INCREASED CORTISOL PRODUCTION THROUGH THE H6PD/11β-HSD1 MACHINERY IN MG^{2+}-DEFICIENT LIVER CELLS

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Background - 1

- The term "metabolic syndrome" (aka "insulin resistance syndrome", or "syndrome X") identifies a pathological condition associate with an increased risk for the development of type 2 diabetes and atherosclerotic vascular disease (including heart disease ar stroke).
- The term *metabolic syndrome* was used the first time by Dr. Haller in 1977 to define a pathological condition characterized by the c presence of obesity, diabetes mellitus, hyperlipoproteinemia, hyperuricemia, and hepatic steatosis in a patient. The same year, D Singer used the term *metabolic syndrome* to define the co-presence of obesity, diabetes mellitus, hyperlipoproteinemia, ar hypertension.
- Several slightly difference definitions exists to clinically diagnose the Metabolic Syndrome. The US National Cholesterol Educatio Program (NCEP) Adult Treatment Panel III (2001) defines metabolic syndrome as a condition presenting at least three of th 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)

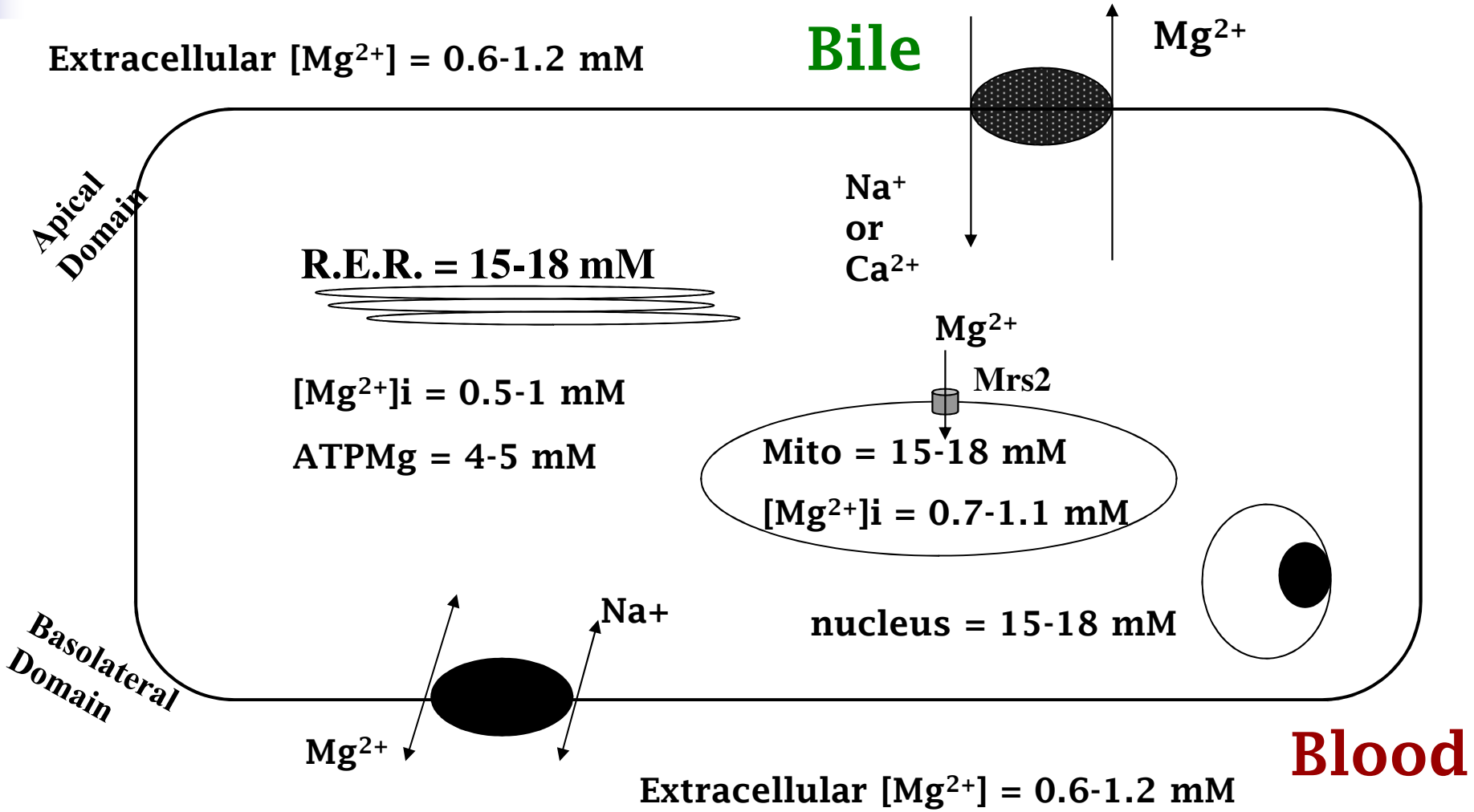
- In 1999, Dr. Resnick reported the presence of hypomagnesaemia and magnesium deficiency under metabolic syndrome condition ('ionic theory' in *Resnick L. The cellular ionic basis of hypertension and allied clinical conditions. Prog Cardiovasc Dis. 199 Jul-Aug;42(1):1-22*.)

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



Mg^{2+}-Regulated Enzymes and Functions within the Hepatocyte

Cytoplasm

- Adenylyl Cyclase
- Aquaporin 3
- ATPases
- Ca2+ channels
- K+ channels
- NBCe1-B
- G-proteins
- 5'-nucleotidase

- PKCε
- ERK1/2

- Glycogen phosphorylase
- Phosphorylase kinase
- Phospho-glucomutase
- Glucose 6-Phosphatase

E.R.

- cADPr-induced Ca^{2+} release
- IP3-induced Ca^{2+} release
- RyR-induced Ca^{2+} release
- SERCA pumps
- G6Pase

Mitochondria

- Acetyl-CoA synthetase
- ANT
- α-ketoglutarate-DH
- Isocitrate carrier
- mitochondrial respiration

Nucleus

- RNAses
- Cyclin D
- Cyclin E

A few examples out of more than 180 enzymes regulated by Mg^{2+}

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.

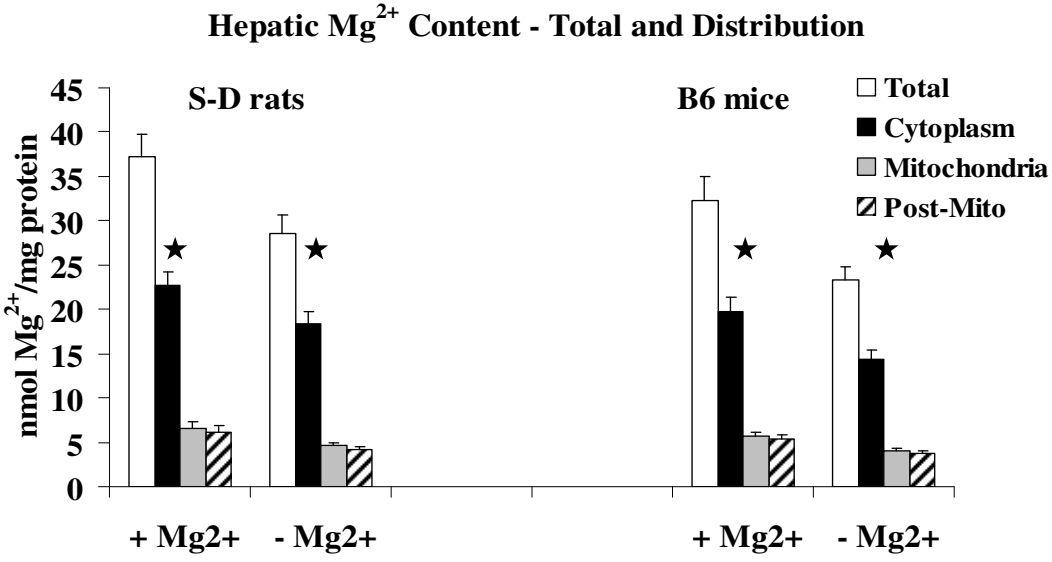
Liver Cation Content Following 2 weeks Exposure to Mg^{2+} Deficient Diet

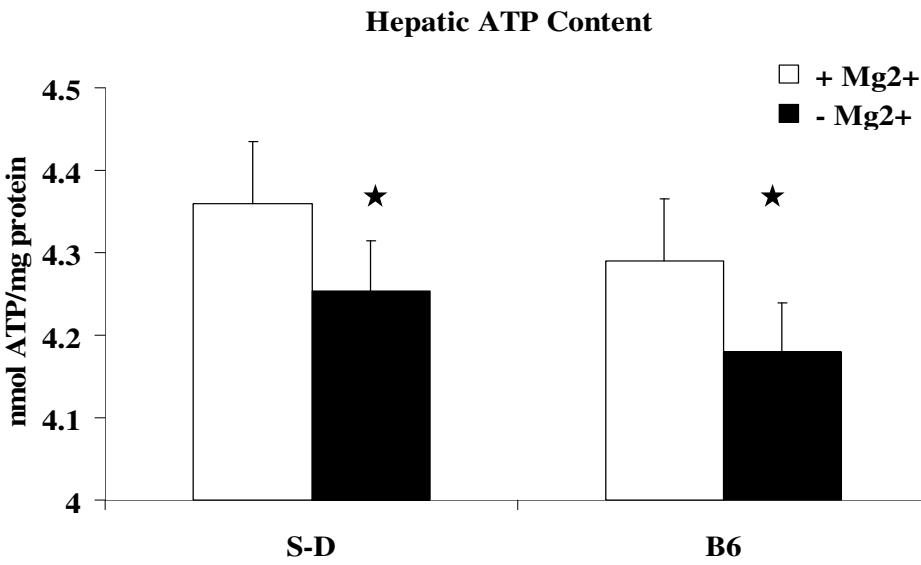
	Na^+	Mg^{2+}	Ca^{2+}	K^+
S-D – normal Diet	251.7±18.9	79.3±4.3	8.8±0.9	1147.3±72.5
S-D – Mg^{2+} Deficient diet	286.3±23.4	58.3±4.8*	10.7±1.1	849.2±41.7*
% Change	**113**	**74**	**122**	**74**
B6 mice – Normal Diet	252.3±21.4	76.4±5.2	7.9±1.2	1271.1±67.4
B6 mice – Mg^{2+} Deficient Diet	301.1±26.8	59.5±3.7*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

