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The Need to Consider Functional Endpoints in Defining Nutrient Requirements

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The first edition of the Recommended Dietary Allowances was published nearly 55 years ago[1]. At that time knowledge about human nutrient requirements was limited, and it was often necessary to extrapolate information from studies in experimental animals. Currently, three different approaches are often used to determine human nutrient requirements, 1) balance studies that measure nutrient losses in relation to intake, 2) depletion/repletion studies in which subjects are maintained on diets low or deficient in a nutrient, followed by correction of the deficit with measured amounts of the nutrient, and 3) the observed intakes of a nutrient by healthy people(Table 1).

Scientific basis of current RDAs is as follows(Table 2). Fractional estimate of expenditures or losses was used for energy, calcium, iron, zinc, copper and folic acid. Balance study was used for protein and magnesium and measurement of customary intakes was used for vitamin E and manganese. Measurement of body pool sizes was used for vitmain C and B_{12}. Static biochemical measurement was used for vitmain A, riboflavin and iodine. Functional biochemical measurement was used for vitamin K, thiamin, niacin. vitamin B_6 and selenium. In balance studies and depletion/repletion studies, nutrient requirements are defined as the amount required in the diet to replace endogenous losses and maintain appropriate levels of that nutrient or a nurient-dependent metabolite in circulation. But, these two approaches fail to consider homeostatic adjustments in nutrient metabolism when dietary intakes are changed. This failure may flaw subsequent estimates of human nutrient requirements(Table 3).

Studies of zinc depletion in humans illustrate how tissue zinc concentrations are maintained within a narrow range when zinc intake decreased sharply. This is most evident from the results of acute zinc depletion study in a group of young men fed a diet virtually free of zinc(0.3mg zinc/d)[2]. Although the

Table 1. Early approaches used to determine Nutrient requirements

- Balance studies that measure nutrient losses in relation to intake.
- Nutrient depletion/repletion studies in which subjects are maintained on diets low or deficient in a nutrient, followed by correction of the deficit with measured amounts of the nutrient.
- Observed intakes of normal, healthy people.

Table 2. Scientific Basis of Current RDA's

Fractional estimate of expenditures or losses :	
	Energy, Calcium, Iron, Zinc, Copper, Folic acid
Balance :	
	Protein, Magnesium
Customary intakes :	
	Vitamin E, Manganese
Body pool sizes :	
	Vitamin C, Vitamin B_{12}
Static biochemical measurement :	
	Vitamin A, Riboflavin, Iodine
Functional biochemical measurement :	
	Vitamin K, Thiamin, Niacin, Vitamin B_6, Selenium

men were unable to achieve zinc balance, their net loss of zinc declined from over 5mg/d at the beginning of the depletion period to about 0.7mg/d at the end of three weeks(Fig. 1, Fig. 2). Another study[3] demonstrated that five of six men achieved zinc balance within nine days when dietary zinc was reduced from 15 to 5.5mg/d(Fig. 3). Stable isotope zinc tracer studies demonstrated that the amount of zinc absorbed declined with the reduction in dietary zinc, but balance was re-established by a concomitant reduction in endogenous fecal zinc excretion[4)5)]. When dietary intake of zinc was high(15mg/d), apparent zinc absorption was 25% and the amount of zinc absorbed was 3.7mg. When zinc intake was low(5.5 mg/d) apparent absorption increased to 53%, but the amount of absorbed zinc decreased to 2.9mg(Table 4). Usually zinc is excreted through feces, urine and skin. Endogenous excretion of zinc to the gastrointestinal tract decreased when dietary intake of zinc was low, but urinary zinc excretion did not(Fig. 4) and subjects could maintain zinc balance over a wide range of zinc intake(Fig. 5). Therefore, it was concluded that a decrease in zinc intake causes loss of functional zinc from compounds with rapid turnover before zinc balance is re-established(Fig. 6).

Table 3. Nutrient requirement

Amount of nutrient required in the diet to replace endogenous losses and/or to maintain appropriate circulating "levels" of that nutrient or a nutrient-dependent metabolite.

