A multidisciplinary reconstruction of Palaeolithic nutrition that holds promise for the prevention and treatment of diseases of civilisation

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Abstract

Evolutionary medicine acknowledges that many chronic degenerative diseases result from conflicts between our rapidly changing environment, our dietary habits included, and our genome, which has remained virtually unchanged since the Palaeolithic era. Reconstruction of the diet before the Agricultural and Industrial Revolutions is therefore indicated, but hampered by the ongoing debate on our ancestors' ecological niche. Arguments and their counterarguments regarding evolutionary medicine are updated and the evidence for the long-reigning hypothesis of human evolution on the arid savanna is weighed against the hypothesis that man evolved in the proximity of water. Evidence from various disciplines is discussed, including the study of palaeo-environments, comparative anatomy, biogeochemistry, archaeology, anthropology, (patho)physiology and epidemiology. Although our ancestors had much lower life expectancies, the current evidence does neither support the misconception that during the Palaeolithic there were no elderly nor that they had poor health. Rather than rejecting the possibility of 'healthy ageing', the default assumption should be that healthy ageing posed an evolutionary advantage for human survival. There is ample evidence that our ancestors lived in a land–water ecosystem and extracted a substantial part of their diets from both terrestrial and aquatic resources. Rather than rejecting this possibility by lack of evidence, the default assumption should be that hominins, living in coastal ecosystems with catchable aquatic resources, consumed these resources. Finally, the composition and merits of so-called 'Palaeolithic diets', based on different hominin niche-reconstructions, are evaluated. The benefits of these diets illustrate that it is time to incorporate this knowledge into dietary recommendations.

Key words: Palaeolithic nutrition: Disease prevention: Disease treatment: Dietary recommendations: Evolutionary medicine: Healthy ageing

Introduction

In the *Origin of Species*[1], Darwin recognised that there are two forces of evolution, i.e. natural selection and the conditions of existence, where the latter was considered the most powerful[2]. For example, important steps in evolution are the origin of eukaryotic life approximately 1·6–2·7 billion years ago[3,4] and the appearance of photosynthetic cyanobacteria that began to oxygenate the atmosphere about 2400 million years ago (Mya)[5]. However, there was relatively little alteration in the design of life forms before the Cambrian explosion about 600 Mya. Only when the oxygen tension in the atmosphere rose above the Pasteur point did aerobic metabolism become thermodynamically possible[6], resulting in an explosion from simple prokaryotics to a diversity of eukaryotic life forms[7].

During the past millions of years of evolution, with relatively little alteration in life forms and environmental circumstances, the human genome has become optimally adapted to its local environment[8–11]. In other words, our genome may have reached a state of homeostasis, defined as the 'optimal interaction between environment and genome' or 'nature in balance with nurture', to support optimal survival for reproductive success. The aetiologies of many typically Western diseases, also known as diseases of affluence or civilisation, have been attributed to the disturbance of this delicate balance, secondary to the rapid changes in the conditions of existence, while our genome has remained basically unchanged since the beginning of the Palaeolithic era. The former include changes in physical activity, stress, sleep duration, environmental pollution and others[12,13],

but one of the most rapidly changing conditions of existence has been the human diet.

Since the onset of the Agricultural Revolution, some 10 thousand years ago (Kya), and notably in the last 200 years following the start of the Industrial Revolution, humans have markedly changed their dietary habits. Consequently, it has been advocated that the current pandemic of diseases of civilisation results in part from the mismatch between the current diet and our Palaeolithic genome. In other words, 'we are what we eat, but we should be what we ate'[14,15]. The ensuing poorly adapted phenotype may find its origin as early as in the fetal period[16,17] and possibly as far back as in the maternal grandmother's womb[18]. This phenotype might be laid down in, inherently labile, epigenetic marks that are meant for the short- and intermediate-term adaptation of a phenotype to the conditions of existence. With clear evolutionary advantages they may become transmitted to the next generations as a memory of the environmental conditions that can be expected after birth[19]. They thereby give rise to a seemingly high contribution of genetics in some of the associated 'typically Western' degenerative diseases, which are in fact complex diseases that by definition do not inherit by Mendel's law, illustrating that epigenetic marks can also become erased.

From a pathophysiological point of view, the poorly adapted phenotype in Western countries, ensuing from the conflict between the changing lifestyle and our Palaeolithic genome, centres on chronic low-grade inflammation and the metabolic syndrome (also named the insulin resistance syndrome), which are risk factors for many of the diseases and conditions typical for affluent countries, such as CVD, type 2 diabetes mellitus, osteoporosis, certain types of cancer (notably colon, breast, prostate), fertility problems (polycystic ovary syndrome), pregnancy complications (gestational diabetes, pre-eclampsia), some psychiatric diseases (major and postpartum depression, schizophrenia, autism) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease)[20–22]. The genetically determined flexibility to adapt to a changing environment appears to have been exceeded and the genetically most vulnerable have become sick first, but ultimately all individuals will become sick with increasing dose and exposure time.

Environment, nutrients and their interaction with the genome

Adjustment of the DNA base sequence is a slow process that in an individual cannot support adaptation to environmental changes occurring at intermediate or rapid pace. Flexibility for rapid adaptation is provided by genetically encoded mechanisms that allow adjustment of phenotype by epigenetics and by the interaction of the environment with sensors, such as those of the sensory organs, but also by the many that remain unnoticed[23–25]. The role

of nutrients in (epi)genetics and their direct interaction with the genome have become increasingly acknowledged[26]. Examples of such nutrients are iodine, Se, vitamins A and D, and n-3-fatty acids, which are direct or indirect ligands of the thyroid hormone receptor (TR), retinoid X receptor (RXR), retinoic acid receptor (RAR), vitamin D receptor (VDR) and PPAR. Homodimerisation and heterodimerisation of these receptors facilitate gene transcription and thereby keep our phenotype optimally adapted to the reigning conditions of existence. The roles of these nutrients, their respective receptors and the interaction between their receptors are indicative of the importance of their dietary presence and of a certain balance between their dietary intakes to arrive at optimal interaction with the genome. Lessons for this optimal interaction, and hence for the development of randomised controlled trials aiming at the study of diet or lifestyle, rather than single nutrients, might derive from knowledge on human evolution and the conditions of existence to which our ancestors have been exposed. These lessons might provide us with valuable information on what we should genuinely define as a 'healthy diet'.

Evolutionary medicine

The concept that a thorough understanding of evolution is important in the prevention and treatment of (human) diseases has long been recognised. For example, in the early 1960s it was stated that 'nothing in biology makes sense except in the light of evolution'[27], while in ethology, a distinction was made between proximate and ultimate (also named evolutionary) causes[28]. Proximate explanations provide a direct mechanism for certain behaviour in an individual organism. They explain how biomolecules induce certain behaviour or, for example, an allergic reaction. Proximate explanations, however, provide insufficient information to answer the question why this behaviour or this allergic reaction occurred. Ultimate explanations provide answers explaining why things happen from an evolutionary point of view. Many, if not all, diseases can become explained by both proximate and ultimate explanations. The science searching for the latter explanations has become known as 'evolutionary medicine'. Unfortunately, modern medicine deals mostly with proximate explanations[29,30], while ultimate explanations seem more prudent targets for long-time disease prevention[29].

The term 'evolutionary medicine' (also named Darwinian medicine) was launched by Randolph M. Nesse and George C. Williams[31,32]. They provided evolutionary answers for the understanding of human diseases. Many diseases do not result from a single biological, anatomical or physiological abnormality, but rather from a complex web of interactions. They often reflect the collateral damage of the survival and reproduction strategies of our genes and the genes of other organisms in our environment.

The resulting disease manifestations include the outcomes of human defence mechanisms to clear foreign pathogens and the collateral damage of conflicts and trade-offs between humans and foreign invaders. Examples often overlooked are coincidence, in which diseases may result from imperfections of human evolution, and exaptation, in which a feature is not acquired in the context of any function to which it might eventually be put[33]. For example, the equilibrium between the not yet full-grown, but yet relatively large, brain of a newborn and the small birth canal in its turn is constrained by an upright posture and provides an example of a trade-off in human evolution. The location of the birth canal in its turn provides an example of an evolutionary coincidence that urges to deal with an, in retrospect, imperfect evolutionary design. These examples illustrate that evolution builds on the past: it is not possible to start a completely new design from scratch, which argues against 'intelligent design'. The most important example of an evolutionary explanation for human disease, however, comes from the mismatch between our slowly adapting genome and the rapidly changing environment, notably our diet.

Evolutionary medicine argues that the chronic degenerative diseases causing most morbidity and mortality in affluent countries occur because of the current mismatch between the rapidly changing conditions of existence and our Palaeolithic genome[34]. These mismatches will persist, notably in the light of our long generation time. The genetic adjustments needed to adapt to the new environment are also unlikely to occur, since the mismatch exerts little selection pressure. That is, they do not cause death before reproductive age, but rather reduce the numbers of years in health at the end of the life cycle[35]. Consequently, evolutionary medicine acknowledges a return to the lifestyle before the onset of the Agricultural Revolution as translated to the culture of the 21th century and as popularised by the expression: 'how to become a 21th century hunter–gatherer'[36]. Skeptics of evolutionary medicine often raise the intuitive criticism that the human ancestor had a very short life expectancy compared with contemporary individuals[35]. Consequently, they argue, there was no selection pressure on longevity or 'healthy ageing', since there were virtually no old people, while the few individuals reaching old (for example, postmenopausal) age provided no evolutionary benefit to younger individuals who were still able to reproduce. The counterargument is multilevelled.

Arguments and counterarguments in evolutionary health promotion

It needs to be emphasised that evolutionary medicine predicts no further increase in life expectancy, but rather a decrease in the numbers in deteriorating health at the end of the life cycle. It has been estimated that the complete elimination of nine leading risk factors in chronic degenerative diseases would increase life expectancy at birth by only 4 years, since these diseases only affect late-life mortality[37]. Second, the increased life expectancy at present originates mostly from the greatly diminished influence of some unfavourable conditions of existence, including (childhood) infections, famine, homicide and tribal wars[34,38] secondary to the high levels of medical sciences and continuing civilisation. Thus, to achieve the average life expectancy of 40 years in a present-day hunter–gatherer society, for every child that does not survive beyond 1 year of age, another should reach the age of 80 years. In fact, about 20% of modern hunter–gatherers reach at least the age of 60 years[39–41]. In other words, the popular argument that very few individuals in these societies live past 50 years[35] is unsupported by ethnographic data. The third, often raised, argument is that due to the higher life expectancy in present-day humans, it is invalid to compare the mortality figures for cancer and degenerative disease of present-day hunter–gatherers (with low life expectancies) with those of Western populations (with a life expectancy of 80 years). However, early biomarkers of degenerative diseases such as obesity, high blood pressure, atherosclerosis and insulin resistance are also less common in younger, age-matched, members of present hunter–gatherer compared with members of affluent societies[9,42], while measurements indicative for 'good health' such as muscular strength and aerobic power are more favourable in the former[43]. Moreover, even the oldest individuals in hunter–gatherer societies appear virtually free from chronic degenerative diseases[44–46]. A fourth counterargument against the assumption that our human ancestors before the Agricultural Revolution died at a young age derives from archaeological records. After the transition from hunting and gathering to farming about 10 Kya, life expectancy dropped from about 40 years (as it is in recently studied hunter–gatherers, but also was among students of the Harvard College Class born in 1880[47]) to about 20 years[48–50]. This seemingly evolutionary disadvantage, secondary to a decrease in nutritional quality, is substantiated by a decrease in general health that has become noticeable from a decrease in final height, while skeletal markers of infection and nutritional stress became more common in archaeological finds[49–52]. These setbacks were eliminated by a net increase in population growth, secondary to an increased productivity per land area that resulted in more energy intake per capita. Life expectancy remained stable throughout the Neolithic until the late 18th century, seldom exceeding 25 years in 'civilised' nations[35]. From this time, improvements in hygiene, food production and manufacturing, energy generation, per capita income, shelter, transportation, clothing and energy intakes substantiated an increase to and beyond the life expectancy that prevailed before the onset of the Agricultural Revolution. Greater energy availability enhanced, for example, the energy requirements of the immune system and for

reproduction, both improving longevity[35,53]. Importantly, it was concluded that medical treatments had little impact on mortality reduction, while public health achievements (sanitation, food and water hygiene, quarantine and immunisations) have critically improved life expectancy. The fifth counterargument is that old people do provide an evolutionary benefit to the younger generations. Male fertility remains largely intact and male provisioning might help in the problem of high female reproductive costs, although the latter is contested[54,55]. The benefits of older females have been put forward in the grandmother hypothesis. This hypothesis, in which the presence of older females within a certain group benefits the reproductive success of their offspring, is supported by studies in human hunter–gatherer[56–62] and primate societies[56,60,63]. Interestingly, the fitness benefits of grandmothering proved insufficient to fully explain the evolution of increased longevity[62], suggesting that other evolutionary benefits, such as grandfathering, might also be involved in the long reproductive and non-reproductive lifespan of *Homo sapiens*. A recent analysis supports such benefits for both older males and females, since the presence of post-reproductive women increased the numbers of newborns by 2·7%, while 18·4% of the infants in a polygamous society in rural Africa were sired by males aged 50 years and above[64]. In support of the statement that 'nothing in biology makes sense except in the light of evolution' we therefore conclude that, unless proven otherwise, the presence of a substantial proportion of older males and postmenopausal females in hunter–gatherer, in contrast to primate societies, should be considered as proof for the evolutionary benefit that these individuals are to their progeny. Finally, we propose that this assumption would only be convincible if these individuals were reasonably fit, thereby supporting the concept of healthy ageing. Hence, healthy ageing seems both supported by ethnographic data and its benefit to hunter–gatherer societies. Other commonly raised arguments against the genome–environment mismatch hypothesis are the potential genetic changes since the Agricultural Revolution, the heterogeneity of ancestral environments and innate human adaptabilty[35]. Counterarguments to these critics have been discussed in great detail elsewhere[35].