***Statistically Significant: p<0.05, n = 8**

Etwebi, et al. manuscript in preparation

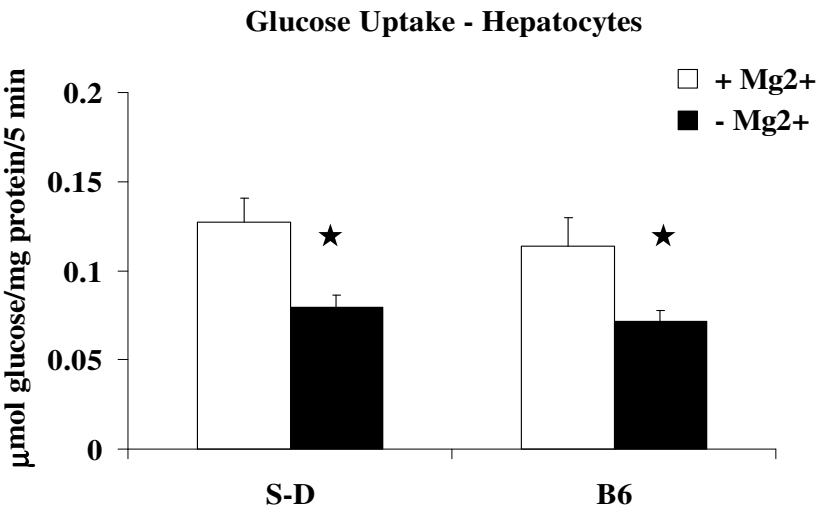
Changes in Hepatic Content and Distribution in Animals on Mg^{2+} Deficient Diet



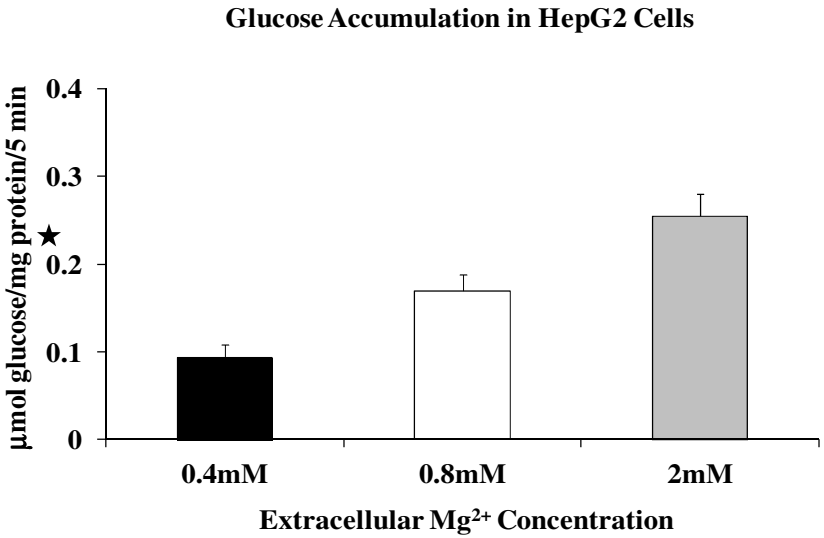


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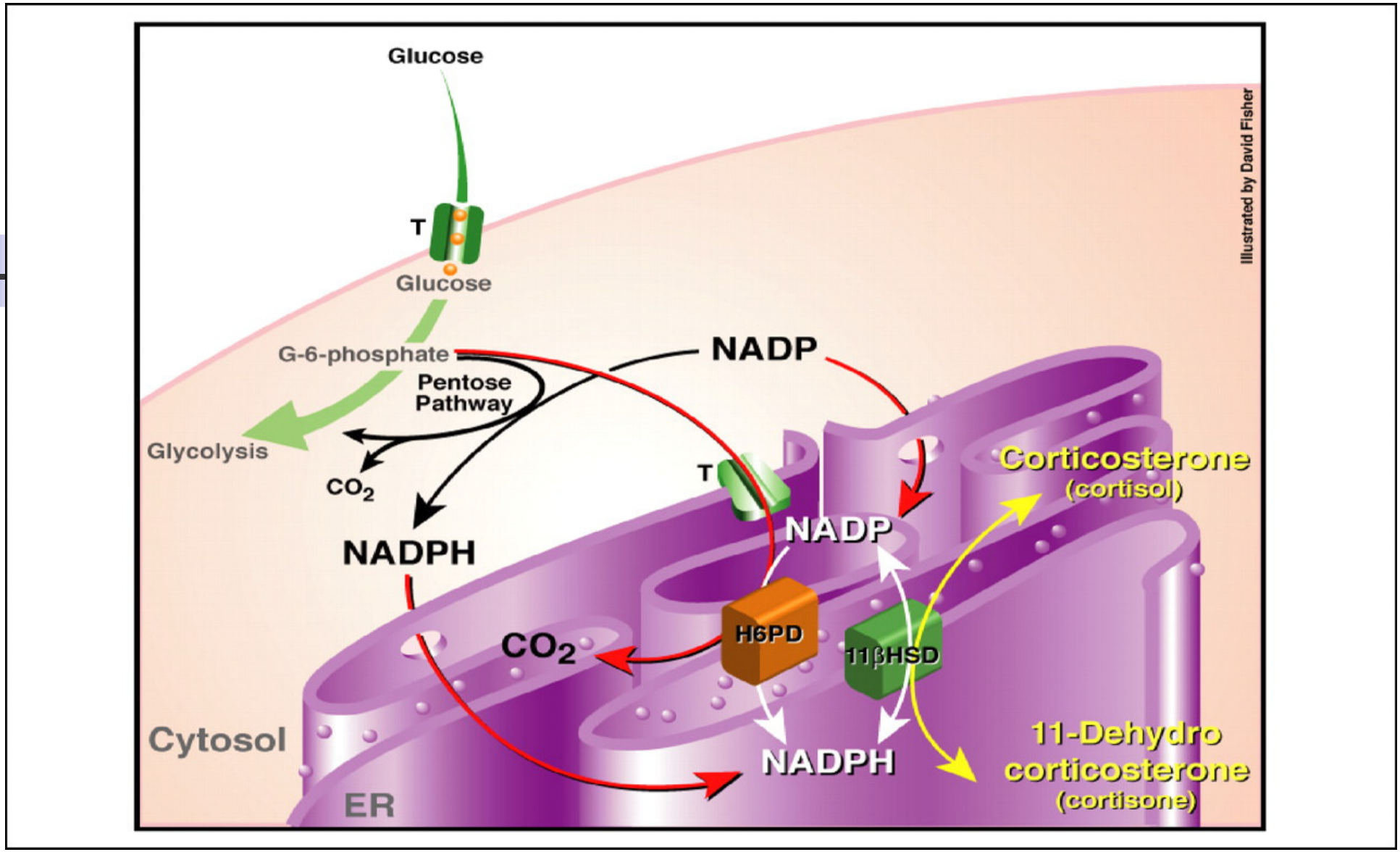
Hepatic Glucose Accumulation in Mg^{2+} Deficient Animals and HepG2 Cells Cultured in Varying $[Mg^{2+}]o$



100 µM [glucose]o

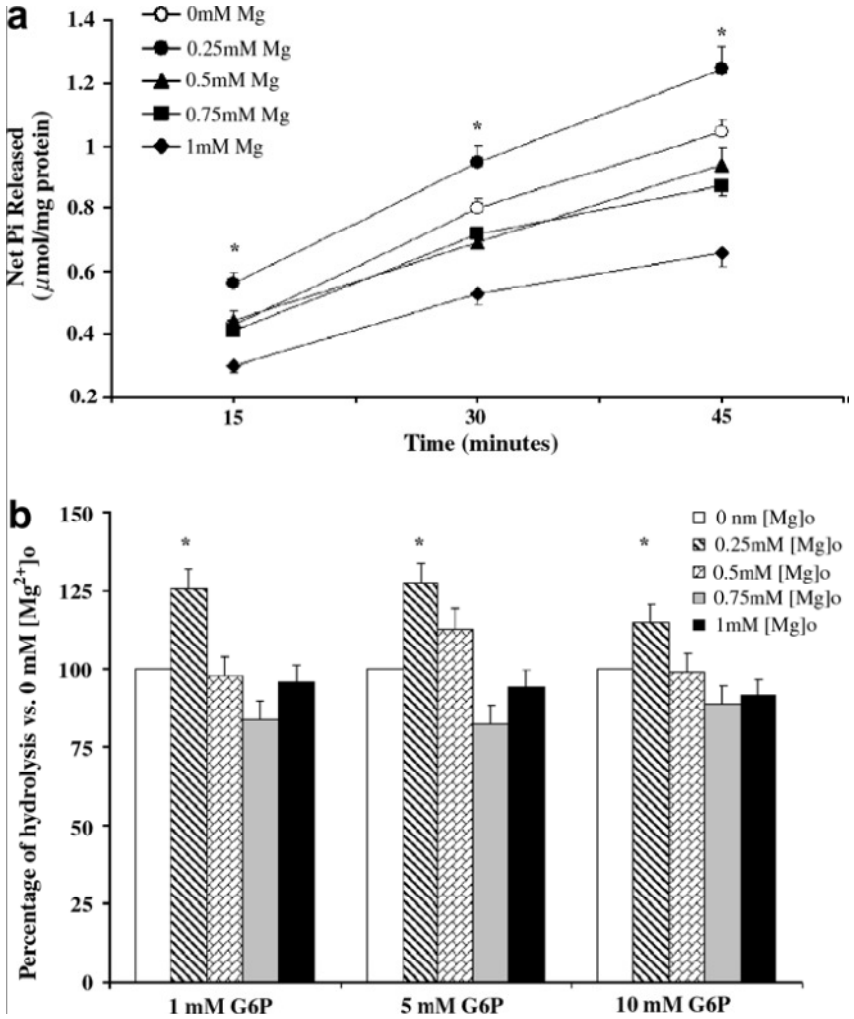


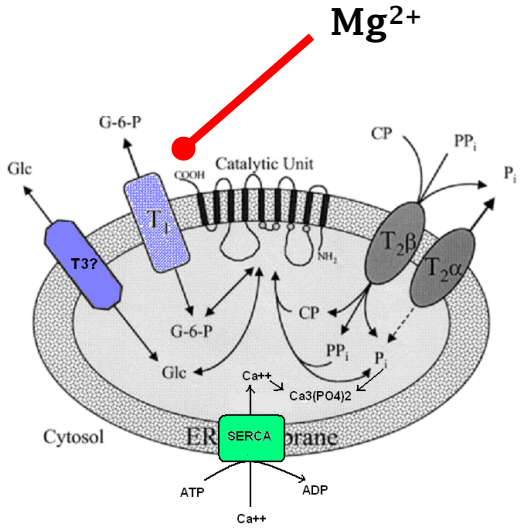
Etwebi, et al. manuscript in preparation



