Stable isotope application in research related to life stages

Wantanee Kriengsinyos, Ph.D, RD, CDT
Institute of Nutrition, Mahidol University
Stable isotope methods

- Isotopes: atoms of an element containing the same number of protons, but different number of neutrons
- Unlike radioisotopes which are unstable, stable isotopes are safe and emit no radiation
- Stable isotopes occur naturally
- Suitable for all ages
- Non-invasive
- Can be used in community settings
The basic principle of all stable isotope techniques is to administrate a stable isotope–labeled compound to the body (ie, orally, intravenously) in “trace” amounts to minimally disturb normal physiology, and to subsequently track the fate of the compound or its catabolic products in breath, tissue, feces, urine, and/or blood.

- heavy isotopes (e.g., $^{58}$Fe, $^{70}$Zn)
- light isotopes (e.g., $^2$H, $^{18}$O)

The noninvasive nature of stable isotope technologies potentially allow for low-hazard and are applicable across all age groups.
Compounds containing stable isotopes can be identified and measured using the molecular weight of the compound. This is because it will differ from that of the original compound.

Using an instrument called a mass spectrometer, researchers can use this weight difference to trace the stable isotopes as they travel through the body and appear in blood, urine, breath, and stool samples.
• Global policy – exclusive breastfeeding for 6 months
  - BF studies to guide national policy
• Food-based guideline eg complementary foods
  - impact on growth/development
• Dietary Reference Intake (DRI)
  - energy requirement; protein/amino acids; micronutrients
Thailand Policies: maternal/child nutrition; IYCF → prevention of obesity and NCDs

Stable isotope techniques for:
- Human milk intake; Body composition
- Total Daily Energy expenditure

Evidence: Exclusivity of breastfeeding; mother/infant nutrient intake; impact of local CF eg fortified broken rice; body fat estimation; physical activity levels
Thailand Policies: From Farm to Fork value chain; food-based strategies for malnutrition

Stable isotope techniques for:
- Bioavailability; Efficacy of intervention
- Interaction with Obesity/NCDs

Evidence: Fortified rice and condiments, bio-fortified crops, legumes, veggies/fruits – increased absorption and improved status
Determination total body water

Estimation of body composition
Deuterium oxide ($^{2}$H$_{2}$O)

- Total body water can be measured by deuterium dilution
- Deuterium oxide is water labelled with $^2$H
- The maximum enrichment of $^2$H in body water is $\sim0.1\%$ or 1000 mg/L (ppm) when a 30 g (FTIR) dose of deuterium oxide is given to an adult
- Deuterium oxide had been used in studies involving humans for over 50 years. No harmful effects have been observed in mammals below 15% enrichment of body water

• Water is found exclusively within the Fat Free Mass (FFM)
• At birth the body is 70-75% water
• The adult body contains 50-60% water, decreasing to less than 40% in obese adults
• In adults FFM contains ~73.2% water
• Measure TBW
• Calculate FFM (TBW / hydration factor)
• A 2 compartment model is used. Body fat is the difference between FFM and body weight
• The enrichment of $^2$H in saliva or urine samples can be measured using an Isotope Ratio Mass Spectrometer (IRMS) or a Fourier Transform Infrared Spectrometer (FTIR)

• FTIR cannot be used for analysis of urine
Calculation of body composition

- TBW is calculated from the volume of distribution ($V_D$) of a dose of deuterium oxide ($^{2}$H$_2$O). $V_D$ is calculated from the enrichment of $^2$H in body water and the dose of D$_2$O consumed.

- If samples are analysed by FTIR

\[
V_D \text{ (kg)} = \frac{\text{dose (mg)}}{\text{enrichment (mg/kg)}}
\]

\[
\text{TBW (kg)} = \frac{V_D}{1.04}
\]

- In adults FFM is assumed to be 73.2% water

\[
\text{FFM (kg)} = \frac{\text{TBW (kg)}}{0.732}
\]

- FM is calculated by difference between body weight and FFM

\[
\text{Fat Mass (kg)} = \text{body weight (kg)} - \text{FFM (kg)}
\]

- Fat mass is often expressed as % body weight
F.1. Deuterium Oxide “Dose-to-Mother” Technique to Assess Intake of Human Milk in Breastfed Infants

1. Mother drinks $^2\text{H}_2\text{O}$
2. Baby consumes $^2\text{H}_2\text{O}$ in human milk
3. Saliva in both mother and baby is enriched with $^2\text{H}_2\text{O}$

Dose-to-mother technique for assessing human milk intake.

