EPIGENOMIC MODIFICATION
OVERVIEW of the METHYL CYCLE
TRANSCRIPTION and TRANSLATION

DNA is rewound
Coding strand
Template strand
DNA is unwound
Matching nucleotide is added
RNA strand created
RNA DNA hybrid region
NTPs

TRANSCRIPTION FACTOR

REGULATE

GENE

ACAGTGA

BINDING SITE

PROTEIN

TRANSCRIPTION

Pre-mRNA
mRNA

RNA processing

DNA polymerase

RNA polymerase

Ribosome

Translation

Polypeptide
mRNA
TRANSCRIPTION FACTOR and PROMOTERS

Sterol Regulatory Element Binding Protein

Nuclear Factor Kappa Beta
Activator Protein-1
Nrf2
## CHANGING EXPRESSION of the HUMAN TEMPLATE

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Process</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instantaneous</td>
<td>Post-Translational Modification</td>
<td>pAMPK → pHMG CoA-Reductase</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Histone Modification</td>
<td>Turmeric Anti-Inflammation</td>
</tr>
<tr>
<td>Lifetime</td>
<td>DNA Methylation</td>
<td>Silencing Oncogenes</td>
</tr>
<tr>
<td>Generational</td>
<td>SNIP Experimentation</td>
<td>MTHFR</td>
</tr>
</tbody>
</table>

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 → imparts (-) charge

Negatively charged histones collapse onto (+) mCpG containing segments of DNA
⇒ Gene Silencing (DNA Polymerase cannot bind promoters)
⇒ 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:

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

Ten eleven translocation enzymes (TETs)

- Oxidize 5mC $\rightarrow$ 5-OHmethylcytosine (5hnC) $\rightarrow$ 5-formylcytosine $\rightarrow$ 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 → 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

⇒ 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 →
• 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
Cancer and age-related illness associated with:
- DNA global hypomethylation $\rightarrow$ Activation of oncogenes
- Promoter hypermethylation $\rightarrow$ 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

<table>
<thead>
<tr>
<th>Diet Component</th>
<th>Methyl Support</th>
<th>High Methyl Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline (gm/kg)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Betaine (gm/kg)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Folic acid (mg/kg)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>B12 (mg/kg)</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Methionine (g/kg)</td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>-</td>
<td>150</td>
</tr>
</tbody>
</table>

Evaluate Avy/a offspring for Agouti phenotype
AGOUTI MOUSE

Coat Color (Y0 = Dark, Y5 = Yellow)

- Standard
- Methy Diet
- High Methyl

% 5-MC Hael LTR

Percent Agouti Coat

Yellow, Slightly mottled, Mottled, Heavily mottled, Pseudo-agouti
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

Coat Color

- BPA: Brown 10%, Yellow 21%
- Control: Brown 18%, Yellow 10%

Diagram showing the proportion of brown and yellow coat colors in BPA and control groups.
ORGANIC POLLUTANTS and AGOUTI EXPRESSION

Coat Color

- BPA: 10% Brown, 21% Yellow
- BPA + Methyl Donors: 17% Brown, 12% Yellow
- BPA + Genistein: 17% Brown, 12% Yellow

Bar charts showing the percentage of brown and yellow coat colors under different conditions.
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 $\rightarrow$ altered expression of multiple genes

Methylation (of CpG islands within) ER gene promoter $\rightarrow$ inactivates ER receptor

Estrogen ligation of promoter methylated ER $\rightarrow$ physiologic effect attenuated/lost

ER receptor present in arterial wall
  - ER activity less in vessels with atherosclerosis

ER receptor negative breast cancer $\approx$ 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

% ER Methylation

HAEC

E1  E2  E3  E4  E5  E6

3.1 kb
1.9 kb
1.2 kb

HASMC

M1  M2  M3  M4

55  99  63  19

Arrowhead
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

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (nmol/l)</td>
<td>10.8</td>
<td>11.8</td>
</tr>
<tr>
<td>B12 (pmol/l)</td>
<td>420</td>
<td>395</td>
</tr>
<tr>
<td>B6 (nmol/l)</td>
<td>54</td>
<td>33</td>
</tr>
</tbody>
</table>

### Homocysteine (umol/l)

<table>
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<tr>
<th></th>
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<th>ERT</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.1</td>
<td></td>
</tr>
</tbody>
</table>

### DNA Methylation (Mononuclear Cells)

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<tr>
<th></th>
<th>Placebo</th>
<th>ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

![Bar chart showing homocysteine levels](chart1.png)

![Bar chart showing DNA methylation levels](chart2.png)
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

**DNA Methylation - Females (%)**

<table>
<thead>
<tr>
<th>Brain Hg (ppm, quartile ranges)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.24</td>
<td>57%</td>
</tr>
<tr>
<td>0.24 - 0.34</td>
<td>59%</td>
</tr>
<tr>
<td>0.35 - 0.52</td>
<td>60%</td>
</tr>
<tr>
<td>&gt; 0.52</td>
<td>58%</td>
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**DNA Methylation - Males (%)**

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<td>0.24 - 0.34</td>
<td>59%</td>
</tr>
<tr>
<td>0.35 - 0.52</td>
<td>56%</td>
</tr>
<tr>
<td>&gt; 0.52</td>
<td>54%</td>
</tr>
</tbody>
</table>
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

Plasma Folate (mcg/l)

- Placebo: Baseline 8.2, Ten Weeks 7.3
- Folic Acid: Baseline 7.4, Ten Weeks 13.5

Red Cell Folate (mcg/l)

- Placebo: Baseline 316, Ten Weeks 305
- Folic Acid: Baseline 282, Ten Weeks 443

Homocysteine (umol/l)

- Placebo: Baseline 11.5, Ten Weeks 12.3
- Folic Acid: Baseline 12.2, Ten Weeks 10.7
FOLIC ACID and DNA METHYLATION

**Methyl* Uptake by WBC DNA**

- **Placebo**
  - Baseline: 678
  - Ten Weeks: 655

- **Folic Acid**
  - Baseline: 748
  - Ten Weeks: 515

**Methyl* Uptake by Colonic DNA**

- **Placebo**
  - Baseline: 473
  - Ten Weeks: 458

- **Folic Acid**
  - Baseline: 602
  - Ten Weeks: 451
### SAMe METHYL TRANSFER REACTIONS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate and Effect</th>
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<tbody>
<tr>
<td>DNA Methyl Transferases</td>
<td>Alters DNA Transcription (Bookmarking)</td>
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<tr>
<td>Synthetic Reactions</td>
<td>Generation of Carnitine</td>
</tr>
<tr>
<td>Protein Methyl Transferases (PRMT)</td>
<td>Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)</td>
</tr>
<tr>
<td>Catechol-(O)-Methyl Transferase</td>
<td>Inactivates Catecholamines</td>
</tr>
<tr>
<td>COMT</td>
<td>Methylates 2-OH and 4-OH Estrogens</td>
</tr>
<tr>
<td>PEMT</td>
<td>Metabolizes Bioflavonoids</td>
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<tr>
<td>Phosphatidylethanolamine N-Methyl Transferase</td>
<td>Generation of Phosphatidylcholine</td>
</tr>
<tr>
<td>GAMT</td>
<td>Generation of Creatine</td>
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<tr>
<td>GNMT</td>
<td>SAMe → 5,10-MethyleneTHF</td>
</tr>
</tbody>
</table>
GLYCINE N-METHYL TRANSFERASE

\[
\text{SAMe} + \text{Glycine} \rightarrow \text{Sarcosine (Methylglycine)} + \text{SAH}\]

\[
\text{GNMT}
\]

\[
\text{Sarcosine} + \text{THF} \rightarrow \text{Glycine} + \text{5,10-Methylene THF}
\]

\[
\text{Sarcosine Dehydrogenase}
\]

\[
\text{SAMe blow off valve} \Rightarrow \text{Shuttles CH}_3 \text{ away from SAMe and towards Methyl-folate/DNA synthesis}
\]
GLYCINE N-METHYL TRANSFERASE

Not directly stimulated by high SAMe

Negatively regulated (inhibited) by Methyl-folate

High SAMe inhibits MTHFR → Low Methyl-folate → Disinhibiton of GNMT

⇒ Shift from SAMe reformation to 5,10-Methylened THF
## SAMe METHYL TRANSFER REACTIONS