McCormick K L et al. J. Biol. Chem. 2006;281:341-347

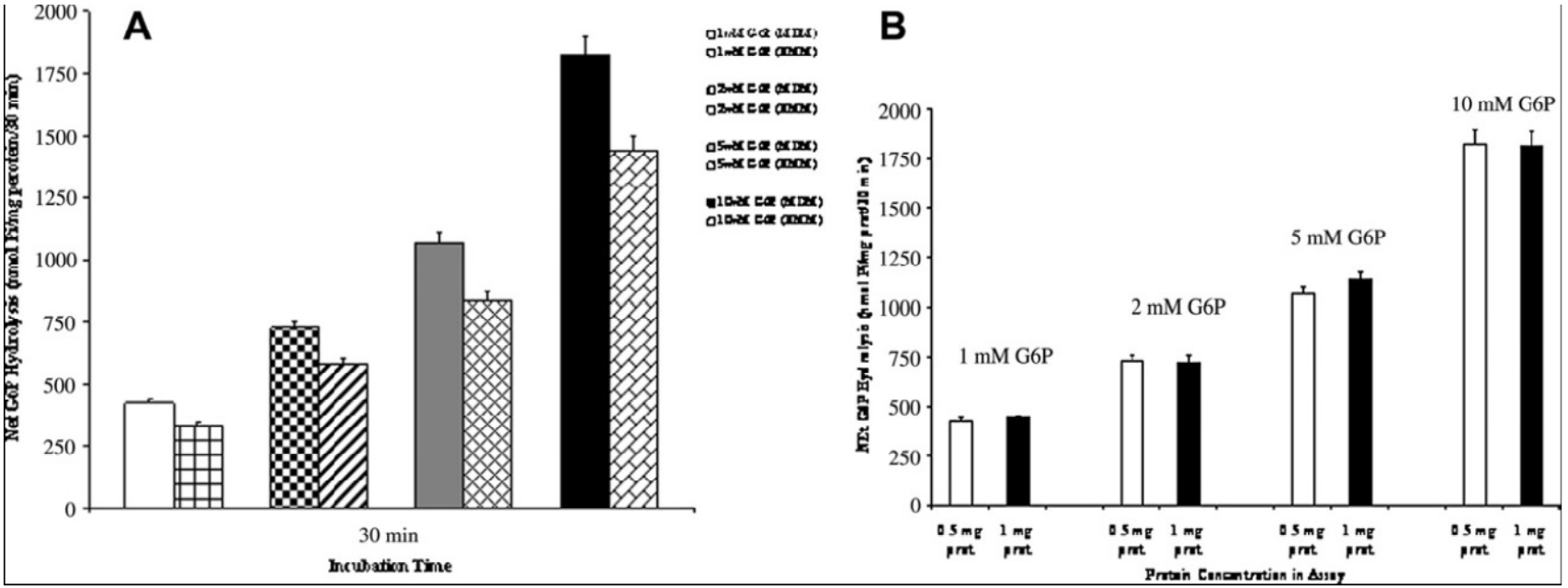
Effect of Mg^{2+} on Glucose 6-Phosphatase in Hepatocyte





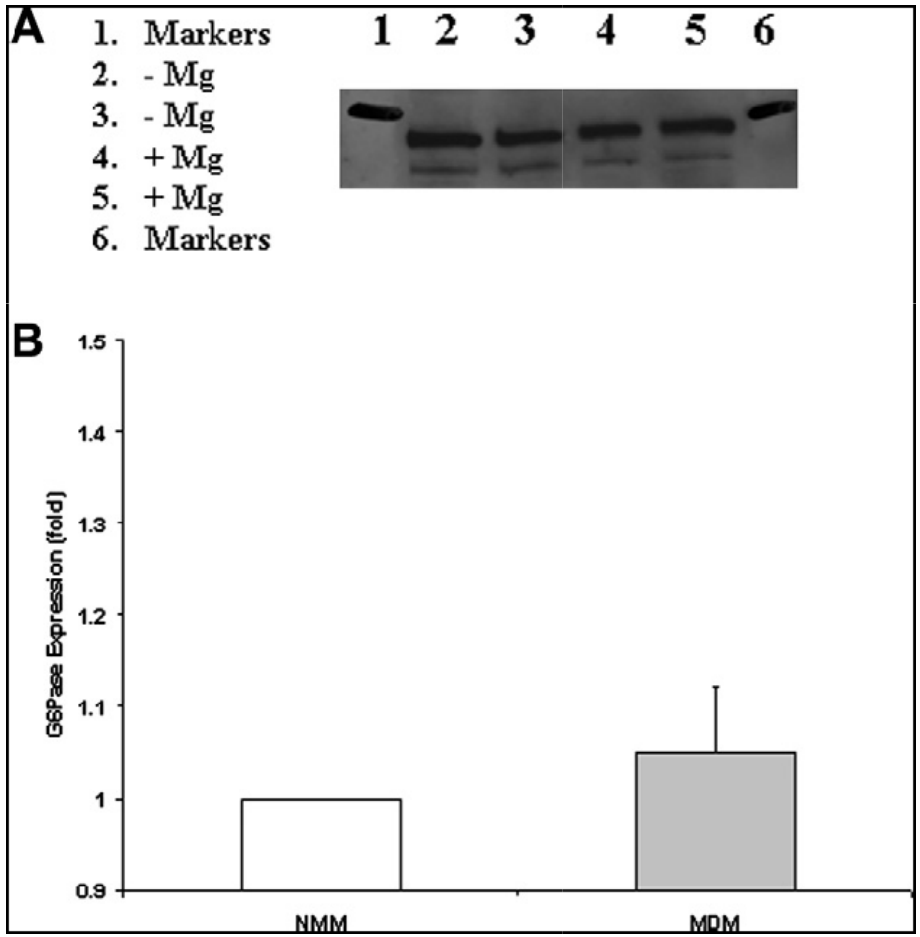
Doleh, Romani, ABB, 2007, 467: 283-290

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

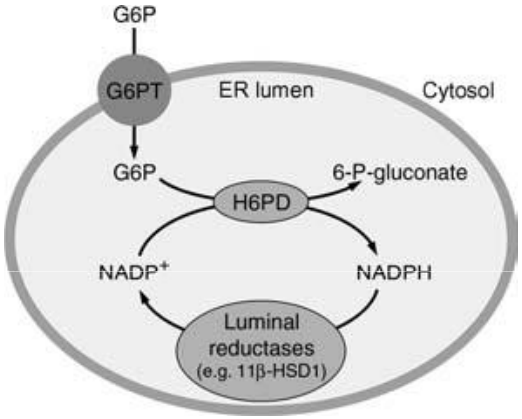


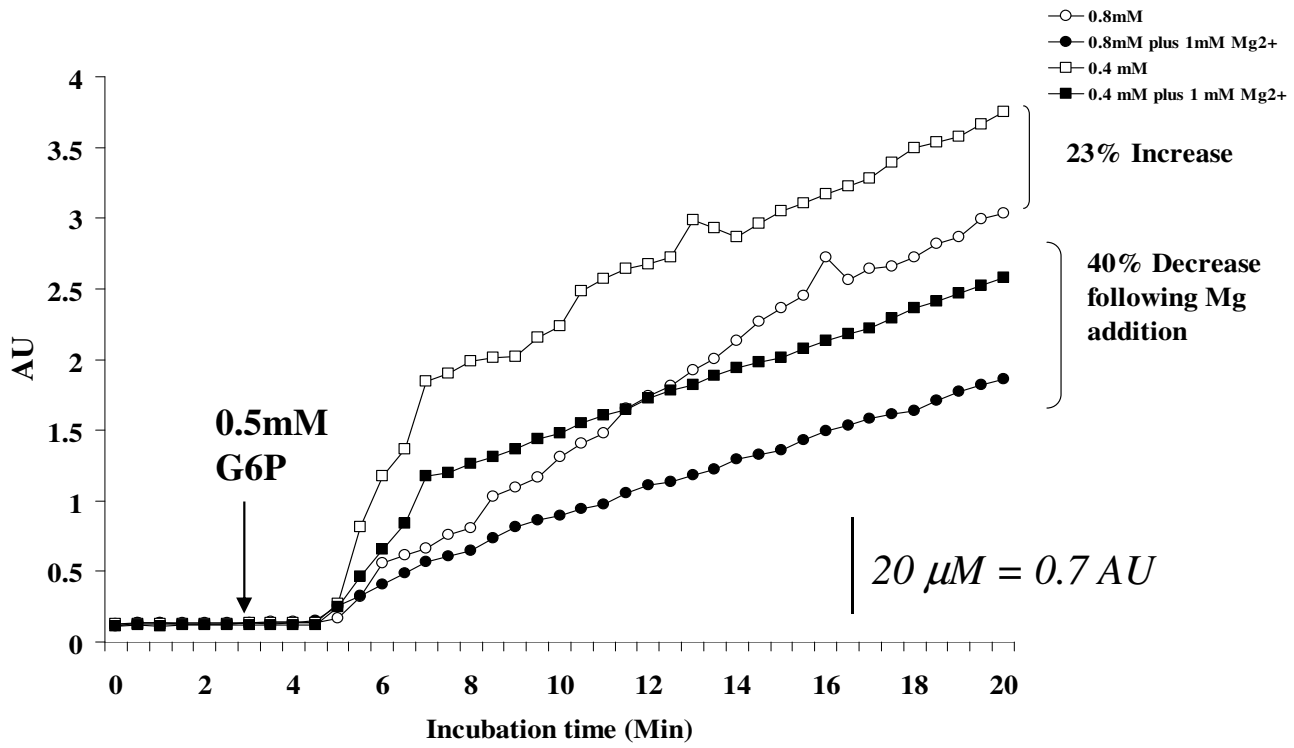
NMM = Normal Mg^{2+} Microsomes

MDM = Mg^{2+} Deficient Microsomes

Barfell et al., ABB, 2011, 509: 157-1

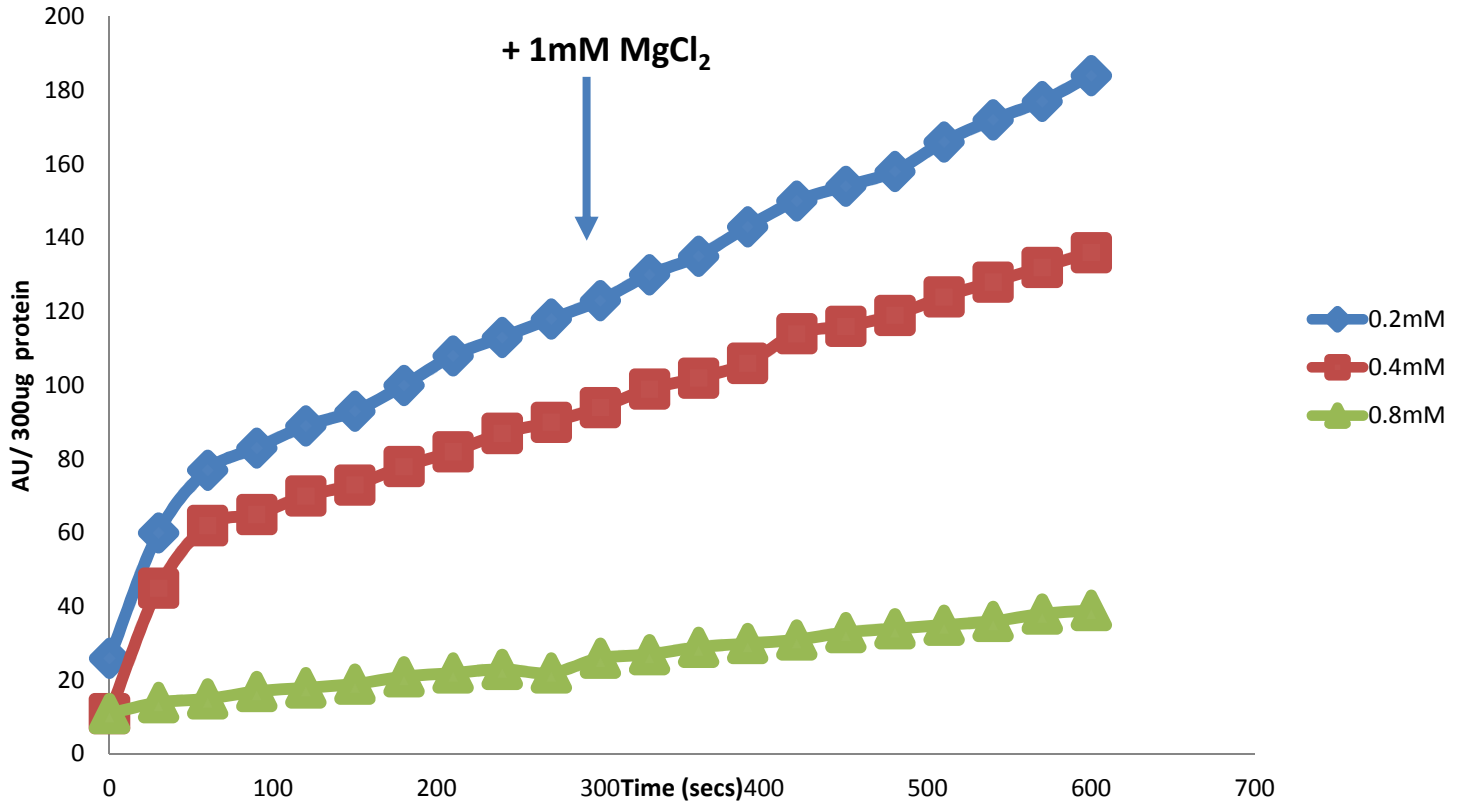
Effect of Mg^{2+} on G6PD activity in HepG2 Cells



