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ARTICLE

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

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^{14}C has long been used as a tracer for quantifying the in vivo human metabolism of food components, biopharmaceuticals, and nutrients. Minute amounts ($\leq 1 \times 10^{-18}$ mol) of ^{14}C can be measured with high-throughput ^{14}C-accelerator mass spectrometry (HT ^{14}C-AMS) in isolated chemical extracts of biological, biomedical, and environmental samples. Availability of in vivo human data sets using a ^{14}C 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 ^{14}C 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 ^{14}C-β-carotene, ^{14}C-α-tocopherol, ^{14}C-lutein, and ^{14}C-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 airline flight across the United States at 37000 ft was 20 μSv.

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

INTRODUCTION

Carbon-14 (^{14}C) is a radioactive isotope of carbon that is produced by nitrogen-14 (^{14}N) capture of thermal neutrons generated from cosmic rays ($^{1}n + {}^{14}N \rightarrow {}^{14}C + {}^{1}H$) (*1*, *2*). The natural abundance of ^{14}C is 1.2×10^{-10}% (*2*, *3*). ^{14}C has long half-life ($t_{1/2}$ = 5730 years), and it decays to nonradioactive ^{14}N through radioactive β-decay, which emits an electron and an antineutrino (*2*−*6*). Due to very low natural abundance and long half-life, ^{14}C is an ideal tracer for environmental as well as in vivo human or animal studies.

Traditionally, ^{14}C has been measured using decay counters such as a liquid scintillation counter (LSC). LSC usually requires a gram-sized carbon sample and long measuring time (>48 h) to obtain a statistical precision of ≤1% (*4*, *5*). Conventional mass spectrometry (MS) was also an alternative technique to detect various isotopes. However, isotopes such as ^{14}C with rare natural abundance cannot be detected for lack of sensitivity of conventional MS (detection limit of ^{14}C/^{12}C ratio using MS, $\geq 1 \times 10^{-7}$, vs ambient level of ^{14}C/^{12}C ratio, 1×10^{-12}) and for isobar interference (^{14}C vs ^{14}N, $^{12}CH_2^-$, ^{12}CD, $^{13}CH_1^-$) (*5*−*9*).

Accelerator mass spectrometry (AMS) is the ultimate technique to measure long-lived isotopes such as ^{14}C due to extremely high selectivity and sensitivity. AMS was first developed in the early 1930s (*10*), but it was not used for ^{14}C measurement until 1977 due to atomic isobar interference with ^{14}N (*11*). Development of the cesium sputter (Cs^+) method to generate $^{14}C^-$ negative ions enabled AMS to be used for radiocarbon dating (*5*, *8*, *12*, *13*). ^{14}N does not form stable $^{14}N^-$ ions.

AMS methods for various radioisotopes were developed to advance specific research objectives: radiocarbon dating (^{14}C), biological/biomedical study (^{3}H, ^{14}C, ^{26}Al, ^{41}Ca, ^{129}I), nuclear weapon testing (^{41}Ca), monitoring migration of nuclear waste from nuclear power plant (^{36}Cl, ^{129}I), hydrogeological study (^{10}He, ^{36}Cl), and exposure dating (^{10}Be, ^{26}Al, ^{36}Cl) (*5*). ^{14}C was the most popular radioisotope measured with AMS (*3*, *5*). AMS measured the ^{14}C/^{12}C or ^{14}C/^{13}C ratio (rather than ^{14}C 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 ^{14}C tracer.