Two compartment steady state model of water flow in a mother–baby pair.
FIG 26. Two compartment steady state model of water flow in a mother–baby pair: $F$ = flow; $m$ = mother; $b$ = baby; $o$ = outside; $V$ = volume TBW; $V_m$ = mother’s TBW volume; $V_b$ = baby’s TBW volume; $F_{mo}$ = from outside to mother; $F_{bo}$ = from outside to baby (non-breast fluid intake); $F_{bm}$ = from mother to baby (breast milk intake); $F_{om}$ = from mother to outside; $F_{ob}$ = from baby to outside.

FIG 2. Disappearance of deuterium from the body water of the mother (●) and appearance in her baby (■).
Infant feeding practices in transition: Breast milk intake, complementary feeding and body composition during infancy (Wanabhorn Tongchom, Ph.D. student)

Breast milk intake and liquid other than breast milk at 6 weeks, 3, and 6 months classified by breastfeeding patterns at 3 months

<table>
<thead>
<tr>
<th>Infant age at 6 weeks, n²</th>
<th>Breastfeeding patterns classified at 3 months of infant age¹</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EBF</td>
<td>PBF</td>
<td>PartBF</td>
<td>p-value⁵</td>
</tr>
<tr>
<td>Breast milk intake⁶ (g/d)</td>
<td>734.8±178.9a</td>
<td>760.3±198.1a</td>
<td>574.9±296.0b</td>
<td>0.001</td>
</tr>
<tr>
<td>Non-milk oral intake (g/d)</td>
<td>85.8±72.2a</td>
<td>151.0±221.6a</td>
<td>323.0±280.4b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infant age at 3 months, n³</td>
<td>26</td>
<td>41</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Breast milk intake⁶ (g/d)</td>
<td>768.4±144.0a</td>
<td>763.5±175.9a</td>
<td>543.4±322.9b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-milk oral intake (g/d)</td>
<td>41.7±37.1a</td>
<td>117.4±173.2a</td>
<td>461.4±467.8b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infant age at 6 months, n⁴</td>
<td>29</td>
<td>41</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Breast milk intake (g/d)</td>
<td>722.5±167.9</td>
<td>698.9±194.5</td>
<td>661.2±198.3</td>
<td>0.533</td>
</tr>
<tr>
<td>Non-milk oral intake (g/d)</td>
<td>185.1±179.0a</td>
<td>249.9±213.3ab</td>
<td>380.3±246.2b</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Body mass index is associated with fat mass in normal, overweight/obese, and stunted preschool children in central Thailand

Tippawan Pongcharoen PhD, Kunchit Judprasong PhD, Siwaporn Jitngarmkusol MSc, Wantanee Kriengsinyos PhD, Pattanee Winichagoon PhD

Work was carried out through the Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

Table 2. Body composition of preschool children by nutritional status of 3-5 y old children

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Normal BMI (n=47)</th>
<th>Stunted</th>
<th>Thin</th>
<th>Overweight/obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body water (kg)</td>
<td>8.6±1.0²</td>
<td>3.9±1.3²</td>
<td>3.4±1.2²</td>
<td>9.2±1.1²</td>
</tr>
<tr>
<td>Total body water (%)</td>
<td>56.4±3.2²</td>
<td>30.4±3.3²</td>
<td>34.8±2.9²</td>
<td>34.5±3.2²</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>4.3±1.1²</td>
<td>2.1±0.9²</td>
<td>2.6±1.1²</td>
<td>4.8±1.3²</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>27.8±4.1²</td>
<td>26.1±3.4²</td>
<td>25.5±3.2²</td>
<td>30.1±4.2²</td>
</tr>
<tr>
<td>Fat mass index (kg/m²)</td>
<td>4.3±0.9²</td>
<td>2.9±0.8²</td>
<td>3.2±1.0²</td>
<td>5.2±1.4²</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>10.9±1.2²</td>
<td>5.8±1.1²</td>
<td>5.4±1.2²</td>
<td>14.0±2.0²</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>72.2±4.1³</td>
<td>73.8±3.2²</td>
<td>76.1±3.4²</td>
<td>59.5±3.9³</td>
</tr>
<tr>
<td>Fat-free mass index (kg/m²)</td>
<td>11.1±0.8²</td>
<td>10.7±0.7²</td>
<td>9.5±0.5²</td>
<td>12.9±1.1³</td>
</tr>
</tbody>
</table>

²ANOVA (Tukey’s post hoc test), values in a row without a common superscript letter differ, p<0.05.

