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Carbon Isotopes Profiles of Human Whole Blood, Plasma, Red Blood Cells, Urine and Feces for Biological/Biomedical ^{14}C-Accelerator Mass Spectrometry Applications

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Abstract

Radiocarbon (^{14}C) is an ideal tracer for in vivo human ADME (absorption, distribution, metabolism, elimination) and PBPK (physiological-based pharmacokinetic) studies. Living plants preferentially incorporate atmospheric $^{14}CO_2$, vs $^{13}CO_2$, vs $^{12}CO_2$, which result in unique signature. Furthermore, plants and the food chains they support also have unique carbon isotope signatures. Humans, at the top of the food chain, consequently acquire isotopic concentrations in the tissues and body fluids depending on their dietary habits. In preparation of ADME and PBPK studies, 12 healthy subjects were recruited. The human baseline (specific to each individual and their diet) total carbon (TC) and carbon isotope ^{13}C (δ^{13}C) and ^{14}C (F_m) were quantified in whole blood (WB), plasma, washed red blood cell (RBC), urine, and feces. TC (mg of C/100μL) in WB, plasma, RBC, urine, and feces were 11.0, 4.37, 7.57, 0.53, and 1.90, respectively. TC in WB, RBC, and feces was higher in men over women, $P < 0.05$. Mean δ^{13}C were ranked low to high as follows, feces < WB = plasma = RBC = urine, $P < 0.0001$. δ^{13}C was not affected by gender. Our analytic method shifted δ^{13}C by only ± 1.0 ‰ ensuring our F_m measurements were accurate and precise. Mean F_m were ranked low to high as follows, plasma = urine < WB = RBC = feces, $P < 0.05$. F_m in feces was higher for men over women, $P < 0.05$. Only in WB, ^{14}C levels (F_m) and TC were correlated with one another ($r = 0.746$, $P < 0.01$). Considering the lag time to incorporate atmospheric ^{14}C into plant foods (vegetarian) and or then into animal foods (non-vegetarian), the measured F_m of WB in our population (recruited April 2009) was 1.0468 ± 0.0022 (mean±SD), the F_m of WB matched the (extrapolated) atmospheric F_m of 1.0477 in 2008. This study is important in presenting a procedure to determine a baseline for a study group for human ADME and PBPK studies using ^{14}C as a tracer.

Keywords

Carbon isotopes; accelerator mass spectrometry; human blood; urine; feces

INTRODUCTION

Carbon is the fourth most abundant element (180 ppm) in the earth's crust.[1] Elemental carbon has two stable isotopes (^{12}C, ^{13}C) and one radioactive isotope (^{14}C). The natural

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abundance of ^{12}C is 98.89 %, ^{13}C is 1.108 %, and ^{14}C is 1×10^{-10} %.[1] The ^{14}C has 6 protons and 8 neutrons and is present in biomass-based carbons but not petroleum-based carbons. Therefore biomass-based versus petroleum-based compounds differ from one another in the ratios of ^{14}C to ^{13}C to ^{12}C. ^{14}C is produced in the upper atmosphere by neutron and nitrogen atom reactions ($^{1}n + {}^{14}N \rightarrow {}^{14}C + {}^{1}H$), and most of the ^{14}C is stored in the oceans.[1-3]

^{14}C is an ideal label/tag compared to ^{13}C for long-term in vivo human ADME (absorption, distribution, metabolism, elimination) and PBPK (physiological-based pharmacokinetic) studies of food components, nutrients and or new drugs for the following four reasons.[1,3-6] First ^{14}C has a low natural abundance. Second, ^{14}C has a long half-life ($t_{1/2}$ = 5,730 yrs). Third ^{14}C is stably incorporated into many natural/organic molecules and or compounds. Fourth, ^{14}C can be measured at attomol levels of sensitivity with Accelerator Mass Spectrometry (AMS) using samples that contain a milligram or less of carbon.

AMS was originally developed and used for radiocarbon dating in geochronology, archaeology, and anthropology. Recently, AMS has found biological and biomedical applications that have already filled important gaps in quantitative understandings of the long-term in vivo human ADME and PBPK of new drugs candidates, nutrients, and preservation of food components.[7-16] Furthermore, the Food and Drug Administration[17] and the European Medicines Agency[18] recently approved ^{14}C (the ideal tracer) for long-term in vivo human ADME and PBPK dosing of humans with ^{14}C-labeled compounds. The doses were to be equal to or less than 1/100th of the therapeutic level or 1/50th of the no-observed adverse effect level from rodent and non-rodent species for two weeks, these doses are now commonly referred to as microdoses.

