CHAPTER 16

COMPARTMENTAL MODELING, STABLE ISOTOPES, AND BALANCE STUDIES

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COMPARTMENTAL APPROACHES TO NUTRITION RESEARCH

The systems studied in human nutrition research are complex collections of biochemical reactions, many of which share substrates, intermediates, and products. In contrast to animal studies, few tissues are available for analysis, thus limiting what can be studied directly in humans. Thus, a method is needed for inferring the dynamics of hard-to-observe physiological phenomena. Further, as information about metabolic processes is acquired, a system of ''bookkeeping'' is required to organize the data in a meaningful way. Compartmental modeling, which has become invaluable in biological investigations, provides such a bookkeeping system (1–3).

A *compartmental model* is a mathematically simulated description of a real-world system in terms of theoretical physiological spaces (such as plasma) and the transfers among them. Each component of the model corresponds to a component of the system being modeled. The relationships among the components of the model correspond to relationships among the components of the system of interest. For example, features of a doll correspond to features of a human. A doll possesses arms, legs, and a head in proportions similar to that of a human. However, not all features of the human are present in the doll. Lungs, for example, are not important for the functioning of a doll and are therefore not included in the doll's construction. Similarly, a compartmental model includes features of the system that are important to the processes of interest but does not include features unrelated to the specific processes of interest. For example, a compartmental model of glucose metabolism will likely not include processes of protein synthesis.

The metabolic fate of nutrients can be studied by evaluating three components: input, intermediate processes, and output (see Figure 16-1). Measuring nutritional or dietary *input* requires food composition, quantities of food eaten, and compliance with the study diet to be well characterized. Typical measures of *intermediary metabolism* can include oxidation rates of nutrients, biochemical transformations, transport, and changes in nutrient stores. *Output* from the system is evaluated by measuring losses from the system, whether as nutrient mass or as energy.

For example, once a food is consumed, a proportion of the minerals in that food is absorbed and passes into the circulation while the nonabsorbed portion is eliminated in feces (see Figure 16-2) (4). Complete elimination of unabsorbed minerals may take 6 to 12 days. Some of the absorbed minerals will pass from the circulation into body tissues; some will be excreted in urine. Another path of excretory loss is from body tissues back into the gut via biliary or

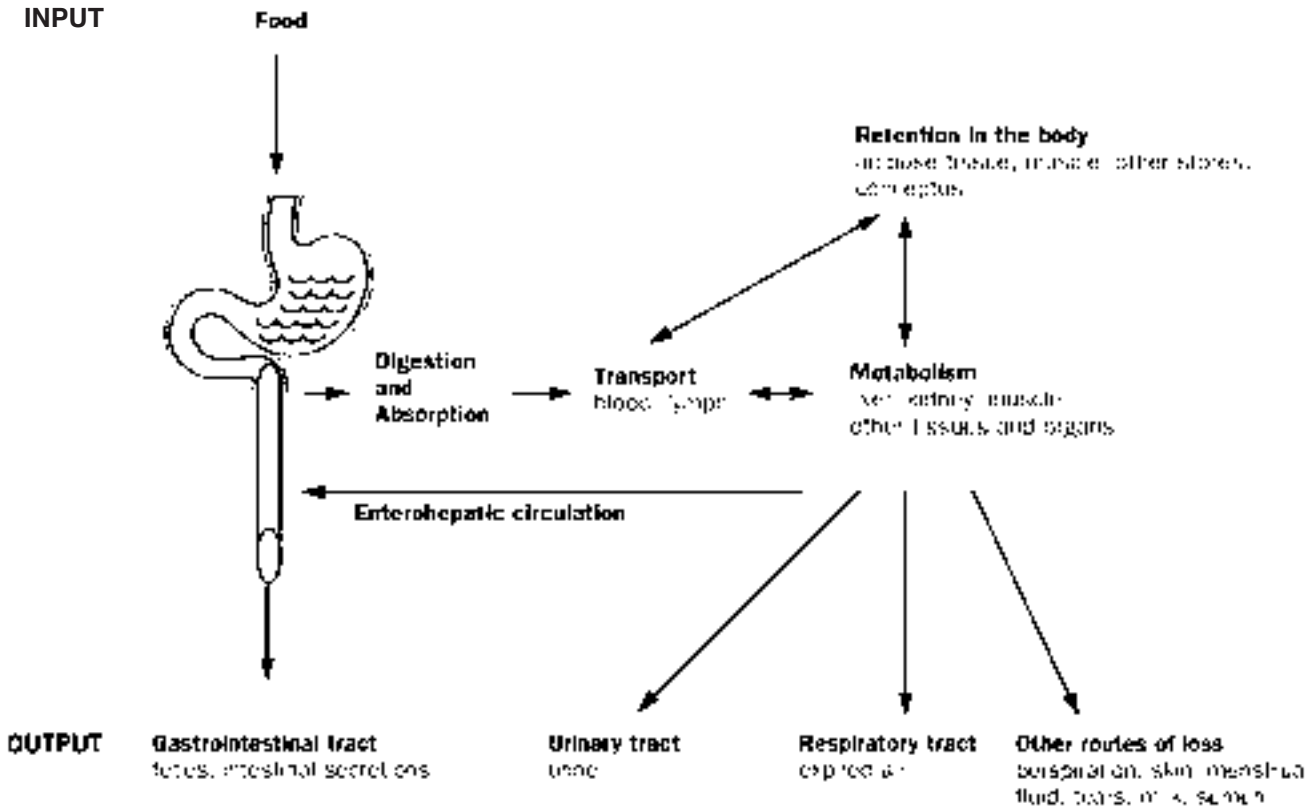

FIGURE 16-1. Physiologic pools in nutrient metabolism. This schematic figure summarizes the movement of nutrients in the body. Mass changes between input and output are the basis for calculating digestibility and nutrient balance.

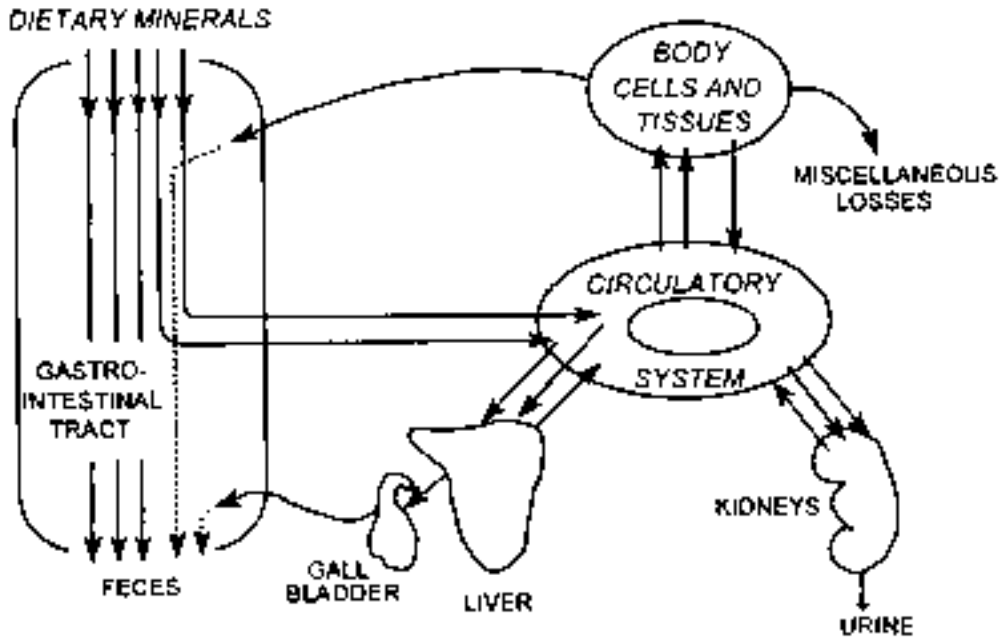

FIGURE 16-2. Absorption, distribution, and elimination of dietary minerals. Solid lines through the gastrointestinal tract represent unabsorbed minerals. Dotted lines represent endogenous minerals that are excreted into the gastrointestinal tract and eliminated with unabsorbed minerals.

