UNCOVERING THE HIDDEN LINKS

NUCLEAR & ISOTOPE TECHNIQUES TARGET NUTRITIONAL NEEDS

BY VENKATESH IYENGAR

Hunger and malnutrition are among the most devastating problems facing the majority of the world's poor and needy. Several United Nations Conferences on Food and Nutrition have highlighted the need for eliminating poverty and malnutrition, especially among women and children.

Nuclear and isotopic techniques are valuable tools in helping to meet the multi-faceted challenges.

Each year 30 million infants in the developing world are born with intra-uterine growth retardation leading to low weight at birth. This represents about 24% of the births in these countries. Nearly 200 million children (more than 150 million in Asia and about 27 million in Africa) under age five are moderately to severely undernourished, while 70 million are severely undernourished. There are projections that about one billion children will be growing up by 2020 with impaired mental development.

Adults suffer as well. About 243 million adults in developing countries are reportedly undernourished, which lowers their work capacity and resistance to infection. Anemia in mothers is pandemic, surpassing 80% in some countries, and is associated with very high rates of maternal mortality. Evidence from both developing and industrialized countries links maternal and early childhood malnutrition to increased susceptibility in adult life to diabetes, heart disease, and hypertension.

A fundamental link is emerging between mother and child malnutrition and the child's subsequent marked sensitivity to abdominal obesity, diabetes, high blood pressure, and coronary heart disease. Added to this is the burden of over-nutrition and obesity that is rapidly emerging in the same developing countries, affecting children and adults alike.

Since the early 1990s, emerging problems of what is called "hidden or silent hunger" have commanded greater attention. The term refers to micronutrient malnutrition found in men, women, and children who are persistently undernourished. According to global estimates, some 2 billion people in more than 100 developing countries suffer from multiple micronutrient deficiencies. More than 90% of pregnant women and preschool children in developing countries show signs of iron deficiency anemia and other related disorders.

Nutritional Disorders Across the Human Lifespan. Malnutrition seriously affects individuals in all stages of human life. Combating nutrition-related disorders that span across the entire life cycle will be the biggest task for scientists in the decades ahead. *(See table, page 18.)*

Malnutrition during pregnancy often results in intrauterine growth retardation (IUGR) of the fetus, resulting in low birth weight of the infant. This directly influences the survival and growth of young children. Poor nutritional status of the mother during lactation further compounds the already existing negative effects on the growing infant. These undernourished children in later years show poor performance at school.

Adolescent nutrition is an area that is just being recognized as one that requires nutritional monitoring.

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Children gain up to 50% of their adult weight, 20% of their adult height, and 50% of their adult skeletal mass during this crucial stage of adolescent life. Energy, protein, calcium and micronutrients, including iron, zinc and folate, are required at maximum levels. Deficits in intake of nutrients could signal the start of nutrition-related disorders in adulthood. Malnourished adults have decreased overall resistance to disease and increased disability at work, eventually affecting the overall quality of life, especially as they join the ranks of the elderly.

Nutrition of the elderly is of special concern because of the high prevalence of osteoporosis. This serious disease is characterized by low bone density and leads to fractures (particularly in postmenopausal women), severely limiting their quality of life. Aging is also associated with changes in body composition, such as a decline in lean body mass, and with an increase in the risks of disease and accidents. Thus, increasing attention to nutritional needs and care of the aged is important to reduce the risk of the onset of osteoporosis and other degenerative diseases.

Pollution Interactions & Related Diseases. The severity of malnutrition is further exacerbated by environmental pollution. Pollutants such as lead and oxides of nitrogen have debilitating effects on people who are already poorly nourished. Pollution, in the larger context, encompasses both anthropogenic and non-anthropogenic factors. Parasitic infestations, and the spread of infections through communicable diseases, form a major segment of the environmental component of nutritional disease.

In Asia, Africa, and Latin America, the malnourished populations suffer from a host of other nutrient deficiencies and are more prone to parasitic infestations (hookworm, amoebiasis) and malaria. The contrast in mortality between people living in developed and developing countries, arising out of the differential load of infections, is a key indicator. *(See graph, page 17.)* Operating in synergism with diarrheal, respiratory and other infections, poor diets in early childhood lead to growth failure, delayed motor and mental development, impaired immunocompetence, and higher risks of complications and deaths from infectious disease.

