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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 δ^t is the measured δ value for carbon or nitrogen at time t, δ^{eq} is the δ value at equilibrium or when all of the new diet has been incorporated into the tissue of interest (when t = infinity), δ^{init} is the δ value at the time of the diet switch (t = 0), and λ 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 ≤90% 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:

$$\left(\frac{|\delta^{init}|-|\delta^{t}|}{|\delta^{init}|-|\delta^{eq}|}\right)\times 100 = \%\, exchange. \quad [5]$$

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 δ^{eq} was reached before the final day of the experiment, I calculated δ^{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 δ^{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):

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

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 δ^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 (δ^{init}) to the final, equilibrium isotope value (δ^{eq}), λ 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 δ^{eq} is the asymptotic isotope value for the tissue once it is in equilibrium with the final diet. Both λ and δ^{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:

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

where *t* is time in days, x is some % turnover, and λ 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 *et al.*, 2008, Martinez del Rio & Anderson-Sprecher, 2008) and that can be done with:

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

where τ 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:

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

where δ^t, *t*, *a*, *c*, and λ 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 λ_2 is the rate of change for the second pool (Tsahar *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:

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

where $\bar{t}_{1/2}$ 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:

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

where $\bar{\tau}$ is the mean retention time (see equation 11) and τ_a and τ_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:

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

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 AIC_c value (pg. 70-72 in Burnham *et al.*, 2002, Martinez del Rio *et al.*, 2008). When the AIC_c 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.

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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_3 plant-based diet to a fish and C_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.