These early approaches failed to consider the impact of homeostatic regulation on nutrient metabolism when the dietary supply of a nutrient is limited.

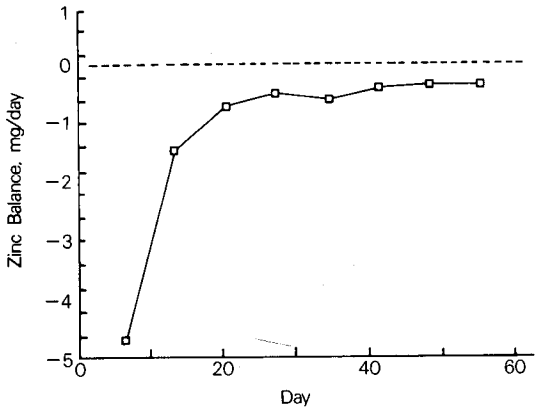

Fig. 1. Zinc balance of men fed 0.3mg zinc per day.

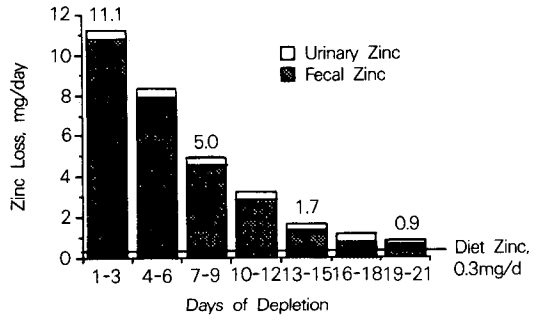

Fig. 2. Effect of a zinc-free diet on zinc losses in healthy young men.

It appears, therefore, that maximal adaptations can maintain zinc balance when low zinc diets are fed,

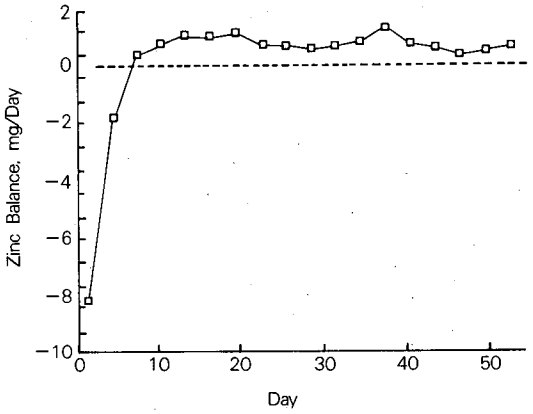

Fig. 3. zinc balance of men fed 5.5mg zinc per day.

Table 4. Apparent zinc absorption(^{70}Zn or ^{67}Zn absorbed)

	Dietary zinc	% absorbed ($\bar{X}\pm$ SEM)	mg absorbed ($\bar{X}\pm$ SEM)
MP I-Day 10	15 mg	24.7 ± 2.2^a	3.7 ± 0.82^a
MP II-Day 13	5.5mg	52.6 ± 4.4^b	2.9 ± 0.59^b
MP II-Day 42	5.5mg	48.9 ± 2.8^b	2.7 ± 0.38^b

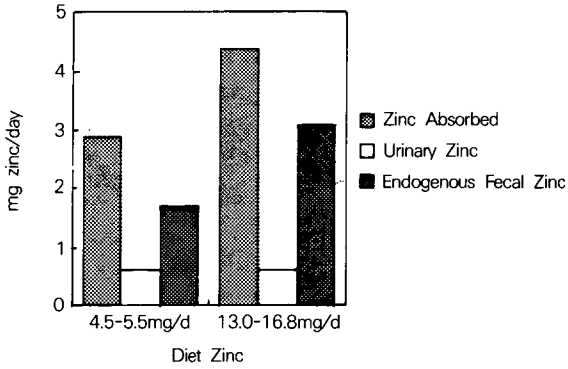

Fig. 4. Changes in zinc absorption and excretion with low and high zinc diets.

