A role for lipids as determinants of evolution and hominid brain development

Michael A Crawford

Abstract

The thrust of this paper is that lipids played a major, as yet unrecognised, role as determinants in evolution. Life originated 2.5 billion years ago during which time there was ample opportunity for DNA modification. Yet there was little change in the life forms seen in the fossil record for the first 2.5 billion years. It was not until the oxygen tension rose to a point where oxygen utilising life forms became thermodynamically possible that change is seen. The sudden appearance of the 32 phyla in the Cambrian fossil record was associated with the rise in the oxygen tension and the appearance of intracellular detail and cell differentiation. That detail was provided by cell membranes in which the lipids were structural essentials. Docosahexaenoic acid (DHA) provided the basic membrane backbone of the novel photoreceptors that converted photons into electricity laying the foundation for the evolution of the nervous system and ultimately the brain. Although there are two closely related fatty acids with only one double bond different, DHA was not replaced despite some 600 million years of genomic change. The second obvious lipid involvement occurred during the Cretaceous. As flowering plants evolved their protected seeds they introduced into the land food web a novel, rich source of omega-6 fatty acids. Coincidentally mammals evolved using the omega-6 fatty acids for the synthesis of powerful adhesion molecules to capture the fertilised egg, and vascular development enabling placentation associated with the switch from egg-laying to mammalian reproduction. With the brain utilising omega-6 and omega-3 fatty acids in a ratio of 2:1 the injection of the omega-6 to an already omega-3 rich food web would have played a critical role in the advance of brain evolution and finally the cerebral expansion in human evolution. Lipids are still modifying the present evolutionary phase of our species with their contribution to a changing panorama of non communicable disease which includes the sharp rise in brain disorders.

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Introduction

This paper will review ideas about the conflict between Darwin's original concept of origin of species and the subsequent disassembly of his broad view into a narrow focus on natural selection. His concept of the conditions of existence as the higher law persists in his writing into the 6th and final edition was discarded by most evolutionary biologists. However, Darwin spent much of the latter part of his life searching for what he called "pangenes" which he felt would explain how the environment interacted with evolution. Darwin's pangenes are now in evidence in the nutritionally and environmentally dependent ligands for nuclear receptors which influence the expression of genetic information. The biological features that can lead to multigenerational changes in gene behaviour referred to as epigenetics is essentially the pangenomic behaviour for which Darwin was searching.

The origin of life and the Cambrian explosion

The stromatolite reef at Pilbara in Western Australia has been dated to 3,43 and 3,35 billion years old when it was submerged in a shallow sea (Allwood *et al.,* 2006). Although there is some debate as to whether or not these stromatolites represent the first signs of the biosphere, it is generally agreed that life had evolved by about 3 billion years ago on this planet. The relatively brief period before the appearance of life can be considered as the period of chemical evolution.

Conceptionally the appreciation of chemical evolution from the origins of matter in a super nova is important because it makes the point so often forgotten, that life is chemistry and physics. It was the appropriate conditions of chemistry that brought life into being. Without the correct conditions this would not have happened. The forces in action are Darwin's "*Conditions of Existence*". It follows that life emerged in an energy and chemically rich environment.

The chemical synthesis of reduced molecules of greater and greater complexity is what would be expected following the creation of elements gifted by a super nova. At the high temperatures of the nuclear furnace of a maturing star and its final super nova, the energy is simply too great for elements to combine. As energy levels subsided and gravity took over the expectation is of element combinations to occur. Oxygen being as ferocious a combiner as it is, the burning of silicone and the like would be predicted and of course that is what happened, otherwise there would be no crust to the earth, Mars or the moon. Similarly, high-energy phosphates, sulphates, oxides of hydrogen, carbonates and a plethora of other carbon compounds would also be predicted. Once oxygen was consumed by burning hydrogen and the many elements, the early atmosphere and aquatic environments were reducing. Given the condensation of such material into a solid planet which would include many if not all elements of the periodic table, seams of elements would form through normal crystallisation mechanisms, water soluble compounds would collect in the primitive lakes and oceans as the surface of the planet cooled. The intense solar radiation, volcanic and electrical storm hold all the necessary stimulants for the increasing complexity of natural chemical interactions until, as has been shown experimentally, the building blocks of life were formed. Viewed from the perspective of chemistry and the conditions of existence, self-replicating systems and then life is predictable and unsurprising (Oparin, 1924). It is indeed curious that the discovery of an amino acid or other biochemicals in outer space or on a meteorite raises such extraordinary headlines in the scientific press. Such molecules are expected to emerge as a consequence of interaction between elements and natural chemical evolution.

