Rebuttal to the *Proceedings of the National Academy of Sciences* June 2013 Papers

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A series of three scientific papers were published this this month in the early edition of the *Proceedings of the National Academy of Sciences* (1-3) evaluating the diet of numerous species of fossilized hominins, bipedal or upright walking apes, who lived in Africa from 4.1 to 1.4 million years ago. The diet of a grass eating baboon was examined as well (4). Many of the authors of these papers are friends and colleagues whose data contribute to our understanding of our remote African ancestors' diets. Collectively, the papers examined the following hominin genus's and the time frame they lived: *Australopithecus* (circa 4 million years ago [MYA]), *Kenyanthropus* (circa 3-3.6 MYA), *Paranthropus* (circa 2.5-1.4 MYA), and early *Homo* (circa 2.3-1.5 MYA).

Before I get into the details of these studies, let me first openly reprimand some of the popular press who have incorrectly interpreted these studies by suggesting that our distant ancestors were regular consumers of grass and grass seeds (cereal grains). For instance, popular blogger Carrie Arnold, titles her write-up (5) of these three scientific studies as, "*Even Our Ancestors Never Really Ate the "Paleo Diet*," and goes on to say, "*Researchers are just beginning to understand what ancient humans ate, and these recent studies show that grasses and grains have been part of the human diet for millions of years*." As I will shortly show you, this statement represents sensationalistic journalism and is patently false, as nowhere in any of these three papers (1-3) is this conclusion reached by any of the authors.

Another piece of inaccurate and hyped journalism (6) by author Chris Joyce at NPR labels his piece, "*Grass: It's What's For Dinner* (*3.5 Million Years Ago*)." Chris then tells us, "*What the tale of the teeth reveals is this: About 3.5 million years ago, our ancestors started switching from the ape diet – leaves and fruit – to grasses and grass-like sedges*." This statement is false and again nowhere in any of these three papers (1-3) is this assumption made by the scientists who wrote these manuscripts. Chris finally gets it right in his following statement, "*Now, one thing this carbon isotope technique can't tell is whether Australopithecus just grazed like a bunch of antelope, or whether they ate the antelope that did the grazing.*" However, in his final paragraph his conclusion again is erroneous: "*So, what to make of this? Well, for one, those who favor a "Paleo diet" that resembles what our early ancestors lived on might consider investing in a lawn mower. After all, lawn grass is probably American's largest un-harvested crop – there's plenty to go around. Why not go back to our roots*?"

Catherine Griffin, a writer for Science World Reports obviously did not carefully read any of these three papers (1-3) because of incorrect statements she has made in her brief article (7), "*Human Ancestors' Ape-like Diet Changed 3.5 Million Years Ago to Grass*". Catherine informs us, "*Feel like eating some grass? Didn't think so – but our ancient ancestors did. About 3.5 million years ago, our human forebears added tropical grasses and sedges to an ape—like diet of leaves and fruits from trees and shrubs.*" She goes on to make other statements like, "*In the end, the scientists found a surprising increase in the consumption of grasses and sedges*" and "*The earliest ancestors that consumed substantial amounts of grass foods . . .*" which were never made in the original scientific papers.

In science, the devil is almost always in the details. Accordingly, all three of these popular science writers have done their readers a disservice by inaccurately reporting the details of these three studies (1-3) and making assumptions about ancient hominin diets that the scientists themselves did not make.

In all three papers the measurement of two stable isotopes of carbon (^{13}C and ^{12}C) were made from samples of enamel in teeth of extinct hominins. From the ratio $^{13}C/^{12}C$ a difference (delta) ($\delta^{13}C$) is calculated relative to a standard value (8). $\delta^{13}C$ values can then be used to determine if the carbon isotopes in the enamel ultimately originated from plants using either the C_3 or C_4 photosynthesis pathways.

In Africa and elsewhere, C_4 plants include grasses and sedges and little else, whereas C_3 plants include trees, shrubs, herbs and bushes. C_4 plants incorporate relatively more ^{13}C into their tissues during photosynthesis than do C_3 plants. Hence, $\delta^{13}C$ values extracted from enamel can reveal the dietary source of the isotopic signature, be it: 1) grasses and sedges, 2) trees, bushes, shrubs, herbs or 3) a combination of both categories of plants.

