Global Metabolic Changes and Cellular Dysfunction in Diamide Challenged G6PD-Deficient Red Blood Cells

Dr. Daniel Tsun-Yee Chiu, Professor

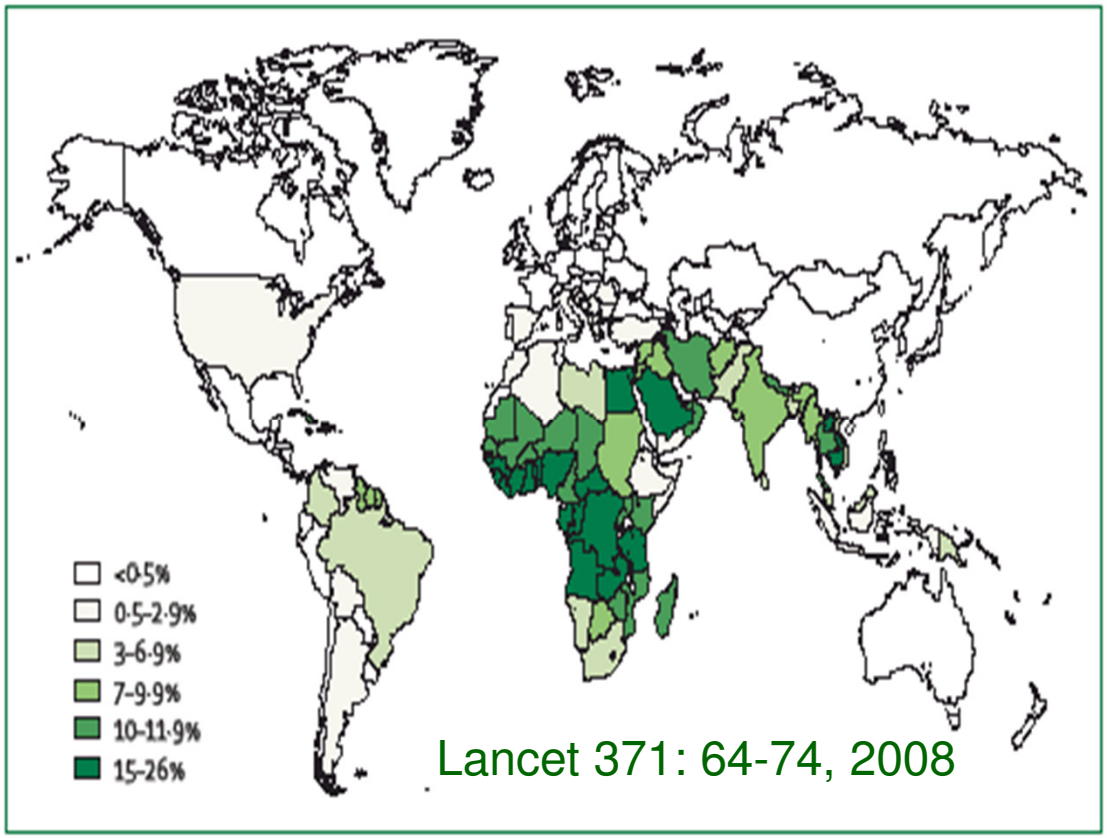
Graduate Institute of Medical Biotechnology,
Chang Gung University, Taiwan
Dept. of Laboratory Medicine,
Chang Gung Memorial Hospital (Linkou), Taiwan

Email: dtychiu@mail.cgu.edu.tw

September 30, 2014

G6PD deficiency (Also known as Favism)

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a most common enzyme deficiency affecting over 400 million people worldwide, causes a spectrum of diseases including, acute and chronic hemolytic anemia, neonatal jaundice and etc.



Lancet 371: 64-74, 2008

J Med Screen 19:103-104, 2012

G6PD deficiency in Taiwan:

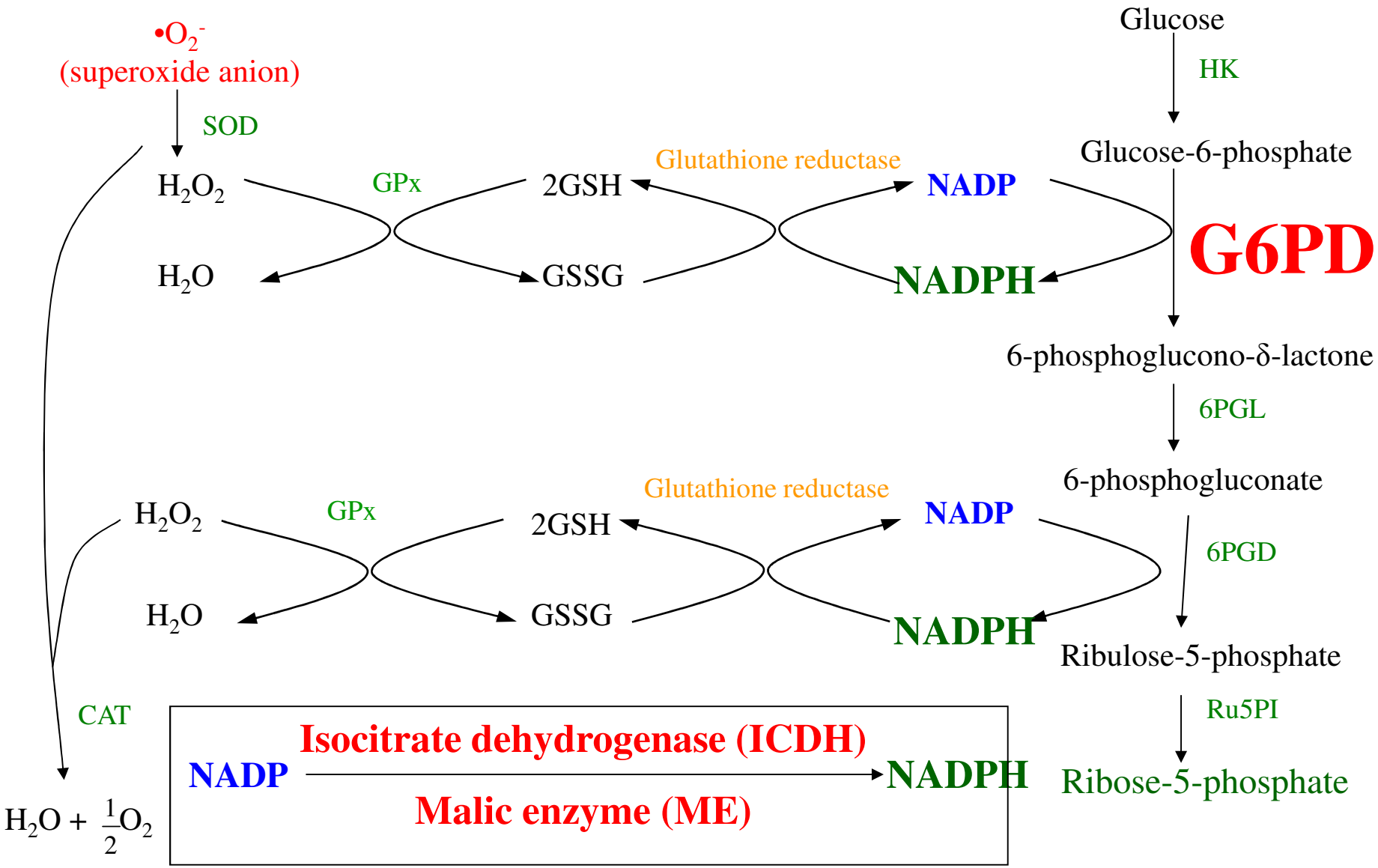
Male 3%

Female 0.9%

Redox Rep 12: 109-18, 2007

Free Rad Res 48: 1028-48, 2014

Biochemical and antioxidant roles of G6PD: to regenerate NADPH and Ribose



Our Previous findings related to NADPH/GSH metabolism in G6PD-deficient cells:

1. NADPH status modulates oxidant sensitivity in normal & G6PD-deficient erythrocytes

   Scott MD et at. Blood 77: 2069-2064, 1991

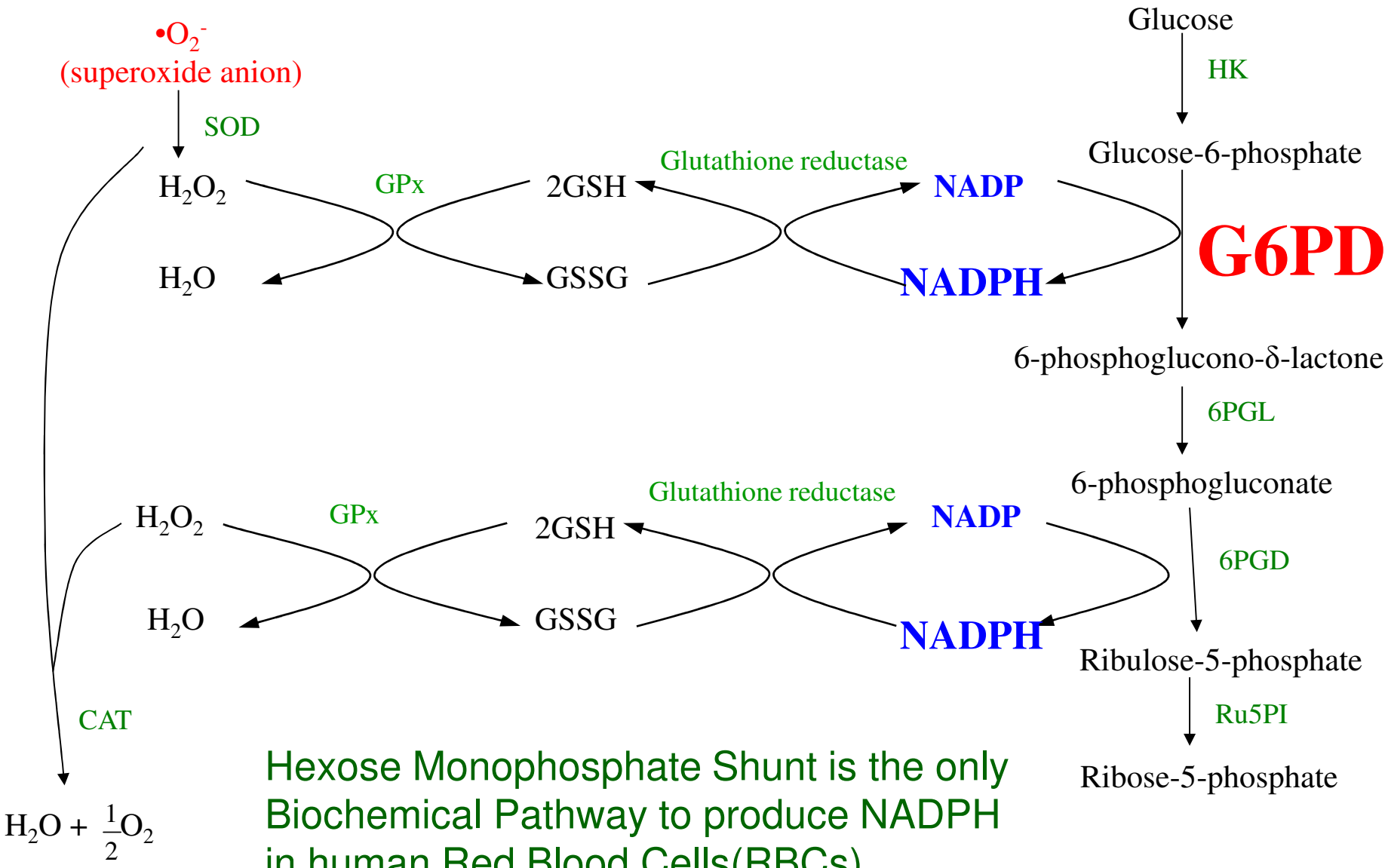
2. Ineffective GSH regeneration enhances G6PD-knockdown Hep G2 cell sensitivity to diamide-induced oxidative damage

   Gao LP et al. Free Rad Biol Med 47: 529-535, 2009

3. Characterization of global metabolic responses of G6PD-deficient hepatoma cells to diamide-induced oxidative stress

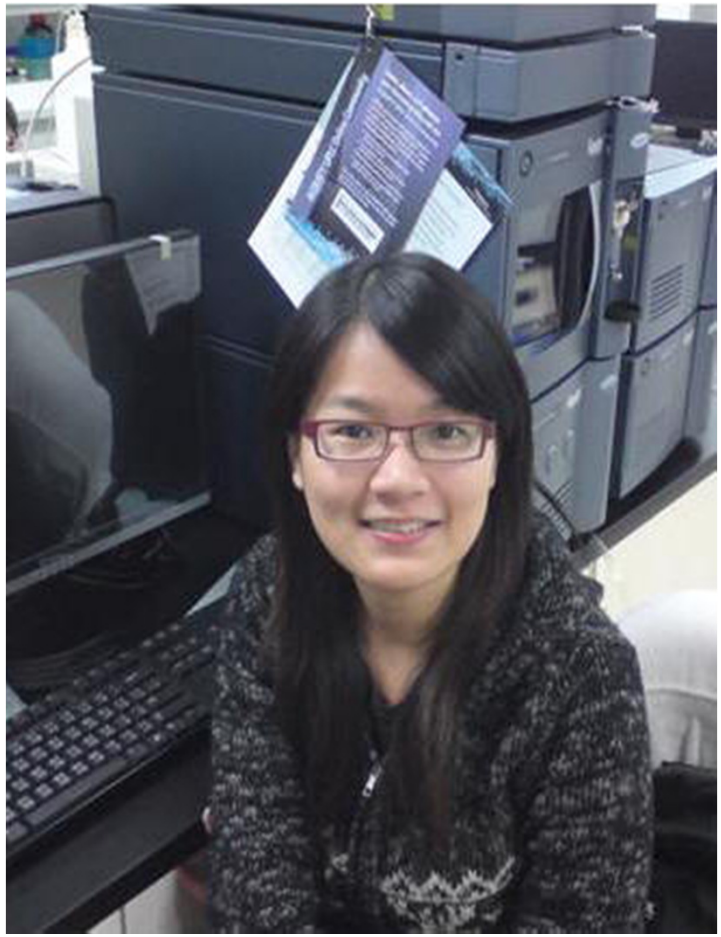
   Ho HY et al. Free Rad Biol Med 54: 71-84, 2013

Antioxidant role of G6PD in Human Red Cells: to regenerate NADPH



Hexose Monophosphate Shunt is the only Biochemical Pathway to produce NADPH in human Red Blood Cells(RBCs)