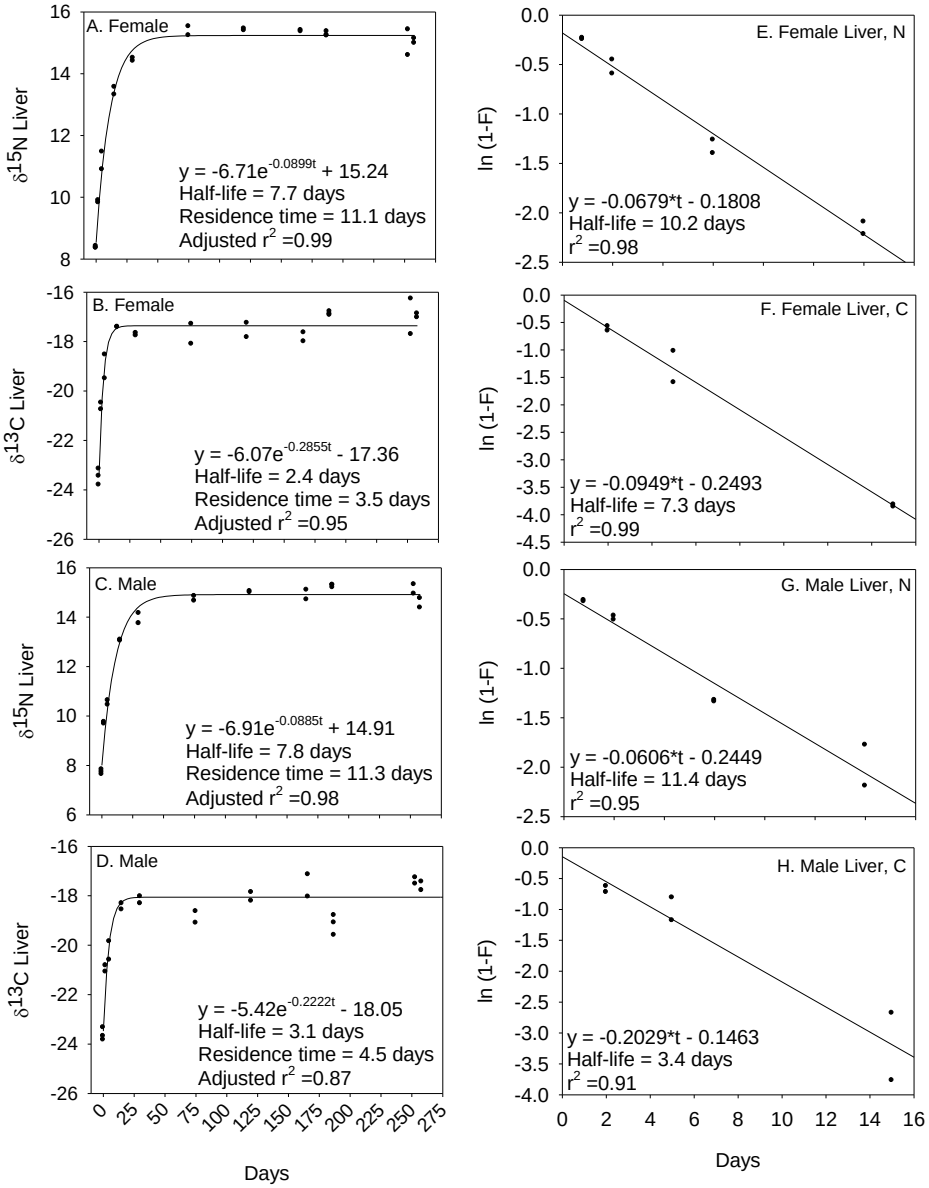

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Figure S2. A-D) Non-linear, 2 pool models for N and C isotope incorporation rates in serum from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. E-L) Reaction progress variable method 2 pool models for 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.

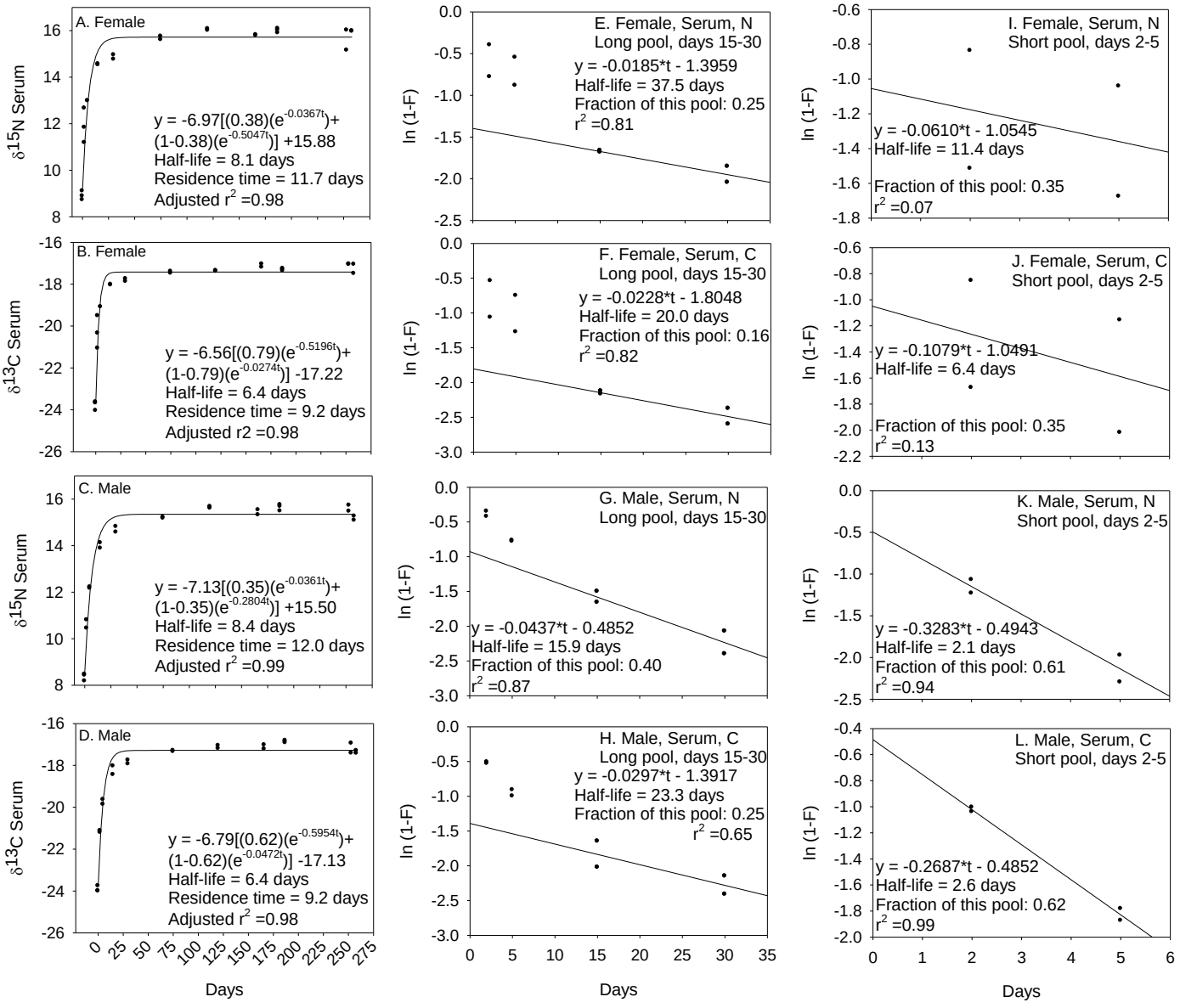

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Figure S3. A-D) Non-linear, 2 pool models for N and C isotope incorporation rates in kidney from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. E-L) Reaction progress variable method 2 pool models for 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.

