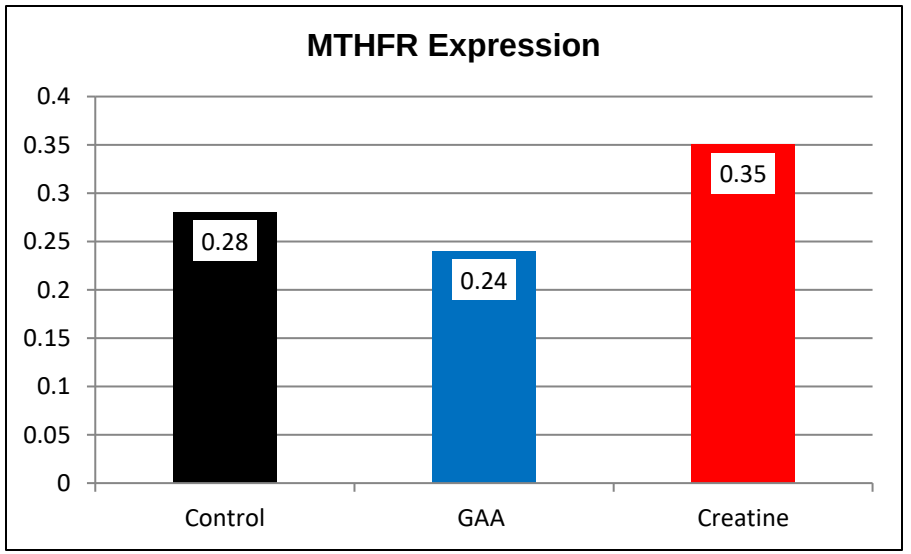
AGAT GAA GAMT → CREATINE and SAH ⇒ Hcy

CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM





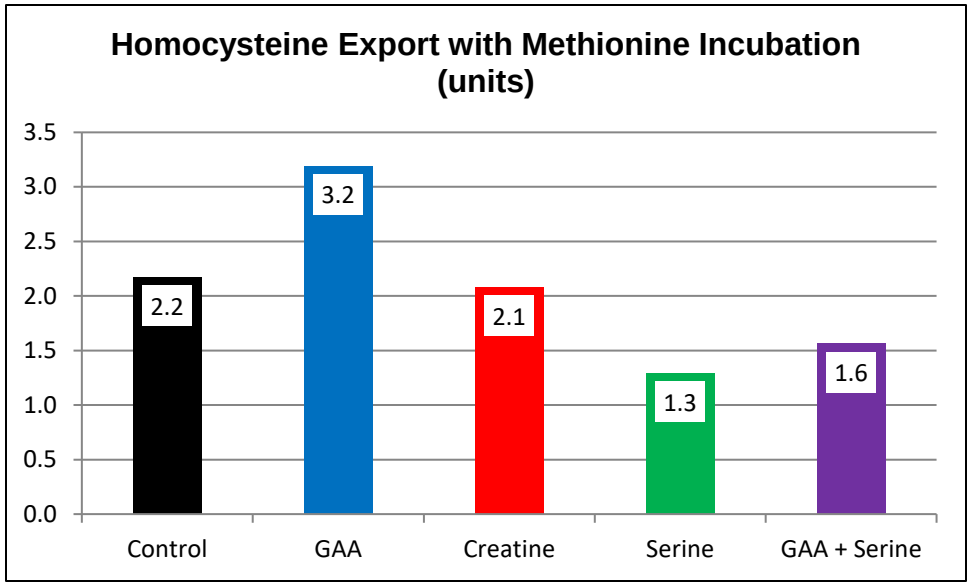


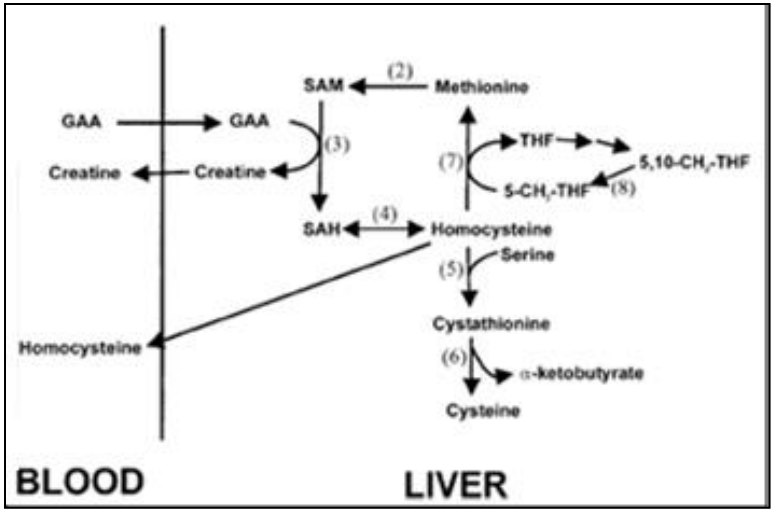


SAMe → GNMT → Sarcosine → 5,10MeTHF

CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

♥ Incubate hepatocytes with Methionine +/- other substances





CREATINE to DECREASE HOMOCYSTEINE

♥ Ten 24-28 year old male athletes

Record at baseline:

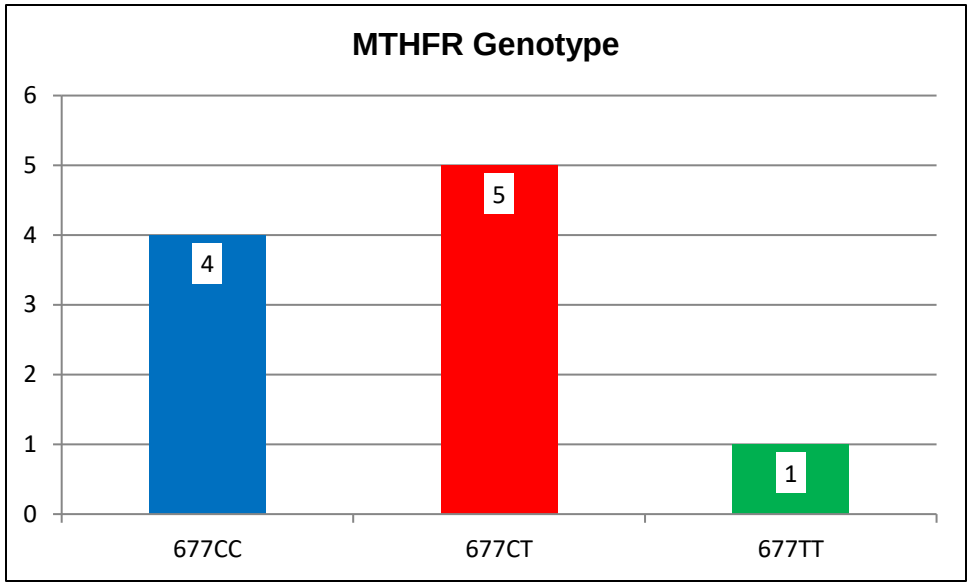
- Homocysteine
- MTHFR genotype

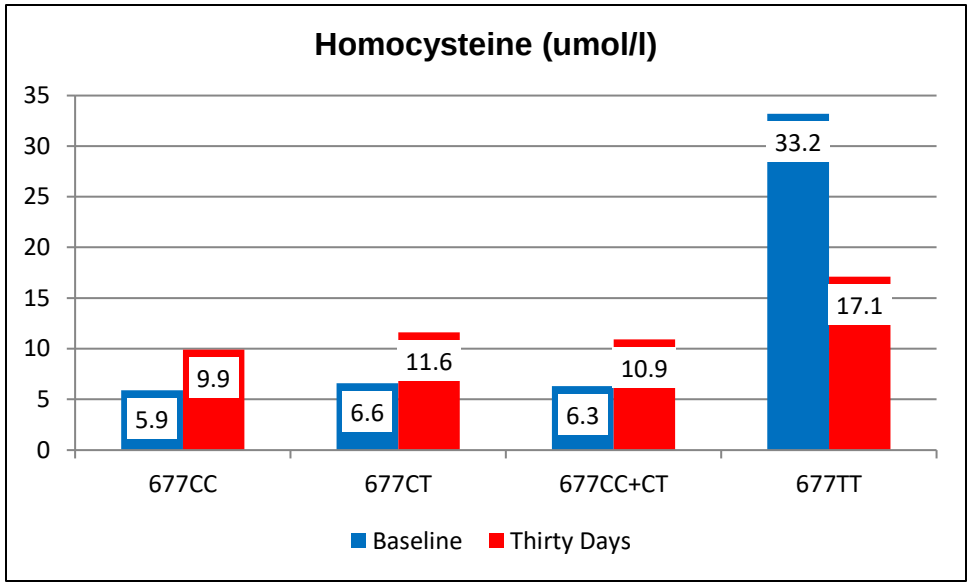
Treat all with creatine 5 gm/day

- No additional B vitamins
- Diet and activity level unchanged

Repeat homocysteine level at 30 days

CREATINE to DECREASE HOMOCYSTEINE





↓ AGAT
less GAA

↓ GAMT
less SAMe → SAH

↑ GNMT
SAMe → 5,10-THF

CREATINE to DECREASE HOMOCYSTEINE

♥ 16 healthy volunteers (young adults – mean age 30 years)
- Mean Homocysteine 6.7 umol/l
- Normal folate, B6, and B12 levels

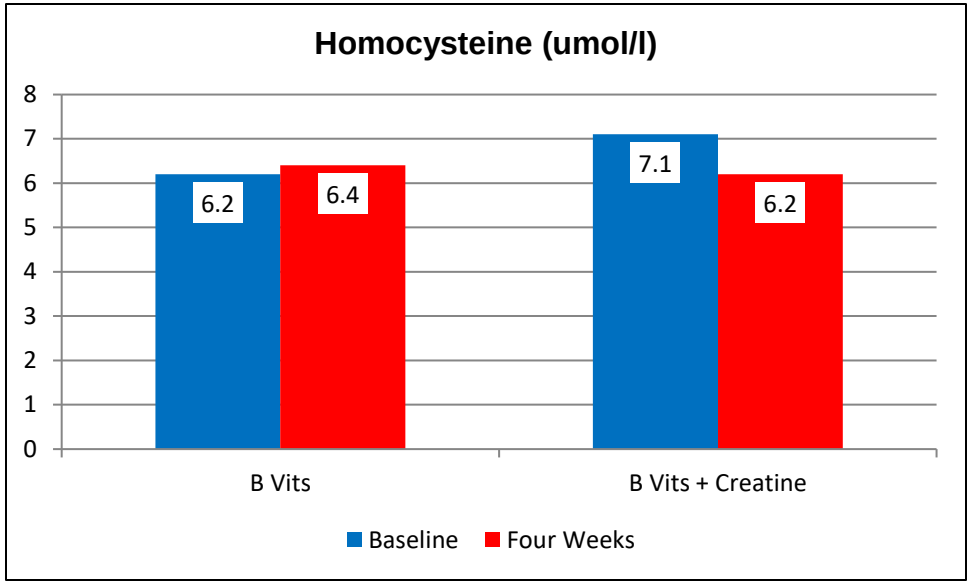
Treat all with Folate 400 mcg, B6 6 mcg, and B12 2 mg over four weeks

Record Homocysteine level

Randomize to receive over an additional four weeks:
- Ongoing B Vitamin supplementation
- B Vitamins + Creatine 2.2-5.1 gm/day (daily creatine excretion x 2)

Repeat homocysteine level at four weeks

CREATINE to DECREASE HOMOCYSTEINE



Creatine group:

- Homocysteine decreased in 7/8
- 18-27% decrease in 4/8

Control group:

- Homocysteine decreased in 3/8
- Only by 1-9%

CREATINE and CONGESTIVE HEART FAILURE

♥ 20 male patients with stable CHF

- Mean age 65 years
- 70% ischemic and 25% dilated, and 5% valve disease
- NYHA 2.9
- Furosemide 260 mg/day

Baseline exercise capacity (handgrip strength and endurance)

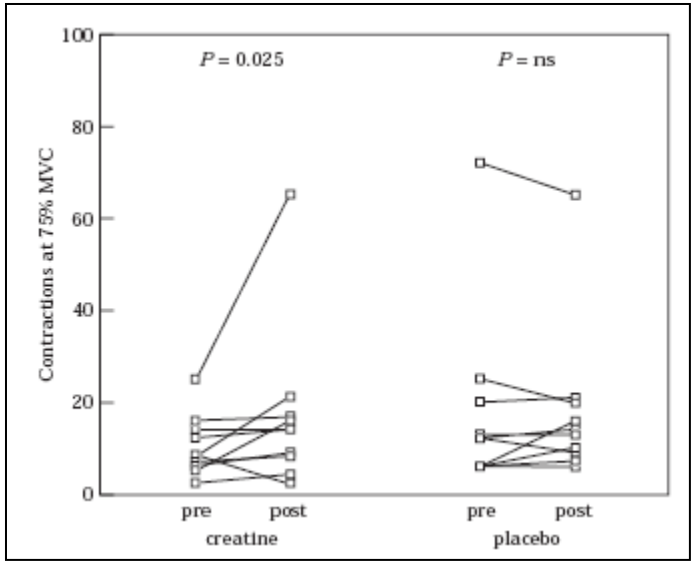
Randomize to receive over five days:

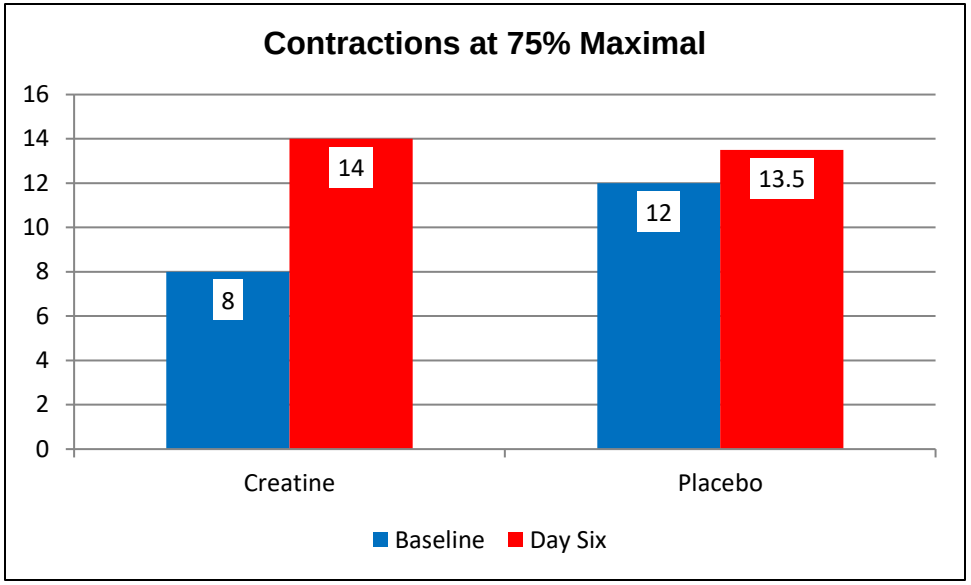
- Creatine 5 gm qid
- Placebo qid

Repeat baseline measurements

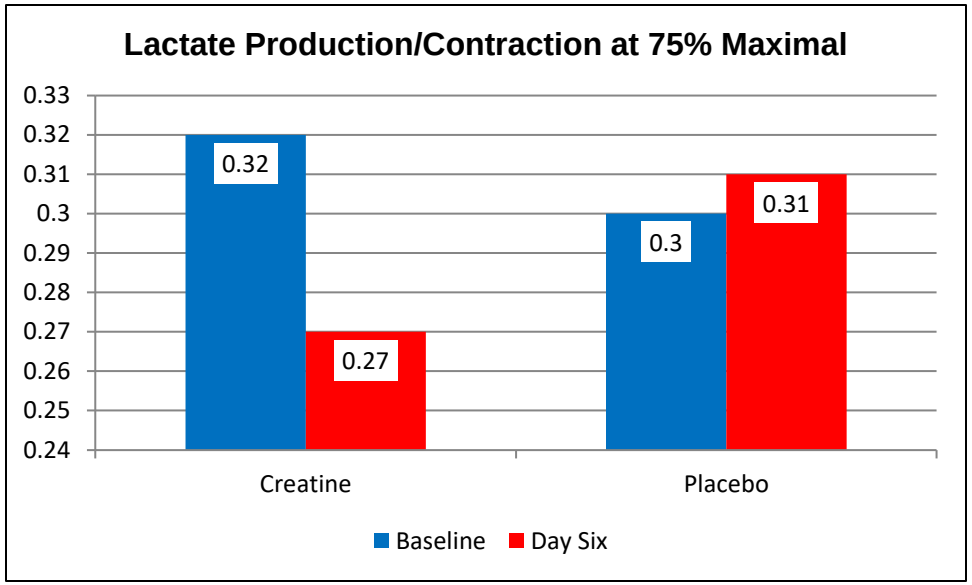
Double blind protocol followed

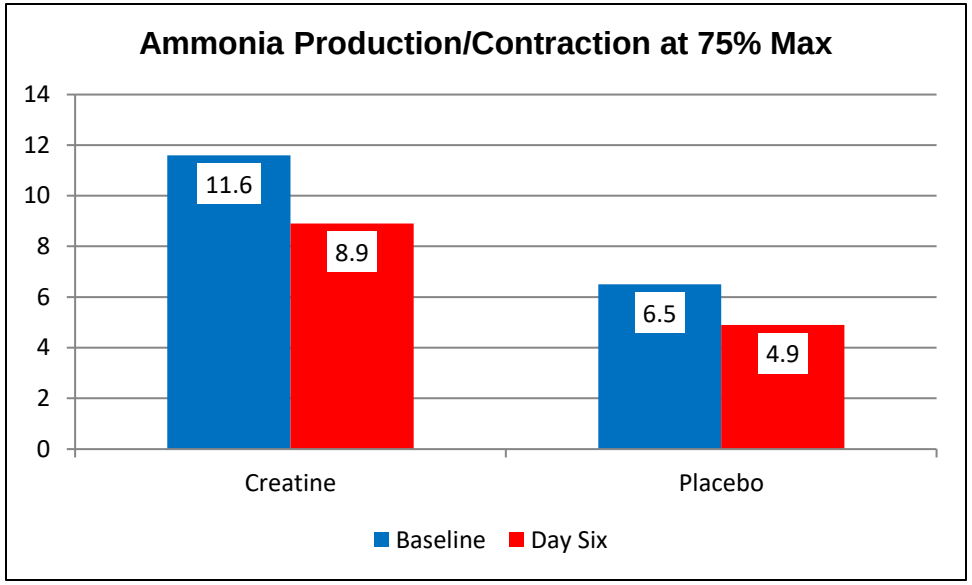
CREATINE and CONGESTIVE HEART FAILURE





CREATINE and CONGESTIVE HEART FAILURE





CREATINE and LIPID CONTROL

♥ 34 subjects with hyperlipidemia

Baseline studies

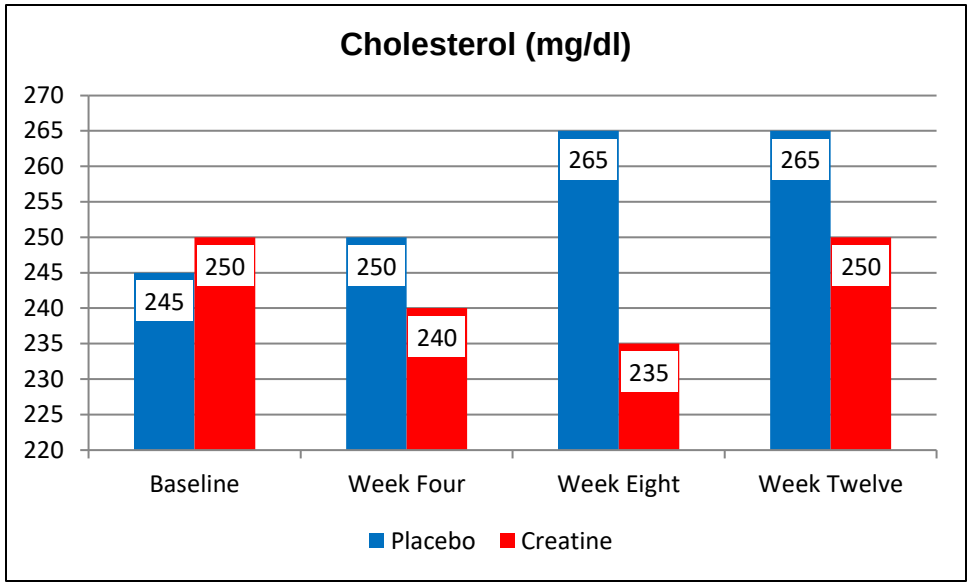
Randomize to receive over eight weeks:

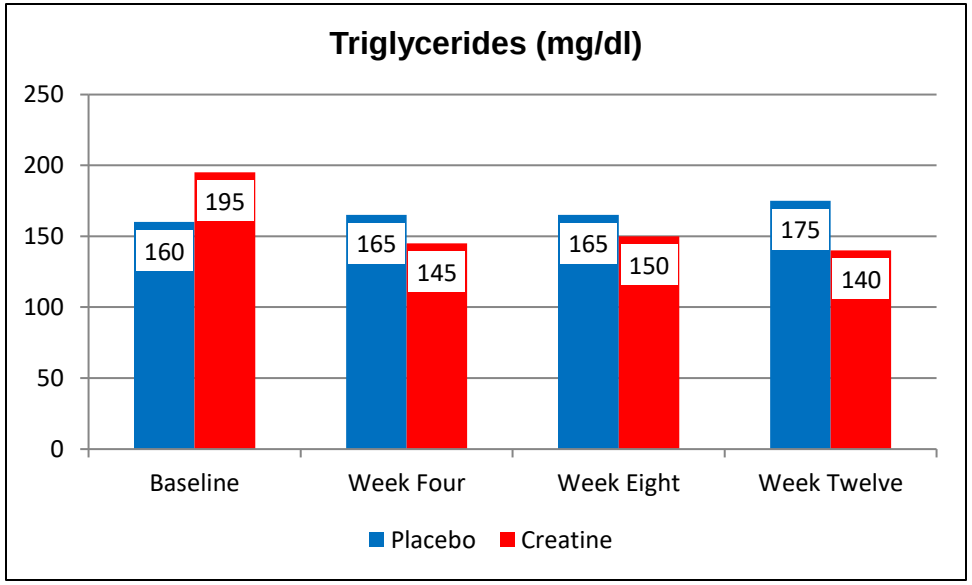
- Creatine 5 gm qid x 5 days with 5 gm bid to follow
- Placebo (flavored) at same schedule

Repeat measurements at weeks four, eight, and twelve

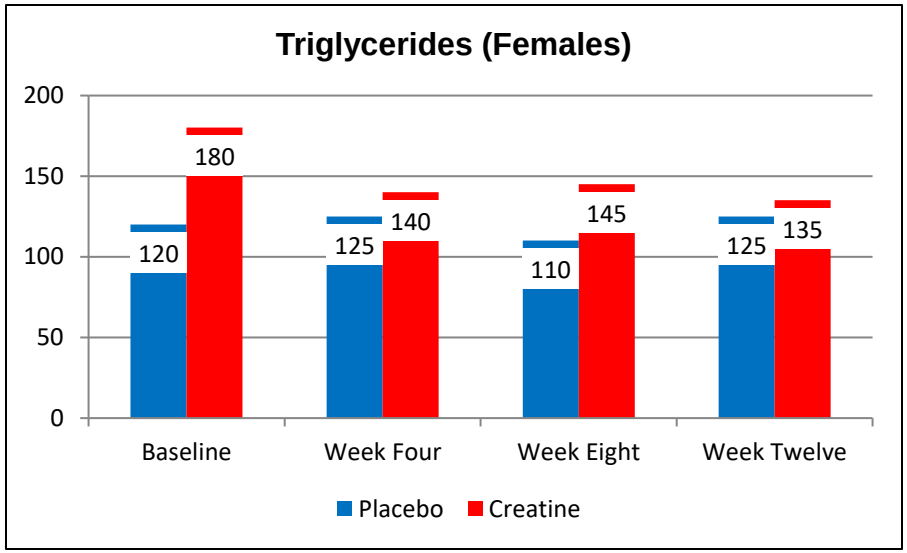
Double blind protocol followed

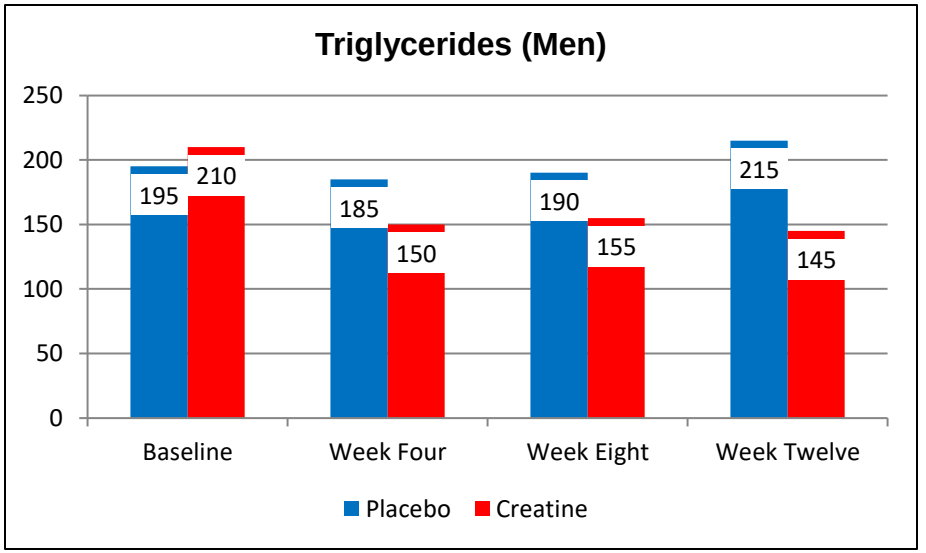
CREATINE and LIPID CONTROL





CREATINE and LIPID CONTROL





- Creatine spares SAMe → Phosphatidylcholine
- Creatine reduces SAH and Homocysteine formation
- Homocysteine
  - ♣ Decreases expression of AMPK
  - ♣ Increases expression of HMG Co-A Reductase
- SAH (lower SAMe:SAH) compromises fatty acid oxidation

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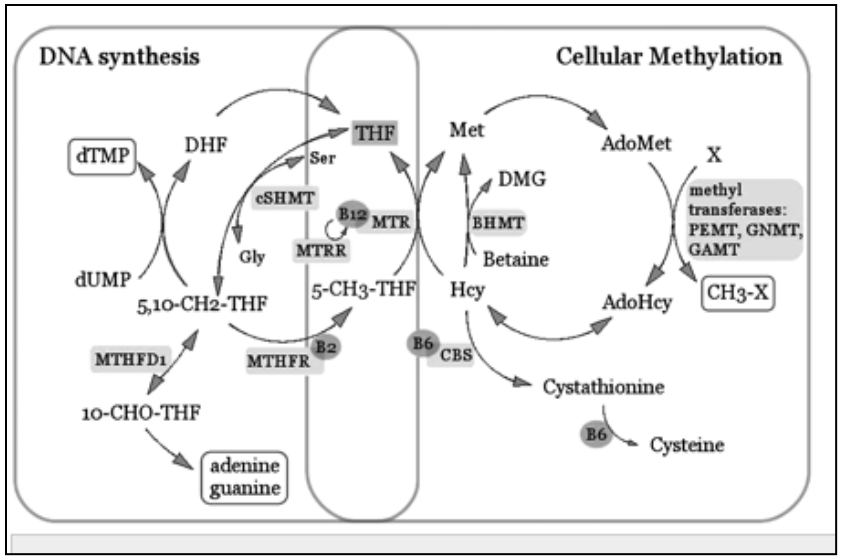
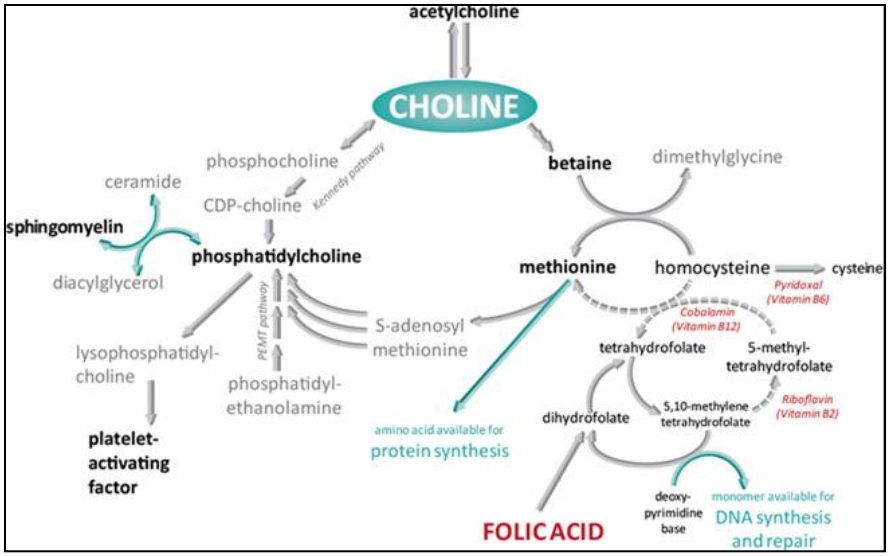
GNMT Glycine-N-Methyl Transferase	SAMe → 5,10-MethyleneTHF

PHOSPHATIDYL ETHANOLAMINE N-METHYL TRANSFERASE

PEMT



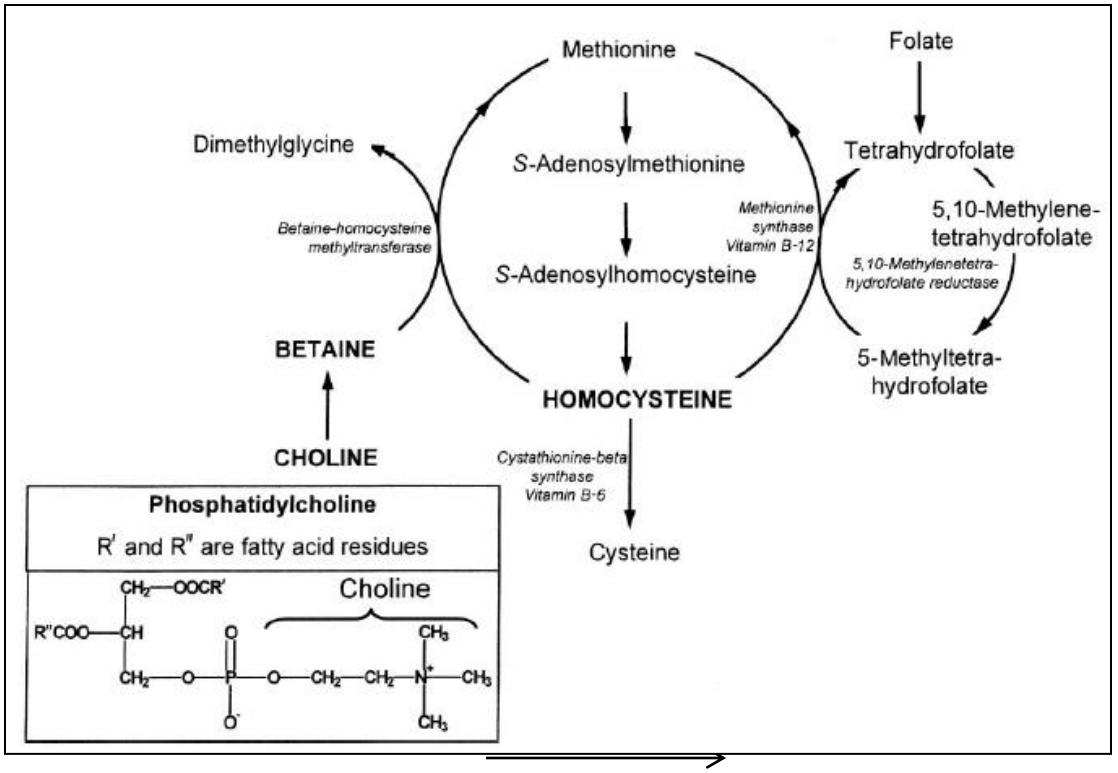


Phosphoethanolamine + 3 SAMes ⟶ Phosphatidylcholine + 3 SAHs

Phosphatidylcholine:

- Cell membrane
- Lipid metabolism
- Acetylcholine

PHOSPHATIDYL ETHANOLAMINE N-METHYL TRANSFERASE



Phosphphatidylcholine $\rightarrow$ Choline $\rightarrow$ Betaine (TMG) $\Rightarrow$ BHMT pathway

Generate metabolic product and promote SAMe reformation

PHOSPHATIDYLCHOLINE and HOMOCYSTEINE

♥ Forty eight healthy men
- None taking B vitamins, PC, choline, or betaine
- None with Hcy > 26 umol/l

Study the 26/48 with elevated Hcy (11-23.1 umol/l)

Mean Hcy 14.7 umol/l

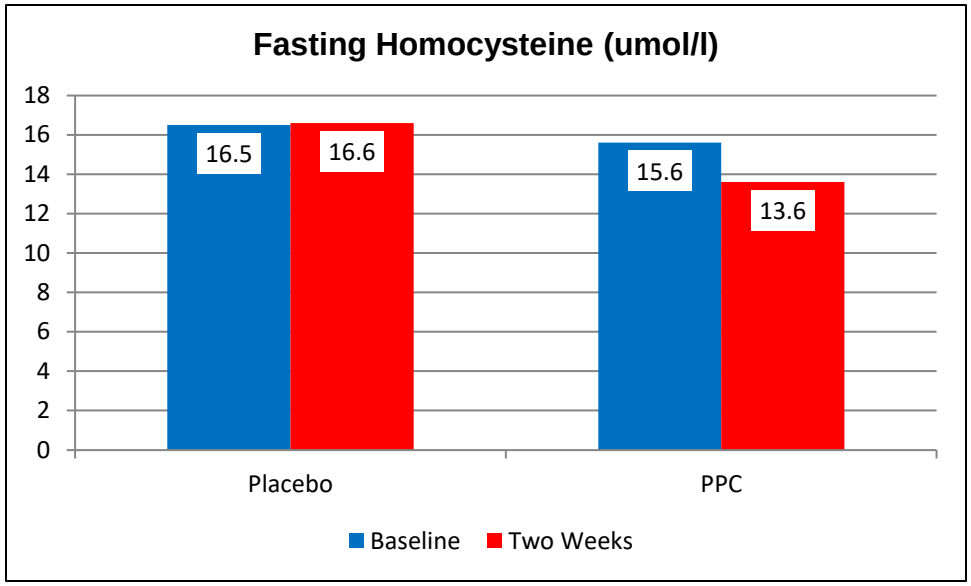
Baseline measurements

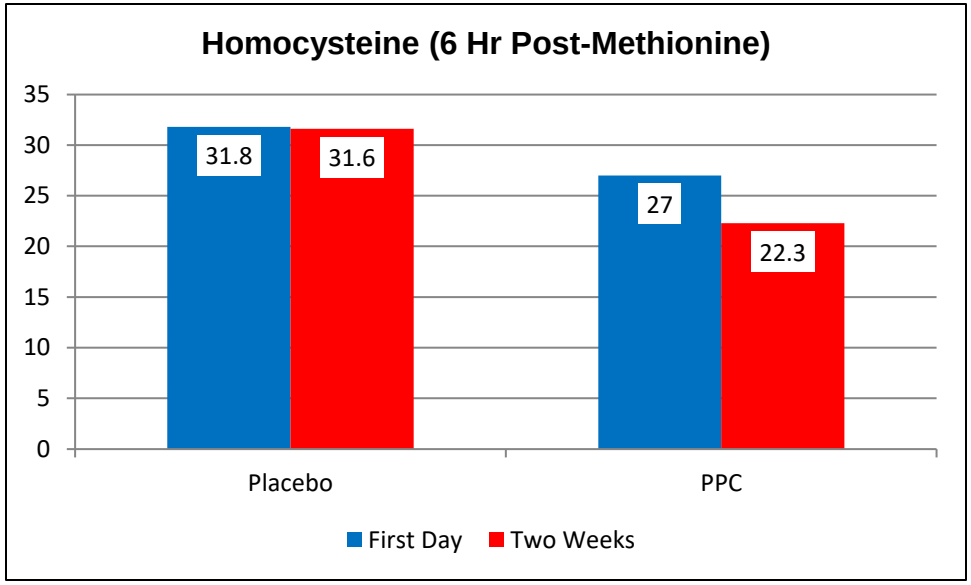
Randomize to receive over two weeks:
- 34 gm PPC (1/2 dose bid with meals) to provide 2.6 gm choline
- 25 gm placebo oil (same fatty acid composition)

Repeat baseline measures and cross over to opposite regimen (after 2 week washout)

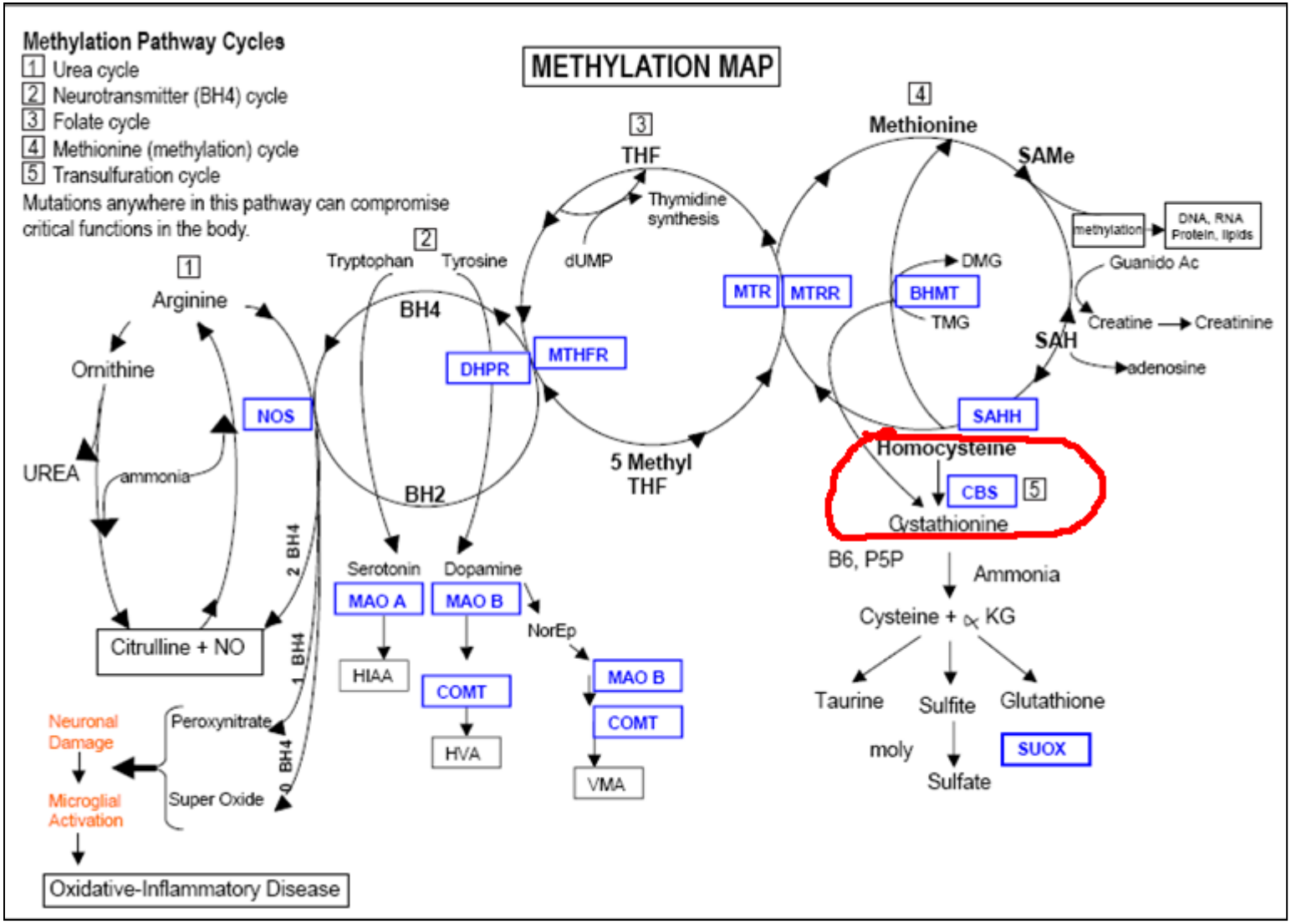
Double blind protocol followed

PHOSPHATIDYLCHOLINE and HOMOCYSTINE



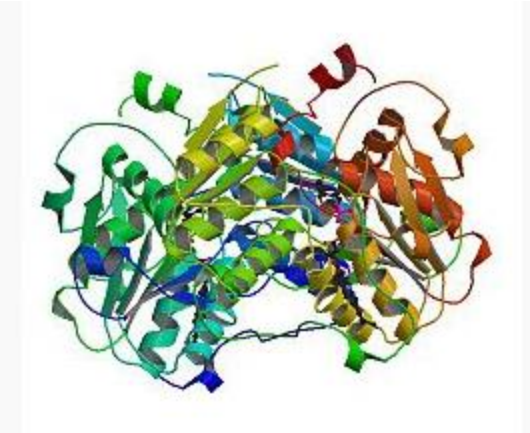


CYSTATHIONINE BETA SYNTHASE (CBS)



Trans-Sulfuration Pathway Gatekeeper

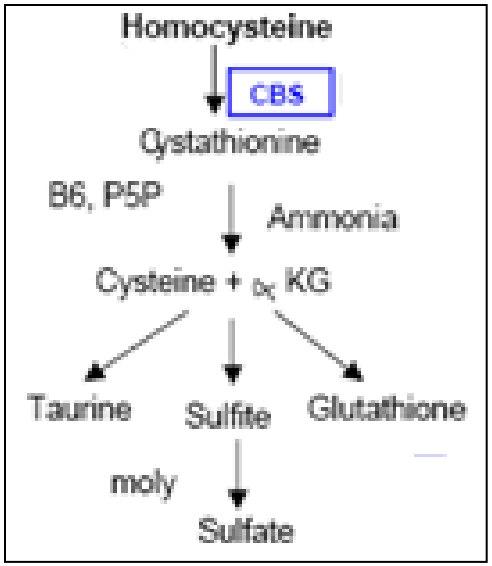
CYSTATHIONINE BETA SYNTHASE (CBS)



Serine + Homocysteine $\xrightarrow{\text{P-5-P}}$ Cystathionine

Cystathionine $\longrightarrow$ Cysteine + Ammonia

Cystathionine Gamma Lyase



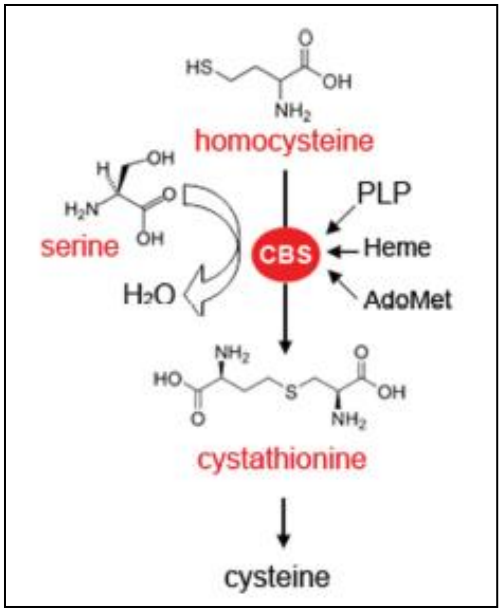
Down stream production of:

- Cysteine and Glutathione
- Taurine and Sulfate
- Hydrogen sulfide
- Alpha-ketobutyrate

CYSTATHIONINE BETA SYNTHASE

Serine + Homocysteine $\xrightarrow{\text{P-5-P}}$ Cystathionine

Cystathionine $\xrightarrow{\text{Cystathionine Gamma Lyase}}$ Cysteine + Ammonia

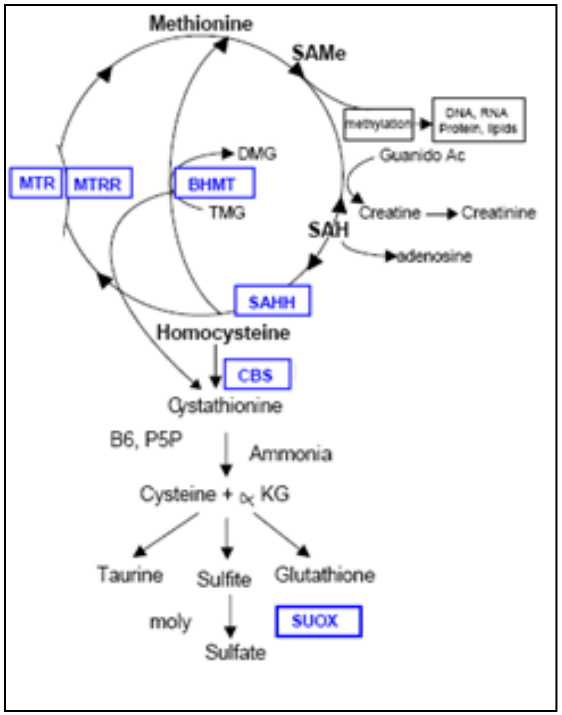


Up regulated by:

- Oxidative stress (H_2O_2)
- Inflammatory cytokines (TNF-alpha)
- SAMe (Methionine load)
- Hyperglycemia
- Serine (Glycine)
- Danshensu

Down regulated by:

- Absence of the above
- Cysteine
- Insulin



CYSTATHIONINE BETA SYNTHASE (CBS)

Serine + Homocysteine $\xrightarrow{\text{P-5-P}}$ Cystathionine

Cystathionine $\xrightarrow[\text{Cystathionine Gamma Lyase}]{}$ Cysteine + Ammonia

CBS Loss of Function (Kilmer McKully MD)

Hcy > 50 $\rightarrow$ High SAH $\rightarrow$ Low SAMe:SAH:

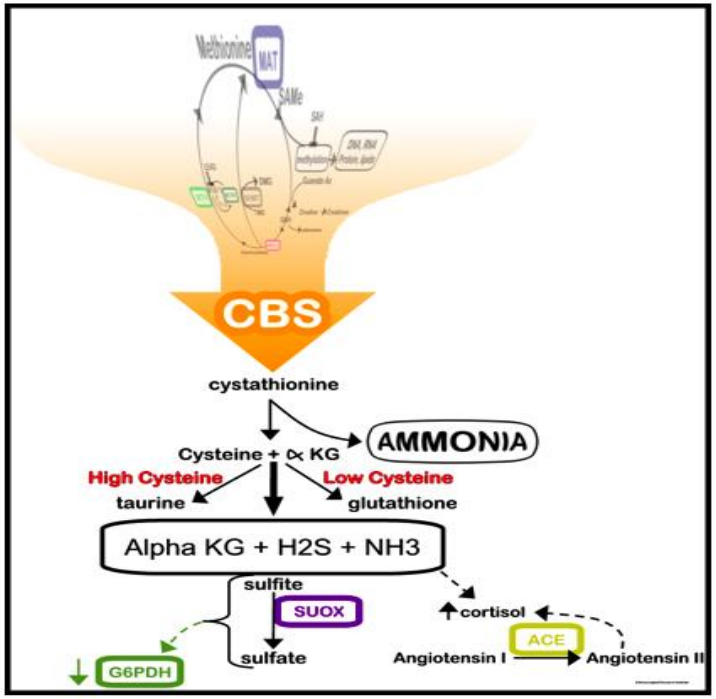
- Methylation blocked
- Low glutathione and cysteine $\rightarrow$ Oxidative stress
- Low taurine and sulfate $\rightarrow$ Impaired detoxification

$\Rightarrow$ Premature atherosclerosis and neurologic disease

CYSTATHIONINE BETA SYNTHASE (CBS)

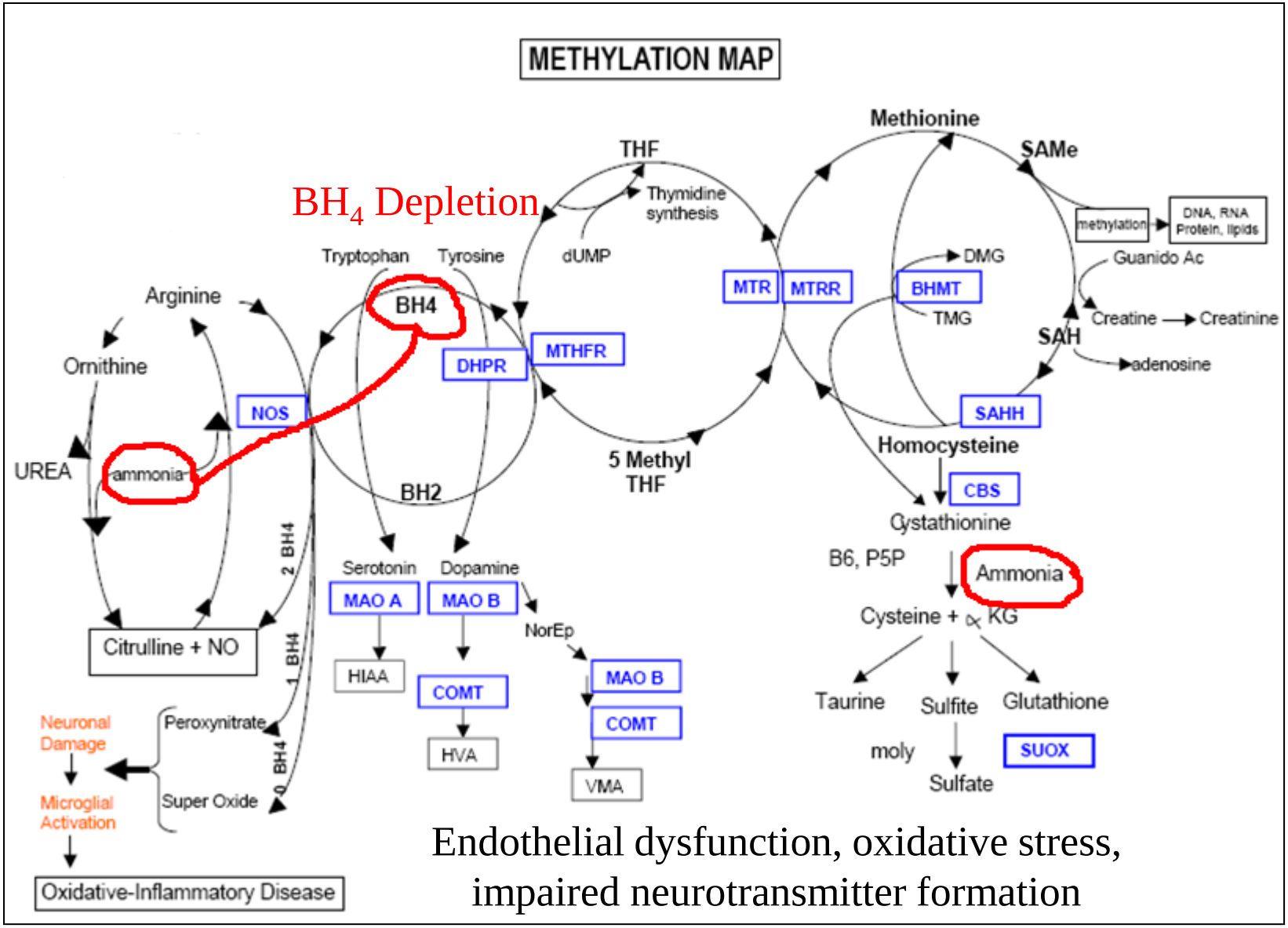
CBS Gain of Function

- Oxidative/Inflammatory stress
- CBS C699T (10-fold up regulation)
- CBS A360A (less powerful)



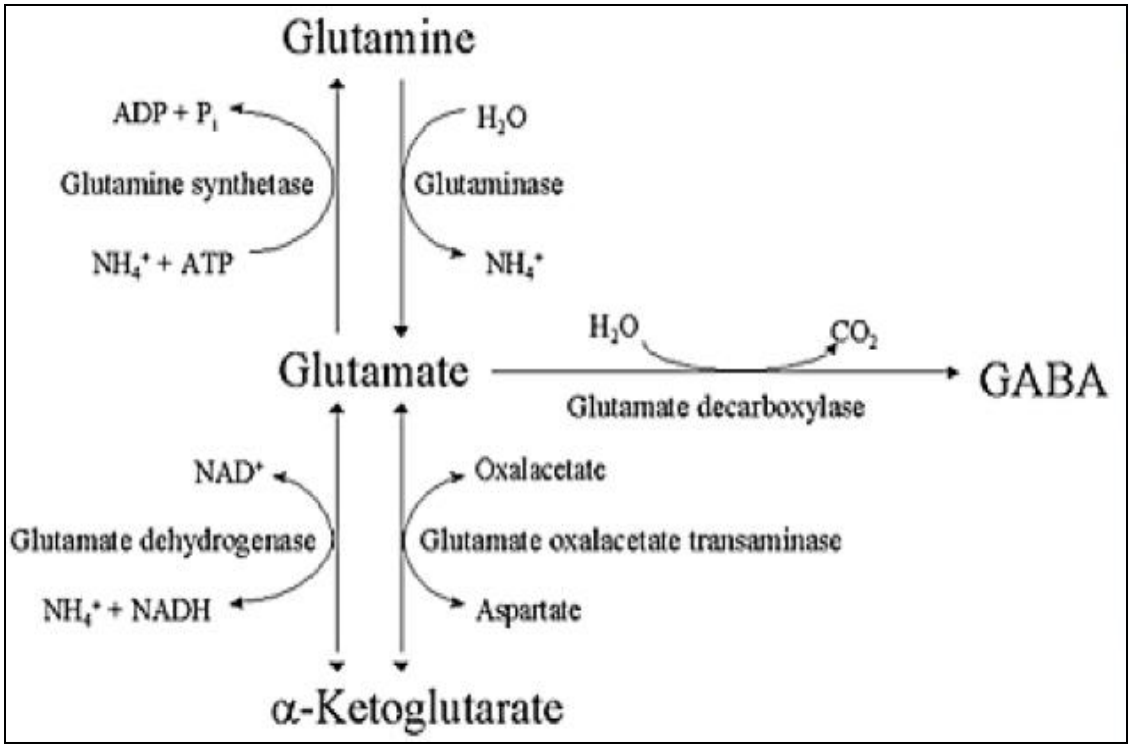
Hcy remethylation (via MTR and BHMT) to SAMe compromised
Excess Sulfite (neurotoxic) and sulfate (fight or flight → RAS)
Hydrogen sulfide → Brain fog and platelet activation
Glutamate → Excitotoxicity
Ammonia → BH4 used up in ammonia metabolism
High cysteine and glutathione → Impaired detoxification (?)
Predisposition to asthma and GERDz

CYSTATHIONINE BETA SYNTHASE (CBS)



Endothelial dysfunction, oxidative stress, impaired neurotransmitter formation

CYSTATHIONINE BETA SYNTHASE (CBS)

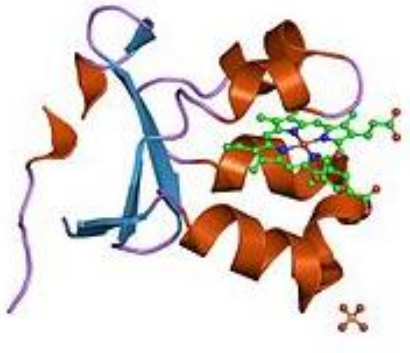


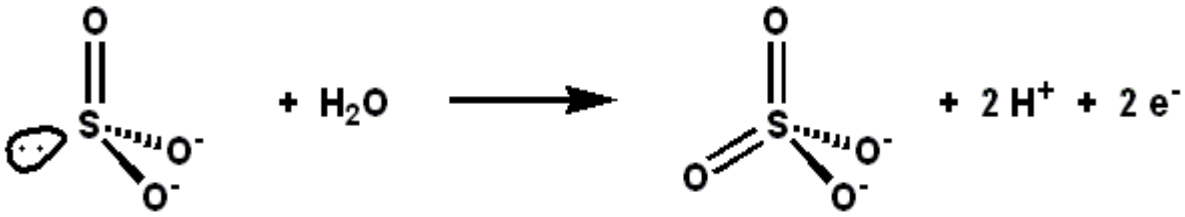
Interconversion compromised by metals (Lead)

Result is Glutamate Excess

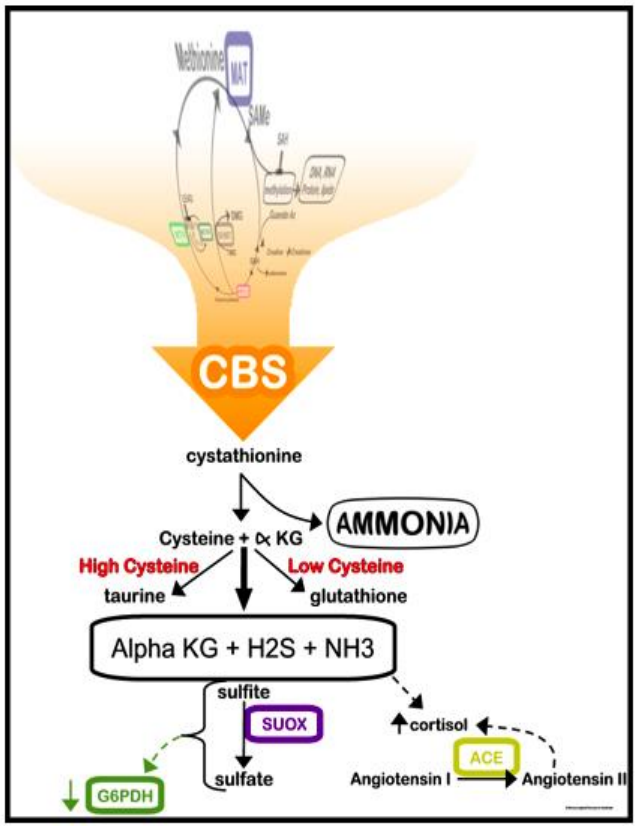
→ MSG-like Excitotoxicity

SULFITE OXIDASE





Sulfite + H2O ⟶ Sulfate



Down regulation not common → Sulfite excess

Co-factor depletion common → Sulfite excess

Molybdenum key co-factor

Boron, Hydroxy-B12, and Vitamin E Succinate accelerate SUOX activity

Support Sulfite Oxidase when CBS up regulated

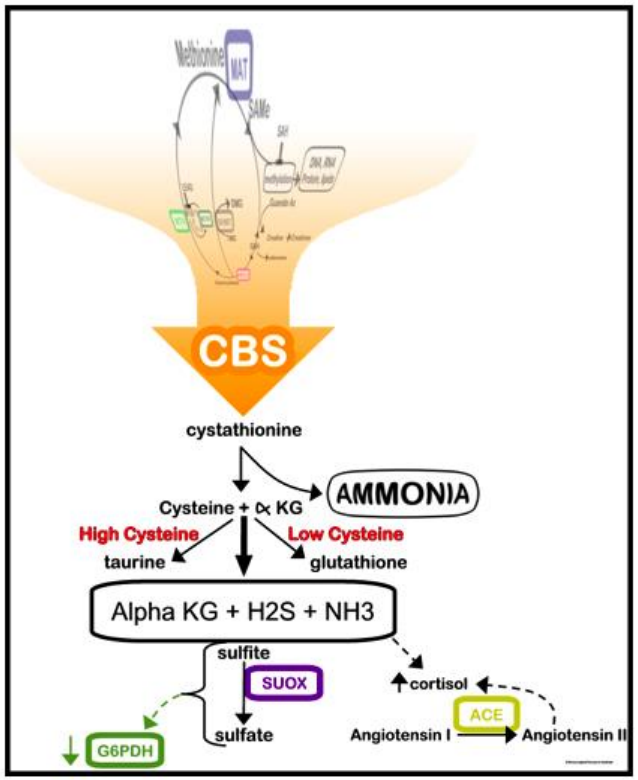
Sulfite worse than Sulfate

SULFATE EXCESS

“Sulfates” ≈ SH-bearing molecules involved in detoxification

High interstitial levels compromise up take across cell membrane →

Impaired endogenous detoxification



Dr. Yasko found

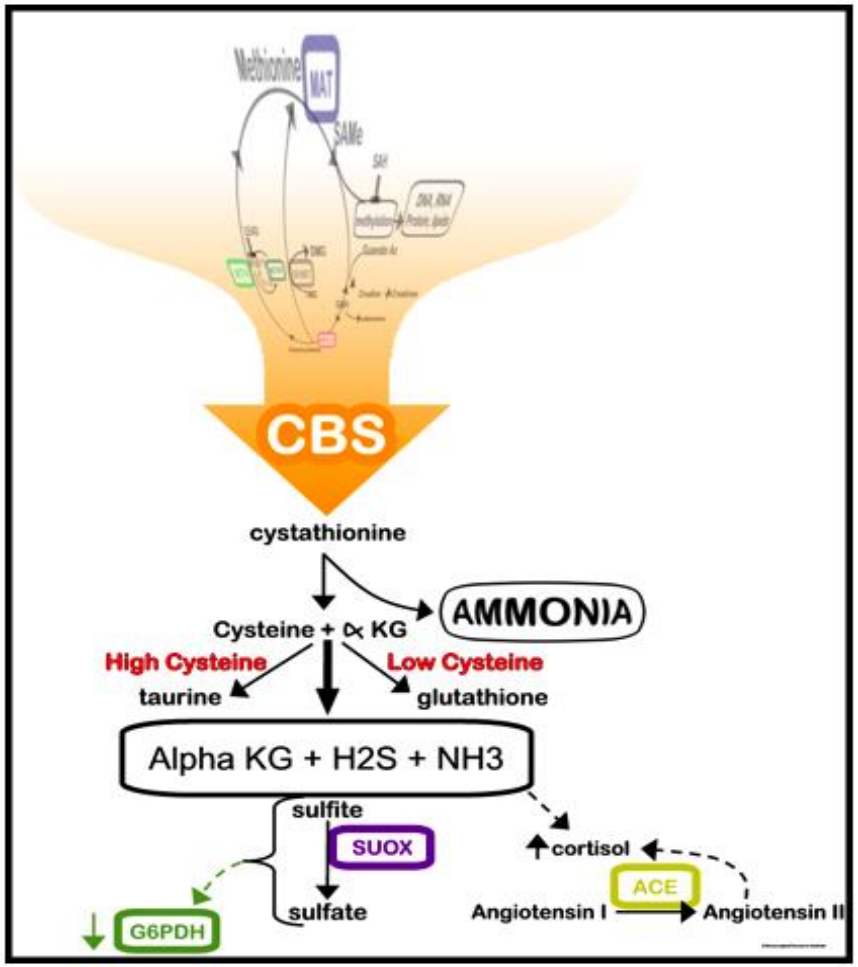
Strong link between

CBS and Autism

and related

Neurodevelopmental Disorders

CYSTATHIONINE BETA SYNTHASE (CBS)



