Analysis of Stable Isotope Data to Estimate Vitamin A Body Stores

Jointly prepared by HarvestPlus, IAEA and USAID

HarvestPlus IAEA USAID FROM THE AMERICAN PEOPLE

IAEA
International Atomic Energy Agency

Analysis of Stable Isotope Data to Estimate Vitamin A Body Stores

Jointly prepared by Harvest Plus, the IAEA and USAID

IAEA
International Atomic Energy Agency

June 2007

The originating Section of this publication in the IAEA was:

Nutritional and Health-related Environmental Studies Section
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
A-1400 Vienna, Austria

ANALYSIS OF STABLE ISOTOPE DATA TO ESTIMATE VITAMIN A BODY STORES
IAEA, VIENNA, 2008

ISBN 978–92–0–100608–0

Printed by the IAEA in Austria
June 2008

FOREWORD

Vitamin A deficiency (VAD) is a serious public health problem in most developing countries. Because of the detrimental effects of vitamin A deficiency on human health, accurate assessment of vitamin A status is necessary to develop and evaluate intervention programmes. The IAEA is providing technical support to its Member States to use stable isotope dilution techniques to develop and evaluate programmes aimed at reducing vitamin A deficiency in populations. The stable isotope dilution technique, in contrast to other methods, have the potential to provide a quantitative estimate of vitamin A concentration across the continuum of status, from deficient to excess vitamin A body stores.

In 2004 the IAEA, the United States Agency for International Development (USAID) and HarvestPlus initiated the Vitamin A Tracer Task Force, made up of international experts. HarvestPlus is a Global Challenge Program of the Consultative Group on International Agricultural Research (CGIAR). It is coordinated by the Centro Internacional de Agricultura Tropical (CIAT), a not-for-profit organization that conducts socially and environmentally progressive research aimed at reducing hunger and poverty and preserving natural resources in developing countries, located in Colombia, and the International Food Policy Research Institute (IFPRI), located in the United States of America and whose mission is to provide policy solutions aimed at reducing hunger and malnutrition in developing countries.

The role of the Vitamin A Tracer Task Force was to prepare three complementary publications on the use of stable isotope dilution techniques to assess vitamin A body stores. The first publication entitled "Appropriate Use of Vitamin A Tracer (Stable Isotope) Methodology" was published in 2004 by USAID/International Life Sciences Institute (ILSI) through the Micronutrient Global Leadership (MGL) project with co-sponsorship of IAEA and HarvestPlus. The second handbook is on "Vitamin A Tracer Dilution Methods to Assess Status and Evaluate Intervention Programmes" and was published in 2005 by HarvestPlus. The publication was endorsed by the IAEA and USAID. This is the third publication of this series that focuses on the use of model-based compartmental analysis of stable isotope data to estimate vitamin A body stores in humans.

The IAEA is grateful to M. H. Green, Pennsylvania State, University, United States of America; J. Balmer Green, Pennsylvania State University, United States of America; and H. Furr, Craft Technologies, United States of America for preparing this publication.

Endorsement of this handbook has been granted by USAID and HarvestPlus.

The IAEA officer responsible for this publication was Najat Mokhtar, Division of Human Health.

EDITORIAL NOTE

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

CONTENTS

1. INTRODUCTION

Vitamin A deficiency (VAD) is a world-wide public health problem that affects millions of people in developing countries [1, 2]. VAD has been recognized as the leading cause of preventable childhood blindness and is associated with infection and high risk of mortality. Women in developing countries are also at risk of VAD especially during pregnancy and lactation. VAD occurs when body stores of vitamin A are depleted to the extent that physiologic function is impaired even when clinical signs are not evident. To ameliorate this situation, simple and accurate techniques for assessing vitamin A status in the field are needed.

Among the many methods that have been proposed and used, the stable isotope dilution technique is the method of choice for both estimating liver vitamin A reserves — i.e. assessing vitamin A status — and evaluating the efficacy and effectiveness of vitamin A interventions to improve status. The stable isotope dilution method [1, 2] involves administering a dose of stable isotope-labelled vitamin A to test subjects and determining the isotope ratio in plasma at specified later times. Total body vitamin A pool size is predicted from these isotope dilution data using a prediction equation developed by Professor James Olson and colleagues [3].

While the isotope dilution technique has been successfully applied by researchers in the field [4−16], several refinements and extensions have been suggested that may greatly add to the reliability and usefulness of the method. These are discussed in this handbook. First, the prediction equation is reviewed and several assumptions implicit in its use are discussed. The potential usefulness of a shortened 3-day sampling protocol instead of the conventional 20- to 28-day protocol that is generally used in vitamin A isotope dilution studies is then reviewed and alternate protocols for estimating vitamin A stores are described. Finally, information on a more sophisticated form of mathematical modelling, referred to as model-based compartmental analysis [17, 18], is presented. This technique relies on a similar but more extensive experimental protocol, and it can provide insights into the quantitative and descriptive aspects of whole-body vitamin A metabolism as well as estimates of vitamin A stores, is presented. A glossary of terms and an example calculation have been provided. This information will help researchers become more familiar with the mathematical basis for stable isotope studies of vitamin A and thus improve the application of these methods.

2. STABLE ISOTOPE DILUTION METHODS FOR ESTIMATING VITAMIN A STATUS

2.1. Estimation of total body vitamin A pool size using the isotope dilution technique: the "Olson equation"

The stable isotope dilution technique is an indirect method for estimating the size of liver vitamin A stores or the exchangeable body pool of vitamin A [1, 2], depending on how the equation is formulated (see below). The technique is based on the principle of isotope dilution, in which a known dose of stable isotope-labelled vitamin A is administered orally and is mixed with the endogenous vitamin A pool; 20 days is considered to be adequate mixing time in both young adults and elderly humans (Figure 1) [5, 7]. At that time, a blood sample is obtained for measurement of the plasma or serum isotopic ratio of labelled to non-labelled retinol. (Plasma or serum can be used; for simplicity, the term "plasma" will be used hereafter.) Total body vitamin A pool size (mmol retinol) is estimated quantitatively using the measured plasma isotopic ratio of labelled to non-labelled retinol and the Olson equation [3]:

$$\text{Total body exchangeable vitamin A pool} = F\ dose \times [S\ a\{(1/D{:}H)-1\}]$$

where F is a factor related to the efficiency of absorption and storage of the orally administered dose; *dose* is the amount of isotope administered (mmol); the factor S corrects for the inequality of the plasma to liver ratio of labelled to non-labelled retinol; the factor a corrects for irreversible loss of labelled vitamin A during the equilibration period; $D{:}H$ is the isotopic ratio of labelled to non-labelled retinol in plasma; and -1 corrects for the contribution of the dose to the total liver vitamin A reserve.

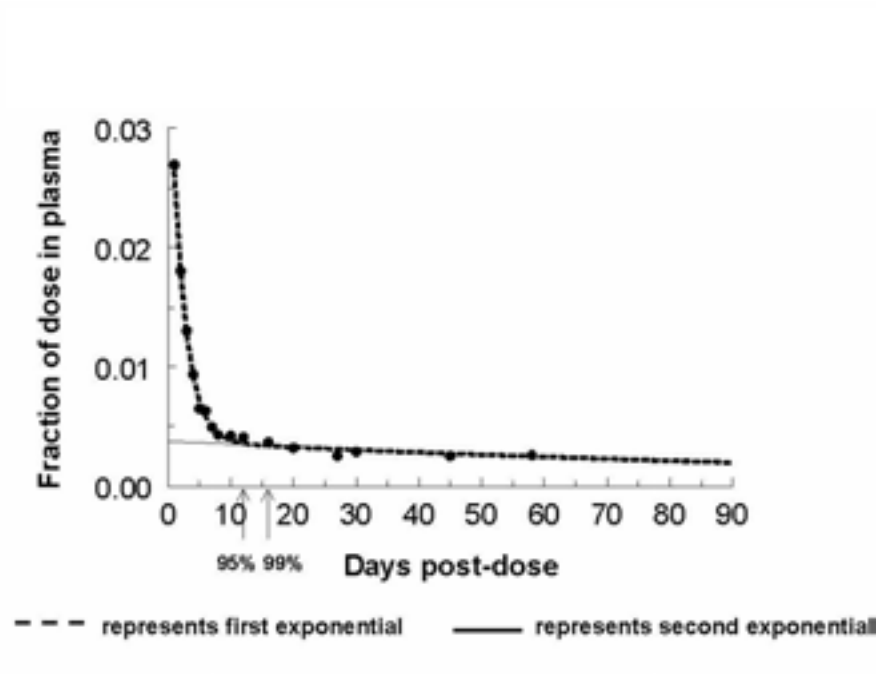

FIG. 1. Estimation of equilibration time based on biexponential equation. Mixing of orally administered labelled vitamin A in humans. Labelled retinyl acetate was administered and plasma isotope kinetics were measured for up to 90 days. The dose mixes with the endogenous vitamin A pool in less than 20 days in adult humans [5].

