
Supplemental Material

Appendix S1 Modeling methods for estimating stable isotope turnover times and AIC values

The reaction progress variable is:

\[
\frac{\delta_t - \delta_{eq}}{\delta_{init} - \delta_{eq}} = e^{-\lambda t}, \quad [1]
\]

where \( \delta_t \) is the measured \( \delta \) value for carbon or nitrogen at time \( t \), \( \delta_{eq} \) is the \( \delta \) value at equilibrium or when all of the new diet has been incorporated into the tissue of interest (when \( t = \text{infinity} \)), \( \delta_{init} \) is the \( \delta \) value at the time of the diet switch \( (t = 0) \), and \( \lambda \) is the fractional rate of isotope incorporation or turnover rate as the tissue changes from the initial diet to the final diet (for complete derivation, see Cerling, Ayliffe, Dearing et al., 2007). The reaction progress variable scales the values between 0 and 1; thus the equation can be written as:

\[
\frac{\delta_t - \delta_{eq}}{\delta_{init} - \delta_{eq}} = (1 - F), \quad [2]
\]

where \( F \) is the fraction of change that has occurred at time \( t \). Combining equations 1 and 2 provides the equation linearizing the data:

\[
\ln(1 - F) = -\lambda t, \quad [3]
\]

which is a straight line in the form:

\[
y = mx + b, \quad [4]
\]

with a slope \( (m) \) of \(-\lambda\) and an intercept \( (b) \) of 0.0. Utilizing these parameters, I then fit a line to the isotope turnover data from each tissue for females and males using a statistical package (SigmaPlot version 10). I report the \( r^2 \) values to indicate the goodness of fit of the linear model to the turnover data. I calculated the parameters of the line starting at the day when there was \(~10\%\) isotope exchange and ending on the day when there was \(\leq90\%\) exchange to avoid errors associated with endpoints in linear equations (see Cerling et al., 2007, Podlesak, McWilliams & Cerling, in review). The days of start and finish varied for each tissue type and to determine

those days, I calculated the % exchange for each day as:

$$\left( \frac{\delta_{\text{init}} - \delta_{\text{eq}}}{\delta_{\text{init}} - \delta_{\text{eq}}} \right) \times 100 = \% \text{ exchange.} \quad [5]$$

I collected data 2 days following a diet switch and therefore missed the ~10% isotope exchange for the tissues with the fastest isotopic turnover (liver, serum, and kidney). Thus, the parameters of the lines for the data from those tissues start on day 2 even though the isotope exchange was greater than 10%. When $\delta_{\text{eq}}$ was reached before the final day of the experiment, I calculated $\delta_{\text{eq}}$ by taking the mean isotope value from the day of first equilibrium to the final day of the experiment. For example, isotopic equilibrium with the fish and C$_4$ plant diet for $\delta^{15}$N from muscle from females was reached on day 120, so $\delta_{\text{eq}}$ was calculated by averaging the $\delta^{15}$N values from days 120-258. The half-life and retention time of the isotope within a tissue can be calculated using equations 10 and 11, respectively, described below. When graphing ln($1 - F$) versus time, an intercept greater than zero indicates there is a delay in the incorporation of dietary isotope into that tissue and the delay can be calculated by dividing the intercept by the slope as:

$$-\frac{b}{\lambda}. \quad [6]$$

An intercept less than zero indicates that multiple source pools of nitrogen or carbon contribute to the isotope signature of a particular tissue. Two pools were indicated for serum and kidney tissue. To determine the isotopic half-lives and fractions of the turnover associated with each pool using the RPVM, I used the equation for a two-pool system (Podlesak et al., in review):

$$\frac{\delta_t - \delta_{\text{eq}}}{\delta_{\text{init}} - \delta_{\text{eq}}} = f_1 e^{-\lambda_1 t} + f_2 e^{-\lambda_2 t}. \quad [7]$$