In the present review, a multidisciplinary approach is used, including palaeo-environmental reconstruction, comparative anatomy, biogeochemistry, archaeology, anthropology, (patho)physiology and epidemiology, to assess the characteristics of the ecosystem that supported human evolution. Based on this assessment, an approximation is made of the dietary composition that derives from this ecosystem. Finally, the potential benefit of a return to this 'Palaeolithic diet' is discussed and an update is provided for the evidence for the positive health effects of these diets.

Human evolution

Hominins are defined as members of the taxon *Hominini*, which comprises modern *Homo sapiens* and its extinct relatives over the past about 7 million years. The oldest-known hominins (Fig. 1) are *Sahelanthropus tchadensis* from Chad (about 7 Mya[65]) and *Orrorin tugenensis* from Kenya (about 6–5·7 Mya[66]). The next oldest are *Ardipithecus kadabba* (Ethiopia, about 5·8 Mya[67]) *and A. ramidus* (Ethiopia, about 4·4 Mya[68]), *Australopithecus anamensis* (Kenya, about 4·1–3·9 Mya[69]), *Au. afarensis* (Ethiopia, Tanzania and maybe Kenya, 3·6–3·0 Mya[70,71]), *Au. bahrelghazali* (Chad, about 3·5 Mya[72]), *Kenyanthropus platyops* (Kenya, about 3·5 Mya[73]), *Au. garbi* (Ethiopia, about 2·5 Mya[74]) and *Au. africanus* (South Africa, about 2·9–2·0 Mya[75]). From these earliest hominins evolved the genera *Paranthropus* (three known subspecies) and *Homo*. The earliest species that have been designated *Homo* are *Homo rudolfensis*, *Homo habilis* and *Homo erectus sensu lato* –including *H. ergaster* (Eastern Africa, about 2–1.8 Mya): these in turn are the presumed ancestors of Asian *H. erectus*, *H. heidelbergensis* (Africa, Eurasia 0·6–0·3 Mya), *H. neanderthalensis* (Eurasia, 0·4–0·03 Mya) and *H. sapiens* (from about 0·2 Mya onwards)[76–78]. The recently discovered *H. floresiensis* (0·095–0·013 Mya[79]) and the previously unknown hominins from Denisova Cave (about 0·05–0·03 Mya[80]) show that in the recent past several different hominin lines co-existed with modern humans.

Africa is now generally accepted as the ancestral homeland of *Homo sapiens*[77,81,82]. In several subsequent out-of-Africa waves[83], hominins of the genus *Homo* colonised Asia, Australia, Europe and finally the Americas (Fig. 2). Archaic *Homo* species reached as far as the island of Flores in South-East Asia, East China and Southern Europe (Spain). *Homo heidelbergensis* remains were found in Africa, Europe and Eastern Asia, while *Homo neanderthalensis* was restricted to Europe, Western Asia and the Levant. At last, in the later out-of-Africa diaspora starting about 100 Kya, *Homo sapiens* finally reached Australia and the Americas, while probably replacing earlier hominins in Africa, Europe and Asia that had left during the earlier out-of-Africa waves. However, there remains some debate[82,84–86] whether or not the gene pool of archaic hominins contributed to that of modern humans. In the replacement theory, archaic hominins make no contribution to the gene pool of modern man, whereas in the hybridisation theories (either through assimilation or gene flow), newly arriving hominins from the later out-of-Africa wave mixed with archaic predecessors. Current evidence from DNA analyses supports the concept that the gene pool of archaic hominins, notably Neanderthals[87], but also Denisovans[80] contributed to the gene pool of *Homo sapiens*.

The African cradle of humankind is supported by micro-satellite studies[88] that reveal that within populations the genetic variation decreases in the following
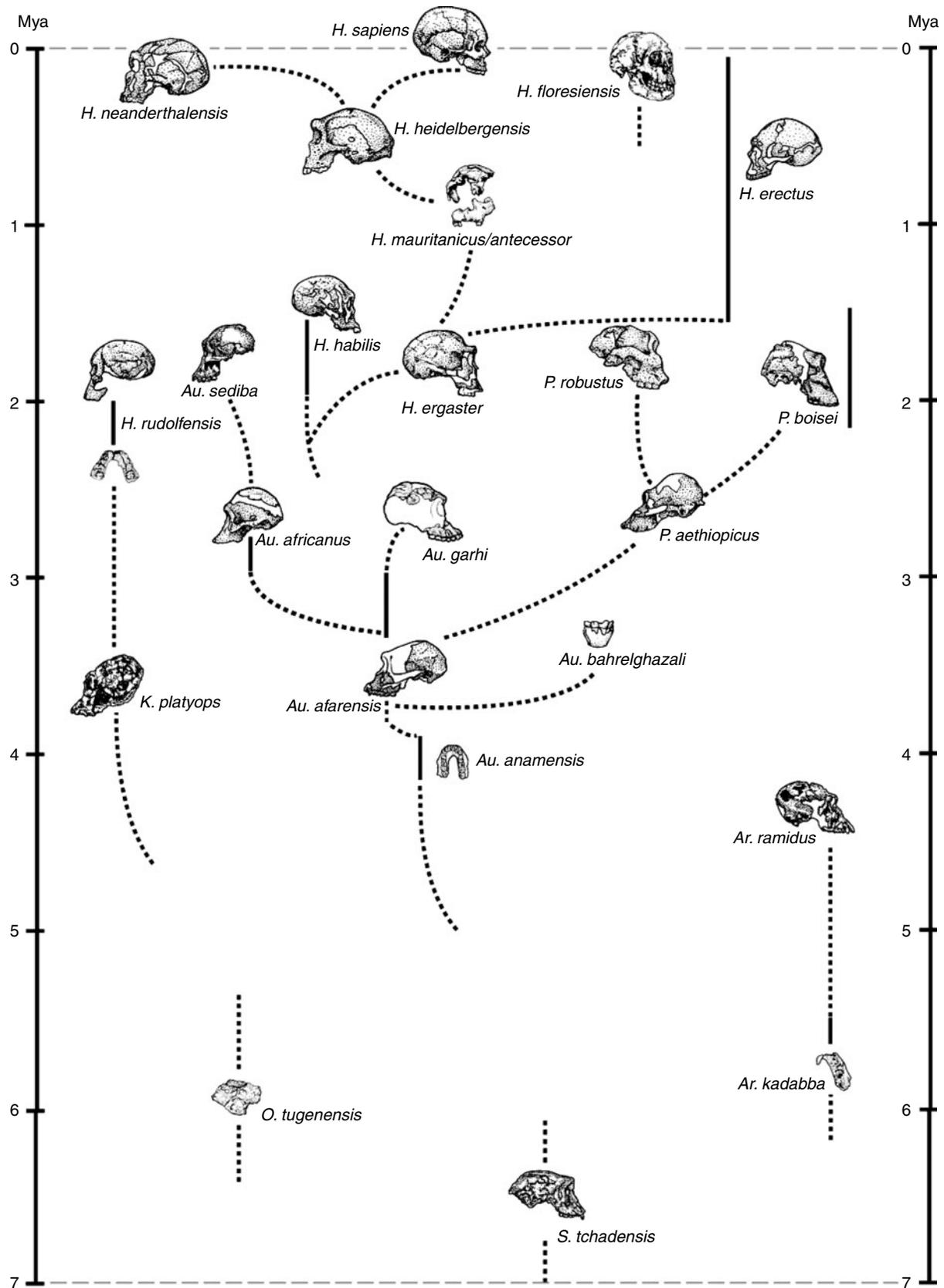
Fig. 1. Scheme of the possible phylogenetic relationships within the family Hominidae. Note that at many time points of evolution, several different hominin species coexisted. Mya, million years ago; *H.*, *Homo*; *Au.*, *Australopithecus*; *K.*, *Kenyanthropus*; *P.*, *Paranthropus*; *Ar.*, *Ardipithecus*; *O.*, *Orrorin*; *S.*, *Sahelanthropus*. © Ian Tattersall, with permission[76].
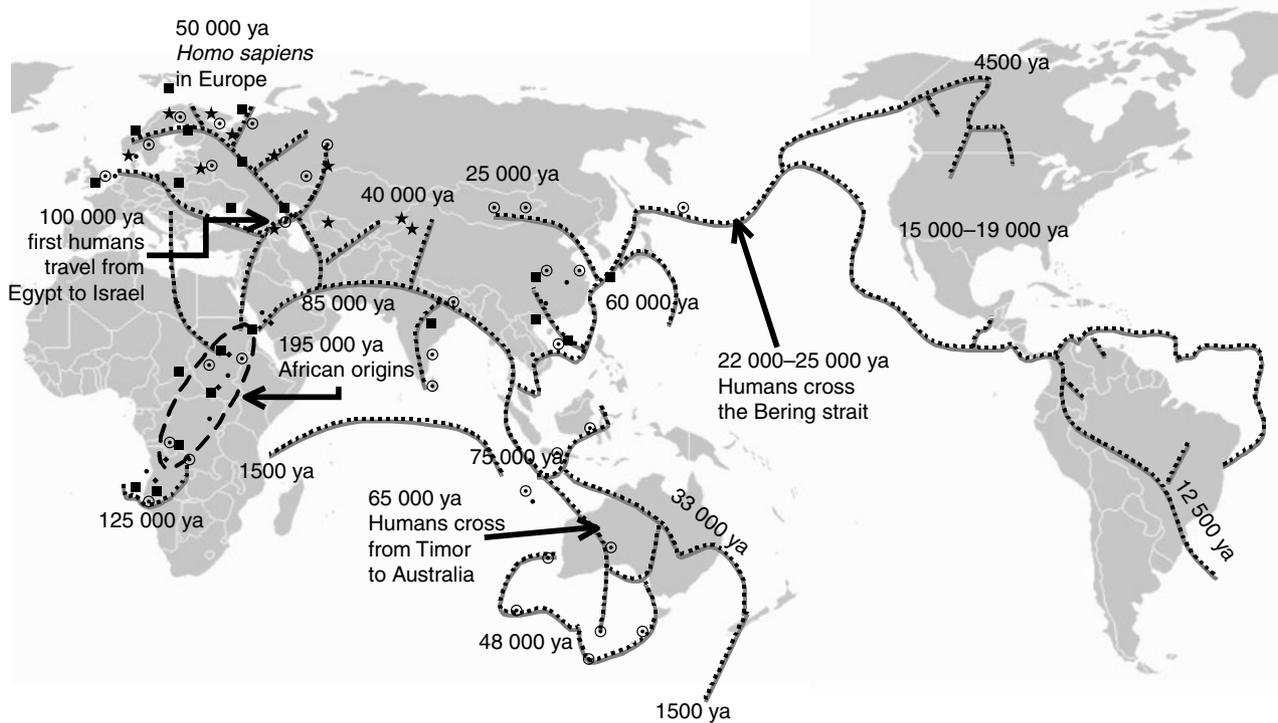
Fig. 2. Coasting out of Africa: following the water in the third out-of-Africa diaspora. Assumed dispersal routes of archaic and anatomically modern man out of Africa and the supportive fossil evidence for hominin presence: (◆), *Australopithecus* sp.; (●), *Homo habilis, erectus, ergaster* or *antecessor*; (■), *H. heidelbergensis*; (★), *H. neanderthalensis*; (◎), *H. sapiens*. ya, Years ago. Source: National Geographic Society 1988, 1997; adapted from www.handprint.com/LS/ANC/disp.html and Oppenheimer[83].

order: sub-Saharan Africa > Eurasia > East Asia > Oceania > America, with the hunter–gatherer Hadzabe of Tanzania separated from the Ju|'hoansi (previously called !Kung) from Botswana by a genetic distance greater than between any other pair of populations[89], which indicates the chronology of continent inhabitation and points to South or East Africa as the cradle of humankind[89,90]. Human evolution was characterised by several large-scale decimations, and it has been estimated that the current world population derives from only 1000 surviving individuals at a certain time point[91]. Such bottlenecks[92], characterised by strong population decrease, or where groups of hominids were separated due to global climate changes, volcanic winters or geographic boundaries as mountain ridges or seas, caused gene flow and genetic drift. As a result, different phenotypic races emerged in different geographic regions[88,92]. However, differences among these populations contribute only 3–5 % to genetic diversity, while within-population differences among individuals account for 93–95 % of genetic variation[93]. In other words, genetically we belong to one species that originally evolved in Africa and that for the great majority genetically still resides in the Palaeolithic era. Most of the current inter-individual genetic differences were already existent when *Homo sapiens* emerged, some 200 Kya[78]. Bipedalism, hairlessness, speech and the ability to store fat differentiate humans from the closest relatives, the primates, but it is the

uniquely large brain, which allowed for symbolic consciousness and pose 'what-if' questions, that finally made humanity[76].

Changing habitat and increasing brain size

It is assumed that during the early stages of human evolution early hominins introduced more animal food into their diets, at the expense of plant foods[94,95]. Subsequent hominins further increased the amount of animal food and consequently the energy density and (micro)nutrient content of their diet, i.e. the dietary quality. While increasing their dietary intake from animal food, early hominins grew taller and increased their brain mass relative to body mass (encephalisation quotient; EQ). Brain mass in primates relates to the number of neurons[96] and global cognition[97], while the human cortex also has more cycles of cell division compared with other primates[98]. During hominin evolution the first significant increase in EQ occurred about 2 Mya (Table 1). From about 2 Mya to 200 Kya the human ancestors tripled their brain size from *Australopithecus* species with an EQ of 1·23–1·92 to an EQ of 1·41–4·26 for the genus *Homo*[99,100]. The increase in brain size and the number of neurons differentiate *Homo* from their closest primate relatives. However, a large brain requires an adaptation or an exaptation to accommodate it, and notably sufficient intake of so-called 'brain-selective nutrients'[100,101] to build and conserve it.

Table 1. The development of brain weight relative to body dimensions*

Species	Brain weight (g)	Brain:body ratio (%)	Relative EQ†
Gorilla gorilla	500	0·3	25
Pongo pygmaeus	400	0·5	32
Pan troglodytes	400	0·9	42
Australopithecus afarensis	455	1·7	41
Australopithecus africanus	450	1·0	44
Paranthropus aethiopicus	405	1·1	44
Paranthropus boisei	510	0·9	46
Paranthropus robustus	520	1·1	50
Homo rudolfensis	750	1·7	59
Homo habilis	600	1·7	57
Homo ergaster	855	–	
Homo erectus	863	1·6	63
Homo heidelbergensis	1200	1·8	74
Homo neanderthalensis	1450	1·9	75
Homo sapiens (Cro-Magnon)	1490	2·4	102
Modern *Homo sapiens*	1360	2·3	100

EQ, encephalisation quotient.
* Adapted from Templeton[85].
† Relative to modern *Homo sapiens*.