Several assumptions facilitate the use of the "Olson equation," and several caveats need to be kept in mind. First, the factor *F* is assumed to be 0.5 based on the work of Bausch and Reitz [19]; i.e. it is assumed that 50% of the oral dose is stored in the liver.

Second, a value of 0.65 is used for the factor *S* based on the mean observed plasma to liver ratio of the specific activities of radiolabelled vitamin A in rats with varying levels of dietary vitamin A intake and liver vitamin A stores [20]. The interindividual variability in these values has not been experimentally verified in humans over a variety of ages and physiological states (e.g. pregnancy or lactation). For better use of isotope dilution techniques, covariates that may act as biomarkers for individuals need to be defined, or isotope dilution methods that do not require such factors need to be developed.

Third, the factor *a* is used to adjust for catabolism of the dose of labelled vitamin A during the mixing period. The value used is based on the half-life of vitamin A turnover and is estimated as 140 days in adults [21]. It is assumed to be independent of the size of the liver reserves of vitamin A, which is unlikely to be true because the system fractional catabolic rate is very sensitive to liver vitamin A stores — as discussed later — and is time-invariant ($a = e^{-kt}$, where k = ln 2/140 days and t = time in days since dose). The isotope dose, however, will not truly equilibrate with the endogenous vitamin A pool if there is continuous intake of unlabelled dietary vitamin A (see below) and catabolism of the dose of labelled vitamin A during the 20-day mixing period.

2.1.1. Total liver vitamin A reserves versus total body vitamin A pool

Estimating liver vitamin A stores is useful because the liver is a major storage site for whole-body vitamin A; thus, liver concentrations of retinyl esters (measured directly by biopsy or indirectly such as by isotope dilution) are considered the most useful indicator of vitamin A status. The total exchangeable body pool of vitamin A (or the total body vitamin A pool) may be smaller or larger than chemically measured liver vitamin A stores because the exchangeable pool includes all vitamin A in the body that is in a kinetic state (i.e. it turns over) and is thus measurable using isotopic methods such as isotope dilution. The total exchangeable body pool of vitamin A will be smaller than total liver vitamin A if the liver has very large stores of vitamin A, some of which is nonexchangeable. The exchangeable pool will be larger than liver vitamin A when there is a significant amount of vitamin A in extrahepatic tissues.

2.1.2. Effect of consumption of vitamin A during isotope dilution studies

When designing an isotope dilution study and considering vitamin A intake, it is important to remember that newly absorbed dietary vitamin A is preferentially secreted from the liver into the blood stream. Consequently, labelled vitamin A in the plasma is diluted to a greater extent than labelled vitamin A in liver stores when subjects consume unlabelled dietary vitamin A during the mixing period. For this reason, in classical isotope dilution studies, subjects ideally consume as little vitamin A as possible after administering the oral dose, while the isotope is mixing with exchangeable vitamin A pools. This creates a quasi closed system with respect to specific activity of tracer versus tracee, and the slope of the curve of fraction of dose remaining in plasma versus time post-mixing will be close to zero (Figure 2). Under these conditions, the isotope dilution in plasma will be most sensitive to liver and total body vitamin A stores. The post-equilibration slope will be close to zero because there is little or no new tracee (unlabelled vitamin A) entering the system to further dilute the tracer. It should also be noted that the specific activity of tracer to tracee (i.e. *D/H* in the Olson equation)

under this condition will be the same in all exchangeable vitamin A pools. Thus, a correction for the difference in specific activity in liver versus plasma (the factor *S* in the Olson equation) is not needed. However, this condition (little or no dietary intake of vitamin A) may not be feasible in free-living subjects.

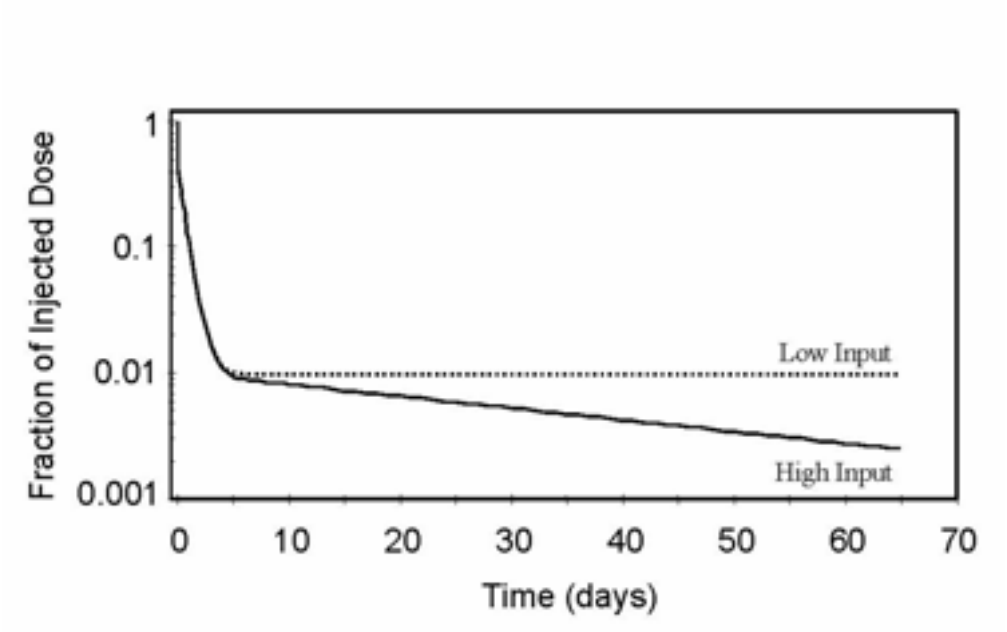

FIG. 2. Effect of dietary vitamin A input on the post-mixing slope of the plasma tracer response curve. This simulation of the fraction of an intravenously administered dose of isotope remaining in plasma versus time after isotope injection shows the influence of vitamin A intake on the slope of the curve during the "equilibration" period. When input to the system is low (i.e. subjects consume as little vitamin A as possible while the isotope is mixing with exchangeable vitamin A pools), the slope of the curve will be close to zero and the isotope dilution in plasma will be most sensitive to liver and total body vitamin A stores. If input is high, the isotope continues to be diluted in plasma with time.

Furthermore, if subjects are either in vitamin A balance or a positive balance during an isotope dilution study, then applying the Olson equation with data collected on day 20 after tracer administration may not give an accurate estimate of liver vitamin A reserves in all subjects. The system fractional catabolic rate for vitamin A varies greatly depending on liver vitamin A stores; i.e. post-mixing slopes for the curves of fraction of ingested dose versus time will be very different as a function of liver vitamin A store. Figure 3 shows data collected in vitamin A kinetic studies in rats with very low (13 nmol), moderate (206 nmol) or high (580 nmol) liver vitamin A levels [22].

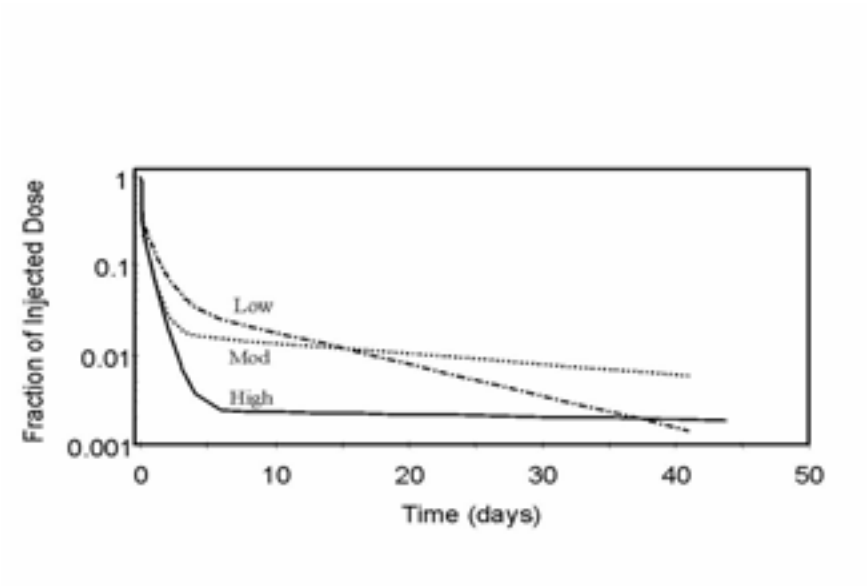

FIG. 3. Effect of liver vitamin A stores on the system fractional catabolic rate for vitamin A. These plots show the plasma tracer response curves after administration of [^{3}H]retinol-labelled plasma to rats with low (13 nmol), moderate (206 nmol) or high (580 nmol) liver vitamin A levels. Note differences in the shapes of the curves both at 3 and 20 days after dose administration. (Adapted [22]).

Fractional catabolic rates averaged 2.8%/day in the rats with high liver vitamin A stores, 5.1%/day in those with moderate reserves, and 5.8%/day in rats with low vitamin A status. [In the latter group, compartmental modelling of the results (see later) predicted that the slow turning-over extra-vascular pool contained 275 nmol of vitamin A or 21 times that measured in the liver.] At ~20 days after dose administration, the fraction of dose remaining in plasma is not very sensitive to liver stores. However, around 3 days after dosing, the fraction of dose in plasma is very sensitive to liver vitamin A levels. This is one reason to develop equations to predict liver vitamin A stores based on a blood sample collected 3 days after dose administration (see discussion below).

