TOPICAL REVIEW

Aerobic metabolism underlies complexity and capacity

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The evolution of biological complexity beyond single-celled organisms was linked temporally with the development of an oxygen atmosphere. Functionally, this linkage can be attributed to oxygen ranking high in both abundance and electronegativity amongst the stable elements of the universe. That is, reduction of oxygen provides for close to the largest possible transfer of energy for each electron transfer reaction. This suggests the general hypothesis that the steep thermodynamic gradient of an oxygen environment was permissive for the development of multicellular complexity. A corollary of this hypothesis is that aerobic metabolism underwrites complex biological function mechanistically at all levels of organization. The strong contemporary functional association of aerobic metabolism with both physical capacity and health is presumably a product of the integral role of oxygen in our evolutionary history. Here we provide arguments from thermodynamics, evolution, metabolic network analysis, clinical observations and animal models that are in accord with the centrality of oxygen in biology.

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Physiological studies frequently deal with the extremes of functional capacity. The extremes are exemplified by fascination with Olympic competitors and the difficulty of those struggling with disease and age-related decline. Combinations of reductionist and integrative (or systems) approaches are often used for discovery of information about these extremes. One integrative method is to utilize scientific laws to generate hypotheses for which tests can be devised.

In general, the laws of thermodynamics provide a statement about the most probable behaviour of an isolated system. Law One has no known exceptions and the operation of Law Two is highly probable for macroscopic systems with masses of more than a few picograms (Landau & Lifshitz, 1980). In deference to the complexity of living systems, it seemed logical to base biological hypotheses upon highly probable states as predicted by thermodynamics. Here we synthesize information from a wide range of disciplines to argue that a central role for oxygen metabolism has high probability for any biological system. Arguments are mostly based upon circumstantial evidence and we provide one remote experimental test.

For our first step, we considered evolution as a thermodynamic event to formulate this hypothesis: The steep thermodynamic gradient of an oxygen environment was permissive for the evolution of multicellular complexity. A corollary of this hypothesis is that aerobic metabolism underwrites complex function mechanistically at all levels of biological organization.

Organisms can be considered as systems in thermodynamic disequilibrium (Qian & Beard, 2005) that exist by the exchange of low-entropy inputs for high-entropy outputs to yield a continuous transfer of energy that can be converted to do work (free energy). This definition presumes that biological properties derive from and operate within the Laws of Thermodynamics. Law One is the conservation of energy, commonly expressed as:

$$\mathrm{d}U = \delta Q - \delta W \quad (1)$$

Law Two is the trend for concentrated energy to disperse with time as measured by entropy (S). Entropy is generally related to the heat term (δQ) by:

$$\mathrm{d}S = \delta Q / T \geq 0 \quad (2)$$

(where $\mathrm{d}U$ is the change in internal energy of the system, δQ is the heat added to the system, δW is the work done by the system, $\mathrm{d}S$ is the change in entropy and T is temperature). The most pertinent concept is that, for an isolated system, a process occurs only if it increases the total entropy of the system.

From this, we presume it axiomatic that a transfer of free energy was necessary and antecedent for the transition from inanimate to animate (biogenesis) and for all subsequent steps of escalating biocomplexity.

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This view implies that the initial and continued driving force behind evolution is related directly to capacity of selectable replicating units to transfer free energy that is obligatory for change (Baldwin & Krebs, 1981). Anaerobic glycolysis apparently yielded enough energy transfer for the evolution of complex pathways for single cell organisms in an atmosphere with essentially zero oxygen. The subsequent build-up of an oxygen environment provided a widened redox potential for development of a highly exergonic metabolism requisite for the development of complex multicellular organisms. For this review, we start historically and then outline examples that suggest oxygen metabolism is a prime mechanistic determinant of our current biological complexity and capacity.

Origin of biological complexity

Single-celled life originated approximately 3.7 billion years ago (Ga) in an anoxic atmosphere by development of glycolytic pathways that are extant for all known organisms (Baldwin & Krebs, 1981; Ronimus & Morgan, 2003). Despite the wide success of glycolysis, there are no known examples of multicellular complex organisms that are exclusively anaerobic (Catling *et al.* 2005). Biocomplexity apparently required the steep thermodynamic gradient of an oxygen environment.

Earth's oxygenation history (Catling *et al.* 2005; Falkowski *et al.* 2005; Holland, 2006) has been assembled from geochemical studies (Fig. 1). Cells capable of anoxygenic photosynthesis were present about 3.3 Ga. These single celled organisms reduced CO_2 into organic fuels by oxidation of molecules such as hydrogen sulphide ($CO_2 + 2\,H_2S \rightarrow CH_2O + 2\,S + H_2O$). By 2.5 Ga, oxygenic photosynthesis ($CO_2 + H_2O \rightarrow CH_2O + O_2$) became established and initiated the Great Oxidation Event (GOE) with atmospheric oxygen increasing to a partial pressure of about 15 mmHg by 2.0 Ga. During the next one billion years aerobic respiration and small, non-complex, multicellular organisms became widespread in an atmosphere of oxygen that remained at about 15 mmHg. From 1.0 to 0.5 Ga atmospheric oxygen rose to its current value of about 150 mmHg. This increase was associated with an escalating development of complexity that included the Cambrian explosion during which all the major animal phyla appeared. Hedges *et al.* (2004) used protein sequence data and molecular clock methods to estimate the timing of the rise in the number of different cell types. Organisms with two to