Buiding a big brain

Compared with other primates, humans have an extraordinarily large brain[102,103]. To understand the expansion of the human brain during evolution, it is important to comprehend its composition and its biochemistry. Brain tissue has a unique profile of long-chain PUFA (LCP)[99]. Comparison of the brain ethanolamine phosphoglycerols of forty-two studied animal species shows an almost identical LCP pattern, independent of the grade of encephalisation, containing approximately equal proportions of arachidonic acid (AA) and DHA. Consequently, for normal neuronal function, mammalian brain tissue appears to have an invariant structural requirement for both AA and DHA. This shows that both these fatty acids are important building blocks for building a big brain and for encephalisation. The weight of a newborn human brain is about 340 g[104] and it contains about 9 g lipid[105]; the brain of a 10-month-old infant is 850 g and contains 52 g lipid. At 3 years, the brain is 1100 g and contains 130 g lipid. Thus, the major part of the human brain spurt occurs postnatally[106], implying that especially the newborn infant has high demands for AA and DHA.

Toothed whales (brain weight 9000 g) and African elephants (4200 g) have brains much larger than humans, but they have lower cognitive abilities and a lower EQ[107]. These observations substantiate an EQ-centred approach to explain variation in cognition between species. Recent analyses, however, have shown remarkable differences between primate and non-primate brains; a primate brain contains many more neurons than a non-primate brain of similar size[96,108,109] and the absolute number of neurons, rather than body relative to brain ratio (EQ), best predicts cognitive ability[97], although it still needs to be determined whether humans have the largest number of brain neurons among all mammals.

From this new neuron-centred view, there seems to be nothing special about the human compared with the primate brain, except for its size[96], which basically determines both the number of neurons and non-neurons[110,111]. Detailed comparisons of human and primate brains have revealed other differences, such as different levels of gene expression[112–114], secondary to chromosomal rearrangements[115], differences in the relative extent of the neocortical areas[96,116], the distribution of cell types[117] and the decrease of brain structure volumes with increasing age in man in contrast to chimpanzees[118,119]. The best predictor of cognitive ability in humans compared with non-primates, however, still needs to be established, but rather than EQ or brain size, the absolute number of neurons seems a prudent candidate[97,108], since there is no clear relationship between neuron number and the absolute brain size among the different animal species[96,108,109].

In contrast to intuitive belief, growing a large brain and a large skull to accommodate it is less difficult to achieve than it seems at first glance. It was recently shown that different levels of expression of a single gene might have resulted in the markedly different beak shapes and lengths of Darwin's finches. Experimental overexpression of the calmodulin gene in chicken embryos resulted in a significant increase in the length of their beaks[120,121]. These experiments suggest that small and seemingly insignificant changes can have profound implications for the evolution of anatomical size and shape and thereby provide great potential for explaining the origins of phenotypic variation[122], including increases in brain and skull size. Analogously, many mutations in humans are associated with either microcephaly[123] or macrocephaly[124], while the growth of the skull in hydrocephaly shows that the increased skull size is secondary to the increase of its

contents, suggesting that brain rather than skull size is the limiting factor here. The evolution of certain genetic variants associated with brain size has accelerated significantly since the divergence from the chimpanzee some 5–6 Mya. A recent variation that occurred 37 Kya has spread more rapidly through the human population than could be explained by genetic drift[125–128], suggesting that it conferred evolutionary advantage.

The anatomical and metabolic changes encoded in the genome (see 'Comparative anatomy') might have provided hominins with the anatomical and energetic opportunity to, over a period of several million years, steadily increase their brain size, but these mutations *per se* did not fulfil the nutrient requirements for brain expansion[101,129–131]. The underlying small number of mutations should rather have been accompanied, and most probably have been preceded, by increased availability of 'brain-specific nutrients' such as LCP for their ultimate conservation through the process of mutation and selection, which basically underlines both Darwin's concept of the crucial importance of 'the conditions of existence' and the secondary role of mutation. An example may come from current knowledge on the sources of AA and DHA. In humans, both AA and DHA can be synthesised from their precursor essential fatty acids α-linolenic acid (ALA) and linoleic acid (LA) (Fig. 3), respectively. ALA and LA are present in various natural food resources. ALA is predominantly found in plant foods, while LA is mainly found in vegetable oils such as sunflower-seed oil. Both AA and DHA may derive from their synthesis from abundantly consumed
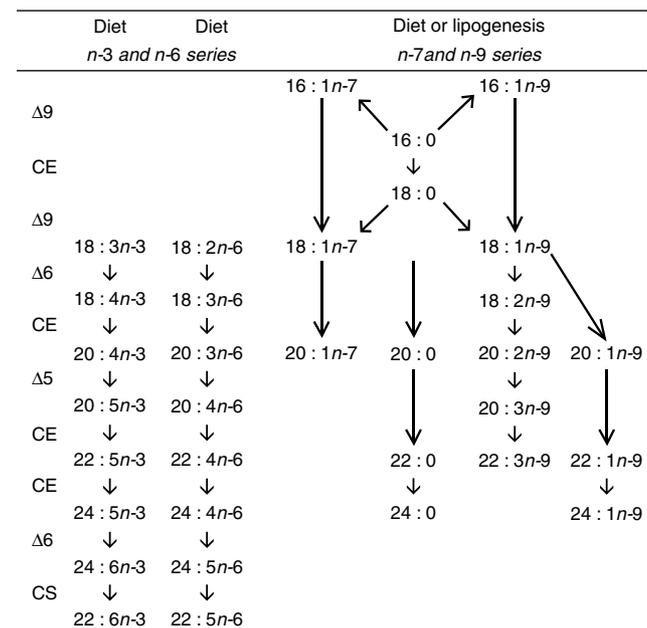

Fig. 3. Metabolism of the parent essential fatty acids and endogenously synthesised fatty acids. Δ9, Δ9-Desaturase; CE, chain elongation; Δ6, Δ6-desaturase; Δ5, Δ5-desaturase; CS, chain shortening through peroxisomal β-oxidation. 18 : 3n-3, α-linolenic acid; 18 : 2n-6, linoleic acid; 18 : 1n-9, oleic acid; 20 : 5n-3, EPA; 20 : 4n-6, arachidonic acid; 20 : 3n-9, mead acid; 22 : 6n-3, DHA.

precursor fatty acids ALA and LA, but in humans and especially neonates, these synthetic activities are insufficient to cope with metabolic demands[132]. Consequently both these LCP, but especially those of the *n*-3 series, need to be present in sufficient quantities in our diet. It is still under debate what dietary resource(s) provided the LCP that enabled us to grow a large brain[101,133–137].

The probability of hunting on the savanna

It has been a longstanding paradigm in palaeoanthropology that early human evolution occurred in a dry and open savanna environment[138–140]. Recent studies from the Afar basin[68,141], although recently contested[142–144], indicated that the habitat of *Ardipithecus ramidus* at about 4·4 Mya was characterised not by savanna but by woodland to grassy woodland conditions. Human characteristics, such as poor water-drinking capacity, excessive urination and transpiration and poor water retention support the argument that we would be poorly adapted savanna dwellers[140].

A second long-reigning paradigm was 'man the hunter', which was the standard version of human origins advocated for many years. Washburn & Lancaster[94] referred at most to our most recent antecessors, *Homo sapiens* and possibly *H. neanderthalensis*, when they claimed that our intellect, interests, emotions and basic social life are evolutionary products of the hunting adaptation. The strongest argument against this hunting paradigm comes from combined studies of past and present-day hunter–gatherer societies indicating that the role of hunting is exaggerated, notably (around the campfire) in hunter–gatherer societies, since the majority of the dietary protein is in reality obtained by women gathering nuts, tubers and small animals[145–147]. Cordain *et al.*[148] showed that only 25–35 % of energy (en%) of subsistence in worldwide hunter–gatherer communities is derived from hunting, while the remainder is derived from both plant and fished food. Thus, while meat from large game may have been the most valued food, it is highly unlikely that it was the most valuable (nutritionally important) food resource from a dietary perspective[41,149]. At present, the niche of early hominins and thus the environment of human evolution, and, most importantly for the present review, the nutritional composition of the early human diet are still heavily debated[150].

Reconstruction of our ancient diet

In the next sections we will discuss various views on (changes in) the hominin ecological niche that over time shaped the human genome to what it currently is.

Palaeo-environments

Sahelanthropus, Orrorin *and* Ardipithecus. In the late Miocene (up to 5·3 Mya), the African continent became

more arid, which resulted in fragmentation of the (sub)tro-
pical forests and the appearance of more open environ-
ments[151]. The widespread dispersal of some of the
earliest hominins such as *Sahelanthropus*[65,152], and *Aus-
tralopithecus bahrelghazali* from Chad, might be explained
by the presence of the relatively low-lying humid East–
West corridor constituted by the remnants of the Creta-
ceous Central African and Sudan Rifts between Western
and Eastern Africa[153,154]. The reconstructed environment
of *Sahelanthropus* (about 7 Mya) suggests a mosaic of
gallery forest at the edge of a deep, well-oxygenated lake,
swampy and vegetated areas, and extensive grasslands[155].
Since there is no indication of carnivore modification or
fluvial transport of its bones, *Sahelanthropus chadensis*
probably lived in this area[156]. The palaeo-environment
of *Orrorin* (about 6 Mya) was probably characterised by
open woodland, with dense stands of trees in the vicinity
and possibly fringing the lake margin and/or streams
that drained into the lake[157]. *Ardipithecus kadabba*
(5·6 Mya) remains are associated with wet and closed,
grassy woodland and forest habitats around lake or river
margins[158]. *Ardipithicus ramidus* (4·4 Mya) lived in or
near a groundwater-supported grassy woodland to
forest[159]. Additionally, the abundance of fossilised shal-
low-water aquatic species such as catfish, barbus, cichlidae
and crocodiles additionally suggests an episodically present
flood-plain environment[159].

Early Australopithecus *species.* *Australopithecus ana-
mensis* appeared at about 4·2 Mya and its environment
was characterised by a mix of wetlands and terrestrial
environments, such as lacustrine and fluvial floodplains,
woodland and gallery forest[156,160–163]. The later *Australo-
pithecus afarensis* survived in a variety of habitats[164], but
apparently thrived better in the more wooded and humid
conditions in the Afar basin than in the relatively dry Lae-
toli area[165]. Stewart[156] pointed out that in Africa the only
environmental constant in hominin sites throughout the
period from 3·4 to 2·9 Mya was a wetlands habitat, charac-
terised by aquatic herbaceous vegetations around lakes
and rivers, with large populations of wetland fauna such
as reduncines and hippopotami. Hence, these wetlands
could have been refuge for early hominins throughout an
extensive period of human evolution.

Paranthropus, late Australopithecus *and* Homo *species.*
About 2·9–2·5 Mya tectonic and global climatic changes
made Africa cooler and drier[166–169]. The great wet forests
of middle Africa retreated and made place for more
savanna grasslands. It is around this time, from about 2·6
Mya onwards, that the first traces of the new hominin
genus *Homo* appeared in the archaeological record[140]. It
has been suggested that alternating wet and dry periods
after 2·7 Mya could have isolated hominin populations
around sources of potable water, while forcing them to
the extremes of their conditions of existence[156] and thus
facilitating specialisation[170], either by adaptation or exap-
tation. Compared with *Australopithecus*, *Paranthropus*

existed in slightly more open habitats, including wetlands
and grasslands, but also in woodland and bushland
areas. The habitats of *Homo* species seem similar to
those utilised by *Paranthropus* species[161], but *Homo*
remains at Olduvai Gorge and Koobi Fora are associated
with well-vegetated swamps, lakes and river margins,
and (semi-) aquatic fauna[171,172]. Only the later *Homo*
species are also found in assemblages that indicate extre-
mely arid and open landscapes such as savanna[161].
Also, it has been suggested that hominins and other
(aquatic) species dispersed throughout Africa along water
systems, while even the last out-of-Africa migration might
have occurred via the 'green Sahara' that existed during
the last interglacial (125 Kya)[156,173].

In conclusion, the palaeo-environmental evidence
suggests that early hominins lived in the proximity of
water. However, it is frequently argued that bones are pre-
ferentially preserved in lake, river or fluvial sediments,
making their recovery in any other than an aquatic setting
unlikely[174]. Alternatively, hominin remains may have
been relocated to the water by carnivores[162], including
crocodiles. Nevertheless, the combined evidence strongly
suggests that early hominins frequented the land–water
ecosystem and thus lived there. Joordens *et al.*[154] pro-
posed, based on comparison with other terrestrial omni-
vores, that the default assumption should be that
hominins living in freshwater or marine coastal ecosystems
with catchable aquatic resources could have consumed
these aquatic resources[154,175].

Comparative anatomy

The diet of our closest relatives. Field studies on our clo-
sest relatives, the extant apes, show that their preferred
food items are primarily fruits and/or leaves and stems
from terrestrial forests. Lowland gorillas, for example,
derive 57 % of their metabolisable energy (en%) from
SCFA derived from colonic fermentation of fibre, 2·5 en%
from fat, 24 en% from protein and 16 en% from carbo-
hydrate[176]. 'Fallback foods' are consumed when preferred
foods are unavailable[177] and are generally composed of
herbaceous plants and high-fibre fruits from aquatic and
terrestrial environments[156]. Like our closest rela-
tives[156,178–181], hominins might have used foods from
the aquatic environment as fallback foods, while, although
speculative, this niche might eventually have proven
favourable with regard to subsequent encephalisation.