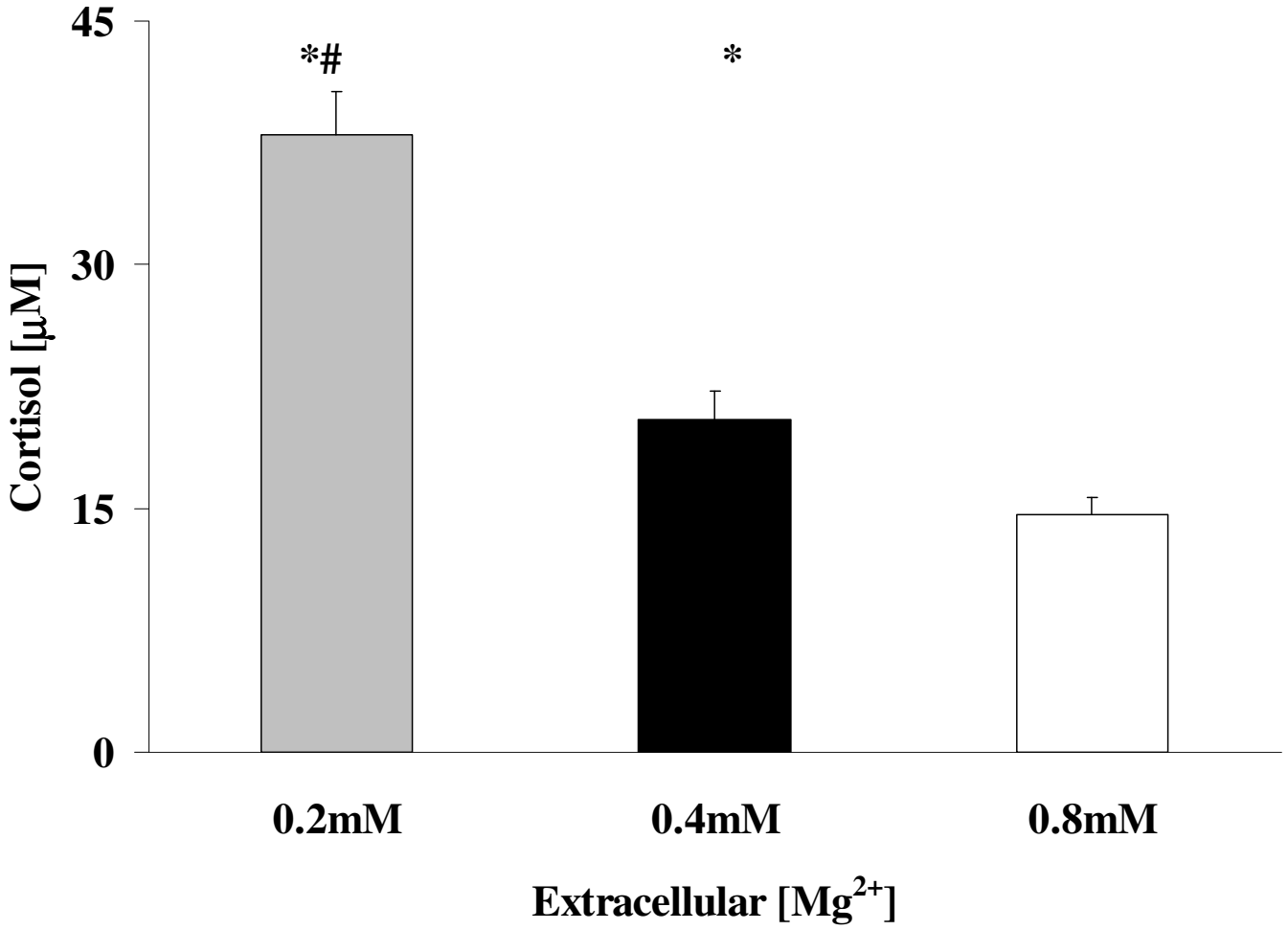


NADPH extinction coefficient = 6220 M^{-1} cm^{-1}

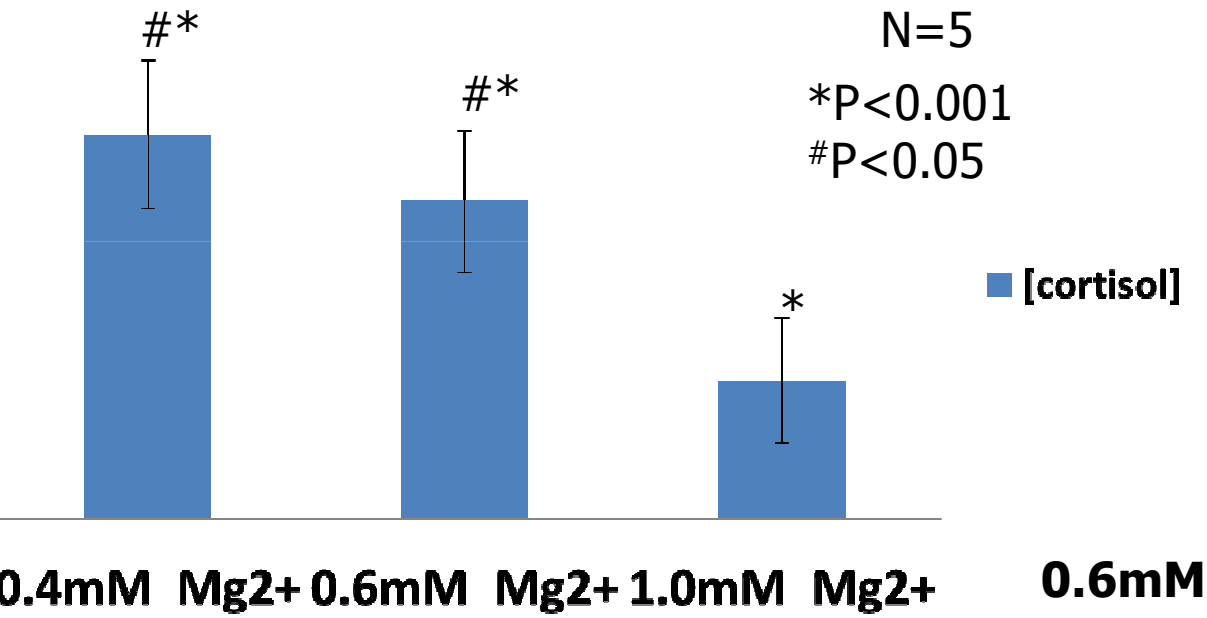
NADPH Production in the presence of Magnesium (Late Addition)

