Online Supplemental Material

Kurle, CM, Koch PL, Tershy, B. Croll, DA

Effects of sex, tissue type, and dietary components on stable isotope discrimination factors ( $\Delta^{15}N$  and  $\Delta^{13}C$ ) in mammalian omnivores

Equations for calculating the fraction of carbon routed to body tissues from dietary lipids and carbohydrates.

Tables S1-S6

# Supplemental Information: Equations to determine the fraction of carbon routed to body tissues from dietary lipids and carbohydrates.

## Equations

1. Start with the % by weight of lipids and carbohydrates and calculate the decimal % dry weight for each.

$$Decimal \% dry weight of lipid = \frac{\% by weight of lipid}{(\% by weight of lipid) + (\% by weight of carbohydrates)} (1)$$

The % by weight of lipid and carbohydrate values are in Table 1 in the manuscript.

2. Calculate the grams of carbon from lipid in 100 grams of dry weight:

```
g C from lipid per 100 g dry weight = [(decimal \% dry weight of lipid)(100)]x (0.75) (2)
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The 0.75 value comes from [1].

3. Do the same for grams of carbon from carbohydrates per 100 grams of dry weight:

g C from carbohydrates per 100 g dry weight = 
$$[(decimal \% dry weight of carbs)(100)]x (0.45) (3)$$

The 0.45 value comes from [1].

4. Calculate the fraction of lipid derived carbon and the fraction of carbohydrate derived carbon:

% carbon from lipid = 
$$\frac{\text{lipid per 100 g dry weight}}{(\text{lipid per 100 g dry weight}) + (\text{carbohydrate per 100 g dry weight})}$$
(4)

5. Determine the  $\delta^{13}$ C value for the entire dietary energy budget:

$$\partial^{13}C_{energy} = \left[ \left( \partial^{13}C_{lipid} \right) \left( \% \ C \ from \ lipid \right) \right] + \left[ \left( \partial^{13}C_{carbohydrate} \right) \left( \% \ C \ from \ carbohydrate \right) \right]$$
(5)

The  $\delta^{13}$ C values for lipids and carbohydrates are in Table 1 in manuscript.

6. Calculate 25% of the  $\delta^{13}C_{energy}$  value to account for the amount of dietary carbon incorporated into the body from energy (lipids and carbohydrates). This is modified from MacAvoy et al. 2005 [2].

$$\partial^{13}C$$
 of dietary energy incorporated into body tissues =  $(\partial^{13}C_{energy})(0.25)$  (6)

7. This value would be added to the  $\delta^{13}$ C value that reflects the proportion of carbon incorporated into the body from the protein components of the diet  $[(\delta^{13}C_{\text{protein}})(0.75)]$  to arrive at an adjusted bulk diet  $\delta^{13}$ C value that accounts for the routing of carbon from dietary protein, carbohydrate, and lipid into the consumer carbon pool.

# Example using the values from the wheat gluten diet in this study

1. Start with the % by weight lipids and carbohydrates and calculate the decimal % dry weight for each.

Decimal % dry weight of lipid = 
$$\frac{8.20}{(8.20) + (53.40)} = 0.1331$$
 (1)

Decimal % dry weight of lipid = 
$$\frac{53.40}{(8.20) + (53.40)} = 0.0.8669$$
 (1)

The % by weight of lipid and carbohydrate values are in Table 1 in the manuscript.

2. Calculate the grams of carbon from lipid in 100 grams of dry weight:

$$g C from lipid per 100 g dry weight = [(0.1331)(100)]x (0.75) = 9.98 (2)$$

The 0.75 value comes from [1].

3. Do the same for grams of carbon from carbohydrates per 100 grams of dry weight:

$$g C from carbohydrates per 100 g dry weight = [(0.8669)(100)]x (0.45) = 39.01 (3)$$

The 0.45 value comes from [1].

4. Calculate the fraction of lipid derived carbon and the fraction of carbohydrate derived carbon:

% carbon from lipid = 
$$\frac{9.98}{(9.98) + (39.01)} = 0.20$$
 (4)  
% carbon from carbohydrates =  $\frac{39.01}{(9.98) + (39.01)} = 0.80$  (4)

5. Determine the  $\delta^{13}$ C value for the entire dietary energy budget:

$$\partial^{13}C_{energy} = [(-28.5\%)(0.20)] + [(-24.9\%)(0.80)] = -25.62\%$$
 (5)

6. Calculate 25% of the  $\delta^{13}C_{energy}$  value to account for the amount of dietary carbon incorporated into the body from energy (lipids and carbohydrates). This is modified from MacAvoy et al. 2005 [2].

$$\partial^{13}C$$
 of dietary energy incorporated into body tissues =  $(-25.62\%)(0.25) = -6.405\%$  (6)

7. This value (-6.045‰) would be added to the  $\delta^{13}$ C value that reflects the proportion of carbon incorporated into the body from the protein components of the diet to arrive at an adjusted bulk diet  $\delta^{13}$ C value that accounts for the routing of carbon from dietary protein, carbohydrate, and lipid into the consumer carbon pool.

To calculate that value for the wheat gluten diet:

 $(\delta^{13}C_{\text{protein}})(0.75) = -19.35\%$ 

-6.045% + -19.35% = -25.8%

#### **Conclusion:**

The adjusted bulk diet  $\delta^{13}$ C value used in the manuscript for the wheat diet, following the protocol described in MacAvoy et al. 2005 [2], was -25.6‰, only 0.2‰ larger than the value

calculated above. The same was true for all diets; the  $\delta^{13}$ C value for the adjusted bulk diet accounting for lipid routing and using the above equations would have resulted in  $\delta^{13}$ C values that were only 0.2‰ larger than what were reported and used in the manuscript, an extremely minimal amount. As accounting for lipid routing this way has not been described or used before, we accounted for carbohydrate routing only (as per MacAvoy et al. 2005 [2]), but are including the above calculations for reference.