Teeth morphology and dental microwear. Comparative
anatomy (Fig. 4) of the hominins might confer some infor-
mation about these fallback and preferred foods of our
ancestors. Dental studies of *Sahelanthropus* (Fig. 1) describe
that the teeth had thick enamel[65], similar to orangutans,
suggesting that it could eat hard and tough foods[182], such
as available from the lakeshore vegetation[156]. *Ardipithecus
ramidus*, however, had thin molar enamel and smaller teeth
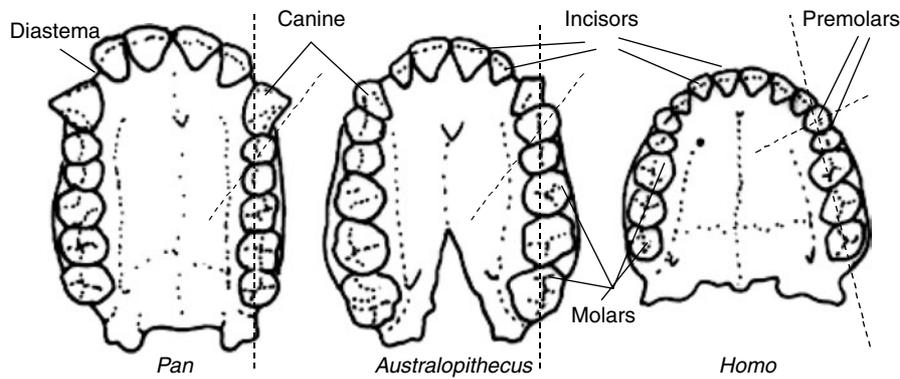compared with later hominins. This dental morphology is

Fig. 4. Lower jaw of a chimpanzee (*Pan troglodytes*), *Australopithecus africanus* and *Homo sapiens*. Note the somewhat human-like shape of the teeth, but ape-like axis in the jaw of *Australopithecus*. © Australian Museum.

consistent with a partially terrestrial, omnivorous/frugivorous niche[183]. Studies on cranio-dental changes such as tooth size, tooth shape, enamel structure and jaw biomechanics indicate that *Australopithecus* and *Paranthropus* had prominent jaws, relatively flat molar teeth, small incisors and thick enamel, suitable for breaking and crushing small hard, brittle foods such as fruits, nuts and underground storage organs[150], but unsuitable for breaking down tough plant foods or tearing meat. Together, this would allow early hominins to eat both hard and soft, abrasive and non-abrasive foods, which suits well for life in a variety of habitats[184].

In addition to studies on teeth morphology, microwear studies are essential. Dental microwear studies analyse tooth-wear, showing evidence for where teeth were actually used for and thus what an animal in reality ate[185]. While adaptive morphology will give important clues about what a species was capable of eating, microwear studies reflect what an animal ate during some point in its lifetime. In these studies, 'complexity' is used as an indicator for hard and brittle items, while 'anisotrophy' is an indicator for tough foods[186].

Most primates show either low complexity combined with high anisotrophy, indicative of consumption of tough foods such as leaves, stems and meat, or high complexity with low anisotrophy associated with hard-brittle foods, such as nuts and seeds[150,187]. *Ardipithecus ramidus*'s preference for an omnivorous/frugivorous diet was confirmed by microwear studies [183], suggesting a diet of fleshy fruits and soft young leaves[150]. Conversely, microwear textures of *Australopithicus afarensis* and *anamensis* show striations rather than pits (low complexity and low anisotrophy), i.e. patterns similar to those of grass-eating and folivorous monkeys instead of the predicted diets predominated by hard and brittle foods[187,188]. *Australopithecus africanus* showed microwear patterns that were more anisotropic, suggestive for consumption of tough leaves, grasses and stems[189]. *Paranthropus robustus*, also know as the 'Nutcracker Man', has enormous, flat, thickly enamelled teeth that are combined with a robust cranium, mandible and powerful chewing muscles, suggestive of breaking

hard and brittle foods[186]. After microwear analysis of its teeth, however, Ungar *et al.*[150,186] showed that *P. robustus* had low complexity and anisotrophy; thus *Paranthropus* might only have consumed mechanically challenging items as fallback foods when preferred foods were unavailable. Similarly, microwear studies support the notion that the diet of *P. boisei* contained large quantities of low-quality vegetation, rather than hard objects[190].

Generally, microwear studies confirm earlier dental topography studies[191], which revealed the incorporation of more fracture-resistant foods, i.e. tougher foods as leaves, woody plants, underground storage organs and animal tissues, in the diet of *Australopithecus africanus* compared with *Australopithecus afarensis* and for *P. robustus* compared with *Australopithecus africanus*[187]. Dental topographic analysis suggested that successive *Homo* species emphasised more on tougher and elastic foods, perhaps including meat[191]. The latter suggestion is in line with the optimal foraging theory, which states that humans prefer foods with high energy density over those with low energy density[192,193].

Microwear studies confirmed that early *Homo*, such as *H. erectus* and *H. habilis*, did not prefer fracture-resistant foods, although some *H. erectus* specimens showed more small pits than *H. habilis* members, suggesting that none of the early *Homo* specialised on very hard-brittle or tough foods, but rather could consume a varied diet[194]. This does not imply that early *Homo* had very broad diets, but rather that early *Homo* was adapted to subsist in a range of different environments, providing evolutionary advantage in the climatic fluctuations and the mosaic of habitats in Africa during the late Pliocene[195]. A study on dental microwear of 300 000-year-old *H. heidelbergensis* teeth from Sima de los Huesos in Spain showed striation patterns that indicated a highly abrasive diet, with substantial dependence on poorly processed plant foods such as roots, stems and seeds[196]. Lalueza *et al.*[197] compared teeth of very recent hunter–gatherers (Inuit, Fuegians, Bushmen, Aborigines, Andamanese, Indians, Veddahs, Tasmanians, Laps and Hindus) with Middle and Upper Pleistocene fossils. Their results indicate that some

Neanderthals resemble carnivorous groups, while archaic *H. sapiens* show a more abrasive diet, partly dependent on vegetable materials.

Overall, remarkably few studies have related microwear patterns to hominin diet for the period between 1·5 Mya and 50 Kya (PS Ungar, personal communication). Studies in more recent hominins, such as from an Upper Palaeolithic site in the Levant (22 500–23 500 before present; BP) showed a high frequency of long narrow scratches and few small pits, suggesting a tough abrasive diet of aquatic foods rather than a diet with hard foods that needed compressive force[198,199]. A study of subsequent local hunter–gatherer (12 500–10 250 BP) and farmers (10 250–7500 BP) living in the Levant showed larger dental pits and wider scratches among the farmers compared with the hunter–gatherers, suggesting that the implementation of agriculture led to a more fracture-resistant diet[199].

Gut morphology, energy expenditure and muscularity. Gut morphology studies[200,201] support the introduction of animal foods, at the expense of vegetable foods, in the diet of early *Homo*. The dominance of the colon (>45 %) in apes indicates adaptation to a diet rich in bulky plant material, such as plant fibre and woody seeds. In contrast, the proportion of the human gut dominated by the small intestine (>56 %) suggests adaptation to a diet that is highly digestible, indicating a closer structural analogy with carnivores than to folivorous or frugivorous mammals. Importantly, the shorter gut in *Homo*, as compared with primates, might have had some other advantage. During the evolution from *Ardipithecus* and *Australopithecines* to early *Homo*, the improvement of dietary quality coincided with an increase in height, the size of our brain and its metabolic activity. However, an increase in body size coincides with increased daily energy demands, notably during gestation[202] and lactation[203]. It was, for example, calculated that daily energy expenditure for a *Homo erectus* female is about 66 % higher compared with an *Australopithicine* female, while being almost 100 % higher in a lactating *Homo erectus* female compared with a non-lactating, non-pregnant *Australopithicine*[204]. These high energy demands might have been met by increased female fat reserves[205,206], such as demonstrated by the presence of female steatopygia in some traditional human populations, such as the Khoisan of southern Africa.

Apart from the extra energy need for reproduction and increasing height, the human brain of a modern adult uses 20–25 en% of the total RMR, while this value is 8–9 en% for a primate[207]. It has been postulated that the extra energy needs did not derive from a general increase in RMR, but partly from a concomitant reduction of the gastrointestinal tract[208] and a reduction in muscularity[207]. These features are combined in the 'expensive tissue hypothesis' of Aiello & Wheeler[208] that points at the observation that the mass of the human gastrointestinal tract is only 60 % of that

expected for a similar-sized primate and that humans are relatively under-muscled compared with other primates[207]. Interestingly, the negative relationship between gut and brain size across anthropoid primates[209] was confirmed in a study with highly encephalised fish[210], whereas it became falsified in mammals[102]. Unfortunately, however, the latter study did not include marine mammals[102], while it is questionable whether with respect to brain development humans adhere to general mammalian rules[103]. Other adaptations might have saved energy expenditure as well. The short human inter-birth interval[204], compared with inter-birth intervals of 4–8 years in the gorilla, chimpanzee and orangutang[211], reduces the most expensive part of reproduction, i.e. lactation[212,213], while the shift from quadrupedal to bipedal locomotion also reduced daily energy expenditure[214]. Together, all of these adaptations allow for an increased daily energy expenditure, including the reallocation of energy to the metabolically active brain, but were only possible after *Homo* included more energy-dense foods into its diet. Brain mass in primates is positively related to dietary quality and inversely to body weight[207]. Generally, a shift towards more energy-dense foods includes a shift from primarily carbohydrate-rich vegetables to fat- and protein-rich animal foods. However, it has also been suggested that a shift from the complex carbohydrates in leafy vegetables towards underground storage organs, such as tubers, might have provided easy-to-digest carbohydrates[200,215] to support a larger hominin body mass.

A phenotypic specialisation on non-preferred resources, without compromising the ability to use preferred resources, is also known as Liem's paradox[216]. This paradox is important to keep in mind during attempts to reconstruct the preferred diet from the available evidence. Interestingly, Stewart[156] recently noted that in the case of *Paranthropus*'s phenotype with regard to its fallback food, there is just as much evidence to talk about a 'Nutcracker Man' as there is to talk about a 'Shellcracker Man'. Thus, a phenotypic characterisation needs support from other studies to confirm adaptation to preferred rather than fallback foods. In other words, it should be noted that not all physical characteristics might be taken as unambiguous proof for the preferred diet of early hominins (Liem's paradox). The increasing absolute body size and brain size and the reduction in gut size, however, do indicate a shift from low- to high-dietary-quality foods for more recent hominins.

Biogeochemistry

Evidence from the strontium:calcium ratio. Based upon the principle 'you are what you eat'[217] several techniques have been developed to study early hominin diets[218]. Trace element studies first started with Sr:Ca ratios, which decrease as an animal moves up the food chain, secondary to the biological discrimination against strontium[219]. A first study[220] suggested that *Paranthropus robustus* (Fig. 1)

had lower Sr:Ca ratios compared with contemporaneous *Papio* (baboon) and *Procavia* (hyrax), suggesting that *Paranthropus* was not an exclusive herbivore. However, a subsequent study showed that two *Homo* specimens had a higher Sr:Ca ratio than *Paranthropus*[221]. Since *Homo* had been assumed to consume more animal foods than *Paranthropus* and thus have a lower Sr:Ca ratio, these higher Sr:Ca ratios needed explanation. For example, consumption of specific foods with a high Sr:Ca ratio might have increased the ratio in *Homo* compared with *Paranthropus*. Foods in the area of research with elevated Sr:Ca ratios were mainly geophytes. These are notably perennial plants with underground food storage organs, such as roots, bulbs, tubers, corms and rhizomes. Consequently, this discrepancy has been attributed to the consumption of underground storage organs by early *Homo*[215,221]. The use of these underground storage organs may have become necessary from the start of a first period of aridity about 2·8 Mya, when forest was replaced by drier woodlands, forcing hominins to search for available resources around water margins[222].

Although promising at first, the use of Sr:Ca ratios was found to suffer from several limitations. For example, a problem is that hominin Sr:Ca ratios in fossilised bones alter with time[223], a process that is known as diagenesis. This problem can be circumvented by the use of tooth enamel, which is less susceptible to diagenesis than bone[223]. Subsequent studies showed that *Australopithecus africanus* had a higher Sr:Ca ratio in its enamel compared with *Paranthropus* and contemporaneous browsers, grazers, baboons and carnivores[224–226]. The interpretation of these findings, however, remains a subject of debate. For example, a group of brown seaweeds and some aquatic plants discriminate against Ca, resulting in an increase in the Sr:Ca ratio, and likewise in the Sr:Ca ratio of the fish feeding on them[226,227]. Similarly, leaves from trees have lower Sr:Ca ratios compared with grasses, which becomes subsequently reflected in the Sr:Ca ratios of browsers and grazers, respectively[228]. Thus, it might be argued that the relatively high Sr:Ca ratios in *Australopithicines* and *Homo* reflect their consumption of aquatic resources or of animals such as insects or other small animals feeding on grasses. For now, the exploration of especially Sr:Ca ratios as a dietary proxy method has largely been stalled[229].

Evidence from the barium:calcium ratio. Another trace element ratio that might provide information on the composition of the early hominin diet is the Ba:Ca ratio, particularly when used in a multiple-element analysis with Sr:Ca and Sr:Ba ratios[224,230]. Combined Ba:Ca and Sr:Ba ratios clearly differentiate grazers from browsers and carnivores in both modern and fossil mammals[230]. Hominins have a lower Ba:Ca and higher Sr:Ba ratio compared with grazers and browsers. *Paranthropus* shows considerable similarity for both ratios with both carnivores and *Papionins* (baboons), while *Australopithecus*

shows an even higher Sr:Ba ratio. The unusual combination of a high Sr:Ca and low Ba:Ca ratio in hominins (and baboons) has been further observed for some animals such as warthogs and mole rats that make extensive use of underground resources[231]. Finally, the high Sr:Ba ratio, as observed in *Australopithecus*, might derive from the consumption of grass seeds, which have Sr:Ba ratios three to four times higher than grass straw, while consumption of these grasses is also consistent with stable-isotope evidence (see below) showing that *Australopithecus* derived a substantial part of its diet from C_4 resources. Although not included in any study so far, Sr:Ba ratios in aquatic foods might add to the understanding of the unusual combination of low Ba:Ca and high Sr:Ba ratios.

