Calculating Radiation Exposures during Use of 14C-Labeled Nutrients, Food Components, and Biopharmaceuticals To Quantify Metabolic Behavior in Humans

SEUNG-HYUN KIM,† PETER B. KELLY,‡ AND ANDREW J. CLIFFORD*,†
*Departments of Nutrition and ‡Chemistry, University of California—Davis, One Shields Avenue, Davis, California 95616

INTRODUCTION

14C has long been used as a tracer for quantifying the in vivo human metabolism of food components, biopharmaceuticals, and nutrients. Minute amounts (≤1 × 10^{-18} mol) of 14C can be measured with high-throughput 14C-accelerator mass spectrometry (HT 14C-AMS) in isolated chemical extracts of biological, biomedical, and environmental samples. Availability of in vivo human data sets using a 14C tracer would enable current concepts of the metabolic behavior of food components, biopharmaceuticals, or nutrients to be organized into models suitable for quantitative hypothesis testing and determination of metabolic parameters. In vivo models are important for specification of intake levels for food components, biopharmaceuticals, and nutrients. Accurate estimation of the radiation exposure from ingested 14C is an essential component of the experimental design. Therefore, this paper illustrates the calculation involved in determining the radiation exposure from a minute dose of orally administered 14C-carotenoids, 14C-α-tocopherol, 14C-lutein, and 14C-folic acid from four prior experiments. The administered doses ranged from 36 to 100 nCi, and radiation exposure ranged from 0.12 to 5.2 μSv to whole body and from 0.2 to 3.4 μSv to liver with consideration of tissue weighting factor and fractional nutrient. In comparison, radiation exposure experienced during a 4 h airplane flight across the United States at 37000 ft was 20 μSv.

KEYWORDS: 14C tracer; radiation exposure calculation; in vivo in human; accelerator mass spectrometry (AMS)

AMS was first developed in the early 1930s (10), but it was not used for 14C measurement until 1977 due to atomic isobar interference with 14N (11). Development of the cesium sputter (Cs^+) method to generate 14C−negative ions enabled AMS to be used for radiocarbon dating (5, 8, 12, 13). 14N does not form stable 14N−ions.

AMS methods for various radioisotopes were developed to advance specific research objectives: radiocarbon dating (14C), biological/biomedical study (4H, 14C, 26Al, 41Ca, 129I), nuclear weapon testing (41Ca), monitoring migration of nuclear waste from nuclear power plant (38Cl, 129I), hydrogeological study (10He, 36Cl), and exposure dating (10Be, 26Al, 36Cl) (5). 14C was the most popular radioisotope measured with AMS (3, 5). AMS measured the 14C/12C or 14C/13C ratio (rather than 14C decay) in samples of interest and thereby increased sensitivity to the attomole (10^{-18}) level, making AMS 1000 times more sensitive than LSC (3, 5, 14). AMS required a milligram or less of carbon (≤mg of C). Sample mass for AMS was 1000 times less than that for LSC (3–5, 8, 14). Furthermore, AMS achieved a precision of ≈1% in 5–10 min (3–6). The great efficiency and sensitivity made AMS the ultimate tool for quantifying metabolic behaviors of food components, nutrients, and biopharmaceuticals using a 14C tracer.

Biological/biomedical applications using AMS were first suggested ≈30 years ago (15), and these applications began in earnest...
in the early 1990s (16). In vivo in human or in vivo in animal
dynamic and kinetic behaviors, absorption, distribution, meta-
bolism, elimination (ADME) of food component, nutrients
(17–21), or environmental chemicals (22–24) using
14C-AMS
have been reported during the past two decades.
For biological/biomedical 14C-AMS
applications, very small
amounts of 14C-labeled compound were administered to humans
and commonly called microdosing in drug development
studies. The U.S. Food and Drug Administration (25) and European
Medicines Agency (26) defined microdosing as only 1% of a
therapeutic dose or ≤100 μg/person. Because pharmacokinetics
(PK) with microdosing was ≈70% equivalent of a PK with
therapeutic dose (27), microdosing combined with AMS enabled
physiological-based (steady-state) kinetic/dynamic behavior
study of food components or nutrients to be conducted. Thus,
AMS
methods led to better understanding of in vivo in human
metabolism of food components or nutrients (14). The combina-
tion of microdosing and AMS also minimized the risk of toxicity,
cost, time, and labor, especially for new drug development in the
pharmaceutical industry (4).

