

# **The Plasma Proteome: A New Potential Approach for Assessing Multiple Micronutrient Deficiencies**

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# Micronutrient Deficiencies Affect the World's Poorest

## Nutrient Deficiencies

- **Vitamin A**
- **Iron**
- **Folate**
- **Iodine**
- **Zinc**

...but also ...

- **Vitamins D, E, K**
- **B-complex**
- **Vitamin C**
- **Selenium**
- **Copper**
- **Others**

...compounded by...

- **Protein-energy deficits**
- **Inflammation**
- **Infection**



Demra Bustee Camp,  
Dhaka 1977; K West.

## Child and Maternal Health Problems

### • **Infant or Child**

- Infection
- Poor growth
- Impaired cognition, motor & behavioral development
- Mortality

### • **Mother**

- Short stature
- Infection/sepsis
- Obstetric problems
- Mortality

**Chronic disease, disability, mortality**

# Vitamin A Interventions Tested in S Asia

- **Newborn vitamin A reduces infant mortality by ~15%**  
Rahmathullah et al BMJ 2003; Klemm R et al Pediatr 2008
- **Preschool vitamin A reduces child mortality by 30%**  
Sommer et al Lancet 1986; Rahmathullah et al NEJM 1990  
West et al Lancet 1991
- **...and hearing loss from ear infection by ~40%**
  - Schmitz J et al Intl J Audiol 2010; Schmitz J et al BMJ 2012
- **In Nepal antenatal vitamin A reduced maternal mortality**
  - West K et al BMJ 1999; Christian P et al Am J Epidemiol 2000
- **...night blindness and morbidity;**  
Christian P et al J Nutr 2000; Christian P et al J Nutr 2000
- **...and improved lung function and innate immunity of offspring**  
Checkley W et al NEJM 2010; Palmer A et al (submitted)

# From 'whither' to 'wither' micronutrient malnutrition?

Zinc



Towards the new millennium: away with

West CE, Hautvast JGAJ Lancet 1997

## prevention of micronutrient deficiencies

tools for policymakers  
and public health workers

IOM1998

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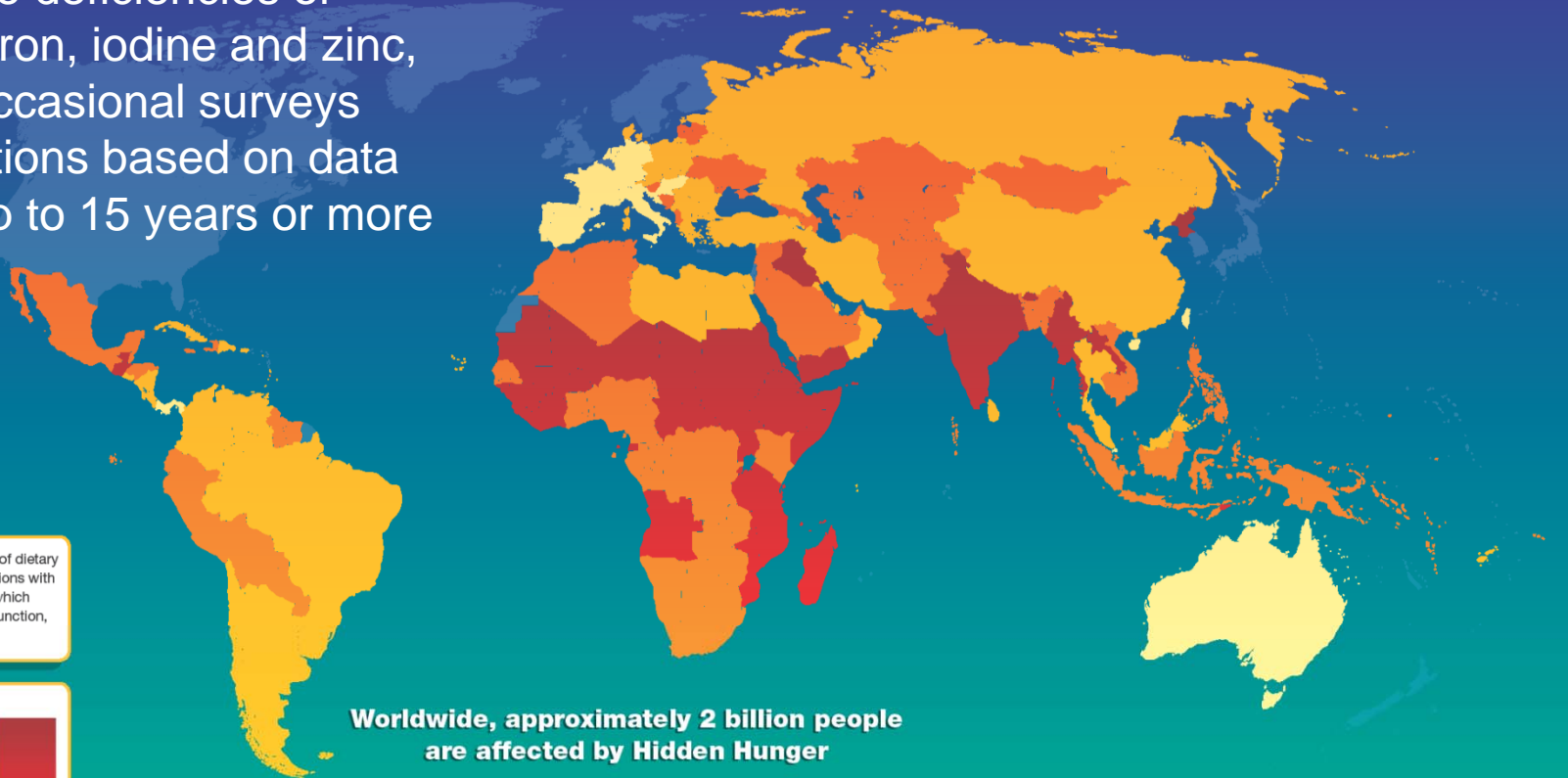