Evidence from the ^{13}C:^{12}C ratio. Fractionation studies of carbon isotopes differentiate between different routes of photosynthesis. While most tropical African woody plants from forests (like fruits, leaves, trees, roots, bushes, shrubs and forbs) use the C_3 photosynthetic pathway, some South African[232] and East African[233] grasses and sedges use the C_4 photosynthetic pathway. C_4 plants such as sedges (for example, *Cyperus papyrus*) typically occur in a mosaic of extensive seasonal and perennial shallow freshwater wetlands that can also be found in savanna and 'bushvelds' receiving summer rainfall[234]. The real impact of C_4 plants occurred with their spread into Eastern and Southern Africa during the Pliocene[235]. Tissues of plants that utilise the C_4 pathway have a relatively high content of the stable carbon isotope ^{13}C (about 1·1% of carbon), since C_3 plants discriminate more strongly against $^{13}CO_2$ during photosynthesis. As a result C_3 and C_4 plants have quite different ^{13}C:^{12}C ratios in their tissues, as have the herbivorous animals that feed on these plants[228,232] and the carnivores that prey on these herbivores[193,206]. Differences are expressed as $\delta^{13}C$ values ($\delta^{13}C = (({}^{13}C{:}^{12}C)_{sample} - ({}^{13}C{:}^{12}C)_{standard}) \times 1000/({}^{13}C{:}^{12}C)_{standard}$) in parts per thousand (‰) relative to the ^{13}C:^{12}C ratio in the reference standard (named Pee Dee Belemnite; PDB), i.e. the carbonate obtained from the fossil of a marine Cretaceous cephalopod (*Belemnitella americana*) which is highly enriched in ^{13}C (^{13}C:^{12}C ratio = 0·0112372). Consequently, most animals have negative $\delta^{13}C$ values (Fig. 5). The $\delta^{13}C$ ranges from -35 to -21‰ (mean -26‰) in C_3 plants and from -14 to -10‰ (mean -12‰) in C_4 plants[225]. Studies that attempted to reconstruct mammalian food webs indicated that carbon is slightly enriched (1–2‰) with each trophic step[193,206]. To facilitate comparison of current and historical animals, $\delta^{13}C$ analyses are predominantly performed in hard tissues, such as bone collagen and enamel (notably apatite), since these constitute the majority of the fossil record. It was shown that enamel mineral is enriched by about 13‰ compared with dietary $\delta^{13}C$[238], while collagen is enriched by about 5‰ compared with dietary $\delta^{13}C$[225]. Collagen from terrestrial mammal C_3 herbivores shows a value of -21‰ (range -22 to -14‰), while in collagen of
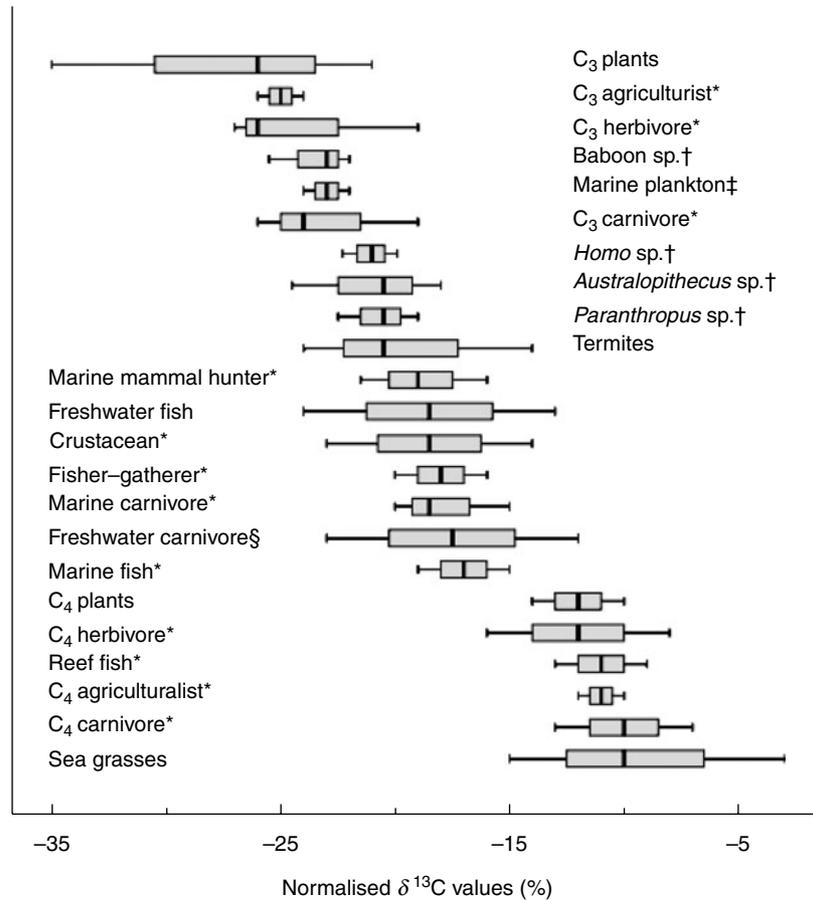
Fig. 5. Normalised collagen $\delta^{13}C$ values (mean and range; in per thousand (‰)) in plankton, crustaceans, sea grasses, C_3 and C_4 plants; of marine crustaceans, fish and freshwater fish and their respective carnivores; of terrestrial C_3 and C_4 herbivores and their carnivores; and of human groups in historic and prehistoric times. * Corrected[229] for collagen (-5‰). † Corrected[238] for enamel (-13‰). ‡ Arbitrary range of $\pm$ 1‰ due to a lack of data. § As predicted from other predator–prey relationships and after correction[239] for tropic level ($+1$‰). Adapted from Ambrose & Deniro[228], Sponheimer *et al.*[231,247], Peters & Vogel[234], Lee-Thorp *et al.*[237,244], Kelly[239], Schoeninger & Deniro[240], Mbabazi *et al.*[241], Schoeninger *et al.*[242,246], Sponheimer & Lee-Thorp[243,248,249] and van der Merwe *et al.*[245].

C_4 herbivores this value is -7‰ (-12 to -6‰). Their respective carnivores show collagen values of -19‰ (-21 to -14‰) and -5‰ (-8 to -2‰), respectively[225]. Marine phytoplankton, which uses the C_3 pathway, shows an average value of -22‰[239]. Collagen of marine fish shows a range from -15‰ to -10‰, while the values in collagen of reef fish range from -8 to -4‰[240]. Marine carnivores have, similar to terrestrial carnivores, intermediate values of -14‰, ranging from -10‰ in collagen of sea otters to -15‰ in collagen of the common dolphin[239,240]. Thus, also the $\delta^{13}C$ values of marine carnivores compare well with those of their prey. Finally, $\delta^{13}C$ values for the muscle of freshwater fish species range from -24 to -13‰, but considerable variation may exist between different lakes[241]. Unfortunately, no $\delta^{13}C$ data are available for terrestrial piscivorous carnivores, but the consistency of the other data suggests that these might be between -24 and -13‰, i.e. comparable with their prey (Fig. 5).

Consistent with the data above, Schoeninger *et al.*[242] showed that European agriculturalists consuming C_3 grasses had much lower $\delta^{13}C$ values in bone collagen (-21 to -19‰) compared with Mesoamerican agriculturalist consuming C_4 maize (-7 to -5‰), while North American and European fisher–gatherers had intermediate values (-15 to -11‰). However, bone collagen proved less reliable to study early hominin diets. For that purpose the $^{13}C:^{12}C$ ratio is preferably measured in tooth enamel. To compare collagen $\delta^{13}C$ values with enamel $\delta^{13}C$ values, an additional (8‰) correction has to be made. Similar to the clear distinctions between bone collagen of modern grazers, browsers and their carnivores, tooth enamel data from fossilised fauna from South Africa showed similar differences for Plio-Pleistocene C_3 feeders (-11.5‰) and C_4 feeders (-0.5‰), with *Australopithecus*, *Paranthropus* and *Homo* taking intermediate positions (-10 to -4‰)[231,234,237,243], which compared well with the values for contemporaneous felids (-10 to -0.5‰)[231,237,244] (Fig. 5). These results clearly demonstrated that a significant proportion of the diets of the early hominins from Swartkrans, Makapansgat and Sterkfontein derived from C_4 resources. Using these data it

was calculated that South African *Paranthropus* derived 14–47 % of its diet from C_4 sources, compared with 5–64 % in *Australopithecus* and 20–35 % in *Homo*[245].

A second study in Olduvai showed that the Tanzanian *Paranthropus boisei* derived 77–81 % and *Homo* 23–49 % from its diet from C_4 resources[245]. The low nutritional value of grasses, and microwear studies (see above) render it unlikely that humans were directly eating grass[231,243]. Analogously, the sizeable carnivory of C_4-consuming mammals (such as cane rats, hyraxes or juvenile bovis) was argued to be practically impossible and thus unable to leave a strong C_4 signature[243]. However, at about 1·8 Mya, there were extensive wetlands in the Olduvai area, where a river from the Ngorongoro mountains entered the area, while at 1·5 Mya the Peninj river produced wetlands near Lake Natron[156]. Some researchers investigated the edible plants in a present-day wetland (Okavango Delta) and found that the rhizomes and culms of three species of C_4 sedges were edible, the most common one of which is *Cyperus papyrus*[245]. However, it seems unlikely that the C_4 signature in all early hominins derived from the consumption of papyrus. It was recently suggested that *P. robustus* and especially *P. boisei* had a diet of primarily C_4 resources, most probably grasses or sedges, from savanna or wetland environments, respectively[190]. Theoretically, a good source of C_4 foods would be a seasonal freshwater wetland with floodplains and perennial marshlands, with an abundance of easy accessible aquatic foods, large aggregations of nesting birds and calving ungulates[234]. Consumption of termites could have contributed to the high C_4 signature observed in hominin fossils (Fig. 5), but it seems unlikely that termites could explain values as high as 50 % of the diet from C_4[243]. Finally, an enamel C_4 signature of −10 to −4 ‰ in hominins, which translates into a soft tissue signature of −23 to −17 ‰ and a collagen signature of −18 to −12 ‰ (see above), might also derive from the consumption of small freshwater aquatic animals or fish, since they compare well with the $\delta^{13}C$ values of −24 to −13 ‰ for freshwater fish[241] and −18 to −9 ‰ in collagen of crustaceans and anthropods[240], respectively. Moreover, $\delta^{13}C$ values for hominins are similar to those reported for marine mammal hunters, freshwater fish, crustaceans, fisher–gatherers, marine and freshwater carnivores and marine fish (Fig. 5).

In agreement with the variability selection hypothesis of Potts[170], which states that large disparities in environmental conditions were responsible for important episodes of adaptive evolution, the wide range in $\delta^{13}C$ values in particularly *Australopithecus* suggests that early hominins utilised a wide range of dietary sources, including C_4 resources. This contrasts with chimpanzees, which, even in the most arid and open areas of their range, are known to consume negligible amounts of C_4 resources, despite their local abundance. Consequently, chimpanzees show very little variability in their $\delta^{13}C$ carbon signature[246,247]. This underscores that even if contemporaneous chimpanzees and early hominins inhabited similar habitats, hominins had broadened their dietary range sufficiently to survive in habitats uninhabitable by chimpanzees. The latter assumption provides an interesting perspective on the recent data, which suggest that C_4 foods were absent in the diet of *Ardipithecus ramidus* at 4·4 Mya. Consequently, it has been proposed that the origins of the introduction of C_4 foods into the hominin diet lie in the period between 3 and 4 Mya[229].

Limited evidence from the $^{15}N:^{14}N$ *ratio.* Another stable-isotope ratio that has received considerable attention is the N isotope ($^{15}N:^{14}N$) ratio. A number of food web studies have shown that each step in the food chain is accompanied by 3–4 ‰ enrichment in $\delta^{15}N$[228,239] and that $\delta^{15}N$ can therefore be useful as a trophic level indicator. Additionally, animals feeding in marine ecosystems have higher values compared with animals feeding on terrestrial resources[242]. For example, North American and European fisher–gatherers and North American marine mammal hunters and salmon fishers had much higher $\delta^{15}N$ values (+13 to +20 ‰) compared with agriculturalists (+6 to +12 ‰). Analyses of phyto- and zooplankton suggest that freshwater organisms have $\delta^{15}N$ values intermediate to terrestrial and marine organisms[242]. $\delta^{15}N$ values are routinely measured in bone collagen, but it has been shown that good-quality collagen (preserving the original $\delta^{15}N$ value) can, and only under favourable conditions, survive up to a maximum of 200 000 years[225]. This limits $\delta^{15}N$ isotopic studies to Late Pleistocene hominins (see below), but with improved technology, future studies using collagen extracted from tooth enamel may expand their application to early hominins[238].

Limited evidence from the $^{18}O:^{16}O$ *ratio.* A final isotope that might provide information about an animal's diet and thermophysiological adaptations is the oxygen isotope ratio ($^{18}O:^{16}O$). More energy is needed to vaporise $H_2^{18}O$ than $H_2^{16}O$. When ocean water evaporates and during evapotranspiration, i.e. the sum of evaporation and plant transpiration from the earth's land surface to the atmosphere, more of the lighter isotope evaporates as $H_2^{16}O$. The ensuing ^{18}O enrichment of transpiring leaves results in ^{18}O enrichment in typical browsers such as kudu and giraffe who rely less on free drinking water and derive most of their water from the consumption of the ^{18}O-enriched plant water. As the ^{16}O-enriched water vapour in clouds moves inland, some of it condenses as rain, during which more of the heavier isotope (as $H_2^{18}O$) rains out, making the $\delta^{18}O$ of coastal rain only slightly less enriched than the original vaporated ocean water, while the $\delta^{18}O$ of the remaining water vapour that eventually comes down is highly negative (i.e. more ^{18}O depleted). Consequently, river water from rain and melting ice is more $\delta^{18}O$ negative than seawater. Roots derive their water from meteoric or underground water that is thus relatively depleted from ^{18}O and so become animals that are consuming these roots[248,249]. Browsers of leaves

undergoing evapotranspiration and consumers of roots may thus be expected to have high and low $\delta^{18}O$ values, respectively.

Australopiths showed lower $\delta^{18}O$ values compared with *Paranthropus*, but the meaning of this difference remains uncertain. However, one might argue that *Australopithecus* preferred less arid conditions compared with *Paranthropus* or was more dependent on seasonal drinking water[231]. Low $\delta^{18}O$ was additionally found in primates and suids, which might be linked to frugivory, although this is not supported by the higher ^{18}O values found in *Ardipithecus ramidus* compared with *Australopithicines*[68,229]. Taken together, the use of $\delta^{18}O$ for exploration of ancient human diets is still in its infancy, but might, especially in combination with other isotope ratios, become more appreciated in the future.

