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# Turnover of stable carbon isotopes in the muscle, liver, and breath $CO_2$ of alpacas (*Lama pacos*)

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**Stable carbon isotope analysis of animal liver and muscle has become a widespread tool for investigating dietary ecology. Nonetheless, stable carbon isotope turnover of these tissues has not been studied in large mammals except with isotopically labelled tracer methodologies, which do not produce carbon half-lives analogous to those derived from naturalistic diet-switch experiments. To address this gap, we studied turnover of carbon isotopes in the liver, muscle, and breath $CO_2$ of alpacas (*Lama pacos*) by switching them from a $C_3$ grass diet to an isonitrogenous $C_4$ grass diet. Breath samples as well as liver and muscle biopsies were collected and analyzed for up to 72 days to monitor the incorporation of the $C_4$-derived carbon. The data suggest half-lives of 2.8, 37.3, and 178.7 days for alpaca breath $CO_2$, liver, and muscle, respectively. Alpaca liver and muscle carbon half-lives are about 6 times longer than those of gerbils, which is about what would be expected given their size. In contrast, breath $CO_2$ turnover does not scale readily with body mass. We also note that the breath $CO_2$ and liver data are better described using a multiple-pool exponential decay model than a single-pool model. Copyright © 2006 John Wiley & Sons, Ltd.**

Stable isotope analysis is now frequently used to investigate wildlife dietary ecology,[1–7] and, in recent years, there has been an increasing emphasis on using stable isotopes to explore dietary changes within individuals or populations over time.[8–12] Some studies have even analyzed several tissues that turn over at different rates simultaneously to reconstruct dietary life-histories.[8,9,13–16] The success of such enterprises, however, is greatly limited by our lack of knowledge of stable carbon isotope turnover in mammalian tissues, in that we have only a general idea of the time period represented in any given sample of large mammal liver, muscle, bone collagen, etc. There is a long history of using isotopically labelled tracers to investigate whole body and organ-specific turnover in a variety of organisms including *Homo sapiens*.[17–22] These studies provide us with a general picture of tissue turnover in a wide variety of mammalian organs, and show, for instance, that liver turnover is much faster than muscle turnover.[19–22] There are a variety of reasons, however, that such studies are of limited utility for ecological applications, the most important of which is that different methods produce highly divergent results. For instance, fractional synthesis rates for liver can differ from 12.1% to 24.7% per day depending on whether one uses the continuous infusion or flooding dose method.[20] Thus, it is not at all clear which synthesis/turnover values are most appropriate for ecological applications. This has been remedied in the ecological literature by switching animals from one diet to another with a naturally distinct isotopic composition, and monitoring the resulting changes in tissue isotopic composition over a period of months.[23–32] While time- and labor-intensive, these studies are directly analogous to the ecological phenomena being investigated (changes in diet between two isotopically distinct foods such as $C_3$ browse and $C_4$ grass) and as they are largely methodologically uniform, they are readily comparable. It is also worth noting that these 'naturalistic' studies consistently suggest much longer stable isotope half-lives than are implied by short-term artificial labelling studies (see[19,21,23,24]), which is another reason that ecologists do not traditionally engage with the isotopic-labelling literature.

Although 'naturalistic' stable isotope turnover studies have been carried out in birds and fish,[24,26–28,31,32] there are no published data on the turnover of stable isotopes in the soft tissues of large mammals that do not rely on artificially labelled tracers. At present, those who are interested in the ecology of large mammals are constrained to rely upon the results of the seminal study by Tieszen *et al.*,[23] which

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examined the carbon isotope turnover in gerbils (*Meriones unguiculatus*). Although this was an excellent study, its applicability to large animals is questionable given the general, but not absolute trend between tissue turnover/synthesis and mass-specific metabolic rate.[31,33,34] Consequently, we would expect stable isotope turnover/synthesis to be much slower in large mammals than in gerbils, as has been observed in studies using artificial tracers.[19,20,22] Here, we address this question by presenting the first data on the turnover of stable isotopes in the muscle, liver and breath $CO_2$ of yearling and adult alpacas (*Lama pacos*) without the use of artificially labelled tracers.