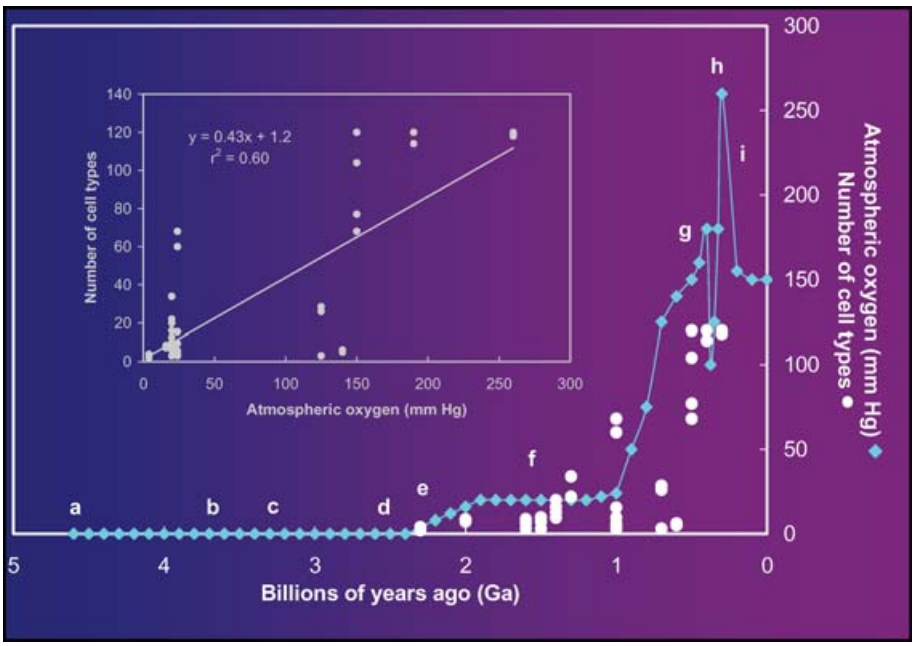

Figure 1. Oxygen and complexity
The blue line is atmospheric oxygen in mmHg as estimated from geochemical studies (Catling *et al.* 2005; Falkowski *et al.* 2005; Holland, 2006). White circles are the number of cell types present as estimated using protein sequence data and molecular clock methods (Hedges *et al.* 2004). Inset regression is number of cell types *versus* atmospheric oxygen. Every 2.32 mmHg increase in oxygen was associated with an increase of one cell type. Lower case letters indicate approximate timing of major events: a,earth formed; b, first living cells; c, anoxygenic photosynthesis; d, oxygenic photosynthesis; e, great oxidation event; f, aerobic respiration widespread; g, Cambrian explosion; h, insect gigantism; i, placentals. The coincident increases in atmospheric oxygen and complexity are suggestive of mechanistic association.

three cell types appeared shortly after the initial increase in atmospheric oxygen (2.3 Ga) with further increases up to 120 cell types by 0.5 Ga (Fig. 1).

Catling *et al.* (2005) use the term 'limited tool kit' to emphasize that formation of life is confined to the use of the 85 stable elements occurring in the universe. As a probability, it seems reasonable to hypothesize that formation of life anywhere would follow the earth's pattern and be established out of the most abundant elements of the universe. The most abundant 10 elements of the universe are hydrogen, helium, oxygen, carbon, neon, nitrogen, magnesium, silicon, sulphur and iron, of which seven are represented in organisms (Klemperer, 2006).

Oxygen is the third most abundant element and has features of special importance with regard to the metabolism of energy transfer. First, molecules of life are presumably carbon-based because of this element's ability to form complex molecules for information storage and energy transfer. Among the elements only nine are more electronegative on the revised Pauling scale (Pauling, 1932; Allred, 1961) than carbon and thus able to serve as an acceptor of electrons from carbon-based fuel substrates. Of these nine (selenium, sulphur, iodine, krypton, bromine, nitrogen, chlorine, oxygen, fluorine) oxygen ranks second only to fluorine in electronegativity and the other elements are either a solid, highly reactive, less abundant, or substantially lower in electronegativity. Thus, reduction of oxygen provides for close to the largest possible transfer of energy for each electron transfer reaction. Second, diatomic ground state triplet oxygen is structurally stable because of non-polar covalent bonds which allow it to accumulate and distribute freely as an atmospheric gas. Third, a terminal oxidant in the form of a gas seems more likely relative to a liquid or solid. A gas would distribute in the atmosphere thus not limiting habitat, be transported internally with higher efficiency because of lower viscosity, and allow for rapid diffusion in a distribution system (Catling *et al.* 2005).

Large-scale evolutionary events

If biocomplexity is linked mechanistically with atmospheric oxygen in our evolutionary history, then specific large-scale examples of this association should be observable. Indeed, the influence of oxygen upon biology is recorded via the global distribution of animal size (polar gigantism), insect gigantism, oxygen incorporation into membrane proteins, terrestrialization of animals (Romer's Gap) and climate change in marine species.

Polar gigantism. The trend of animals to be larger at higher latitudes is termed polar gigantism and has not been adequately explained by low temperature or metabolism. Investigation of gigantism requires evaluation of widely distributed taxa with extensive species representation at numerous latitudes. Chapelle & Peck (1999) measured body length in 1853 species of benthic amphipod crustaceans from 12 sites worldwide that included polar, tropical, marine and freshwater environments. They found a strong association between maximum body length and oxygen content ($r^2 = 0.98$, $P < 0.0001$) and not a significant association between minimum size and oxygen content ($r^2 = 0.16$, $P = 0.195$) of water. Peck & Chapelle (2003) also found that crustaceans at high altitude (Lake Titicaca in the Andes Mountains, 3809 m) have a maximal length 2–4 times smaller than observed at other low-salinity sites closer to sea level. Consistent with these influences of oxygen in natural environments, Frazier *et al.* (2001) report that hypoxia (10%) decreases and hyperoxia (40%) increases body mass of *Drosophila melanogaster* in laboratory conditions. These observations support the idea that oxygen availability mechanistically underlies polar gigantism.

Insect gigantism. Geophysical analyses suggest the presence of an atmospheric oxygen pulse (Fig. 1, point h) about 300 million years ago (Berner *et al.* 2000). Driven by plant terrestrialization, atmospheric oxygen may have reached 35% (260 mmHg) at the peak. Coincident with this rise and fall in atmospheric oxygen was a dramatic rise and fall in the size of insects that has been termed the era of insect gigantism (Dudley, 2000). The pulse in atmospheric oxygen apparently presented a new environment for evolutionary change in body size and locomotor capacity via enhanced flux within diffusion-limited tracheal systems and increased aerodynamic forces associated with denser air.

Oxygen incorporation into membrane proteins. Part of the transition from procaryote to eucaryote complexity involved the development of intracellular compartments bounded by selective membrane barriers. Acquisti *et al.* (2007) demonstrate the usefulness of an atomic analysis of proteins as compared to function commonly assumed to be associated with amino acid sequence. They found that the time of appearance of cellular compartmentalization correlates with atmospheric oxygen concentration. Transmembrane proteins initially excluded oxygen in ancient ancestral taxa when atmospheric oxygen was close to zero but this constraint decreased when atmospheric oxygen levels rose. That is, the relative number of transmembrane proteins containing amino side chains high in oxygen content correlated with historical atmospheric oxygen in 19 taxa (ranging from *Halobacterium* sp. to *H. sapiens*). They hypothesized that oxygen-rich protein domains were selectively excluded under low levels of oxygen because the reducing atmosphere would have

made such structures unstable. Thus, it appears that atmospheric oxygen concentration influenced the timing of the evolution of cellular complexity associated with compartmentalization.