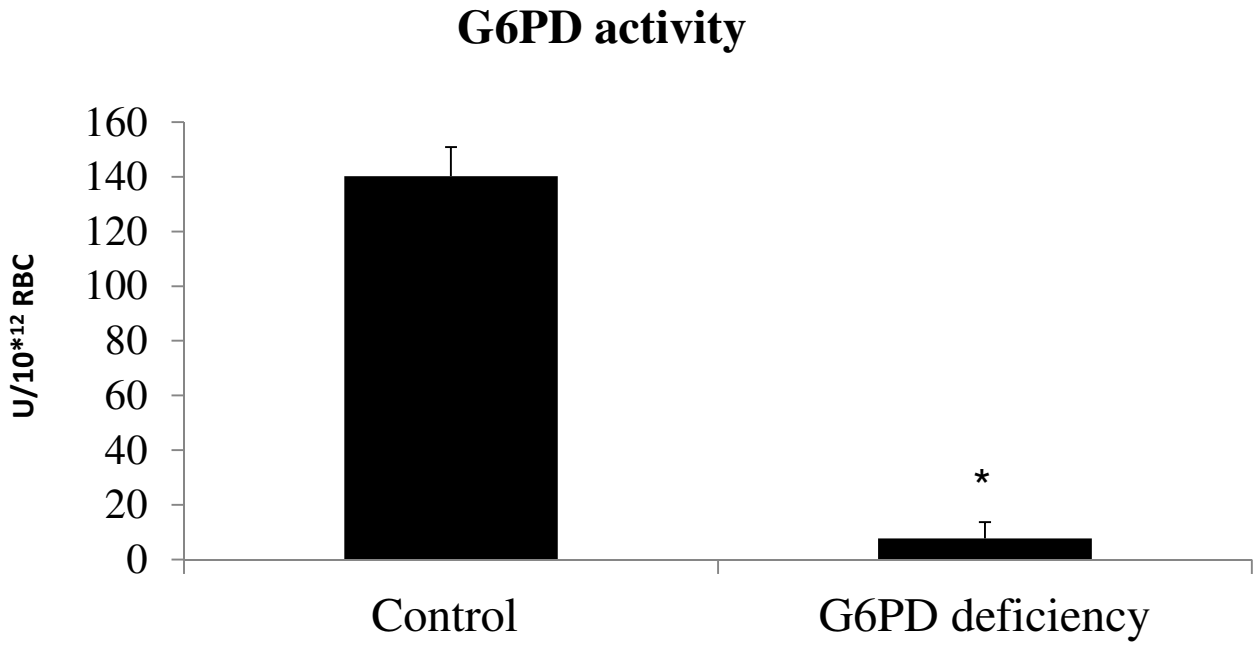
Metabonomic Profiles in Human Red Blood Cells from patients with G6PD Deficient upon Oxidant Challenge



Tang SY (唐湘瑜)

(Manuscript in preparation and is part of her Ph.D thesis)

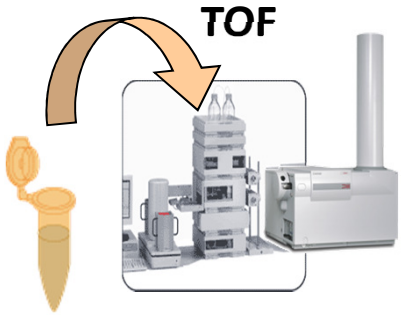
G6PD activity in normal and G6PD deficient whole blood



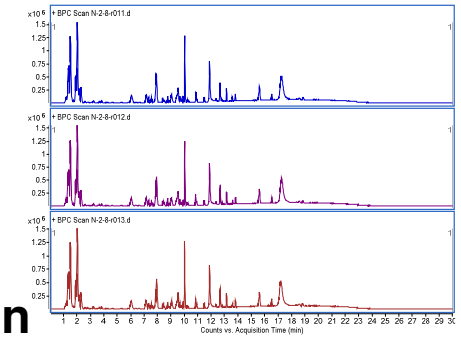
G6PD activity in G6PD deficient RBCs (n=11) and control RBCs (n=11). Data was shown as U/ 10^{12} of RBC numbers. *P<0.05, patients vs control samples.

Typical workflow for MS-based metabolic profiling

Sample collection and pretreatment

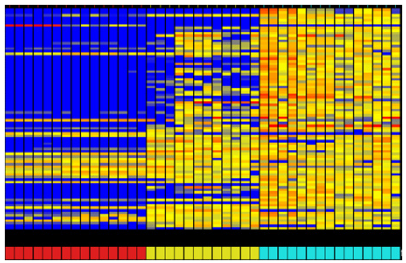


Data collection

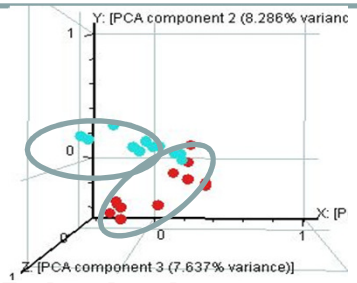


Data analysis

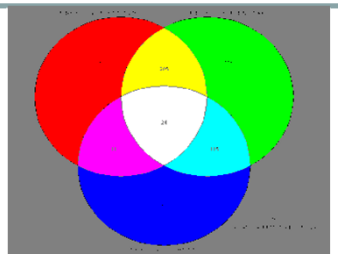
Statistical analysis / Bioinformatics



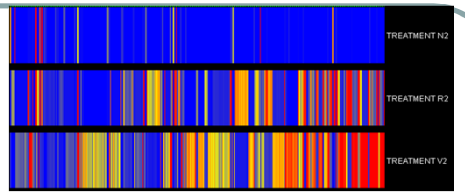
Condition tree (Clustering)



Principal component analysis (PCA)



Volcano plot



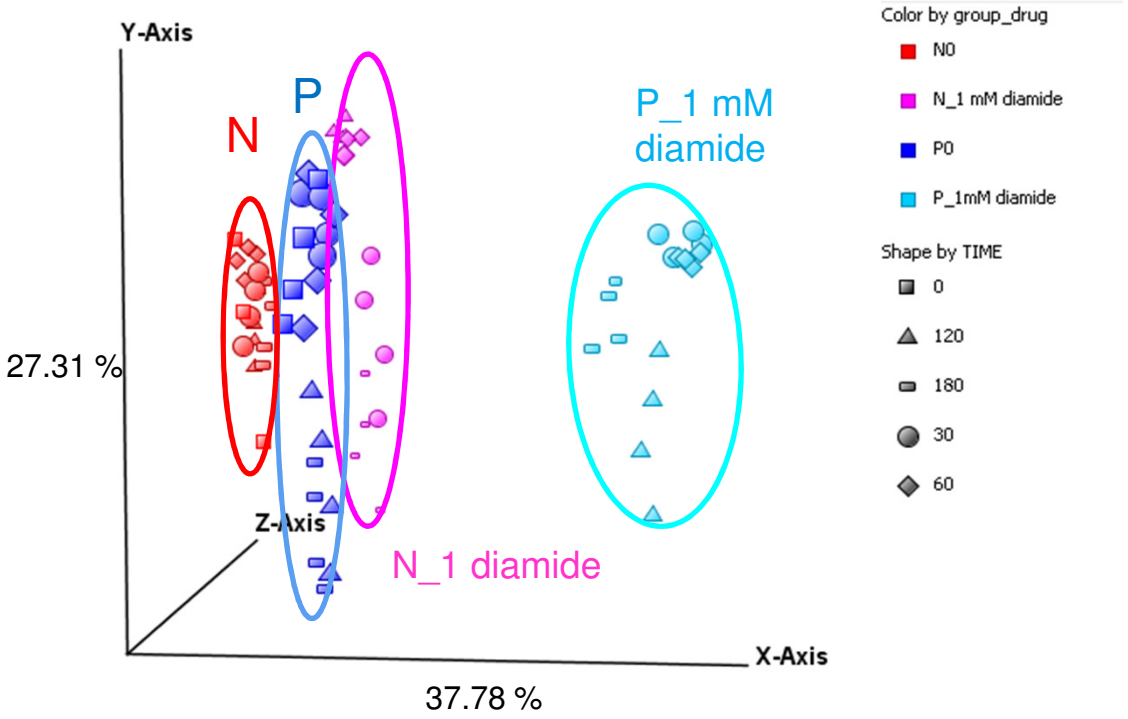
Heat map (ANOVA)

Metabolome: Metabolic pathways and interaction

Targeted Metabolite identification

Function and dysfunction

Principal component analysis (PCA) in G6PD deficient and control RBCs with or without diamide-treatment



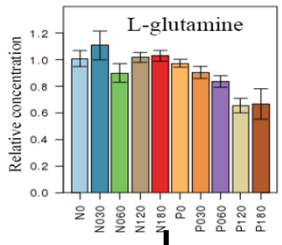
Principal component analysis (PCA) of metabolomes in control and G6PD deficient RBCs with or without diamide treatment. Both groups were un- or treated with 1mM of diamide for various time period. Features were acquired in ESI positive ion mode.

