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THE IMPORTANCE OF DIETARY CARBOHYDRATE IN HUMAN EVOLUTION

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ABSTRACT

We propose that plant foods containing high quantities of starch were essential for the evolution of the human phenotype during the Pleistocene. Although previous studies have highlighted a stone tool-mediated shift from primarily plant-based to primarily meat-based diets as critical in the development of the brain and other human traits, we argue that digestible carbohydrates were also necessary to accommodate the increased metabolic demands of a growing brain. Furthermore, we acknowledge the adaptive role cooking played in improving the digestibility and palatability of key carbohydrates. We provide evidence that cooked starch, a source of preformed glucose, greatly increased energy availability to human tissues with high glucose demands, such as the brain, red blood cells, and the developing fetus. We also highlight the auxiliary role copy number variation in the salivary amylase genes may have played in increasing the importance of starch in human evolution following the origins of cooking. Salivary amylases are largely ineffective on raw crystalline starch, but cooking substantially increases both their energy-yielding potential and glycemia. Although uncertainties remain regarding the antiquity of cooking and the origins of salivary amylase gene copy number variation, the hypothesis we present makes a testable prediction that these events are correlated.

INTRODUCTION

THE global increase in the incidence of obesity and diet-related metabolic diseases has intensified interest in ancestral or "Paleolithic" diets. Surprisingly, however, there is little clear agreement on what quantitatively constitutes a healthy diet, or indeed a Paleolithic diet, with much conflicting information disseminated to the public. Yet it is clear that—to a first order of approximation—our physiology should be optimized to the diet that we have experienced during our evolutionary past. This has led researchers to seek a better understanding of the foods eaten during key stages of hominin evolution and by modern hunter-gatherers (Eaton et al. 1988; Lindeberg et al. 2003; Cordain et al. 2005). Key features in human evolution that are considered directly linked to alterations in dietary composition include: changes in tooth morphology and enamel striation patterns (Ungar and Sponheimer 2011); a reduction in the size of the digestive tract, achieved by 1.8 million years ago (Aiello and Wheeler 1995); an increase in brain size, which began around 2 million years ago but accelerated in the Middle Pleistocene, from around 800,000 years ago (Lee and Wolpoff 2009); and increased aerobic capacity by around 2 million years ago (Bramble and Lieberman 2004).

Although the transition from a fibrous-plant based diet to a predominantly meat-based diet has been argued as a major driver of these evolutionary changes (Aiello and Wheeler 1995; Milton 2003; Snodgrass et al. 2009), reconstructing ancestral dietary composition is difficult and requires the integration of multiple lines of evidence, including the chronology of human brain and body size changes over the last 2 million years, food source availability, food remains found at archeological sites, stable isotopes (Sponheimer et al. 2013) and other biomolecular markers, evaluation of human physiology and nutritional requirements, and information on modern hunter-gatherer diets.

Here, we argue that plant carbohydrates and meat were both necessary and complementary dietary components in hominin evolution. We agree with Conklin-Brittain et al. (2002) and Wrangham (2009) that concentrated starch from plant foods was essential to meet the substantially increased metabolic demands of an enlarged brain and we argue that these foods were also essential to support successful reproduction and increased aerobic capacity (Bramble and Lieberman 2004). However, although the development of cooking has been proposed at around 1.8 million years ago (Wrangham et al. 1999; Wrangham and Conklin-Brittain 2003; Wrangham 2007, 2009; Wrangham and Carmody 2010), empirical ev-

idence is lacking until considerably later. We hypothesize that the multiplication of the salivary amylase (*AMY1*), and possibly pancreatic amylase (*AMY2*), genes would have become selectively advantageous only when cooking became widespread. Although the exact date of the multiplication of the *AMY1* genes remains unknown, it is thought to be less than 1 million years ago (Samuelson et al. 1996; Lazaridis et al. 2014). Raw starches are only poorly digested by salivary amylases, but their energy-yielding potential is substantially increased when cooked (Butterworth et al. 2011). We propose that after cooking became widespread, starch digestion became the rate-limiting step in starch utilization, and the coevolution of cooking and copy number variation (CNV) in the *AMY1* (and possibly *AMY2*) gene(s) increased availability of preformed dietary glucose, permitting the acceleration in brain size increase observed from the Middle Pleistocene onward.

To evaluate the importance of dietary carbohydrate in human evolution we consider estimates for the timing of controlled use of fire for cooking, the physiological need for preformed glucose from the diet, the effectiveness of α-amylases in digesting raw and cooked starches, and the origins of CNV in salivary amylase genes. We contend that in terms of energy supplied to an increasingly large brain, as well as to other glucose-dependent tissues, consumption of increased amounts of starch may have provided a substantial evolutionary advantage to Mid-to-Late Pleistocene omnivorous hominins.