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gastrointestinal (GI) secretions. Some of the endogenous minerals can be reabsorbed, but the majority pass into the feces. Once a mineral is absorbed into the body, it mixes with minerals from meals consumed recently and weeks, months, or years earlier. Thus, minerals excreted in feces, urine, and other routes are from a combination of recent and earlier intakes.

Whole-body nutrient economy is difficult to study in human subjects and generally requires the use of *balance study* methodology. The fundamental components of a balance study are the measurements of nutrient intake and nutrient output. This is a classic technique in human nutrition research. In fact, balance study methodology underlies several important lines of evidence for the determination of nutrient requirements (5–8): clinical or biochemical measures of nutrient status in relation to intake; biochemical measures of tissue saturation or of adequacy of molecular function in relation to intake; and knowledge of nutrient homeostasis and exchanges between body pools in relation to intake.

There also is a long tradition of using balance studies in animal nutrition research in order to link requirements for growth, maintenance, pregnancy, or lactation to the development of nutritionally adequate diets (9). Balance studies can be appreciated in a broader context by viewing them as a specialized form of compartmental modeling research, having the input and output compartments particularly well defined.

A recently developed methodology for enhancing compartmental studies uses *stable isotopes* (10). These naturally occurring, nonradioactive isotopes can be used to investigate the metabolism of energy, water, macronutrients, and micronutrients. Oral and intravenous doses of a nutrient can be tracked and distinguished from endogenous amounts of the nutrient already present in the body. Isotopic tracers thus permit additional information to be obtained on the metabolic fate of nutrients. When partnered with up-to-date analytical techniques and mathematical modeling methods, stable isotopes provide new research approaches for understanding nutrient transfer among different tissues and organs and *in vivo* metabolic processes.

This chapter discusses several types of compartmental modeling studies and the kinds of research questions such studies can answer. The focus of the information is on practical aspects to consider in study design and implementation.

Creating a Compartmental Model

Compartmental models consist of *pools* (compartments) of metabolites and *flows* (interactions) among pools. A pool is a kinetically distinct, homogeneous, well-mixed form of an analyte in the body. The compartments may or may not be associated with a physical space. For example, vitamin A in the liver is physiologically distinct (ie, found in a different physical location) from vitamin A in the plasma. In contrast, water in the extracellular space and water in the plasma are physically distinct but not kinetically distinct (ie, having different half-lives) because the two body spaces exchange water freely. Moreover, two analytes may exist in the same physical space but be represented by distinct compartments in a model if their kinetic behavior differs. For example, vitamin A in the form of retinyl ester behaves differently than vitamin A bound to retinol-binding protein; therefore, these two would be represented as separate compartments even though they are both forms of vitamin A existing in the same physical space (the plasma).

The compartments interact by exchanging the analyte(s) of interest. The exchanges are represented by mathematical equations whose variables are the masses in the compartments and whose parameters describe the rate of exchange of material from the donor compartment to the recipient compartment. Material can flow into the system from the environment as oral ingestion or infusions, and material can leave the system irreversibly by urine or fecal loss, by metabolism, or by other means of loss. Flows between compartments may occur through simple partitioning of an analyte into different physical spaces or through processes of state conversions such as oxidation, binding, or delipidation.

An example of this concept is shown in Figure 16-3. In this two-compartment model of zinc kinetics, P (or p) represents the plasma pool; E (or e) represents the exchangeable pool (ie, the pool that exchanges between plasma and other tissues), Q (or q) represents isotope transported out of the system, and k is the rate constant representing the fraction of the pool that is replaced within a given time (t) (11). The fluxes between compartments can be described mathematically using differential equations. A good discussion of this topic is provided in Wolfe (5).

If the flows *into* the compartment balance the flows *out* of the compartment, the system is in steady state and there is no change in the pool size in any compartment. In states

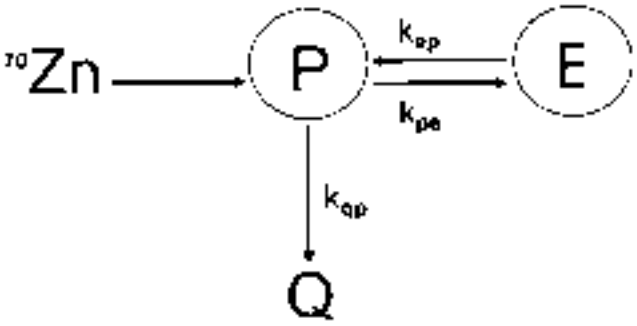

FIGURE 16-3. A simple two-compartment model of zinc kinetics. The two compartments shown are plasma *(P)* and an exchangeable pool *(E)*. Isotope transported out of the model system is represented as *Q*. The rate constant, *K,* represents the fraction of the pool that is replaced within a given time.

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of nutrient balance, the input mass (shown in Figure 16-3 as an intravenous dose of ^{70}Zn) will equal the output mass (shown in Figure 16-3 as Q).

The first step in creating a compartmental model is to create a schematic of the system of interest based on previous knowledge of the system. The schematic should include all measurable analyte pools and other pools that are important to the research question posed. Once the important pools are delineated, arrows are drawn among the pools to represent flows of material. The flows are then described by mathematical equations. Collaboration with an experienced mathematical modeler is essential to ensure that the correct equations are used and to avoid pitfalls in the assumptions used in deriving the model.

The mathematical equation that describes a particular flow from one compartment to another has a form that is determined by the characteristics of the exchange. Using this concept the measurement of size and rate of turnover of exchangeable body pools of an element can be derived. Thus far kinetic modeling has been used to examine many nutrients, including zinc (12), selenium (13), calcium (14), copper (15), vitamin A (16), and beta-carotene (17) metabolism. In some cases, such as in Figure 16-4, human whole-body copper metabolism, a model can be developed using kinetic modeling and balance study methodology. This figure shows a compartmental model of copper metabolism in adult men using enriched ^{65}Cu. The model predicted that enriched copper-65 masses were converted to total copper masses as in the following equations (14):

$$\text{Total copper-65} = \frac{\text{Predicted mass of enriched copper-65}}{\text{Copper-65 enrichment}}$$

$$\text{Total Cu} = \frac{\text{Total copper-65}}{\text{0.3083 copper-65 in total Cu}}$$

Scott and Turnlund used ^{65}Cu in this study to develop a model of copper metabolism, the first in humans. The compartmental model was developed using CONSAM version 30.1 (18). The study was conducted in 5 young men who lived in a metabolic research unit for a 90-day period during which three different dietary levels of copper were fed. Copper-65 was administered orally 4 times and intravenously 3 times throughout the study. The model developed was simple, it used a simple recycling design, and it demonstrated that the amount of dietary copper consumed influences the flow of copper from one liver compartment to one plasma compartment and from that plasma compartment to the ''other tissues'' compartment. In this five-compartment (two plasma, two liver, and one ''other tissues'') model, tissue uptake of oral and intravenous copper differs, with the flow from plasma to a liver compartment varying with the route of administration. The circles represent compartments, the squares are delay elements, the triangle is the sum of the indicated compartments, and * is the site of the Cu tracer input. The authors hypothesized that plasma compartment 6 represents nonceruloplasmin-bound copper, whereas compartment 8 contains ceruloplasmin-bound copper. This model predicts that 65% of plasma copper is bound to cer-

