INCREASED CORTISOL PRODUCTION THROUGH THE H6PD/11β-HSD1 MACHINERY IN Mg²⁺-DEFICIENT LIVER CELLS

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NIAAA
DIABETES ASSOCIATION OF GREATER CLEVELAND

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The term "metabolic syndrome" (aka "insulin resistance syndrome", or "syndrome X") identifies a pathological condition associated with an increased risk for the development of type 2 diabetes and atherosclerotic vascular disease (including heart disease and stroke).

The term metabolic syndrome was used the first time by Dr. Haller in 1977 to define a pathological condition characterized by the co-presence of obesity, diabetes mellitus, hyperlipoproteinemia, hyperuricemia, and hepatic steatosis in a patient. The same year, Dr. Singer used the term metabolic syndrome to define the co-presence of obesity, diabetes mellitus, hyperlipoproteinemia, and hypertension.

Several slightly different definitions exist to clinically diagnose the Metabolic Syndrome. The US National Cholesterol Education Program (NCEP) Adult Treatment Panel III (2001) defines metabolic syndrome as a condition presenting at least three of the following:

- Central obesity: waist circumference ≥ 102 cm or 40 inches (male), ≥ 88 cm or 35 inches (female)
- Dyslipidemia: TG ≥ 1.7 mmol/L (150 mg/dl)
- Dyslipidemia: HDL-C < 40 mg/dL (male), < 50 mg/dL (female)
- Blood pressure ≥ 130/85 mmHg (or treated for hypertension)
- Fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dl)

In 1999, Dr. Resnick reported the presence of hypomagnesaemia and magnesium deficiency under metabolic syndrome conditions ("ionic theory" in Resnick L. The cellular ionic basis of hypertension and allied clinical conditions. Prog Cardiovasc Dis. 1999 Jul-Aug;42(1):1-22.)
Working Hypothesis

- Currently, dietary magnesium content in the typical Western diet is ~35% to 40% lower than in the early 80s’ (http://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/).
- The change in dietary Mg\(^{2+}\) content coincides in time with the increased incidence of obesity and type 2 diabetes in the general population.
- The present study was undertaken to test the hypothesis that dietary magnesium deficiency induces metabolic changes within the hepatocytes that favor obesity and promote the onset of the metabolic syndrome and its complications.
Mg$^{2+}$ Distribution within the Hepatocyte

Extracellular [Mg$^{2+}$] = 0.6-1.2 mM

R.E.R. = 15-18 mM

[\text{Mg}^{2+}]_i = 0.5-1 \text{ mM}

\text{ATPMg} = 4-5 \text{ mM}

Mito = 15-18 mM

[\text{Mg}^{2+}]_i = 0.7-1.1 \text{ mM}

nucleus = 15-18 mM

Extracellular [\text{Mg}^{2+}] = 0.6-1.2 \text{ mM}

\text{Bile}

\text{Mg}^{2+}

\text{Na}^+

\text{or Ca}^{2+}

\text{Blood}

\text{Mito}

\text{Mrs2}
# Mg²⁺-Regulated Enzymes and Functions within the Hepatocyte

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>E.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylyl Cyclase</td>
<td>cADPr-induced Ca²⁺ release</td>
</tr>
<tr>
<td>Aquaporin 3</td>
<td>IP3-induced Ca²⁺ release</td>
</tr>
<tr>
<td>ATPases</td>
<td>RyR-induced Ca²⁺ release</td>
</tr>
<tr>
<td>Ca²⁺ channels</td>
<td>SERCA pumps</td>
</tr>
<tr>
<td>K⁺ channels</td>
<td>G6Pase</td>
</tr>
<tr>
<td>NBCe1-B</td>
<td></td>
</tr>
<tr>
<td>G-proteins</td>
<td></td>
</tr>
<tr>
<td>5’-nucleotidase</td>
<td></td>
</tr>
<tr>
<td>PKCε</td>
<td></td>
</tr>
<tr>
<td>ERK1/2</td>
<td></td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td></td>
</tr>
<tr>
<td>Phosphorylase kinase</td>
<td></td>
</tr>
<tr>
<td>Phospho-glucomutase</td>
<td></td>
</tr>
<tr>
<td>Glucose 6-Phosphatase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitochondria</td>
</tr>
<tr>
<td></td>
<td>Acetyl-CoA synthetase</td>
</tr>
<tr>
<td></td>
<td>ANT</td>
</tr>
<tr>
<td></td>
<td>α-ketoglutarate-DH</td>
</tr>
<tr>
<td></td>
<td>Isocitrate carrier</td>
</tr>
<tr>
<td></td>
<td>mitochondrial respiration</td>
</tr>
<tr>
<td></td>
<td>Nucleus</td>
</tr>
<tr>
<td></td>
<td>RNAses</td>
</tr>
<tr>
<td></td>
<td>Cyclin D</td>
</tr>
<tr>
<td></td>
<td>Cyclin E</td>
</tr>
</tbody>
</table>