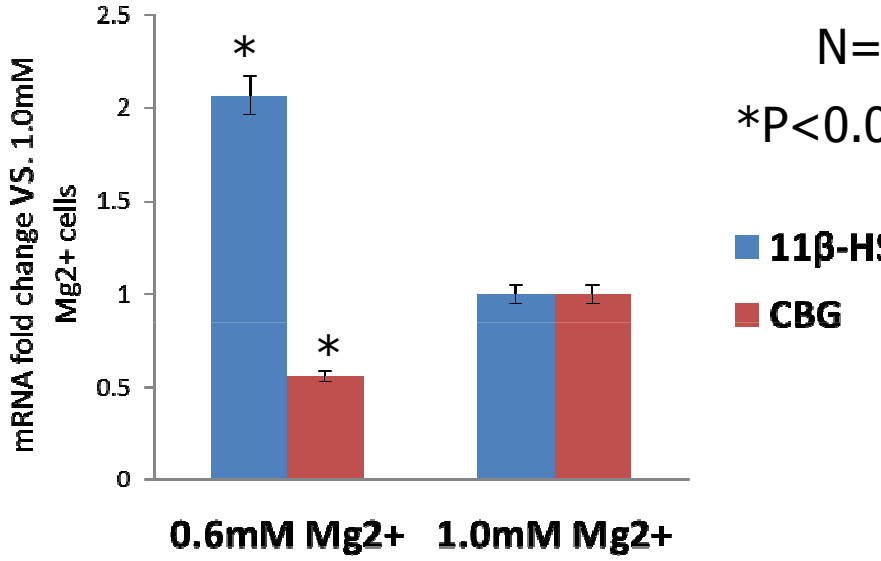


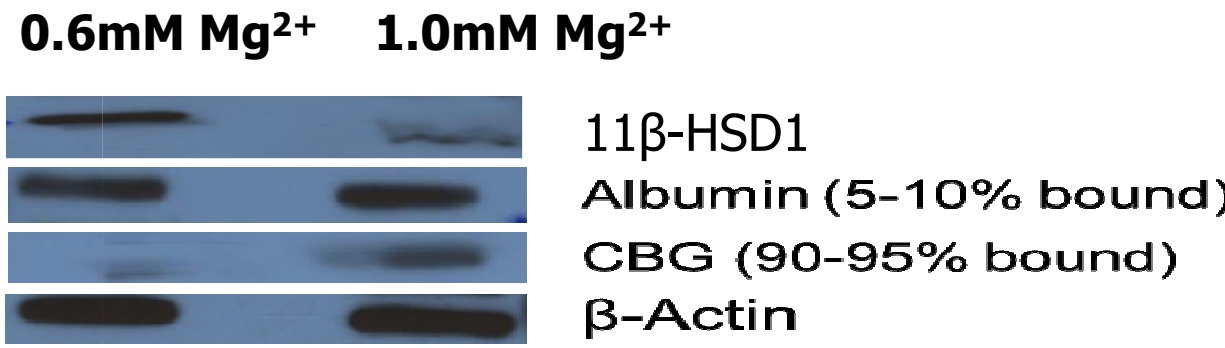
Cortisol Production in HepG2 Cells



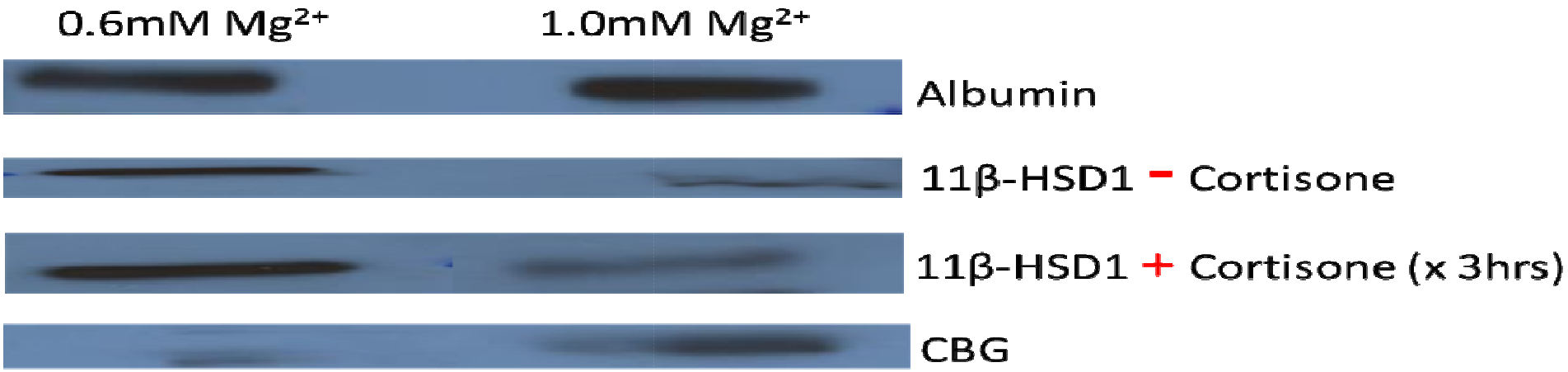
Mg^{2+} deficiency Increases Cortisol Production

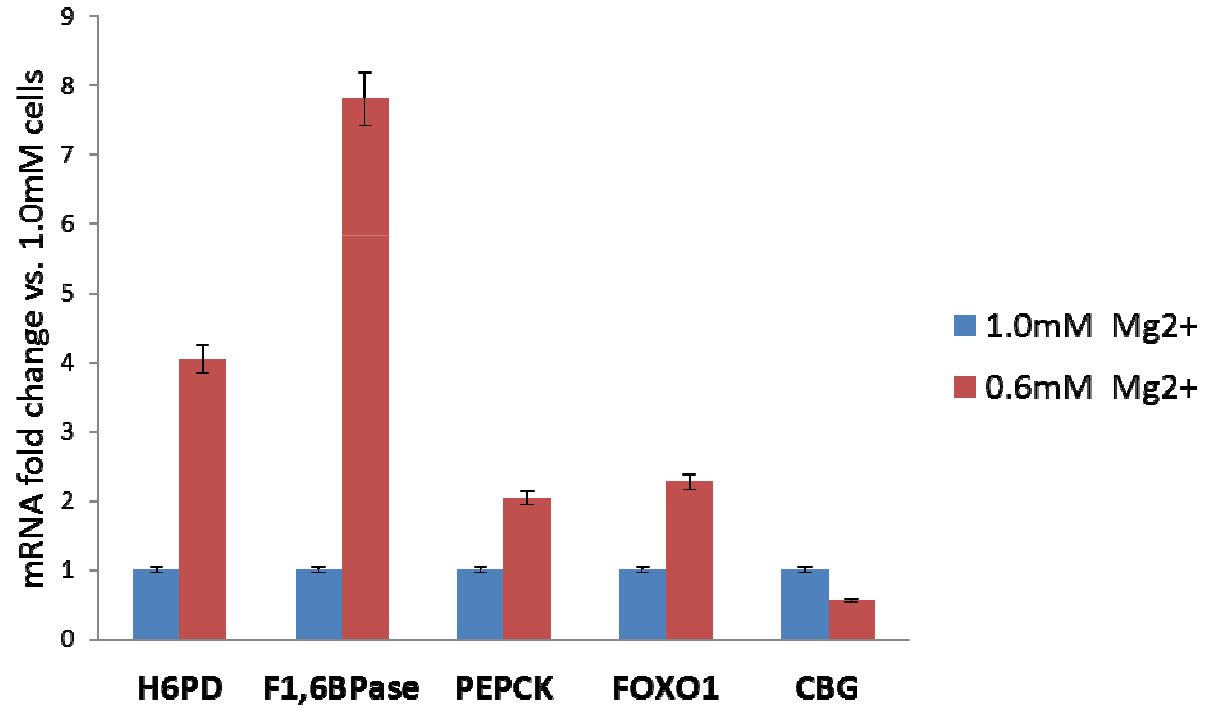


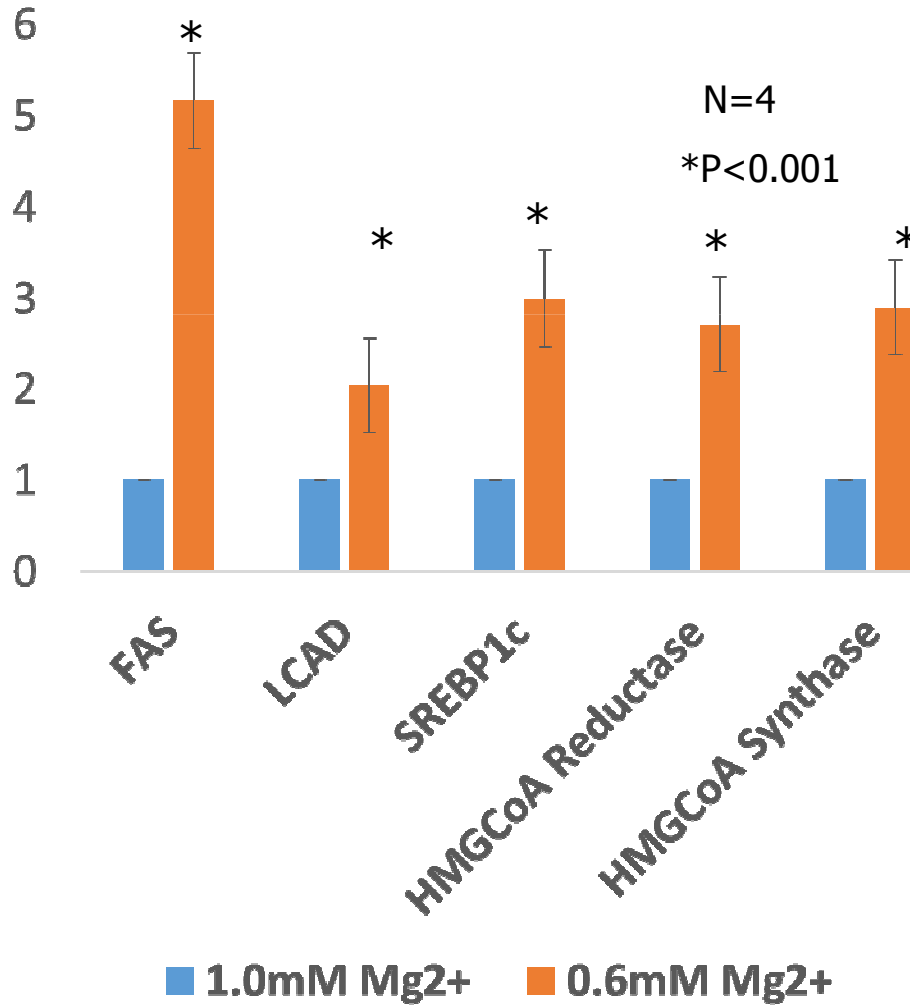




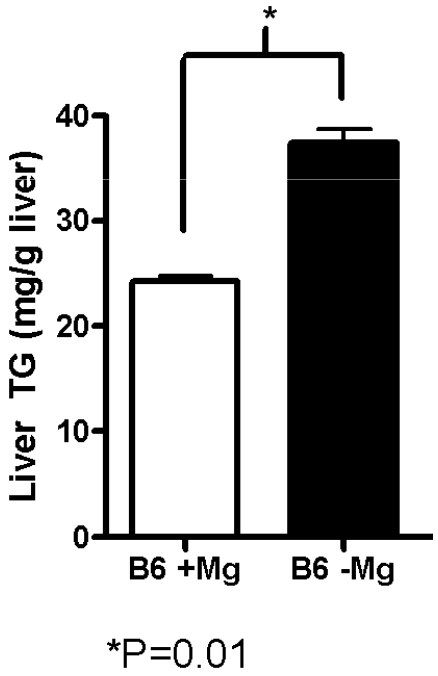
Mg^{2+} deficiency affects protein expression of CBG, 11β-HSD1, and Albumin



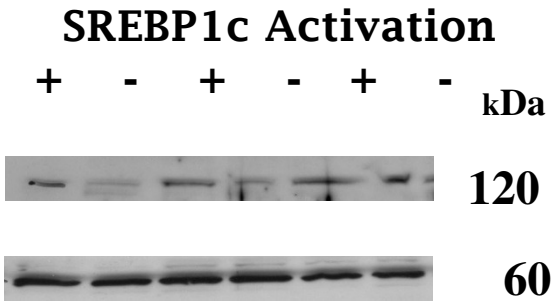

mRNA fold change vs. 1.0mM cells
0
1
2
3
4
5
6
7
8
9
H6PD
F1,6BPase
PEPCK
FOXO1
CBG
1.0mM Mg2+
0.6mM Mg2+


N=4
*P<0.001
0
1
2
3
4
5
6
*
FAS
LCAD
SREBP1c
HMGCoA Reductase
HMGCoA Synthase
1.0mM Mg2+
0.6mM Mg2+

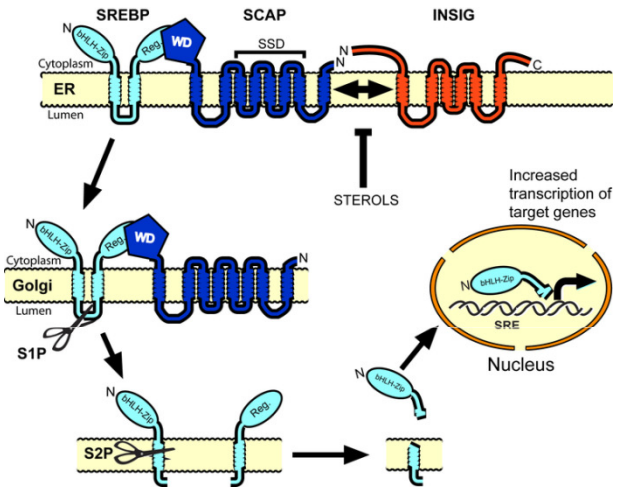
Activation of FAs Metabolism in Animals Exposed t Mg^{2+} Deficient Diet