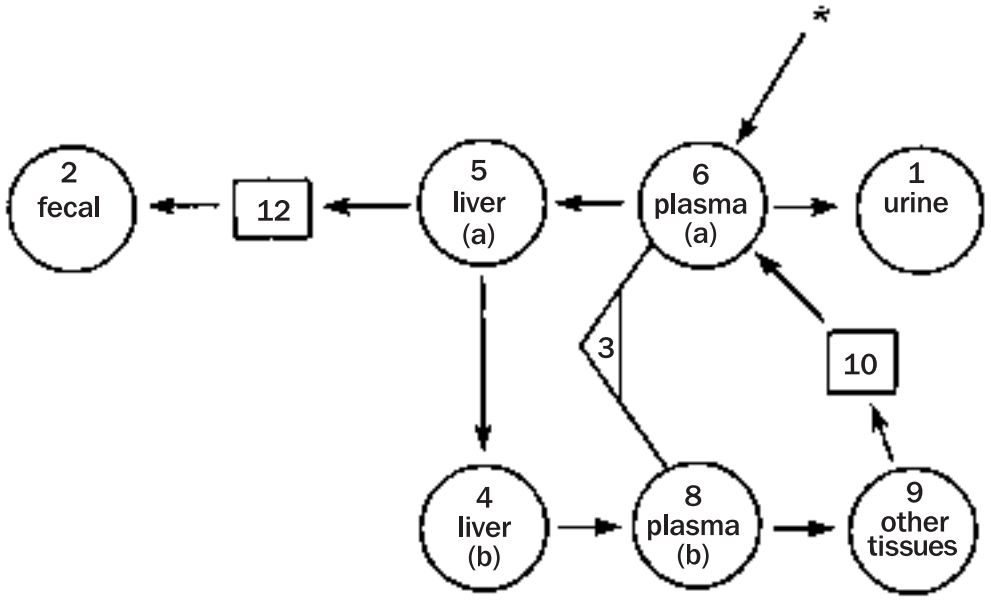

FIGURE 16-4. Schematic representation of a compartmental model of copper metabolism in adult men. Circles represent compartments, squares are delay elements, the triangle is the sum of the indicated compartments, and the asterisk (*) is the site of input for the copper tracer.

Reprinted from *Journal of Nutritional Biochemistry,* vol 5, KC Scott and JR Turnlund, Compartmental model of copper metabolism in adult men, pp 342–350, 1994, with permission from Elsevier Science.

ulophasmin compared to 56% to 68% calculated from data. Similarly, the model predicted 4 ± 1% of total body copper would be present in plasma, very close to the 2% to 6% expected.

From this example, it is evident that the mathematics of compartmental modeling is complex and requires specially trained investigators. Jacquez (19) provides a good general introduction to compartmental modeling. Collins (20) reviews books, journals, and software packages focused on modeling. A complete and detailed description of the steps involved in the development of a compartmental model is described in Novotny et al (21). Software packages, such as Simulation, Analysis, and Modeling (SAAM) and CONSAM (the interactive conversational SAAM) (17) are now used not only to assist in data analysis but also to indicate parameters that must be incorporated into the basic experimental design of the study.

The power of computer mathematic simulation enables the modeler to develop a unified hypothesis of the functioning of the many complex processes that compose the system of interest. The model is then tested against experimental observation to be sure that the model mimics the system for every known situation.

Under ideal circumstances, once the model's behavior does not deviate from experimental observation in any case tested, the model can be used to investigate aspects of the system that otherwise would be experimentally inaccessible.

Balance Studies

It is assumed that a healthy adult who eats an adequate diet can thereby maintain equilibrium or balance for essential nutrients. This means that over a period of time, the amount of each of these substances that enters the body is equaled by the amount that leaves the body. By convention, when intake and output are equal, the subject is considered to be ''in balance,'' a state in which nutrient or energy requirement have been met (no net change). When output exceeds input, the subject is in ''negative balance,'' a state leading to depletion. When output is less than intake, the subject is in ''positive balance,'' a state of nutrient accrual, growth, weight gain, anabolism, or repletion of stores. Equilibrium can be disturbed by changes in food intake, medications, physical activity, or disease, or by physiological states such as growth, pregnancy, and lactation.

For some nutrients, such as water, minerals, protein, fat, and energy, measuring nutrient or energy balance has provided an important means of understanding absorption and bioavailability. Balance studies of nutrients such as energy, nitrogen, and water also can provide clues to an individual's physiological state, such as growth. However, for other nutrients, a strictly defined concept of balance is less meaningful. For example, some of the vitamins (such as biotin, pantothenic acid, and vitamin K) (8) can be synthesized *in vivo* from intestinal bacteria, and diet only supplies part of total ''input''; as a result, it is not possible to obtain precise quantitative data on the mass balance of these compounds. Instead, in this case, balance studies may provide qualitative data about metabolism and requirements.

Specific time sequences of balance states can be established to address certain research questions. For example, nutrient requirements often are investigated using the following time sequence: negative balance is induced by feeding a diet known to provide inadequate levels of the nutrient under study; the diet then is altered until the point of balance (repletion or correction of deficiency) is established; and finally a state of positive balance is induced in which nutrient stores are established and intake exceeds requirement (22).

The balance technique is both time-consuming and labor-intensive. To measure balance precisely, one must determine the difference between the intake of nutrient and the amount excreted through urine, feces, and other routes, such as exhaled air, skin, hair, perspiration, menses, and other secretions. (In practice, measurement of total excretion is not always attempted because such collection conditions require complete research control that only a metabolic research unit can provide.) An additional complication is that nutrients undergo metabolic changes in the body, and the amount excreted as such may not equal the intake. Errors associated with the measurement of intake and output thus can cause a loss of precision and make it difficult to interpret the data.

Calculating Nutrient Balance and Availability

Nutrient balance (Formula 1) typically is calculated as the difference between the amount consumed and the amount excreted (23, 24):

(1) $$\text{Balance} = \text{Intake} - \text{Excreted}$$

Nutrient digestibility (Formula 2) also can be calculated from balance study data. In the simplest form, *true digestibility* is nutrient balance expressed as a fraction (or percent) of the amount consumed:

(2) $$\text{Digestibility} = \frac{\text{Intake} - (\text{Total excreted} - \text{Endogenous fecal excretion})}{\text{Intake}}$$

Because it is often difficult to separate the contribution of unabsorbed nutrients from the endogenous secretions and bacterial microflora of the gastrointestinal tract, a more common approach is to measure *apparent digestibility* and ignore the contributions of endogenous production.

Particularly in measuring the energy content of food or nutrients, further distinctions in apparent digestibility can be made if output from fecal and urinary sources is measured through bomb calorimetry. When only the energy output from feces is measured through bomb calorimetry, the data allow the calculation of the *digestible energy content* (Formula 3):

(3) Digestible energy =

$$\frac{\text{Intake energy} - \text{Excreted energy (feces)}}{\text{Intake energy}}$$

When the energy content of both feces and urine is measured through bomb calorimetry, the *metabolizable energy content* (Formula 4) of the diet can be determined:

(4) Metabolizable energy =

$$\frac{\text{Intake energy} - \text{Excreted energy (feces)} - \text{Excreted energy (urine)}}{\text{Intake energy}}$$

Net energy (Formula 5) is determined as the difference between metabolizable energy intake and heat increment (or the *thermic effect of food*):

(5) Net energy = Metabolizable energy – Heat increment

The net energy is the energy available for productive purposes (growth, tissue repair, lactation, or pregnancy). Heat increment is the amount of energy released during digestive and metabolic processes associated with digestion and storage. The heat increment of a test meal can be measured by 6-hour calorimetry after ingestion of a test meal; however, this approach is limited to short-term measures.

Net energy (NE) (Formula 6) can also be determined as the ratio of the change (Δ) in retained energy (RE) at two (or more) levels of energy intake to the change in energy intake (IE).

(6)

$$NE = \frac{\Delta RE}{\Delta IE}$$

Availability of a nutrient can be assessed by its digestibility (Formula 2), metabolizability (Formula 4), or net energy (Formula 5 or 6). These measures are most useful as direct comparisons between diets. Increasing digestibility is indicative of greater nutrient or energy availability. Although some foods may have the same digestibility, they may have different net energies as a consequence of nutrient interactions or of differences in the cost of digestion or metabolism. These formulas can be used to aid in the interpretation of differences that might be observed among diets containing various levels of fiber, energy, protein, fat, or carbohydrate.