Another critical issue is food safety, which touches several aspects surrounding the nutrition-health-disease domain. The study of nutritional toxicology is fast becoming a global challenge. Inter-element interactions of lead and iron (anemia), mercury and iron, arsenic and selenium, selenium and iodine, cadmium and zinc, are becoming an area of concern. So is placental transfer of nutrients and toxicants from the mother to the fetus.

Children are more susceptible to the effects of lead and a combination of iron deficiency and lead toxicity in them can have devastating effects. Higher levels of lead may be absorbed in children with iron deficiency anemia compared to those who are iron sufficient.

Besides heavy metals, nutrient interactions with pesticides, oxides of nitrogen, tobacco, and alcohol are important to understand.

The combination of pollution and malnutrition affects an individual's quality of life, causing disability and morbidity. This is often expressed as Disability-Adjusted Life Years (DALY), which is a composite measure of the time lost due to premature mortality and time lived with disability. The higher the magnitude of the DALYs, the greater the burden of disease. A significant fraction of the DALYs worldwide is contributed by infectious and parasitic diseases. On a regional basis, over 40% DALY due to infectious and parasitic disease is seen in sub-Saharan Africa as opposed to less than 3% in the European region.

RESPONDING TO THE NEEDS

Global nutrition problems raise a host of questions and warrant action by the international community of scientists, nutritionists, physicians and other medical professionals. What steps should be taken to remedy this situation? How can this be accomplished economically? How can progress be monitored? What is the role of technology in the overall monitoring process? The last question, which is most relevant to this article, is of particular importance to the IAEA and its support of nutrition programmes.

The IAEA's activities in human nutrition were initiated to apply nuclear and related isotopic techniques for solving problems prevalent in developing countries.

Among the numerous applications available, isotopic techniques are uniquely well suited to targeting and tracking progress in food and nutrition development programmes. These are tools that help evaluate nutritional status of individuals and populations, measure nutrient requirements and the uptake and bio-availability of vitamins and minerals.

The IAEA's efforts help to:

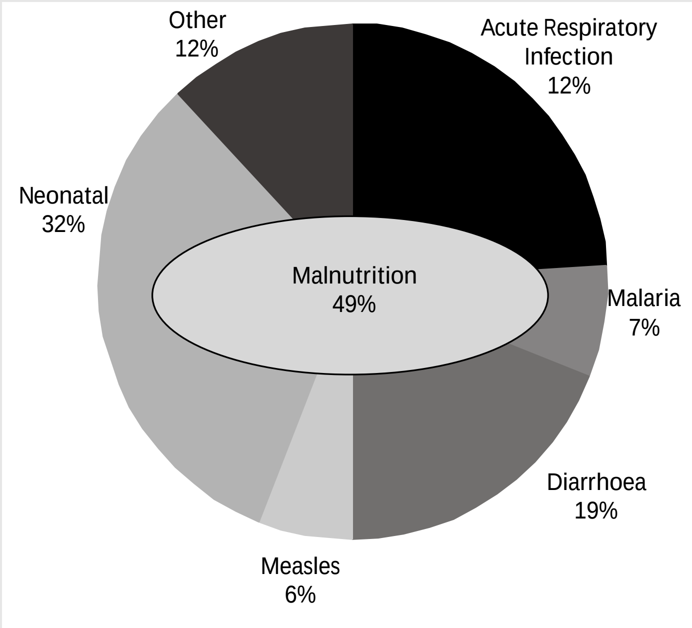
- verify the nature of the nutrition problem and the efficacy of specific interventions;
- implement nutrition intervention programmes by monitoring effectiveness and reducing programme costs;
- guide in the processing of local foods for optimal nutritional value;
- serve as early indicators of important long-term health improvements; and
- strengthen capacity building in developing countries.

Health and nutrition monitoring has been strengthened through extensive nuclear and isotope applications in industrialized countries to analyze human energy requirements, body composition, and the metabolism of important nutrients such as protein, fat, vitamins and minerals. The information has led directly to many improvements in nutrition and health. The challenge is to extend benefits to the developing world.

