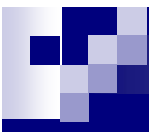


RISK ASSESSMENT OF ESSENTIAL ELEMENTS: COPPER: OVERVIEW AND UPDATE

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“Given the importance of copper as an essential mineral for human health, it is conceivable that this and other minerals with health significance should be approached differently from nonessential minerals”

M. Olivares and R. Uauy (1996)



OBJECTIVE

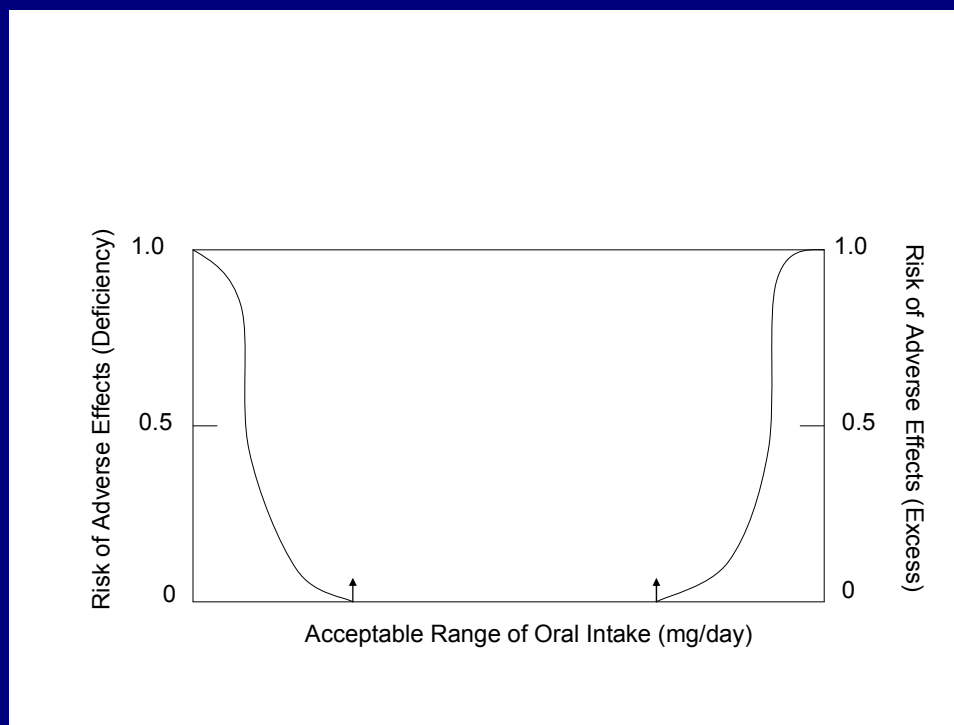
- Overview and update of the Cu data base
- Health issues surrounding copper-induced toxicity and deficiency
- Challenges for developing risk assessment methodologies, strategies and policies that are public health protective
- Health is not only absence of disease but reduction in risk of developing disease



Conceptual Framework for Essential Metals Risk Assessment

- Different from nonessential metal or chemical risk assessment
- Essential: zero intake \neq optimal
- Homeostatically regulated over a range of intakes
- Basal vs. normative requirements – sufficient nutrient to prevent pathology + provide tissue stores (protective buffer)
- Bioavailability: intake \neq absorption

A Theoretical U-Shaped Dose-Response Curve



What is Copper?

- Atomic # 29, mass = 63.546
- Member of 3rd transition series (w Zn, Mn, Fe, Ni)
- Isotopes:
 - Two stable ^{63}Cu , ^{65}Cu
 - Two radioactive ^{64}Cu , ^{67}Cu
- Three oxidation states: Cu^0 (metal), Cu^{1+} (cuprous), Cu^{2+} (cupric)
- Redox cycling=essentiality
- Redox cycling=potential toxicity