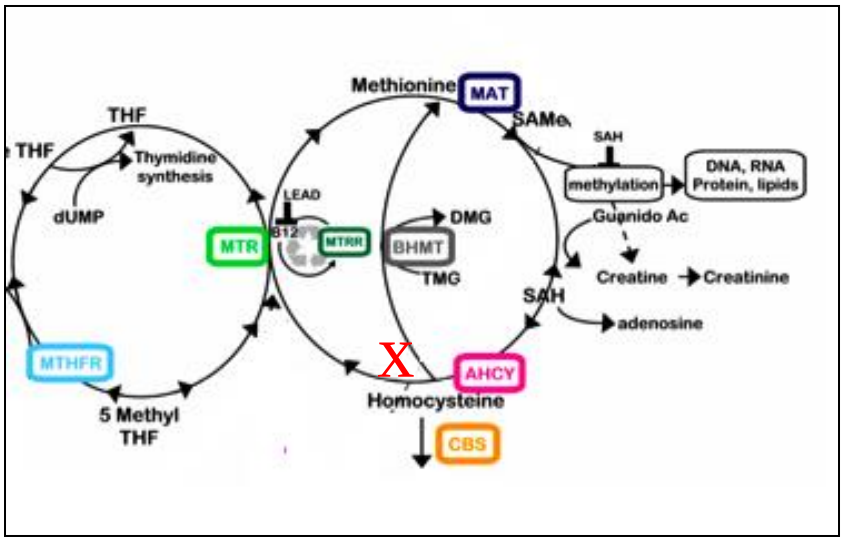
1. Most important abnormality

2. Most challenging to address

3. BHMT and MTHFR A1298C amplify pathophysiology ⇒

CBS C699T or CBS A360A

BETAINE-HOMOCYSTEINE METHYLTRANSFERASE (BHMT)



Homocysteine + Trimethylglycine $\longrightarrow$ Methionine + Dimethylglycine
BHMT (Zn)

"Back Door Reaction"

Direct remethylation of Homocysteine to Methionine

"Pulls" Homocysteine away from CBS "Drain"

BHMT defects thus "Push" Homocysteine down the CBS "Drain"

BETAINE and HOMOCYSTEINE

♥ 132 healthy subjects
- None taking B vitamins
- None with Hcy > 26 umol/l

Study the 76/132 with highest Hcy (8.4-22.2 umol/l)

Mean Hcy 10.7 umol/l

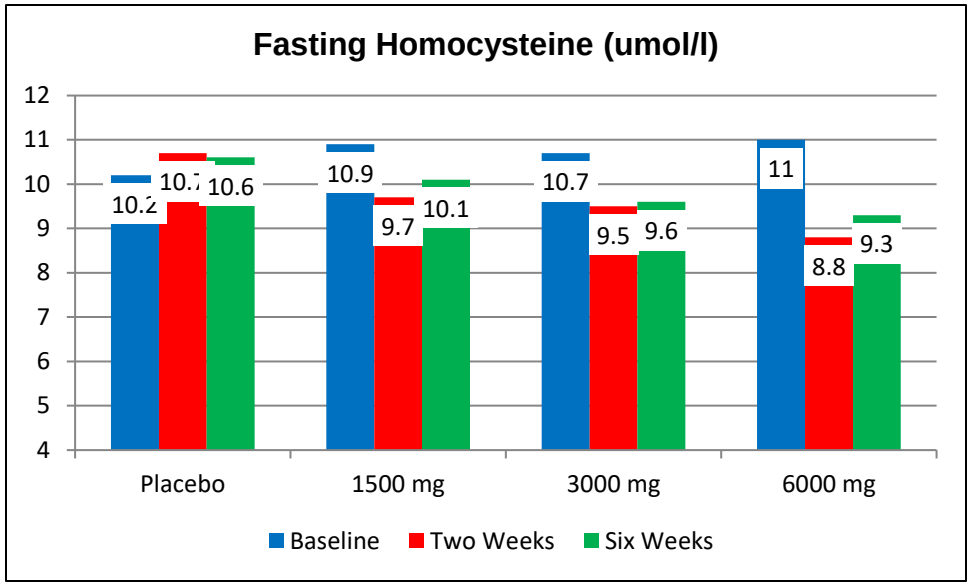
Baseline measurements after one week run in period

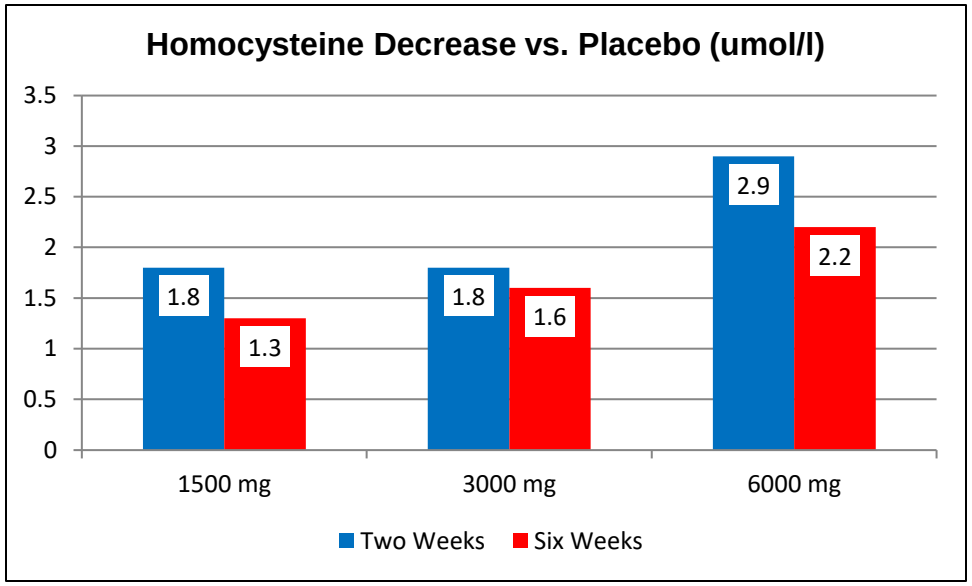
Randomize to receive over six weeks (1/2 dose in water bid):
- Placebo
- Betaine 1500 mg
- Betaine 3000 mg
- Betaine 6000 mg

Repeat measurements at weeks two and six

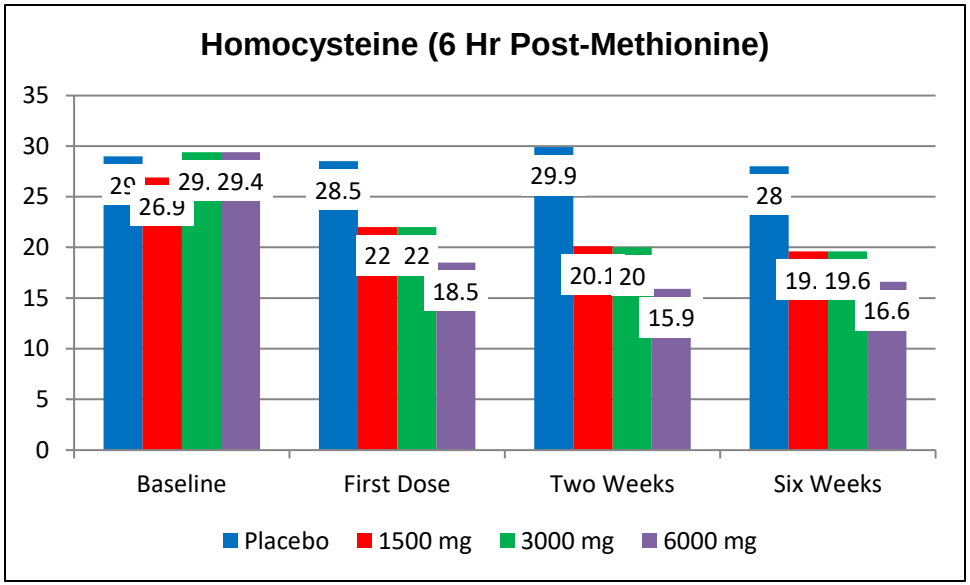
Double blind protocol followed

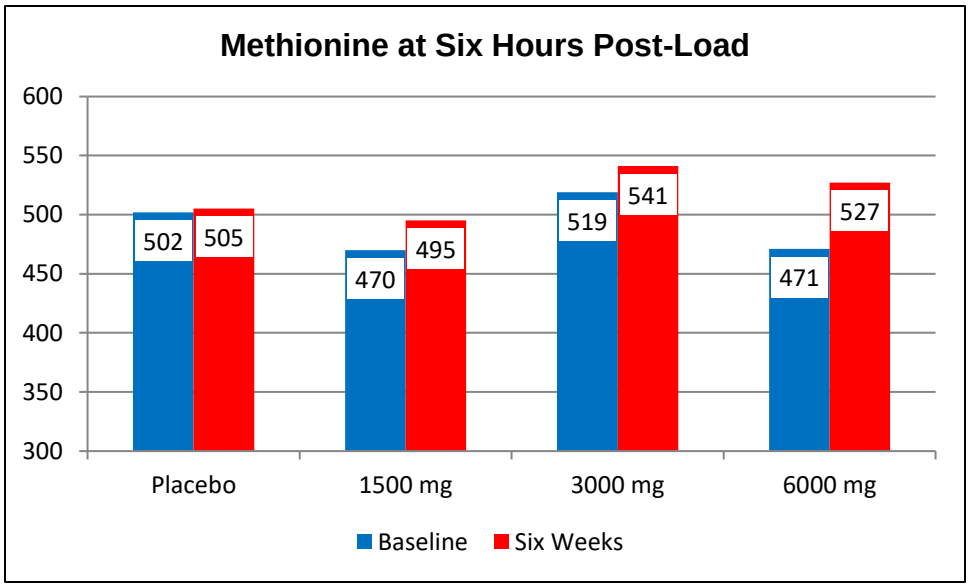
BETAINE and HOMOCYSTEINE

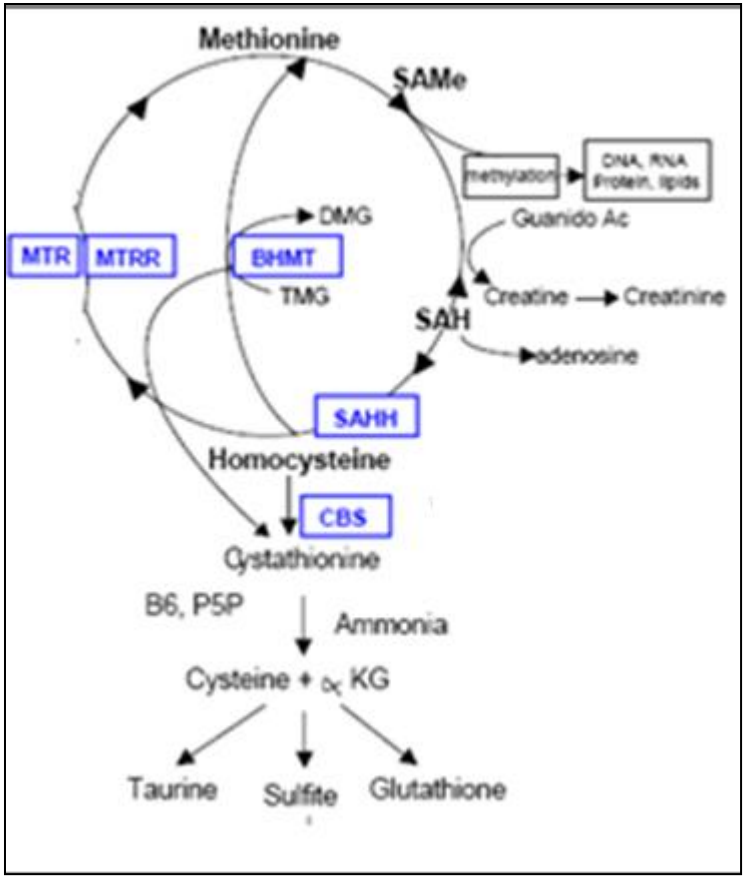




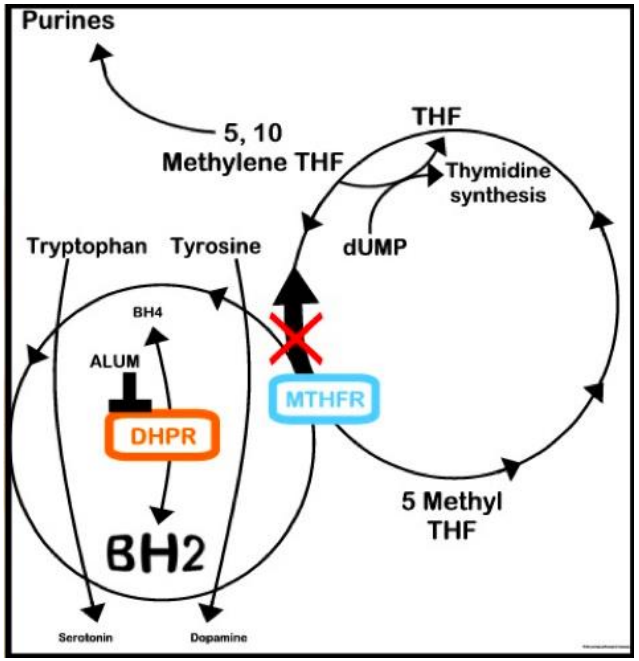
BETAINE and HOMOCYSTEINE







5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (A1298C)



Compromises “backward” conversion of

5-Methyl Folate (5-Methyl THF) $\xrightarrow{\text{MTHFR}}$ 5,10-Methylene Tetrahydrofolate

5-Methyl Folate + BH2 $\longrightarrow$ 5,10-Methylene THF + BH4

MTHFR A1298C aggravates CBS up regulation induced BH4 depletion

RECOGNITION of CBS UP REGUALTIONS

Low Homocysteine

- Normal Homocysteine with MTHFR and MTRR abnormalities

Sickest functionally ill patients:

- Autistic spectrum disorders
- Multiple chemical sensitivities
- Fibromyalgia and chronic fatigue

Sensitivities to:

- Alcohol and high sulfite/sulfate foods/supplements/pharmaceuticals
- MSG
- DMSA and DMPS
- B vitamins
- Post-prandial arrhythmia

Lab tip offs:

- Low molybdenum, serine, and B6
- Elevated taurine, cysteine, glutamate, and ammonia
- Elevated tyrosine, phenylalanine, and tryptophan with
- Low dopamine, norepinephrine, or serotonin or low HVA and VMA

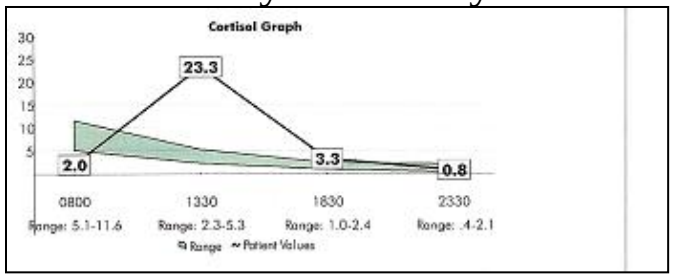
64 y/o female with “Lone Atrial Fib”

Structurally normal heart, normotensive, & no sleep apnea

Sensitive to MSG and “allergic” to sulfa

GERDz and tendency to asthma

Homocysteine only 6.6



Element	Reference Range	Reference Range
Lead	0.018	<= 0.048 mcg/g
Mercury	0.0062	<= 0.0039 mcg/g
Antimony	0.001	<= 0.002 mcg/g
Arsenic	0.021	<= 0.071 mcg/g
Cadmium	0.000	<= 0.001 mcg/g
Tin	<dl	<= 0.0009 mcg/g

Nutritionally Essential Amino Acids

Amino Acid		Reference Range
Arginine	15	10-64
Histidine	258	296-1,136
Isoleucine	66	24-69
Leucine	42	30-87
Lysine	49	45-286
Methionine	95	30-82
Phenylalanine	49	26-71
Taurine	1,757	68-538
Threonine	122	65-252
Tryptophan	82	28-111
Valine	29	23-61

Nonessential Protein Amino Acids

Amino Acid		Reference Range
Alanine	154	146-486
Asparagine	71	49-152
Aspartic Acid	80	35-86
Cystathionine	100	21-78
Cystine	37	26-78
γ-Aminobutyric Acid	9	<= 31
Glutamic Acid	21	5-21
Glutamine	333	172-570
Proline	10	2-18
Tyrosine	67	33-124

Intermediary Metabolites

B Vitamin Markers		Reference Range
α-Aminoadipic Acid	31	11-73
α-Amino-N-butyric Acid	20	9-49
β-Aminoisobutyric Acid	181	22-192
Cystathionine	<5	6-33
3-Methylhistidine	233	131-318
Urea Cycle Markers		
Ammonia	35.0	14.0-49.0 mmol/g creatinine
Citrulline	49	12-45
Ornithine	8	4-21
Urea *	386	168-485 mmol/g creatinine
Glycine/Serine Metabolites		
Glycine	1,160	639-3,306
Serine	256	187-568
Ethanolamine	218	208-514
Phosphoethanolamine	36	18-70
Phosphocreatine	39	28-63
Sarcosine	35	<= 48

Oxidative Stress Markers

		Reference Range
Glutathione (whole blood)	1,681	<=869 micromol/L
Lipid Peroxides (urine)	8.1	<=10.0 micromol/g Creat.
8-OHdG (urine)	13	<=16 mcg/g Creat.
Coenzyme Q10, Ubiquinone (plasma)	0.66	0.43-1.49 mcg/mL

CBS MANAGEMENT

- Decrease ammonia production/absorption (spare BH4)

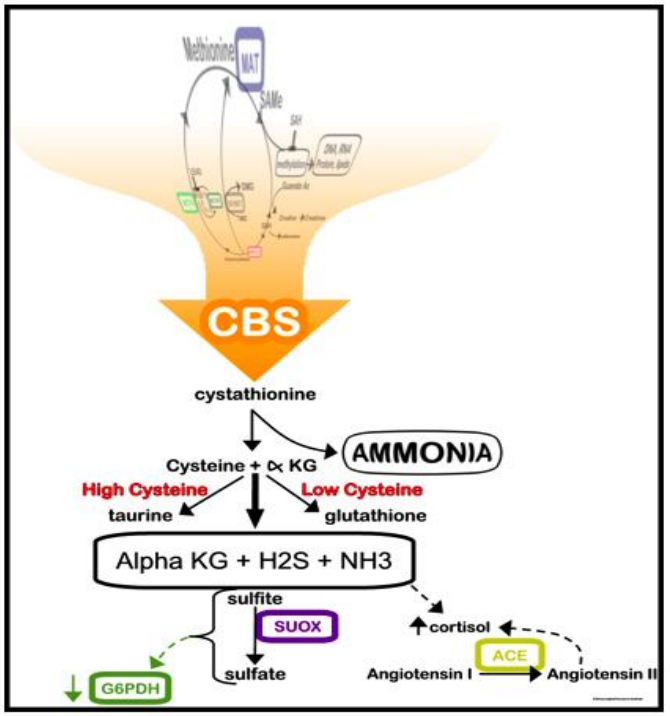
Restrict animal protein "Nothing with Eyes" diet
  Caveat #1 → Protein malnutrition
  Caveat #2 → Weight gain and insulin insensitivity

Charcoal at bedtime (Magnesium prn constipation)

Yucca with food and resolve dysbiosis

Ammonia/CBS Support siRNA Products

Urea Cycle stimulation with LOLA ⇒



Use ammonia neutralizing supplements to liberalize dietary protein restriction

Asses efficacy with 24 hour urine for ammonia and taurine

Clinical judgment important

HEPATIC ENCEPHALOPATHY

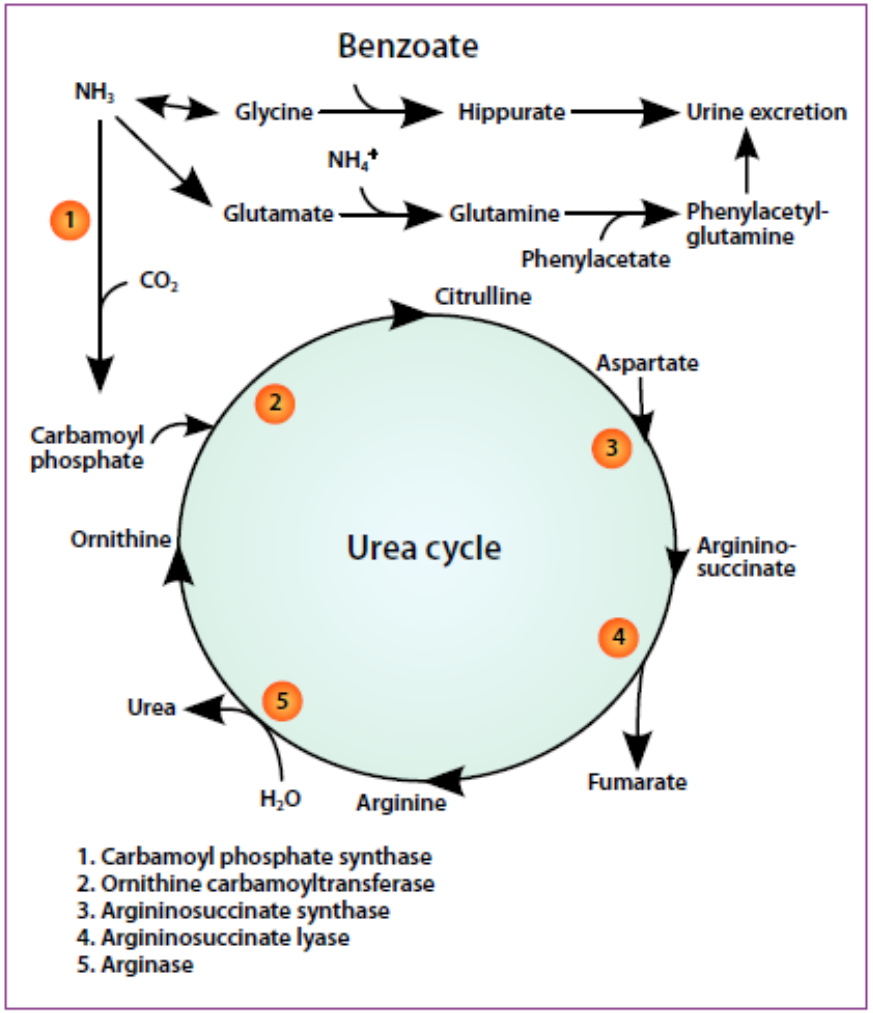
Low protein diet (protein malnutrition)

Decrease ammonia absorption

- Lactulose
- Rifaxamin

Increase ammonia metabolism

- IV Phenylbutyrate
- Oral Sodium Benzoate
- L-Ornithine/L-Aspartate



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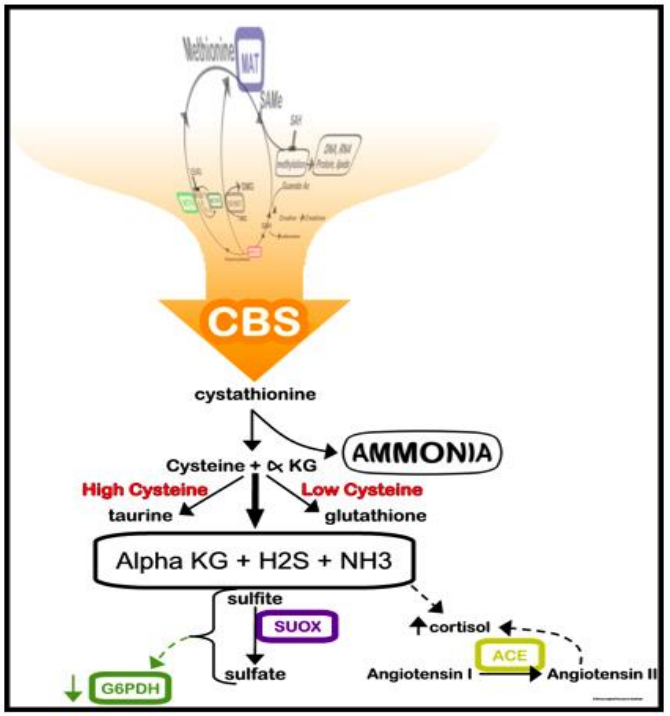

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Clinical judgment important

CBS MANAGEMENT

- Decrease sulfate burden

Restrict animal protein “Nothing with Eyes” diet

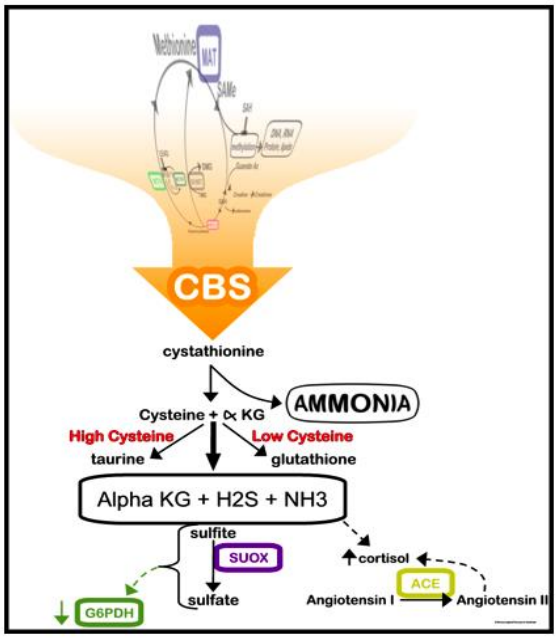
Limit high sulfur/sulfate/sulfite foods

Limit high sulfate nutritionals and pharmaceuticals ⇒

CBS Support siRNA Products

Botanical/Homeopathic Charged Sulphur Detox

Monitor urine sulfate (and sulfite if SUOX +/-)



CBS MANAGEMENT

Supplements High In Sulfur			
Taurine	Cysteine	Methionine	Glutathione
Glucosamine Sulfate		Chondroitin Sulfate and MSM	
Epsom Salts		Magnesium Sulfate Cream	
Canned meats, Aged game		Homemade yeast breads	
DMSA and DMPS (Metal Chelators)		Milk thistle, Beyond C, and Heparin	

Foods High In Sulfur			
Vegetables:			
Garlic, Onion Family	Kale	Collards	Pickles
Cabbage	Brussel Sprouts	Kohlrabi	Broccoli
Cauliflower	Bok Choy	Mizuna	Broccoli Rabe
Chinese Cabbage	Napa Cabbage	Turnip / Rutabaga	Canola / Rape Seeds; Greens
Mustard Seeds	Tatsoi	Radish	Daikon
Horseradish	Japanese Horseradish	Arugula	Watercress
Peas	Spinach		
Fruits:			
Raspberry	Cranberry	Currents	All Dried Fruit
Others:			
Vinegar (especially if prepared from wine)			
Alcohol Beverages (especially wine; not vodka - beer is less of an issue, especially German beer)			
Soft Drinks	Animal Products	Dairy	Eggs
Brazil Nuts	Peanuts	Soy	

Sulfamethoxazole/Trimethoprim, diuretics other than spironolactone

Alcohol (except potato based Vodka)

DMSA and DMPS

Sulfites and Chronic Disease, by Rick Williams

CBS MANAGEMENT

- Decrease sulfate burden

Restrict animal protein “Nothing with Eyes” diet

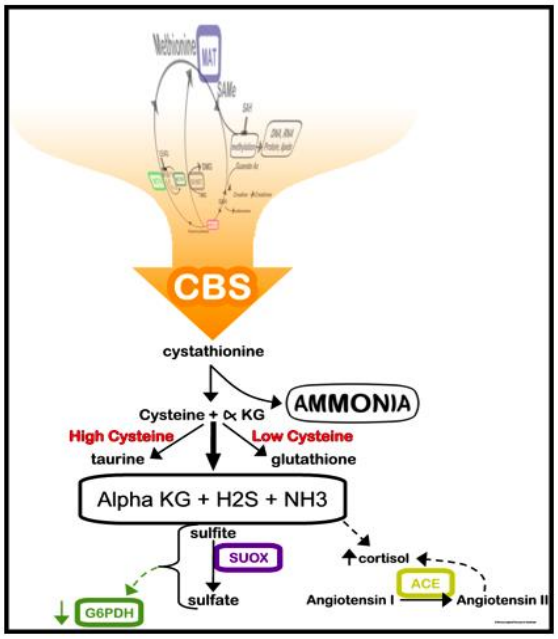
Limit high sulfur/sulfate/sulfite foods

Limit high sulfate nutritionals and pharmaceuticals

CBS Support siRNA Products

Botanical/Homeopathic Charged Sulphur Detox

Monitor urine sulfate (and sulfite if SUOX +/-)



CBS MANAGEMENT

- Support Sulfite Oxidase (SUOX)

Molybdenum 150 mcg/day

- Minimize dairy (Xanthine Oxidase)

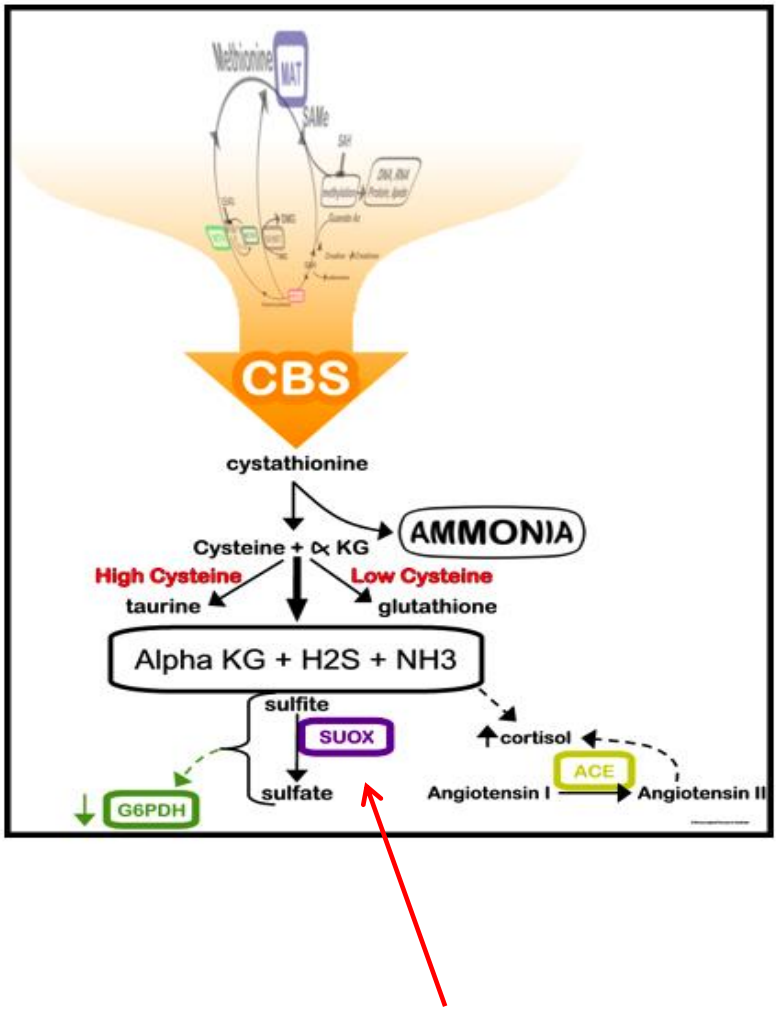
Hydroxy-B12 2000 mcg/day sl

Boron 3 mg/day

Vitamin E Succinate 400 IU/day

Limit B-6 (P-5-P less of an issue)?

Monitor urine sulfite & sulfate if SUOX +/-



CBS MANAGEMENT

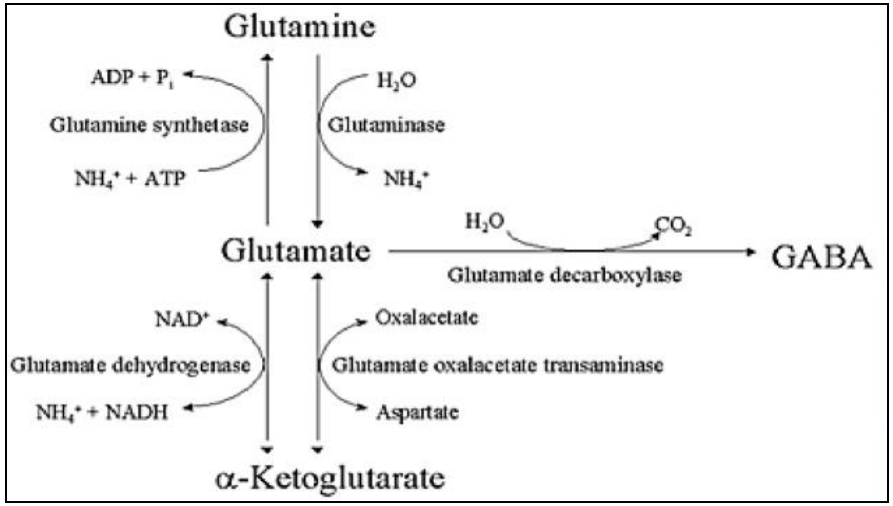
- Rebalance GABA:Glutamate

Avoid MSG and Excitotoxic foodstuffs ⇒

Supplement with:

- GABA 500-1000 mg bid (if COMT +/+ or +/-)
- Zen (GABA + Theanine) if COMT WT

Remove metals (comprise GABA:Glutamate)



CBS MANAGEMENT

Sources of Excitotoxins			
Glutamate	Glutamic acid, glutamine, and MSG. High levels are found in foods such as peas, tomatoes, parmesan cheese, milk, mushrooms, fish, and many vegetables		
Aspartate	Aspartame, NutraSweet		
Other "Names" for Excitotoxins			
Monosodium Glutamate		Glutamate	Natural Flavor(s)
Maltodextrin	Carrageenan	Gelatin	Spice(s)
Seasoning(s)	Seasoned Salt	Dough Conditioner(s) Isolate	
Autolyzed Yeast	Autolyzed Yeast Extract	Autolyzed Anything	Broth
Stock	Soup Base	Chicken/Pork/Beef "Flavoring"	
Hydrolyzed Vegetable Protein (HPV)		Hydrolyzed Plant Protein	
Hydrolyzed Oat Flour		Hydrolyzed Anything	Yeast Extract
Sodium Caseinate	Calcium Caseinate	Caseinate	Disodium Guanylate
Disodium Inosinate	Disodium Caseinate	Hydrolyzed Protein	Chicken/Pork/Beef "Base"
Bouillon	Vegetable Gum	Plant Protein Extract	Smoke Flavoring(s)
Malted Barley Flour	Malt Extract	Malt Flavoring(s)	Malted Barley
Malted Anything	Textured Protein	Guar Gum	Soy Extract
Soy Protein	Soy Protein Concentrate	Soy Sauce	Whey Protein
Whey Protein Isolate	Whey Protein Concentrate		L-Cysteine
Ajinomoto	Kombu Extract	Natural Flavoring(s)	Barley Malt
Foods with MSG (Monosodium Glutamate)			
Hydrolyzed Protein	Hydrolyzed Oat Flour	Sodium Caseinate / Calcium Caseinate	
Gelatin	Glutamic Acid	Monosodium Glutamate	
Autolyzed Yeast or Yeast Extract			
Possible Sources of MSG			
Textured Protein	Carrageenan Or Vegetable Gum	Seasonings Or Spices	Flavorings Or Natural Flavorings
Chicken, Beef, Pork, Smoke Flavorings	Bouillon, Broth, Or Stock	Barley Malt, Malt Extract, Malt Flavoring	Whey Protein, Whey Protein Isolate, Or Concentrate

CBS MANAGEMENT

Other Sources of MSG			
Food From Fast-Food Chains		OTC Medications	Chicken Pox Vaccine
NutraSweet	Binders and Fillers in Supplements		Prescription Medications
Foods with Glutamates			
Doritos	Pringles	KFC Fried Chicken	Boar's Head Cold Cuts/Hot Dogs
Progresso Soups	Lipton Soups/Sauces	Gravy Master	Planter's Salted Peanuts
Sausages / Processed Meats / Cold Cuts	Processed Cheese Spread	Molasses	Supermarket Turkey And Chicken (Injected)
Restaurant Gravy	Ramen Noodles	Bouillon	Instant Soup Mixes / Stocks
Salad Dressings / Croutons	Salty, Powdered Dry Food Mixes	Flavored Potato Chips	Restaurants Soups Made From Soup Base
Gelatin	Soy Sauce	Worcestershire Sauce	Kombu Extract
Dry Milk Or Whey Powder	Dough Conditioners	Body Builder Protein Mixes	Parmesan Cheese
Fresh Produce Sprayed With Auxigro In The Field	Some Spices	Skim, 1%, 2%, Non-Fat, Or Dry Milk	Whipped Cream Topping Substitutes
Non-Dairy Creamers	Chocolates / Candy Bars	Low-Fat / Diet Foods	Cereals
Baked Goods From Bakeries	Frostings And Fillings	Catsup	Mayonnaise
Chili Sauce	Mustards	Pickles	Bottled Spaghetti Sauce
Citric Acid Made From Processed Corn	Canned And Smoked Tuna, Oysters, Clams	Barbeque Sauce	Canned, Frozen, Or Dry Entrees And Potpies
Fresh And Frozen Pizza	Flavored Teas, Sodas	Seasoned Anything	Some Bagged Salads And Vegetables
Tomato Sauce / Stewed Tomatoes	Egg Substitute	Flour	Canned Refried Beans
Tofu And Other Fermented Soy Products	Table Salts	Anything With Corn Syrup Added	Anything With Milk Solids
Anything Fermented	Anything Vitamin Enriched	Anything Protein Fortified	Anything Enzyme Modified
Anything Ultra-Pasteurized	Carmel Flavoring/Coloring	Pectin	Cornstarch
Flowing Agents	Xanthan Gum / Other "Gums"	L-Cysteine	

CBS MANAGEMENT

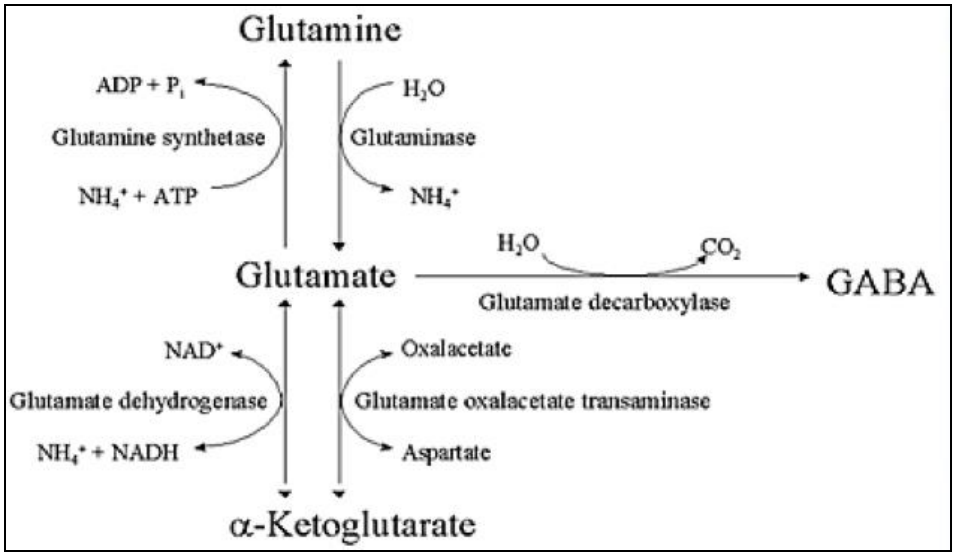
- Rebalance GABA:Glutamate

Avoid MSG and Excitotoxic foodstuffs

Supplement with:

- GABA 500-1000 mg bid (if COMT +/+ or +/-)
- Zen (GABA + Theanine) if COMT WT

Remove metals (comprise GABA:Glutamate)



CBS MANAGEMENT

- Support BHMT

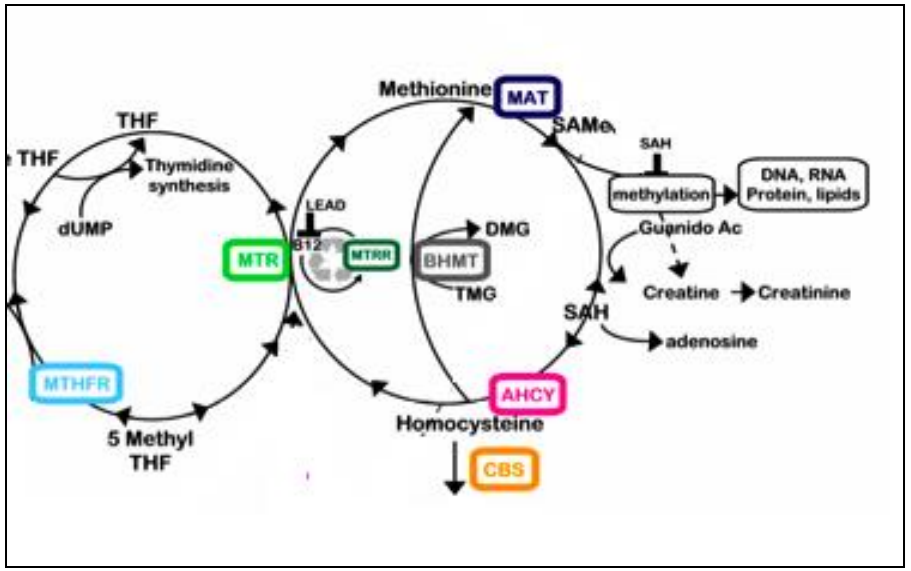
Trimethylglycine (TMG - Betaine) and Zinc (BHMT co-factor)

- Caution if COMT +/- or +/+

Phosphatidylcholine (CV, hepatic, and neurologic disease)

- 30% SAMe “spent” in Phosphatidylcholine biosynthesis

Phosphatidylserine (Modulates high cortisol)



CBS MANAGEMENT

- Mineral support

Most of our patients are mineral deficient

Carry out 24 hour urine minerals or RBC mineral assessment

Be liberal with mineral supplementation

Repeat mineral assessment periodically

- Glutathione support

Biologically contraindicated?

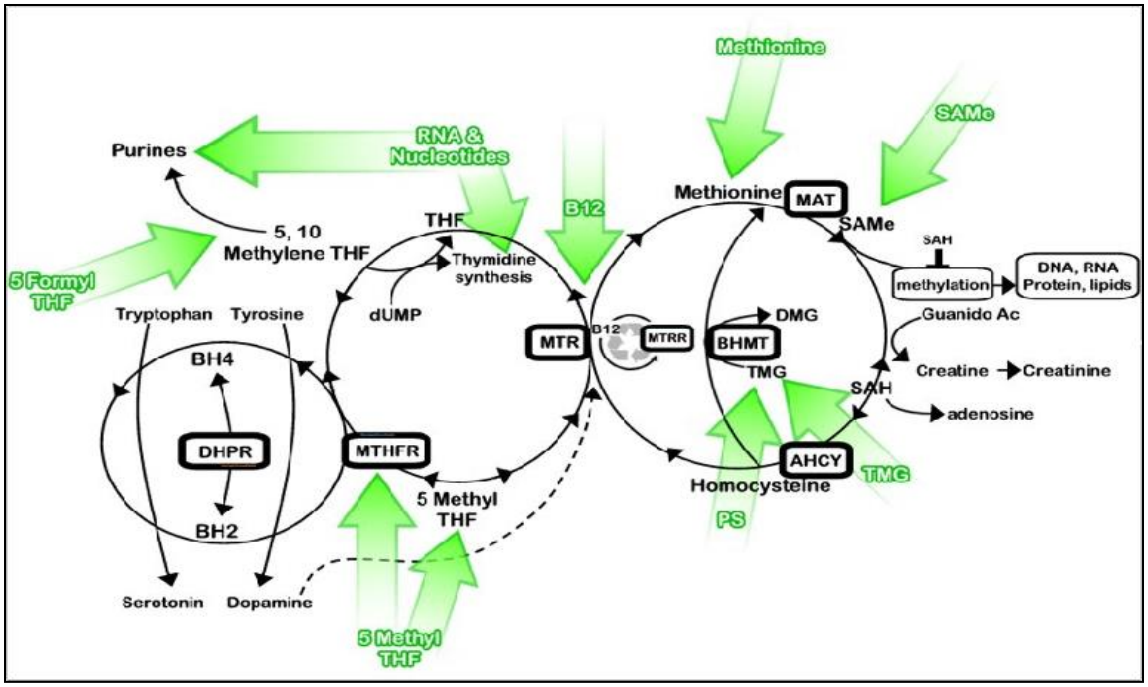
- N-Acetyl Cysteine, Lipoic Acid, & Glutathione contain SH groups

Needless acupuncture Glutathione patch

- One patch every day or every other day (alternate with Carnosine)
- One patch, 6 hours/day, 6 days/week
- Watch for detox phenomena

CBS MANAGEMENT

Delay introduction of other Methyl Cycle supplements until CBS is under control



Methyl-Folate, Methyl-B12, and/or BH4

when Sulfate levels are High

→ Honeymoon followed by a Crash

CBS MANAGEMENT

When urine sulfate and ammonia are under control:

- Lab evaluation
- Clinical judgment
- Genotype often $\neq$ phenotype

Methyl-B12

Methyl-Folate

BH4

Start low and go slow

Watch urine sulfate levels and clinical response

Adverse clinical response?:

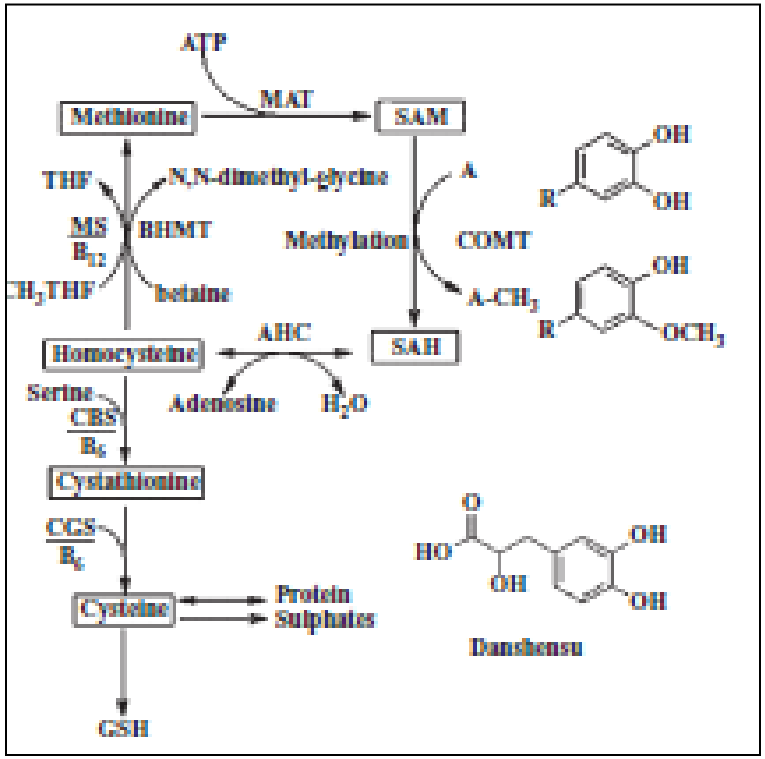
- Sulfate/ammonia overload
- Detox phenomena

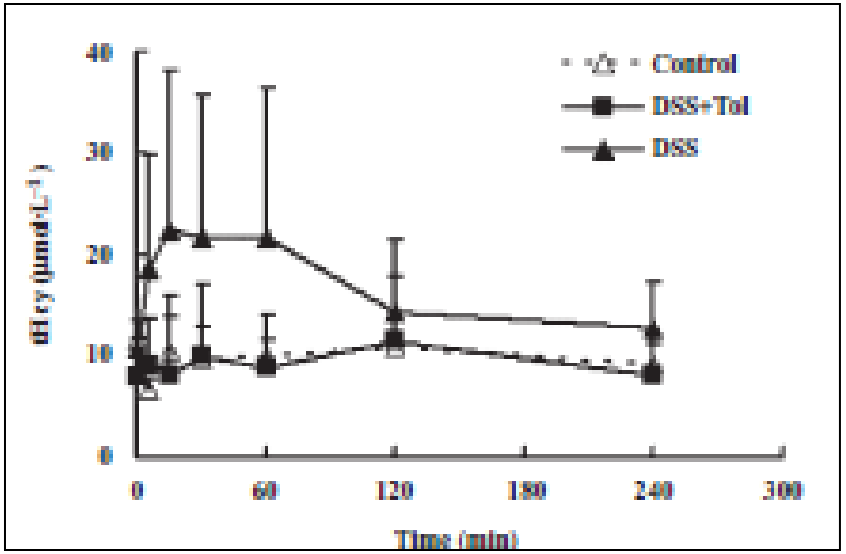
DANSHENSU STIMULATES CBS

♥ Male Sprague-Dawley rats (180-250 gm.)

IV administration of:

- Saline
- Danshensu 20 mg/kg
- Danshensu + Tolcapone (COMT inhibitor) 10 mg/kg

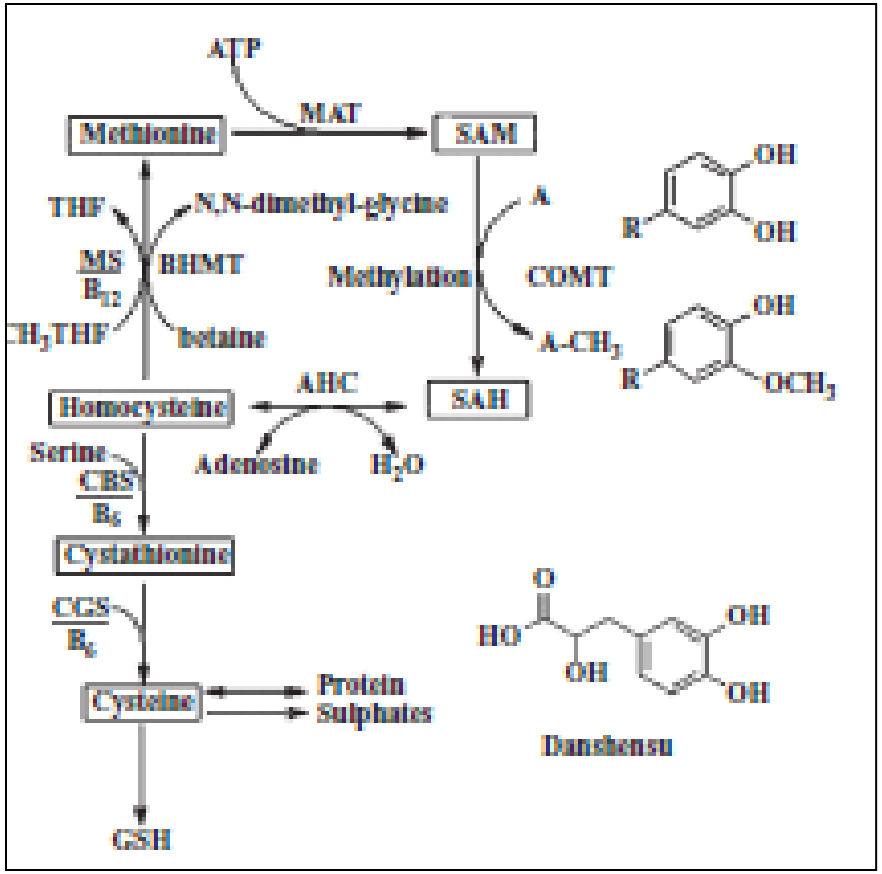


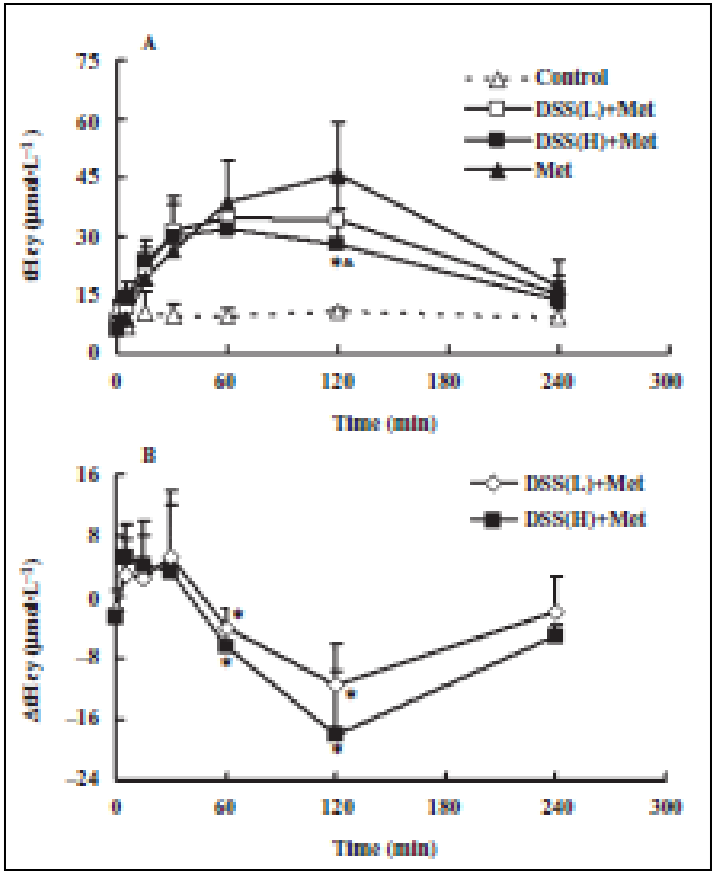


DANSHENSU STIMULATES CBS

IV administration of:

- Saline
- Methionine 0.8 mmol/kg
- Methionine plus Danshensu 10 or 20 mg/kg

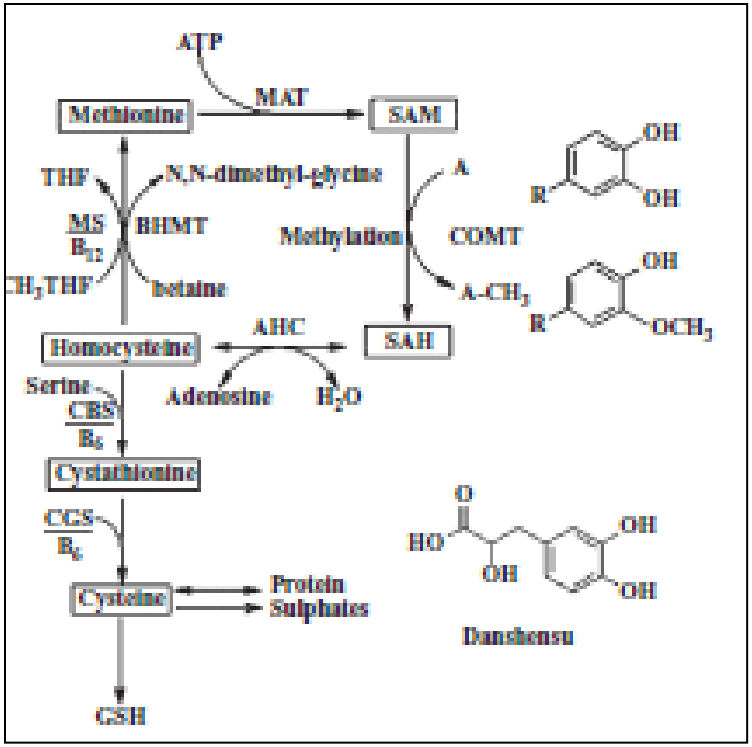


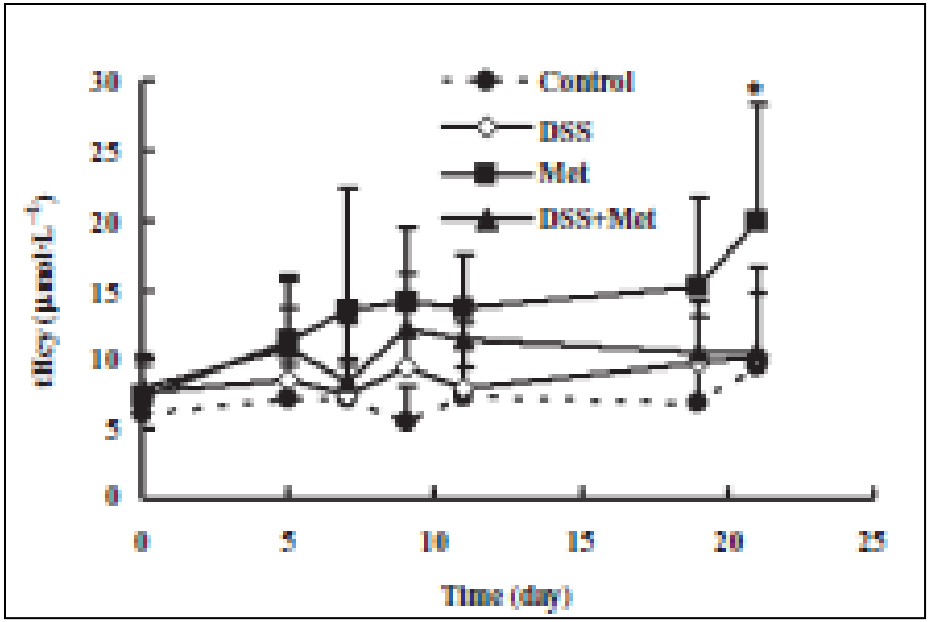


DANSHENSU STIMULATES CBS

Daily IP administration of:

- Saline
- Danshensu 5 mg/kg
- Methionine 0.8 mmol/kg
- Methionine plus Danshensu

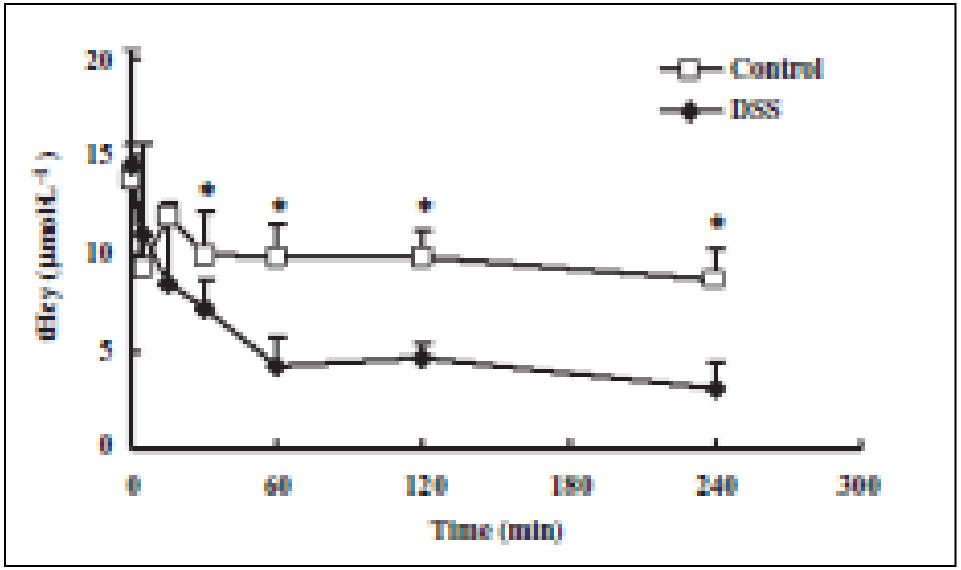




DANSHENSU STIMULATES CBS

IP administration of methionine 0.8 mmol/kg/day over three weeks:
Then administer single ip dose of:

- Saline
- Danshensu 20 mg/kg



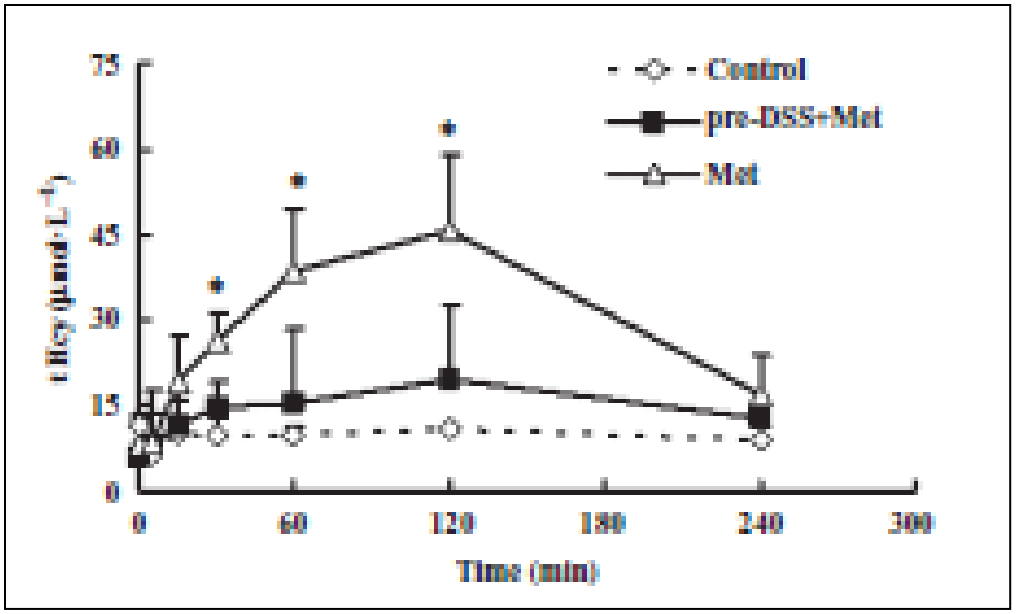
DANSHENSU STIMULATES CBS

IP administration over three weeks with:

- Saline
- Danshensu 5 mg/kg/day

Three day washout

Then administer single ip dose of methionine 0.8 mmol/kg/day

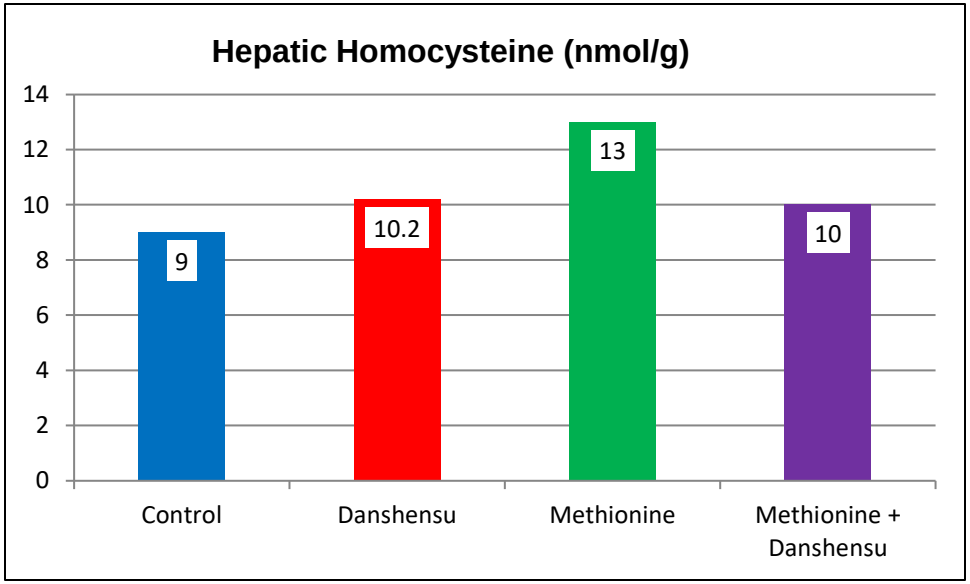


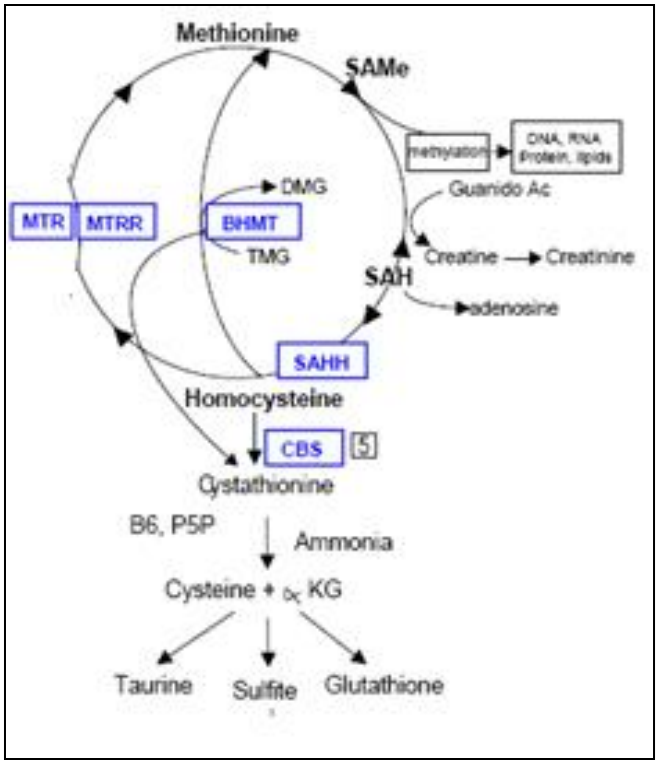
DANSHENSU STIMULATES CBS

Danshensu lowers (elevated) homocysteine rapidly and persistently – How?

