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Diet in Vitamin A Research

A. Catharine Ross^{*,1}

¹Department of Nutritional Sciences and Huck Institute for the Life Sciences, Pennsylvania State University

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Introduction

Diet provides all vertebrates with the macronutrients needed for energy production and tissue anabolism, with minerals, such as calcium and phosphorus, that serve a structural role, and with numerous micronutrients that play an essential role as cofactors in metabolism and as regulators of metabolic functions. Thus, a properly formulated diet is essential for practically all in vivo research. In the early 1900s, McCollum and Davis and Osborne and Mendel demonstrated the nutritional requirement for “fat-soluble A” and deduced several of the vitamin’s most important effects. It is now well established that all vertebrates require vitamin A for adequate growth, cell and tissue differentiation, vision, development and function of the immune system, and survival. Although the amount of vitamin A (retinol) required for these functions, in the range of $\mu\text{g/day}$ (1), is a trace component of the diet, its biological impact is wide ranging.

This chapter focuses on the practical use of diet in retinoid research from two perspectives: human diets, and animal (rodent) diets. In human research on vitamin A, vitamin A intake is seldom controlled, although this has been done in a few studies. However, the assessment of vitamin A intake is important in population-based and epidemiological research. Humans consume a variety of foods and thus obtain their vitamin A in multiple forms--preformed retinol and provitamin A carotenoids-- and the variable consumption of different foods contributes significantly to the problem of analyzing how much vitamin A has been consumed. In contrast, for animal studies diet is a controllable factor and the diet is usually the same over time. The amount of vitamin A present in the diet establishes the animal’s vitamin A status, which, in turn, can determine outcomes, such as levels of gene expression and rates of metabolic processes. However, many standard animal diets are nonpurified and the vitamin A content can be variable. It is hoped that a discussion of diets in human and animal research will assist investigators interested in the molecular biology of retinoids in making the best decisions regarding the design of their future human and animal research.

Human studies -- controlling and assessing the diet

Controlling the diet

In human studies, the intake of vitamin A has only rarely been strictly controlled, but some recent studies provide insight into the planning and care required. In a study of adult, male Bangladeshi volunteers who initially had low vitamin A status (plasma retinol of 0.51 to

Address correspondence to: A. Catharine Ross, Ph.D., Department of Nutritional Sciences, Pennsylvania State University, University Park, PA 16802, Tel.: +01 814 865 4721; fax: +01 814 865 4723, acr6@psu.edu.

1.22 $\mu\text{mol/L}$ at the beginning of the study), Haskell et al. (2) fed diets low in vitamin A, to which were added graded supplements of retinol to produce differences in the total body vitamin A pool size. All meals were provided at the study's research center for a period of 129 days. The basal diet consisted primarily of rice and lentils with small amounts of curried meats (mutton, chicken, and fish), vegetables (cabbage, cauliflower, white squash, and white potato), and fruit (banana), all with low vitamin A content. This basal diet was estimated to provide the equivalent of 0.94 μmol retinol/d (270 μg RE (RE=retinol equivalents)/d. In another stable isotope dilution study that was conducted in the U.S. to evaluate intestinal and postintestinal β -carotene conversion, Tang et al. (3) fed controlled diets to older men and women while subjects resided in their research facility for the first 10 days of the study. For days 11–57 of the study the subjects returned to their homes. They were provided with instructions from the study dietitian to consume only fruits and vegetables from a list low carotene foods, and were counseled to abstain from multivitamins, minerals, nutritional supplements, fortified cereals, and fish liver oil. Recently, Ahmad et al. (4) conducted a controlled feeding study in 36 healthy Bangladeshi men (20–30 y of age), designed to assess whether total body vitamin A pool size, determined by a deuterium-labeled retinol dilution technique, predicted immune response to immunization. This study used a 2-month residential period in which a low-vitamin A basal diet was fed, modeled on the traditional Bangladeshi diet that provided the equivalent of 40 μg retinol/d. These studies illustrate the logistical details required to carefully control vitamin A intake in humans.

Assessing the diet

By far the majority of human studies have relied on methods for assessing vitamin A or carotenoid intake in community-dwelling subjects. Subjects have either consumed their usual diet without any control, or they have been instructed to follow dietary advice in terms of avoiding, limiting, or consuming certain foods. A number of epidemiological investigations have investigated the intake of dietary vitamin A intake, either as preformed retinol or provitamin A carotenoids, or both, in studies of cross-sectional, case-control, or prospective designs (5–8),

Two main approaches to the collection of dietary information have been developed: *i*) dietary recordings followed by nutrient analysis, or recalls; and *ii*) questionnaires, often focused on the frequency of intake of certain foods high in vitamin A or carotenoids. Either assessment method is complicated by the multiple forms of vitamin A present in the foods in most diets. Preformed vitamin A is found as retinol or retinyl esters in foods of animal origin--typically, milk, cheese, and liver, but also including preformed vitamin A present in some nutritional supplements and fortified foods such as enriched breakfast cereals). Provitamin A carotenoids are present in numerous vegetables, being highest in green leafy and yellow vegetables, and certain fruits, such as mangoes and oranges. To assess total vitamin A intake, retinol and carotenoids must be calculated separately (1), followed by conversion to a basic unit.