# Worldwide, Approximately 2 Billion People are Affected by Hidden Hunger

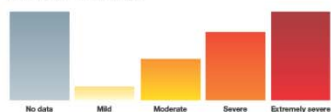
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Restricted to deficiencies of vitamin A, iron, iodine and zinc, based on occasional surveys and imputations based on data spanning up to 15 years or more



**Hidden Hunger** refers to a lack or loss of dietary quality that leaves individuals or populations with deficiencies in essential micronutrients which negatively impact on health, cognition, function, survival, and economic potential.

Severity of Hidden Hunger



Severity of Hidden Hunger was determined based on the proportion of under-fives affected by anemia, vitamin A deficiency, stunting as an indicator of zinc deficiency and school-aged children affected by goiter.

UNICEF State of the World's Children 2008, WHO Global database on vitamin A deficiency and anemia, iodine deficiency

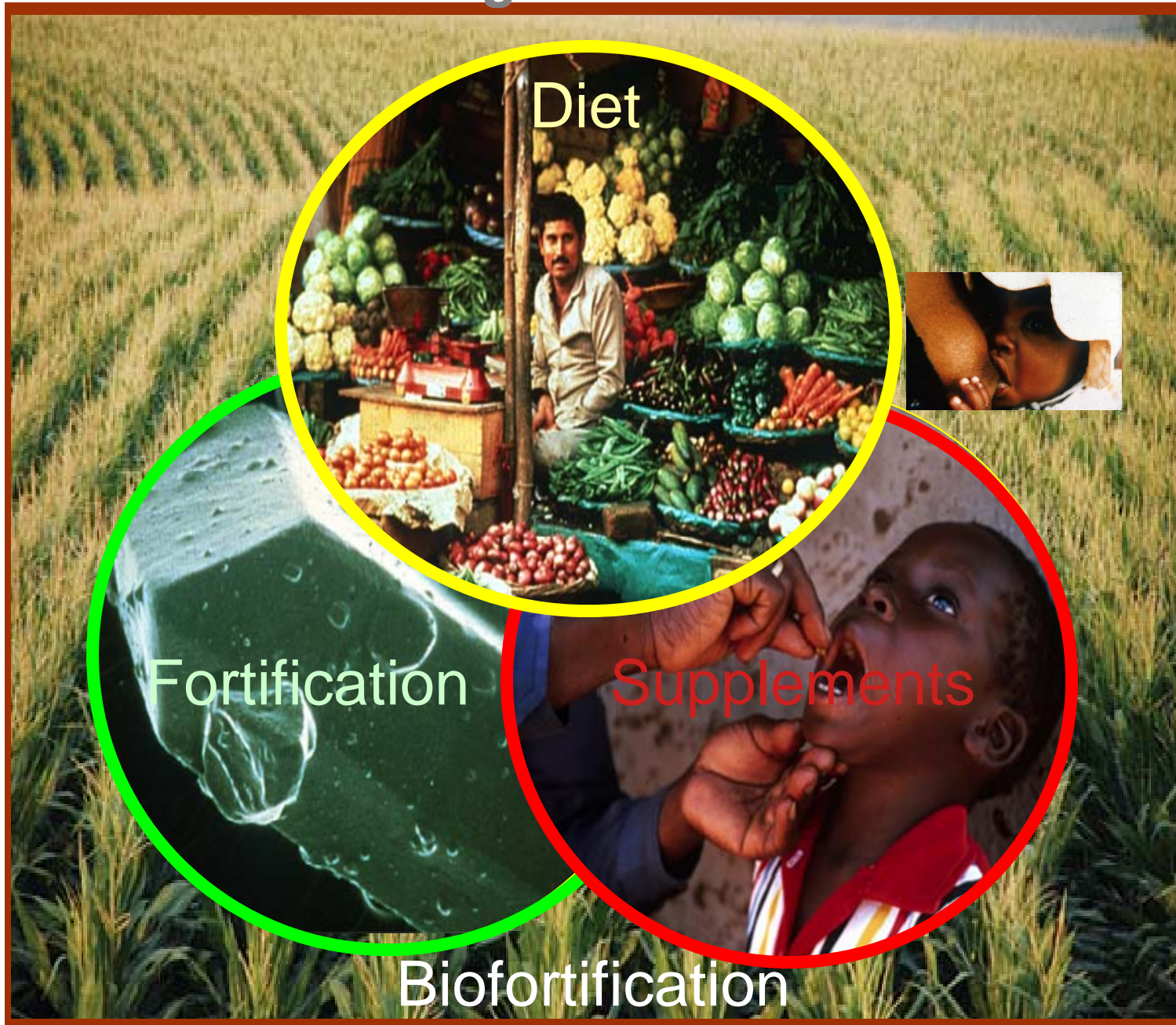
**Worldwide, approximately 2 billion people are affected by Hidden Hunger**