*P=0.01

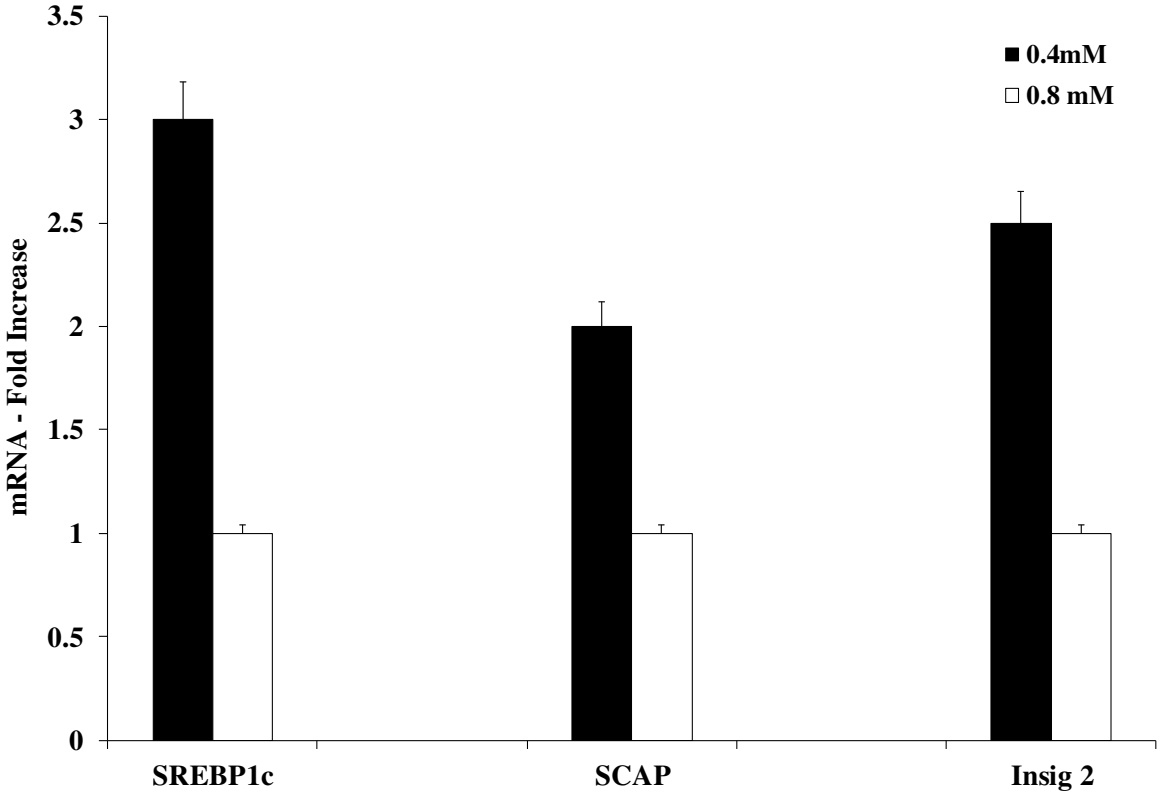


2 weeks



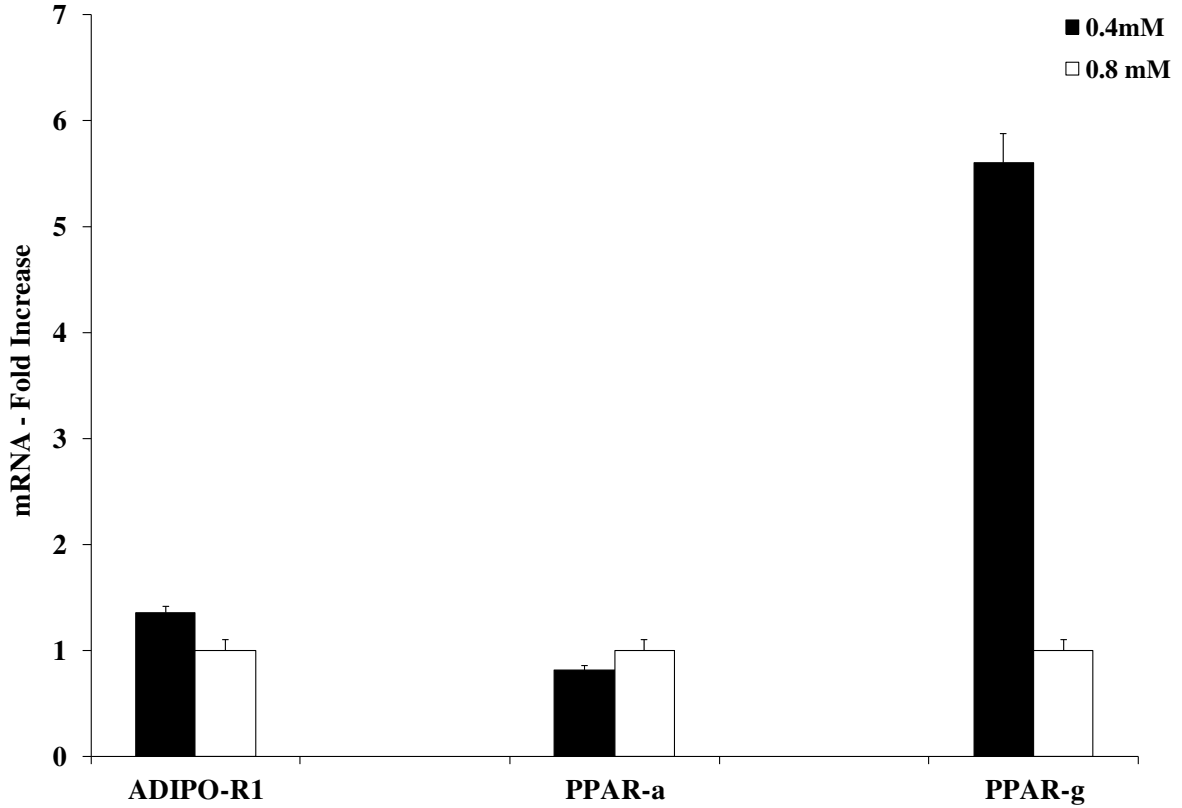
Etwebi, et al. manuscript in preparation