Units—The units used to describe vitamin A have changed over time and can be confusing. Nutritional units currently in use include the International Unit (IU), the Retinol Equivalent (RE), and for human diets only, the Retinol Activity Equivalent (RAE) (1). One IU (also equal to 1 USP unit) is equivalent to 0.3 μg all-trans-retinol (molecular weight 286.6), 0.55 μg retinyl palmitate (molecular weight 525), and 0.6 μg of beta-carotene. The current unit for human research, established by the Institute of Medicine (IOM) in 2001, is the Retinol Activity Equivalent (RAE). The nutritional equivalency among retinol, β -carotene in supplements (e.g., in oily solution from which it is readily absorbed), β -carotene in foods (embedded in food matrix and therefore less bioavailable), and provitamin A carotenoids other than β -carotene (α -carotene or β -cryptoxanthin in foods) is listed in Table 1. The new

RAE unit was established because research on carotenoids bioavailability in human subjects had established that carotenoids in foods are converted into retinol less efficiently than was previously thought (1). In analyzing dietary data and comparing study results, careful attention must be given to the form of vitamin A in foods consumed as well as the units in which vitamin A is reported in tables of food composition.

Methods of Assessment

In the recording method, subjects are required to keep a food intake diary (essentially a log in which the subject records the types of foods and amounts consumed) for a specified period of time, which may be as little as a day or much longer. Food records may be based on real-time recordings, meal by meal, or on a retrospective recall of food consumption during a stated period of time. Recalls may be conducted as interviews, by telephone, or in person, or the subjects themselves may record their intake after the fact. Obtaining accurate information on the amount of the foods consumed is very problematic, as people are seldom aware of the weight or volume of the foods they consume, and often confuse the standard serving size with their usual “helping size” which may be larger. Diet records and recalls can be improved by the use of measuring cups, scoops, food models or photographs to illustrate portion size. Mixed dishes are also problematic, as the recipe must essentially be deconstructed and analyzed to obtain good estimates of the item’s nutrient contents. All together, recording and recall methods are more difficult than might be assumed. Strengths and weaknesses of dietary recall methods have been reviewed (9, 10). Factors contributing to the imprecision of dietary records or recalls include: a tendency for subjects to forget or underreport the foods they have consumed; variations in nutrient intake over time that may not be recalled in the recording period; and limitations in the food composition databases that must be used to translate food consumption into nutrient intake. For vitamin A, certain factors affect the quality of food records. It is known that vitamin A intake in humans has wider day to day variations than for some other nutrients; thus many days of food records were needed to attain a good correlation between FFQ data and amounts of usual vitamin A intake (10).

A second approach to estimating nutrient intakes is to assess how often certain foods are consumed using a food frequency questionnaire, FFQ. Commonly used FFQs have been designed to assess diet and disease risk in large surveys and in multi-ethnic studies (10). Information from FFQs may be used to establish qualitative trends, such as dietary patterns (11), or they may be used in a quantitative manner by approximating not only how often certain foods were consumed but also approximately how much was consumed. Inquires are made about the frequency of intake (daily, weekly, etc.) and are generally focused on those types of foods that are likely to contribute a substantial amount to the total intake of the particular nutrient of interest. In the case of vitamin A, FFQ queries should focus on the intake of green leafy and yellow vegetables, tomatoes and tomato-based products, oranges and orange juice, mangoes, eggs, milk (and whether the milk is vitamin A enriched), certain fortified breakfast cereals that may contain vitamin A, and fish, meat, and especially liver. Some FFQs have been simplified to include a shorter list of foods or food groups richest in the nutrient of interest, with the information gained being less precise than from a more detailed or quantitative FFQ.

Supplement use as a factor in dietary vitamin A intake

Vitamin-mineral supplement use is an important component of vitamin A intake in developed countries. Dietary intake and vitamin-mineral supplement use were determined in the Hawaii-Los Angeles Multi-Ethnic Cohort study, which includes 215,823 adults who were aged 45 y at baseline in 1993–1996. Murphy et al. (12) concluded that 48% of men and 56% of women reported using a multivitamin supplement at least once weekly for the past

year. For vitamin A, the percentage of the population with an adequate intake (comparable to the RDA), increased by 16% for men and 14% for women when supplements were included along with intake from foods, while the prevalence of vitamin A intakes greater than the Upper Level (UL, 3000 μg of retinol/day for adults) was 15.6% in men and 15.7% in women. An analysis of data from the 2002 Feeding Infants and Toddlers Study (13) also showed 30% higher vitamin A intakes in supplement users compared to nonusers. Thus, supplement use needs to be factored in to accurately assess vitamin A intake in human studies (9).