## Global Hidden Hunger Map



**...BUT ...Hidden Hunger is as Hidden Today as Twenty Years Ago**

# Preventive Strategies Exist: But for Which Nutrients?

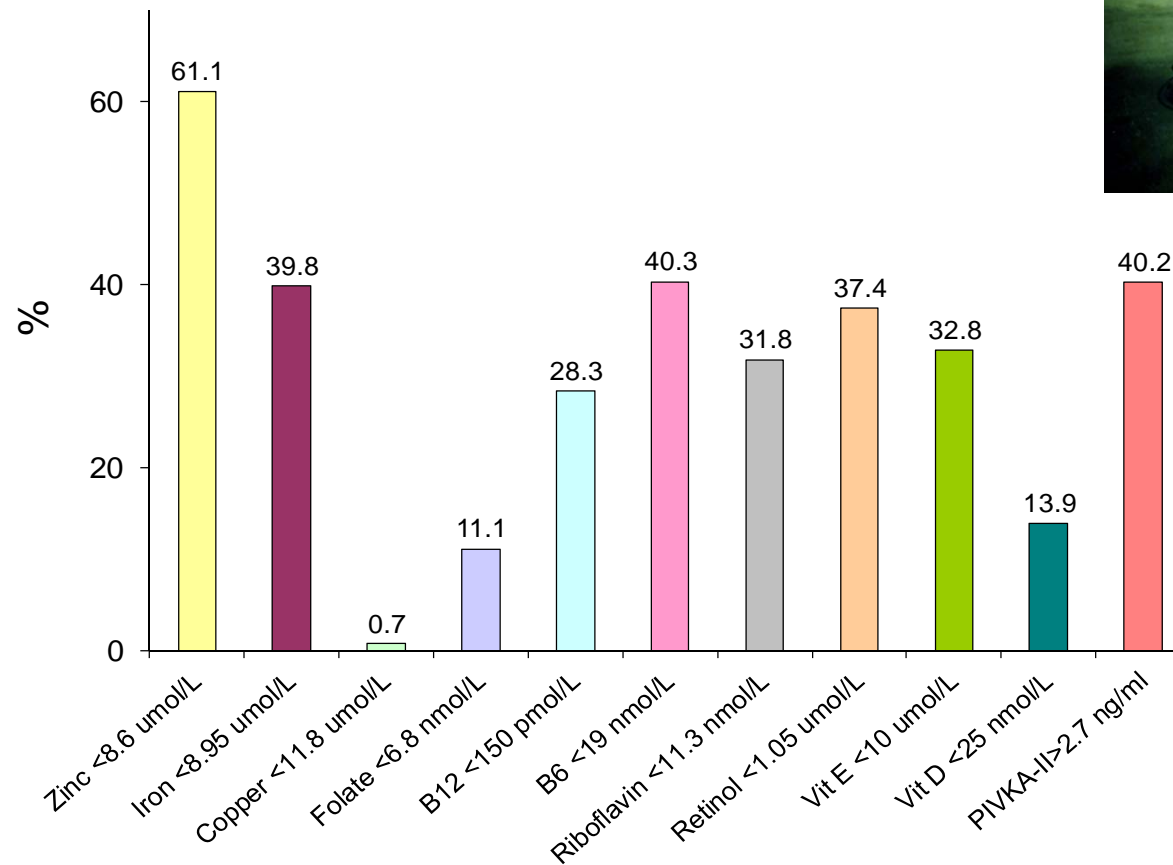


Vitamins:  
A,D,E,K  
B-complex  
C

Minerals:  
Iron  
Zinc,  
Selenium

35-40  
essential/  
semi-  
essential  
nutrients

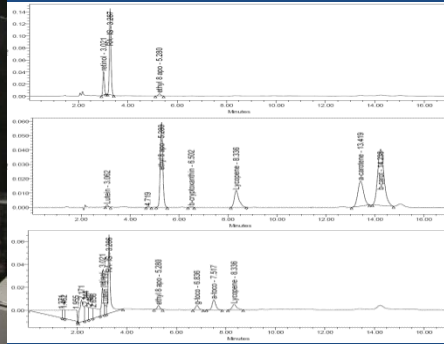
# Prevalence of Low Micronutrient Status during 1<sup>st</sup> Trimester of Pregnancy in Sarlahi District, Nepal (NNIPS-3)