## EXPERIMENTAL

Ten male alpacas, five adults and five yearlings, were fed a $C_3$ grass diet (*Festuca arundinacea*) on a small pasture at Brigham Young University for a period of at least 1 year to allow their tissues to equilibrate with their diets. The alpacas were then placed in enclosures and fed a diet based on the same $C_3$ grass ($\delta^{13}C = -25.4 \pm 0.4$‰ (standard deviation), crude protein (CP) = 13%) for a 60-day acclimation period. After the acclimation period, they were placed on an isotopically disparate but isonitrogenous $C_4$ grass (*Cynodon dactylon*) diet ($\delta^{13}C = -13.6 \pm 0.4$‰ (standard deviation), CP = 13%) for a period of 72 days, after which the experiment was terminated. Food and water were available *ad libitum* throughout the acclimation and experimental periods.

Breath $CO_2$, liver, and muscle samples were collected from all individuals just prior to the $C_3$ to $C_4$ diet switch (day 0). Further breath and liver samples were collected at 0.25, 0.5, 1, 2, 3, 5, 7, 14, 21, and 35 days after the diet switch. Liver samples were collected again at the termination of the experiment (day 72). Muscle samples were collected at the same intervals as liver, only starting at day 3. Breath $CO_2$ was collected in plastic syringes and passed through an alcohol trap (−50°C) to remove water before being stored in 10-mL head-space vials. Liver and semitendinosus muscle biopsies were collected with biopsy needles, freeze-dried, and then were placed in tin capsules for stable isotope ratio analysis. Following Tieszen *et al.*,[23] we did not remove lipids from the samples, so as to maximize comparability with the only other published mammalian dataset for these tissues (but see Voight *et al.*[29] for isotopic turnover in bats).

The carbon isotope composition of breath $CO_2$ was determined by injecting samples into a gas chromatography (GC) column connected to a continuous flow isotope ratio mass spectrometer (Finnigan, Bremen, Germany). The standard deviation of replicate measurements of a working standard (~2% $CO_2$ and ~98% $N_2$) injected alongside the experimental samples was 0.2‰. Muscle and liver samples were combusted in an automated Carlo-Erba device (Milan, Italy) and stable carbon isotopes were analyzed using a continuous flow isotope ratio mass spectrometer (Finnigan). Working standards run concurrently with the experimental samples had standard deviations of 0.1‰.

Following previous work on stable isotope turnover,[23–25,29,32] the mean values for each sampling interval were fitted using SigmaPlot 9.0 to a constrained single-pool exponential decay equation of the form $y = a + be^{ct}$, where $y$ is the relevant $\delta$ value at time $t$, $a$ the asymptotic stable isotope composition, $b$ the overall change in stable isotope compositions of the two diets, and $c$ the unknown turnover constant. We assumed that the asymptotic stable carbon isotope compositions were enriched by 11.8‰ compared with the initial values, as that is the difference between the $C_3$ and $C_4$ diets. Half-life estimates were then made using the equation $\ln(0.5)/c$. We looked for differences in the $\delta^{13}C$ of liver, muscle, and breath $CO_2$ using analysis of variance (ANOVA), and then looked for differences in the $\delta^{13}C$ of each sampling interval (day) for liver, muscle and breath $CO_2$ separately using Fisher's PLSD test. All $\delta^{13}C$ values are presented ± standard deviation.

## RESULTS

ANOVA showed that the stable isotope compositions of adult and yearling breath $CO_2$, liver, and muscle differed neither on day 0 (just prior to the diet switch) nor on day 72 of the experiment ($P > 0.40$). As a result, we pooled the data for juveniles and adults for all further analyses. On day 0, liver $\delta^{13}C$ ($x = -25.0 \pm 0.1$‰) was statistically indistinguishable from that of the diet ($P = 0.37$), while muscle ($x = -23.9 \pm 0.2$‰) and breath $CO_2$ ($x = -23.0 \pm 2.3$‰) were enriched by 1.5‰ and 2.4‰, respectively ($P < 0.0001$). All of these enrichments are similar to those of large ungulates that have been on a single hay diet for their entire lives (Sponheimer, unpublished data), thus we feel that breath $CO_2$, liver, and muscle stable isotope compositions were largely equilibrated with dietary compositions on day 0 (which was 14 months after the animals began the diet).