Potential uses of dietary information in molecular research

In general, dietary assessment involving vitamin A has not yet received much attention in molecular biological research. However, for other nutrients, such as iron and folate acid, interactions of diet and genotype are now well documented (14–16). As genetic factors modifying vitamin A metabolism are identified, research may turn to determining the interactions of genotype and dietary vitamin A. Interestingly, Berson (17) has noted that nutritional approaches have been effective in treating certain diseases of the retina, for example, the night blindness associated with Sorsby fundus dystrophy can be reversed over the short term with vitamin A, and has concluded that “risk-factor analyses of well-defined populations followed over time with food frequency questionnaires in conjunction with careful assessments of visual function may reveal other dietary constituents that can modify the course of degenerative diseases of the retina.” The proven benefit of antioxidant supplementation, including carotenoids, for age-related macular degeneration in the Age-Related Eye Disease Study (AREDS) also suggests that the interaction of diet or supplementation and genetic risk factors should be examined more closely (18).

Diets for research in animals

In small animals used for research, diet can be used to create conditions that cannot be studied in humans. For vitamin A, this includes studies of vitamin A status ranging from deficiency to toxicity, developmental studies, physiological studies of nutrient metabolism in genetic models (expressing transgenes or null deletions), pharmacological studies with natural and synthetic retinoids, and a variety of studies related to preclinical testing.

The diets fed to most rodents housed in research facilities are nonpurified diets. In contrast, most research diets are, or should be, purified. The characteristics of these types of diets will be discussed first, and then some recommendations will be proposed for diets appropriate for different types of animal studies. It should be noted that diets and nutrient recommendations for animals are almost always expressed in amounts (mass) per weight of diet (e.g., g/kg or g %), not in an amount per day as for humans.

Nonpurified diets

The primary ingredients in nonpurified diets come from natural sources. Most nonpurified diets are comprised of a mixture of grains (corn, wheat, barley, sorghum, alfalfa, soybean meal, as examples) and other products (animal or vegetable fats). Feed manufacturers offer a range of such diets, formulated for the growth and reproductive needs of particular species. These diets are suitable for feeding production animals and companion animals as a regular feeds, and are the typical diet of research animals unless special diets are indicated. Nonpurified diets are formulated to provide at least a minimal amount of all essential nutrients—protein, fat, fiber, and vitamins and minerals—which must fall within certain ranges. They are sometimes classified as open or closed formula. For open formula diets, the composition is made available to the potential user and the diet must be formulated as

specified. For closed formula diets, although specified tolerances must be maintained the exact composition of the diet is known only to the manufacturer (19).

For research requiring standardization of vitamin A, grain-based diets are not sufficiently uniform over time, nor is the actual vitamin A content of the diet known, other than that it meets a minimal standard. The amount of carotenoids present in grains such as corn and grasses such as alfalfa can vary by strain, seasonal, or geographical origin. Rodents are generally very efficient at converting these carotenoids to retinol within the intestine and thus variations in the amount of provitamin A in the diet can be expected to lead to differences in the vitamin A content of the animal's vitamin A-storing tissues. Manufacturers may add vitamin A as retinol to nonpurified diets to meet a certain level. A chapter on "Label Review" of the Feed Inspector's Manual, Association of American Feed Control Officials Inspection and Sampling Committee (20) refers to feed labeling for vitamin A as: "Vitamin A, other than precursors of vitamin A, in International Units per pound." This implies that precursors of vitamin A (carotenoids) in the feed are not counted on the product label. In addition, manufacturing allowances can also affect vitamin A content. Manufacturers are allowed to add more than the stated amount of certain nutrients, to compensate for "shelf life" (possible loss or deterioration prior to consumption). However, if the diet is carefully stored and is used quickly, the amount of the nutrient is unlikely to have decayed substantially and the amount the animals ingest could be higher than the amount calculated from the product label and the quantity of diet consumed. Manufacturers may also increase the level of micronutrients in diets designed to be autoclaved. A summary of 13 studies on the retention of vitamins after steam autoclaving reported a range of 23–95% retention for vitamin A, with >80% retention in 8 of the studies (21). Thus, the amount lost during autoclaving does not appear to be high. Diets used in transgenic mouse facilities are likely to be of the autoclavable type (22), and thus animals housed in such facilities may be ingesting even higher amounts of vitamin A (and other micronutrients) from nonpurified diets labeled "autoclavable" than even from regular nonpurified diets, which are already high in vitamin A. Overall, the types and amounts of vitamin A present in nonpurified diets are essentially unknown and the use of such diets in research, except for general maintenance of rodent colonies, should be discouraged.