Some Key Copper Enzymes

	Function
Amine oxidases	Oxidation of biogenic amines
Ceruloplasmin (Ferroxidase I) [Cp]	Plasma transporter of Fe and Cu
Cytochrome <i>c</i> oxidase [CCO]	Mitochondrial electron transport
Dopamine- β -hydroxylase	Catecholamine metabolism
Haphaestin	Transmucosal Fe transporter
Lysyl oxidase	Collagen/elastin cross-linking
PAM	Peptide/neuropeptide amidation
Superoxide dismutase [SOD]	Free-radical scavenging



Cu Kinetics

- Fractional g.i. absorption – primary site is duodenum
- Bioavailability – varies inversely w amt. ingested, affected by dietary matrix (e.g., Zn, ascorbate, protein, sugars, fat)
- Portal circulation to liver – site of Cu incorporation into Cp for systemic distribution, excess stored in metallothionin (MT) and lysosomes, excess excreted in bile
- Intake \neq Bioavailability

Cu Transport

- Tightly regulated and coordinated
- Specific Transporters - DMT, ATP7A, Ctr1, ATP7B, others
- Specific Metallochaperones include:
 - Atox1 (HAH1) → to transporter ATPases (A and B)
 - COX17 (yeast) → to mitochondria for incorporation into CCO
 - CCS → to cytosol for incorporation into Cu,Zn SOD



Results??

Homeostatic control:

Precise orchestration of Cu transport to sites of enzyme synthesis/function

Processes ensure that unbound ionic Cu (toxic moiety) does not exist unless homeostatic capacity is exceeded



Inborn Errors of Cu Metabolism

Menkes Disease (MNK): Cu deficiency

- X-linked, freq $\approx 1/300,000$, ATP7A defect

Wilson Disease (WD): Cu overload

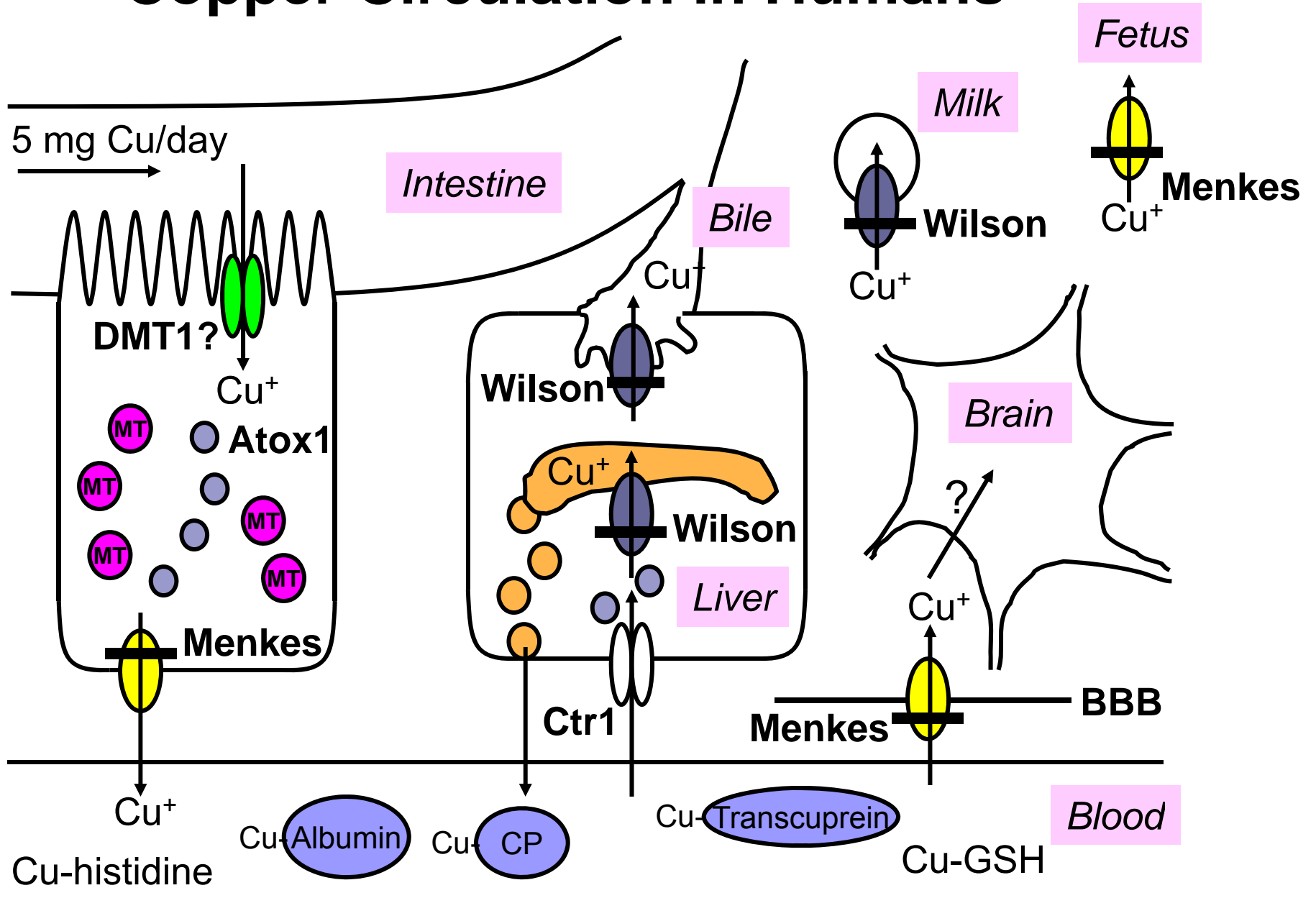
- Autosomal recessive, freq $\approx 1/30,000$, ATP7B defect, severe liver damage
- Treatable with Cu chelation and diet control