In contrast, when performing a kinetic study for model-based compartmental analysis (see below), it is ideal if subjects are in a steady state with respect to vitamin A (i.e. they are absorbing as much vitamin A as they are irreversibly utilizing). In this situation, the post-mixing slope of the plasma tracer response curve will be a function of the system fractional catabolic rate, and the calculated total traced mass will approximate the body vitamin A exchangeable pool. If subjects are not consuming vitamin A, or if they are consuming less than they are utilizing, then the total traced mass will overestimate the body vitamin A store because the post-mixing slope will be less than that for subjects in balance.

2.1.3. Effect of inflammation on the isotope ratio

The evaluation of acute phase proteins, e.g. C-reactive protein, in human studies using isotope dilution methods is recommended to determine if any underlying infections exist [2]. The effect of inflammation induced using lipopolysaccharide or recombinant human interleukin-6 on the plasma isotopic ratio has been modelled in rats (Gieng, S.H., et al., J. Lipid Res., in press 2007). During inflammation, hepatic retinol mobilization is significantly reduced. This phenomenon also negatively impacts the fraction of isotopically labelled dose in the plasma

compartment. Therefore, the plasma isotopic ratio determined during acute inflammation would overestimate total body reserves of vitamin A using the Olson equation.

2.2. Isotope dilution methods for estimating total body vitamin A pool size based on sampling at 3 days

In most isotope dilution studies, the plasma sample for determining isotope ratio is obtained about 20 days after administration of the oral dose. This time is based on the data [5] determining that the mixing time for isotopic vitamin A was 16-17 days in adults (Figure 1). Because the length of time between dosing and sampling may pose challenges in field studies, the observation [23] that liver vitamin A levels can be predicted over a wide range of vitamin A status in rats based on plasma isotope dilution data obtained 3 days after oral administration of [^{3}H]retinol suggested that earlier sampling times may also be useful in humans. A 3-day sampling protocol was tested in elderly people in Guatemala [7]; results (D:H ratio at 3 days) were compared with vitamin A stores predicted by the Olson equation using a sample obtained 20 days after dosing. A significant inverse correlation ($r = -0.75$, $P = 0.002$; Spearman correlation coefficient $\rho = -0.81$, $P = 0.004$) was observed. In a subsequent study [12], the researchers developed a prediction equation that allows estimation of total body vitamin A stores in adults based on isotope dilution in a sample obtained 3 days after oral isotope administration. Because factors other than liver stores of vitamin A (e.g. inflammation) can influence the ratio of tracer to tracee 3 days after dosing, it is prudent to also determine the isotope ratio on day 1 when the ratio is peaking in plasma. This will provide a qualitative estimate of absorption efficiency and liver processing and secretion of the dose into plasma. That is, if the day 1 ratio is lower than expected, this information will be useful if the day 3 ratio is low.

Further work is needed to validate the potential usefulness of a 3-day sampling protocol for determining total body vitamin A pool size in different populations. The shorter study time is preferable for field studies because it will be easier for subjects to be available at this 3 rather than 20 days and smaller doses of labelled vitamin A will be required, which will reduce the isotope costs. In addition, use of a 3-day prediction equation will eliminate the need for two of the three assumptions (the factors *S* and *a*) required in applying the Olson equation: specific activities in plasma and liver will be approximately equal on day 3, eliminating the need for *S*, and irreversible loss will be negligible at this early time, eliminating the need for the factor *a*.

2.3. Estimates of total exchangeable vitamin A pool based on model-based compartmental analysis

The compartmental modelling methods that are discussed in detail later are another indirect technique for estimating total body exchangeable pools of vitamin A pool or total traced mass. Figure 4 shows the association between total traced mass and chemically measured liver vitamin A in rats [24]. As liver stores increase from the deficient state, extrahepatic pools of vitamin A are exchangeable with plasma and part of the total traced mass. When stores are very large, non-exchangeable pools of vitamin A exist in the liver [25]; these are not included in the total traced mass.

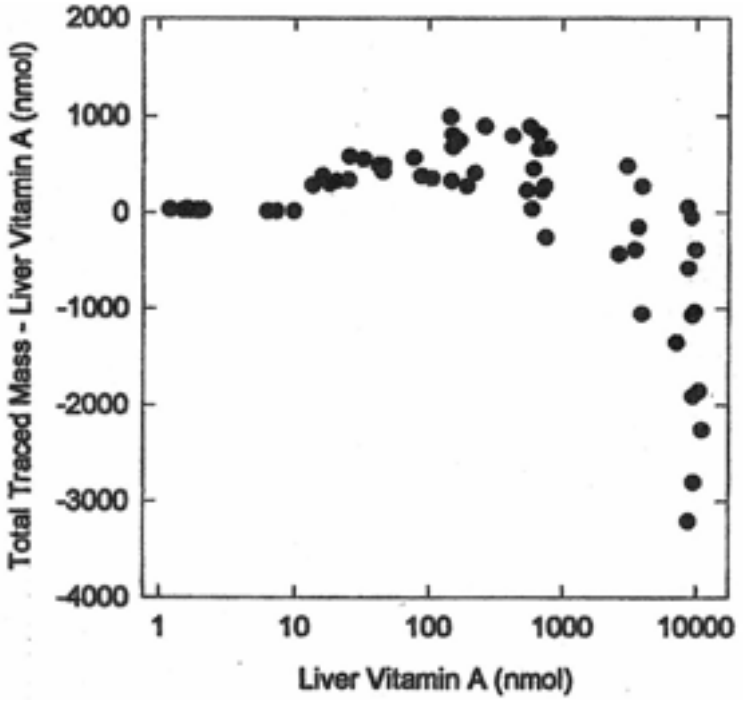

FIG. 4. Association between total traced mass and chemically measured liver vitamin A in rats. Data on liver vitamin A levels in rats over a wide range from very low (~3 nmol) to very high (~10,000 nmol) [24] are plotted against total traced mass determined by model-based compartmental analysis. When liver stores are very high, some of the hepatic vitamin A is contained in non-exchangeable pools that will not be included in the estimate of total traced mass.

3. USE OF MODEL-BASED COMPARTMENTAL ANALYSIS TO STUDY VITAMIN A STATUS AND METABOLISM IN HUMANS

3.1. Overview

Model-based compartmental analysis [17, 18, 24, 26, 27] is a form of mathematical modelling that provides quantitative, descriptive, and predictive information about vitamin A metabolism, total traced mass, dietary input, and utilization. Like the stable isotope dilution technique, the experimental protocol for model-based compartmental analysis requires the administration of labelled vitamin A and the collection of blood samples, albeit more frequently and over longer time. In view of this overlap, and because of the additional useful information that can be obtained by applying model-based compartmental analysis to stable isotope studies of vitamin A, it is recommended that the duration and sampling protocols for some isotope dilution experiments be extended for two reasons: first, to obtain more information about vitamin A metabolism and, second, to enhance optimization of the isotope dilution procedures.

Not unexpectedly, more applications have been made of model-based compartmental analysis to describe and quantitate vitamin A metabolism in rats than in humans, and the rat has served as a very good model for human vitamin A metabolism. The technique has been used to study vitamin A metabolism at different levels of status [22, 28, 29], in different organs [30, 31], and under different treatments [31, 32, 33]. These studies have provided several unique insights to vitamin A metabolism, including the extensive recycling of retinol among tissues and plasma before irreversible utilization, the contribution of non-hepatic tissues to plasma retinol input, and the importance of extra-hepatic tissues in vitamin A storage. In addition, they have provided information on parameters of vitamin A metabolism: size of compartments and total traced mass, utilization rate and other transfer rates, residence and transit times, the fraction of the vitamin A pool lost each day, the number of times retinol recycles to plasma before irreversible loss, and the time it takes for retinol to recycle to plasma.

Although few human kinetic studies have been explicitly designed for analysis by model-based compartmental analysis, the technique has been retrospectively applied in several instances [34, 35]. It has also been used to analyse carotene metabolism in humans [36, 37, 38]. Applications of model-based compartmental analysis to data from tracer label studies is likely to enable estimates to be made for important parameters of human vitamin A metabolism, including disposal rates and dietary requirements for vitamin A in different physiological states, including childhood, pregnancy, and lactation. Although the application of model-based compartmental analysis requires optimization of experimental design before the study begins, an extended study duration and the collection of more blood samples than are needed for isotope dilution *per se* (see below), followed by sophisticated computer analyses, the information gained justifies the additional effort.

