

## Invited Commentary

### Where does our protein carbon come from?

It is always pleasing in science when a study that is directed to a rather specific end, in an apparently far-flung field, manages to illuminate basic aspects of mainstream research. So it is here, where the concerns of those engaged in using the fossil record to reconstruct ancient diet lead on to feeding experiments on laboratory rats and the isotopic analysis of the allocation of dietary carbon to protein.

Procuring adequate nutrition has been a dominant force in evolution, and a great deal of archaeological research is devoted to the implications and indirect effects of so much effort. Most archaeological evidence, however, misses the diet itself, leaving the remains – discarded bone, burnt scrapings in cooking pots – to be intensely scrutinised. But what does record the diet, of course, is the bone into which food has been transformed, and bones survive tolerably well in the archaeological record, often preserving much of their collagen content for tens of millennia. Therefore the study of bone chemistry in relation to palaeo-diet has beckoned ever since suitable techniques have been available (DeNiro & Epstein, 1978; Lambert *et al.* 1990; Schwarcz & Schoeninger, 1991). This area of interest can contribute, through the analysis of animal bone and teeth, in important ways also to the study of palaeo-ecology (Koch, 1998) and is now being applied to analysing contemporary foodwebs, for example in the context of bird migration (Hobson, 1999).

The approach depends on using isotopic values of C (and N, though only C is considered here) as tracers from food source to body tissue (in this, as in most cases, the slowly turning over collagen in bone). Food sources become isotopically distinct through the effects of isotopic fractionation during metabolism, whenever there is isotopic discrimination at a branching point in the flux of metabolite C.

For C, the overwhelming example occurs during photosynthesis, whereby most terrestrial plants are depleted in the heavier isotope ( $^{13}\text{C}$ ) by about 1.5‰ (written as 15‰ or 15 per mil) compared with atmospheric  $\text{CO}_2$ . However, plants using the C4 (Hatch–Slack) pathway, and those synthesizing in aquatic – especially marine – environments, show less depletion (only about 5‰), and diets based on one or the other can be easily distinguished by mass-spectrometric measurements, which can have a very high precision.

Although metabolism within organisms can potentially greatly complicate this simple start – there is a 20–25‰ range in plant-synthesised amino acids, for example – overall some simple behaviour fortunately emerges. This has been enshrined in the adage ‘You are what you eat plus five per mil’ because, for quite a wide range of vertebrates, the  $^{13}\text{C}:^{12}\text{C}$  ratio of bone collagen, as measured in bulk, is found to reflect the dietary average  $^{13}\text{C}:^{12}\text{C}$ , albeit with an offset of about 5‰.

Such a generalisation has been applied in many archaeological and palaeo-ecological situations. For example, concomitant with the appearance of the Neolithic in north-west Europe, a rather sharp change from a largely marine-based economy to agriculture has been demonstrated (Richards *et al.* 2003). The collagen of many Mesolithic (pre-Neolithic) people had an isotopic content similar to that of the fish they ate. Such diets were probably high in protein, and it is easy to see how fish protein might be registered strongly in the collagen of the consumer. This is not so obviously the case for cereals, however. The domestication of maize (a C4 plant) in Mexico, and its subsequent spread and cultivation in South and North America, has been extensively documented from bone collagen analysis (Van der Merwe & Vogel, 1978; Pearsall & Piperno, 1990). But as maize was not necessarily the main source of dietary protein, detecting maize-labelled C atoms in bone collagen leads one to consider how they got there.

Two extreme views might be taken: first, that all collagen protein C is derived only from dietary protein, and second, that all collagen protein C is from all sources of dietary C (and therefore dominated by the carbohydrate in most diets, except for extreme hunters like the Inuit and perhaps, of course, our Palaeolithic ancestors). In reality, collagen non-synthesisable (essential) amino acids must be diet-derived, and once individual amino acids are considered, it becomes necessary to make measurements at that level in order to track the origins of each (or at least most) of them. This is what Jim *et al.* (2006), in an article published in this issue of the *British Journal of Nutrition*, have done, using rats raised on a variety of isotopically labelled diets in which protein and carbohydrate (from C3 or C4 sources) can be distinguished; the paper relates to the consequences of their findings.

There are some caveats, however; rats are not man (although the research group has found broadly similar results with pigs); rat bone collagen mainly reflects growth and therefore rather different metabolic demands; a diet of 20% protein (presumably as weight of C) is not very natural for rats. On the other hand, the findings make sense and are probably cautiously generalisable to both palaeo-dietary interpretation and, perhaps of more interest to *British Journal of Nutrition* readers, the interpretation of contemporary dietary behaviour. They show that the essential amino acids are pretty well carried through to collagen without significant further isotopic discrimination. (One place where this might occur is in the branch point to catabolism, for example in the gut (Wu, 1998)). This accounts for approximately 20% of the collagen C.

At the other extreme, Jim *et al.* show that collagen alanine, and aspartic and glutamic acids, are rather insensitive to isotopic changes in dietary protein, whereas proline, with glycine close behind, behaves as if derived only from the diet. In all, Jim *et al.* estimate some 50% of collagen C for these rats was synthesised, from Krebs cycling and glycolytic metabolism, in the rat itself. Such direct insight is very welcome, although the results are not surprising: dietary glutamate and aspartate are mainly catabolised in the gut (Wu, 1998), whereas alanine synthesis in both gut and muscle from glycolysis is the main contribution to its circulation. Collagen synthesis makes unusual demands locally on both proline (together with hydroxyproline) and glycine, and perhaps synthesis proceeds when these are in abundant supply.

What are the consequences of this study? In one way it will help to underpin the use of 'bulk collagen'  $\delta^{13}\text{C}$  values by providing data for modelling situations in which animal and plant foodstuffs provide different signals. For example, the Mesolithic fish-subsisting people mentioned previously have collagen in which virtually all C must have come from protein (because if only, say, 50% came from protein, their marine food intake would have to be unfeasibly high, with the danger of protein poisoning). On the other hand, there is debate (Hedges, 2004) over how sensitively small amounts of marine protein are registered, because the collagen isotopic composition would be less responsive (to protein sources) if most non-essential amino acids were being synthesised from the total C (i.e. plant carbohydrate) in a low-protein diet. In any case, these issues can surely be further dissected with individual amino acid data.

However, reconstructing the dietary origin of a collagen amino acid will require knowledge of at least three unknowns: the amino acid  $\delta^{13}\text{C}$  in the food protein (which may be possible to estimate); the contribution of carbohydrate C to a given amino acid (i.e. what the paper by Jim *et al.* is all about); the isotopic fractionation involved in synthesising the amino acid from carbon catabolic pathways (which may turn out to be a consistent value). Until we know how these behave, reconstruction will be hampered.

Meanwhile, there is surely scope for new information – under what conditions might collagen synthesis, or perhaps protein synthesis generally, choose to incorporate dietary amino acids, or choose to incorporate synthesised amino acids? Is this important for those of us who study the diets of the past, but equally worth asking, is this relevant to the study of contemporary human diet? The connection between carbohydrate and amino acid metabolism is becoming increasingly prominent in studies of metabolic changes during starvation and infectious stress, and in obesity and

diabetes (Fraysn, 2003). Much industrial-culture diet embodies protein and carbohydrate from many sources, including those with a range of isotopic compositions, although this is less true for those of the developing world. It is worth considering if samples of, for example, hair would contain suitable information at the amino acid isotopic level to learn more about how individuals allocate their resources under varied conditions of stress, such as from malnutrition, immunological reaction or overeating. An advantage, as with archaeology, is that one could work at the level of natural populations.

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