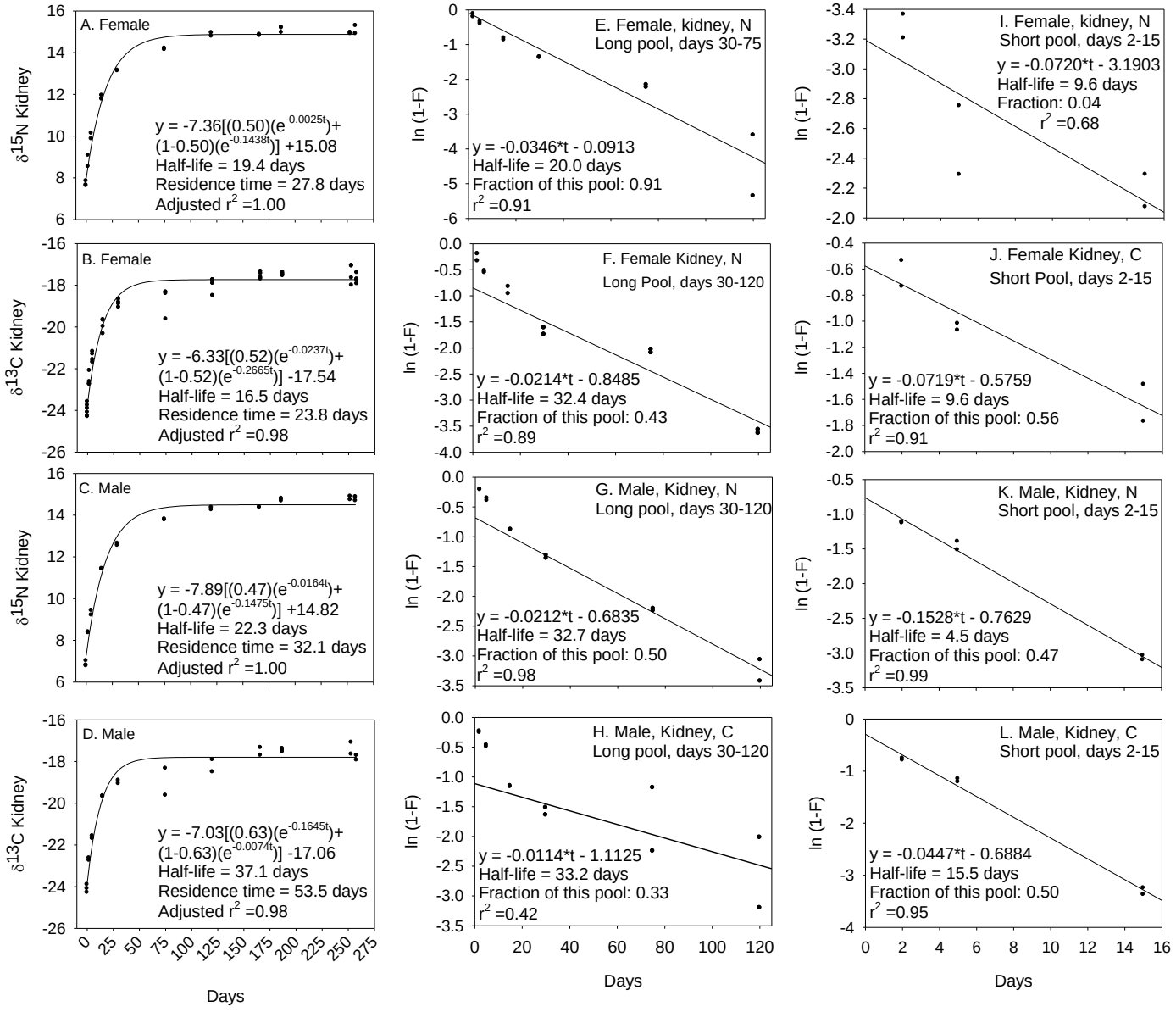

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Figure S4. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in RBC from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