Early Hominins and the Adoption of Cooking

Physical remains of early hominins are rare and there is considerable uncertainty regarding their ecologies and evolutionary relationships (Lovejoy 2009; White et al. 2009; Lordkipanidze et al. 2013). *Ardipithecus* was partially bipedal at 4.4 million years ago (White et al. 2009), although whether it was a direct human ancestor is unclear (Wood and Harrison 2011). It is also uncertain which of the many paleospecies of *Australopithecus* was a direct ancestor to *Homo habilis*, who lived from around 2.4 to 1.4 million years ago (Berger et al. 2010). Recent finds at Dmanisi in Georgia suggest that one species, *Homo erectus,* spread out of Africa at least 1.8 million years ago (Lordkipanidze et al. 2013).

Regional paleospecies, including *Homo neanderthalensis*, survived in Europe until somewhere between 41,000–39,000 years ago (Higham et al. 2014), although the possibility remains that *H. neanderthalensis* persisted in southern Iberia until 28,000 years ago (Finlayson et al. 2006), while in Asia, the purported *Homo floresiensis* is thought to have survived until around 17,000 years ago (Morwood and Jungers 2009). The earliest known date for *Homo sapiens* is 195,000 years ago in Ethiopia (McDougall et al. 2005).

Several key changes in hominin morphology are associated with the emergence of *H. erectus*: height and body weight increased substantially, teeth reduced in size, changes in skeletal structure suggest a reduction in gut size, and a doubling of cranial capacity indicates increased brain size between 2 million and 500,000 years ago. These changes are proposed to have occurred with a shift from a high-volume, low-energy diet to a low-volume, high-energy diet (Aiello and Wheeler 1995; Leonard et al. 2003, 2007; Milton 2003; Snodgrass et al. 2009). By 1.8 million years ago the hominin gut is thought to have been effectively modern in shape with a reduced stomach and long small intestine (Aiello and Wheeler 1995). Cranial capacity increased relatively slowly at first to reach around 600 cc by 1.8 million years ago. The rate of increase then accelerated from around 800 kya, and this has been linked to the adoption of cooking (Aiello and Wheeler 1995). Increased body size, particularly the relative size of females and the presence of fat deposits in infants, would have required increased dietary intake to meet additional daily energy expenditure, although a larger body size may have improved the ability of early *Homo* to procure food (Leonard and Robertson 1997; Aiello and Key 2002; Steudel-Numbers 2006). Encephalization has also been proposed as a secondary change, occurring after dietary and other morphological changes (Antón and Snodgrass 2012).

There was a generalized trend toward a drier climate during the Late Pliocene and

Early Pleistocene—as changes in the hominin body were occurring—with a corresponding increase in open grasslands during this period. The climate probably fluctuated between moist and dry periods (Potts 2012), suggesting a need for mobility and dietary flexibility; the ability to deal with "ecological shocks" is considered fundamental to the development of the early hominins (Wells 2012a,b). The evolution of human characteristics, including omnivory, incremental encephalization, and the ability to colonize varied environments, is proposed to have been the result of positive feedback based on ecological adaptability (Wells 2012a,b). Increased mobility and habitat range have been linked to an increase in carnivory (O'Connell et al. 1999; Aiello and Key 2002), with consumption of meat suggested to act as a buffer against environmental change and to support expansion into unfamiliar environments (Potts 2012). However, we propose that high-starch plant foods would have been a plentiful, reliable, and important part of the diet.

Humans are the only species that cook food and this is considered to have been a transformational event in human evolution (Wrangham et al. 1999; Wrangham 2007). Suggestive evidence for an association between humans and fire is found in a number of early African sites, including Wonderwerk Cave (Berna et al. 2012), Swartkrans (Brain and Sillent 1988), Chesowanja, Kenya (Gowlett et al. 1981), and Koobi Fora (Bellomo 1994; Rowlett 2000), dating to at least 1.6 million years ago (Roebroeks and Villa 2011). The site of Gesher Benot Ya'aqov, in Israel, which dates to around 780,000 BP, has charcoal, plant remains, and burned microartifacts in concentrations that the excavators believe suggests evidence for hearths (Alperson-Afil 2008). However, secure evidence of the use of fire for cooking at these sites is still lacking, and it has been suggested that there is no secure evidence for the use of fire in Europe until 300,000–400,000 years ago (Roebroeks and Villa 2011). The well-known site of Schöningen provides tentative evidence for controlled use of fire at 400,000 BP (Thieme 1997), although both the evidence for use of fire and this date are currently being reevaluated (Balter 2014). Qesem Cave in Israel currently has the earliest secure evidence for of fire use, displaying indications of fire from 400,000 BP and repeatedly used hearths from 300,000 BP (Shahack-Gross et al. 2014).

Although the timing of widespread cooking is not known, Wrangham and Conklin-Brittain (2003) argue that it was long enough ago to allow for biological adaptations to take place, including changes in digestive anatomy around 1.8 million years ago, reduction in tooth size, and reduced capacity for digestion of raw, fibrous foods. They further propose that cooked foods were soft enough to be palatable by infants, potentially leading to earlier weaning and shorter interbirth intervals (also see Carmody et al. 2011).