Jiang et al, J Nutr 2005



# Plasma Micronutrient Analyses

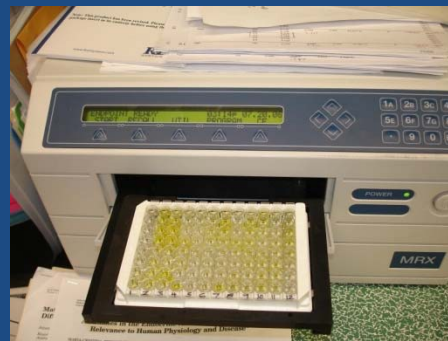


- **HPLC**

- Vitamins A/E/carotenoids

- **Immunoassays**

- Folate, B12, ferritin, thyroglobulin, C-reactive protein, homocysteine (chemiluminescent)
- Vitamin D, transferrin receptor (Ramco, ELISA)
- AGP (RID)



- **Graphite furnace AAS**

- Copper
- Zinc
- Selenium



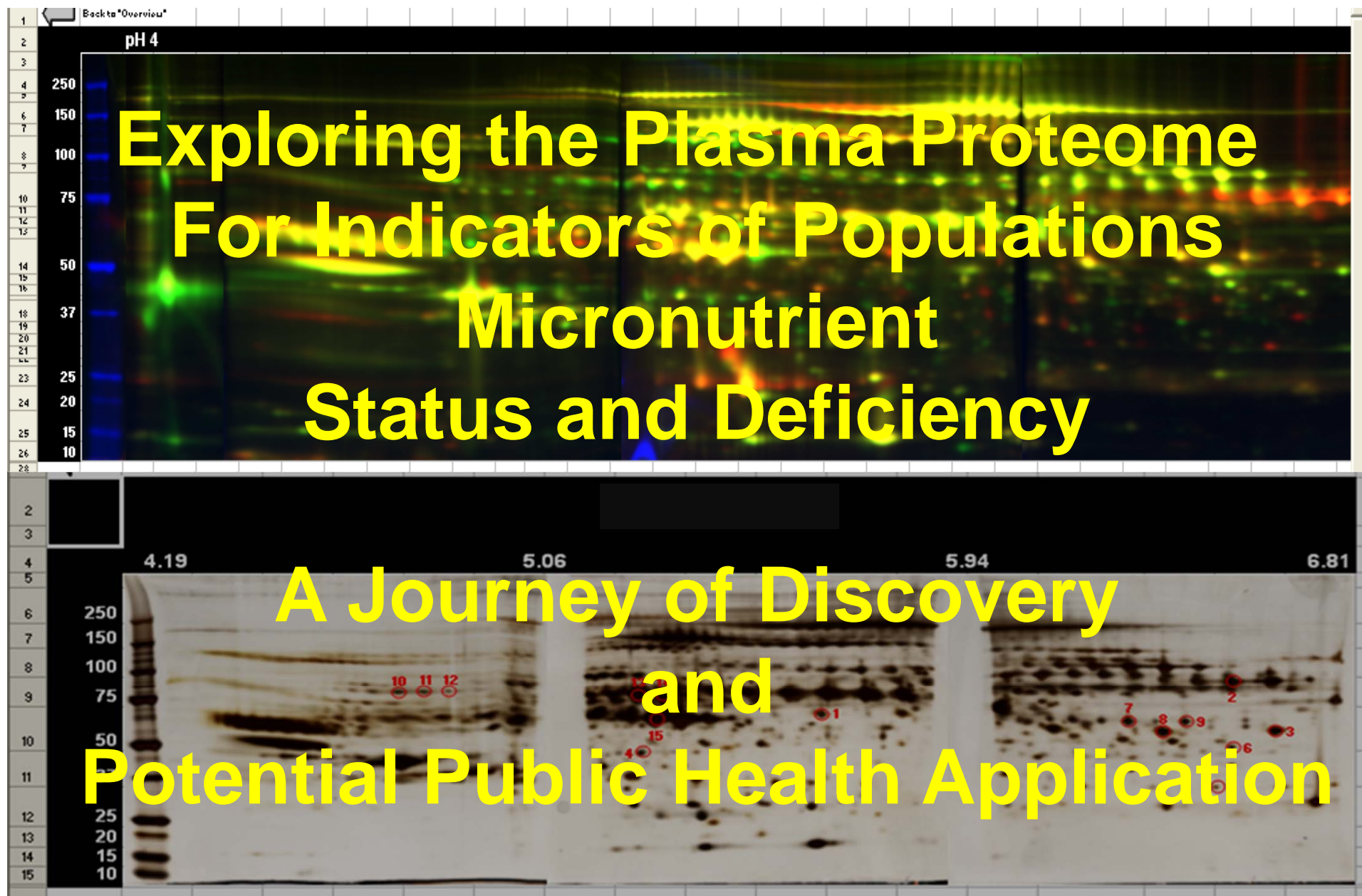


## A need to assess population deficiencies ...

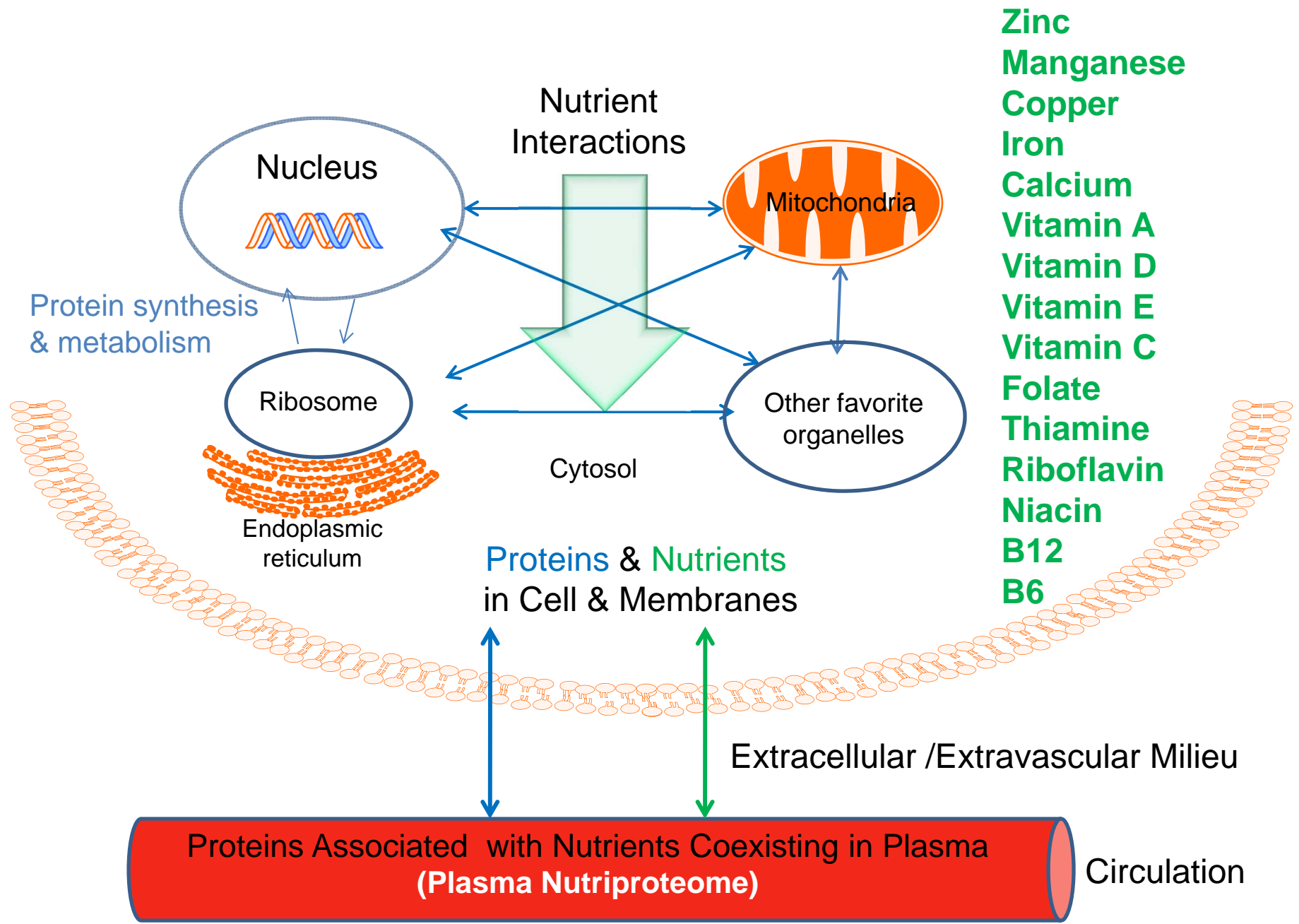
- Precisely (with sufficient sample size)
- Efficiently (for **multiple nutrients** at same time)
- Cheaply (to assess large samples, repeatedly)
- Accurately (relative to “gold standards”)
- Locally (in affected countries)
- Easily (requiring minimal technical skills)
- Rapidly (in weeks vs years)

... to detect, monitor and target, implement and evaluate evidence-driven programs.

... preferably on a single platform (which doesn't exist!)



Supported by the Bill and Melinda Gates Foundation (OPPGH5241)