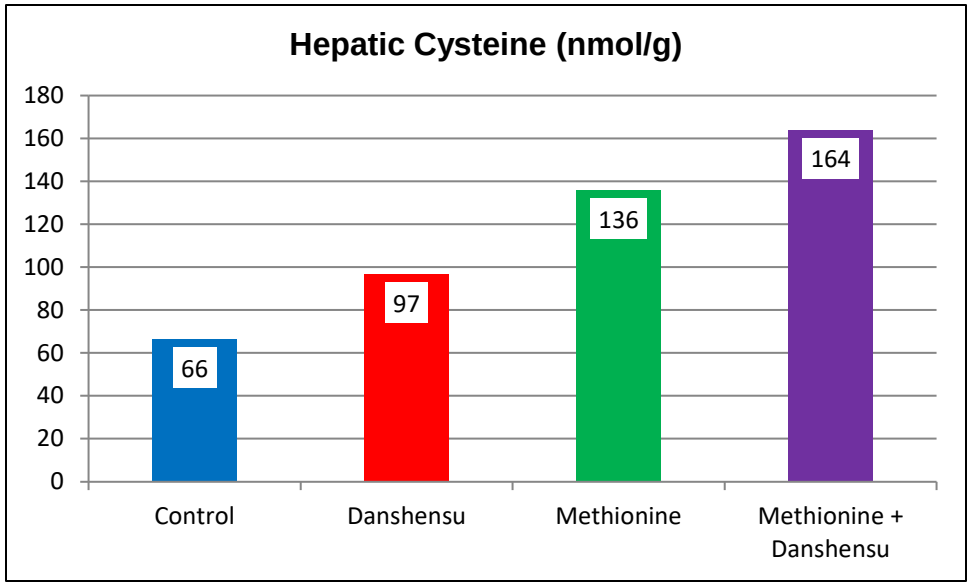
IP administration over three weeks with:

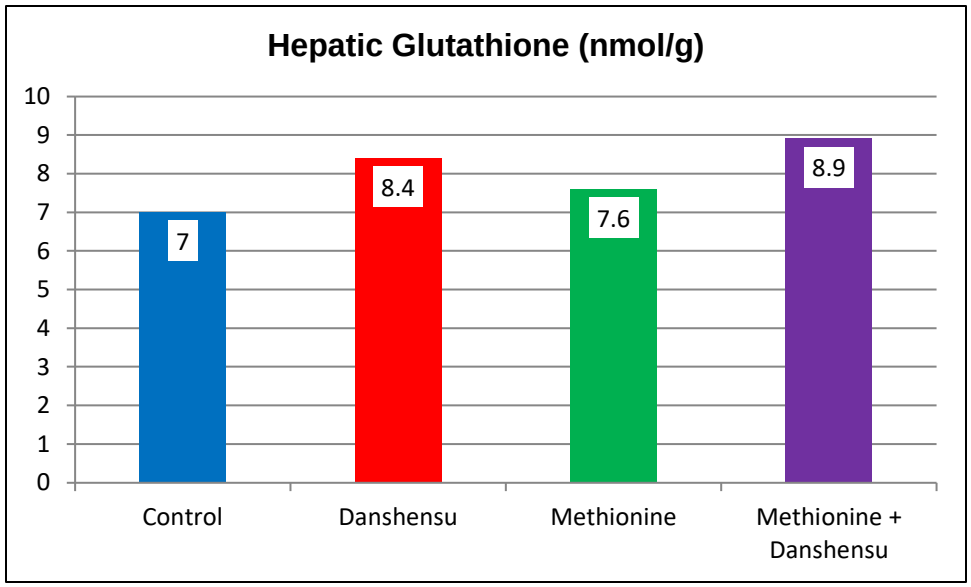
- Saline
- Danshensu 5 mg/kg/day
- Methionine 0.8 mmol/kg/day
- Danshensu + methionine:

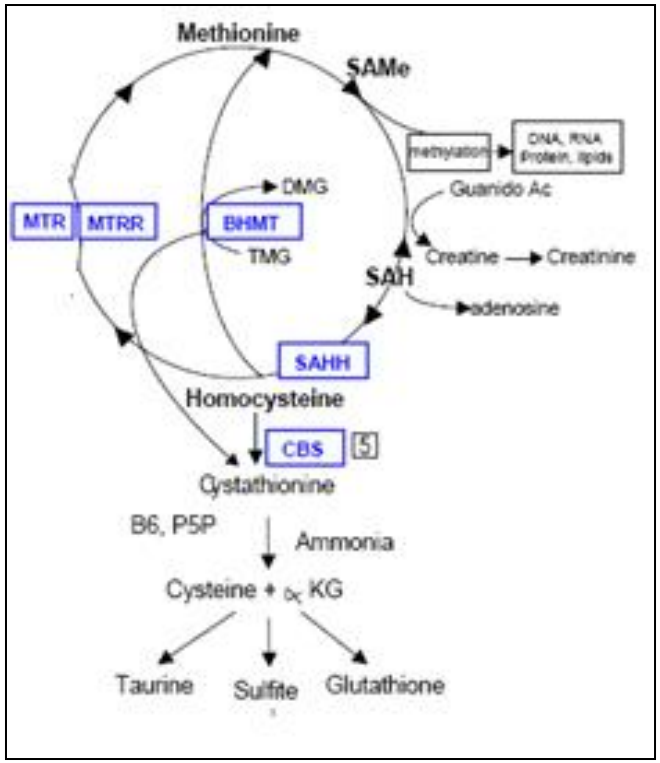




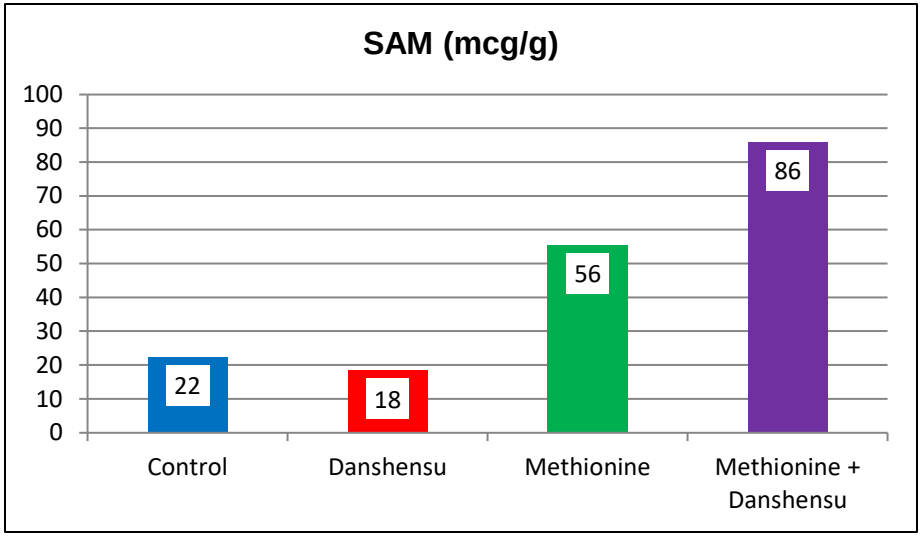
DANSHENSU STIMULATES CBS

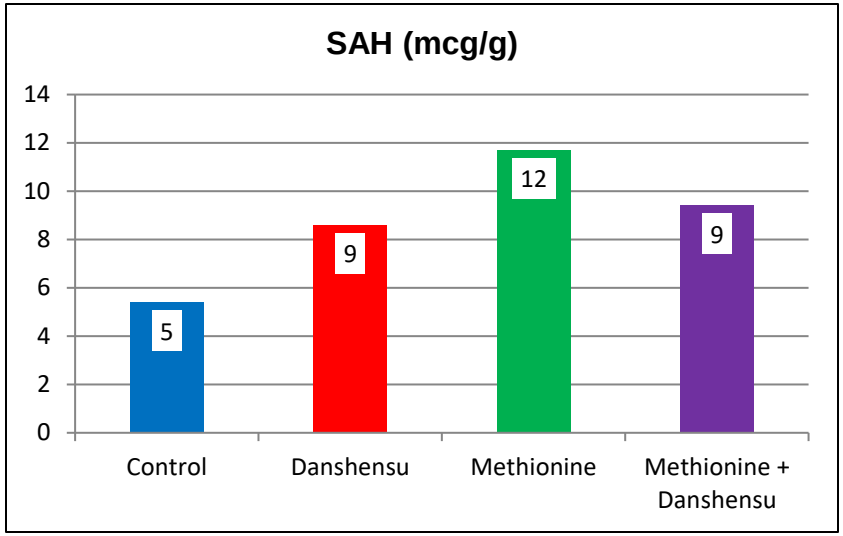


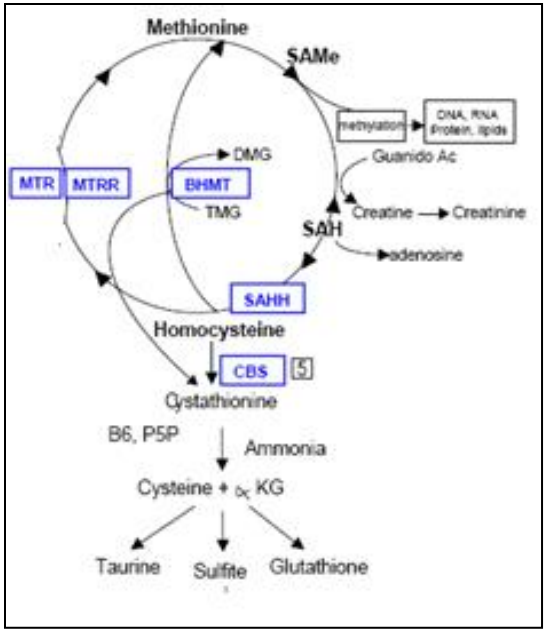


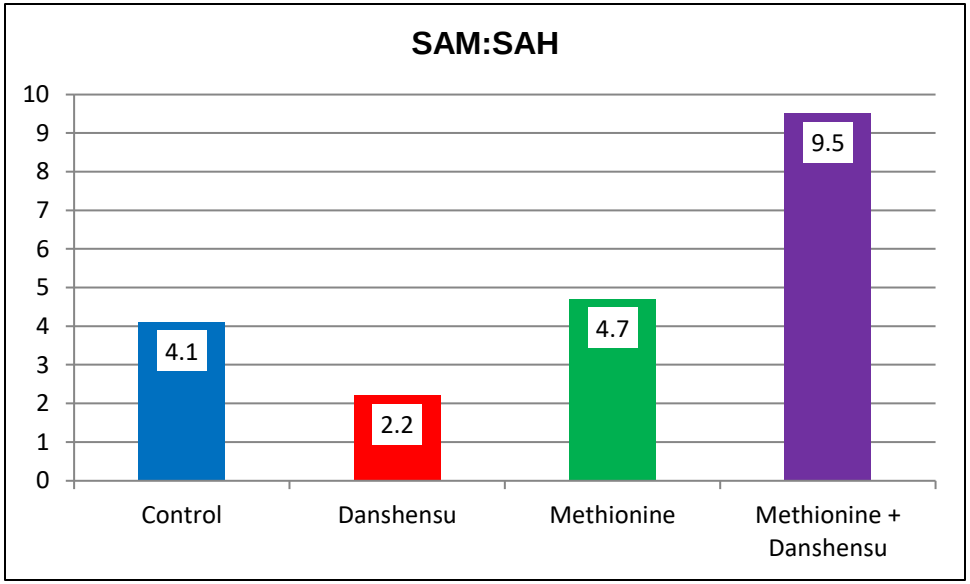


DANSHENSU STIMULATES CBS





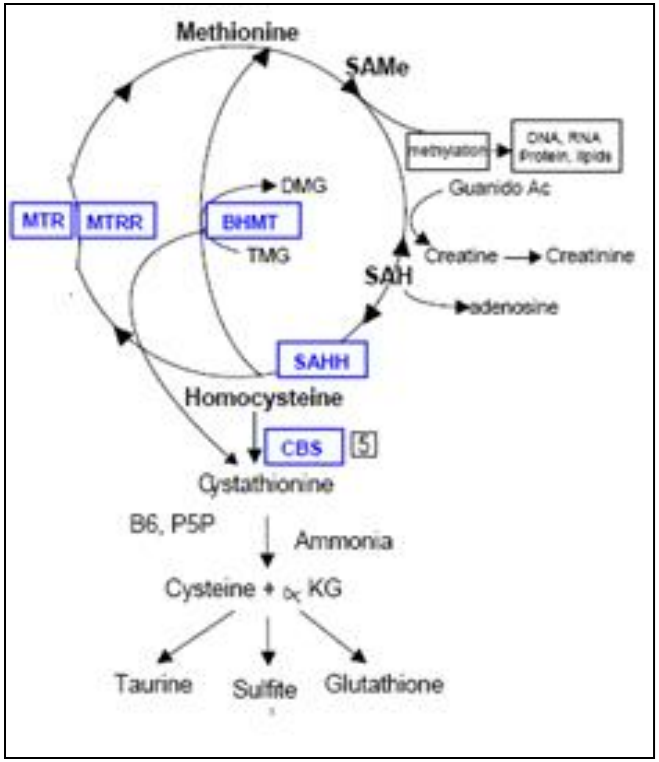




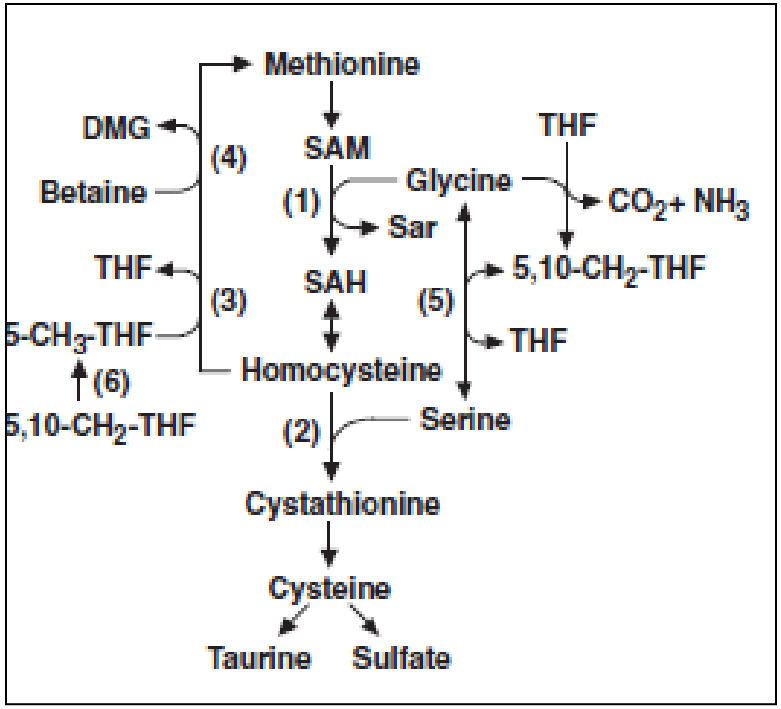
DANSHENSU STIMULATES CBS

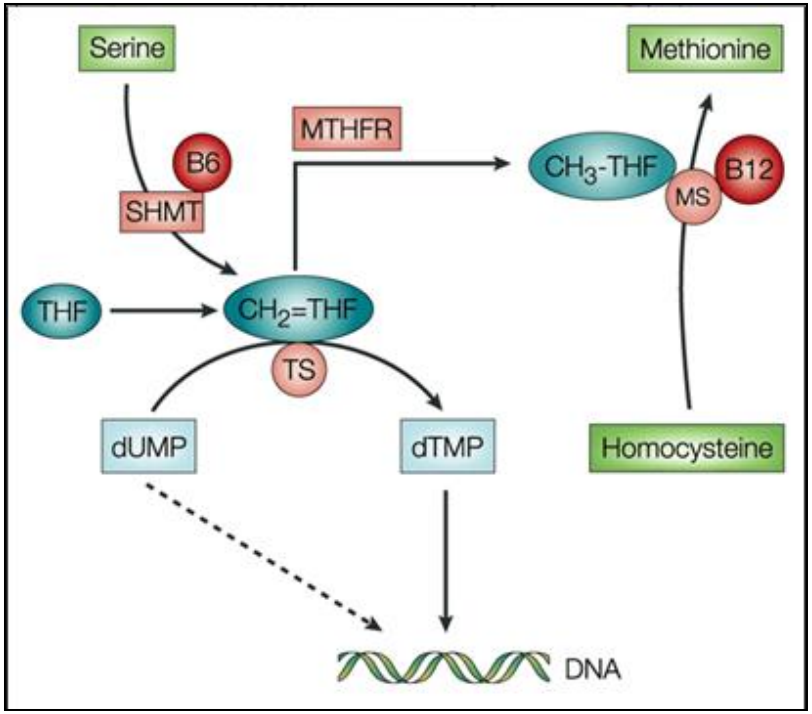
Danshensu effect on Homocysteine:

- Short term rise in homocysteine (COMT-methylation)
- Rapid activation of CBS system
- Sustained increased expression of CBS enzymes
- Response related to burden placed on CBS system



GLYCINE, SERINE, and HOMOCYSTEINE



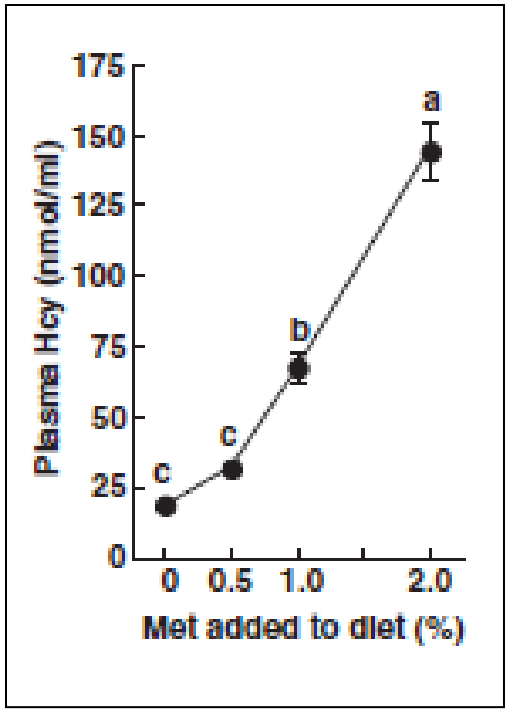


GLYCINE, SERINE, and HOMOCYSTEINE

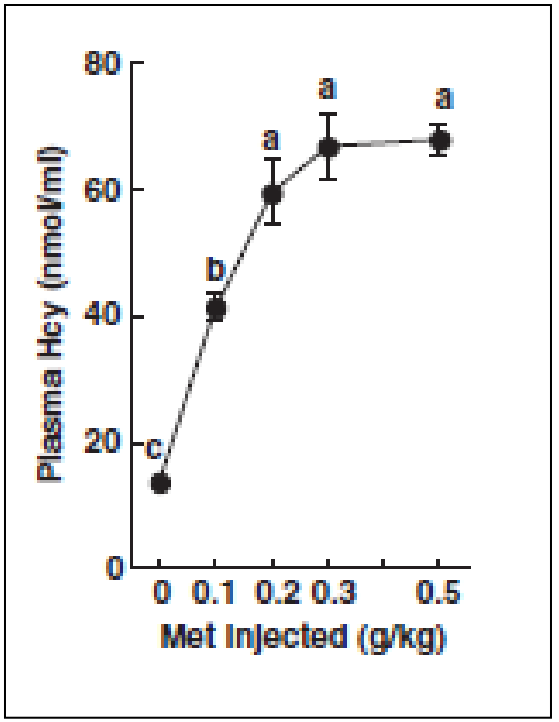
♥ Six week old Wistar rats

Provide ad lib over 10 days:

- Standard chow
- Chow + 0.5, **1**, or 2% methionine



Standard chow over 7 days;
then ip methionine at
100, 200, **300**, or 500 mg/kg

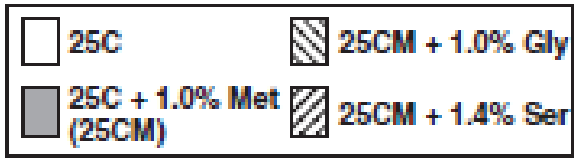


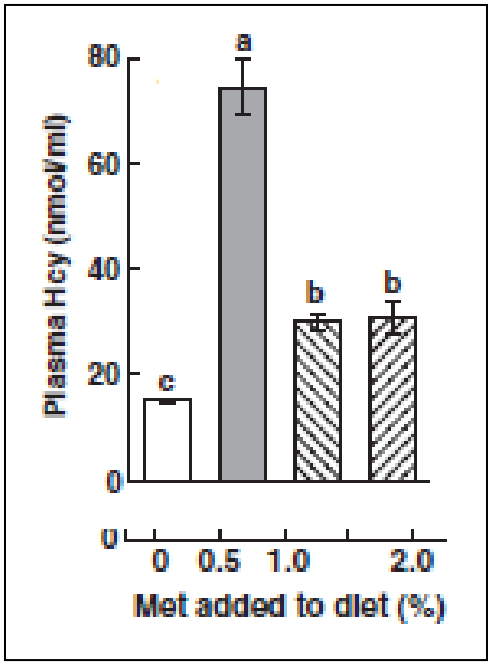
GLYCINE, SERINE, and HOMOCYSTEINE

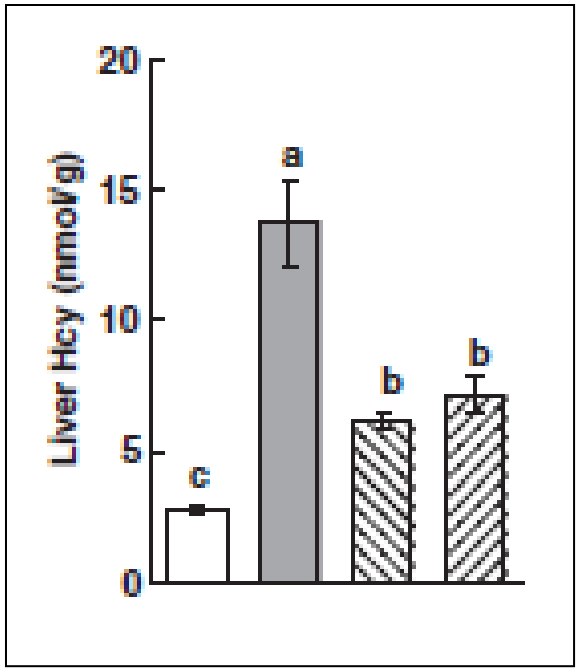
♥ Six week old Wistar rats

Provide ad lib over 7 days:

- Standard chow
- Chow + 1% methionine
- Chow + 1% methionine + 1% glycine
- Chow + 1% methionine + 1.4% serine





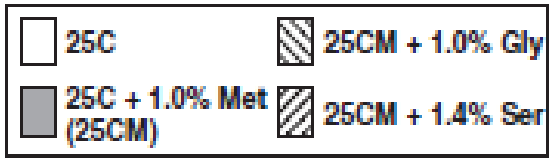


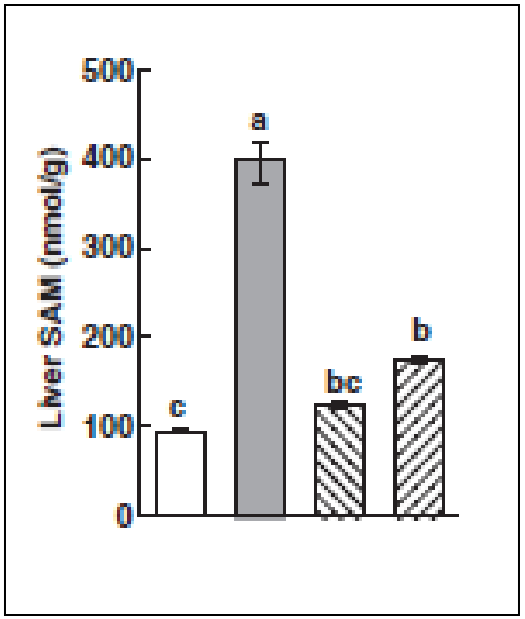
GLYCINE, SERINE, and HOMOCYSTEINE

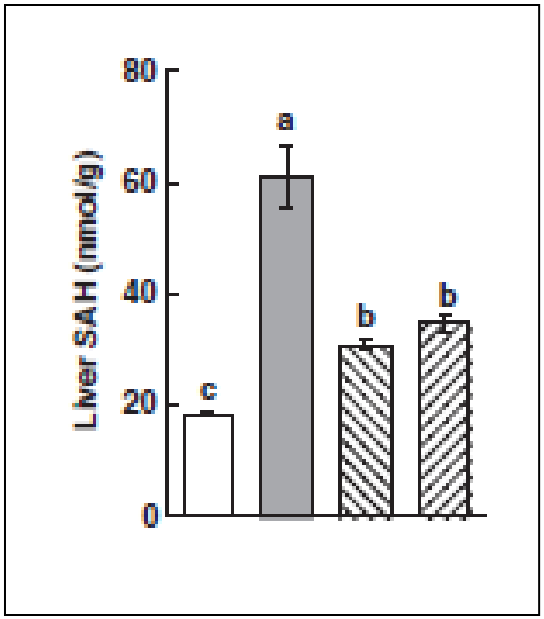
♥ Six week old Wistar rats

Provide ad lib over 7 days:

- Standard chow
- Chow + 1% methionine
- Chow + 1% methionine + 1% glycine
- Chow + 1% methionine + 1.4% serine





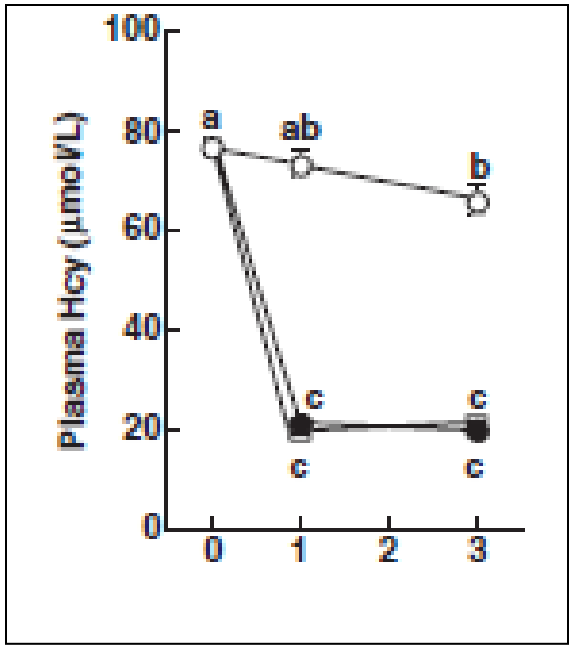


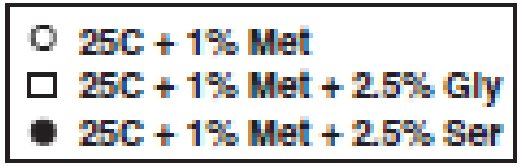
GLYCINE, SERINE, and HOMOCYSTEINE

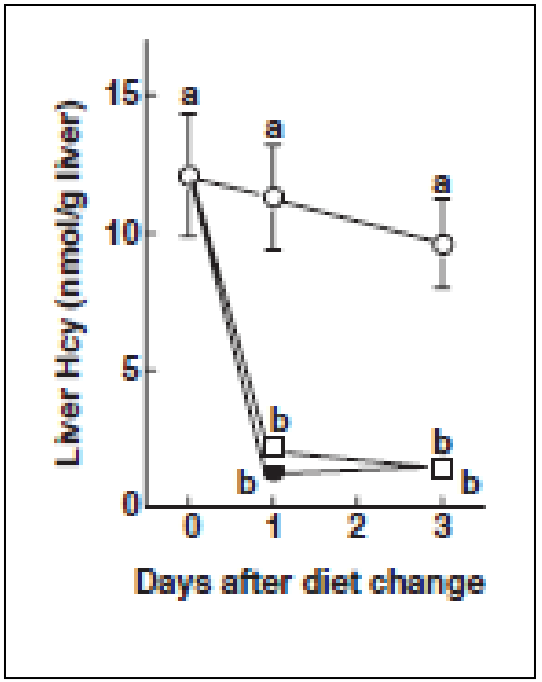
♥ Six week old Wistar rats

All receive chow + 1% methionine, followed by 3 additional days of:

- Chow + 1% methionine
- Chow + 1% methionine + 2.5% glycine
- Chow + 1% methionine + 2.5% serine





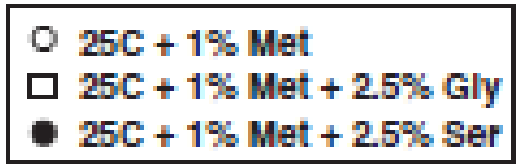


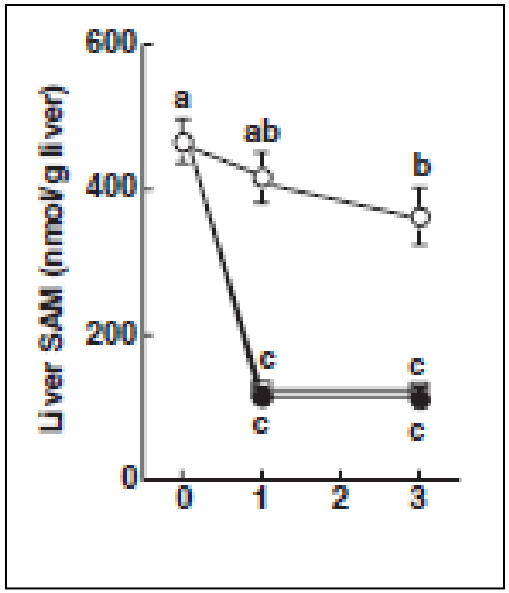
GLYCINE, SERINE, and HOMOCYSTEINE

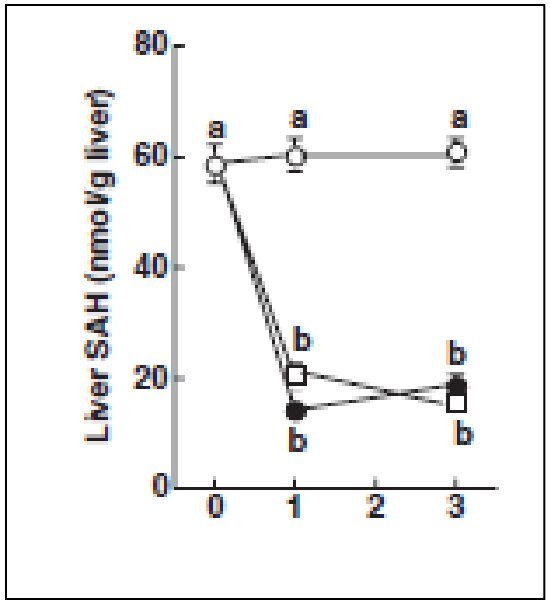
♥ Six week old Wistar rats

All receive chow + 1% methionine, followed by 3 additional days of:

- Chow + 1% methionine
- Chow + 1% methionine + 2.5% glycine
- Chow + 1% methionine + 2.5% serine





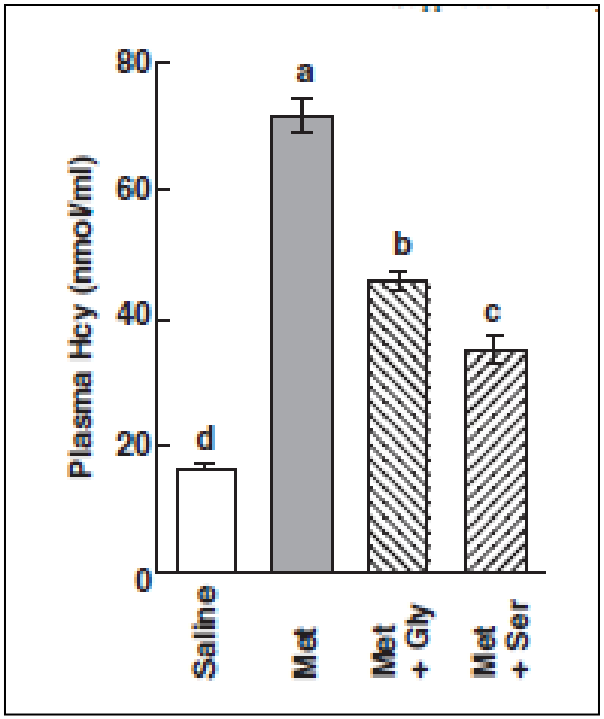


GLYCINE, SERINE, and HOMOCYSTEINE

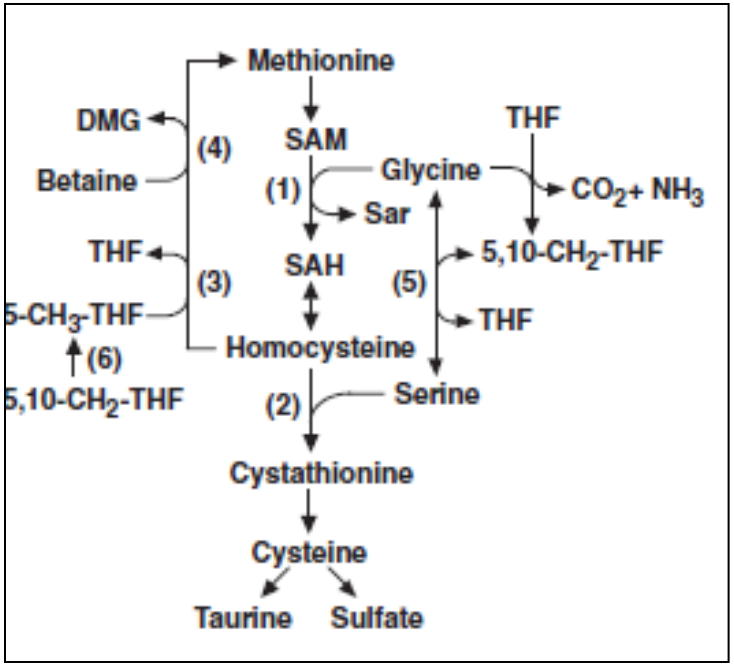
♥ Six week old Wistar rats

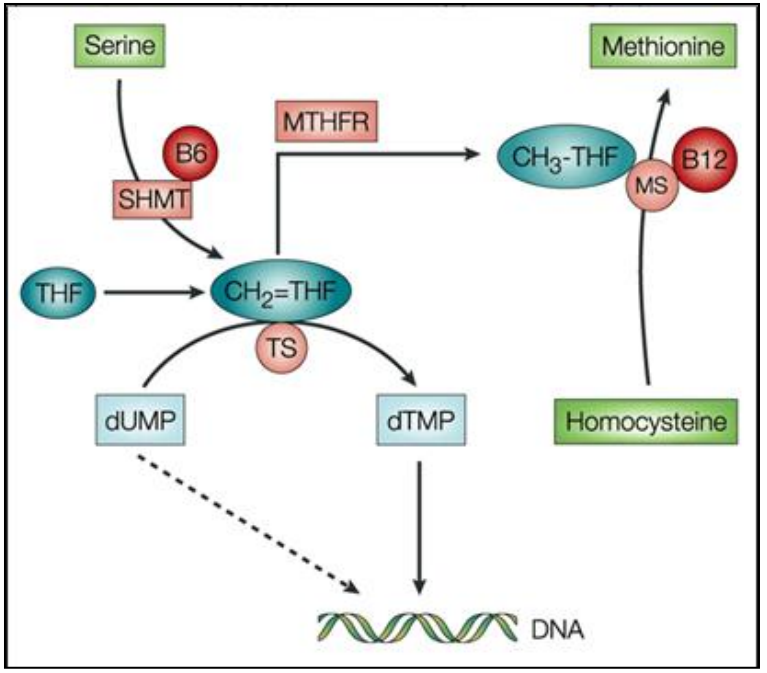
All receive standard chow over 7 days, followed by ip administration of:

- Saline
- Methionine 300 mg/kg
- Methionine 300 mg/kg + glycine 300 mg/kg
- Methionine 300 mg/kg + serine 420 mg/kg



GLYCINE, SERINE, and HOMOCYSTEINE





Serine:

- Stimulates CBS to metabolize Hcy
- Stimulates SHMT to generate 5,10-THF to load MTHFR →Methyl-folate for MTR
- Stimulates SHMT to generate glycine

Glycine:

- Lowers SAM via GNMT to relieve repression of MTHFR →Methyl-folate for MTR
- Via GNMT generates 5,10-Methylene-THF to load MTHFR →Methyl-folate for MTR
- Can be converted into serine

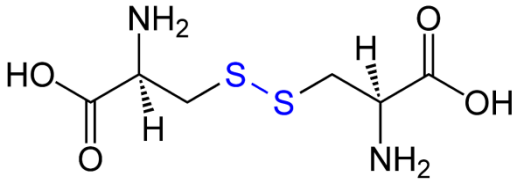
SERINE, CYSTINE, and HOMOCYSTEINE

♥ Twenty four healthy men

- Normal homocysteine, folate, and lipid values

One separate days (one week apart) provide each subject a morning meal:

- Low-protein meal fortified with 30 mg/kg methionine
- Low-protein meal + methionine + 60mg/kg serine
- Low-protein meal + methionine + 12 mg/kg cystine
- High protein meal containing:
  - ♦ 30 mg/kg methionine,
  - ♦ 60 mg/kg serine, and
  - ♦ 12 mg/kg cystine



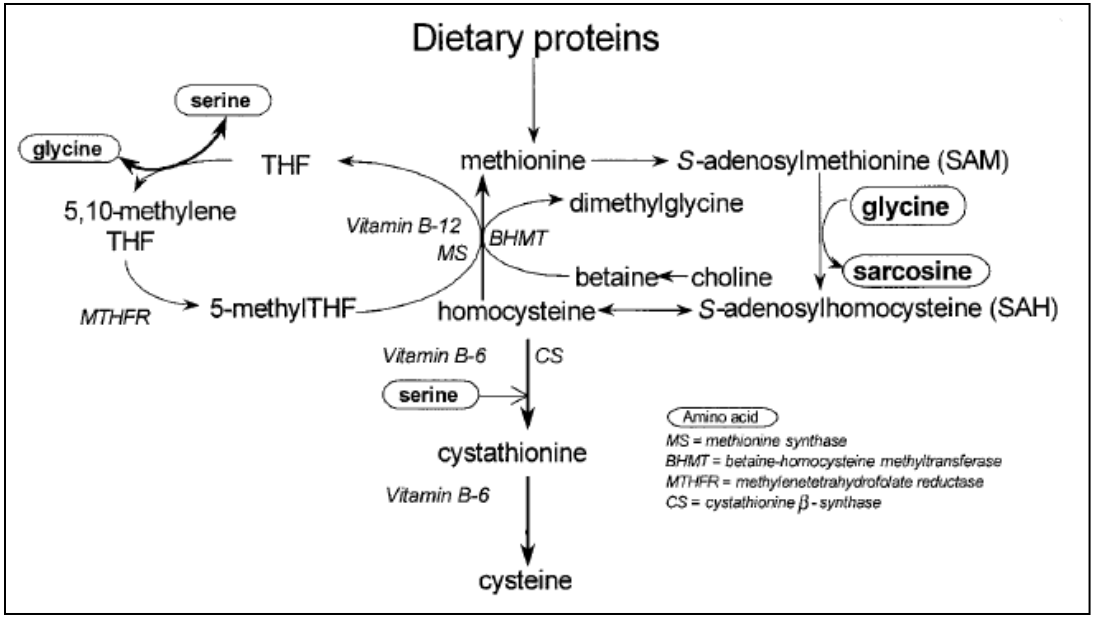
cystine

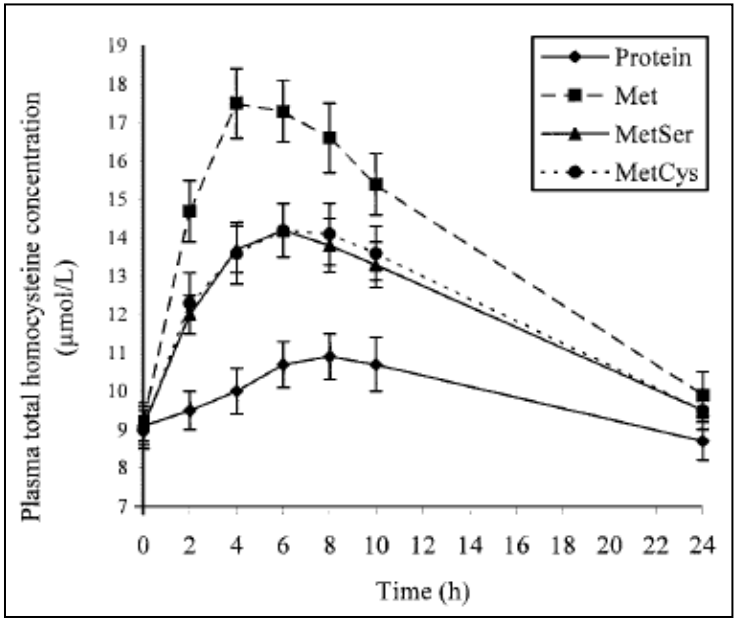
All receive identical low protein lunch and dinners

Measure serum homocysteine 2 → 24 hours following AM test meal intake

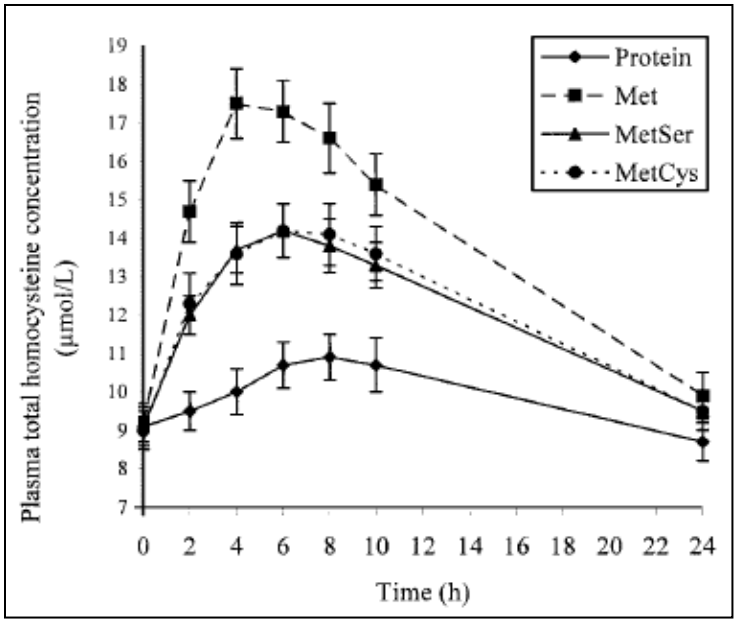
Randomized cross-over protocol utilized

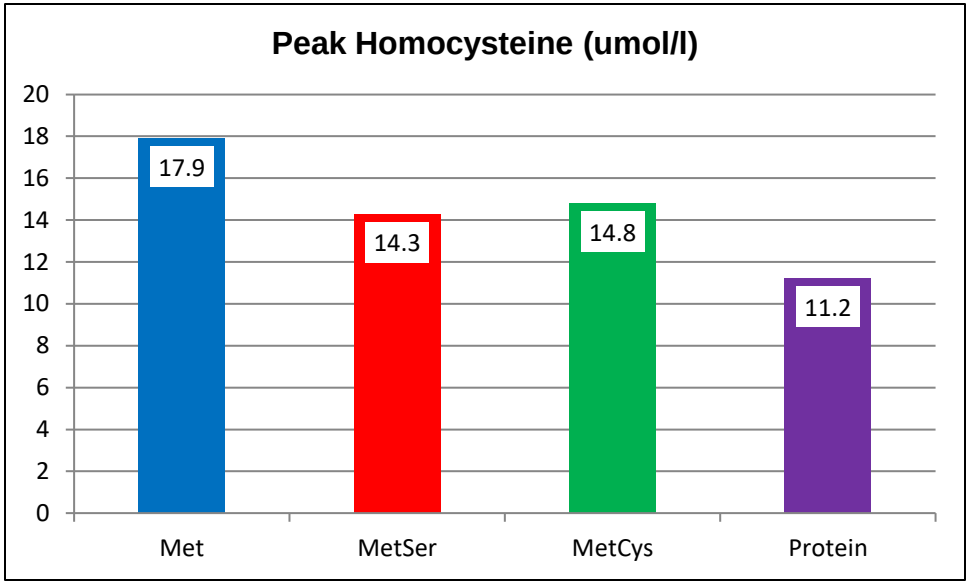
SERINE, CYSTINE, and HOMOCYSTEINE

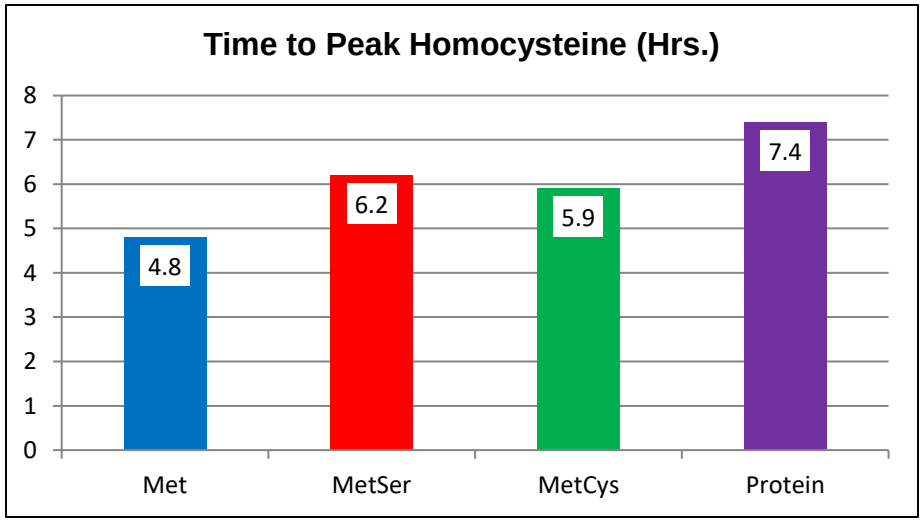


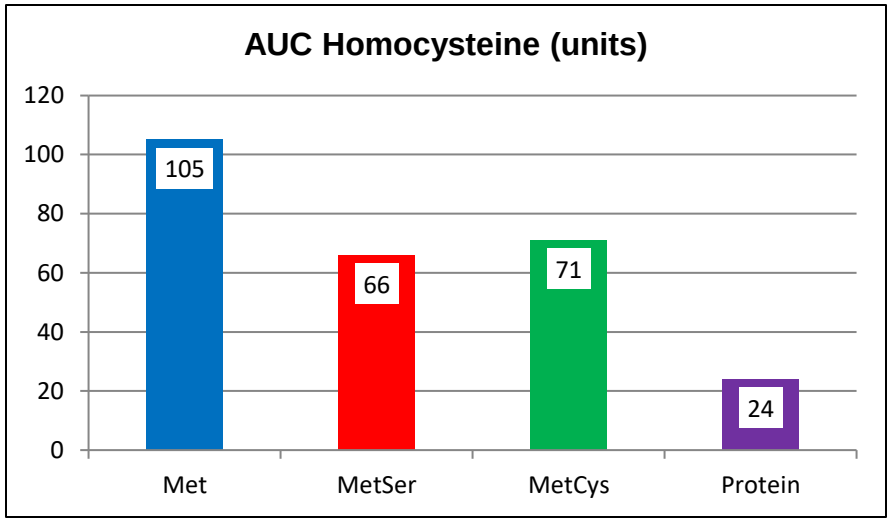


SERINE, CYSTINE, and HOMOCYSTEINE

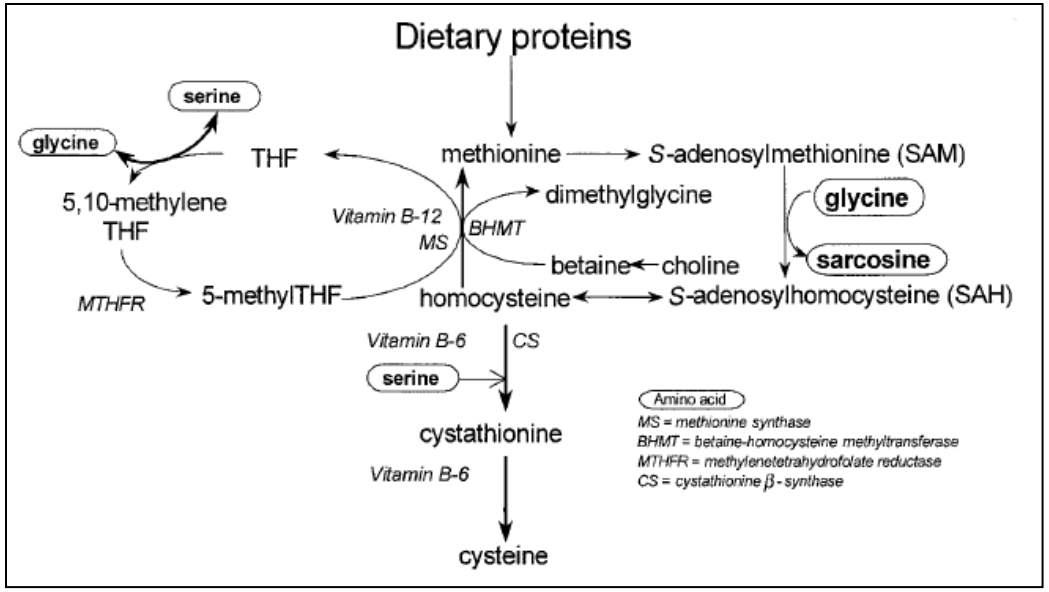


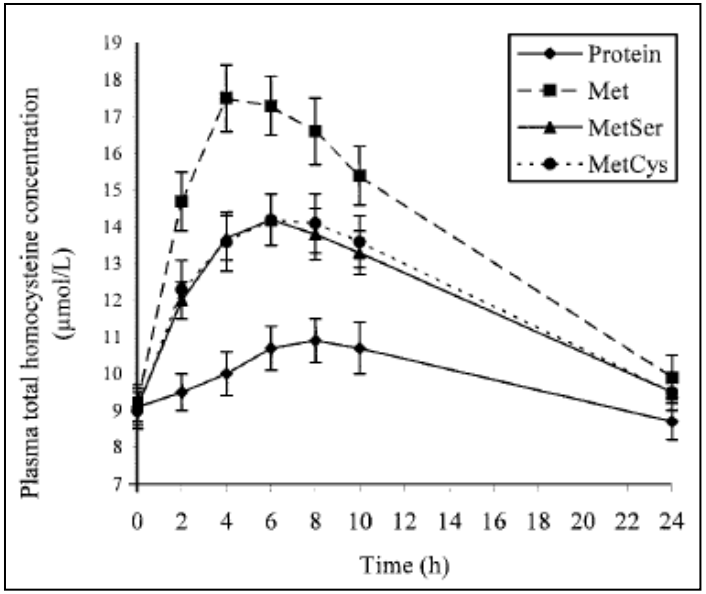






SERINE, CYSTINE, and HOMOCYSTEINE





Serine → Stimulates SHMT and CBS

Full Meal:

- Slower absorption of methionine
- Serine and cysteine within test meal
- More methionine directed to protein synthesis (concomitant AAs)

Cysteine:

- Inhibits CBS pathway but homocysteine decreases?
- Increases remethylation pathways (MTR and BHMT)
- Cysteine-Homocysteine disulfide formation → Enhanced clearance

NAC, FOLATE, HOMOCYSTEINE, and ENDOTHELIAL FN

♥ 60 hyperhomocysteinemic coronary patients
- Homocysteine > 15 umol/l
- > 50% stenosis one or more vessels

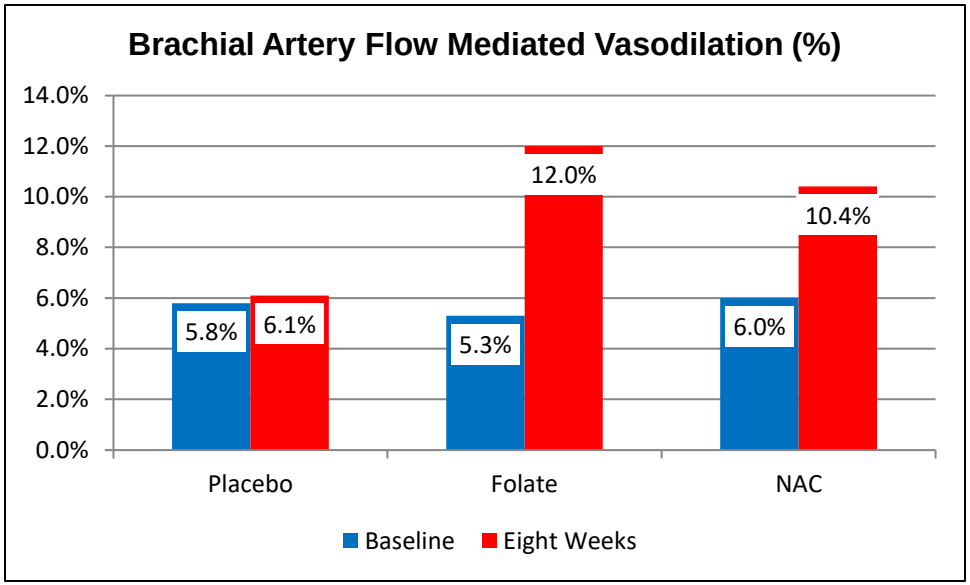
Baseline measurements:
- Fasting homocysteine
- Endothelial function (BA FMD)

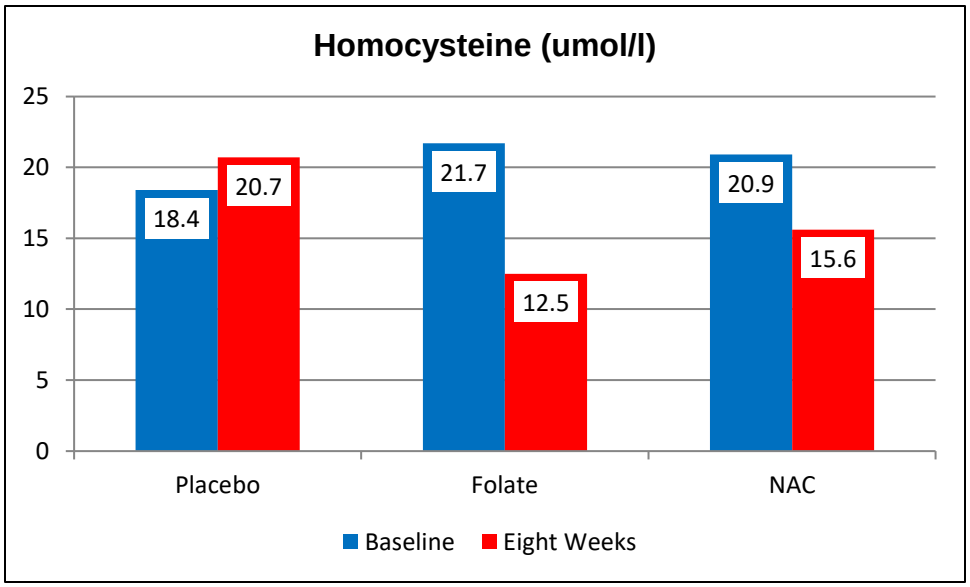
Randomize to receive over eight weeks
- Folic acid 5 mg/day
- N-Acetylcysteine 600 mg/day

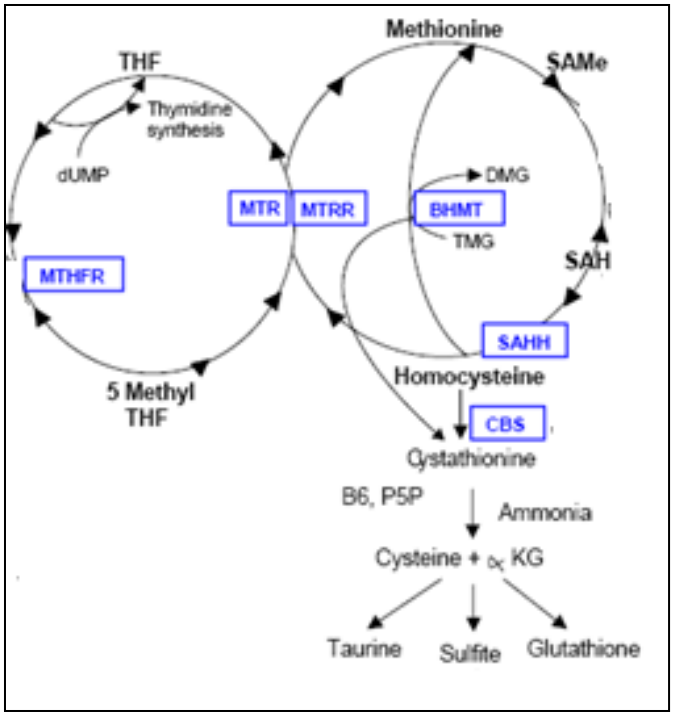
Repeat baseline measurements

Double blind protocol followed

NAC, FOLATE, HOMOCYSTEINE, and ENDOTHELIAL FN







N-ACETYL CYSTEINE MECHANISM of ACTION

♥ 40 healthy volunteers
- Mean age 44
- 20 male and 20 female

Baseline studies

Randomize to receive over four weeks:
- No therapy
- NAC 600 mg daily
- NAC 1,800 mg once daily