Ancestral Diet

Plants produce a wide range of carbohydrates to serve as energy reserves or for structural functions. Reserve carbohydrates can be deposited in underground storage organs (USOs) such as roots, tubers, and rhizomes, or above ground in seeds, certain fruits and nuts, and in the inner bark of some trees. Starch constitutes up to 80% of the dry weight of edible roots and tubers and, if left undisturbed in the ground, they remain stable and can be harvested as needed over a period of months. USOs can also be dried to increase durability and portability, and have been proposed as important foods for early hominins (Laden and Wrangham 2005). The ability to exploit starch-rich roots and tubers in early hominin diets is considered a potentially crucial step in differentiating early Australopithecines from other hominids and to have permitted expansion into new habitats (Wrangham et al. 1999; Laden and Wrangham 2005). The consumption of USOs could also explain differences in dentition between early hominins and African apes (Laden and Wrangham 2005). USO-rich aquatic habitats such as deltas have been proposed as an intermediate niche in the adaptation of early hominins to savanna habitats, with the need to forage in shallow water promoting bipedality (Wrangham 2005, 2009). O'Connell et al. (1999, 2002) suggest that postmenopausal females played a central role in foraging

for USOs and food sharing, which directly enabled younger female relatives to reproduce more frequently. They further proposed that meat formed an irregular component of the diet and that hunting by early hominins may have been as much to do with status as nutrition, something that has also been proposed for chimpanzees (Nishida et al. 1992; Stanford 1998). Although meat may have been a preferred food, the energy expenditure required to obtain it may have been far greater than that used for collecting tubers from a reliable source (Carmody et al. 2011).

Many other lines of evidence support consumption of starchy USOs by early hominins. Correlation of evidence for C_4 plants in the diet of *Australopithecus africanus* and *Paranthropus robustus,* and specific-use wear traces on teeth are proposed to be indicative of consumption of sedge corms (Dominy et al. 2008; Ungar and Sponheimer 2011; Grine et al. 2012; Ungar et al. 2012). A C_4 signal identified in the tooth enamel of a 3-million-year-old *Australopithecus bahrelghazali* from Chad has been interpreted as evidence for exploitation of Cyperaceae sedge tubers (Lee-Thorp et al. 2012). Evidence of abundant suids in many African hominin sites has been taken to suggest that USOs, the predominant food source for these animals, were plentiful (Reed and Rector 2007). The presence of palms in the Olduvai Gorge region date from around 1.8 mya (Albert et al. 2009); palms often have abundant edible starch in their trunks, and some species also produce dates. The roots of lilies (Liliaceae), rushes (Juncaceae), and sedges (Cyperaceae) have also been identified at Olduvai Gorge from a horizon dated to between 1.89 and 1.75 million years ago (Bamford et al. 2008). Edible USOs from these monocotyledons, along with grasses (Poaceae) identified at the same sites, offer evidence for the abundance of edible starch at a time that hominins were present.

Faunal remains (with cut marks suggesting butchery) survive in numerous Middle Pleistocene sites (Stiner et al. 2009; Yravedra et al. 2010) and have been interpreted as evidence of early hominin meat consumption. In contrast, evidence for plant foods rarely survives, making it difficult to estimate their contribution and to reconstruct ancestral diet on the basis of physical remains alone. We agree with Wrangham (2009) that the reduction in gut size is more likely to have occurred due to a gradual replacement of fibrous plants by higher energy-yielding plant foods, including starchy tubers.

Human Nutritional Requirements

The main dietary source of the monosaccharide glucose is from the digestion of glycemic carbohydrates (also referred to as available carbohydrates). Available carbohydrates comprise 40–75% of modern dietary energy intake (FAO 1998), of which starch is the most abundant. Starch is digested slowly and incompletely in raw crystalline form, but more rapidly after cooking. Plants also contain many other carbohydrates, including cellulose and other structural polysaccharides in cell walls, and various types of mono-, di-, and oligosaccharides. However, many polysaccharides and oligosaccharides are considered unavailable because they are not hydrolyzed by human upper gut enzymes, and pass into the large intestine, where some are fermented by gut microbiota. Among the products of these fermentations are the short-chain fatty acids, butyrate, propionate, and acetate, which can be absorbed from the gut and provide 5–10% of the energy requirement of adults (McNeil 1984).

Modern humans require a reliable source of glycemic carbohydrate to support the normal functioning of our brain, kidney medulla, red blood cells, and reproductive tissues. The brain alone accounts for 20–25% of adult basal metabolic expenditure (Fonseca-Azevedo and Herculano-Houzel 2012). In addition to the demands of the brain, red blood cells require approximately 20 g glucose per day directly from the bloodstream (Mulquiney et al. 1999). Under normal circumstances, a glucose requirement of approximately 170 g/day is met by a mixture of dietary carbohydrate and gluconeogenesis from noncarbohydrate sources, such as the glycerol moiety of fats, some amino acids (e.g., alanine), or absorbed propionate from gut fermentations of dietary carbohydrates (Wong et al. 2006).