Inborn Errors of Cu Metabolism

- WD diagnosis: low Cp, high ratio of non-Cp Cu:Cp Cu, elevated liver Cu, Kayser-Fleischer rings
 - WD HZ: WD HZ, freq \approx 1/90 are carriers
 - Susceptible to Cu overload???
- No evidence



Copper Circulation in Humans





Other Copper-Related Hereditary Syndromes

- Indian childhood cirrhosis (ICC), Non-Indian childhood cirrhosis (NICC), Idiopathic copper toxicosis (ICT)
- Syndromes similar in etiology and presentation
- Have both genetic component (unidentified) and contribution from elevated intake
- High frequency of parental consanguinity



Deficiency: Effects Evaluation

Humans: Case reports, case series

Depletion-repletion clinical studies

Dose metric: mg/day

Responses: negative Cu balance, alterations in phospholipids, glucose/insulin, immune parameters, cuproenzyme levels, cardiac



Deficiency: Effects Evaluation

Animals: Anemia, “Swayback disease” (zoonotic ataxia) (1930’s).

Short-term experiments: 1 severely deficient dose, developing young (gestation, lactation and/or postweaning). Dose metric = mg Cu/kg feed (e.g. CuD = 0.6, CuA = 6).

Responses: target-organ specific (blood, heart, brain/behavior, immune), cuproenzyme levels

More recently, studies of marginal Cu def., longer dur.



Deficiency: Effects Evaluation

In both humans and animals, major target organs

- blood and hematopoietic system
- cardiovascular system
- connective tissue and bone,
- nervous system
- immune system
- teratogenicity



Deficiency: Effects Evaluation Summary

- Clinically-evident copper deficiency considered to be very rare except in premature low-birth weight infants, malnourished infants, adults receiving TPN without added Cu.
- Marginal deficiency thought to occur extensively but no available biomarkers of marginal Cu status to confirm.

Deficiency: Effects Evaluation

NEW DATA

Adult onset Cu-deficiency myeloneuropathy

- Resembles subacute combined degeneration of Vit B₁₂ def.
- May also present with neuromuscular degeneration similar to ALS
- Clear presentation, diagnostics, progression
- Cu status not assessed in initial diagnostic workup. Often misdiagnosis (e.g. ALS) or no diagnosis (idiopathic neuropathy)
- Myeloneuropathy progressive.