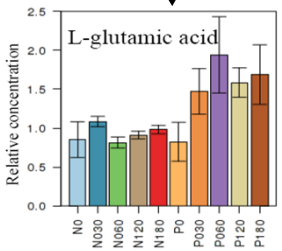
Altered glutathione metabolism in G6PD deficient RBCs leading to the formation of ophthalmic acid upon diamide treatment

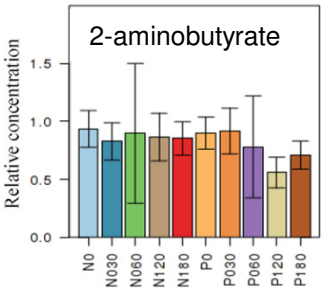
Normal GSH Synthetic Pathway

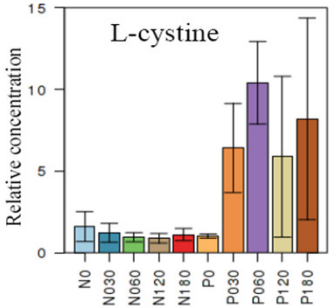
Altered GSH Synthetic Pathway

Methionine Cycle → → → Cysteine

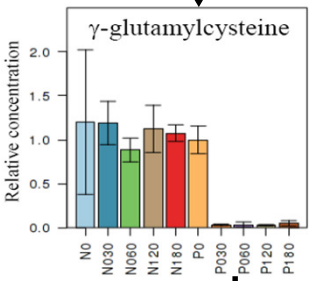


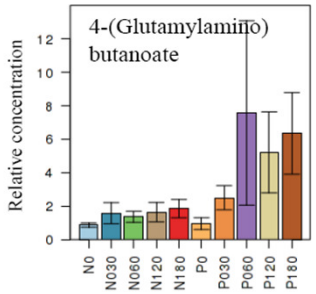


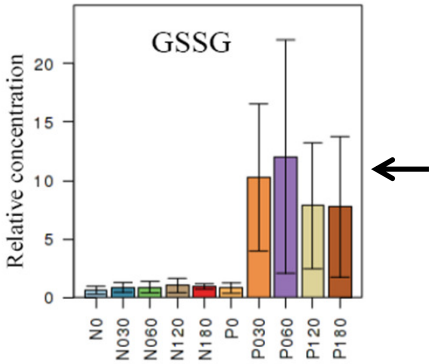


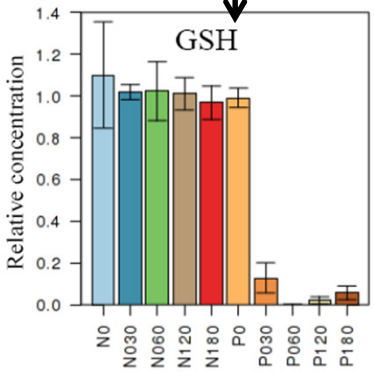


γ-glutamylcysteine synthetase

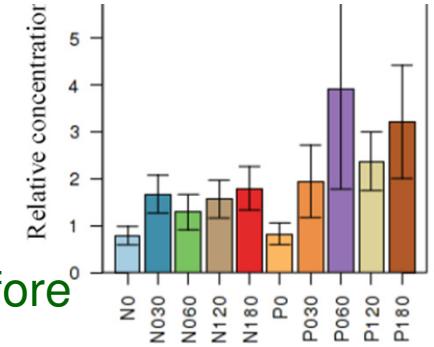








Ophthalmic acid

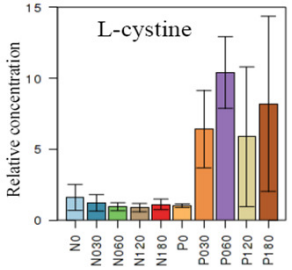


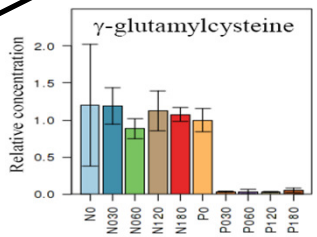
Ophthalmic acid has never been reported in human RBCs before

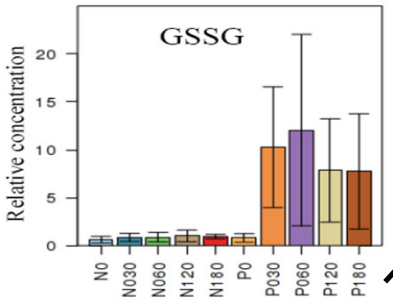
Such alterations are mainly due to the shunting from GSH regeneration via the glutathione reductase system to GSH synthesis via γ-glutamylcysteine synthetase