Romer's Gap. The fossil record provides evidence that vertebrates started terrestrialization about 415 million years ago (Ma). Subsequently, the record declined and essentially disappeared for the interval of 360–345 Ma. This 15 million year decline in vertebrate colonization of land is known as Romer's Gap and until recently it was unknown if this interruption was due to unfavourable fossilization conditions, collection failure or an interval of low diversity. Ward *et al.* (2006) tested the hypothesis that Romer's Gap is accounted for by environmental factors by examining the ranges of terrestrial arthropods over the same time interval. They demonstrate that the geochronological range of terrestrial arthropods has a pattern similar to that of vertebrates (Fig. 2). That is, few new taxa developed for both limbed vertebrates and arthropods during Romer's Gap. For mechanistic explanation, they provide a model demonstrating that Romer's Gap coincided with and is explained by low atmospheric oxygen. Their model supports the general hypothesis that atmospheric oxygen was a major driver of successful terrestrialization for arthropods and vertebrates.

Climate change. Rapid global warming presents the challenge of understanding mechanistically the influence of thermal increases for individual organisms as they relate to the overall ecosystem. Our general thermodynamic arguments predict that thermal change, like essentially any challenge, will operate largely via influences on aerobic metabolism. Warming is particularly critical for fish because it produces oxygen limitations by both forced increases in oxygen metabolism (demand) and by decreases in oxygen solubility of water (supply). Portner &

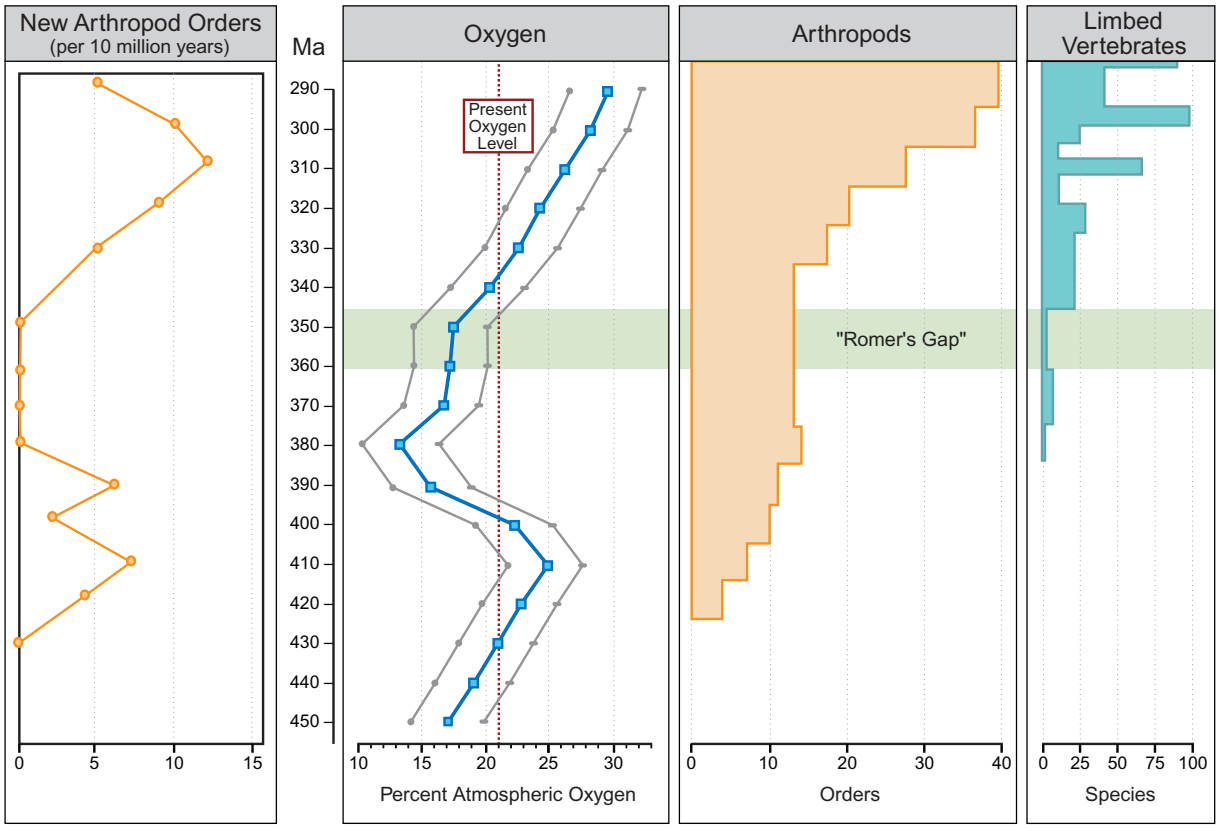

Figure 2. Romer's Gap
Diversity data for arthropods and limbed vertebrates shown in 10 Ma bins, plotted against atmospheric O_2 levels (±3% error margins). Over the study interval O_2 levels rise significantly above 21%, then subside to < 15%, before a subsequent and sustained increase. The first phase of land colonization by arthropods apparently is tied to rising atmospheric O_2 with a slight time lag. Although both arthropod and limbed vertebrate clades survive the low-O_2 interval, they do so at low standing diversity. The clades that do survive are composed of long-lived taxa. No new arthropod and very few digitized vertebrate taxa originate during the low-O_2 interval. A more dramatic second phase of land colonization is linked to a second, more elevated rise in atmospheric O_2. Figure from Ward *et al.* (2006) with permission from the American Association for the Advancement of Science.

Table 1. Substrates with the largest number of network links (data from Jeong *et al*. (2000; supplement 1)

1	H_2O	10	NADH	19	Phosphorenolpyruvate
2	ADP	11	CO_2	20	Acetyl-CoA
3	Phosphate	12	NH_4	21	H^+
4	ATP	13	CoA	22	Uridine
5	L-Glutamate	14	AMP	23	Cytidine
6	$NADP^+$	15	Pyruvate	24	UMP
7	Phosphate	16	Glutamine	25	CMP
8	NAD^+	17	Oxoglutarate	26	Glycerol
9	NADPH	18	Alpha-D-glucose 1-P		

Knust (2007) utilized data for marine fish and invertebrates from varying climates to generate the hypothesis that, 'as a unifying principle', the first mechanism to restrict animal tolerance to thermal extremes would be a mismatch between tissue oxygen demand and the capacity to supply oxygen to tissue. As proof of principle, they studied eelpouts (*Zoarces viviparous*) from the North and Baltic Seas to discover that thermally limited oxygen delivery closely matches environmental temperatures beyond which growth performance and abundance decrease. From this, they predict that deficits in aerobic capacity in warming seas will be the first process to cause extinction or relocation of organisms to cooler waters.

