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Exposure to cold but not exercise increases carbon turnover rates in specific tissues of a passerine

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SUMMARY

Carbon turnover differs between tissues within an animal, but the extent to which ecologically relevant increases in metabolism affect carbon turnover rates is largely unknown. We tested the energy expenditure and protein turnover hypotheses that predict increased carbon turnover, either in association with increased daily energy expenditure, or in concert with tissue-specific increased protein metabolism. We used stable-isotope-labeled diets to quantify the rate of carbon turnover in 12 different tissues for three groups of zebra finches (*Taeniopygia guttata*): cold-exposed birds kept at ambient temperatures below their thermoneutral zone, exercised birds that were flown for 2h per day in a flight arena, and control birds that were kept at ambient temperatures within their thermoneutral zone and that were not exercised. We found that increases in metabolism associated with cold-exposure but not exercise produced measurable increases in carbon turnover rate of, on average, 2.4±0.3 days for pectoral muscle, gizzard, pancreas and heart, even though daily energy intake was similar for exercised and cold-exposed birds. This evidence does not support the energy expenditure hypothesis, and we invoke two physiological processes related to protein metabolism that can explain these treatment effects: organ mass increase and tissue-specific increase in activity. Such changes in carbon turnover rate associated with cold temperatures translate into substantial variation in the estimated time window for which resource use is estimated and this has important ecological relevance.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/213/3/526/DC1

Key words: thermoregulation, stable isotopes, protein turnover, energy metabolism, ^{13}C incorporation, mean carbon retention time.

INTRODUCTION

The pioneering research by Tieszen et al. (Tieszen et al., 1983) revealed that carbon turnover in animals differs between organs and tissues. Carbon turnover rates for small intestine, liver, and kidney were consistently higher than for red blood cells and skeletal muscles in mammals and birds of different sizes (Tieszen et al., 1983; Hobson and Clark, 1992; MacAvoy et al., 2005; Arneson et al., 2006; Sponheimer et al., 2006; Carleton et al., 2008; Bauchinger and McWilliams, 2009). Sponheimer et al. (Sponheimer et al., 2006) related carbon turnover of skeletal muscles to that of the liver and noted that this relative carbon turnover is more or less constant, even between different taxa. Other recent work revealed that the rate of carbon turnover into whole blood for various animals scales with body mass to the approximately –1/4 power (Carleton et al., 2005). This same quarter-power allometric relationship between rate of carbon turnover and animal body mass was confirmed for red blood cells, whole blood, leg muscle, and liver (Bauchinger and McWilliams, 2009). Although the available data are scant, especially for the later two tissues, the prediction is that rate of carbon turnover for all tissues scales similarly with body mass (Bauchinger and McWilliams, 2009).

What mechanisms account for this quarter-power scaling of carbon turnover and animal body mass? Tieszen et al. (Tieszen et al., 1983) used the term 'metabolically more active' to explain why a tissue such as liver incorporates carbon more quickly than skeletal

muscles, and this has led to the hypothesis that organisms and tissues with high metabolic rate will have faster rates of carbon incorporation (Klaassen et al., 2004; McAvoy et al., 2006). In such cases, a positive correlation between metabolic rate and tissue turnover rate is usually used as evidence to support the energy expenditure hypothesis. Here we adopt the interpretation of Carleton and Martinez del Rio (Carleton and Martinez del Rio, 2005), Carleton et al. (Carleton et al., 2008), Martinez del Rio et al. (Martinez del Rio et al., 2008) and Wolf et al. (Wolf et al., 2009) that this phrase was not meant to imply that carbon turnover rate was related to tissue metabolic rate, but rather that more rapid rate of syntheses and degradation of proteins and thus protein turnover increased carbon incorporation rates. Three recent studies provide evidence in support of the protein turnover hypothesis. Cold-acclimated house sparrows (*Passer domesticus*) almost doubled their metabolic rate to satisfy the higher thermoregulatory demands, but the higher metabolic rate was associated with only minor changes in the rate of carbon turnover, and the incorporation rate of nitrogen remained unaffected (Carleton and Martinez del Rio, 2005). Rosy starlings (*Sturnus roseus*) exercised for 34 days also did not show the predicted increase in rate of carbon turnover into red blood cells [RBC (Hobson and Yohannes, 2007)]. Carbon turnover rates for selected house sparrow tissues were fastest for intestine and liver and slowest for skeletal muscle, and this rank order corresponded to that of protein turnover (Carleton et al., 2008).

Our experiment was designed to determine whether increased whole-animal energy expenditure of zebra finches (*Taeniopygia guttata*) associated with thermoregulation in the cold or flying results in increased carbon turnover rate of all or only some tissues. If cold-acclimated and exercised birds have equally higher whole-animal energy expenditure as control birds, but tissue-specific turnover rates differ between treatment groups, then such evidence would not support the energy expenditure hypothesis. If tissue-specific turnover rates are consistently in the same rank-order as tissue-specific protein turnover rate (Waterlow, 2006) regardless of differences in whole-animal energy expenditure, then such evidence would support the protein turnover hypothesis. This experimental design also allows us to detect organ-specific responses to the different treatments. For example, rate of carbon turnover in flight-exercised birds may increase most in organs such as pectoral muscle and the heart rather than, for example, leg muscle, because of their different use during powered flight, whereas these organ-specific differences may not occur in cold-exposed birds sitting in cages. We chose an ambient temperature for the cold group so that energy and food intake of the cold group was similar to that of the exercise group. This allowed us to determine whether differences in carbon turnover between treatment groups were related to exercise, energy/food intake, or some combination of both.

MATERIALS AND METHODS
Animals and diets
We randomly assigned 87 adult zebra finches to three experimental conditions: control group (*N*=30), exercise group (*N*=28) and cold group (*N*=29). Birds were kept in single-sex groups of four to six individuals under a 12h:12h L:D regime, in cages (45cm×90cm×45cm), at an air temperature of 32±2°C (mean ± standard deviation) for control group and exercise group, and at 15±1°C for the cold group. The air temperature for the control and exercise groups was within the thermoneutral zone (29.5–40°C) of the zebra finch (Calder, 1964). Birds were initially fed a C_4-mixed seed diet ($\delta^{13}C$=–16.3±0.33‰ $\delta^{13}C$; Blattner no. 1140103, Ermengerst, Germany) for at least 3months prior to onset of treatment. Food of all birds was changed to a C_3 single-seed diet ($\delta^{13}C$=–26.3±0.50‰ $\delta^{13}C$; Blattner no. 160505, Ermengerst, Germany) and the day of this diet shift is subsequently referred to as experimental day 0. Both the C_4 and C_3 diets were enriched (2g kg^{-1} seeds) with a mineral and vitamin mixture (Günter Enderie, Nektron-MSA Lot-No 363850 and Nektron-S Lot-No 364636; Blattner, Ermengerst, Germany). Birds were supplied with food and water *ad libitum* each day, apart from the 2h during the daily experimental treatment (see below). Morning body mass was recorded to the nearest 0.01g weekly throughout the entire experimental period (Kern balance, 440-33N, Balingen, Baden-Württemberg, Germany).

Experimental treatments
Starting 2weeks before the diet shift (day–14), birds were either subjected to an exercise treatment (exercise group), a cold treatment (cold group) or remained without exercise within the thermoneutral zone (control group). Treatments lasted for 10weeks (until experimental day 56) for the exercise and cold group, whereas the control group birds were maintained until day 256 post-diet shift to establish carbon isotope values of each tissue for birds fully acclimated to the C_3-diet. Exercised birds were subjected to flight training for 1h twice a day for 6weeks, while control and cold group birds remained in their cages. Food was removed from all cages while exercise group birds were trained and were without access to food and water. During each 1h flying period, exercised birds flew in a flock around a flight aviary (6m×3m×2.5m) that had perches in opposite corners and a wall that partially divided the aviary lengthwise. A person continuously walked clockwise around the partition 300 times, resulting in 600 flights of 6m for each bird each hour. This type of exercise is energetically expensive because costs of passerine flight are among the highest reported for endurance exercise in vertebrates, and the cost of burst flight, like that which occurs immediately after take-off, is three times higher than that of sustained flight (Nudds and Bryant, 2000).