Biological/biomedical applications using AMS were first suggested ≈30 years ago (*15*), and these applications began in earnest

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Table 1. Summary of Our Prior ^{14}C-Labeled Nutrient Studies (*18*−*21*) and Daily Reference Nutrient Amounts

				α-tocopherol (*20*)		
	unit	β-carotene (*18*)	lutein (*19*)	RRR[a]	all-rac[a]	folic acid (*21*)
molecular weight	g/mol	536.873	568.9	430.71	430.71	441.4
specific activity, SA	Ci/mol	98.8(*18*)	0.2888	56	60	1.24[b]
gender		male (n = 4) female (n = 4)	female (n = 1)	male (n = 1)	male (n = 1)	male (n = 7) female (n = 6)
subject	kg	76.7 ± 23.2	64.1	79.5	79.5	78.3 ± 0.28
body mass index	kg/m^2	25.0 ± 4.2	27.6	23.8	23.8	26.5 ± 5.9
age	year	35 ± 8.2	45	36	36	32 ± 0.38
^{14}C dose (*18*−*21*)	nmol [nCi]	1.01 [100]	125 [36]	1.821 [101.5]	1.667 [99.98]	0.5 [100]
ratio (mol/mol) between ^{14}C dose (*18*−*21*) and Recommended Daily Allowance (RDA) or average intake (AI) in the U.S.		1/6544	1/3 through 1/28	1/19124	1/20891	1/1812 (>19 years) 1/2719 (pregnancy)
reference nutrient amount/day						
RDA or AI in the U.S.	mmol/day	0.0066 (AI) (*17*)	0.0003−0.0035 (AI) (*38*)	0.0348 (>19 years) (RDA) (*39*)		0.0009 (>19 years) 0.0014 (pregnancy) (RDA) (*40*)
therapeutic level	mmol/day	≈0.32 (*39*)	0.011−0.018 (*38*)	0.12−7.43 (*41*)		0.0005−0.022 (*41*)
tolerable upper intake level/day	mmol/day	nd[c] (*39*)	nd (*39*)	2.32 (>19 years) (*39*)		0.0023 (>19 years) (*40*)

[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 ^{14}C-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 (UL) for carotenoids when it reviewed these compounds in 2000.

in the early 1990s (*16*). In vivo in human or in vivo in animal dynamic and kinetic behaviors, absorption, distribution, metabolism, elimination (ADME) of food component, nutrients (*17*−*21*), or environmental chemicals (*22*−*24*) using ^{14}C-AMS have been reported during the past two decades.

For biological/biomedical ^{14}C-AMS applications, very small amounts of ^{14}C-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 combination 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 ^{14}C-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 ^{14}C radiation exposure during the conduct of our four prior studies (*18*−*21*). The method took into account ^{14}C dose level, body mass, biological half-life of ^{14}C-labeled nutrients, tissue weighting factor (radiation exposure in an organ and/or tissue relative 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 20−37.5 kg/m^2 were recruited (**Table 1**). Written informed consent was obtained from volunteers per University of California—Davis (UCD) and Lawrence Livermore National 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 ^{14}C dose, nutrient dose, etc. in each study (*18*−*21*). Nutrients were chemically (β-carotene, α-tocopherol) or biologically (lutein, folic acid) labeled with ^{14}C. ^{14}C-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 ^{14}C-nutrient studies (*18*−*21*). A small ratio (mol/mol) of ^{14}C-nutrient dose to Recommend Dietary Allowances (RDA) was necessary for the physiological-based (steady-state) ^{14}C-tracer study. The RDAs of β-carotene and lutein were not decided yet, so U.S. average intake (AI) was considered for steady-state ^{14}C-tracer study.

Sample Collections. Prior to ^{14}C-nutrient dose, fasting plasma, urine, and feces were collected for ^{14}C baselines of each human subject. Serial plasmas, urines, and feces were collected over time since ^{14}C-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 ^{14}C-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 ^{14}C-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 ^{14}C-nutrient dosing. Durations of β-carotene, lutein, α-tocopherol, and folate studies were 5, 3, 3, and 6 months, respectively. When 100 nCi of ^{14}C was dosed to the 70 kg human subject, collected samples had 0.0014 nCi of ^{14}C/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), enabling waste to be disposed of as nonradioactive waste.