There is debate on whether dietary carbohydrates are actually essential for human nutrition. In the absence of dietary carbohydrate, or during starvation, gluconeogenesis alone is usually not sufficient and the brain begins to utilize ketones, a byproduct of high levels of fat oxidation (Westman 2002). Under these conditions, about 80% of the brain's energy needs can be met from ketones but to maintain normal brain function in individuals adapted to an essentially carbohydrate-free diet there remains an absolute requirement for 30–50 g (Institute of Medicine 2006) of dietary glycemic carbohydrate per day to fill the gap between gluconeogenic capacity and the brain's requirement for glucose (Macdonald 1988). A daily carbohydrate intake of about 50–100 g is considered essential to prevent ketosis in adults (Institute of Medicine 2006), and is consistent with a more realistic recommendation for the practical minimal requirement of 150 g/day of glycemic carbohydrate intake beyond the ages of 3 to 4 years (Bier et al. 1999). Up to the age of 3, while brain size increases rapidly, the recommendation is that at least one-third of dietary energy should be supplied from carbohydrates (Bier et al. 1999). The additional need for pregnancy and lactation was not considered.

It has been suggested that early *Homo* acquired the capacity for endurance running, considered essential to exhaust prey or outpace other scavengers in hunting, by 2 million years ago (Bramble and Lieberman 2004). Although the need for thermoregulation during sustained exercise has been explored (Wheeler 1991a,b; Liebenberg 2006; Ruxton and Wilkinson 2011), the energy source for prolonged high level aerobic activity is also an important consideration. Glucose is the only energy source for sustaining running speeds above 70% of maximal oxygen consumption (Romijn et al. 1993).

In healthy individuals, blood glucose levels are maintained within a narrow range to avoid the physiological consequences of hypo- or hyperglycemia (Sanders and Lupton 2012). In the fed state—up to five hours after a meal—glucose enters the bloodstream following digestion of various dietary carbohydrates. Some glucose is oxidized immediately to provide energy, with the remainder used to build reserves of the polymer glycogen in muscle and liver tissues. Muscle glycogen provides a short-term energy reserve for muscles, while liver glycogen maintains glucose homeostasis in the bloodstream. In the fasting or postabsorptive state, glycogen is mobilized to provide glycemic carbohydrate for the brain, red blood cells, and other tissues that cannot use fatty acids as their energy source. Total body reserves of glycogen are limited, providing glycemic carbohydrate for only 18–24 hours of fasting (Macdonald 1988). In more prolonged fasting, or under starvation conditions, there is considerable loss of tissue protein, which is broken down out of necessity to provide the amino acids for gluconeogenesis. Indeed, death due to starvation is as much a consequence of critical loss of tissue protein to supply energy to the brain as it is the reduction in the body's fat stores (Institute of Medicine 2006). In an evolutionary context, large stores of glycogen must be generated in order to provide sources of glucose for periods of sustained fasting or hardship. To build these reserves, the diet must consistently provide energy surplus to basal metabolic requirements.

There is a limit, considered to be 35–40%, to the amount of energy requirements humans can derive from proteins; above this ceiling protein toxicity can occur, which can cause death quite rapidly (Speth 2010). It has been suggested from evidence of the dietary practices of hunter-gatherer populations, both present day and historical, that humans avoid diets that exceed this limit for protein (Speth 1989; Cordain et al. 2000). The diets of traditional Arctic populations are sometimes given as examples of successful high-protein diets (Lindeberg 2009). An estimate of their dietary composition suggested that about 50% of the calories come from fat, 30–35% from protein (or around 300 g per day and lower for pregnant women; Speth 2012), and 15–20% from carbohydrate principally in the form of glycogen from the meat they consume (Ho et al. 1972). Ethnographic evidence demonstrates that nutrients, including essential vitamins,

minerals, and carbohydrates, were obtained from eating the stomach contents of terrestrial prey animals, and tundra plants and kelp (Kuhnleini and Soueida 1992; Speth 2012). Meat frozen soon after slaughter will retain much of its muscle glycogen (Varmin and Sutherland 1995), providing another source of available carbohydrate. It is likely that circumpolar peoples ate more carbohydrate than is generally thought (Rabinowitch 1936; Sinclair 1953). Nevertheless, Inuit have enlarged livers with an increased capacity for gluconeogenesis, and have greater capacity for excreting urea to remove ammonia, a toxic byproduct of protein breakdown (Kaleta et al. 2012). Indeed, a recent study has identified high frequencies of a nonsynonymous G>A transition (rs80356779) leading to a Pro479Leu change in the *CPT1A* gene—a key regulator of mitochondrial long-chain fatty-acid oxidation—in modern circum-Arctic populations (Clemente et al. 2014). Interestingly, the derived A-allele has been shown to associate with hypoketotic hypoglycemia and high infant mortality. Ethnographic texts record the Inuit habit of snacking frequently (Klutschak 1987). This custom may well be a direct consequence of the rs80356779 'A' allele gene as fasting, even for several hours, can be deleterious for people with this allele. The high frequency of the *CPT1A* Pro479Leu change in circum-Arctic therefore suggests that it is an important adaptation to high meat, low-carbohydrate diets.