Once life formed, the earth's life history is usu-

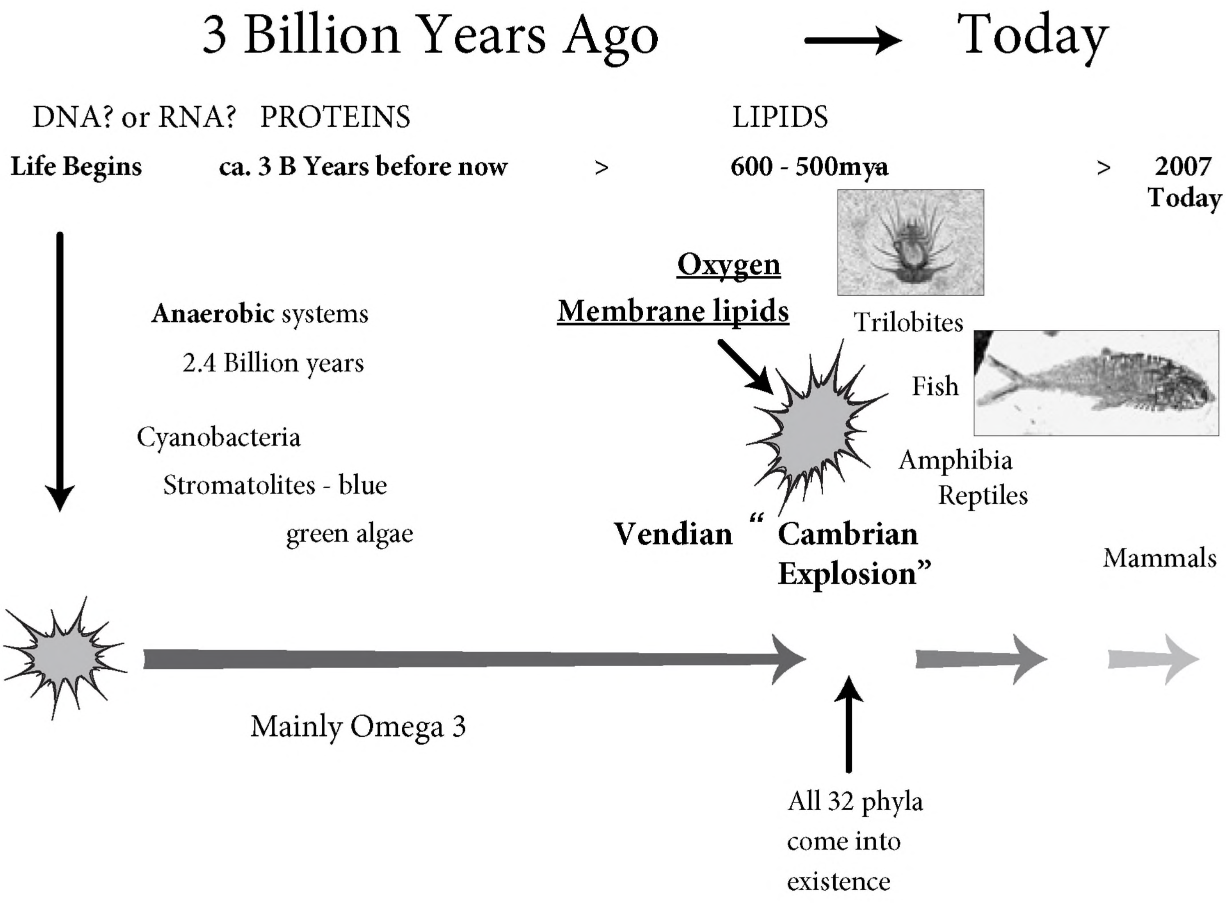

Diagram: A rough sketch of the time lines of the evolution of life ally divided sharply into two parts, to which I will add a third. These three parts again reflect chemistry as a driving force:

1. During the first 2.5 billlion years, the only fossils found in abundance in the pre-Cambrian era are the stromatolites. This vast pre-Cambrian era was dominated by the Cyanophyta generally attributed to the blue-green algae. Notably, they show no intracellular detail. Their photosynthetic systems synthesize proteins, nucleic acids and other chemicals in an essentially reducing atmosphere. We can expect some polyenoic fatty acids to have been made and that these would likely to be of the omega-3 variety.

2. The second part is the Vendian followed by the Cambrian era of about 600 million years ago. It was then that the oxygen tension rose above the Pasteur point at which aerobic metabolism becomes thermodynamically possible (Fischer, 1965). The 32 phyla we know today suddenly explode into the fossil record in a short period of time. These fossils provide considerable intracellular detail.

3. We shall add a third part; the evolution of flowering plants and the new reproductive system of the mammals. This latter period laid a new chemistry making possible as will be asserted, the evolution of the human brain.

Each of the three phases is an example of a change in chemistry and in evolutionary direction and style. In the first instance chemistry became biochemistry. In the second, biochemistry shifted up through eight gears in efficiency to aerobic metabolism giving birth to 32 phyla. In the third, biochemistry made a lateral shift to a change in the reproduction system that would lead to mass extinctions of many species with the dominance of one.

The evolution of complex life forms

In the first phase of prokaryote life, the biochemistry of nucleic acids and proteins held sway and there was little change on the design of the life forms despite the 2.5 billion year time period offering ample opportunity for the rate of mutational change to provide for substantial modification. There is of course evidence of variation but it is limited. There is however no evidence of such events as witnessed in the Cambrian explosion. This 2.5 billion year stasis is powerful evidence for Darwin's view that there were two forces in evolution; "natural selection and conditions of existence". Of the two, he wrote, the latter was the most powerful. The absence of an appropriate oxygen tension was a condition of existence which did not permit the advance to more complex life. When the Pasteur point was breached, all the 32 phyla we have today appear in the fossil record with a remarkable suddenness leading to this phase being described as the Cambrian explosion. This is, again, clear evidence of the importance of the "Conditions of Existence".

Of special interest in the eukaryotic evolution were the appearance of intracellular structures in the fossils and the emergence of differentiated cells. The intracellular detail is made largely of membrane lipid bilayers and embedded proteins. The organisation of cellular structures was made possible by membrane lipids. With the 32 phyla emerging in the Cambrian explosion it seems likely that it was not only the rise in the oxygen tension that was important but also the cell structural complexity in which the lipids would have played an important role in the genesis of specialisation and then speciation.

Was increased complexity of lipids responsible for cell structures and specialisation amongst the eukaryotes?

The nucleic acids and the proteins derived from them would have been in abundance during the first 2.5 billion years. The state of the lipids can only be a topic of conjecture. Were there highly unsaturated fatty acids available prior to the Cambrian explosion? Fresh water algae operate more anaerobically than sea water algae and synthesise little of the long-chain super-unsaturated, polyunsaturated fatty acids which have 20 and 22 carbons or more, with 3-6 double bonds (SUFA). Moreover, anaerobic systems, as in the gut flora or rumen, use unsaturated fatty acids as hydrogen donors suggesting that in the anaerobic phase of life, the highly unsaturated fatty acids might have been rare.

Although one can only speculate, the complexity of the lipids for structures is likely to have been greatly enhanced by aerobic metabolism. The synthesis of the double bonds in DHA requires 6 oxygens without including the energy requirement for the chain elongations. These lipids are today used for the organisation of complex cellular structures, as in the reticulo-endothelium, electron transport systems, nuclear envelope and plasma membranes which accommodate receptors, transporters, signaling systems and antioxidant enzymes. Oxidative metabolism and with it the possibility of desaturating fatty acids and producing the SUFA in quantity would have provided a great and novel wealth of architectural possibilities to engage in sophisticated organisation and function not previously possible. A largely anaerobic system would be unlikely to produce SUFA in quantity. Hence one has to consider that sophisticated membrane lipids rich in SUFA was also a feature of the Cambrian explosion making possible sophisticated membrane systems.