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<td>Metabolizes Bioflavonoids</td>
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<tr>
<td>PEMT Phosphatidylethanolamine N-Methyl Transferase</td>
<td>Generation of Phosphatidylcholine</td>
</tr>
<tr>
<td>GAMT Guanidinoacetate N-Methyl Transferase</td>
<td>Generation of Creatine</td>
</tr>
<tr>
<td>GNMT Glycine-N-Methyl Transferase</td>
<td>SAMe → 5,10-MethyleneTHF</td>
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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...

\[
\text{ATP} \rightarrow \text{Work} + \text{ADP} + \text{P}_i \rightarrow \text{ADP} + \text{P}_i + \text{energy} \rightarrow \text{More ATP}
\]

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

\[
\text{ATP} \rightarrow \text{Work} + \text{ADP} + \text{P}_i \rightarrow \text{ADP} + \text{P}_i + \text{no energy} \rightarrow \text{no more ATP}
\]

\[
\text{PCr} + \text{ADP} \rightarrow \text{Cr} + \text{ATP}
\]
\[
\text{ADP} + \text{ADP} \rightarrow \text{ATP} + \text{AMP}
\]
\[
\text{AMP} \rightarrow \text{Adenosine} + \text{Pi}
\]

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

**Plasma Creatine (mM)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Creatine (mM)</td>
<td>0.06</td>
<td>0.35</td>
<td>0.31</td>
</tr>
</tbody>
</table>

**Muscle Creatine Metabolites (umol/g muscle)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td>9.6</td>
<td>13.3</td>
<td>14</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>12.2</td>
<td>13.1</td>
<td>15.2</td>
</tr>
<tr>
<td>Total Creatine</td>
<td>21.8</td>
<td>26.4</td>
<td>29.2</td>
</tr>
</tbody>
</table>

**Muscle Adenine Nucleotides (umol/g muscle)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>5.5</td>
<td>5.4</td>
<td>6.2</td>
</tr>
<tr>
<td>ADP</td>
<td>0.96</td>
<td>0.9</td>
<td>0.96</td>
</tr>
</tbody>
</table>
CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

![Graph showing serum homocysteine levels for control, GAA, and Creatine.](image)

AGAT        GAA   GAMT → CREATINE and SAH ⇒ Hcy
CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

Cystathionine β-Synthase (CBS) Expression

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression</td>
<td>5.4</td>
<td>7.2</td>
<td>3.8</td>
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</tbody>
</table>

Methionine Synthase (MTR) Expression

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression</td>
<td>0.23</td>
<td>0.31</td>
<td>0.23</td>
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</tbody>
</table>

MTHFR Expression

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GAA</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression</td>
<td>0.28</td>
<td>0.24</td>
<td>0.35</td>
</tr>
</tbody>
</table>

SAMe → GNMT → Sarcosine → 5,10MeTHF
CREATINE vs. GUANIDINOACETATE and Hcy METABOLISM

♥ Incubate hepatocytes with Methionine +/- other substances

**Homocysteine Export with Methionine Incubation (units)**

![Graph showing homocysteine export with methionine incubation](image)

- **Control**: 2.2 units
- **GAA**: 3.2 units
- **Creatine**: 2.1 units
- **Serine**: 1.3 units
- **GAA + Serine**: 1.6 units

**Diagram showing blood-liver metabolism pathways**
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

MTHFR Genotype

Homocysteine (umol/l)

↓ 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

Contractions at 75% Maximal

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day Six</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Placebo</td>
<td>12</td>
<td>13.5</td>
</tr>
</tbody>
</table>

$P = 0.025$ (Creatine vs. Placebo)

$P = ns$ (Placebo pre vs. post)

$P = ns$ (Creatine pre vs. post)
CREATINE and CONGESTIVE HEART FAILURE

Lactate Production/Contraction at 75% Maximal

Ammonia Production/Contraction at 75% Max
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

**Cholesterol (mg/dl)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>245</td>
<td>250</td>
</tr>
<tr>
<td>Week Four</td>
<td>250</td>
<td>240</td>
</tr>
<tr>
<td>Week Eight</td>
<td>265</td>
<td>235</td>
</tr>
<tr>
<td>Week Twelve</td>
<td>265</td>
<td>250</td>
</tr>
</tbody>
</table>

**Triglycerides (mg/dl)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>160</td>
<td>195</td>
</tr>
<tr>
<td>Week Four</td>
<td>165</td>
<td>145</td>
</tr>
<tr>
<td>Week Eight</td>
<td>165</td>
<td>150</td>
</tr>
<tr>
<td>Week Twelve</td>
<td>175</td>
<td>140</td>
</tr>
</tbody>
</table>
• 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
# SAMe METHYL TRANSFER REACTIONS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate and Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Methyl Transferases</td>
<td>Alters DNA Transcription (Bookmarking)</td>
</tr>
<tr>
<td>Synthetic Reactions</td>
<td>Generation of Carnitine</td>
</tr>
<tr>
<td>Protein Methyl Transferases (PRMT)</td>
<td>Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)</td>
</tr>
<tr>
<td>Catechol-(O)-Methyl Transferase</td>
<td>Inactivates Catecholamines</td>
</tr>
<tr>
<td>COMT</td>
<td>Methylates 2-OH and 4-OH Estrogens</td>
</tr>
<tr>
<td>PEMT</td>
<td>Metabolizes Bioflavonoids</td>
</tr>
<tr>
<td>Phosphatidylethanolamine N-Methyl Transferase</td>
<td>Generation of Phosphatidylcholine</td>
</tr>
<tr>
<td>GAMT</td>
<td>Generation of Creatine</td>
</tr>
<tr>
<td>Guanidinacetate N-Methyl Transferase</td>
<td></td>
</tr>
<tr>
<td>GNMT</td>
<td>SAMe → 5,10-MethyleneTHF</td>
</tr>
</tbody>
</table>
PHOSPHATIDYL ETHANOLAMINE N-METHYL TRANSFERASE

PEMT

Phosphoethanolamine + 3 SAMes → Phosphatidylcholine + 3 SAHs

Phosphatidylcholine:
- Cell membrane
- Lipid metabolism
- Acetylcholine
Phosphatidyl ethanolamine N-methyl transferase

Phosphatidylcholine $\rightarrow$ 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

**Fasting Homocysteine (umol/l)**

- **Placebo**: Baseline - 16.5; Two Weeks - 16.6
- **PPC**: Baseline - 15.6; Two Weeks - 13.6

**Homocysteine (6 Hr Post-Methionine)**

- **Placebo**: First Day - 31.8; Two Weeks - 31.6
- **PPC**: First Day - 27; Two Weeks - 22.3
Trans-Sulfuration Pathway Gatekeeper
CYSTATHIONINE BETA SYNTHASE (CBS)

Serine + Homocysteine $\rightarrow$ Cystathionine

$\rightarrow$ P-5-P

Cystathionine $\rightarrow$ Cysteine + Ammonia

Cystathionine Gamma Lyase

Down stream production of:
- Cysteine and Glutathione
- Taurine and Sulfate
- Hydrogen sulfide
- Alpha-ketobutyrate
CYSTATHIONINE BETA SYNTTHASE

Serine + Homocysteine $\rightarrow$ Cystathionine P-5-P

Cystathionine $\rightarrow$ Cysteine + Ammonia

Cystathionine Gamma Lyase

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 $\rightarrow$ Cystathionine

P-5-P

Cystathionine $\rightarrow$ Cysteine + Ammonia

Cystathionine Gamma Lyase

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)

BH₄ Depletion

Endothelial dysfunction, oxidative stress, impaired neurotransmitter formation
Interconversion compromised by metals (Lead)

Result is Glutamate Excess

→ MSG-like Excitotoxicity
SULFITE OXIDASE

Sulfite $+$ H$_2$O $\rightarrow$ Sulfate

Down regulation not common $\rightarrow$ Sulfite excess

Co-factor depletion common $\rightarrow$ 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 uptake across cell membrane →

Impaired endogenous detoxification

Dr. Yasko found

Strong link between

CBS and Autism

and related

Neurodevelopmental Disorders
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 $\rightarrow$ 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

**Fasting Homocysteine (umol/l)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Two Weeks</th>
<th>Six Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>10.2</td>
<td>10.7</td>
<td>10.7</td>
</tr>
<tr>
<td>1500 mg</td>
<td>10.9</td>
<td>9.7</td>
<td>9.5</td>
</tr>
<tr>
<td>3000 mg</td>
<td>10.7</td>
<td>9.6</td>
<td>8.8</td>
</tr>
<tr>
<td>6000 mg</td>
<td>11.0</td>
<td>8.8</td>
<td>9.3</td>
</tr>
</tbody>
</table>