When there are 2 pools contributing to the isotope turnover, graph ln(1-F) versus time for all data from the day of ~10% turnover to the day of first equilibrium (in this case, days 2 to 30 for
serum and days 2 to 120 for kidney). Then fit a regression line to the data for the long pool only (days 15 to 30 for serum and days 30 to 120 for kidney; see Figures 2 E-H and 3 E-H); the slope of the line for the long pool is the rate constant used to determine the isotope half-life or retention time (see equations 10 and 11 below) and the intercept allows for the calculation of the fractional component of the pool:

\[ e^{f_1}, \quad [8] \]

where \( f_1 \) is the intercept of the regression line for the long pool. The (1-F) values of the long pool are then subtracted from the total (1-F) to give the first residual plots. A regression line is fit to the residual plot data and the slope and intercept of that line are used to determine the half-lives and fractional contribution of the short pool to the isotope turnover as described above (for complete derivations for multiple pool calculations using the RPVM, see Cerling et al., 2007, Podlesak et al., in review).

I fit single compartment exponential equations to the diet turnover data of the form:

\[ \delta^t = ae^{-\lambda t} + \delta^{eq}, \quad [9] \]

where \( \delta^t \) is the isotope value of the tissue in question for a particular time \( t \), \( a \) is the total change in isotope value when the tissue has changed from initial (\( \delta^{init} \)) to the final, equilibrium isotope value (\( \delta^{eq} \)), \( \lambda \) is the rate constant or fractional rate of isotope incorporation or turnover rate as the tissue changes from the initial diet to the final diet, and \( \delta^{eq} \) is the asymptotic isotope value for the tissue once it is in equilibrium with the final diet. Both \( \lambda \) and \( \delta^{eq} \) are derived from the data. I fit Equation 9 to the data using the nonlinear least squares routine with the curve fitting tool in MATLAB (version 7.3.0.267, R2006b) which provided 95% confidence intervals for all parameters and adjusted \( r^2 \) values to indicate the goodness of fit of the model to the data.

The length of time required for \( x\% \) turnover of carbon and nitrogen in each tissue was
calculated by rearranging the terms in the exponential equation and solving for \( t \):

\[
t = \frac{\ln \left(1 - \frac{x}{100}\right)}{\lambda}, \tag{10}
\]

where \( t \) is time in days, \( x \) is some \% turnover, and \( \lambda \) is the fractional rate of incorporation. To calculate the half-lives of tissue nitrogen or carbon, \( i.e. \) the time required for half the existing tissue to reflect the isotope signature of the new diet, we solved the equation for \( x = 50 \) (signifying a 50\% exchange of isotopes). Alternatively, some studies prefer to calculate the amount of time the isotopes from a particular diet remain within a particular tissue (residence or retention time) (Tsahar, Wolf, Ishaki \textit{et al.}, 2008, Martinez del Rio & Anderson-Sprecher, 2008) and that can be done with:

\[
\tau = \frac{1}{\lambda}, \tag{11}
\]

where \( \tau \) is the residence or retention time of an isotope.

As stated above, when graphing \( \ln(1 - F) \) versus time using the RPVM, an intercept less than zero indicates that multiple source pools of nitrogen or carbon may contribute to the isotope signature of a particular tissue. When the results from the RPVM indicated a multiple compartment model may be appropriate, I fit the data to a two compartment nonlinear model of the form:

\[
\delta^t = a[p(e^{-\lambda t}) + (1 - p)(e^{-\lambda_2 t})] + c, \tag{12}
\]

where \( \delta^t, t, a, c, \) and \( \lambda \) are described with equation 9 above and \( p \) and \( 1-p \) are the fractional sizes of the first and second compartments or pools, respectively, and \( \lambda_2 \) is the rate of change for the second pool (Tsahar \textit{et al.}, 2008). I fit Equation 12 to the data using the nonlinear least squares routine with the curve fitting tool in MATLAB (version 7.3.0.267, R2006b) which provided 95\% confidence intervals for all parameters and adjusted \( r^2 \) values to indicate the goodness of fit of