The typical radiation exposure experienced during a 4 h airplane
ride at 37000 feet altitude was 20 μSv (28). The radiation exposure
of 20 μSv was considered to be an acceptable level of exposure in
the United States, so 20 μSv is used as a reference level. Human
radiation exposure calculation from the administered
14C-labeled food components or nutrients is an integral part of protocols
for biological/biomedical AMS
applications. Therefore, the present
study reported a practical and complete method for calculating the
14C radiation exposure during the conduct of our four prior
studies (18–21). The method took into account 14C dose level,
body mass, biological half-life of 14C-labeled nutrients, tissue
weighting factor (radiation exposure in an organ and/or tissue rela-
tive to that of the whole body mass in dimensionless multiplicative
factor), and fractional nutrient content in each organ or tissue.

MATERIALS AND METHODS

Subject Selection. Human subjects who were healthy nonsmoking
adults aged 20–60 years with a BMI of 20–35.5 kg/m² were recruited
(Table 1). Written informed consent was obtained from volunteers per
University of California–Davis (UCD) and Lawrence Livermore Nat-
onal Laboratory (LLNL) Human Subjects Review Committees. All studies
were performed with Good Clinical Practice Guidelines (ver. 1989) and
the ethical guidelines of the 1975 Declaration of Helsinki (18–21).

Dose Administration. Table 1 summarizes the 14C dose, nutrient dose,
etc. in each study (18–21). Nutrients were chemically (β-carotene,
α-tocopherol) or biologically (lutein, folic acid) labeled with
14C. 14C-Nutrients (36–100 nCi) were orally administered to human subjects
with breakfast. Oral dose administration was also approved by the UCD
and LLNL Human Subjects Review Committees prior to 14C-nutrient
studies (18–21). A small ratio (mol/mol) of 14C-nutrient dose to Recom-
manded Dietary Allowances (RDA) was necessary for the physiological-
based (steady-state) 14C-tracer study. The RDAs of β-carotene and lutein
were not decided yet, so U.S. average intake (AI) was considered for
steady-state 14C-tracer study.

Sample Collections. Prior to 14C-nutrient dose, fasting plasma,
urine, and feces were collected for 14C baselines of each human subject.
Serial plasma, urines, and feces were collected over time since
14C-nutrient dose (18–21). A predose baseline blood sample was collected.
Additional blood samples were usually collected at every 0.5 h interval
from 0 to 12 h, hourly from 12 to 16 h, and then at 24 and 36 h after
14C-nutrient dose. Subsequent blood samples were collected daily from
2 to 7 days, every other day from 7 to 14 days, then weekly, biweekly
and/or monthly for the remainder of the study. In addition, prior to
dosing 14C-nutrient in each study, a complete collection of feces and
urine was also taken to establish baseline values. Serial collections
of feces and urine usually continued for 14 and 21 days, respectively,
after the day of 14C-nutrient dosing. Durations of β-carotene, lutein,
α-tocopherol, and folate studies were 5, 3, and 6 months, respec-
tively. When 100 nCi of 14C was dosed to the 70 kg human subject,
colleced samples had 0.0014 nCi of 14C/g, a level considered to be
nonradioactive according to the U.S. Code of Federal Regulations,
Title 10, Section 20.2005, 1991 (cutoff, ≤50 nCi/g or 1 μCi/year),

| Table 1. Summary of Our Prior 14C-Labeled Nutrient Studies (18–21) and Daily Reference Nutrient Amounts |
|---------------------------------|-----------------|-----------------|-----------------|
| unit                           | β-carotene (16) | lutein (19)     | α-tocopherol (20) |
| molecular weight              | g/mol           | 538.73          | 430.71          | 441.4 |
| specific activity, SA         | Ci/mol          | 98.8(18)        | 56              | 1.24  |
| gender                        | male (n = 4)    | 0.2688          | female (n = 1)  | male (n = 1) |
| body mass index               | kg              | 76.7 ± 22.2     | 79.5            | 73.8 ± 0.28 |
| body mass index               | kg/m²           | 25.0 ± 4.2      | 23.8            | 28.5 ± 5.9 |
| age                           | year            | 35 ± 8.2        | 36              | 32 ± 3.8 |
| 14C dose (18–21)              | ±mol/µCi        | ±1.01[100]      | ±125[36]        | ±1.82[101.5] |
| ratio (mol/mol) between 14C dose (18–21) and Recommended Daily Allowance (RDA) or average intake (AI) in the U.S. reference nutrient amount/day |
| therapeutic level             | mmol/day        | ±0.066          | ±0.0003–0.0035  | ±0.0348 (19 years) |
| tolerable upper intake level/day | mmol/day     | ±0.011–0.018 (38) | ±2.2 (19 years) | ±2.23 (19 years) |
|                           | n.d.           | ±2.1             | ±0.022 (41)     | ±0.023 (19 years) |