Atmospheric levels of ^{14}C doubled (over 1950s levels) because of nuclear weapons testing in the early 1960s whereas atmospheric levels of ^{12}C and ^{13}C have been relatively stable. Living plants incorporate atmospheric $^{14}CO_2$, $^{13}CO_2$, and $^{12}CO_2$ in proportion to the natural abundance of the C-isotopes and to the extent that plants, their individual tissues, and the food chains they support discriminate against heavier C-isotopes (isotope fractionation). So the ratio of $^{14}C/^{13}C$ (that AMS measures) in living organisms reflect ambient atmospheric ^{14}C levels, with small variation among organs, and dietary habit etc.

A search of the scientific literature concerning baseline/ambient levels of carbon isotopes and/or profiles in human fluids and tissues revealed only three citations.[5,19,20] Blood plasma drawn in early Y 2004 from humans at various US locations had average F_m values of 1.086 ± 0.014.[20] ^{13}C and ^{14}C levels in bloods of humans deceased in Y 2006 had an average $\delta^{13}C$ of −19.13 ± 1.15 and an average F_m value of 1.0607 ± 0.0069.[19] ^{14}C levels (in units of F_m) in whole blood (WB), plasma, and blood clots of humans which were collected in Japan in Y 2007 averaged the F_m of 1.0814 ± 0.0838, 1.0861 ± 0.0821, and 1.1667 ± 0.0428, respectively.[5] The calculated values[5] were based on the carbon content of 11 % for WB, 4.4 % for plasma, and 15 % for the blood clots. Finally, we found no values for ^{13}C and ^{14}C levels for urine or feces from humans. Therefore, the present study reports baseline (specific to each individual and their diet) total carbon (TC) contents, ^{13}C levels, and ^{14}C levels in fasting WB, plasma, and red blood cell (RBC), and in urine and feces from twelve healthy humans. This report is important because it provides baseline reference values of carbon isotopes contents and profiles for human ADME and PBPK studies using high-throughput (HT)-bio-^{14}C-AMS.

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MATERIALS and METHODS

Subject Selection

Healthy, non-smoking subjects (6 men and 6 women) were participated in this study. The range of their age was 19 – 39 y and their body mass index (BMI) ranged from 18.9 – 24.7 kg/m^2 (Table 1). This study was approved by the Institutional Review Board (IRB) and informed consent was obtained from all subjects. The study was conducted following Good Clinical Practice guidelines (Ver. 1989) and the ethical guidelines of the 1975 Declaration of Helsinki.

Sample Collections

Fasting WB was collected in Vacutainer containing K_2EDTA (BD Diagnostic). Plasma and RBC were collected from one K_2EDTA Vacutainer where the original total volume was marked then promptly centrifuged at 1380 × g for 15 min (Fisher Scientific Centrific model 228 centrifuge, Labequip). The plasma aliquot was extracted after centrifugation. The remaining RBC were washed twice with a phosphate buffered saline (10 mM K_2HPO_4, 2.7 mM KCl, and 137 mM NaCl , pH 7.4 at 25 °C) then reconstituted to the original volume with the phosphate buffered saline, mixed, and stored in small aliquot (washed RBC).

A baseline 24-h collection of urine in 2 L Urisafe containers (Fisher Scientific, Fairlawn, NJ) was obtained from each subject. A baseline feces collection was in 4-mm-thick Stomacher bags (Fisher Scientific, Fairlawn, NJ). Feces were homogenized for 2 min with methanol (1:2, feces: methanol, w/v). Only urine and feces were collected for three week in order to examine the day to day variability in carbon content in each subject. All samples were stored in − 80 °C until analyzed for TC, ^{13}C, and ^{14}C.

Analysis of TC, ^{13}C, and ^{14}C

TC contents in all samples were measured as previously described[21] using a Model 1112 carbon/nitrogen elemental analyzer (Thermo Finnegan, Rodano, Italy). TC contents were expressed as mg of carbon (C)/100 μL of samples.

^{13}C concentrations were measured as previously described[22] using a Europa 20/20 isotope ratio mass spectrometer (IR-MS, Sercon Ltd., Cheshire, UK). ^{13}C concentrations were expressed as the change (δ) per mil (‰) difference between the $^{13}C/^{12}C$ ratio in the sample and that in a known laboratory reference standard according to the following formula: $\delta^{13}C = [(^{13}C/^{12}C \text{ of sample}/^{13}C/^{12}C \text{ of VPDB-standard}) - 1] \times 10^3$ ‰, so the ^{13}C enrichment was expressed relative to that of a well-known laboratory reference standard (Vienna-Pee Dee Belemnite, VPDB). The measured $\delta^{13}C$sample values are used in the calculation of F_m reported in this study.