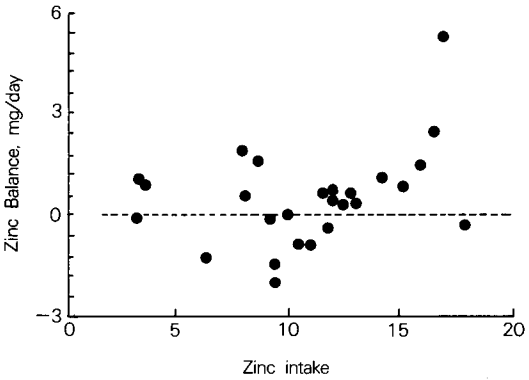

Fig. 5. Crude zinc balance.

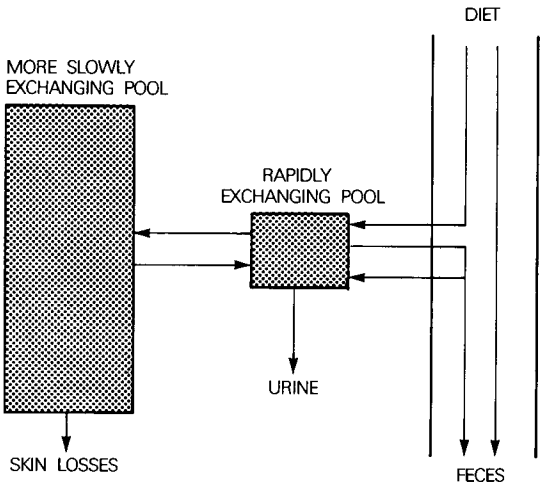

Fig. 6. Model of zinc homeostasis.

but this may not be without some functional consequences. For example, the men fed 5.5mg zinc/d achieved zinc balance, but showed biochemical changes such as decreased concentrations of serum albumin, pre-albumin, retinol-binding protein, thyroid stimulating hormone and free thyroxine and increased fasting blood glucose level[6](Table 5). These changes occurred with only aloss of 59mg of tissue zinc and no changes in urinary zinc excretion or

Table 5. Biochemical changes in men fed 5.5mg Zn/day

	Baseline	Depletion	p
Prealbumin, mg/dl	37.2	32.2	<0.05
Retinol-binding protein, mg/dl	6.55	6.02	<0.05
Thyroxine, ug/dl	8.5	7.0	0.052
Free T4, ug/dl	1.45	1.23	<0.05
T3, ng/dl	192	196	NS
TSH, ulU/ml	3.16	2.32	0.054
BMR, kcal/kg/hr	1.00	0.91	<0.05
Fasting RQ	0.95	0.86	<0.05
Fasting glucose, mg/dl	92	101	<0.05
Fasting insulin, uU/ml	26.7	26.7	NS

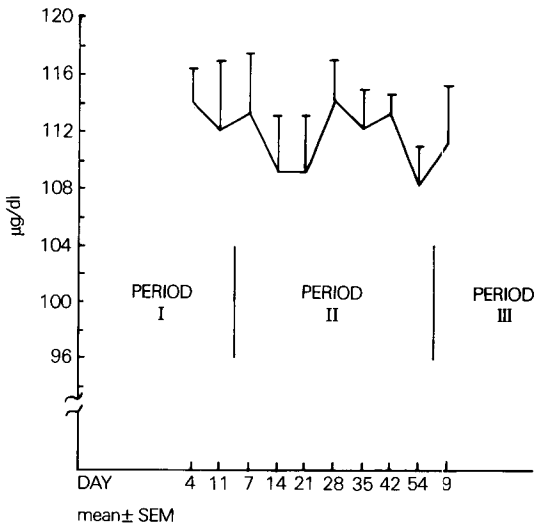

Fig. 7. Serum zinc.

plasma zinc concentrations(Fig. 7). Men on the diet of 0.3mg zinc/d adapted to minimize fecal excretion of zinc, but showed biochemical changes of zinc deficiency and clinical changes such as skin lesions, increased energy to maintain weight, poor appetite, diarrhea and sore throats(Table 6, 7). In a study of lactating Amazonian women who were consuming 8.5mg zinc/d[7], positive zinc balance was achieved by absorbing between 60 and 85% of the zinc intake. But, when those women were given zinc supplements, no decline in the concentration of zinc in breast milk was observed during the first 120 days of lactation and higher levels of milk retinol were supported [8]. Also, the incidence of diarrhea in the infants declined[8]. It appears, therefore, that zinc balance dose not imply optimal function. Possibly, when dietary zinc is very low, zinc-containing compounds that turn over rapidly are lost leading to functional changes(Fig. 8).

