Suppression of statin effectiveness by copper and zinc in yeast and human cells

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Exogenous Metabolites Influence Drug Function

- Grapefruit
- Cytochrome P450
- Vitamin K
- Warfarin
- Tyramine
- MAO inhibitors
Statins are Important for Preventing Heart Disease

![Chemical structure of lovastatin](image)

- acetyl-CoA → HMG-CoA → HMG-CoA reductase → mevalonic acid → cholesterol
Statins are Important for Preventing Heart Disease

- poorly understood pleiotropic effects
- significant (though rare) side effects
- very large patient population (~30 million in the U.S.)
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Known metabolites (vitamin D) impact statin function
1. Identification of Metal-Statin Interactions in a Yeast-Based Screen

2. Ergosterol and Intermediates are Impacted by Statin and Metal

3. Altered Gene Expression Induced by Statin and Metal

4. An Integrated Genomic and Metabolomic Model for Metal-Statin Interaction
The budding yeast *S. cerevisiae* is an ideal model system because many features of cholesterol biosynthesis are conserved from yeast to humans.
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A Yeast-Based Assay for Statin-Metabolite Interactions

Quantify drug-metabolite interactions as epistasis:
$$\varepsilon = W_{\text{drug+metabolite}} - W_{\text{metabolite}} \times W_{\text{drug}}$$

$$\varepsilon < 0$$ implies synthetic lethality
$$\varepsilon > 0$$ implies rescue

Measure fitness for each condition:
$$W = \frac{T50_{\text{treated}}}{T50_{\text{vehicle}}}$$
A Small, Nutritionally-Focused Library of Metabolites

<table>
<thead>
<tr>
<th>Coenzymes &amp; Cofactors</th>
<th>Nutriceuticals</th>
<th>Nutrients</th>
<th>Amino Acids</th>
<th>Metals</th>
</tr>
</thead>
<tbody>
<tr>
<td>coenzyme A</td>
<td>resveratrol</td>
<td>myoinositol</td>
<td>arginine</td>
<td>lithium chloride</td>
</tr>
<tr>
<td>coenzyme Q10</td>
<td>curcumin</td>
<td>taurine</td>
<td>glutamate</td>
<td>zinc chloride</td>
</tr>
<tr>
<td>vitamin B12</td>
<td>caffeine</td>
<td>5-hydroxytryptophan</td>
<td>tyrosine</td>
<td>zinc sulfate</td>
</tr>
<tr>
<td>methylcobalamin</td>
<td>genistein</td>
<td>ascorbic acid</td>
<td></td>
<td>iron sulfate</td>
</tr>
<tr>
<td>NADH</td>
<td>daidzein</td>
<td>propionic acid</td>
<td></td>
<td>manganese chloride</td>
</tr>
<tr>
<td>NADPH</td>
<td>carotene</td>
<td>linolenic acid</td>
<td></td>
<td>copper chloride</td>
</tr>
<tr>
<td>FADH</td>
<td>docosahexaenoic acid</td>
<td>mevalonic acid</td>
<td></td>
<td>copper sulfate</td>
</tr>
<tr>
<td>pyridoxal phosphate</td>
<td>eicosapentaenoic acid</td>
<td>ergosterol</td>
<td></td>
<td>sodium selenite</td>
</tr>
<tr>
<td>thiamine pyrophosphate</td>
<td></td>
<td>astaxanthin</td>
<td></td>
<td>calcium chloride</td>
</tr>
<tr>
<td>biotin</td>
<td></td>
<td>betaine</td>
<td></td>
<td>chromium chloride</td>
</tr>
<tr>
<td>nicotinic acid</td>
<td></td>
<td>citrate</td>
<td></td>
<td>nickel sulfate</td>
</tr>
<tr>
<td>riboflavin</td>
<td></td>
<td>glutathione (reduced)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetrahydrobiopterin</td>
<td></td>
<td>pyruvic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>thiamine pyrophosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pantothenic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>folinic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>folic acid</td>
<td></td>
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</tbody>
</table>

Each metabolite was used at the highest possible non-toxic concentration, which was typically low mM.
One day’s worth of growth data. Note positive epistatic effect in highlighted plots.
Most Metabolites do not Influence Statin Effectiveness

\[ \varepsilon = W_{\text{drug+metabolite}} - W_{\text{metabolite}} \times W_{\text{drug}} \]

\( \varepsilon < 0 \) implies synthetic lethality
\( \varepsilon > 0 \) implies rescue
Divalent Metal Ions Rescue Statin-Mediated Growth Inhibition
Divalent Metal Ions Rescue Statin-Mediated Growth Inhibition

- other metals (Ca, Li, Ni, Cd, Cr) had no effect

- hydrogen peroxide did not rescue statin toxicity

- effects were strain/mating type independent
Cu and Zn Exhibit a Narrow Dose-Response Curve
Cu and Zn Rescue Involves Ergosterol Biosynthesis