The peroxidation paradox and Alzheimer's disease

Another way of considering the transition from the prokaryotes to eukaryotes is that the chemistry of the former was based on the robust chemistry of the nucleic acids. The proteins, which were less robust, possessed a new dimension of complexity with their chemistry being dependent on arrangement of the nucleotides of the DNA. The eight gear shift of energy efficiency with aerobic metabolism brought lipid chemistry into play, introducing a new order of complexity of molecules. The paradox was synthesis of reduced molecules in the pre-Cambrian era was challenged by the novel use of oxygen as the dynamic driver of the new aerobic life forms. Whilst being favourable to the eukaryotes, oxygen was none the less toxic to the reduced systems.

The eukaryotes embraced the reduced cellular interior using the many reduced molecules such as ascorbic acid, carotenoids and tocophopherols for protection which are now referred to as antioxidants. The puzzle still lies with the super-unsaturated fatty acids, DHA in particular. Despite its susceptibility to peroxidation it was actually used in the cell membranes where there is the greatest use of oxygen as in the photoreceptor, brain and mitochondria where hydrogen peroxide is a routine end product. A plausible explanation for such a paradox is that these highly unsaturated fatty acids stimulate the expression of antioxidant enzymes (Phylactos *et al.,* 1994) and indeed their own protective molecules (Lukiw *et al* ., 2005).

Although it can be argued that the reduced life forms never truly died, eukaryote evolution introduced the clear concept of death. Despite 600 million years of evolution no solution to death was discovered. A plausible reason is that the substance responsible for this new evolutionary life style was also responsible for death. The many new research centres for ageing usually with a focus on Alzheimer's disease consider this principle as there is already evidence of peroxidative damage as a component of cause and conversely the protective nature of seafood and omega-3 DHA in alleged cause and prevention (Bazan, 2007; Butterfield and Sultana, 2007; Moreira *et al.*, 2007).

The protein-lipid interface

The cooperation between lipids and protein in the organisation as the cell membrane bilayer introduced the ability to specialise in a manner not seen if the previous 2.5 billion years or so. Specialisation of function of the plasma membrane was accompanied by the proliferation of unstable molecules with increasing complexity. The language of DNA has 4 words (nucleotides), that of proteins 20 words (amino acids). There are 64 main lipid words (molecular species) and several hundred minor words which some say are more than 1,000 in number. One can argue that this higher degree of structural diversity was essential to accommodate the stereochemistry of the more sophisticated proteins and in particular offered a supporting medium for the lipophilic proteins thus expanding the biological repertoire. The nucleic acid protein interplay in the largely aqueous medium of the prokaryotes would have been less versatile than in the eukaryote system with lipid systems to accommodate lipid protein interactions.

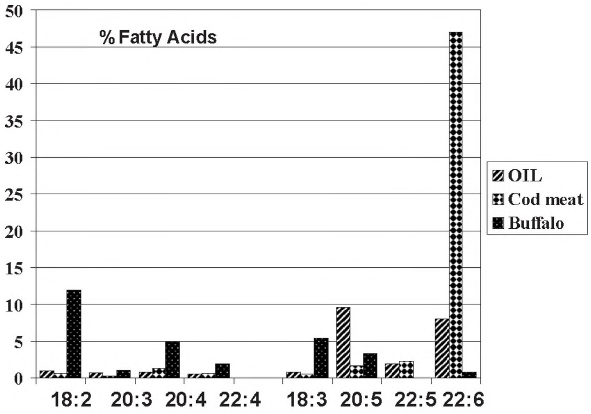

Fig. 1. Fatty-acid composition of cod muscle compared with cod liver oil and buffalo meat.

The cell membrane is the first point of contact between the cell and its external environment. The DNA determines the nature of the protein which cannot change in composition to the diet. However, we know cell behaviour responds to diet, temperature and pressure. In animals, the variable in the membrane is the lipid.

So it is the lipids that will respond to dietary influences and talk to the proteins which in turn influence the cell nucleus. Thus the variety of ways in which Nature could experiment with the design of organism would be greatly enhanced, particularly in view of the wide variety of temperatures and pressures as well as local chemical, energetic, and hence nutritional differences associated with the changing behaviour of the sun and radiation penetration, volcanic activity and as some claim greater intensity of meteorite bombardment. The latter two factors would have been much more active in the pre-Cambrian so again one comes back to the innovation of oxygen and lipids as the novelty opening the wider possibilities of gene environment interaction seen in the Cambrian explosion.

Vision as a trigger for neural development

Relevant to the species that ultimately led to human evolution was vision. The more efficient energy system of the aerobic metabolism led to the DHA/mitochondrial rich photoreceptor. For the previous 2.5 billion years, photon reception had converted photon energy to carbohydrates, proteins etc. In switching to the conversion of photons to "electricity", neural transmission was born, a nervous system evolved and then finally the brain.

The key structures of the neural signaling systems are lipid rich (60% of dry matter). The lipids involved are enriched with long-chain fatty acids

(20 carbon chain length and longer). Of special interest are the super-unsaturated fatty acids, particularly all-*cis*-docosa-4,7,10,13,16,19-hexaenoic acid (DHA). Although vision as we know it today is thought of in the context of eyes many other invertebrates that do not have obvious eyes have photo-sensitive systems. Figure 1 compares the composition of fish oil with cod muscle lipid and that of a land-based animal, the buffalo (*Syncerus caffer*). The cod muscle phospholipid content of DHA closely approximates the proportions used in the photoreceptor and synapse.

Lipid and cell function

The plasma membrane, long considered as a simple barrier between the extracellular and intracellular compartments, is being slowly recognised as playing a pivotal role in many physiological processes that respond to the communications from the environment to the cells. Lipids are closely associated with membrane proteins. Alteration of their composition alters protein function. Moreover, influencing their function alters signaling as well as shifting the dynamics of the individual fatty acids acting as ligands for nuclear receptors (Chawla *et al.,* 2001). The synthesis of lipid-derived second messengers with "activation of protein phosphorylation cascades has emerged as one of the fundamental mechanisms of signal transduction in animal cells" (Hindenes *et al.,* 2000, Underhaug Gjerde *et al.,* 2004, Rozengurt *et al.,* 2005).