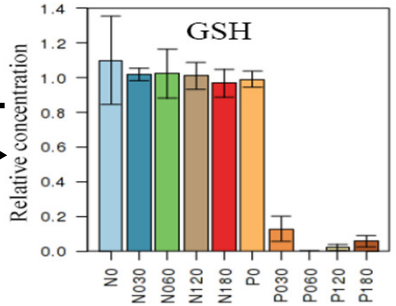
Normal GSH Synthetic Pathway

Methionine Cycle → → → Cysteine









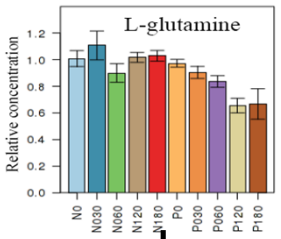
GR

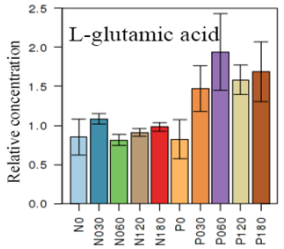
NADPH

NADP

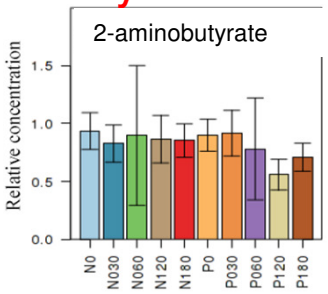
G6PD

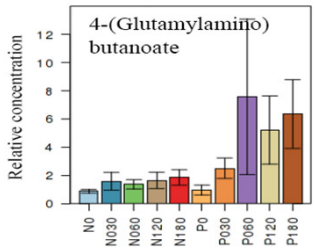
Altered GSH Synthetic Pathway



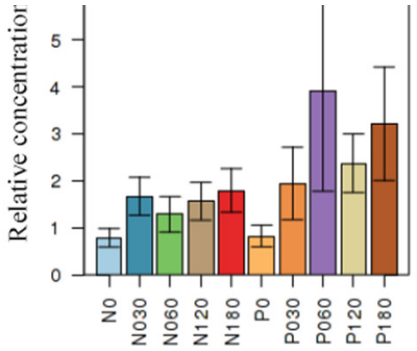


γ-glutamylcysteine synthetase



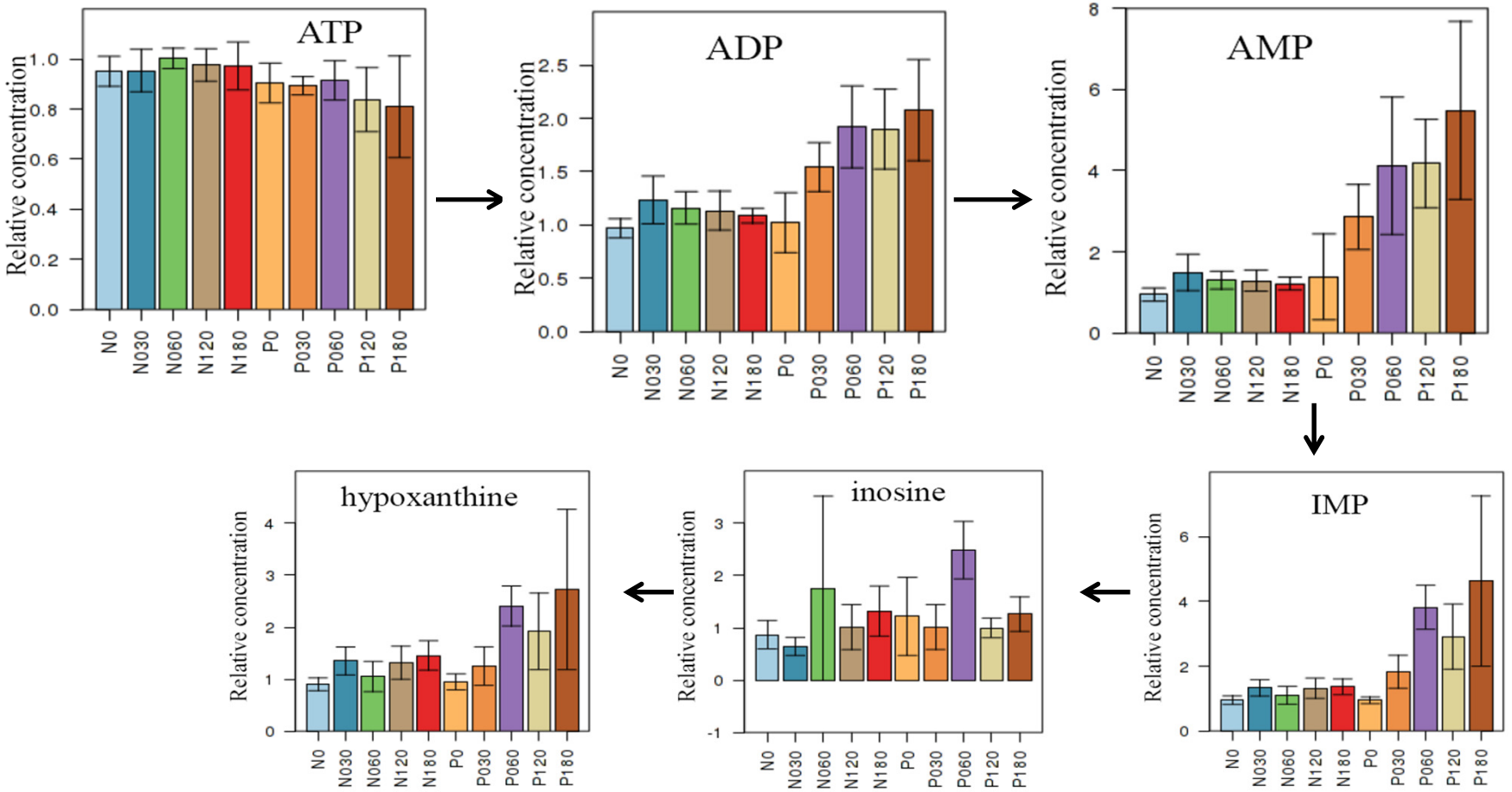


Ophthalmic acid



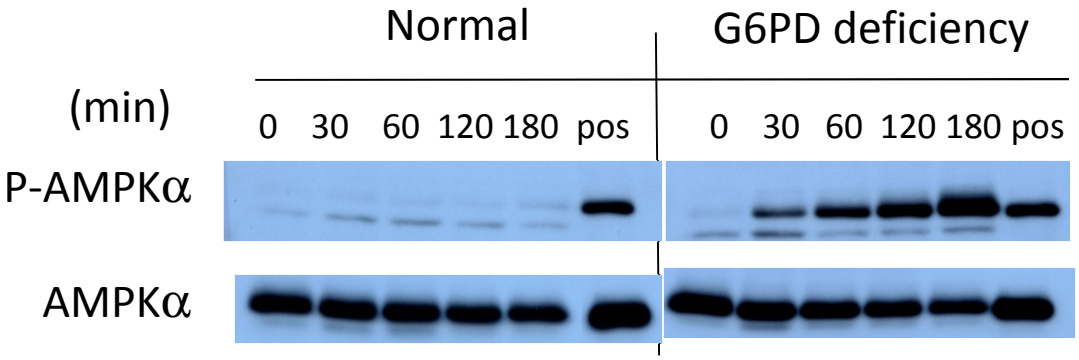
Shunting from GSH regeneration to synthesis

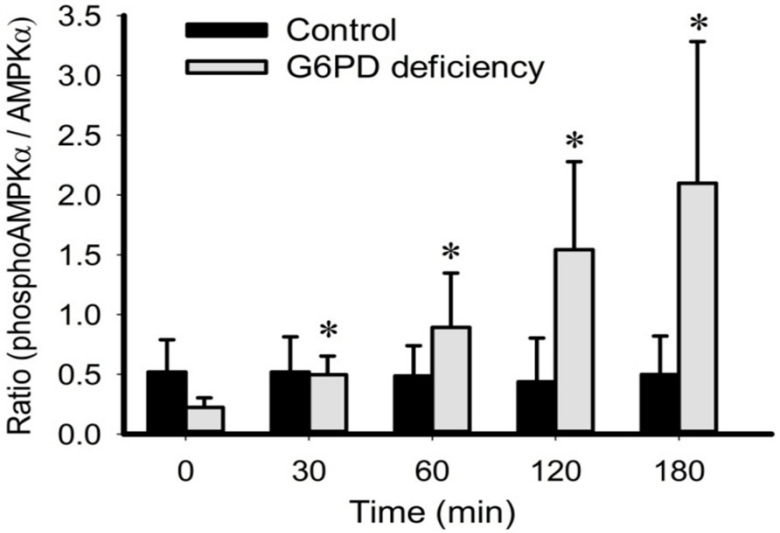
Shunting from GSH regeneration to GSH synthesis is accompanied by Exhaustive Energy Consumption in G6PD deficient RBCs upon diamide treatment