3.2. Compartmental modelling of human vitamin A metabolism

To apply model-based compartmental analysis, the researcher carries out a long-term *in vivo* kinetic study in which a series of blood samples is collected after the administration of a non-perturbing dose of labelled vitamin A. The data are plotted as a fraction of the ingested dose remaining in plasma as a function of time after dose administration (Figure 5). Data analysis requires modelling software (e.g. the Simulation, Analysis, and Modeling [SAAM] program;

see later) that can handle a conceptual compartmental model of vitamin A metabolism (Figure 6) to quantify the transfer of vitamin A between compartments, the masses of vitamin A in the various compartments, and other parameters characterizing the system (e.g. vitamin A utilization or disposal rate, transit time, and recycling characteristics; see below).

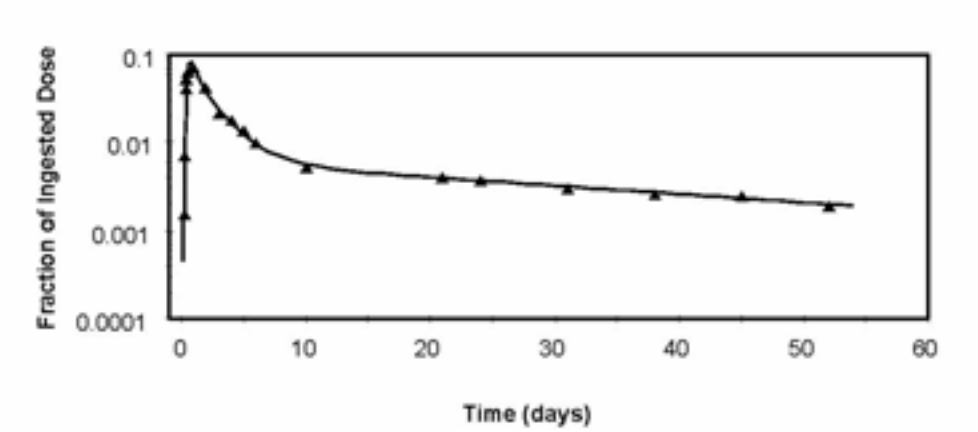

FIG. 5. Vitamin A kinetics in a human subject. Data are fraction of the ingested dose remaining in plasma as a function of time after administration of octadeuterated retinyl acetate to a woman [39, 40]. Triangles show the observed data; the plot is the simulation of the 7-compartment model (Figure 6) developed to fit the data using WinSAAM.

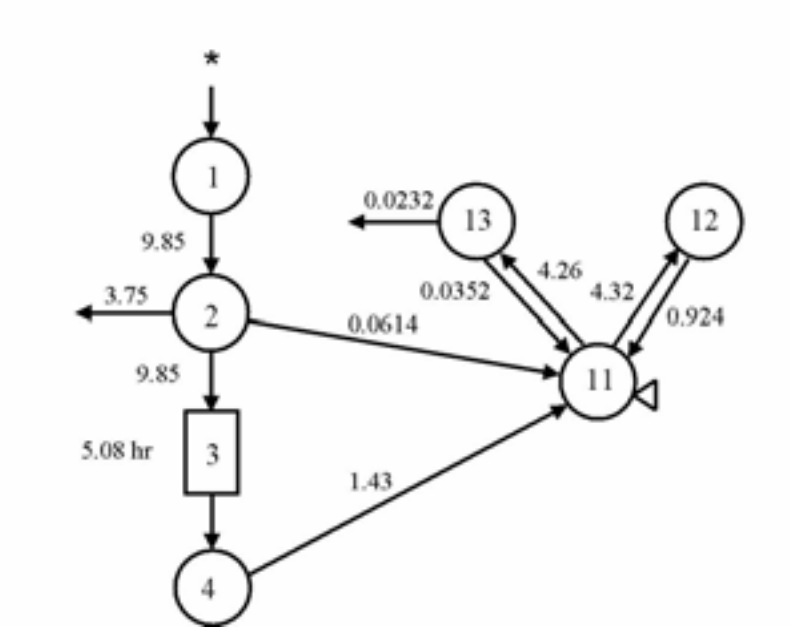

FIG. 6. Compartmental model for vitamin A metabolism. Data for one subject [39, 40] on fraction of an oral dose of octadeuterated retinyl acetate versus time after dose administration were fit to a 7-compartment model using WinSAAM. Circles represent compartments, the rectangle is a delay element; arrows show the interconnectivities between compartments; the asterisk shows the site of input of the oral dose and the triangle shows the site of sampling. Compartments 1, 2, and 4 and delay element 3 correspond to the absorption and initial hepatic metabolism of the dose; compartment 11 represents plasma retinol in its physiological transport complex; compartment 12 is a faster turning-over extra-vascular pool of vitamin A and compartment 13 is a slow turning-over extra-vascular pool of vitamin A, presumably mainly the retinyl ester storage pool in the liver. The model parameters associated with the arrows are fractional transfer coefficients [L(I,J)s, or the fraction of compartment J's tracer transferred to compartment I each day] and the delay time (hours) for delay element 3.

The first application of model-based compartmental analysis to vitamin A metabolism in humans was described by Green and Green [34]. In studies of plasma retinol-binding protein in 1965–1966, Goodman and colleagues collected data on the long-term (up to 240 days) kinetics of plasma retinol in 3 human subjects who had received an intravenous dose of their own plasma that had been labelled in vitro with 15-[^{14}C]retinol. Data for each subject were fit to a 3 compartment model in which one compartment represented the plasma retinol pool, a second represented slowly turning-over vitamin A stores (mainly in the liver), and the third corresponded to faster turning-over extra-vascular pools of vitamin A. Using a feature called "multiple studies" in SAAM, the best-fit 3 compartment models for the 3 subjects were modelled together to determine a population working hypothesis model of vitamin A kinetics. The model predicted that the average molecule of labelled retinol spent 5.4 hours in plasma before leaving reversibly or irreversibly ("transit time;" see later). This is somewhat longer than the 1.2–3.4 hours that has been observed in rats [41]. The average retinol molecule spent 0.86 days in plasma before irreversible loss ("residence time") and 105 days in the whole body. This plasma residence time is similar to the value found for rats. The model also predicted that the average retinol molecule recycled to plasma 3 times before irreversible utilization (compared with 9–13 times in rats). The vitamin A utilization rate in these presumably well-nourished subjects was estimated to be 6 μmol/day, almost twice the RDA of 3.14 μmol/day for an adult human male. The model predicted that 26% of plasma retinol turnover was irreversibly utilized and 74% recycled to plasma; of the plasma retinol turnover, 40% was predicted to go to slowly turning-over vitamin A pools and 60% to the fast turning-over pools. Assuming that the slow turning-over compartment contains mainly liver vitamin A stores, liver vitamin A was estimated as 990 μmol [495 μmol/kg liver or 141 μg retinol equivalents (RE)/g] in these subjects. This estimate agreed well with a mean liver vitamin A content of 441 μmol/kg or 126 μg RE/g determined in an autopsy study of 101 accident victims in the United States of America [42].

Von Reinersdorff et al. [35] applied the technique of model-based compartmental analysis to data on plasma retinol kinetics in a human subject followed for 7 days after administration of an oral dose of 105 μmol of [8,9,19-^{13}C]retinyl palmitate. Despite the study's limitations (including the short length of the study, the large mass of the oral dose, and the unknowns of the extent of dose absorption and the size of liver vitamin A stores), a useful compartmental model was developed. It included several compartments related to absorption of the dose and initial liver clearance, a compartment representing plasma retinol, and two tissue compartments (liver and extra-hepatic tissues) as described above. The model predicted a vitamin A utilization rate of 4 μmol/day compared with the ~50 μmol of retinol per day passing through the plasma compartment. Also, in this well-nourished subject ~80% of the absorbed dose was predicted to be contained in the liver vitamin A storage pool at 7 days after dose administration.

More recently [43] model-based compartmental analysis was applied to data from a human subject [39, 40]. Twenty data points (for the fraction of the ingested dose of octadeuterated retinol remaining in plasma for 52 days after dose administration) were fit to a 7-compartment model (Figures 5 and 6) — similar to the one developed for the von Reinersdorff data. The model predicted that this healthy, normal-weight, 67 year-old woman had a vitamin A store of 387 μmol. If this were all in the liver, it would be equivalent to 235 μmol/kg liver (67.2 μg RE/g). The vitamin A disposal rate was estimated to be 9 μmol/day or 2.6 times the RDA. The fractional catabolic rate was 2.3%/day of body stores. The plasma transit time was predicted to be 2.8 hours; residence times were 0.59 day (plasma) and 46.5 days (whole body). The average retinol molecule was recycled to plasma 4.1 times before irreversible loss and it took, on average, 11.2 days for a retinol molecule to recycle to plasma. The plasma

retinol turnover rate was 45.5 μmol/day, and only 20% of that turnover (9.1 μmol/day or 2600 μg RE/day) was irreversibly lost. The model also predicted that it took 26 hours from the time of ingestion of the label until the average retinol molecule was secreted from the liver bound to retinol-binding protein. In addition, the model predicted that by day 3, only 2% of the dose had been irreversibly lost following absorption. This again points to the fact that the factor *a* in the Olson equation will not be needed if sampling were done on day 3.