Fats, mostly from marine sources, are the major source of energy for nonglucose-dependent tissues, thereby sparing glucose for those tissues that require it, such as the brain. Although a very high fat intake will prevent protein toxicity and provide energy for metabolism, it comes at the cost of high levels ketones in the blood, which can compromise reproductive function (Kim and Felig 1972). Traditional Arctic Inuit populations are known to have had a relatively low birth rate and a highly seasonal pattern of reproduction, which continued after widespread contact. Whether the low and seasonal birth rate was associated with seasonally varied diet, such as possibly reduced access to plant foods and carbohydrates in winter, or the presence at high frequency of the *CPT1A* Pro479Leu change, or whether there are other social and cultural explanations, are matters for speculation (Condon 1982; Scheffel 1988).

Glucose is the main energy source for fetal growth, and low glucose availability can compromise fetal survival (Herrera 2000; Baumann et al. 2002). Pregnant females have a minimal requirement for 70–130 g/day of preformed glucose or glucose equivalents to maintain optimum cognitive function in the mother and to nourish the fetus (Institute of Medicine 2006). Although increased gluconeogenesis is one of the first metabolic adaptations to pregnancy, maternal glucose levels decline in early pregnancy (Butte 2000; Metzker 2010; Thame et al. 2010). As pregnancy progresses, intrinsic insulin resistance develops in order to redirect glucose away from the mother and toward the growing fetus (Butte 2000). In healthy pregnant women, fetal growth is directly correlated with maternal glucose concentration (Metzger et al. 2009); larger infants are born to women with higher blood glucose (Butte 2000), while a link has been made between maternal gestational ketonemia and a reduced offspring IQ (Rizzo et al. 1991). Tests of glucose deprivation in animals have repeatedly demonstrated a link between maternal glucose levels and offspring viability (Romsos et al. 1981; Taylor et al. 1983; Koski and Hill 1986). Offspring survival can also be affected by the increased demand for glucose during lactation. At peak lactation, mammary glands require an additional 70 g glucose/day for synthesis of lactose, the main sugar in milk (Institute of Medicine 2006).

One argument for increased meat consumption as the catalyst for brain expansion, and for the development of certain patterns of social organization, is founded on the dietary need for very long-chain omega-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA; (Kaplan et al. 2000; Bunn 2007; Leonard et al. 2007; Brenna and Carlson 2014). These fatty acids are critical structural components of brain, retina, and other nerve tissues, and are essential for the growth and functional development of the brain in infants, and for normal brain func-

tion in adults (Innis 2008). Dietary DHA availability has been proposed as a limiting factor in brain evolution (Leonard et al. 2007). Although meat is a source of these essential fatty acids (Howe et al. 2006), DHA can also be obtained directly from other dietary sources, or it can be synthesized from other fatty acids such as α-linolenic acid (ALA), which is present in oils from ocean fish, eggs, seed oils, and various leafy plant foods (Brenna and Carlson 2014). The potential evolutionary value of plant-sourced omega-3 fatty acids has been highlighted previously (Kuipers et al. 2010). More DHA may be needed in the modern diet than was in prehistory, due to the abundance in the modern Western diet of omega-6 fatty acids, which inhibit a key enzyme step in the conversion of ALA to DHA (Simopoulos 2006).

Starch as a Glycemic Carbohydrate

Starch is composed of a mixture of two polymers, amylose and amylopectin, and is packed into discrete, stable, semicrystalline granules (Copeland et al. 2009; Butterworth et al. 2011). Native starch granules vary considerably in their physicochemical properties according to botanical source, including size and shape, amylose-to-amylopectin ratio, fine structure of the starch polymer molecules, crystallinity, porosity, and structural inhomogeneities (Copeland et al. 2009). Although these properties can influence the accessibility to digestion by α-amylases to some extent, intact native granules are nevertheless digested slowly in comparison to granules that have been partially gelatinized by hydrothermal treatment, such as cooking (Tester et al. 2006; Butterworth et al. 2011; Wang and Copeland 2013). For example, gelatinization increases enzymic digestibility of potato starch by around twentyfold (Cummings and Englyst 1995). Additionally, cooking disrupts the integrity of plant cell walls so that the starch granules are more accessible to α-amylase digestion, and the digestion products more readily released from the food matrix (Singh et al. 2013). Consequently, cooking greatly increases the glucose-releasing potential of starchy plant materials in the gut, and so provides improved support for the energy needs of a large human brain and other glucose-dependent tissues (Butterworth et al. 2011).