Graphitization. All reagents and materials for sample preparation (*18*−*21*) and graphitization/^{14}C-AMS measurement (*29*−*31*) were previously described and are summarized below. High-throughput (HT) ^{14}C-AMS applications usually required 1 mg of C sample, so carbon content in the sample of interest was measured (*32*). Prior to ^{14}C-AMS measurement, samples of interest were converted to elemental carbon, usually graphite-like materials. Sample preparation (often called graphitization) consisted of oxidation (combustion: sample → CO_2) and reduction (graphitization: $CO_2 \rightarrow C_{\text{graphite-like materials}}$) steps. We have previously used three different graphitization methods for β-carotene (*31*), lutein (*31*), α-tocopherol (*29*, *30*), and folic acid (*29*) studies. Recently, we optimized our graphitization method (maximum 270 samples/day) for accurate and precise HT-^{14}C-AMS measurement (*33*). More information about graphitization is included in the Supporting Information (Figure 1; Table 1).

^{14}C-AMS Measurement. One million voltage AMS at the Center for AMS at LLNL (see Supporting Information, Figure 2) measured

Table 2. Estimated Radiation Exposure from ^{14}C-Labeled Nutrients over the Biological Mean-Life ($\tau_{biological}$) as a Function of Time since Dose

				α-tocopherol (*20*)		
	unit	β-carotene (*18*)	lutein (*19*)	RRR	all-rac	folic acid (*21*)
subject body weight	kg	76.8 ± 23.2 (*n* = 8)	64.1 (*n* = 1)	79.5 (*n* = 1)	79.5 (*n* = 1)	78.3 ± 22.3 (*n* = 13)
dose	nCi	100	36	101.5	99.95	100
half-life ($t_{1/2}$)	day	25	14	2.5	2.5	100
mean-life ($\tau_{biological} = t_{1/2}$/Ln 2)	day	36.1	20.2	3.6	3.6	144.3
tissue weighting factor, W_T, liver		0.04	0.04	0.04	0.04	0.04
fractional nutrient, FN, liver		0.8 (as carotene) 0.9 (as retinol)	0.5	0.8	0.8	0.333
radiation exposure[a]						
whole body, WB	μSv	1.3 ± 0.4	0.3	0.12	0.12	5.2 ± 1.5
liver, 1.5 kg[b]	μSv	63.3 (as carotene) 354 (as retinol)	12.7	6.4	6.3	253
liver, 1.5 kg[c]	μSv	2.0 (as carotene) 12.8 (as retinol)	0.3	0.2	0.2	3.4

[a] Nutrient absorption is assumed 100%. [b] Radiation exposure regardless of W_T and FN. [c] Radiation exposure regard of W_T (ICRP 103, 2008) and FN. The dimensionless multiplicative factors (W_T and FN) are 1 for WB.

^{14}C/^{13}C ratios from graphitized solid samples. Measured ^{14}C/^{13}C ratios of samples were normalized with one of the popular AMS standards such as oxalic acid, NIST SRM 4990B/4990C, or sucrose, IAEA-C6 (*34*, *35*). Finally, ^{14}C level in samples was defined as "Modern or Fraction Modern (F_m)". A current/natural ^{14}C level in living organisms is about 1.1 F_m (= 107.6 amol of ^{14}C/mg of C, 6.72 fCi/mg of C, or 14.92 dpm/g of C) (*30*, *36*).

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

$$\text{exposure} = \frac{E_d}{M_d} \times \text{dose} \times \int_0^\infty e^{t/\tau_{bio}}\, dt = \frac{E_d}{M_d} \times \tau_{bio} \times \text{dose} \times W_T \times \text{FN} \quad (1)$$

where E_d is energy deposition per decay as joules (8.3 fJ/decay), M_d is the affected mass (kg), dose is the amount of radioactivity in dpm, τ_{bio} is the biological mean-life of ^{14}C-labeled compound in minutes or seconds, W_T 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_T and the FN (*3*). The formula (eq **1**) for radiation exposure estimation was modified by considering W_T 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 ^{14}C-β-carotene, which had the τ_{bio} 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_T 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 ^{14}C-food component and ^{14}C-nutrient studies are available as Supporting Information (Table 2).