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



Soltani & Etwebi, manuscript in preparation

Effect of Mg^{2+} on PPAR Expression in Hepatocytes

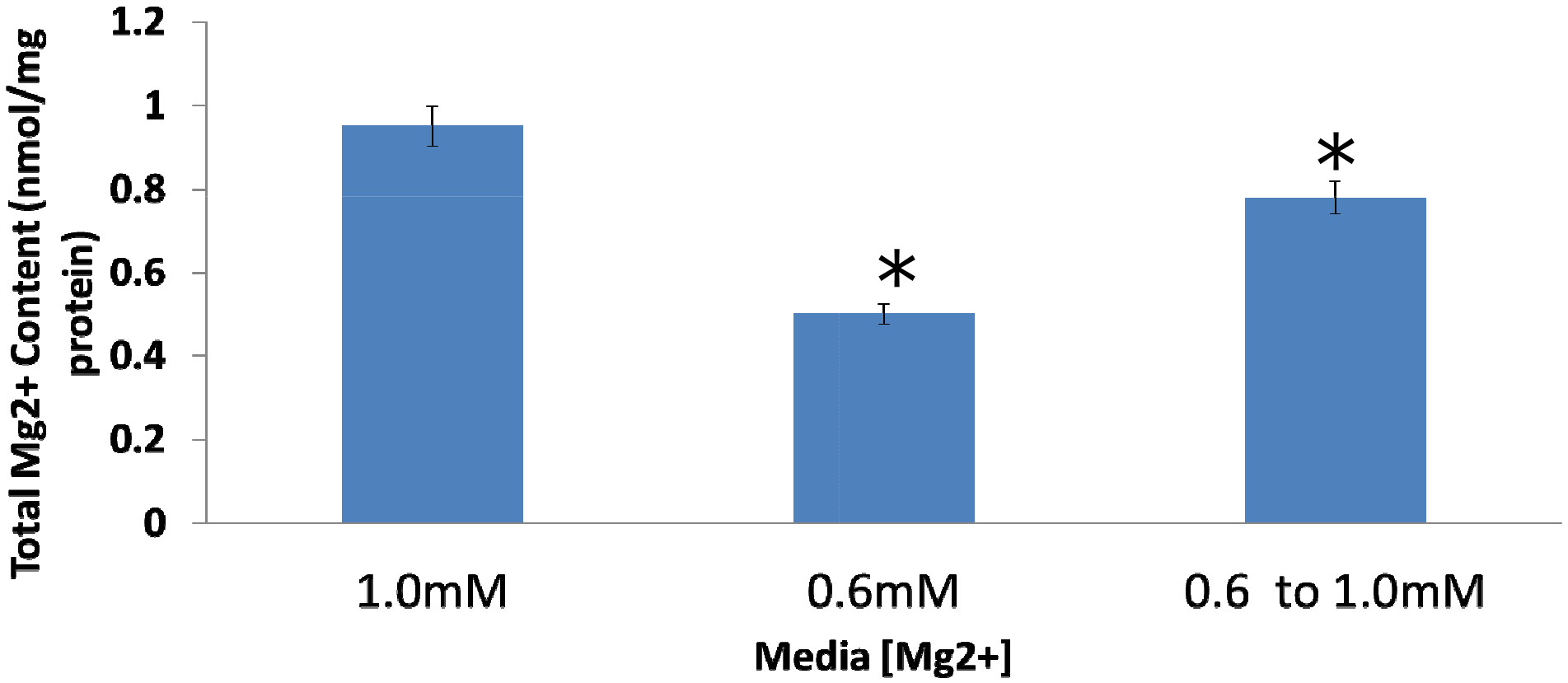


Soltani & Etwebi, manuscript in preparation

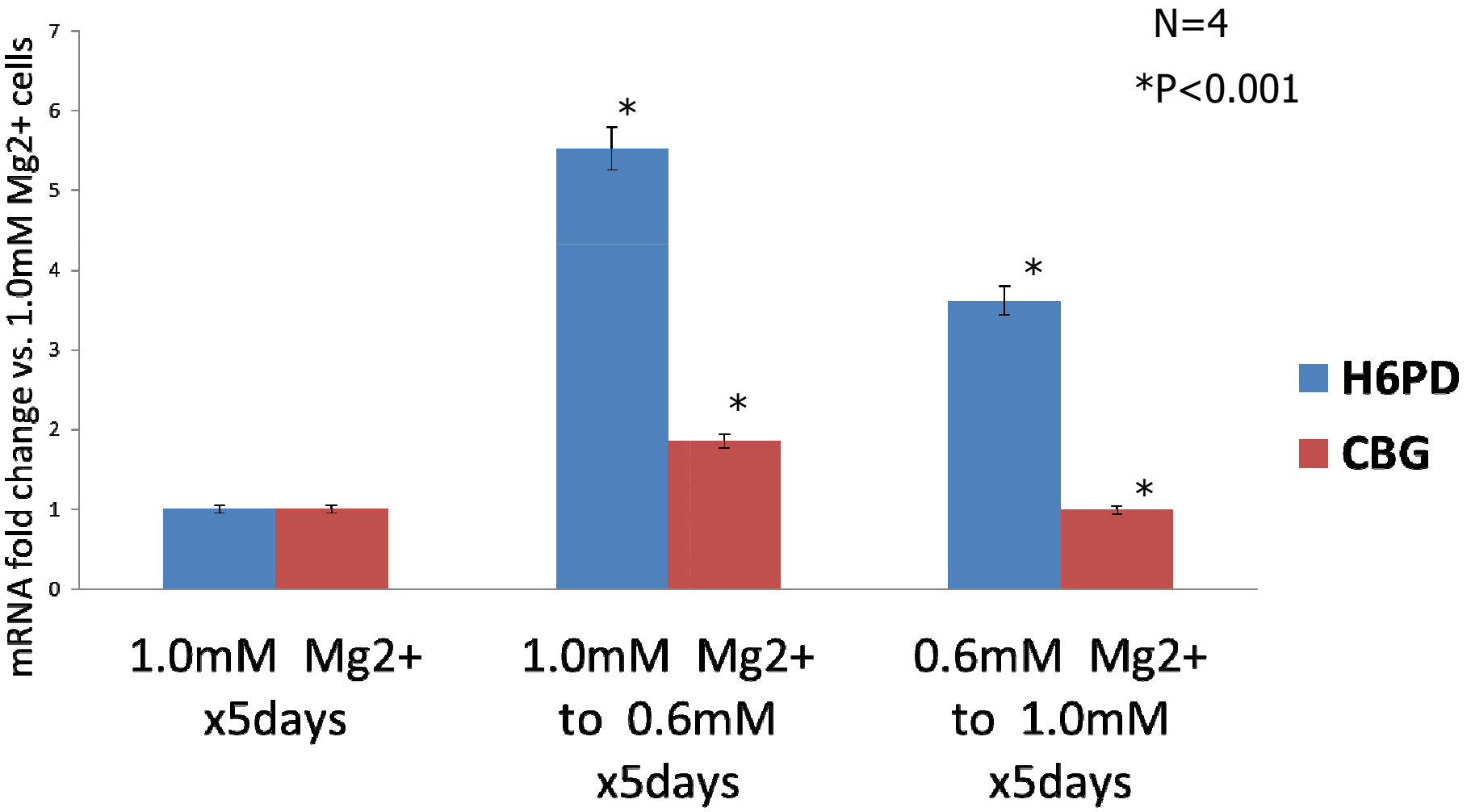
Mg^{2+} Recovers after 5 days

N=3

*P<0.0



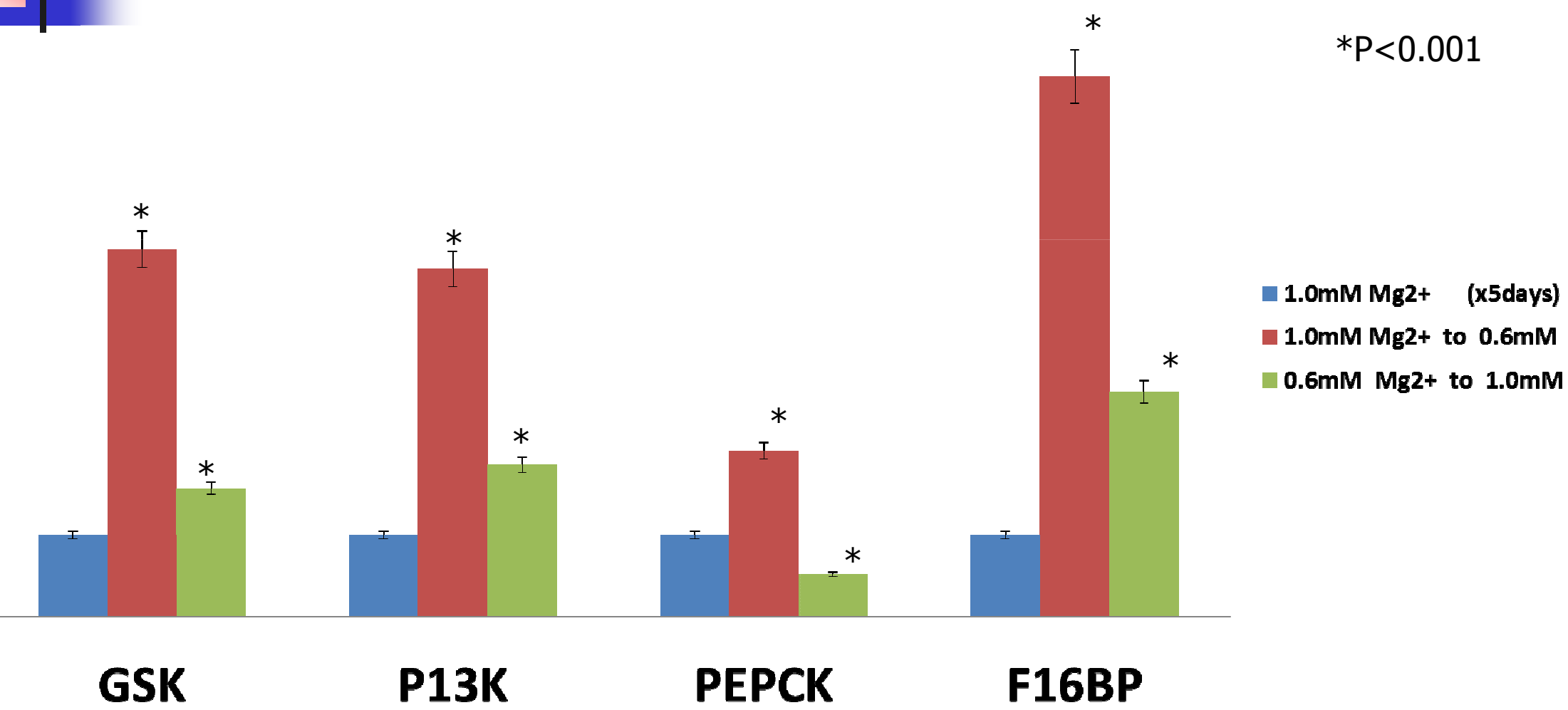
Genes Recover from Mg^{2+} Deficiency - #1

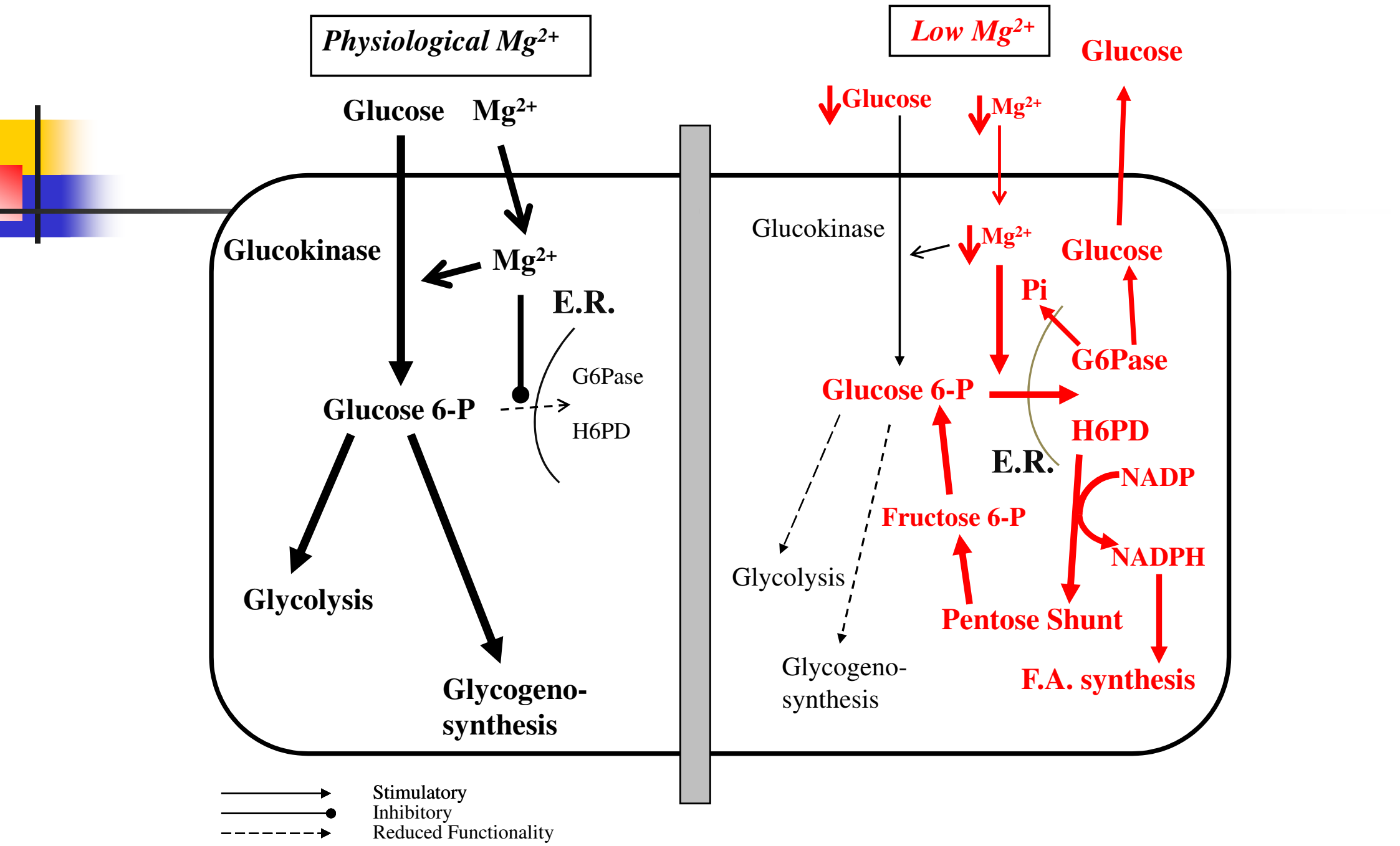


Genes Recover from Mg^{2+} Recovery - #2

N=4

*P<0.001




Physiological Mg2+
Glucose
Mg2+
Glucokinase
Mg2+
E.R.
G6Pase
H6PD
Glucose 6-P
Glycolysis
Glycogeno-
synthesis
Low Mg2+
Glucose
Glucose
Mg2+
Glucokinase
Mg2+
Glucose
Pi
G6Pase
Glucose 6-P
H6PD
E.R.
NADP
Fructose 6-P
NADPH
Glycolysis
Pentose Shunt
Glycogeno-
synthesis
F.A. synthesis
Stimulatory
Inhibitory
Reduced Functionality