Salivary Amylases

Humans have two types of α-amylases, one expressed in salivary glands, and the other expressed in the pancreas and secreted into the small intestine. Both types of amylases hydrolyse only α-1,4 glucodisic bonds, which account for the majority of linkages between the glucosyl moieties in starch (and glycogen). Salivary amylase begins starch hydrolysis in the oral cavity. Chewing comminutes the food and provides good mixing of enzyme and substrate, which affords the enzyme protection inside the bolus and allows digestion to continue to some extent in the low pH environment of the stomach (Butterworth et al. 2011). Starch digestion is continued by pancreatic amylases in the duodenum, and by various α-1,4 and 1,6 glucosidases, which break down branched chains and convert oligosaccharides into glucose for absorption into the bloodstream. Young infants have minimal pancreatic amylase activity. When nondairy foods are introduced into the diet following weaning, a large part of starch digestion, possibly 50%, is accomplished by salivary amylases (Butterworth et al. 2011). In contrast, a much greater proportion of the starch molecules are broken down in the duodenum of adults (Lee et al. 2013).

Human amylase genes are located in a complex multigene cluster, which has evolved as a result of two independent retroviral insertion events, as well as a number of unequal homologous recombination events (Gumucio et al. 1988; Samuelson et al. 1988; Groot et al. 1989a). Pancreatic amylases are encoded by the *AMY2A* and *AMY2B* genes, and salivary amylase by the *AMY1A*, *AMY1B*, and *AMY1C* genes (Groot et al. 1988, 1991). There is also an inactive pseudogene, *AMYP1*, located within the amylase gene cluster. The haplotype structures of this multigene family are complex and incompletely ascertained, but it is known that there is significant variability in the total number of *AMY1* genes (Gumucio et al. 1988; Samuelson et al. 1988; Groot et al. 1989a; Lafrate et al. 2004; Perry

et al. 2007; Carpenter et al. 2015), as well as some variability in the copy number of *AMY2* genes (Carpenter et al. 2015). Humans are unusual in that they have high levels of the salivary α-amylase, apparently due mostly to multiple copies of *AMY1* genes. Among primates, multiple copy numbers of *AMY1* genes have been identified only in *H. sapiens* (Groot et al. 1989b; Bank et al. 1992; Samuelson et al. 1996). Furthermore, a strong correlation has been shown between *AMY1* copy number and the concentration of amylase in saliva (Bank et al. 1992; Perry et al. 2007) as well as oral starch digestion rate (Mandel et al. 2010). Young infants, prior to weaning in particular, who have a greater requirement for preformed glucose (Bier et al. 1999) and for whom salivary amylases have greater importance in starch digestion (Lee et al. 2013), may have benefitted from multiple copies of the *AMY1* genes. Interestingly, domestic dogs have been found to have multiple copies of the pancreatic amylase gene *AMY2B* (dogs do not express amylase in the saliva; Simpson et al. 1984), suggesting parallel adaptation in dogs and humans to diets rich in cooked and, therefore, highly digestible starches (Axelsson et al. 2013).

The evolution of amylase gene cluster haplotypes has not been fully elucidated, but appears to be a result of multiple structural rearrangements and retroviral insertions occurring over millions of years. Comparative genomic studies of humans and other primates indicate that the first major step in primate amylase gene evolution was a tandem duplication of an ancestral *AMY2B*-like gene to give rise to *AMY2A* and *AMY2B* precursor genes of the pancreatic amylases (Samuelson et al. 1990, 1996). The first retroviral insertion in the amylase gene cluster is thought to have occurred around 43 mya, and from this point, precursors for *AMY2A* and *AMY2B* evolved separately. *AMY2A* then experienced a second upstream retroviral insertion around 39 mya, which was subsequently duplicated, giving rise to *AMY2A* and *AMY1* precursors. It is the juxtaposition of these independently inserted retroviral sequences that transformed the expression of *AMY1* precursors from the pancreas to the salivary glands. Excision of the retroviral element upstream of the *AMY2A* precursor gene reverted its expression back to the pancreas. The *AMY1* precursor is then thought to have undergone a triplication event resulting in the three salivary amylase genes: *AMY1A*, *AMY1B*, and *AMY1C*. Following this, duplications of a cassette contain *AMY1A*, *AMY1B*, and the inactive pseudogene, *AMYP1*, gave rise to much of the *AMY1* CNV observed in human populations today. However, it should be noted that more complex amylase gene cluster haplotypes are now apparent, including those with CNV in *AMY2* (Carpenter et al. 2015).