On one hand changes in membrane structure contribute to the transcellular transfer of biological information. On the other hand the plasma membrane directly participates in intracellular signaling to the nucleus. The major actors implicated in these responses are in the variety of the polar phosphoglycerides, their composition and relationship to and balance with the non-polar lipids that constitute the rafts and caveolae and the domains around the lipophilic proteins. Evidence now exists for functional roles for individual molecular species of the phospholipids. Phosphatidylserine (PS) exposure on the cell surface accounts for the alteration of activities of several membrane proteins, including P2X7 as well as Ca^{2+} and Na^{+} transport through the P2X7 channel. Moreover, highly specific molecular species and their derivatives have now been identified in signaling as with arachidonyl-stearoyl diacyl glycerol derived from phosphatidylinositol (PI) activating protein kinase C (Hindenes *et al.*, 2000).

Recent work has identified the topology of almost all the inner membrane proteins in *Escherichia coli* and advances in nuclear magnetic resonance spectroscopy now allow the determination of alpha-helical membrane protein structures at high resolution. There is a view that these transmembrane proteins in eukaryotes stride across the membrane without any direct connection with the lipids of the bilayer. The role of the lipid is explained as exerting lateral pressure on the protein with the degree of desaturation determining the liquidity of the system. However, one has to ask why would a protein sit in the bilayer and not anywhere else? Two plausible explanations come to mind. First, one end of the protein has an affinity to the extracellular or outer membrane environment whilst the other has an affinity for the opposite, inner membrane environment. These opposites could be in response to hydrogen ion, electrochemical gradients or differences in K^{+} and Na^{+} concentrations.

Trans-membrane proteins are commonly depicted as ribbons or cylinders. They are of course not like that. They are an arrangement of peptide groups, aromatic and heterocyclic structures all of which are polarisable. The likelihood is that the lattice network of the lipids, with their twisting and writhing unsaturated acyl groups will provide opportunities for a three dimensional marriage between the lipid and the protein. It is almost a certainty that an optimised relationship will be in place in the bilayer. This is not a lock and key situation but then nor is it a stick in the sand. There has to be a thermodynamic relationship between the protein and the surrounding lipid which will be arranged to conform to the lowest energy in relation to the protein three-dimensional structure. The possibility of hydrogen bonding cannot be excluded.

Generally speaking, proteins can be separated from the membrane with mild conditions. By contrast, lipophilic proteins are difficult to crystallise and few structural determinations have been made, testifying to a strong protein-lipid interaction. So in effect there is a strong and weak force operating. One reason why the membrane protein lipid interaction is so poorly understood is because artificial membranes and reconstitution studies are frequently done with unreal membrane lipids e.g. studies of the hydrophilic loops in LacY from *E. coli* reconstituted in liposomes of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). The former does not naturally occur in mammalian phosphoglycerides.

The significance of microdomains influencing specific directions of signaling is also being recognised. Trans-membrane signaling requires modular interactions between signaling proteins, phosphorylation or dephosphorylation of the interacting protein partners and temporary elaboration of supramolecular structures, to convey the molecular information from the cell surface to the nucleus. Raft-based signaling pathways in T-lymphocytes have led to the suggestion of specific signaling compartments in raft microdomains. Moreover it has now become possible to visualize membrane proteins and lipids with atomic force microscopy (AFM) in living cells which describes a quite clear domain geography in the plasma membrane (Mouritsen, 2005).

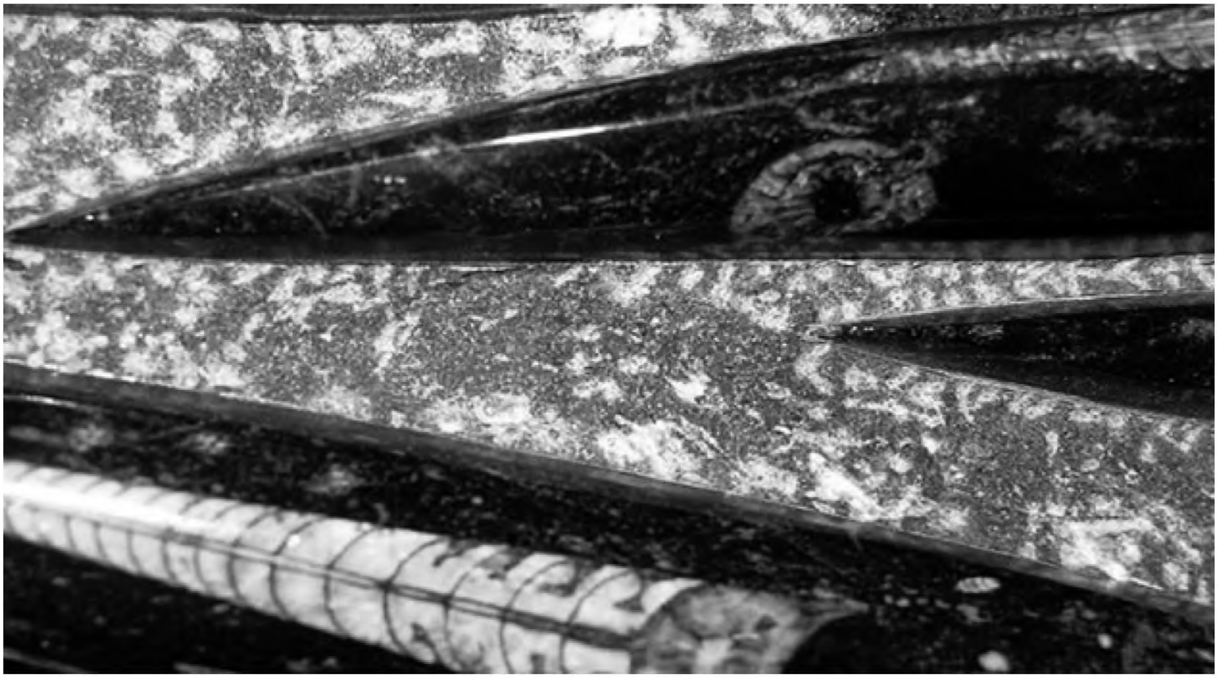

Fig. 2. The eye of orthocerus looking out from about 400 million years ago.