A dramatic increase in AMP level

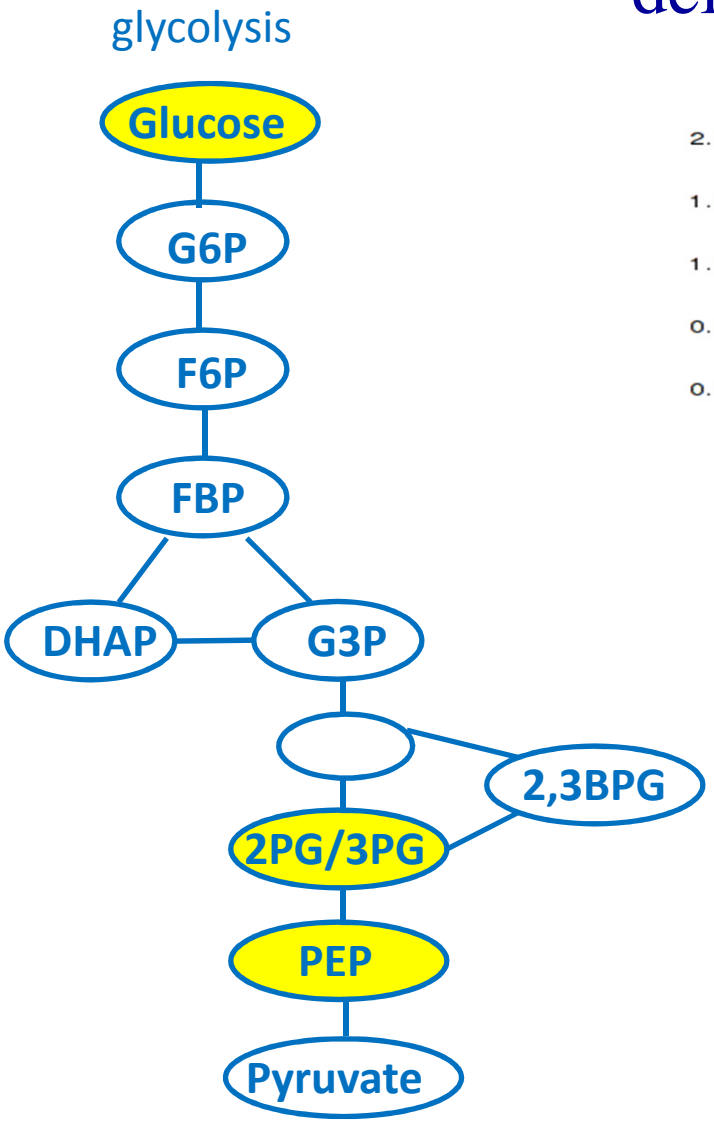
AMP accumulation due to exhaustive ATP consumption activates AMP protein kinase (AMPK)

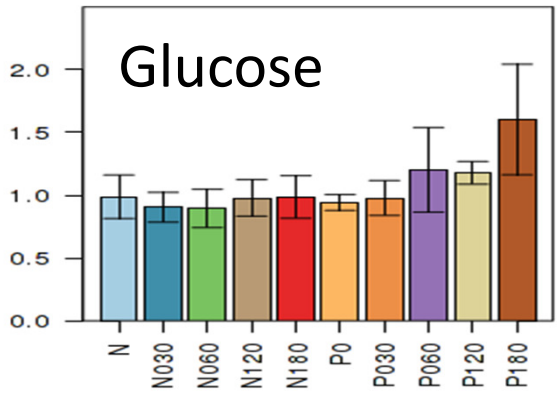


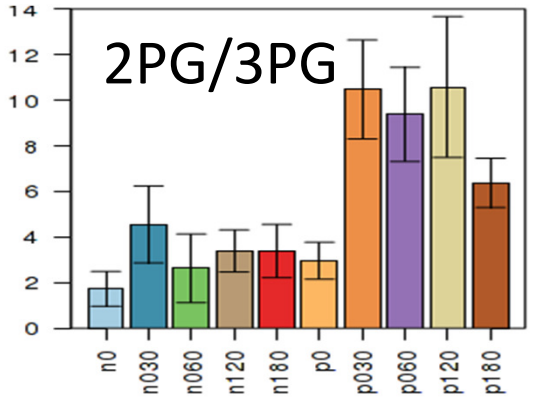


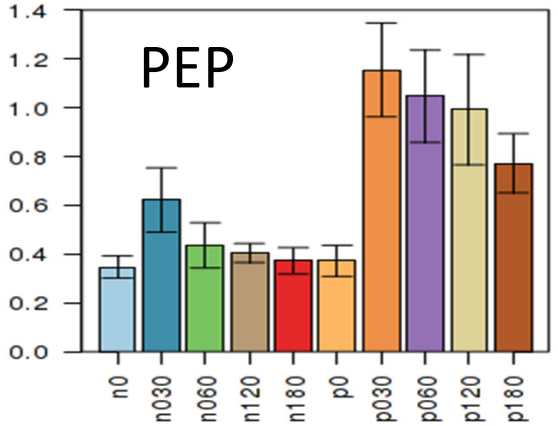
Level of phospho AMPK alpha and total AMPK alpha protein in RBCs from normal and G6PD deficient. RBCs from normal and G6PD-deficient individuals were treated with 1 mM DIA for 0 min, 30 min, 60 min, 120 min, or 180 min, and detected by immunoblotting