Such formulas have been used in past investigations to determine protein and energy balance or requirements. In one study, through the usage of balance, digestibility, and metabolizable energy formulas, Calloway and Kretsch (25) examined protein and energy use. In the same study these investigators evaluated also whether absorption of protein and energy are different between poor rural Guatemalan inhabitants and healthy North Americans. Current and future investigative work in nutrition requirements or bioavailability evaluations will continue to rely on the usage of formulas, such as those provided above, to address many unresolved intake adequacy concerns.

Practical Considerations

Balance studies are carried out by keeping constant all possible influences that might disturb the balance and by varying or controlling only the one factor under investigation. It is essential that precise control and measurement of intake and output be made because small changes, rather than gross changes, are often expected. A slight error of overestimation of intake and a slight underestimation of excretion will cause a compounded error in the final balance calculation because ''the two losses are additive, not self-canceling, as is generally supposed'' (26).

Statistical issues for balance studies are not unique to this type of investigation. (See Chapter 2, ''Statistical Aspects of Controlled Diet Studies.'') In general, the experimental sequence of depletion, repletion, and balance states suggests using analysis techniques suited to designs in which the participant serves as his or her own control. This is particularly important because balance studies tend to enroll small numbers of participants and maximum statistical power must be gained from the design. Along with protecting against selection bias, randomized assignment of subjects to treatment sequences will allow adjustment for carryover effects. Repeated crossover treatments may also serve this purpose. If it is necessary to compare multiple dietary treatments, the protocol can be designed to combine the features of parallel-arm assignments with those of crossover designs to allow satisfactory comparison of treatment groups.

Adaptation to the Study Diet

Adaptation to a new diet is essential to allow for microbial changes in the gastrointestinal tract and to allow for enzymatic or secretory changes that may occur. Typically, subjects are allowed to adapt to a new diet for several days, but the length of the adaptation period often depends on the nutrient being altered. For many nutrients, an adaptation period of several weeks is required for metabolic changes to reach the new norm (27). An appropriate adaptation period is especially important when changes are made in the type or amount of dietary fiber. The symbiotic microbial population of the gastrointestinal tract, which ferments the fiber, must be provided enough time to adapt to the new diet.

When a change from a diet high in a substance to a diet low in the same substance takes place, there will be a transitional period before a new equilibrium is established. This adjustment period is best disregarded in evaluating the level of the new equilibrium, but important information can be gained from evaluating the rate of change and variability.

Sources of Error in Estimating Intake

The principal investigator and the research dietitian must be vigilant to see that errors are not introduced into balance studies. Some typical sources of error associated with nutrient intake and output are outlined here.

Controlling and Measuring Intake

A crucial factor in measuring nutrient balance is the accurate measurement of nutrient intake through accurate characterization of the ingested diet. Underestimating intake can produce a false negative balance, whereas overestimating intake can cause the incorrect conclusion of positive nutrient balance.

Maintaining a constant diet, and thus a consistent and predictable food and nutrient intake, is another important aspect of balance studies. Because changes in intake of some nutrients can alter rate of passage and hence absorption, care must be given to maintaining a constant level of intake prior to and during the balance period. It is best if the experimental and the control diets are closely matched for all nutrients except the nutrient to be investigated. When the diet consists of conventional foods, the greatest error in estimating nutrient intake is caused by day-to-day variations in the composition of the foods. This variation stems from the chemical variability of the food items as well as variation in weighing of food items. Use of formula diets or pureed/blended diets minimizes both types of errors. (Also see Chapter 14, ''Planning and Producing Formula Diets.'') If the amount of variation is expected to be high and unpredictable, it may be necessary to purchase food from specific production lots and also to collect food samples daily for chemical analysis. (Also see Chapter 22, ''Validating Diet Composition by Chemical Analysis.'')

For vitamin studies, unless nutrient retention values are available for the cooking method used, it is advisable to calculate the nutrient content of the diet using data for cooked forms of the food. Laboratory assay of the diet, as mentioned earlier, will yield yet more accurate data.

Food Preparation

Food preparation for balance studies must achieve the highest possible degree of precision because the state of nutrient balance is calculated by comparing the absolute nutrient intake with the absolute nutrient output (ie, loss). Thus it is critical that balance study diets deliver the specified *absolute* levels of nutrients, whereas for other study diets such precision may not be necessary or practical.

Basic techniques for balance diets are similar to those for other research diets. See Chapters 11 (''Designing Research Diets''), 12 (''Producing Research Diets''), and 13 (''Delivering Research Diets''). Additional precision is gained by using foods that have very consistent nutrient content, calculating the diet with database values for the foods in the forms that will be weighed out (ie, cooked or raw), and evalauating the potential for nutrient loss each time foods are transferred (ie, from storage containers to cookware to tableware to participant).

Extra precautions are required to prevent environmental contamination in trace mineral studies. Supplies and equipement (such as cookware, food storage containers, detergents, water for cooking and washing, and tableware, including disposable paper and plastic items) must be selected, and perhaps tested as well, to ensure that study minerals are not released into the food.

Plate Residues

Invisible ''returns'' will remain even after meticulous scraping with a spatula. Isaksson and Sjogren (28, 29) found daily mean losses for nitrogen to be 0.1 g, calcium to be 20 mg, sodium to be 2.7 mEq, and potassium to be 1.1 mEq. These losses can be much higher if the participant neglects to scrape and rinse the plates and glasses and drink the resulting rinse water. This error can be reduced by carefully explaining eating procedures to the participants before the start of the study and by establishing a system to check the participants' dishes for visible food particles after each meal. (Also see the discussion of eating techniques in Chapter 13, ''Delivering Research Diets.'')

Unauthorized Food Intake

Unauthorized food intake and omission of study foods are common problems during the initial days of a study as participants adjust to the study protocol. Hunger due to inaccurate calorie adjustments promotes consumption of unauthorized foods. Occasionally, deliberate or even malicious intake or omission of foods occurs—particularly when a participant's dissatisfaction with any part of the routine is not appreciated quickly enough by the staff. (Also see Chapter 13, ''Delivering Research Diets.'')

Sources of Error in Estimating Output

The two primary routes of loss for most nutrients are feces and urine. For some nutrients, there may be other appreciable routes of loss that need to be accounted for in order to obtain accurate balance measurements. For example, sodium loss associated with sweating can be significant, especially during short-term studies.

Fecal Collections

When feces represent a significant route of loss of the nutrient of interest (eg, macronutrients, some minerals), it is imperative to collect samples for analysis. Feces can be collected into plastic disposable freezer containers or into self-sealing plastic bags. Once collected, feces can be stored in Styrofoam coolers containing dry ice until they can be brought to the facility for additional processing, storage, pooling, and analysis. Temporary storage on wet ice is acceptable if the analyte of interest is relatively stable. Wet ice may not provide a cold enough holding temperature to minimize microbial fermentation, however, which can compromise the analysis of certain nutrients (eg, fiber, fatty acids). Thus, nutrient changes can occur after defecation and before proper sample storage.

The required length of time for collection depends on the menu cycle and the rate of food passage. If menus do not change from day-to-day, then the length of collection can be as short as 3 to 4 days, after an appropriate adaptation

period. However, even when diet is relatively constant, there still might be physiologic variation in absorption and excretion. Therefore, when nutrient balance over an entire menu cycle is the research outcome of interest, it is preferable to collect all feces resulting from that menu cycle. Thus, if the menu cycle is 7 days long, then the subjects should consume all 7 days of meals, and the corresponding feces should be included in the fecal composite.

The length of time between bowel movements as well as the mineral or other nutrient content for each bowel movement varies. This variability causes fluctuation in the balance value of nutrients commonly excreted in feces (calcium, magnesium, phosphorus, and trace minerals). To obtain a reliable measure of fecal output, one can use an inert fecal marker such as polyethylene glycol (PEG) (30, 31). (Also see Chapter 24, ''Biological Sample Collection and Markers of Dietary Compliance.'')