a The study design was a test and retest in one subject. b SA was determined by using a mix of 0.5 nmol of 14C-folic acid and 79.5 nmol of nonlabeled cold folic acid. c The Institute of Medicine at the National Academy of Sciences did not establish a Tolerable Upper Intake Level for carotenoids when it reviewed these compounds in 2000.

14C-AMS Measurement. One million voltaje AMS at the Center for AMS at LLNL (see Supporting Information, Figure 2) measured
Table 2. Estimated Radiation Exposure from 

| subject body weight | unit | 
|---------------------|------| 
| dose | nCi | 
| half-life (t<sub>b</sub>) | day | 
| mean-life (t<sub>biological</sub> = t<sub>b</sub>/ln 2) | day | 
| tissue weighting factor, W<sub>t</sub>, liver | | 
| fractional nutrient, FN, liver | | 

| 
| β-carotene (18) | 
| lutein (19) | 
| RRR | 
| all-rac | 
| folic acid (21) | 

| 76.8 ± 23.2 | 64.1 (n = 8) | 79.5 (n = 1) | 79.5 (n = 1) | 78.3 ± 22.3 (n = 13) | 0.8 | 0.5 | 0.8 | 0.8 | 0.8 | 0.8 |
| 100 | 36 | 14 | 2.5 | 2.5 | 100 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| 25 | 20.2 | 3.6 | 3.6 | 144.3 | 0.8 | 0.9 | 
| 0.8 (as carotene) | 0.5 | 0.8 | 0.8 | 0.33 |

| radiation exposure<sup>a</sup> | whole body, WB | μSv | 1.3 ± 0.4 | 0.3 | 0.12 | 0.12 | 5.2 ± 1.5 | 0.3 |
| 1.5 kg<sup>b</sup> | μSv | 63.3 (as carotene) | 12.7 | 6.4 | 6.3 | 253 | 0.2 |
| 1.5 kg<sup>c</sup> | μSv | 2.0 (as carotene) | 12.8 | 3.4 |

<sup>a</sup>Nutrient absorption is assumed 100%.<sup>b</sup> Radiation exposure regardless of W<sub>t</sub> and FN.<sup>c</sup> Radiation exposure regard of W<sub>t</sub> (ICRP 103, 2008) and FN. The dimensionless multiplicative factors (W<sub>t</sub> and FN) are 1 for WB.

14C/13C ratios from graphitized solid samples. Measured 14C/13C ratios of samples were normalized with one of the popular AMS standards such as oxalic acid, NIST SRM 4990B/4990C, or sucrose, IAEA-C6 (24, 35). Finally, 14C level in samples was defined as “Modern or Fraction Modern (F<sub>M</sub>).” A current/natural 14C level in living organisms is about 1.1 F<sub>M</sub> (= 107.6 amol of 14C/mg of C, 6.72 fCi/mg of C, or 14.92 dpm/g of C (30, 36).

**Human Radiation Exposure Calculation from 14C Dose.** Radiation exposure from 14C-labeled nutrient was calculated (3) as:

\[
\text{exposure} = \frac{E_d}{M_d} \times \text{dose} \times \int_0^{t_{\beta decay}} e^{\frac{-t}{\tau_{\beta decay}}} dt = \frac{E_d}{M_d} \times \tau_{\beta decay} \times \text{dose} \times W_T \times FN
\]

where E<sub>d</sub> is energy deposition per decay as joules (8.3 fJ/decay), M<sub>d</sub> is the affected mass (kg), dose is the amount of radioactivity in dpm, τ<sub>β decay</sub> is the biological mean-life of 14C-labeled compound in minutes or seconds, W<sub>T</sub> is tissue weighting factor (radiation exposure in an organ and/or tissue relative to that of the whole body (WB) in dimensionless multiplicative factor (see Supporting Information, Table 2) (37)), and FN is the fractional nutrient content in each organ or tissue (see Supporting Information, Table 2).