Isotopic data for more recent hominins. It would be of high interest to explore the hominin diet during the last spurt of encephalisation between 1·9 Mya to 100 Kya, when brain size tripled in size to volumes between 1200 and 1490 cm^3 for *Homo erectus*, *H. heidelbergensis*, *H. neanderthalensis* and modern *H. sapiens*[100]. Isotopic data for this period are, however, absent. Due to the limited preservation of collagen beyond 200 000 years, and the near absence of C_4 plants in Europe, these answers will have to come from further studies with tooth enamel in Africa and Asia. So far, there is no isotope evidence for the diet of *Homo* between 1·5 Mya up to 50 Kya (MP Richards, personal communication).

Dietary information from more recent humans comes from data on $\delta^{13}C$, supplemented with data on $\delta^{15}N$ and the $^{13}C{:}^{15}N$ ratio. The ^{15}N-isotope values of bone collagen[242] for differentiation between aquatic and agricultural diets were additionally verified by the study of the sulfur isotope ratios ($^{34}S{:}^{32}S$), since high intakes of marine organisms also result in higher $\delta^{34}S$ values[250]. Combined isotope studies reveal high intakes of animal protein, with substantial proportions derived from freshwater fish by Upper and Middle Palaeolithic (40–12 Kya) humans in Eurasia, indicating that in some populations about 30 % of dietary protein came from marine sources[250–256]. In contrast, isotopic evidence indicates that Neanderthals were top-level carnivores that obtained most of their dietary protein from large terrestrial herbivores, although even Neanderthals certainly exploited shellfish such as clams, oysters, mussels and fish on occasion[250–252]. At the onset of the Neolithic period (5200 years ago), there was a rapid and complete change from aquatic- to terrestrial-derived proteins among both coastal and inland Britons compared with Mesolithic (9000–5200 years ago) British humans[254], which coincides precisely with the local onset of the Agricultural Revolution in Europe.

Conclusions from isotope studies. The isotope systems that have been studied thus far in hominin bone and teeth provide evidence that early hominins were opportunistic feeders[257]. The spread of C_4 foods in East Africa, and subsequently in the hominin food chain between 3 and 4 Mya, is in agreement with a niche of early hominins that locates close to the water. This conception is in agreement with the palaeo-environmental evidence. However, many questions still remain unanswered. With regard to the possible niche in the water–land interface, it seems interesting to include aquatic as well as terrestrial piscivorous animals into future studies. The data of combined studies of early hominins and the more recent hominins suggest a gradual increase in dietary animal protein, a part of which may derive from aquatic resources. In the more recent human ancestors, a substantial part of the dietary protein was irrefutably derived from marine resources, and this habit was only abandoned in some cases after the introduction of agriculture at the onset of the Neolithic[254].

Archeology

The oldest stone tools found so far are dated to 2·6 Mya[258,259] and it has been suggested that these were used for flesh removal and percussion on long bones for marrow access. From this time onward stone tools were apparently used for defleshing and butchering of large animals. However, again there is a pitfall in putting too much emphasis on the association between stone tools and hunting and butchering of large animals as the sole food source of the human ancestors, especially with regard to brain foods such as LCP. As stated by Liem's paradox; the apparently overwhelming evidence for the consumption of bone marrow, or even brain from cracked skulls, by the findings of cut marks on animal bone may not be evidence for the primary food resources of human ancestors, but only for its fallback food. Bones, especially long bones, are also better preserved than vegetable material. Moreover, cut marks on bone are easier ascribed to human utilisation than any nearby found fossilised fish bones or molluscan shells that only seldomly bear cut marks[260,261] and are often not even examined. Hence, while human remains are nearly always found in the vicinity of water and the fossil record of nearby found fish is extensive[74,262], the exploitation of aquatic resources is difficult to relate to early man[263].

The present review is about the diet that allowed early humans to increase their brain size and thereby become intelligent enough to develop, for example, symbolic thinking and the controlled use of fire. Hunting and/or scavenging is often invoked as an important source of LCP, but, as pointed out by Crawford[2], even in the more recent certainly 'hunting' ancestors 'a [scavenged] small brain was not going to go far among the ladies [and children] even if it was still in an edible condition when they [the male hunters] got it back [from the savanna]'[2], not even in the scenario[264] that we were specialised, as suggested[265], in endurance running. Apart from organ tissue (liver and brain) and bone

marrow (whether scavenged or hunted), fish, shellfish and other aquatic foods are also mentioned as rich sources of the nutrients involved in brain expansion[99,130,266]. Therefore, the question arises whether the archeological evidence for human habitation in the land–water ecosystem only represents facilitated fossilisation or indicates the true ecological niche. The following section will focus on comparable evidence for the concurrent exploitation of aquatic resources.

Living in the water–land ecosystem. Because sea levels have risen up to 150 m in the past 17 000 years, a substantial part of the evidence for the exploitation of aquatic resources is hidden below sea level, if not permanently destroyed by the water[267,268]. However, in Kenya, a site in East Turkana provides solid evidence that at about 1·95 Mya hominins enjoyed carcasses of both terrestrial and aquatic animals including turtles, crocodiles and fish, which were associated with Oldowan artifacts[261]. More ambiguous evidence for the exploitation of freshwater fish, crocodiles, turtles, amphibians and molluscs by *Homo habilis* in the Olduvai Gorge in Tanzania goes back as far as 1·8–1·1 Mya[267,269]. Subsequent tentative evidence from the Olduvai Gorge dates the use of similar aquatic resources by *Homo erectus* to 1·1–0·8 Mya[267,269]. Also the out-of-Africa diaspora probably took place largely via the coastlines[81], even after the crossing of the Bering Strait into North America[270] (Fig. 2). In Koa Pah Nam, Thailand, 700 000-year-old piles of freshwater oyster shells were associated with *Homo erectus*[271,272]. In Holon, Israel, freshwater turtles, shells and hippopotamus bones were associated with *Homo erectus* and dated to 500–400 Kya[273]. *Homo erectus* fossils associated with seal remains in Mas del Caves (Lunel-Viel, France) were dated to 400 Kya[274].

The archeological evidence for aquatic resource use increases with the appearance of archaic *Homo sapiens*[267]. Although dominated by land mammal bones, 400 000–200 000-year-old remains from penguin and cormorants in Duinefontein, South Africa were associated with early *Homo*[275]. Shellfish and possibly fish remains, dated 300–230 Kya, were associated with the French coastal campsite at Terra Amata[276,277], while marine shellfish and associated early human remains, dated 186–127 Kya, were found in Lazaret, France[274]. Marean *et al.*[278] found evidence for the inclusion of marine resources, at 164 Kya, in the diet of anatomically modern humans from the Pinnacle Point Caves (South Africa). At the Eritrea Red Sea coast, Middle Stone Age artifacts on a fossil reef support the view that early humans exploited near-shore marine food resources by at least 125 Kya[279]. In several North African sites, dated to 40–150 Kya[267], human remains were associated with shell middens and aquatic resources such as aquatic snails, monk seals, mussels and crabs. Several European sites, dated to 30–125 Kya, are comparable with archeological sites that reveal evidence ranging from thick layers of

mussels and large heaps of marine shells in Gibraltar[267] to diverse marine shells in Italy[280], and to a casual description of the presence of marine shells of unknown density in Gruta da Figueira in Portugal[267]. Further evidence for the use of shellfish, sea mammals and flightless birds comes from: Klasies River Mouth (South Africa) dated between 130 and 55 Kya[267,281,282]; from Boegoeberg, where 130 000–40 000-year-old shell middens and cormorant bones were associated with *Homo sapiens*[267,283]; from Herolds Bay Cave, where 120 000–80 000-year-old shell middens, shellfish, mussels and otter remains were associated with human hearths[267]; from Die Kelders (75–55 Kya), where abundant remains of sea mammals, birds and shellfish were found in cave deposits[267,284]; and from Hoedjies Punt (70–60 Kya), Sea Harvest (70–60 Kya) and Blombos Cave (60–50 Kya) for the use of shellfish, sea mammals and fish[267,285]. From this period onwards, human settlements are strongly associated with the exploitation of aquatic resources[267,268,282,286–288]. Evidence for more sophisticated fishing by use of barbed bone harpoon points dates back to 90–75 Kya in Katanda, Semlike River, Zaire[289,290] and to 70 Kya in South Africa[291]. Finally, indications for seafaring are dated to 42–15 Kya[292–294]. Possibly, seafaring dates as far back as 800 Kya, as indicated by the finding of *Homo erectus* stone tools at the Indonesian island of Flores, which is located on the other side of a deep sea strait[79,295–299]. In general, many archeological sites are found along channels, lake- and seashores[99,266,267,282] and reveal aquatic fauna, such as catfish, crocodile and hippopotamus[300], but its proves difficult to relate their possible utilisation to our early ancestors.

Several events within the time span of the past about 2 million years have been attributed to the increase in brain size and intelligence. The introduction of meat in the hominin diet, which resulted in a higher dietary quality, has been discussed above. Claims for controlled fire in the Olduvai Gorge (Tanzania) and Koobi Fora (Kenya) go as far back as 1·5 Mya[215,301,302]. Evidence for cooking is as old as 250 Kya[301], but possibly dates back to 800 Kya[303], when indications of controlled fire were found to be present. However, recently it has been concluded that solid evidence for systematic use of fire is only found from 400 to 300 Kya onwards[304]. Evidence that cooking provided increased dietary quality was recently provided by Wrangham *et al.*[215]. According to archeological evidence, this could only have played an important role since the appearance of *Homo sapiens*[215,301] and Neanderthals[305]. Also, the inclusion of aquatic resources as an attributor to human brain evolution has been suggested[2,99–101,130,266,306–308], but remains a matter of debate[134,135,268,309].

From hunting–gathering to agriculture. The hunter–gatherer lifestyle continued worldwide for several millions of years and ended quite abruptly with the introduction of agriculture. The first indications for the abandonment

of the hunter–gatherer lifestyle towards settlement come from a 23 000 year-old fisher–hunter–gatherer's camp at the shore of the Sea of Galilee[310,311]. The associated return from diets containing substantial amounts of protein (from hunting and gathering) back to substantial amounts of carbohydrates is supported by indications for the ground collecting of wild cereals[312]. This was slowly followed by the large-scale utilisation of cereals starting with the onset of the Agricultural Revolution some 10 Kya.

As indicated above (see 'Biogeochemistry'), there is much controversy about the diet of the earliest humans and until now it is often stated that fishing was only introduced until more recently. From an anthropological perspective this might be true, since certain types (for example, deepwater) of fishing require advanced techniques[294]. However, from a nutritional point of view, 'fishing' might include anything from collecting sessile shellfish to the seasonal hand capture or clubbing of migrating or spawning fish in very shallow water. Since fresh drinking water is the single most important aquatic resource for humans, hominins probably observed predators and scavengers feeding on aquatic animals. This makes it unlikely that they would not have participated in opportunistic harvesting of the shallow-water flora and fauna, such as molluscs, crabs, sea urchins, barnacles, shrimp, fish, fish roe or spawn, amphibians, reptiles, small mammals, birds or weeds[175,267]. There are many indications suggesting that the evolution of early *Homo* and its development to *Homo sapiens* did not take place in the 'classical' hot, arid and waterless savanna, but occurred in African ecosystems that were notably located in places where the land meets the water (with the land ecosystem possibly consisting of – depending on rainfall – wooded grasslands). Compared with terrestrial hunting and/or scavenging in the savanna, food from this land–water ecosystem is relatively easy to obtain and is rich in the aforementioned combination of haem-Fe, iodine, Se, vitamins A and D, and long-chain *n*-3-fatty acids[100,101,266].

In conclusion, there is ample archeological evidence for a shift from the consumption of plant towards animal foods. Second, although there is an extensive archeological record for aquatic fossils (representing possible food) in the vicinity of human remains, their co-occurence is usually attributed to the preferential conservation of human remains in the vicinity of water. The present review provides support for the notion that the exploitation of these aquatic resources by hominins in coastal areas should be the default assumption, unless proven otherwise[154]. For a long time period in hominin evolution, hominins derived large amounts of energy from (terrestrial and aquatic) animal fat and protein. This habit became reversed only by the onset of the Neolithic Revolution in the Middle East starting about 10 Kya.

Anthropology

The hunter–gatherer diet. The *Homo* genus has been on earth for at least 2·4 million years[313] and for over 99 % of this period has lived as hunter–gatherers[314]. Surprisingly, very little information is available on the macro- and micro-nutrient compositions of their diet in this extended and important period of human evolution[34,148]. Since the onset of agriculture, about 10 Kya, agriculturalists and nomadic pastoralists have been expanding at the expense of hunter–gatherers[314], with agricultural densities increasing by a factor of 10–1000 compared with the highest hunter densities. For this reason, present-day hunter–gatherers are often found in marginal environments, unattractive for crop cultivation or animal husbandry.

In order to study the original hunter–gatherer way of life, it is appropriate to aim at the few hunter–gatherer communities living in the richer environments that bear closer resemblance to those in which the evolution of the genus *Homo* probably took place. Most studies on hunter–gatherers and their diets are, however, performed by anthropologists[315], whose primary interests are different from those of nutritionists. Anthropologists would, for example, conclude that 'fishing was so unimportant as to be a type of food collection'[316], or consider collecting both small land fauna and shellfish[314] as part of 'gathering', whereas from a nutritional point of view considerable differences exist in energy density, macro- and micro-nutrient composition between plants, terrestrial and aquatic animal foods.