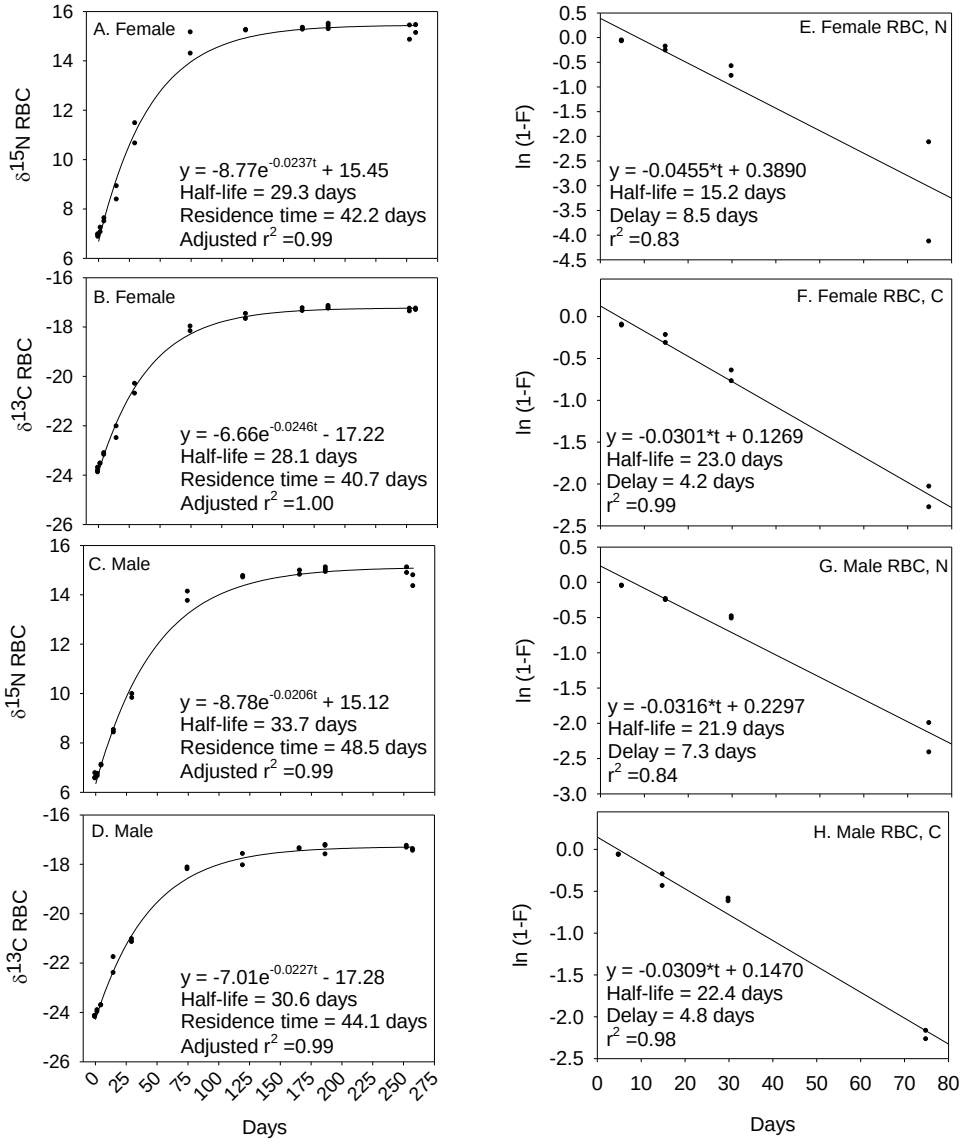

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Figure S5. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in muscle from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.