## The plasma proteomics approach to population micronutrient assessment is based on an ...

- **Axiom:** All nutrients interact with proteins during digestion, absorption, transport, cellular uptake, storage, metabolism, function and excretion: each has a proteome
- **Assumption:** The abundance of of plasma proteins correlated with nutrient indicators offers a valid reflection of micronutrient nutriture



# Goal: In rural 6-8 yr old NNIPS-3 children ...

- Assess micronutrient and inflammation status by conventional nutritional biochemical methods
- Measure relative abundance of the plasma proteome by tandem MS and bioinformatics
- Identify a plasma “nutriproteome”: (proteins correlated with micronutrient levels), and...
- Predict multiple micronutrient status from these nutrient-linked proteins



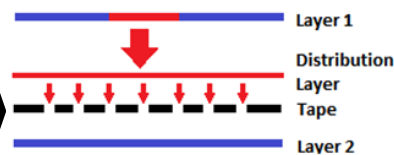
# Why? Need to Take the Lab to the Field

Conventional laboratory-based micronutrient measurement

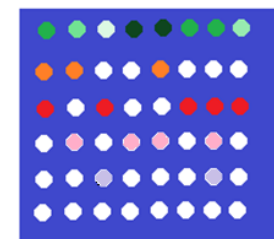


Paper-based microfluidic device to quantify plasma micronutrients & related proteins

Side View



Blue = Hydrophobic region



Vit A indicators  
Vit E indicators  
Vit K indicators  
Folate indicators  
Iron indicators  
Etc.