Typical homeostatic responses to reduced intake of some nutrients are known(Table 8). Potential homeostatic responses to a reduction in nutrient intake are increased net absorption(increased fractional absorption and reduced endogenous gastrointestinal excretion), reduced clearance from the blood by the kidney and a decline in urinary excretion, reduced utilization or turnover rate and a decline in the excretion of metabolic end-products and mobilization of reserves or stores leading to a negative balance (Table 9).

Although homeostatic adjustments to low intakes often lead to changes in endogenous nutrient losses, many recommended dietary allowances are based

Table 6. Biochemical changes in men fed 0.3mg zinc/day

	Basal	End of depletion	p
Albumin, g/dl	4.47	4.36	<0.05
Retinol BP, ml/dl	4.86	4.64	<0.05
LDH, mu/ml	160	133	<0.05
I ALD, units	33.6	25.4	<0.05
Uric acid, mg/dl	4.75	4.29	<0.05
Glucose tolerance, AUC	22,771	23,208	<0.005

Table 7. Clinical changes in men fed 0.3mg/day

- Skin lesions : Patches of dry skin to severe acne.
- Increased energy to maintain weight.
- Poor appetite.
- Diarrhea.
- Sore throats

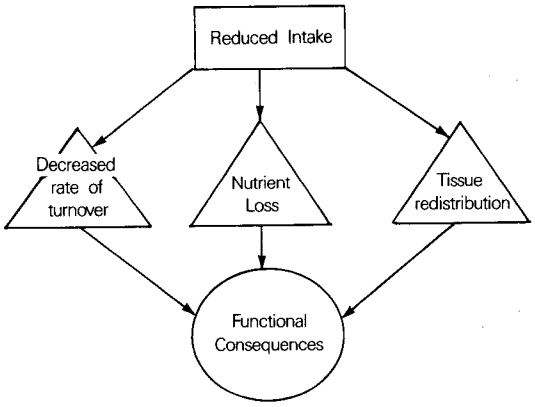

Fig. 8. Model of a homeostatic response to a low nutrient intake.

Table 8. Typical homeostatic responses

Water	Decreased urinary excretion
Energy	Mobilization of fat reserves
Protein	Reduced urinary N excretion
Electrolytes, I^-, FI^-	Decreased urinary excretion
Essential cations	Increased net absorption
Fat-soluble vitamins	Mobilization of reserves/ stores
Water-soluble vitamins	Decreassed utilization ; reduced clearance from the blood

Table 9. Potential homeostatic responses to a reduction in nutrient intake

- Increased net absorption(increased fractional absorption and reduced endogenous GI excretion)
- Reduced clearance from the blood by the kidney and a decline in urinary excretion
- Reduced utilization or turnover rate and a decline in the excretion of metabolic end-products
- Mobilization of reserves or stores leading to a negative balance

on quantitative estimates of the amount needed in the diet to replace endogenous losses. For example, estimated minimum requirement of folic acid is 1 μg/kg based on the rate of folate loss when none is consumed[9](Table 10). RDA was set at 3μg/kg or 200μg/d for men and 180μg/d for women. The RDAs are about 50% less than the amounts recommended previously. Potential flaws in the recommendation are as follows. There is no allowance for reductions in loss when none is fed. There is no consideration of folate intakes associated with good function. And there is no consideration of variation in bioavailability depending on the diet and food source. Therefore, the approach may underestimate the true dietary requirement for folic acid.