the model to the data. This method provides information necessary to compute the half-lives and retention times of the isotopes in each pool using the methods described in equations 10 and 11, respectively. The overall mean half-life for a 2 pool non-linear model can be obtained using:

\[ t_{1/2} = pt_{1/2a} + (1 - p)t_{1/2b}, \quad [13] \]

where \( t_{1/2} \) is the overall mean half-life (see equation 10) and \( t_{1/2a} \) and \( t_{1/2b} \) are the half-lives for the first and second pools described in equation 12. The overall mean retention time for the system can be obtained with:

\[ \tau = p\tau_a + (1 - p)\tau_b, \quad [14] \]

where \( \tau \) is the mean retention time (see equation 11) and \( \tau_a \) and \( \tau_b \) are the retention times for the first and second pools described in equation 12 (Tsahar et al., 2008, Martinez del Rio et al., 2008).

The overall mean half-life for a 2 pool RPVM model can be obtained using:

\[ t_{1/2} = f_1 t_{1/2a} + f_2 t_{1/2b}, \quad [15] \]

where \( f_1 \) and \( f_2 \) are the fractions of isotopic turnover attributed to pools 1 and 2, respectively.

As mentioned above, I missed some of the initial isotope turnover for serum and kidney. Therefore, the fractions of the pools contributing to the overall isotope turnover for some cases do not equal 100\% (see Figures 2 and 3) and therefore it is not possible to obtain the complete overall mean isotope half-lives for all of my data. However, the amount of time that I missed and that contributed to the initial turnover was likely very small as it occurred between days 0 and 2 of the turnover experiment. Therefore my estimates of overall mean isotope turnover are likely fairly reliable. The overall mean retention time for the 2 pool RPVM can be obtained using equation 14 and substituting \( f_1 \) and \( f_2 \) for \( p \) and \( 1-p \).

In general, the more parameters used for a model, the better the fit as reflected by the

coefficient of determination ($r^2$) values. To avoid erroneously choosing the 2 compartment model due to possible over-parameterization, I used an approach described for use in stable isotope incorporation studies by Martinez del Rio and Anderson-Sprecher (2008) using parsimonious models that avoid under- and over-fitting and provide a quantifiable approach to choosing the best model given the data available (Anderson & Burnham, 2001, Martinez del Rio et al., 2008, Burnham & Anderson, 2002, Hobbs & Hilborn, 2006, Stephens, Buskirk & Martinez del Rio, 2007, Tsahar et al., 2008). When multiple compartment models seemed appropriate given the results from the RPVM, I fit a single and dual compartment model to the data and estimated the Akaike Information criterion (AIC) for each model. I used the small sample AIC (AICc) because the ratio of data points (n) to the number of parameters in the model (K) is small (Burnham et al., 2002, Martinez del Rio et al., 2008):

$$AICc = AIC + \frac{2K(K + 1)}{n - K - 1}, \quad [16]$$

where

$$AIC = nLog(\hat{\sigma}^2) + 2K, \quad [17]$$

and $\hat{\sigma}^2$ is the sum of squares due to error (SSE) which is provided in the output of the non-linear fitting procedure run in the computer program of choice. The best model is the one with the lowest AICc value (pg. 70-72 in Burnham et al., 2002, Martinez del Rio et al., 2008). When the AICc values are very close between the 2 models, one must determine if there are differences in the estimations of retention times and half-lives between the 2 models and decide which model makes most biological sense.