Table S1. Essential amino acid contents of the four experimental diets and the minimum amino acid requirements for maintenance and growth of an adult rat (measured in g/kg of diet)<sup>1</sup> [3, 4]. Amino acids marked with an asterisk are only essential in certain cases. All other dietary components are listed in Table 1.

Amino acid		Ι	Diet Type		Maintenance	Growth
	Wheat	Fish	Wheat/	Wheat/fish/		
	gluten		fish	casein/egg		
Arginine <sup>*</sup>	9.6	16.1	11.8	12.6	NA	4.3
Cystine <sup>*</sup>	3.9	3.0	3.7	4.0	1.2	NA
Histidine <sup>*</sup>	4.8	5.4	5.1	5.4	0.7 to 0.8	2.8
Isoleucine	9.6	10.9	10.3	11.3	2.4 to 3.1	6.2
Leucine	15.6	17.4	16.6	18.4	1.0 to 1.8	10.7
Lysine	9.2	18.1	9.7	12.2	0.5 to 1.1	9.2
Methionine	6.9	8.7	6.9	8.2	1.7	NA
Phenylalanine	11.1	9.1	10.8	11.5	0.8	NA
Threonine	6.4	10.3	7.0	8.7	1.6 to 1.8	6.2
Tryptophan	2.4	2.7	2.6	2.9	0.4 to 0.5	2.0
Tyrosine <sup>*</sup>	6.3	7.3	6.8	8.1	0.6	NA
Valine	9.6	12.1	10.6	12.7	1.5 to 2.3	7.4
Cystine and						
Methionine						
Together <sup>2</sup>	10.8	11.7	10.6	12.2	2.3	9.8

 $^1$ [3, 4]  $^2\text{Cystine}$  may supply up to 50% of the methionine plus cystine requirement on a weight basis [3].

Table S2. Carbon trophic discrimination factors ( $\Delta^{13}$ C, reported in ‰ ± SD) between bulk diet and tissues for rats held on 4 experimental diets (see Table 1 for diet details). Tissues are listed in order of isotope turnover time from fastest (liver) to slowest (fur) [5]. Significant differences in  $\Delta^{13}$ C values between sexes held on the same diets are denoted by an asterisk (\*). N = 3 females and 3 males per diet. The  $\Delta^{13}$ C values that consider differential routing of carbon from dietary  $\delta^{13}$ C values that consider differential routing of carbon from dietary protein and carbohydrates sources (see Methods).

Tissue	Whea	at Diet	Fish	Diet
	Female	Male	Female	Male
	$\Delta^{13}$ C	$\Delta^{13}$ C	$\Delta^{13}$ C	$\Delta^{13}$ C
Liver	+1.0±0.3	$+0.8\pm0.8$	-0.7±0.1	-1.2±0.4
Serum	$+1.3\pm0.1$	$+1.1\pm0.0$	-1.0±0.2	$-0.8\pm0.2$
Kidney	$+1.0\pm0.1$	$+1.1\pm0.1$	-0.8±0.2	$-0.8\pm0.1$
RBC	$+1.0\pm0.1$	$+0.9\pm0.1$	-0.9±0.1	-1.0±0.1
Muscle	$+1.4\pm0.1$	$+1.4\pm0.2$	-0.4±0.1	$-0.4\pm0.1$
Fur	$+3.2\pm0.2$	$+2.9\pm0.1$	$+0.6\pm0.1$	$+0.4\pm0.2$
	Wheat /	Fish Diet	Wheat/Fish/Casei	n/Egg White Diet
Liver	$+1.1\pm0.5$	$+0.4{\pm}1.1$	-0.7±0.0	-0.6±0.1
Serum	$+1.9\pm0.1$	$+1.9\pm0.2$	-1.7±0.0	-1.6±0.1
Kidney	$+1.9\pm0.2$	$+1.5\pm0.3$	-1.4±0.1	-1.6±0.1
RBC	$+2.0\pm0.1$	$+1.6\pm0.3$	-2.2±0.1	$-2.3\pm0.1$
Muscle	+2.3±0.1	$+1.8\pm0.4$	-1.4±0.0	-1.3±0.1
Fur	$+3.6\pm0.1^{*}$	$+3.1\pm0.2^{*}$	$-0.5\pm0.1^{*}$	$-0.6\pm0.1^*$

Table S3. Stable isotope values ( $\delta^{15}$ N and  $\delta^{13}$ C, reported in  $\% \pm$  SD) from rat tissues held on four experimental diets for 276-278 days. Significant differences ( $p \le 0.05$ ) in  $\delta^{15}$ N and  $\delta^{13}$ C values (as shown by t-tests,  $-2.4 \le t \le 11.7$ , all n=3, all df = 4, 0.01 >  $p \le 0.90$ ) between sexes held on the same diets are denoted by an asterisk (\*).