Investigations can be carried out in key areas of health and nutrition. These include:

MALNUTRITION'S TOLL ON CHILDREN

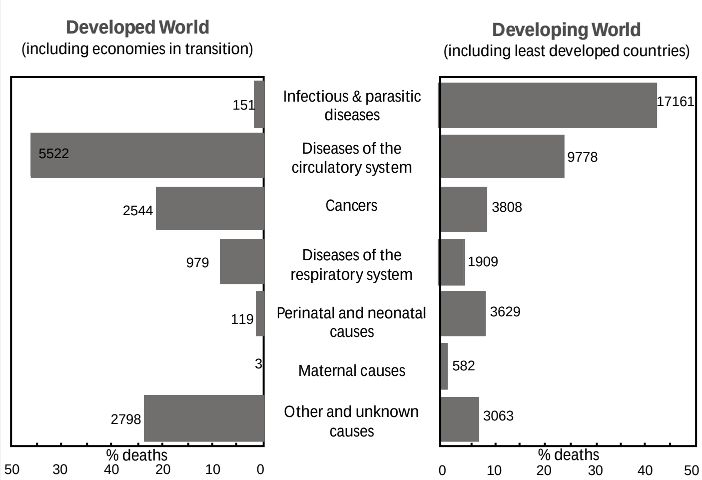
DISTRIBUTION OF 10.4 MILLION DEATHS IN DEVELOPING COUNTRIES AMONG CHILDREN LESS THAN 5 YEARS OLD



Source: WHO

CONTRASTING CAUSES

CAUSES OF DEATH IN THE DEVELOPING & DEVELOPED WORLD



Note: The figures given in thousands refer to the total annual deaths from each cause in either the developed or developing world. Thus, although the proportion of total deaths from diseases of the circulatory system is higher within the developed world, the 5.5 million deaths are far outstripped by the 9.8 million deaths from the same diseases in the developing world.

Source: *The World Health Report, 1997,* WHO, Geneva

MALNUTRITION
ACROSS THE HUMAN LIFESPAN

Life Stage	Nutritional Disorders	Main Consequences	Applicable Nuclear Techniques
Embryo/Foetus	Intrauterine Growth Retardation, Iodine Deficiency Disorders, Folate Deficiency	• Low birth weight • Brain damage • Neural tube defect • Still births	• RIA (T_3, T_4, TSH, folate)
Neonate	Low birth weight, Iodine Deficiency Disorders	• Growth retardation • Developmental retardation • Brain damage • Continuing malnutrition	• RIA (T_3, T_4, TSH, folate) • Deuterium labeled water (breast milk intake) • Stable isotopes (micronutrients) • ^{13}C and ^{15}N labeled substrates (macronutrients)
Infant & Young Child	Protein-Energy Malnutrition, Iodine Deficiency Disorders, Vitamin A Deficiency, Iron Deficiency Anemia,	• Developmental retardation • Increased risk of infection • High risk of death • Blindness • Anemia • Growth retardation	• RIA (ferritin, folate, T_3, T_4, TSH and other hormones) • Deuterium labeled water (breast milk intake) • Stable isotopes (micronutrients, e.g. ^{57}Fe, ^{67}Zn) • ^{13}C labeled substrates (macronutrients, Helicobacter pylori)
Adolescent	Protein-Energy Malnutrition, Iodine Deficiency Disorders, Iron Deficiency Anemia, Folate Deficiency, Calcium Deficiency	• Delayed growth spurt • Stunted height • Delayed/retarded intellectual development • Goitre • Increased risk of infection • Blindness • Anemia • Inadequate bone mineralization	• RIA (ferritin, folate, T_3, T_4, TSH and other hormones) • Doubly labeled water (energy expenditure) • Stable isotopes (micronutrients) • ^{13}C labeled substrates (macronutrients, Helicobacter pylori) • DEXA (bone density, body composition)
Pregnant & Lactating Mothers	Protein-Energy Malnutrition, Iodine Deficiency Disorders, Vitamin A Deficiency, Iron Deficiency Anemia, Folate Deficiency, Calcium Deficiency	• Maternal anemia • Maternal mortality • Increased risk of infection • Night blindness/blindness • Low birth weight/ high risk death rate for fetus	• RIA (ferritin, folate, T_3, T_4, TSH and other hormones) • Deuterium labeled water (breast milk intake) • Stable isotopes (micronutrients) • ^{13}C labeled substrates (macronutrients, Helicobacter pylori) • DEXA (bone density, body composition)
Adults	Protein-Energy Malnutrition, Iron Deficiency Anemia, Obesity, Cancer	• Thinness • Lethargy • Obesity • Heart disease • Diabetes • Hypertension/stroke • Anemia	• RIA (ferritin, hormones e.g. insulin) • Doubly labeled water (energy expenditure) • Stable isotopes (micronutrients) • ^{13}C labeled substrates (macronutrients, Helicobacter pylori) • DEXA (body composition)
Elderly	Protein-Energy Malnutrition, Iron Deficiency Anemia, Obesity, Cancer, Osteoporosis	• Thinness • Obesity • Spine and hip fractures & Accidents • Heart disease • Diabetes	• RIA (ferritin, hormones) • Doubly labeled water (energy expenditure) • Stable isotopes (micronutrients) • ^{13}C labeled substrates (macronutrients, Helicobacter pylori) • DEXA (bone density, body composition) • Deuterium labeled water (body composition)