Hunting v. *gathering.* Studies on food procurement of present-day hunter–gatherer societies show, in terms of energy gain *v.* expenditure, the advantage of hunting compared with plant foraging[192]. Nevertheless, three distinct studies[41,148,314] showed that hunting makes up only about 35 % of the subsistence base for worldwide hunter–gatherers, independent of latitude or environment. However, collection of small land fauna and shellfish was included as gathering in these studies. While gathering evidently played an important role over the whole of human evolution, hunting, although introduced later, coincided with 'a major leap for mankind' and has ever since played the most dominating cultural role. While hunting may have overtaken gathering in cultural importance, gathering continued to play a very important nutritional role, because: (i) gathering still contributes about 65 % to the subsistence base; (ii) many micronutrients derive only from plant sources; (iii) gathering of, for example, shellfish provides a substantial amount of LCP and other nutrients essential for brain development; and (iv) gathering plays an important cultural role since women, children and grandparents can participate[56,57,317].

Contrary to common belief, hunting in present-day hunter–gatherers is still not very successful: the probability for a kill in !Kung bushmen is only 23 %[314] and the subsistence of Hadza, as described by Marlowe[41] and

Woodburn[145], is composed of 75–80% of plant foods. Conversely, studies of North American hunting–gathering societies describe the dietary role of shellfish as similar to 'bread and butter', being the staple food[318] in these societies. The anthropological remark[314] that for many studied hunter–gatherer tribes 'fishing was only a type of food collection' also adds to the notion that the collection of aquatic foods might have preceded scavenging and hunting. Collecting aquatic foods is still daily practice in Eastern Africa and picking up, clubbing or spearing stranded aquatic animals seems much easier and safer than either scavenging or hunting game on the Serengeti plains.

We conclude that gathering plays, and most likely always played, the major role in food procurement of humans. Although hunting doubtlessly leaves the most prominent signature in the archaeological record, gathering of vegetables and the collection of animal, notably aquatic, resources (regardless of whether their collection is considered as either hunting or gathering), seems much easier compared with hunting on the hot and arid savanna. We suggest that it seems fair to consider these types of foods as an important part of the human diet, unless proven otherwise[154]. Conversely, while hunting might have played a much more important role at higher latitudes, dietary resources in these ecosystems are rich in *n*-3-fatty acids (for example, fatty fish and large aquatic mammals), while the hominin invasion of these biomes occurred only after the development of more developed hunting skills.

(Patho)physiology

Brain-selective nutrients. Nutrients and other environmental factors are increasingly recognised to influence epigenetic marks[319–323], either directly or indirectly via many bodily sensors. Food from the diverse East African aquatic ecosystems is rich in haem-Fe, iodine, Se, vitamins A and D, and *n*-3 fatty acids from both vegetable origin and fish[101]. All of these nutrients seem to act at the crossroad of metabolism and inflammation[24]. For example, PPAR[324,325] are lipid-driven nuclear receptors with key cellular functions in metabolism and inflammation[26]. TR[326], VDR[327], RXR and RAR[328] are other examples of nuclear transcription factors that serve functions as ligand-driven sensors. The iodine- and Se-dependent hormone triiodothyronine (T_3)[329–331] is a ligand of TR[326], many fatty acids and their derivatives are ligands of PPAR[332], the vitamin D-derived 1,25-dihydroxyvitamin D hormone is a ligand of the VDR[333], 9-*cis*-retinoic acid and the fish oil fatty acid DHA are ligands of RXR[328], while RAR interacts with vitamin A (retinol) and many of its derivatives such as all-*trans*-retinoic acid, retinal and retinyl acetate[328]. The ligated nuclear transcription factors usually do not support transcription by themselves, but need to homodimerise or heterodimerise notably with

RXR to facilitate gene transcription. Examples of the latter are TR/RXR, PPAR/RXR, VDR/RXR and RAR/RXR. It has become clear that their modes of action illustrate the need of balance between, for example, iodine, Se, fish oil fatty acids and vitamins A and D, a balance that is notably found in the land–water ecosystem.

Deficiencies of the above 'brain-selective nutrients' are among the most widely encountered in the current world population[101,334]. While iodide is added to table salt in many countries, margarines and milk have become popular food products for fortification with vitamins A and D. After discussing some general health differences between traditionally living individuals and those living in Westernised countries, we focus on the importance of LCP and notably those of the *n*-3-series, as examples of the above-mentioned nutrients that are especially abundant in the land–water ecosystem.

Hunter–gatherer v. *'Western' physiology*. There are many differences in health indicators between traditionally living individuals and those living in Western societies. For instance, primary and secondary intervention trials with statins indicate lowest CHD risk at an LDL-cholesterol of 500–700 mg/l (1·3–1·8 mmol/l), which is consistent with levels encountered in primates in the wild and hunter–gatherer populations with few deaths from CVD[335–339]. Another example of the healthy lifestyle of present-day hunter–gatherers comes from the observed 'insulinopenia' or 'impaired insulin secretion' following an oral glucose tolerance test (Fig. 6) in Central African Pygmies and Kalahari Bushmen[340,341], respectively. As opposed to the 'impairments' noted by these authors, it may also be argued that these researchers were actually witnessing an insulin sensitivity that has become sporadic in Western countries as a consequence of the decrease in physical activity and fitness, increase in fat mass and as a result of the quantity and quality of the foods consumed[36,133,342]. The current consensus is that 'fat is bad' and especially saturated fats have become associated with CVD[343–345]. However, traditional Maasai consumed diets high in protein and fat (milk and meat) and low in carbohydrates[346,347]. They had high intakes of saturated fat and cholesterol, showed extensive atherosclerosis with lipid infiltration and fibrous changes, but had very few complicated lesions, and were virtually devoid of CVD[348]. The average total and LDL-cholesterol in these societies was low and did not increase with age[348]. Finally, the physical fitness of individuals in such traditional societies, such as the Maasai, is often remarkable[337].

In Kalahari Desert Bushmen and Central African Pygmies, observers could not find any case of high blood pressure and blood pressure did not increase with age[335,349]. Dental surveys of Kalahari Bushmen[350] and other hunter–gatherers[49] showed a remarkable absence of caries. The absence was explained by the repetitive annual abstinence of fermentable sugars in their diet, with a consequent inability to build a cariogenic oral
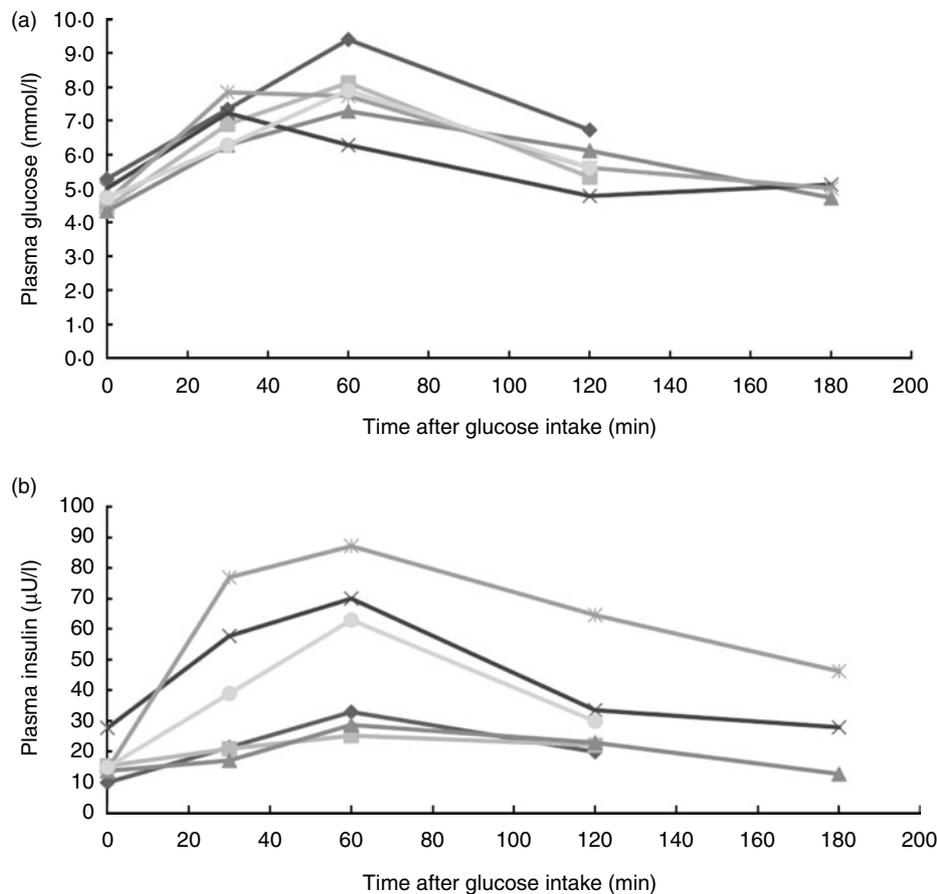
Fig. 6. 'Abnormal' insulin response but normal glucose response after oral glucose tolerance test in African Bushmen and Pygmies, compared with Western controls. (a) Plasma glucose response after an oral glucose load of 50 g (Bushmen (♦) and white controls (○)) or 100 g (Pygmy (■), Pygmy with 2 weeks' daily supplementation of 150 g carbohydrates before testing (▲), Bantu (X) and American controls (✳)). Of note is that Bushmen and Pygmies have significantly lower body weights as compared with Bantu and white and American controls (average weight Bushmen/Pygmy males 46 kg, females 38 kg; controls 65 kg), while each group received the same unadjusted loading dose of 50 g or 100 g glucose. (b) The so-called 'abnormal' insulin response or 'impaired' insulin secretion as observed by the authors in both Bushmen and Pygmies[340,341].

Lactobacillus flora[350]. Inhabitants of Kitava (Trobriand Islands, Papua New Guinea) have high intakes (70 en%) of carbohydrates from yams, high intakes of SFA from coconuts, and a high fish intake[44,45,351]. Although both high intakes of carbohydrates and saturated fat have been related to the metabolic syndrome and CVD, these traditional Kitavians do not show symptoms of either the metabolic syndrome and are virtually free from the Western diseases that ensue from it.

Evidence-based medicine as applied to long-chain PUFA in CVD and depression. Despite some compelling examples of the healthy lifestyles of traditional populations, current dietary recommendations derive preferably from randomised clinical trials with single nutrients and preferably hard endpoints[352]. This approach clearly oversimplifies the effects of dietary nutrients[353], since neither macronutrients, nor micronutrients, are consumed in isolation and their effects may be the result of a complex web of interactions between all the nutrients present in the biological systems that we consume, such as a banana or a fish.

The current recommendations from many nutritional boards for a daily intake of 450 mg EPA + DHA in adults derive from epidemiological data that demonstrated a negative association of fish consumption with CHD[354–357] that has subsequently become supported by landmark trials with ALA[358] and fish oil[359–361] in CVD. However, not all trials in CVD have been positive[362]. In addition, a negative association was observed for fish consumption and depression[363–365] and for homicide mortality[366]. The causality of these relationships was supported by some, but not all, trials with fish oil in depression[367–371], while a recent meta-analysis demonstrated the beneficial effect of EPA supplements with ≥ 60 % EPA of total EPA + DHA in a dose range of 200–2200 mg/d of EPA in excess of DHA[372].

The influence of polymorphisms in the genome is increasingly recognised, but seldom interpreted in an evolutionary context. As argued above, most polymorphisms were already amongst us when *Homo sapiens* emerged, some 200 Kya, while that also holds true for most, if not all, currently identified 'disease susceptibility genes' that are usually abundant but confer low risk[373].

A loss-of-function mutation in a specific biosynthetic pathway might be an evolutionary advantage if the specific endproduct has been a consistent part of the diet, such as is probably applicable to all vitamins, for example, vitamin C[374,375]. Applied to our LCP status, it is nowadays well established that all humans synthesise DHA with difficulty[376,377]. Analogously, the recently discovered polymorphisms of fatty acid desaturases 1 (FADS1; also named Δ-5 desaturase) and FADS2 (Δ-6 desaturase) with lower activities in their conversion of the parent essential fatty acid to LCP suggest that from at least the time of their appearance, the dietary intakes of AA, EPA and DHA have been of sufficient magnitude to balance the LCP *n*-3:LCP *n*-6 ratio[378,379] to maintain good health.

Long-chain PUFA benefits in pregnancy and early life. Another indication for the importance of LCP comes from the higher LCP contents in the fetal circulation compared with the maternal circulation, a process named biomagnification[380–382], which occurs at the expense of the maternal LCP status[383,384]. The decreasing maternal *n*-3 LCP status during pregnancy in Western countries is associated with postpartum depression[363,364], although intervention studies with LCP in postpartum depression have been negative so far[370,371,385,386]. However, a positive effect was seen for *n*-3 LCP supplementation on depression during pregnancy[387] and it has been advocated to start supplementation earlier in pregnancy and with higher dosages[388].

Maternal LCP intakes have also been related to infant health. AA and DHA in premature and low-birth-weight infants correlated positively with anthropometrics, AA to increased birth weight[389] and DHA to prolonged gestation[390–392]. Studies with supplementation of DHA during pregnancy yielded, for example, evidence for: (i) the maturation of the brain, visual system and retina of the newborn at 2·5 and 4 months, but not at 6 months[393–397]; (ii) increased problem solving at 9 months but no difference in memory[398]; and (iii) superior eye–hand coordination at 2·5 years[399] and higher intelligence quotient at 4 years[400] but not at 7 years of age[401]. In contrast to the inconclusive human studies, animal studies and combined human and animal studies showed abnormal behaviour together with disturbed cognition at lower brain DHA levels[402]. The importance of dietary AA during pregnancy seems less pronounced, but a positive association between umbilical AA and neonatal neurological development[389] and a lower venous AA for those with slightly abnormal neurological development[403] has been shown. A reduced DHA status in the brain is associated with a mildly increased AA status[404], which is in its turn associated with low-grade inflammation[405].

Infant health starts with maternal health; thus dietary recommendations issued for pregnant women indirectly also apply to their infants. The recommendation for adults to consume 450 mg DHA + EPA per d translates into a DHA composition in breast milk of about 0·79 %[406].

However, current recommendations for the composition of infant formulae derive mainly from the range of human milk fatty acid compositions as observed in Western countries, which in their turn derive from women with recorded intakes below the 450 mg recommended daily intake of EPA + DHA[407,408].