HMG-CoA → mevalonic acid
  ↓
  prenylation → lanosterol
  ↓
  ketoconazole → C-14 demethylase → ergosterol

lovastatin → HMG-CoA reductase

HMG-CoA reductase
Cu and Zn Rescue Involves Ergosterol Biosynthesis

HMG-CoA → mevalonic acid → lanosterol → ergosterol

lovastatin inhibits HMG-CoA reductase
ketoconazole inhibits C-14 demethylase
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Cu and Zn Induce Ergosterol Synthesis

spectrophotometric quantification of ergosterol shows that Cu and Zn induce ergosterol synthesis
In order to correlate growth rates with gene expression, I calculated doubling times for each condition. Growth rates used were 1/doubling time.
GC/GC-MS to Identify Ergosterol Intermediates

**Procedure**
- Growth in the presence of statin and/or metal
- Extraction with heptane to isolate hydrophobic metabolites
- GC/GC-MS quantification of intermediates/ergosterol
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We used standards and chemical library information to identify ergosterol and eight intermediates
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Cu and Zn Alter Intermediate Levels
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Procedure

- Growth in the presence of statin and/or metal
- Rapid RNA extraction
- Microarray expression analysis (randomized technical replicates, 3 biological replicates)
- Identification of genes co-regulated by statin and metal using a linear-model
- Hierarchical clustering revealed six major clusters (2 metal responsive, 4 statin responsive)
Genes Responsive Primarily to Cu and Zn
Genes Responsive Primarily to Cu and Zn

- ion binding
- oxidative phosphorylation
- amino acid biosynthesis
Genes Responsive Primarily to Cu and Zn

- Ion binding
- Oxidative phosphorylation
- Amino acid biosynthesis

- Cation transport
Genes Responsive Primarily to Statin
Genes Responsive Primarily to Statin

sterol metabolism
response to stress
lipid biosynthesis
Genes Responsive Primarily to Statin

- sterol metabolism
- response to stress
- lipid biosynthesis

no significant terms
Genes Responsive Primarily to Statin

- sterol metabolism
- response to stress
- lipid biosynthesis

- no significant terms

- amino acid metabolism
- nucleotide metabolism
Changes in Expression are not Due to Altered Growth Rate

This experiment

ESR induced

ESR repressed
Changes in Expression are not Due to Altered Growth Rate

Of the 321 genes found to be significantly up- or downregulated, only 17 were growth rate-dependent in Brauer et al.

Genes Synergistically Upregulated by Both Statin and Metal
Genes Synergistically Upregulated by Both Statin and Metal

<table>
<thead>
<tr>
<th>Term name</th>
<th>% Query</th>
<th>% Total</th>
<th>P-value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>sterol biosynthetic process</td>
<td>5 of 26</td>
<td>36 of 6345</td>
<td>8.33E-05</td>
<td>ERG5, DAP1, ERG11, CYB5, ERG1</td>
</tr>
<tr>
<td>lipid metabolic process</td>
<td>7 of 26</td>
<td>272 of 6345</td>
<td>2.51E-02</td>
<td>ERG5, DAP1, ERG11, PLB3, CYB5, YPC1, ERG1</td>
</tr>
</tbody>
</table>
Genes Synergistically Upregulated by Both Statin and Metal

4 of the 5 sterol biosynthesis genes are involved in oxidoreductive reactions.
Genes Synergistically Upregulated by Both Statin and Metal

4 of the 5 sterol biosynthesis genes are involved in oxidoreductive reactions

4 of the 5 sterol biosynthesis genes have Sfp1, Hap1 and Yap1 transcription factor binding sites
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4 of the 5 sterol biosynthesis genes have Sfp1, Hap1 and Yap1 transcription factor binding sites.

Only a few of these factors’ other targets are differentially expressed (Sfp1, 6%; Hap1, 13%; Yap1, 7%)
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Ergosterol Biosynthetic Gene Expression Correlates with Metabolite Levels
Ergosterol Biosynthetic Gene Expression Correlates with Metabolite Levels
Cu and Zn Rescue Growth in Cultured HeLa Cells

Mammalian sterol homeostasis is achieved by SREBP s, unlike yeast which use Upc2/Ecm22.
Conclusions

‣ Metabolite exposure in the context of drug treatment is an effective way to analyze drug-metabolite interactions

‣ The interaction between lovastatin and metals occurs on the level of the entire sterol biosynthetic pathway

‣ Nutritive metal intake may have importance for human health
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Statin Reduces Ergosterol Levels
Ergosterol Levels Are Rescued by Metal Treatment

Spectrophotometric determination of ergosterol
ΔPDR5 Strain Enhances Statin Effectiveness
Statin does not Induce Mitochondrial Loss