Notes: DEXA = Dual Energy X-Ray Absorptiometry; RIA = Radioimmunoassay
TSH = Thyroid Stimulating Hormone T_4 = Thyroxin

Source: Modified from WHO/NHD/99.9

NUCLEAR & ISOTOPE TECHNIQUES IN HEALTH & NUTRITIONAL STUDIES

Assessment of Micronutrient Malnutrition. Stable isotopes provide the only direct way for the measurement of iron uptake and bio-availability. They have come to be regarded as a kind of "gold standard" for iron studies in humans and other studies in nutrient bioavailability (e.g. iron, zinc, and vitamin A).

Isotope dilution methods are used in the assessment of vitamin A status. The principle relies on labelled carotenoid conversions to vitamin A, which can be traced with carbon-13 carotenoids. This way, vitamin A pool sizes are measured by the dilution of an oral ingested tracer into the different body pools. This technique has potential applications in measuring the effectiveness of vitamin A and carotenoid supplementation and fortification regimes in nutrition studies.

Another powerful use for stable isotopes is in the assessment of trace element bio-availablity and pool sizes for trace elements, such as iron (iron-57 and iron-58) and zinc (zinc-67 and zinc-70). The uptake of these labelled micronutrients can be traced *in vivo*. This approach has proved valuable in measuring the effectiveness of nutrition supplementation or fortification trials in several developing countries.

Detection of Infections. Carbon-13 breath tests are used to examine bacterial colonization in the stomach by *Helicobacter pylori*. The test measures the production rate of $^{13}CO_2$ in expired air, followed by oral ingestion of ^{13}C-labelled urea. The tests using stable isotopes are reliable and non-invasive tools that can be safely applied in children wherever high rates of infection and malnutrition are observed. Importantly, the cost of the equipment is not very expensive and can be within the reach of many developing countries.

Estimation of Total Energy Expenditure. The doubly-labelled water method ($^2H_2{}^{18}O$) is the only technique that can accurately determine the energy needs of people in their own environments and is one of the most reliable methods for determining food energy intake. This method is gaining wider acceptance since it is inexpensive, accurate, and can be applied under field conditions.

When doubly labelled water is administered to a subject, both isotopes mix with body water and are eliminated in body fluids over a period of days. The turnover of body water can be estimated from the daily measurements of hydrogen-2 (deuterium) concentration in urine or saliva samples. When the samples are analysed for oxygen-18, the values will reflect a more rapid excretion rate than that for hydrogen-2 because the oxygen-18 is also incorporated into exhaled carbon dioxide. The difference in excretion rates between oxygen-18 and hydrogen-2 tracers thus reflects the volume of carbon dioxide produced over the period of observation. This parameter can be used to calculate the total energy expenditure of a subject.

Determination of Lean Body Mass. A tracer dose of water labelled with hydrogen-2 and oxygen-18 is administered and allowed to equilibrate for four to six hours. The isotope concentration in saliva or urine will reflect the dilution undergone by the isotope. When the lean body mass is calculated, the difference in body weight is the amount of adipose (fatty) tissue.