Purified diets

Purified diets, also known as semisynthetic diets, are made of refined ingredients, including isolated proteins, refined sugars and oils, and purified sources of vitamins and minerals. They are formulated to minimize nutrient variability. Purified, fixed-formula diets are typically prepared with a particular type of protein, casein or whey protein isolated from milk, soy protein or another protein, a particular oil or fat (corn oil, soybean oil, canola oil, etc., or a known blend), certain carbohydrates (dextrose (glucose), maltose, sucrose, corn starch, or another carbohydrate, and cellulose), and all of the essential vitamins and minerals added in purified form and in exact amounts. Such diets are meant to be stored carefully (e.g., refrigerated or frozen in closed bags or bins to prevent oxidation and light exposure) and used soon after purchase; the amounts of the nutrients they contain should be exactly those specified on the formula sheet.

AIN-76 and AIN-93 diets as classical purified diets

Since the 1970s, the American Institute for Nutrition (AIN), now the American Society for Nutrition, has sponsored the testing of purified diets for rodent research. The AIN-76 diet (23), and the slightly modified AIN-76A diet (24), were used extensively by nutritional scientists and others, around the world for two decades. Having a common reproducible diet greatly aided comparisons among studies. Wise (25) has discussed several practical issues in

preparing this diet, including the order of addition of ingredients, which should be understood by researchers planning to prepare animal diets in their laboratory.

Two new formulations were published in 1993, based on new science and testing in rats for growth and reproduction. AIN-93G (G for growth) was designed for feeding to young animals during rapid growth, and for pregnancy and lactation), and AIN-93M (M for maintenance), for which the optimal protein intake is lower, to mature animals. As described by Reeves (26), the criteria used for the AIN-93 formulations were *i*) the diets can be made from purified ingredients; *ii*) they conform to or exceed the nutrient requirements suggested by the NRC, 1978 and 1995 (27, 28)); *iii*) they can be made from readily available components at a reasonable cost; *iv*) the compositions are consistent and reproducible, and *v*) the diets can be used over a wide range of applications. Some major differences were made in the formulation of AIN-93G diet, compared with AIN-76A diet, to increase the amount of the essential n-3 fatty acid, linolenic acid; substitute cornstarch for sucrose to reduce dental caries; reduce the amount of phosphorus to help eliminate the problem of kidney calcification in female rats, which had become apparent with AIN-76; substitute L-cystine for DL-methionine as the amino acid supplement for casein, known to be deficient in the sulfur amino acids; lower the manganese concentration; increase the amounts of vitamin E, vitamin K and vitamin B-12; and add the trace minerals molybdenum, silicon, fluoride, nickel, boron, lithium and vanadium to the mineral mix. For the AIN-93M maintenance diet, the amount of fat is reduced to 40 g/kg (4% from 7% in the AIN-93G formula), and the amount of casein to 140 g/kg from 200 g/kg in the AIN-93G diet, because a lower protein diet was beneficial for maintenance. The energy distribution and ingredients in the AIN-93G diet are listed in Table 2. The AIN-93 diets contain 4,000 IU (1,200 µg) retinol/kg of diet, with retinol added to the vitamin mix in the form of gelatin-stabilized retinyl palmitate for greater diet stability.

Certain purified diets cannot be prepared in solid form. A commonly used liquid diet used in research on alcohol, and vitamin A-alcohol interactions, is the Lieber-DeCarli diet containing ethanol, 1 kcal/g (36% of kcal) of liquid diet (29). The ingredients for this diet can be purchased as a solid mix, minus ethanol, and blended in the laboratory to contain 36% of calories (or a modified level) from ethanol. The vitamin A content is 6,000 IU/L (1800 µg/L). Given that the AIN-93 solid diet has an energy density of ~4 kcal/g and contains 1.2 µg retinol/g, equal to 0.3 µg retinol/kcal, the Lieber-DeCarli diet with 1.8 µg retinol/kcal provides more vitamin A per calorie.

Custom modifications of dietary vitamin A to control vitamin A status

Custom diets are purified diets tailored to the needs of the users. Vitamin A-deficient diets and diets with different, specified levels of vitamin A fall into this category. In the 1980s, our laboratory began studies of vitamin A depletion and repletion, and later of a range of vitamin A status, using diets first based on AIN-76 and later on AIN-93G, with AIN-93M used for mature animals in a long-term study of aging (30–32). Table 2 provides a summary of the AIN-93G diet and its modification to obtain 5 different “levels” of vitamin A status in rats, ranging from “low marginal” to “supplemented.” We increased the concentration of vitamin A in the Adequate diet to 4 µg/g (from 1.2 in the AIN-93 formula) after finding that plasma retinol and liver retinyl esters were relatively low if the animals were fed AIN-93 diet for several months. Rats fed the vitamin A-Adequate diet with 4 µg retinol/g had liver total retinol concentrations of 362 nmol/g (104 µg/g) at 3 months of age (32), well within the range considered adequate in humans (--).