**Homocysteine Decrease vs. Placebo (umol/l)**

<table>
<thead>
<tr>
<th></th>
<th>Two Weeks</th>
<th>Six Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500 mg</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>3000 mg</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>6000 mg</td>
<td>2.9</td>
<td>2.2</td>
</tr>
</tbody>
</table>
BETAINE and HOMOCYSTEINE

**Homocysteine (6 Hr Post-Methionine)**

- **Baseline**: Placebo 29, 1500 mg 29.4, 3000 mg 28.5, 6000 mg 29.9
- **First Dose**: Placebo 26.9, 1500 mg 22, 3000 mg 18.5, 6000 mg 20.1
- **Two Weeks**: Placebo 29.9, 1500 mg 19.9, 3000 mg 15.9, 6000 mg 19.6
- **Six Weeks**: Placebo 28, 1500 mg 19.6, 3000 mg 16.6, 6000 mg 16.6

**Methionine at Six Hours Post-Load**

- **Baselines**: Placebo 502, 1500 mg 495, 3000 mg 519, 6000 mg 541
- **Six Weeks**: Placebo 505, 1500 mg 470, 3000 mg 527, 6000 mg 471

Bar charts show changes in homocysteine and methionine levels over time with different doses of betaine.
TMG in ALCOHOLIC LIVER DISEASE

♥ Male Sprague-Dawley rats (180 gm)

Treat over four weeks with:
- Standard chow and water
- Standard chow with 0.5% betaine
- High ethanol diet
- Ethanol diet with 0.5% betaine

Sacrifice and evaluate liver morphology and Methyl Cycle parameters
TMG in ALCOHOLIC LIVER DISEASE

### Body Weight (gm)

- **Control**: 184, Baseline; 220, Four Weeks
- **Control + TMG**: 182, Baseline; 222, Four Weeks
- **Alcohol**: 188, Baseline; 163, Four Weeks
- **Alcohol + TMG**: 183, Baseline; 181, Four Weeks

### Liver Weight (gm/100 gm body wgt.)

- **Control**: 2.8
- **Control + TMG**: 3.1
- **Alcohol**: 4.3
- **Alcohol + TMG**: 4.2
TMG in ALCOHOLIC LIVER DISEASE

Liver Methionine Synthase (MTR) Activity

Liver BHMT Activity

Liver Betaine (umol/liver tissue)

Liver SAMe (nmol/liver tissue)
TMG in ALCOHOLIC LIVER DISEASE

Histology – TMG protected vs. fatty liver
5,10-METHYLENE TETRAHYDROFOLATE REDUCTASE (A1298C)

Compromises “backward” conversion of

5-Methyl Folate (5-Methyl THF) → 5,10-Methylene Tetrahydrofolate
MTHFR
5-Methyl Folate + BH2 → 5,10-Methylene THF + BH4

MTHFR A1298C aggravates CBS up regulation induced BH4 depletion
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
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
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

Assess efficacy with 24 hour urine for ammonia and taurine

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 +/-)
<table>
<thead>
<tr>
<th>CBS MANAGEMENT</th>
</tr>
</thead>
</table>

**Supplements High In Sulfur**

<table>
<thead>
<tr>
<th>Taurine</th>
<th>Cysteine</th>
<th>Methionine</th>
<th>Glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine Sulfate</td>
<td>Cysteine</td>
<td>Methionine</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Epsom Salts</td>
<td>Magnesium Sulfate Cream</td>
<td>Glutathione</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Canned meats, Aged game</td>
<td>Homemade yeast breads</td>
<td>Glutathione</td>
<td>Glutathione</td>
</tr>
<tr>
<td>DMSA and DMPS (Metal Chelators)</td>
<td>Milk thistle, Beyond C, and Heparin</td>
<td>Glutathione</td>
<td>Glutathione</td>
</tr>
</tbody>
</table>

**Foods High In Sulfur**

<table>
<thead>
<tr>
<th>Vegetables:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic, Onion Family</td>
<td>Kale</td>
<td>Collards</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Brussel Sprouts</td>
<td>Kohlrabi</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Bok Choy</td>
<td>Mizuna</td>
</tr>
<tr>
<td>Chinese Cabbage</td>
<td>Napa Cabbage</td>
<td>Turnip / Rutabaga</td>
</tr>
<tr>
<td>Mustard Seeds</td>
<td>Tatsoi</td>
<td>Radish</td>
</tr>
<tr>
<td>Horseradish</td>
<td>Japanese Horseradish</td>
<td>Arugula</td>
</tr>
<tr>
<td>Peas</td>
<td>Spinach</td>
<td></td>
</tr>
</tbody>
</table>

**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)
### Sources of Excitotoxins

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Glutamic acid, glutamine, and MSG. High levels are found in foods such as peas, tomatoes, parmesan cheese, milk, mushrooms, fish, and many vegetables</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>Aspartame, NutraSweet</td>
<td></td>
</tr>
</tbody>
</table>

### Other “Names” for Excitotoxins

<table>
<thead>
<tr>
<th>Monosodium Glutamate</th>
<th>Glutamate</th>
<th>Natural Flavor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>Carrageenan</td>
<td>Gelatin</td>
</tr>
<tr>
<td>Seasoning(s)</td>
<td>Seasoned Salt</td>
<td>Dough Conditioner(s) Isolate</td>
</tr>
<tr>
<td>Autolyzed Yeast</td>
<td>Autolyzed Yeast Extract</td>
<td>Autolyzed Anything</td>
</tr>
<tr>
<td>Stock</td>
<td>Soup Base</td>
<td>Chicken/Pork/Beef “Flavoring”</td>
</tr>
<tr>
<td>Hydrolyzed Vegetable Protein (HPV)</td>
<td>Hydrolyzed Plant Protein</td>
<td>Hydrolyzed Anything</td>
</tr>
<tr>
<td>Hydrolyzed Oat Flour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Caseinate</td>
<td>Calcium Caseinate</td>
<td>Caseinate</td>
</tr>
<tr>
<td>Disodium Inosinate</td>
<td>Disodium Caseinate</td>
<td>Hydrolyzed Protein</td>
</tr>
<tr>
<td>Bouillon</td>
<td>Vegetable Gum</td>
<td>Plant Protein Extract</td>
</tr>
<tr>
<td>Malted Barley Flour</td>
<td>Malt Extract</td>
<td>Malt Flavoring(s)</td>
</tr>
<tr>
<td>Malted Anything</td>
<td>Textured Protein</td>
<td>Guar Gum</td>
</tr>
<tr>
<td>Soy Protein</td>
<td>Soy Protein Concentrate</td>
<td>Soy Sauce</td>
</tr>
<tr>
<td>Whey Protein Isolate</td>
<td>Whey Protein Concentrate</td>
<td></td>
</tr>
<tr>
<td>Ajinomoto</td>
<td>Kombu Extract</td>
<td>Natural Flavoring(s)</td>
</tr>
</tbody>
</table>

### Foods with MSG (Monosodium Glutamate)

<table>
<thead>
<tr>
<th>Hydrolyzed Protein</th>
<th>Hydrolyzed Oat Flour</th>
<th>Sodium Caseinate / Calcium Caseinate</th>
<th>Monosodium Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>Glutamic Acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Possible Sources of MSG

<table>
<thead>
<tr>
<th>Textured Protein</th>
<th>Carrageenan Or Vegetable Gum</th>
<th>Seasonings Or Spices</th>
<th>Flavorings Or Natural Flavorings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken, Beef, Pork, Smoke Flavorings</td>
<td>Bouillon, Broth, Or Stock</td>
<td>Barley Malt, Malt Extract, Malt Flavoring</td>
<td>Whey Protein, Whey Protein Isolate, Or Concentrate</td>
</tr>
</tbody>
</table>
### Other Sources of MSG