Tissue		Whea	t Diet			F	Fish Diet	
	Fer	nale	Male			male	Male	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}$ N	$\delta^{13}C$	$\delta^{15}$ N	$\delta^{13}C$
Liver	$8.0 \pm 0.2$	$-24.3 \pm 0.0$	$7.8 \pm 0.5$	$-24.5 \pm 0.1$	$15.7 \pm 0.1^{*}$	$-16.9 \pm 0.1$	$15.1 \pm 0.1^{*}$	$-17.4 \pm 0.1$
Serum	$8.5\pm0.1^*$	$-24.0\pm0.1$	$8.3\pm0.0^{*}$	$-24.2 \pm 0.0$	$16.2 \pm 0.1^{*}$	$-17.2 \pm 0.1$	$15.7 \pm 0.2^{*}$	$-17.0 \pm 0.1$
Kidney	$7.6\pm0.0^{*}$	$-24.3\pm0.1$	$7.0 \pm 0.1^{*}$	$-24.2 \pm 0.1$	$15.7 \pm 0.1^{*}$	$-17.0 \pm 0.1$	$15.0 \pm 0.1^{*}$	$-17.0\pm0.0$
RBC	$7.0 \pm 0.1^{*}$	$-24.4\pm0.1$	$6.5 \pm 0.1^{*}$	$-24.3\pm0.1$	$15.5 \pm 0.1^{*}$	$-17.1 \pm 0.0$	$15.0 \pm 0.1^{*}$	$-17.2 \pm 0.0$
Muscle	$7.3\pm0.0^{*}$	$-23.9\pm0.0$	$6.8 \pm 0.1^{*}$	$-23.8 \pm 0.1$	$15.7 \pm 0.1^{*}$	$-16.6\pm0.0$	$15.0 \pm 0.0^{*}$	$-16.6\pm0.0$
Fur	$7.3 \pm 0.1$	$-22.1\pm0.1$	$7.1 \pm 0.0$	$-22.4 \pm 0.1$	$16.4\pm0.1$	$-15.6\pm0.1$	$16.4\pm0.1$	$-15.6\pm0.1$
		Wheat/H	Fish Diet			Wheat/Fish/C		et
Liver	$10.5 \pm 0.1^{*}$	$-23.4 \pm 0.3$	$10.0 \pm 0.1^{*}$	$-24.1 \pm 0.6$	$10.6 \pm 0.1^{*}$	$-17.4 \pm 0.0$	$10.1 \pm 0.0^{*}$	$-17.3 \pm 0.1$
Serum	$11.0 \pm 0.1^{*}$	$-22.6\pm0.1$	$10.6 \pm 0.1^{*}$	$-22.6 \pm 0.1$	$11.2\pm0.1^{*}$	$-18.4 \pm 0.0$	$10.6 \pm 0.0^{*}$	$\textbf{-18.3}\pm0.1$
Kidney	$10.1 \pm 0.1^{*}$	$-22.6\pm0.1$	$9.4 \pm 0.1^{*}$	$-23.0 \pm 0.2$	$9.9\pm0.1^{*}$	$-18.2 \pm 0.1$	$9.3\pm0.0^{*}$	$\textbf{-18.3}\pm0.1$
RBC	$9.6 \pm 0.0^{*}$	$-22.5\pm0.1$	$9.2 \pm 0.0^{*}$	$-22.9\pm0.2$	$9.8\pm0.1$	$-19.0\pm0.1$	$9.4\pm0.0$	$-19.0\pm0.0$
Muscle	$10.1 \pm 0.1^{*}$	$-22.2 \pm 0.1$	$9.3\pm0.2^{*}$	$-22.7 \pm 0.0$	$9.9\pm0.2$	$-18.1 \pm 0.0$	$9.5\pm0.1$	$-18.1 \pm 0.0$
Fur	$10.0\pm0.1$	$-20.9 \pm 0.1^{*}$	$9.7\pm0.1$	$-21.4 \pm 0.1^{*}$	$10.4\pm0.1$	$-17.2 \pm 0.0^{*}$	$10.2\pm0.0$	$-17.4 \pm 0.0^{*}$

Table S4. F-values from ANOVAs and *p*-values from Post-hoc Tukey's Honestly Significant Difference tests demonstrating the differences in the  $\Delta^{15}$ N and  $\Delta^{13}$ C values among tissue types from rats held on 1 of 4 diets: Wheat (wheat gluten, beet sugar, cottonseed oil (sugar and oil from C<sub>3</sub> plants)), Fish (fish meal, cane sugar, corn oil (sugar and oil from C<sub>4</sub> plants)), Wheat/Fish (wheat gluten, fish meal, beet sugar, cottonseed oil), and Wheat/Fish/Egg/Milk (wheat gluten, fish meal, cow casein, chicken egg whites). All ANOVA *p*-values < 0.05.

Diet	Sex	Isotope	F <sub>5,12</sub>	Tissues		<i>p</i> -values			
Wheat	Female	Ν	88.4		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.00				
				Kidney	0.00	0.00			
				RBC	0.00	0.00	0.00		
				Muscle	0.00	0.00	0.05	0.01	
				Fur	0.00	0.00	0.05	0.01	1.00
Wheat	Female	С	90.0		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.29				
				Kidney	1.00	0.20			
				RBC	1.00	0.42	0.99		
				Muscle	0.00	0.98	0.07	0.16	
				Fur	0.00	0.00	0.00	0.00	0.00
Wheat	Male	Ν	46.7		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.03				
				Kidney	0.00	0.00			
				RBC	0.00	0.00	0.04		
				Muscle	0.00	0.00	0.81	0.30	
				Fur	0.00	0.00	0.97	0.01	0.40
Wheat	Male	С	16.4		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.79				
				Kidney	0.81	1.00			
				RBC	0.99	0.98	0.99		
				Muscle	0.22	0.85	0.83	0.47	
				Fur	0.00	0.00	0.00	0.00	0.00
Fish	Female	Ν	11.2		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.05		-		
				Kidney	1.00	0.05			
				RBC	0.67	0.01	0.74		
				Muscle	1.00	0.04	0.00	0.84	
				Fur	0.01	0.77	0.00	0.00	0.00
Fish	Female	С	58.4		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.26		•		
				Kidney	0.96	0.66			
				RBC	0.51	0.99	0.91		
				Muscle	0.16	0.00	0.04	0.01	
				Fur	0.00	0.00	0.00	0.00	0.00
Fish	Male	Ν	21.7		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.00				
				Kidney	1.00	0.00			
				RBC	1.00	0.00	1.00		
				Muscle	1.00	0.00	1.00	1.00	
				Fur	0.00	0.33	0.00	0.00	0.00

Table S4. F-values from ANOVAs and *p*-values from Post-hoc Tukey's Honestly Significant Difference tests demonstrating the differences in the  $\Delta^{15}$ N and  $\Delta^{13}$ C values among tissue types from rats held on 1 of 4 diets: Wheat (wheat gluten, beet sugar, cottonseed oil (sugar and oil from C<sub>3</sub> plants)), Fish (fish meal, cane sugar, corn oil (sugar and oil from C<sub>4</sub> plants)), Wheat/Fish (wheat gluten, fish meal, beet sugar, cottonseed oil), and Wheat/Fish/Egg/Milk (wheat gluten, fish meal, cow casein, chicken egg whites). All ANOVA *p*-values < 0.05.