Tissue sampling
We designed the tissue sampling scheme so that we could accurately estimate carbon turnover rate of 12 tissues from exercised, cold-acclimated and control birds. Three to eight birds from each experimental group were killed on the mornings of days 0, 1, 2, 4, 8, 16, 32, 56, and for the control group an additional eight birds were sampled on days 128 and 256 to obtain asymptotic carbon isotopic values for tissues with slower carbon turnover rate. Sampling on day 0 was done immediately before the diet shift, thus these tissues were from birds fed only the C_4 diet for at least 3months. Sampled birds were weighed to the nearest 0.1g, decapitated, and selected tissues (see below) were removed within 10min. Blood was collected immediately upon decapitation, centrifuged for 10min (Rotilabo mini-centrifuge, Carl Roth, Karlsruhe, Baden-Württemberg, Germany) for separation of red blood cells (RBC) from plasma and RBC were kept for further analysis. All tissue samples were dried, homogenized using a pestle and mortar, and fat was extracted for 24h in a Soxhlet apparatus (Merck, Whitehouse Station, NJ, USA; petroleum benzene, boiling range 40–60°C) in order to determine carbon isotopic value of fat-free tissue. Fat-free tissues were stored in Eppendorf tubes and then stable isotope were analyzed with a Carlo-Erba (Lakewood, NJ, USA) NA 1500 Series II Elemental Analyzer, attached to a continuous-flow isotope ratio Micromass Optima spectrometer (Beverly, MA, USA; CF-IRMS) (Podlesak and McWilliams, 2006). On sampling day 32, eight birds from each group were killed to determine the effect of exercise and cold treatments on organ mass of birds. We measured to the nearest 0.0001g (Sartorious balance, type 2432) the wet tissue mass of pectoral muscle (hereafter flight muscle) and muscles of the tibiotarsus (hereafter leg muscle), proventriculus, gizzard, small intestine, pancreas, heart, liver, kidney and brain. Tissues were dried at 60°C to constant mass for determination of dry tissue mass. Tarsus length of all individuals was measured using calipers and used as an index of bird size.

Food and feces measurement
We measured daily food intake and excreta (feces plus uric acid) production of experimental and control birds (6–8 individuals per group) between days 8 and 28 of the experiment. Birds were housed individually for two consecutive days during which we measured daily food intake by subtracting the remaining food in the cage in the evening from the amount of food offered in the morning. Bird excreta were collected from plastic-coated paper and quantified as excreta produced during 24h. Food and excreta were dried at 60°C and then energy density of food and excreta was measured using bomb calorimetry (Bomb calorimeter, Model C7000, IKA-Analysetechnik, Heitersheim, Germany). Zebra finches remove the husk and only ingest the seed. We quantified the energy content of the husk fraction and calculated the energy intake for the food

without husk. We calculated apparent metabolizable energy intake (AMEI) per day for experimental groups as:

$$AMEI = (E_{intake} \times m_{intake}) - (E_{excreta} \times m_{excreta}), \qquad (1)$$

where (E_{intake}; in $kJ\,g^{-1}$ food) is the energy density of food corrected for the husks, ($E_{excreta}$; in $kJ\,g^{-1}$ excreta) is the energy density of excreta, and m_{intake} and $m_{excreta}$ are daily food intake ($g\,day^{-1}$) and daily excreta production ($g\,day^{-1}$), respectively (Servello et al., 2005).

Carbon turnover

Several recent studies have emphasized that isotopic incorporation into some tissues is best described by multi-compartment models rather than standard one-compartment models with first-order rate kinetics (Cerling et al., 2007; Carleton et al., 2008; Martinez del Rio and Anderson-Sprecher, 2008; Martinez del Rio et al., 2008; Kurle, 2009; Wolf et al., 2009), so we empirically determined the best-fit model for each tissue as recommended (e.g. Carleton et al., 2008; Bauchinger and McWilliams, 2009). We fitted carbon isotopic values for each organ and experimental group over time since diet switch to a one- and a two-compartment non-linear model to assess which of the models was best supported. The one-compartment model was a standard first-order kinetic function: $y_t = y_\infty + ae^{(-t/\tau)}$, where y_t is the measured $\delta^{13}C$ of tissue in ‰ at time t; y_∞ is the estimated final $\delta^{13}C$ of tissue after the switch from a C_4 to a C_3 diet in parts per thousand; a is the estimated range in $\delta^{13}C$ between diets in parts per thousand; τ is the estimated average carbon retention time in days (a measure used to describe the rate of carbon turnover); and t is time in days since the diet switch (Martinez del Rio and Wolf, 2005). The two-compartment model was similar to that recommended by Martinez del Rio and Anderson-Sprecher (Martinez del Rio and Anderson-Sprecher, 2008) and used by Carleton et al. (Carleton et al., 2008): $y_t = y_\infty + a(p_1)e^{(-t/\tau 1)} + a(1-p_2)e^{(-t/\tau 2)}$, where p is the fractional size of each 'pool' (Cerling et al., 2007) or 'phase' (Martinez del Rio and Anderson-Sprecher, 2008). In our application of two-compartment models, p_1 and p_2 are used to describe the fractional size of pools one and two, and τ_1 and τ_2 give the associated average carbon retention time of each pool. We estimated the average carbon retention time for one-compartment models as $\tau_{one-comp}$ and that for two-compartment models as: $\tau_{two-comp} = p\tau_1 + (1-p)\tau_2$ (Carleton et al., 2008).

For control birds, we used the measured carbon isotope values for each organ from birds sampled on day 0 to day 256. For exercised and cold group birds, we used the measured carbon isotope values for each organ from control birds sampled on day 0, immediately before the diet shift, the appropriate experimental birds sampled on day 1 to day 56, and control group birds sampled on days 128 and 256. This procedure assumes that treatment birds were initially similar to controls and that 128 days is adequate time for carbon isotope values of these organs to reach asymptotic values regardless of treatment condition. This assumption is justified given published estimates of carbon turnover rate (Carleton et al., 2008) as well as our own (Bauchinger and McWilliams, 2009), which suggest that >90% of tissue carbon is replaced by 128 days in all organs except for skin and brain. The non-linear regression algorithms always found a locally optimal one- and two-compartment model for all tissues. However, brain and skin carbon values in control birds had not reached an asymptote after 256 days and this resulted in unreasonable estimates of final $\delta^{13}C$ of these tissues (y_∞) that were more negative than that of diet ($\delta^{13}C = -26.3 \pm 0.50$‰). Thus, for brain and skin we report parameter estimates from two-compartment models with these constraints:

$y_\infty > -24.5$‰ $\delta^{13}C$ and a >11.5‰ $\delta^{13}C$ (assuming ~1.5‰ discrimination from diet). For the data set of small intestine exercise group and flight muscle cold group we removed one outlier defined by the program (studentised residual ≥2.5), after which curve fitting could be performed without adding any constraints. Likewise, we removed one outlier for the pancreas and skin data set from sampling day 0.

We compared AIC (Akaike information criterion) for small sample sizes [ΔAIC_c (Burnham and Anderson, 2002)] for one- versus two-compartment models for each tissue to define the best-supported model. Burnham and Anderson (Burnham and Anderson, 2002) used $\Delta AICc = AIC_i - AIC_{min}$ as an estimate of the information loss of a model ($_i$) compared with the estimated best model ($_{min}$). The further $\Delta AICc$ deviates from 0, the less supported is the respective model. In the range 0–2, both models have substantial support, in the range 4–7 model (i) has considerably less support and models with values above 10 are not supported (Burnham and Anderson 2002). When $\Delta AICc > 2$, we chose the model with the higher level of support. If $\Delta AICc$ for a given tissue indicated high level of support for only one of the used models for at least one of the groups we used this model for parameter estimation for all three groups, even if $\Delta AICc$ indicated equally high support for both models. This procedure allowed us to compare τ-values estimated for a specific tissue from the same model for all three groups. Choosing the same model for parameter estimation is important because two-compartment models tend to result in higher τ-values compared with one-compartment models, especially for tissues with a faster carbon turnover rate (Carleton et al., 2008; Bauchinger and McWilliams, 2009). We regressed average retention time estimates from one- and two-compartment models to compare turnover rate estimates between models.

Standard errors for τ from the two-compartment models were derived according to Martínez del Rio and Anderson-Sprecher (Martínez del Rio and Anderson-Sprecher, 2008), and we estimated the 95% confidence interval of the estimates as the critical value of the t-distribution with $\alpha = 0.05$ multiplied by the standars error of the estimate (Sokal and Rohlf, 2000). Wilcoxon signed-rank test was used to test for a consistent difference in τ between groups across all ten tissues. By use of this test we could control for the heterogeneity in τ-values across tissues and each tissue is weighed similarly. We used ANCOVA to test for differences in τ between groups for each of the ten tissues [Zar pp. 370-371 (Zar, 1999)].