^{14}C concentrations were measured as previously described.[22-25] Prior to ^{14}C measurement, all samples of interest must be converted to elemental carbon such as graphite and/or graphite-like materials[25], called graphitization.[24] The graphitized samples were packed into AMS target holders and ^{14}C concentrations were measured at the center for AMS, Lawrence Livermore National Laboratory. In this report, the ^{14}C concentrations were expressed in units of "Fraction Modern" (F_m), a sample having a F_m of 1.0 would contain 97.89 femtomole (fmol) ^{14}C/g of C, 6.11 pico curie (pCi)/g of C or 13.56 disintegrations per minute (dpm)/g of C in that sample.[3] Therefore, if a 0.025 mL aliquot of plasma contained 1 mg of C and had an F_m = 1.1, then 1.0 mL of that plasma would contain 4.3 fmol ^{14}C/mL plasma (1.1 F_m × 97.8 fmol ^{14}C/g of C × 0.04 g of C/mL plasma) or 0.59664 dpm/mL plasma (1.1 F_m × 13.56 dpm/g of C × 0.04 g of C/mL plasma). The measured ambient F_m varies with year of sampling. An example of a recent ambient F_m is the mean atmospheric

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value of 1.0599 measured at the High Alpine Research Station (Swiss Alps, 46°33′N, 7°59′E, 3450 m a.s.l.) in Y 2006.[26]

In the present study, AMS measures the ratios of $^{14}C/^{13}C$ in the sample of interest and in the AMS standard Oxalic acid II (OX-2, NIST 4990C). The atmospheric $^{14}CO_2$ in 1950 is the defined absolute reference F_m of 1.0. The defined absolute reference ^{14}C-radioactivity is 0.7459 that of the OX-2 ^{14}C-radioactivity. Thus 0.7459 appears in the F_m calculation.[27] The Modern (time zero) is defined as 1950.

The ^{13}C content of OX-2 (−17.8 ‰) differs from the constant atmospheric ^{13}C content (−25 ‰) due to isotopic fractionation in the biological system used to generate the OX-2.[27] Thus the adjustment (0.975/0.9822) appears in the equation below. So, the biological isotopic fractionation in the biological sample is normalized to a constant atmospheric ^{13}C content by $[0.975/(1 + \delta^{13}C_{sample}/1000)]$.

In the present study, AMS measures the ratio of $^{14}C/^{13}C$ in sample of interest and $^{14}C/^{13}C$ in the OX-2 standard. The $\delta^{13}C_{sample}$ was measured by the IR-MS described above.

$$F_m = \frac{^{14}C_{sample}}{^{14}C_{1950}} = \left\{ \frac{\left[\frac{0.975}{1+(\delta)^{13}C_{sample}/1000} \right] \times \left(^{14}C/^{13}C \right)_{sample}}{0.7459 \times \frac{0.975}{0.9822} \times \left(^{14}C/^{13}C \right)_{OX-2}} \right\}$$

The $^{14}C_{1950}$ is a hypothetical atmospheric carbon-14 level in 1950 normalized to ^{13}C of −25 ‰. Thus F_m is the ^{14}C ratio in the sample of interest relative to the defined standard.[27]

Finally, the data were analyzed using analysis of variance (ANOVA). The dependent variables were TC content; $\delta^{13}C$ levels before graphitization; $\delta^{13}C$ levels after graphitization; and F_m values. The independent variables were the sample types (fasting whole blood; fasting plasma; fasting washed RBC; urine; and feces) and gender of the study population. Differences among treatments were evaluated using Fisher's Protected Least Significant Difference (PLSD). The results were presented as mean ± standard deviation (mean ± SD).

RESULTS

Table 2 shows the concentration of TC (mg of C/100μL) in WB, plasma, RBC, urine, and feces. WB, plasma, and RBC were collected at a fasting state and analyzed in duplicate. A wide subject by subject variation was observed in urine and feces that were collected over a three week period. There was a 2.39 fold difference between subjects in terms of urine carbon and a 1.64 fold difference in fecal carbon. TC content ranked from low to high as follows: urine (0.53 %) < feces (1.90 %) < plasma (4.37 %) < RBC (7.57 %) < WB (11.0 %), $P < 0.0001$. WB, RBC, and feces in males had a higher level of TC than females $P < 0.05$, while plasma and urine were not gender dependent. TC content in WB, RBC, and plasma had smaller daily differences, so 11 μL of WB, 15 μL of washed RBC, and 25 μL of plasma reliably provided one mg of C, which was needed for accurate and precise HT-bio-^{14}C-AMS measurement.[28] TC content in the washed RBC (7.57 %) was one half the content in the non-washed RBC (17 %)[3] because the washed RBC was diluted with a phosphate buffered saline in the present study. Although the TC in urine and feces varied among subjects and even varied from day to day in the same subject ($P < 0.0001$), an aliquot of ≈ 200 μL of urine and ≈ 60 μL of fecal homogenate provided one mg of C, which was usually needed for accurate and precise HT-bio-^{14}C-AMS measurement.[28]

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Table 3 summarizes ^{13}C levels ($\delta^{13}C$) in WB, plasma, RBC, urine, and feces before and after graphitization process. Mean $\delta^{13}C$ values before and after graphitization were ranked from low to high as follows: feces < WB = plasma = RBC = urine, $P < 0.0001$. The amount of $\delta^{13}C$ in samples before graphitization differed among subjects ($P < 0.0001$). Since this difference was independent of gender, dietary habits could be responsible for this variation. The correlation between TC and $\delta^{13}C$ (for all samples) was not statistically significant.