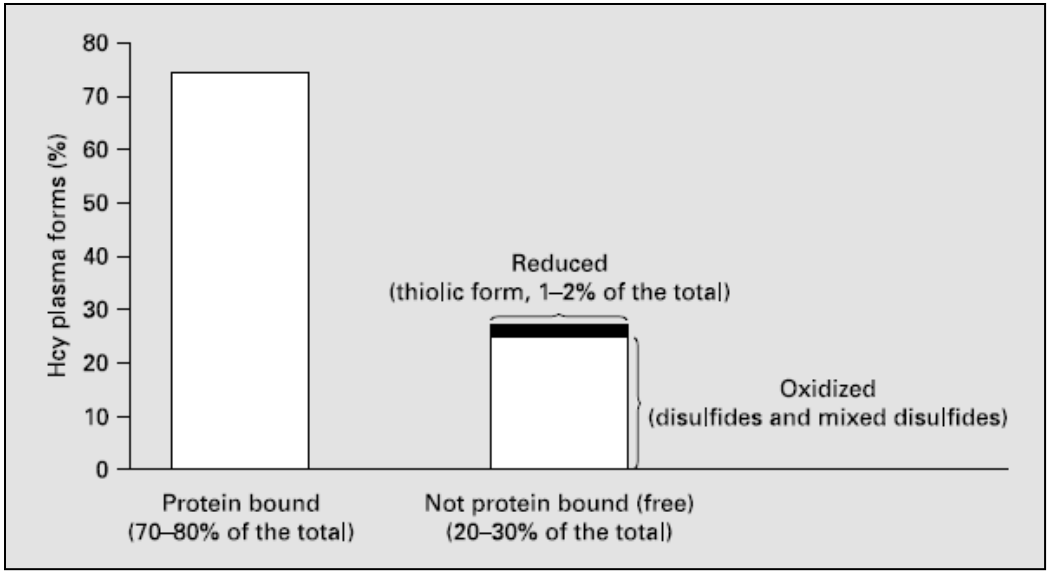
Repeat baseline studies

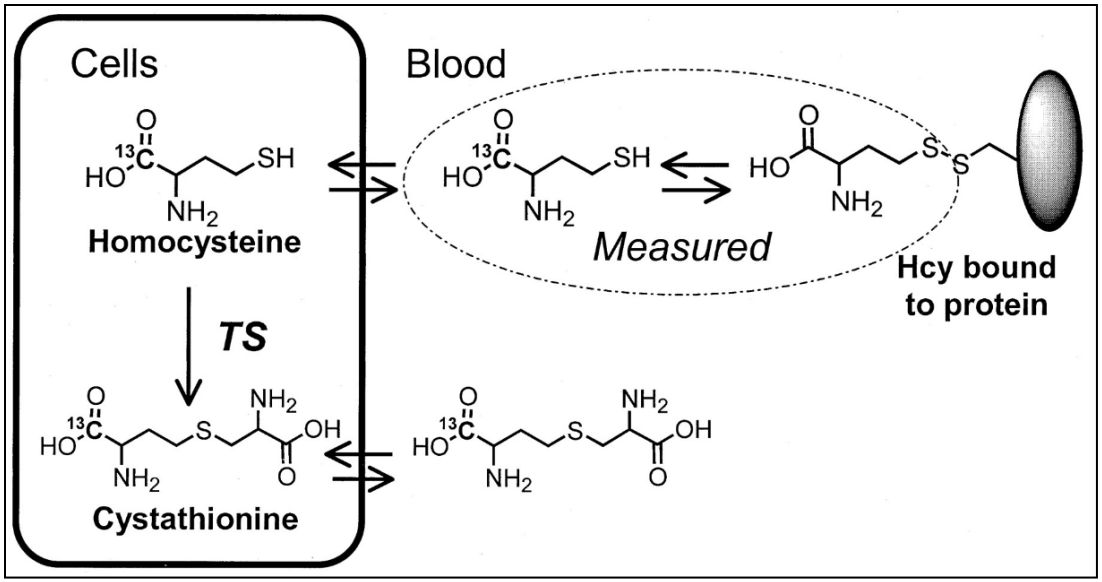
Washout over four weeks

Treat all with NAC 1,800 mg/day over eight weeks and repeat baseline studies

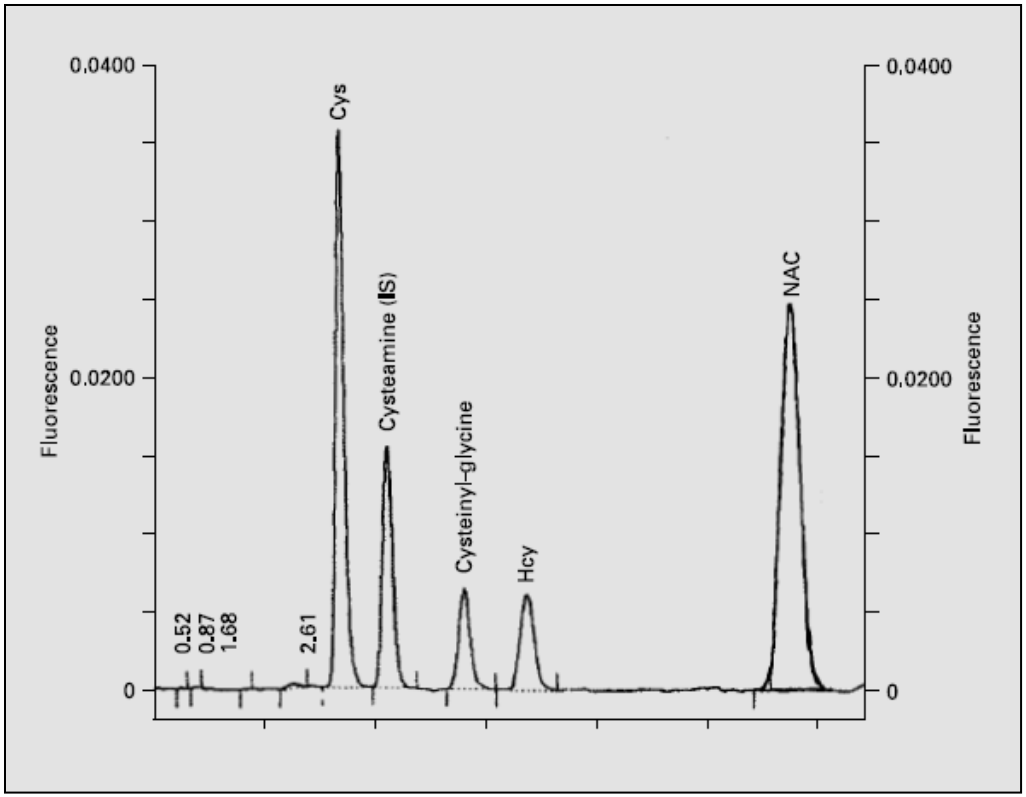
Double blind protocol followed

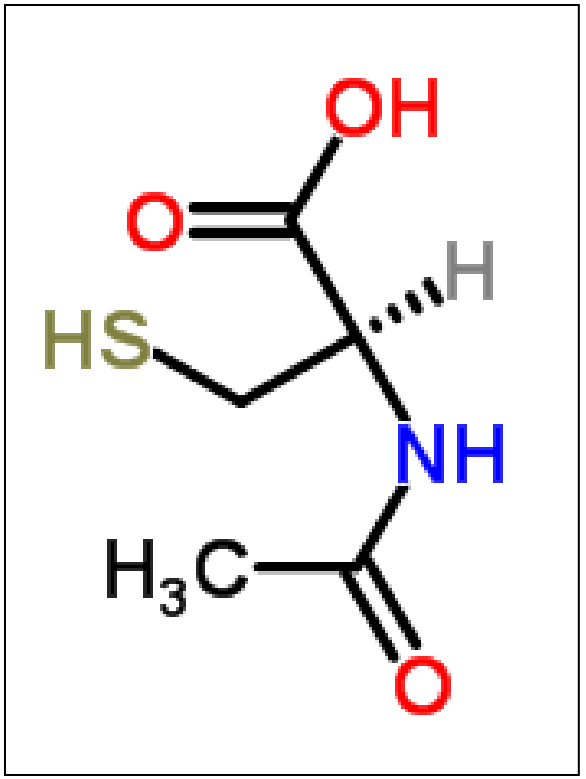
N-ACETYL CYSTEINE MECHANISM of ACTION





N-ACETYL CYSTEINE MECHANISM of ACTION

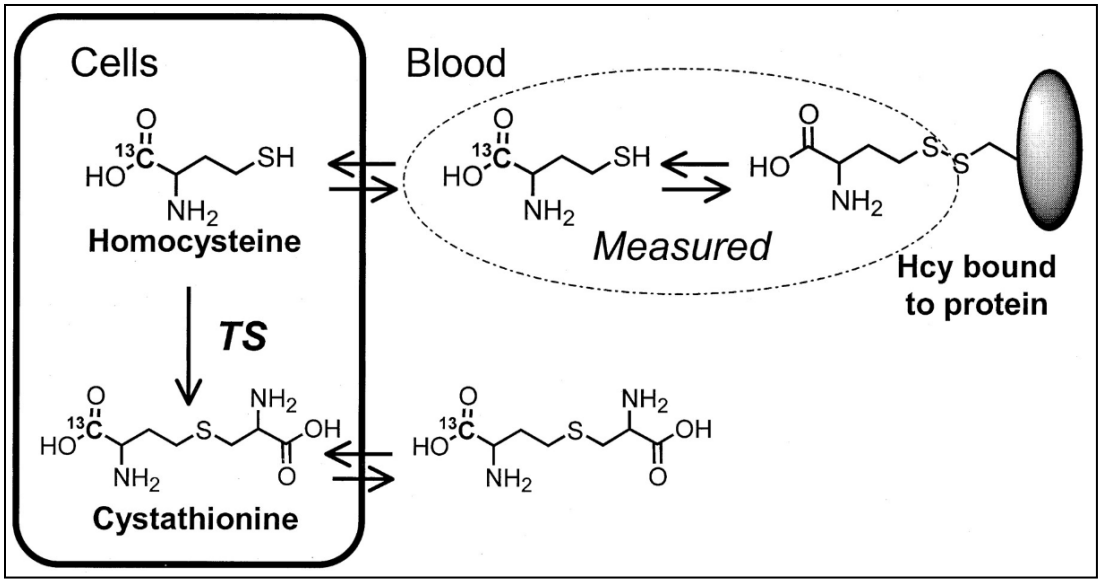


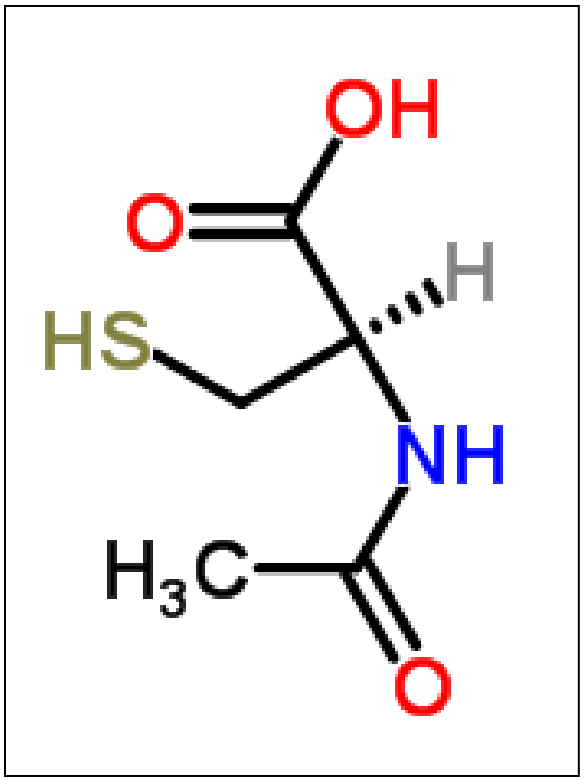


Glutathione precursor (renal & hepatic disease, nitrate tolerance; acetaminophen OD)

Mucolytic agent (splices SH bonds within mucus macromolecules)

N-ACETYL CYSTEINE MECHANISM of ACTION



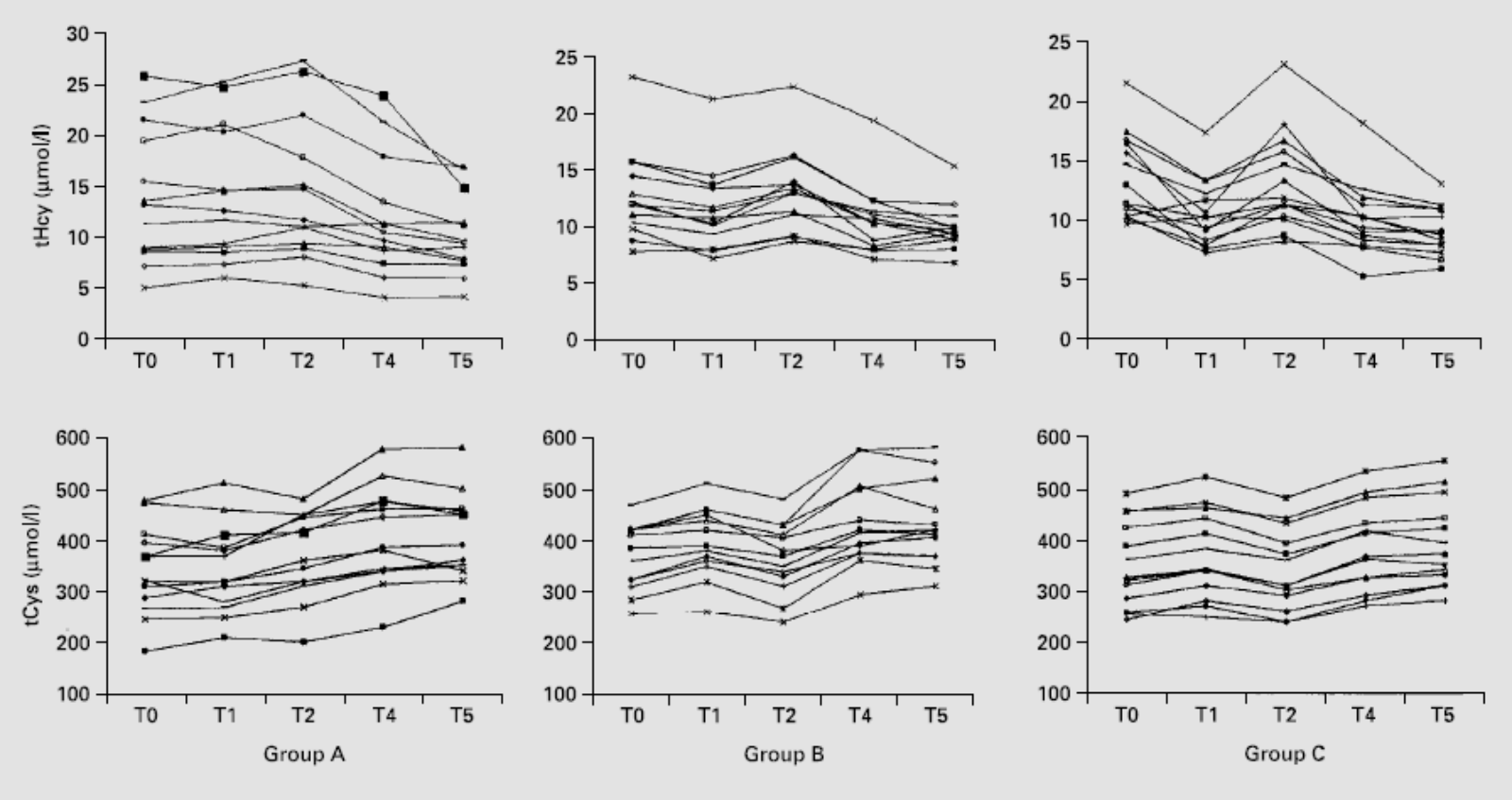


Could NAC increase unbound proportion of Homocysteine?

Would this increase metabolism of plasma homocysteine
and/or
enhance renal excretion?

N-ACETYL CYSTEINE MECHANISM of ACTION

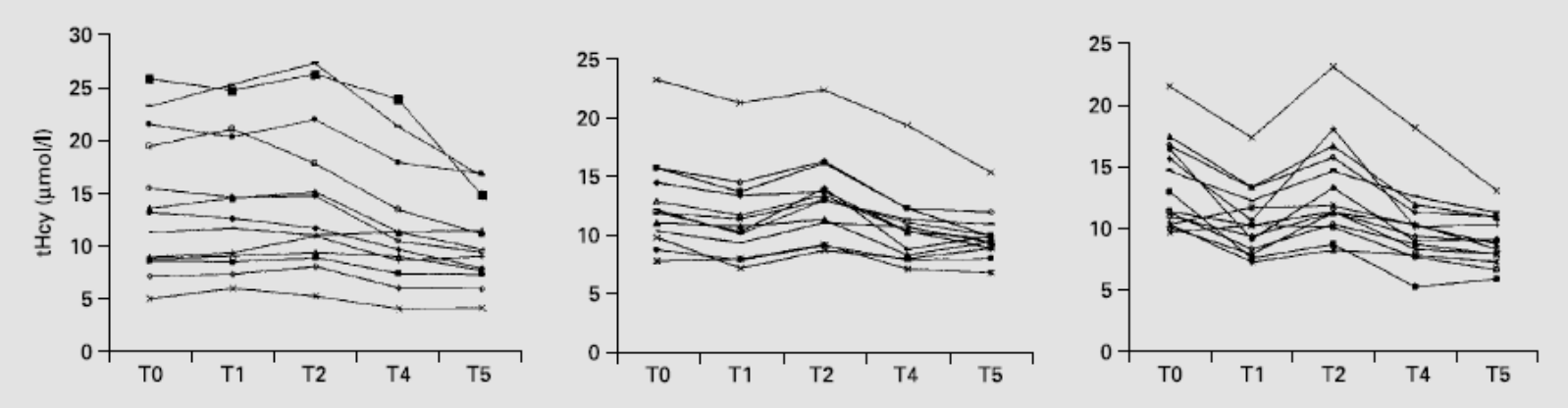
Homocysteine (total plasma)



Controls NAC 900 mg NAC 1,800 mg

All subjects received 1,800 mg/day weeks 8→ 12

N-ACETYL CYSTEINE MECHANISM of ACTION



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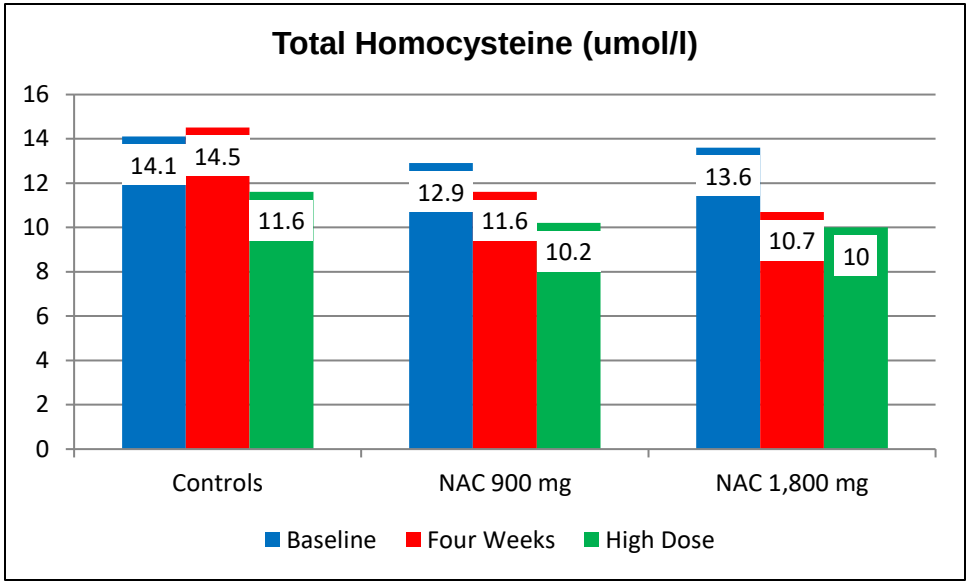
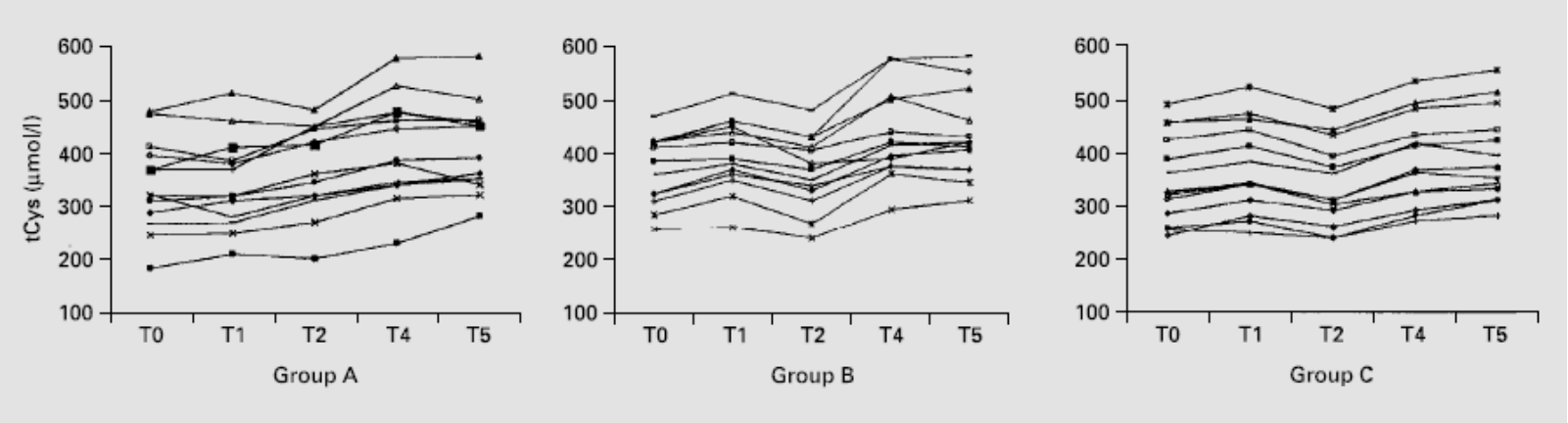
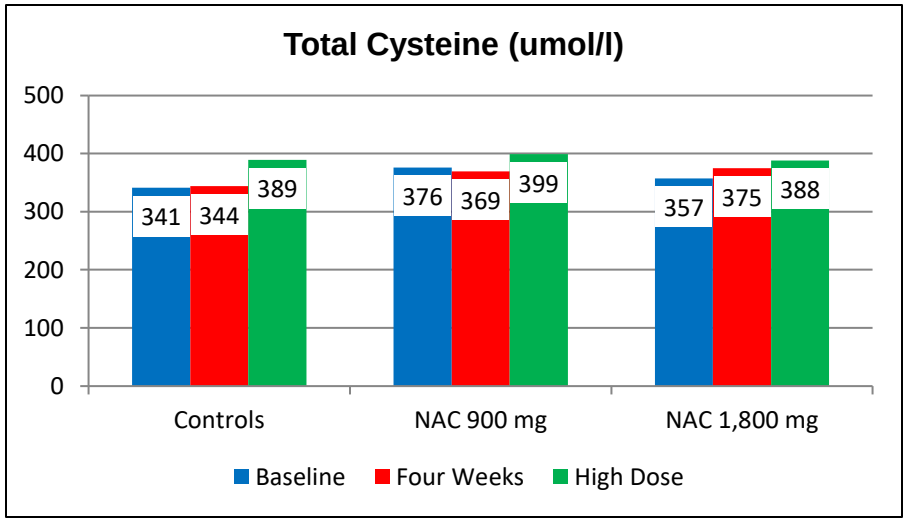
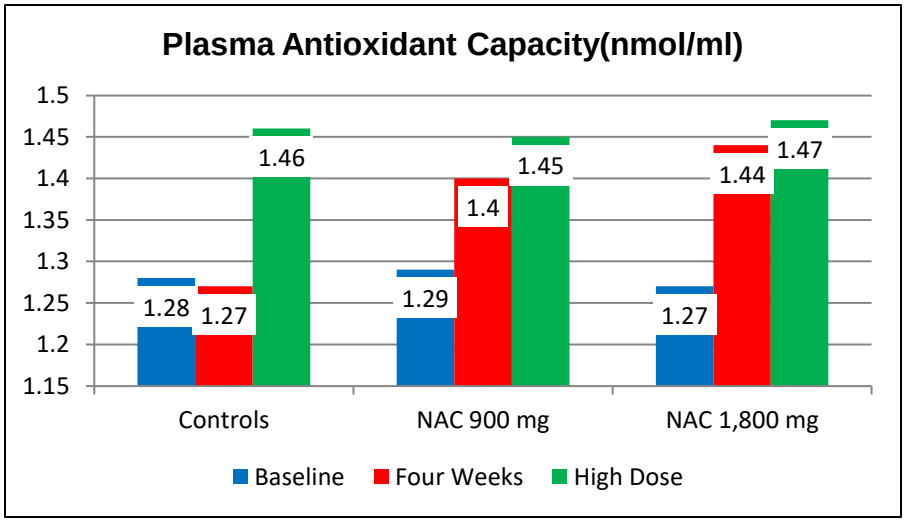


N-ACETYL CYSTEINE MECHANISM of ACTION

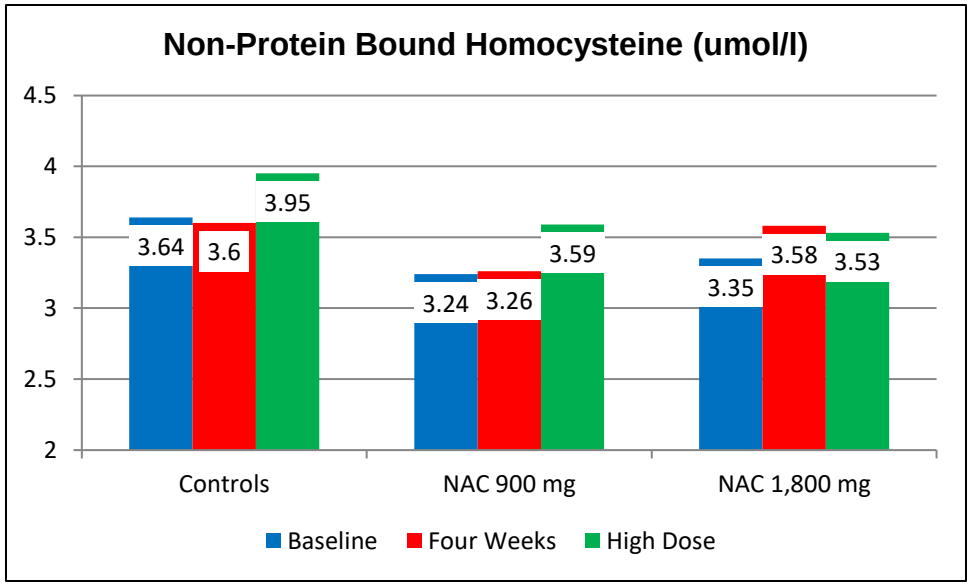


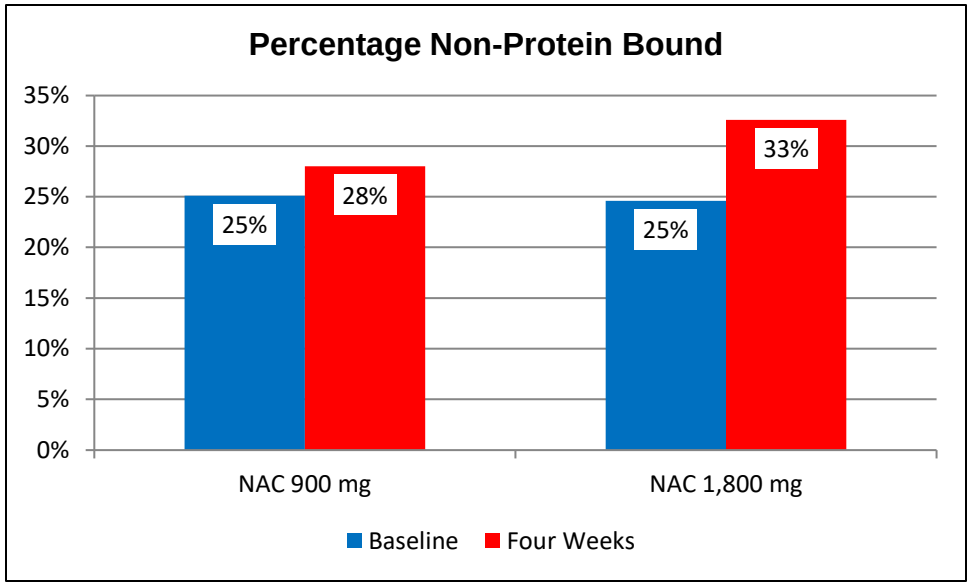
Controls NAC 900 mg NAC 1,800 mg





N-ACETYL CYSTEINE MECHANISM of ACTION





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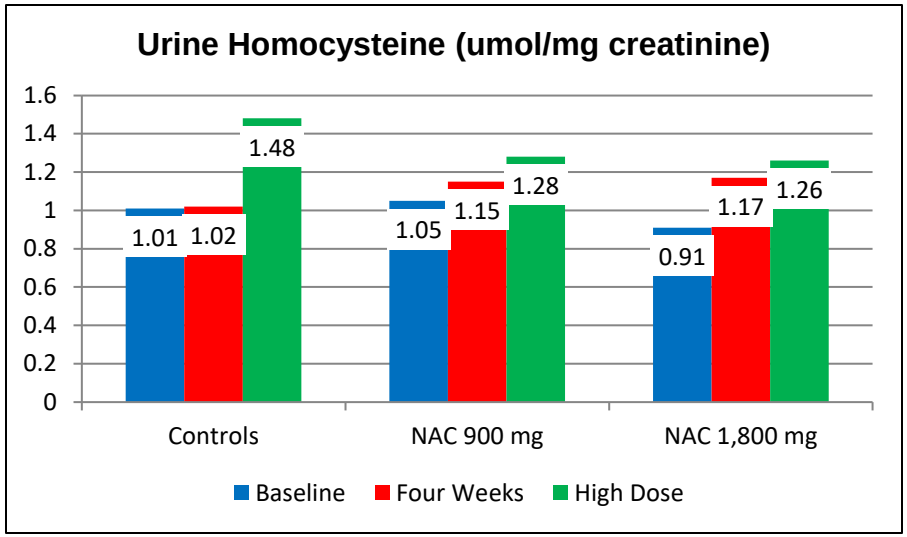
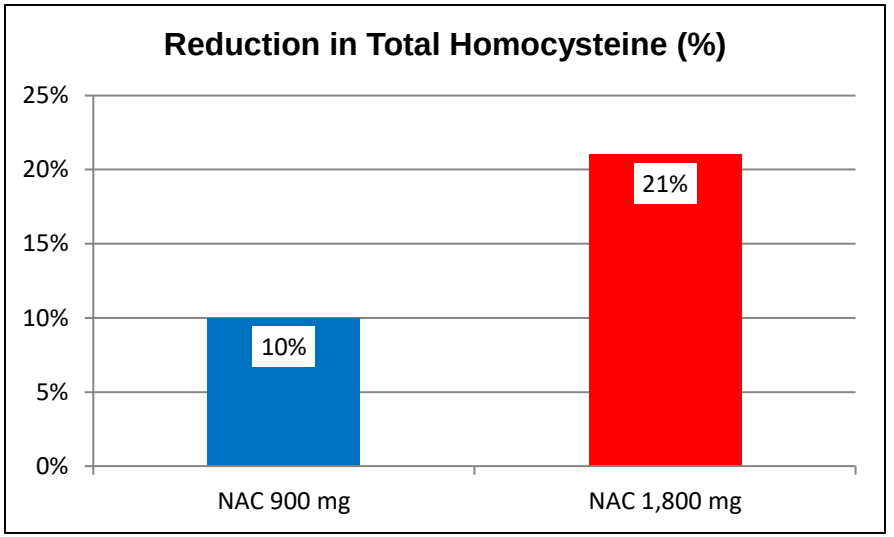




Homocysteine = Free Homocysteine (30%) + Protein Bound Homocysteine (70%)

Homocysteine and N-Acetylcysteine both bear a –SH group

N-Acetylcysteine splices Homocysteine from –SH groups on circulating proteins

Homocysteine-SH-SH-N-Acetylcysteine

- More readily cleared by kidneys
- Possibly easier to metabolize

FISH OIL and HOMOCYSTEINE CONTROL

♥ 24 diabetic subjects with suboptimal lipid control
- Twelve months of Pravastatin 20 mg and Fenofibrate 200 mg/day
- Lipid values above target goals
- All on Metformin (mean dose 1500 mg/day)

Baseline studies

Add 3.6 gm/day omega-3 (57% EPA and 29% DHA) to medical program

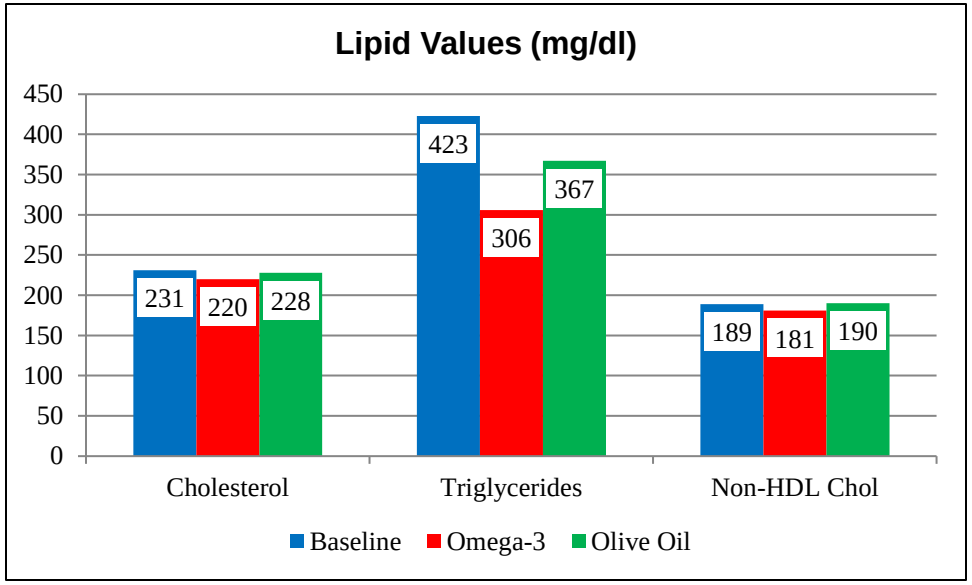
Repeat baseline studies at three months

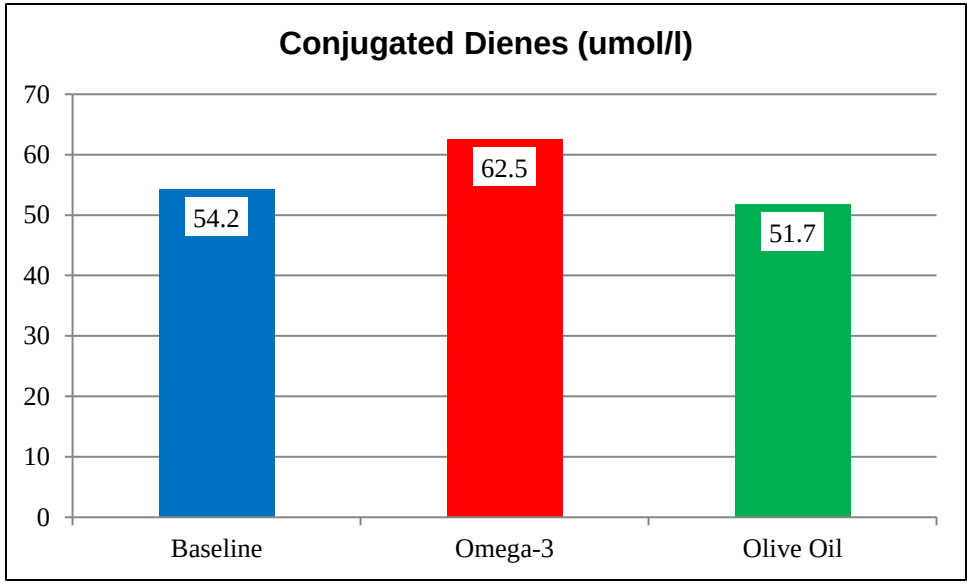
Then cross-over to 3.6 gm/day of olive oil for three months

Repeat measurements

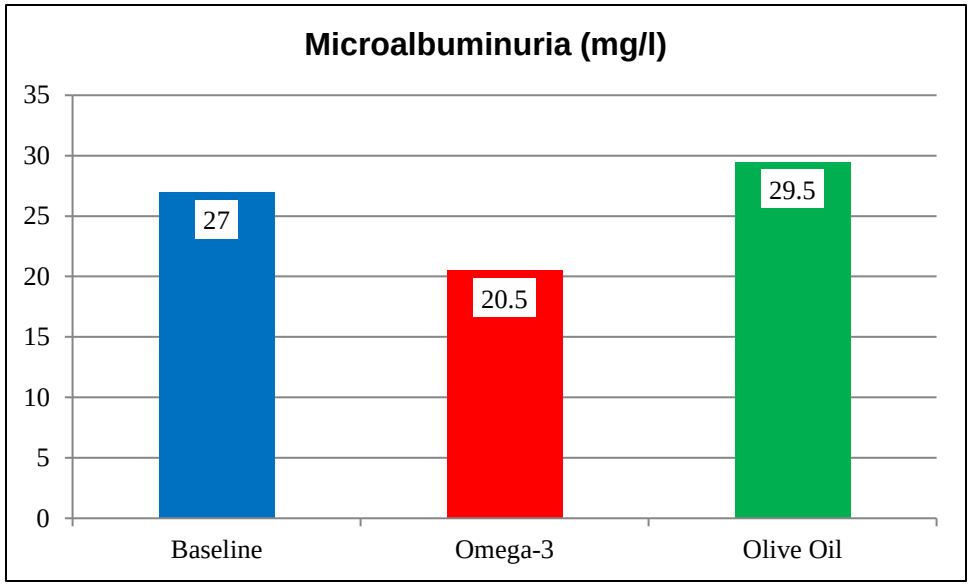
Single blind protocol followed

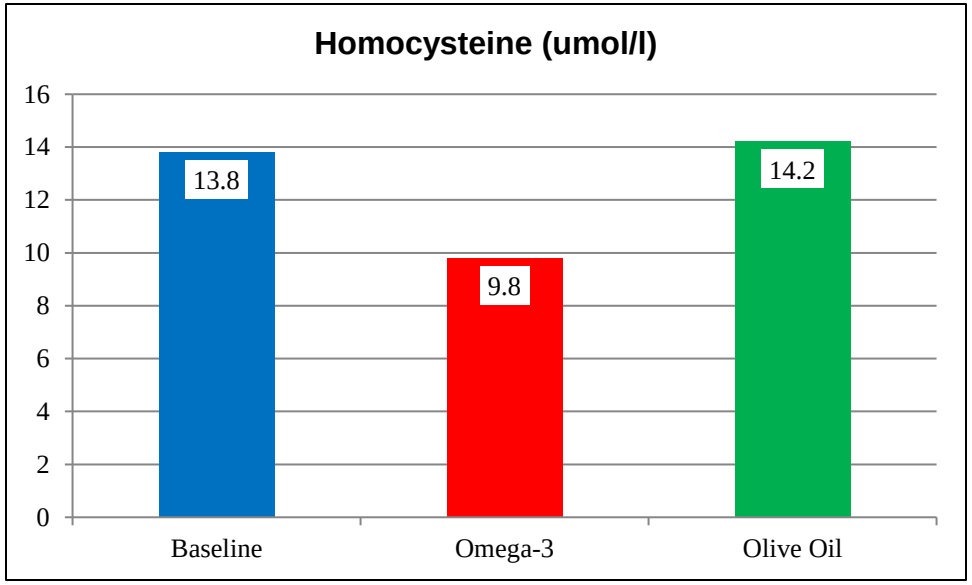
FISH OIL and HOMOCYSTEINE CONTROL

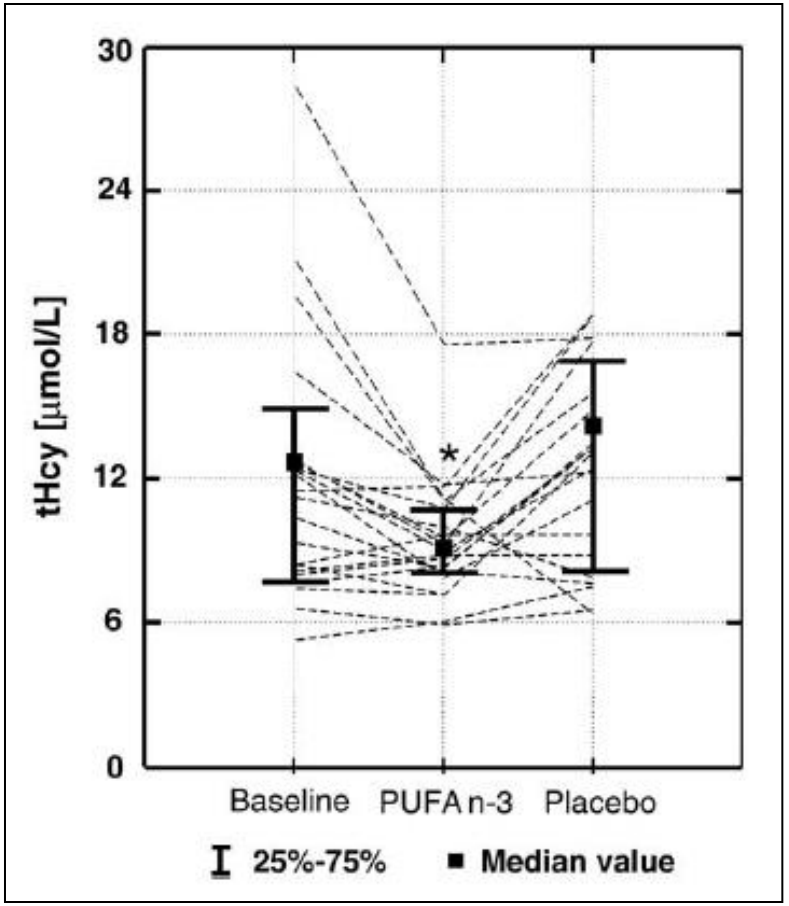




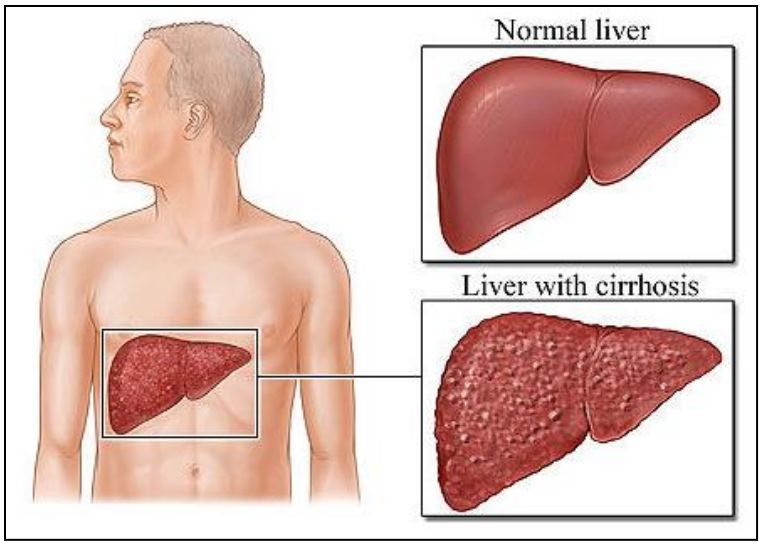
FISH OIL and HOMOCYSTEINE CONTROL

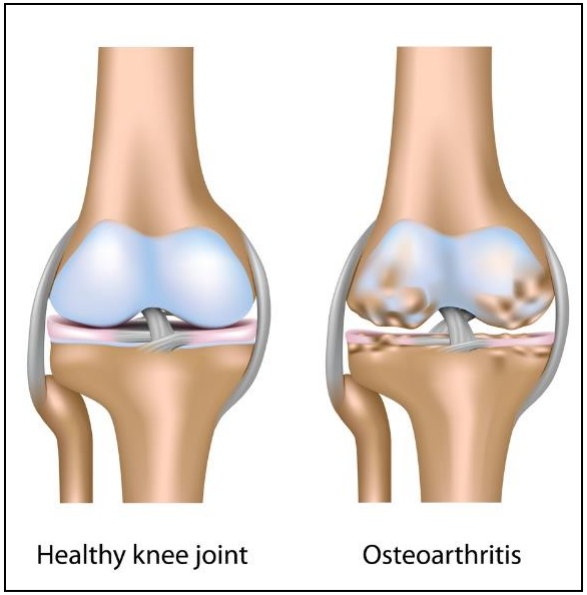


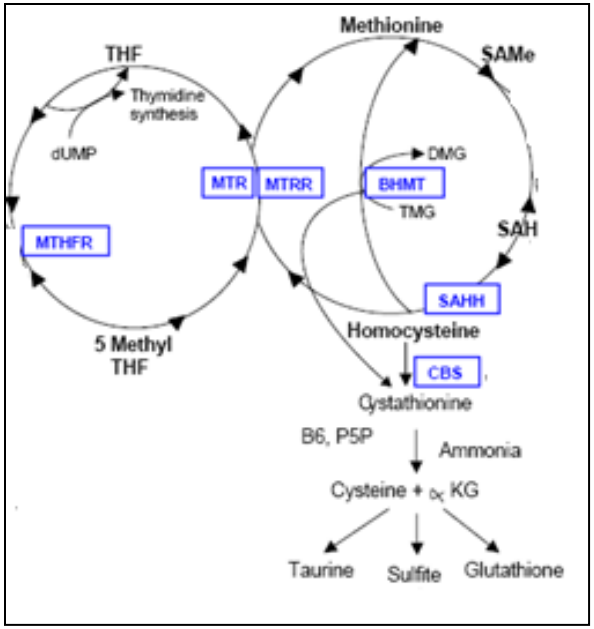


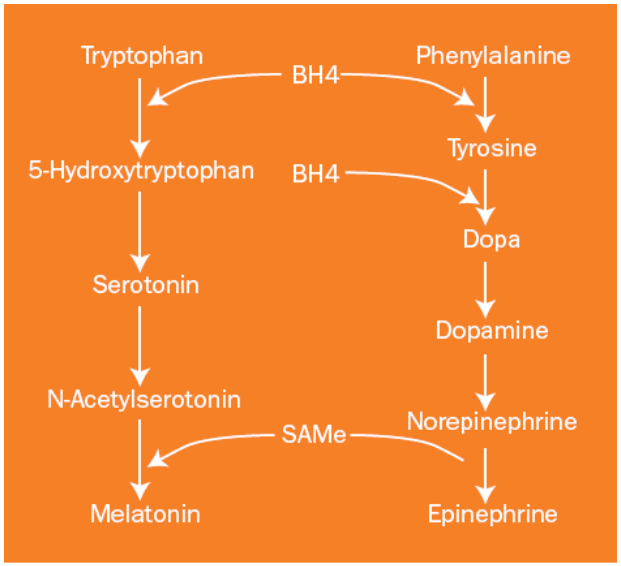


SAMe as a THERAPEUTIC AGENT









SAMe and FOLATES in DEPRESSION

1/4th US population will experience depression within their lifetime

Folate deficiency:

- Increases risk of depression
- Present outright in 1/3rd depressed
- Reduced response to SSRIs
- Longer duration of symptoms
- Lower CSF folate, SAMe, and neurotransmitter levels

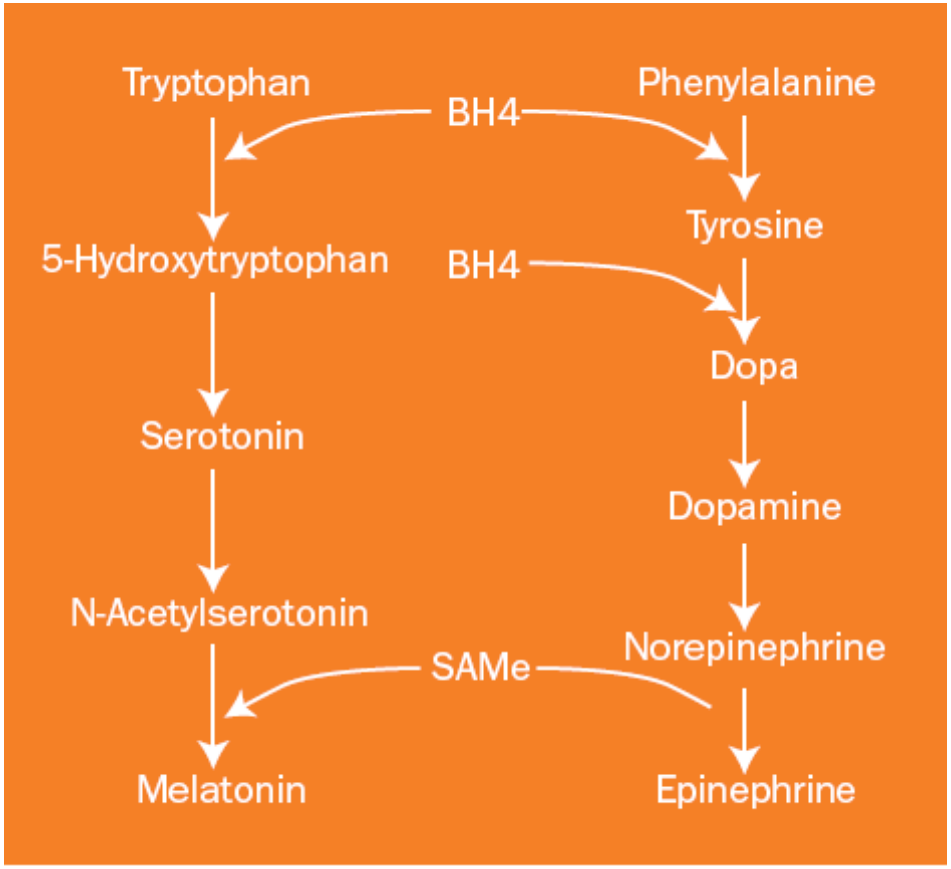
Institutionalized patients:

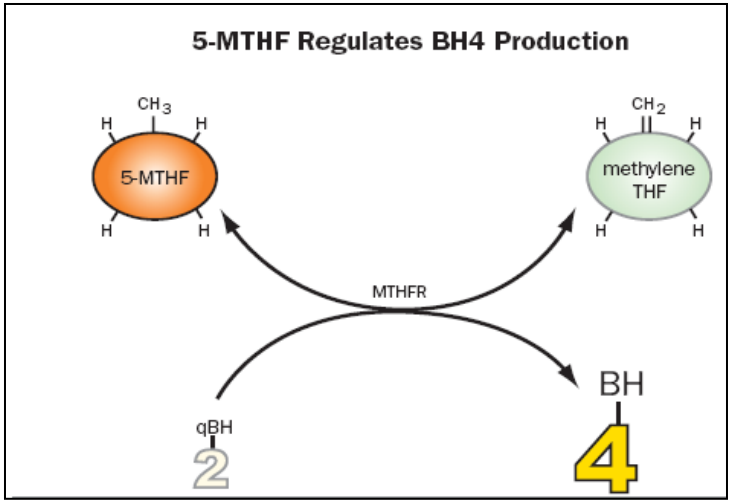
- Low folate ≈ depression risk
- Low B12 folate ≈ psychosis risk

Homocysteine:

- Fifth quintile homocysteine doubles risk
- Elevated in 52% depressed patients
- 36% risk increase if MTHFR TT vs. CC

SAMe in DEPRESSION





Methyl-folate ⇒ BH4

Methyl-folate ⇒ SAMe

Folinic acid and Methyl-folate enhances response to SSRIs

Methyl-folate (high dose – up to 50 mg) mono-therapy effective

FOLATE and CSF NEUROTRANSMITTER LEVELS

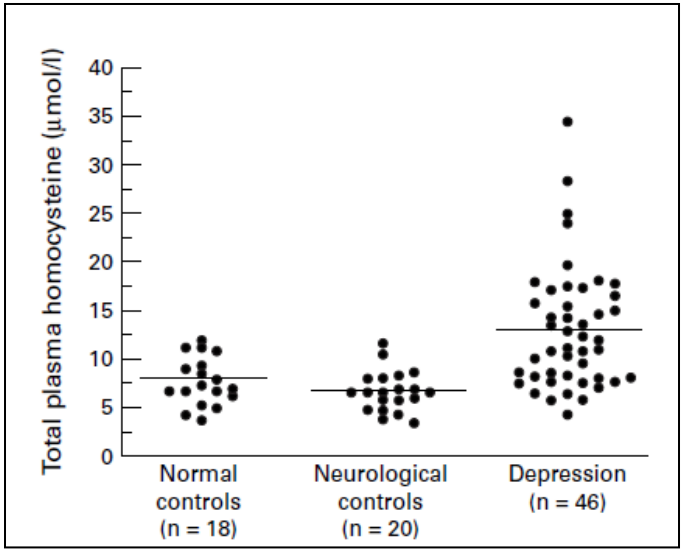
♥ 84 subjects

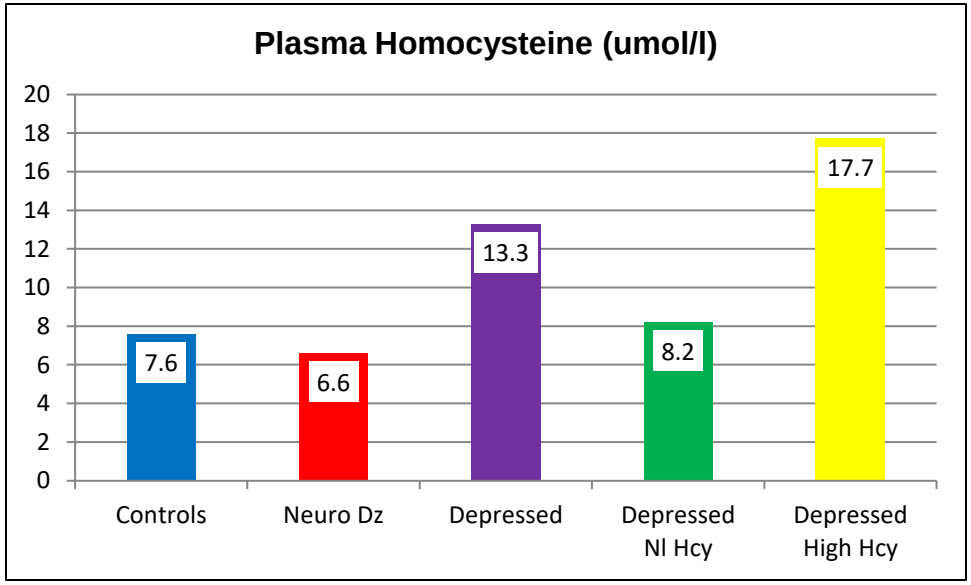
- 46 inpatients with severe depression
- 20 subjects with neurological disorders
- 18 healthy volunteers

Record:

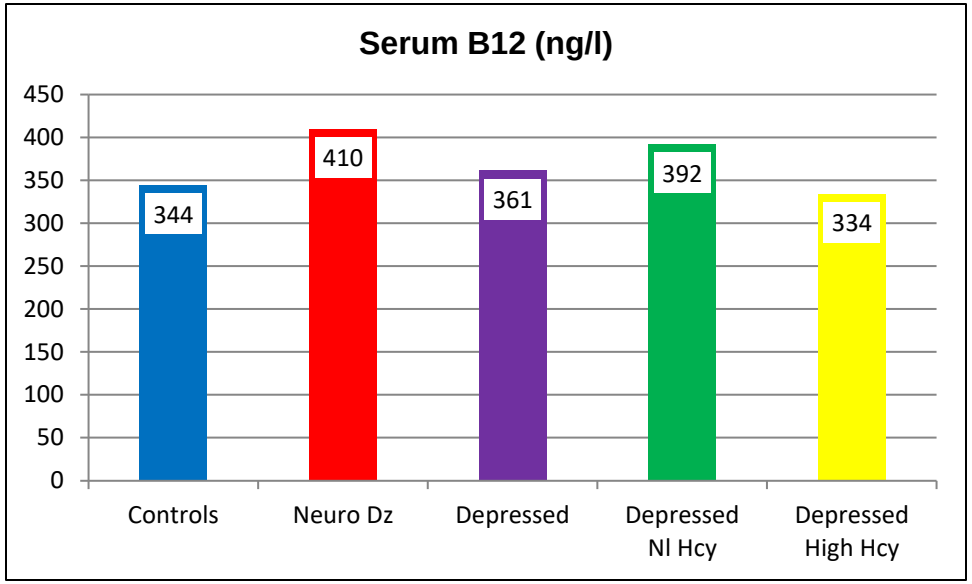
- Plasma folate and B12 and RBC folate
- Homocysteine
- CSF folate
- CSF SAMe
- CSF monoamine neurotransmitter metabolites
    - ♦ 5-HIAA (5-hydroxyindoleacetic acid)
    - ♦ HVA (homovanillic acid)
    - ♦ MHPG (3-methoxy-4-hydroxyphenyl glycol)

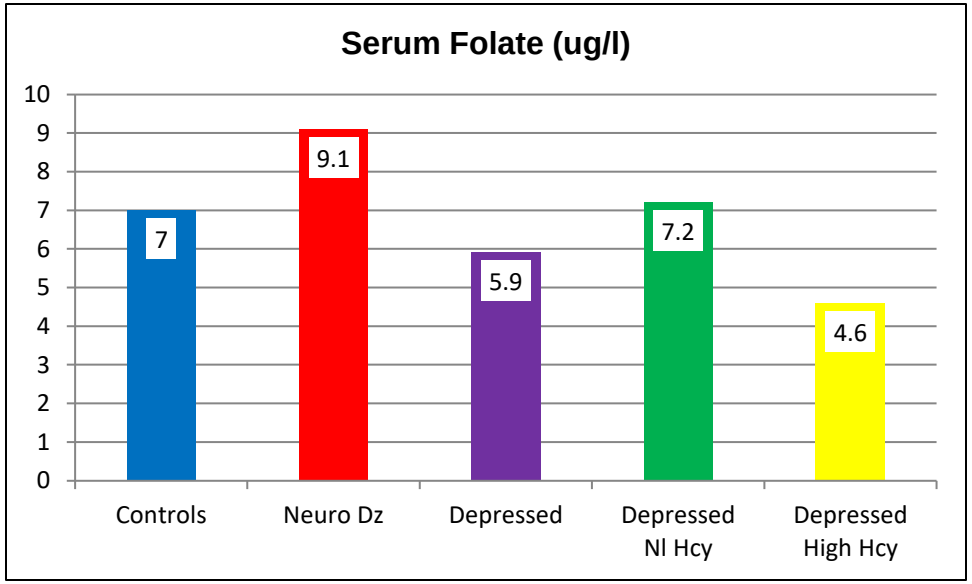
FOLATE and CSF NEUROTRANSMITTER LEVELS



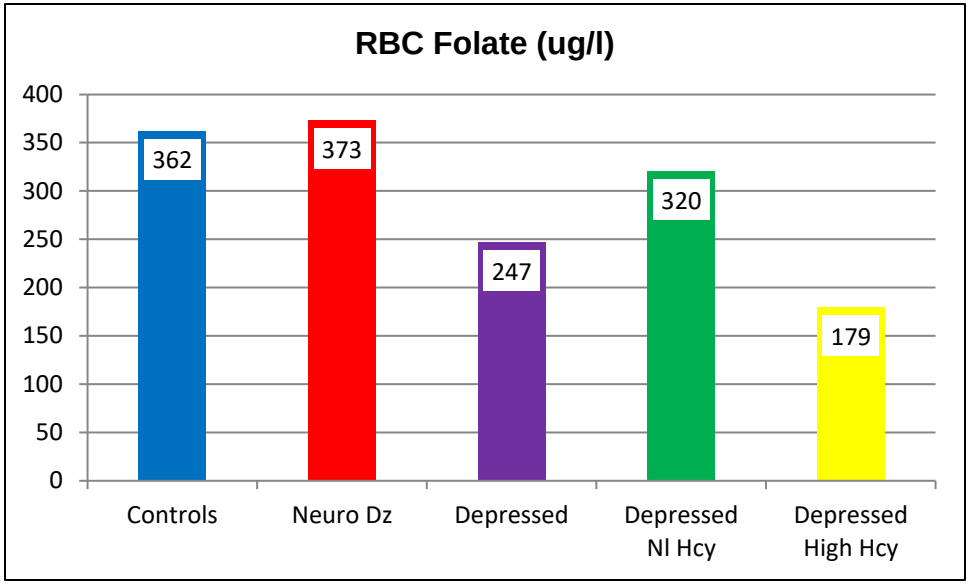


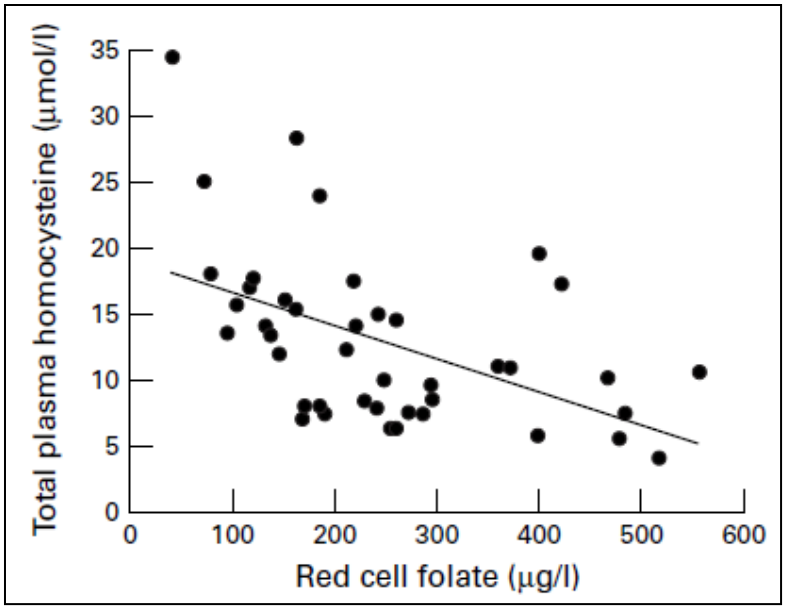
FOLATE and CSF NEUROTRANSMITTER LEVELS



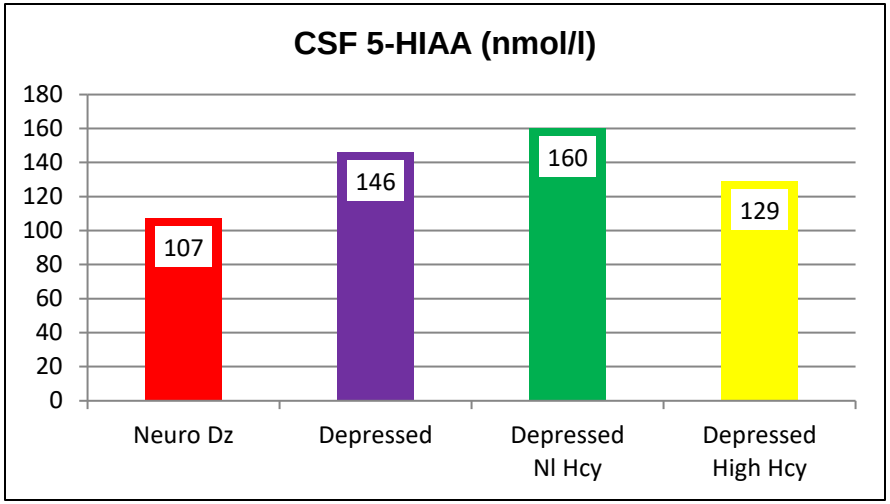


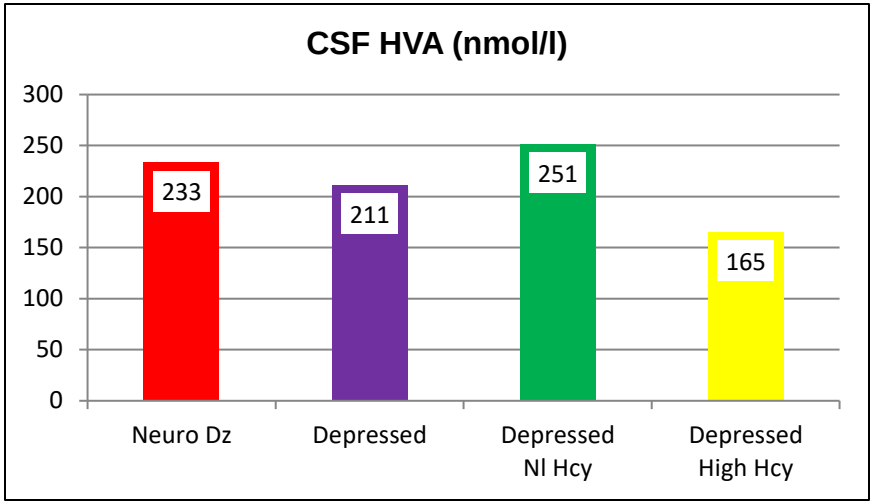
FOLATE and CSF NEUROTRANSMITTER LEVELS

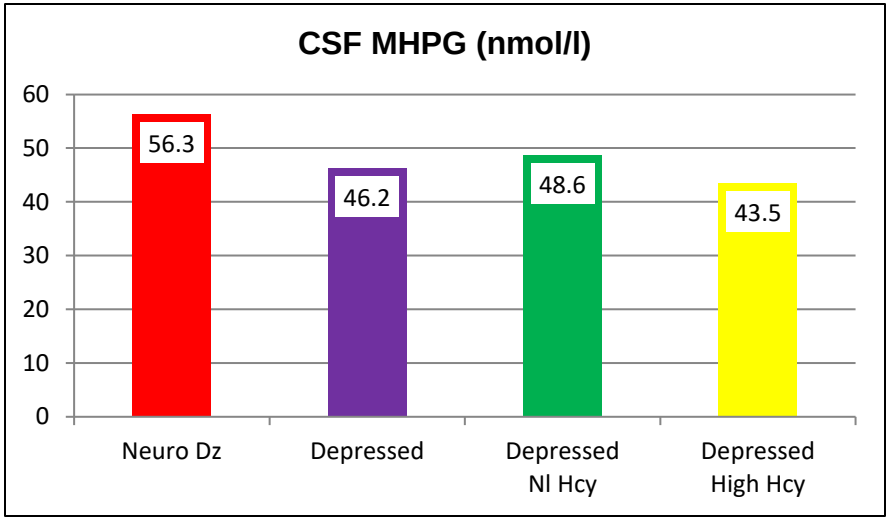


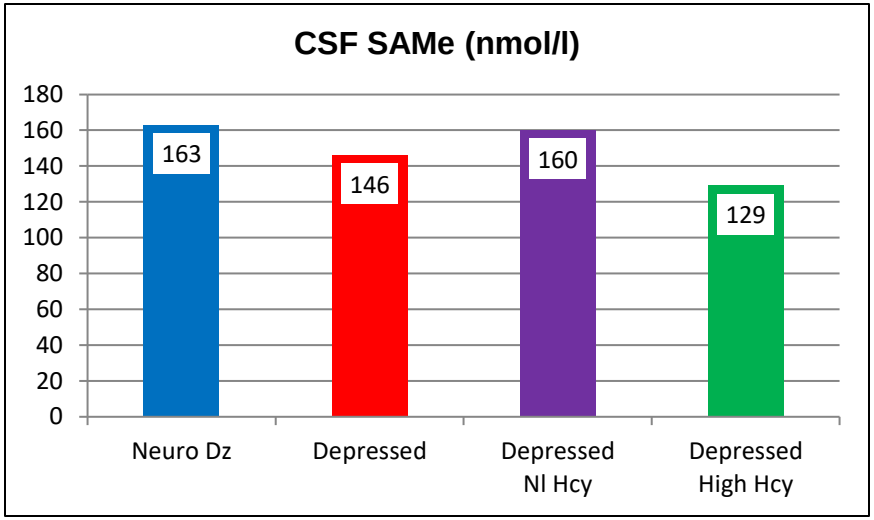


FOLATE and CSF NEUROTRANSMITTER LEVELS





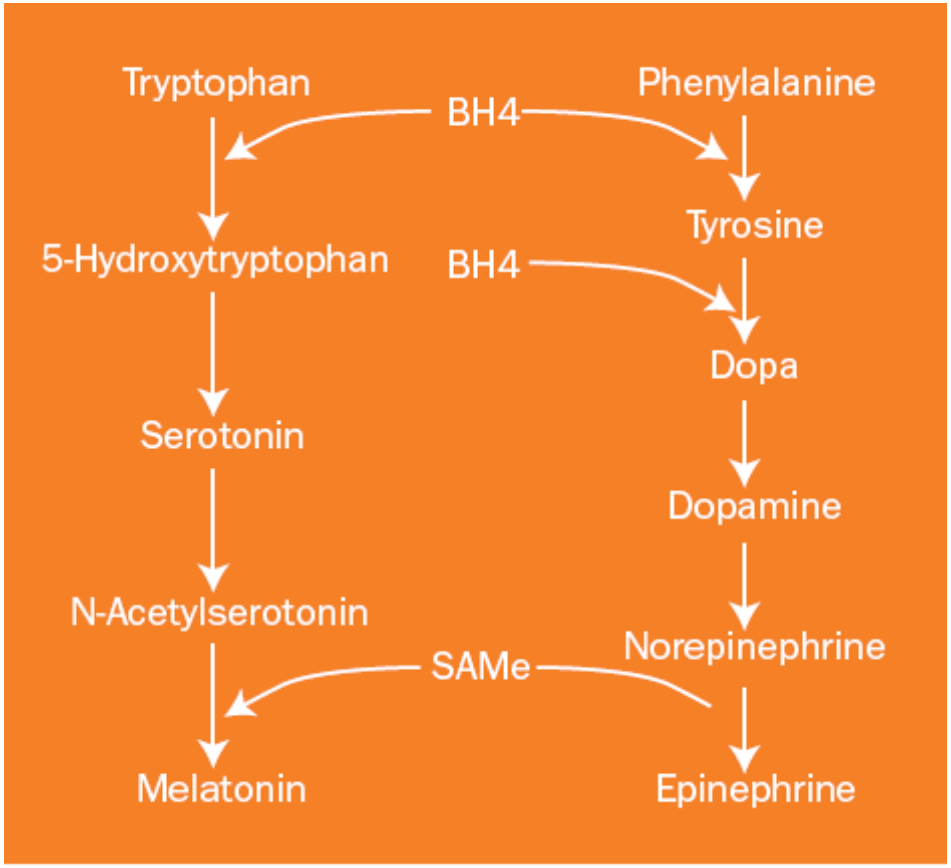




FOLATE and CSF NEUROTRANSMITTER LEVELS

- 1/3rd of severely depressed are folate deficient ≈ Elevated homocysteine

- Lower CSF monoamine metabolites and SAMe



SAMe in TREATMENT RESISTANT DEPRESSION

♥ 30 patients with inadequately controlled depression:

- HAM-D score of ≥ 14
- Adequate and stable dose of SSRI or Venlafaxine
  - ♦ Fluoxetine, Paroxetine, Citalopram ≥ 20 mg
  - ♦ Escitalopram ≥ 10 mg ♦ Sertraline ≥ 50 mg
  - ♦ Venlafaxine ≥ 75 mg
- Mean duration 20 months
- Mean lifetime major depression episodes of four

Baseline measurements

Treat all over six weeks with:

- SAMe tosylate 400 mg bid
- Increase to 800 mg bid at 4 weeks
- Drug and/or SAMe dose decreases permitted

Response – 50% reduction in HAM-D score vs. baseline
Remission – HAM-D score ≤ 7

SAMe in TREATMENT RESISTANT DEPRESSION

77% completed six week trial

Side-effects nuisance in nature

7% discontinued due to intolerance

Homocysteine fell from 8.2 to 7.8 umol/l

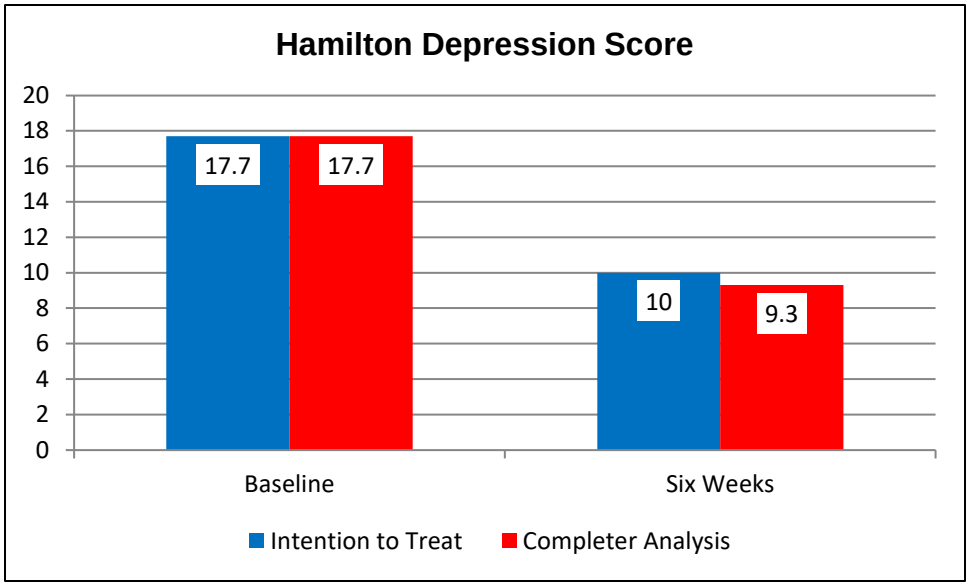
Weight without change

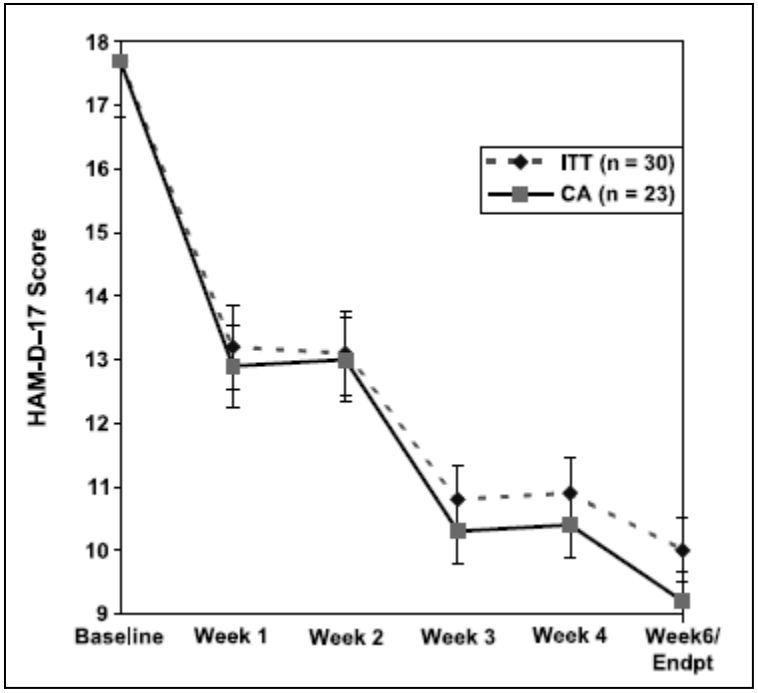
TABLE 1. Common Side Effects of SAMe 800 to 1600 mg/d*

Gastrointestinal	**n**
Constipation	50.0% (15)
Gastrointestinal upset	16.6% (4)
Diarrhea/gas	16.6% (4)
Decreased appetite	6.6% (2)
Musculoskeletal/Nervous	**n**
Headaches	13.3% (4)
Activation (anxiety, irritability)	10.0% (3)
Fatigue, sedation	10.0% (3)
Sleep disturbance	6.6% (2)

*Defined as reported by 5% or more of the sample (n = 30).