We used ANOVA to test for differences in food intake and AMEI between the groups and ANCOVA with correction for tarsus length as measurement of structural size to test for differences in body mass and tissue mass between groups. All statistical analyses were performed using SIGMAPLOT (version 10.0) and SYSTAT (version 12.0).

RESULTS
Body mass and tissue mass
Exercise, cold and control treatment had no significant effect on size-corrected body mass measured on day 32 and birds on average maintained body mass during the 6 weeks of exercise, cold, or control treatment (Table 1). Of the ten tissues for which we measured dry mass, only gizzard and pancreas were heavier in cold-acclimated birds compared with exercised birds (Table 1).

Food intake and AMEI
Daily food intake was significantly different between the three groups (ANOVA $F_{2,31} = 51.0$, $P < 0.001$) with cold-acclimated and exercised birds eating 108% and 149%, respectively, more on average than control birds (Fig. 1). Daily feces production was

Table 1. Dry tissue mass and body mass for the three experimental groups sampled at day 32 following six weeks of treatment, i.e. control, flight exercise, cold exposure

	Control group I Mean (±95 CI)	Exercise group II Mean (±95 CI)	Cold group III Mean (±95 CI)	Corrected model		Tarsus (covariate)		Group (factor)	
				F-value	P-value	F-value	P-value	F-value	P-value
M_b	14.95 (±0.99)	14.35 (±0.80)	14.97 (±1.97)	**12.9**	**0.000**	**37.7**	**0.000**	1.1	0.35
$\Delta M_{b\ (treatment)}$[†]	−0.50 (±0.42)	−0.19 (±0.46)	−0.06 (±0.66)	1.3	0.31				
Carcass*	1.968 (±0.283)	2.003 (±0.266)	1.946 (±0.267)	2.6	0.08				
Small intestine	0.028 (±0.005)	0.025 (±0.005)	0.031 (±0.005)	2.0	0.15				
Gizzard	0.053 (±0.010)[a,b]	0.050 (±0.006)[a]	0.067 (±0.010)[b]	**5.9**	**0.005**	**5.4**	**0.031**	**5.6**	**0.012**
Kidney	0.015 (±0.001)	0.014 (±0.003)	0.016 (±0.003)	2.0	0.15				
Liver	0.035 (±0.013)	0.034 (±0.008)	0.037 (±0.011)	1.1	0.38				
Pancreas	0.020 (±0.003)[a,b]	0.016 (±0.003)[a]	0.021 (±0.003)[b]	**4.4**	**0.016**	**4.5**	**0.046**	**4.0**	**0.034**
Proventriculus	0.007 (±0.002)	0.006 (±0.002)	0.007 (±0.002)	2.1	0.13				
Heart	0.024 (±0.004)	0.026 (±0.002)	0.024 (±0.003)	2.4	0.10				
Brain	0.051 (±0.004)	0.050 (±0.005)	0.055 (±0.004)	1.3	0.32				
Flight muscle	0.399 (±0.041)	0.408 (±0.035)	0.403 (±0.055)	**7.3**	**0.002**	**21.8**	**0.000**	0.1	0.89
Leg muscle	0.055 (±0.006)	0.057 (±0.003)	0.059 (±0.009)	**12.1**	**0.000**	**34.1**	**0.000**	0.4	0.69
N (d.f.)	8	8	8	3 (23)		1 (23)		2 (23)	

M_b, body mass in grams.

Statistical results are presented for general linear model ANOVA, for the covariate tarsus and the difference between groups. Different letters indicate significant differences between the respective groups based on Schéffé *post-hoc* tests ($P<0.05$).

*Lean dry mass after removal of the analysed tissues.

[†]Change after 6 weeks of treatment.

Values in bold indicate statistically significant results.

$0.31\pm0.14\,\mathrm{g\,day^{-1}}$ for the control group, $0.36\pm0.03\,\mathrm{g\,day^{-1}}$ for the exercise group and $0.48\pm0.12\,\mathrm{g\,day^{-1}}$ for the cold group. Energy density of the seeds without husk was calculated as $19.3\,\mathrm{kJ\,g^{-1}}$ given that energy density of seeds including husks was $18.8\pm1.0\,\mathrm{kJ\,g^{-1}}$ (N=3), energy density of the husks was $15.1\pm0.4\,\mathrm{kJ\,g^{-1}}$, and mean mass of husks was 13.6% of total seed mass. Energy density of feces was $17.51\pm1.54\,\mathrm{kJ\,g^{-1}}$ for the control group, $18.13\pm0.54\,\mathrm{kJ\,g^{-1}}$ for the exercise group, and $17.59\pm0.37\,\mathrm{kJ\,g^{-1}}$ for the cold group. Apparent metabolizable energy intake was significantly lower in control compared with treatment birds (exercise and cold group; ANOVA, $F_{2,21}$=21.9, $P<0.001$; Fig. 1).

One-compartment model *versus* two-compartment model

The rate of carbon isotope incorporation into 11 of the 12 tissues was best described by two-compartment models (i.e. ΔAICc values >2) whereas that of RBC was best described by one-compartment models (i.e. ΔAICc values <2; Table 2, supplementary material Fig. S1A,B). The choice of a one- or two-compartment model was ambiguous for heart, brain, flight muscle, leg muscle and skin from control birds, and for gizzard from exercised birds; however, the two-compartment model best described rate of carbon incorporation from the other two groups of birds for these same tissues (Table 2). Therefore, we used the parameter estimates from the two-compartment model for all groups for all tissues except RBC so that turnover rates for a given tissue were comparable between groups (see all details for both models in supplementary material Table S1).

Fitting one-and two-compartment models to the carbon isotope incorporation into brain and skin was more complicated because we needed to include additional constraints (y_∞>−24.5‰ δ^{13}C and a >11.5‰ δ^{13}C) in order for the models to produce biologically reasonable estimates of asymptotic δ^{13}C. Despite these additional constraints, and unlike all other tissues, the resulting estimates of turnover rate from the one- and two-compartment models were remarkably different (e.g. 18.5 and 65.5 days for brain, respectively; Table 2). These inconsistent results probably occurred because more than 256 days is required to reliably estimate the final carbon tissue value of brain and skin (y_∞; supplementary material Table S1, Fig S1A,B).

Average carbon retention time (τ)

Carbon turnover estimated as mean retention time (τ) ranged from ~7 days for small intestine, 8–12 days for gizzard, pancreas, kidney and liver, 10–18 days for proventriculus and heart, 16–24 days for RBC and flight muscle, and 26–29 days for leg muscle (Table 2). Cold-acclimation consistently affected carbon turnover of tissues whereas exercise did not (Fig. 2). τ for all ten tissues was consistently fastest for the cold group, with significant differences between control and cold groups, and between exercise and cold groups (Wilcoxon signed rank test, both cases $P<0.01$), but was not different between control and exercise groups (P=0.33). Testing for a tissue-specific difference in carbon turnover rates between the groups revealed that gizzard, heart, flight muscle and pancreas were the only tissues with significantly faster turnover rates in cold group birds (Fig. 3), although the same trend was apparent for all other tissues except leg muscle (Table 2). We calculated a pooled τ across groups for tissues that were not significantly different between the groups, namely, small intestine, kidney, liver, proventriculus, RBC and leg muscle (Fig. 3).

DISCUSSION

We predicted a uniform faster carbon turnover for all tissues in response to exercise and cold treatment based on the energy expenditure hypothesis (Tieszen et al., 1983; McAvoy et al., 2006). Exercise did not consistently increase the rate of carbon incorporation for any of the ten tissues, even though the exercised birds doubled AMEI compared with control birds. By contrast, cold exposure consistently increased carbon turnover across tissues and especially for flight muscle, heart, gizzard and pancreas. Given that cold-exposed and exercised birds had no difference in AMEI, these results are not consistent with the hypothesis that increased whole-animal energy expenditure in turn increases tissue-specific carbon turnover. We consider our results as more consistent with the protein turnover hypothesis.