Measurement of Overall Nitrogen Flux. The nitrogen-flux balance stumbles in periods of stress, then the catabolic processes predominate over synthetic processes and a negative balance is the result. Whole body protein turnover is measured by administration of a single oral dose of an amino acid, or preferably a protein, labelled with nitrogen-15. Urine is collected for nine to twelve hours and the amounts of tracer nitrogen in urinary NH_3 and in the urea are determined. These two values provide a reliable estimation of whole-body protein turnover that is insensitive to changes in nonprotein-nitrogen metabolism.

Nutrient Absorption and Utilization. Weaning infants often have periods of infection leading to diarrhoea. During these periods nutrient intake is insufficient to maintain infant growth – and regeneration of small intestinal capacity is essential. When rice labelled with carbon-13 (exposed to $^{13}CO_2$ during periods of photosynthesis) is cooked and eaten, digestion and absorption of the starch can be detected from the appearance of labelled $^{13}CO_2$ in breath samples. The degree of malabsorption can be estimated from the recovery of tracer carbon in total stool carbon.

Analysis of Foods. Neutron activation analysis (NAA) is very effective due to its exceptional sensitivity and the possibility of simultaneous determination of several trace elements. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) also offers multielement determinations. Application of NAA-related methods is particularly attractive for developing countries since many research reactors are available to provide the needed neutron source.

Air Pollution Measurement. Nuclear and related analytical techniques are uniquely suited for conducting non-destructive multi-element analyses of air particulate matter collected on filters. Commonly used techniques include: ICP-MS, NAA, Particle-Induced X-ray Emission, Particle-Induced Gamma-ray Emission, Energy Dispersive X-ray Fluorescence Analysis, and Total Reflection X-ray Fluorescence.

- Assessment of Nutritional Status and Nutrient Requirements;
- Assessment of Micronutrient Malnutrition;
- Detection of Infections;
- Analysis of Foods;
- Air Pollution Measurement.

(See box on page 19 for examples of applications.)

STABLE ISOTOPES IN IAEA-SUPPORTED NUTRITION STUDIES

Many factors are important to nutritional assessments of infants, children, pregnant women, and nursing mothers, as well as people who subsist on marginal food supplies. They include the measurement of breast-milk intake, energy expenditure, micronutrient status, macronutrient utilization, and body composition. Stable isotopic tracers are safe and non-invasive techniques. They emit no externally measurable radiation and their presence in excess of natural levels is detectable only by changes in the ratio of minor isotope to major isotope. The ratio is measured by an isotope ratio mass spectrometer in which heavy and light forms of the same molecule undergo separation and quantification.

The costs of analysis are indeed a cause for concern since the instrumentation and expertise required are not available in many countries. However, stable isotopes for IAEA-supported studies are made available under the project, and in some cases assistance is also provided for analysis in an IAEA recognized laboratory. The underlying idea is that these studies would encourage the strengthening of infrastructure at national facilities for subsequent investigations.

The major isotopes (hydrogen-1, carbon-12, nitrogen-14, or oxygen-18) are always accompanied by a constant proportion of minor heavier isotopes whose individual abundance range from 0.02% to 1.11%. An inventory of the human body shows that a 50-kilogram individual has an aggregate of 225 grams of hydrogen-2, carbon-13, nitrogen-15, oxygen-17, and oxygen-18. Although there are variations in the respective ratios of minor to major isotopes, each major isotope has a characteristic baseline abundance to which tracer measurements are referred. Stable isotopes can be administered orally and the metabolic products into which they enter (e.g. body water, respiratory carbon dioxide, urea) can be sampled in breath, saliva, milk, urine, and stool.

There are several strategic applications of nuclear and isotopic techniques supported by the IAEA. Indeed some of these techniques possess very special features and specificity, thus rendering them unique in their suitability for studies in specific fields of human nutrition.

To date, isotopic strategies evolved through IAEA efforts are practiced in more than 50 Member States. A few examples are cited below:

Technical Cooperation Projects. A regional technical cooperation programme involving Argentina, Brazil, Chile, Cuba, and Mexico applied isotopes for evaluating nutrition intervention programmes. The national project in Chile completed a study using isotope techniques for various needs. Scientists measured iron bio-availability in fortified milk of the National Complementary Food Programme, the bio-availability of zinc and body composition in children, and the body composition and energy expenditure in pre-school children.