The language of DNA, proteins and lipids

There is a paucity of systematic information on lipids that are proximal and distal to membrane proteins. This is because of the difficulty of identifying the positions and measuring the relative effects of the lipids. There is a large number of lipid molecular species (words). A single double bond makes the difference to be or not to be a signaling molecule. This is not a liquidity issue as the difference made between say 4, 5, or 6 double bonds is minimal and insufficient to explain the striking conservation of molecules like DHA in signaling systems (Bloom *et al.*, 1999). The wealth of lipid molecular species is not unlike verbal language where several words can have similar meaning although a difference in subtle nuance. At the same time alteration of a single word in a sentence can change its function, for example replace "no" by "yes" or docosapentaenoic acid (DPA) by docosahexaenoic acid (DHA). Similarly the number of words in the language is an important determinant of the quality of expression and meaning. In that sense, the quality of the lipid language is undoubtedly of the highest biological order. The seductive robustness of the DNA is testified by the attempts to analyse the DNA of dinosaurs and mammoths which died out several thousand if not million years ago. By contrast the lipids most concentrated in signaling sites contain molecules most susceptible to peroxidative destruction.

Despite the wide genomic changes over the last 600 million years, DHA has been used in the photoreceptor and synaptic structures in the dynoglagelates, cephalpods, fish, amphibia, reptiles, birds and mammals, cf. Fig. 2. This extreme conservation is despite the fact that its immediate precursor all-*cis*-docosa-7,10,13,16,19-pentaenoic acid (ω3DPA - C22:5ω3), cf. Fig. 3, would have been more abundant, more readily synthesised and less susceptible to peroxidation (Bloom *et al.*, 1999). The omega-6 DPA would have also had the potential to replace DHA; however, it is seldom seen in natural products. It is the omega-6 product that appears in experimental omega-3 deficiencies. By contrast the omega-3 DPA is the major omega-3 in the cell lipids of the large herbivorous mammals (Crawford *et al.*, 1969) yet in none was the DHA replaced in the brain.

One could argue that for 600 million years whilst the genomes changed, the gene for DHA remained fixed; if so why? However, there are genes for enzymes involved in fatty acid synthesis but no known "gene for DHA". Conversely, as DHA stimulates the expression of over 107 genes involved in neural development, function and metabolism (Kitajka *et al.,* 2004), was the story the other way around? Was it the unique properties of DHA which determined the neural gene performance so rigidly for 600 million years? Nor can one neglect the role of rhodopsin which has similarly remained at the focal point of photoreception with its single molecule retinal from the lipid soluble vitamin A as the actual photon receptor. This remarkable consistency down some 600 million years during which genomics was in a fluid state of change, will have some, as yet unrecognised meaning for the manner in which cellular design was articulated during evolution of the species. At one conceptual level the protein could have been a determinant of the rigidity of structure and function.

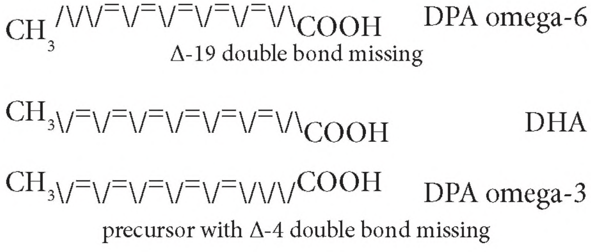

Fig. 3. DHA C22:6ω3 compared to two C22:5 fatty acids with only one double bond different, yet neither replaced DHA in 600 million years of evolution.

However there are two arguments against the protein determinacy. Firstly, the expectation of evolutionary change through measurements made on DNA modifications works in some but not all situations. Rhodopsin is not known for its variability throughout different species.

Secondly, analysis of the 450 million year puffer fish genome and that of other species describes a remarkable consistency for conserved gene families which, as an example, encodes trans-membrane proteins with fibronectin, immunoglobulin, leucine rich repeat domains (FIGLER) and the plasma clotting factors of 38 to 99% overall amino acid identity with their human counterpart (Munfus *et al.,* 2007). Hence some factor other than the standard rate of DNA mutation was operating to conserve certain functions. The 600 million year track record of DHA in visual and other signaling structures provides compelling evidence that the lipids were determinants of evolution.

The end of the Cretaceous and the evolution of mammals

The collapse of the dinosaurs is attributed to volcanism or a meteorite impact which invokes dramatic catastrophe as a sudden explanatory event. There could have been a simpler, Malthusian answer at work. A 120 ton dinosaur would consume 1,146,531 calories per day, on the assumption it was not far off current estimates. This calculation might be in error by several 10,000s or more but the giant dinosaurs would still have exercised a phenomenal rate of food consumption. The likelihood that the vegetation could keep up with this rate of consumption in face of a population explosion as suggested by the large number of fossils is questionable.

There are arguments that the giant carnivorous species should have kept the population in check but two interesting facts remain that are relevant to the subsequent phase of evolution. First, many of the plant systems escaped extinction. The late Early Silurian is associated with fossils of one of the earliest known fossil of vascular plant on land Cooksonia. That is about 425 million years ago. It was a small plant of only a few centimetres high. Propagation was by spores dispersed by the wind. The Triassic Period, 248-208 million years ago was connected with the evolution of the giant ginkgos, ferns and their allies and the giant reptiles. Ginkgos grow in many places and line the peripheral road that surrounds the Emperor's Palace in Tokyo for example. However, they are "bonsai" ginkgos compared to what they were 100 million years ago. The same applies to the ferns and their allies, except when you see them on the slopes of mountains whose trace elements have been replenished by volcanism as for example the higher slopes of the Ruwenzori or indeed in parts of New Zealand. This principle is well illustrated with for example the dramatic eruption of Mount St. Helens in 1980. Having destroyed much plant life even in the proudly well kept gardens of Portland, Oregon, the following years saw spectacular blossoms in Portland gardens and astonishing regeneration of the mountain slopes.

Secondly, the converse is the fact that the age of the dinosaurs was the age of the giant plants which lived in a moist, fertile soil. The dinosaurs when eating them would urinate and defecate into the swamps with the elements being lost in the run off into the rivers and oceans. Whether or not the carnivores could keep the population in check, the prodigious amount of vegetation eaten and the excretion of the trace elements would surely have depleted the soil so that the plant life would loose its giantness. Hence two natural forces were operating in opposite directions, the body size of the reptiles and the fertility of the soil. When demand outstripped the supply, collapse of the dinosaurs would have been inevitable. It may be that volcanism or meteorite impact was involved in the final demise. However, it may not have been necessary.