A functional approach is used as the basis of the selenium recommendation and the selenium RDA may be closer to actual needs for the population than that of folic acid(Table 11). The selenium RDA is based on the dietary need to maintain a selenium-dependent function, plasma glutathione peroxidase activity[9]. The activity of this enzyme, measured in adult Chinese men who routinely consumed diets that were low in selenium, plateaued when selenium intake reached 30μg/d[10]. To cover individual variation, 40μg/d was suggested as the requirement for the Chinese population ; the U.S. RDA was set at 70μg/d for men and 55μg/d for women to assure an adequate intake for individuals with larger body sizes and to provide for individual variability[9].

Since homeostatic adjustments reduce endogenous losses with lower intakes, future RDAs should be based on dietary requirements for maintenance of nutrient functions rather than replacement of endogenous losses(Table 12). A functional approach requires information from two types of nutritional studies. First, specific, sensitive end-points need to be identified from comprehensive human metabolic experimental studies of nutrient metabolism, function and homeostasis. Second, the dietary requirement of population to maintain these functions must be established in epidemiological studies(Table 13). Functional end-points may be more sensitive indicators of nutrient requirements than are metabolic ba-

Table 10. Recommended dietary allowance for folic acid

Estimated minimum requirement : 1ug/kg
−Based on rate of loss when none is fed
RDA set at 3ug/kg, or 200ug/d for men and 180ug/d for women
−Allows for an estimated 50% bioavailability
−30% added for individual variability
Potential flaws in the recommendation :
−No allowance for reductions in loss when none is fed.
−No consideration of folate intakes associated with good function.
−No consideration of variation in bioavailability depending on the diet and food source.

Table 11. Selenium dietary requirement : model of an epidemiological study of intake and function

• Selenium supplementation study in adult men living in an endemic area for Keshan disease in China(Yang et al., 1987).
• Monitored plasma glutathione peroxidase activity for several months.
• Plasma glutathione peroxidase activity was maximazed by 40ug selenium/day.
• Dietary requirement modified for body size and individual variation in selenium requirements to establish the RDA.
• RDA for U.S. men : 70ug/d ; women : 55ug/d.

Table 12. Conclusions

• Homeostatic mechanisms alter rates of nutrient utilization or losses and allow balance to be established over a wide range of intakes.
• Functional changes are often associated with adaptation to low intakes.
• Functional endpoints may be more sensitive indicators of nutrient requirements than are metabolic balances or static endpoints.

lances or static end-points. Optimal functional end-points of nutrient requirements should be nutrient-specific, respond promptly to small changes in nutritional status and have well-established normal ranges(Table 14). Areas of competency related to dietary intakes are disease reponse, reproductive competence, cognitive function, work output and social and behavioral function(Table 15). Currently, very few nutrient-specific, functional end-points have been defined. Solomons and Allen[11] identified seven different categories of nutrient function(structural integrity, host defense, transport, hemostasis, reproduction, nerve function and work capacity), but few of the functions(e.g, platelet aggregation, sperm count, dark adaptation) in those categories were nutrient-specific and sensitive to small changes in intake

Table 13. A two-step approach for determining nutrient requirements of a population

- Identify functional endpoints of nutritional status.
  - Comprehensive experimental studies of nutrient metabolism, function and homeostasis in humans
- Relate dietary intakes of a population to those functional endpoints.
  - Epidemiological studies of diet intake and nutrient function

Table 14. Optimal functional endpoints of nutrient requirements

- Are nutrient-specific
- Respond promptly to small changes in nutritional status
- Have well-established normal ranges

Table 15. Areas of competency related to dietary intakes : National Academy of Sciences committee on international nutrition programs, 1978

- Disease response
- Reproductive competence
- Cognitive function
- Work output
- Social and behavioral function

(Table 16). More specific categories are listed by Gibson(Table 17). To establish nutrient requirements based on functional end-points, therefore, static measures of nutritional status need to be measured

Table 16. Categories of nutrient function : Solomons and Allen, nutrition reviews, 1983