As expected, by day 72, liver ($x = -18.3 \pm 0.6$‰) and muscle ($x = -21.2 \pm 0.3$‰) $\delta^{13}C$ values were significantly different from day 0 values ($P < 0.0001$), demonstrating incorporation of carbon from the experimental diet. Indeed, breath $CO_2$, liver, and muscle $\delta^{13}C$ differed significantly ($P < 0.05$) from day 0 values by day 0.5 ($x = -19.8 \pm 2.9‰_{\text{breath CO2}}$), day 2 ($x = -24.4 \pm 0.3‰_{\text{liver}}$), and day 14 ($x = -23.2‰ \pm 0.3‰_{\text{muscle}}$). The liver and muscle data fit single-pool exponential decay models quite well (liver $R^2 = 0.90$; muscle $R^2 = 0.99$), although the breath $CO_2$ data fit less well ($R^2 = 0.76$; (Figs. 1(a)–(c)). The half-lives for carbon in breath, liver, and muscle are estimated to be 2.8, 37.3, and 178.7 days, respectively.

## DISCUSSION

### Soft-tissue turnover

It was our goal in this study to determine if carbon isotope turnover in the tissues of alpacas is similar to that in gerbils, or if it is much lower given the large difference in their mass-specific metabolic rates. The soft-tissue half-lives produced here are much longer than those published for birds[24,32] and gerbils.[23] More specifically, our half-life estimates for both liver and muscle carbon are about 6 times greater than those reported for gerbils ($HL_{liver} = 6.4$ days; $HL_{muscle} = 27.6$ days),[23] and 15 times greater than those for Japanese quail ($HL_{liver} = 2.5$ days; $HL_{muscle} = 12.4$ days).[24] This difference between alpacas and gerbils is consistent





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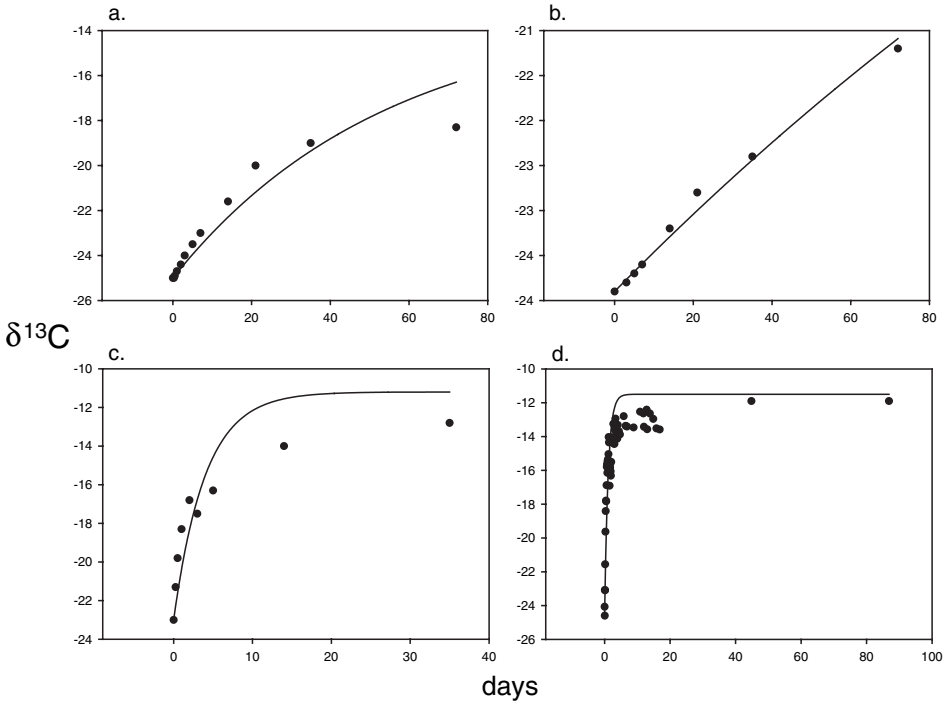