A few examples out of more than 180 enzymes regulated by Mg²⁺
Experimental Approaches

- Male Sprague-Dawley rats (200-220 g bw) or male B6 mice (25-28 g bw) were exposed to a pellet diet containing ~35% less magnesium for 2 weeks.

- Livers were explanted and flash-frozen in liquid nitrogen. Hepatic cations and ATP contents were measured by atomic absorbance spectrophotometry and luciferin-luciferase assay, respectively.

- Hepatocytes were isolated by collagenase digestion and tested in vitro for glucose accumulation, ER enzymatic activities, NADPH production, mRNA and gene product (WB) expression, and cortisone to cortisol conversion.

- HepG2 cells (ATCC) were maintained in culture in the presence of 0.4mM and 0.8mM extracellular Mg\(^{2+}\). Cells were harvested at confluence and used experiments similar to those described above for freshly isolated hepatocytes.
Liver Cation Content
Following 2 weeks Exposure to Mg\(^{2+}\) Deficient Diet

<table>
<thead>
<tr>
<th></th>
<th>Na(^+)</th>
<th>Mg(^{2+})</th>
<th>Ca(^{2+})</th>
<th>K(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-D – normal Diet</td>
<td>251.7±18.9</td>
<td>79.3±4.3</td>
<td>8.8±0.9</td>
<td>1147.3±72.5</td>
</tr>
<tr>
<td>S-D – Mg(^{2+}) Deficient diet</td>
<td>286.3±23.4</td>
<td>58.3±4.8*</td>
<td>10.7±1.1</td>
<td>849.2±41.7*</td>
</tr>
<tr>
<td>% Change</td>
<td>113</td>
<td>74</td>
<td>122</td>
<td>74</td>
</tr>
<tr>
<td>B6 mice – Normal Diet</td>
<td>252.3±21.4</td>
<td>76.4±5.2</td>
<td>7.9±1.2</td>
<td>1271.1±67.4</td>
</tr>
<tr>
<td>B6 mice – Mg(^{2+}) Deficient Diet</td>
<td>301.1±26.8</td>
<td>59.5±3.7*</td>
<td>9.9±0.7</td>
<td>918.5±37.2*</td>
</tr>
<tr>
<td>% Change</td>
<td>119</td>
<td>78</td>
<td>125</td>
<td>72</td>
</tr>
</tbody>
</table>

*Statistically Significant: \(p<0.05\), \(n = 8\)

Etwebi, et al. manuscript in preparation
Changes in Hepatic Content and Distribution in Animals on Mg\textsuperscript{2+} Deficient Diet

Hepatic Mg\textsuperscript{2+} Content - Total and Distribution

<table>
<thead>
<tr>
<th></th>
<th>S-D rats</th>
<th>B6 mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ Mg\textsuperscript{2+}</td>
<td>- Mg\textsuperscript{2+}</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Post-Mito</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Hepatic ATP Content

<table>
<thead>
<tr>
<th></th>
<th>S-D</th>
<th>B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Mg\textsuperscript{2+}</td>
<td>4.4</td>
<td>4.3</td>
</tr>
<tr>
<td>- Mg\textsuperscript{2+}</td>
<td>4.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Etwebi, et al. manuscript in preparation
Hepatic Glucose Accumulation in Mg$^{2+}$ Deficient Animals and HepG2 Cells Cultured in Varying [Mg$^{2+}$]o

Etwebi, et al. manuscript in preparation
Effect of Mg\(^{2+}\) on Glucose 6-Phosphatase in Hepatocytes

Doleh, Romani, ABB, 2007, 467: 283-290
G6Pase Enzymatic Activity in Liver Microsomes from Mg$^{2+}$ Deficient Rats

Barfell et al., ABB, 2011, 509: 157-163
G6Pase Expression in Liver Microsomes from Mg\(^{2+}\) Deficient Rats

**NMM** = Normal Mg\(^{2+}\) Microsomes

**MDM** = Mg\(^{2+}\) Deficient Microsomes

Barfell et al., ABB, 2011, 509: 157-16
Effect of Mg\(^{2+}\) on G6PD activity in HepG2 Cells