Nontoxic dye markers can be used also to demarcate the beginning and end of the fecal collection period and to identify stool samples corresponding to specific days of the diet. Brilliant blue, carmine (red), or indigo carmine (blue) dyes are commonly used fecal markers. These markers can be given to the subjects in gelatin capsules. Small amounts of marker (approximately 200 mg) are sufficient for detection in the feces. Participants typically swallow the dye capsules at two time points, one marking the beginning of the test period and the second marking the end of the test period. Transit time (ie, from the time the food with the marker is eaten to the time the corresponding stool is produced) varies among individuals with a usual range of 1 to 4 days. All stool samples are collected from the time the dye marker first appears until the appearance of the second marker. The duration of collection varies among studies; for calcium balance, a 72-hour collection has been recommended (30).

Although these markers are used to help visually detect when collection should begin, they are often not the most reliable indicators to use. Color-blind subjects find the markers difficult to see. Also, depending on the foods consumed by a subject, the colors of the markers may blend with the excreted waste.

The failure to collect all feces during a study is extremely difficult to prove. Inadequate fecal collections can cause errors in balance determinations for elements in which the net absorption is small compared to the intake.

Urine Collections

Urine can contain appreciable amounts of urea, some minerals, and vitamins. Thus, total urine collections are necessary for balance measurements of many nutrients. However, for free-living studies in which complete collections are not possible, timed sample urine collections may be useful. Sometimes timed or random urine samples are necessary to check whether energy or total food intake is sufficient. In such a case, the urine is checked for ketone bodies. In the case of doubly labeled water methodology, post-dose urine samples are collected at set times on the day of dosing and for 14 days afterward. In metabolic studies where complete collections are possible, urine would be collected for 24 hours daily.

In these studies, there is considerable individual variation in urine output, and there can be significant intraindividual variation as well. In normal healthy individuals, daily urine volume can range from 1,000 ml to 3,000 ml or more, depending on daily fluid intake. It is important to provide enough collection containers. For example, a subject making 24-hour urine collections returned completely full 1-gallon containers of urine daily. Upon questioning, it was learned that he was disposing of additional urine. The subject, who was employed in a service job requiring many hours in transit, habitually consumed unusually large volumes of fluid as he drove to his multiple job sites.

Once collected, samples can be stored on ice in coolers or on dry ice. For nitrogen balance studies, it might be necessary to acidify (usually with hydrochloric acid) urine after collection to minimize loss of ammonia generated from urea. Ammonia loss will result in a false positive balance of nitrogen and energy balance.

Errors caused by inadequate collection of urine are difficult to demonstrate. One solution to ensure completeness of urine collection is to feed para-amino-benzoic acid (PABA) and use this marker as a urine completeness validity check. (See Chapter 24, ''Biological Sample Collection and Markers of Dietary Compliance.'') Measures of compliance with urinary collection also can be based on a metabolic constant. Creatinine, for example, is excreted in proportion to muscle mass. However, a 5% to 10% day-to-day variation in urinary creatinine excretion is common (some of this variation may be caused by diet, such as the level of meat intake), and this reduces the precision of creatinine determination as an index of completeness of urinary collection.

Another technique to check for consumption compliance is through the urinary osmolality test. The osmolar load of urine is based on compounds, such as nitrogen, sodium, and potassium, derived from foods. A lower than predicted value for urinary osmolality suggests the subject did not eat all of the food provided; a higher value suggests the subject ate unauthorized foods. The disadvantage of this method is low precision and that it requires performing more than 6 days of 24-hour urine collection. (See Chaper 24, ''Laboratory Quality Control in Dietary Trials.'')

Other Sources of Nutrient Loss

As illustrated in Figure 16–1, there are many other potential routes of nutrient loss in addition to feces and urine. These include blood (phlebotomy, menses, injury), sweat, tears, saliva, hair, skin and dermis, and pulmonary exhalations. In most cases, these losses are negligible and difficult to measure.

Take, for example, the small but cumulative losses of nitrogen from various routes. If in determining nitrogen balance, the dermal and miscellaneous losses are disregarded, the total error in balance is about 0.5 g nitrogen per day for

sedentary men in a comfortable environment. Nutrient losses in sweat and desquamated cells of hair, skin, and nails can be determined to account for the dermal losses. Calloway, Odell, and Margen (32) found that an average value of this error is 149 ± 51 mg of nitrogen per day for sedentary men. Nitrogen losses in tooth brushing, toilet tissues, plate wastes, and exhaled ammonia were found to amount to about 115 mg per day. The loss of nutrients from blood varies widely from study to study depending on the amount of blood drawn during the study, but Calloway, Odell, and Margen found a loss of 32 mg N/100 ml of blood. In addition, these investigators found other sources of nitrogen loss to include saliva (0.9 mg N/g), semen (27 mg N/ejaculate), and menstrual fluid (900 mg N/period).

Typical Applications of Balance Methodology

Zinc, Copper, and Iron Balance in Elderly Males

Turnlund et al (33) conducted a study of zinc, iron, and copper balance in elderly men to evaluate the adequacy of dietary recommendations for these elements for the elderly. The researchers attempted to eliminate a number of sources of error thought to contribute to unreliable and inconsistent results of balance studies by including long adaptation periods on constant mineral intake and balance periods of 21 days. The mineral balance study was part of a larger study on the protein, energy, and mineral requirements of elderly men.

The study comprised two 6-week metabolic periods. The only difference in the two metabolic periods was the protein intake: 9 g to 19 g of nitrogen per day in period 1 dependent on habitual protein intake, and 70 mg nitrogen/kg body weight in period 2. Two 3-week balance studies for zinc, copper, and iron were carried out in 6 elderly males who were confined to a metabolic unit for a total of 12 weeks with constant dietary mineral intake. The diet used in the study was adequate in all nutrients and consisted of formula drinks, peaches, low-protein rusks with margarine, tea, coffee, and vitamin supplements. The nutrients of interest were made into solutions that were added to the formula drinks.

The subjects were observed during meals and care was taken to ensure complete consumption of all food offered. The subjects rinsed all dishes with deionized water and rinse water was consumed. Twenty-four-hour urine collections were collected in polyethylene bottles, and feces were collected for 3-day periods in polyethylene containers. Feces were homogenized in a colloid mill and acidified with 1% high-purity hydrochloric acid (HCL). Urine was acidified also with 1% high-purity HCL. Sweat and integumenatry collections were made for two 3-day periods. Subjects bathed and rinsed with deionized water containing 25% polyoxyethlene-23-lauryl ether before collections and wore cotton suits of long underwear that were covered with disposable, plastic-coated coveralls to avoid contamination of the underwear. The clothing was pretreated by soaking in 0.1 N HCL for 3 days; after wear, clothes were removed and soaked again in 0.1 N HCL that had been tested for zinc, copper, and iron levels. The bathwater and laundry soaking solutions were combined and aliquots taken for analysis. Blood was drawn during screening of subjects and at the end of the 6th and 12th weeks of the study.

In this study, mean zinc and copper balance for the 6 males was close to 0:0.1 mg/day for zinc and 0.06 mg/day for copper. (See Tables 16-1 through 16-4: zinc balance and serum zinc table, copper balance, serum copper, and ceruloplasmin table, and iron balance and blood parameters.) Iron balance appeared to be negative (mean of –0.44 mg/day) despite improved blood iron parameters for the majority of the subjects. Determinations of zinc, copper, and iron

TABLE 16-1

Intake and Excretion of Zinc, Copper, and Iron in Elderly Men

	Dietary Intake (mg/day)[1]	Fecal Excretion (mg/day)	Urinary Excretion (mg/day)
Zinc			
Metabolic period 1	15.4 ± 0.3	14.1 ± 0.4	0.7 ± 0.1
Metabolic period 2	15.5 ± 0.3	14.6 ± 0.5	0.8 ± 0.1
Copper			
Metabolic period 1	3.24 ± 0.14	3.18 ± 0.14	<0.015
Metabolic period 2	3.28 ± 0.11	3.22 ± 0.06	<0.015
Iron			
Metabolic period 1	10.0	9.87 ± 0.29	<0.1
Metabolic period 2	10.0	10.4 ± 0.16	<0.1

[1]Values represent mean ± SEM. (There was no SEM reported for iron intake.)