Radiation exposure was calculated previously without considering W<sub>T</sub> and the FN (3). The formula (eq 1) for radiation exposure estimation was modified by considering W<sub>T</sub> for each organ as well as different FN. The formula (eq 1) can more completely calculate radiation exposure over the biological mean-life. For example, if 100 nCi of 14C-β-carotene, which had the τ<sub>β decay</sub> of 36.1 days (equals to 51984 min), was administered to a human subject of 61.2 kg (see Supporting Information, Table 2), the radiation exposures to WB and liver (≈1.5 kg) were calculated by considering W<sub>T</sub> and FN as follows:

\[
\text{exposure to WB} = \frac{8.3 \text{ fJ/decay}}{61.2 \text{ kg}} \times 51984 \text{ min} \times \left( 100 \text{ nCi} \times \frac{2200 \text{ dpm}}{1 \text{nCi}} \right) \times 1 \times 1 = 1.6 \mu\text{Sv}
\]

\[
\text{exposure to liver} = \frac{8.3 \text{ fJ/decay}}{\approx 1.5 \text{ kg}} \times 51984 \text{ min} \times \left( 100 \text{ nCi} \times \frac{2200 \text{ dpm}}{1 \text{nCi}} \right) \times 0.04 \times 0.8 = 2.0 \mu\text{Sv}
\]

All radiation exposure calculations from 14C-food component and 14C-nutrient studies are available as Supporting Information (Table 2).

**14C Dosing Calculation for Detecting Optimal 14C Concentration with 14C-AMS.** A 14C dose estimation was required for determining the optimal 14C range for the 14C-AMS, which ranged from 0.01 to 100 μCi (C18/C19 34, 35), and the expected 14C dose (nCi) was estimated using the Modern of samples (37):

\[
\text{expected 14C dose (nCi)} = \text{expected Modern (of samples)} \times \frac{6.11 \text{ fCi/mg of C} \times \text{sample volume or mass}}{1 \text{ mg of C} \times \text{sample volume or mass}} \times \frac{1 \text{ mg of C}}{25 \mu\text{L of plasma} \times 42 \text{ L (70 kg human) \times 10^6}} \approx 103 \text{ nCi}
\]

Therefore, each radiation exposure of 100 nCi was increased 50 times higher than that to a 70 kg human subject, a 25 μL plasma sample would be about 10 Modern. The formula (eq 2) can be used to estimate the amount of 14C dose. For more complete estimation, the bioavailability and body distribution of the food component or nutrient should be considered (3).

**RESULTS AND DISCUSSION**

**Calculating Human Radiation Exposure from 14C-Nutrients.** The dose of 14C-nutrients and radiation exposures were estimated over the biological mean-life (t<sub>biological</sub>) (Table 2). The 14C-nutrients containing 36−101.5 nCi were orally ingested at breakfast by human subjects weighing 52−116 kg. Biological half-lives (t<sub>1/2</sub>) of these nutrients ranged from 2.5 to 100 days.

Radiation exposure to WB ranged from 0.12 to 5.2 μSv, which was 4−170 times lower than that experienced during a 4 h flight (20 μSv) (28). In general, radiation exposure increased as the biological mean-life of nutrient increased and as the organ or tissue mass decreased. Assuming 100% of the body’s stored 14C-nutrients was housed in liver regardless of the W<sub>T</sub> and FN, then radiation exposure to a 1.5 kg liver was calculated to be 50 times higher than that to a 70 kg WB. If all of a 100 nCi 14C-β-carotene dose was converted to 14C-retinol and stored in the liver, the radiation exposure to liver was increased to a maximum 354 μSv (Table 2). Sequestration of 100% of the 14C label in the liver represents the worst-case scenario.
Table 3. Estimated and Measured $^{14}$C Levels from $^{14}$C-Labeled Nutrients as a Function of Time since Dose