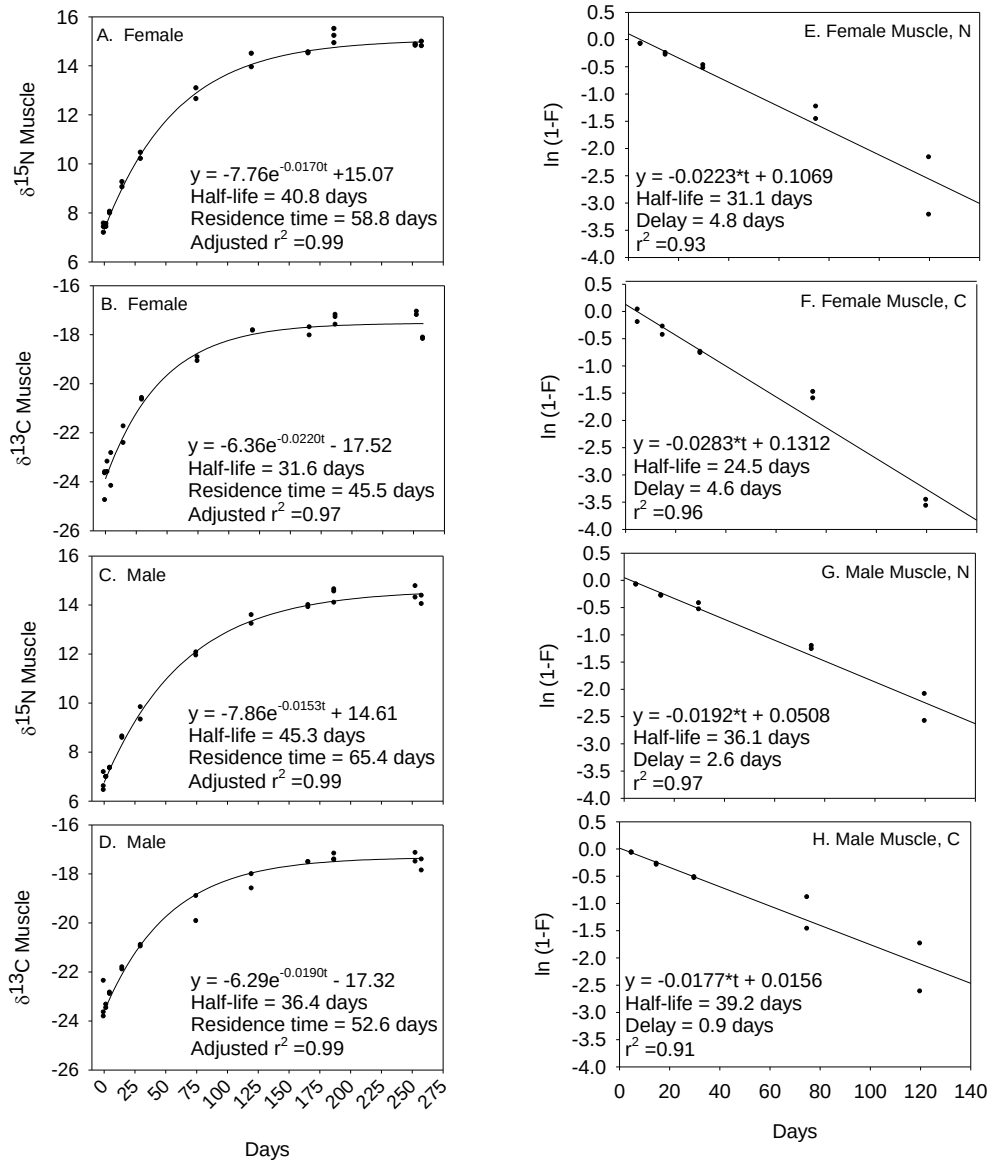

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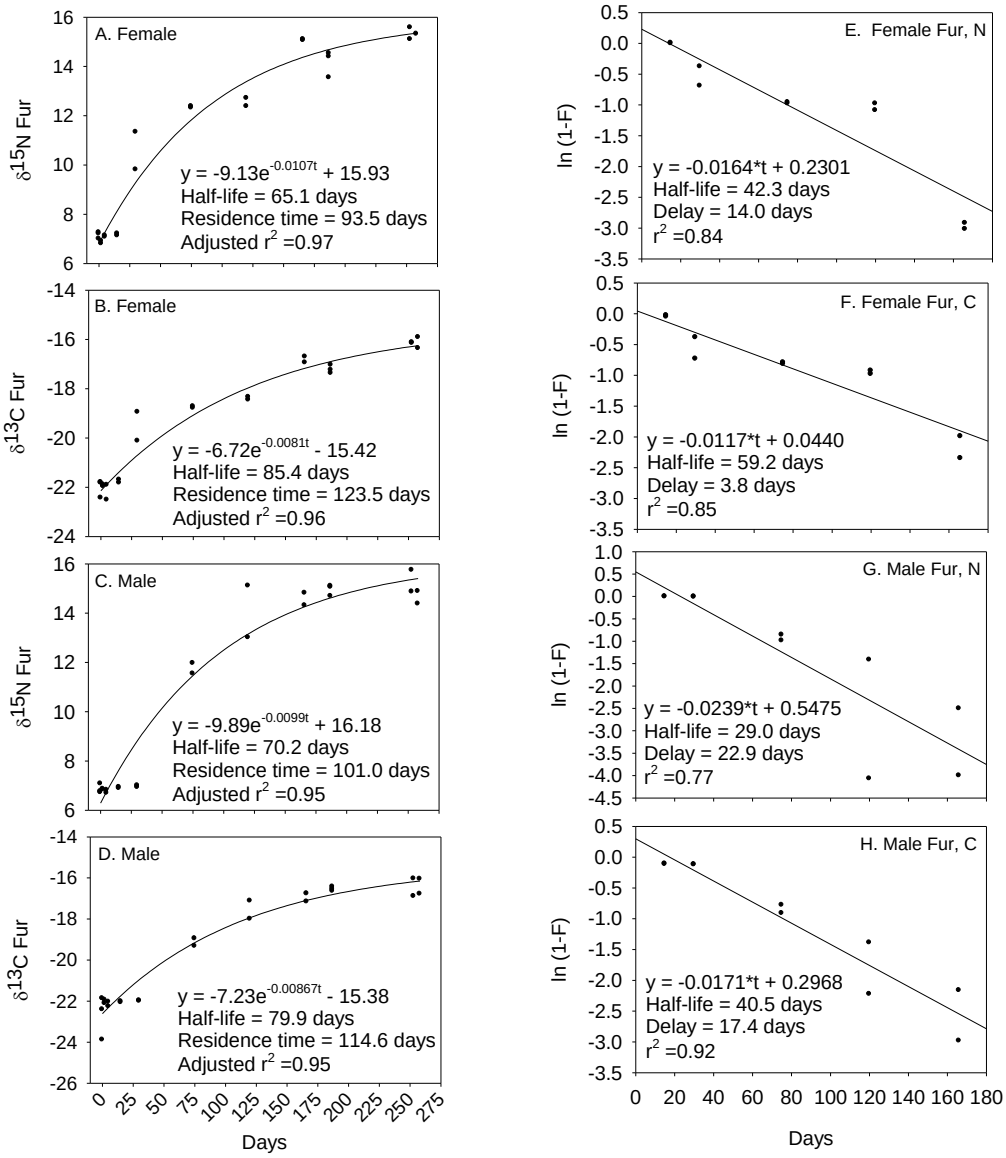
Figure S6. A-D) Non-linear, 1 compartment models for N and C isotope incorporation rates in fur from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. E-H) Reaction progress variable method models for the same data. Data are graphed as individual rats; n = 2 rats for each sex on each day except day 187 where n = 3.



LITERATURE CITED

Anderson, D. & Burnham, K. (2001) Commentary on models in ecology. *Bulletin - Ecological Society of America* **82**, 160-161.

Burnham, K. & Anderson, D. (2002) *Model selection and multimodel inference.* Springer, New York, NY.

Cerling, T., Ayliffe, L., Dearing, M., Ehleringer, J.R., Passey, B., Podlesak, D.W., Torregrossa, A. & West, A. (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. *Oecologia* **151**, 175-189.

Hobbs, T. & Hilborn, R. (2006) Alternatives to statistical hypothesis testing in ecology: a guide to self-teaching. *Ecological Applications* **16**, 5-19.

Martinez Del Rio, C. & Anderson-Sprecher, R. (2008) Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia* **156**, 765-772.

Podlesak, D., Mcwilliams, S. & Cerling, T. (in review) Estimating turnover of carbon and nitrogen in the red-blood cells of birds using stable isotopes and its implications for diet reconstruction.

Stephens, P., Buskirk, S. & Martinez Del Rio, C. (2007) Inference in ecology and evolution. *Trends in Ecology & Evolution* **22**, 192-197.

Tsahar, E., Wolf, N., Ishaki, I., Arad, Z. & Martinez Del Rio, C. (2008) Dietary protein influences the rate of ^{15}N incorporation in blood cells and plasma of Yellow-vented bulbuls (*Pycnonotus xanthopygos*). *Journal of Experimental Biology* **211**, 459-465.