^{14}C Dosing Calculation for Detecting Optimal ^{14}C Concentration with ^{14}C-AMS. A ^{14}C dose estimation was required for determining the optimal ^{14}C range for the ^{14}C-AMS, which ranged from 0.01 to 100 Modern with resolution of ≤0.05 Modern to the ambient Modern (baseline). The ^{14}C dose level was calculated as follows: (*3*):

$$\text{expected } ^{14}\text{C dose (nCi)} = \text{expected Modern (of samples)} \times 6.11\frac{\text{fCi}}{\text{mgC}} \times \frac{1\ \text{mg of C}}{\text{sample volume or mass}} \times \text{total sample volume or mass} \times 10^6 \quad (2)$$

In general, biological/biomedical AMS easily measured 10 Modern in sample containing 1 mg of C. For example, if 10 Modern was from 25 μL of plasma from a 70 kg human subject, if the ^{14}C-compound was water-soluble, which can be distributed to WB water (maximum 42 L), and if absorption is 100%, then the ^{14}C dose level can be calculated as

$$\text{expected } ^{14}\text{C dose (nCi)} = 10\ \text{Modern} \times 6.11\frac{\text{fCi}}{\text{mg of C}} \times \frac{1\text{mgC}}{25\ \mu\text{L of plasma}} \times 42\ \text{L (in 70 kg human)} \times 10^6 \approx 103\ \text{nCi}$$

Therefore, when the water-soluble ^{14}C compound of ≈100 nCi was dosed 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 ^{14}C 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 ^{14}C-Nutrients. The dose of ^{14}C-nutrients and radiation exposures were estimated over the biological mean-life ($\tau_{biological}$) (**Table 2**). The ^{14}C-nutrients containing 36−101.5 nCi were orally ingested at breakfast by human subjects weighing 52−116 kg. Biological half-lives ($t_{1/2}$) 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 ^{14}C-nutrients was housed in liver regardless of the W_T 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 ^{14}C-β-carotene dose was converted to ^{14}C-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 ^{14}C 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

				α-tocopherol (*20*)		
	unit	β-carotene (*18*)	lutein (*19*)	RRR	all-rac	folic acid (*21*)
dose	nCi	100	36	101.5	99.95	100
estimated maximum ^{14}C level/mg of C (in 25 μL 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 ± 1.6
feces[b]	Modern	≥35	54	18	11	53 ± 50
urine[b]	Modern	≈53	1.0	0.6	1.8	66 ± 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 μSv, which was lower than that experienced during a 4 h flight at 37000 ft altitude (20 μSv). 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 μSv 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 α-, β-, or γ-decay. People are exposed to various radiation sources during their lifetime, and the limit of annual radiation exposure differed between WB, organ, or tissue (see Supporting Information Table 3). ^{14}C emitted an electron for each β-decay, the average energy of which was 8.3 fJ/decay (*3*). Thus, the reference human (70 kg, ≈ 1.1 Modern, assumed 23% carbon content) was naturally/constantly exposed to ≈1.7 nSv/h at the ambient ^{14}C level

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

$$= \frac{120\text{nJ}}{\text{hour}}, \text{accordingly} \frac{120\ \text{nJ}}{\text{hour}} \div 70\ \text{kg} = \frac{1.7\ \text{nSv}}{\text{hour}}$$

where J/kg = Sv) (*3*).

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*). Therefore, full dynamic/kinetic modeling was required for the more exact calculation of human radiation exposure. Furthermore, correction of radiation exposure due to radioisotope decay was not necessary when $\tau_{isotope}$ was much longer than τ_{bio}. In addition, considering W_T and FN enabled more complete calculation than a prior calculation (*3*) 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 ≤1/1812 (*17*, *18*, *20*, *21*), which was even smaller than the microdosing 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 $\leq$20% bioavailability required a larger dose of $\approx$200 nCi to study ^{14}C-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 and fractional nutrient factor, the radiation exposures to WB (0.12−5.2 μSv) or to liver (0.2−3.4 μSv) were much lower than that from a 4 h aircraft flight (20 μSv). Furthermore, ^{14}C-doses of 36−100 nCi were measured in the range of 0.01−100 Modern in plasma, urine, and feces aliquots that contained 1 mg of C. All materials and samples from graphitization and ^{14}C-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.

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