After graphitization, our analytic method shifted $\delta^{13}C$ (up or down from baseline) by only 1.0 ‰ ensuring our F_m measurements were accurate and precise. The regression analysis of $\delta^{13}C$ before and after graphitization showed a $\delta^{13}C$ of urine was slightly less well fitted ($r^2 = 0.94$) against the line of identity ($y = x$, $r^2 = 1.0$) than was WB ($r^2 = 0.98$), RBC ($r^2 = 0.98$), plasma ($r^2 = 0.99$), and feces ($r^2 = 0.98$). Variations in the graphitization yield (81 ± 17 %) caused by other components like sodium or amines in urine may have contributed to the lowered r^2 value in urine. Regardless of the observed $\delta^{13}C$ shift in urine, the accuracy and precision of ^{14}C-bio-AMS measurement was not affected.

Table 4 shows the correlation of $\delta^{13}C$ between WB, RBC, plasma, urine, and feces prior to graphitization only. Any isotopic fractionation effect during the graphitization was eliminated for the correlation analysis. Blood and blood fractions (WB, plasma, and RBC) were highly correlated with one another ($r = 0.95 – 0.99$, $P < 0.0001$), but not with urine ($r = 0.63 – 0.66$, $P < 0.001$) nor with feces ($r = 0.53 – 0.57$, $P < 0.01$). Feces was more highly correlated with urine ($r = 0.77$, $P < 0.0001$) than blood and blood fractions (WB, RBC, plasma). Differences in $\delta^{13}C$ between WB, plasma, RBC, urine, and feces could be due to different metabolic system, turnover system/time, etc. in human.

Figure 1 shows a bomb curve adopted from Levin and Kromer (2004)[29] and Levin et al. (2008).[26] The bomb curve (insert, Figure 4) showed the atmospheric ^{14}C level from Y 1959 to 2006 calendar years. The enlarged bomb curve shows the atmospheric ^{14}C level from Y 1991 to 2006 calendar years (red rectangle area in the insert). The atmospheric CO_2 kinetics was a complex process, and using the bomb curve to extrapolate the present atmospheric ^{14}C level was not straight forward. In summary, fitting the full dataset from the peak at Y 1963 to 2006 calendar year, the second-order polynomial extrapolation overestimated contemporary atmospheric ^{14}C level, while a linear extrapolation underestimated it. The present study used the dataset that included the period Y 1991 to 2006 only. The regression analysis (exponential, second-order polynomial, and linear regression) showed no difference between the regression methods for the time period of interest. The present study used a linear regression to extrapolate atmospheric ^{14}C level from 2007 to 2010 calendar years. The mean atmospheric ^{14}C measured in 2006 was 1.0599 F_m. Mean atmospheric ^{14}C extrapolated was 1.0477 F_m in 2008 and 1.0420 F_m in 2009. The estimated atmospheric ^{14}C concentration was then compared with ^{14}C level in WB, plasma, RBC, urine, and feces that were collected in April 2009 as seen in Table 5.

Table 5 shows the mean F_m in WB, plasma, RBC, urine, and feces. The F_m ranking from low to high was: plasma = urine < WB = RBC = feces, with $P < 0.05$. Individual variation in F_m of WB, plasma, RBC, urine, and feces was observed ($P < 0.05$), whereas the F_m of feces was only higher in men than women, $P < 0.05$. In addition, ^{14}C levels (F_m) and TC were highly correlated only in WB ($r = 0.746$, $P < 0.01$). Considering the lag time to incorporate atmospheric ^{14}C into plant foods (vegetarian) then into meat supply (non-vegetarian), the measured F_m of WB in our population (recruited April 2009) was 1.0468 ± 0.0022 (mean ±SD). This matched the extrapolated atmospheric F_m of 1.0477 in 2008.[26,29] For the same subjects, the measured F_m (1.0320) of plasma was similar to the atmospheric ^{14}C level (1.0363 F_m) in 2010. In contrast, the measured F_m (1.0597) of RBC was matched to the mean atmospheric ^{14}C level (1.0599 F_m) measured in 2006.

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DISCUSSION

Elemental carbon consisted of three natural isotopes (^{12}C, ^{13}C, ^{14}C), and twelve human-made isotopes (^{8}C – ^{22}C). Among various carbon isotopes, ^{11}C, ^{13}C, and ^{14}C are the most popular isotopes for in vivo tracer studies in human and/or non-human models. Generally, ^{11}C was measured by positron emission tomography (PET), ^{13}C by conventional MS/isotopic ratio MS (IRMS), and ^{14}C by AMS (/or decay counter).