Based on the sequence similarity among the three human *AMY1* genes, it has been estimated that they have been evolving separately for less than 1 million years (Gumucio et al. 1988; Samuelson et al. 1990). This date may be an underestimate if the region has been subjected to positive selection or gene conversion. However, while read-depth analysis of ancient DNA samples has shown that *AMY1* copy number in prefarming Europeans is comparable to that found in modern populations (Lazaridis et al. 2014; Olalde et al. 2014), an *AMY1* diploid copy number of 2 has been identified in *H. neanderthalensis* (N=1) and Denisovans (N=1) (Lazaridis et al. 2014). This latter observation does not exclude the possibility of *AMY1* CNV in the common ancestors of *H. sapiens* and *H. neanderthalensis,* since between species gene coalescence dates predate species divergence dates, often by some considerable margin. Additionally, *AMY1* CNV could have been lost in the largely carnivorous Neanderthal lineage by relaxation of selective constraint. Nonetheless, this observation does provide some indication that the evolution of high-copy number *AMY1* haplotypes may postdate the divergence of modern humans from *H. neanderthalensis* and Denisovans.

The observed *AMY1* diploid copy number of 2 in *H. neanderthalensis* specimens (Lazaridis et al. 2014) does not exclude the possibility of *AMY1* CNV in this species, since only one individual has been genotyped at this locus. However, if specieswide, this absence could be explained by the particular ecology of *H. neanderthalensis*. Although there is

growing evidence to suggest that the diet of Neanderthals (Henry et al. 2011; Hardy et al. 2012) and earlier Lower Paleolithic hominins (Hardy et al. 2015) included plants, stable isotope analyses indicate a mainly carnivorous diet for Neanderthals; a wider range of isotopic values have been observed in contemporary Middle Pleistocene *H. sapiens* (Richards and Trinkaus 2009), indicating that considerable differences in the levels of starch consumption existed between these two species. The high glucose demands of the large Neanderthal brain could be met with a meat/fat-rich diet by switching to gluconeogenesis. Although this pathway is inherently less efficient, a recent study of the *CPT1A* gene—a key regulator of mitochondrial long-chain fatty-acid oxidation—in modern Arctic populations (Clemente et al. 2014) shows that this switch can evolve very rapidly. Indeed, the diet-driven, rapid evolution of other genes involved in fatty acid metabolism has also been shown recently in the carnivorous polar bear lineage, since its split from omnivorous brown bears (Liu et al. 2014).

This evolution of *AMY1* gene CNV and its associated increase in oral starch digestion rates (Mandel et al. 2010) appears to overlap the time frame for hominin adoption of fire for cooking. It has been proposed that high *AMY* copy number is correlated with the quantity of starch in ancestral diets among modern human populations (Perry et al. 2007), although concerns remain about data quality and the assignment of test populations as "high" or "low" starch in that study (Carpenter et al. 2015). We hypothesize that cooking-driven increase in the availability of digestible starches—probably arising in the Middle Pleistocene—would have resulted in salivary (and possibly pancreatic) amylase levels becoming the rate-limiting step in starch digestion, and so favoring the evolution of *AMY1* (and possibly *AMY2*) CNV. The coevolution of these two traits would have resulted in the increased availability of preformed glucose necessary to support the energy requirements of an enlarging brain.

Cultural adaptation is more rapid, and in many cases more flexible, than biological adaptation (Richerson and Boyd 2008). It is our contention that changes in starch consumption—driven in part by the cultural transition to controlled fire use and cooking—is likely to have either activated positive selection on standing variation in *AMY1* (and possibly *AMY2*) CNV, or favored de novo *AMY1* CNV after cooking was adopted. Dietary coevolution between culture and genes is strongly supported in the case of milk consumption (Holden and Mace 1997), whereby the development of dairying practices during the early Neolithic led to increased milk consumption (Evershed et al. 2008), and the evolution and spread of lactase persistence (Swallow 2003; Itan et al. 2009, 2010; Gerbault et al. 2011).

Other digestible plant carbohydrates would have been available in the diet, but their contribution of preformed glucose would have been small and highly variable. Honey, which contains 36% glucose, 44% fructose, and 20% water (Murray et al. 2001), is a preferred food among some modern hunter-gatherer populations, such as the Hadza and the Aché, due to its sweetness and energy density (Kaplan et al. 1984; Skinner 1991; Berbesque et al. 2011). The importance of honey during hominin evolution has been discussed (Crittenden 2011), however, it may not have been widely available year-round in sufficient quantities to meet the need for glycemic carbohydrate of large groups of individuals (estimated at 50 g per day per person).