Unfortunately, a number of fundamental limitations exist with $\delta^{13}C$ analysis to evaluate diet. $\delta^{13}C$ measurements cannot determine the exact species of either C_3 or C_4 plants that were consumed, **but more importantly $\delta^{13}C$ values cannot distinguish if the C_3 or C_4 signatures originated from the direct consumption of plants or from the indirect consumption of animals that consumed these plants**. In all three studies (1-3), this crucial point was brought out again and again by the authors. Apparently, the popular science writers covering these papers missed it. The data from all three papers (1-3) corroborates the increasing body of literature (8) demonstrating an increased C_4 signature in the enamel of African hominins starting about 3.5 MYA, but whether or not it resulted from increased consumption of animal or plant foods or both is unknown. The authors of one of these three scientific papers (1) put it best, "*The ^{13}C-enriched resources that hominins ate remain unknown and must await additional integration of existing paleodietary proxy data and new research on the distribution, abundance, nutrition and mechanical properties of C_4 (and CAM) plants.*"

I would like to point out a number of logical shortcomings with any interpretation of the hominin C_4 data suggesting that it originated primarily from increased consumption of either grass leaves, grass seeds (cereal grains) and sedges rather than from consumption of animals (grazers) that ate grasses and grains. The point in time (~3.5 MYA) at which the C_4 signature begins to increase occurs simultaneously with the earliest known use (before 3.39 MYA) of stone tools to cut flesh from animal carcasses and to extract marrow from their bones (9). Such hominin dietary practices have also been documented by 2.5 MYA (10) and appear to be widely employed by 2.0 MYA (11) and by 1.5 MYA (12). Hence by triangulating these indisputable archaeological facts with stable carbon isotope data, it is virtually certain that $\delta^{13}C$ values in hominin enamel were enriched partially or perhaps mainly from increasing consumption of animals that ate C_4 plants.

Other lines of evidence indicate that early African hominins were increasingly consuming more animal foods during the same time interval (3.5 MYA to 1.5 MYA) that $\delta^{13}C$ had become enriched. Aiello and Wheeler (13) have shown that the mass of the human gastrointestinal tract is only about

60% of that expected for a similar-sized primate. Consequently, the increase in brain size that occurred in hominins starting ~2.5 MYA was balanced by an almost identical reduction in the size of the gastrointestinal tract (13). The selective pressures that simultaneously allowed for both a reduction in gut size and an increase in brain size are attributed to an improvement in dietary quality (DQ) that occurred largely as a result of increased consumption of animal foods by *Australopithecine* species prior to the emergence of the first members of *Homo* (13-15). Because a diet with an increased DQ contains less structural plant parts and more animal material (16), its nutrient and energy density is greater. Hence the greater DQ of animal foods permitted relaxation of the selective pressures in hominins that formerly selected for a large, metabolically active gut necessary to process low DQ foods, which in turn permitted the natural selection of a large metabolically active brain (13, 14). Grass leaves and seeds maintain a low DQ (15), and are high in fiber and cellulose and are indigestible in their raw, unprocessed state in modern humans (17). Accordingly, the proposition that increased consumption of grass leaves and seeds were the C_4 source in hominin enamel, is inconsistent with the evolutionary gut/brain metabolic tradeoff (13-15). Selective pressures that reduce the size and metabolic activity of the gut require more energetically dense foods like meat and marrow – not energy poor, high cellulose and high fiber foods like grasses and sedges.

In addition to their low DQ, grass leaves and seeds are devoid of long chain fatty acids of both the omega 6 family (arachidonic acid, 20:4n6) and omega 3 family (docosahexanoic acid, 22:6n3), as are all plant foods (15). These fatty acids are necessary structural elements required for the synthesis of brain and neural tissues and cannot be produced endogenously in sufficient quantities to relax the selective pressures normally constraining encephalization (brain volume expansion relative to body weight). Therefore, exogenous sources of these two fatty acids must be obtained through diet in hominins to permit the evolution of large metabolically active brains (15, 18-21). Likely candidate animal foods which simultaneously increased the DQ and provided arachidonic acid (AA) and docosahexanoic acid (DHA) were scavenged de-fleshed long bones (which contain marrow – a high fat food) and skulls (which contain brains – high in AA and DHA) from carnivore kills (15). These foods along with meats from grazing animals likely represent the dominant dietary source for the increasing C_4 signature in our African ancestors.