Diamide treatment enhances glycolytic activities in G6PD deficient RBCs

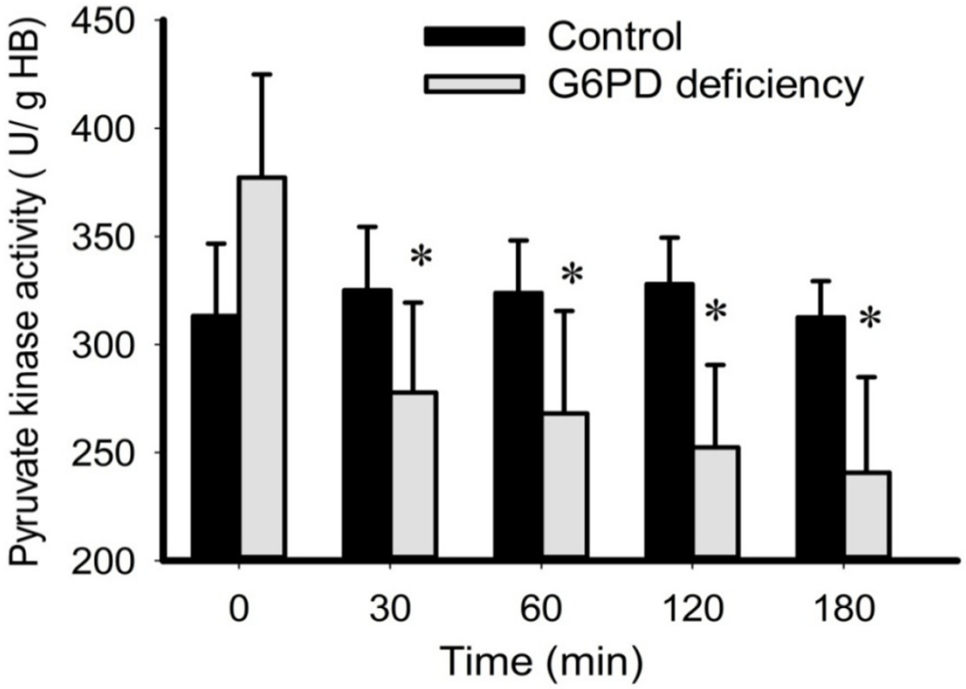








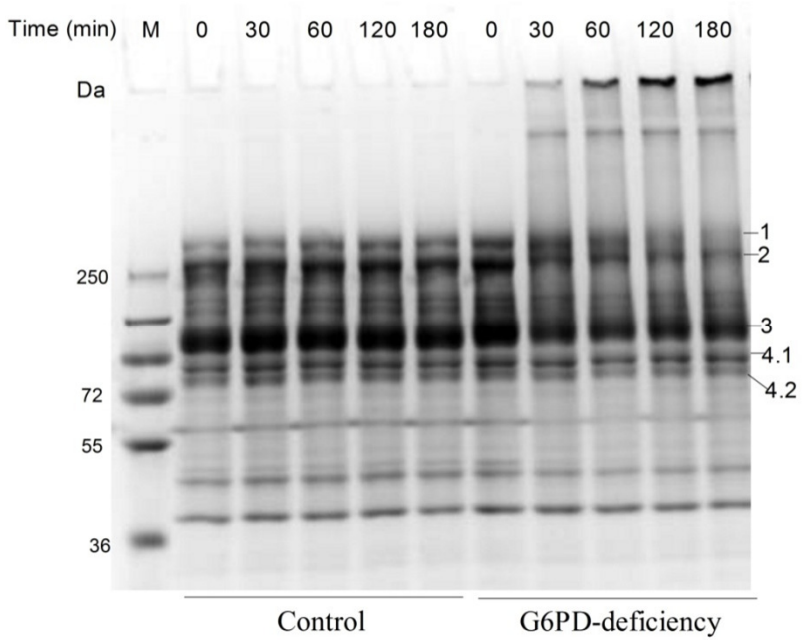
Pyruvate Kinase (PK) was blocked in G6PD-deficient RBCs upon diamide treatment



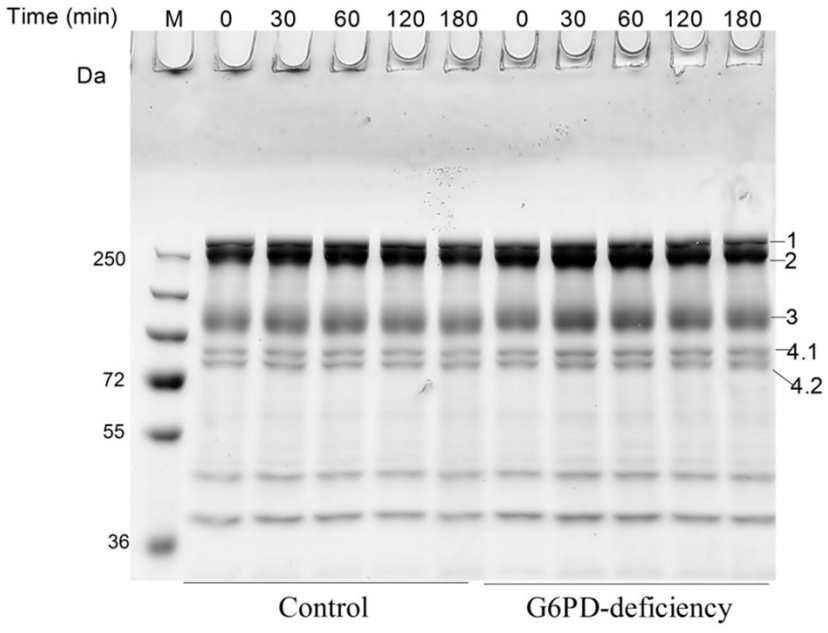
Control and G6PD deficient RBCs were treated with 1 mM diamide for various time periods. After lysing the cells, pyruvate kinase activity was assayed (mean± SD) and analyzed by Student's t test, n=4.
*indicates $p<0.05$.

Linking metabolic alterations to functional abnormalities 1:

Defective GSH metabolism with the appearance of high-molecular weight protein aggregates in G6PD deficient RBCs upon oxidant treatment



diamide

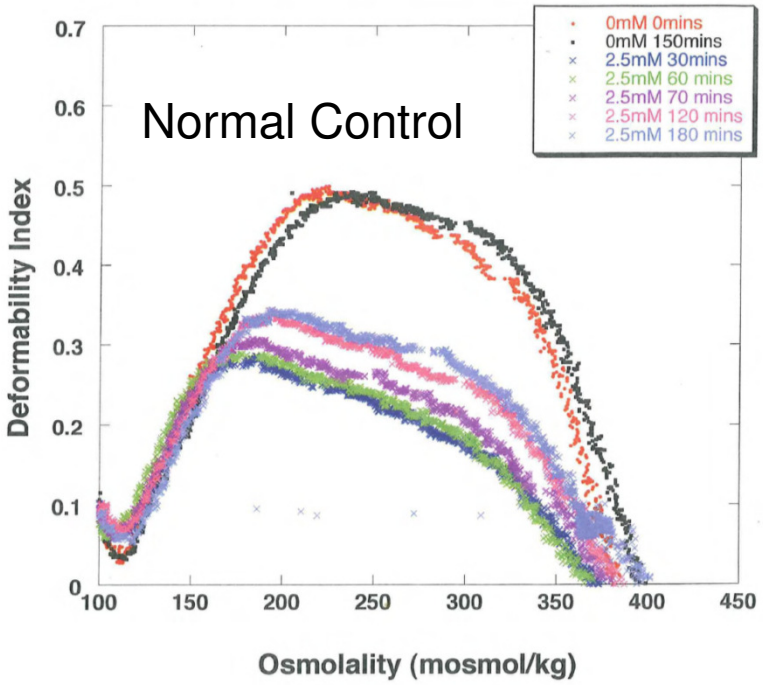


diamide + DTT

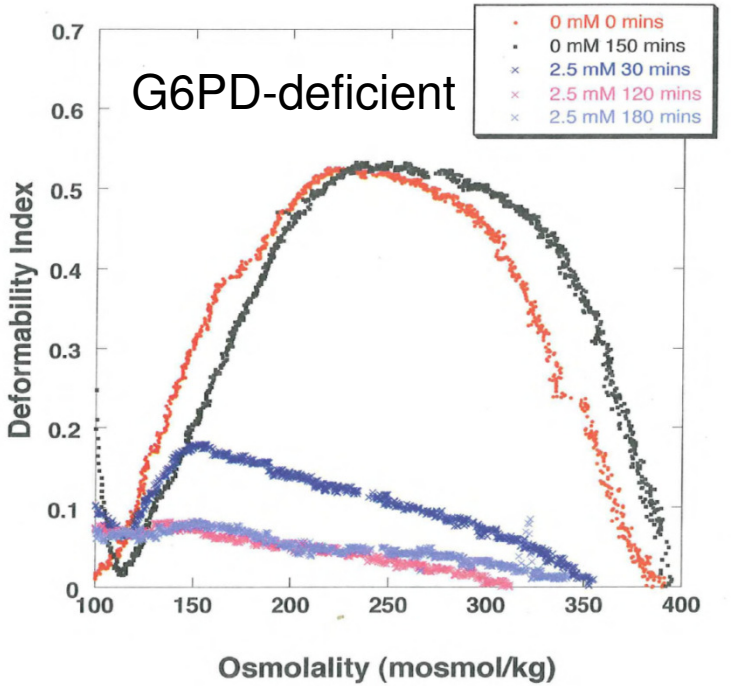
Modification of RBC proteins after 1 mM diamide treatment. SDS–PAGE analysis revealed that treatment with diamide (left panel) induced the appearance of high-molecular weight protein aggregates. The oxidized protein can be restored by DTT treatment (right panel)

Linking metabolic alterations to functional abnormalities 2:

Dramatic and Irreversible decrease in deformability of G6PD-deficient RBCs upon oxidant treatment



A- Effect of Diamide on the deformability of normal RBCs.



B- Effect of Diamide on the deformability of G6PD-deficient RBCs.