Male		F <sub>5,12</sub>			<i>p</i> -values			
Male	С	30.6		Liver	Serum	Kidney	RBC	Muscle
			Serum	0.23				
			Kidney	0.29	1.00			
			RBC	0.85	0.80	0.88		
			Muscle	0.00	0.15	0.12	0.02	
			Fur	0.00	0.00	0.00	0.00	0.00
Female	Ν	29.7		Liver	Serum	Kidney	RBC	Muscle
			Serum	0.02		-		
			Kidney	0.04	0.00			
			RBC	0.00	0.00	0.05		
			Muscle	0.02	0.00	0.00	0.08	
			Fur	0.01	0.00	0.95	0.19	0.99
Female	С	39.6		Liver	Serum	Kidney	RBC	Muscle
			Serum	0.01				
			Kidney	0.02	1.00			
			RBC	0.01	1.00	0.96		
			Muscle	0.00	0.53	0.32	0.75	
			Fur	0.00	0.00	0.00	0.00	0.00
Male	Ν	76.0		Liver	Serum	Kidney	RBC	Muscle
			Serum	0.00		-		
			Kidney	0.00	0.00			
			RBC	0.00	0.00	0.47		
			Muscle	0.00	0.00	0.97	0.87	
			Fur	0.05	0.00	0.02	0.00	0.01
Male	С	8.5		Liver	Serum		RBC	Muscle
			Serum	0.04				
			Kidney	0.16	0.94			
			RBC	0.13	0.97	1.00		
			Muscle	0.05	1.00	0.98	0.99	
			Fur	0.00	0.11	0.02	0.03	0.07
Female	Ν	21.0		Liver	Serum		RBC	Muscle
			Serum	0.02				
			Kidney	0.02	0.00			
			•			0.98		
							0.98	
			Fur					0.10
Female	С	310.3					RBC	Muscle
			Serum			- 5	-	
					0.00			
						0.00		
							0.00	
			Fur	0.00	0.00	0.00	0.00	0.00
	Female	FemaleCMaleNMaleCFemaleN	FemaleC39.6MaleN76.0MaleC8.5FemaleN21.0	Kidney RBC Muscle FurFemaleN29.7FemaleN29.7FemaleN29.7FemaleNSerum Kidney RBC Muscle FurFemaleC39.6FemaleC39.6FemaleN76.0MaleN76.0MaleN76.0MaleNSerum Kidney RBC Muscle FurMaleN76.0FemaleNSerum Kidney RBC Muscle FurMaleC8.5FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleFurFemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleN21.0FemaleNFurFemaleNFurFemale	Kidney0.29RBC0.85Muscle0.00FemaleN29.7FemaleN29.7FemaleN29.7Kidney0.02Kidney0.04RBC0.00Muscle0.01FemaleC39.6FemaleC39.6FemaleC39.6FemaleC39.6FemaleC39.6MaleN76.0MaleN76.0MaleN76.0MaleN76.0MaleN76.0MaleN76.0FermaleLiverSerum0.00MaleC8.5MaleC8.5MaleC8.5FermaleN21.0FermaleN21.0FermaleN21.0FermaleC310.3FermaleC310.3FermaleC310.3FermaleC310.4Kidney0.00Kidney0.00Fur0.00Fur0.00Fur0.01Kidney0.02Fur0.02Fur0.03Fur0.04Kidney0.02Fur0.02Fur0.03Fur0.04Kidney0.02Fur0.03Fur0.04Fur0.05 <td>Kidney         0.29         1.00           RBC         0.85         0.80           Muscle         0.00         0.015           Fur         0.00         0.00           Female         N         29.7         Liver         Serum           Serum         0.02         Kidney         0.04         0.00           Female         N         29.7         Kidney         0.02         0.00           Rerum         0.02         Kidney         0.02         0.00           Muscle         0.02         0.00         Muscle         0.02         0.00           Female         C         39.6         Liver         Serum         0.01         1.00           Female         C         39.6         Kidney         0.02         1.00           Male         N         76.0         Liver         Serum           Muscle</td> <td>Kidney         0.29         1.00           RBC         0.85         0.80         0.88           Muscle         0.00         0.00         0.00           Female         N         29.7         Liver         Serum         0.02           Female         N         29.7         Kidney         0.00         0.00           Female         N         29.7         Kidney         0.02         0.00           Rerum         0.02         0.00         0.00         0.05           Muscle         0.00         0.00         0.05           Muscle         0.02         0.00         0.00           Female         C         39.6         Liver         Serum         0.00           Female         C         39.6         Liver         Serum         0.00         0.00           Male         N         76.0         Liver         Serum         0.00         0.00           Male         N         76.0         Kidney         0.00         0.00         0.01           Male         N         76.0         Kidney         0.00         0.00         0.02           Male         N         8.5         Liver</td> <td>Kidney         <math>0.29</math> <math>1.00</math>           RBC         <math>0.85</math> <math>0.80</math> <math>0.88</math>           Muscle         <math>0.00</math> <math>0.15</math> <math>0.12</math> <math>0.02</math>           Fur         <math>0.00</math> <math>0.00</math> <math>0.00</math> <math>0.00</math>           Female         N         <math>29.7</math>         Liver         <math>Serum</math> <math>Kidney</math> <math>0.04</math> <math>0.01</math>           Female         N         <math>29.7</math>         Kidney         <math>0.04</math> <math>0.00</math> <math>0.00</math> <math>0.03</math>           Female         N         <math>29.7</math>         Kidney         <math>0.02</math> <math>0.00</math> <math>0.00</math> <math>0.08</math>           