The above three examples illustrate the wealth of information about vitamin A metabolism that can be obtained by expanding the design of isotope dilution studies in humans so that sufficient data are obtained for mathematical modelling (specifically, model-based compartmental analysis). Clearly it is neither desirable nor feasible to apply model-based compartmental analysis to all human studies involving the stable isotope dilution technique, but it is clearly worth identifying those where this can be accomplished. Data from such studies could also be used to develop and validate equations for predicting total body vitamin A pool size based on a single blood sample obtained 3 days after oral administration of a stable isotope of vitamin A.

3.3. Requirements for design of human vitamin A model-based compartmental analysis studies

Several criteria must be considered in designing an *in vivo* vitamin A kinetic study for model-based compartmental analysis, including dose preparation and subject selection as discussed in regard to the isotope dilution technique [1]. For example, at this stage in the development of the model, it is important that the mass of isotopic tracer in the dose does not perturb the vitamin A tracee system because straight-forward compartmental analysis assumes a "steady state" with respect to vitamin A. More sophisticated applications available in SAAM can accommodate a non-steady state. At the same time, however, the amount of tracer must be adequate to ensure detection in plasma for ~60 days after administration; thus, the study must be long enough to define the long-term kinetics of plasma retinol tracer response. It also highlights the need for continued development of more sensitive analytical methods. The use of ^{13}C-vitamin A with analysis by isotope-ratio/combustion/gas chromatography/mass spectroscopy gives advantages over ^{2}H-vitamin A in this respect [2]. A long study duration is needed so that the isotope can thoroughly mix with the body's exchangeable pools of vitamin A; at that point and thereafter, a semi-log plot of plasma tracer concentration versus time will show its final terminal slope. This point is crucial for any experiment in which the area under the plasma tracer response curve will be calculated because, mathematically for certain applications (e.g. calculating system residence time), that requires integrating the response from time zero to infinity.

An adequate number of samples must be collected during the study to accurately define the plasma tracer response profile (see below). Based on the human studies done to date, when the dose was administered orally, between 24 and 27 blood samples must be collected at critical times during a 60–day experiment. (Fewer samples would be needed if the dose could be given intravenously, as discussed below.) The sampling schedule may be determined by sensitivity analysis [18] or a geometric progression [44] using the equation

$$T_{i+1} = T_i \, (T_N / T_1) \, e^{\,1/(n-1)}$$

where T_N is the time of the final blood sample (i.e. the end of the experiment), T_1 is the time of the first blood sample, and n is the number of blood samples. Time points will be more

concentrated at early times after dosing and more widely spaced as the study progresses; the key is to select times at which the model parameters are sensitive to the data so that a unique model can be identified. In general 3 data points are needed to identify each model parameter, giving 2 degrees of freedom for regression analysis during modelling. Thus for the model shown in Figure 6, which has a maximum of 8 parameters, 24 blood samples are needed. Obviously both the number of samples required and the timing might be experimental stumbling blocks in human studies conducted in the field. The identifiability of the model may also be limited by uncertainties about absorption efficiency of an orally administered dose (see below).

Once plasma samples are analysed for tracer and tracee, data are converted to fraction of the oral dose administered versus time after dosing and analysed using appropriate modelling software. A compartmental model is postulated based on current knowledge of the system under study. The observed data and model parameters estimated from the proposed model are entered into a programme such as SAAM. The programme sets up a set of differential equations based on the proposed model and solves them over the time frame specified. The results allow a comparison of the data with the proposed model by providing graphic and tabular information on goodness of fit. The modeler then iteratively adjusts the model parameters and the model structure in physiologically reasonable ways to arrive at agreement between the observed data and the model simulation.

The modelling software used in both the rat and human studies described above is WinSAAM, the Windows version of the Simulation, Analysis, and Modeling (SAAM) computer programme [18] or its earlier versions [45]. This software was originally designed by Mones Berman to study the metabolism of blood-borne components of physiological interest. It has been applied to many nutrients other than vitamin A [17, 18] and has greatly extended knowledge of these systems. WinSAAM is used to set up and solve the linear first-order ordinary differential equations describing the system kinetics, allowing comparison of a model solution with the data; weighted nonlinear regression analysis is used by SAAM to obtain final values for the model parameters (fractional transfer coefficients; see below) and their statistical uncertainties [18]. Other model-derived parameters can then be calculated from the fractional transfer coefficients and the estimated plasma retinol pool size by requesting a steady state solution to the model. Besides SAAM, other programmes have been developed for modelling physiological systems [46].

In addition to the criteria discussed above, it is best if researchers are familiar with the modelling software even if compartmental analysis will be done by a colleague who has expertise in the method. This guarantees that the researchers are sensitive to particular requirements of the modelling process such as the need for a relatively constant dietary intake of vitamin A and provitamin A carotenoids and a relatively constant body weight, both of which impact the assumption of steady state. Plasma retinol concentration must be determined periodically during the kinetic study; values are averaged over the study duration so that plasma retinol pool size can be estimated using the equation: plasma retinol pool size (μmol) = plasma retinol concentration (μmol/L) * estimated plasma volume (L), where plasma volume in adults may be approximated as body weight (kg) * 0.0435 L plasma/kg body weight [40]. Plasma retinol pool size is used by the modelling software to estimate other steady state model parameters (see below).

3.4. Parameters obtainable using model-based compartmental analysis (nomenclature)

After the modeler has developed a model that fits the data reasonably well, WinSAAM can generate final values for the *fractional transfer coefficients* associated with the model. Fractional transfer coefficients [L(I,J)s; day^{-1}] are the fraction of material in compartment J that is transferred to compartment I per unit time. These L(I,J)s are the numeric values associated with the vectors ('arrows') in the model schematic (Figure 6). Using these values and an estimate of the plasma retinol pool size (as discussed above), and assuming the subject is in a steady state with respect to vitamin A, the SAAM programme can be used to calculate a number of other parameters related to vitamin A metabolism; see [47] for more details.

- *Transfer rates* [R(I,J)s; μmol/day] are the rate of transfer of tracee (unlabelled vitamin A) from compartment J to compartment I per unit time and are computed as the product of L(I,J) and M(J), where M(J) is the mass (μmol) of tracee in compartment J. The sum of the M(J)s would estimate the *total traced mass* of vitamin A in the body.
- The *vitamin A disposal rate or utilization rate* is one of the transfer rates and is defined as the rate at which material leaves the system irreversibly (μmol/day).
- The *input rate* or dietary vitamin A absorption rate [U(I); μmol/day] is the amount of new material that enters the system each day. The amount of dietary vitamin A consumed would be the input rate divided by the absorption efficiency.
- The *fractional catabolic rate* is the fraction of the pool of vitamin A in compartment I that is irreversibly utilized each day.
- The *residence time* [T(I,J); day] is the mean of the distribution of times that retinol molecules spend in compartment I from the time of entering the system via compartment J until leaving compartment I irreversibly.
- The *transit time* [t(I); day or hour] is the mean of the distribution of times that retinol molecules entering compartment I spend there during a single transit before leaving reversibly or irreversibly.
- The *recycle number* [υ(I)] is the number of times a retinol molecule recycles through compartment I before irreversibly exiting that compartment.
- The *recycling time* [tt(I); day] is the mean of the distribution of times that retinol molecules leaving compartment I spend in other compartments before recycling to compartment I.

3.5. Estimating vitamin A absorption efficiency

One important difference between most of the vitamin A modelling studies conducted in humans and the experiments done in rats is the route of dose administration. In rats the label has been usually incorporated *in vivo* into its physiological transport complex (i.e. retinol/retinol-binding protein/transthyretin) using donor animals [26]. The retinol-labelled plasma dose is then administered intravenously, bypassing the processes of vitamin A intestinal absorption, chylomicron catabolism, and initial hepatic metabolism. In general, the oral route is more feasible for human studies but, because absorption efficiency (i.e. the true amount of tracer dose that enters the plasma) is unknown, oral administration introduces additional assumptions that must be considered during the modelling process.

When the dose of stable isotope-labelled vitamin A is administered orally, the dose goes through the process of intestinal absorption, similar to that for dietary vitamin A or carotenoids. Surprisingly little quantitative data are available on vitamin A absorption and carotenoid bioefficacy in humans (for review see Blomhoff et al. [48]). In the rat, vitamin A

absorption has been determined to be 76 ± 5 % (mean ± SEM, n=11) in lymph duct-cannulated rats [49]. Because it is not feasible to use this method to measure absorption efficiency in humans, an alternate technique may be to collect feces for several days after the dose is administered and then determine the fraction of the dose that was not absorbed. This approach is complicated by possible problems due to incomplete sample collection and extraction of feces, as well as possible degradation of the tracer dose by bacteria in the lower intestine.

Obtaining reliable estimates of vitamin A absorption in individuals with differing vitamin A status, physiological states (e.g. pregnancy and lactation), ages, and genders, may require new techniques. An experimental approach using two isotope labels has been proposed and tested using a rat model [50]. The dual label technique involves administering a known amount of one label (e.g. tetradeuterated retinyl acetate in oil) as part of a meal to maximize absorption. Approximately 2 hours later, when chylomicron production and secretion is maximal, a known amount of a second label (e.g. octadeuterated retinyl acetate) is administered intravenously in artificial chylomicrons [51, 52]. Absorption efficiency is determined by measuring the ratio of the two isotopes in plasma once the ratio has stabilized. This may require 12 to 15 days in rats but may occur sooner in humans given artificial chylomicrons intravenously. The ratio 'fraction of oral dose in plasma/fraction of intravenous dose in plasma' gives the fraction of the oral dose absorbed (i.e. the intravenous dose represents 100% absorption).