Interestingly, the depletion of the soil fertility would have added fertility to the oceans. Although the planet has lost its giants on land, we still have giants in the oceans: the blue whale. The nutrition of the whales provides another link to the determinacy of the lipids which will be returned to later.

This portrayal of the pre-history is somewhat simplistic. Nonetheless, a key issue of the collapse of the dinosaurs is that the gentler flowering plants, which in order to survive in the more depleted soil systems, developed well protected seeds. Seed bearing plants had been evolving for some time and so also was the co-evolution of the mammals and pollinating insects.

The Cretaceous, omega-6 fatty acids and mammals

The evolution of the mammals towards the end of the Cretaceous period resulted in a leap in relative brain size compared to any living system in the previous 500 million years. The cod may lay up to a million eggs at a time, whilst the land based reptiles would lay 12 or more. In each case the offspring get one dose of brain-specific lipid nutrients. The biological breakthrough for the evolution of larger brains came with the evolution of the placenta which perfuses the new off-spring with brain specific nutrients (Crawford, 2000). A model of the switch to the placenta can be constructed from the involvement of the arachidonic acid derived adhesion molecules attaching the fertilized egg to the uterine wall. The process of placentation begins with implantation of the blastocyst beneath the uterine epithelium and differentiation of trophoblast cell lineage into embryonic and extra-embryonic structures of conceptus (Cross, 1998). This invasive behaviour follows a precise chronology of vascular events during the first weeks of gestation (Kingdom *et al.,* 2000a; 2000b). These events involve placental tissue angiogenesis, organogenesis and progressive establishment of the two vascular circulations within the placenta in preparation for the second phase of pregnancy of fetal growth (Dantzer *et al.*, 2000). The placenta is a vascular system with the maternal blood circulating on one side of a membrane and that of the embryo/fetus on the other, i.e. the novelty in the evolution of placental mammals was a sudden increase in vasculogenesis.

This change in reproductive plan is unlikely to have been the result of a single mutation. It is more likely to have resulted from conditions which favoured vascularisation rather than calcification

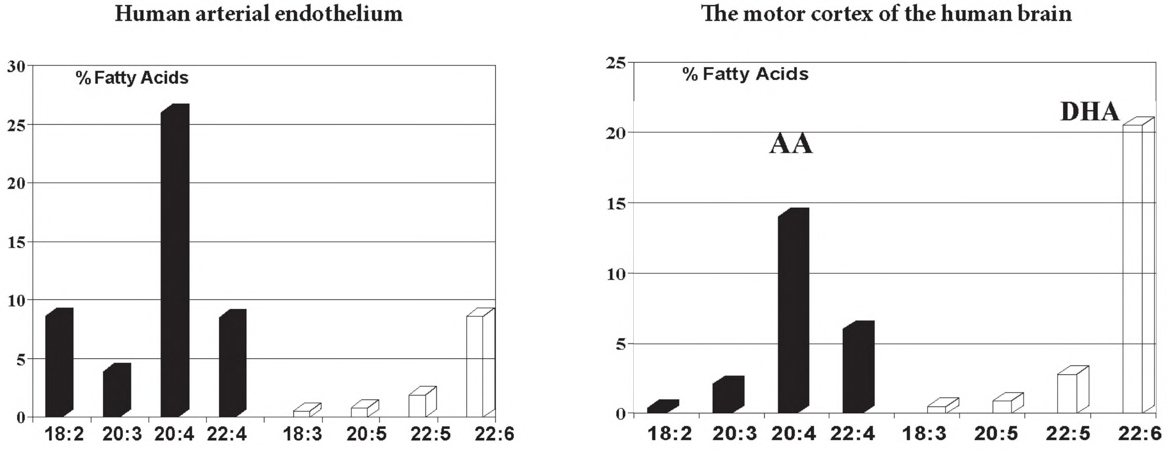

Fig. 4. Inner cell membrane lipid (ethanolamine phosphoglycerides) in the artery and in the brain. ■: ω-6 □: ω-3

(egg laying). Imprinting of the genetic mechanism would have followed. The end result leaves little doubt. The placenta is an extensive and extremely fast growing vascular network. In all mammals, this processing by the placenta supervises the early phases of brain development that eventually reached a peak in *H. sapiens.* The continuous perfusing of the product of conception provided a substantial biological advantage for brain growth compared to the one shot, external egg. The question to be asked is what was it that enabled the development of the placenta to take place?

The interesting question is how did the system of reproduction change from egg laying to placental mammals?

The difference between laying the egg externally and the entrapment of the embryo which adheres to the wall of the uterus could be explained by the several adhesion and angiogenic molecules that would have arisen along side the increasing concentrations of arachidonic acid (AA). The hypotheses we suggest is that this novelty in evolution was driven by omega-6 fatty acids and in particular AA. There is much money made and publicity given today about the value of fish oils, eicosapentaenoic acid (EPA) in particular, to suppress AA activity. Hence significant amounts of AA would have been necessary to change the biochemical scene. The inner cell membrane lipid of the human endothelium is AA rich as opposed to that of the brain which is DHA rich, cf. Fig. 4. The omega-3 fatty acids would have dominated the food chain during previous evolutionary epochs as the first 3 billion years of life was fed from photosynthesis of the marine type and on land by photosynthetic green leaves, resulted in an omega-3 rich food chain.

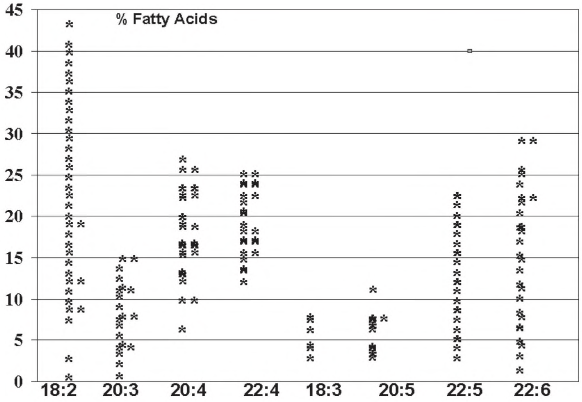

Fig. 5. Liver essential fatty-acid composition (ethanolamine phosphoglycerides).