<table>
<thead>
<tr>
<th>Foods From Fast-Food Chains</th>
<th>OTC Medications</th>
<th>Chicken Pox Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NutraSweet</td>
<td>Binders and Fillers in Supplements</td>
<td>Prescription Medications</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Foods with Glutamates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doritos</td>
</tr>
<tr>
<td>Progresso Soups</td>
</tr>
<tr>
<td>Sausages / Processed Meats / Cold Cuts</td>
</tr>
<tr>
<td>Restaurant Gravy</td>
</tr>
<tr>
<td>Salad Dressings / Cretons</td>
</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Dry Milk Or Whey Powder</td>
</tr>
<tr>
<td>Fresh Produce Sprayed With</td>
</tr>
<tr>
<td>Avocados In The Field</td>
</tr>
<tr>
<td>Non-Dairy Creamers</td>
</tr>
<tr>
<td>Baked Goods From Bakeries</td>
</tr>
<tr>
<td>Chili Sauce</td>
</tr>
<tr>
<td>Citric Acid Made From Processed Corn</td>
</tr>
<tr>
<td>Fresh And Frozen Pizza</td>
</tr>
<tr>
<td>Tomato Sauce / Stewed Tomatoes</td>
</tr>
<tr>
<td>Tofu And Other Fermented Soy Products</td>
</tr>
<tr>
<td>Anything Fermented</td>
</tr>
<tr>
<td>Anything Ultra-Pasteurized</td>
</tr>
<tr>
<td>Flowing Agents</td>
</tr>
</tbody>
</table>
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 ≠ 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 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:

![Graph showing hepatic homocysteine levels](image)
DANSHENsu STIMULATES CBS

**Hepatic Cysteine (nmol/g)**

- Control: 66
- Danshensu: 97
- Methionine: 136
- Methionine + Danshensu: 164

**Hepatic Glutathione (nmol/g)**

- Control: 7
- Danshensu: 8.4
- Methionine: 7.6
- Methionine + Danshensu: 8.9
DANSHENSU STIMULATES CBS

**SAM (mcg/g)**

- Control: 22
- Danshensu: 18
- Methionine: 56
- Methionine + Danshensu: 86

**SAH (mcg/g)**

- Control: 5
- Danshensu: 9
- Methionine: 12
- Methionine + Danshensu: 9

**SAM:SAH**

- Control: 4.1
- Danshensu: 2.2
- Methionine: 4.7
- Methionine + Danshensu: 9.5
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

![Graphs showing changes in plasma and liver Hcy levels across different diets and time points.](image-url)
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

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

![Graph showing plasma total homocysteine concentration over time for Met, MetSer, MetCys, and Protein.](image)

**Peak Homocysteine (umol/l)**

- Met: 17.9
- MetSer: 14.3
- MetCys: 14.8
- Protein: 11.2

**Time to Peak Homocysteine (Hrs.)**

- Met: 4.8
- MetSer: 6.2
- MetCys: 5.9
- Protein: 7.4

**AUC Homocysteine (units)**

- Met: 105
- MetSer: 66
- MetCys: 71
- Protein: 24
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
Brachial Artery Flow Mediated Vasodilation (%)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Eight Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5.8%</td>
<td>6.1%</td>
</tr>
<tr>
<td>Folate</td>
<td>5.3%</td>
<td>6.0%</td>
</tr>
<tr>
<td>NAC</td>
<td>6.0%</td>
<td>10.4%</td>
</tr>
</tbody>
</table>

Homocysteine (umol/l)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Eight Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>18.4</td>
<td>20.7</td>
</tr>
<tr>
<td>Folate</td>
<td>21.7</td>
<td>12.5</td>
</tr>
<tr>
<td>NAC</td>
<td>20.9</td>
<td>15.6</td>
</tr>
</tbody>
</table>

NAC, FOLATE, HOMOCYSTEINE, and ENDOTHELIAL FN
N-ACETYLCYSTEINE MECHANISM of ACTION

1. 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-ACETYLATED CYSTEINE MECHANISM of ACTION

**Cells**
- Homocysteine
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
- Cystathionine
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C

**Blood**
- Homocysteine
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
- Cystathionine
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C
  - $^{13}$C

**TS**
- Translation

**Hcy bound to protein**
- Measured

**Diagram**
- Protein bound (70–80% of the total)
- Not protein bound (free) (20–30% of the total)
- Reduced (thiol form, 1–2% of the total)
- Oxidized (disulfides and mixed disulfides)
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

Controls                     NAC 900 mg                NAC 1,800 mg

Total Homocysteine (umol/l)

Controls                     NAC 900 mg                NAC 1,800 mg

Baseline                     Four Weeks                  High Dose
N-ACETYLYL CYSTEINE MECHANISM of ACTION

Controls
NAC 900 mg
NAC 1,800 mg

Total Cysteine (umol/l)

Baseline Four Weeks High Dose

Plasma Antioxidant Capacity(nmol/ml)

Baseline Four Weeks High Dose
N-ACETYLY CYSTEINE MECHANISM of ACTION

**Non-Protein Bound Homocysteine (umol/l)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Four Weeks</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3.64</td>
<td>3.95</td>
<td>3.53</td>
</tr>
<tr>
<td>NAC 900 mg</td>
<td>3.24</td>
<td>3.26</td>
<td>3.58</td>
</tr>
<tr>
<td>NAC 1,800 mg</td>
<td>3.35</td>
<td>3.35</td>
<td>3.53</td>
</tr>
</tbody>
</table>

**Percentage Non-Protein Bound**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Four Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC 900 mg</td>
<td>25%</td>
<td>28%</td>
</tr>
<tr>
<td>NAC 1,800 mg</td>
<td>25%</td>
<td>33%</td>
</tr>
</tbody>
</table>
N-ACETYL CYSTEINE MECHANISM of ACTION

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

**Lipid Values (mg/dl)**

- **Cholesterol**: Baseline - 231, Omega-3 - 220, Olive Oil - 228
- **Triglycerides**: Baseline - 423, Omega-3 - 306, Olive Oil - 367
- **Non-HDL Chol**: Baseline - 189, Omega-3 - 181, Olive Oil - 190

**Conjugated Dienes (umol/l)**

- **Baseline**: 54.2
- **Omega-3**: 62.5
- **Olive Oil**: 51.7
FISH OIL and HOMOCYSTEINE CONTROL

**Microalbuminuria (mg/l)**

- Baseline: 27
- Omega-3: 20.5
- Olive Oil: 29.5

**Homocysteine (umol/l)**

- Baseline: 13.8
- Omega-3: 9.8
- Olive Oil: 14.2
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

Folinic acid and Methyl-folate enhances response to SSRIs

Methyl-folate $\Rightarrow$ BH4

Methyl-folate $\Rightarrow$ SAMe

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

![Graph showing plasma homocysteine levels in different groups: Normal controls (n=18), Neurological controls (n=20), Depression (n=46). The graph indicates higher levels of plasma homocysteine in depressed individuals compared to controls.]

**Plasma Homocysteine (umol/l)**

- Controls: 7.6
- Neuro Dz: 6.6
- Depressed: 13.3
- Depressed NI Hcy: 8.2
- Depressed High Hcy: 17.7
FOLATE and CSF NEUROTRANSMITTER LEVELS

Serum B12 (ng/l)

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>Neuro Dz</th>
<th>Depressed</th>
<th>Depressed NI Hcy</th>
<th>Depressed High Hcy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum B12 (ng/l)</td>
<td>344</td>
<td>410</td>
<td>361</td>
<td>392</td>
<td>334</td>
</tr>
</tbody>
</table>

Serum Folate (ug/l)

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>Neuro Dz</th>
<th>Depressed</th>
<th>Depressed NI Hcy</th>
<th>Depressed High Hcy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Folate (ug/l)</td>
<td>7</td>
<td>9.1</td>
<td>5.9</td>
<td>7.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>
FOLATE and CSF NEUROTRANSMITTER LEVELS

![Graph showing RBC Folate levels]
FOLATE and CSF NEUROTRANSMITTER LEVELS

CSF 5-HIAA (nmol/l)

- Neuro Dz: 107
- Depressed: 146
- Depressed NI Hcy: 160
- Depressed High Hcy: 129

CSF HVA (nmol/l)

- Neuro Dz: 233
- Depressed: 211
- Depressed NI Hcy: 251
- Depressed High Hcy: 165

CSF MHPG (nmol/l)

- Neuro Dz: 56.3
- Depressed: 46.2
- Depressed NI Hcy: 48.6
- Depressed High Hcy: 43.5

CSF SAMe (nmol/l)

- Neuro Dz: 163
- Depressed: 146
- Depressed NI Hcy: 160
- Depressed High Hcy: 129
FOLATE and CSF NEUROTRANSMITTER LEVELS