**Figure 1.** Alpaca liver (a), muscle (b), and breath $CO_2$ (c) $\delta^{13}C$ from day 0 to the end of collection (days). Horse breath $CO_2$ $\delta^{13}$ C (d) is also shown from day 0 to day 85 of a similar experiment.

with the published relationship between whole body protein turnover and body weight in mammals (16.6 body weight $(kg)^{0.74} = g/day$),[25] which predicts that gerbil whole body protein turnover (2.95 g/day or 3.0% of body mass/day) should be about five times faster than alpaca whole body protein turnover (312.1 g/day or 0.6% of body mass/day). Thus, it is clear that body mass must be accounted for, and that this should be reasonably simple given the abundant data available on the relationship between body mass and metabolic rate/protein turnover.[33–38]

Contrary to our expectations, we observed no differences in tissue turnover between juveniles and adults. This is probably due to our having used yearlings (~1 year old), which had already gained much of their adult body weight, rather than more rapidly growing infants. Had we used younger individuals, differences in tissue turnover rates would almost certainly have been evident. Nonetheless, this negative result is of some significance for wildlife studies, as it suggests that adult and yearling stable isotope compositions will provide dietary information for the same time period, and that age is less of a confounding variable (except for the very young) than was previously supposed.

## Breath $CO_2$ turnover

We are not aware of any published data on small mammal breath $CO_2$ turnover, but there are data on breath $CO_2$ turnover in birds (for a discussion of breath $CO_2$ enrichment, see[39–41]). Yellow-rumped warblers (*Dendroica coronata*) and pigeons (*Columba livia*) both have breath $CO_2$ half-lives of slightly less than 0.2 days,[27,32] which is a great deal faster than the equivalent half-life for alpacas (2.8 days). While the much slower breath $CO_2$ turnover in alpacas is at least partially a function of body size, it is also the product of its specialized pseudoruminant digestive system, which allows the digestion of low-quality foods, but at the cost of markedly reduced food intake and passage rates.[42–44] Indeed, when non-ruminant horses (which are almost ten times heavier than alpacas) were switched from a pure $C_3$ to a pure $C_4$ diet, their breath $CO_2$ half-life was more similar to that of birds than that of alpacas (~0.8 days; Fig. 1(d)). This is because the mean retention time of alpacas is much longer than that of horses (or these birds), so that alpacas continued to digest significant quantities of the pre-experiment $C_3$ diet for over a week, while the pre-experiment diet was largely eliminated from the digestive system of horses within days. More specifically, the half-life of carbon in horse feces is only 21.5 h, while it is 83.4 h in alpacas (data from Sponheimer *et al.*[45]). Hence, differences in digestive physiology can, at least to some extent, override the expected relationship between breath $CO_2$ half-life and body size. It is important to note, however, that this difference in carbon turnover between mammals with different digestive physiologies is not found in all tissues. For example, despite large differences in breath $CO_2$ turnover, the half-lives of carbon in shaved hair patches of alpacas and horses are both about 50 days (Sponheimer, unpublished data), and are consonant with the reported half-life for gerbil hair (47.5 days).[23]

Despite the much slower turnover times reported here, there is an important point of concordance between this and the gerbil and quail studies.[23,24] In all cases, the muscle to liver carbon turnover ratios (MLCT = muscle half-life/liver half-life) are between 4.3 and 5.0 (Table 1). Thus, it appears that even among animals as taxonomically distinct as gerbils and quail, and as disparate in body size as gerbils and alpacas, liver carbon generally turns over between 4 and 5 times more quickly than muscle carbon. Consequently, it is likely that one can accurately estimate liver turnover for a wide variety of taxa using minimally