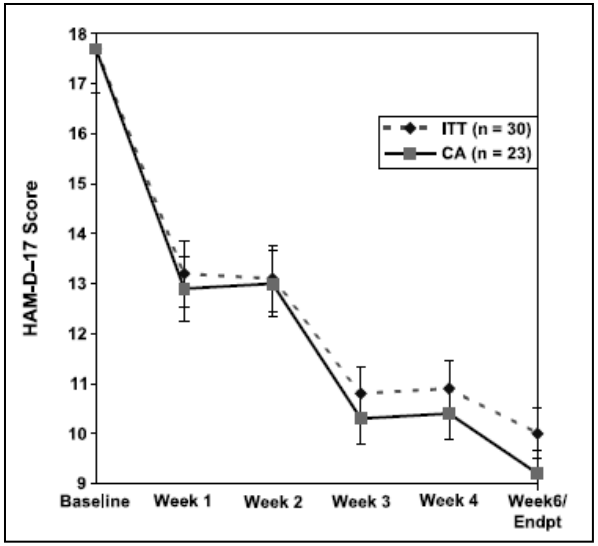
SAMe in TREATMENT RESISTANT DEPRESSION

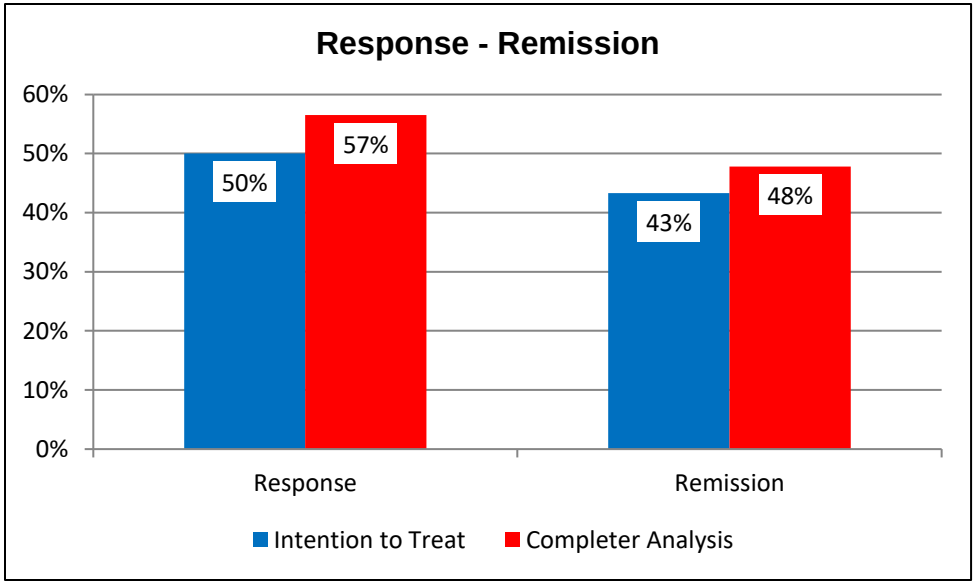




	Baseline	Six Weeks
MGH Sexual Function Scale	22.8	20.6
Montgomery Depression Rating	23.2	13.9
Beck Depression Inventory	18.8	12.2

SAMe in TREATMENT RESISTANT DEPRESSION



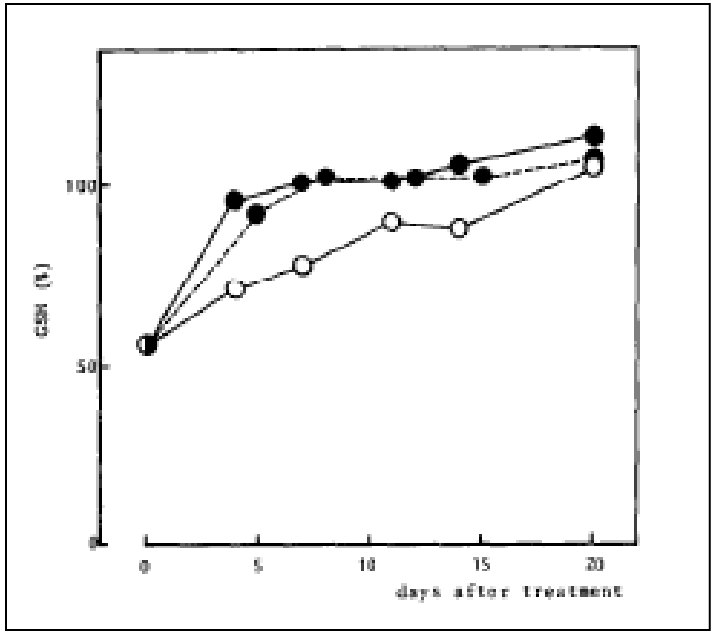


SAMe in ACUTE LEAD TOXICITY

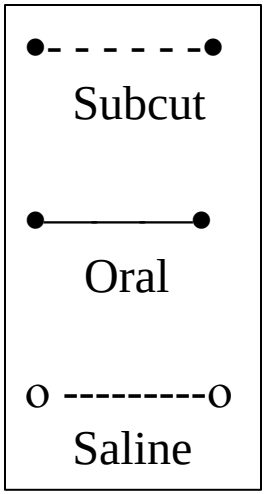
♥ Mice exposed to acute lead toxicity

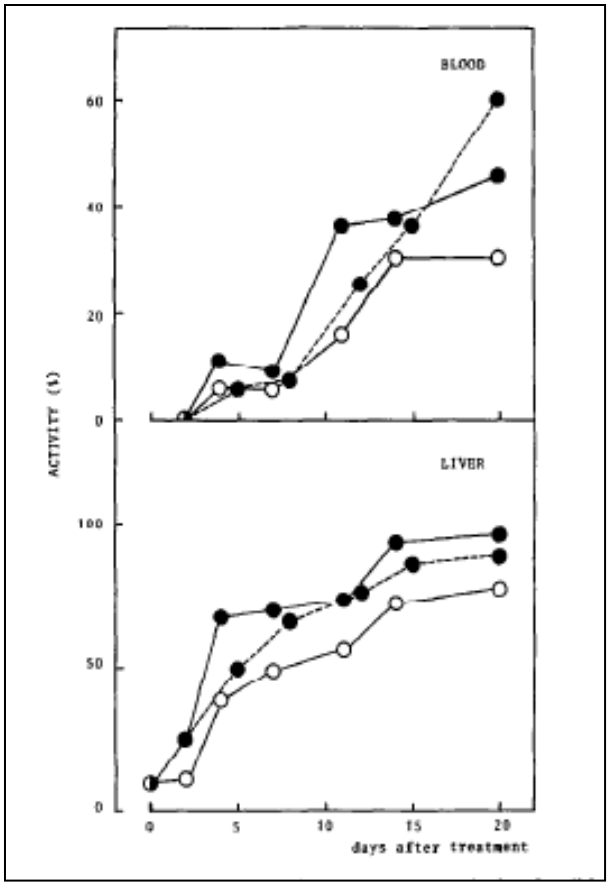
One hour post-final lead injection begin 20 day program of:

- SAMe 20 mg/kg subcut daily
- SAMe 80 mg/kg po
- Saline subcut or po



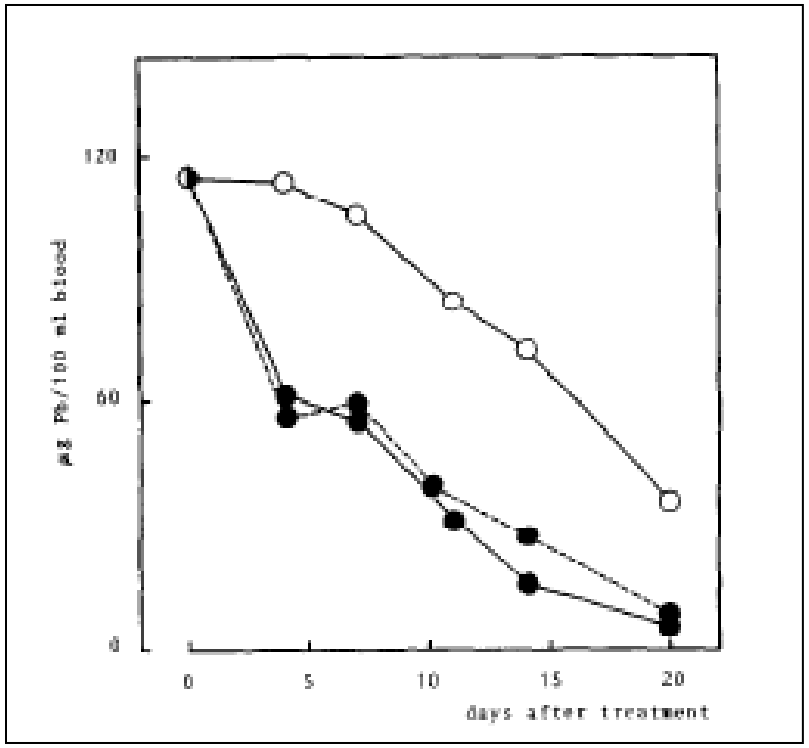
Glutathione



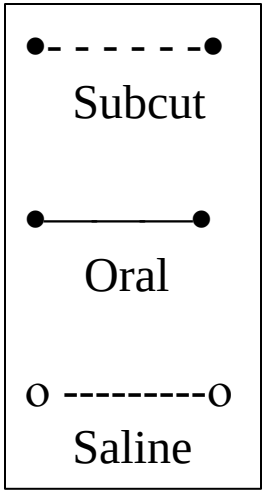


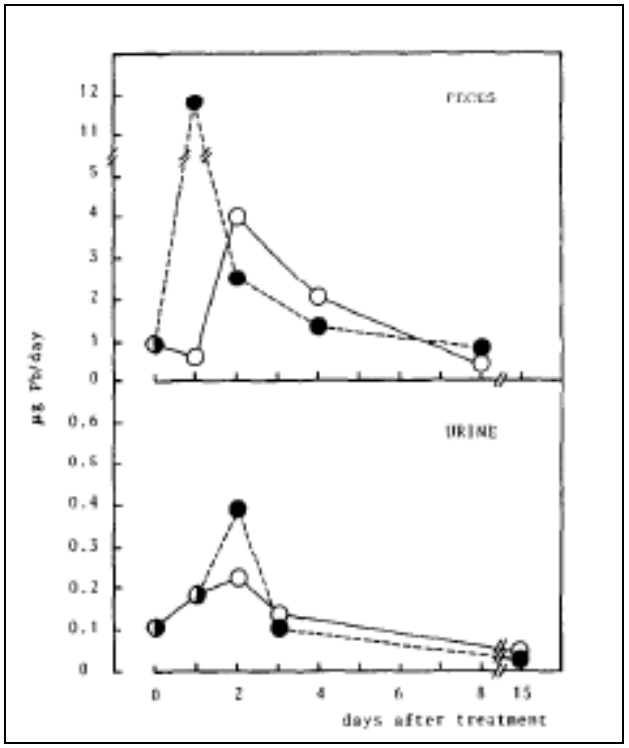
δ-Aminolevulinate Dehydrase

SAMe in ACUTE LEAD TOXICITY



Blood Lead





Fecal and Urine Lead

SAMe in CHRONIC LEAD TOXICITY

♥ Ten patients with chronic lead toxicity

Five receive 12 mg/kg IV daily over 20 days

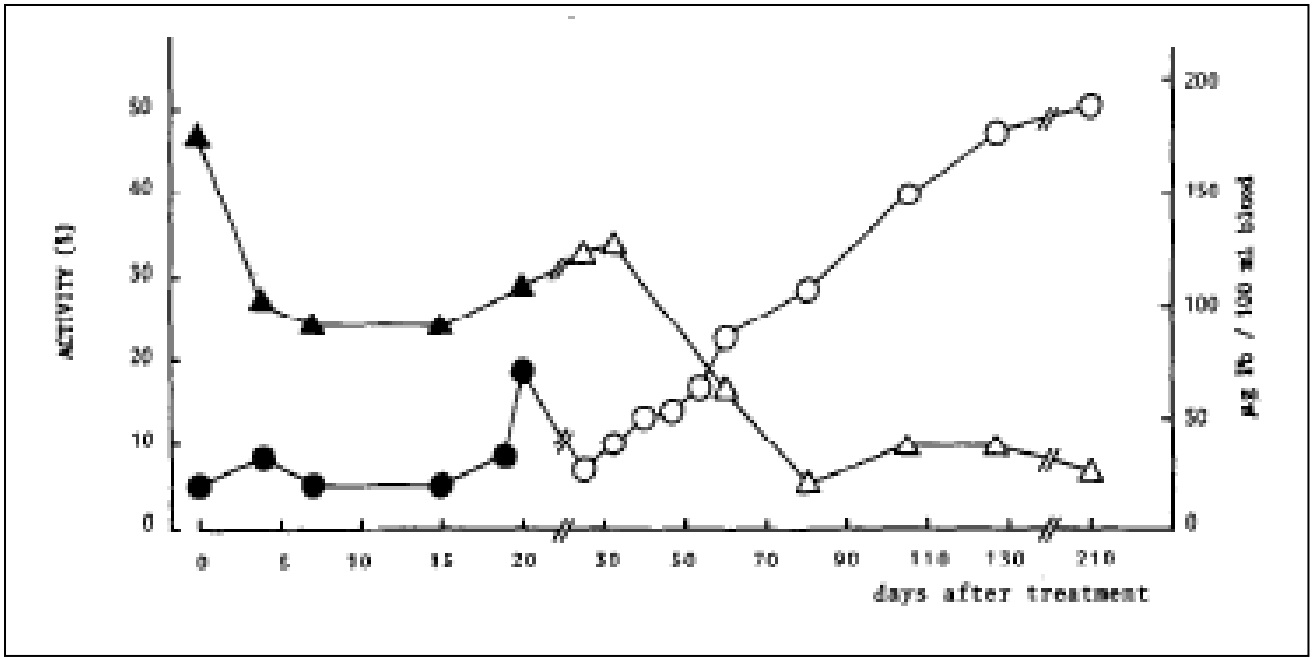
Five receive 25-30 mg/kg po (divided into three doses/day) over 20 days

SAMe in CHRONIC LEAD TOXICITY

♥ 26 year old female architect involved in ceramic business

ICU by 2 weeks→ SAM 12 mg/kg IV daily over 20 days

δ-ALA



Lead

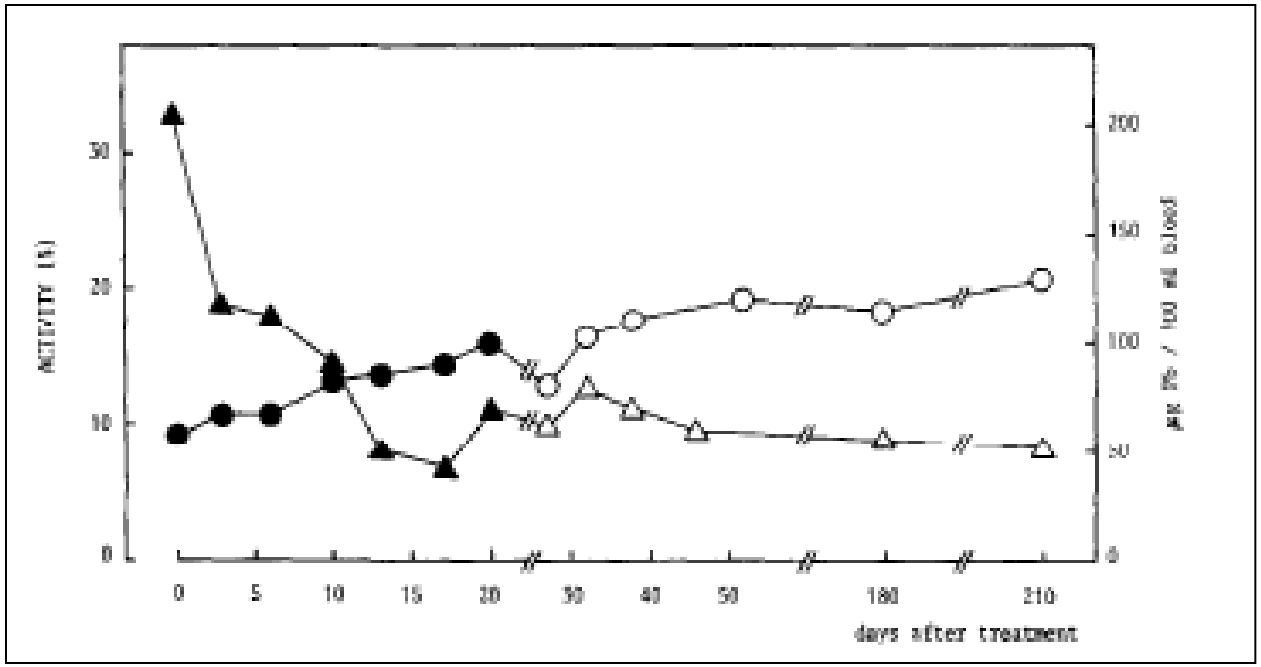
Note lead rebound post-SAM therapy → Brief drop in δ-ALA

SAMe in CHRONIC LEAD TOXICITY

♥ 65 year old male plumber

SAM 25-30 mg/kg po (divided into three doses/day) over 20 days

δ-ALA



Lead

SAMe in LEAD TOXICITY

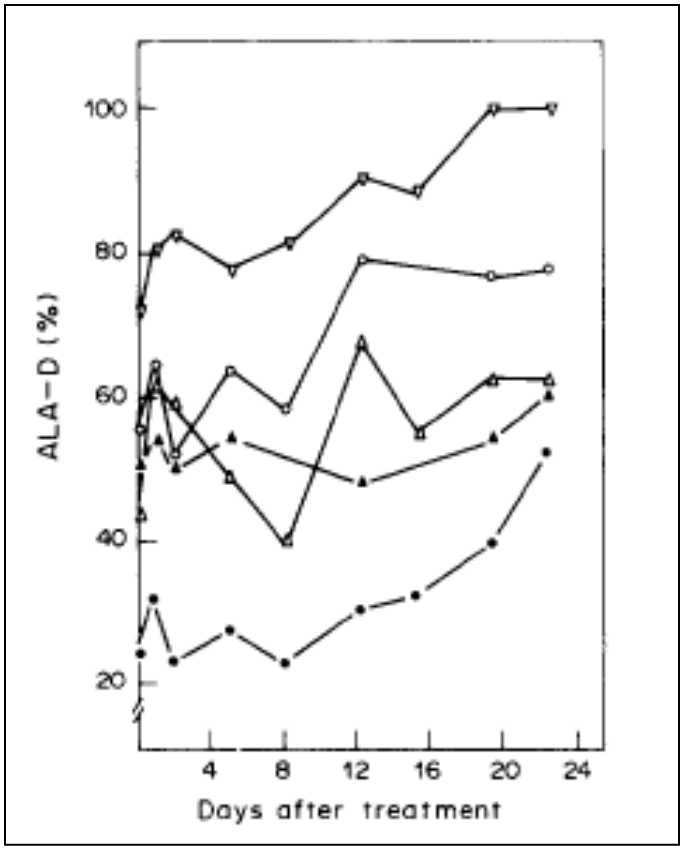
♥ 5/250 patients hospitalized with lead toxicity
- Lived near clandestine smelter
- Members of same family

Baseline studies
- Blood lead
- ALA-D

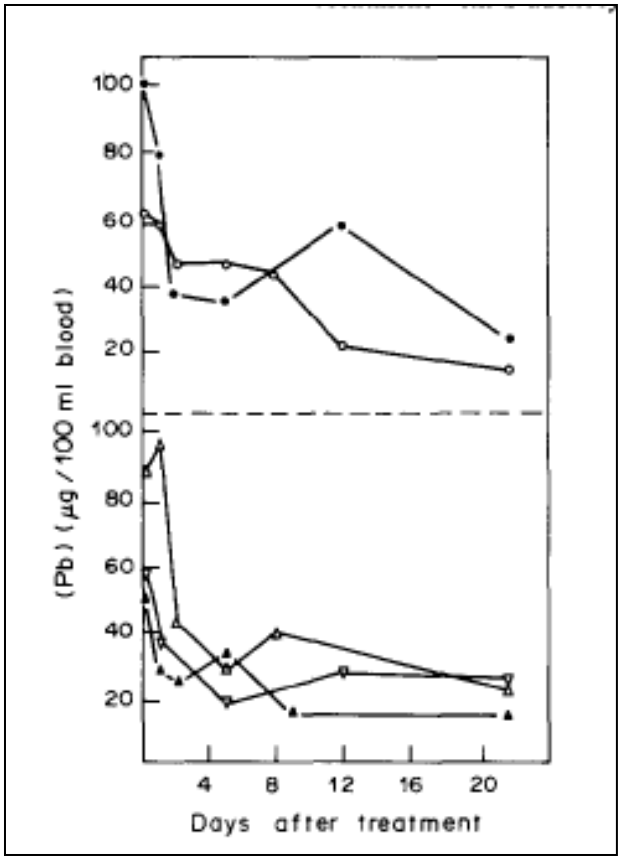
Treat over 22 days with IV SAM (12 mg/kg in 250 cc NS over four hours)

Monitor lab and clinical status

SAMe in LEAD TOXICITY



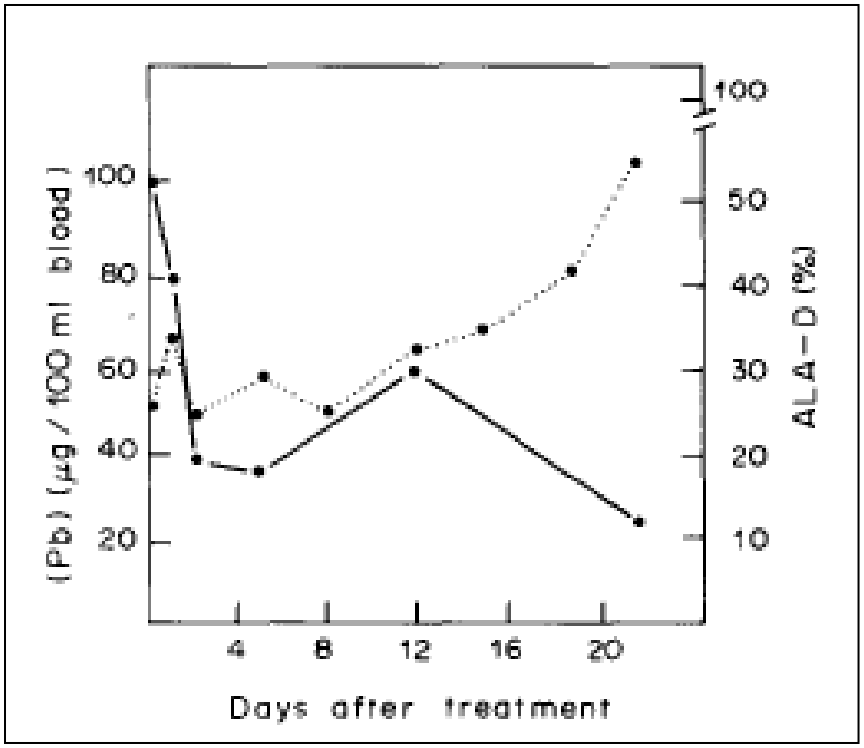
Erythrocyte
δ- Aminolevulinate Dehydrase



Blood Lead

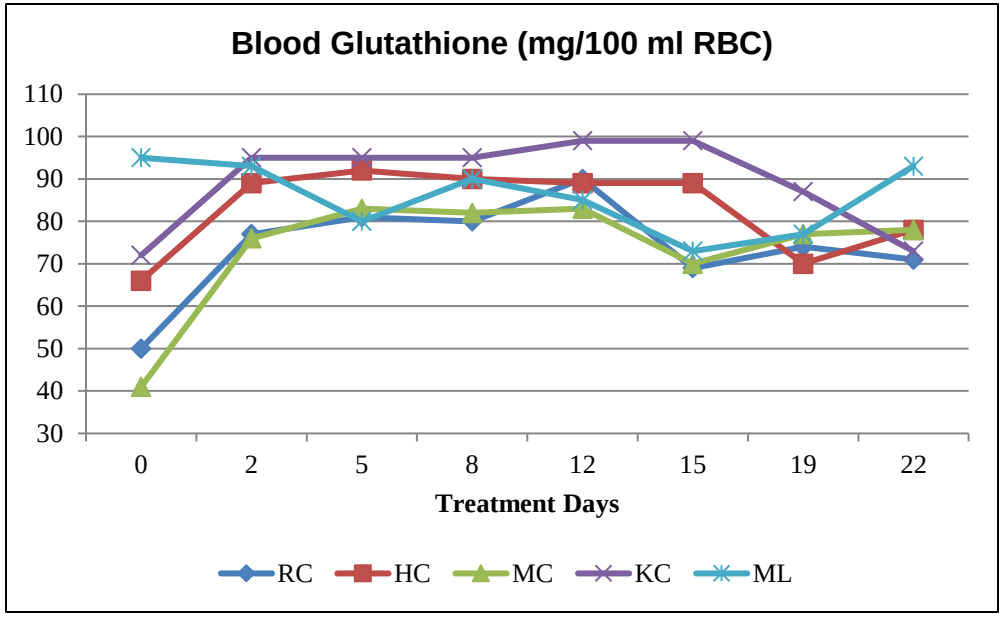
SAMe in LEAD TOXICITY

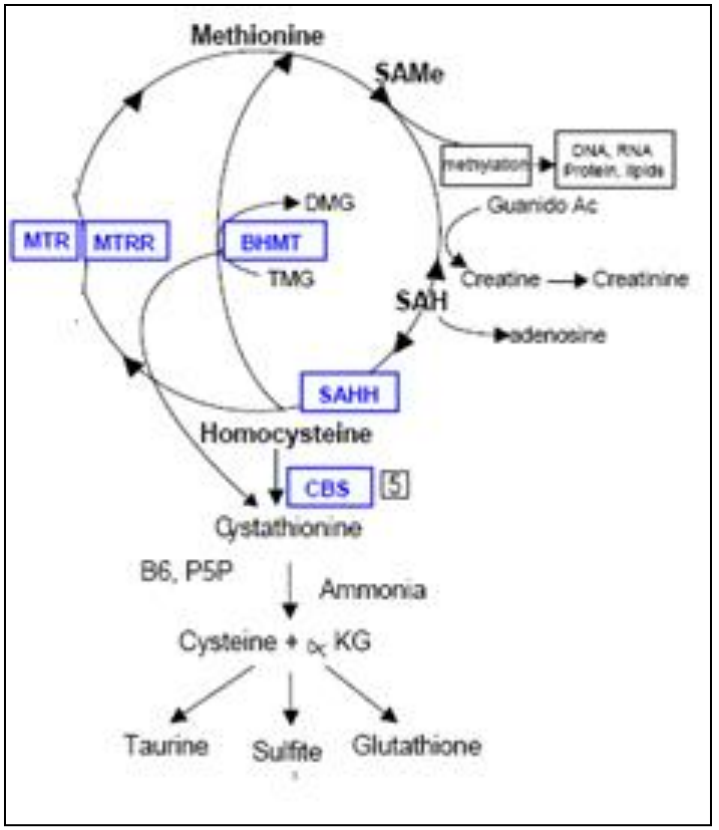
Lead



δ-ALA

SAMe in LEAD TOXICITY





SAMe EFFECT on GLUTATHIONE in LIVER DISEASE

♥ Liver biopsy

♦15 controls undergoing laparoscopy for non-liver indication

♦17 subjects with alcoholic liver disease
- Etoh 150 gm/day x ≥ 3 years

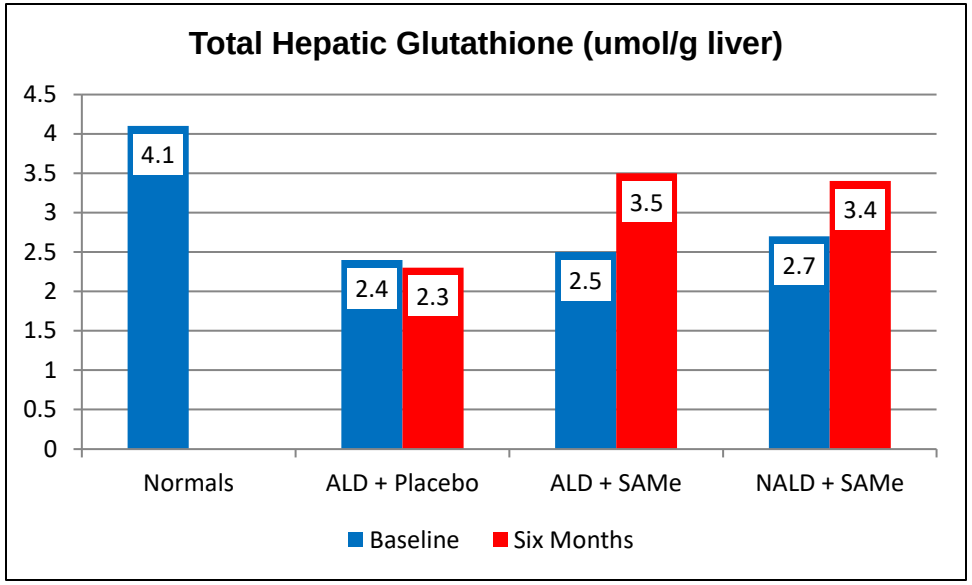
Randomize patients to receive over six months:
- ♦ SAMe 400 mg tid
- ♦ Placebo tid

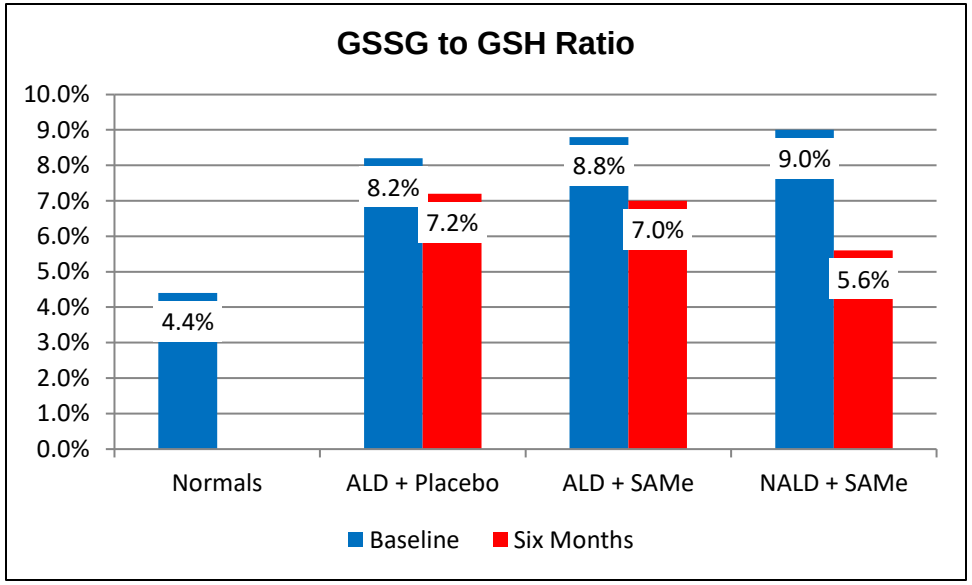
Abstinence during 6 month study advised
- ♣ 3 in each group abstinent

♦7 with non-alcoholic liver disease; all receive SAMe

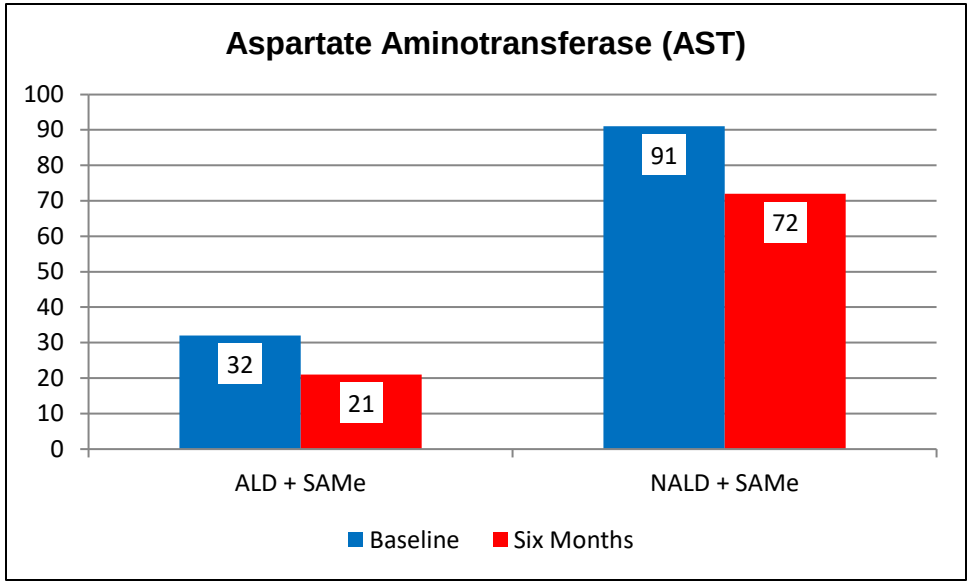
Repeat liver biopsy at six months in patients

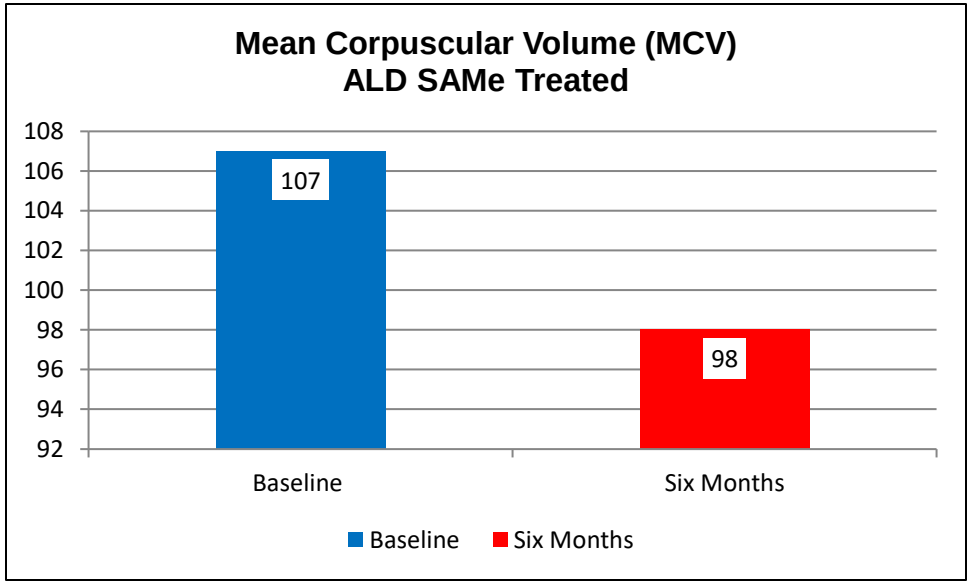
SAMe EFFECT on GLUTATHIONE in LIVER DISEASE





SAMe EFFECT on GLUTATHIONE in LIVER DISEASE





SAMe vs. CELECOXIB in DJD

♥ 56 subjects with DJD of knee(s):
- ≥ 40 years of age
- ACR criteria

Baseline measurements

Randomize to receive over eight weeks:
- SAMe 600 mg bid
- Celecoxib (COX-2 inhibitor) 200 mg/day

Repeat baseline studies

Cross-over to opposite treatment after one week washout

Double blind protocol followed

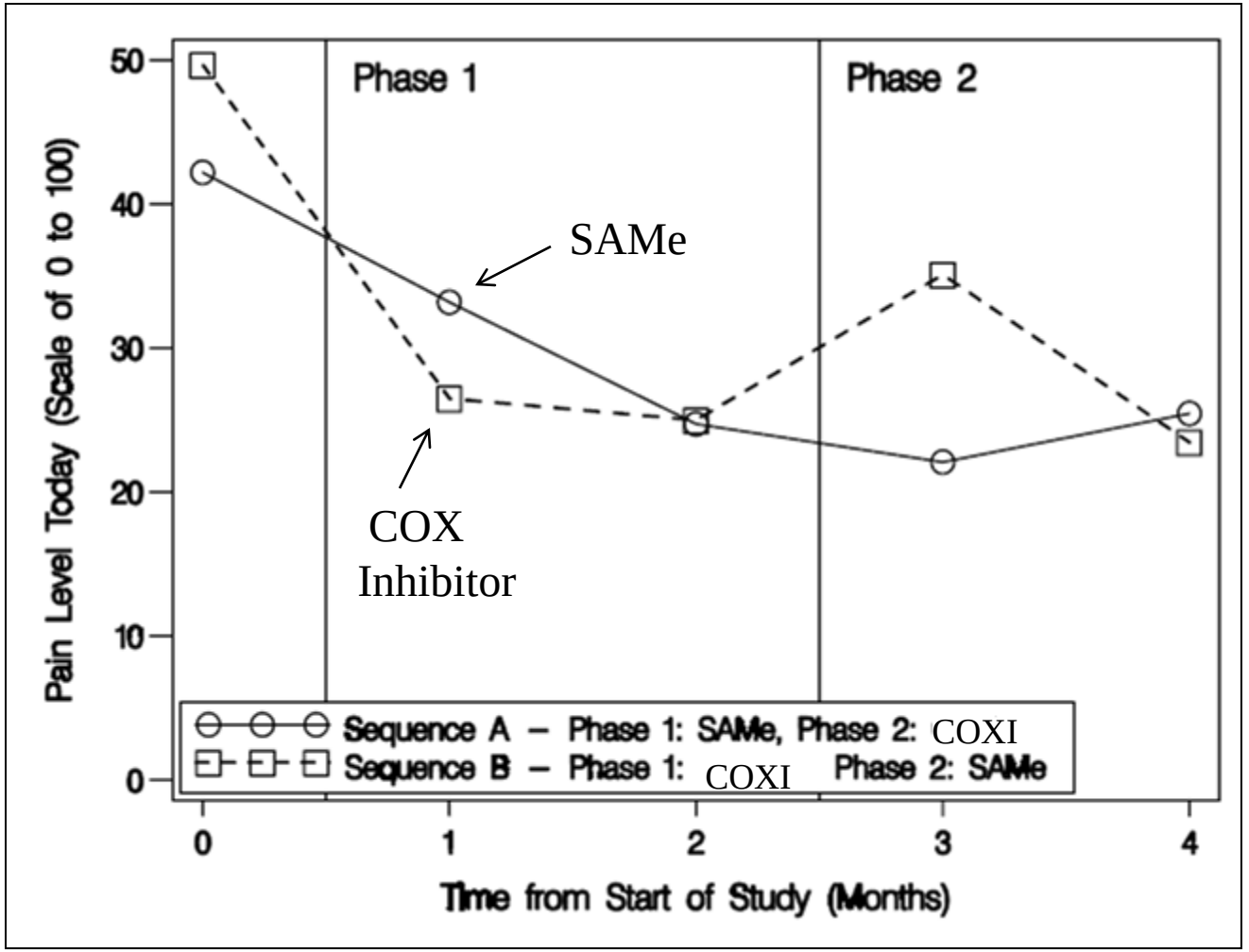
SAMe vs. CELECOXIB in DJD

Adverse Effects

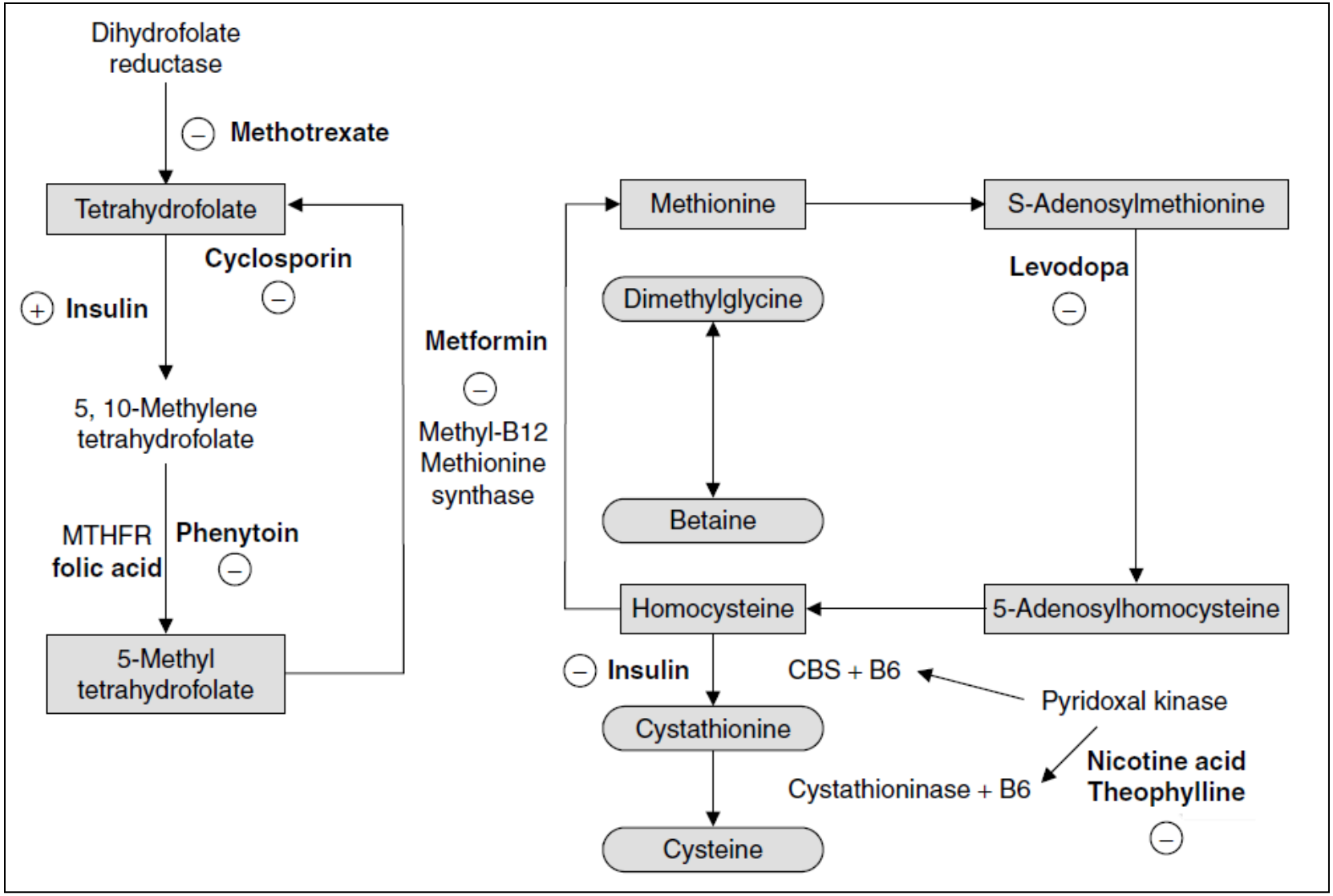
	SAMe	Celecoxib
Patients	36	46
GI	4	6
Anxiety	5	4
Dyspepsia	1	3

SAMe vs. CELECOXIB in DJD

Pain Rating – Visual Analog Scale (VAS)



METHYL THIEVES and IATROGENIC HHcy



METHYL THIEVES and IATROGENIC HHcy

Drug	Hcy	Mechanism
Fibrates	↑	Decrease in GFR; depletes TMG
Cholestyramine	↑	Blunts folate and B12 absorption
Niacin	↑	Uses methyl groups; blocks B6 synthesis
Metformin	↑	Blocks B12 absorption
Insulin	↓	↑ MTHFR > ↓ CBS
Estradiol	↓ or↑	↑ PEMT and depletes B6
Testosterone	↑	↑ Need for creatine
Methotrexate	↑	Blocks DHFR
Dilantin	↑	↓ MTHFR and MTR
Carbamazepine	↑	Folate depletion
Cyclosporin	↑	Decrease GFR and ↓ MTHFR
Levodopa	↑	Generation of SAH
NAC	↓	Thiol-disulfide exchange

METHYL THIEVES and IATROGENIC HHcy

Drug	Hcy	Mechanism
PPIs and H_2 Blockers	↑	Blocks B12 absorption
OCPs	↑	↓ B12, B6, folate, riboflavin, Vit C, & Zn
Alcohol	↑	↓ MTR; compromises folate metabolism
Mercury	↑	↓ MTR
Lead	↑	Enzyme dysfunction
Aluminum	↑	Enzyme dysfunction
Cadmium	↑	Enzyme dysfunction
Organic Pollutants	↑	Enzyme dysfunction
Diuretics	↑	Lowers GFR; depletes B Vits
Spironolactone	No Δ	No effect
β-Blockers	↓	Uncertain mechanism

FENOFIBRATE, GEMFIBROZIL, and HOMOCYSTEINE

♥ 22 male subjects with untreated hypertriglyceridaemia
- None with creatinine > 110 umol/l (1.24 mg/dl)
- None with thyroid disease

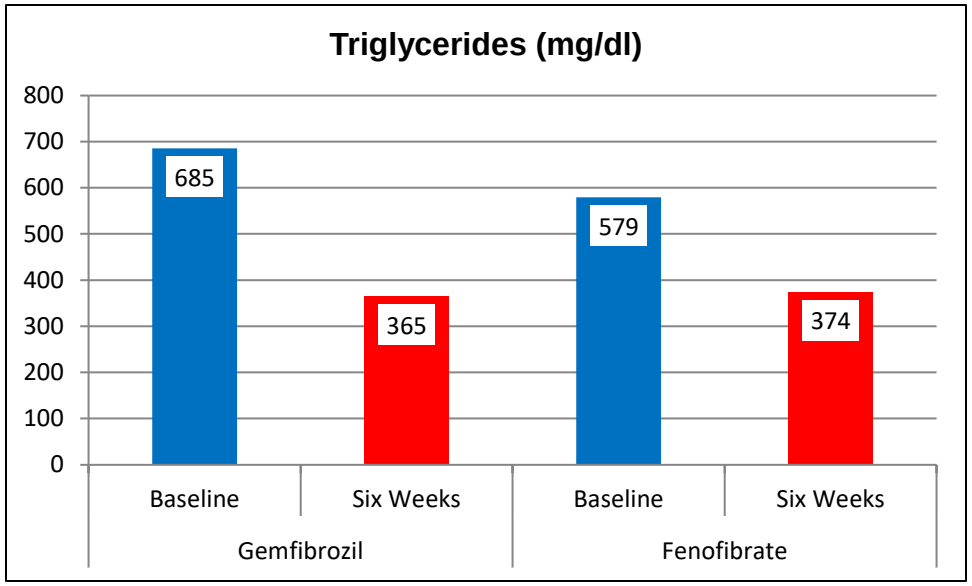
Baseline studies

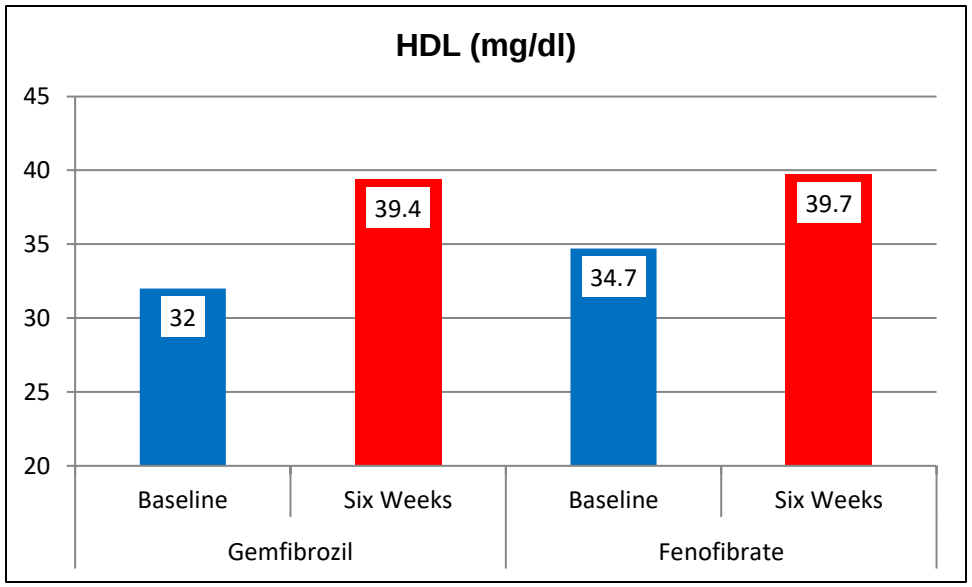
Randomize to receive over six weeks:
- Fenofibrate 200 mg/day
- Gemfibrozil 900 mg/day

Washout over six weeks then cross-over to opposite treatment

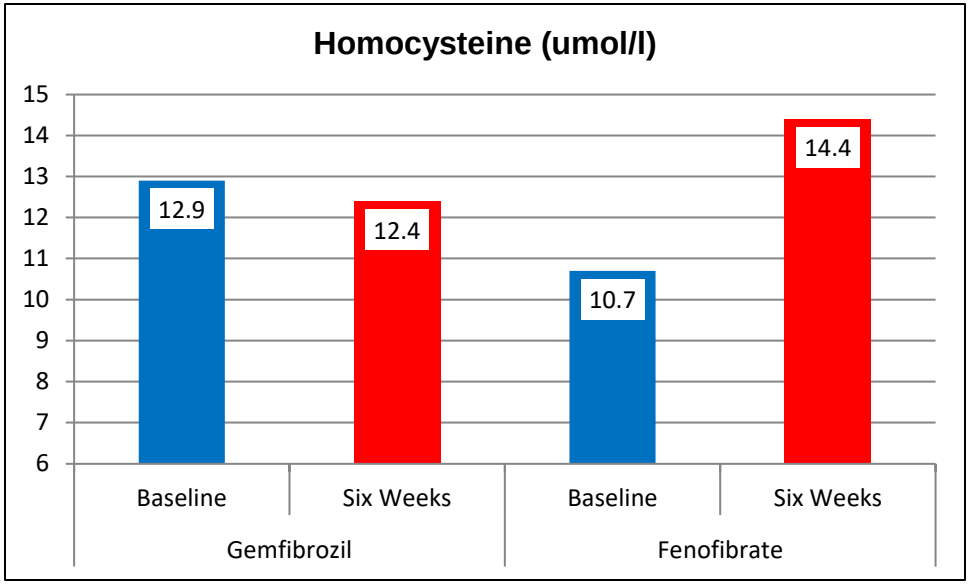
Repeat baseline studies at 6, 12, and 18 weeks

FENOFIBRATE, GEMFIBROZIL, and HOMOCYSTEINE





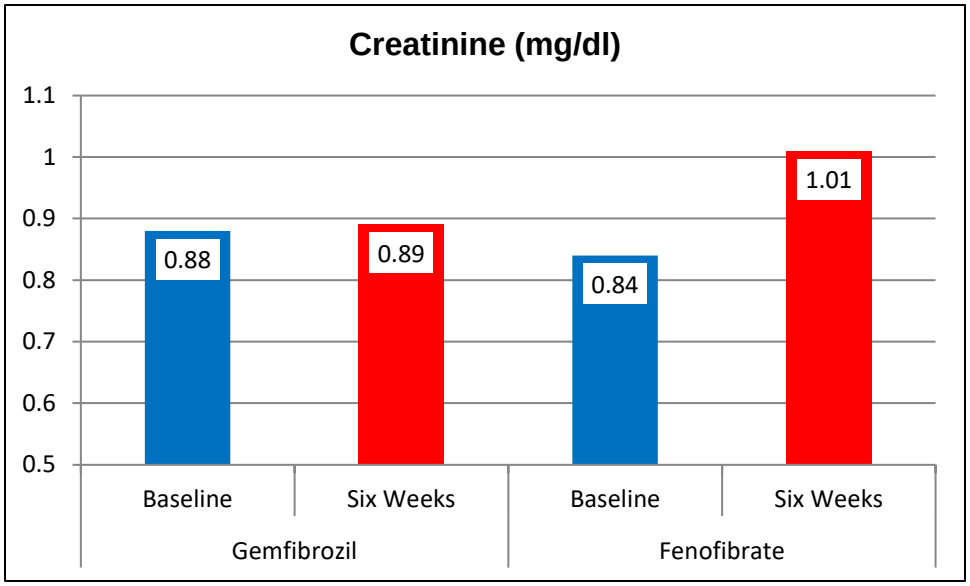
FENOFIBRATE, GEMFIBROZIL, and HOMOCYSTEINE

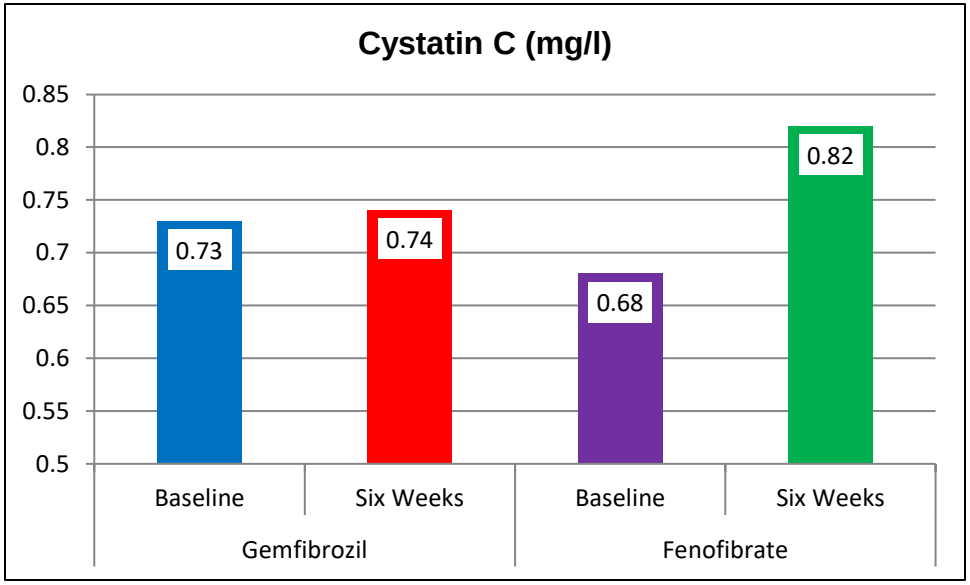


	Gemfibrozil (900 mg/day)			Fenofibrate (200 mg/day)			p* tr gemf
	Before	After	p*	Before	After	p*	
Triglycerides (mmol/L)	7·7 (2·5–53·2)	4·1 (1·1–24·6)	0·046	6·5 (2·3–60·7)	4·2 (0·3–15·8)	0·001	0·21
HDL-cholesterol (mmol/L)	0·83 (0·46–1·89)	1·02 (0·43–1·71)	0·010	0·90 (0·40–1·69)	1·03 (0·54–1·80)	0·001	0·26
Total homocysteine (µmol/L)	12·9 (7·1–23·6)	12·4 (6·3–29·5)	0·592	10·7 (4·4–24·8)	14·4 (7·7–23·1)	0·001	0·00
Creatinine (µmol/L)	79 (50–110)	80 (55–109)	0·626	75 (41–107)	90 (61–120)	<0·001	0·00
Cystatin C (mg/L)	0·73 (0·57–1·48)	0·74 (0·59–1·71)	0·237	0·68 (0·54–1·56)	0·82 (0·63–1·56)	<0·001	0·04
Vitamin B6 (ng/mL)	10·1 (4·2–33·6)	11·7 (4·3–35·4)	0·948	11·3 (4·2–30·2)	11·1 (4·8–39·0)	0·029	0·02
Folate (ng/mL)	8·4 (3·6–11·6)	8·6 (3·6–11·1)	0·809	9·2 (2·6–12·4)	9·4 (3·6–12·2)	0·983	0·35
Cobalamin (pg/mL)	367 (170–1145)	382 (189–964)	0·481	386 (215–758)	421 (217–1087)	0·351	0·84
Creatine kinase (U/L)	123 (53–310)	120 (35–589)	0·775	124 (51–243)	131 (42–592)	0·656	0·65

All values are medians with 5th and 95th percentiles. *According to the Wilcoxon signed-rank test.