Toxicity: Effects Evaluation

Humans: Case reports

Metabolic-unit studies

Acute exposure studies

Population-based studies (M. Araya)

Dose metric = mg Cu/day, mg Cu/L



Toxicity: Effects Evaluation

Animals:

- Single-dose studies targeting liver and kidney
- One guidelines subchronic toxicity study

NEW DATA

- Two-gen reproductive toxicity study
- Unpublished rabbit dev. tox study
- Infant rhesus monkey study (Araya)

Dose metric: mg Cu/kg feed, mg Cu/L water, mg Cu/day → mg Cu/kg bw/day



Toxicity: Effects Evaluation

- Acute toxicity: Gastrointestinal in humans (nausea and vomiting), repeated acute dosing → right-shift in LOAEL and NOAEL
- Repeated-dose toxicity: Primary target organ in humans and animals is the liver, other organs include kidney and g.i.t.



Toxicity: Effects Evaluation

- Animal studies (Haywood) show almost complete regression & regeneration in liver and kidney histopathology over dosing period
Appears to be considerable adaptation to copper loading over a range of high doses
- Liver Cu content remains elevated



Dose-Response Assessment/Modeling

- **Conceptual Approach:** Homeostatic model providing an acceptable range of oral intake (AROI) to meet nutritional requirements and avoid toxicity (WHO 2002)
- **Common Currency:** Risk-risk or risk-benefit comparison – decreasing the risk of one adverse health effect (deficiency) balanced against increasing risk of another adverse health effect (toxicity) (Renwick et al. 2004)



Dose-Response Assessment/Modeling

Key Considerations:

- Quality of studies (toxicity, deficiency)
- Use of biological endpoints of comparable functional significance to define AROI boundaries (or make other adjustments)
- Knowledge of homeostatic mechanisms & of coefficient of variation (CV) of susceptibility within human population



Dose-Response Assessment/Modeling

- Appropriate dose metric (mg/day, mg/kg/d)
- Appropriate response metric (defining acceptable level of response)
- Incorporation of variability
- Incorporation of uncertainty
- CV and/or uncertainty factors (UFs) ?
- Similar modeling approach for both D and T

Essential Metal Requirements

- “Requirement for what?”
- Related to specified criteria of adequacy
 - Prevention of clinical disease?
 - Health benefits at intakes $>$ RDA?
 - Highest intake level without appreciable risk of adverse effects ? (UL)

Severity Scores for Cu-Associated Outcomes

<<<<<Deficiency			<Homeostasis>		Excess >>>>>		
4	3	2	1	1	2	3	4
Gross Deficiency	Metabolic Perturbation	Loss of Cu-dependent Enzyme Activity	Molecular Manifestation	Molecular Manifestation	Loss of Cu-Dependent Enzyme Function	Metabolic Perturbation	Gross Excess

Severity Scoring: Deficiency

Severity Score	Types of End Points
1D	Cu body burden; MT; urine Cu levels
2D	Loss of Cu-dependent enzyme function (SOD), changes in blood cell #s or function,
3D	Severe body wt, changes, organ wt. changes, plasma glucose/insulin, minor histopathology, white blood cell activities/count
4D	Death, gross histopathology, reproductive/development changes

Severity Scoring: Excess

Severity Score	Types of End Points
1E	Cu body burden; MT; urine Cu levels
2E	Changes in cholesterol/triglycerides; lge Cu burdens; nausea, vomiting, diarrhea; enzyme changes without histopathology
3E	Lge body weight changes; anemia; hemolysis; vitamin/mineral levels; liver enzymes; inflammation; organ weights
4E	death; gross histopathology; reproductive function changes



Summary

Rich Cu data base, excellent case study for RA

Areas of research:

Biomarkers – sensitive, specific, dose-responsive, predictive of adverse clinical outcome; few good biomarkers of marginal Cu status

Exposure – Recent multi-route studies (Sadhra et al. 2006, Georgopoulos et al., 2007) show deficiency more of a concern than toxicity