The evolution of the mammals coincided with the evolution of flowering plants with protected seeds. The seeds contained oil stored as energy for the growth of the new plant in the next season. For the most part the seed oil contained linoleic acid, the parent omega-6 fatty acid. Previously the fish living in an omega-3 rich environment required omega-3 fatty acids. A new evolutionary break point emerged alongside the introduction of a rich source of omega-6.

Whereas the omega-3 fatty acids are essential for the reproduction of fish, omega-6 is required for mammalian reproduction. The omega-6 family includes eicosatrienoic and eicosatetraenoic (AA) acids. Both these membrane fatty acids are precursors for vasodilatory, anti-platelet adhesion and anti-thrombotic eicosanoids. At the same time AA is the precursor for adhesion, thrombogenic and smooth muscle tone molecules. Moreover, the membrane lipids of the vascular endothelium and the placenta itself are very rich in AA. To test if this high concentration of AA in the placenta was either a collection during the course of pregnancy or intrinsic we studied the earliest placentas from elective abortions. In fact the proportions of AA in the membrane phosphoglycerides were higher at the beginning of pregnancy than at term, demonstrating that these high concentrations were intrinsic and not acquired (Bitsanis *et al.*, 2006). Indeed, the concentrations of AA in the earliest placentae were actually the highest we have seen in any mammalian, tissue phosphoglyceride.

Studies on the lipid language of the liver lipids cf. Fig. 5, in some 42 mammals described a striking species variability which was clearly dependent on the type of food eaten and velocity of body growth: the faster the rate of body growth the less DHA was synthesised. Even the rat pup brain incorporates DHA early and preferentially to its synthesis with an order of magnitude greater efficiency (Sinclair and Crawford, 1972a; 1972b).

If you are a large fast-growing mammal, the only solution to accumulating DHA is to eat it. Fish and sea food being the richest source suggests that the evolution of *H. sapiens* would have had a substantial advantage from being coastal over others who were living inland (Crawford, 1992; Leigh Broadhurst *et al.,* 1998; 2002; Crawford *et al.,* 1999). All the large inland mammals and primates simply loose DHA and brain capacity shrinks as they evolved larger and larger bodies with increased growth velocities. Small mammals such as the squirrel, *Sciurus carolinensis*, and cebus monkey, *Cebus capucinus*, have about 2.5% of their body weight as brain. The chimpanzee, *Pan troglodytes*, has 0.4%, the much bigger rhinoceros, *Perissodactyla rhinocerotidae*, less than 0.02%. The only parallel example of a large mammal with a relatively large brain body weight ratio is the dolphin, *Tursiops truncates*, which has a brain weight of 1.8 kg, a ratio of just over 1%, the sperm whale has 8 kg of brain. Albeit in a very large body it is massively greater than any seen on land. The dolphin 1.8 kg compares with the brain weight of a similar sized-lands mammal as for example that of the zebra, *Equus quagga*, is only about 350g. Modern humans with 1.4 kg have a brain capacity of 2% which is a bit smaller than that of the squirrel. So the chimpanzee losing out with 1 kg less of brain compared to *H. sapiens* can be explained by the lack of availability of preformed DHA in its chosen food web. The dolphin on the other hand is obviously constrained by the low availability of arachidonic acid as both AA and DHA would have been required to the successful evolution of the brain (see Fig. 6). The point is that *H. sapiens* does not really have a large brain compared to a squirrel.

What *H. sapiens* did was to find an ecological niche which enabled both body size and brain size to advance in a harmony of growth. The only niche that satisfies the biochemical requirements is the land-water interface, which during the period of evolution would have been the richest food resource on the planet.

The fatty-acid composition of the motor cortex of the brain in 42 species, cf. Fig. 6, shows that it is closely identical regardless of species and dietary strategies (Svennerholm, 1968; Crawford and Sinclair, 1972; Crawford *et al.*, 1993; 1976; Williams *et al.,* 1987). The variable is the extent to which the brain evolved not the chemistry. Variation is a determinant of function (Messeri *et al.,* 1975). In this context AA and its elongation product contribute quantitatively to neural structures similar to the total omega-3 content. Moreover, there is more long chain omega-6 than omega-3 in the brain where the DHA is mostly concentrated in the synapses where it is preferentially taken up (Suzuki

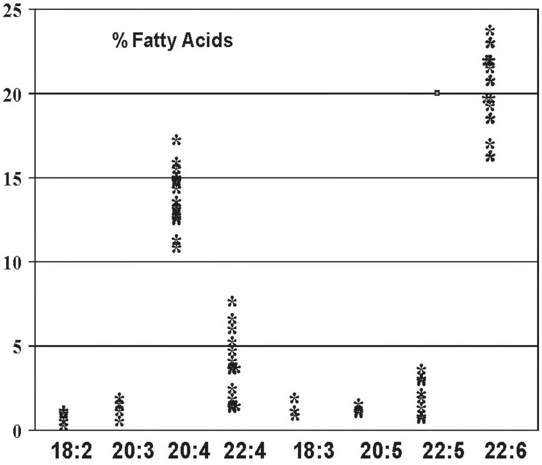

Fig. 6. Brain essential fatty-acid composition (ethanolamine phosphoglycerides). Adapted form Crawford *et al.* (1976) and subsequent publications and data.

et al., 1997). Consequently the addition of a rich source of omega-6 fatty acids in the build up to mammalian evolution by the Cretaceous period the evolution of the omega-6 requiring mammals, vascular and cerebral expansion is unlikely to be a coincidence. It is more likely that the disparate evolutionary paths of relative brain size on land and in the sea were defined by the availability of AA and DHA preformed together, with their associated micronutrients such as iodine, selenium, copper and zinc etc. Today, according to the World Heath Organisation, there are about 1.6 billion people at risk to iodine deficiency disease, the simplest way to produce mentally retarded children. That is nearly a fifth of the human population and the people at risk are inland communities. As the same communities are those at high risk to vitamin A deficiency it would be unsurprising of they we also relatively deficient of omega-3 fatty acids, DHA in particular.

A good test of the concept of lipids as evolutionary determinants is the radiation of the land mammals from their omega-6 food web into the omega-3 rich marine environment which started over 50 million years ago. We reported significant amounts of AA in the muscle, liver and brain of dolphins (Williams *et al.*, 1987). However, the dolphin was one of the most recent migrations into the sea, the gray whale one of the most ancient. The Table 1 below shows results from work being done on the migration of the gray whale from the Bering Sea in the Arctic to their breeding lagoons in Bahía Magdalena (Caraveo-Patiño *et al.*, 2008).