Once proteins are known to predict micronutrient status, then portable devices can be made that measure **ONLY** proteins. This will take young scientists to develop such nano-, microfluidic or other devices.



## Statistical Inference from Multiple iTRAQ Experiments without Using Common Reference Standards

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175018

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## The Plasma Proteome Identifies Expected and Novel Proteins Correlated with Micronutrient Status in Undernourished Nepalese Children<sup>1–4</sup>

Robert N. Cole,<sup>3,5–7</sup> Ingo Ruczinski,<sup>8</sup> Kerry Schulze,<sup>6,9</sup> Parul Christian,<sup>6,9</sup> Shelley Herbrich,<sup>8</sup> Lee Wu,<sup>6,9</sup> Lauren R. DeVine,<sup>5,7</sup> Robert N. O'Meally,<sup>5,7</sup> Sudeep Shrestha,<sup>6,9</sup> Tatiana N. Boronina,<sup>5,7</sup> James D. Yager,<sup>6,10</sup> John Groopman,<sup>6,10</sup> and Keith P. West Jr.<sup>6,9\*</sup>

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# Plasma Proteome Analyses (n=500)

Measure **micronutrient indicators** in plasma

Deplete plasma of 6 highly abundant proteins:  
analyze low abundant proteins (LAPs)

albumin, haptoglobin, IgG, IgA,  
anti-trypsin, transferrin

Detect differences in the relative abundance of individual LAPs

8-plex iTRAQ Tandem MS  
(isotope Tags for Relative and  
Absolute Quantification)

Correlate **differential protein expression** and **nutrient concentration** data  
to identify candidate biomarkers (individual or clusters)