| dose | unit | $\beta$-carotene (18) | lutein (19) | RRR | all-rac | $\alpha$-tocopherol (20) | folic acid (21) |
|------|------|----------------------|-------------|------|--------|------------------------|-----------------|
| estimated maximum $^{14}$C level/mg of C | nCi | 100 | 36 | 101.5 | 99.95 | 100 |
| (in 25 $\mu$L of plasma)$^a$ | | | | | | |
| distributed in plasma of 3 L | Modern | 137 | 49 | 139 | 137 | 137 |
| distributed in interstitial water of 18 L | Modern | 23 | 8 | 23 | 23 | 23 |
| distributed in WB water of 42 L | Modern | 10 | 4 | 10 | 10 | 10 |
| measured maximum $^{14}$C level/mg of C | | | | | | |
| plasma | Modern | 13 | 3.8 | 21 | 14 | 4.7 $\pm$ 1.6 |
| feces$^b$ | Modern | $\geq$35 | 54 | 18 | 11 | 53 $\pm$ 50 |
| urine$^b$ | Modern | $\approx$53 | 1.0 | 0.6 | 1.8 | 66 $\pm$ 22 |

$^a$ Assumed 100% absorption of $^{14}$C-nutrient since dose. $^b$ Except for feces and urine samples in folic acid study (21), some feces and urine samples (18–21) that were suspected to have high $^{14}$C levels were diluted with a TRIB for optimal $^{14}$C-AMS measurement (0.01–100 Modern range).

By considering the $W_T$ and FN, radiation exposures to liver (Table 2) were calculated to range from 0.2 to 12.8 $\mu$Sv, which was lower than that experienced during a 4-h flight at 37000 ft altitude (20 $\mu$Sv). Although $^{14}$C-retinol had a longer half-life (140 days), radiation exposure to liver from $^{14}$C-retinol was calculated to be 12.8 $\mu$Sv when the $W_T$ (0.04 in liver) and FN (0.9 in liver) were considered. Consequently, radiation exposure from dose with 100 nCi $^{14}$C was equal to or less than that experienced from common/natural radiation (see Supporting Information, Table 3).

Radioactive isotopes emit energy depending on $\alpha$-, $\beta$-, or $\gamma$-decay. People are exposed to various radiation sources during their lifetime, and the annual radiation exposure differed between WB, organ, or tissue (see Supporting Information Table 3). $^{14}$C emitted an electron for each $\beta$-decay ($3\beta$). Thus, the reference human (70 kg, $\approx$ 1.1 Modern, assumed 23% carbon content) was naturally/constantly exposed to $\approx$1.7 nSv/h at the ambient $^{14}$C level

$$1.1 \text{ Modern} = \frac{14.96 \text{ dpm/gC}}{gC} \times \frac{23 \text{ gC/100 g}}{100 \text{ g}} \times 70 \text{ kg} \times \frac{8.3 \text{ fJ/dpm}}{36 \text{ fJ/hour}} \times \frac{60 \text{ min}}{1 \text{ hour}}$$

$$= \frac{120 \text{nJ/hour}}{120 \text{nJ/hour}} \times \frac{70 \text{ kg}}{70 \text{ kg}} = 1.7 \text{ nSv/hour}$$

where J/kg = Sv ($3\beta$).

Deposition of the radiation energy from the administered $^{14}$C-nutrient was neither complete nor constant throughout the human body during the study because the $^{14}$C-nutrient was metabolized, stored, and eventually excreted. The radiation exposure from ingested $^{14}$C-nutrients or food components can be calculated using an exponential elimination model as a function of time since dose, as long as the biological mean-lives of the $^{14}$C-labeled nutrients were shorter than the expected human life span ($3\beta$). Therefore, full dynamic/kinetic modeling was required for the more exact calculation of human radiation exposure. Furthermore, correction of radiation exposure due to radio-isotope decay was not necessary when $t_{\text{isotope}}$ was much longer than $t_{\text{bio}}$. In addition, considering $W_T$ and FN enabled more complete calculation than a prior calculation ($3\beta$) of radiation exposure to individual organs and tissues. In the present study, radiation exposure to liver was 30–75 times lower when the $W_T$ and FN of each nutrient were considered (Table 2). Finally, radiation exposures to WB and liver from all $^{14}$C-nutrients (Table 2) were lower than natural/human-made radiation exposure.