• 1/3\textsuperscript{rd} of severely depressed are folate deficient \(\approx\) Elevated homocysteine

• Lower CSF monoamine metabolites and SAMe
SAMe in TREATMENT RESISTANT DEPRESSION

♥ 30 patients with inadequately controlled depression:
  • HAM-D score of $\geq 14$
  • Adequate and stable dose of SSRI or Venlafaxine
    ♦ Fluoxetine, Paroxetine, Citalopram $\geq 20$ mg
    ♦ Escitalopram $\geq 10$ mg
    ♦ Sertraline $\geq 50$ mg
    ♦ Venlafaxine $\geq 75$ 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 $\leq 7$
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>
<thead>
<tr>
<th>TABLE 1. Common Side Effects of SAMe 800 to 1600 mg/d*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Constipation</td>
</tr>
<tr>
<td>Gastrointestinal upset</td>
</tr>
<tr>
<td>Diarrhea/gas</td>
</tr>
<tr>
<td>Decreased appetite</td>
</tr>
<tr>
<td>Musculoskeletal/Nervous</td>
</tr>
<tr>
<td>Headaches</td>
</tr>
<tr>
<td>Activation (anxiety, irritability)</td>
</tr>
<tr>
<td>Fatigue, sedation</td>
</tr>
<tr>
<td>Sleep disturbance</td>
</tr>
</tbody>
</table>

*Defined as reported by 5% or more of the sample (n = 30).
SAMe in TREATMENT RESISTANT DEPRESSION

### Hamilton Depression Score

- **Baseline**: 17.7
- **Six Weeks**: 9.3

### MGH Sexual Function Scale

- **Baseline**: 22.8
- **Six Weeks**: 20.6

### Montgomery Depression Rating

- **Baseline**: 23.2
- **Six Weeks**: 13.9

### Beck Depression Inventory

- **Baseline**: 18.8
- **Six Weeks**: 12.2
SAMe in TREATMENT RESISTANT DEPRESSION

---

**Response - Remission**

<table>
<thead>
<tr>
<th></th>
<th>Intention to Treat</th>
<th>Completer Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>50%</td>
<td>57%</td>
</tr>
<tr>
<td>Remission</td>
<td>43%</td>
<td>48%</td>
</tr>
</tbody>
</table>
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

Subcut

Oral

Saline
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

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

![Graph showing δ-ALA and Lead levels over time](image)
SAMe in CHRONIC LEAD TOXICITY

65 year old male plumber

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

\[ \Delta-\text{ALA} \quad \text{Lead} \]
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

![Graph showing the relationship between Lead (Pb) and ALA-D (%)]

- **Lead (Pb) (µg/100 ml blood)**
- **ALA-D (%)**

Days after treatment
SAMe in LEAD TOXICITY

Blood Glutathione (mg/100 ml RBC)

- Treatment Days
- Blood Glutathione levels over time for different groups labeled RC, HC, MC, KC, ML.

Diagram showing the metabolism of SAMe, including pathways for methionine, homocysteine, and glutathione synthesis and catabolism.
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

### Total Hepatic Glutathione (umol/g liver)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>Six Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>ALD + Placebo</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>ALD + SAMe</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>NALD + SAMe</td>
<td>2.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

### GSSG to GSH Ratio

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>Six Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>4.4%</td>
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</tr>
<tr>
<td>ALD + Placebo</td>
<td>8.2%</td>
<td>7.2%</td>
</tr>
<tr>
<td>ALD + SAMe</td>
<td>8.8%</td>
<td>7.0%</td>
</tr>
<tr>
<td>NALD + SAMe</td>
<td>9.0%</td>
<td>5.6%</td>
</tr>
</tbody>
</table>
SAMe EFFECT on GLUTATHIONE in LIVER DISEASE

Aspartate Aminotransferase (AST)

Mean Corpuscular Volume (MCV)
ALD SAMe Treated
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

<table>
<thead>
<tr>
<th></th>
<th>SAMe</th>
<th>Celecoxib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>GI</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Anxiety</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
SAMe vs. CELECOXIB in DJD

Pain Rating – Visual Analog Scale (VAS)

[Graph showing pain levels over time for SAMe and COXI (COX Inhibitor) in two phases of the study.]
# METHYL THIEVES and IATROGENIC HHcy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hcy</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrates</td>
<td>↑</td>
<td>Decrease in GFR; depletes TMG</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>↑</td>
<td>Blunts folate and B12 absorption</td>
</tr>
<tr>
<td>Niacin</td>
<td>↑</td>
<td>Uses methyl groups; blocks B6 synthesis</td>
</tr>
<tr>
<td>Metformin</td>
<td>↑</td>
<td>Blocks B12 absorption</td>
</tr>
<tr>
<td>Insulin</td>
<td>↓</td>
<td>↑ MTHFR &gt; ↓ CBS</td>
</tr>
<tr>
<td>Estradiol</td>
<td>↓ or↑</td>
<td>↑ PEMT and depletes B6</td>
</tr>
<tr>
<td>Testosterone</td>
<td>↑</td>
<td>↑ Need for creatine</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>↑</td>
<td>Blocks DHFR</td>
</tr>
<tr>
<td>Dilantin</td>
<td>↑</td>
<td>↓ MTHFR and MTR</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>↑</td>
<td>Folate depletion</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>↑</td>
<td>Decrease GFR and ↓ MTHFR</td>
</tr>
<tr>
<td>Levodopa</td>
<td>↑</td>
<td>Generation of SAH</td>
</tr>
<tr>
<td>NAC</td>
<td>↓</td>
<td>Thiol-disulfide exchange</td>
</tr>
</tbody>
</table>
## METHYL THIEVES and IATROGENIC HHcy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hcy</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPIs and H₂ Blockers</td>
<td>↑</td>
<td>Blocks B12 absorption</td>
</tr>
<tr>
<td>OCPs</td>
<td>↑</td>
<td>↓ B12, B6, folate, riboflavin, Vit C, &amp; Zn</td>
</tr>
<tr>
<td>Alcohol</td>
<td>↑</td>
<td>↓ MTR; compromises folate metabolism</td>
</tr>
<tr>
<td>Mercury</td>
<td>↑</td>
<td>↓ MTR</td>
</tr>
<tr>
<td>Lead</td>
<td>↑</td>
<td>Enzyme dysfunction</td>
</tr>
<tr>
<td>Aluminum</td>
<td>↑</td>
<td>Enzyme dysfunction</td>
</tr>
<tr>
<td>Cadmium</td>
<td>↑</td>
<td>Enzyme dysfunction</td>
</tr>
<tr>
<td>Organic Pollutants</td>
<td>↑</td>
<td>Enzyme dysfunction</td>
</tr>
<tr>
<td>Diuretics</td>
<td>↑</td>
<td>Lowers GFR; depletes B Vits</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>No Δ</td>
<td>No effect</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>↓</td>
<td>Uncertain mechanism</td>
</tr>
</tbody>
</table>
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

**Triglycerides (mg/dl)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Six Weeks</th>
<th>Baseline</th>
<th>Six Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemfibrozil</td>
<td>685</td>
<td>365</td>
<td>579</td>
<td>374</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HDL (mg/dl)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Six Weeks</th>
<th>Baseline</th>
<th>Six Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemfibrozil</td>
<td>32</td>
<td>39.4</td>
<td>34.7</td>
<td>39.7</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FENOFIBRATE, GEMFIBROZIL, and HOMOCYSTEINE

![Graph showing the change in Homocysteine (umol/l) over Baseline and Six Weeks for Gemfibrozil and Fenofibrate.]