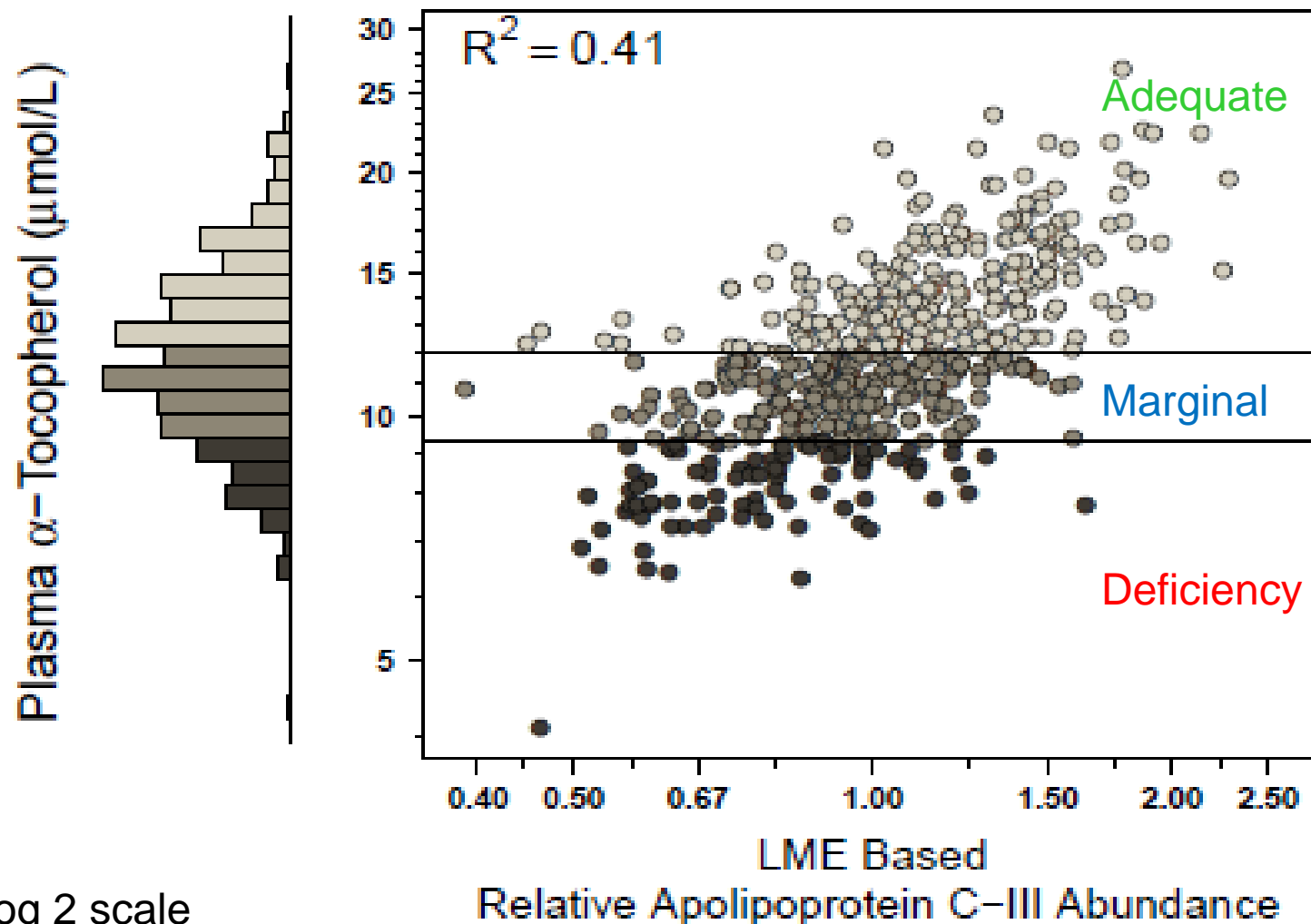
# ***Proof of Concept***

## **Five Micronutrient:Protein Dyads**

***Aim: Show that relative abundance of proteins that transport nutrients strongly correlate: i.e.,***

- Vitamin E: Apolipoprotein C-III
- Vitamin A: Retinol-binding protein 4
- Vitamin D: Vitamin D binding protein
- Selenium: Selenoprotein P isoform 1
- Copper: Ceruloplasmin

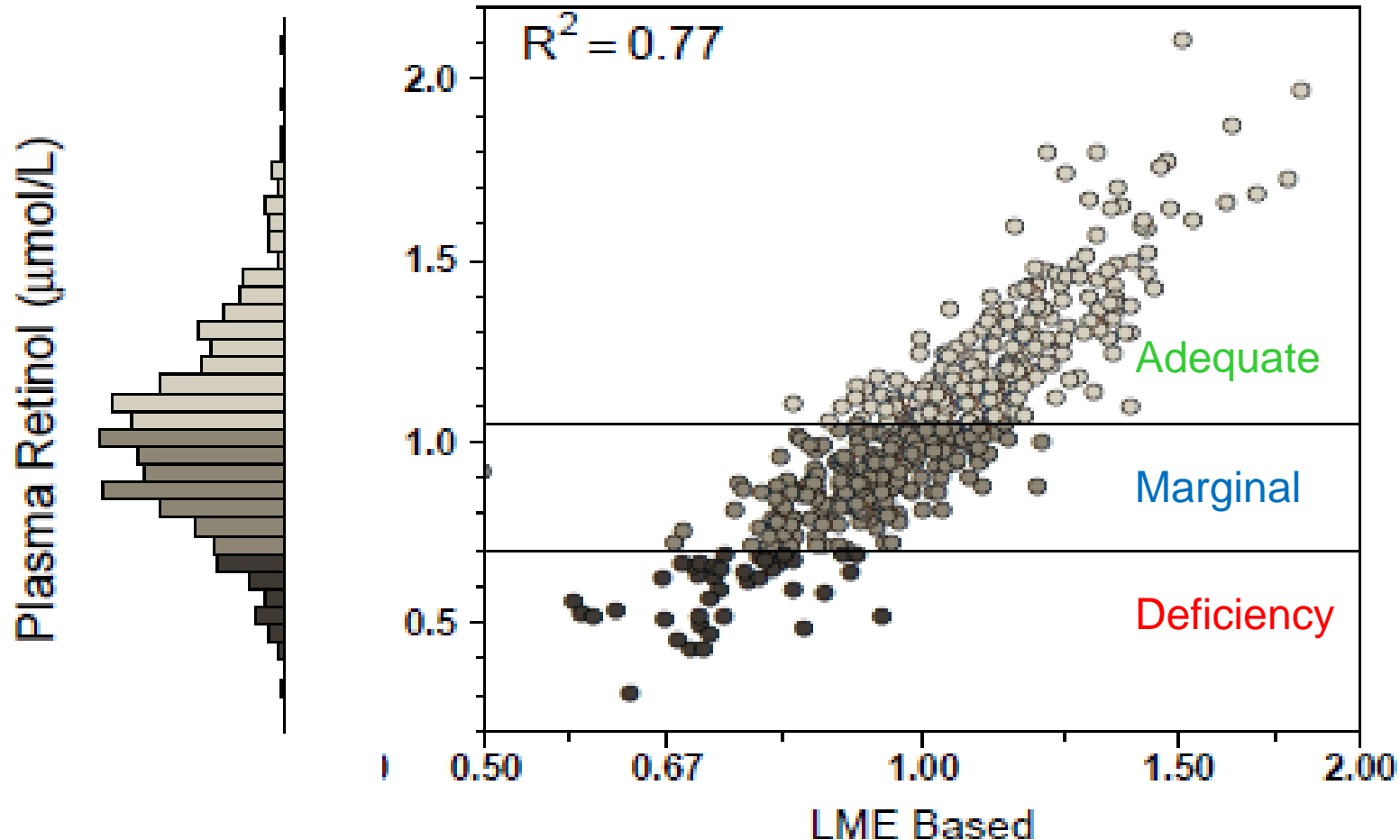
# Plasma Vitamin E Concentration\* and Relative Abundance of Apo C-III (n=500)