Source: Turnlund J, Costa F, and Margen S. Zinc, copper, and iron balance in elderly men. *Am J Clin Nutr.* 1981;34:2641–2647. © *Am J Clin Nutr,* American Society for Clinical Nutrition.

TABLE 16-2

Zinc Balance and Serum Zinc Concentration in Elderly Men

	Subject						
	1	3	5	6	7	8	Mean
Balance (mg/day)							
Metabolic period 1	−0.5	+1.8	+0.7	+0.3			
Metabolic period 2	−0.8	+1.1	−1.1	+1.3	−1.0	+1.0	0.1 ± 0.5
Serum Zn (μg/ml)							
Prestudy	0.7	0.9	1.0	0.6	0.7	0.8	
End study	0.9	1.0	1.2	1.1	0.9	0.9	
Change	+0.2	+0.1	+0.2	+0.5	+0.2	+0.1	0.2 ± 0.1[1]

[1]Mean ± SE of change.
Source: Turnlund J, Costa F, and Margen S. Zinc, copper, and iron balance in elderly men. *Am J Clin Nutr.* 1981;34:2641–2647. © *Am J Clin Nutr,* American Society for Clinical Nutrition.

TABLE 16-3

Copper Balance, Serum Copper, and Ceruloplasmin Concentration in Elderly Men

	Subject						
	1	3	5	6	7	8	Mean
Balance (mg/day)							
Metabolic period 1	−0.27	+0.08	+0.15	+0.07			
Metabolic period 2	−0.02	+0.16	−0.17	+0.22	+0.12	+0.04	0.06 ± 0.06
Serum Cu (μg/ml)							
Prestudy	1.3	1.2	1.0	1.0	0.4	1.0	
End study	1.7	1.1	0.8	0.9	0.9	1.0	
Change	+0.4	−0.1	−0.2	−0.1	+0.5	0	0.1 ± 0.1[1]
Ceruloplasmin (mg/dl)							
Prestudy	41	37	28	32	32	34	
End study	43	26	23	30	24	35	
Change	+2	−9	−5	−2	−8	+1	−3.5 ± 1.9[1]

[1]Mean change ± SE of change.
Source: Turnlund J, Costa F, and Margen S. Zinc, copper, and iron balance in elderly men. *Am J Clin Nutr.* 1981;34:2641–2647. © *Am J Clin Nutr,* American Society for Clinical Nutrition.

losses from sweat and the integument were not considered as reliable because for some subjects the bath and laundry water had a higher mineral content *before* washing and laundering occurred (ie, the ''blank'' samples) than did the water samples collected *afterward*.

The authors concluded that these losses were relatively small in most subjects and did not contribute significantly to trace mineral losses. They also concluded that daily intakes of 15 mg zinc and 3 mg copper were approximately sufficient to maintain balance in the studied sample of elderly men. Iron intake of 10 mg/day resulted in an average negative balance, although 5 of 6 subjects had improved markers of iron status during the study. This finding highlights the complexities of interpreting balance data for individuals, in contrast with balance data for the entire experimental group.

Nitrogen Balance Studies

Protein status is determined by conducting nitrogen balance studies. Proteins are nitrogenous compounds, and nitrogen serves as a surrogate for protein. Each 100 g of protein contains 16 g of nitrogen. Thus, food protein can be mathematically converted to nitrogen (N = Protein × 0.16); food, urine, or fecal nitrogen can be mathematically converted to protein (Protein = N × 6.25).

Urinary nitrogen (primarily from urea) typically represents about 90% of the dietary protein intake. Nitrogen bal-

TABLE 16-4

Iron Balance and Blood Parameters in Elderly Men

	Subject						
	1	3	5	6	7	8	Mean ± SEM
Balance (mg/day)							
Metabolic period 1	−1.04	+0.20	−0.08	+0.08			
Metabolic period 2	−0.59	−0.13	−0.80	−0.13	−0.01	−0.95	−0.44 ± 0.16
(Serum Fe (μg/ml)							
Prestudy	0.5		0.9	1.3	1.3	0.6	
End study	0.6	1.6	2.0	1.4	1.4	1.7	
Change	+0.1		+1.1	+0.1	+0.1	+1.1	+0.5 ± 0.2[1]
Hb (g/dl)							
Prestudy	12.6	13.9	14.1	13.4	15.1	11.6	
End study	12.5	16.3	16.3	14.1	15.9	12.8	
Change	−0.1	+2.4	+2.2	+1.3	+0.8	+1.2	+1.3 ± 0.4[1]
Hematocrit (%)							
Prestudy	38.8	41.2	41.4	39.9	43.6	34.8	
End study	37.7	47.1	46.9	41.5	47.2	39.8	
Change	−1.1	+5.9	+5.5	+1.6	+3.6	+5.0	+3.4 ± 111[1]
Total iron-binding capacity (μg/dl)							
Prestudy	333		273	240	225	393	
End study	264	285	255	230	225	288	
Change	−69		−18	−10	0	−105	−40 ± 20[1]
Saturation (%)							
Prestudy	15		34	59	57	16	
End study	23	54	78	56	63	60	
Change	+8		+44	−3	+6	+44	+20 ± 10[1]
Serum transferrin (mg/dl)							
Prestudy	265	290	223	240	200	366	
End study	295	320	255	230	255	335	
Change	+30	+30	+32	−10	+55	−31	+18 ± 13[1]
Total serum protein (g/dl)							
Prestudy	7.0	7.1	7.2	7.4	8.1	7.8	
End study	7.3	7.7	7.9	7.6	8.5	7.3	
Change	+0.3	+0.6	+0.7	+0.2	+0.4	−0.5	+0.3 ± 0.2[1]

[1]Change ± SE of change.

Source: Turnlund J, Costa F, and Margen S. Zinc, copper, and iron balance in elderly men. *Am J Clin Nutr.* 1981;34:2641–2647. © *Am J Clin Nutr,* American Society for Clinical Nutrition.

ance can thus be approximated by analyzing 24-hour urine collections for nitrogen and comparing to the nitrogen content of the diets consumed. As with minerals, the remaining nitrogen is excreted in the stool and sweat. In some nitrogen balance studies, the protein and nonprotein nitrogen pool present in the blood should also be considered; phlebotomy procedures will alter the size of this pool. Collected blood can be weighed after it is drawn into a syringe and the nitrogen loss estimated and subtracted from the intake (32).

Fecal nitrogen losses can sometimes be large dependent on the diet being consumed. However, large losses do not necessarily mean negative balance status. Past investigations have reported that rural Guatemalans with low D-xylose absorption values have large fecal losses of nitrogen, fat, and total energy. However, in spite of large fecal nitrogen loss, subjects could be in nitrogen equilibrium. Calloway and Kretsch (25) fed healthy men a fiber-free formula diet providing egg protein (0.57 g/kg) and a rural Guatemalan diet providing 0.875 g protein per kg body weight. The Guatemalan diet consisted of black beans, corn tortillas that were lime-treated, white rice, sweet wheat rolls, white cheese, dried whole egg, butterfat, chicken consommé, frozen sum-

TABLE 16-5

Stable Isotopes Commonly Used in Nutrition Research[1]

Element	Stable Isotope (Mass Number)	Protons (atomic Number)	Neutrons	Natural Abundance (%)
Hydrogen	1	1	0	99.98
	2	1	1	0.02
Carbon	12	6	6	98.89
	13	6	7	1.11
Nitrogen	14	7	7	99.63
	15	7	8	0.37
Oxygen	16	8	8	99.76
	17	8	9	0.04
	18	8	10	0.20
Sulfur	32	16	16	95.00
	33	16	17	0.76
	34	16	18	4.20
Iron	54	26	28	5.82
	56	26	30	91.66
	57	26	31	2.19
	58	26	32	0.33
Zinc	64	30	34	48.86
	66	30	36	27.81
	67	30	37	4.11
	68	30	38	18.57
	70	30	40	0.62
Selenium	74	34	40	0.87
	76	34	42	9.02
	77	34	43	7.58
	78	34	44	23.52
	80	34	46	49.82
	82	34	48	9.19

From Wolfe RR. *Radioactive and Stable Isotope Tracers in Biomedicine*. New York, NY: Wiley-Liss; 1992. Copyright © 1992 John Wiley & Sons. Adapted with permission of Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc.