Using MS (/or IRMS) for ^{13}C avoids the radiation exposure and the cost of disposing radioactive waste.[30] The MS (/or IRMS) also measures a ratio of ^{13}C-tracer and tracee, and can also determine metabolites profiles, simultaneously. However, due to ^{13}C detection limit of MS (/or IRMS), ^{13}C-MS applications need to administer relatively large amounts of ^{13}C-labeled compounds which led to a non-steady-state condition in vivo in human. In contrast, although the PET or AMS can measure very small amounts of ^{11}C or ^{14}C with only one microdosing, administration of ^{11}C and ^{14}C to human are limited because of human health caused by radiation exposure.

^{13}C level in humans or animals varied with dietary habits (vegetarian, semi-vegetarian, and non-vegetarian) and dietary source (C3 plants *vs*. C4 plants, terrestrial-based sources *vs*. marine-based sources, etc.). In the present study, differences in the $\delta^{13}C$ values in WB, plasma, RBC, urine, and feces were statistically significant ($P < 0.0001$). Furthermore, $\delta^{13}C$ in WB, plasma, RBC, urine, and feces were also statistically differed between subjects, suggesting that subjects in our population consumed food from different dietary sources and had different dietary habits. For example, the $\delta^{13}C$ values of subjects 6 and 7 in the present study were similar to one another in that their menu included moose meat almost daily. The $\delta^{13}C$ values of subjects 4, 5, and 10 were similar to one another in that their menu was a mix (burrito, pizza, beer, soda etc.). Finally, subject 11 consumed Spanish wine on a daily basis, and for this subject the wine might account for the lower $\delta^{13}C$ values in urine and feces.

In general, persons who ate marine-based foods or C4 plants like corn reflected higher $\delta^{13}C$ values than those ($\delta^{13}C$) of persons with diets based on C3 plant-based foods.[19] A prior study[31] reported that elders who lived in Southwest Alaska had lower ^{13}C level in RBC compared to ^{13}C level of the younger population that lived at same region. This difference was from different dietary habits/sources between the elder population (more intake of marine-based foods) and younger population (more intake of certain marker foods).[31] Furthermore, dietary protein source was also affected to ^{13}C level in human hair protein, and omnivores had higher ^{13}C level than vegetarians.[32] Thus, individuals who participated in this study might consume more terrestrial-based C3 plants, meat, or etc. instead of the consumption of C4 plants or marine-based foods. The $\delta^{13}C$ of feces strongly reflected ^{13}C levels of dietary sources compared to those of WB, plasma, RBC, and urine, because of its direct tie to dietary intake.

The applications of ^{11}C-PET and ^{14}C-AMS have greatly improved ADME, PK, and mass balance studies of nutrients, biopharmaceuticals, and drug (/or candidates) in vivo in human. Prior to development of AMS, ^{14}C applications in vivo in humans were limited due to potential of the high radiation exposure caused by a requirement for large ^{14}C dose. Recent developments of high accuracy, precision, and sensitivity of PET and AMS have led to an increased use of ^{11}C-PET and ^{14}C-AMS applications. Significantly, ^{14}C-AMS has been applied to long-term tracer studies in vivo in humans with only one time microdosing.

The ^{14}C level in living creatures reflects the atmospheric input of carbon in the form of CO_2 through photosynthesis into the food chain. Nydal et al. (1971)[33] reported a good agreement in ^{14}C level between human blood and human hair. ^{14}C level in human blood plasma which was collected in various US areas in early Y 2004 was 1.086 ± 0.014 F_m. And, natural ^{14}C

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level in plants and animals was ≈ 1.075 F_m in Y 2005. These ^{14}C values were also well matched to the ^{14}C levels in the atmosphere at the same time.[20]

The ^{14}C level in the atmosphere decreased exponentially (mean life ≈ 16 yrs) since atmospheric bomb tests were discontinued.[34] The ^{14}C level in the atmosphere slowly declined for the past 20 years at an average rate ≈ 0.0061 F_m per year. In general, extrapolation using a linear regression fit underestimated the ^{14}C level in the atmosphere, while a second-order polynomial regression slightly overestimated the ^{14}C level.[19] The atmospheric ^{14}C level in Y 2009 was extrapolated to 1.0420 F_m with a linear regression fit, when the present study truncated the dataset to include only Y 1991 to 2006.[26,29]

The ^{14}C level differed between atmosphere and human or animal (organs/tissues) because of their lag time (i.e. 1.1 year for blood, 1.8 year for lung, much greater time for collagen within cartilage) caused by tissue turnover (i.e. life-time of RBC: 120 days) and food dietary issues.[34-38] For example, ^{14}C level in herbivores show a slight delay compared to that of atmosphere owing to the fast turnover time of their primary carbon source. In contrast, ^{14}C level in humans or animals (omnivores or carnivores) further delayed compared to that of atmosphere because of longer turnover time of their carbon source.[34] Therefore, ^{14}C level of human blood collected in Y 2009 was close to that (1.0477 F_m) in atmosphere in Y 2008. Of course, ^{14}C level of humans was also reflected by ^{14}C level in dietary sources at cultivation/ harvest year.