Protocols for induction of vitamin A deficiency

The time required to induce vitamin A deficiency depends on the level of preexisting vitamin A storage and the animal's rate of growth. To obtain a reproducible time course in the development of vitamin A deficiency, we begin feeding vitamin A-deficient diet (Table 2) to the lactating mothers of nursing pups; this significantly reduces the transfer of vitamin A from mother to pups (33, 34). The pups are then weaned onto the same vitamin A-deficient diet, or onto a vitamin A-containing diet according to the study's design. With this protocol, growing rats fed the vitamin A-deficient diet are biochemically depleted of vitamin A (liver total retinol concentration of $< 5 \mu\text{g/g}$ tissue; plasma retinol $< 0.3 \mu\text{M}$) by 7 weeks of age for males and 8 weeks of age for females. External signs of vitamin A deficiency become apparent approximately a week later for both sexes. Signs include slowing or cessation of growth, somewhat rough fur, and occasionally a red crust (porphyrins from dried tears) around the eyes and/or nares. A staggered gait and locomotor impairment is seen rather late in the course of vitamin A deficiency. Locomotor dysfunction has been attributed to changes in striatal neurons, implicating vitamin A in the maintenance of basal ganglia motor function in the adult rat brain (35). Internally, nearly all of the animal's visceral fat is depleted and thus the adrenal glands can be easily seen in the remnants of perirenal fat. The thymus may be smaller and less white in color. If weanling rats are purchased from suppliers and then fed a vitamin A deficient diet, the time course of depletion is likely to be longer and not easily predictable, as the young animals will have received more vitamin A from their dams, and they most likely will have begun to consume crumbs of their mother's nonpurified diet before weaning. Thus these animals will have accumulated vitamin A in their tissues prior to the time of shipment.

Mice are well known to be difficult to deplete of vitamin A. A deficient state which impaired immune response was produced in mice by vitamin A deficient diet (36) in a manner similar to the rat studies, above. In studies of development, Morriss-Kay and Sokolova (37) noted finding a low incidence of mild effects is in the first litter of mice fed a vitamin A-deficient diet, with a higher incidence of more severe effects observed in the second litter, reflecting a greater degree of maternal deficiency during the second pregnancy. Clagett-Dame and coworkers developed a dietary strategy to produce vitamin A deficiency at specific times later in pregnancy (38). In their procedure, female mice were first made deficient in vitamin A, then mated with vitamin A-adequate males. Pregnant females were fed vitamin A-deficient diet supplemented with $12 \mu\text{g tRA/g}$ of diet ($\sim 230 \mu\text{g tRA/rat/day}$) to assure normal fetal development to mid-pregnancy. The tRA was then withdrawn at specific times, after which the mothers and fetuses rapidly became retinoid deficient. The approach enabled the investigators to achieve tight control over the timing of the deficiency state of the animal, owing to the rapid turnover of RA. They observed gross abnormalities, including defects in eye development, in embryos at day E12.5.

One reason for the difficulty of depleting mice of vitamin A may be related to the practice of animal research facilities, especially those housing transgenic animals, to feed an autoclavable nonpurified diet with extra vitamin A (see above). Another reason could be related to the efficiency of recycling retinoids. Both mice and rats practice coprophagy (in which feces are consumed), which may serve to conserve retinol even after a vitamin A-deficient diet has been imposed. Young mice are said to practice "vigorous coprophagy" (39), which may increase the recycling and delay the onset of deficiency. The type of housing used for mice (typically shoebox-type cages with multiple animals per cage) may enable more recycling of feces, although this is a conjecture. Hanging wire cages, sometimes used for rats, reduce but do not totally eliminate coprophagy, but this type of caging is seldom used for mice.

We have fed the same vitamin A-deficient and vitamin A-adequate diets shown in Table 2 to mice, with similar effects on plasma and liver vitamin A as in rats. However external signs of vitamin A deficiency (body condition) were not as readily apparent in mice as in rats.

Diets differing in macronutrient contents—The diets shown in Table 2 are all equal in energy density. When protein, carbohydrate or fat contents are altered, the energy density of the diet is also altered. Thus the amounts of the vitamin and mineral mixes also must be adjusted if a constant intake per kcal is to be maintained. Clinton and Visek (40) provides an excellent example of the formulation of purified diets that differ in protein or fat, while maintaining micronutrients at a constant level per kcal. These or similar diets could be modified for studies vitamin A metabolism related to obesity, metabolic syndrome, etc., to assure comparable micronutrient intakes across all diet groups.

Practical considerations for diet preparation and storage—Nonpurified diets are formulated with a low moisture level to improve stability. The diet is typically extruded in the form of hard pellets, which simplifies the feeding of animals and generally reduces waste. Diet is often added to the cage unit in amounts that will last several days. By contrast, purified diets are more labile and should be stored in a closed container in a cold room, kept dark and protected from oxidation. Diets with a very high fat content should be kept frozen. These diets should be fed in amounts that animals will consume in a day or two, and then replaced as needed.