Elsewhere, the first phase of a regional project in East Asia and Pacific yielded practical results. Studies measured the effectiveness of multi-nutrient supplementation using stable isotopic techniques to assess zinc and iron bio-availability in seven participant countries, China, Indonesia, Malaysia, Pakistan, Philippines,Thailand, and Viet Nam.

Additionally, to target malnutrition, the IAEA has been developing non-invasive isotopic tracer techniques for measuring whole body vitamin A under conditions of supplementation (Ghana, Peru), food fortification (Peru, Israel) and dietary improvement (China, Thailand, Philippines and India). Problems of vitamin A nutrition in children and pregnant or lactating women are the primary focus.

Coordinated Research Projects (CRPs). A CRP on osteoporosis examined differences in bone mineral density (BMD)of young adults across a range of races in a total of 3752 subjects recruited at 11 centres in nine countries. Highly significant differences in mean weight, height, and BMD between countries was found. Following adjustment for age, weight and height, differences in BMD persisted between centres for both men and women. Significant differences existed in young adult bone mass which, if persisting into old age, may contribute to two- to threefold fold difference in fracture risk.

A CRP on the physiological modelling of a Reference Asian Man involved several Asian countries and generated reliable data sets for dietary intake that all can use. Among other applications, this will enhance their ability in resolving national problems of radiological protection. It will also strengthen capabilities to address nutritional issues through improved reference values that were derived for a number of additional elements and reference material matrices.

Another project focused on the isotopic evaluations of maternal and child nutrition to help prevent stunted growth. Pakistan and participating countries in Latin America refined and applied isotopic techniques in field studies. The techniques also are being used in a CRP to evaluate the monitoring of infant growth, in collaboration with the WHO Growth Monitoring Programme.

Significant progress through field work also has been made against persistent diarrhoea, which accounts for a significant percentage of infant diarrhoeal deaths in many countries. The work involved countries from Africa, Asia and Latin America which joined a CRP on *Helicobacter pylori* infection and malnutrition particularly among the young. Isotopic techniques using carbon-13 breath tests were successfully used.Investigations covered bacterial colonization and digestion and absorption of nutrients to examine the significance of *Helicobacter pylori* and its consequence on poor nutrient assimilation in young children.

FUTURE PROSPECTS

A consultant's meeting called by the IAEA in December 2000 offered insights into the future applications of stable isotopes in nutrition research. Novel applications were identified by improving existing techniques to extend the usefulness of stable isotope techniques in mineral and trace element nutrition research and to allow their use more routinely. Examples include:

- Recent investigations have shown that calcium in the skeleton can be labeled using the isotope calcium-41. This offers the unique opportunity to look at calcium losses and balance in bone directly via urinary excretion of the isotopic label.
- Based on the simultaneous excretion of an oral and an intravenously given label, the urinary monitoring technique has been validated for determination of true calcium absorption; attempts are being made to validate this technique for urinary monitoring for zinc and magnesium.
- Stable isotope techniques are used routinely to assess the absorption of iron, copper, zinc, selenium, calcium, and magnesium from test meals. It is possible to extend the range of application to other elements, such as molybdenum, nickel, vanadium, tin, and boron, to better understand their biological functions. Semi-stable, very long-lived radionuclides (aluminium-26, manganese-53 and iodine-129) can be used for mono-isotopic elements.
- Use of stable isotope techniques for absorption studies is not limited to those that are essential to the human body. They can also be used for toxicological studies of lead, mercury, and chromium. For heavy metals, stable isotope techniques have been used in humans to study cadmium absorption.
- Impressive progress is seen in the instrumentation of Isotope Ratio Mass Spectrometry (IRMS) incorporating a gas-chromatographic interface. This improvement facilitates specific compounds to be converted to carbon dioxide, hydrogen or nitrogen yielding isotope ratio measurements that are specific to compounds. This is expected to open new and exciting applications in nutritional sciences.

Advances in the field will extend the many beneficial applications of isotope techniques for improving human nutrition and health. These techniques have only begun to be applied in developing countries where they can not only benefit millions, through improved nutrition, but serve as specific indicators of broader social and economic advances. In many cases, stable isotopes provide the only direct way for gaining information that can directly affect the lives and well-being of men, women, and children everywhere. ❐