Table 2. Mean ^{13}C retention time and Akaike information criteria for 12 tissues of the three experimental groups (control group, exercise group and cold group) estimated by one-compartment and two-compartment models that were fitted for tissue-specific $\delta^{13}C$ values collected over 256 days after a diet shift

Tissue	Group	$\tau_{one\ comp}$	$\tau_{two\ comp}$	$AIC_{c\ one\ comp}$	$AIC_{c\ two\ comp}$	$\Delta\Delta_{one-two}$	F-value[‡]	P-value
Small intestine	Control	3.8	**8.0**	96.8	91.8	5.0	1.0	0.715
	Exercise	3.2	**6.5**	117.1	105.9	11.2		
	Cold	2.3	**6.0**	83.6	69.7	13.9		
Gizzard	Control	7.6	**10.0[a]**	98.9	88.3	10.6	5.4	0.012
	Exercise	8.4	**10.2[a]**	106.3	104.5	1.8		
	Cold	4.2	**7.2[b]**	88.6	82.8	5.8		
Pancreas	Control	6.8	**10.6[a]**	92.7	69.6	38.1	5.2	0.014
	Exercise	6.7	**10.8[a]**	105.5	95.3	31.4		
	Cold	4.6	**8.2[b]**	88.8	65.2	40.7		
Kidney	Control	9.2	**11.6**	90.0	74.8	15.0	0.6	1.000
	Exercise	7.5	**9.7**	74.7	64.1	10.6		
	Cold	7.2	**9.4**	86.7	81.6	5.1		
Liver	Control	5.8	**11.9**	107.0	83.2	23.9	0.8	0.941
	Exercise	5.8	**12.2**	97.9	79.0	18.9		
	Cold	4.4	**9.6**	79.3	56.4	22.9		
Proventriculus	Control	7.4	**13.3**	100.5	71.7	28.8	0.1	1.000
	Exercise	6.4	**17.0**	113.5	89.2	24.4		
	Cold	5.2	**10.1**	90.5	75.1	15.4		
Heart	Control	17.2	**17.0[a]**	77.5	76.9	0.6	9.8	<0.001
	Exercise	14.8	**18.1[a]**	96.1	89.2	6.9		
	Cold	12.0	**15.0[b]**	89.5	63.9	25.6		
Brain*	Control	18.5	**65.5**	117.2	116.7	0.6	–	–
	Exercise	22.6	**26.0**	119.0	115.8	3.1		
	Cold	14.5	**19.2**	113.6	105.6	8.1		
RBC[†]	Control	**19.3**	19.5	108.4	113.6	−5.2	2.7	0.151
	Exercise	**16.8**	17.0	107.5	111.1	−3.6		
	Cold	**15.9**	15.9	98.6	105.2	−6.6		
Flight muscle	Control	20.9	**21.0[a]**	92.8	92.6	0.3	10.1	<0.001
	Exercise	20.3	**24.2[a]**	103.9	92.2	11.7		
	Cold	15.0	**18.8[b]**	105.0	80.8	24.3		
Leg muscle	Control	26.0	**25.9**	108.6	106.7	1.9	2.2	0.235
	Exercise	28.3	**29.4**	105.2	102.0	3.2		
	Cold	21.9	**26.8**	111.5	100.5	11.0		
Skin*	Control	24.2	**25.0**	117.4	116.7	0.7	–	–
	Exercise	28.7	**82.1**	136.5	124.8	11.7		
	Cold	5.8	**99.8**	125.8	105.2	20.6		

τ, mean ^{13}C retention time (in days); AIC, Akaike information criterion.

*Tissues with large differences between $\tau_{one\ comp}$ and $\tau_{two\ comp}$ and heterogeneous results between the treatment groups, see Results for details. No statistical comparison performed.

[†]Average retention time from the one-compartment model was used because $\Delta_{one-two}<2$.

[‡]Numerator d.f.=2 in all cases, denominator d.f.=96–104 depending on sample size.

For each tissue $\Delta_{one-two}$ (=$AIC_{c\ one\ comp}$–$AIC_{c\ two\ comp}$) was used to determine the model with the highest level of support (Burham and Anderson, 2002) and the associated average retention time ($\tau_{one\ comp}$ or $\tau_{two\ comp}$) used for subsequent analyses (in bold). Different letters indicate significant differences between the respective groups with $P''0.012$.

Response to exercise

We found that exercise had no effect on the carbon incorporation rate or mass of the 10 sampled tissues (Tables 1, 2 and Fig. 3). Hobson and Yohannes (Hobson and Yohannes, 2007) also found that carbon incorporation rate of RBC in rosy starlings did not increase in response to exercise. By contrast, repeated daily exercise reduced flight muscle mass in European starlings (*Sturnus vulgaris*) (Swaddle and Biewener, 2000). They suggested that the reduced muscle mass enhanced flight efficiency and was associated with compositional changes of the muscle that are necessary to maintain power output. Compositional changes are reported for flight muscle in various birds in response to egg laying, fasting, sustained migratory flights, or in preparation for migration (Marsh, 1981; Marsh, 1984; Marsh, 1985; Lundgren and Kiessling, 1985; Lundgren and Kiessling, 1986; Jones, 1991; Swain, 1992; Bauchinger and Biebach, 2001) and such changes, if occurring, may alter whole-muscle carbon turnover because certain components (e.g.

sarcoplasmic and myofibrillar proteins) differ in carbon turnover rate (Goldstein and Reddy, 1967). We suspect that our 2 h daily exercise was not strong enough to induce changes in composition and mass of the tissues including the flight muscle.

Response to cold

Exposure to temperatures below the thermoneutral zone results in increased heat production and this may account for the observed increase in carbon turnover rate in cold-exposed birds. Birds generate heat primarily in three ways (Rashotte et al., 1999; Eduardo et al., 2001): as a byproduct of metabolism associated with locomotion, as a byproduct of digestion, and shivering thermogenesis which occurs mainly in skeletal muscles (Carey et al., 1978; Marsh and Dawson, 1982; Raimbault et al., 2001). Birds must use shivering thermogenesis because they lack the uncoupling protein 1, which enables mammals to perform non-shivering thermogenesis in adipose tissue (brown fat) (Mezentseva et al.,
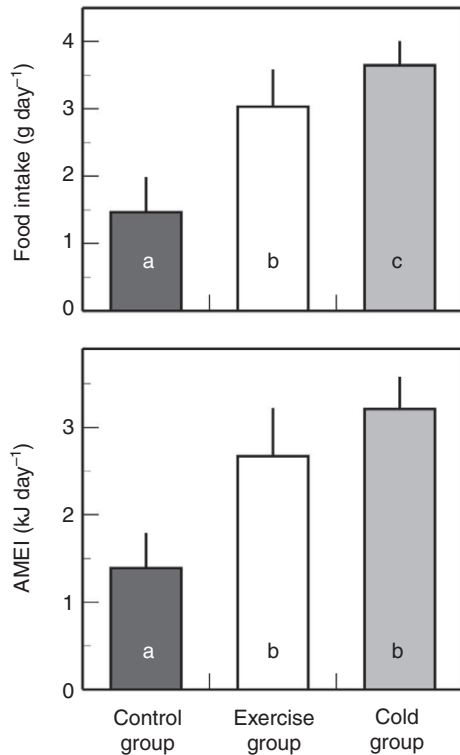
Fig. 1. Mean food intake (upper graph) and mean apparent metabolizable energy intake (AMEI; lower graph) plotted for the three groups. Error bars indicate the standard deviation. Different letters indicate significant differences between the groups based on ANOVA followed by *post-hoc* Scheffé test.

Fig. 2. Difference between average carbon retention time (τ) in each tissue examined for each pair of the three experimental groups. (Upper panel) Differences in τ between control group and exercise group, with negative values indicating a faster carbon retention time for exercise group. (Middle panel) Differences in τ between the control group and cold group showing a faster carbon retention time in nine out of ten tissues. (Lower panel) Differences in τ between exercise group and cold group showing a faster carbon retention time in all ten tissues. Statistical details in the panels refer to Wilcoxon signed-rank test for the respective two groups.

2008). The cold group birds ate more each day than control birds, indicating that this cold exposure affected the energy metabolism of the animals. Rashotte et al. (Rashotte et al., 1999) found that heat production from shivering thermogenesis was higher in fasted than in fed pigeons (*Columba livia*). Shivering thermogenesis in birds is associated with increased mitochondrial volume and density and capillary diameter of the flight muscle (Mathieu-Costello et al., 1998), as well as increased activity of cytochrome *c* oxidase and state-4 respiration in flight muscle and liver (Zheng et al., 2008). Such an increase in sarcoplasmic proteins relative to myofibrillar proteins would result in more rapid tissue-specific rates of carbon turnover (Goldstein and Reddy, 1967). This indicates that more tissues are subjected to changes in the specific activity level besides the skeletal muscles, the tissue typically analyzed in studies on cold exposure. We propose that the increased demands associated with shivering thermogenesis significantly affected protein metabolism and hence carbon turnover rate of tissues in cold-exposed birds.