Until studies can be done using an intravenous dose of labelled retinol and/or until more data on vitamin A absorption are available, the problems associated with oral dose administration may be minimized by collecting a sufficient number of plasma samples during the absorptive phase (e.g. 0.5–8 hours after dose administration) so that these data can be incorporated into the model to represent the absorptive phase. Early plasma samples will need to be analyzed for retinyl esters as well as for retinol to discern newly absorbed retinyl esters versus newly secreted retinol: RBP.

3.6. Specific populations and special cases

3.6.1. Aggregated data sets

In many cases (e.g. modelling studies in children), it will not be feasible to obtain enough blood samples from one individual to identify a unique model. A solution to this problem may be to develop a "super-child" model, based on the ideas of Landaw and DiStefano [53] that have been applied to rats [28, 29, 31]. Specifically, several plasma samples are collected from different individuals within a population at specified times and the data modelled as one or more single datasets. For example, 2 blood samples were collected from participating preschool-age children in Peru during the 75 days following administration of [$^2H_4$]-retinol acetate [11]. A sample was collected from all children on day 3. Another sample was collected from each child on 1 of 23 time points over the 75 days. The net result was that samples were available for ~5 children at each time point. If 2 additional samples had been collected from each child, there would have been sufficient data to construct a composite super-child "population" model. Inclusion of a sample from all children on day 3 is useful because the plasma isotope ratio at that time is correlated with predicted liver vitamin A concentrations [11], allowing categorization of children into low, moderate, and high vitamin A status.

3.6.2. Studies in pregnant/lactating women and growing children

Assessing vitamin A status, or modelling whole-body vitamin A metabolism, will be more complicated in pregnant and lactating women as well as in growing infants/children because the subjects may not be in a steady state with respect to vitamin A or plasma volume. Although the SAAM programme can accommodate cases of non-steady state, the analysis requires more sophisticated methods and expertise. In addition, it is likely that whole-body vitamin A utilization will be affected by transfer of the vitamin to the fetus or milk. At present information on how these processes might affect the isotope dilution method does not exist.

3.6.3. Studies in subjects who have used vitamin A supplements for long periods of time

Some vitamin A in individuals with very large vitamin A stores may be contained in non-exchangeable pools. The latter will not be measured by either the isotope dilution technique or by model-based compartmental analysis. This limitation may not be of practical concern because presumably this vitamin A is not involved in whole-body vitamin A kinetics. However, the non-detection may be of physiological importance if the subject is approaching vitamin A toxicity.

3.6.4. Inflammation-induced hyporetinolemia

Retinol-binding protein is a negative acute phase protein (i.e. the concentration of RBP in plasma drops during inflammation), leading to an inflammation-induced hyporetinolemia. It is not known what effect inflammation will have on the plasma tracer:tracee ratio (i.e. the specific activity) on day 3 in stable isotope dilution studies. From what is known about vitamin A metabolism, the vitamin A that enters plasma from hepatocytes will have a lower specific activity than plasma retinol due to dilution by unlabelled dietary vitamin A. During inflammation, the influx of retinol bound to RBP into plasma is reduced. The influx of retinol into plasma from hepatic stellate cells (the cellular site of vitamin A storage) will have a higher specific activity than plasma retinol. This input is also reduced during acute phase inflammation; the reduction is thought to be due to a reduced availability of *apo* RBP that is necessary for secretion of retinol from stellate cells into plasma. Recent work (Gieng, S.H., et al., J. Lipid Res., in press 2007) has shown that the plasma retinol specific activity drops during inflammation. This means the Olson equation will overestimate the total body exchangeable vitamin A pool unless a correction was made based on the day 1 sample.

3.6.5. Non-steady state kinetics of vitamin A metabolism: dose response tests

If dietary vitamin A intake and absorption do not match vitamin A utilization, the system is not in steady state. This situation can also be modelled by appropriate software, such as SAAM and WinSAAM, but it is considerably more complicated. An additional application of non-steady state kinetics is in modelling the dose-response assays for vitamin A status using the relative dose response (RDR) or modified relative dose response (MRDR) tests. Mathematical modelling of these results may lead to refinement in their application and interpretation.

4. FUTURE DIRECTIONS AND CONCLUSIONS

4.1. Interval analysis

In the Olson equation, the coefficients *F*, *s*, and *a* are not constants but unknowns that may be estimated within certain confidence intervals. For example, it would be useful to consider using restrained multiple regression methods to define a confidence interval for liver stores; this would be more realistic than assigning a single value. Because this is not a very well known statistical method, statisticians will need to be consulted and the literature reviewed to learn how to apply constrained multiple regression to the isotope dilution technique.

4.2. Outreach: mathematical modelling workshops

It is likely that the use of stable isotope dilution techniques to determine vitamin A status or the efficacy of interventions in improving vitamin A status will increase in the coming years. As is evident in this report and the others in this series [1, 2], such studies require careful attention to experimental design, subject selection, and analytical methods. In addition, as proposed here, a lot of useful information about vitamin A metabolism may be gained by making relatively small adjustments to the design of some isotope dilution studies so that sufficient data are obtained to apply model-based compartmental analysis. For the latter, researchers will need to be able to analyse their data mathematically. At present, few nutrition scientists are experts in model-based compartmental analysis.

In contrast to the analytical equipment required to analyse biological samples collected in human vitamin A stable isotope studies, the technical support required for mathematical modelling is minimal. The only equipment needed is a computer with adequate processing speed and memory. The SAAM software is freely available for downloading (www.WinSAAM.com), thanks to continued development and support by Ray Boston (University of Pennsylvania School of Veterinary Medicine). It is important to reiterate that, ideally, researchers should decide at the experimental design stage that data will be analysed by model-based compartmental analysis because this influences the sampling schedule, study duration, and subject management.

Interested scientists can learn the tools of model-based compartmental analysis by working with an expert (e.g. during a sabbatical leave). Alternately, it may be expeditious to develop modelling workshops aimed at teaching this technique to selected scientists in developing countries. An intensive 1-week workshop for a small group of researchers with some background in calculus and an interest in mathematical modelling may be feasible. Participants can be introduced to the concepts of model-based compartmental analysis using examples from the published literature and tutorials that have been developed for the SAAM programme. Scientists can bring their own data to use as examples during the workshops. Expert follow-up and more sophisticated applications can be provided by teleconferencing and electronic communications. Note that the use of model-based compartmental analysis is routinely and successfully taught to graduate students and faculty at Pennsylvania State University but a long-term commitment is required. Building on the basics learned in a workshop, self-study can be very useful; for example, it may be possible to obtain a manual of tutorials developed approximately 20 years ago to accompany CONSAM, the predecessor of WinSAAM. However, it is wise to acknowledge at the outset that, unlike many laboratory techniques that can be easily learned, mastering the use of model-based compartmental analysis is more challenging. Nevertheless, the pay-back is well worth the investment, both

for the individual scientist and in terms of the information about vitamin A metabolism that will be gained.

4.3. Concluding comments

Although biological scientists may not always feel comfortable applying the techniques of mathematical modelling to their work, it is clear that in the vitamin A field, such methods hold promise for improving the assessment of vitamin A status, evaluating vitamin A intervention programmes, and learning more about the mechanisms that regulate whole-body vitamin A metabolism. Much useful information has been gained by applying the stable isotope dilution method in human populations. Future work needs to tackle the underlying assumptions in the Olson equation, the vagaries of vitamin A absorption, application in various populations, and the potential usefulness of a shortened study protocol. Furthermore, researchers can consider expanding the design of some isotope dilution studies to collect enough data to develop whole-body models of vitamin A metabolism using model-based compartmental analysis. All of these approaches will provide useful information that may help to design more effective strategies for alleviating vitamin A deficiency worldwide.