Ferm         <math>0.03</math> <math>0.02</math> <math>0.00</math> <math>0.00</math> <math>0.08</math>           Ferm         <math>0.01</math> <math>0.00</math> <math>0.00</math> <math>0.08</math>           Fur         <math>0.01</math> <math>1.00</math> <math>0.96</math> <math>1.00</math>           Male         N         <math>76.0</math>         Liver         Serum         <math>Kidney</math> <math>0.02</math>           Male         N         <math>76.0</math>         Liver         Serum         <math>Kidney</math> <math>0.00</math>           Male         N         <math>76.0</math>         Liver         Serum         <math>Kidney</math></td>	Kidney         0.29         1.00           RBC         0.85         0.80           Muscle         0.00         0.015           Fur         0.00         0.00           Female         N         29.7         Liver         Serum           Serum         0.02         Kidney         0.04         0.00           Female         N         29.7         Kidney         0.02         0.00           Rerum         0.02         Kidney         0.02         0.00           Muscle         0.02         0.00         Muscle         0.02         0.00           Female         C         39.6         Liver         Serum         0.01         1.00           Female         C         39.6         Kidney         0.02         1.00           Male         N         76.0         Liver         Serum           Muscle	Kidney         0.29         1.00           RBC         0.85         0.80         0.88           Muscle         0.00         0.00         0.00           Female         N         29.7         Liver         Serum         0.02           Female         N         29.7         Kidney         0.00         0.00           Female         N         29.7         Kidney         0.02         0.00           Rerum         0.02         0.00         0.00         0.05           Muscle         0.00         0.00         0.05           Muscle         0.02         0.00         0.00           Female         C         39.6         Liver         Serum         0.00           Female         C         39.6         Liver         Serum         0.00         0.00           Male         N         76.0         Liver         Serum         0.00         0.00           Male         N         76.0         Kidney         0.00         0.00         0.01           Male         N         76.0         Kidney         0.00         0.00         0.02           Male         N         8.5         Liver	Kidney $0.29$ $1.00$ RBC $0.85$ $0.80$ $0.88$ Muscle $0.00$ $0.15$ $0.12$ $0.02$ Fur $0.00$ $0.00$ $0.00$ $0.00$ Female         N $29.7$ Liver $Serum$ $Kidney$ $0.04$ $0.01$ Female         N $29.7$ Kidney $0.04$ $0.00$ $0.00$ $0.03$ Female         N $29.7$ Kidney $0.02$ $0.00$ $0.00$ $0.08$ Ferm $0.03$ $0.02$ $0.00$ $0.00$ $0.08$ Ferm $0.01$ $0.00$ $0.00$ $0.08$ Fur $0.01$ $1.00$ $0.96$ $1.00$ Male         N $76.0$ Liver         Serum $Kidney$ $0.02$ Male         N $76.0$ Liver         Serum $Kidney$ $0.00$ Male         N $76.0$ Liver         Serum $Kidney$

Table S4. F-values from ANOVAs and *p*-values from Post-hoc Tukey's Honestly Significant Difference tests demonstrating the differences in the  $\Delta^{15}$ N and  $\Delta^{13}$ C values among tissue types from rats held on 1 of 4 diets: Wheat (wheat gluten, beet sugar, cottonseed oil (sugar and oil from C<sub>3</sub> plants)), Fish (fish meal, cane sugar, corn oil (sugar and oil from C<sub>4</sub> plants)), Wheat/Fish (wheat gluten, fish meal, beet sugar, cottonseed oil), and Wheat/Fish/Egg/Milk (wheat gluten, fish meal, cow casein, chicken egg whites). All ANOVA *p*-values < 0.05.

Diet	Sex	Isotope	F <sub>5,12</sub>	Tissues					
Wheat/Fish/Egg/Milk	Male	Ν	97.6		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.00				
				Kidney	0.00	0.00			
				RBC	0.00	0.00	0.47		
				Muscle	0.00	0.00	0.16	0.96	
				Fur	0.57	0.00	0.00	0.00	0.00
Wheat/Fish/Egg/Milk	Male	С	198.2		Liver	Serum	Kidney	RBC	Muscle
				Serum	0.00				
				Kidney	0.00	1.00			
				RBC	0.00	0.01	0.00		
				Muscle	0.00	0.03	0.02	0.00	
				Fur	0.96	0.00	0.00	0.00	0.00

Table S5. The *p*-values from ANOVA and Tukey post-hoc tests for differences in discrimination factors ( $\Delta^{15}$ N and  $\Delta^{13}$ C) between diet types for 6 tissues from rats held on 1 of 4 diets: Plant (wheat gluten, beet sugar, cottonseed oil (sugar and oil from C<sub>3</sub> plants)), Fish (fish meal, cane sugar, corn oil (sugar and oil from C<sub>4</sub> plants)), Plant/Fish (wheat gluten, fish meal, beet sugar, cottonseed oil), and Plant/Fish/Egg/Milk (wheat gluten, fish meal, cow casein, chicken egg whites, cane sugar, corn oil).