In the present study, the mean ^{14}C level in WB, RBC, plasma, urine, and feces showed statistically significant differences ($P < 0.05$). The ^{14}C level in the WB matched the atmospheric level in Y 2008. This was consistent with a lag time of ^{14}C level between atmosphere and human blood. Some subjects had ^{14}C levels (in plasma, RBC, urine, and feces) that were slightly higher or slightly lower than the expected during Y 2008 to 2009 calendar years, these differences could be accounted for by human to human variations caused by different dietary habit/sources, delay time, tissue turnover, some combinations of the above, and others. Our results were also consistent with a prior report[38] which reported that an individual's diet can play as an important role in establishing radiocarbon levels in vivo in human.

In conclusion, the content of carbon isotopes in human WB, RBC, plasma, urine, and feces was affected by dietary source/habit of each subject in our population. In the present study, TC in human's WB, plasma, RBC, urine, and feces ranged from 0.53 to 11.0 mg of C/100 μL. ^{13}C level ($\delta^{13}C$) in human's WB, plasma, RBC, urine, and feces ranged from −23.58 to −19.92 ‰, and ^{14}C level (F_m) in human's WB, RBC, plasma, urine, and feces also varied from 1.0320 to 1.0597 F_m. TC content in WB, RBC, and feces had gender effect, $P < 0.05$. And there was no gender effec on ^{13}C levels in the samples, however there was a gender effect in ^{14}C levels (F_m) only in the feces ($P < 0.05$). Thus the observed gender effect is probably related to diet. In addition, TC content was correlated with ^{14}C levels (F_m) only in WB ($r = 0.746$, $P < 0.01$). Therefore, accurate and precise HT- bio-^{14}C-AMS measurements would require baseline TC, ^{13}C, and ^{14}C levels in human samples prior to the ^{14}C tracer study in vivo in human.

Finally, this report is important because it provides baseline reference values of carbon isotopes contents and profiles, which are individual and diet specific for human ADME and PBPK studies using high-throughput (HT)-bio-^{14}C-AMS.

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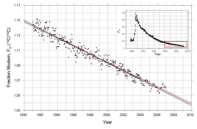

Figure 1.
The bomb curve shows the change of atmospheric ^{14}C level from Y 1959 to 2006. The bomb curve (insert) was adopted from data of Levin and Kromer (2004)[29] and Levin et al (2008).[26] The enlarged bomb curve shows the ^{14}C level in atmosphere from Y 1991 to 2006. The atmospheric ^{14}C level was extrapolated up to the Y 2010 with a linear regression fit.

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Table 1

Characteristics of twelve human subjects that participated in the present study.

Subject	Gender	Age, y	BMI, kg/m^2	Height, cm	Weight, kg	Packed Cell Volume (PCV), %
2	Female	25.0	18.9	162.6	49.9	39.9
3	Female	23.0	20.0	170.2	58.1	36.7
4	Male	22.0	23.0	180.3	74.8	38.6
5	Male	23.0	24.4	185.4	83.9	39.2
6	Female	26.0	21.0	167.6	59.0	43.6
7	Male	25.0	22.9	175.3	70.3	46.0
8	Male	39.0	22.1	188.0	78.0	44.1
9	Male	32.0	24.7	177.8	78.0	43.5
10	Male	23.0	23.1	182.9	77.1	40.2
11	Female	29.0	21.0	167.6	59.0	39.9
12	Female	39.0	19.7	170.2	57.2	39.2
13	Female	19.0	21.0	167.6	59.0	36.6
Range (highest/lowest)		2.05	1.25	1.16	1.44	1.26
Mean ± SD						
Female	n = 6	26.8 ± 6.8	20.3 ± 0.9^a	167.6 ± 2.8^a	57.0 ± 3.6^a	39.3 ± 2.6^a
Male	n = 6	27.3 ± 6.8	23.4 ± 1.0^b	181.6 ± 4.8^b	77.0 ± 4.5^b	41.9 ± 3.0^b
Total	n = 12	27.1 ± 6.5	21.8 ± 1.8	174.6 ± 8.2	67.0 ± 11.1	40.6 ± 3.0

^{a} ^{b}Females had lower BMI, Height, Weight, and Packed Cell Volumes than did males $P < 0.05$.

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Table 2

The total carbon (TC) contents of whole blood (WB), plasma, red blood cell (RBC), urine, and feces from twelve human subjects.