- Structural integrity
  Erythrocyte fragility, Lipoprotein peroxidation
- Host defense
  Leukocyte chemotaxis, delayed cutaneous hypersensitivity
- Transport
  Retinol relative dose response, ^{65}Zn uptake by erythrocytes
- Hemostasis
  Platelet aggregation
- Reproduction
  Sperm count
- Nerve function
  Dark adaptation, Taste acuity
- Work capacity
  VO_2 Max, Heart Rate

Table 17. Functional tests of nutritional status : Gibson, Principles of nutrtional assessment, 1990

- Abnormal metabolic products arising from suboptimal intakes.
  - xanthurenic acid excretion in B-6 deficiency
- Changes in blood components or enzyme activities.
  - hemoglobin concentrations
  - erythrocyte glutathione peroxidase activity
- *In vitro* tests of *in vivo* functions.
  - leukocyte chemotaxis
  - d-uridine suppression test
- Induced responses or load tests.
  - histidine load test
  - retinol relative dose response test
- Spontaneous *in vivo* responses.
  - dark adaptation
  - taste acuity
- Growth or developmental responses.
  - sexual maturation

along with function. For example, zinc requirements could be based on intakes supporting appropriate plasma zinc concentrations and platelet aggregation.

Finally, requirements of individuals need to be converted to dietary recommendations for populations(Table 18). This step requires epidemiological studies of dietary intake, nutrient function and static concentrations. Objective of these studies is to define a range of intakes through which there is a low risk of either inadequate or detrimentally high intakes. The epidemiological approach requires sound estimates of nutrient intake and valid functional endpoints. The sound estimate of nutrient intake could be determined by accurate measurement of food consumption and good food composition data. Recommendations for a population are generally greater than that of a small number of individuals due to differences in genetics, dietary sources(bioavailability), physiology(age, health, substance abuse), lifestyle or occupation(physical acitivity) and climate (heat or cold)[12](Table 19). Also, these epidemiological studies need to be done in populations where some indviduals have insufficient intakes in order to define a recommendation that is not in excess of the true need.

Table 18. Relate dietary intake of a population to nutrient function

• Requires epidemiological studies
• Objective : Define a range of intakes through which there is a low risk of either inadequate or detrimentally high intakes.
Requirements of the epidemiological approach
• Sound estimates of nutrient intake
−accurate determinations of food consumption
−good food composition data
• Valid functional endpoints
−nutrient-specific
−sensitive to small changes in nutrient status
−measureable in a field setting

Table 19. Causes of variations in nutrient requirements in a population

Due to differences in :
−Dietary source(bioavailability)
−Physiology(age, health, substance abuse)
−Lifestyle or occupation(physical activity)
−Climate(heat)
−Genetic variation

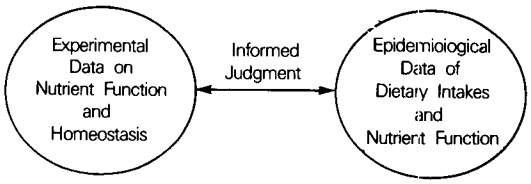

Fig. 9. Model for establishing dietary recommendations.

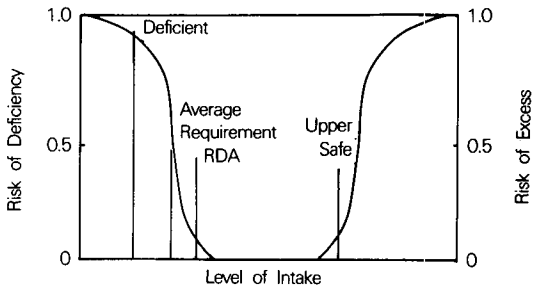

Fig. 10. RDA's.

In summary, a new paradigm for determining RDAs is indicated. That should include two tracks of research, 1) human metabolic studies of changes in nutrient function and homeostasis with changes in intake and 2) epidemiological studies of nutrient intake, status and function. Using informed judgement, Food and Nutrition Board RDA Committees can then establish standards for nutrient intakes that support metabolic function as well as maintain homeostasis(Fig. 9, 10).

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