\* Log 2 scale

Cole R, Ruczinski I, Schulze K et al J Nutr 2013 doi: 10.3945/jn.113.175018

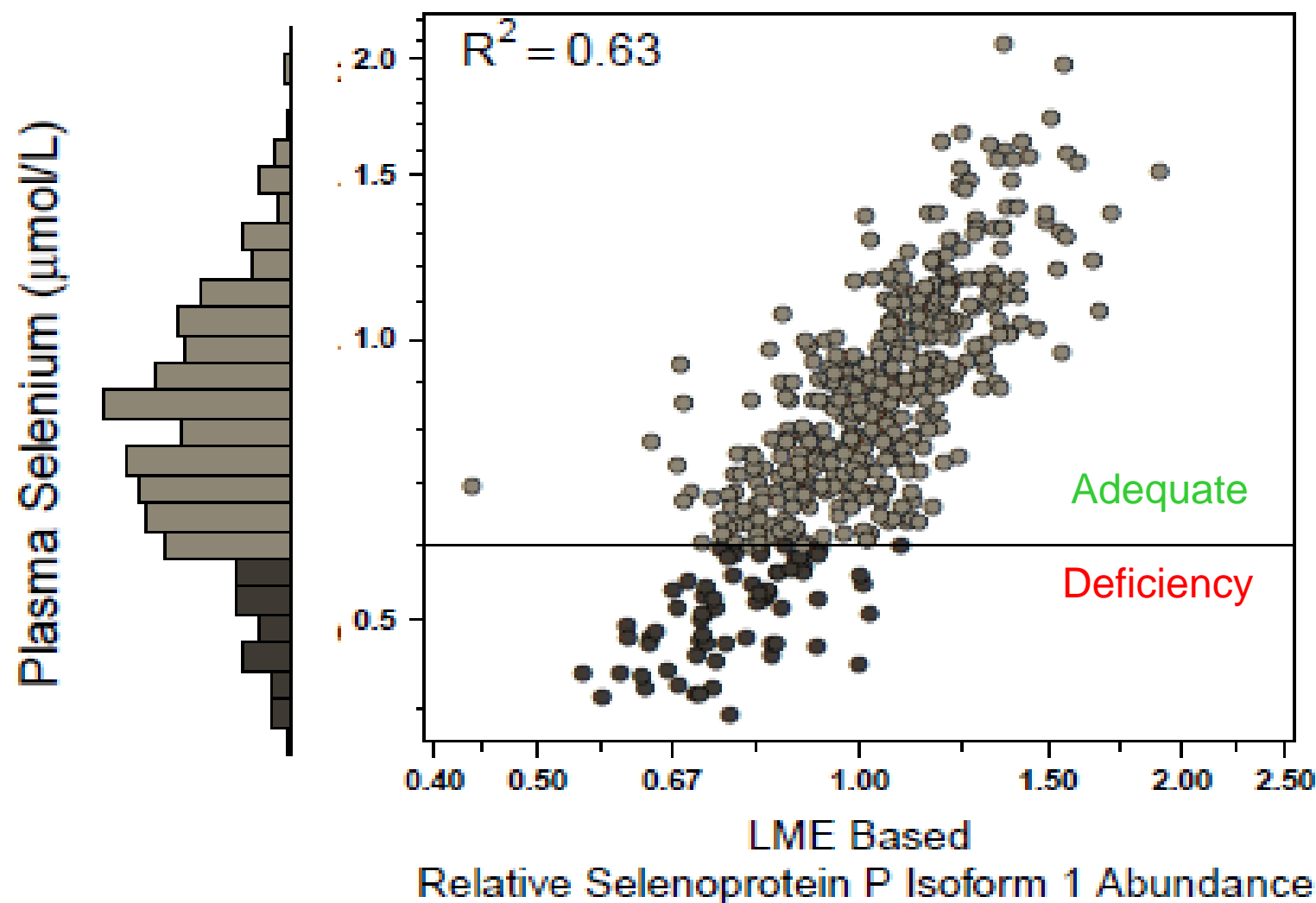
# Plasma Retinol Concentration and Relative Abundance of RBP 4 (n=500)



Hebrich S et al, J Proteome Res 2013 [Relative RBP4 Abundance](#)

Cole R, Ruczinski I, Schulze K et al J Nutr 2013 doi: 10.3945/jn.113.175018

# Plasma (log2) Selenium Concentration and Relative Abundance of Selenoprotein P 1 (SEPP1)





## **Revealing and Preventing Hidden Hunger will require evidence of ...**

- Nutritional “plane” of a population
- Number, type, severity of deficiencies
- Dietary patterns of high risk groups
- Dominant diseases and environmental exposures in a population
- Agriculture-to-Diet-to-Nutrition Pathways
- Resources available for prevention

# Acknowledgements

Johns Hopkins Nutriproteomics Research Team  
Nepal Nutrition Intervention Project – Sarlahi (NNIPS)

## Original NNIPS-3 Trial (2000-1):

USAID, Washington DC

Bill and Melinda Gates Foundation (GH 614)

## NNIPS Follow-up Cohort Studies:

Bill and Melinda Gates Foundation (GH 614)

## Plasma Nutriproteomics Study:

Bill and Melinda Gates Foundation (GH 5241)

**THANK YOU**