Network analyses

The importance of energy transfer pathways is demonstrated in three large-scale unbiased evaluations of biological connectivity. First, Barabasi and colleagues (Jeong *et al.* 2000) interrogated the topological properties of the core metabolic network of 43 different organisms (all three domains of life represented) based on data deposited in the WIT (What Is There) database (currently merged into PUMA2). This integrated database predicts the existence of a given metabolic pathway on the basis of the annotated genome of an organism combined with established data from the biochemical literature. A metabolic network is built up of nodes that are connected to one another through links, which are the actual metabolic reactions. This network was systematically investigated to quantify the topological properties of metabolic networks using the tools of graph theory and statistical mechanics. Analysis yielded two primary conclusions: (1) biochemical reactions connect through nodes as scale-free networks. That is, the number of connections per node approximates a power law, with a few very highly connected nodes, and (2) the most highly associated nodes were for pathways associated with energy transfer (Table 1).

Second, Barrett *et al.* (2005) used a genome-scale in silico reconstruction of the integrated transcriptional regulatory and metabolic network for *Escherichia coli* (iMC1010$_{v1}$) to computationally assess growth phenotypes. The visualized structure showed that the regulatory network governing metabolism in *E. coli* responds primarily to the available electron acceptor and to the presence of glucose as the carbon source. The result that the terminal electron acceptor and carbon source are what primarily organize the iMC1010^{v1} network in *E. coli* is consistent with function based upon non-equilibrium thermodynamics.

Third, to understand the changes in biochemistry and enzymology that accompanied adaptation to atmospheric O_2 Raymond & Segre (2006) integrated network analysis with information on enzyme evolution to infer how oxygen availability changed the architecture of metabolic networks. They evaluated the effect that the presence or absence of common biomolecules (e.g. oxygen) has on the complexity, size and connectivity of metabolic networks. This was achieved using a heuristic developed by Ebenhoh *et al.* 2004) and referred to as metabolic network expansion. A set of pre-specified 'seed' compounds was allowed to react according to enzymatic reaction rules as enumerated, for example, by the Kyoto Encyclopaedia of Genes and Genomes database (KEGG). A reaction could occur only if all of its reactants were present in the seed set. Once all possible reactions have been carried out, the products of those reactions then join the seed compounds, potentially allowing new reactions to occur. This process was iterated until no new products were generated and thus no new reactions possible (convergence). Their analysis revealed the existence of four discrete groups of networks of increasing complexity, with transitions between groups being contingent on the presence of these key metabolites: NAD^+, S-adenosyl methionine, Coenzyme A, ATP, O_2, CO_2, NH_3, pyruvate or 2-oxoglutarate. The most complex group IV reactions were associated almost exclusively with the presence of O_2 and had as many as 1000 reactions more than those of the largest networks achieved in the absence of O_2 (Fig. 3). These data support the general idea that O_2 availability was coupled to an increase in network complexity that might have been important in the evolutionary adaptation to an oxic atmosphere and the subsequent development of complex multicellular life.

A quantitative model links energy flux with rate of evolution

Gillooly *et al.* (2005) present a model that predicts heterogeneity in rates of molecular evolution by utilizing principles of allometry, biochemical kinetics and neutral theory of evolution. The model quantifies the relationship between rates of energy flux and genetic change based upon the effects of body size and temperature on metabolic rate. They define mass-specific metabolic rate (B) as it varies with body size (M) and temperature (T), as

$$B = b_0 M^{-1/4} e^{-E/kT} \quad (3)$$

where, b_0 is a coefficient and the body size term ($M^{-1/4}$) accounts for the fractal geometry of biological exchange surfaces and distribution networks. The Boltzmann factor ($e^{-E/kT}$) underlies the temperature dependence of metabolic rate, where E is an average activation energy for the biochemical reactions of metabolism (-0.65 eV), k is Boltzmann's constant, and T is temperature in degrees Kelvin. This equation explains most of the variation in the metabolic rates of organisms (Gillooly *et al.* 2001). They started with an expression of metabolic rate because it presumably has prime influence on the two factors thought to control mutation rate: (1) free radical production, and (2) generation time.

The metabolic rate (B) equation was combined with two assumptions to characterize the rates of molecular evolution. The first assumption is that evolution operates via nucleotide substitutions caused by neutral mutations that randomly drift to fixation. The second assumption is that point mutations produce nucleotide substitutions at a rate proportional to B. These two assumptions imply that the number of nucleotide substitutions per site per unit of time (α), varies with body size (M) and temperature (T) as

$$\alpha = f v B = f v b_0 M^{-1/4} e^{-E/kT} \quad (4)$$

where f is the proportion of point mutations that are selectively neutral and v is the number of point mutations per site per unit of metabolic energy expended by a unit mass of tissue.

Thus, fv is the neutral mutation rate per unit of mass-specific metabolic energy. If the temperature and body size dependence of nucleotide substitution rate is

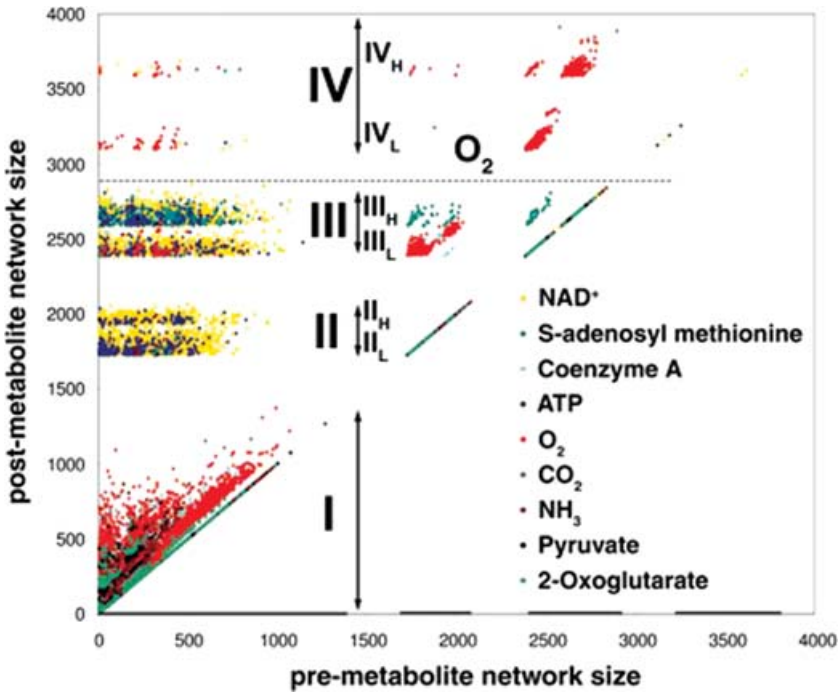

Figure 3. Oxygen and biocomplexity
The effect of metabolites on the number of reactions in metabolic networks, as computed with a network expansion algorithm. Each point represents two consecutively generated networks: the first network, whose size is the *x*-axis value, is generated from a randomly chosen set of seed metabolites, and the second network, whose size is the *y*-axis value, is generated from that same seed set amended with the addition of one of the nine indicated metabolites. Points are colour coded as based on the amended metabolite. All networks occupy four broadly similar groups that result from often very different but chemically interconvertible seed sets. Only networks that include O_2 as a metabolite transitioned into the most complex group IV reactions. The group IV had about 1000 more reactions than those of the networks achieved in the absence of O_2. Figure from Raymond & Segre (2006) and reproduced with permission from the American Association for the Advancement of Science.