APPENDIX
EXAMPLE CALCULATION

Example of estimation of total body vitamin A pool size using the "Olson equation"

Olson equation:

Total body exchangeable vitamin A pool = *F dose* [*S a*{(1/*D:H*)-1}]

(1) *F* is a factor for efficiency of absorption and storage of the orally administered dose

(2) *Dose* is the amount of isotopically labelled vitamin A administered orally (μmol)

(3) *S* is a factor that corrects for the inequality of the plasma to liver ratio of labelled to non-labelled retinol

(4) *a* is a factor that corrects for irreversible loss of labelled vitamin A during the mixing period; specifically, $a = e^{-kt}$, where *k* is the estimated system fractional catabolic rate, and *t* is time, expressed as days since dose

(5) *D:H* is the plasma isotopic ratio of labelled to non-labelled retinol

(6) -1 corrects for the contribution of the dose of labelled vitamin A to the total body vitamin A pool

Assumed values for factors F, S, and a:

$F = 0.50$
$S = 0.65$
$a = e^{-kt}$, where $k = 0.005$, t = days since dose

The example below is for an adult subject who received an oral dose of 17.2 μmol of [$^2H_4$] vitamin A acetate (5.7 mg) ([$^2H_4$]-vitamin A = 5000 μg retinol activity equivalents); the subject's plasma isotopic ratio of labelled to non-labelled retinol was 0.050, 20 days after dosing:

Total body exchangeable vitamin A pool = $0.50 \times 17.2\ \mu mol \times [0.65 \times e^{(-0.005*20)} \times \{(1/0.05)-1\}]$

$= 8.6 \quad [0.65 \times e^{(-0.1000)} \times \{(20)-1\}]$

$= 8.6 \quad \times [0.65 \times 0.9048 \times 19]$

$= 8.6 \quad \times [11.17]$

= 96.1 μmol or 0.0961 mmol or 27.5 mg vitamin A

molecular weight of [$^2H_4$]-retinyl acetate = 332
molecular weight of [$^2H_4$]-retinol = 290
molecular weight of unlabelled retinol = 286
retinol activity equivalent = 1 μg retinol

REFERENCES

[1] VITAMIN A TRACER TASK FORCE, Appropriate Use of Vitamin A Tracer (Stable Isotope) Methodology, USAID and ILSI, (Eds.), (2004).

[2] HASKELL, M.J., et al., Handbook on vitamin A tracer dilution methods to assess status and evaluate intervention programmes, HarvestPlus Technical Monograph 5, Washington DC, USA, (2005).

[3] FURR, H.C., et al., Vitamin A concentrations in liver determined by isotope dilution assay with tetradeuterated vitamin A and by biopsy in generally health adult humans, Am. J. Clin. Nutr. **49** (1989) 713-716.

[4] HASKELL, M.J., et al., Assessment of vitamin A status by the deuterated-retinol-dilution technique and comparison with hepatic vitamin A concentration in Bangladeshi surgical patients, Am. J. Clin. Nutr. **66** (1997) 67-74.

[5] HASKELL, M.J., et al., Plasma kinetics of an oral dose of [$^2H_4$]retinyl acetate in human subjects with estimated low or high total body stores of vitamin A, Am. J. Clin. Nutr. **68** (1998) 90-95.

[6] HASKELL, M.J., et al., Use of the deuterated-retinol-dilution technique to assess total-body vitamin A stores of adult volunteers consuming different amounts of vitamin A, Am. J. Clin. Nutr. **70** (1999) 874-880.

[7] RIBAYA-MERCADO, J.D., et al., Assessment of total body stores of vitamin A in Guatemalan elderly by the deuterated-retinol-dilution method, Am. J. Clin. Nutr. **69** (1999) 278-284.

[8] TANG, G., et al., Green and yellow vegetables can maintain body stores of vitamin A in Chinese children, Am. J. Clin. Nutr. **70** (1999) 1069-1076.

[9] RIBAYA-MERCADO, J.D., et al., Bioconversion of plant caratenoids to vitaimn A in Filipino school-aged children varies inversely with vitamin A status, Am. J. Clin. Nutr. **72** (2000) 455-465.

[10] TANG, G., et al., Use of a short-term isotope-dilution method for determining the vitamin A status of children, Am. J. Clin. Nutr. **76** (2002) 413-418.

[11] HASKELL, M.J., et al., Population-based plasma kinetics of an oral dose of [$^2H_4$]retinyl acetate among preschool-aged Peruvian children, Am. J. Clin. Nutr. **77** (2003) 681-686.

[12] RIBAYA-MERCADO, J.D., et al., Quantitative assessment of total body stores of vitamin A in adults with the use of a 3-d deuterated-retinol-dilution procedure, Am. J. Clin. Nutr. **77** (2003) 694-699.

[13] RIBAYA-MERCADO, J.D., SOLOMONS, N.W., Deuterated-retinol-dilution approach for longitudinal monitoring of health and nutritional interventions involving vitamin A, Sight Life Newsletter **2** (2003) 3-4.

[14] HASKELL, M.J., et al., Daily consumption of Indian spinach (*Basella alba*) or sweet potatoes has a positive impact on total body vitamin A stores of Bangladeshi men, Am. J. Clin. Nutr. **80** (2004) 705-714.

[15] RIBAYA-MERCADO, J.D., et al., Dietary vitamin A intakes of Filipino elders with adequate or low liver vitamin A concentrations as assessed by the deuterated-retinol-dilution method: implications for dietary requirements, Am. J. Clin. Nutr. **79** (2004) 633-641.

[16] RIBAYA-MERCADO, J.D., et al., Use of the deuterated-retinol-dilution technique to monitor vitamin A status of Nicaraguan schoolchildren 1 y after initiation of the Nicaraguan national programme of sugar fortification with vitamin A, Am. J. Clin. Nutr. **80** (2004) 1291-1298.

[17] GREEN, M.H., GREEN, J.B., The application of compartmental analysis to research in nutrition, Ann. Rev. Nutr. **10** (1990) 41-61.

[18] WASTNEY, M.E., et al., Investigating Biological Systems Using Modeling, San Diego: Academic Press, (1999).
[19] BAUSCH, J., REITZ, P., Method for the assessment of vitamin A liver stores, Acta Vitaminol. Enzymol. **31** (1977) 99-112.
[20] HICKS, V.A., GUNNING, D.B., OLSON, J.A., Metabolism, plasma transport and biliary excretion of radioactive vitamin A and its metabolites as a function of liver reserves of vitamin A in the rat, J. Nutr. **114** (1984) 1327-1333.
[21] SAUBERLICH, H.E., et al., Vitamin A metabolism and requirements in the human studied with the use of labelled retinol, Vitam. Horm. **32** (1974) 251-275.
[22] GREEN, M.H., GREEN, J.B., Vitamin A intake and status influence retinol balance, utilization and dynamics in rats, J. Nutr. **124** (1994) 2477-2485.
[23] ADAMS, W.R., GREEN, M.H., Prediction of liver vitamin A in rats by an oral isotope dilution technique, J. Nutr. **124** (1994) 1265-1270.
[24] GREEN, M.H., GREEN, J.B., Quantitative and conceptual contributions of mathematical modelling to current views on vitamin A metabolism, biochemistry, and nutrition, Adv. Food Nutr. Res. **40** (1996) 3-24.
[25] WAKE, K., Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs, Int. Rev. Cytol. **66** (1980) 303-353.
[26] GREEN, M.H., GREEN, J.B., Experimental and kinetic methods for studying vitamin A dynamics in vivo, Meth. Enzymol. **190** (1990) 304-317.
[27] GREEN, M.H., Introduction to modeling, J. Nutr. **122** (1992) 690-694.
[28] GREEN, M.H., UHL, L., GREEN, J.B., A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores, J. Lipid Res. **26** (1985) 806-818.
[29] LEWIS, K.C., et al., Retinol metabolism in rats with low vitamin A status: a compartmental model, J. Lipid Res. **31** (1990) 1535-1548.
[30] GREEN, M.H., GREEN, J.B., LEWIS, K.C., Model-based compartmental analysis of retinol kinetics in organs of rats at different levels of vitamin A status, in LIVREA, M., PACKER, L., (Eds.), Retinoids: New Trends in Research and Clinical Applications, New York: Marcel Dekker (1993) 185-204.
[31] CIFELLI, C.J., GREEN, J.B., GREEN, M.H., Dietary retinoic acid alters vitamin A kinetics in both the whole body and in specific organs of rats with low vitamin A status, J. Nutr. **135** (2005) 746-752.
[32] KELLEY, S.K., et al., Use of model-based compartmental analysis to study effects of 2, 3, 7, 8-tetrachlorodibenzo p dioxin on vitamin A kinetics in rats, Toxicol. Sci. **44** (1998) 1-13.
[33] JANG, J.-T., et al., Kinetic analysis shows that iron deficiency decreases liver vitamin A mobilization in rats, J. Nutr. **130** (2000) 1291-1296.
[34] GREEN, M.H., GREEN, J.B., Dynamics and control of plasma retinol, in: BLOMHOFF, R., (Ed.), Vitamin A in Health and Disease, New York: Marcel Dekker (1994) 119-133.
[35] VON REINERSDORFF, D., GREEN, M.H., GREEN, J.B., Development of a compartmental model describing the dynamics of vitamin A metabolism in men, Adv. Exp. Bio. Med. **445** (1998) 207-224.
[36] NOVOTNY, J.A., et al., Compartmental analysis of the dynamics of Beta-carotene metabolism in an adult volunteer, J. Lipid Res. **36** (1995) 1825-1838.
[37] NOVOTNY, J.A., et al., Mathematical modeling in nutrition: constructing a physiologic compartmental model of the dynamics of Beta-carotene metabolism, Adv. Food Nutr. Res. **40** (1996) 25-54.