Purified diets of the AIN type can be prepared in pelleted form. However, some diet formulas, like very low fat diets, are difficult to form into pellets and thus must be fed in powdered form using a glass cup that fits inside the animal's cage. Since the incisors of rodents grow continuously, care should be taken to observe the teeth of animals fed soft diets and to trim the teeth as necessary (41).

Summary—Controlled, purified diets are fundamental for reproducible vitamin A research in rodents. The use of nonpurified diets described as autoclavable and containing increased levels of micronutrients may be contributing to high tissue vitamin A in rodent studies. The use of a purified diet, such as AIN-93, is recommended for retinoid research because the diet is defined, the onset of vitamin A deficiency is reproducible, and graded levels of vitamin A can be added to control the animal's vitamin A status. Supplementing the purified vitamin A-deficient diet with tRA is a strategy for maintaining most retinoid-dependent tissues in an adequate state, so that a rapid, synchronous onset of retinoid deficiency occurs when RA is withdrawn. Such procedures have proved useful in studies of retinoid-dependent development in the later stages of pregnancy.

Abbreviations

AIN	American Institute of Nutrition
IOM	Institute of Medicine
NRC	National Research Council
RA	retinoic acid
RDA	recommended dietary allowance

References

1. Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington: National Academy Press; 2001.
2. Haskell MJ, Mazumder RN, Peerson JM, Jones AD, Wahed MA, Mahalanabis D, Brown KH. 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.* 1999; 70:874–780. [PubMed: 10539748]
3. Tang GW, Qin J, Dolnikowski GG, Russell RM. 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.* 2003 Aug;78:259–66. [PubMed: 12885706]
4. Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. Men with low vitamin A stores respond adequately to primary yellow fever and secondary tetanus toxoid vaccination. *J Nutr.* 2008; 138:2276–83. [PubMed: 18936231]
5. Hargreaves MK, Baquet C, Gamshadzahi A. Diet, nutritional status, and cancer risk in American blacks. *Nutr Cancer.* 1989; 12:1–28. [PubMed: 2652094]
6. Fontham ETH, Pickle LW, Haenszel W, Correa P, Lin Y, Falk RT. Dietary vitamins A and C and lung cancer risk in Louisiana. *Cancer.* 1988; 62:2267–73. [PubMed: 3179940]
7. Paganini-Hill A, Ross RK, Henderson BE. Vitamin A, b-carotene, and the risk of cancer. *JNCI.* 1988; 80:76. [PubMed: 3343688]
8. Ross, AC. Vitamin A and cancer. In: Carroll, KK.; Kritchevsky, D., editors. Nutrition and disease update Cancer. Champaign, IL: AOCS Press; 1994. p. 27-109.
9. Smiciklas-Wright, H.; Mitchell, DC.; Ledikwe, JH. Dietary intake assessment: methods for adults. In: Berdanier, CD.; Dwyer, JT.; Feldman, EB., editors. Handbook of nutrition and food. 2. Boca Raton, FL: CRC Press; 2007.
10. Reeves, RS.; Pace, PW. Use of food frequency questionnaires in minority populations. In: Berdanier, CD.; Dwyer, JT.; Feldman, EB., editors. Handbook of nutrition and food. 2. Boca Raton, FL: CRC Press; 2007.
11. Haskell MJ, Lembcke JL, Salazar M, Green MH, Peerson JM, Brown KH. Population-based plasma kinetics of an oral dose of [2H4]retinyl acetate among preschool-aged, Peruvian children. *Am J Clin Nutr.* 2003; 77:681–6. [PubMed: 12600861]
12. Murphy SP, White KK, Park S-Y, Sharma S. Multivitamin-multimineral supplements' effect on total nutrient intake. *Am J Clin Nutr.* 2007; 85(suppl):280S–4S. [PubMed: 17209210]
13. Briefel R, Hanson C, Fox MK, Novak T, Ziegler P. Feeding Infants and Toddlers Study: Do Vitamin and Mineral Supplements Contribute to Nutrient Adequacy or Excess among US Infants and Toddlers? *J Am Diet Assoc.* 2006; 106:S52–S65. [PubMed: 16376630]
14. Mithen R. Effect of genotype on micronutrient absorption and metabolism: a review of iron, copper, iodine and selenium, and folates. *Int J Vitam Nutr Res.* 2007; 77:205–16. [PubMed: 18214022]
15. Powers HJ. Interaction among folate, riboflavin, genotype, and cancer, with reference to colorectal and cervical cancer. *J Nutr.* 2005; 135(Suppl):2960S–6S. [PubMed: 16317155]
16. Friso S, Choi SW. Gene-nutrient interactions in one-carbon metabolism. *Curr Drug Metab.* 2005; 6:37–46. [PubMed: 15720206]
17. Berson EL. Nutrition and retinal degenerations. *Int Ophthalmol Clin.* 2000; 40:93–111. [PubMed: 11064860]
18. Coleman H, Chew E. Nutritional supplementation in age-related macular degeneration. *Curr Opin Ophthalmol.* 2007;18.
19. Newberne PM, Fox JG. Nutritional adequacy and quality control of rodent diets. *Lab Anim Sci.* 1980; 30:352–65. [PubMed: 6892043]
20. Feed Inspector's Manual. 2. Association of American Feed Control Officials Inspection and Sampling Committee; 2000. Chapter 4, Label Review.