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Six Weeks</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemfibrozil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>7.7 (2.5-53.2)</td>
<td>4.1 (1.1-24.6)</td>
<td>0.046</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.83 (0.46-1.89)</td>
<td>1.02 (0.43-1.71)</td>
<td>0.010</td>
</tr>
<tr>
<td>Total homocysteine</td>
<td>12.9 (7.1-23.6)</td>
<td>12.4 (6.3-29.5)</td>
<td>0.592</td>
</tr>
<tr>
<td>Creatinine</td>
<td>79 (50-110)</td>
<td>80 (55-109)</td>
<td>0.626</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.73 (0.57-1.48)</td>
<td>0.74 (0.59-1.71)</td>
<td>0.237</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>10.1 (4.2-33.6)</td>
<td>11.7 (4.3-35.4)</td>
<td>0.948</td>
</tr>
<tr>
<td>Folate</td>
<td>8.4 (3.6-11.6)</td>
<td>8.6 (3.6-11.1)</td>
<td>0.809</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>367 (170-1145)</td>
<td>382 (189-964)</td>
<td>0.481</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>123 (53-310)</td>
<td>120 (35-589)</td>
<td>0.775</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>6.5 (2.3-60.7)</td>
<td>4.2 (0.3-15.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.90 (0.40-1.69)</td>
<td>1.03 (0.54-1.80)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total homocysteine</td>
<td>10.7 (4.4-24.8)</td>
<td>14.4 (7.7-23.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>75 (41-107)</td>
<td>90 (61-120)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.68 (0.54-1.56)</td>
<td>0.82 (0.63-1.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>11.3 (4.2-30.2)</td>
<td>11.1 (4.8-39.0)</td>
<td>0.029</td>
</tr>
<tr>
<td>Folate</td>
<td>9.2 (2.6-12.4)</td>
<td>9.4 (3.6-12.2)</td>
<td>0.983</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>386 (215-758)</td>
<td>421 (217-1087)</td>
<td>0.351</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>124 (51-243)</td>
<td>131 (42-592)</td>
<td>0.656</td>
</tr>
</tbody>
</table>

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

**Creatinine (mg/dl)**

- **Baseline**
  - Gemfibrozil: 0.88
  - Fenofibrate: 0.84

- **Six Weeks**
  - Gemfibrozil: 0.89
  - Fenofibrate: 1.01

**Cystatin C (mg/l)**

- **Baseline**
  - Gemfibrozil: 0.73
  - Fenofibrate: 0.68

- **Six Weeks**
  - Gemfibrozil: 0.74
  - Fenofibrate: 0.82
Subjects with ACS - Measure TMG, DMG, and Hcy and correlate with outcome

5th quintile Homocysteine $\rightarrow$ Increased risk of MI, CHF, and mortality

5th quintile DMG $\rightarrow$ Increased risk of MI, CHF, and mortality

1st quintile TMG $\rightarrow$ Increased risk of MI

5th quintile TMG $\rightarrow$ Increased risk of CHF
  ♦ High TMG $\approx$ impaired BHMT activity

1st and 5th quintile TMG $\rightarrow$ 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 via PPARα (except Gemfibrozil):
- Decrease GFR
- Deplete TMG
  \Rightarrow 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

The graph shows the changes in homocysteine levels (umol/l) over time for Spironolactone and Metoprolol.

- **Spironolactone**:
  - Baseline: 13.6
  - One Month: 13.1
  - Five Months: 13.3

- **Metoprolol**:
  - Baseline: 13.5
  - One Month: 12.4
  - Five Months: 11.9
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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Level/kg Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>47 mg</td>
</tr>
<tr>
<td>Methionine</td>
<td>2 gm</td>
</tr>
<tr>
<td>B-6</td>
<td>11 mg</td>
</tr>
<tr>
<td>Folate</td>
<td>1 mg</td>
</tr>
<tr>
<td>B-12</td>
<td>15 mcg</td>
</tr>
</tbody>
</table>

Food Intake and Weight Gain vs. Niacin Intake

- Weight Gain (gm)
- Food Intake (gm/day)
- Liver Weight (gm)

<table>
<thead>
<tr>
<th>Niacin Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 mg</td>
</tr>
<tr>
<td>400 mg/kg</td>
</tr>
<tr>
<td>4000 mg/kg</td>
</tr>
</tbody>
</table>
NIACIN RELATED HYPERHOMOCYSTEINEMIA

**Lipid Values (mg/dl)**

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>LDL</th>
<th>Triglycerides</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>93</td>
<td>19</td>
<td>159</td>
<td>42</td>
</tr>
<tr>
<td>400</td>
<td>85</td>
<td>8</td>
<td>141</td>
<td>50</td>
</tr>
<tr>
<td>4000 mg/kg</td>
<td>73</td>
<td>4</td>
<td>50</td>
<td>46</td>
</tr>
</tbody>
</table>

- Blue: 47
- Red: 400
- Green: 4000 mg/kg
NIACIN RELATED HYPERHOMOCYSTEINEMIA

Plasma Amino Acids (umol/l) vs. Niacin Intake

Urine Amino Acids (umol/l) vs. Niacin Intake
NIACIN RELATED HYPERHOMOCYSTEINEMIA

Plasma Vitamin Levels vs. Niacin Intake

Niacin

Utilizes CH₃ 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

**Lipid Values (mg/dl)**

- Cholesterol: 47 mg/kg
- LDL: 4000 mg/kg
- Triglycerides: 4000 mg/kg + B6 10 mg
- HDL: 47 mg/kg

**Food Intake and Weight Gain vs. Niacin Intake**

- Weight Gain (gm)
  - 47 mg/kg: 164
  - 4000 mg/kg: 147
  - 4000 mg/kg + B6 10 mg: 160

- Food Intake (gm/day)
  - 47 mg/kg: 19.4
  - 4000 mg/kg: 16.3
  - 4000 mg/kg + B6 10 mg: 19
B6 in Niacin Related Hyperhomocysteinemia

Plasma Amino Acids (umol/l) vs. Niacin Intake

Urine Amino Acids (umol/l) vs. Niacin Intake

Methionine | Cysteine | Homocysteine
---|---|---
47 mg/kg | 4000 mg/kg | 4000 mg/kg + B6 10 mg

Methionine

Cysteine

Homocysteine

Methionine

Cysteine

Homocysteine

47 mg/kg | 4000 mg/kg | 4000 mg/kg + B6 10 mg

MTR | MTRR | BHMT

SAH

CREATINE

SAMe

DNA, RNA

Protein, lipids

Creatine

Creatinine

Guanido Ac

Adenine

Taurine

Sulfite

Glutathione

Ammonia

Cysteine + α-KG

B6, P5P
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

Fasting Homocysteine (umol/l)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>11.1</td>
<td>11.4</td>
</tr>
<tr>
<td>ERT</td>
<td>11.8</td>
<td>10.1</td>
</tr>
<tr>
<td>HRT</td>
<td>11.2</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Fasting Homocysteine Percent Reduction

<table>
<thead>
<tr>
<th></th>
<th>ERT</th>
<th>HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ESTRADIOL, HOMOCYSTEINE, B6, and CBS

![Graph showing homocysteine levels in different conditions]

**Homocysteine (umol/l)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Placebo Baseline</th>
<th>Placebo 12 Weeks</th>
<th>ERT Baseline</th>
<th>ERT 12 Weeks</th>
<th>HRT Baseline</th>
<th>HRT 12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>11.1</td>
<td>11.4</td>
<td>11.8</td>
<td>10.1</td>
<td>11.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Post-Methionine</td>
<td>36.6</td>
<td>36.6</td>
<td>33.1</td>
<td>43.1</td>
<td>36.8</td>
<td>47.9</td>
</tr>
<tr>
<td>Difference</td>
<td>47.7</td>
<td>48</td>
<td>44.9</td>
<td>53.2</td>
<td>48</td>
<td>57.6</td>
</tr>
</tbody>
</table>

**Diagram showing folate cycle and homocysteine metabolism**

- **Folate cycle**
  - Methylene-THF
  - MTHFR
  - B12
  - Methionine
  - dimethylglycine
  - Metabolized in the transmethylation pathway

- **Homocysteine metabolism**
  - CBS
  - S-Adenosylhomocysteine
  - Metabolized in the transsulfuration pathway

- **Transsulfuration pathway**
  - Cystathionine
  - Sulfate + H2O
  - Glutathione

- **Remethylation pathway**
  - Dietary protein

**Phosphatidylcholine**

- R' and R'' are fatty acid residues

**Methionine cycle**

- Methionine
- dimethylglycine
- S-Adenosylmethionine (SAM)
- Metabolized in the transmethylation pathway

**Methylation cycle**

- Folate
- Tetrahydrofolate
- 5,10-Methylene-tetrahydrofolate
- 5-Methyltetrahydrofolate

**S-Adenosylmethionine cycle**

- Beta-alanine
- Betaine-homocysteine methyltransferase
- Methionine synthase

**Cystathionine synthesis cycle**

- CHOLINE
- Cystathionine beta-synthase
- Vitamin B6
ESTRADIOL, HOMOCYSTEINE, B6, and CBS

Homocysteine (umol/l)