We highlight two different physiological processes known to be associated with heat production that can directly affect rate of carbon turnover for specific tissues and that can explain our results: phenotypic mass increase and elevated tissue-specific activity. Increasing the size of organs involves constructing proteins and increased activity of tissues requires more proteins associated with metabolism. The latter is often associated with structural and compositional changes of tissues that can include replacement of components with faster turnover rates [e.g. sarcoplasmic *versus* myofibrillar proteins (Bauchinger and Biebach, 2001)].
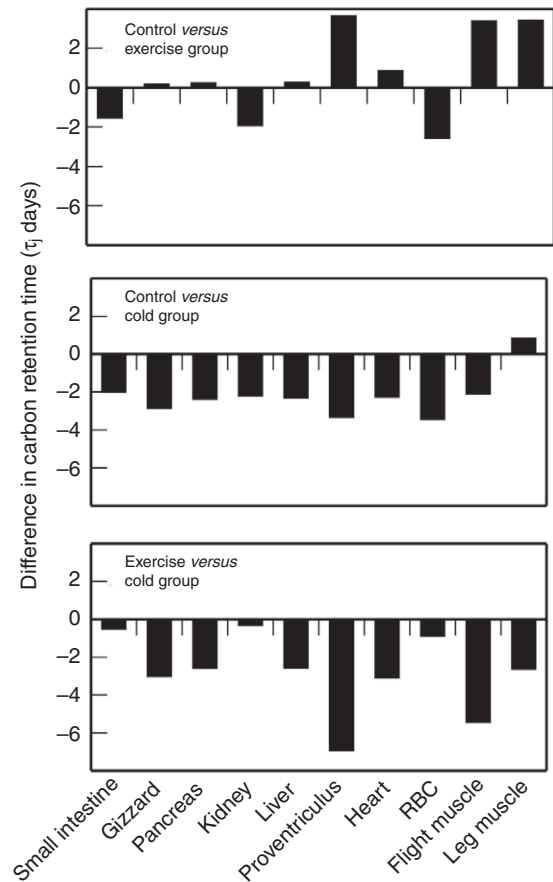
Ten-weeks exposure to constant cold temperature resulted in a significantly higher food intake, faster carbon turnover in all tissues, and an increase in mass of pancreas and gizzard in zebra finches. Recall that cold-exposed birds ate significantly more than exercised birds although AMEI was not different between these two groups. The 100–150% increase in food intake of treatment birds in our study was similar in extent to that of free-living birds in winter compared with summer in juniper titmouse [*Baeolophus griseus* (Cooper, 2000)]. The simultaneous increase in mass and carbon turnover of the gizzard and pancreas in cold-exposed birds is probably a consequence of increased activity and protein synthesis of tissues associated with digestion.

The increase in pancreas and gizzard mass of cold-acclimated birds provides another example of phenotypic flexibility in digestive organs of birds in response to increased demands (reviewed by Piersma, 2002; McWilliams and Karasov, 2005; Karasov and McWilliams, 2005). Zebra finches exposed to the cold ate more than exercised and control birds, had higher AMEI than control birds, and had heavier pancreas and gizzard than exercised and control birds. In general, birds exposed to the cold increase food intake to
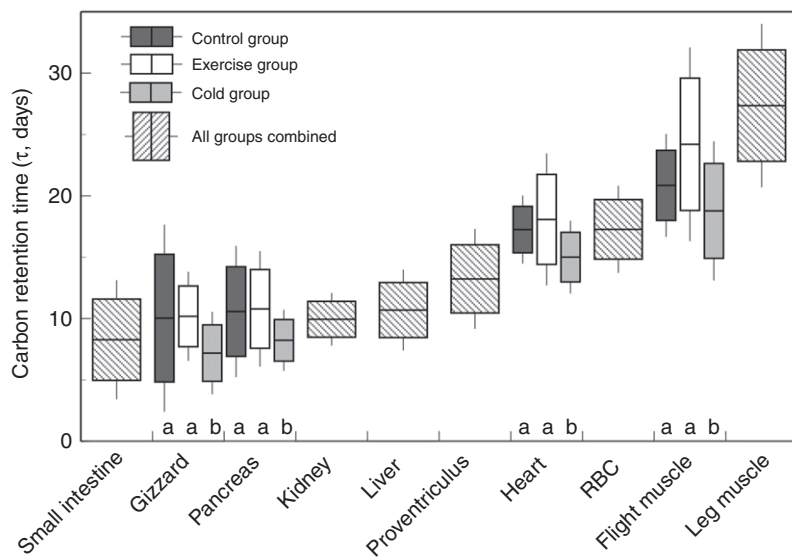
Fig. 3. Average carbon retention time (τ) in days for 10 different organs in zebra finches. Data are presented for all groups combined (hatched boxes) when no statistical difference was calculated. When average retention time did significantly differ all three groups are plotted separately (dark grey, control group; white, exercise group; light grey, cold group). Identical letters indicate that organs were not significantly different. Box plots show 25% and 75% confidence intervals, error bars refer to 5% and 95% confidence intervals.

compensate for increased requirements of thermoregulation and this is associated with increased mass of a suite of digestive organs (McWilliams et al., 1999; McWilliams and Karasov, 2005; Karasov and McWilliams, 2005; Zheng et al., 2008). Increased rate of tissue synthesis rather than a reduction in tissue breakdown usually produces organ mass change (Swick and Benevenga, 1967) and this can produce the observed higher rate of carbon incorporation. However, other digestive organs (e.g. liver, small intestine) in zebra finches did not change in mass with increased demand, which is inconsistent with most other cold-acclimation studies of birds (e.g. Zheng et al., 2008). These contrasting results probably occur because of differences between studies in the extent of cold. The zebra finches in our study were exposed to 15°C primarily because we attempted to match food intake of birds exercised for 2h each day with that of our cold-exposed birds. Most other studies of cold-acclimation in birds used much lower temperatures [e.g. –15°C for sparrows (Zheng et al., 2008), –20°C for waxwings (McWilliams et al., 1999)] that require birds to eat more and thus cause more extensive changes in mass of many digestive organs.

Here we show that phenotypic flexibility in mass of pancreas and gizzard was associated with increased rate of carbon turnover. Interestingly, other digestive organs (e.g. liver, small intestine) in zebra finches did not change in mass with increased demand while the rate of carbon turnover of these organs consistently increased in cold-exposed birds. The significant effects of cold acclimation on carbon turnover rate of certain tissues that we report are consistent with the few other studies that have been conducted. House sparrows had slightly shorter carbon half-life of RBC (14.7days) when maintained at 5°C ambient temperature compared with 16.5days at 22°C, which was considered to be the result of increased protein turnover (Carleton and Martínez del Rio, 2005). We did not detect a significant difference in RBC turnover rate for zebra finches kept at 15°C (16.8days) compared with unexercised birds at 32°C (19.3days). Thus, increased rate of carbon turnover may be a general response to increased thermogenic demands, and phenotypic flexibility in whole-organ mass may occur only with more extreme cold.

Transfer of heat within the bird's body would also be facilitated by an increase in the capacity of the cardiovascular system including increased heart muscle activity and supply of blood vessels. Pigeons exposed to cold temperature increased capillary density in the flight muscle (Mathieu-Costello et al., 1998). Rats increased heart rate after 1week of cold exposure (Chambers et al., 2000) and chronic exposure to cold ambient temperature resulted as well in an increased cytochrome oxidase activity in various rat tissues, including the heart, liver, kidney and skeletal muscle (Terblanche et al., 2000). We found that cold-exposed zebra finches increased carbon turnover rate of the heart, which may provide another example of how increased tissue-specific activity affects carbon turnover. Increased cardiovascular support in response to cold-exposure seems necessary to meet the elevated demands in resource supply for mass increase, increased turnover and shivering thermogenesis in the flight muscle. Furthermore, a higher amount of carrier proteins in the plasma fraction of the blood can be expected in response to higher energy metabolism during cold exposure, but unfortunately, neither study quantified the carbon turnover of plasma proteins.