Depending on latitude, fruits and berries can supply moderate amounts of carbohydrate-based energy during parts of the year (Hurtado et al. 1985); for example, Cordain et al. (2000) suggested that as much as 41% of foods collected by Australian aborigines were fruits. Uncultivated fruits are, however, typically not as rich in sugars as those obtained from modern domesticated varieties. Even today, berries typically contain only 3–4 g sugars per 100 g edible portion and would need to be eaten in huge quantities (about 3 kg) to provide 130 g glucose equivalents per day. Sucrose, the main sugar in fruit, is digested by sucrase-isomaltase to produce glucose and fructose in equal proportion. Fructose can be absorbed as a source of energy and some may be converted indi-

rectly to glucose via a pathway that requires multiple enzymic steps (Delarue et al. 1993; Elliott et al. 2002). However, although fructose could have had a glucose-sparing effect, its relatively low abundance in food sources, even when eaten in large quantities, would have meant the contribution to glycemic carbohydrates was small. Fruits and berries may have supplemented the diet depending on climate and seasonality, in particular by providing essential vitamins, but they are unlikely to have provided a consistent contribution to carbohydrate energy requirements. Apart from fruits, simple sugars do not occur widely in plant food sources. Other carbohydrates of limited botanical distribution include raffinose (galactosylsucrose) oligosaccharides, which constitute about 5–8% of the dry matter of some legume grains (for example, peas, beans, and lentils) and fructooligosaccharides and fructans, including inulins (60–70% of the dry matter of onions, Jerusalem artichoke, *Agave*, and other species). These carbohydrates are not digested in the upper gut and do not contribute preformed glucose. Trehalose, a glucose disaccharide, occurs widely in insects and fungi (including mushrooms) but is uncommon in plants (Avigad 1990). Most humans maintain functional trehalase activity (Murray et al. 2000), but the low concentration of trehalose in most foods containing it suggests that it only provided a small proportion of the preformed glucose requirements in ancestral humans.

High *AMY1* CNV may not only be related to human adaptation to increased digestible starch availability, but also to the presence of plant secondary metabolites, particularly polyphenols such as tannins. Tannins, and polyphenolic compounds more generally, can act as ubiquitous, nonspecific inhibitors of digestion enzymes and glucose transporters (Manzano and Williamson 2010; Mimsek et al. 2014), thereby reducing the rate of digestion and absorption of key nutrients, particularly glucose (Zucker 1983). Tannin-rich food has been shown to affect the activity of salivary amylase in some animals by increasing it in some cases (Ahmed et al. 1991; Da Costa et al. 2008; Lamy et al. 2010), although the inverse has been observed in cockerels (Ahmed et al. 1991).

Plant flavonoids are also known to inhibit amylase activity as well as α-glucosidase activity in brush border enzymes (Kim et al. 2000; Tadera et al. 2006; Shen et al. 2012; Barrett et al. 2013). Two secondary advantages of increased salivary amylase activity may have been to render some plant foods more palatable by mopping up polyphenols, and to counterbalance amylase inhibition.

Conclusion

The rapid growth in hominin brain size during the Middle Pleistocene will have required an increased supply of preformed glucose. Such increased demands can be met through a range of biologically and culturally driven dietary adaptations. Noting that there is considerable overlap in date estimates for the origins of controlled fire use and the origins of *AMY1* CNV, we hypothesize a gene-culture coadaptation scenario whereby cooking starch-rich plant foods coevolved with increased salivary amylase activity in the human lineage. Without cooking, the consumption of starch-rich plant foods is unlikely to have met the high demands for preformed glucose noted in modern humans. Likewise, the improved accessibility of starch to α-amylases through cooking would, in turn, have led to an increased advantage for high levels of salivary amylase expression, particularly in infants. Carmody and Wrangham (2009) highlight the increased speed of digestibility and consequent energy gain provided by starch that has been thermally processed; however, *AMY1* expression is also required for this to be effective. In addition to the increased energy availability from starch, other advantages of the coevolution of cooking and *AMY1* expression include a reduction in chewing time, increased palatability and digestibility of polyphenol-rich plant foods, and improved reproductive function; a reliable supply of glycemic carbohydrate is likely to have sustainably supported fetal growth, provided the extra caloric intake needed during lactation, and improved infant survival. The regular consumption of starchy plant foods offers a coherent explanation for the provision of energy to the developing brain during the Late Pliocene and Early

Pleistocene while the development of cooking, and a concomitant increases in salivary amylase expression, explains how the rapid increases in brain size from the Middle Pleistocene onward were energetically affordable.

Testing this hypothesis will require a convergence of information from archeology, genetics, and human physiology. Despite a number of high-profile studies on the amylase gene cluster, it is becoming clear that the locus remains incompletely characterized, that published estimates of *AMY1* CNV are problematic, and that the structural nature of that CNV is more complex than previously thought (Carpenter et al. 2015). A more complete understanding of amylase gene CNV, and additional ancient DNA data for this locus, will provide more reliable and precise estimates of when *AMY1* CNV arose and was subjected to natural selection. It is more difficult to predict when a broad archeological consensus will be reached on the timing of widespread adoption of fire use for cooking. However, it seems likely that developments in the archeological sciences will help to improve estimates considerably in the coming years. Regarding the importance of preformed glucose, this is currently a very active area of research and evidence for the essential nature of glucose in brain growth and function, and in fetal development, is likely to increase rapidly. Although our hypothesis is potentially falsifiable with new data from the research areas outlined in this article, it is consistent with the current lines of evidence discussed, and illustrates the synergies of addressing this complex topic using data from different disciplines.

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