FENOFIBRATE, GEMFIBROZIL, and HOMOCYSTEINE





TMG and POST-MI OUTCOMES

♥ Subjects with ACS - Measure TMG, DMG, and Hcy and correlate with outcome

5^{th} quintile Homocysteine → Increased risk of MI, CHF, and mortality

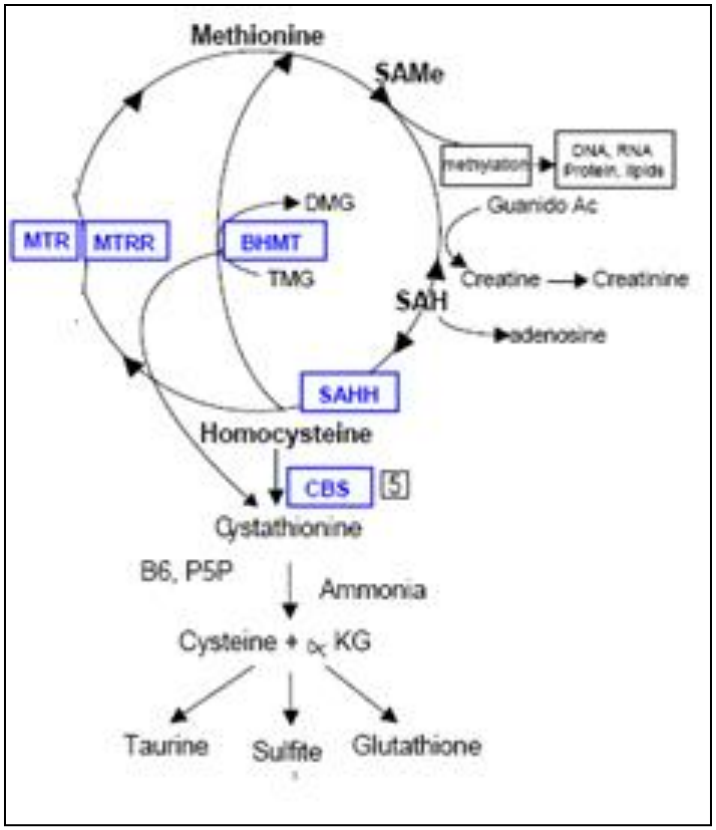
5^{th} quintile DMG → Increased risk of MI, CHF, and mortality

1^{st} quintile TMG → Increased risk of MI

5^{th} quintile TMG → Increased risk of CHF

- ♦ High TMG ≈ impaired BHMT activity

1^{st} and 5^{th} quintile TMG → Increased BNP



FIBRATES DEPLETE TMG and INCREASE HOMOCYSTEINE

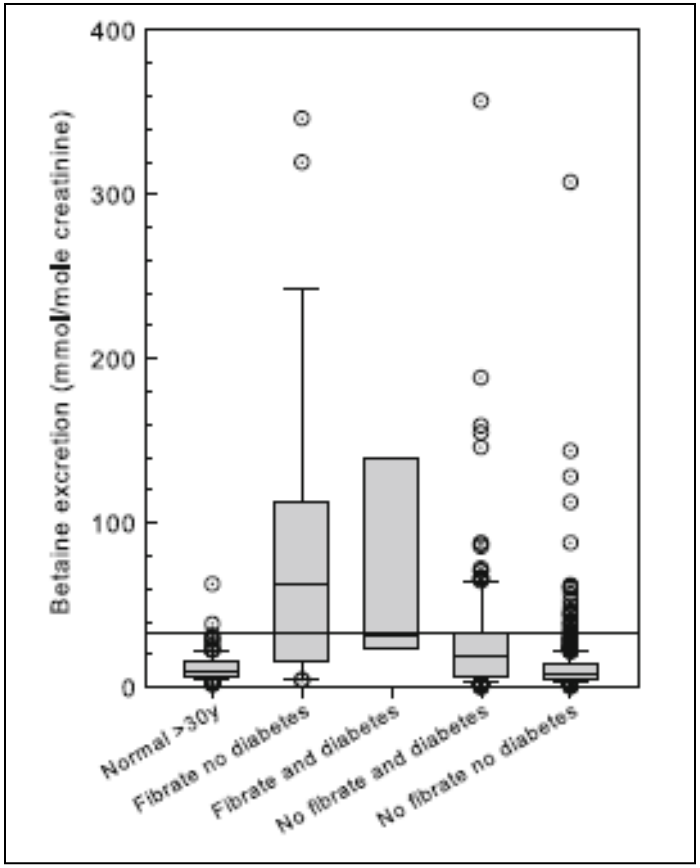
♥ Subjects with varying metabolic health

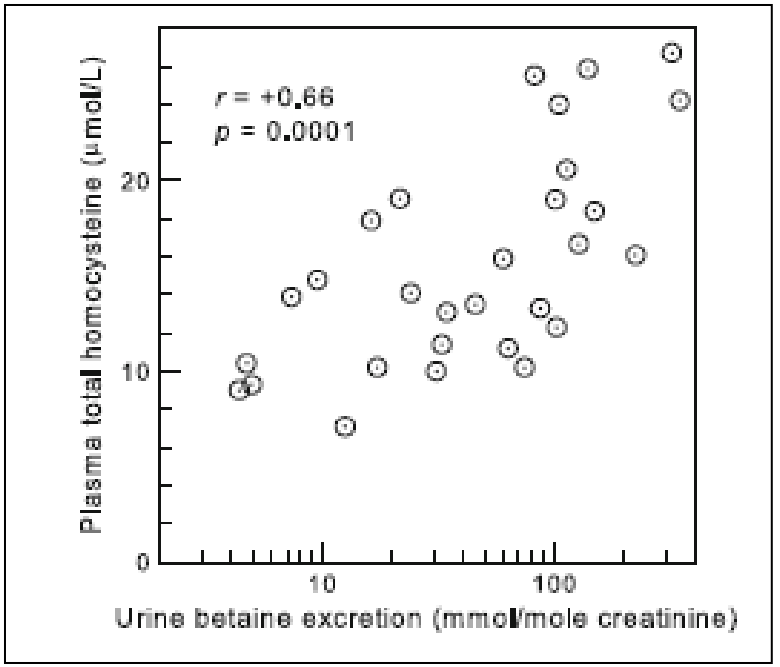
- Healthy volunteers
- Non-diabetics on Fibrate for hyperlipidemia
- Diabetic patients on Fibrate for hyperlipidemia
- Diabetic patients not on Fibrate
- Non-diabetics not on Fibrate

Measure renal excretion of betaine (TMG)

Correlate renal excretion of betaine with plasma homocysteine

FIBRATES DEPLETE TMG and INCREASE HOMOCYSTEINE

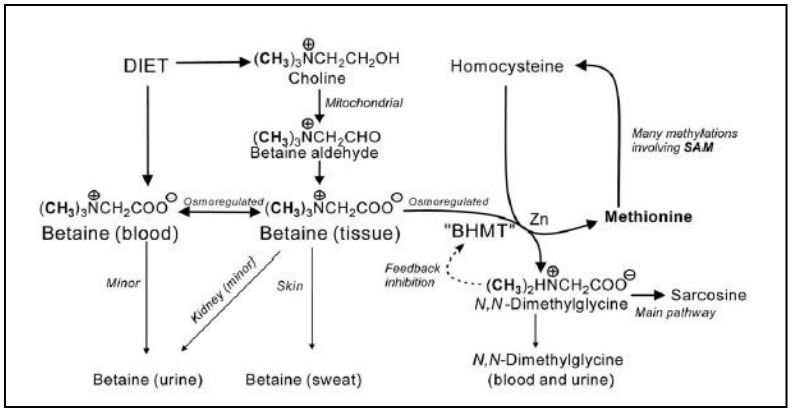




Fibrates via PPARα (except Gemfibrozil):

- Decrease GFR
- Deplete TMG

  ⇒ Increase homocysteine



BETA BLOCKERS, SPIRONOLACTONE, and HOMOCYSTEINE

- ♥ 65 subjects with newly diagnosed hypertension
  - No prior meds
  - Otherwise good health

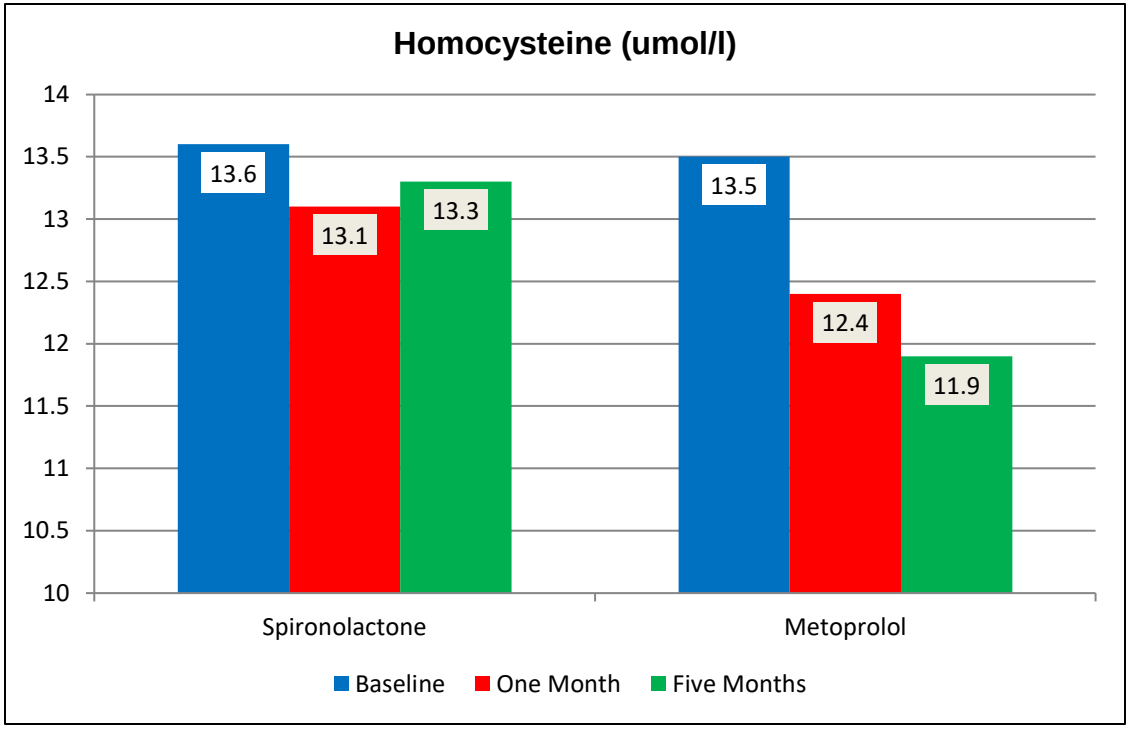
Baseline plasma homocysteine, folate, and B12

Randomize to receive over five months:

- Spironolactone 50 mg/day
- Metoprolol 100 mg/day
  - ♦ Double dose if BP control inadequate
    - ♣ 41% spironolactone went to 100 mg
    - ♣ 34% metoprolol advanced to 200 mg

Repeat baseline studies at 1 and 5 months

BETA BLOCKERS, SPIRONOLACTONE, and HOMOCYSTEINE



NIACIN RELATED HYPERHOMOCYSTEINEMIA

♥ Male Sprague-Dawley rats (120-150 gm.)

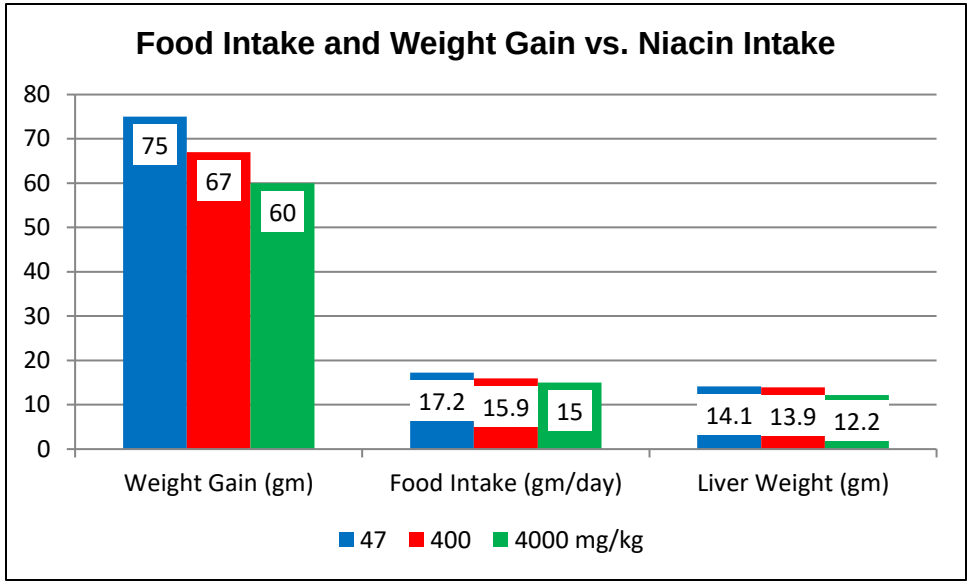
Standard chow (Niacin 47 mg/kg chow)

Supplement chow with:

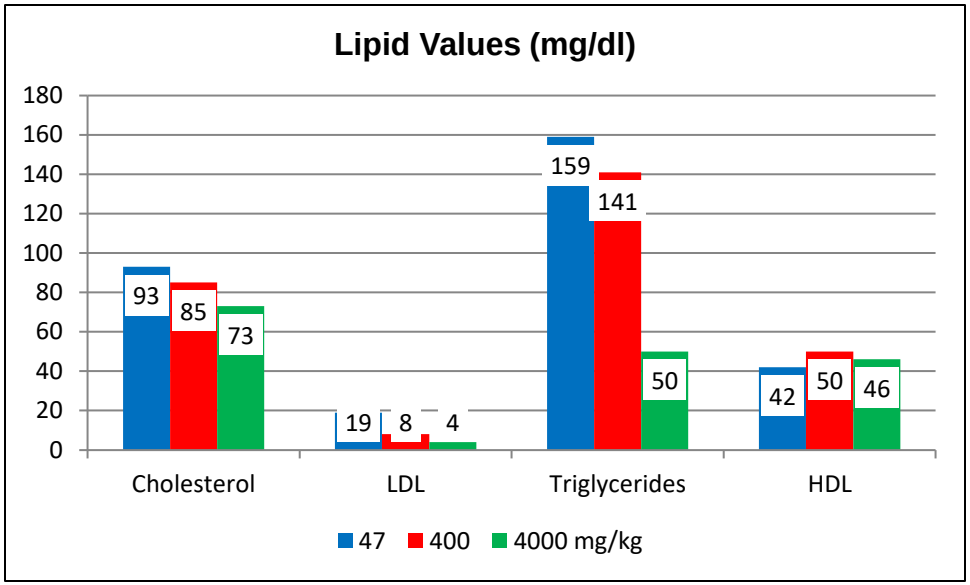
- Niacin 400 mg/kg
- Niacin 4000 mg/kg

Evaluate at six weeks

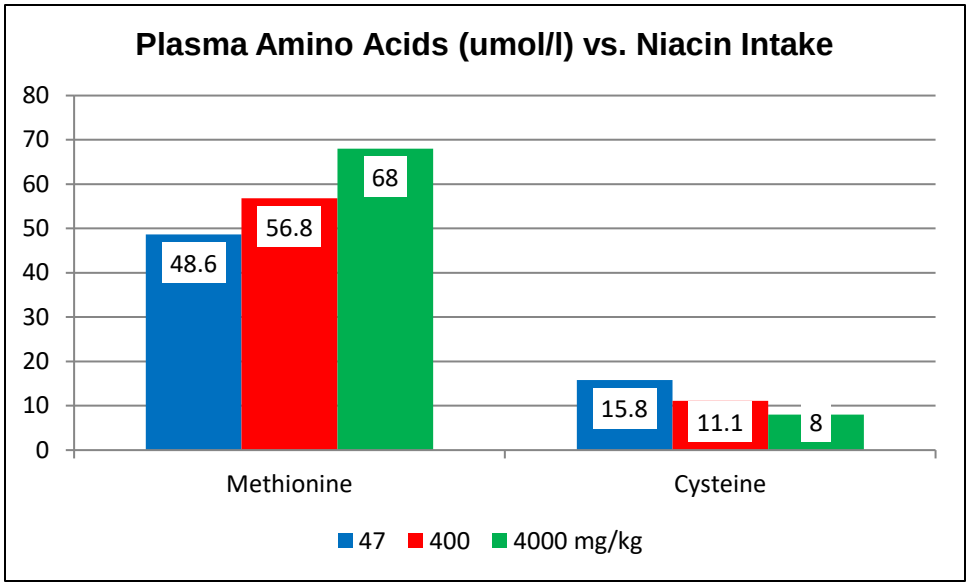
Nutrient	Level/kg Chow
Niacin	47 mg
Methionine	2 gm
B-6	11 mg
Folate	1 mg
B-12	15 mcg

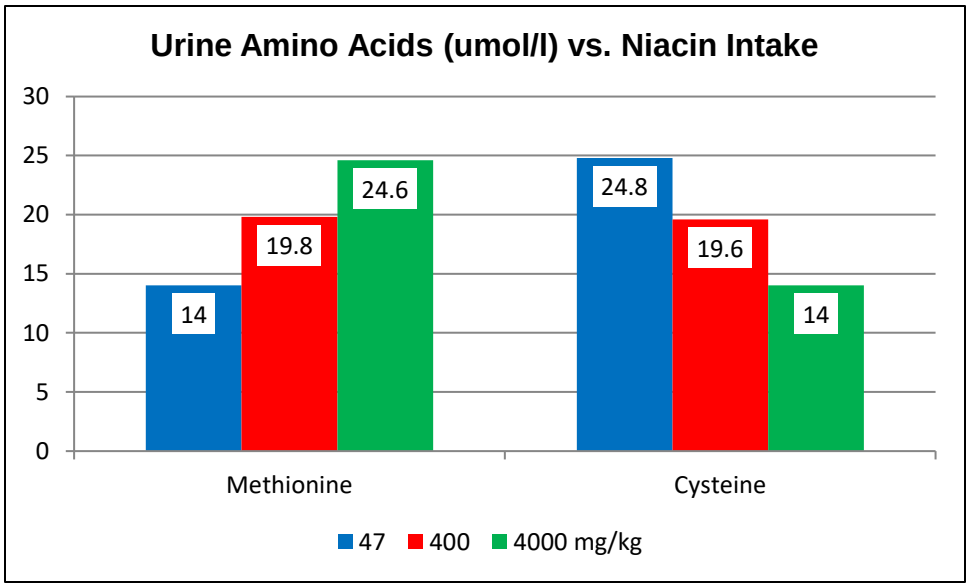


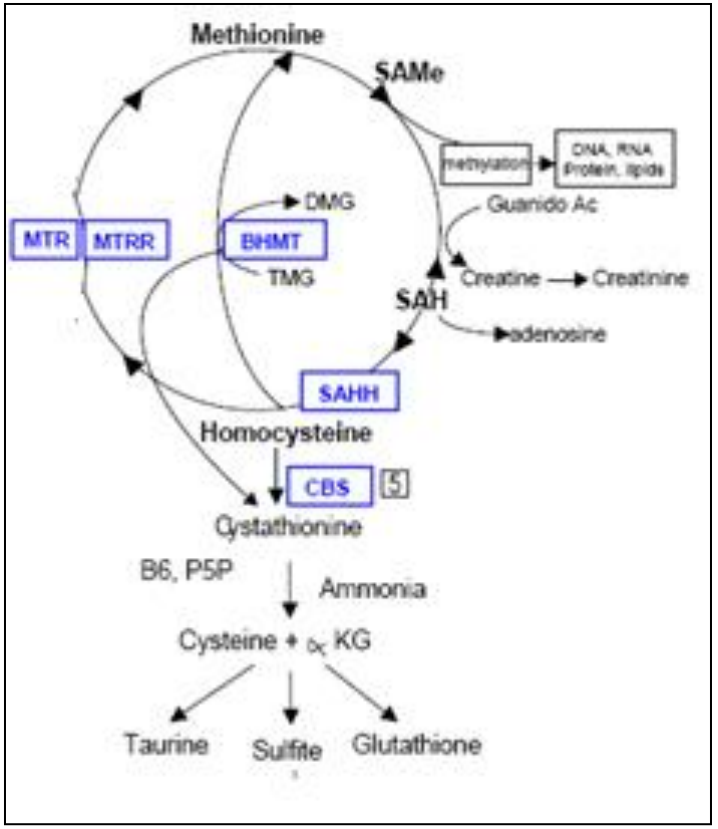
NIACIN RELATED HYPERHOMOCYSTEINEMIA



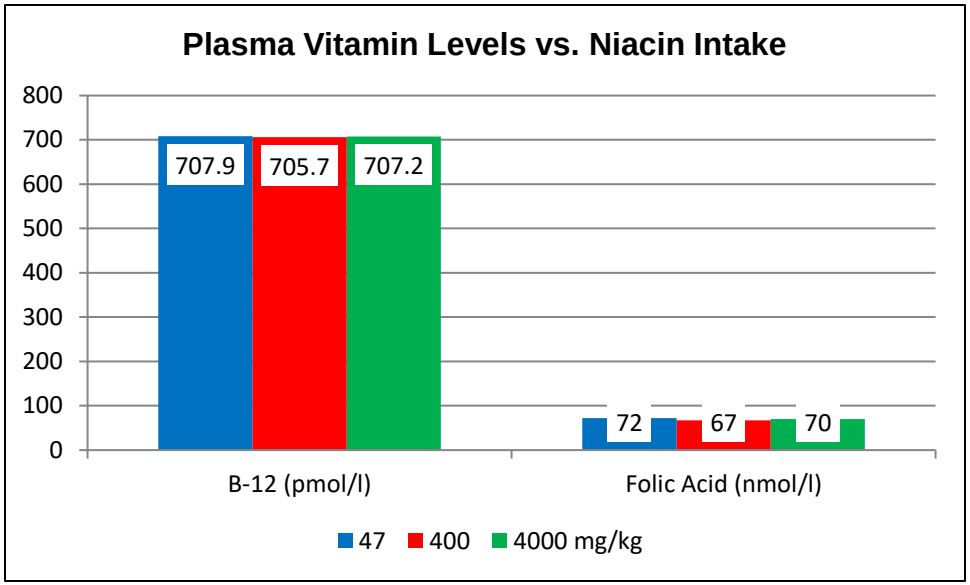
NIACIN RELATED HYPERHOMOCYSTEINEMIA

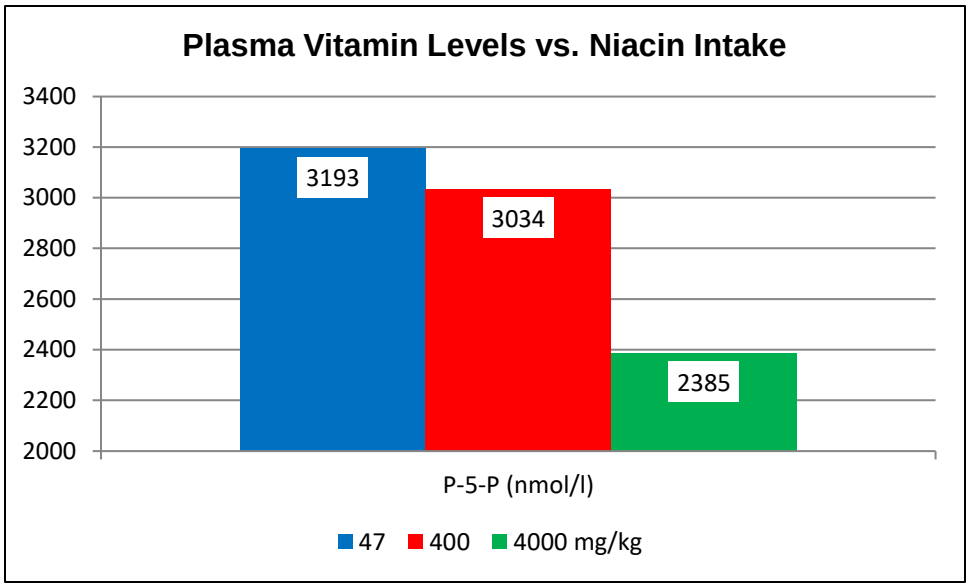


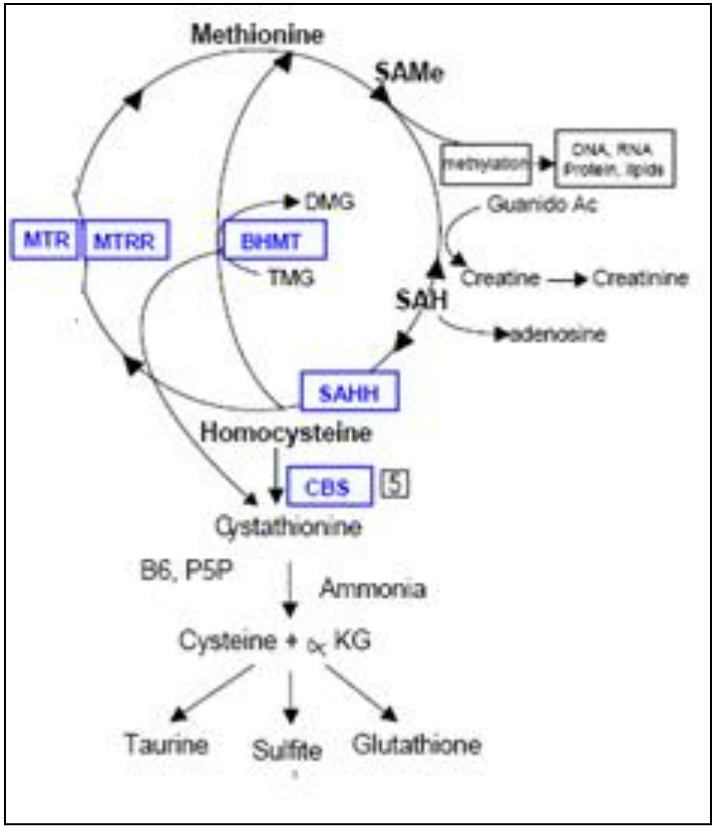




NIACIN RELATED HYPERHOMOCYSTEINEMIA







Niacin

Utilizes CH_3 in metabolism

Depletes B6

B6 in NIACIN RELATED HYPERHOMOCYSTEINEMIA

♥ Male Sprague-Dawley rats (120-150 gm.)

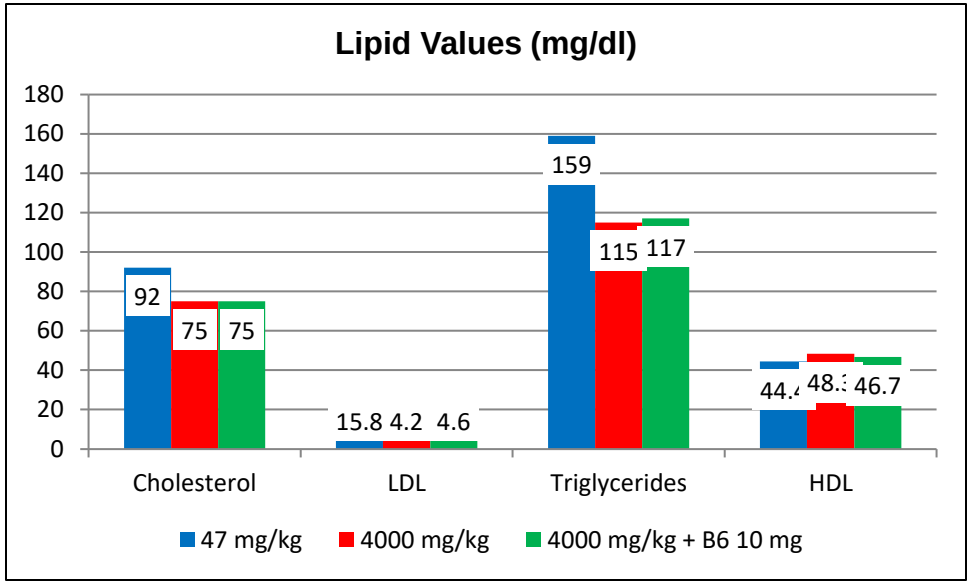
Standard chow (Niacin 47 mg/kg chow) and water

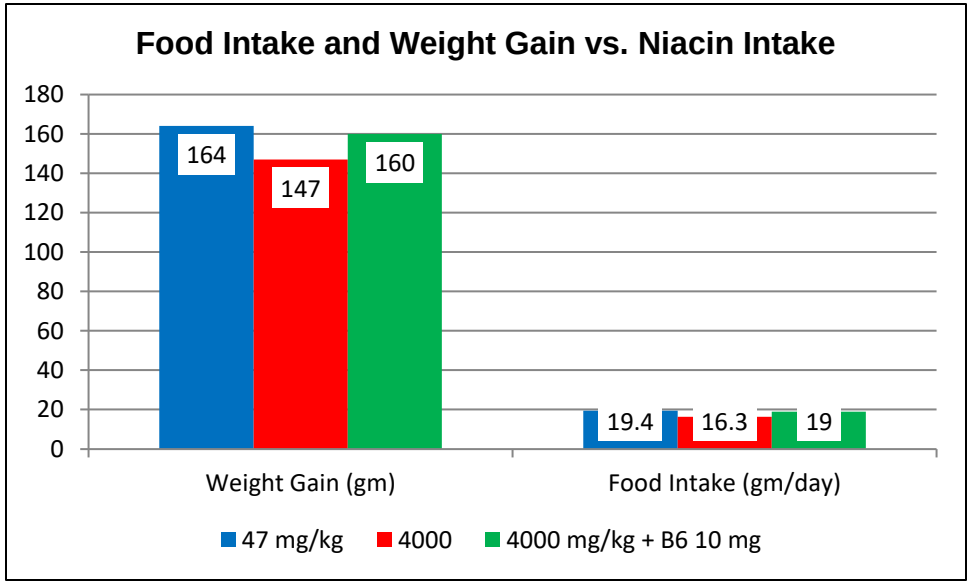
Supplement chow to provide:

- Niacin 4000 mg/kg
- Niacin 4000 mg/kg + B6 10 mg/kg

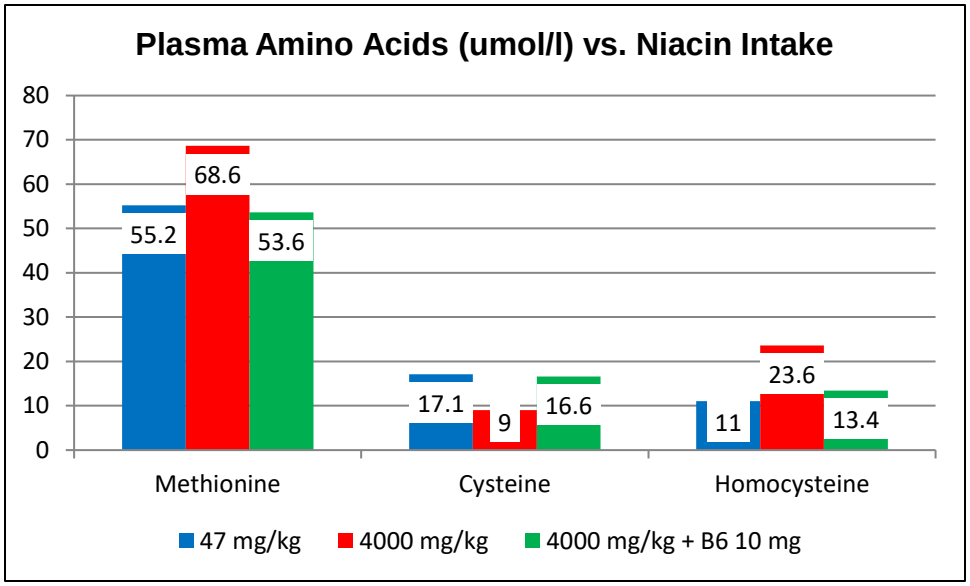
Evaluate at six weeks

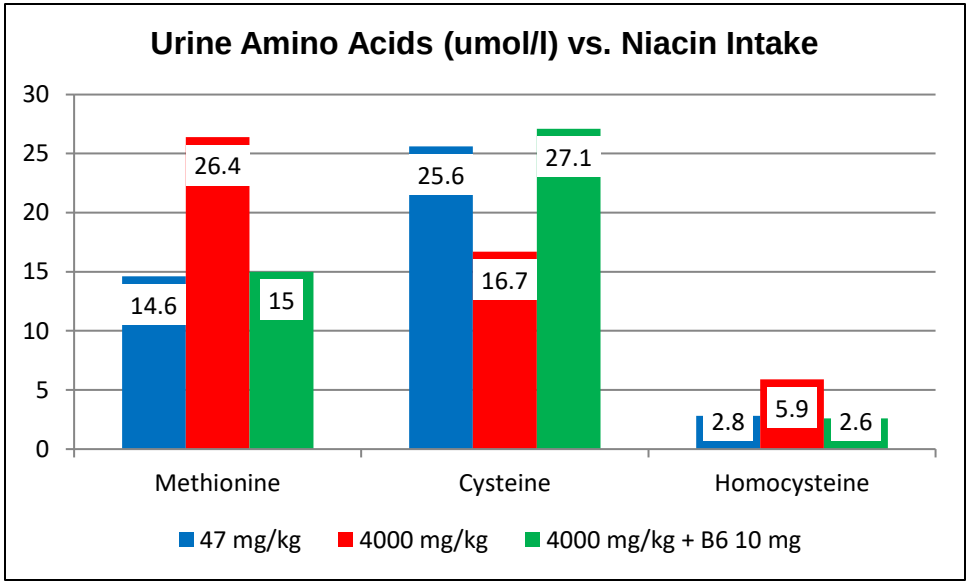
B6 in NIACIN RELATED HYPERHOMOCYSTEINEMIA

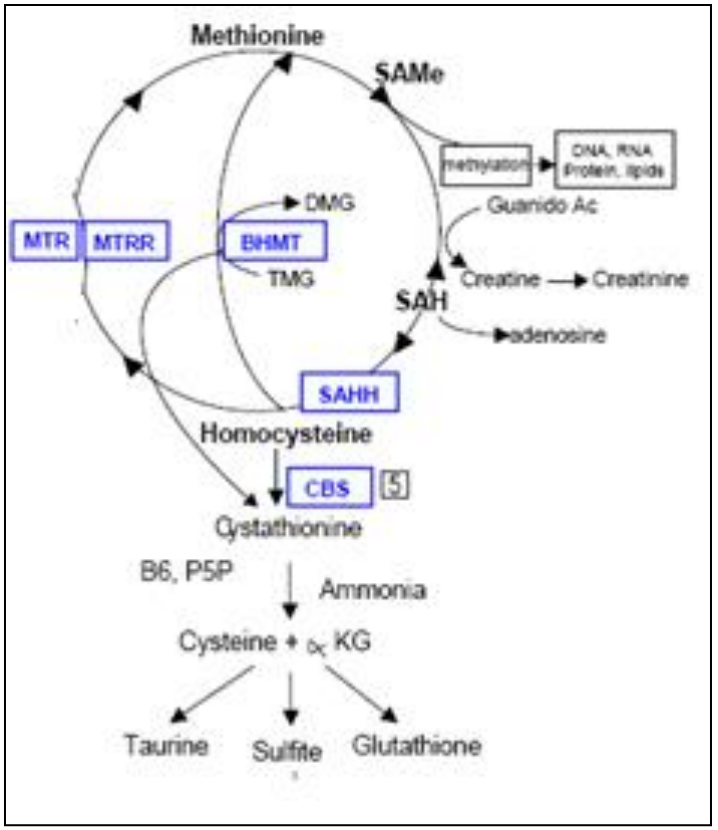




B6 in NIACIN RELATED HYPERHOMOCYSTEINEMIA







ESTRADIOL, HOMOCYSTEINE, B6, and CBS

♥ 25 post-menopausal women with homocysteine > 10 umol/l

Baseline studies

Randomize to receive over twelve weeks:

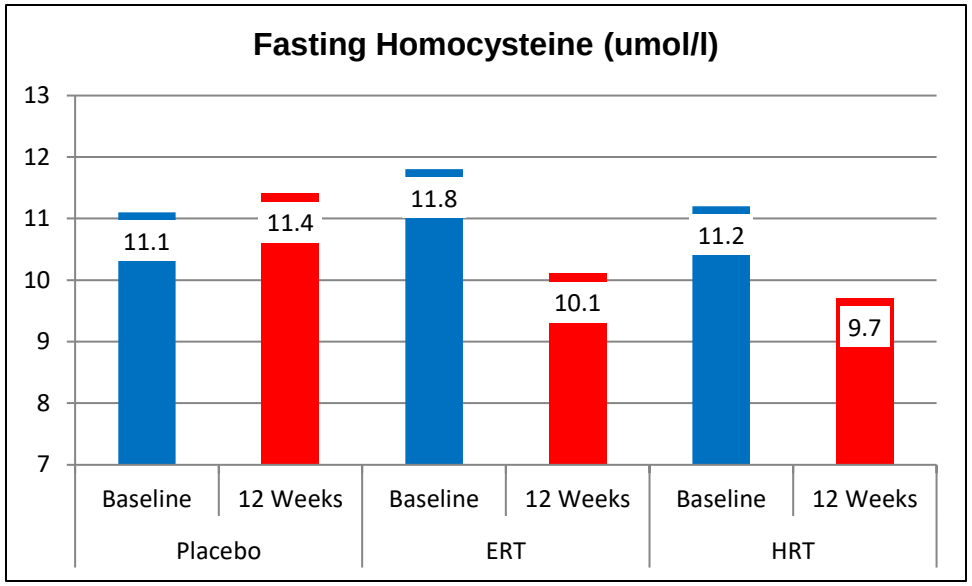
- Placebo
- Estradiol 4 mg/day po
- Estradiol 4 mg/day + Dydrogesterone 10 mg/day

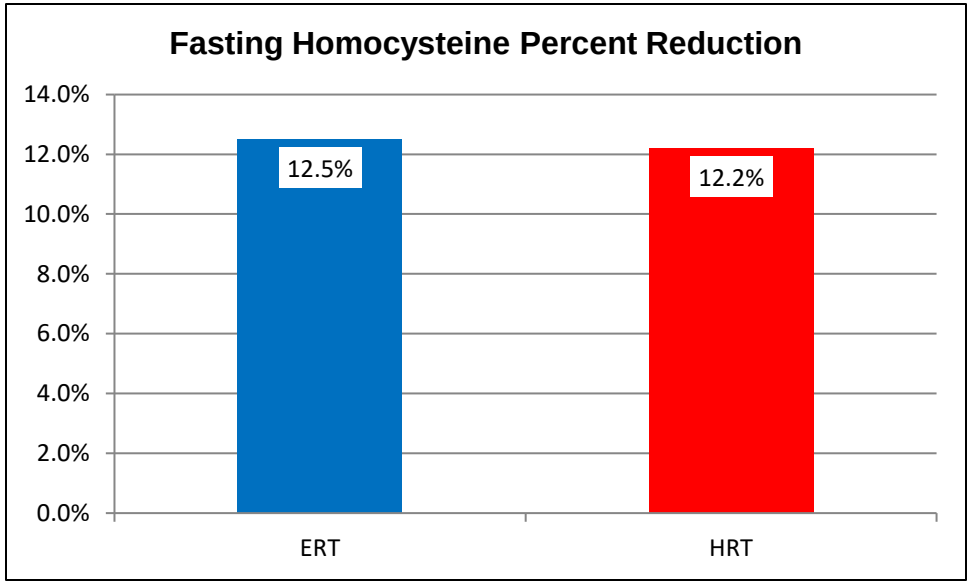
Repeat baseline studies

Estradiol only group received Estradiol + Dydrogesterone x 14 days

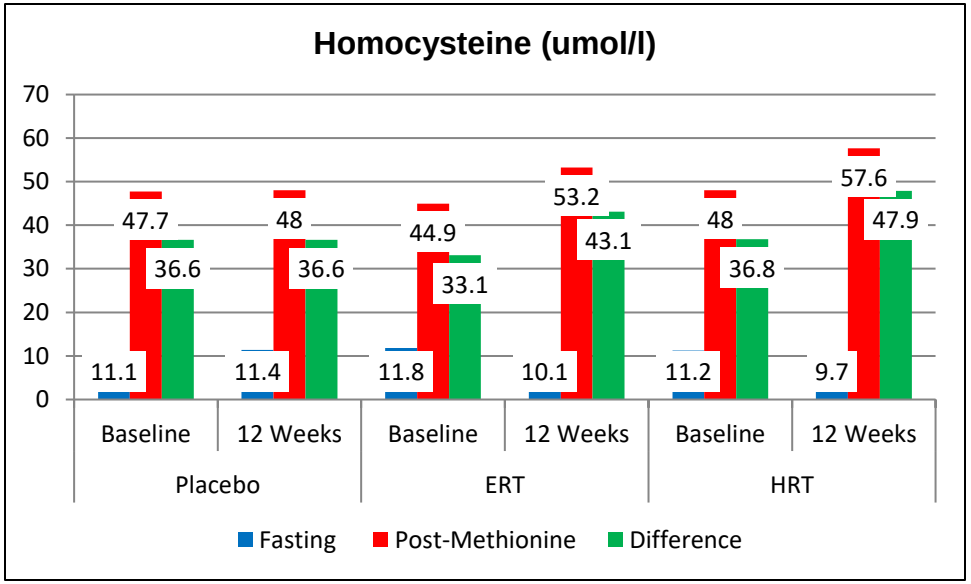
Double blind protocol followed

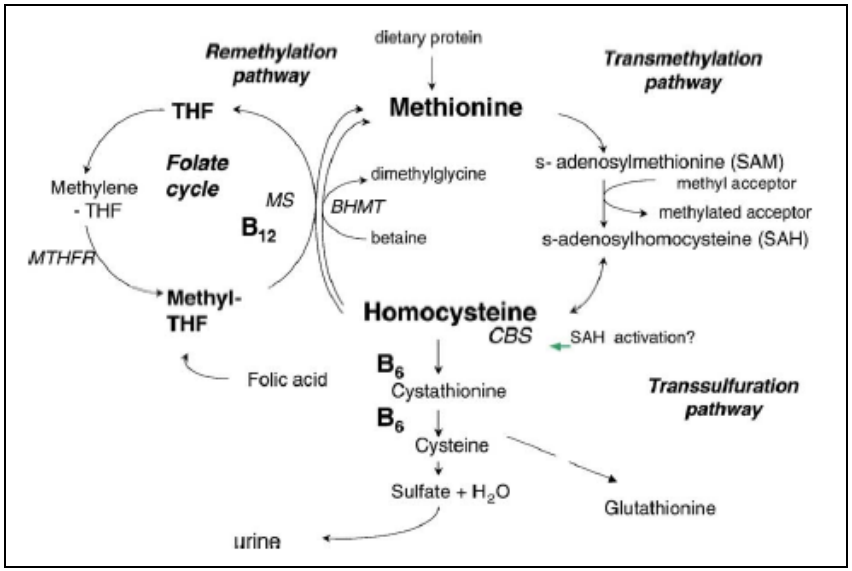
ESTRADIOL, HOMOCYSTEINE, B6, and CBS

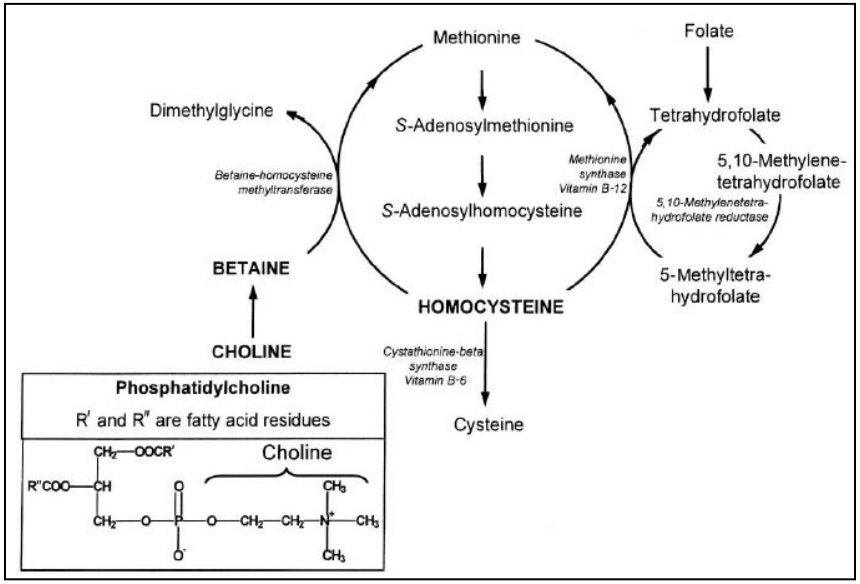




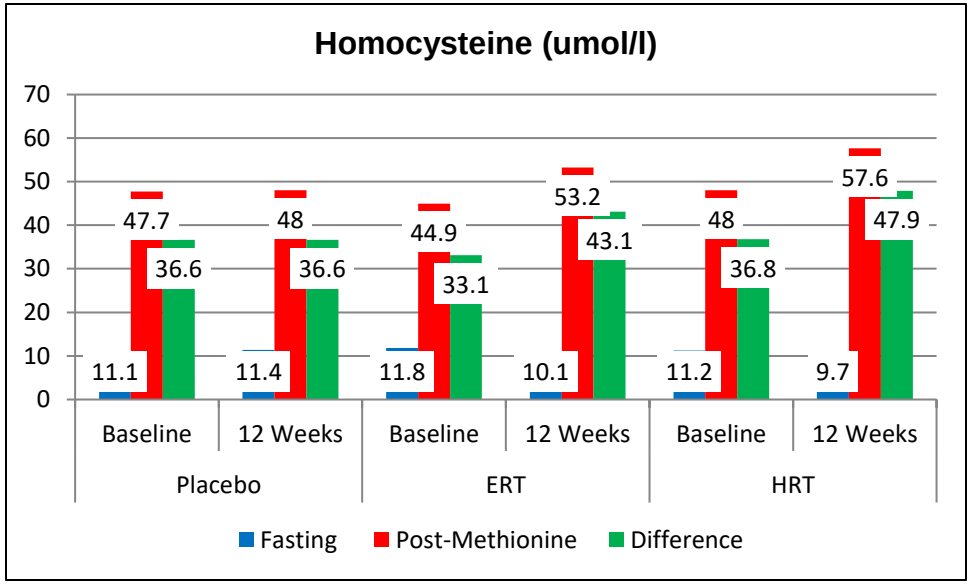
ESTRADIOL, HOMOCYSTEINE, B6, and CBS

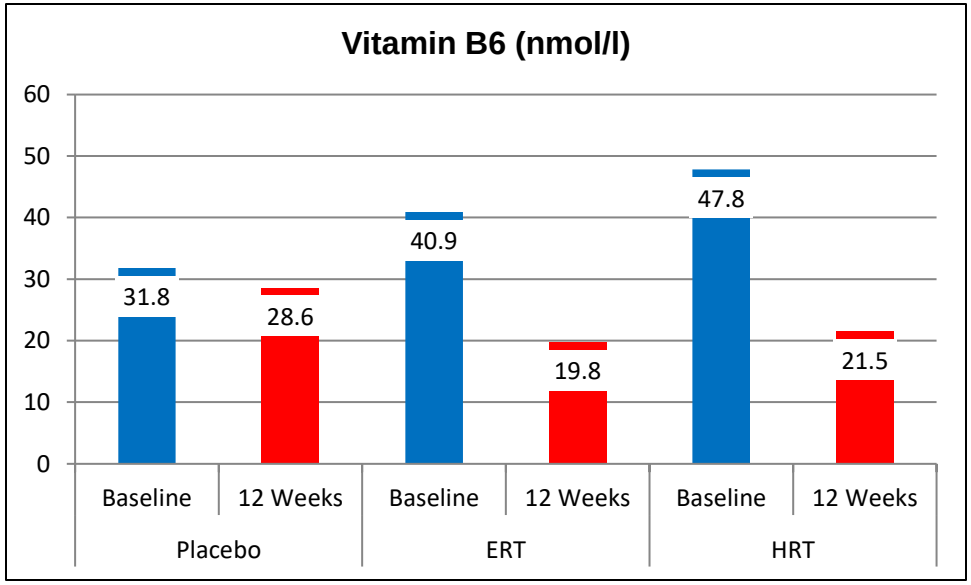




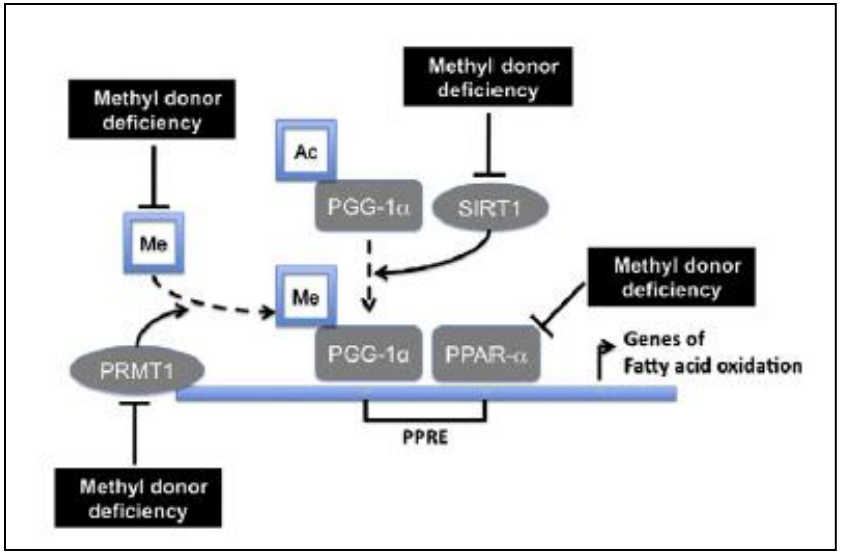


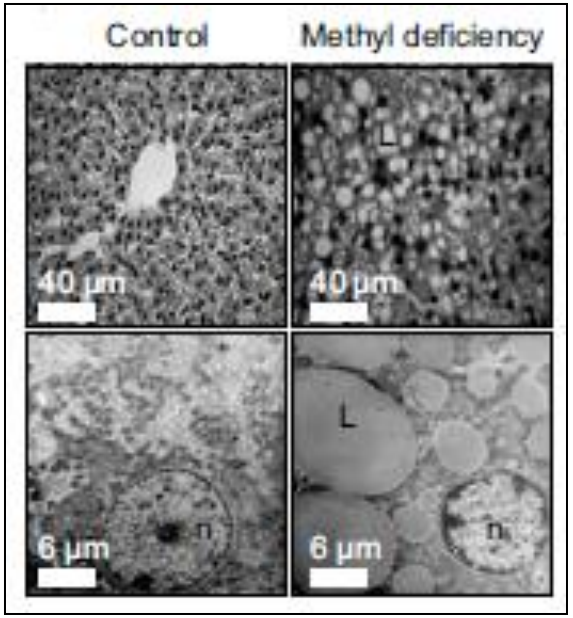
ESTRADIOL, HOMOCYSTEINE, B6, and CBS

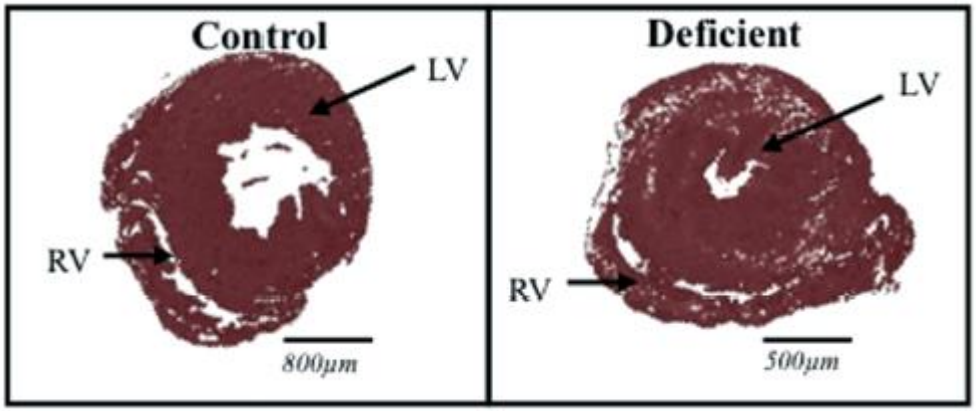


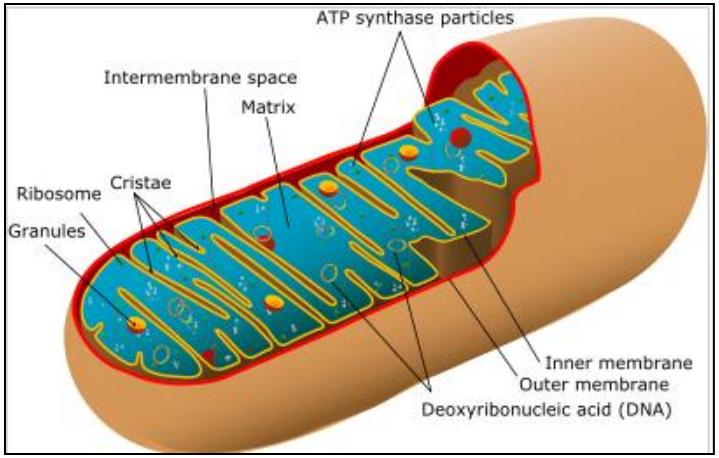


FATTY ACID OXIDATIVE METABOLISM





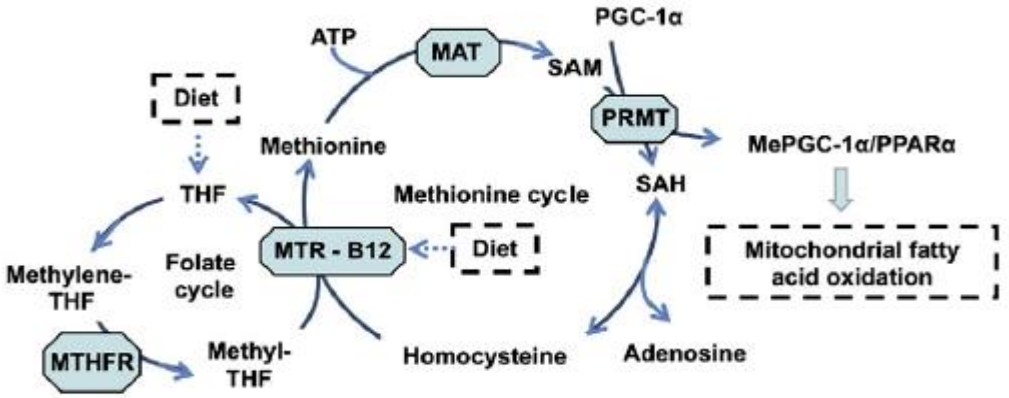


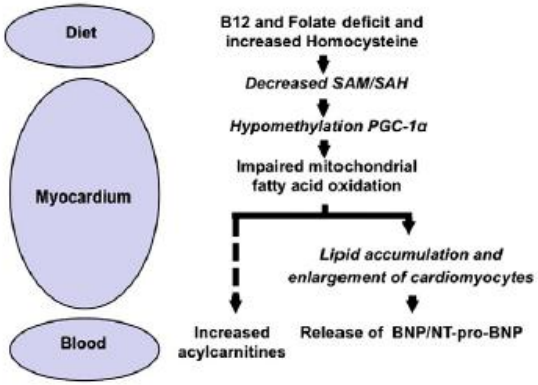


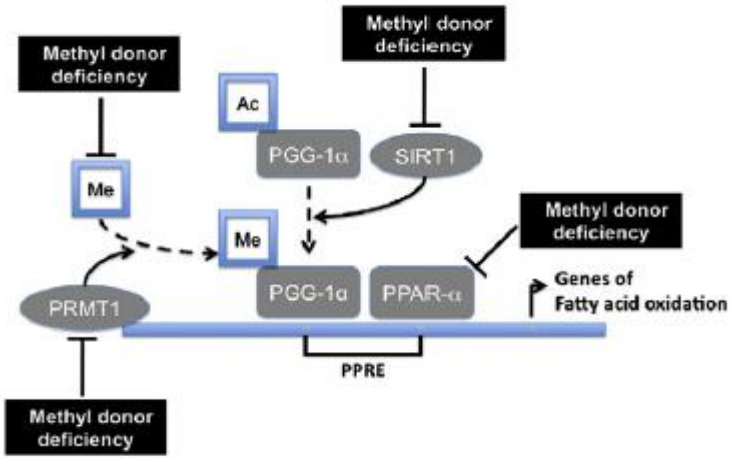
SAMe METHYL TRANSFER REACTIONS

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
COMT	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

SAMe and POST-TRANSLATIONAL ENZYME MODIFICATION







SAMe + PGC-1α → MePGC-1α
PRMT

SAMe → HDAC SIRT

AcPGC-1α → PGC-1α
SIRT1

MePGC-1→ PPARα, ERα, ERRα & HNF-4α

→ Fatty Acid Oxidation ⇒ ATP

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

METHYL DEFICIENCY and FATTY LIVER

♥ Female Wistar rats

One month prior to mating → weaning of pups place dams on:

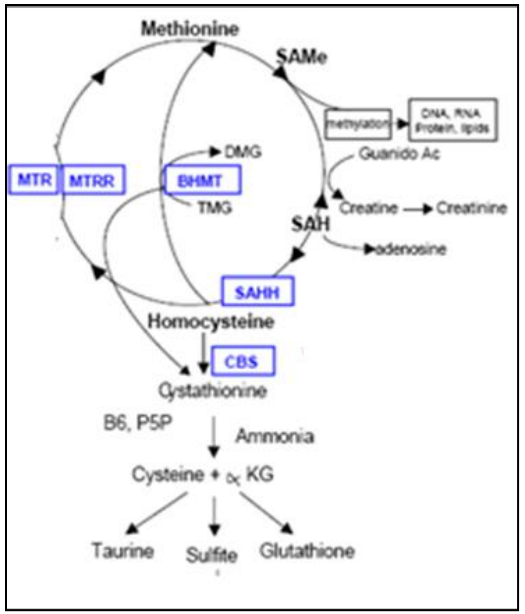
- Standard chow
- B12 and folate-free chow

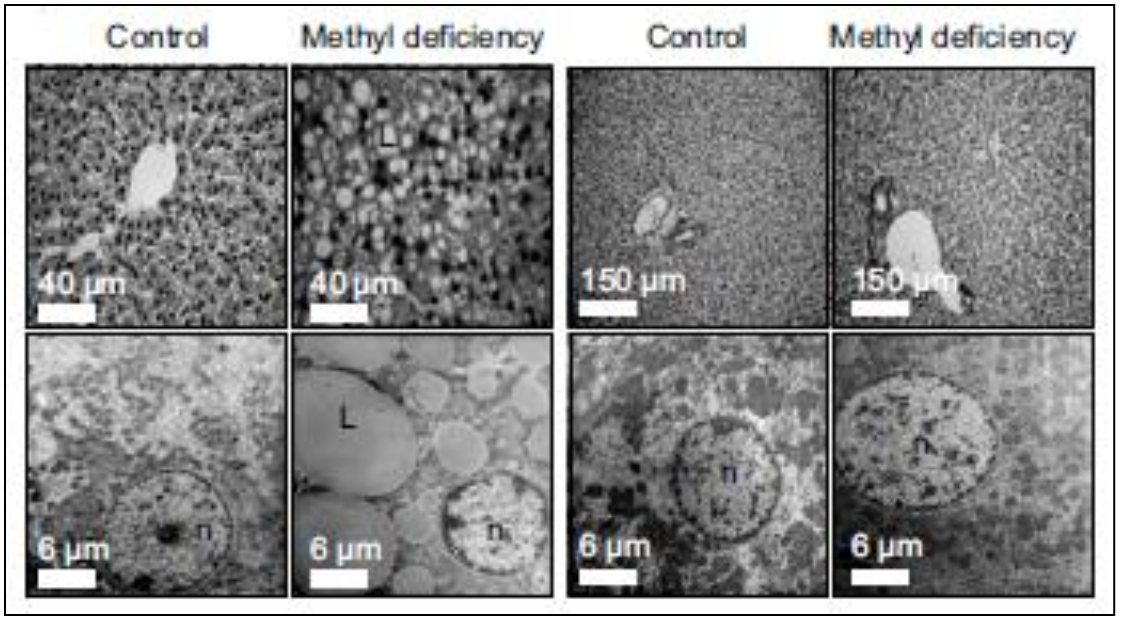
Evaluate pups at 21 days (and at 80 days with B Vitamin replete diet)