controlled by B, then $f\,v$ is a constant and independent of M and T. Gillooly *et al.* (2005) provide extensive data from mitochondrial and nuclear genomes that are in accord with their model predictions. By accounting for the effects of body size and temperature on metabolic rate, their model explains heterogeneity in rates of nucleotide substitution for different genes, taxa and thermal environments. Importantly, this model suggests a single molecular clock that operates per unit of mass-specific metabolic energy rather than per unit of time. The general contention is that body size and temperature control the rate of evolution through their effects on metabolism. Our view is similar, except reverses cause and effect. That is, metabolism is the outcome of thermodynamic driven evolution, not the mediator.

Clinical observations

Above we argue that the steep thermodynamic gradient of an oxygen environment was the driving force for the evolution of multicellular complexity and that aerobic metabolism must underlie complex function mechanistically at all levels of biological organization. As a logical extension of these ideas, we propose that the aetiology of complex disease must also be tightly associated with oxygen metabolism. In accord with this thesis, clinical studies reveal a strong statistical link between low aerobic capacity and all-cause mortality. That is, large-scale clinical investigations demonstrate dysfunctional oxygen and energy metabolism in essentially all complex diseases.

In a broad perspective study, Myers *et al.* (2002) evaluated the predictive power of exercise capacity relative to other clinical variables. For 6.2 years they followed 6213 men referred for treadmill exercise testing for clinical reasons. Subjects were classified into two groups: 3679 had an abnormal exercise-test result and/or a history of cardiovascular disease and 2534 had a normal exercise-test result and no history of cardiovascular disease. Exercise capacity was measured in metabolic equivalents (MET) and overall mortality was the end point. In both healthy subjects and those with cardiovascular disease, the peak exercise capacity was a stronger predictor of an increased risk of death compared with established risk factors such as hypertension, smoking, diabetes, ST-segment depression, or the development of arrhythmias during exercise. In all subgroups, the risk of death from any cause in subjects whose exercise capacity was less than 5 MET was roughly double that of subjects whose exercise capacity was more than 8 MET (Fig. 4). Each 1 MET increase in aerobic exercise capacity was associated with a 12% increase in survival. The conclusion is that exercise capacity appears to be a more powerful predictor of mortality relative to other established risk factors for complex disease.

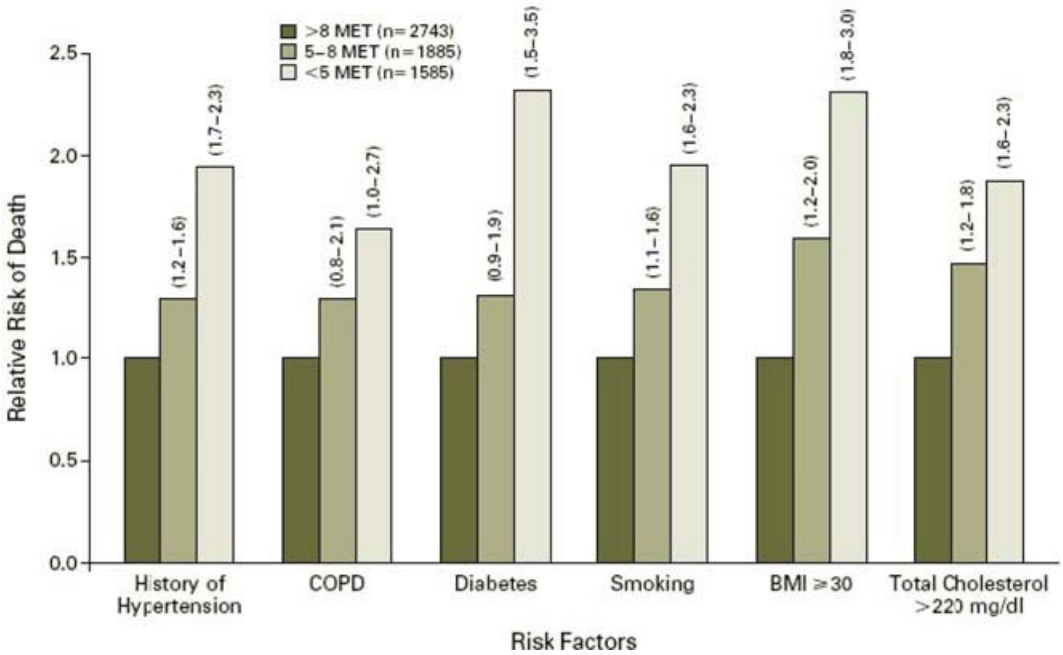

Figure 4. Aerobic capacity increases survival
Relative risks of dying from any cause among subjects with the indicated risk factors who achieved an exercise capacity of less than 5 MET or 5–8 MET, relative to subjects whose exercise capacity was more than 8 MET. Numbers in parentheses are 95% confidence intervals. BMI, body-mass index; COPD, chronic obstructive pulmonary disease; and MET, metabolic equivalent. Figure from Myers *et al.* (2002) and reproduced with permission from the author and the Massachusetts Medical Society.

Type 2 diabetes. Numerous studies have linked declines in aerobic capacity and mitochondrial function with type 2 diabetes. Petersen *et al.* (2003) reported that elders were markedly insulin resistant relative to young. This resistance, attributable to reduced insulin-stimulated muscle glucose metabolism, was associated with a 40% reduction in mitochondrial oxidative and phosphorylation activity. Mootha *et al.* (2003) applied Gene Set Enrichment Analysis (GSEA) and found that the expression of genes involved in oxidative phosphorylation was co-coordinately decreased in muscle from humans with type 2 diabetes. Expression of these genes was: (a) high at sites of insulin-mediated glucose disposal, (b) activated by a transcriptional coactivator of energy metabolism pathways (PGC-1α) and (c) correlated with total-body aerobic capacity. In accord with these clinical findings, studies in mice show that disruption of the nuclear gene encoding mitochondrial transcription factor A (Tfam) produces early onset diabetes, depletion of mitochondrial DNA and decreased oxidative phosphorylation in pancreatic islet cells (Silva *et al.* 2000).