One- versus two-compartment model
The recent findings from a number of other investigations (Cerling et al., 2007; Martinez del Rio and Anderson-Sprecher 2008; Carleton et al. 2008; Bauchinger and McWilliams, 2009) and our present study make it clear that one- and multi-compartment models must be compared and that information from theoretical criteria are useful for determining the best supported model(s) given data on isotope incorporation over time. Isotope incorporation over time was best described by the two-compartment model for nine of the ten tissues, and only for RBC was the one-compartment model best (Table 1, supplementary material Table S1, Fig. S1A,B). These results are consistent with those of Carleton et al., (Carleton et al., 2008) and Bauchinger and McWilliams (Bauchinger and McWilliams, 2009). In general, estimated τ from the two models was most different for faster turnover splanchnic tissues (e.g. intestine, liver, heart) compared with structural tissues with slower turnover rates (e.g. flight and leg muscle; supplementary material Fig. S2). The rank order of carbon turnover rate between tissues was evident regardless of the model used, although the absolute values of τ for a given tissue and treatment group were often quite different for the two models. Most importantly for the present study, our conclusions about how exercise and cold affected carbon turnover rate for a given tissue did not depend on the model used. Specifically, cold-acclimated birds had consistently more rapid carbon turnover for

the four tissues for which we detected significant treatment effects (i.e. gizzard, pancreas, heart, flight muscle) regardless of whether one- or two-compartment model estimates of τ were used. This does not imply that model selection is trivial given that we detected substantial differences in fit of the different models given the same data. RBC, together with plasma, are the only tissues with continuous turnover that can be sampled easily and non-destructively from wild animals. Our results confirm those of Carleton et al. (Carleton et al., 2008) who showed that rate of carbon turnover in RBC was best described by a one-compartment model. Here we also show that the one-compartment model best describes rate of carbon turnover regardless of whether birds are exercised or acclimated to cold temperatures. Additional studies such as ours are needed before we can conclude that the dynamics of isotopic turnover for most tissues are consistently best described by two-compartment models with RBC as the notable exception.

Conclusion

Quantifying isotopic incorporation into tissue has developed into an intensively used tool to investigate spatiotemporal resource and habitat use (for reviews, see DeNiro and Epstein, 1978; Gannes et al., 1997; Hobson, 1999; West et al., 2006; Inger and Bearhop, 2008). Tissues that continuously incorporate exogenous carbon and other elements (e.g. the ten tissues in our study) are commonly used to answer questions about the timing and location of resource use of a variety of animals but such applications require knowledge about tissue-specific turnover rates (Phillips and Eldredge, 2006; Martínez del Rio et al., 2008; Wolf et al., 2009; Bauchinger and McWilliams, 2009). We have shown that ecologically relevant increases in metabolism associated with cold exposure but not exercise produced measurable increases in carbon turnover rate of, on average, 2.4±0.3 days for pectoral muscle, gizzard, pancreas and heart. Such changes in turnover rate translate into a 19% variation in the estimated time window for which resource use is estimated (29% for gizzard, 23% for pancreas, 12% for heart and 11% for flight muscle). These effects of moderately cold temperatures are ecologically relevant in that circannual and circadian ambient temperature ranges are more extreme in the temperate zone than in the tropics and can vary substantially within the annual cycle and life history strategy of an animal.

Cold-exposure but not exercise affected carbon turnover rate even though daily energy intake was similar for exercised and cold-exposed birds. The energy expenditure hypothesis predicts that similar increases in daily energy expenditure produce similar increases in carbon turnover, although this hypothesis has been challenged and increasing energy expenditure is suggested to represent only a by-product of elevated protein turnover (Carleton and Martínez del Rio, 2005; Martínez del Rio et al., 2008; Wolf et al., 2009; Bauchinger and McWilliams, 2009; MacAvoy et al., 2006). Our data do not support this energy expenditure hypothesis, but support the protein turnover hypothesis.

In view of the protein turnover hypothesis we suggest two different physiological processes associated with the obvious requirements for heat production in response to cold exposure that directly affect the rate of carbon turnover for a specific tissue: phenotypic mass change and higher tissue-specific activity. We can directly demonstrate phenotypic mass increase within this study. Increased protein synthesis rather than decreased protein breakdown serves as the most likely explanation for phenotypic mass increase (Swick and Benevenga, 1976), which can explain increased carbon turnover. Higher tissue-specific activity is a well documented and common phenomenon among animals that require increased heat production due to cold exposure. Structural and compositional changes of the flight muscle in response to cold exposure are known to increase the amount of enzymes, mitochondrial activity and density, and capillaries. The involved proteins show faster protein turnover compared with myofibrillar proteins (Goldstein and Reddy, 1967). The observed faster carbon turnover would thus be a consequence of a higher proportion of proteins with faster turnover.

Based on the accumulating support for the protein turnover hypothesis we can predict that specific ecological conditions that are associated with increased protein turnover, such as gestation, migration, egg laying and molt will positively affect carbon turnover. These ecological conditions are not only characterized by increased protein turnover, but are typically also associated with increased energy metabolism (Hammond and Diamond, 1997). Therefore, one of the challenges for understanding how ecologically relevant conditions affect carbon turnover is the disentangling of the effect of protein turnover and energy metabolism.