[1]An example of a commonly used radioactive isotope in nutrition research is ^{3}H (tritium). The three isotopes of this nuclide are unstable and undergo spontaneous changes that result in the generation of radioactive energy.

mer squash, canned pumpkin, frozen banana, lemon juice, sugar, and coffee powder. For comparison, an egg formula diet was fed at the higher protein level with and without oat bran. Complete 24-hour urine and fecal collections were carried out throughout the study. Urine and fecal samples were analyzed for nitrogen and energy contents. Diets were separately analyzed for all nutrients under study. Metabolic balances were computed from analyzed dietary intakes and the average urinary and fecal excretion. Routine hematological parameters were measured. Nitrogen lost was estimated for each blood sample taken.

Study results showed that by the addition of oat bran, fecal excretion of dry matter and energy doubled and digestibility of energy and protein was reduced by up to 4%. Fecal dry matter, nitrogen, and energy excretions in the Guatemalan diet were nearly four times as high as with the formula diet. These investigators found that protein digestibility of the Guatemalan diet was 78% and total digestible energy was 92%. At 0.875 g of protein per kg, the intake level maintained nitrogen balance in spite of large fecal losses of nitrogen and energy.

Stable Isotope Studies

The stable isotopes used in nutrition research are naturally occurring elements that are found in relatively low concentrations in the environment (see Table 16-5). For example, most of the oxygen in the environment (99.76% natural abundance) contains 8 protons and 8 neutrons (oxygen 16, ^{16}O). The commonly used stable isotope, ^{18}O, contains 8 protons and 10 neutrons and has a natural abundance of

0.204%. Because the mass of these two isotopes is different, the enrichment of ^{18}O can be measured with a mass spectrometer. Stable isotope research relies on enriching the background concentration of a naturally occurring atom and measuring changes in enrichment.

Stable isotopes are fundamentally different from radioactive isotopes. The nucleus of a radioactive isotope is unstable and spontaneously emits particles and electromagnetic radiation. Unlike the nucleus of a radioactive isotope, the nucleus of a stable isotope does not change or decay with time. A neutron in the nucleus of radioactive isotopes breaks down by releasing particles and energy, and in this process the radioactive isotope is converted to another element. For example, a uranium-238 nucleus releases a stream of helium-4 nuclei, and the remaining fragment is a thorium-234 nucleus. The energy carried in the released particles can be damaging to body components, such as DNA, and therefore radioactive isotopes are rarely used in human studies.

Modeling Nutrient Metabolism with Stable Isotopes

Use of stable isotopes in research endeavors is costly because the synthesis of compounds labeled at a specific location is a technically complicated process. Compartmental modeling has provided an effective means of analyzing data from stable isotope studies and maximizing the amount of information these expensive experiments can yield.

Once the preliminary equations have been derived, as described earlier in this chapter, the model's initial conditions are determined. The initial conditions are estimates of the state of the system at the beginning of the experiment. Initial conditions include values for pool sizes and estimates of transfer coefficients (rate coefficients between compartments). The initial pool sizes for the stable isotope are usually set to zero because the isotopes used are in low abundance and thus negligible in the subject when the study begins, and the initial pool sizes for the system at steady state are entered based on previously published literature values.

The initial conditions combined with the estimates of transfer coefficients provide the means for calculating flows of material through the system for each time interval. The stable isotope dose or infusion is mathematically entered into the system; then the equations are solved systematically in proper order to yield the mass of isotope moving to and from each compartment in each time interval. The calculations are reiterated in sequence to produce a model time course of the stable isotope mass in the various compartments. The model-predicted masses of the stable isotope are compared to those measured experimentally. Differences between model prediction and experimental measurement are used to modify the model parameters and structure to improve the model prediction. Once the model prediction is close to the experimental measurements, a least-squares fitting routine is performed to minimize the difference between the model prediction and the experimental measurement.

The results of the model include rates of flows among compartments, sizes of storage pools of the analyte in the body, other steady-state pool sizes of the analyte, daily irreversible utilization (from which we can learn nutrient requirement), levels of absorption of a nutrient from the GI tract, and other information about nutrient metabolism. The model therefore provides access to information that was previously inaccessible.

Logistical Aspects of Stable Isotope Studies

Stable isotope experiments and their accompanying compartmental models should be planned simultaneously so that the model requirements can guide the experimental design. An initial plan of the modeling strategy can help determine sampling sites, sampling frequencies, means for optimizing the observed response of the labeled nutrient, and so on. In human studies, sampling sites are chosen to be relatively noninvasive. Collections commonly include urine, feces, and plasma. These need only be collected if the nutrient is expected to appear in each of these types of samples. Fat and muscle biopsies can also be collected, if necessary, but with limited frequency. Viable cells can be collected from the oral (buccal) epithelium or from feces. The specific sites sampled in a given study are based on the expected appearance of the labeled nutrient in those locations.

Sampling frequency must be increased during times when the mass of labeled nutrient is changing rapidly in the sampled pools. For example, several hours after ingestion of a labeled nutrient, there may be a rapid rise of that nutrient in the plasma. Samples must be collected sufficiently often to clearly define the rise and fall of the nutrient level in the plasma. At later time points, when the level of labeled nutrient is changing less rapidly, the sampling frequency can be reduced.

The dose of the isotope to be used in a study depends on the natural abundance of the enriched and reference isotopes. It is also dependent on the content of the element expected in samples to be analyzed, the length of time detectable enrichment is required, and the precision of the analysis technique used to determine the isotope ratios. When extrinsic labeling with stable isotopes is used to measure absorption, it is important that the label be administered in a manner that creates optimal conditions for isotopic exchange.

The stable isotope-labeled nutrient can be delivered to the subject in several ways (11). The isotope-labeled nutrient can be enclosed in a small gelatin capsule for ingestion (21), mixed into a food or drink (34), or injected into a vein (either as a bolus or at a constant rate over several minutes or hours) (35). When the test nutrient is mixed with a food, it is important that no residue remain in the food container; the container must be rinsed with water, which is then consumed by the subject or wiped clean with bread that is subsequently eaten. Some studies serve the stable isotopes in formula or blended foods. Others use solid foods but in homogeneous

forms. The isotope solution is usually added during preparation of the test foods or dripped onto the meal components and mixed carefully with them.

Labeling is usually conducted several hours before serving the food in order to allow sufficient time for equilibrium to occur. Another method is to administer the isotopes in a larger volume of solution together with the test meal. This technique, however, might not be the best design when studying gastrointestinal uptake, because solutions are often more efficiently absorbed than are solid foods.

Sometimes the dose is provided through *intrinsically labeled* food, rather than using an oral dose (*extrinsic labeling* method). Plants are labeled intrinsically by incorporating the isotope into the growing plant; animal products are labeled by injection or via the feed provided to the animal. Examples of intrinsically labeled foods include peas labeled with iron-57, spinach with iron-57, goat's milk with zinc-67, and chicken meat and eggs with iron-57 and zinc-67. Some groups have reported differences in absorption between intrinsic and extrinsic tags (36, 37). The reasons for the discrepancy are unknown. Nevertheless, most recent studies in humans have revealed good agreement between intrinsically and extrinsically added tracers of Zn, Ca, Mg, and Cu (38–40). (See Chapter 12, ''Producing Research Diets,'' for additional discussion of isotopically labeled foods.)