Dose Calculation for Optimal $^{14}$C-AMS Measurement of 1 mg of C Size Samples. Table 3 summarizes the calculated and measured maximum $^{14}$C levels in human plasma, feces, or urine samples that contained 1 mg of C. The physiological-based $^{14}$C-nutrient study required a dose with the same or smaller amount of $^{14}$C-nutrient relative to its RDA or AI. The ratio (mol/mol) of $^{14}$C-nutrient to the RDA or AI was $\approx$1/1812 (17, 18, 20, 21), which was even smaller than the microradosing level, 1/100 (25, 26). The ratio (mol/mol) of $^{14}$C-lutein to its AI ranged from 1/3 to 1/28 (19), due to the low specific activity of $^{14}$C-lutein ingested (Table 1), and even this ratio (1/3 to 1/28) was suitable for physiological-based pharmacokinetics and ADME measurements (Table 1).

Peak $^{14}$C levels in plasma, urine, and feces occurred within the first 5 days as a function of time since dosing. For human subjects dosed with 36–100 nCi of $^{14}$C, the calculated peak of $^{14}$C in plasma (mg of C) ranged from 49 to 137 Modern for a 3 L plasma pool, from 8 to 23 Modern for an 18 L interstitial water pool, and from 4 to 10 Modern for a 42 L WB water pool. The measured level of $^{14}$C in plasma (mg of C), however, varied over a 10–30-fold lower range, because $^{14}$C-nutrients were distributed, metabolized, and excreted (Table 3). The measured peak range (3.8–21 Modern) of $^{14}$C in plasma was within the optimal $^{14}$C-AMS range of 0.01–100 Modern.

In general, the maximum $^{14}$C level in feces and in urine was higher than the maximum $^{14}$C level in plasma. Therefore, feces and urine samples containing >100 Modern usually needed to be diluted with tributyrin, which was devoid of $^{14}$C. Thus, the levels of $^{14}$C in the diluted feces and urine were within the optimal $^{14}$C-AMS working range. Masses or volumes of the feces and of urine collections were difficult to control compared to those of human blood. Furthermore, feces and urine collections varied widely in their carbon content compared to human blood. Consequently, the Modern values of feces and urine were higher and more variable than those of human blood, due to the variable concentrations of carbon in feces/urines and the first-pass elimination of $^{14}$C-nutrient in feces. Although maximum $^{14}$C levels in feces and urine were variable depending on ADME, nutrient hydrophobicity, subject traits, etc., dosing of ≤100 nCi was suitable for $^{14}$C-AMS, the optimal range of which was 0.01–100 Modern.

In fact, determination of $^{14}$C-dose amount for optimal $^{14}$C-AMS measurement was difficult because that determination needed to consider the hydrophilic, hydrophobic, and bioavailability characteristics of nutrients, human body weight, and sensitivity of AMS. Most nutrients would be transferred, partitioned, or distributed among body organs (liver, gut, etc.), intracellular (21 L), interstitial (18 L), intravascular fluids (3 L), and WB water (42 L). If water-soluble compounds were distributed in WB water of 42 L, ≥100 nCi of $^{14}$C would peak at about
10 Modern in 25 μL of plasma (which contained 1 mg of C), even though the bioavailability of nutrients was variable. On the other hand, very hydrophobic compounds or compounds with ≤20% bioavailability required a larger dose of ∼200 nCi to study 14C-nutrients with long biological half-life (3).

Even though the administered doses ranged from 36 to 100 nCi in our four prior studies, by considering tissue weighting factor materials and samples from graphitization and 14C-AMS measurement can be considered nonradioactive waste materials, at significant cost saving for disposal.

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Supporting Information Available: Two figures, three tables, and supporting text and references. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Kamen, M. D. Early history of carbon-14: discovery of this supremely important tracer was expected in the physical sense but not in the chemical sense. Science 1963, 140, 584–590.

(2) Pierson, H. O. The element carbon. In Handbook of Carbon, Graphite, Diamond, and fullerene. Properties, Applications, and Energy (Noyes Publications: Park Ridge, NJ, 1993); pp 20–21.

(3) Vogel, J. S.; Love, A. H. Quantitating isotopic molecular labels with accelerator mass spectrometry. Methods Enzymol. 2005, 402, 402–422.

(4) Salehpour, M.; Possnert, G.; Bryhni, H. Subattonle sensitivity in AMS detection at natural concentration.

(5) Hellborg, R.; Skog, G. Accelerator mass spectrometry. Mass Spectrom. Rev. 2008, 27, 398–427.

(6) Tuniz, C.; Bird, J. R.; Fink, D.; Herzog, G. F. Performance of AMS systems. In Accelerator Mass Spectrometry. Ultrasensitive Analysis for Global Science; CRC Press: New York, 1998; pp 21–31.