	Blubber biopsies n=6			Liver n=2		Muscle biopsies n=4			
Fatty Acid	Triglyceride			PC	PE	PC		PE	
18:2 ω6	1.8	±	0.4	0.39	0.22	0.39	±0.02	0.17	±0.06
18:3 ω3		nd		0.02	0.02	0.01	±0.02	0.02	±0.02
20:4 ω6	1.5	±	0.3	5.76	16.93	4.72	±0.20	10.08	±0.49
20:5 ω3	18.1	±	7.9	2.71	5.52	7.47	±0.37	15.44	±0.33
22:6 ω3	7	±	2.8	6.44	8.07	1.18	±0.03	3.49	±0.31

Table 1. Gray whale muscle and liver contain significant amounts of AA (data from a paper by Caraveo-Patiño *et al.* (2008)).

As the cold polar food web where the whales spend most of the summer are particularly omega-3 rich, the observations by Caraveo-Patiño pose the question from where are they getting their AA which exceeds the DHA in liver and muscle inner cell membrane lipid the ethanolamine phosphoglycerides (PE). The AA is less but still prominent in the largely outer membrane lipid, the choline phosphoglycerides (PC).

These whales are large animals and the proportion of AA in an adult gray whale works out at about 0.2 ton of AA rich lipid with about 1/10 as AA. Caraveo-Patiño *et al.* (2008) speculate that the reason for this longest of migrations is to obtain AA which is present in significant amounts in the warm water food web. This surprising conservation of AA in marine mammals again emphasises the role of lipids in determining evolutionary directions. What is interesting about this example is that the lipid requirements are not only constraining the biochemistry but also the non-genomic behaviour in the migration of this large mammal. Note that the blubber contains little detectable AA which is all sequestered for cell membranes.

The evidence on (i) the extreme conservation of DHA in the photoreceptor, neurones and synapse taken together with (ii) the coincidence evolution of omega-6 fatty acids with the mammals raise interesting possibilities that the lipids were actually determinants of evolution. Similarly, the use of AA for mammalian reproduction has been conserved for the best part of 200 million years and in the case of the gray whale, conserved despite living in an omega-3 rich environment for over 50 million years. That is, lipid conservation outstretches the genomic changes over the last 600 million years with DHA and the last 100-200 million years for AA in mammalian evolution. No matter what genomic changes occurred in the 600 million years since the Cambrian explosion, gene mutation did not find an alternative to DHA. So was it the other way round? Was the genomic capability constrained by the lipidomics and other environmental factors? Are these constraints, examples of Darwin's "conditions of existence"?

This evidence bears strongly on the final evolution of the human brain which could not have been on the savannahs of Africa and had to be at the land-water interface to satisfy these powerful twin requirements for AA and DHA. The land water ecological niche provided the best of two world's omega-6 from land and omega-3 from the marine and fresh water systems. As Philip Tobias said at his seminal "Dual Congress" on biology and paeleo-anthropology, in Sun City, South Africa in 1998 "wherever man was evolving, he had to have water to drink".

It is clear that the constraints by chemistry and physics on evolution are not just confined to lipids. Many other examples can be found such as the use of iron in heme proteins and indeed was a thesis of Ernst Baldwin (1947) then professor of biochemistry in London and discussed at a PNAS conference in 1954 (Woodring, 1954). In 1954 the influence of nutrition on the cell membrane and its impact on signaling and gene expression was unknown. The evolutionary paradigm was the Weismann (1893) "all sufficiency of natural selection" so people bent over backwards to interpret systems in this context. Stephen J. Gould raised a serious question about the all sufficiency paradigm pointing out the sudden breaks in evolution followed by long periods of stasis, "punctuated evolution" which seemed inconsistent with the Weisman view adopted by many modern evolutionary biologists (Gould, 1982). It therefore seems that there were forces at work in evolution which as Darwin claimed were more powerful than natural selection and by implication than genomics. This force was Darwin's "conditions of existence" and his pangenomics which now finds evidence in epigenetics and the influence of external conditions on membrane physics, signaling and gene expression. The compelling fact about the lipids is to repeat what has been said, the DNA does not change in a century so nor do the proteins, it is the membrane lipids that changed and were associated with a change in size shape and disease pattern in one century and with still more to come. The introduction of cell complexity and organisation depending on the elaboration of complex lipid membranes in the Cambrian explosion, suggests that lipids have played a pivotal role in evolution since then.

The implication for nutrition, food and health policy in this century and the questions raised by the dependence of the human brain and vascular systems on specialised lipids cannot be underestimated. With brain disorders having overtaken all other burdens of ill health in the EU and predicted by the Global Forum of Health (www.g;obalforumhealth.org) to be in the top three burdens of ill health worldwide by 2020 the gravity of the present situation is supreme. At stake is the future of children yet to be born.

Summary

Unlike DNA, lipid composition varies with nourishment and environment (chemistry, temperature and pressure). The DNA does not change in composition in response to diet so neither does protein composition change. The cell factor that readily changes in response to both environment and diet is the lipid component. Just as the environmental medium set the unchanging life forms for the first 2.5 billion years of prokaryotic life on the planet, is it possible that the influence of environment and the chemistry of the food web on lipids affected protein function, signaling and gene expression in a manner that actually set the boundaries and paths for evolution? In that way the lipids would have been regulating and defining the subsequent aerobic evolutionary gene potential and metabolic pathways consistent with Darwin's concept of "conditions of existence". If so, then with the changing lipid environment since the domestication of plants and animals in the Fertile Crescent 10,000 years ago, it is likely that human evolution is still being manipulated by lipidomics. The changing pattern of non-communicable disease is evidence of this evolutionary process in action. There is little doubt in Europe and America people have changed in shape, size and disease pattern in one century. That change is not due to a change in genome. The continued evolutionary change of *Homo sapiens* is beginning to be suggested by others (World Science, 2007) as affecting physical and mental states. The concern today is the rise in brain disorders which have now outstripped all other burdens of ill health in the European Union and pose what is by far the most serious threat in relation to health in society. This discussion raises concerns about present and hence future food and aquatic policies which have yet to take into consideration the impact of nutrition on neural development and degeneration.

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