Parameters*	Control	Methyl donor deficiency	*p* value
Body weight (g)	40.4 ± 0.6	20.0 ± 1.6	<0.001
Liver (g)	1.5 ± 0.1	1.6 ± 0.1	0.471
Index liver weight/body weight	3.6 ± 0.1	8.5 ± 0.1	<0.001
Glucose (mg/dl)	138.3 ± 3.8	75.4 ± 5.5	<0.001
Vitamin B12 (pmol/L)	322.3 ± 56.0	127.1 ± 165.8	<0.001
Folate (nmol/L)	74.8 ± 18.5	35.4 ± 16.3	<0.001
Homocysteine (µmol/L)	6.28 ± 0.92	17.36 ± 5.80	<0.001
Insuline (µU/ml)	26.1 ± 4.0	22.5 ± 2.6	0.4750
Total cholesterol (mmol/L)	2.7 ± 0.2	4.6 ± 0.3	<0.001
Triglycerides (mmol/L)	0.4 ± 0.1	0.7 ± 0.1	<0.001
Free fatty acids (mmol/L)	200.6 ± 34.9	884.8 ± 178.4	<0.001
AST (IU/L)	185.8 ± 13.4	1007.2 ± 270.1	<0.001

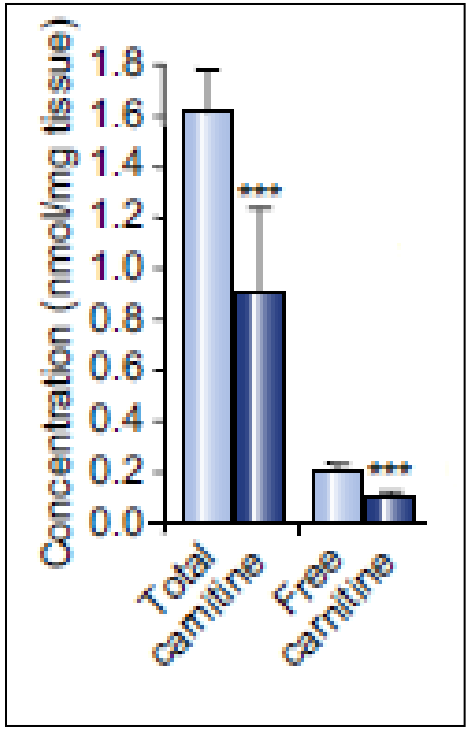
METHYL DEFICIENCY and FATTY LIVER

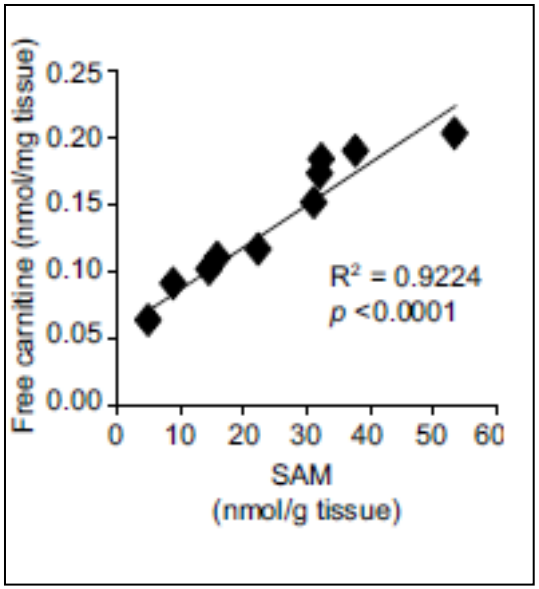
Parameters*	Control	Methyl donor deficiency	*p* value
Vitamin B12 (pmol/mg protein)	2.3 ± 0.5	2.5 ± 0.5	0.614
Folate (nmol/mg protein)	1.2 ± 0.2	0.2 ± 0.1	0.001
SAM (nmol/g tissue)	37.8 ± 9.3	13.9 ± 6.6	0.001
SAH (nmol/g tissue)	11.2 ± 3.2	10.3 ± 5.3	0.769
SAM/SAH ratio	3.4 ± 0.6	1.8 ± 1.3	0.038
MTR (nmol/h/mg protein)	2.5 ± 0.3	0.6 ± 0.1	0.008
BHMT (nmol/h/mg protein)	7.0 ± 0.3	7.8 ± 1.4	0.691
Total lipids (µg/mg tissue)	65.9 ± 10.5	391.1 ± 183.4	<0.001
Cholesterol (µg/mg tissue)	0.3 ± 0.2	0.4 ± 0.2	0.271
Triglycerides (µg/mg tissue)	3.5 ± 1.2	21.3 ± 8.2	0.010

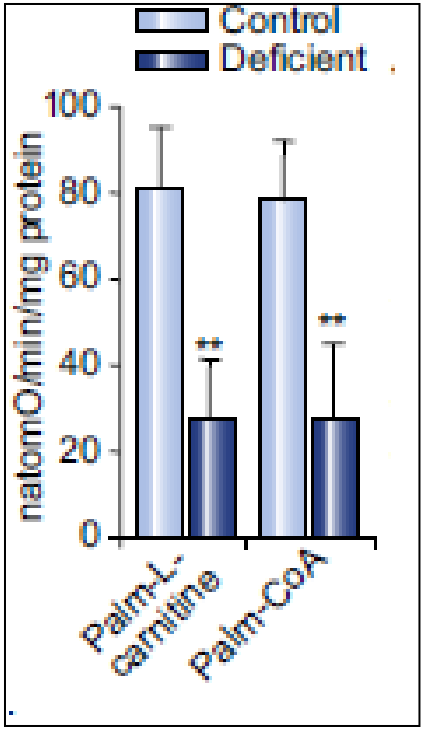




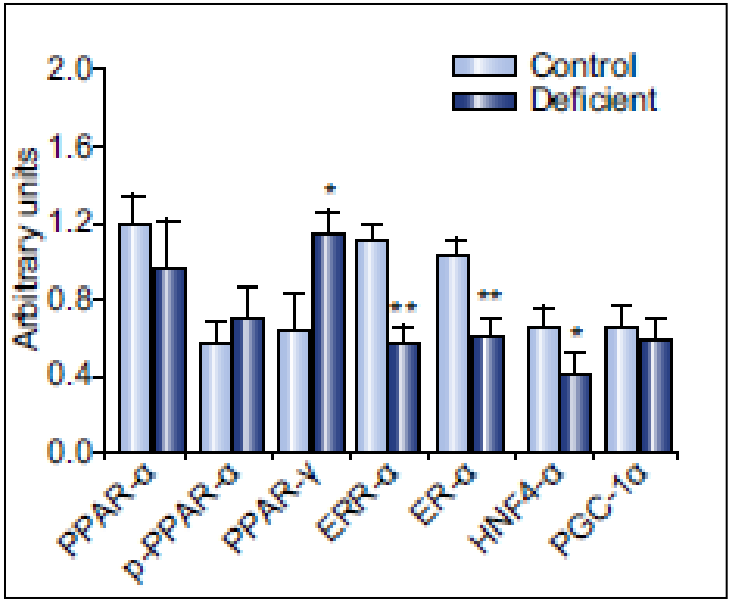
METHYL DEFICIENCY and FATTY LIVER

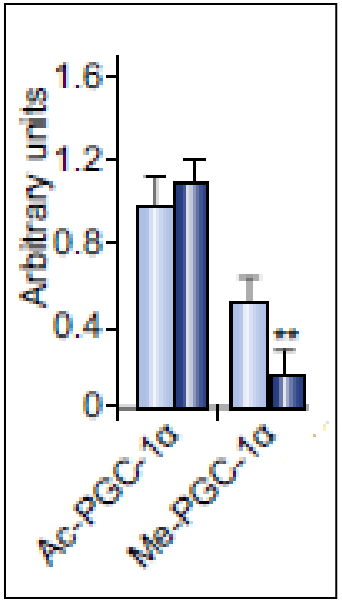


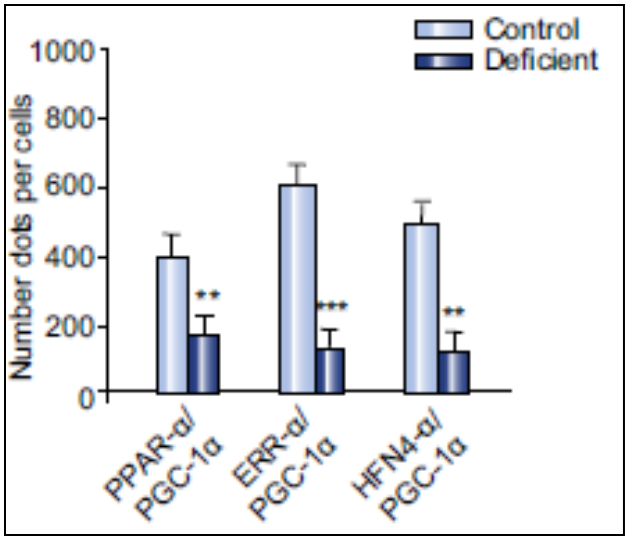


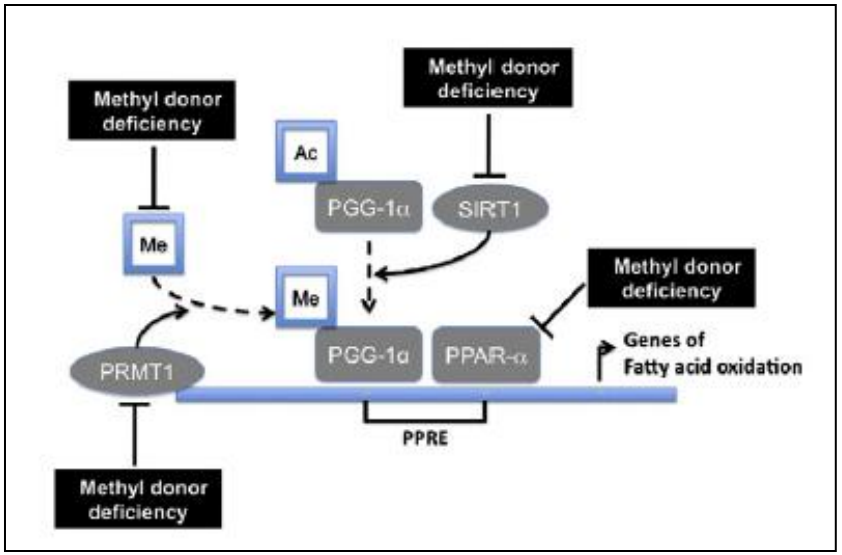


METHYL DEFICIENCY and FATTY LIVER









METHYL DONOR DEFICIENCY CARDIOMYOPATHY

♥ Female Wistar rats

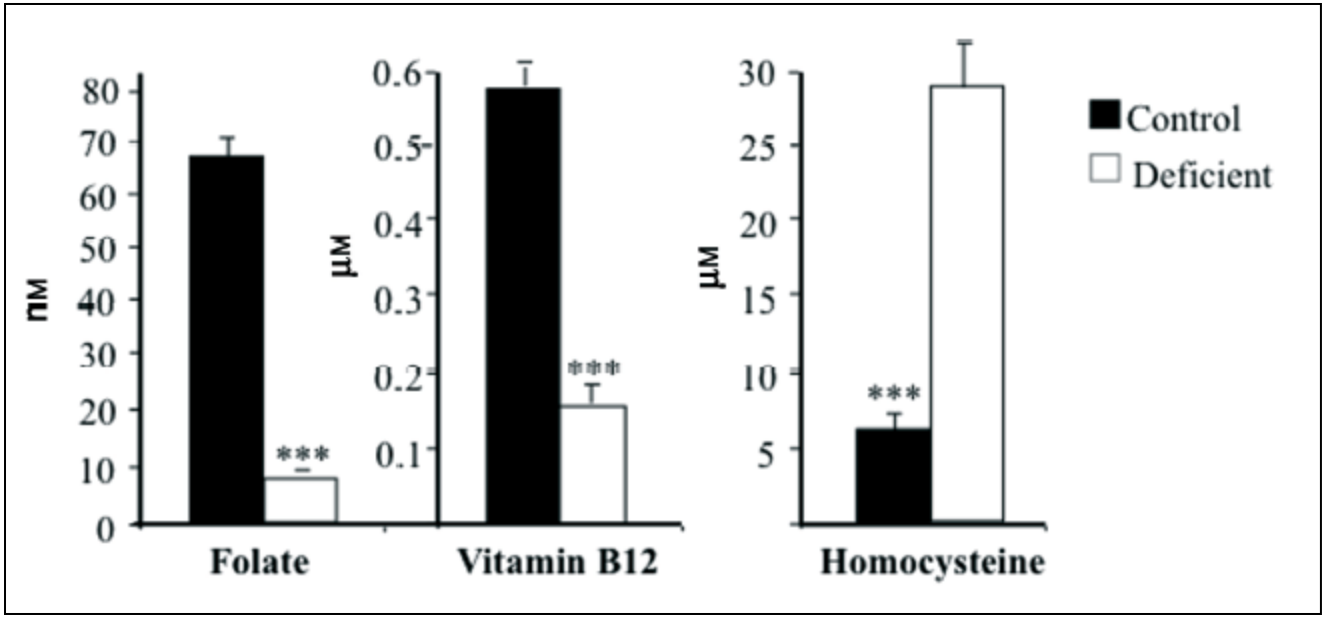
One month prior to mating → weaning of pups place dams on:

- Standard chow
- B12, folate, and choline deficient chow

Evaluate pups at 21 days

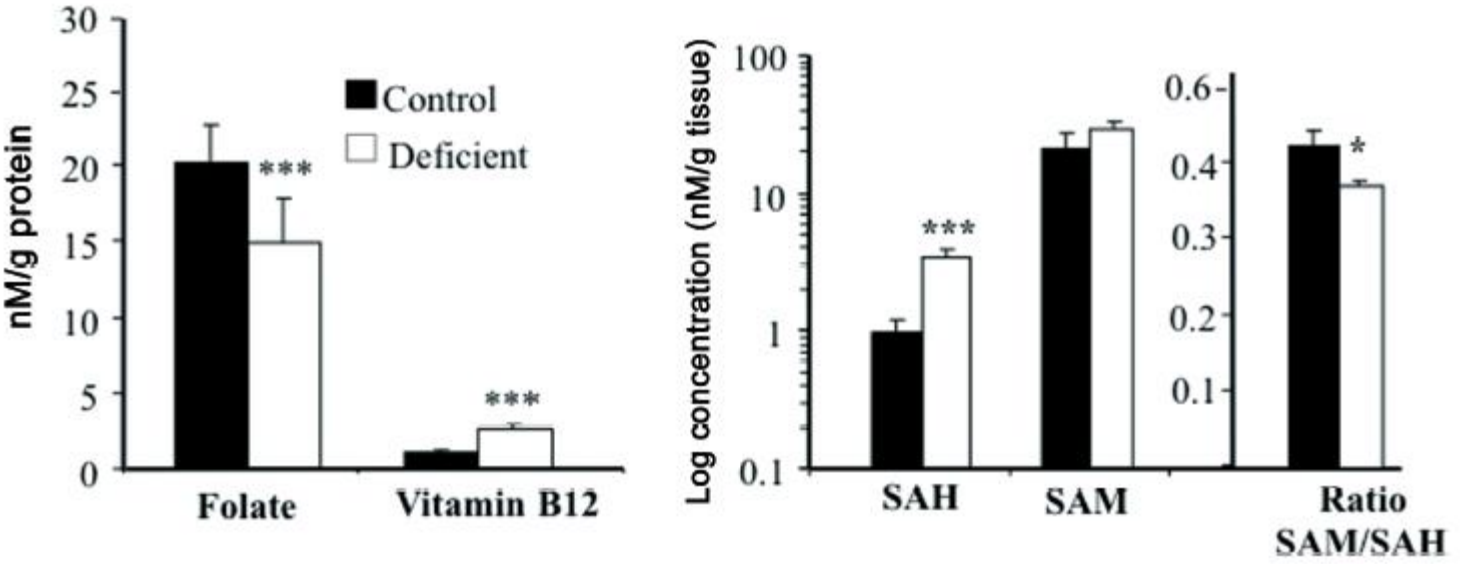
METHYL DONOR DEFICIENCY CARDIOMYOPATHY

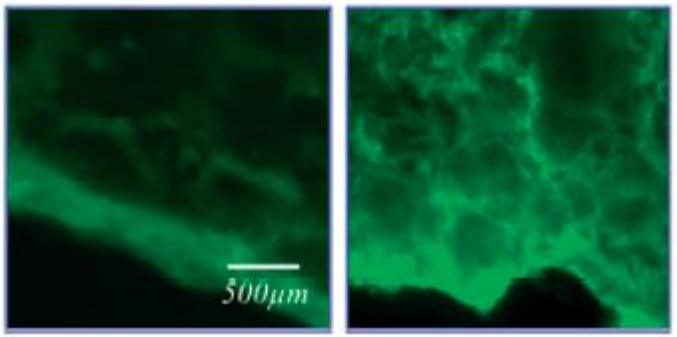
Pup plasma values



METHYL DONOR DEFICIENCY CARDIOMYOPATHY

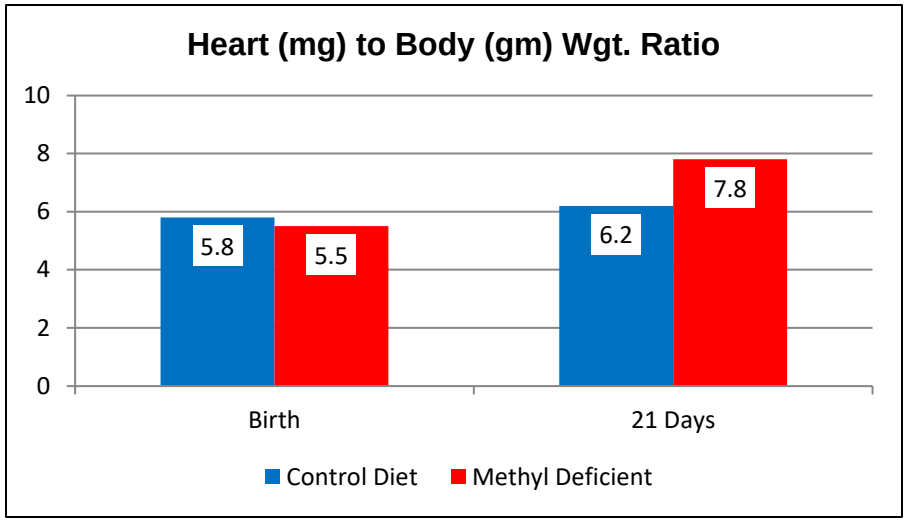
Pup myocardial values

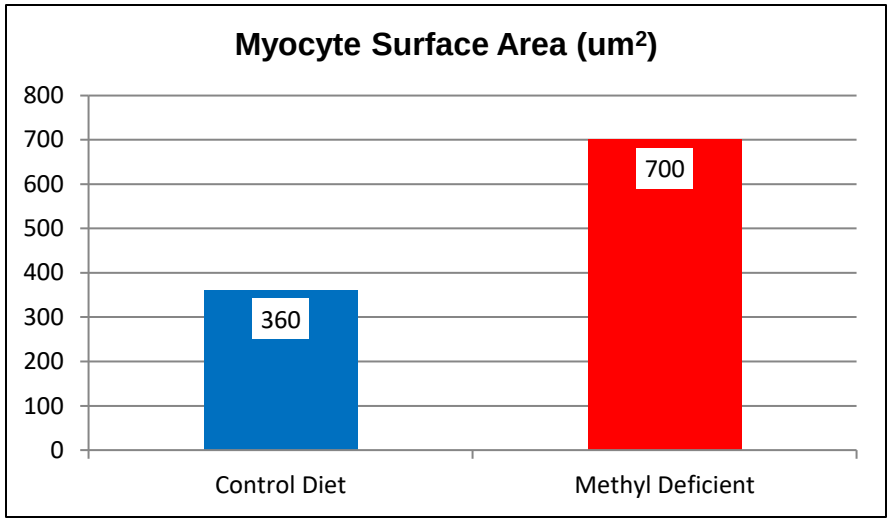


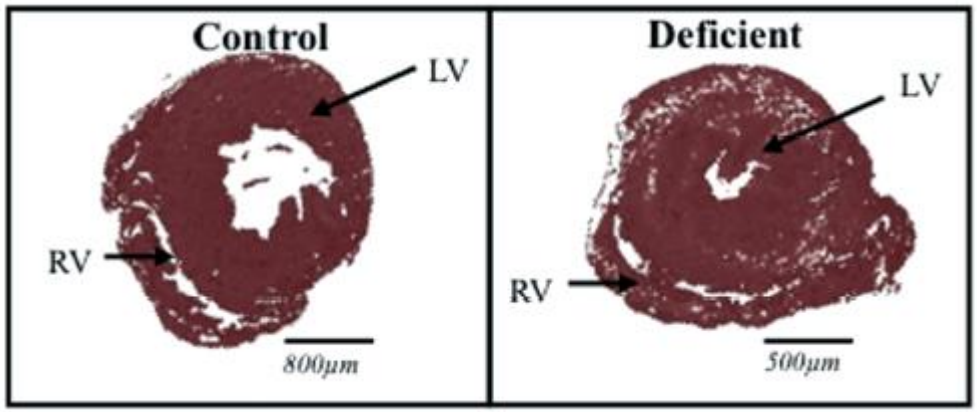


Homocysteine

METHYL DONOR DEFICIENCY CARDIOMYOPATHY

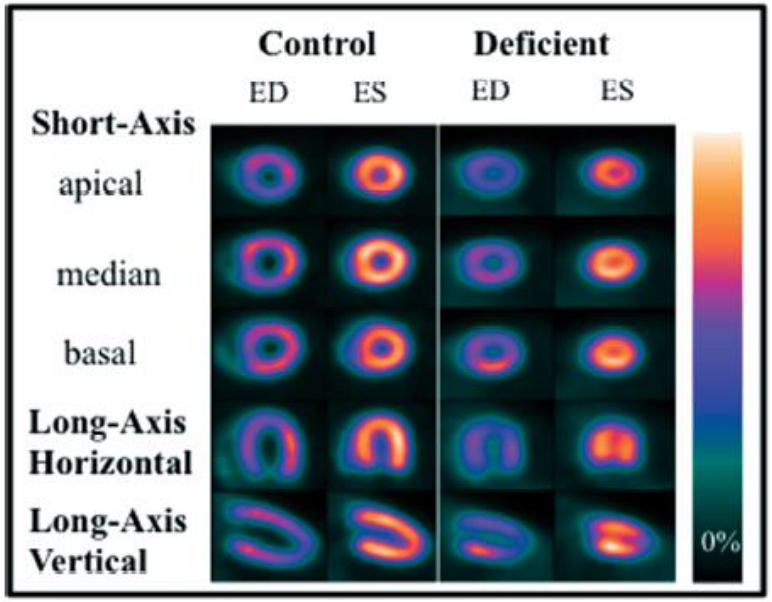






EF 63%

EF 70%



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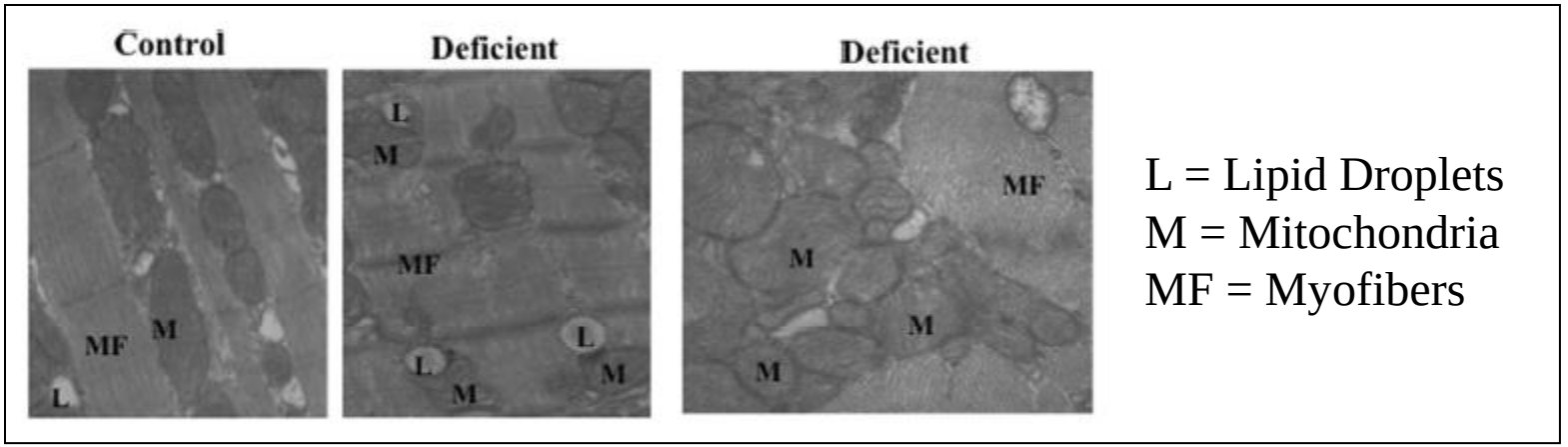

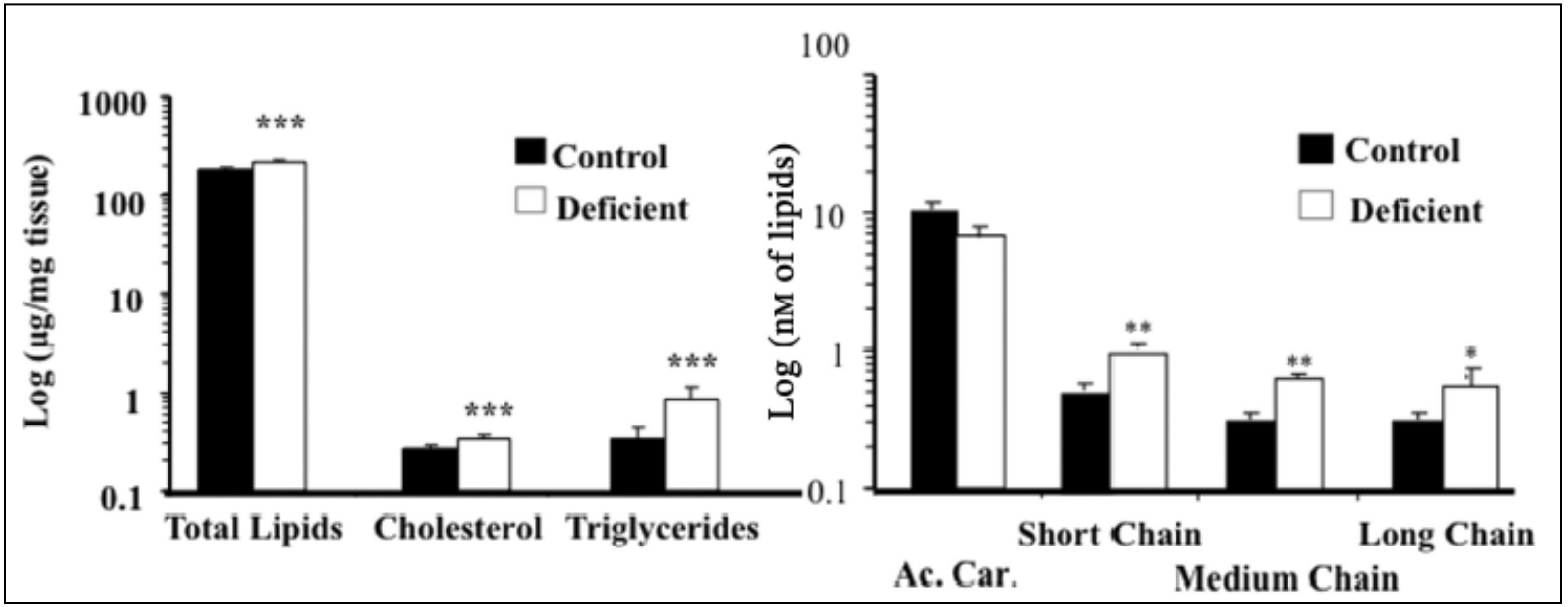

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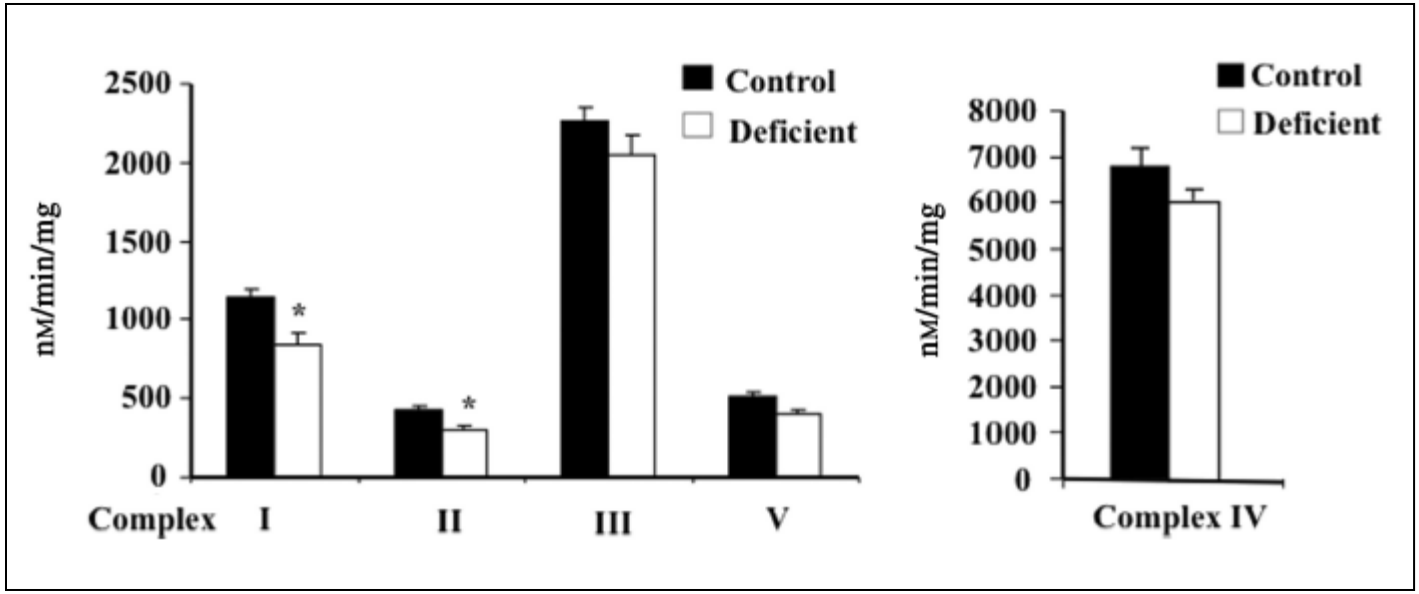

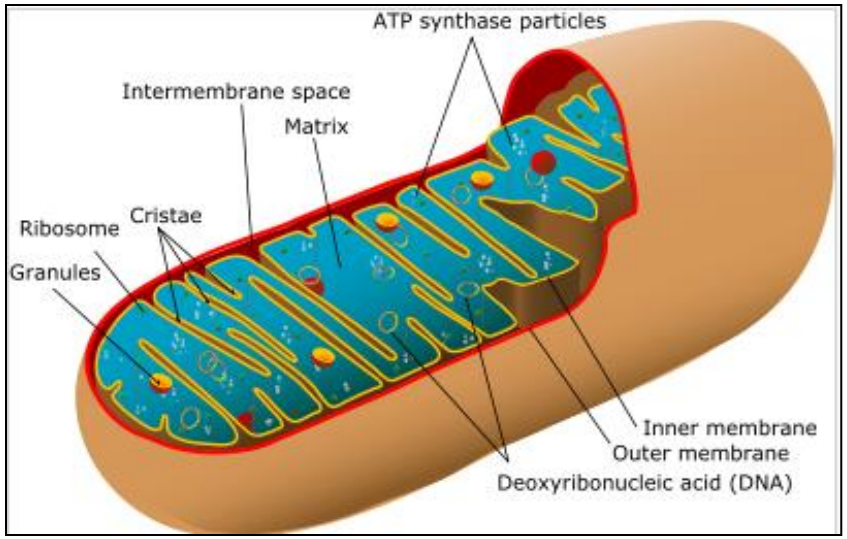

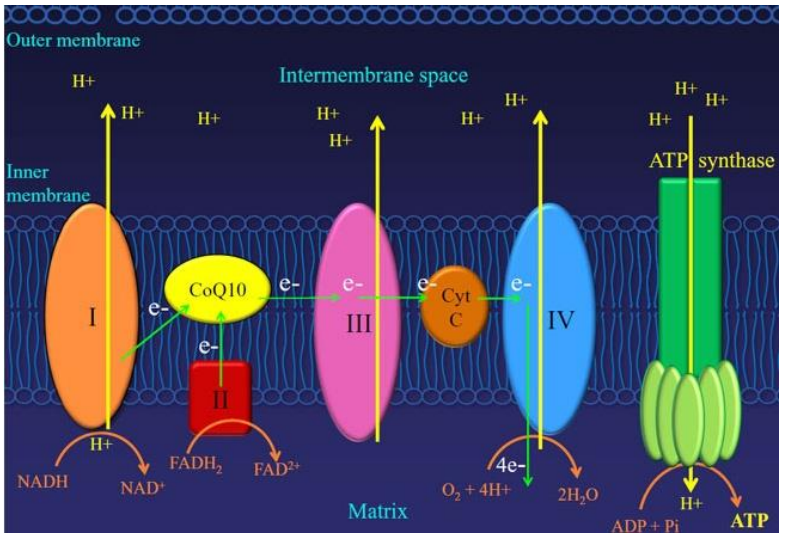

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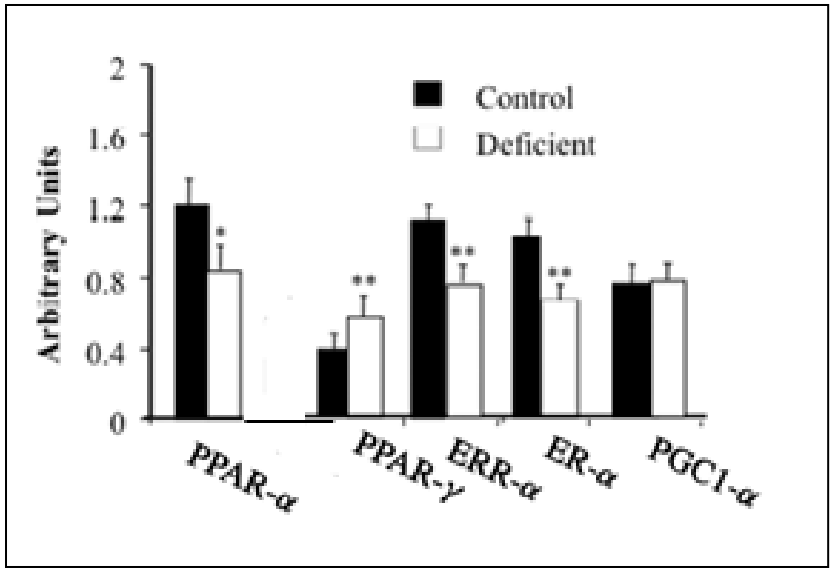
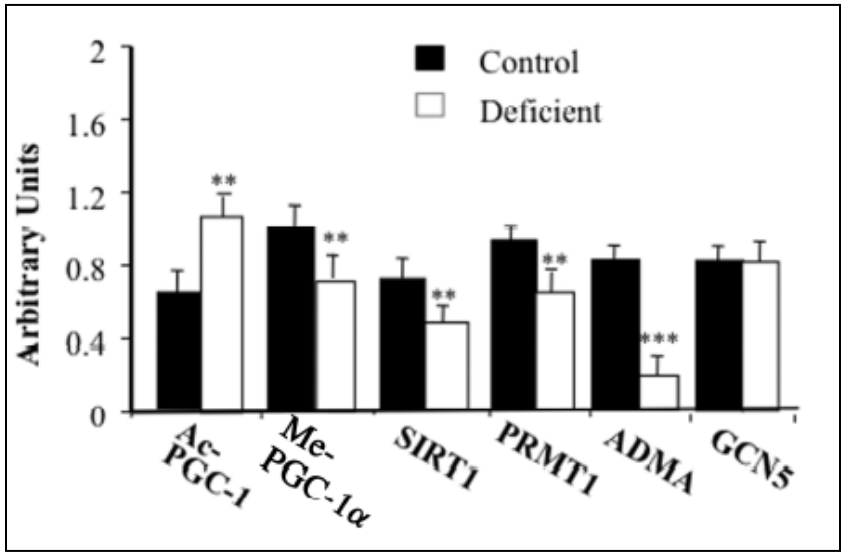




ELEVATED HOMOCYSTEINE in CHF

♥ 108 consecutive subjects presenting with CHF (CADz or DC)

- LVEF < 45%
- NYHA II-IV symptoms
- Symptoms > six months duration

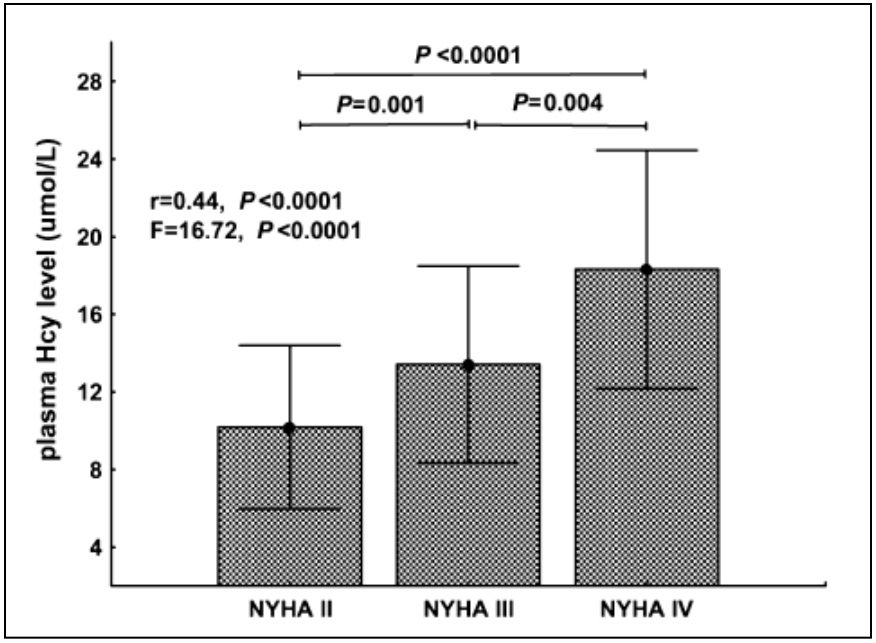
Baseline data collection

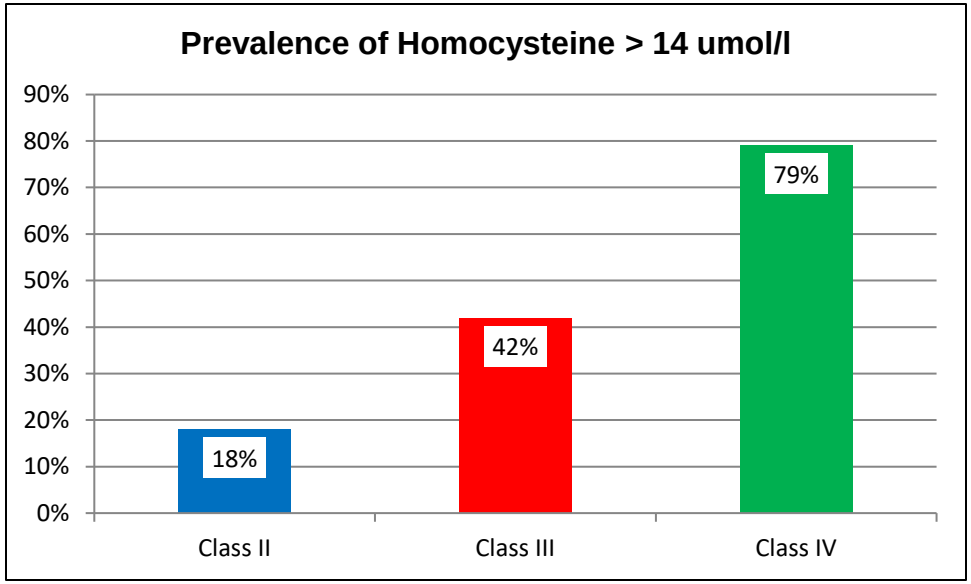
Follow for three years

Mean homocysteine 12.5 umol/l

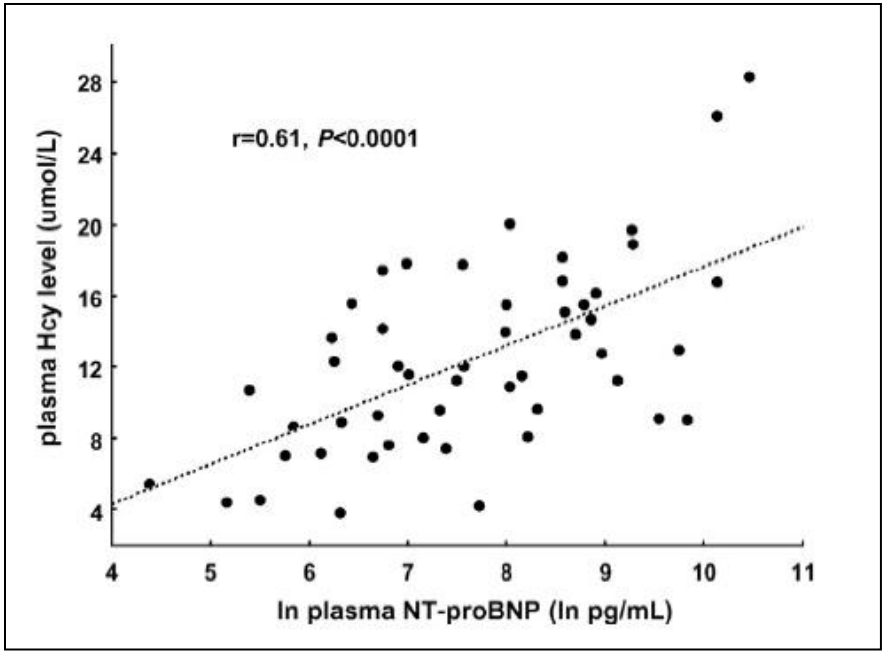
- Range 2.3-28.3
- HHcy (> 14) in 35%

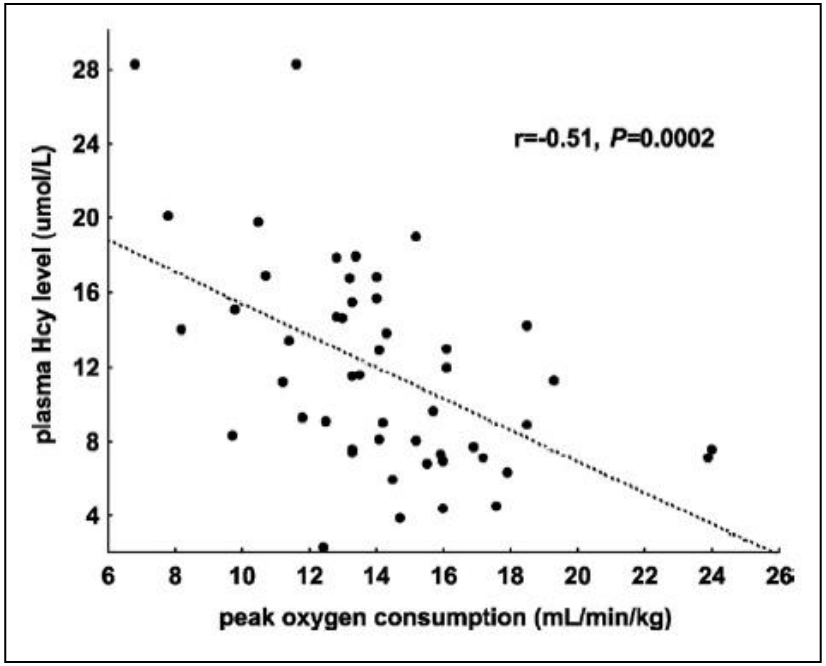
ELEVATED HOMOCYSTEINE in CHF



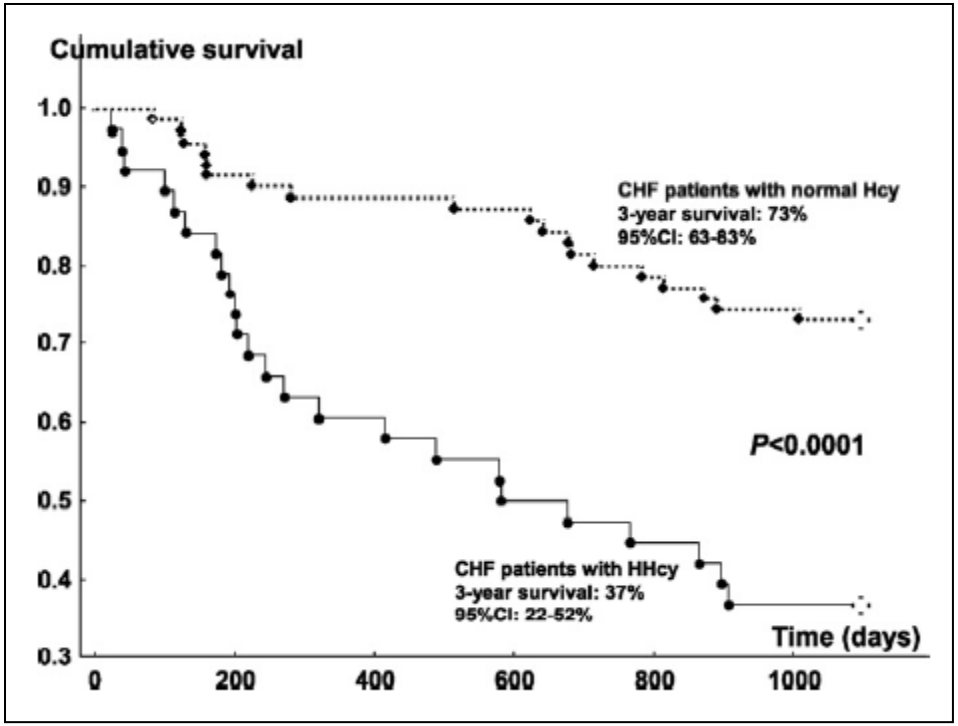


ELEVATED HOMOCYSTEINE in CHF





ELEVATED HOMOCYSTEINE in CHF



HHcy strongest predictor of mortality (LVEF and albumin)

3-year survival 37% vs. 73% (level < 14)

HOMOCYSTEINE and BNP

♥ 358 patients undergoing angiography for stable CV symptoms

Coronary narrowing ≥ 50%	68%
EF < 40% (mean 44%)	47%
Pro-BNP > 250 pg/ml (mean 2308)	65%
Hcy > 12 mmol/l (mean 16.2)	88%
B-12 < 150 pmol/l (mean 347)	13%
MMA > 0.5 mmol/l (mean 0.51)	29%
Folate < 7 nmol/l (mean 11.4)	25%

HOMOCYSTEINE and NT-pro-BNP

♥ 662 older Sicilians (60-85 years) volunteering for screening study

Hy CV Dz	29%
BNP > 100 pg/ml (mean 43)	9%
Hcy > 12 mmol/l (mean 15.8)	74%
B-12 < 150 pmol/l (mean 350)	10%
MMA > 0.5 mmol/l (mean 0.74)	8%
Folate < 7 nmol/l (mean 13.8)	13%

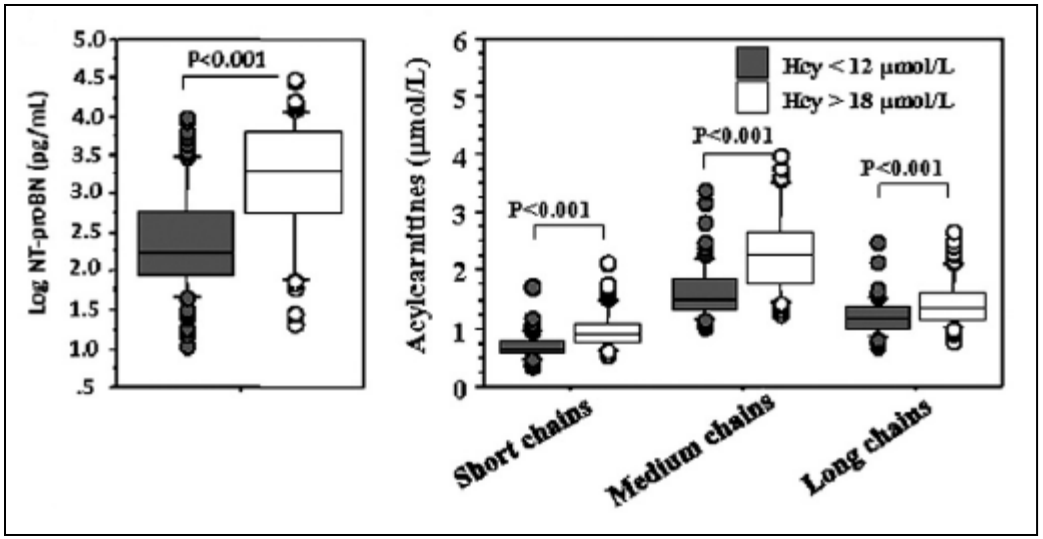
HOMOCYSTEINE and BNP

	Cath Patients	Screening Subjects
Elevated BNP	65%	9%
Hcy Mean	16.2	15.8
Elevated Hcy	88%	74%
Folate	11.4	13.8
Low Folate	25%	13%
MMA	0.51	0.74
Elevated MMA	29%	8%
B-12	347	350
Low B12	13%	10%

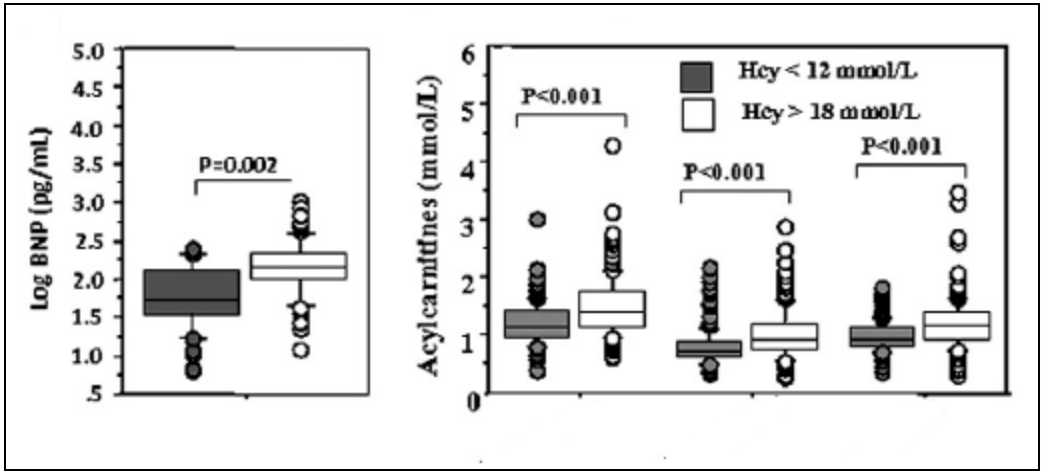
Low B12 Present	MMA 5.8	MMA 0.23
Low Folate Present	Hcy 19.3	15.3

HOMOCYSTEINE and BNP

Cath Patients (1st vs. 4th Hcy Quartile)

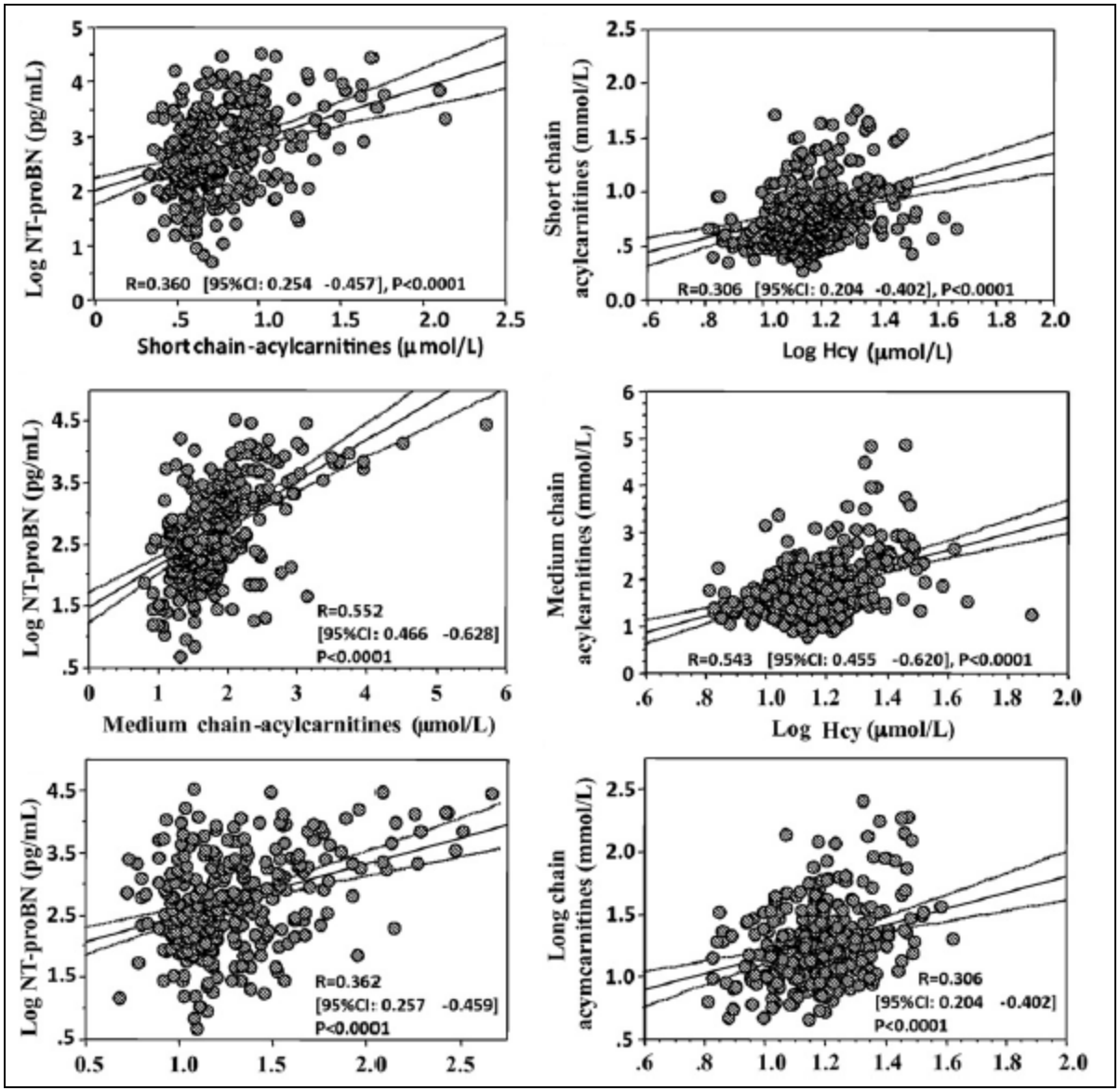


Screening Subjects (1st vs. 4th Hcy Quartile)



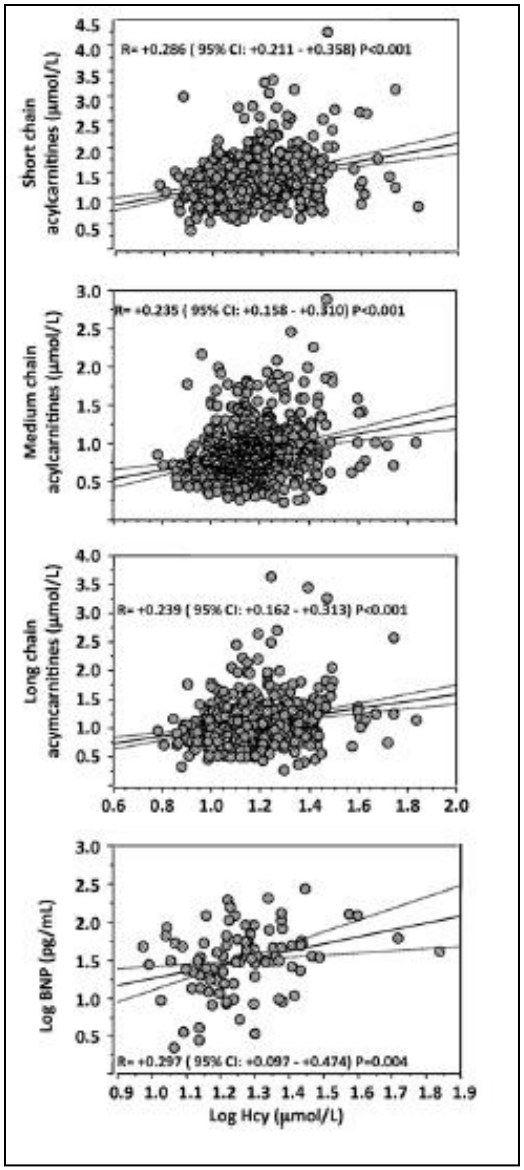
HOMOCYSTEINE and BNP

Cath Patients

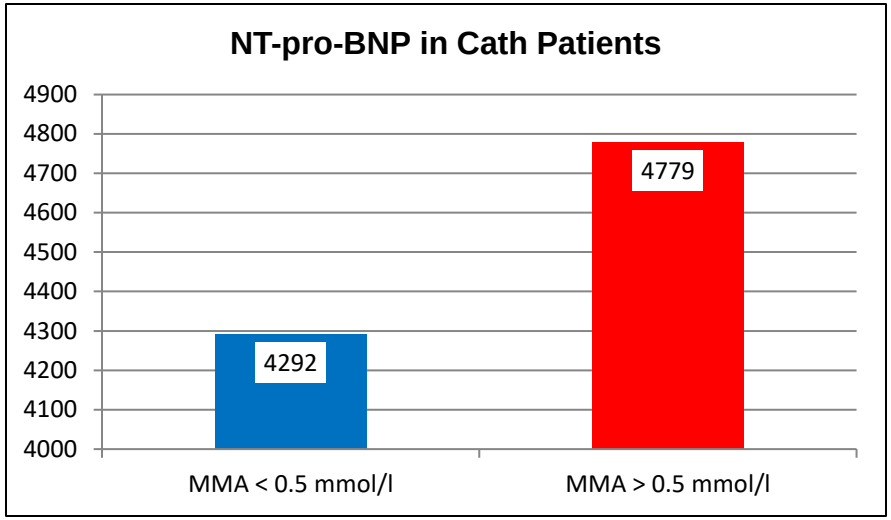


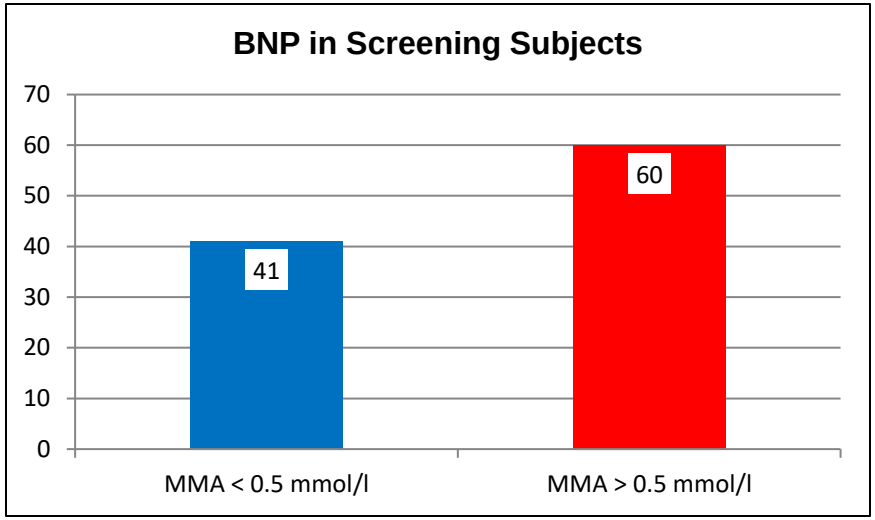
HOMOCYSTEINE and BNP

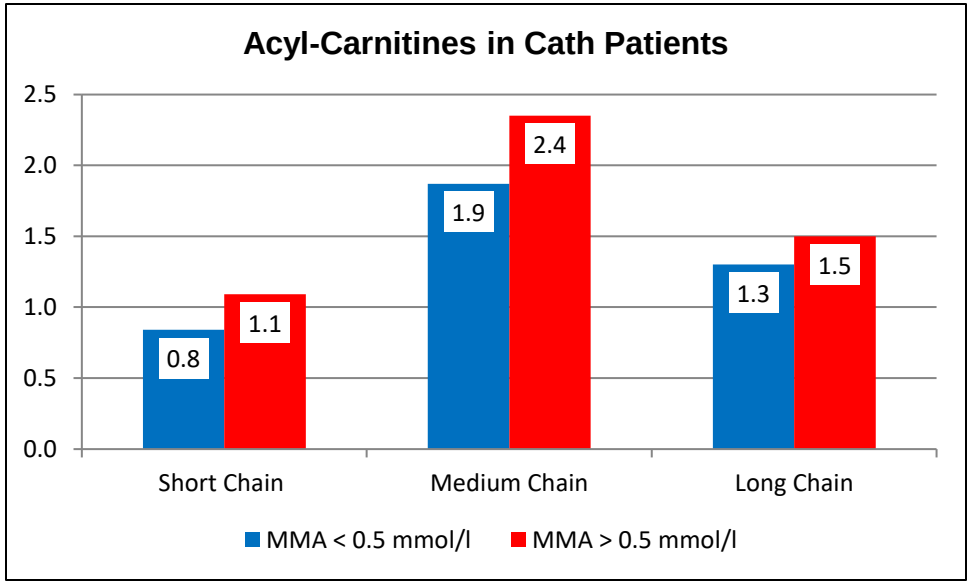
Screening Subjects



HOMOCYSTEINE and BNP



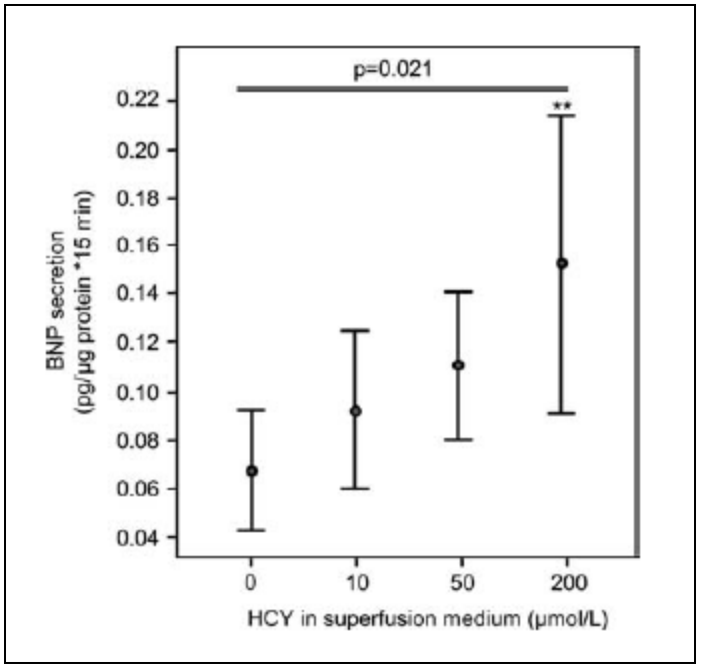




HOMOCYSTEINE INCREASED BNP

♥ Rat LV slices in superfusion chamber

Increase concentration of Hcy in superfusate



B VITAMIN THERAPY in HEART FAILURE

♥ 28 patient with CHF (ischemic etiology)