(7) Vogel, J. S.; Tortelautb, K. W.; Farbel, R.; Nelson, D. E. Accelerator mass spectrometry, isotope quantification at attomole sensitivity. Anal. Chem. 1995, 67, 353A–359A.

(8) Vogel, J. S.; Tortelautb, K. W. Accelerator mass spectrometry as a biological tool for nutritional research. In Mathematical Modeling in Experimental Nutrition AEMB; Clifford, A. J., Muller, H.-G., Eds.; Plenum Press: New York, 1998; Vol. 445, pp 397–410.

(9) Aiiken, M. J. Archaeological involvements of physics. Phys. Rep. Rev. Sect.: Phys. Lett. 1978, 40, 278–351.

(10) Wilson, R. R.; Littauer, R. In Accelerator: Machines of Nuclear Physics, 1st Ed.; Anchor Books: New York, 1960; p 60.

(11) Muller, R. A. Radiosotope dating with a cyclotron. Science 1977, 196, 189–194.

(12) Nelson, D. E.; Korteling, R. G.; Stottle, W. R. Carbon-14: direct detection at natural concentration. Science 1977, 198, 508–510.

(13) Bennett, C. L.; Beukens, R. P.; Clover, M. R.; Gove, H. E.; Liebert, R. B.; Litherland, A. E.; Purser, K. H.; Sondheim, W. R. Radio carbon dating using electrostatic accelerator: negative ions provide key. Science 1977, 198, 508–510.

(14) de Moura, F. F.; Burri, B. J.; Clifford, A. J. Accelerator mass spectrometry in the study of vitamins ans mineral metabolism in humans. In Handbook of Vitamins; Zempleni, J., Rucker, R. B., McCormick, D. B., Suttie, J. W., Eds.; CRC Press/Taylor and Francis Group: Boca Raton, FL, 2007; pp 545–557.

(15) Keilson, J.; Waterhouse, C. First Conference on Radiocarbon Dating with Accelerators; Grove, H. E., Ed.; University of Rochester: Rochester, NY, 1978.

(16) Tortelautb, K. W.; Felton, J. S.; Gledhill, B. L.; Vogel, J. S.; Southon, J. R.; Caffee, M. W.; Finkle, R. C.; Nelson, D. E.; Proctor, J. D.; Davis, J. C. Accelerator mass spectrometry in biomedical dosimetry: relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA. Proc. Natl. Acad. Sci. U.S.A. 1990, 87, 5288–5292.

(17) Ho, C. C.; de Moura, F. F.; Kim, S. H.; Clifford, A. J. Exentral cleavage of β-carotene in vivo in a healthy man. Am. J. Clin. Nutr. 2007, 85, 770–777.

(18) Ho, C. C.; de Moura, F. F.; Kim, S. H.; Burri, B. J.; Clifford, A. J. A minute dose of 14C-β-carotene is absorbed and converted to retinoids in humans. J. Nutr. 2009, 139 (8), 1480–1486.

(19) de Moura, F. F.; Ho, C. C.; Getaechw, G.; Hickenbottom, S.; Clifford, A. J. Kinetics of 14C distribution after tracer dose of 14C-lutein in an adult woman. Lipids 2005, 40, 1069–1073.

(20) Clifford, A. J.; de Moura, F. F.; Ho, C. C.; Chuang, J. C.; Follett, J.; Fadel, J. G.; Novotny, J. A. Feasibility study quantifying in vivo human α-tocopherol metabolism. Am. J. Clin. Nutr. 2006, 84, 1430–1441.

(21) Lin, Y.; Ducker, S. R.; Follett, J. R.; Fadel, J. G.; Arjomand, A.; Schneider, P. D.; Miller, J. W.; Green, R.; Buchholz, B. A.; Vogel, J. S.; Phair, R. D.; Clifford, A. J. Quantitation of in vivo human folate metabolism. Am. J. Clin. Nutr. 2004, 80, 680–691.

(22) Turtelautb, K. W.; Dingley, K. H.; Curtis, K. D.; Maifali, M. A.; Turesky, R. J.; Colin Garner, R.; Felton, J. S.; Lang, N. P. Macromolecular adduct formation and metabolism of heterocyclic amines in humans and rodents at low doses. Cancer Lett. 1999, 147, 149–155.