Vitamin B6 (nmol/l)
FATTY ACID OXIDATIVE METABOLISM

- **Methyl donor deficiency**
- **Ac**
- **PGG-1α**
- **SIRT1**
- **PRMT1**
- **PPRE**
- **PPAR-α**

**Genes of Fatty acid oxidation**

**Control** vs. **Methyl deficiency**

- **LV**
- **RV**

**Intermembrane space**
- **Matrix**
- **Cristae**
- **Ribosome**
- **Granules**
- **ATP synthase particles**
- **Inner membrane**
- **Outer membrane**
- **Deoxyribonucleic acid (DNA)**
# SAMe METHYL TRANSFER REACTIONS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate and Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA Methyl Transferases</strong></td>
<td>Alters DNA Transcription (Bookmarking)</td>
</tr>
<tr>
<td><strong>Synthetic Reactions</strong></td>
<td>Generation of Carnitine</td>
</tr>
<tr>
<td><strong>Protein Methyl Transferases (PRMT)</strong></td>
<td>Alters Enzyme Activity (PGC-1α → PPARα → FA Oxidation)</td>
</tr>
<tr>
<td><strong>Catechol-O-Methyl Transferase</strong></td>
<td>Inactivates Catecholamines</td>
</tr>
<tr>
<td><strong>COMT</strong></td>
<td>Methylation 2-OH and 4-OH Estrogens</td>
</tr>
<tr>
<td><strong>PEMT</strong></td>
<td>Metabolizes Bioflavonoids</td>
</tr>
<tr>
<td><strong>GAMT</strong></td>
<td>Generation of Phosphatidylcholine</td>
</tr>
<tr>
<td><strong>GNMT</strong></td>
<td>Generation of Creatine</td>
</tr>
<tr>
<td><strong>SAMe METHYL TRANSFER REACTIONS</strong></td>
<td><strong>SAMe → 5,10-MethylenetTHF</strong></td>
</tr>
</tbody>
</table>
SAMe and POST-TRANSLATIONAL ENZYME MODIFICATION

\[
\begin{align*}
&\text{MTHFR} \rightarrow \text{Methyl-THF} \\
&\text{Methyl-THF} \rightarrow \text{Homocysteine} \\
&\text{Homocysteine} \rightarrow \text{ATP} \\
&\text{ATP} \rightarrow \text{SAM} \\
&\text{SAM} \rightarrow \text{PRMT} \\
&\text{PRMT} \rightarrow \text{Methionine cycle} \\
&\text{Methionine cycle} \rightarrow \text{THF} \\
&\text{THF} \rightarrow \text{Diet} \\
&\text{Diet} \rightarrow \text{MTR - B12} \\
&\text{MTR - B12} \rightarrow \text{Methionine} \\
&\text{Methionine} \rightarrow \text{SAMe} \\
&\text{SAMe} \rightarrow \text{PGC-1\(\alpha\)} \\
&\text{PGC-1\(\alpha\)} \rightarrow \text{MePGC-1\(\alpha\)} \\
&\text{MePGC-1\(\alpha\)} \rightarrow \text{PPAR\(\alpha\)}, \text{ER\(\alpha\)}, \text{ERR\(\alpha\)} & \text{& HNF-4\(\alpha\)} \\
\end{align*}
\]

Protein Arginine Methyl Transferase → Fatty Acid Oxidation ⇒ ATP
Histone Deacetylase
Peroxisome Proliferator-Activated Receptor-Gamma Co-Activator-1

SAMe + PGC-1\(\alpha\) → MePGC-1\(\alpha\)
PRMT

SAMe → HDAC SIRT

AcPGC-1\(\alpha\) → PGC-1\(\alpha\)
SIRT1

MePGC-1 → PPAR\(\alpha\), ER\(\alpha\), ERR\(\alpha\) & HNF-4\(\alpha\)
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)

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

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

*Note: SAMH is not mentioned in the table.
METHYL DEFICIENCY and FATTY LIVER
METHYL DEFICIENCY and FATTY LIVER

Diagram showing changes in various proteins under control and deficient conditions.

Graphs depicting quantitative analysis of different proteins under control and deficient conditions.

Summary: The diagram and graphs illustrate the impact of methyl deficiency on fatty liver, highlighting changes in key proteins and their activities under control and deficient conditions.
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

![Graph showing plasma values of folate, vitamin B12, and homocysteine between control and deficient groups.](image)
METHYL DONOR DEFICIENCY CARDIOMYOPATHY

Pup myocardial values

[Graphs and images showing folate and vitamin B12 levels, SAH and SAM concentrations, and homocysteine images with scale bar 500μm]
METHYL DONOR DEFICIENCY CARDIOMYOPATHY

Heart (mg) to Body (gm) Wgt. Ratio

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Methyl Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>5.8</td>
<td>5.5</td>
</tr>
<tr>
<td>21 Days</td>
<td>6.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Myocyte Surface Area (um²)

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Methyl Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>360</td>
<td>700</td>
</tr>
</tbody>
</table>

EF 63%  EF 70%
METHYL DONOR DEFICIENCY CARDIOMYOPATHY

L = Lipid Droplets
M = Mitochondria
MF = Myofibers
METHYL DONOR DEFICIENCY CARDIOMYOPATHY

Diagram showing the electron transport chain in the mitochondria, with labels for different components such as ATP synthase particles, intermembrane space, matrix, cristae, ribosome, granules, inner membrane, outer membrane, and deoxyribonucleic acid (DNA).
METHYL DONOR DEFICIENCY CARDIOMYOPATHY
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

Prevalence of Homocysteine > 14 umol/l

- NYHA II: 18%
- NYHA III: 42%
- NYHA IV: 79%
ELEVATED HOMOCYSTEINE in CHF

**Graph 1:**
- Y-axis: Plasma Hcy level (umol/L)
- X-axis: In plasma NT-proBNP (In pg/mL)
- Correlation coefficient: $r=0.61$, $P<0.0001$

**Graph 2:**
- Y-axis: Plasma Hcy level (umol/L)
- X-axis: Peak oxygen consumption (mL/min/kg)
- Correlation coefficient: $r=-0.51$, $P=0.0002$
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

<table>
<thead>
<tr>
<th>Coronary narrowing ≥ 50%</th>
<th>68%</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF &lt; 40% (mean 44%)</td>
<td>47%</td>
</tr>
<tr>
<td>Pro-BNP &gt; 250 pg/ml (mean 2308)</td>
<td>65%</td>
</tr>
<tr>
<td>Hcy &gt; 12 mmol/l (mean 16.2)</td>
<td>88%</td>
</tr>
<tr>
<td>B-12 &lt; 150 pmol/l (mean 347)</td>
<td>13%</td>
</tr>
<tr>
<td>MMA &gt; 0.5 mmol/l (mean 0.51)</td>
<td>29%</td>
</tr>
<tr>
<td>Folate &lt; 7 nmol/l (mean 11.4)</td>
<td>25%</td>
</tr>
</tbody>
</table>
HOMOCYSTEINE and NT-pro-BNP

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

<table>
<thead>
<tr>
<th>Hy CV Dz</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP &gt; 100 pg/ml (mean 43)</td>
<td>9%</td>
</tr>
<tr>
<td>Hcy &gt; 12 mmol/l (mean 15.8)</td>
<td>74%</td>
</tr>
<tr>
<td>B-12 &lt; 150 pmol/l (mean 350)</td>
<td>10%</td>
</tr>
<tr>
<td>MMA &gt; 0.5 mmol/l (mean 0.74)</td>
<td>8%</td>
</tr>
<tr>
<td>Folate &lt; 7 nmol/l (mean 13.8)</td>
<td>13%</td>
</tr>
</tbody>
</table>
## HOMOCYSTEINE and BNP