Cardiac arrhythmias. Acute coronary artery occlusion that leads to ventricular fibrillation is a leading cause of death, especially in developed countries (Rodgers *et al.* 2004). While the incidence of sudden cardiac death is decreased by regular physical activity (Ekelund *et al.* 1988), the exact mechanism by which occlusion produces cardiac arrhythmias is not known. The work of Akar *et al.* (2005) suggests that ischaemia-related electrophysiological alterations and arrhythmias in intact hearts are at least in part a consequence of the failure of the cellular mitochondrial network to maintain inner membrane electrical potential. They found that preventing membrane depolarization by blocking the mitochondrial benzodiazepine receptor stabilized the action potentials of metabolically stressed cardiomyocytes, blunted ischaemia-induced action potential shortening, improved post-ischaemic recovery of the action potential, and prevented the occurrence of spontaneous arrhythmias upon reperfusion of the heart. In contrast, facilitating inner membrane depolarization with a mitochondrial benzodiazepine receptor agonist accelerated ischaemia-induced changes in action potentials, created regions of conduction block, and promoted sustained arrhythmias upon reperfusion. These findings support the hypothesis that mitochondrial function is critical for normal cardiac electrical activity.

Inflammatory response. Inflammation is a common feature of essentially all complex diseases. Calvano *et al.* (2005) used the Ingenuity Pathways Knowledge Base (KB) tool for evaluation of genome-wide expression arrays to identify functional networks responsible for the systemic activation and spontaneous resolution of a well-defined inflammatory challenge. The KB tool is based on findings presented in peer-reviewed scientific publications that were encoded into an ontology by scientific content and modelling experts. The tool computes a molecular network of physical, transcriptional and enzymatic interactions between mammalian orthologues (the 'interactome') based upon input from high-throughput platforms such as expression arrays. Gene expression in leucocytes was measured before and at 2, 4, 6, 9 and 24 h after the intravenous administration of bacterial endotoxin to four healthy human subjects. Four control subjects were treated the same, but were not administered endotoxin. Calvano *et al.* (2005) found that the response to acute systemic inflammation included widespread suppression at the transcriptional level of mitochondrial energy production. This result is consistent with the observation of reduced energy substrates in burn injury (Padfield *et al.* 2005), endotoxaemia (Crouser *et al.* 2002), and critically ill patients and animal models of sepsis (Brealey *et al.* 2002).

Longevity. Longevity and capacity at a given age can perhaps be considered the most relevant clinical phenotypes. Zahn *et al.* (2006) compared transcriptional profiles across ageing in humans, mouse and *Drosophila.* Although expression changes were species specific (private) for several pathways, only the electron transport pathway was decreased in association with ageing in all three species (public). These results suggest that changes in electron transport pathways may be the common signature that underlies ageing.

Cancer. Although physical exercise and aerobic capacity are associated with reduced cancer risk, the mechanisms are unknown (Bernstein *et al.* 2005). Recent work by Matoba *et al.* (2006), however, might provide some insight. It has long been known that cancer cells down-regulate aerobic respiration and preferentially utilize glycolytic pathways for energy transfer (Warburg effect). The debate is over whether the Warburg effect represents a fundamental and required feature of cancer or is just a by-product of a cell's transformation into cancer. Matoba *et al.* hypothesized that this shift to anaerobic metabolism must be mediated by a path commonly altered in cancer cells. Protein 53 (p53), a transcriptional factor best known as a tumour suppressor was selected as a candidate because of its high mutation frequency in cancers. They found a decrease in aerobic respiration in mouse liver mitochondria that correlated with graded disruption in going from the wild type ($p53^{+/+}$), to the heterozygotic ($p53^{+/-}$) and homozygotic ($p53^{-/-}$) conditions. In addition, they demonstrated that SCO2 (synthesis of Cytochrome c oxidase 2) mediates the down-

stream effects of p53 upon the COX (cytochrome c oxidase) complex. The work of Matoba *et al.* (2006) does not resolve the Warburg debate, but as a minimum, it defines a cancer-integrated 'switch' mechanism for conversion from aerobic to glycolytic metabolism.

Animal models of capacity

Our goal from about 20 years ago was to create an animal model that emulates the polygenic nature of a complex disease such as type 2 diabetes or hypertension. We thought that a mechanistically correct animal model would have high utility for invasive and efficient exploration antecedent to highly focused studies in humans. Development of such a model proved somewhat intractable and we rejected every known approach as not effective. Those we deem as flawed paths include: (1) Chemical and physical manoeuvers, such as administration of streptozotocin to mimic diabetes mellitus or ligation of coronary arteries to emulate arterial disease represent responses to injury, not the progression of disease. (2) Single or multiple gene knockout models are problematic because complex diseases generally result from expression of combinations of allelic variants sensitive to a given environment. That is, gene knockout only reveals essentiality of a gene and biological reorganization subsequent to its loss. (3) Mutagenic approaches, such as that produced by administration of the gametic mutagen ENU (ethylnitrosourea), are random and do not define allelic variants actually associated with disease. (4) Ostensibly, it seems that disease models produced by selective breeding would be highly useful. Yet, selection based upon measurable disease traits does not guarantee inclusion of the full complement of underlying mechanisms. This problem is amplified because chronic diseases emerge not as discrete events, but as complexes, such as the metabolic syndrome.

These seemingly intractable problems led us to search for more meaningful approaches to the development of animal models of complex diseases. We wanted to define the broadest possible feature mechanistically underlying the polygenic condition of complex disease. A paper by Baldwin & Krebs (1981) entitled: 'The evolution of metabolic cycles' triggered our view that evolution was a thermodynamic event related to the more optimal use of resources. That is, at every moment, selection weighs the benefit of a change for its value in energy transfer. Once we knew to focus on evolution as a thermodynamic event, the centrality of oxygen metabolism for underwriting complexity and disease was obvious. We now had fundamental ideas explanatory for the strong statistical linkage between low aerobic capacity and disease risks in man. As a remote test for these connections, we hypothesized that artificial selection of rats based on low and high intrinsic aerobic treadmill running exercise capacity would also yield models that contrast for disease risks.

Artificial selection means breeding individuals expressing the extreme values of a phenotype and is one of the more powerful tools of biology. Generating strains for the low and high extremes of a trait produces somewhat ideal models because contrasting allelic variation for the trait will be concentrated from one generation to the next. A phenotypic response to selection is possible if sufficient additive genetic variance (variance associated with the average effects of substituting one allele for another) exists in a population for that trait. Based on Fisher's 1930 Theorem of Natural Selection (Fisher, 1930), traits associated with evolutionary fitness are predicted to demonstrate less additive genetic variance because of more pressure from natural selection (Mousseau & Roff, 1987). Despite this, we decided to apply two-way (divergent) artificial selection for aerobic capacity in a mammalian species because of oxygen's central role in the evolution of complexity. The assumption was that enough additive genetic variance was available for aerobic function to allow responses to selection. We chose to first select for the simpler intrinsic component of aerobic function rather than the more complex adaptational component.