21. Tobin, G.; Stevens, KA.; Russell, RM. Nutrition. In: Fox, JG.; Barthold, SW.; Davisson, MT.; Newcomer, CE.; Quimby, FW.; Smith, AL., editors. The mouse in biomedical research. Academic Press; 2007. p. 321-84.
22. Lipman, NS. Design and analysis of research facilities for mice. In: Fox, JG.; Barthold, SW.; Davisson, MT.; Newcomer, CE.; Quimby, FW.; Smith, AL., editors. The mouse in biomedical research. 2. Academic Press; 2007.
23. American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J Nutr. 1977;107.
24. American Institute of Nutrition. Second report of the ad hoc committee on standards for nutritional studies. J Nutr. 1980; 110:1726.
25. Wise, A. Diet formulation and experimental design for laboratory animal studies. In: Rowland, IR., editor. Nutrition, toxicity and cancer. CRC Press; 1991. p. 27-53.
26. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr. 1997; 127:838S-41S. [PubMed: 9164249]
27. National Research Council. Nutrient Requirements of Laboratory Animals. 3. 1978.
28. National Research Council. Nutrient Requirements of Laboratory Animals. 4. 1995.
29. Lieber CS, DeCarli LM. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. Alcohol Clin Exp Res. 1982;523-31. [PubMed: 6758624]
30. Zolfaghari R, Ross AC. Chronic vitamin A intake affects the expression of mRNA for apolipoprotein A-I, but not for nuclear retinoid receptors, in liver of young and aging Lewis rats. Archives of Biochemistry and Biophysics. 1995; 323:258-64. [PubMed: 7487086]
31. Dawson HD, Li NQ, DeCicco KL, Nibert JA, Ross AC. Chronic marginal vitamin A status reduces natural killer cell number and function in aging Lewis rats. J Nutr. 1999; 129:1510-7. [PubMed: 10419983]
32. Dawson HD, Yamamoto J, Zolfaghari R, Rosales F, Dietz J, Shimada T, Li N-Q, Ross AC. Regulation of hepatic vitamin A storage in a rat model of controlled vitamin A status during aging. J Nutr. 2000; 130:1280-6. [PubMed: 10801930]
33. Davila ME, Norris L, Cleary MP, Ross AC. Vitamin A during lactation: Relationship of maternal diet to milk vitamin A content and to the vitamin A status of lactating rats and their pups. J Nutr. 1985; 115:1033-41. [PubMed: 4020482]
34. Akohoue SA, Green JB, Green MH. Dietary vitamin A has both chronic and acute effects of vitamin A indices in lactating rats and their offspring. J Nutr. 2006; 136:128-32. [PubMed: 16365071]
35. Cartaa M, Stancampianoa R, Troncib E, Colluc M, Usiellod A, Morellib M, Faddaa F. Vitamin A deficiency induces motor impairments and striatal cholinergic dysfunction in rats. Neuroscience. 2006; 139:1163-72. [PubMed: 16530976]
36. Smith SM, Levy NL, Hayes CE. Impaired immunity in vitamin A deficient mice. J Nutr. 1987; 117:857-65. [PubMed: 3495650]
37. Morriss-Kay GM, Sokolova N. Embryonic development and pattern formation. FASEB J. 1996; 10:961-8. [PubMed: 8801178]
38. Clagett-Dame M, DeLuca HF. The role of vitamin A in mammalian reproduction and embryonic development. Annu Rev Nutr. 2002; 22:347-81. [PubMed: 12055350]
39. Brown CJ, Donnelly TM. Rodent husbandry and care. VetClin North America: Exotic An Practice. 2004; 7:201-25.
40. Clinton SK, Imrey PB, Alster JM, Simon J, Truex CR, Visek WJ. The combined effects of dietary protein and fat on 7,12-dimethylbenz(a)anthracene-induced breast cancer in rats. J Nutr. 1984; 114:1213-23. [PubMed: 6429293]
41. Donnelly, TM.; Brown, CJ. Rodent Husbandry and Care. 2009. <http://www.veterinarypartner.com/Content.plx?P=A&A=2497&S=4>
42. World Health Organization. Technical report series, No 329. Geneva: 1966. WHO Expert Committee on Biological Standardization Eighteenth Report.