Subject	Gender	TC content using elemental analyzer, mg of C/100μL				
		WB†	Plasma†	RBC†	Urine‡	Feces‡
		Values based on one fasting blood from each subject, analyzed in duplicate			Values based on 24 to 32 urine and 11 to 32 feces collections, analyzed in duplicate	
2	Female	10.89	4.38	7.29	0.31 ± 0.16, n = 24	1.75 ± 0.50, n = 13
3	Female	10.96	4.47	7.36	0.35 ± 0.23, n = 26	1.34 ± 0.73, n = 11
4	Male	11.33	4.34	7.63	0.59 ± 0.27, n = 28	2.20 ± 0.33, n = 15
5	Male	11.11	4.26	7.47	0.52 ± 0.26, n = 32	1.93 ± 0.49, n = 23
6	Female	10.16	4.82	7.10	0.67 ± 0.27, n = 28	1.45 ± 0.37, n = 32
7	Male	11.69	4.34	8.43	0.68 ± 0.24, n = 29	2.45 ± 0.77, n = 19
8	Male	11.56	4.09	8.18	0.53 ± 0.28, n = 28	2.51 ± 0.35, n = 17
9	Male	11.09	4.50	8.17	0.44 ± 0.19, n = 30	2.19 ± 0.42, n = 19
10	Male	11.18	4.54	7.57	0.74 ± 0.34, n = 29	1.45 ± 0.36, n = 28
11	Female	11.13	4.35	7.65	0.37 ± 0.15, n = 28	1.78 ± 0.53, n = 16
12	Female	10.19	4.14	6.87	0.42 ± 0.23, n = 28	1.58 ± 0.32, n = 24
13	Female	10.43	4.15	7.16	0.73 ± 0.31, n = 29	2.12 ± 0.66, n = 20
Range (highest/lowest)		1.15	1.24	1.18	2.39	1.64
Mean ± SD						
Female	n=6	10.63 ± 0.42^{az}	4.19 ± 0.30^{x}	7.24 ± 0.26^{ay}	0.48 ± 0.18^{v}	1.67 ± 0.28^{aw}
Male	n=6	11.33 ± 0.25^{bz}	4.23 ± 0.26^{x}	7.91 ± 0.40^{by}	0.58 ± 0.11^{v}	2.12 ± 0.39^{bw}
Total	n=12	10.98 ± 0.49^{z}	4.37 ± 0.20^{x}	7.57 ± 0.48^{y}	0.53 ± 0.15^{v}	1.90 ± 0.40^{xw}

†Fasting whole blood was drawn into tubes containing K_2EDTA. One aliquot of whole blood was removed. Plasma was separated by centrifugation and removed, and RBC were washed twice then reconstituted. Each whole blood, plasma, and washed RBC was analyzed in duplicate.

‡The subjects collected 24-32 urine samples and collected 11-32 fecal samples over the three week period. All urine and feces were analyzed. The day to day variation seen in a subject and the variation between subjects were high in urine and feces.

$v - z$Values with different superscripts differed from one another samples ($P < 0.0001$).

$a - b$WB, RBC, and feces from males have a higher level of total carbon over those from females, $P < 0.05$.

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Table 3

The ^{13}C levels ($\delta^{13}C$) of whole blood (WB), plasma, red blood cell (RBC), urine, and feces from twelve subjects. Each value is a mean of a duplicate analysis using IR-MS. Each value is based on a fasting blood, blood fractions, urine, and feces from each subject, analyzed in duplicate before and after graphitization.

Subject	Gender	^{13}C level using IR-MS, $\delta^{13}C = [(^{13}C/^{12}C \text{ of sample})/(^{13}C/^{12}C \text{ of VPDB-standard}) - 1] \times 10^3$ ‰									
		WB		Plasma		RBC		Urine		Feces	
		Before	After	Before	After	Before	After	Before	After	Before	After
2	Female	−21.27	−21.32	−21.39	−21.17	−21.43	−21.25	−21.34	−21.82	−23.18	−23.31
3	Female	−19.94	−20.06	−20.20	−20.03	−20.13	−20.09	−21.15	−21.63	−24.52	−24.63
4	Male	−19.13	−19.37	−19.56	−19.38	−19.23	−19.22	−20.11	−20.84	−21.61	−22.06
5	Male	−19.09	−19.23	−19.44	−19.25	−19.21	−19.16	−19.17	−19.90	−21.81	−22.35
6	Female	−20.04	−20.21	−20.36	−20.25	−20.06	−20.06	−19.66	−20.75	−23.36	−23.57
7	Male	−20.25	−20.41	−20.59	−20.38	−20.40	−20.36	−20.81	−21.08	−23.43	−23.86
8	Male	−20.24	−20.46	−20.55	−20.37	−20.39	−20.38	−21.02	−21.67	−25.09	−25.41
9	Male	−20.30	−20.65	−20.83	−20.73	−20.20	−20.34	−21.05	−21.70	−24.15	−24.34
10	Male	−18.74	−18.98	−18.96	−18.86	−18.91	−18.97	−18.88	−19.56	−21.91	−22.31
11	Female	−19.91	−20.18	−20.30	−20.20	−19.90	−19.99	−22.47	−23.34	−25.07	−25.29
12	Female	−19.77	−20.13	−20.01	−19.88	−19.98	−20.06	−20.74	−20.98	−24.63	−24.97
13	Female	−20.37	−20.64	−20.78	−20.75	−20.24	−20.37	−20.54	−21.28	−24.24	−24.39
Means ± SD											
Before	n=12	−19.92 ± 0.68^{y}		−20.25 ± 0.67^{y}		−20.01 ± 0.67^{y}		−20.58 ± 1.00^{y}		−23.58 ± 1.25^{x}	
After	n=12	−20.14 ± 0.67^{y}		−20.10 ± 0.67^{y}		−20.02 ± 0.64^{y}		−21.21 ± 0.97^{y}		−23.87 ±1.17^{x}	
Total	n=24	−20.03 ± 0.67^{y}		−20.18 ± 0.66^{y}		−20.01 ± 0.64^{y}		−20.90 ± 1.02^{y}		−23.73 ± 1.20^{x}	
Regression†		y=0.97x –0.91, R^2=0.98		y=0.99x – 0.01, R^2=0.99		y=0.95x – 1.04, R^2=0.98		y=0.95x – 1.71, R^2=0.94		y=0.93x – 1.99, R^2=0.98	