In 1996 Koch & Britton (2001) started large-scale selective breeding to develop strains of rats that contrast for intrinsic (i.e. untrained) aerobic treadmill running capacity. The founder population was 96 male and 96 female genetically heterogeneous rats (N:NIH stock). The 13 lowest- and 13 highest-capacity rats of each sex were selected from the founder population and randomly paired for mating. Running capacity was assessed by using an incremental velocity treadmill running protocol when the animals were 11 weeks of age (Koch & Britton, 2001).

Physiological traits. As is true for just about any complex trait, there was little doubt that divergent selection pressure would produce strains that differed for endurance running capacity. After 11 generations of selection, the low-capacity runners (LCR) and high-capacity runners (HCR) differed by 347% in aerobic running capacity (Fig. 5). We continued the selection and at 21 generations (completed in June, 2007) of selection the LCR and HCR differed by 461% in aerobic running capacity.

The exact timing and nature of emergent functional features across generations of selection were not predictable. In general, it appears that selection produced increases in endurance capacity by influencing peripheral and central components differentially across time (Gonzalez *et al.* 2006) (Fig. 6). After seven generations of selection maximal O_2 uptake ($\dot{V}_{O_2,max}$) was 12% greater in HCR compared with LCR. This difference was due exclusively to a greater O_2 uptake and utilization by skeletal muscle of HCR, without differences between the lines in

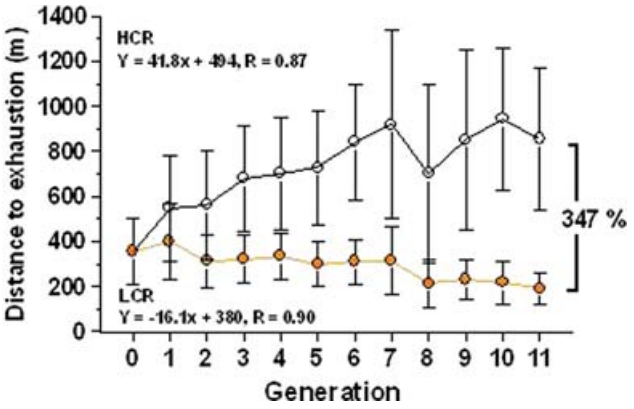

Figure 5. Models of aerobic capacity
Response to selection for aerobic treadmill running capacity across 11 generations ($n = 2912$ rats). On average the LCR rats decreased 16 m per generation and the HCR rats gained 40 m per generation in distance run to exhaustion. Values are mean ±1 S.D. Figure from Wisloff *et al.* (2005) and reproduced with permission from the American Association for the Advancement of Science.

O_2 delivery ($\dot{Q}_{O_2,max}$) to muscle by the cardiopulmonary system. At generation 15 $\dot{V}_{O_2,max}$ was 50% higher in HCR than LCR. The greater $\dot{V}_{O_2,max}$ in HCR was accompanied by a 41% increase in $\dot{Q}_{O_2,max}$ in HCR *versus* LCR. The greater $\dot{V}_{O_2,max}$ of HCR at generation 15 was due to a 48% greater stroke volume relative to the LCR. Although tissue O_2 diffusive conductance continued to increase in HCR, tissue O_2 extraction was not significantly different from LCR at generation 15, presumably because of the offsetting effect of greater HCR blood flow on tissue O_2 extraction. These results indicate that continuing divergence in $\dot{V}_{O_2,max}$ between lines occurs largely as a consequence of changes in the capacity to deliver O_2 to the exercising muscle.

Disease risks. The first test of the hypothesis that artificial selection of rats based on low (LCR) and high (HCR) intrinsic aerobic treadmill running exercise capacity would also yield models that contrast for disease risks was conducted in rats from generations 10 and 11 of selection (Wisloff *et al.* 2005). The LCR scored higher on cardiovascular risks and features of the metabolic syndrome, including higher blood pressure, insulin, random glucose, fasting glucose, free fatty acids, visceral adiposity and triglycerides when young adults. The HCR measured higher for health factors such as $\dot{V}_{O_2,max}$, heart function, endothelial nitric oxide formation, economy of oxygen use and levels of transcription factors and oxidative enzymes

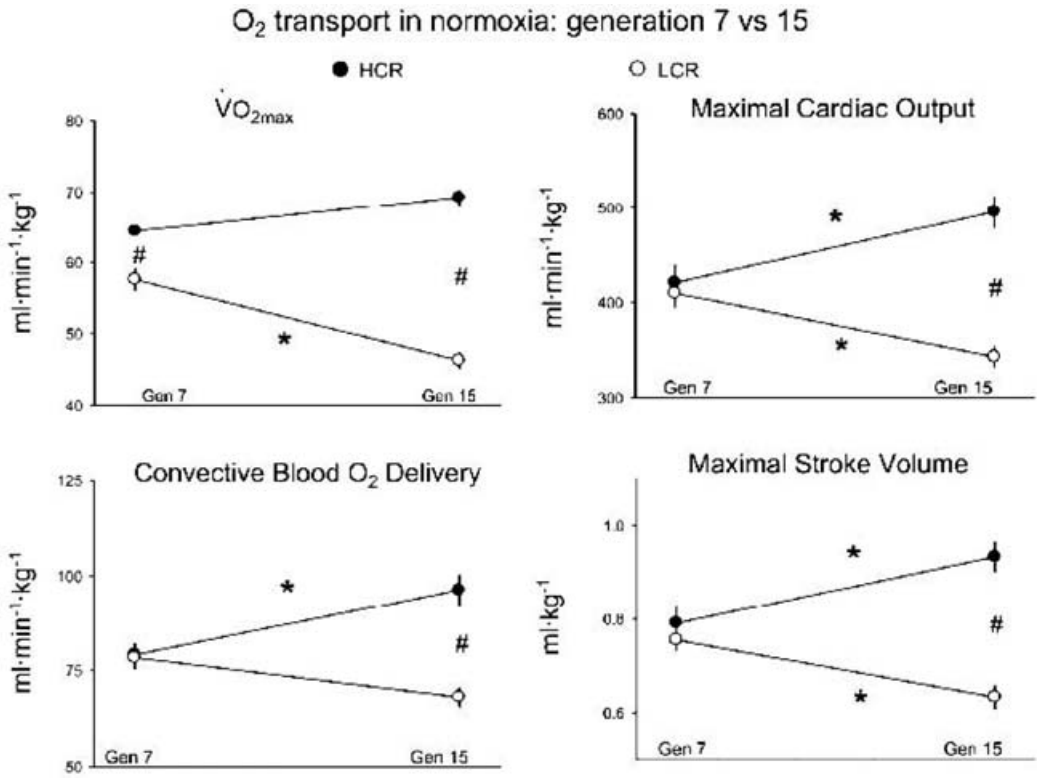

Figure 6. Phenotypes of intrinsic aerobic capacity
O_2 transport and haemodynamic variables during maximal exercise after 7 and 15 generations of selection for aerobic capacity. At generation 7 $\dot{V}_{O_2,max}$ was 12% higher in HCR compared with LCR and was accounted for exclusively by a greater O_2 uptake and utilization by skeletal muscle in the HCR. By generation 15 substantial differences in convective blood flow delivery to the periphery contributed substantially to the 41% greater $\dot{V}_{O_2,max}$ in the HCR relative to LCR. $^{\#}P < 0.05$ HCR *versus* LCR; $^{*}P < 0.05$ generation 7 *versus* generation 15. Figure from Gonzalez *et al.* 2006) and reprinted with permission from the American Physiological Society.