Tissue	Sex	Isotope	F <sub>3.8</sub>	ANOVA <i>p</i> -value	Diet	Tukey p	-values	5
Fur	Female	N	68.1	0.00		Wheat	Fish	Wheat/Fish
					Fish	0.00		
					Wheat/Fish	0.05	0.00	
					Wheat/Fish/Egg/Milk	0.01	0.00	0.67
						Wheat	Fish	Wheat/Fish
Fur	Female	С	246.4	0.00	Fish	0.00		
					Wheat/Fish	0.00	0.00	
					Wheat/Fish/Egg/Milk	0.00	0.56	0.00
						Wheat	Fish	Wheat/Fish
Fur	Male	Ν	42.7	0.00	Fish	0.00		11000011000
1 41	ivitale	11	12.7	0.00	Wheat/Fish	0.06	0.00	
					Wheat/Fish/Egg/Milk	0.00	0.00	0.71
					11 Hour 1 Ishi 1565 White	Wheat	Fish	Wheat/Fish
Fur	Male	С	140.2	0.00	Fish	0.00	1 1511	vv neu/ 1 15h
i ui	maie	C	170.2	0.00	Wheat/Fish	0.00	0.00	
					Wheat/Fish/Egg/Milk	0.00	0.00	0.00
					Wheat I isit Egg/ willk	Wheat	Fish	Wheat/Fish
Kidney	Female	Ν	9.8	0.01	Fish	0.03	1 1511	w neat/1/1811
Kiulicy	remate	1 N	2.0	0.01	Wheat/Fish	0.03	0.15	
					Wheat/Fish/Egg/Milk	0.00	0.15	0.08
					w neat/FISH/Egg/WillK	Wheat	Fish	Wheat/Fish
Vidnau	Female	С	02.0	0.00	Fish	0.02	FISH	wheat/Fish
Kidney	remaie	C	92.0	0.00	Wheat/Fish		0.00	
						0.00		0.00
					Wheat/Fish/Egg/Milk	0.69	0.00	0.00
17.1	1.1	NT	07.0	0.00	<b>T</b> ' 1	Wheat	Fish	Wheat/Fish
Kidney	Male	Ν	27.0	0.00	Fish	0.00	0.00	
					Wheat/Fish	0.36	0.02	0.01
					Wheat/Fish/Egg/Milk	0.07	0.00	0.01
77' 1	1.6.1	G	24.0	0.00		Wheat	Fish	Wheat/Fish
Kidney	Male	С	34.0	0.00	Fish	0.01	0.00	
					Wheat/Fish	0.00	0.00	
					Wheat/Fish/Egg/Milk	0.52	0.07	0.00
		<b>.</b> -	<b>-</b> -	a		Wheat	Fish	Wheat/Fish
Liver	Female	Ν	3.5	0.07	Fish	0.25		
					Wheat/Fish	0.23	1.00	
					Wheat/Fish/Egg/Milk	0.98	0.15	0.13
						Wheat	Fish	Wheat/Fish
Liver	Female	С	11.9	0.00	Fish	0.33		
					Wheat/Fish	0.14	0.01	
					Wheat/Fish/Egg/Milk	0.03	0.00	0.70
						Wheat	Fish	Wheat/Fish
Liver	Male	Ν	2.4	0.15	Fish	0.42		
					Wheat/Fish	1.00	0.51	
					Wheat/Fish/Egg/Milk	0.20	0.93	0.25
						Wheat	Fish	Wheat/Fish
Liver	Male	С	3.4	0.07	Fish	0.69		
					Wheat/Fish	1.00	0.66	
					Wheat/Fish/Egg/Milk	0.23	0.05	0.27
					. 00	-		

Table S5. The *p*-values from ANOVA and Tukey post-hoc tests for differences in discrimination factors ( $\Delta^{15}$ N and  $\Delta^{13}$ C) between diet types for 6 tissues from rats held on 1 of 4 diets: Plant (wheat gluten, beet sugar, cottonseed oil (sugar and oil from C<sub>3</sub> plants)), Fish (fish meal, cane sugar, corn oil (sugar and oil from C<sub>4</sub> plants)), Plant/Fish (wheat gluten, fish meal, beet sugar, cottonseed oil), and Plant/Fish/Egg/Milk (wheat gluten, fish meal, cow casein, chicken egg whites, cane sugar, corn oil).

Muscle         Female         N         9.4         0.01         Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish         Wheat Wheat/Fish Wheat/Fish         Wheat Wheat/Fish Wheat/Fish           Muscle         Female         C         259.2         0.00         Fish Wheat/Fish Wheat/Fish Wheat/Fish Wheat/Fish         0.00         0.03         0.00           Muscle         Male         N         20.7         0.00         Fish Wheat/Fish         0.00         0.03         0.03           Muscle         Male         N         20.7         0.00         Fish Wheat/Fish         0.00         0.34           Muscle         Male         N         20.7         0.00         Fish Wheat/Fish         0.02         0.00           Serum         Female         N         1.69         0.25         Fish Wheat/Fish Wheat/Fish         0.24         0.99           Serum         Female         C         103.0         0.00         Fish         0.00         0.00           Serum         Male         N         3.7         0.06         Fish         0.00         0.00           Serum         Male         N         28.3         0.00         Fish         0.00	Tissue	Sex	Isotope	F <sub>3.8</sub>	ANOVA <i>p</i> -value	Diet	Tukey p	-values	3
Wnear, Fish         0.16         0.21           Musele         Female         R         259.2         0.00         Fish         0.00         Wheat/Fish/Egr/Milk         0.00         0.00           Musele         Male         Male         P         259.2         0.00         Fish         0.00         0.00           Musele         Male         Male         P         20.7         0.00         Fish         0.00         0.00           Musele         Male         Male         P         9.00         Fish         0.00         0.01           Musele         Male         Male         1.69         0.25         Fish         0.01         Wheat/Fish           Serum         Female         R         1.69         0.25         Fish         0.01         Useat/Fish           Serum         Female         R         1.69         0.25         Fish         0.01         Useat/Fish/Egr/Milk         0.16         0.95         0.85           Serum         Female         Female         R         1.69         0.60         Fish         0.01         Useat/Fish/Egr/Milk         0.02         0.02           Serum         Male         Male         R         R <td></td> <td></td> <td>•</td> <td>210</td> <td>*</td> <td></td> <td></td> <td></td> <td></td>			•	210	*				
Image         Image <th< td=""><td>Muscle</td><td>Female</td><td>Ν</td><td>9.4</td><td>0.01</td><td>Fish</td><td>0.01</td><td></td><td></td></th<>	Muscle	Female	Ν	9.4	0.01	Fish	0.01		
Musele         Female         Female<						Wheat/Fish	0.16	0.21	
Muscle         Female         Female<						Wheat/Fish/Egg/Milk	1.00	0.01	0.18
Muscle         Wale         N         20.7         Mode         Wheat/Fish/Eg/Mik         0.00         0.03         0.00           Muscle         Male         N         20.7         0.00         Fish         0.00         0.01         0.01           Muscle         Male         N         20.7         0.00         Fish         0.00         0.34           Muscle         Male         C         19.1         0.00         Fish         0.00         0.34           Muscle         Male         Male         C         19.1         0.00         Fish         0.00         0.34           Muscle         Male         Male         C         19.1         0.00         Fish         0.02         0.00           Serum         Female         N         1.69         0.25         Fish         0.34         0.95         0.55           Serum         Female         N         1.69         0.25         Fish         0.01         1.00         0.01         1.00         0.01         1.00         0.01         1.01         0.01         1.01         0.01         1.01         0.01         1.01         0.01         1.01         0.01         0.01         1.01							Wheat	Fish	Wheat/Fish