The same paradox holds for other fatty acids in breast milk. For instance, there are few recommendations for the medium-chain SFA (MCSFA) content of human milk. High MCSFA contents in some traditional societies derive from their high intakes of 12 : 0 and 14 : 0 from coconuts[409]. Conversely, the high MCSFA contents in Western populations are primarily influenced by maternal carbohydrate intakes[410], since the mammary gland has the unique ability to convert glucose into MCSFA (6 : 0–14 : 0), mainly lauric (12 : 0) and myristic (14 : 0) acids. However, women with regular consumption of coconuts have a much higher 12 : 0:14 : 0 ratio compared with women with high carbohydrate intakes. Both MCSFA are readily absorbed in the gastrointestinal tract, while antiviral as well as antibacterial properties have been attributed to some MCSFA, but mainly to 12 : 0[411,412].

The PUFA content of Western milk has increased over the last decades[413,414]. While the human milk LA content in the USA increased by at least 250 %, its DHA content decreased by almost 50 %[414]. The (from an evolutionary point of view) abnormally high LA intake is, despite a lack of evidence[415], advocated for cardiovascular health[416]. The resulting high LA status is likely to interfere with both the incorporation of AA and DHA into phospholipids and also inhibits their synthesis from their parent essential fatty acid[417]. Major differences are noted in the comparison of the human milk fatty acid compositions of Western mothers compared with some traditional African women[409,418], with unknown consequences for infant health or the occurrence of disease at adult age (i.e. the 'Barker hypothesis')[16,419]. It has been proposed that the high concentrations of EPA, DHA as well as AA in human milk, such as described for many fish-consuming societies[409,420,421], might be a more appropriate reflection of the Palaeolithic breast milk composition and may therefore constitute a better reference for infant formulae than do Western human milks[422].

The influence of environment. It is estimated that 70 % of all cases of stroke and colon cancer, 80 % of all CVD and 90 % of all cases of type 2 diabetes mellitus have been caused by lifestyle and could have been prevented by paying more attention to modifiable behaviour factors, including specific aspects of diet, overweight, inactivity and smoking[423]. The mismatch between the human diet and the Palaeolithic genome might therefore be responsible for many typically Western diseases. In addition to the evidence from many other disciplines, evidence from (patho)physiology and epidemiology adds to the notion that a great deal of information on healthy diets might derive from the study of the diets of the early human

ancestors. The metabolic syndrome, characterised by impaired insulin sensitivity, is at the centre of many diseases of civilisation. High intakes of refined carbohydrates as well as low intakes of LCP have been implicated in the development of insulin resistance. As such, low carbohydrate intakes[424,425] and high LCP[8,34,426,427] intakes by the early human ancestor might explain in part the low incidence of diseases of civilisation in current hunter–gatherer societies. The available evidence from pathophysiology and epidemiology supports the hypothesis that the land–water ecosystem contributed important and indispensable nutrients to evolving hominins.

Dietary reconstruction of the nutrients available in Eastern Africa

The debate on the ecological niche of human ancestors is unlikely to reach a consensus shortly. The millions of years of human evolution concurred with marked and abrupt climatic changes, which renders a single ecological niche of human ancestry unlikely. However, it is at the same time clear that in a short period of time humans have made tremendous changes in their lifestyle, their diet included, that lie at the basis of the diseases of Western civilisation. This prompted various investigators to reconstruct the possible compositions of diets that could have been consumed by our Palaeolithic ancestors. Their studies are, for example, based on the plausibility that before the Agricultural Revolution, when humans lived as hunter–gatherers, cereals were no appreciable part of the diet and that wild animals living in the Eastern African savanna and in Eastern African aquatic ecosystems have different fatty acid compositions compared with the domesticated animals that have now become staple foods. For example, the lean savanna animals that inhabit the Eastern African plains have much lower fat contents, and the available fat is much more enriched in PUFA[428]. Similarly, high-latitude (fatty) fish have much higher EPA and DHA contents, but lower AA contents compared with low-latitude (lean) fish from tropical waters[429–432].

Eaton & Konner[8] where the first to use this approach in reconstructing a Palaeolithic diet; their pioneer study was published in the *New England Journal of Medicine* in 1985. The authors estimated that late Palaeolithic humans consumed diets containing 35% meat and 65% vegetable foods, containing 34 en% from protein, 45 en% from carbohydrate and 21 en% from fat, while the ratio between polyunsaturated and saturated fat equalled 1·41 and their fibre intake amounted to 46 g/d[8]. These outcomes contrasted with the average American diet at the time, that consisted of 12 en% protein, 46 en% carbohydrate and 42 en% fat, with a polyunsaturated:saturated fat ratio of 0·44 and a fibre intake of 20 g/d. After 25 years of additional study, Konner & Eaton confirmed their previous findings by estimating that the Palaeolithic diet provided 25–30 en% protein, 35–40 en% carbohydrate and 20–35 en% fat[433],

while the polyunsaturated:saturated fat ratio was 1·40[434]. Moreover, they concluded that 'it has become clear since our initial publications that marine, lacustrine, and riverine species were important sources of animal flesh during the evolution of modern *Homo sapiens*, and may have played a role in the evolution of brain ontogeny'[433]. In addition to the earlier studies, they also estimated the vitamin and mineral composition of a Palaeolithic diet, showing higher contents of folate, riboflavin, thiamin, vitamins A and E, Ca, Mg, P, Zn, and notably ascorbate, vitamin D (sunlight), Cu, Fe, Mn and K, while the Palaeolithic diet contained much lower Na compared with contemporary US intakes and recommendations[433–435]. In a subsequent study they estimated that in different ancient hunting and gathering populations, fatty acid intakes would have ranged from 5·19 to 20·6 g LA/d, 0·26 to 4·8 g AA/d, 3·45 to 25·2 g ALA/d and 0·03 to 1·52 g DHA/d, which contrasted with the much higher LA (22·5 g/d) and lower ALA (1·2 g/d), AA (0·6 g/d) and DHA (0·08 g/d) intakes as observed in current Western populations[34].

In a meticulous analysis of worldwide hunter–gatherer diets, Cordain *et al.*[148,436] estimated that the most plausible percentages of total energy from dietary macronutrients would be 19–35 en% from protein, 22–40 en% from carbohydrate and 28–58 en% from fat, which reflects a markedly higher contribution of dietary fat, a similar amount of protein, but a lower contribution of carbohydrates, compared with earlier estimates from Eaton & Konner[8,434]. The main differences were explained by the assumption that, wherever it was ecologically possible, hunter–gatherers would have consumed 45–65% of total energy from animal foods[148], while in the earlier estimations[8,434] only 35% derived from animal foods. These higher animal food intakes were explained by their inclusion of both worldwide hunting and fishing hunting–gathering societies into their new calculation models[148], also including mounted and arctic hunters. Those latter possibilities, however, seem insignificant with regard to early human evolution, which explains why they seem to overestimate the amount of the diet that is derived from animal foods. For example, Marlowe[41] estimated that in a warm-climate sample about 53% of the diet derives from gathering, 26% from hunting and 21% from fishing (i.e. about 47% from hunting).

To subsequently investigate the nutrient compositions of such diets, fish consumption was incorporated as a separate variable to plant and meat consumption in the earlier models, since aquatic and terrestrial animals have markedly different fatty acid compositions. In this most recent analysis[437], 12 500 kJ (3000 kcal) Palaeolithic diets were investigated with plant:animal food intake ratios ranging from 70:30 to 30:70 en%/en% under the conditions of four different foraging strategies in which the animal part ranged from exclusive meat consumption including the selective consumption of energy- and LCP-rich fat from bone marrow and brain, respectively[427], to the

consumption of an entirely aquatic diet in an Eastern African water–land ecosystem[438,439]. It was found that that the energy intakes from the macronutrients were: 25–29 en% (range 8–35) from protein, 39–40 en% (range 19–48) from carbohydrate and 30–39 en% (range 20–72) from fat. Dietary LA ranged from 1·7 to 6·2 en%/d, AA from 1·15 to 10·7 g/d, ALA from 2·1 to 5·8 en%/d and EPA + DHA intakes from 0·87 to 28·3 g/d[437]. From these data, despite their wide range in outcomes, it can again be concluded that there are substantial differences with respect to the average composition of the current Western diet, notably because of its higher proportions of carbohydrates and LA, and its much lower protein and ALA and LCP contents. It became also conceivable that ancestors living in the East African water–land ecosystem had daily intakes of gram amounts of EPA + DHA. As such, these n-3 LCP intakes were comparable with those of the traditionally living Eskimos in Greenland, who because of their low CVD risk[354,355] initiated the current interest in the role of n-3 LCP in both primary and secondary prevention of CVD. In addition to these n-3 fatty acids, the water–land ecosystem is also a rich source of haem-Fe, iodine, Se and the vitamins A and D[101], which have important functions and interactions in gene transcription and metabolism[24,26,440].

Dietary changes since the Agricultural Revolution

Whatever the specific composition and wide range of early hunter–gatherer diets, the current consensus is that our diet has changed markedly from the time of large-scale utilisation of cereals and animal domestication (i.e. the Agricultural Revolution) starting some 10 Kya. Contrary to earlier belief, the advent of agriculture coincided with an overall decline in nutrition and general health, but at the same time provided an evolutionary advantage since it increased birth rates and thereby promoted net population growth[49,50].

While the decline of nutritional quality and general health started with the onset of the Agricultural Revolution, these processes became even more pronounced with the advent of the Industrial Revolution some 100–200 years ago[9,11,133]. Among the many dietary and lifestyle changes (Fig. 7) are: a grossly decreased n-3:n-6 fatty acid ratio, the combined high intakes of SFA and carbohydrates[441–443], the introduction of industrially produced *trans*-fatty acids, reduced intakes of n-3 and n-6 LCP, reduced exposure to sunlight, low intakes of vitamins D and K, disbalanced antioxidant status and high intakes of carbohydrates with high glycaemic indices and loads, such as sucrose and industrially produced high-fructose maize syrup[36,133,444,445]. Many of these changes act in concert, which points at the serious limitations of conclusions from contemporary investigations that study the many nutrients in isolation and form the basis of modern nutritional guidelines. An example is the interaction of dietary carbohydrates with SFA[441–443,446].
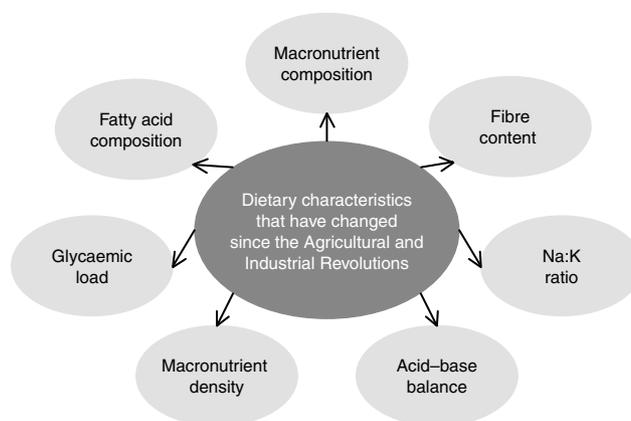

Fig. 7. The seven dietary characteristics that have been changed since the Agricultural and Industrial Revolutions. Adapted from Muskiet[24].

Potential benefits of a Palaeolithic diet

Evidence for the beneficial effects of Palaeolithic diets may derive from their influence on weight reduction and classical coronary artery disease risk factors. In an uncontrolled study with healthy adults, Osterdahl et al.[447] showed a decrease in weight, BMI and waist circumference after 3 weeks *ad libitum* consumption of a Palaeolithic-like diet (i.e. 6627 kJ/d (1584 kcal/d); carbohydrate 40, protein 24, fat 36 en%), compared with their baseline usual diet (10 368 kJ/d (2478 kcal/d); carbohydrate 54, protein 14, fat 30 en%). Additionally, they showed favourable effects on systolic blood pressure and plasminogen activator inhibitor-1. Jönsson et al.[448] performed a cross-over study of 2 × 3 months in type 2 diabetic patients receiving a Palaeolithic diet (6615 kJ/d (1581 kcal/d); carbohydrate 32, protein 24, fat 39 en%) or a diabetes diet (7858 kJ/d (1878 kcal/d); carbohydrate 42, protein 20, fat 34 en%). They showed a reduction of body weight, BMI and waist circumference and lower HbA_{1c}, TAG and diastolic blood pressure, and higher HDL-cholesterol after consumption of the Palaeolithic diet.

In a randomised trial in patients with IHD plus glucose intolerance or type 2 diabetes, Lindeberg et al.[449] showed a reduced energy intake after *ad libitum* consumption of a Palaeolithic diet (5623 kJ/d (1344 kcal/d); carbohydrate 40, protein 28, fat 27 en%) as compared with an *ad libitum* Mediterranean-like Consensus diet (7510 kJ/d (1795 kcal/d); carbohydrate 52, protein 21, fat 25 en%). They also observed a larger improvement in glucose tolerance in the Palaeolithic diet group, independent of decreased waist circumference. The most convincing evidence so far derives from an uncontrolled trial[450] showing that 10 d consumption of an isoenergetic Palaeolithic diet (11 301 kJ/d (2701 kcal/d); carbohydrate 38, protein 30, fat 32 en%) improved blood pressure, arterial distensibility, insulin sensitivity and total, HDL- and LDL-cholesterol in healthy sedentary human subjects, when compared with their baseline usual diet (9924 kJ/d (2372 kcal/d), carbohydrate 44, protein 18, fat 38 en%).

Importantly, there were no changes in energy intakes, activity levels and body weight, which indicates that the improved coronary artery disease risk profile was unrelated to weight reduction or other well-known determinants.

Conclusions

The optimal nutrient combination to support good health can be expected to reflect a certain balance. This balance is present in the foods that were consumed by Palaeolithic and possibly by pre-Palaeolithic ancestors, because it is this balance on which the human genome has evolved. This genome has been shaped by millions of years of evolution, during which it adapted to the conditions of existence, including the diet. There are ample indications from many disciplines that the human ancestors evolved in a water–land interface that provided food from both terrestrial and aquatic resources. For instance, the availability of both *n*-3 and *n*-6 LCP from the aquatic food chain was one of the many factors that provided early humans with the unique combination of brain-selective nutrients for brain growth[2]. The recent deviation from this Palaeolithic diet and lifestyle in general might be at the basis of many, if not all, current diseases of civilisation. Detailed studies with respect to the health effects of the diets of these earlier ancestors are therefore warranted.

Acknowledgements

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