required for mitochondrial function in skeletal muscle. Further study revealed that LCR rats from generations 15 and 17 were more susceptible to ischaemia-mediated cardiac ventricular tachycardia relative to HCR (Lujan *et al.* 2006). This differential in cardiac electrophysiology is important because acute coronary artery occlusion that leads to ventricular arrhythmias is the leading cause of death in humans in developed countries (Akar *et al.* 2005).

Because of the interaction of environments with genetic predisposition to disease, it was of interest to determine if the LCR and HCR respond differently to clinically relevant changes in environments. In the first test of this possibility, Noland *et al.* (2007) evaluated the influence of a high fat diet (HFD) on weight gain patterns, insulin sensitivity and fatty acid oxidative capacity in sedentary male rats. LCR rats fed normal chow were heavier, hypertriglyceridaemic, less insulin sensitive, and had lower skeletal muscle oxidative capacity compared with HCR rats. LCR rats on a HFD gained more weight, fat mass and their insulin resistant condition was exacerbated (Fig. 7), despite consuming similar amounts of energy as chow-fed controls. Remarkably, these metabolic variables remained unaltered in HCR rats when shifted from normal chow to HFD.

Current results suggest the LCR and HCR can serve as models that contrast for disease risks and indirectly support a mechanistic role for oxygen metabolism. As a more direct test of the hypothesis that inefficient energy metabolism in blood vessels can promote vascular disease, Bernal-Mizrachi *et al.* (2005) generated mice with doxycycline-inducible expression of the mitochondrial uncoupling protein-1 (UCP1) in the artery wall. They found that UCP1 expression in aortic smooth muscle cells causes hypertension and increases dietary atherosclerosis without affecting cholesterol levels. UCP1 expression also increased superoxide production and decreased the availability of nitric oxide, suggestive of oxidative stress.

Conclusion

Numerous observations from a variety of perspectives are consistent with a central role for oxygen as a determinant of organismal complexity. An atmosphere with oxygen may be uniquely essential for development of complex life anywhere because it is stable, easy to transport and has a high capacity for energy transfer via redox reactions (Catling *et al.* 2005). The general hypothesis that the steep thermodynamic gradient of an oxygen environment was permissive for the evolution of multicellular complexity is in accord with the principles of thermodynamics. The strong linkage of disease with low aerobic capacity is consistent with a pivotal role of oxygen in our evolutionary history. Nevertheless, even if these clues about the critical role of oxygen are correct, recognizing the mechanistic footprint of oxygen in our evolutionary path remains a challenge.

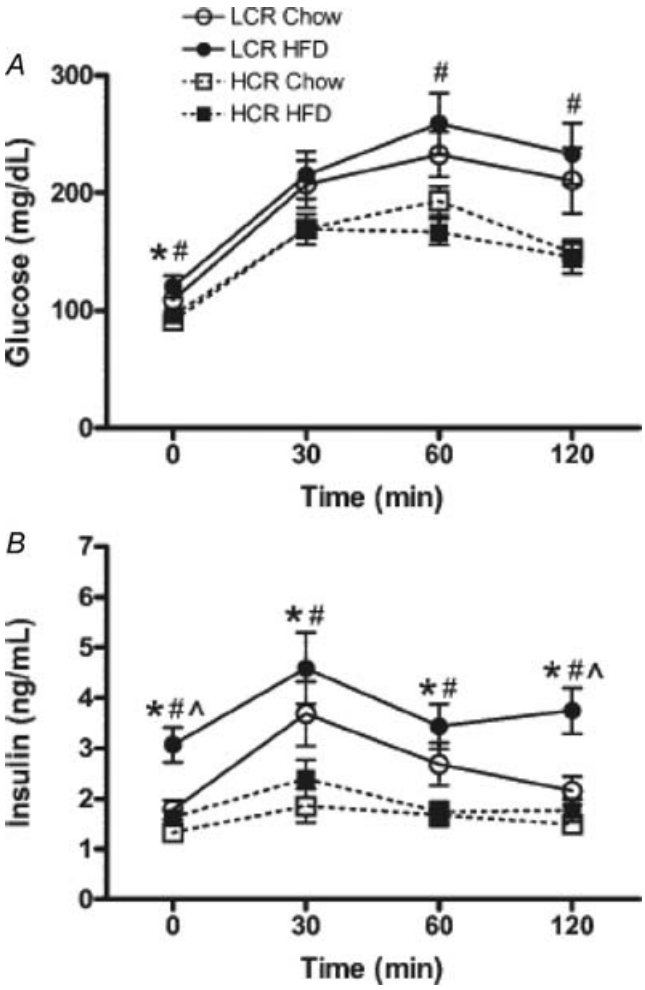

Figure 7. LCR insulin resistant relative to HCR
Blood glucose and serum insulin were measured in response to an oral glucose challenge (2 g glucose (kg body wt)$^{-1}$) in HCR and LCR rats before and after 12 weeks on a high-fat diet (HFD). Basal (time zero) glucose (*A*) and serum insulin (*B*) values were significantly higher in LCR than HCR rats in both the normal chow and HFD conditions. Although the HFD did not alter resting glucose values in either strain, serum insulin levels were elevated exclusively in LCR rats. The insulin response throughout the glucose challenge was significantly higher in LCR rats at all time points for both the chow and HFD (*B*). The HFD had no effect on insulin in HCR rats. These data demonstrate that LCR rats are more prone to the exacerbation of an insulin-resistant condition when exposed to an HFD. HCR rats seem to be protected against the development of impaired glucose tolerance on an HFD. Figure symbols reflect significant differences for a given time point. $P < 0.05$, LCR *versus* HCR on chow (*), LCR *versus* HCR on a high-fat diet ($^{\#}$), and chow *versus* high-fat diet within the LCR strain (·). Data are means ± 1 S.E.M. Figure from Noland *et al.* (2007) and reproduced with permission from the American Physiological Society.

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