- LVEF ≤ 35%
- Mean age 75.4 years
- ACEI 100%, Statin 96%, β-Blocker 82%, Furosemide 64 mg/day

Baseline measurements; then randomize to one year of:

- Placebo
- Multinutrient supplementation

Table 1 Doses of micronutrients, RDI, and upper safe daily intakes

Nutrient	Daily dose (four capsules)	RDI	Upper safe limit for total daily intake
Calcium	250 mg	800 mg	2500 mg
Magnesium	150 mg[a]	300 mg	700 mg
Zinc	15 mg	15 mg	30 mg
Copper	1.2 mg	1.2 mg	9 mg
Selenium	50 μg	65 μg	450 μg
Vitamin A	800 μg	800 μg	3300 μg
Thiamine	200 mg[a]	1.4 mg	No limit
Riboflavin	2 mg	1.5 mg	No limit
Vitamin B_6	200 mg[a]	2 mg	300 mg
Folate	5 mg[a]	200 μg	No limit
Vitamin B_{12}	200 μg	1 μg	No limit
Vitamin C	500 mg[a]	60 mg	2000 mg
Vitamin E	400 mg[a]	10 mg	900 mg
Vitamin D	10 μg[a]	5 μg	25 μg
Co-enzyme Q10	150 mg[a]	15 mg	No limit

B VITAMIN THERAPY in HEART FAILURE

Mortality:

1/14 placebo (pneumonia)

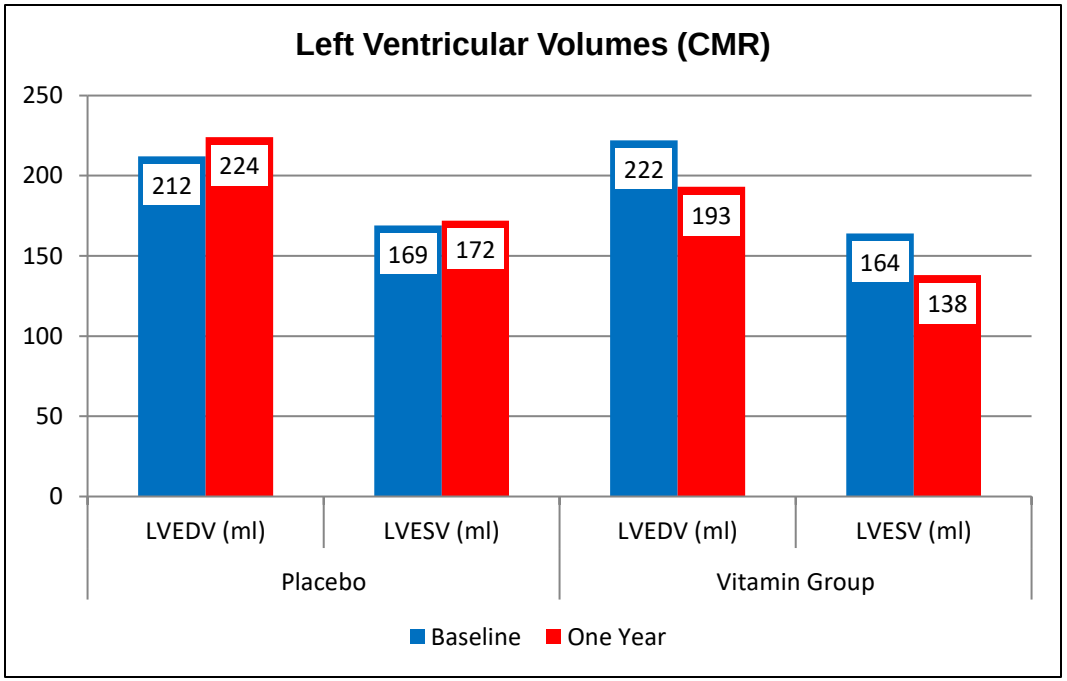
1/14 Vitamin (DVT $\rightarrow$ infection)

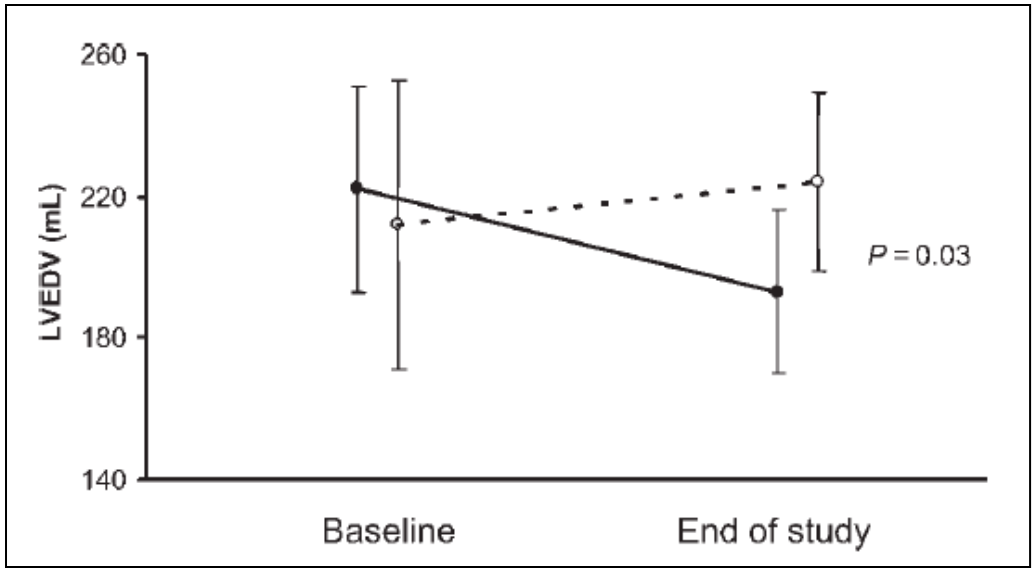
Furosemide dose:

64 $\rightarrow$ 56 mg/day Vitamin Group

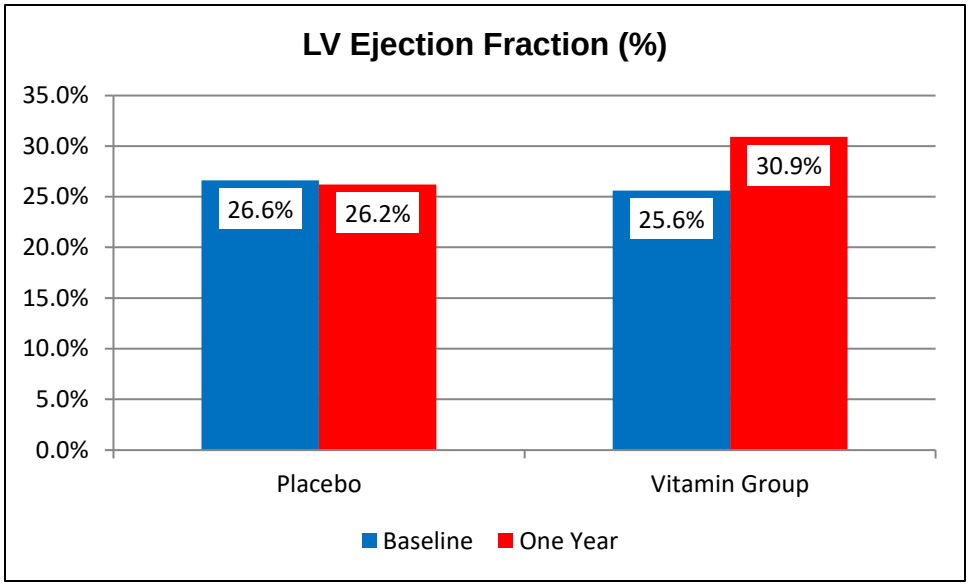
65 $\rightarrow$ 67 mg/day placebo

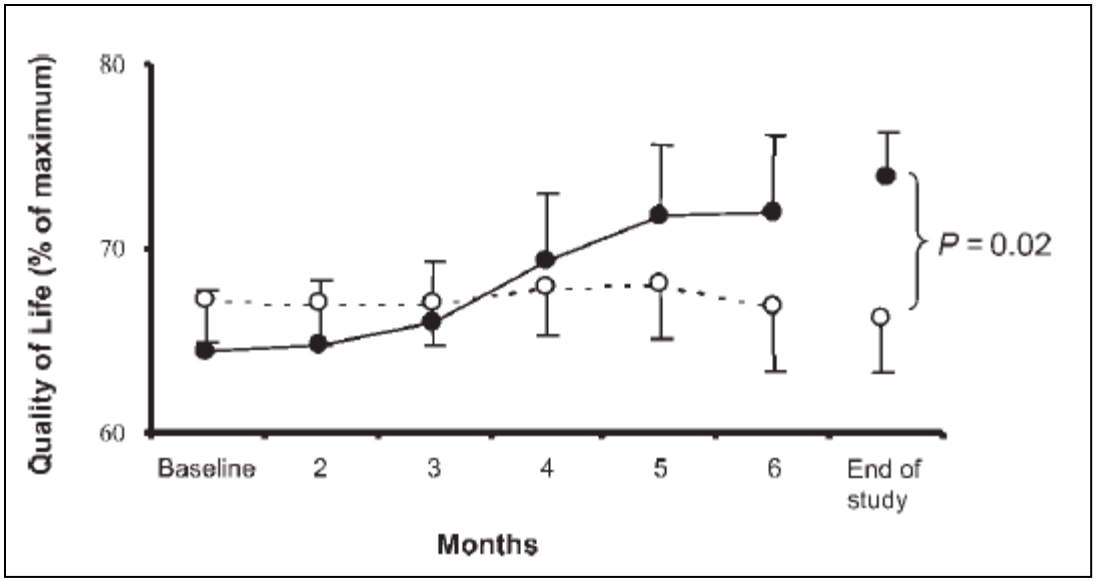
B VITAMIN THERAPY in HEART FAILURE





B VITAMIN THERAPY in HEART FAILURE





B VITAMIN THERAPY in CHF

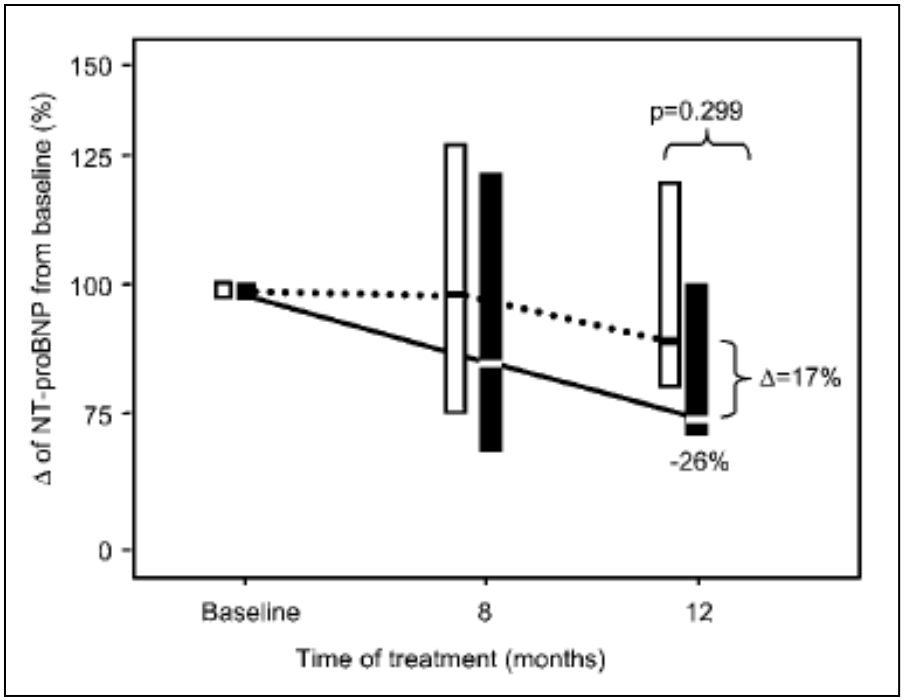
♥ 18 subjects with NT-proBNP > 200 pg/l

Baseline measurements

Randomize to receive over one year:

- 2.5 mg folate, 500 mcg B12, and 25 mg B6
- Placebo

→ 25% decrease in Hcy
⇒ 26% decrease in NT-proBNP



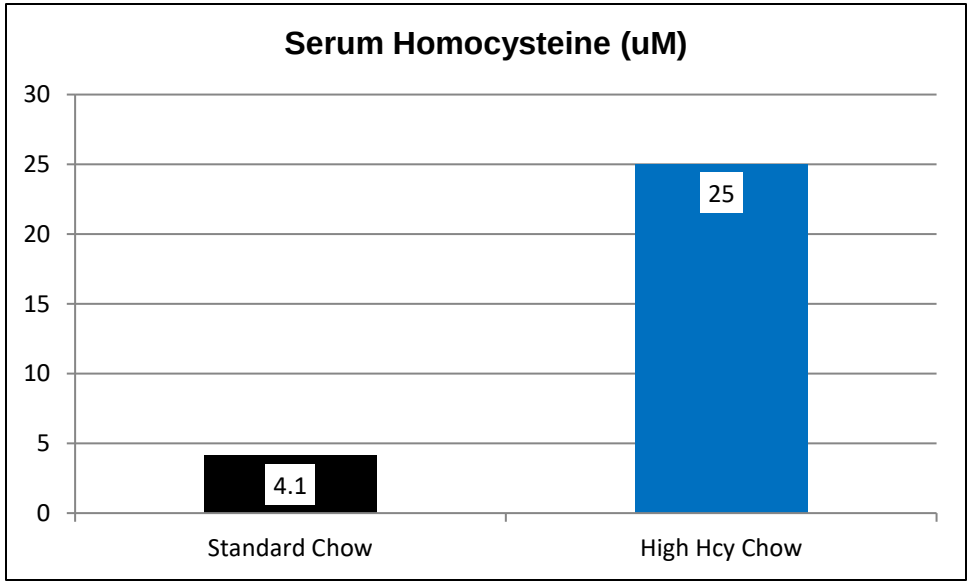
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

♥ Male Sprague-Dawley rats

Baseline measurements and then feed over four weeks:

- Standard rat chow (0.7% methionine) → Hcy 4.1 uM
- Standard chow + Berberine 5 mg/kg i.p. (final five days)
- High methionine (1.7%) chow → Hcy 25 uM
- High methionine chow + Berberine 5 mg/kg i.p. (final five days)

Study effect on lipids and gene expression



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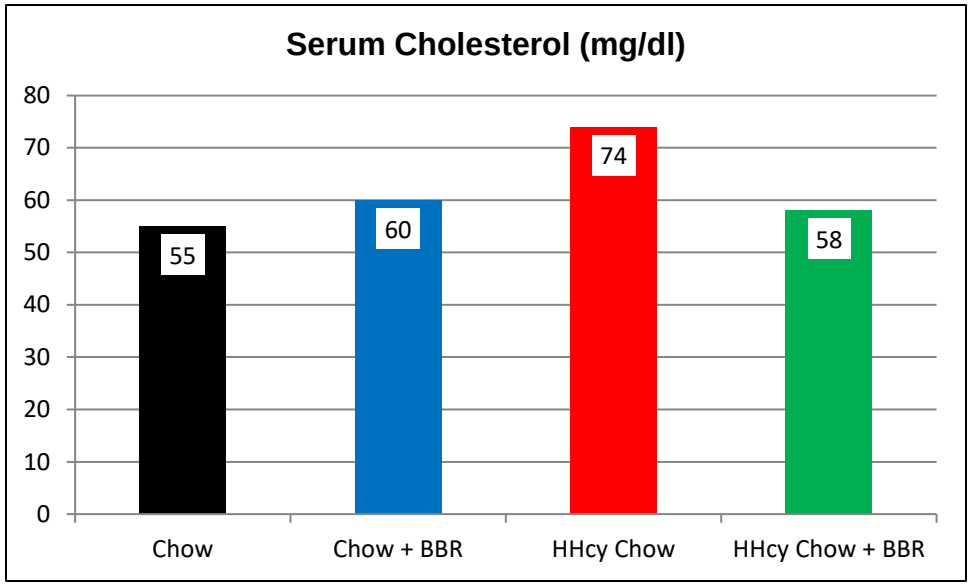

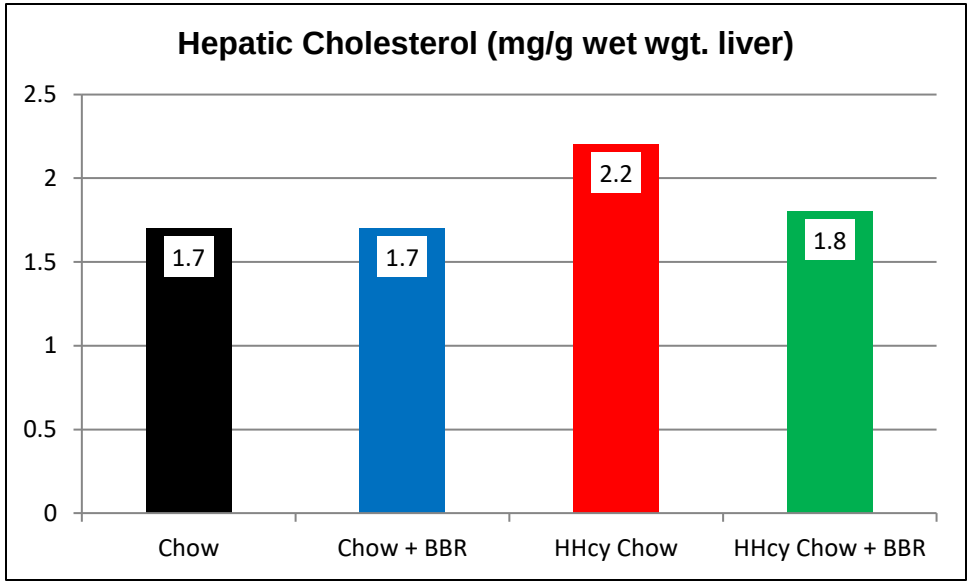

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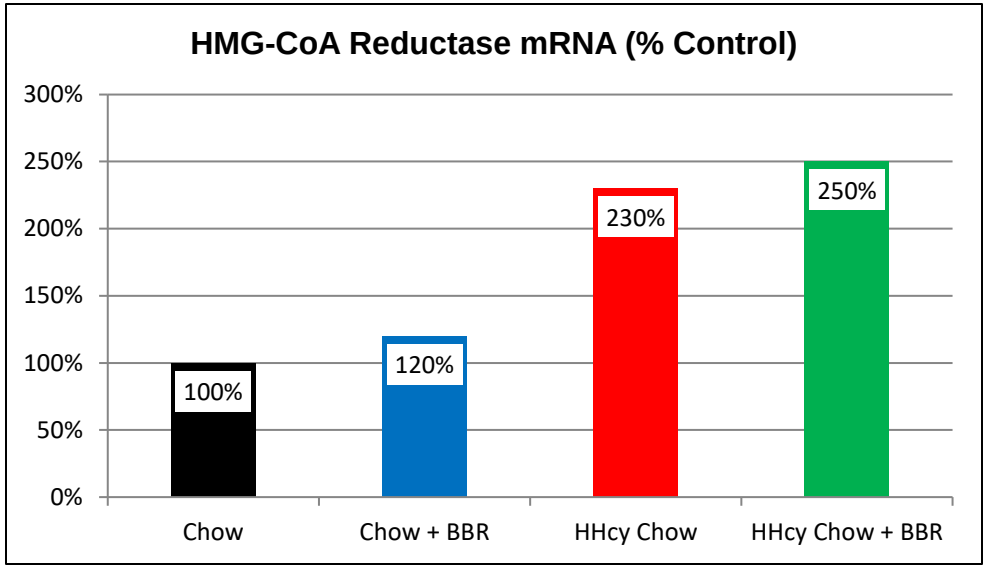

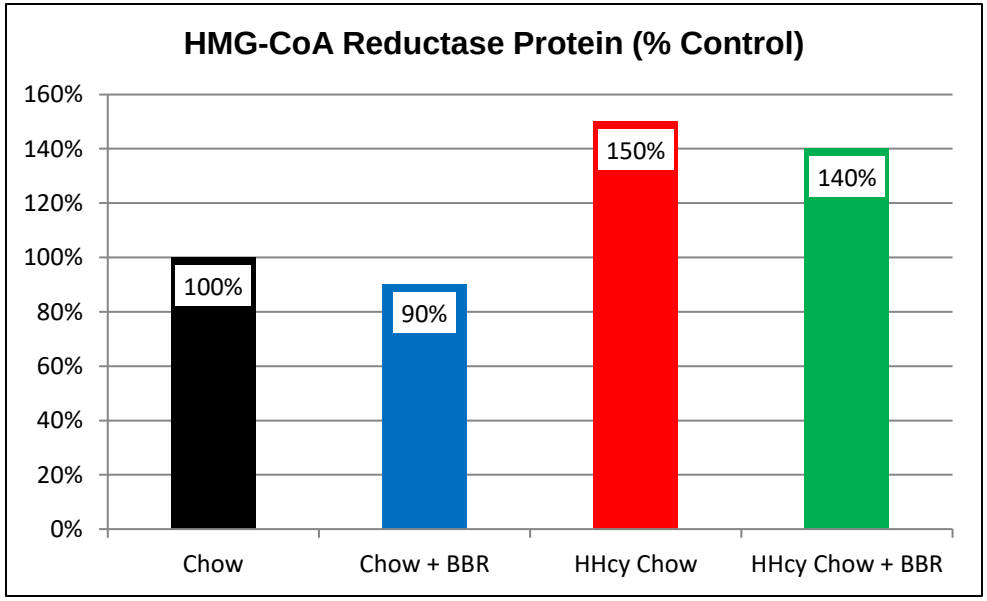

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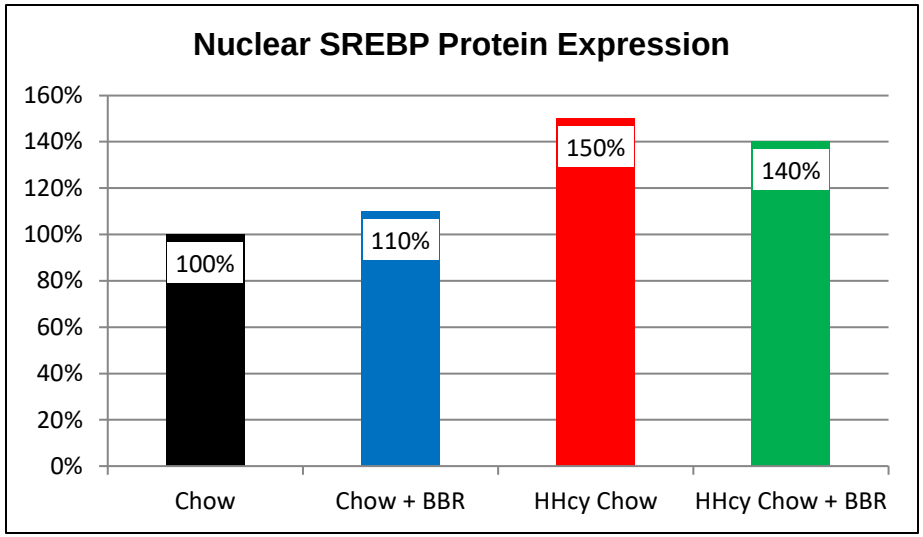

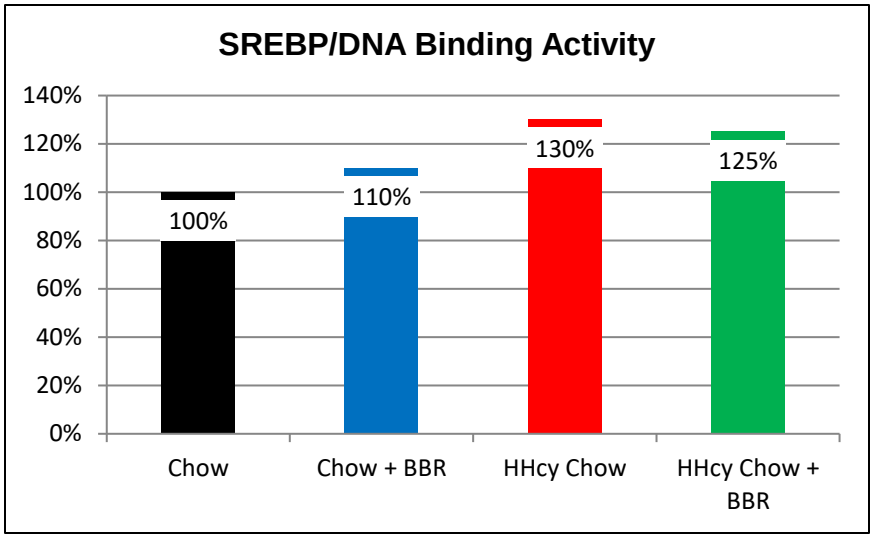

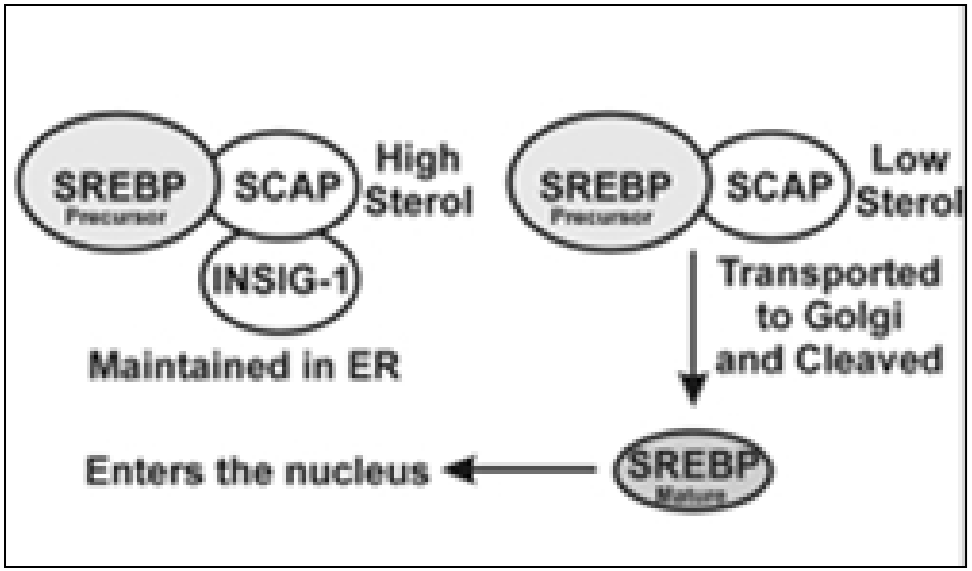

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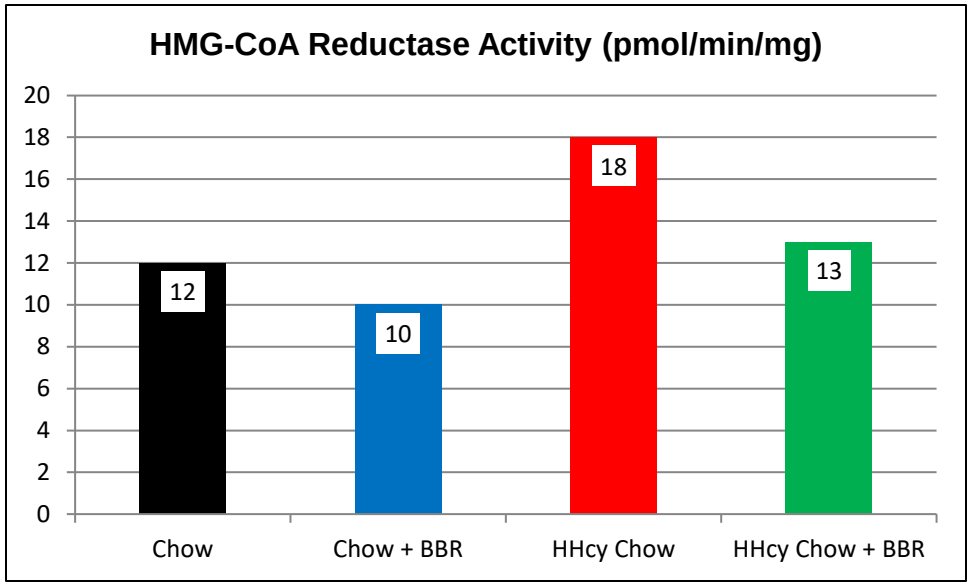

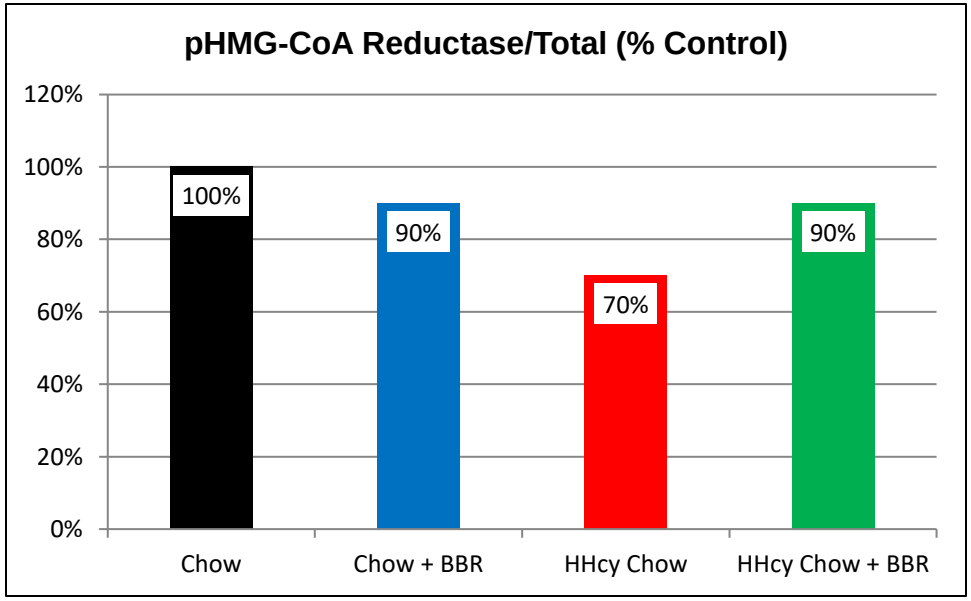

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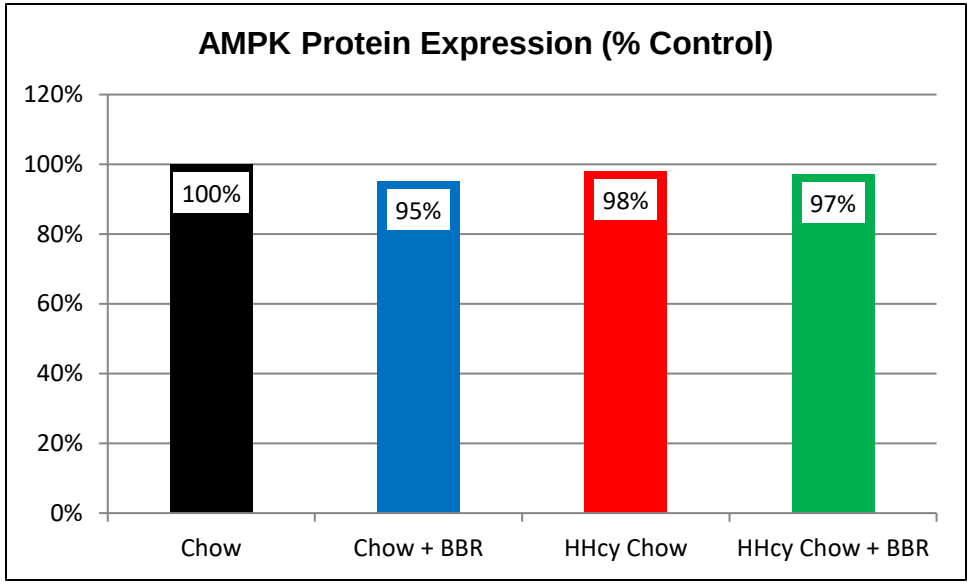

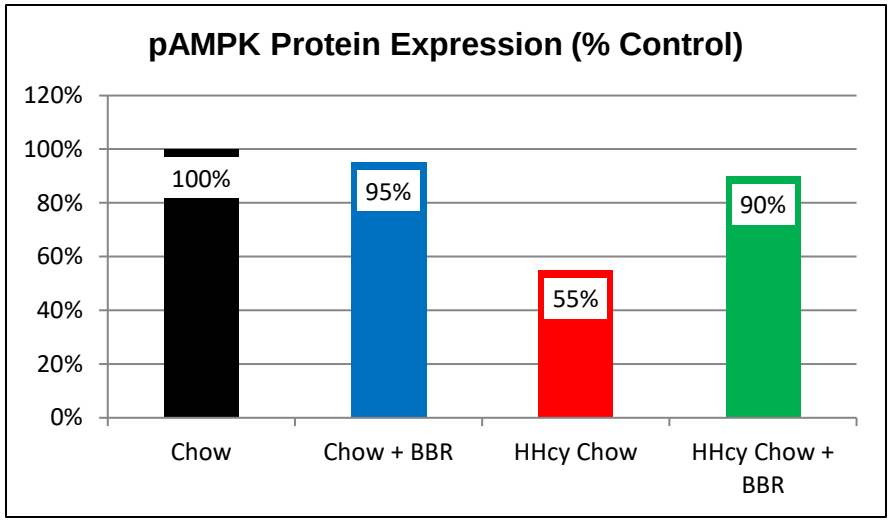

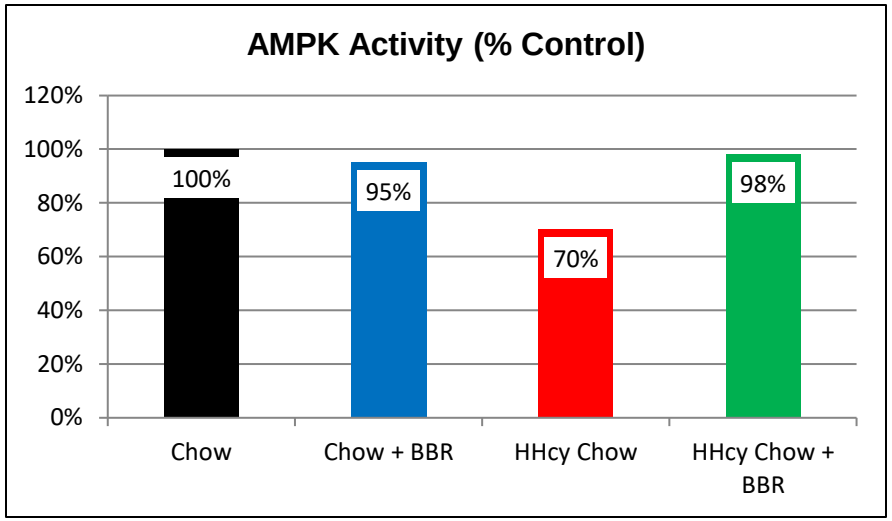

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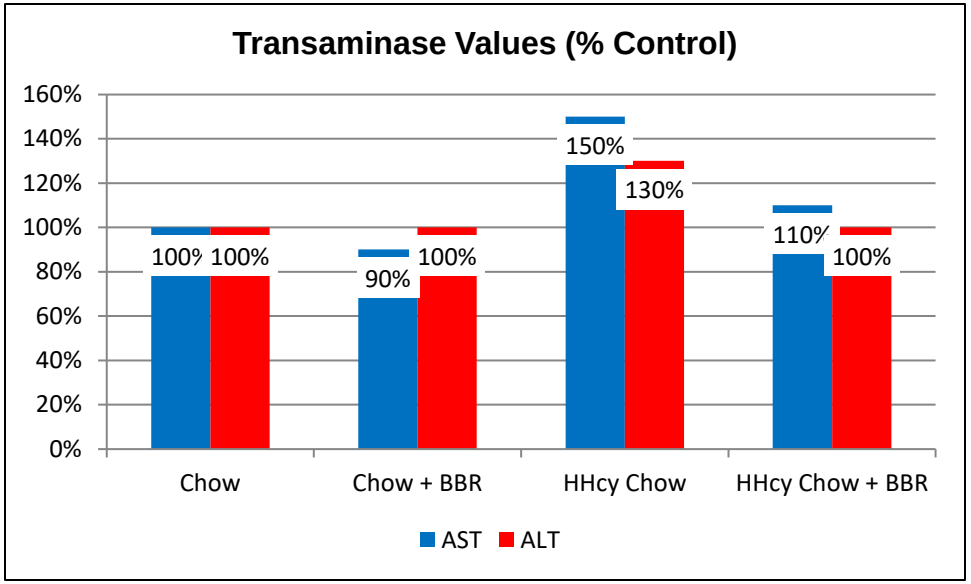


Hcy (mimics low hepatic cholesterol) → SREBP → HMG-CoA Reductase
Hcy → dephosphorylates AMPK → dephosphorylation of HMG-CoA
⇒ Increased cholesterol generation, secretion, and fatty liver

Berberine → pAMPK → pHMG-CoA
⇒ Decreased cholesterol generation, secretion, and resolution of fatty liver

BERBERINE, HOMOCYSTEINE, and CHOLESTEROL

♥ Male Sprague - Dawley rats:

Control rats: Free access to standard rat chow and water over 16 weeks
(12% fat, 62% carb, and 16% protein)

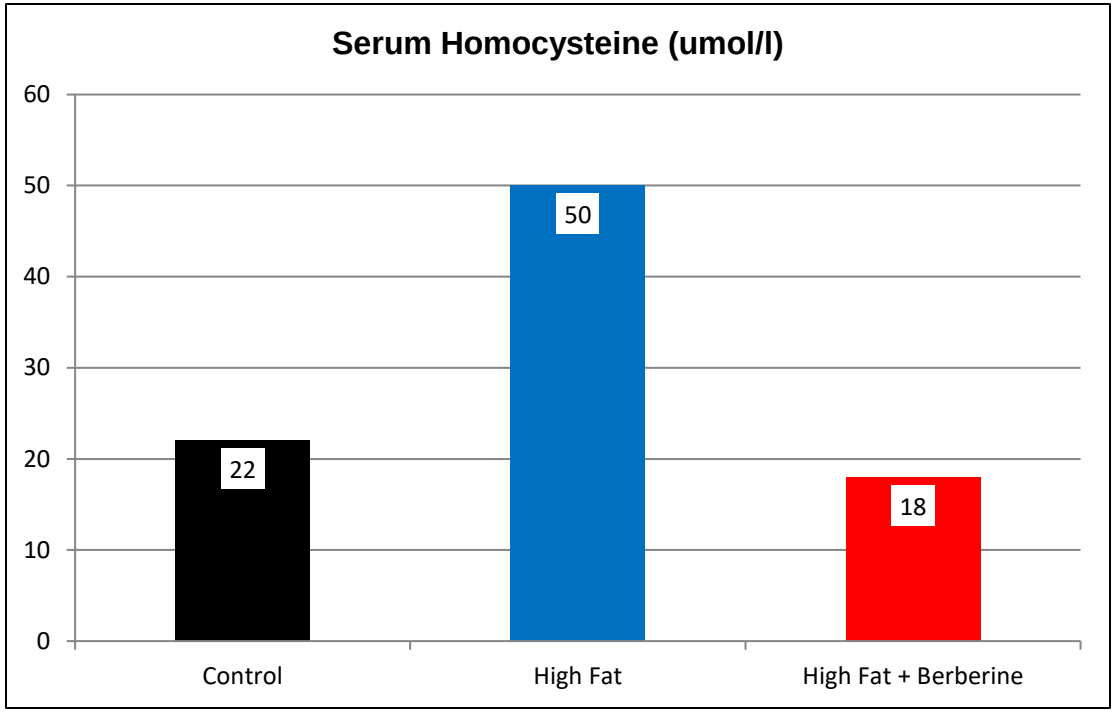
High fat rats: Free access to high fat chow and water
(51% fat, 33% carb, and 16% protein)

Berberine rats :

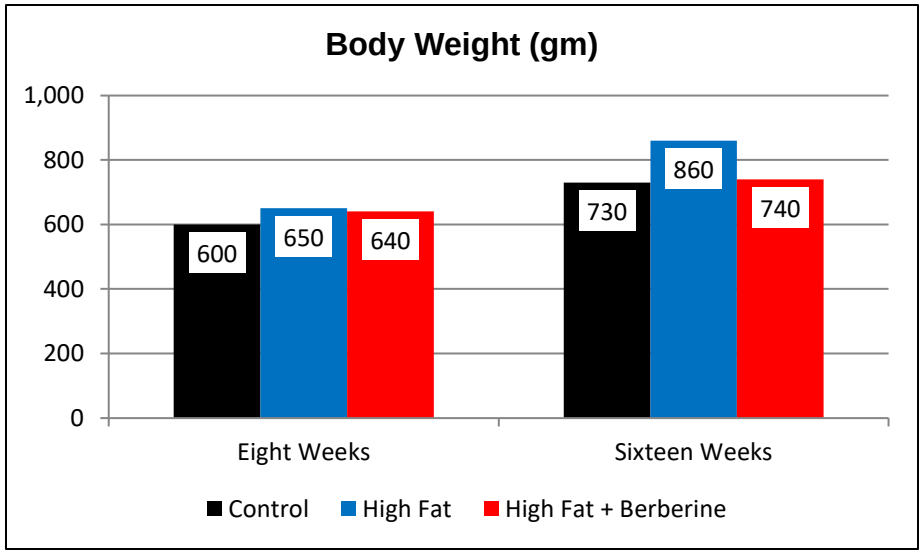
- High fat diet over 12 weeks
- At 8 weeks add Berberine 200 mg/kg/day to high fat diet

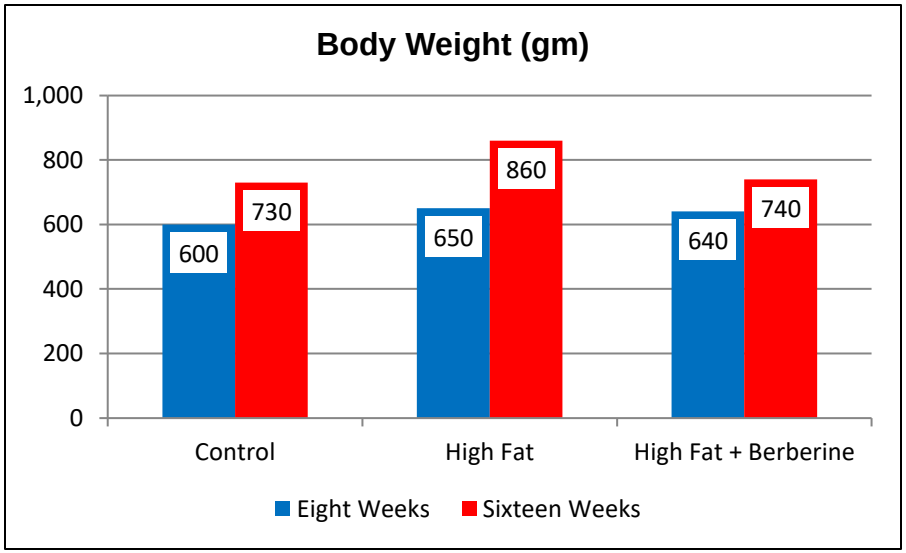
Sacrifice all and evaluate liver status and labs at 12 weeks

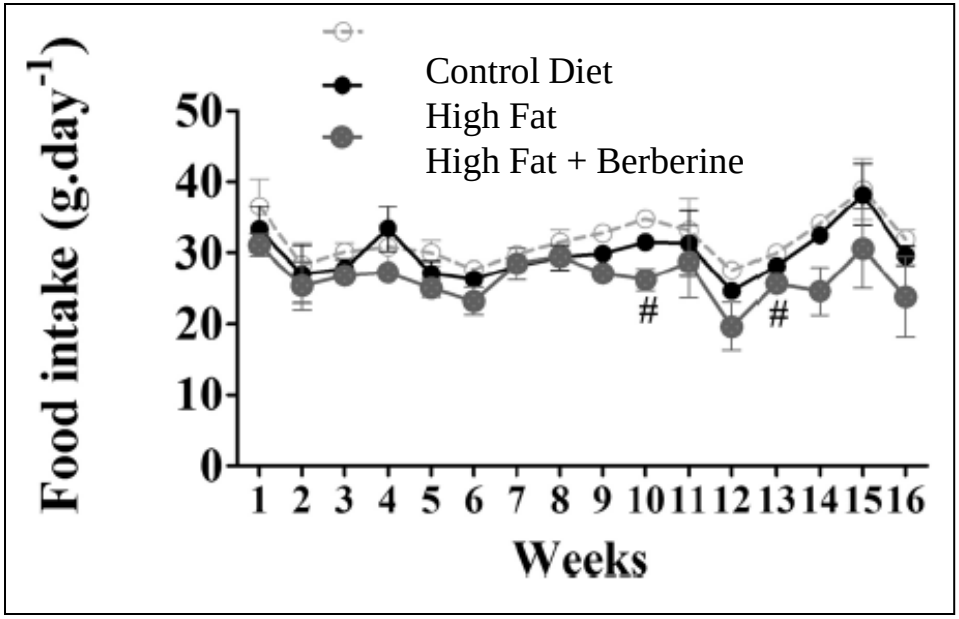
BERBERINE, HOMOCYSTEINE, and CHOLESTEROL



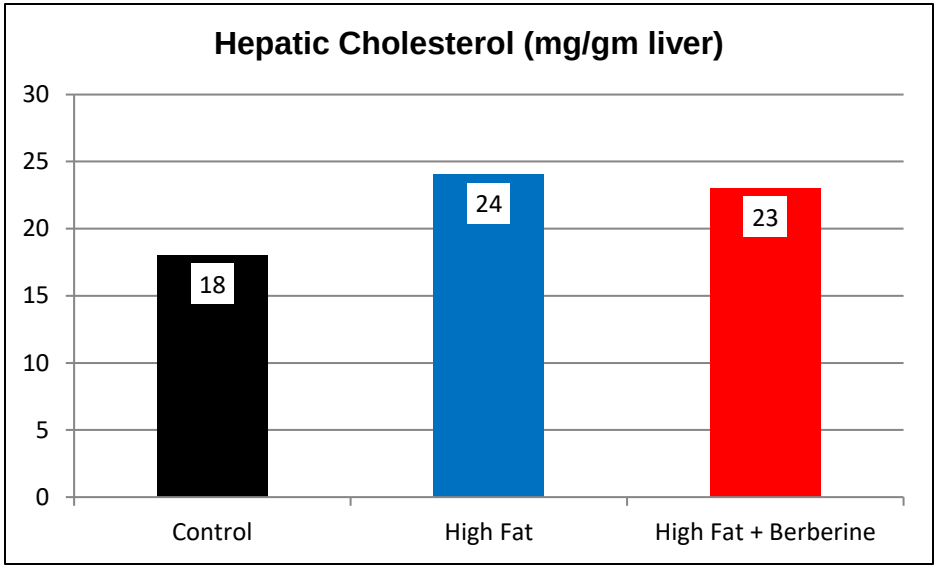
BERBERINE, HOMOCYSTEINE, and CHOLESTEROL

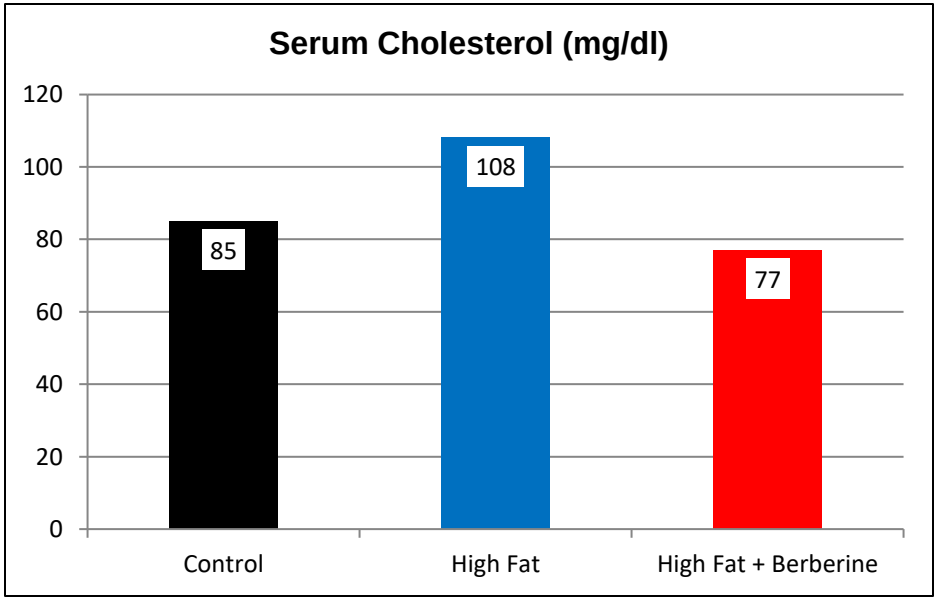




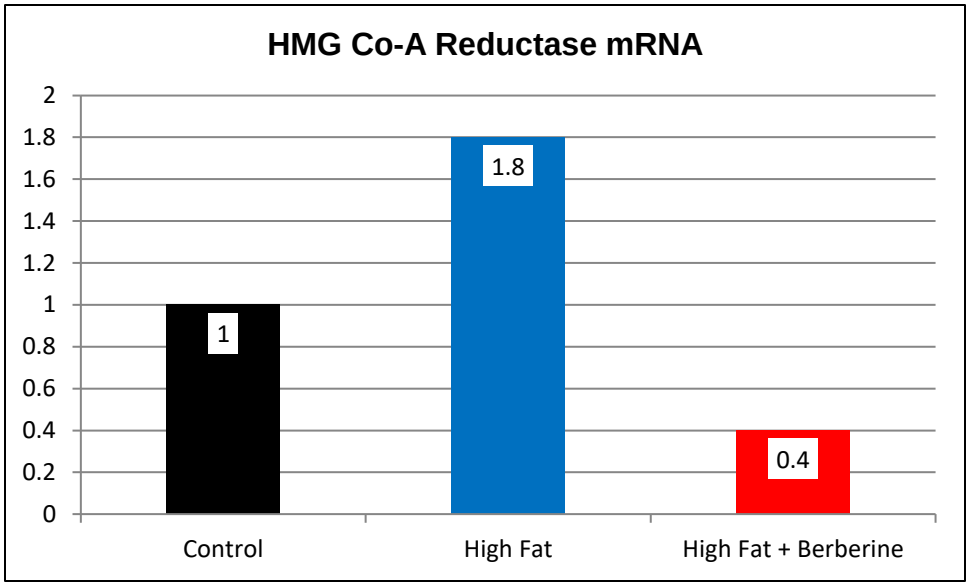


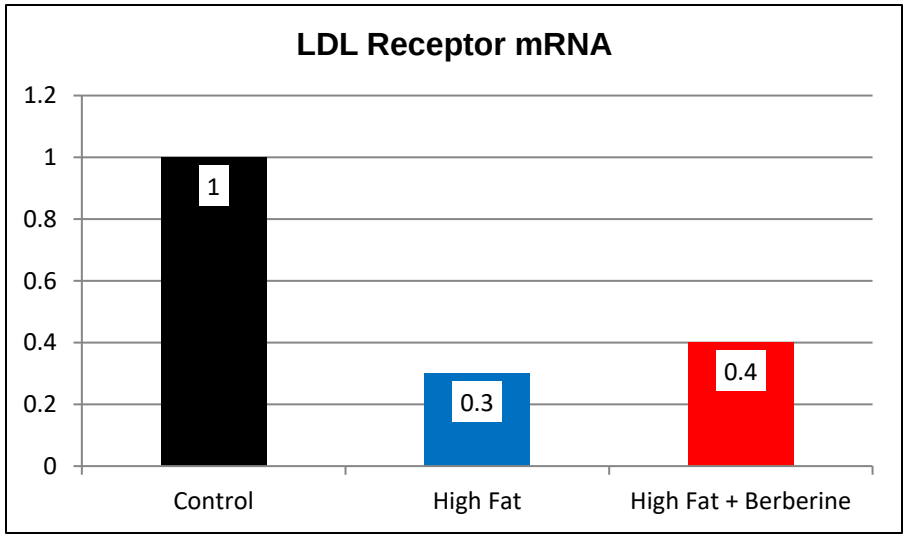
BERBERINE, HOMOCYSTEINE, and CHOLESTEROL

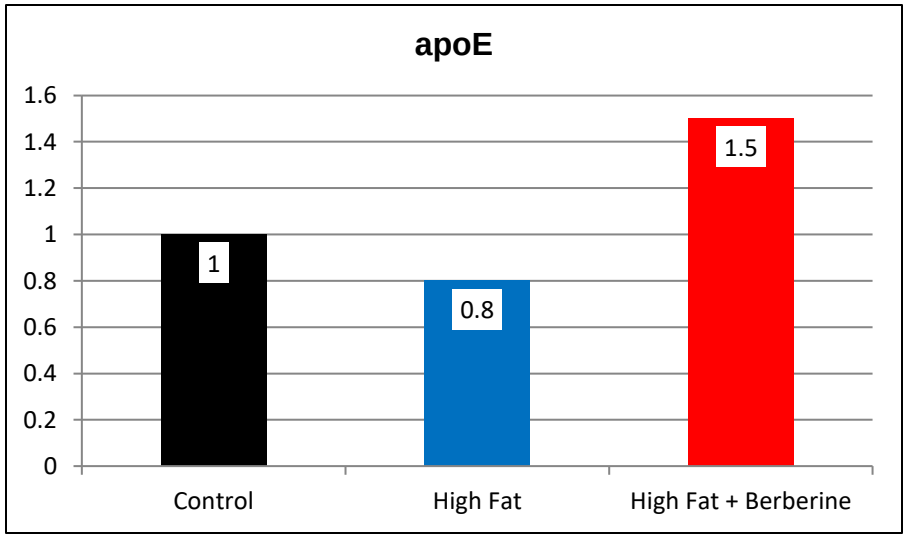




BERBERINE, HOMOCYSTEINE, and CHOLESTEROL







BERBERINE and HOMOCYSTEINE

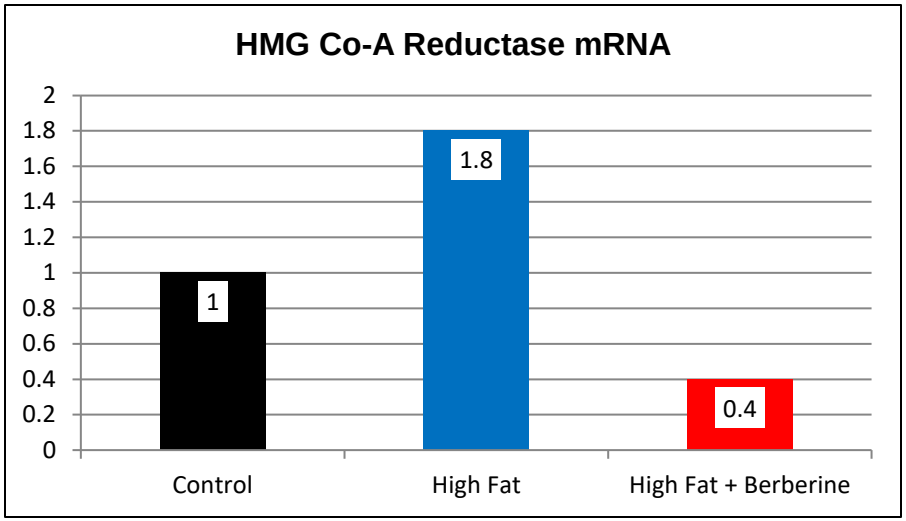
High Fat Diet Increases:

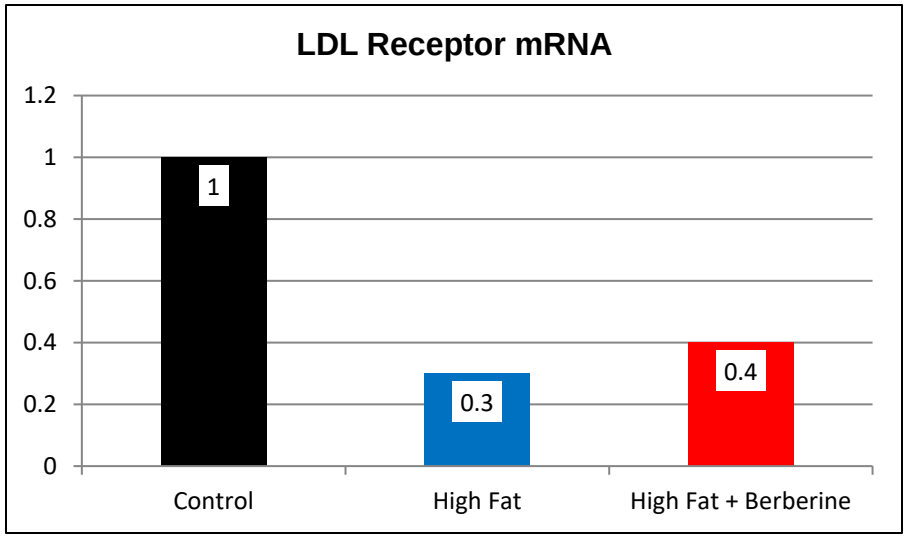
- Homocysteine
- Body weight
- Cholesterol generation

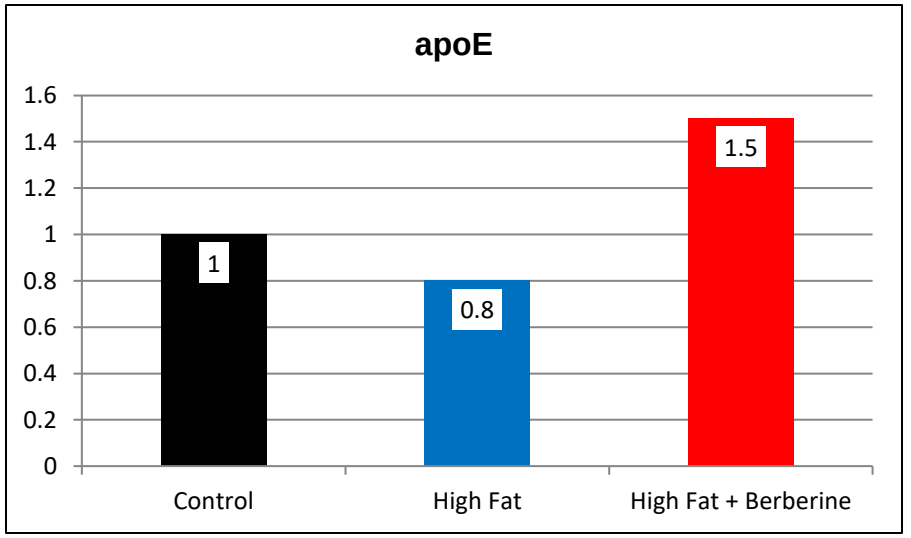
High Fat Diet Decreases:

- LDL R expression
- apoE

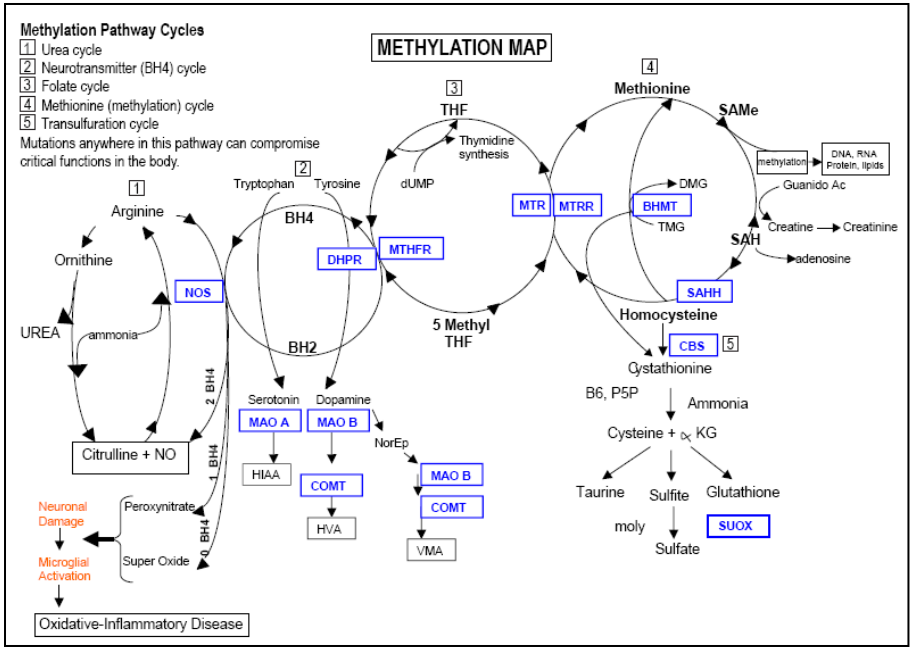
Adverse effects neutralized by Berberine







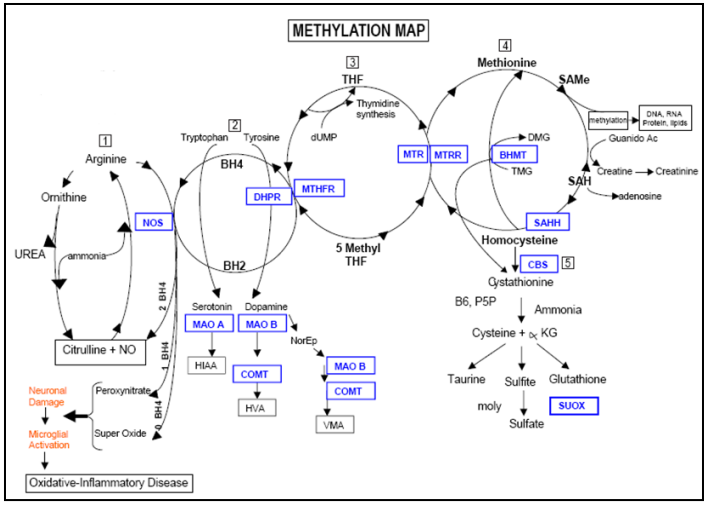
FUNCTIONS of the METHYL CYCLE



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

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

METHYL CYCLE KEY POINTS

Be suspicious of Methyl Cycle Defects

Your sickest patients likely harbor a CBS Up Regulation

Most of us need Methyl-Folate and Methyl-B12

B Vitamin Sensitivity → Consider CBS and/or COMT abnormalities

Pertinent Testing :

- DNA testing ($550 with or $98 without interpretation)
- Support website for hard copies and brief SNIP interpretation
- Nutritional assessment (organic acids and nutritional minerals)
- Methyl Cycle intermediates: SAMe, SAH, THF, methyl-folate, folinic acid, methionine, cystathionine, cysteine, & glutathione
- Toxic burden assessment

Go out and help people who you couldn't help before!

REVIEW of the METHYL CYCLE