Figures S1-S6 Stable isotope turnover model graphs for 6 tissues from captive female and male rats

Figure S1. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in liver from captive rats switched on day 0 from a C\textsubscript{3} plant-based diet to a fish and C\textsubscript{4} plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

\[
y = -6.71e^{0.0699t} + 15.24
\]
Half-life = 7.7 days
Residence time = 11.1 days
Adjusted \(r^2 = 0.99\)

\[
y = -6.07e^{0.0855t} - 17.36
\]
Half-life = 2.4 days
Residence time = 3.5 days
Adjusted \(r^2 = 0.95\)

\[
y = -6.91e^{0.0885t} + 14.91
\]
Half-life = 7.8 days
Residence time = 11.3 days
Adjusted \(r^2 = 0.98\)

\[
y = -5.42e^{0.2222t} - 18.05
\]
Half-life = 3.1 days
Residence time = 4.5 days
Adjusted \(r^2 = 0.87\)

\[
y = -6.079t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.0679t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.2029t - 0.1463
\]
Half-life = 3.4 days
\(r^2 = 0.91\)

\[
y = -6.079t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.0679t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.2029t - 0.1463
\]
Half-life = 3.4 days
\(r^2 = 0.91\)

\[
y = -6.079t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.0679t - 0.1808
\]
Half-life = 10.2 days
\(r^2 = 0.98\)

\[
y = -0.2029t - 0.1463
\]
Half-life = 3.4 days
\(r^2 = 0.91\)
Figure S2. A-D) Non-linear, 2 pool models for N and C isotope incorporation rates in serum from captive rats switched on day 0 from a C3 plant-based diet to a fish and C4 plant-based diet. E-L) Reaction progress variable method 2 pool models for the same data. E-H) Graphs show data from days 2-30 from the RPVM, but only the data from days 15 to the turnover equilibrium on day 30 are included in the regression line in order to compute the half-lives and pool fractions for the long isotope turnover pools. I-L) Graphs use data from days 2-5 to compute the half-lives and pool fractions of the short isotope turnover pools; data are the first residuals derived by subtracting (1-F) values of the long component from the total (1-F) values shown in the companion graphs E-H. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

Adjusted $r^2 = 0.94$

Fraction of this pool: 0.61

Half-life = 2.1 days

Residence time = 11.7 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.99$

Adjusted $r^2 = 0.62$

Fraction of this pool: 0.25

Half-life = 8.4 days

Residence time = 12.0 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.82$

Fraction of this pool: 0.16

Half-life = 20.0 days

Residence time = 9.2 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.81$

Fraction of this pool: 0.61

Half-life = 2.1 days

Residence time = 11.7 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.99$

Fraction of this pool: 0.62

Half-life = 11.4 days

Residence time = 9.2 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.62$

Fraction of this pool: 0.35

Half-life = 23.3 days

Residence time = 12.0 days

Adjusted $r^2 = 0.99$

Adjusted $r^2 = 0.62$

Fraction of this pool: 0.25

Half-life = 37.5 days

Residence time = 15.9 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.98$

Fraction of this pool: 0.25

Half-life = 23.3 days

Residence time = 15.9 days

Adjusted $r^2 = 0.98$

Adjusted $r^2 = 0.87$

Fraction of this pool: 0.16

Half-life = 20.0 days

Residence time = 9.2 days

Adjusted $r^2 = 0.98$

Figure S3. A-D) Non-linear, 2 pool models for N and C isotope incorporation rates in kidney from captive rats switched on day 0 from a C3 plant-based diet to a fish and C3 plant-based diet. E-L) Reaction progress variable method 2 pool models for the same data. E-H) Graphs show data from days 2-120 from the RPVM, but only the data from days 30 to the turnover equilibrium on day 120 are included in the regression line in order to compute the half-lives and pool fractions for the long isotope turnover pools.  I-L) Graphs use data from days 2-15 to compute the half-lives and pool fractions of the short isotope turnover pools; data are the first residuals derived by subtracting (1-F) values of the long component from the total (1-F) values shown in the companion graphs E-H. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

Figure S4. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in RBC from captive rats switched on day 0 from a C₃ plant-based diet to a fish and C₄ plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.
Figure S5. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in muscle from captive rats switched on day 0 from a C₃ plant-based diet to a fish and C₄ plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

Figure S6. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in fur from captive rats switched on day 0 from a C₃ plant-based diet to a fish and C₄ plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.
LITERATURE CITED