[38] SWANSON, J.E., et al., Experimental approaches to the study of Beta-carotene metabolism: potential of a ^{13}C tracer approach to modeling Beta-carotene kinetics in humans, Adv. Food Nutr. Res. **40** (1996) 55-76.
[39] TANG, G., et al., Short term (intestinal) and long term (postintestinal) conversion of Beta-carotene to retinol in adults as assessed by a stable-isotope reference method, Am. J. Clin. Nutr. **78** (2003) 259-266.
[40] WANG, Z., et al., Beta-Carotene-vitamin A equivalence in Chinese adults assessed by an isotope dilution technique, Brit. J. Nutr. **91** (2004) 121-123.
[41] GREEN, M.H., GREEN, J.B., LEWIS, K.C., Variation in retinol utilization rate with vitamin A status in the rat, Nutr. **117** (1987) 694-703.
[42] UNDERWOOD, B.A., et al., Liver stores of vitamin A in a normal population dying suddenly or rapidly from unnatural causes in New York City, Am. J. Clin. Nutr. **23** (1970) 1037-1042.
[43] FURR, H.C., et al., Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans, Public Health Nutr. **8** (2005) 596-607.
[44] DISTEFANO, J. III., Optimized blood sampling protocols and sequential design of kinetic experiments, Am. J. Physiol. **240** (1981) R259-R265.
[45] BERMAN, M., WEISS, M.F., SAAM Manual, DHEW Publ. (NIH) 78-180. Washington D.C.: U.S. Government Printing Office, Washington D.C., USA, (1978).
[46] NOVOTNY, J.A., GREEN, M.H., BOSTON, R.C., (Eds.), Mathematical Modelling in Nutrition and the Health Sciences, New York: Kluwer Academic/Plenum Publishers (2003).
[47] KELLEY, S.K., GREEN, M.H., Plasma retinol is a major determinant of vitamin A utilization in rats, J. Nutr. **128** (1998) 1767-1773.
[48] BLOMHOFF, R., et al., Vitamin A metabolism: new perspectives on absorption, transport and storage, Physiol. Rev. **71** (1991) 951-990.
[49] ALLEN L.E., GREEN M.H., GREEN, J.B., Correspondence re: DEW, S.E., et al., Effects of pharmacological retinoids on several vitamin A-metabolizing enzymes, Cancer Res. **54** (1994) 3319-3320.
[50] GREEN, M.H., GREEN, J.B., Use of a plasma dual isotope ratio method to measure vitamin A absorption, FASEB J. **11** (1997) A142 (abs).
[51] REDGRAVE, T.G., et al., Clearance from plasma of triacylglycerol and cholesteryl ester after intravenous injection of chylomicron-like lipid emulsions in rats and man, Biochem. J. **290** (1993) 843-847.
[52] REDGRAVE, T.G., et al., Chylomicron remnant metabolism in familial dyslipidemias studied with a remnant-like emulsion breath test, J. Lipid Res. **42** (2001) 710-715.
[53] LANDAW, E., DISTEFANO, J. III., Multiexponential, multicompartmental, and noncompartmental modelling. II. Data analysis and statistical considerations, Am. J. Physiol. **246** (1984) R665-R677.

GLOSSARY

Compartmental analysis — a form of mathematical modelling that has been fruitfully applied to describing and quantifying biological systems. In model-based compartmental analysis, the system (e.g. whole-body vitamin A metabolism) is lumped into kinetically-distinct compartments which may or may not have known physiological correspondence. Following an *in vivo* kinetic study, the data are compared to the postulated model; adjustments are made in the model and the kinetic parameters (fractional transfer coefficients) to obtain a good fit. Using the fractional transfer coefficients, transfer rates (including disposal rate), compartment masses and several time parameters can be estimated.

Fractional catabolic rate [FCR(I,J)] — the fraction of the mass in compartment I that irreversibly leaves compartment I per unit time after entering the system via compartment J.

Fractional transfer coefficient [(L(I,J)] — The fraction of compartment J's tracer or mass transferred to compartment I each day. L(I,J)s are the values assigned to the vectors (arrows) entering and leaving compartments in the model.

Interval analysis — a sophisticated form of statistic analysis in which the βs in a regression model are expressed as an interval instead of a fixed value. The solution then provides a confidence interval for the variable (e.g. vitamin A stores).

Isotope dilution — a principle used to estimate the total body pool of vitamin A. To apply isotope dilution to estimate the vitamin A pool size, a known amount of stable isotope-labelled vitamin A is administered orally to experimental animals or human subjects. Over time (about 20 days in adult humans), the isotope mixes with the endogenous vitamin A pool; the ratio of labelled to non-labelled vitamin A in plasma reflects the dilution of the dose and thus can be used to estimate the total exchangeable pool of vitamin A.

Isotope ratio — in the context of stable isotope dilution methods for estimating the body vitamin A pool, this is the ratio of labelled retinol to non-labelled retinol in plasma after administration of a stable isotope of vitamin A. The isotope ratio is determined by mass spectrometry.

Kinetics — in biology, the temporal and spatial interrelationships for a particular compound in a system. For tracer experiments, the term describes the movement of tracer in the system.

Olson equation — an equation developed by James Olson and colleagues [3] to estimate total body vitamin A pool size following oral administration of stable isotope-labelled vitamin A:

$$\text{Total body exchangeable vitamin A pool (mmol)} = F\ dose\ [S\ a\{(1/D{:}H)-1\}]$$

where F is a factor related to the efficiency of absorption and storage of the orally administered dose and is assumed to be 0.5 (i.e. 50% of the oral dose is stored in the liver at the time of plasma sampling); *dose* is the amount of isotope administered (mmol); the factor S corrects for the inequality of the plasma to liver ratio of labelled to non-labelled retinol and is assumed to be 0.65 based on work in rats [20]; the factor a corrects for irreversible loss of labelled vitamin A during the equilibration period and is based on the half-life of vitamin A turnover (140 days in adults [21]; ($a = e^{-kt}$, where k = ln 2/140 days and t = time in days since dose administration); $D{:}H$ is the isotopic ratio of labelled (here, deuterated) to non-labelled

retinol in plasma; and *-1* corrects for the contribution of the dose to the total liver vitamin A reserve.

Residence time [T(I,J)] — the average of the distribution of times that a molecule of retinol spends in compartment I before irreversibly leaving that compartment after entering the system via compartment J. In other words, residence time is equivalent to the total time a tracee molecule will spend in a given compartment.

SAAM — The *S*imulation, *A*nalysis *a*nd *M*odeling software that has been continuously developed since the 1960s to apply the methods of model-based compartmental analysis to describe and quantitate the kinetic behavior of entities in biological systems of interest. The programme fits tracer and tracee data collected in an *in vivo* or *in vitro* kinetic study to a postulated compartmental model and it uses weighted nonlinear regression analysis to estimate the kinetic parameters that describe the model.

Stable isotope — a non-radioactive form of a compound that is useful as a "tracer" when administered in experimental studies. In the case of vitamin A, stable isotopic forms of both carbon and hydrogen have been fruitfully used: for ^{13}C-labelled vitamin A compounds, 2 to 10 carbons have been labelled; for ^{2}H, 4 to 8 hydrogens in the molecule may be labelled.

Steady state / non-steady state — descriptors for the system under study (e.g. whole-body vitamin A). In the steady state, whole body vitamin A is in balance (input = output); in the non-steady state, something is changing during the timeframe of the study (e.g. liver vitamin A might be decreasing or increasing over time).

Tracer/tracee — in biology, an ideal tracer is a radioactive or stable isotopic form of the compound of interest (the tracee or traced substance) which is easily detectable, follows the same kinetics as the tracee, and does not perturb the mass or kinetics of the system.

Transfer rate [R(I,J)] — the amount of vitamin A (nmol) transferred from compartment J to compartment I each day. R(I,J)s are calculated as the product of L(I,J) and the appropriate compartment mass [M(J)].

Transit time [t(I)] — the mean of the distribution of times that molecules entering compartment I spend there during a single transit before leaving reversibly or irreversibly. Transit time is the same as turnover time and is the inverse of the sum of the fractional transfer coefficients leaving compartment I [t(I) = 1 / L(I,I)].

CONTRIBUTORS TO DRAFTING AND REVIEW

Furr, H.C.	Craft Technologies, United States of America
Green, J.B.	Department of Nutritional Sciences, Pennsylvania State University, United States of America
Green, M.H.	Department of Nutritional Sciences, Pennsylvania State University, United States of America
Haskell, M.	Department of Nutrition, University of California-Davis, United States of America
Mokhtar, N.	International Atomic Energy Agency
Nestel, P.	Institute of Human Nutrition, University of Southampton, United Kingdom
Ribaya-Mercado, J.	The Jean Mayer USDA Human Nutrition Research Center, Tufts University, United States of America
Tanumihardjo, S.	Department of Nutritional Sciences, University of Wisconsin-Madison, United States of America
Wasantwisut, E.	Institute of Nutrition, Mahidol University, Thailand
Blaner, W.S.	Columbia University, United States of America
Thurnham, D.	University of Ulster, United Kingdom

Consultants Meetings
2–3 February 2004, Washington D.C., United States of America
2–3 September 2004,Vienna, Austria