†The regression was indicated to the difference of $\delta^{13}C$ between before graphitization and after graphitization compared to the line of identity (y=x, R^2 =1).

$^{x-y}$Feces had a lower $\delta^{13}C$ value than all other samples ($P < 0.0001$), irrespective of whether the feces were before or after graphitization. Gender differences were not significant to all samples, $P > 0.05$.

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Table 4

Correlation matrix of ^{13}C levels ($\delta^{13}C$) in whole blood (WB), plasma, red blood cell (RBC), urine, and feces collected from twelve human subjects.

	WB	Plasma	RBC	Urine	Feces
WB		0.987^{*****}	0.986^{*****}	0.643^{*}	0.555^{ns}
Plasma			0.952^{*****}	0.666^{*}	0.573^{*}
RBC				0.628^{*}	0.533^{ns}
Urine					0.768^{**}
Feces					

ns non-significant,

* 5 % level,

** 1 % level,

*** 0.1 % level,

**** 0.01 % level,

***** < 0.0001 % level

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Table 5

The ^{14}C levels (F_m) of whole blood (WB), plasma, red blood cell (RBC), urine, and feces from twelve subjects. Each value is a mean of a duplicate analysis using ^{14}C-AMS.

Subject	Gender	1.0 F_m = 97.8 fmol ^{14}C/g of C = 6.11 pCi/g of C = 13.56 dpm/g of C				
		WB	Plasma	RBC	Urine	Feces
		Each value is based on a fasting blood, blood fractions, urine, and feces from each subject, analyzed in duplicate after graphitization				
2	Female	1.0502	1.0452	1.0519	1.0503	1.0476
3	Female	1.0573	1.0399	1.0564	1.0499	1.0582
4	Male	1.0433	1.0333	1.0606	0.9376	1.0515
5	Male	1.0384	1.0416	1.0559	1.0206	1.0553
6	Female	1.0380	1.0286	1.0672	1.0208	1.0503
7	Male	1.0414	1.0452	1.0755	1.0415	1.0653
8	Male	1.0481	1.0269	1.0686	1.0468	1.0587
9	Male	1.0389	1.0247	1.0588	1.0362	1.0651
10	Male	1.0504	1.0323	1.0561	1.0442	1.0590
11	Female	1.0377	1.0278	1.0552	1.0634	1.0511
12	Female	1.0464	1.0281	1.0565	1.0412	1.0436
13	Female	1.0713	1.0261	1.0536	1.0135	1.0439
Range (highest/lowest)		1.0324	1.0200	1.0224	1.1202	1.0208
Means ± SD						
Female	n = 6	1.0502 ± 0.0128^{y}	1.0326 ± 0.0079^{x}	1.0568 ± 0.0054^{y}	1.0399 ± 0.0191^{x}	1.0491 ± 0.0054^{ay}
Male	n = 6	1.0434 ± 0.0049^{y}	1.0340 ± 0.0080^{x}	1.0626 ± 0.0078^{y}	1.0212 ± 0.0420^{x}	1.0592 ± 0.0054^{by}
Total	n = 12	1.0468 ± 0.0099^{y}	1.0333 ± 0.0076^{x}	1.0597 ± 0.0071^{y}	1.0305 ± 0.0326^{x}	1.0541 ± 0.0074^{y}

$^{x\,y}$Average F_m values of plasma and urine were lower than those of WB, RBC, and feces ($P < 0.05$).

$^{a\,b}$Average F_m value of feces was higher for men over women ($P < 0.05$).