<table>
<thead>
<tr>
<th></th>
<th>Cath Patients</th>
<th>Screening Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated BNP</td>
<td>65%</td>
<td>9%</td>
</tr>
<tr>
<td>Hcy Mean</td>
<td>16.2</td>
<td>15.8</td>
</tr>
<tr>
<td>Elevated Hcy</td>
<td>88%</td>
<td>74%</td>
</tr>
<tr>
<td>Folate</td>
<td>11.4</td>
<td>13.8</td>
</tr>
<tr>
<td>Low Folate</td>
<td>25%</td>
<td>13%</td>
</tr>
<tr>
<td>MMA</td>
<td>0.51</td>
<td>0.74</td>
</tr>
<tr>
<td>Elevated MMA</td>
<td>29%</td>
<td>8%</td>
</tr>
<tr>
<td>B-12</td>
<td>347</td>
<td>350</td>
</tr>
<tr>
<td>Low B12</td>
<td>13%</td>
<td>10%</td>
</tr>
</tbody>
</table>

| 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

- Log NT-proBN (pg/mL) vs. Short chain-acylcarnitines (µmol/L)
  - R = 0.360, [95% CI: 0.254 - 0.457], P < 0.0001
- Log NT-proBN (pg/mL) vs. Long chain-acylcarnitines (µmol/L)
  - R = 0.362, [95% CI: 0.257 - 0.459], P < 0.0001
- Short chain acylcarnitines (µmol/L) vs. Log Hcy (µmol/L)
  - R = 0.306, [95% CI: 0.204 - 0.402], P < 0.0001
- Medium chain acylcarnitines (µmol/L) vs. Log Hcy (µmol/L)
  - R = 0.543, [95% CI: 0.455 - 0.620], P < 0.0001
- Long chain acylcarnitines (µmol/L) vs. Log Hcy (µmol/L)
  - R = 0.552, [95% CI: 0.466 - 0.628], P < 0.0001
HOMOCYSTEINE and BNP
Screening Subjects
HOMOCYSTEINE and BNP

NT-pro-BNP in Cath Patients

<table>
<thead>
<tr>
<th>MMA &lt; 0.5 mmol/l</th>
<th>MMA &gt; 0.5 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>4292</td>
<td>4779</td>
</tr>
</tbody>
</table>

BNP in Screening Subjects

<table>
<thead>
<tr>
<th>MMA &lt; 0.5 mmol/l</th>
<th>MMA &gt; 0.5 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>60</td>
</tr>
</tbody>
</table>

Acyl-Carnitines in Cath Patients

<table>
<thead>
<tr>
<th>Chain</th>
<th>MMA &lt; 0.5 mmol/l</th>
<th>MMA &gt; 0.5 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Chain</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Medium Chain</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Long Chain</td>
<td>1.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>
HOMOCYSTEINE INCREASED BNP

♥ Rat LV slices in superfusion chamber

Increase concentration of Hcy in superfusate
B VITAMIN THERAPY in HEART FAILURE

- 28 patients 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>
<thead>
<tr>
<th>Nutrient</th>
<th>Daily dose (four capsules)</th>
<th>RDI</th>
<th>Upper safe limit for total daily intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>250 mg</td>
<td>800 mg</td>
<td>2500 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>150 mg*</td>
<td>300 mg</td>
<td>700 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>15 mg</td>
<td>15 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>1.2 mg</td>
<td>1.2 mg</td>
<td>9 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>50 μg</td>
<td>65 μg</td>
<td>450 μg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>800 μg</td>
<td>800 μg</td>
<td>3300 μg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>200 mg*</td>
<td>1.4 mg</td>
<td>No limit</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>2 mg</td>
<td>1.5 mg</td>
<td>No limit</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>200 mg*</td>
<td>2 mg</td>
<td>300 mg</td>
</tr>
<tr>
<td>Folate</td>
<td>5 mg*</td>
<td>200 μg</td>
<td>No limit</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>200 μg</td>
<td>1 μg</td>
<td>No limit</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>500 mg*</td>
<td>60 mg</td>
<td>2000 mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>400 mg*</td>
<td>10 mg</td>
<td>900 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>10 μg*</td>
<td>5 μg</td>
<td>25 μg</td>
</tr>
<tr>
<td>Co-enzyme Q10</td>
<td>150 mg*</td>
<td>15 mg</td>
<td>No limit</td>
</tr>
</tbody>
</table>
B VITAMIN THERAPY in HEART FAILURE

Mortality:
1/14 placebo (pneumonia)
1/14 Vitamin (DVT → infection)

Furosemide dose:
64 → 56 mg/day Vitamin Group
65 → 67 mg/day placebo
B VITAMIN THERAPY in HEART FAILURE

Left Ventricular Volumes (CMR)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (ml)</td>
<td>212</td>
<td>224</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>169</td>
<td>172</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>222</td>
<td>193</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>164</td>
<td>138</td>
</tr>
</tbody>
</table>

Placebo vs Vitamin Group

Baseline vs One Year

LVEDV (ml)

Baseline

End of study

P = 0.03
B VITAMIN THERAPY in HEART FAILURE

**LV Ejection Fraction (%)**

- **Placebo**
  - Baseline: 26.6%
  - One Year: 26.2%

- **Vitamin Group**
  - Baseline: 25.6%
  - One Year: 30.9%

**Quality of Life (as % of maximum)**

- **Baseline**
- 2 months
- 3 months
- 4 months
- 5 months
- 6 months
- End of study

**P = 0.02**
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
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

**Serum Cholesterol (mg/dl)**

- Chow: 55
- Chow + BBR: 60
- HHcy Chow: 74
- HHcy Chow + BBR: 58

**Hepatic Cholesterol (mg/g wet wgt. liver)**

- Chow: 1.7
- Chow + BBR: 1.7
- HHcy Chow: 2.2
- HHcy Chow + BBR: 1.8
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

HMG-CoA Reductase mRNA (% Control)

- Chow: 100%
- Chow + BBR: 120%
- HHcy Chow: 230%
- HHcy Chow + BBR: 250%

HMG-CoA Reductase Protein (% Control)

- Chow: 100%
- Chow + BBR: 90%
- HHcy Chow: 150%
- HHcy Chow + BBR: 140%
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

**Nuclear SREBP Protein Expression**

- Chow: 100%
- Chow + BBR: 110%
- HHcy Chow: 150%
- HHcy Chow + BBR: 140%

**SREBP/DNA Binding Activity**

- Chow: 100%
- Chow + BBR: 110%
- HHcy Chow: 130%
- HHcy Chow + BBR: 125%

Diagram illustrating the interaction between SREBP, SCAP, High Sterol, INSIG-1, Low Sterol, and the Golgi pathway.
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

**HMG-CoA Reductase Activity (pmol/min/mg)**

- Chow: 12 pmol/min/mg
- Chow + BBR: 10 pmol/min/mg
- HHcy Chow: 18 pmol/min/mg
- HHcy Chow + BBR: 13 pmol/min/mg

**pHMG-CoA Reductase/Total (% Control)**

- Chow: 100%
- Chow + BBR: 90%
- HHcy Chow: 70%
- HHcy Chow + BBR: 90%
BERBERINE and Hcy INDUCED HYPERLIPIDEMIA

AMPK Protein Expression (% Control)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Chow + BBR</th>
<th>HHcy Chow</th>
<th>HHcy Chow + BBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Protein Expression</td>
<td>100%</td>
<td>95%</td>
<td>98%</td>
<td>97%</td>
</tr>
</tbody>
</table>

pAMPK Protein Expression (% Control)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Chow + BBR</th>
<th>HHcy Chow</th>
<th>HHcy Chow + BBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAMPK Protein Expression</td>
<td>100%</td>
<td>95%</td>
<td>90%</td>
<td>70%</td>
</tr>
</tbody>
</table>

AMPK Activity (% Control)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Chow + BBR</th>
<th>HHcy Chow</th>
<th>HHcy Chow + BBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK Activity</td>
<td>100%</td>
<td>95%</td>
<td>70%</td>
<td>98%</td>
</tr>
</tbody>
</table>
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

![Bar chart showing Serum Homocysteine (umol/l) levels for different groups: Control, High Fat, and High Fat + Berberine. The chart indicates that the Control group has the lowest level at 22 umol/l, the High Fat group has the highest level at 50 umol/l, and the High Fat + Berberine group has a level of 18 umol/l.]
Berberine, Homocysteine, and Cholesterol

**Hepatic Cholesterol (mg/gm liver)**

- Control: 18 mg/gm
- High Fat: 24 mg/gm
- High Fat + Berberine: 23 mg/gm

**Serum Cholesterol (mg/dl)**

- Control: 85 mg/dl
- High Fat: 108 mg/dl
- High Fat + Berberine: 77 mg/dl
BERBERINE, HOMOCYSTEINE, and CHOLESTEROL

**HMG Co-A Reductase mRNA**

- Control: 1
- High Fat: 1.8
- High Fat + Berberine: 0.4

**LDL Receptor mRNA**

- Control: 1
- High Fat: 0.3
- High Fat + Berberine: 0.4

**apoE**

- Control: 1
- High Fat: 0.8
- High Fat + Berberine: 1.5
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
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