The naturally occurring stable isotope of carbon, carbon-13, is naturally concentrated by a certain plants, notably sugarcane and corn (5). Food products derived from these plants also are rich in carbon-13. Researchers using this isotope for metabolic studies may wish to avoid confounding by providing a diet devoid of these foods. (Also see Chapter 14, ''Planning and Producing Formula Diets,'' for a discussion of this issue.)

The investigator has considerable flexibility in selecting which isotope to enrich. The number of choices is limited only by the number of isotopes the element has. Usually the rare isotopes are used for enrichment, but very rare isotopes are not always preferred because of analytical difficulties. For minimal analytical error, the level of isotope signal should be equal to twice (or more) the level of background variance. Van Dokkum et al (11) provide detailed information about the selection and preparation of isotopes such as calcium, iron, and molybdenum.

Safety Issues

Because the composition of the nucleus of a stable isotope does not change or decay, there is no harm from radioactivity. Some commonly used stable isotopes may have biological effects; however, effects are not observed until these isotopes have been administered at high doses. For example, deuterium oxide (water with deuterium replacing hydrogen) is harmless in small amounts but may cause dizziness or changes in growth rate when consumed in large doses (41). When used in humans as *in vivo* tracers, stable isotopes do not present identifiable risks. Further, the isotope itself does not require special means of disposal.

However, when studies use stable isotopes in a research protocol, it may be advantageous to avoid using the term *isotope* for describing the research methods to subjects. Some subjects may interpret this term analogous to radiation, and it may evoke ungrounded fear. The terminology that has been successfully used in describing the use of hydrogen-2 (deuterium) and oxygen-18 to subjects is:

> Heavy water weighs slightly more than the water you normally drink, but it contains exactly the same elements and number of atoms as all other water (two atoms of the element hydrogen and one atom of the element oxygen). But in heavy water, some of the hydrogen and oxygen atoms weigh more than in the water that you normally drink. Heavy water occurs naturally, so you consume a small amount of it in all of the water you drink. By giving you a cup of water with a higher level of heavy water than you normally drink, we can use very sensitive instruments to measure the amount eliminated in your urine. (Adapted from an IRB-approved protocol used by the Diet and Human Performance Laboratory, USDA Beltsville Human Nutrition Research Center, Beltsville, Md.)

Typical Applications of Stable-isotope Methodology

Using Doubly Labeled Water to Measure Energy Expenditure

One example of the important application of stable isotopes in nutrition is the measurement of energy expenditure of free-living subjects using the doubly labeled water method (42). The doubly labeled water method was developed to study energy expenditure in laboratory rodents during the 1950s (43, 44). Its application for use in humans gained popularity beginning in the 1970s. With the advent of better and less expensive analyses, this method has become an important tool for energy metabolism research.

The method is based on measuring the turnover of ^{2}H (deuterium) and ^{18}O. Both isotopes are administered in the form of water. The ^{18}O is eliminated in equal amounts as both water and carbon dioxide (the enzyme carbonic anhydrase keeps the ^{18}O in equilibrium between CO_2 and H_2O). The deuterium is eliminated as water. Thus, the difference in the rates of elimination is the rate of carbon dioxide production. Based on estimates of or measured food or respiratory quotient (CO_2 production $\div$ O_2 consumption), the rate of oxygen consumption can be calculated. Energy expenditure is calculated using standard equations based on the rate of carbon dioxide production and oxygen consumption.

Typically, a sample of blood or urine is collected to measure background isotopic enrichment and the dose is administered. Samples of urine (spot samples usually collected once daily) are collected for 14 days (approximately two half-lives for the isotopes) and the isotopic enrichment of deuterium and ^{18}O are determined in these samples. This method requires little intervention, and it is easy for subjects to complete the collections.

Using Stable Isotopes to Enhance Balance Studies

Compartmental modeling with stable isotopes can be a useful adjunct to balance studies. The selenium metabolism studies of Patterson et al provide a good example of how these techniques can be combined (45). In this case, an oral dose of selenium-74 was given to the study participants. Fecal and urine samples were collected and analyzed for selenium-74. Plasma samples were also collected and analyzed for the stable isotope. The data collected from the balance portion of the study were combined with the data from the plasma samples, and a model of selenium metabolism was developed. The model was able to predict the rates of movement of selenium through body pools, levels of storage at steady state, and rates of elimination of selenium from the body.

Stable isotope tracers and compartmental modeling have been used to enhance studies of whole-body copper metabolism in humans. In a series of labeled copper studies including young men, elderly men, young women, and pregnant women conducted at the Western Human Nutrition Research Center, Turnlund et al were able to show that copper absorption is influenced markedly by the amount of dietary copper (15, 31, 46–50). These studies showed that the absolute amount of copper absorbed increases as the amount in the diet increases; when intake is low, however, compensation occurs and absorption is much more efficient, with a higher percentage being absorbed. This result suggests that the amount of dietary copper is the primary factor influencing absorption. In addition, these studies showed that endogenous copper excretion is markedly affected by dietary copper intake and fecal copper losses reflect dietary copper.

Thus, copper turnover is high when intake is high. But in contrast, the studies found that urinary copper changes little or not at all with increased dietary copper. This means that when copper intake is high, more is excreted through feces but not urine. Whereas urinary copper does not change with high intakes of copper, it does drop significantly with a low-copper diet. Finally, through the use of isotopes these studies found that several indexes of copper status also change with changes in copper intake. Plasma copper, ceruloplasmin concentration and activity, and urinary copper declined significantly with a low copper intake of 0.38 mg per day.

Vitamin A and beta-carotene metabolism in humans has also been studied using kinetic models based on the use of stable isotopes (51). Burri and Park hypothesized that preformed vitamin A from meat and milk sources might be metabolized differently than vitamin A derived from beta-carotene. A total of 14 healthy adult women, living in a metabolic unit, participated in these studies. Subjects were given one of two treatments: a bolus dose of deuterated retinyl acetate in a capsule containing corn oil and d-4-retinyl acetate, or a bolus dose of d-8-beta-carotene in a capsule containing corn oil. Timed blood samples were collected up to 57 days depending on the isotope measured. Serum levels of deuterated stable isotopes of retinol (d-4-retinol) and beta-carotene (d-8-beta-carotene) were measured using a high-precision technique of isotope-ratio mass spectrometry. Compartmental models of vitamin A and beta-carotene metabolism in women fed known concentrations of vitamins and carotenoids were developed.

Dietary changes of beta-carotene intake did not influence the turnover rate of retinol in the four-compartment model developed. However, steady-state masses and residence times of retinol changes occurred in several compartments. In the working compartmental model for beta-carotene, the kinetics of d-4-retinol formed from beta-carotene is more complicated than the preformed d-4-retinol. In addition, investigators suggested that d-8-beta-carotene readily converts into d-4-retinol with high interindividual variability.

Conclusion

Compartmental models, in conjunction with stable isotope methodology, can be expected to continue to provide an expanding variety of data about nutrient metabolism in humans. Their major advantage as tracers in modeling studies is that they do not decay. Thus, samples can be stored indefinitely, as long as the nutrient of interest does not deteriorate in the biological sample. Stable isotopes of several elements also can be administered simultaneously to develop multicompartmental models of intermediary metabolism and nutrient interactions. Isotopes provide a unique methodologic advantage in that specific foods can be given appropriate intrinsic labels, while entire meals can be tagged with other extrinsic labels, and the two types of labeling doses do not interfere with one another.

Traditional balance study methodologies, in combination with the newer techniques of compartmental kinetic modeling and stable isotope applications, have the potential to advance our understanding of energy balance, vitamin kinetics and metabolism, vitamin and mineral interactions, and interactions between micronutrients and macronutrients. Stable isotopes are completely safe, so they open up the opportunity to conduct experiments in people of diverse ages and physiological conditions. Refinement of these approaches for research on infants, children, adolescents, the elderly, and pregnant or lactating women thus may be a means of obtaining the long-sought data needed to establish nutrient requirements for these population groups.

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