(23) Dingley, K. H.; Freeman, S. P. H. T.; Nelson, D. O.; Garner, R. C.; Turtelautb, K. W. Covalent binding of 2-amino-3,8-dimethylimidazo[4,5-f]quinoline to albumin and hemoglobin at environmentally relevant doses. Comparison of human subjects and F344 rats. Drug Metab. Dispos. 1998, 26, 825–828.

(24) Turtelautb, K. W.; Mauhie, R. J.; Dingley, K. H.; Vogel, J. S.; Frantz, C. C.; Garner, R. C.; Shen, N. MelQx-DNA adduct formation in rodent and human tissues at low doses. Mutat. Res. 1997, 376, 243–252.

(25) Food and Drug Administration, U.S. Department of Health and Human Services Guidelines for Industry Investigators and Reviewers, Exploratory IND Studies, F. R., MD, USA, January 2006.

(26) Position Paper on Nonclinical Safety Studies to Support Clinical Trials with a Single Microdose. Position paper CPMP/SWP/2599, E. L., UK, 23 June 2004.

(27) Garner, R. C.; Lappin, G. Commentary: The phase 0 microdosing concept. Br. J. Clin. Pharmacol. 2006, 61, 367–370.

(28) Friedberg, W.; Copeland, K.; Duke, F. E.; O’Brien, K.; Darden, E. B. Radiation exposure during air travel: guideline provided by the Federal Aviation Administration for air carrier crews. Health Phys. 2000, 79, 591–595.

(29) Vogel, J. S. Rapid production of graphite without contamination for biomedical AMS. Radiocarbon 1992, 34, 344–350.

(30) Ognibene, T. J.; Bench, G.; Vogel, J. S.; Peaslee, G. F.; Murov, S. A high-throughput method for the conversion of CO2 obtained from biochemical samples to graphite in septa-sealed vials for quantification of 14C via accelerator mass spectrometry. Anal. Chem. 2003, 75, 2192–2196.

(31) Getachew, G.; Kim, S. H.; Burri, B. J.; Kelly, P. B.; Haack, K. W.; Ognibene, T. J.; Buchholz, B. A.; Vogel, J. S.; Modrow, J.; Clifford, A. J. How to convert biological carbon into graphite for AMS. Radiocarbon 2006, 48, 325–336.

(32) Pella, E. Elemental organic analysis. Am. Lab. 1990, 22, 116–125.

(33) Kim, S. H.; Kelly, P. B.; Clifford, A. J. Biological/biomedical accelerator mass spectrometry targets. 1. Optimizing the CO2 reduction step using zinc dust. Anal. Chem. 2008, 80, 7651–7660.

(34) Kim, S. H.; Kelly, P. B.; Clifford, A. J. Accelerator mass spectrometry targets of submilligrams carbonaceous samples using high-throughput Zn reduction method. Anal. Chem. 2009, 81, 5949–5954.

(35) Donahue, D. J.; Linick, T. W.; Jull, A. J. T. Isotope-ratio and background corrections for accelerator mass spectrometry radiocarbon measurements. Radiocarbon 1990, 32, 135–142.

(36) Stuiver, M.; Polach, H. A. Discussion reporting of 14C data. Radiocarbon 1977, 19, 355–363.
(37) ICRP Publishing, Icrp, C.R.P.I.C.R.P. In ICRP 103: The 2007 Recom mendation of the International Commission on Radiological Protection; Valentin, J., Ed.; Pergamon Press: New York, 2007; Vol. 37, p 56.

(38) Lewis, B. Promoting eye and skin health through intake of the natural carotenoid lutein. In Wild-Type Food in Health Promotion and Disease Prevention; De Meester, F., Watson, R. R., Eds.; Humana Press: Totowa, NJ, 2008; pp 331–342.

(39) Board, N. A. o. S. I. o. M. F. a. N. Vitamin E and β-carotene and other carotenoids. In Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids; National Academy Press: Washington, DC, 2000; pp 240, 250, 366.

(40) Board, N. A. o. S. I. o. M. F. a. N. Folate. In Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline; National Academy Press: Washington, DC, 1998; pp 228, 233, 238, 281.

(41) Gerber, J. M. Vitamins. In Handbook of Preventive and Therapeutic Nutrition; Jones and Bartlett Publishers: Sudbury, MA, 1993; pp 261–279.

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