BMI is appropriate for reflecting adiposity in normal and overweight/obese children, but not undernourished preschool children.
• DLW is a mixture of $^2$H-labelled water and $^{18}$O-labelled water

• DLW is the reference method for measuring TEE in free-living conditions while people go about their normal daily activities

• In weight-stable adults

  
  energy intake = energy expenditure

• DLW is the reference method for measuring energy intake. DLW can be used to validate dietary intake methodology
Free living daily energy expenditure by the doubly labelled water method ($^{2}\text{H}_{2}\text{^{18}O}$)
Fig. 1. Schematic diagram of the changes in isotopic enrichments of deuterium and oxygen-18 in the body of an animal following an injection. Isotopes flood the body water leading to a steep increase in enrichment until an equilibrium is reached. Following this equilibrium, the isotopes are eliminated down exponential routes back to the background levels. Because oxygen-18 is eliminated in both water and CO₂, its enrichment declines faster than that of deuterium, which to a first approximation is eliminated only in water. The difference in the isotope elimination rates provides a quantitative estimate
Body composition and Energy Expenditure

- Body composition of preschool children using stable isotope
- Establishing equation for body composition in school-aged children
- Ethnic differences in body fat distribution among Asian pre-pubertal children: A cross-sectional multicenter study
- Body composition and energy expenditure of children in the project Childhood Obesity
- Total energy expenditure in adults/elderly with different PAL

Capacity building (IAEA) on using stable isotope (IRMS) and other methods (Bod Pod, DXA)
BIOAVAILABILITY

(what we get from what we have taken in)

• That which becomes bioavailable
• The fraction (or percentage) of nutrient absorbed that is useful to the body
• The degree to which an absorbed nutrient is available to the system
Iron bioavailability in 8–24-month-old Thai children from a micronutrient-fortified quick-cooking rice containing ferric ammonium citrate or a mixture of ferrous sulphate and ferric sodium ethylenediaminetetraacetic acid

Visit Chavasit, Suparat Porasuphatana, Umaporn Suthutvoravut, Christoph Zeder, and Richard Hurrell

- Iron absorption from the rice containing FAC or FeSO₄ + NaFeEDTA was sufficiently high to be used in its formulation
- Mean fraction iron absorption was 5.8 ± 1.9 % from FAC and 10.3 ± 1.9 % from FeSO₄ + NaFeEDTA
- The relative bioavailability of FAC was 83%
- The relative bioavailability of FeSO₄ + NaFeEDTA was 145 %
Main points:

- **Objectives**: To measure iron absorption of fish sauce fortified with ferrous sulfate, ferric ammonium citrate or ferrous lactate acid; To identify the effect of added citric acid on iron absorption from ferrous sulfate fortified fish sauce.

- **Design**: Iron absorption from the intrinsically labeled compounds was determined via erythrocyte incorporation of isotope labels ($^{57}$Fe and $^{58}$Fe).

- **Results**: Iron absorption was 50-100% higher from ferrous sulfate fortified fish sauce than from fish sauce fortified with ferric ammonium citrate or ferrous lactate. In the presence of citric acid as a chelator, ferrous sulfate would appear to be a useful fortificant for fish sauce.
**Table 1** Composition of test meals

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test meal A: Rice + vegetable soup + fish sauce (12 ml) + water (200 g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ $^{57}$Fe-ferrous lactate</td>
<td>+ $^{57}$Fe-ferric ammonium citrate (4.52 ± 0.01 mg $^{57}$Fe)</td>
<td>+ $^{57}$Fe-ferrous sulfate (4.10 ± 0.15 mg $^{57}$Fe)</td>
</tr>
<tr>
<td>(3.74 ± 0.02 mg $^{57}$Fe) + 36 mg citric acid</td>
<td>(57$^{Fe}$) + 36 mg citric acid</td>
<td></td>
</tr>
<tr>
<td><strong>Test meal B: Rice + vegetable soup + fish sauce (12 ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ $^{58}$Fe-ferrous sulfate</td>
<td>+ $^{58}$Fe-ferrous sulfate</td>
<td>+ $^{58}$Fe-ferrous sulfate</td>
</tr>
<tr>
<td>(3.98 ± 0.01 mg $^{58}$Fe) + 36 mg citric acid</td>
<td>(4.52 ± 0.01 mg $^{58}$Fe) + 36 mg citric acid</td>
<td>(3.98 ± 0.03 mg $^{58}$Fe) + 36 mg citric acid</td>
</tr>
</tbody>
</table>

*Iron compounds and citric acid were contained in the fish sauce in all meals except meal A study 3, where the iron compound was added to the water. Isotope doses are given as the mean ± s.d.
Chili, but Not Turmeric, Inhibits Iron Absorption in Young Women from an Iron-Fortified Composite Meal