Another nutritional point lends little support to the notion that the increasing C_4 signature in hominins starting 3.5 MYA resulted from direct consumption of grass leaves or seeds. All great apes (chimps, gorillas, orangutans and gibbons) living in their native environment bear $\delta^{13}C$ values indicative of near total reliance upon C_3 plants. Only a single higher primate, a baboon species, *Theropithecus gelada*, consumes grass leaves and seeds as their primary dietary source. Accordingly, this baboon maintains a carbon isotopic signature that is nearly 100 % C_4 derived (4).

High reliance upon grass and grass seeds in *Theropithecus gelada* or in any hominin requires a number of evolutionary adaptations in the digestive tract to accommodate these low quality, high cellulose foods – none of which have been observed in contemporary humans. All vertebrates lack the enzyme cellulase which is required to breakdown cellulose and hemicellulose found in grass leaves and seeds into glucose. Mammals that rely heavily upon grass and grass seed consumption for their sustenance have evolved large hindguts (caecums) or a four compartment stomach (ruminants) containing enormous quantities of microflora which have the capacity to ferment and breakdown cellulose, hemicellulose, starches and proteins into simpler compounds which can then be assimilated and metabolized by the host animal. In the case of *Theropithecus gelada* (the grass eating baboon), it has evolved a large hindgut where microbial fermentation of grass takes place (22). In contemporary humans, and in the hominin line that led to *Homo*, there is no credible evidence that gut morphology became larger and more metabolically active to support fermentation of cellulose in the caecum, but rather the opposite (13, 14). Hence, without the evolution of hindgut fermentation, efficient consumption of grass and grass seeds would have been impossible in any hominin species.

Other comparative physiological data between modern humans and the grass eating baboon (*Theropithecus gelada*) support the notion that the increasing C_4 signature in evolving African hominins was not a result of grass or sedge consumption. Dicots or C_3 plants produce compounds called tannins which act as a chemical defense system that discourage animals from eating them. Monocots or C_4 plants (such as grass and sedges) do not synthesize tannins (23). Over the course of evolution, mammals that consume tannin containing C_3 plants have evolved measures to counter the adverse effects of tannins. The most important of these mechanisms are salivary proteins that act as a defense against dietary tannins (24). These proline rich salivary proteins (PRPs) bind tannins and form stable complexes which prevent tannins from producing adverse health effects (24-27).

Species that usually ingest tannin containing foods as part of their natural diets produce high levels of PRPs, whereas species not exposed to tannins produce little or no PRPs (24). In this regard, the saliva of the grass (C_4) eating baboon (*Theropithecus gelada*) produces a saliva devoid of PRPs (23). In contrast, modern humans synthesize a saliva containing abundant concentrations of PRPs (25-27) which have been suggested to result from the long evolutionary history of fruit and vegetable (C_3 plants) consumption in human ancestors (25). If ancestral African hominins had intensely exploited C_4 plants (grasses and sedges) for millions of years, then it might be expected that the line of hominins that led to *Homo* and modern humans would also maintain low concentrations of salivary PRPs similar to *Theropithecus gelada*. Data in contemporary *Homo sapiens* do not support this conclusion.

In summary, recent comprehensive analyses (1-3) of $\delta^{13}C$ values in the enamel of African hominins from 4.1 to 1.5 MYA support the conclusion that plants of C_4 origin were ultimately responsible for this isotopic signature. Nevertheless, when the isotopic data is triangulated from archaeological, physiological and nutrition evidence, it is apparent that the C_4 signature in ancestral African hominin enamel almost certainly is resultant from increased consumption of animals that consumed C_4 plants.

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