Both GSH and ATP depletions can contribute to the dramatic reduction of deformability in G6PD-deficient RBCs leading to a rapid removal of these RBCs from circulation.

Summary & Conclusion from our metabonomic study

1. Diamide treatment induces major alterations in GSH related metabolites in G6PD deficient RBCs including the appearance of unusual metabolites such as opthalmic acid which has never been reported in human RBCs before.
2. Such impairment in GSH related metabolism is mainly due to the shunting from GSH regeneration to GSH synthesis and is accompanied by exhaustive ATP consumption and enhanced glycolytic activities in G6PD deficient RBCs. Unfortunately, the last step in glycolysis catalyzed by pyruvate kinase(PK) to produce ATP is blocked in G6PD-deficient RBCs due to the inactivation of PK by diamide.
3. Changes in metabolic activities cause functional defects such as membrane protein aggregation and decreased in RBC deformability of G6PD-deficient RBCs and these new findings provide additional explanation concerning acute hemolytic anemia in G6PD-deficient patients upon encountering oxidative stress such as favism and infection.

In conclusion, this metabonomic study shows that G6PD-deficient RBCs desperately struggle to maintain redox homeostasis upon oxidant challenge to avoid cell death but without success.

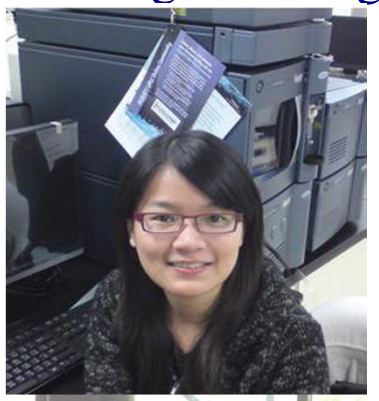
Acknowledgement

Dr. Mei-Ling Cheng, Associate Prof., Chang Gung Univ.
Dr. Hung-Yao Ho, Associate Prof., Chang Gung Univ.

Other Collaborators of Chang Gung
Prof. Ming-Shi Shiao
Dr. Chih-Ching Wu, Assistant Prof. CGU
Prof. SJ Lo,

Students
Hsin-Yi Lin,
Yu-Chia La

Dr. Shin-Ru Lin,
Hsiang-Yu Tang

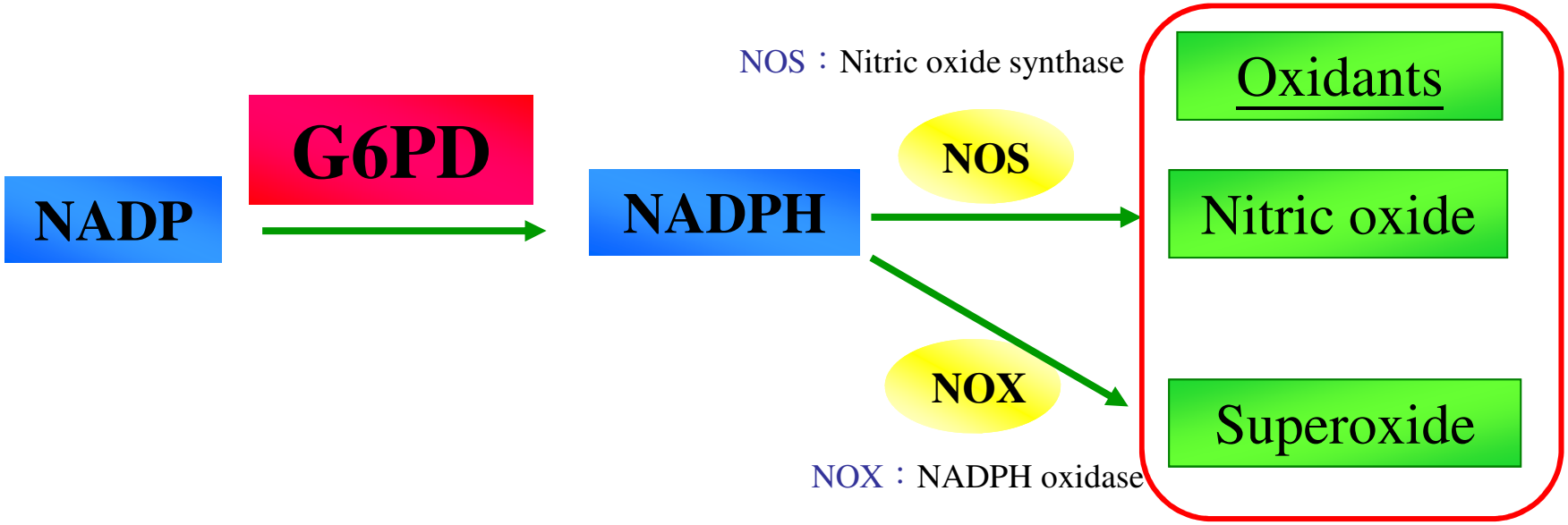


Postdoctoral Fellow
Dr. Yi-Hsuan Wu
Research Assistants
Yi-Yun Chiu, Hui-Ya Liu

Collaborators of other Institutes
Dr. Li-Ping Gao (Lanzhou Univ., China)
Dr. Chang-Jun Lin (Lanzhou Univ., China)
Prof.. Arnold Stern (NYU, USA)
Prof. Frans Kuypers(CHORI, Oakland/UC Berkeley, USA)

Dr. Hung Chi Yang

Pro-oxidant role of G6PD : Provides substrate to generate free radicals



Sample	Nitrite production
	LPS (5 μg/ml)
Normal ($n=4$)	252 ± 135
G6PD-deficient ($n=3$)	70 ± 26

The results are shown as mean ± S.D.

(% of resting cells)

Decreased NO & Superoxide production **FEBS Lett .** 436:411-4, 1998

But

Effective Neutrophil Extra-cellular Trap Formation **Free Rad Res** 47:699-709, 2013

G6PD deficiency-induced cellular abnormalities & related clinical problems (beyond RBCs)

G6PD Deficiency (Redox Report 12: 109, 2007; Free Rad Res 48: 1028, 2014)

Decreased NADPH Production

Redox Imbalance

Decreased NO & Superoxide production

FEBS Letters 436:411-414, 1998

But Effective Neutrophil Extra-cellular Trap Formation
Free Rad Res 47:699, 2013

Increased susceptibility to:
1.Corona Virus infection.
J Infect Dis. 197:812-6, 2008
2. **Enterovirus infection** .
J Gen. Virol.89:2080-9, 2008
3. Protection by EGCG
J Agr Food Chem 57:6140-7, 2009

Accelerated Senescence
Free Rad Biol Med 29: 156-169, 2000;
FEBS Letters 475: 257-262, 2000

Retarded Cell Growth
Free Rad Biol Med 29: 156-169, 2000

Increased susceptibility to certain diseases
Jpn J Canc Res 92: 576-581, 2001
Endocrine 19: 191-196, 2002
[**Ophthalmic Epid.** 13: 109-114, 2006]

Increased Susceptibility to Oxidative Insult
Free Rad Biol Med 36: 580, 2004; **Cytometry** Part A 69: 1054, 2006
J Agr Food Chem 54: 1638, 2006; **Free Radic Res**. 41:571, 2007
Free Rad Biol Med 47: 529, 2009

Alterations in 1. Signal transduction (MAPK) Free Rad Biol Med 49: 361, 2010
2. Proteomic J Proteom Res 12: 3434, 2013
3.Metabonomic Free Rad Biol Med 54**:** 71, 2013
4. C. elegans model. Cell Death Disease 4, e616, 2013