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**Table 1.** Half-life and muscle/liver carbon turnover ratio (MLCT) data from this and previous studies. Single-pool half-life (SPHL) calculations are in days. Multiple-pool half-lives (their fractions are in parentheses) are also in days. No multiple-pool data presented for Ref. 24 because raw data were not included in the publication

| | SPHL | Pool 1 | Pool 2 | Pool 3 |
|---|---|---|---|---|
| Tieszen *et al.*[23] | | | | |
| Gerbil liver | 6.4 | 3.4 (0.62) | 35 (0.39) | |
| Gerbil muscle | 27.6 | 5.6 (0.22) | 45 (0.77) | |
| MLCT | 4.3 | | | |
| Hobson and Clark[24] | | | | |
| Quail liver | 2.5 | | | |
| Quail muscle | 12.4 | | | |
| MLCT | 5.0 | | | |
| Ayliffe *et al.*[30] | | | | |
| Horse hair | NA | 0.5 (0.41) | 4 (0.15) | 140 (0.44) |
| Horse breath | NA | 0.2 (0.67) | 3 (0.17) | 50 (0.16) |
| MLCT | NA | | | |
| This study | | | | |
| Alpaca liver | 37.3 | 11 (.45) | 200 (0.55) | |
| Alpaca muscle | 178.7 | 10 (0.03) | 215 (0.97) | |
| Alpaca breath | 2.8 | 0.4 (0.46) | 10 (0.19) | 26 (0.35) |
| MLCT | 4.8 | | | |

invasive muscle biopsies. It would be intriguing to see the degree to which such relationships could be established for other organs.

## Single-pool versus multiple-pool models

Despite the ubiquity of single-pool exponential decay models in the ecologically oriented stable isotope literature,[23–25,27–29,31,32] there are a number of reasons to believe that stable isotope turnover rates are not always best described in this fashion. One reason is that single-pool exponential decay models do not adequately describe some datasets. Schmidt *et al.*,[25] for example, showed that single-pool exponential models provided a very poor fit for $\delta^{13}C$ turnover in earthworm (*Lumbricus festivus*) mucus. Secondly, even when single-pool models fit datasets adequately, the data may sometimes be better described using multiple-pool models. For instance, although breath $CO_2$ and hair carbon turnover in horses can be described reasonably well using single-pool models ($R^2 > 0.90$), it is better described using three-pool models.[30]

Applying multiple-exponential models as described in Ayliffe *et al.*[30] demonstrates that the same is true for at least some of the data presented here. The alpaca breath $CO_2$ data are much better described using a three-pool model ($R^2 = 0.99$) than a single-pool model ($R^2 = 0.73$) (Table 1; Fig. 2(a)). As can be seen in Fig. 2(a), predicted $\delta^{13}C$ values using the single-pool model are depleted by ~2‰ compared with observed values on day 1, yet enriched by nearly 2‰ on day 5. Thus, the single-pool model significantly underestimates isotopic change early in the experiment, but then overestimates the pace of change shortly thereafter. The multiple-pool model, in contrast, recognizes three separate pools contributing to $\delta^{13}C$ with half-lives of 0.4, 10, and 26 days, respectively (with fractions of 46%, 19%, and 35% in turn), and its predicted values never differ from observed values by more than 0.6‰, and usually differ by much less.

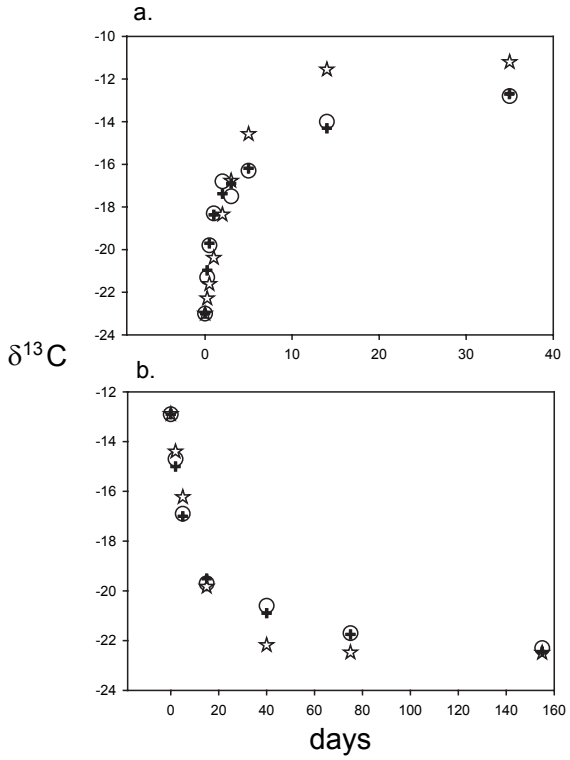


**Figure 2.** (a) Alpaca liver $\delta^{13}C$ from day 0 to day 35 of the experiment. Observed values appear as circles and predicted values from single- and multiple-pool models are shown as stars and crosses, respectively. (b) Gerbil liver $\delta^{13}C$ data from Tieszen *et al.*,[23] with markers as above.