Muscle         Male         N         P         20.7         0.00         Fish         0.00           Muscle         Male         N         20.7         0.00         Fish         0.00           Muscle         Male         N         20.7         0.00         Fish         0.00         0.01           Muscle         Male         C         19.1         0.00         Fish         0.00         0.01           Muscle         Male         C         19.1         0.00         Fish         0.02         0.01           Muscle         Male         C         19.1         0.00         Fish         0.02         0.01           Serum         Female         N         1.69         0.25         Fish         0.14         0.05         0.05           Serum         Female         C         163.0         0.00         Fish         0.14         0.10         0.00           Serum         Male         N         3.7         0.00         Fish         0.00         0.00           Serum         Male         N         3.7         0.06         Fish         0.00         0.00           Serum         Male         N         3	Muscle	Female	С	259.2	0.00	Fish	0.00		
Muse         Male         N         20.7         0.00         Fish         0.00         V           Musel         N         20.7         0.00         Fish         0.01         0.01           Musel         Male         P         P         P         P         Weal/Fish         0.01         0.01           Musel         Male         P         P         P         P         P         P         P           Musel         Male         P         P         P         P         P         P         P         P           Musel         Male         P         P         P         P         P         P         P         P         P           Serum         Female         P         <						Wheat/Fish	0.00	0.00	
Muse         Male         N         20.7         0.00         Fish         0.00         V           Musel         N         20.7         0.00         Fish         0.01         0.01           Musel         Male         P         P         P         P         Weal/Fish         0.01         0.01           Musel         Male         P         P         P         P         P         P         P           Musel         Male         P         P         P         P         P         P         P         P           Musel         Male         P         P         P         P         P         P         P         P         P           Serum         Female         P         <						Wheat/Fish/Egg/Milk	0.03	0.03	0.00
Wheat/Fish/Egr/Mik         0.10         0.00         0.34         0.00         0.34           Muscle         Male         C         19.1         0.00         Fish         0.01         Wheat/Fish/Egr/Mik         0.02         0.01           Serum         Female         N         1.69         0.02         Wheat/Fish         0.02         0.01           Serum         Female         N         1.69         0.25         Fish         0.34         0.02         0.01           Serum         Female         N         1.69         0.25         Fish         0.34         0.02         0.02           Serum         Female         N         1.69         0.25         Fish         0.34         0.99         0.85           Serum         Female         N         1.69         0.00         Fish         0.01         0.02         0.00           Serum         Male         N         3.7         0.06         Fish         0.00         0.01         Wheat/Fish         0.00         0.01           Serum         Male         N         3.7         0.06         Fish         0.00         0.00         0.00           Serum         Male         N							Wheat	Fish	Wheat/Fish
Muscle         Male         C         19.1         0.00         Fish         0.02         0.00           Muscle         Male         C         19.1         0.00         Fish         0.02         0.0           Serum         Female         N         1.69         0.25         Fish         0.24         0.48         0.00           Serum         Female         N         1.69         0.25         Fish         0.24         0.48         0.00           Serum         Female         N         1.69         0.25         Fish         0.24         0.95         0.85           Serum         Female         N         1.69         0.00         Fish         0.00	Muscle	Male	Ν	20.7	0.00	Fish	0.00		
Muscle         Male         C         19.1         0.00         Fish         0.02         0.00           Muscle         Male         C         19.1         0.00         Fish         0.02         0.00           Serum         Female         N         1.69         0.25         Fish         0.24         0.48         0.00           Serum         Female         N         1.69         0.25         Fish         0.24         0.49         0.00           Serum         Female         N         1.69         0.25         Fish         0.24         0.95         0.85           Serum         Female         N         1.69         0.05         Wheat/Fish         0.24         0.95         0.85           Serum         Female         C         103.0         0.00         Fish         0.00						Wheat/Fish	0.10	0.01	
Muscle         Male         C         19.1         0.00         Fish Wheat/Fish (0.06)         Wheat/Fish (0.06)         Fish (0.06)         0.00           Serum         Female         N         1.69         0.25         Fish (0.34)         0.44         0.48         0.00           Serum         Female         N         1.69         0.25         Fish (0.44)         0.44         0.99           Serum         Female         N         1.69         0.25         Fish (0.00)         0.34         0.95         0.85           Serum         Female         C         103.0         0.00         Fish         0.01         0.95         0.85           Serum         Female         C         103.0         0.00         Fish         0.00         0.00         0.00           Serum         Male         N         3.7         0.06         Fish         0.02         0.00           Serum         Male         N         3.7         0.06         Fish         0.99         0.00           Serum         Male         C         79.9         0.00         Fish         0.00         0.00           Serum         Male         C         79.9         0.00         <						Wheat/Fish/Egg/Milk			0.34