NADPH extinction coefficient = 6220 M\(^{-1}\) cm\(^{-1}\)
NADPH Production in the presence of Magnesium (Late Addition)

+ 1mM MgCl₂

[Graph showing the production of NADPH at different magnesium concentrations over time]
Cortisol Production in HepG2 Cells

![Graph showing cortisol production at different extracellular magnesium concentrations (0.2M, 0.4M, 0.8M) with significant differences indicated by asterisks (*) and hash signs (#).]
Mg²⁺ deficiency Increases Cortisol Production

N=5

*P<0.001

#P<0.05

0.6mM Mg²⁺ 1.0mM Mg²⁺

N=3

*P<0.001

11β-HSD1

Albumin (5-10% bound)

CBG (90-95% bound)

β–Actin
Mg\(^{2+}\) deficiency affects protein expression of CBG, 11\(\beta\)-HSD1, and Albumin.
Activation of FAs Metabolism in Animals Exposed to Mg$^{2+}$ Deficient Diet

SREBP1c Activation

+ - + - + -  kDa

120  60

2 weeks

*P=0.01

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Effect of Mg$^{2+}$ on SREBP1c Activation in Hepatocytes

Soltani & Etwebi, manuscript in preparation
Effect of Mg\(^{2+}\) on PPAR Expression in Hepatocytes

Soltani & Etwebi, manuscript in preparation
**Mg^{2+}** Recovers after 5 days

- **N=3**
- **P<0.001**
Genes Recover from Mg\textsuperscript{2+} Deficiency - #1

\[ *P<0.001 \text{ N=4} \]

N=4

*P<0.001

<table>
<thead>
<tr>
<th>Condition</th>
<th>mRNA fold change vs. 1.0mM Mg\textsuperscript{2+} cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0mM Mg\textsuperscript{2+} x5days</td>
<td><img src="image1.png" alt="Graph" /></td>
</tr>
<tr>
<td>1.0mM Mg\textsuperscript{2+} to 0.6mM x5days</td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
<tr>
<td>0.6mM Mg\textsuperscript{2+} to 1.0mM x5days</td>
<td><img src="image3.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

Legend:
- **H6PD**
- **CBG**
Genes Recover from Mg\(^{2+}\) Recovery - #2

- **P<0.001 N=4**

- GSK
- P13K
- PEPCK
- F16BP

- 1.0mM Mg\(^{2+}\) (x5days)
- 1.0mM Mg\(^{2+}\) to 0.6mM
- 0.6mM Mg\(^{2+}\) to 1.0mM
Conclusion

- Cytosolic magnesium concentration regulates the amount of G6P entering into the E.R.

- A decrease in intracellular magnesium enhances the entry of G6P into the E.R. and favors its conversion to 6-PGL via G6PD, resulting in an increased production of NADPH and a stimulation of the Pentose Phosphate shunt.

- In the presence of cortisone, endoluminal NADPH is oxidized to NADP with an increased production of cortisol via the 11-β-OHSD1.

- As a consequence, gluconeogenic genes and PPAR-γ/SREBP1c regulated genes also become up-regulated.
Future Directions

- Determine how magnesium deficiency promotes H6PD and 11β-HSD1 upregulation.
- Determine whether the increase in cortisol induces insulin resistance.
- Validate the effect of Mg²⁺ deficiency on NADPH production and cortisol conversion in hepatocytes from normal and obese (or diabetic) patients.
THANK YOU

Questions are guaranteed in life; Answers aren't.
Cell Membrane

Glucose

Glut2

GlucoKinase

G6P

Glycolysis

Pyruvate

Mg

G6P = Pi + G

Glucose 6 Phosphatase

ER

I.R.

cytoplasm

Glut2

Insulin

Mitochondrion

PDH Phosphatase

PDH

Acetyl-CoA

β-oxidation

FAs synthesis

Cholesterol synthesis

Mitochondrion

Hexose 6 Phosphate Dehydrogenase

G6P + NADP = NADPH + 6-phosphogluconolactone

Glycogen

Dehydrogenase

G6P + NADP = NADPH + 6-phosphogluconolactone

Pentose Shunt
Glucose

Glucokinase

Glucose 6-P

G6PD

Fructose 6-P

Pentose Shunt

G6Pase

E.R.

Futile Cycle

Glycolysis

Glycogen synthesis

H6PD

G6PD