This result is similar to that of one of our previous studies,[30] in which we found three pools contributing to horse breath $CO_2$ with half-lives of 0.2, 3, and 50 days (with fractions of 67%, 17%, and 16%), although, as expected given the differences in digestive physiology, the fast turnover pools were even faster in horses. As we discussed at some length in the horse study,[30] the strong fit for the three-pool model is to be expected given the contribution from slow (e.g., muscle), intermediate (e.g., spleen), and fast (e.g., bicarbonate) turnover pools to breath $CO_2$.

The alpaca liver $\delta^{13}C$ data are also better described using a two-pool ($R^2 = 0.99$) than a one-pool model ($R^2 = 0.90$) (Table 1), but single- and multiple-pool models describe the muscle turnover data equally well ($R^2 > 0.99$), with residuals never exceeding 0.2‰. Multiple-pool models may also better fit data from some previous studies than do single-pool models. For instance, liver $\delta^{13}C$ data from Tieszen *et al.*[23] fit a multiple-pool model ($R^2 = 0.99$) better than a model with only a single pool ($R^2 = 0.93$) (Table 1; Fig. 2(b)). The single-pool model predicted values differing from the observed values by as much as 1.6‰ by day 40, whereas residuals for a two-pool model (with half-lives of 3.4 and 35 days, both faster and slower than the 6.4 days previously reported[23]) never exceed 0.2‰. In fact, we have found that when higher-resolution data are available, protein turnover is, not surprisingly, better described by a three-pool model,[30] which is also consistent with the findings of isotope-labelling studies.[19]

Detailed information on muscle, liver, and breath stable isotope turnover is necessary if they are to be used to good





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effect as dietary archives. Although at present single-pool exponential decay models are the standard for describing stable isotope turnover, in some cases multiple-pool models may be more physiologically revealing and accurate. This increase in accuracy is extremely important for wildlife applications where trophic behavior is reconstructed from high-resolution dietary archives such as tail hair.[12] However, proper application of these models requires knowledge of the precise nature of each pool *in vivo*, lest we sacrifice biological reality for mathematical contrivance. Thus, additional studies designed to better our understanding of what each pool represents are warranted.

Until that time, comparisons of half-lives estimated using single-pool models do allow for simple and useful comparison of intraspecific (within a species) and interspecific (between species) stable isotope turnover. For instance, we have shown here that the half-lives of carbon in alpaca liver and muscle are about 6 times greater than those in gerbils, and about 15 times greater than those in Japanese quail. This finding has important implications for dietary interpretation of liver and muscle $\delta^{13}C$ in large mammals. Approximately 90% of an animal's dietary stable isotope composition will be reflected in its tissues within 3 half-lives. Thus, quail and gerbil muscle carbon will largely reflect dietary carbon isotope compositions within ~40 and ~80 days, respectively. The alpaca data suggest, however, that large mammal muscle carbon will not reflect 90% of a new dietary signal until a period of well over a year. Thus, while quail and gerbil muscle can be readily used to investigate short-term dietary change (~1 to 3 months), the $\delta^{13}C$ of large mammal muscle will be relatively insensitive to short-term dietary change, rather reflecting long-term dietary averages. From a practical standpoint, this means that one cannot assume that similar methods can be used to investigate the dietary ecology of large and small taxa. For instance, if one is interested in investigating an animal's recent diet, muscle might well be appropriate when studying rodents, but not when studying larger mammals. Nevertheless, it appears that large mammal liver and small mammal muscle integrate dietary information over a similar period, and might be fruitfully juxtaposed when attempting to study recent trophic behavior in mammalian communities. Future studies of other dietary archives (e.g., plasma lipids) through the use of compound-specific techniques (GC/C/IRMS) should make it possible to address ecological questions at even shorter temporal scales.

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