MuscleMaleC19.10.00Fish Wheat/Fish/Eg/Milk0.02 0.020.01SerumFemaleN1.690.25Kish0.440.480.00SerumFemaleN1.690.25Fish0.340.740.740.74SerumFemaleN1.690.25Kish0.240.950.85SerumFemaleC103.00.00Fish0.000.000.00SerumFemaleC103.00.00Fish0.000.000.00SerumMaleN3.70.06Fish0.010.020.00SerumMaleN3.70.06Fish0.930.000.02SerumMaleN3.70.06Fish0.010.020.00SerumMaleN3.70.06Fish0.010.020.00SerumMaleN2.8.30.00Fish0.000.010.01RerumMaleC79.90.00Fish0.000.010.01RerumFemaleN28.30.00Fish0.000.010.01RerumFemaleN28.30.00Fish0.000.010.01RerumFemaleN28.40.00Fish0.000.010.01RerumFemaleN28.40.00Fish0.000.010.01									
Serum         Female         N         1.69         0.25         Wheat/Fish/Egg/Mik         0.04         0.44         0.44         0.44           Serum         Female         N         1.69         0.25         Fish         0.12         Wheat/Fish           Serum         Female         N         1.69         0.25         Fish         0.14         0.79           Serum         Female         C         103.0         0.00         Fish         0.10         0.00         0.15           Serum         Female         C         103.0         0.00         Fish         0.00         0.00         0.00           Serum         Male         N         3.7         0.06         Fish         0.93         0.00           Serum         Male         N         3.7         0.06         Fish         0.91         0.25           Serum         Male         R         A         7.7         9.99         0.00         Fish         0.00         0.00           Serum         Male         C         9.79.9         0.00         Fish         0.00         0.00           Male         Female         N         28.3         0.00         Fish	Muscle	Male	С	19.1	0.00	Fish			
SerueFemaleNA.690.25Wheat/Fish/Egg/Mik0.440.480.00SerueFemaleN1.690.25Fish0.240.950.85SerueFemaleC103.00.00Fish0.010.950.85SerueFemaleC103.00.00Fish0.000.000.00SerueFemaleN0.370.00Fish0.000.000.00SerueMaleN3.70.06Fish0.010.020.00SerueMaleN3.70.06Fish0.910.290.00SerueMaleN3.70.06Fish0.910.290.00SerueMaleN3.70.06Fish0.910.290.00SerueMaleN2.779.90.00Fish0.010.290.00SerueMaleN28.30.00Fish0.000.010.010.01RBCFemaleN28.30.00Fish0.010.010.010.01RBCFemaleN324.20.00Fish0.000.010.010.01RBCFemaleN45.50.00Fish0.000.030.010.01RBCFemaleN45.50.00Fish0.000.010.010.01RBCFemaleNA5.50.00Fish <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>0.00</td> <td></td>								0.00	
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SerumFemaleN1.690.25Fish Wheat/Fish/Egg/Mil0.34USerumFemaleC103.00.00Wheat/Fish/Egg/Mil0.610.990.85SerumFemaleC103.00.00Fish0.00Wheat/FishSerumFemaleC103.00.00Wheat/Fish0.000.00SerumMaleN3.70.06Fish0.010.02SerumMaleN3.70.06Fish0.910.62SerumMaleC79.90.00Wheat/Fish/Egg/Mil0.100.62SerumMaleC79.90.00Fish0.000.00SerumMaleC79.90.00Fish0.000.00SerumMaleC79.90.00Fish0.000.00SerumMaleC79.90.00Fish0.000.00SerumMaleFish0.000.000.000.00SerumMaleFish0.000.000.000.00SerumFemaleN28.30.00Fish0.000.00RBCFemaleFish0.000.000.000.00RBCFemaleFish0.000.000.000.00RBCFemaleFish0.000.000.000.00RBCFemaleFish0.000.000.010.00RBC						00			
Serum         Female         C         103.0         0.00         Fish         0.00         0.00           Serum         Female         C         103.0         0.00         Fish         0.00         0.00           Serum         Female         C         103.0         0.00         Fish         0.00         0.00           Serum         Male         N         A.7         0.06         Fish         0.00         0.00           Serum         Male         N         3.7         0.06         Fish         0.01         0.20         0.00           Serum         Male         N         3.7         0.06         Fish         0.01         0.20         0.00           Serum         Male         N         A.7         0.06         Fish         0.01         0.29         0.00           Serum         Male         C         79.9         0.00         Fish         0.00         0.00         0.00           Serum         Male         Female         N         28.3         0.00         Fish         0.00         0.00         0.00           Matrix         Female         R         28.3         0.00         Fish         0.00	Serum	Female	Ν	1.69	0.25	Fish			
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						Wheat/Fish/Egg/Milk	0.00	0.86	0.00

Table S6. Nitrogen and carbon trophic discrimination factors ( $\Delta^{15}N$  and  $\Delta^{13}C$ , reported in  $\% \pm$  SD) between diet (adjusted diet for C; see methods) and tissues for all rats (females and males together) held on 4 experimental diets (see Table 1 for diet details). Tissues are listed in order of isotope turnover time from fastest (liver) to slowest (fur)<sup>1</sup>. N = 6 animals (3 females and 3 males) per diet. The  $\Delta^{13}C$  values between the  $\delta^{13}C_{\text{bulk diet}}$  and  $\delta^{13}C_{\text{rat tissues}}$  are reported in Table S2.

Tissue	Whea	t Diet	Fish	Diet
	All	Rats		Rats
	$\Delta^{15}$ N	$\Delta^{13}C$	$\Delta^{15}$ N	$\Delta^{13}$ C
Liver	+3.1±0.2	+1.2±0.5	+3.1±0.4	$+0.7\pm0.3$
Serum	$+3.6\pm0.2$	+1.5±0.1	$+3.7\pm0.3$	$+0.7\pm0.2$
Kidney	$+2.5\pm0.4$	+1.3±0.1	$+3.1\pm0.4$	$+0.8\pm0.1$
RBC	$+1.9\pm0.3$	+1.3±0.1	$+3.0\pm0.3$	$+0.6\pm0.1$
Muscle	$+2.3\pm0.3$	$+1.7\pm0.1$	$+3.1\pm0.4$	$+1.2\pm0.1$
Fur	$+2.4\pm0.2$	$+3.4\pm0.2$	$+3.9\pm0.3$	$+2.1\pm0.1$
	Wheat /I	Fish Diet	Wheat/Fi	sh/Casein/
			Egg W	hite Diet
Liver	$+3.2\pm0.3$	+1.5±0.9	$+2.9\pm0.3$	$+2.2\pm0.1$
Serum	$+3.7\pm0.2$	$+2.6\pm0.2$	$+3.5\pm0.4$	$+1.2\pm0.1$
Kidney	$+2.7\pm0.4$	$+2.4\pm0.3$	$+2.2\pm0.4$	+1.3±0.1
RBC	$+2.4\pm0.2$	$+2.5\pm0.3$	+2.2±0.2	$+0.5\pm0.1$
Muscle	$+2.6\pm0.4$	$+2.7\pm0.4$	+2.3±0.4	$+1.4\pm0.1$
Fur	$+2.8\pm0.2$	+4.1±0.3	$+2.9\pm0.2$	+2.2±0.1

<sup>1</sup>[50]

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