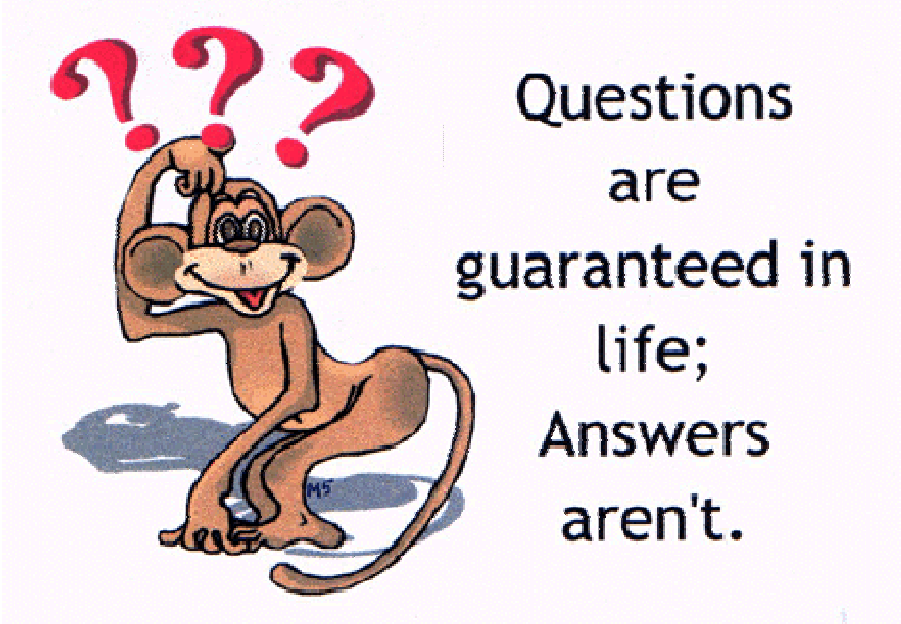
Conclusion

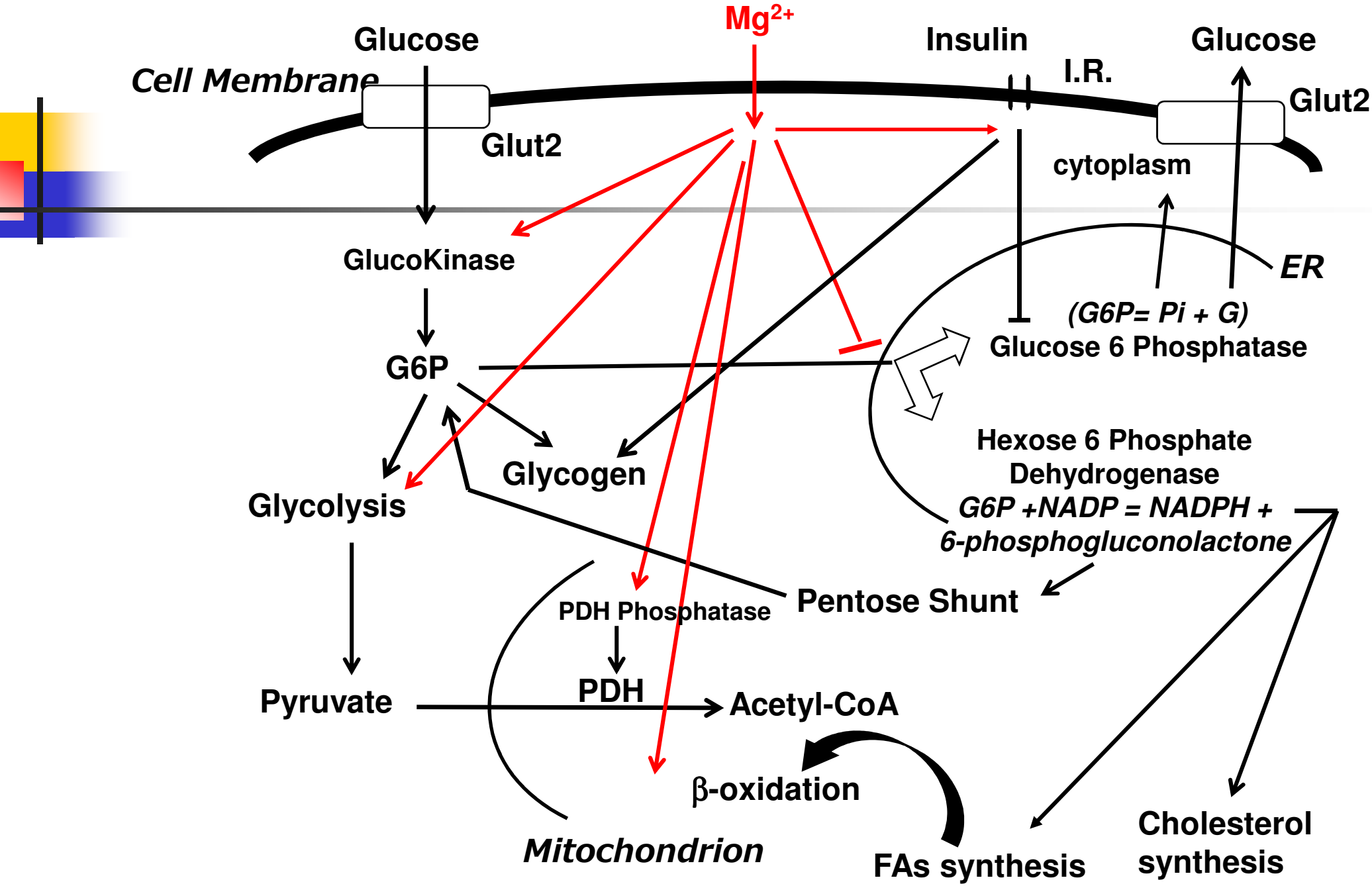
- Cytosolic magnesium concentration regulates the amount of G6P entering into the E.R.
- A decrease in intracellular magnesium enhances the entry of G6P into the E.R. and favors its conversion to 6-PGL via G6PD, resulting in an increased production of NADPH and a stimulation of the Pentose Phosphate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

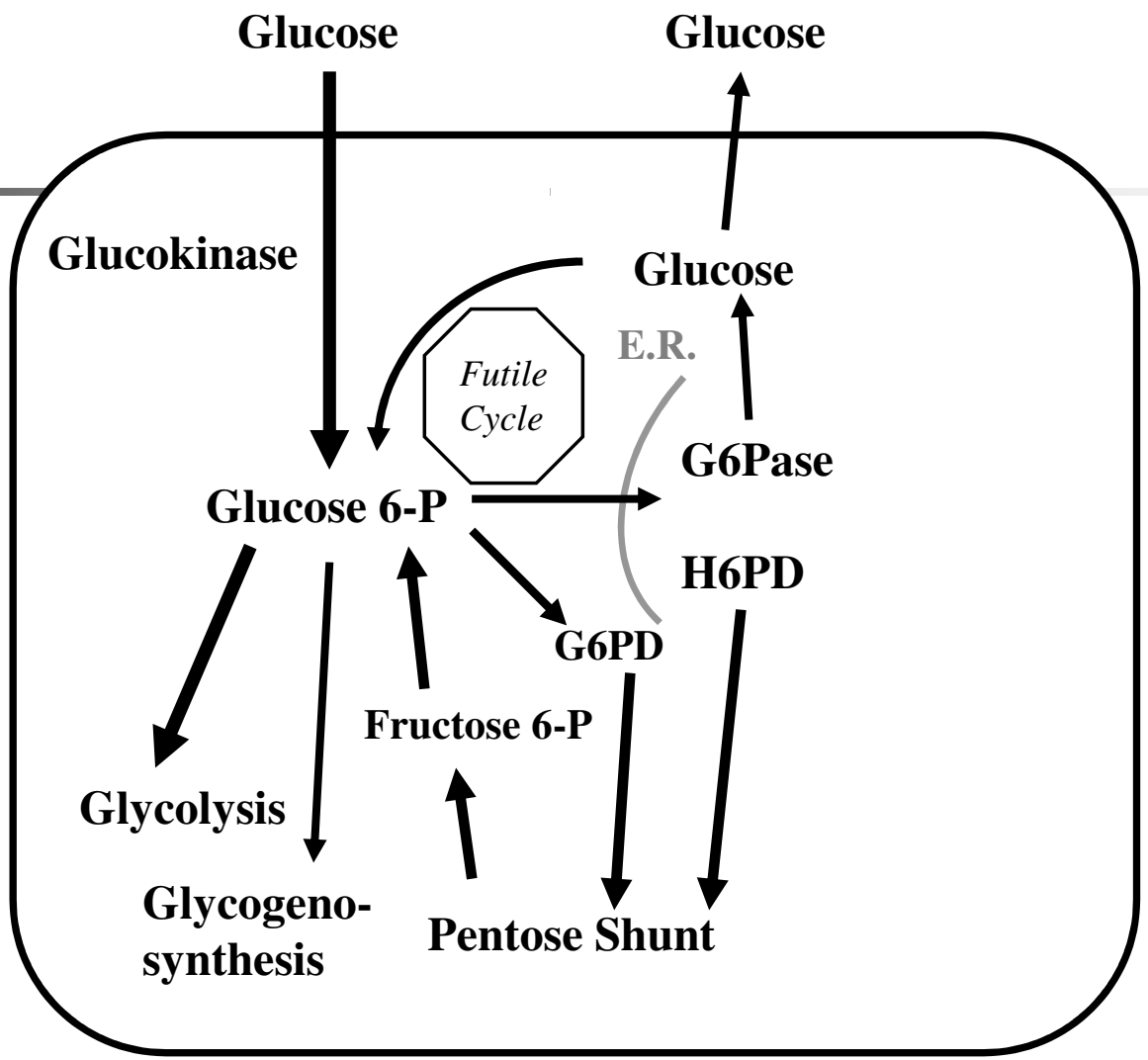
Future Directions

- Determine how magnesium deficiency promotes H6PD and 11β-HSD1 upregulatio
- Determine whether the increase in cortisol induces insulin resistance
- Validate the effect of Mg^{2+} deficiency on NADPH production and cortis conversion in hepatocytes from normal and obese (or diabetic) patients

THANK YOU




Mg2+
Glucose
Insulin
Glucose
Cell Membrane
I.R.
Glut2
Glut2
cytoplasm
GlucoKinase
ER
(G6P= Pi + G)
Glucose 6 Phosphatase
G6P
Hexose 6 Phosphate
Dehydrogenase
Glycogen
Glycolysis
G6P +NADP = NADPH +
6-phosphogluconolactone
Pentose Shunt
PDH Phosphatase
PDH
Pyruvate
Acetyl-CoA
β-oxidation
Mitochondrion
FAs synthesis
Cholesterol
synthesis


Glucose
Glucose
Glucokinase
Glucose
E.R.
Futile
Cycle
G6Pase
Glucose 6-P
H6PD
G6PD
Fructose 6-P
Glycolysis
Glycogeno-
synthesis
Pentose Shunt