<table>
<thead>
<tr>
<th>Study</th>
<th>Test meal</th>
<th>Composition²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>Basic meal + ( {^{57}}\text{FeSO}_4 ) (3.92 ± 0.01 mg ( {^{57}}\text{Fe} )) or ( {^{58}}\text{FeSO}_4 ) (4.09 ± 0.05 mg ( {^{58}}\text{Fe} ))</td>
</tr>
<tr>
<td></td>
<td>R + C</td>
<td>Basic meal + ( {^{57}}\text{FeSO}_4 ) (3.92 ± 0.01 mg ( {^{57}}\text{Fe} )) or ( {^{58}}\text{FeSO}_4 ) (4.08 ± 0.05 mg ( {^{58}}\text{Fe} )) + chili (4.23 ± 0.05 g)</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>Basic meal + ( {^{57}}\text{FeSO}_4 ) (3.92 ± 0.02 mg ( {^{57}}\text{Fe} )) or ( {^{58}}\text{FeSO}_4 ) (4.10 ± 0.01 mg ( {^{58}}\text{Fe} ))</td>
</tr>
<tr>
<td></td>
<td>R + T</td>
<td>Basic meal + ( {^{57}}\text{FeSO}_4 ) (3.92 ± 0.02 mg ( {^{57}}\text{Fe} )) or ( {^{58}}\text{FeSO}_4 ) (4.10 ± 0.01 mg ( {^{58}}\text{Fe} )) + turmeric (0.501 ± 0.001 g)</td>
</tr>
<tr>
<td>3.1</td>
<td>R</td>
<td>Basic meal + ( {^{58}}\text{FeSO}_4 ) (4.11 ± 0.01 mg ( {^{58}}\text{Fe} ))</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Basic meal + ( {^{57}}\text{Ferric pyrophosphate} ) (4.20 ± 0.21 mg ( {^{57}}\text{Fe} ))</td>
</tr>
<tr>
<td>3.2</td>
<td>R</td>
<td>Basic meal + ( {^{58}}\text{FeSO}_4 ) (4.09 ± 0.04 mg ( {^{58}}\text{Fe} ))</td>
</tr>
<tr>
<td></td>
<td>P + C</td>
<td>Basic meal + ( {^{57}}\text{Ferric pyrophosphate} ) (4.05 ± 0.09 mg ( {^{57}}\text{Fe} )) + chili (4.25 ± 0.03 g)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, \( n = 10 \)/study.

² The basic meal consisted of rice, vegetable soup, and distilled water.
**TABLE 3**: Effect of chili (studies 1 and 3) and turmeric (study 2) ingestion on iron absorption in women after a basic test meal

<table>
<thead>
<tr>
<th>Study</th>
<th>Plasma ferritin</th>
<th>Test meal</th>
<th>Fe absorption</th>
<th>RBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>R basic meal + FeSO₄</td>
<td>9.7% (3.9, 24.4)</td>
<td>–</td>
</tr>
<tr>
<td>(28, 119)</td>
<td>R+C basic meal + FeSO₄ + chili</td>
<td>6.0% (2.2, 16.5)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>R basic meal + FeSO₄</td>
<td>8.7% (3.8, 19.9)</td>
<td>–</td>
</tr>
<tr>
<td>(13, 262)</td>
<td>R+T basic meal + FeSO₄ + turmeric</td>
<td>8.9% (3.3, 24.1)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>49</td>
<td>R basic meal + FeSO₄</td>
<td>10.4% (3.8, 28.7)</td>
<td>6.4</td>
</tr>
<tr>
<td>(14, 125)</td>
<td>P basic meal + ferric pyrophosphate</td>
<td>0.7% (0.3, 1.5)</td>
<td>(4.5, 13.3)</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>R basic meal + FeSO₄</td>
<td>9.9% (3.9, 24.6)</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>P+C basic meal + ferric pyrophosphate + chili</td>
<td>0.5% (0.3, 1.0)</td>
<td>(1.9, 16.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Calcium bioavailability of winged bean, young pod was 71% of milk
Calcium bioavailability of ivygourd was 86% of milk
**Objective:** The objective of this study in relation to vitamin D status.

**Methods:** We measured fractional calcium absorption in 19 postmenopausal Thai women (aged 52–63 y) with low parathyroid hormone (PTH) and serum 25-hydroxyvitamin D levels. A calcium isotope method based on calcium ingested in random order: a green leafy vegetable, milk, and cassia. Women received intravenous $^{42}$Ca and calcium supplements.