Finally, the temporal pattern of our treatment, i.e. constant colder temperature *versus* flight activity that was performed only for 2 h a day, may explain the different pattern of responses in carbon turnover. Chronic cold exposure is known to be a stronger stimulus than daily exercise training for tissue-specific adaptations such as mass phenotypic change and mitochondrial volume density (Schaeffer et al., 2003). Possibly, vertebrate morphology and physiology accommodates performance without further adjustments when confronted with moderate requirements or short bouts of increased metabolism, whereas only severe exercise or continuously increased activity requires adjustment of the machinery. It would be interesting to study how prolonged exercise may affect carbon turnover especially given that we detected significant changes in carbon turnover rate only in the cold-acclimated birds that were continuously exposed to cold temperatures. Perhaps intermittent exercise or cold exposure provides the opportunity for compensatory responses during unexposed periods, whereas continuous exposure restricts this ability to compensate.
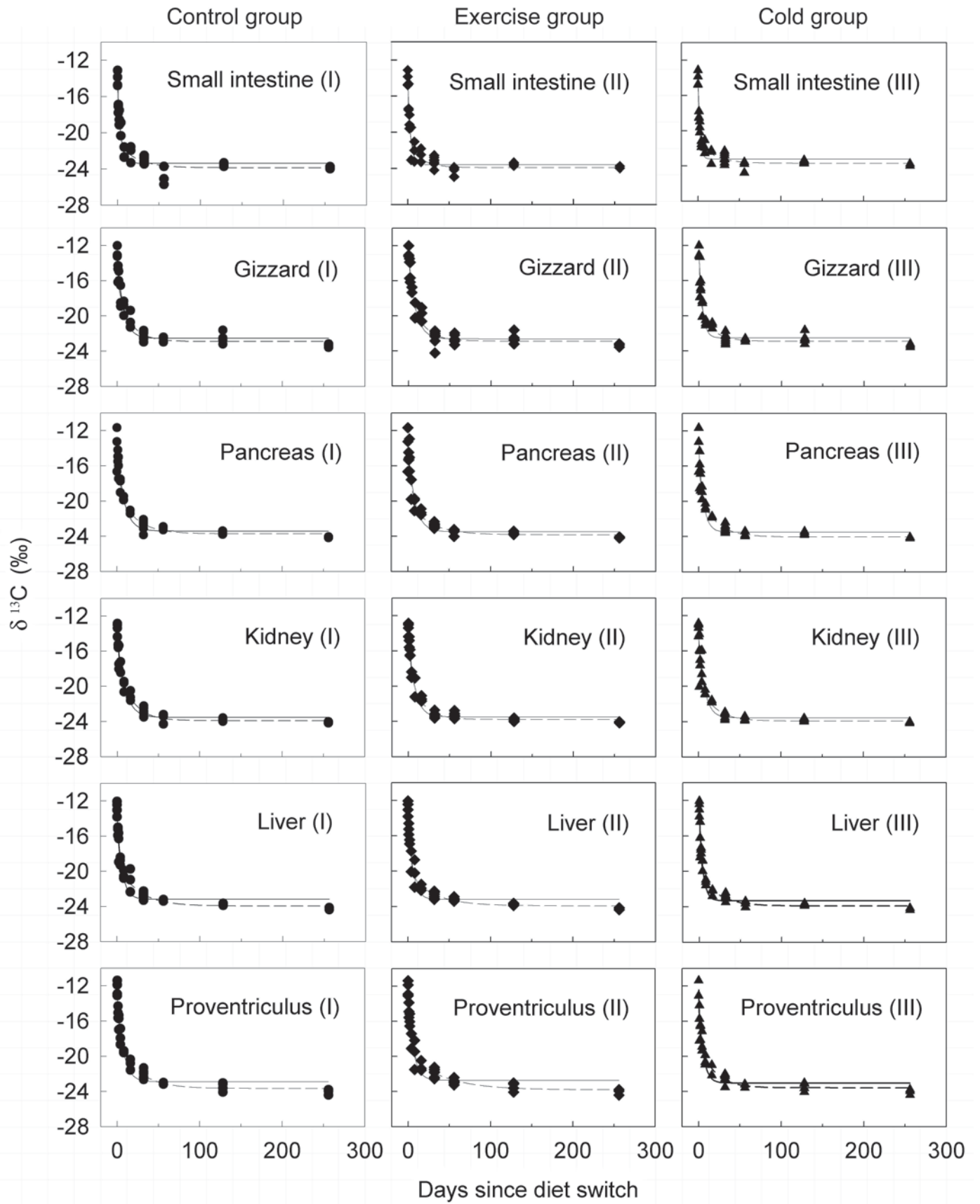
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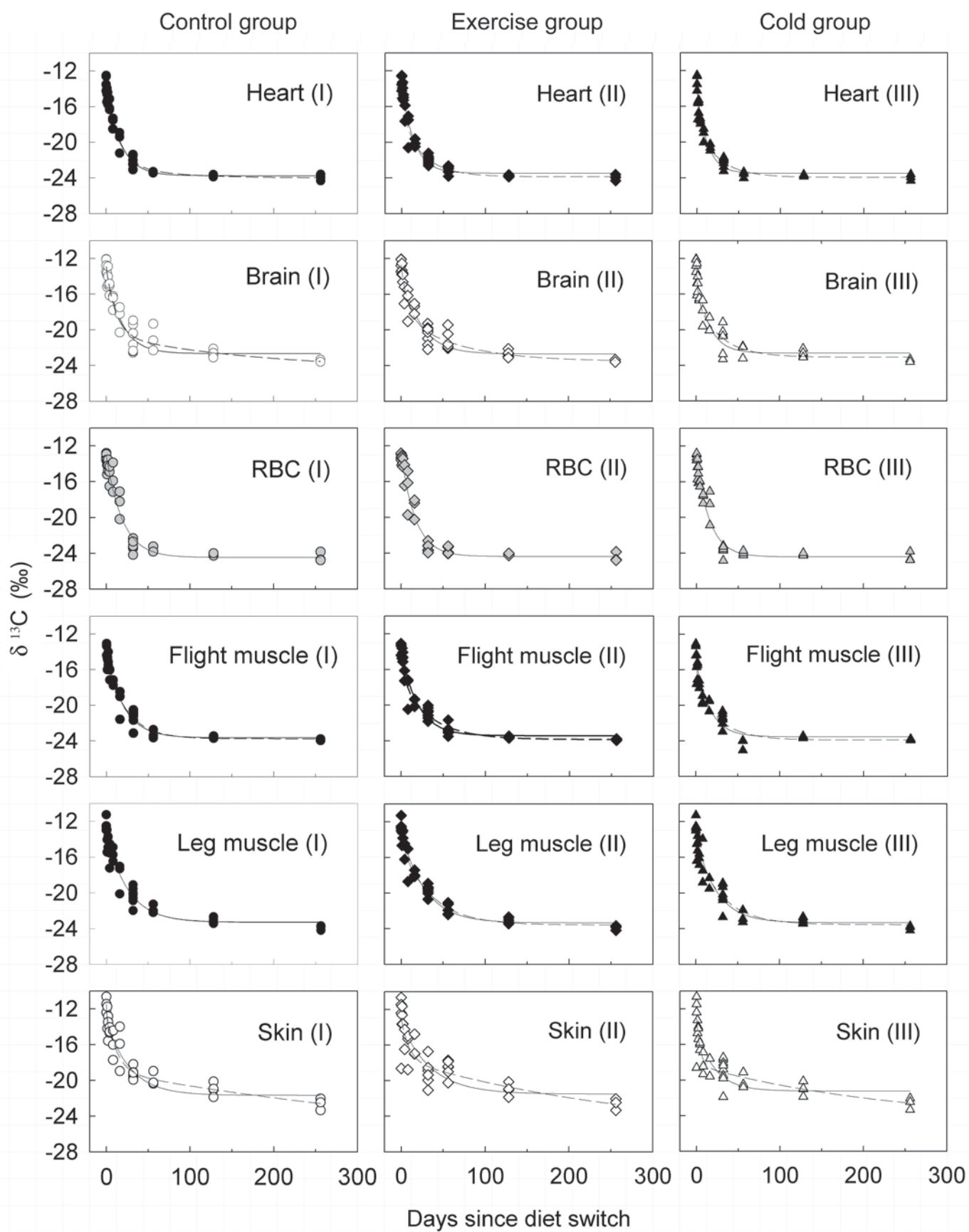**Arneson, L. S., MacAvoy, S. E. and Basset, E.** (2006). Metabolic protein replacement drives tissue turnover in adult mice. *Can. J. Zool.* **84**, 992-1002.

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Control group   Exercise group   Cold group

Small intestine (I)   Small intestine (II)   Small intestine (III)

Gizzard (I)   Gizzard (II)   Gizzard (III)

Pancreas (I)   Pancreas (II)   Pancreas (III)

Kidney (I)   Kidney (II)   Kidney (III)

Liver (I)   Liver (II)   Liver (III)

Proventriculus (I)   Proventriculus (II)   Proventriculus (III)

$\delta^{13}C$ (‰)

Days since diet switch

Control group   Exercise group   Cold group

Heart (I)   Heart (II)   Heart (III)

Brain (I)   Brain (II)   Brain (III)

RBC (I)   RBC (II)   RBC (III)

Flight muscle (I)   Flight muscle (II)   Flight muscle (III)

Leg muscle (I)   Leg muscle (II)   Leg muscle (III)

Skin (I)   Skin (II)   Skin (III)

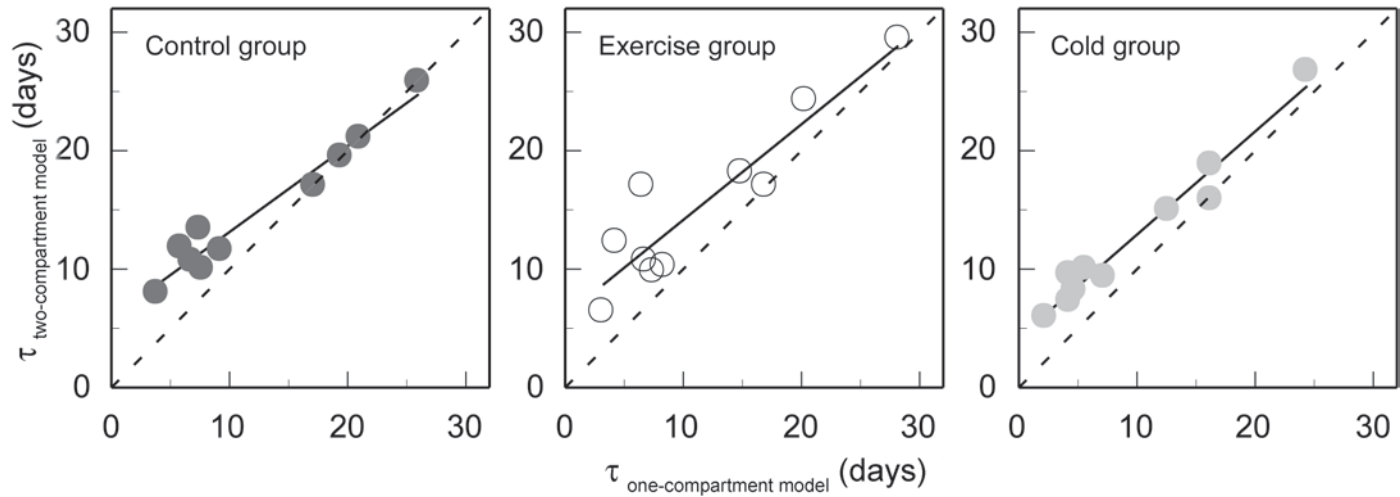$\delta\ ^{13}C\ (‰)$

Days since diet switch

Table S1. Incorporation of ^{13}C into 12 tissues for the three experimental groups described by one-compartment and two-compartment models that were fitted for tissue specific $\delta^{13}C$ values collected over 256 days after a diet shift