Table 1

Comparison of the 1989 National Research Council and 2001 Institute of Medicine Interconversion of Vitamin A and Carotenoid Units

NRC, 1989	IOM, 2001
1 retinol equivalent ($\mu\text{g RE}$)	1 retinol equivalent ($\mu\text{g RE}$)
= 1 μg of all-trans-retinol	= 1 μg of all-trans-retinol
= 2 μg of supplemental all-trans- β carotene	= 2 μg of supplemental all-trans- β carotene
=6 μg dietary β -carotene	=12 μg dietary β -carotene
=12 μg other dietary provitamin A carotenoids	=24 μg other dietary provitamin A carotenoids

Note: 1 μg retinol = 3.33 vitamin A activity from retinol (42); 10 IU β -carotene = 3.33 IU retinol (42). From (1).

Table 2
Composition of diets used for vitamin A depletion and long-term maintenance of selected vitamin A status in rats

	AIN-93G Growing Rodent Diet (26)	Modifications to control the level of vitamin A ¹			
		Vitamin A-deficient	Low marginal	Marginal	Adequate (used as Control)
Energy distribution:					
Protein (kcal %) ² :	20.3	20.3	20.3	20.3	20.3
Carbohydrate (kcal %):	63.9	63.9	63.9	63.9	63.9
Fat (kcal %):	15.8	15.8	15.8	15.8	15.8
Energy density (kcal/g):	4.00	4.00	4.00	4.00	4.00
Final Vitamin A concentration: µg retinol/g diet		0	0.35	0.73	4
Coding colors ³ :	(None)	"White"	"Purple"	"Green"	"Pink"
FD&C Red Dye #40	0	0	0.025		0.025
FD&C Blue Dye #1	0	0	0.025	0.025	
FD&C Yellow Dye #5	0	0		0.025	0.025
Ingredients:					
Casein, Lactic, g/kg ⁴		200	200	200	200
L-Cysteine, g/kg		3	3	3	3
Corn Starch		397.5	397.5	397.5	397.5
Maltodextrin		132	132	132	132
Sucrose		100	100	100	100
Fiber: Cellulose, BW220		50	50	50	50
Soybean Oil ⁵		70	70	70	70
Antioxidant: Tert-Butylhydroquinone		0.014	0.014	0.014	0.014
Mineral Mix (S10022G) ⁶	35	35	35	35	35
Vitamin A-free Vitamin Mix (V13002) ⁷	0	10	10	10	10
Std. Vitamin Mix (V10037)	10				
Vitamin A (Retinyl palmitate) Concentrate, 500,000 USP units/g ⁸		0	0.0027	0.0049	0.0278
Choline bitartrate		2.5	2.5	2.5	2.5

Energy distribution: Total, g	AIN-93G Growing Rodent Diet (26)	Modifications to control the level of vitamin A ¹				
		Vitamin A-deficient	Low marginal	Marginal	Adequate (used as Control)	Supplemented
		1000	1000	1000	1000	1000

¹ Any reputable diet manufacturer can produce the AIN diet or modifications thereof. We have purchased the diets indicated from Research Diets, Inc. The ingredient product numbers are those of this manufacturer.

² Maintenance formula is modified to 14% protein, 73% carbohydrate, and 4% fat (g %), for mature animals.

³ Food-grade dyes are used to visually code the diets.

⁴ Standard casein is used. Vitamin-free (vitamin tested) casein and alcohol-stripped casein are more expensive and were not found to be necessary since their retinol content is extremely low.

⁵ Tocopherol-stripped soybean oil is not necessary unless the diet also must be limited in tocopherol. Soybean oil is not a source of vitamin A or carotene.

⁶ The complete mineral mix is that reported by Reeves (26). 35 g of this mix is added per kg of diet.

⁷ The vitamin A-free vitamin A, per kg of mix, contains: Vitamin D3 (100,000 IU/g), 1.0 g; Vitamin E acetate (500 IU/g), 15 g; Vitamin K as phylloquinone, 0.075 g; Biotin (1%), 2.0 g; Cyanocobalamin, (0.1%), 2.5 g; Folic acid, 0.2 g; Nicotinic acid, 3.0 g; Pantothenate, calcium, 1.6 g; Pyridoxine-HCl, 0.7 g; Riboflavin, 0.6 g; Thiamin HCl, 0.6 g; powdered sucrose 972.7 g; total: 1 kg. 10 g of this mix is added per kg of diet. (Modified from (26)).

⁸ 1 USP unit = 1 IU = 0.55 µg of retinyl palmitate/g of the concentrate.

⁹ Diets labeled Marginal, Adequate and Supplemented diet (25 µg/g diet) were fed to rats for up to 20 months in a long-term aging study. See (31, 32) for plasma and liver vitamin A levels in young, middle-aged and old rats.