**TABLE 2** Fractional calcium absorption from milk and cassia in 19 postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Milk</th>
<th>Cassia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium content of test meal, mg</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fractional absorption, %</td>
<td>47.8 ± 12.8</td>
<td>42.6 ± 12.3*</td>
</tr>
<tr>
<td>$V_a^2$ from test meal, mg/d</td>
<td>135 ± 36.3</td>
<td>120 ± 34.9*</td>
</tr>
<tr>
<td>24-h Calcium intake, mg</td>
<td>137 ± 12.8</td>
<td>122 ± 12.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, $n = 19$. *Significantly different from milk values, $P = 0.006$ (paired t test). $Ca_i$ calcium intake; $V_a$ true calcium absorption.

2 Fractional absorption $\times Ca_i$. 

• Extruded rice with 10 mg of iron, 9 mg of zinc and 1.05 mg of VA/g feeding schoolchildren for 2 months.

• A stable isotope dilution technique with labeled 13C2-retinyl acetate was used before and at the end of the feeding period to estimate total body reserves of vitamin A.
Stable isotope: Metabolic studies

- Stable isotope labelled glucose, fatty acids and amino acids can be used to measure the rate of synthesis of carbohydrates, lipids and proteins.
- $^2$H and $^{13}$C labels are most commonly used.
- The labelled compound is given intravenously, and blood or tissue samples are collected at intervals.
- These methods are much more invasive.
- Samples are analysed by mass spectrometry techniques (GC/MS, LC/MS or IRMS) after initial processing.
- Sample preparation often takes several days.
Using IRMS on studying amino acid requirement and metabolism

• Lysine requirement in healthy adults using the indicator amino acid oxidation technique: emphasis on gender ($^{13}$C-phenylalanine)

• Long-term effects of histidine depletion on whole-body protein metabolism in healthy adults ($^{13}$C-phenylalanine and $^{15}$N-glycine)
To find the best condition for intrinsically-labelled mungbean with D$_2$O

**5 treatments**
- Control
- 25% flowering I
- 50% flowering I
- 75% flowering I
- 100% flowering I

Labelled by 20% of D$_2$O
*(Deuterium oxide)*

Bioavailability of protein in mungbean meal

30 D First FI
35 D 50 % FI
40 D 100 % FI
50 D First FII
55 D 50% FII
60 D 100% FII
75 D Fully repine FII

Flowering I
Fruit ripening I
Flowering II
Fruit ripening II
Test meal composition

- Mungbean protein labelled with deuterium
- Reference protein (Spirulina) labelled with $^{13}$C
- $^{13}$C$_6$-phenylalanine
- Na$^{13}$C-bicarbonate (only first small meal)
- Others: rice, vegetable oil, meat, vegetable, condiments prepared in usual way

<table>
<thead>
<tr>
<th>Time to serve (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
</tr>
</tbody>
</table>

Analysis
• Stable isotopes are very useful tools which are used extensively in scientific research. Within the field of nutritional studies stable isotopes are used for studying the flow of nutrients through the human body. Since they are safe and non-radioactive they can even be used in infants and pregnant women.

• Stable isotopically-labeled compounds are used for in vivo studies of both nutrition and metabolism mechanisms. Labels, whether $^{13}$C, D, or $^{15}$N, offer researchers a way to track the mechanisms by which organisms metabolize nutrients.

• Stable isotope labeling techniques have been used to assess carbohydrate digestion and fermentation, protein-derived amino acid bioavailability and requirements, micronutrient bioavailability and to track microbe-microbe and microbe-host interactions at the single cell level.
Technical support/capacity development: Built to Last-
Institute of Nutrition, Mahidol University, Thailand

• **Technical Cooperation at regional /national levels:** priority agenda, networking

• **Human resource development:** Expert Mission; Scientific visits; Workshops and trainings; doctoral CRP

• **Instrumentation and Facilities:** Regional or Sub-regional RESOURCE Centers
Human Health Programme

From prevention to diagnosis and therapy

The Human Health Programme provides a holistic approach to the prevention, diagnosis and treatment of non-communicable diseases, covering four main support areas: nutrition; diagnosis and follow-up; radiation oncology and radiotherapy; and quality assurance.

Good nutrition is the foundation of human wellbeing, a value also recognized by the United Nations Sustainable Development Goals. The IAEA Human Health Programme, as part of its work on prevention and healthy living, supports the Goals through the application of nuclear techniques, in particular using stable isotopes. The Programme focuses on:

- infant and young child nutrition;
- childhood obesity;
- maternal and adolescent nutrition;
- nutrition and ageing;
- diet quality; and
- the assessment of environmental health effects.
The use of stable isotope techniques in human nutrition research offer strong and concrete evidence beyond conventional approaches in order to appropriate guide policy and program decision to alleviate malnutrition in all its forms.
HANK YOU