Tissue	Group	One-compartment model[1] $y_t = y_\infty + a\,e^{(-t/\tau\,\text{one-comp})}$						Two-compartment model[2] $y_t = y_\infty + a\,(p)\,e^{(-t/\tau 1)} + a\,(1-p)\,e^{(-t/\tau 2)}$											
		y_∞	s.e.m. y_∞	a	s.e.m. a	$\tau_{\text{one comp.}}$	s.e.m. $\tau_{\text{one comp.}}$	y_∞	s.e.m. y_∞	a	s.e.m. a	p	s.e.m. p	τ_1	s.e.m. τ_1	τ_2	s.e.m. τ_2	$\tau_{\text{two comp.}}$	s.e.m. $\tau_{\text{two comp}}$
Small intestine[2]	Control	−23.36	0.19	8.85	8.85	3.8	0.5	−23.86	0.29	9.60	0.46	0.30	0.11	21.6	11.9	2.2	0.61	8.0	2.5
	Exercise	−23.61	0.21	9.41	0.51	3.2	0.5	−23.88	0.32	9.89	0.53	0.25	0.13	19.9	15.2	2.1	0.6	6.5	2.6
	Cold	−23.23	0.15	8.89	0.38	2.3	0.2	−23.71	0.21	9.56	0.36	0.26	0.06	19.1	8.0	1.4	0.3	6.0	1.7
Gizzard[2]	Control	−22.53	0.21	9.20	0.37	7.6	0.9	−22.88	0.23	10.26	0.42	0.62	0.11	15.1	3.7	1.6	0.8	10.0	3.8
	Exercise	−22.64	0.20	9.61	0.37	8.4	1.0	−22.90	0.26	10.32	0.47	0.60	0.23	15.2	5.7	2.7	1.8	10.2	1.8
	Cold	−22.53	0.16	9.80	0.37	4.2	0.4	−22.90	0.23	10.40	0.39	0.30	0.14	17.7	9.1	2.6	0.7	7.2	1.7
Pancreas[2]	Control	−23.11	0.20	10.04	0.38	6.8	0.8	−23.69	0.21	11.44	0.38	0.52	0.08	18.4	3.8	2.0	0.6	10.6	2.6
	Exercise	−23.26	0.20	10.49	0.42	6.7	0.8	−23.81	0.30	11.53	0.50	0.38	0.14	22.9	10.0	3.3	1.1	10.8	2.3
	Cold	−23.35	0.18	10.50	0.43	4.6	0.5	−23.80	0.20	11.65	0.38	0.44	0.08	16.5	4.0	1.8	0.4	8.2	1.2
Kidney[2]	Control	−23.53	0.19	9.35	0.32	9.2	1.0	−23.89	0.20	10.47	0.35	0.68	0.08	16.3	2.8	1.6	0.7	11.6	3.0
	Exercise	−23.49	0.14	9.76	0.26	7.5	0.7	−23.78	0.16	10.47	0.29	0.54	0.15	15.4	4.1	2.8	1.0	9.7	1.1
	Cold	−23.58	0.17	9.83	0.33	6.9	0.7	−23.91	0.22	10.50	0.39	0.49	0.21	15.9	6.2	3.1	1.4	9.4	1.5
Liver[2]	Control	−23.16	0.22	9.68	0.43	5.8	0.7	−23.93	0.24	11.10	0.40	0.45	0.07	23.9	6.0	2.1	0.5	11.9	1.9
	Exercise	−23.21	0.17	9.86	0.35	5.8	0.6	−23.92	0.23	11.03	0.36	0.35	0.08	29.8	9.5	2.9	0.6	12.2	2.3
	Cold	−23.34	0.15	10.20	0.32	4.4	0.4	−23.96	0.18	11.11	0.29	0.28	0.06	27.5	8.8	2.7	0.4	9.6	1.7
Proventriculus[2]	Control	−22.90	0.21	9.74	0.38	7.4	0.9	−23.66	0.21	11.23	0.34	0.50	0.07	2.3	0.5	24.6	5.0	13.3	1.7
	Exercise	−22.73	0.21	9.81	0.42	6.4	0.8	−23.81	0.31	11.42	0.44	0.36	0.06	41.3	14.0	3.0	0.6	17.0	3.9
	Cold	−23.04	0.19	9.98	0.49	5.2	0.6	−23.65	0.23	11.35	0.49	0.39	0.08	22.2	6.5	2.2	0.6	10.1	1.8
Heart[2]	Control	−23.75	0.20	9.99	0.27	17.2	1.4	−23.83	0.20	10.60	0.37	0.91	0.04	18.7	1.7	0.2	2.0	17.0	1.4
	Exercise	−23.49	0.22	9.85	0.31	14.8	1.5	−23.89	0.27	10.75	0.39	0.41	0.18	4.7	2.5	27.4	8.8	18.1	2.7
	Cold	−23.48	0.21	9.31	0.33	12.0	1.3	−23.91	0.19	10.61	0.31	0.64	0.07	22.2	3.4	2.2	0.7	15.0	1.5
Brain[2]	Control	−22.66	0.35	9.48	0.46	18.5	2.6	−24.42	3.50	11.50	3.46	0.34	0.13	172.7	349.6	11.5	3.7	65.5	131.0
	Exercise	−22.70	0.34	9.51	0.43	22.6	2.9	−23.11	0.41	10.64	0.59	0.70	0.12	35.4	9.8	3.4	2.5	26.0	4.6
	Cold	−22.60	0.33	9.23	0.46	14.5	2.3	−23.12	0.38	10.57	0.57	0.57	0.16	31.2	12.2	3.4	2.0	19.2	4.2
RBC[1]	Control	−24.20	0.41	11.10	0.53	19.3	2.6	−24.48	2.6	11.42	*	0.00	*	19.5	*	19.5	*	19.5	*
	Exercise	−24.13	0.34	11.29	0.47	16.8	2.2	−24.37	2.2	11.55	*	1.00	*	17.0	*	−	*	17.0	*
	Cold	−24.43	0.28	11.05	0.38	15.9	1.6	−24.43	1.6	11.05	*	1.00	*	15.9	*	−	*	15.9	*
Flight muscle[2]	Control	−23.63	0.26	9.43	0.33	20.9	2.1	−23.74	0.27	10.14	0.46	0.86	0.07	24.1	3.3	1.3	1.4	21.0	2.3
	Exercise	−23.44	0.27	9.38	0.35	20.3	2.2	−23.87	0.31	10.52	0.43	0.62	0.12	36.3	10.2	4.4	2.1	24.2	3.9
	Cold	−23.39	0.30	8.84	0.41	15.0	2.1	−23.84	0.26	10.36	0.41	0.58	0.09	30.4	7.1	2.6	1.0	18.8	2.8
Leg muscle[2]	Control	−23.32	0.35	9.99	0.42	26.0	2.9	−23.47	0.35	11.02	0.57	0.84	0.06	30.6	4.5	1.0	1.0	25.9	3.1
	Exercise	−23.36	0.31	10.23	0.38	28.3	2.8	−23.60	0.34	11.17	0.50	0.81	0.08	35.8	5.9	2.4	1.9	29.4	3.5
	Cold	−23.19	0.38	9.71	0.49	21.9	3.1	−23.69	0.42	11.38	0.57	0.60	0.10	42.2	13.4	3.3	1.6	26.8	5.6
Skin[2]	Control	−21.52	0.46	8.86	0.56	24.2	4.1	−21.78	0.49	10.27	0.81	0.76	0.10	32.3	8.0	1.4	1.3	25.0	5.0
	Exercise	−21.22	0.57	8.34	0.68	28.7	6.1	−23.73	2.37	12.17	2.38	0.57	0.07	141.4	111.6	4.4	1.9	82.1	67.3
	Cold	−20.17	0.34	8.36	0.73	5.8	1.3	−24.37	3.91	12.91	3.90	0.47	0.13	207.0	241.6	3.3	0.8	99.8	139.2

Based on $\Delta_{\text{one–two}}$ (=$AIC_{c\,\text{one comp.}} - AIC_{c\,\text{two comp.}}$) presented in Table 2 we used average retention time ($\tau_{\text{one comp.}}$) for RBC, and ($\tau_{\text{two comp.}}$) for all other tissues to perform subsequent analyses. y_t is the measured $\delta^{13}C$ value of the tissue at time t.

t is the time in days.

y_∞ is the estimated final $\delta^{13}C$ value.

a is the estimated range in tissue $\delta^{13}C$ between diets.

p_1 and p_2 are the fractional size of pools one and two for the two-compartment model.

$\tau_{\text{one comp.}}$ is the estimated average carbon retention time for the one-compartment model.

τ_1 and τ_2 are the estimated average carbon retention times for 1st and 2nd pool of the two-compartment model.

$\tau_{\text{two comp.}}$ is the average carbon retention time of the two-compartment model: $\tau_{\text{two comp.}} = p\tau_1 + (1-p)\tau_2$ (Carleton et al., 2008).

[1] Average retention time from the one-compartment model was used because $\Delta_{1-2} < 2$.

[2] Average retention time from the two-compartment model was used because $\Delta_{1-2} > 2$.

*Nan error, no adequate curve fitting possible, p_1 and p_2 indicate this is a single pool model.